THE Society for Food Science & Technology

Journal of Food Science

A Publication of the Institute of Food Technologists

Rheology and Oxidative Stability of Whey Protein Isolate-Stabilized Menhaden Oil-in-Water Emulsions as a Function of Heat Treatment

CHANGHUI SUN AND SUNDARAM GUNASEKARAN

ABSTRACT: Menhaden oil-in-water emulsions (20%, v/v) were stabilized by 2 wt% whey protein isolate (WPI) with 0.2 wt% xanthan gum (XG) in the presence of 10 mM $CaCl_2$ and 200 μM EDTA at pH 7. Droplet size, lipid oxidation, and rheological properties of the emulsions were investigated as a function of heating temperature and time. During heating, droplet size reached a maximum at 70 °C and then decreased at 90 °C, which can be attributed to both heating effect on increased hydrophobic attractions and the influence of $CaCl_2$ on decreased electrostatic repulsions. Combination of effects of EDTA and heat treatment contributed to oxidative stability of the heated emulsions. The rheological data indicate that the WPI/XG-stabilized emulsions undergo a state transition from being viscous like to an elastic like upon substantial thermal treatment. Heating below 70 °C or for less than 10 min at 70 °C favors droplet aggregation while heating at 90 °C or for 15 min or longer at 70 °C facilitates WPI adsorption and rearrangement. WPI adsorption leads to the formation of protein network around the droplet surface, which promotes oxidative stability of menhaden oil. Heating also aggravates thermodynamic incompatibility between XG and WPI, which contributes to droplet aggregation and the accumulation of more WPI around the droplet surfaces as well.

Keywords: droplet aggregation, droplet size, lipid oxidation, rheology, thermodynamic incompatibility, whey protein isolate, xanthan gum

Introduction

Proteins and polysaccharides are two of the most important functional biopolymers added in emulsion-based food products to control the stability, texture, shelf life, and overall appearance of food emulsions (Dickinson and McClements 1995). Whey protein isolate (WPI) is an excellent emulsifier and widely used in food emulsions due to its surface-active property. WPI is adsorbed at the oil–water interface to form a protective film and provides structural support for oil droplets through a combination of electrostatic and steric interactions (Reiffers-Magnani and others 2000; Djordjevic and others 2004; Gwartney and others 2004). Xanthan gum (XG) is an extracellular polysaccharide produced by *Xanthomonas campestris* and is a good choice to stabilize food emulsions due to its desirable stabilizing and antioxidant properties as we previously reported (Sun and others 2007). XG is a nonadsorbing polysaccharide since the individual molecules do not physically associate with each other (Euston and others 2002). One of the most remarkable properties of XG is its pseudoplasticity: when XG is added to liquids, it provides a large increase in the viscosity at rest and yet the liquids can be easily poured. Unlike many other hydrocolloids, XG is very stable under a wide range of temperatures and pHs.

Ca^{2+} is nutritionally valuable in food products with a typical concentration ranging from 10 to 30 mM (Keowmaneechai and McClements 2006). However, the addition of Ca^{2+} may stabilize or destabilize oil-in-water (O/W) emulsions depending on the concentration of added Ca^{2+} and the protein. Dickinson and Golding (1998) and Dickinson and others (2003) reported that moderate concentration of Ca^{2+} may inhibit depletion flocculation completely because addition of Ca^{2+} may substantially reduce the osmotic pressure of protein in the aqueous phase, leading to the free energy of depletion interaction too small to flocculate. Ethylenediaminetetraacetate (EDTA), a chelating agent, is one of the most commonly used food ingredients for sequestering multivalent ions (Miller 1996; Keowmaneechai and McClements 2002). EDTA is probably the most successful and effective antioxidant used in O/W emulsions, which acts by preventing metal redox cycling, occupying metal coordination sites, and steric hindrance of interactions between lipids and metals (McClements and Decker 2000; Alamed and others 2006). However, addition of Ca^{2+} in O/W emulsions may decrease the effectiveness of EDTA in chelating iron, a dominant prooxidant, because Ca^{2+} can compete to bind EDTA with iron (Keowmaneechai and McClements 2006). Sun (2006) reported that addition of 10 mM $CaCl_2$ and 200 μM EDTA before heating protected oil droplets against oxidation and stabilized the emulsions via ionic bonding and electrostatic screening.

During the preparation of emulsions-based food products, heating is almost inevitably required at some stage from mixing of the ingredients to packaging of the final product. Such thermal treatments can result in protein denaturation in protein-based food emulsions due to the conformational changes that expose nonpolar and reactive thiol groups and cause protein aggregation via noncovalent interactions and covalent disulphide bond formation (Verheul and Roefs 1996). Main forces involved in the stability of protein structure are hydrogen bonds, electrostatic interaction, hydrophobic effects, and disulfide bonds. Heating of protein solutions may weaken hydrogen bonds and electrostatic interactions but strengthen hydrophobic effects (Kim and others 2005). Partial denaturation of WPI usually improves its emulsifying capacity due to an increase in the surface hydrophobicity and molecular flexibility (Kato and others 1983). However, extensive denaturation

MS 20090607 Submitted 6/28/2009, Accepted 9/15/2009. *Authors are with Food & Bioprocess Engineering Laboratory, Biological Systems Engineering Dept., Univ. of Wisconsin-Madison, Madison, WI 53706, U.S.A. Direct inquiries to author Gