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Influence of pH, Emulsifier Concentration, and Homogenization Condition on the Production of Stable Oil-in-Water Emulsion Droplets Coated with Fish Gelatin

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Abstract An oil-in-water (O/W) emulsion [20 wt% corn oil, 0.5–6.0 wt% fish gelatin (FG), pH 3.0] was produced by high pressure homogenization, and the influence of pH, protein concentration, and homogenization condition on the formation of FG-stabilized emulsions was assessed by measuring particle size distribution, electrical charge, creaming stability, microstructure, and free FG concentration in the emulsions. Optical microscopy indicated that there were some large droplets (d>10 μm) in all FG-emulsions, nevertheless, the amount of large droplets tended to decrease with increasing FG concentration. More than 90% of FG was present free in the continuous phase of the emulsions. To facilitate droplet disruption and prevent droplet coalescence within the homogenizer, homogenization time was adjusted in O/W emulsions stabilized by 2.0 or 4.0 wt% FG. However, the increase in the number of pass rather promoted droplet coalescence. This study has shown that the FG may have some limited use as a protein emulsifier in O/W emulsions.

Keywords: oil-in-water emulsion, fish gelatin, droplet aggregation, high pressure homogenization, emulsion stability

Introduction

Proteins extracted from various natural sources can be used as emulsifiers because of their ability to facilitate the formation, improve the stability, and produce desirable physicochemical properties in oil-in-water (O/W) emulsions (1-3). Recently, there has been growing interest in the use of natural protein emulsifiers as replacements for the synthetic emulsifiers currently used in many foods (4-10). Thus, a wide variety of natural proteins including soy, whey, casein, fish, meat, and plant proteins have been screened for utilization as emulsifiers in emulsion-based food products (1-3). Here, this study focused on the utilization of fish gelatin (FG) as an emulsifier in O/W emulsions.

Gelatin is a relatively high molecular weight protein derived from animal collagen, e.g., pig, cow, or fish (11). It is prepared by hydrolyzing collagen by boiling in the presence of either acid (Type A gelatin) or alkaline (Type B gelatin). One of the major advantages of using Type A gelatin over Type B gelatin is that it should be possible to create O/W emulsion droplets that have a positive charge over most of pH range typically found in foods, because of higher isoelectric point (pI) of Type A gelatin (ca.7–9) than that of Type B gelatin (ca. 5). At pH values below their pI, proteins form positively charged interfacial membranes around oil droplets that electrostatically repel any positive transition metal ions (Fe^{2+}/Fe^{3+}, Cu^{+}/Cu^{2+}) present in the aqueous phase, thus could prevent the ions from catalyzing oxidation of lipids contained within the droplets. Compared with Type A gelatin, many other conventional protein emulsifiers such as soy, casein, or whey proteins have pI values in the pH range of 4.5–5.5 (12) and thus cationic O/W emulsions droplets could be produced only at relatively low pH values not at a pH above 5. The higher pH values of many foods would mean that these emulsions would be anionic and thus would attract prooxidative metals resulting in rapid oxidative deterioration of lipids within the emulsion droplets. Consequently, Type A gelatin should provide emulsion based food products with higher oxidative stability than most of other protein emulsifiers should. Among gelatins, FG could be obtained from low value byproducts of fishing industry that are currently discarded (e.g., fish skin, bones, heads, and tails) because they are considered unsuitable for human consumption, thus utilization of FG could increase economic value of them and finally contribute to the development of fishing industry. For these reasons, Type A FG was selected as a candidate protein for screening emulsifying property. However, although the FG used in this study is classified into Type A gelatin, its pI turned out to be lower than those of other Type A gelatins (see Result and Discussion).

To determine its performance as an emulsifier it is critically important whether the FG could produce physically as well as oxidatively stable emulsion. In my previous study, when FG was used in conjunction with anionic surfactant sodium dodecyl sulfate (SDS) by layer-by-layer deposition technique (i.e., O/W emulsion containing emulsion droplets coated by SDS-FG multi-layer membrane was prepared), it provided better stability against droplet aggregation than showed by SDS single membrane (13). Nevertheless, utilization of gelatin as a single emulsifier has been controversial. Some previous studies have shown that gelatin is surface-active thus it is capable of acting as an emulsifier in O/W emulsions (14, 15). In other study, FG has been shown to have good emulsifying properties, however, it often produces large droplets during homo-

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genization (16). Therefore, this study determined whether the FG that showed good physical stability in multi-layer membrane emulsion system could also produce physically stable emulsions when it used as a single emulsifier by assessing the influence of pH and protein concentration on the emulsion formation, otherwise whether the adjustment of homogenization condition could improve the physical instability observed in the FG-stabilized emulsions.

Materials and Methods

Materials Food/pharmaceutical grade FG (Norland dried fish gelatin, solids: 85% min; total ash: 2.0% max; heavy metals: <10 ppm; arsenic: <0.8 ppm; chromium: <10 ppm; lead: <1.5 ppm; sulfur dioxide: <50 ppm) was kindly provided by Norland Products Inc. (Lot # 1253KD; Cranbury, NJ, USA). As stated by the manufacturer, this product is Type A gelatin produced by the hydrolysis of collagen from the skins of deep water fish such as cod, haddock, and pollock, and contains approximately 60 hydroxyproline and 96 proline residues per 1,000 total residues. FG has similar characteristics to animal gelatin with the exception that it gels at lower temperatures (typically <10°C). Its average molecular weight and pI value were reported to be ca. 55 kDa and pH 7, respectively. Analytical grade sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), imidazole, and sodium azide (NaN_3) were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Acetic acid was purchased from Fisher Science (Chicago, IL, USA). Corn oil was purchased from a local supermarket and used without further purification. Distilled and deionized water was used for the preparation of all solutions.

Preparation of solutions and emulsions A buffer solution was prepared by dispersing 10 mM imidazole and acetic acid into water and then adjusting the pH to 3.0 using 1 M HCl. FG solutions used for the determination of optimum pH at which stable FG-coated emulsion was created were prepared by dispersing 1.0 wt% FG into 10 mM imidazole/acetate buffer (pH 3) and diluting to 0.1 wt% with the same buffer, and then they were adjusted to pH 3 to 8 using 1M HCl or 1 M NaOH. FG solutions used for the preparation of FG-stabilized emulsions were prepared by dispersing the desired amount (0.625–7.5 wt%) of the protein powder into buffer solution (10 mM imidazole/acetate buffer, pH 3) and stirring overnight at room temperature to ensure complete hydration. The pH of the FG solutions was adjusted back to pH 3.0 using 1 M HCl if required.

O/W emulsions were prepared by blending 20 wt% corn oil and 80 wt% FG solutions together using a high-speed blender (M133/1281-0; Biospec Products, Inc., Bartlesville, OK, USA) for 2 min. These coarse emulsions were then passed through a 2-stage high-pressure valve homogenizer (LAB 1000; APV-Gaulin, Wilmington, MA, USA) 3 times: 4,500 psi the first stage, 500 psi the second stage. Sodium azide (0.02 wt%) was added to the emulsions as an antimicrobial agent. The emulsions (20 wt% corn oil, 0.5–6.0 (0.5, 1.0, 1.5, 2.0, 4.0, and 6.0) wt% FG, pH 3.0) were then stored at ambient temperature for 24 hr before being analyzed.

Particle size determination To avoid multiple scattering effects, FG-stabilized emulsions (FG emulsions) were diluted to a droplet concentration of approximately 0.005 wt% using 10 mM imidazole/acetate buffer solution (pH 3) and stirred continuously throughout the measurements to ensure the samples were homogenous. The particle size distribution of the emulsions was then measured using a laser light scattering instrument (Mastersizer MSS; Malvern Instruments, Worcestershire, UK). This instrument measures the angular dependence of the intensity of laser light (λ=632.8 nm) scattered by a dilute emulsion, and then finds the particle size distribution that gives the best fit between experimental measurements and predictions based on light scattering theory. The mean particle size was reported as the surface-weighted mean diameter, d_{32} ($=\Sigma n_i d_i^3/\Sigma n_i d_i^2$) or the volume-weighted mean diameter, d_{43} ($=\Sigma n_i d_i^4/\Sigma n_i d_i^3$), where n_i is the number of particles with diameter d_i. It should be noted that the d_{43} value is more sensitive to the presence of large particles than the d_{32} value, and therefore it can give a good indication of droplet aggregation. All measurements were made on 2 freshly prepared samples and results are reported as averages.

ζ-Potential measurements ζ-Potential of the 0.1 wt% FG solutions was measured without further dilution. Emulsions, prior to analysis, were diluted to a droplet concentration of approximately 0.006 wt% with the same buffer solution used for the preparation of the sample. Then, the ζ-potential was determined using a particle electrophoresis instrument (ZEM5002; Zetamaster, Malvern Instruments) that measures the direction and velocity of droplet movement in the applied electric field. The ζ-potential provides an estimate of the *net* charge on a particle measured at the 'shear plane', which depends on the charge on the actual particle (in this case droplets or emulsifiers) plus the charge associated with any ions that move along with the particle in the electric field. An individual ζ-potential measurement was determined from the average of five readings taken per sample.

Optical microscopy Emulsions were gently agitated in a glass test tube before analysis to ensure that they were homogenous. A drop of emulsion was placed on a microscope slide and then covered with a cover slip. The microstructure of the emulsion was then observed using conventional optical microscopy (Nikon Microscope Eclipse E400; Nikon Corporation, Tokyo, Japan). The images were acquired using a CCD camera (CCD-300T-RC; Dage-MTI, Inc., Michigan City, IN, USA) connected to Digital Image Processing Software (Micro Video Instruments Inc., Avon, MA, USA) installed on a computer.

Creaming stability measurements Ten g of emulsion were transferred into a test tube (i.d. 15 mm, height 125 mm), tightly sealed with a plastic cap, and then stored for 1 month at room temperature. Emulsions tend to be separated into an optically opaque cream layer at the top and a transparent (or turbid) layer at the bottom with time. The extent of creaming [serum (%)=$(H_S/H_E)\times 100$] was characterized by measuring total height of the emulsion (H_E) and the height of the serum layer (H_S) every 1 week.

The % serum provided indirect information about the extent of droplet aggregation in an emulsion. All measurements were made on at least 2 freshly prepared samples.

Free protein measurements The concentration of free protein in the continuous phase of the emulsions was determined by Lowry method (17). Emulsions were centrifuged at 288,520×g for 40 min at 25°C (Sorvall Centrifuges T-1270; Du Pont Co., Wilmington, DE, USA) to separate the oil droplets from the serum phase. The serum phase was collected using a syringe and then diluted with 10 mM imidazole/acetate buffer solution to the proper concentration for spectrophotometer measurement. One mL of Lowry stock reagent (2% Na_2CO_3, 1% $CuSO_4$, 2% $NaKC_4H_4O_6$; 49:0.5:0.5; v/v/v) was added to 100 μL of diluted serum phase and thoroughly mixed using a vortex, then left to stand for 30 min. One-hundred μL of folin's reagent diluted in distilled water (1:1, v/v) was then added to the solution, which was then mixed and left to stand for 30 min. The absorbance was measured at 595 nm in a spectrophotometer (UV-2101 PC; Shimadzu Corp., Kyoto, Japan). The protein concentration of the emulsion serum was then determined using a calibration curve of absorbance vs. protein concentration produced using FG as the standard.

Influence of homogenization condition on the stability of FG emulsions The influence of homogenization condition on the mean particle diameters, particle size distribution, creaming stability, and microstructure of the emulsions (20 wt% corn oil, pH 3) stabilized by 2.0 or 4.0 wt% of FG was examined. The homogenization condition was adjusted by increasing number of passes (3, 5, 7, and 10 passes) through a homogenizer at a constant pressure of 5,000 psi. O/W emulsion samples (20 wt% corn oil, 2.0 or 4.0 wt% FG, 10 mM imidazole/acetate, pH 3) depending on the homogenization condition were prepared as described in the emulsions preparation section. Then, the emulsion samples (10 g) were transferred into glass test tubes (i.d. 15 mm, height 125 mm) and stored at room temperature for 24 hr before being analyzed.

Statistical analysis Experiments were performed twice using freshly prepared samples. Averages and standard deviations were calculated from these duplicate measurements.

Results and Discussion

Influence of pH on net electrical charge of FG-dispersed buffer solution Influence of pH (3 to 8) on the ζ-potential of FG solutions that were prepared using 10 mM imidazole/acetate buffer was examined (Fig. 1). The ζ-potential of the FG solutions went from positive (12.8 ±3.8 mV) to negative (−7.9±2.5 mV) as the pH was increased from 3 to 8. The ζ-potential vs. pH measurements suggested that the pI value of the FG was at between 4.0 and 4.5. The value is appreciably lower than the pI value (pH 7) reported by the FG supplier. The absolute value of the ζ-potential across the whole pH range from 3 to 8 was highest at pH 3 and lowest at pH 4.5, which is very important observation for the preparation

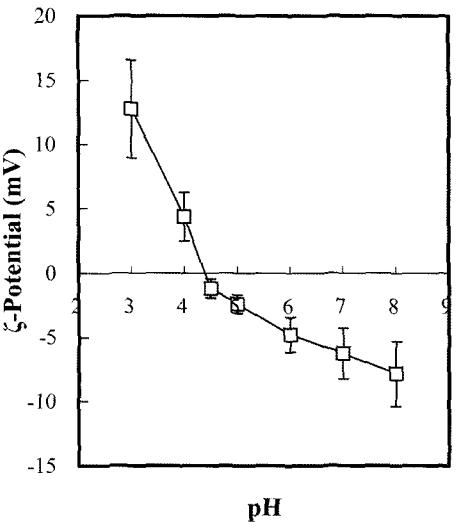

Fig. 1. Influence of pH on net electrical charge (ζ-potential) of FG dissolved in 10 mM imidazole/acetate buffer.

of emulsions stabilized by protein. Protein-stabilized emulsions are particularly sensitive to pH and tend to flocculate at pH values close to the isoelectric point of the adsorbed proteins, and the interfacial membranes formed by proteins are usually relatively thin and electrically charged, hence, the major mechanism preventing droplet flocculation in protein stabilized emulsions is electrostatic repulsion, rather than steric repulsion (2). Therefore, pH 3 was selected for the preparation of FG-stabilized emulsions since pH 3 was far enough from the pI of FG and the absolute electrical charge of FG solution was highest at the pH 3.

Influence of emulsifier concentration on the production of FG-stabilized emulsions Influence of FG concentration (0.5 to 6.0 wt%) on the formation and stability of corn oil-in-water emulsions produced by 2-stage high pressure valve homogenization was examined.

The ζ-potential of the droplets in the FG emulsions was positive at pH 3.0 (Fig. 2). Separately, corn oil-in-water emulsions stabilized by 0.5−3.0 (0.5, 1.0, 1.5, 2.0, and 3.0) wt% FG were also prepared at pH 6.0 where ζ-potential of the emulsion droplets was negative close to zero (Fig. 2). The results could be attributed to the fact that the net electrical charge of adsorbed FG particle on the surface of the oil droplets was positive at pH 3.0 and negative at pH 6.0 (Fig. 1). It suggests that the measurement of electrical charge of protein solution over a range of pH could be a good indicator for selecting optimum pH at which stable emulsions coated by the protein are well created. There was no significant change in ζ-potential with FG concentration, with the average over all FG concen- trations being ζ=18.2±0.8 mV (i.e., less than 5% of relative standard deviation) for the FG-stabilized emulsions. The fact that the emulsion droplets were coated by a biopolymer with an electrical charge suggests that electrostatic repulsion may play an important role in stabilizing them against droplet aggregation (3). However, the magnitude of the droplet ζ-potential of the FG emulsions was relatively low ($|\zeta|$ <20 mV), so that the

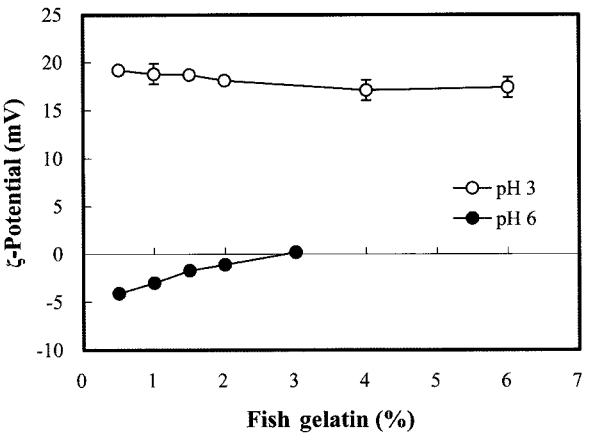

Fig. 2. Influence of FG concentration on electrical charge of oil droplets in FG-stabilized oil-in-water emulsions (20 wt% corn oil, 10 mM imidazole/acetate buffer).

electrostatic repulsion between the droplets might not be sufficient to overcome the attractive droplet-droplet interactions (e.g., van der Waals and hydrophobic attraction). Nevertheless, FG-stabilized emulsions showed good creaming stability, which suggests that factors other than electrostatic interaction might be involved in stabilizing the emulsions against droplet aggregation.

The volume-surface mean particle diameter (d_{32}, which is more sensitive to the presence of small particles) of the emulsions was relatively small (<0.4 μm) and independent of FG concentration: 0.27±0.05 μm (Fig. 3A). On the other hand, there was a fairly steep decrease in the volume-weighted mean particle diameter (d_{43}, which is more sensitive to the presence of large particles) when the FG concentration was increased from 0.5 to 2.0 wt%, after which the mean particle diameter slowly decreased and eventually reached a relatively constant value: 0.35 μm (Fig. 3B). At the FG concentrations of ≤2.0 wt% (where the ratio of FG to dispersed phase was ≤1:10), the particle size distributions were either bimodal or multi-modal, consisting of a major peak corresponding to a large fraction of relatively small droplets and minor peaks corresponding to small fractions of relatively large particles (Fig. 3C). The small population of large droplets observed in the emulsions stabilized by 0.5–2.0 wt% FG may have been due to insufficient FG coverage for the entire oil-water interface created during homogenization, or because some droplet coalescence occurred during or after homogenization. On the other hand, the particle size distributions of FG emulsions appeared mono-modal at higher FG concentrations (here 4.0 and 6.0 wt%, which correspond 1:5 and 1:3.3 of the ratio of FG to disperse phase respectively). This is a typical phenomenon of protein-stabilized emulsions, and possible reasons to account for

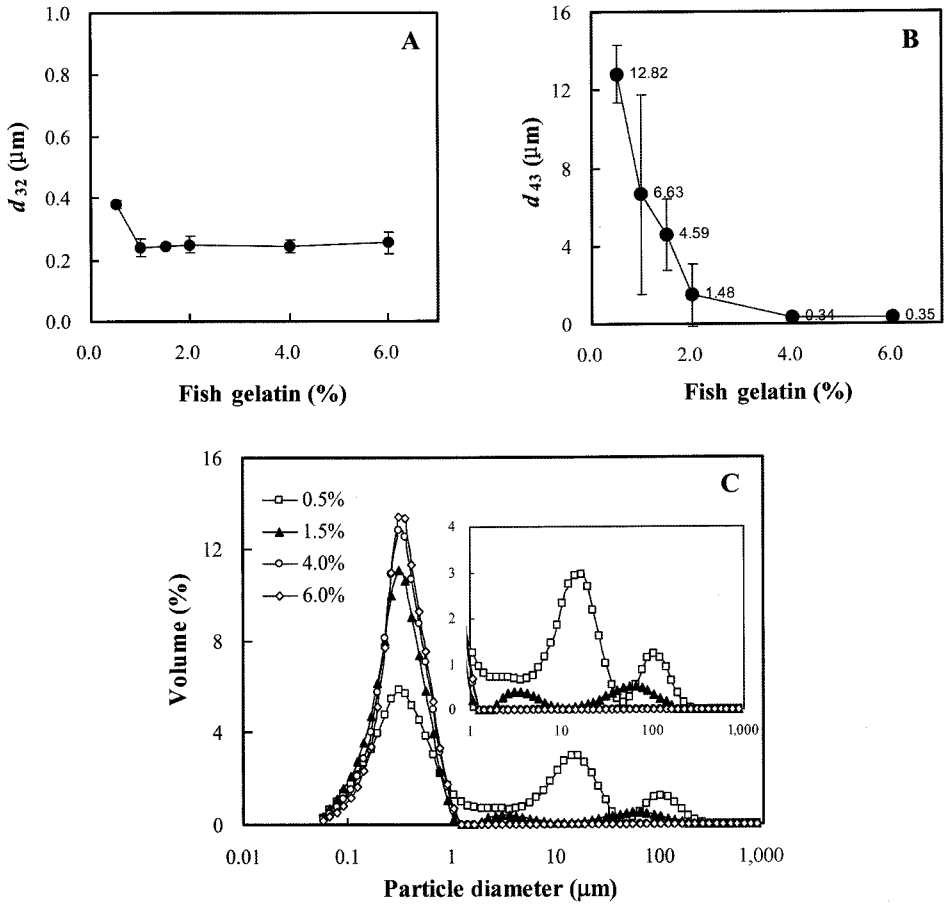

Fig. 3. Influence of FG concentration on mean particle diameters d_{32} (A) and d_{43} (B), and particle size distribution (C) of 20 wt% corn oil-in-water emulsion stabilized by 0.5–6.0 wt% FG.

the observed decrease in mean droplet size with increasing protein concentration have been well documented in the literatures (3, 18, 19): (i) the total droplet surface area that could be stabilized by the protein increased; (ii) the rate at which the droplet surfaces were covered with protein increased; (iii) the frequency of droplet collisions decreased due to the increase in aqueous phase viscosity.

My previous studies with laser diffraction suggested that measured particle size of droplets in diluted emulsions did not always reflect actual droplet characteristics of original emulsions that were not diluted (19, 20), which was attributed to sampling errors associated with the laser diffraction instrument. Therefore, a range of different analytical techniques should be employed to accurately characterize the stability of emulsions prone to droplet aggregation. Here, creaming stability and optical microscopy measurements were conducted additionally to provide information about droplet characteristics of original emulsions. No creaming was observed in all FG emulsions during the experimental period of 1 month regardless of FG concentration (Fig. 4). Initially, this result seemed surprising because laser diffraction measurement indicated the presence of large droplets (i.e., droplet aggregation, either droplet flocculation or droplet coalescence) in the emulsions stabilized by ≤2.0 wt% of FG (Fig. 3B and 3C). According to Stoke's law emulsion droplets move upward with increasing time and then form a creamed layer, during which process larger droplets tend to cream more rapidly than the smaller ones (3). In typical conditions for measuring creaming stability, one month is enough time to monitor any changes. There are a couple of possible reasons to explain the unexpected good creaming stability of the FG emulsions: (i) FG formed a high viscosity solution that slowed down droplet movement; (ii) the droplets were aggregated into a particle network that prevented them from moving; (iii) the disperse phase volume fraction that is associated with droplet concentration exceeded a critical value where the droplets became so closely packed that they couldn't easily move past each other.

Optical microscopy indicated that there were some large

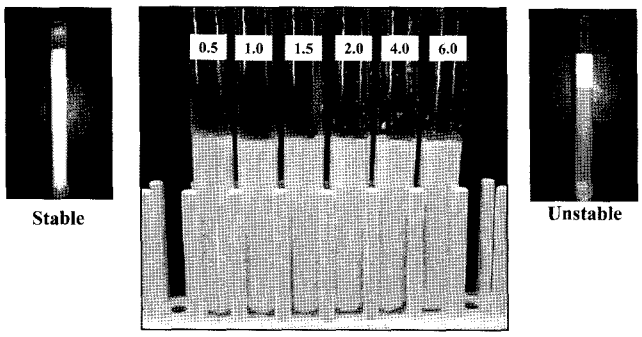

Fig. 4. Influence of FG concentration (0.5–6.0 wt% FG) on creaming stability of FG-stabilized emulsions. Pictures were taken at 1 month after preparation, and no creaming was observed visually in all FG-stabilized emulsions. Typical pictures of creaming were cited from my previous work (20) for comparison purpose ('Stable' emulsion with no serum layer, and 'Unstable' emulsion that are separated into cream layer at the top and serum layer at the bottom).

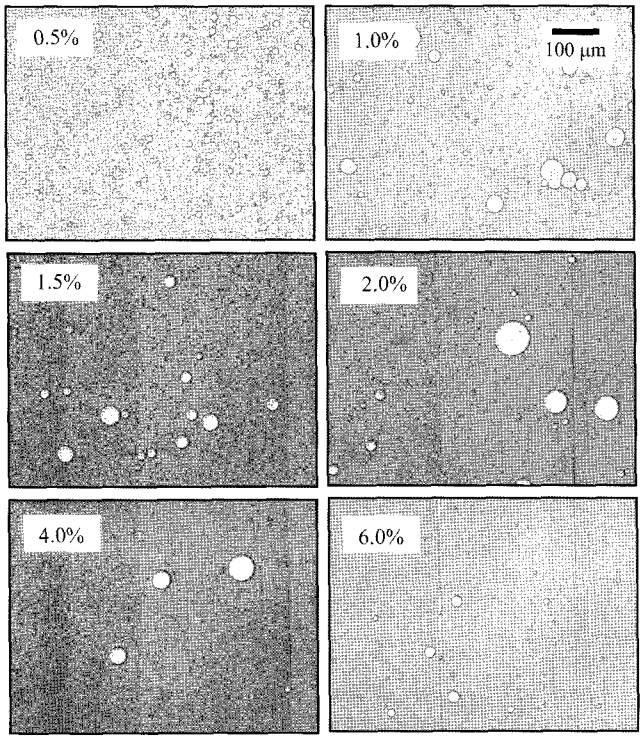

Fig. 5. Photomicrographs of 20 wt% corn oil-in-water emulsions stabilized with fish gelatin (0.5–6.0 wt% FG). More than 6 pictures were taken per each emulsion and a representative one was presented.

droplets (d>10 μm) in all FG-stabilized emulsions regardless of FG concentration (Fig. 5). Initially, I assumed that the FG contained in the emulsions was not sufficient to completely cover all of the oil droplet surface created within the homogenizer because large populations of relatively large droplets observed in the emulsions stabilized by 0.5 wt% FG were appreciably decreased with increasing FG concentration (Fig. 3B and 5) and because the decrease in emulsion droplet size with increasing emulsifier concentration had been well established (3, 18, 19). However, this doesn't always seem likely in FG-stabilized emulsions because there was little difference in mean particle size (Fig. 3) and microstructure (Fig. 5) between emulsions stabilized by 4 and 6 wt% FG, which suggested that FG concentration (or the ratio of FG to dispersed phase) was not a critically limiting factor determining droplet size in the FG emulsions. Some previous studies also have shown that gelatin often produces relatively large droplet size during homogenization even at high concentration (14, 16). For all of the FG emulsions measured, more than 90% of FG added as an emulsifier was present free in the continuous phase of the emulsions (Fig. 6), which indicated that only small amounts of FG were present at the oil-water interface when the droplets were saturated. There are a number of possible reasons to explain the above observations: (i) the adsorption time taken for the FG to move from the continuous phase to the droplet surface was not fast enough to prevent oil droplets from merging together, thus total droplet surface area that could be adsorbed by FG were decreased; (ii) FG molecule was not efficiently adsorbed to the surface of a

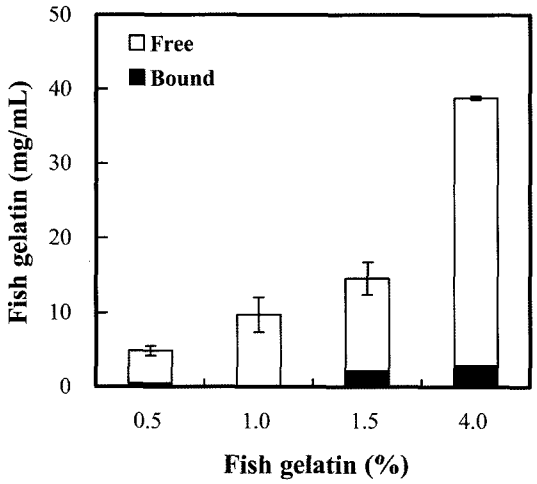

Fig. 6. Free FG protein in the continuous phase of FG-stabilized emulsions depending on FG concentration. Based on the microstructures of FG emulsions presented in Fig. 5, emulsions stabilized by 0.5, 1.0, 1.5, and 4.0 wt% FG were selected as representatives for the measurements.

droplet during an encounter between it and oil droplet because of its relatively high hydrophilic character. Previous study has shown that hydrophobically modified gelatin by attachment of non-polar side-groups produces smaller droplets than non-modified gelatin does (21); (iii) the FG membrane surrounding oil droplets was not effective at protecting the droplets against coalescence after emulsification. All of these three factors depend on the structural and physicochemical properties of FG; (iv) there was no sufficiently intense disruptive forces that cause the larger droplets to be broken down to smaller ones, however, which is unlikely (see below). This fourth factor depends primarily on the energy input of the homogenizer that could be enhanced by increasing the homogenization pressure or recirculating the emulsion through the homogenizer.

Overall, there was always droplet aggregates in the O/W emulsions stabilized by FG. Nevertheless, relatively stable emulsions where the majority of oil droplets were small could be formed by at least 4 wt% FG (see Fig. 3 and 5): i.e., the ratio of FG to disperse phase should be ≥1:5. This ratio value is relatively high compared to most other food protein emulsifiers which have less than 1:10 (3).

Influence of homogenization condition on the stability of FG emulsions Free FG measurements indicated that there were sufficient emulsifiers to cover the surfaces of the oil droplets formed in a homogenizer (Fig. 6), thus it was determined if the droplets size of the FG emulsion could be reduced by increasing the duration of disruptive energy supplied during homogenization. In particular, the influence of homogenization time (or number of pass through a homogenizer) on the stability and properties of the FG emulsions was examined by measuring mean particle diameters (Table 1), particle size distribution (Fig. 7), microstructure (Fig. 8), and creaming stability of the emulsions.

Under most circumstances there is a decrease in droplet

Table 1. Influence of homogenization condition on mean particle diameters and particle size distribution of the emulsions stabilized by FG

FG (%)	No. of pass[1)]	d_{32} (μm)	d_{43} (μm)	PSD[2)]
2.0	3	0.23	1.05	t
	5	0.27	0.57	t
	7	0.24	3.72	t
	10	0.19	2.46	t
4.0	3	0.22	0.33	m
	5	0.28	0.32	m
	7	0.26	0.48	b
	10	0.21	0.47	t

[1)]Number of passes through 2-stage high pressure valve homogenizer at 5,000 psi.
[2)]Particle size distribution; m, b, and t represent monomodal, bimodal, and trimodal distribution, respectively.

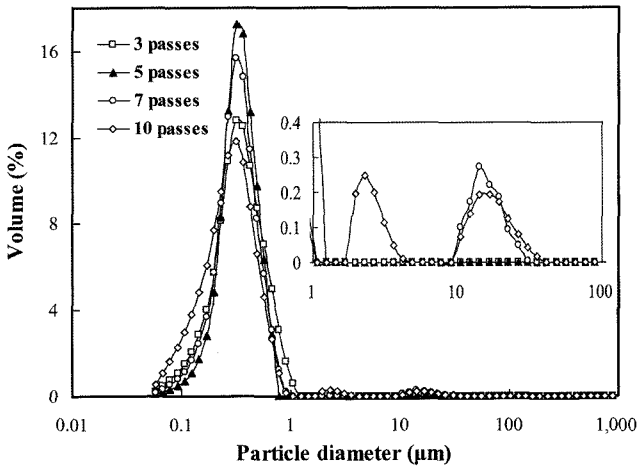

Fig. 7. Influence of number of pass through the homogenizer on particle size distribution of FG-stabilized emulsions (20 wt% corn oil, 4.0 wt% FG, 5,000 psi).

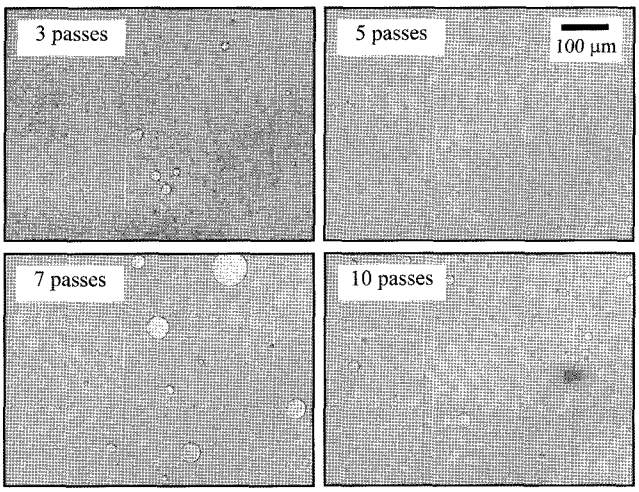

Fig. 8. Influence of number of pass through the homogenizer on microstructures of emulsions stabilized by 4.0 wt% FG (20 wt% corn oil, 5,000 psi).

size as the energy input is increased because droplets tend to be disrupted when the disruptive forces generated within the homogenizer exceed the interfacial forces that hold the droplets together (3). Nevertheless, there were increases in droplet sizes of the emulsions stabilized by 2.0 or 4.0 wt% FG as the number of pass through the high pressure valve homogenizer increased (Table 1 and Fig. 8). For the emulsions containing 4.0 wt% FG, particle size distributions turned into multi-modal from mono-modal as the number of pass increased (Fig. 7). It could be attributed that the adsorption of FG might have been relatively slow compared to the duration of the applied stresses and droplet encounter frequency and/or the interfacial membrane surrounding oil droplets might not have been sufficiently strong to protect droplets from coalescing with one another during repeated droplet-droplet collisions: i.e., as the homogenization proceeded, some of the FG molecules on an oil droplet might have been dragged along the interface, leaving some regions of the oil droplet where there is an excess of FG and other regions where there is a depletion of FG. Two FG-depleted regions on different droplets might have come into close proximity and coalesced during a droplet-droplet encounter. This mechanism of coalescence has been reported in some protein-stabilized emulsions where the interfacial membranes are highly cohesive and particularly under high applied mechanical stresses (2, 3). No creaming was observed in all FG emulsions depending on the homogenization condition.

In conclusion, the aim of this study was to determine if O/W emulsions stabilized by FG that has relatively high hydrophilic character could be created using a high pressure valve homogenizer. Therefore we examined potential factors (aqueous phase pH, FG concentration, and homogenization time) influencing on the preparation of stable FG-emulsions. Laser diffraction instrument indicated that the emulsions with mono-modal particle size distributions and small mean droplet diameters (d_{43}=0.35 μm) could be produced at FG concentrations ≥4.0 wt%, which concentrations correspond ≥1:5 of FG:disperse phase. However, small fractions of relatively large droplets (>10 μm) were observed under microscopy even at these relatively high FG concentrations. In an attempt to prevent droplet coalescence and facilitate droplet disruption while preparing the FG-stabilized emulsions, homogenization time was adjusted. However, increase in the number of pass through the homogenizer rather promoted droplet coalescence in the FG emulsions. Most of FG added as emulsifiers turned out to be present free in the continuous phase of the FG-stabilized emulsions, which suggested that the surface load reflecting the effectiveness of a protein as an emulsifier should be low in the FG emulsions. This study suggests that the FG may have some limited use as a single protein emulsifier in O/W emulsions, however, good creaming stability observed in the FG-emulsions implicates that the FG may contribute to the improvement of emulsion stability when it is used in conjunction with surface active emulsifiers.

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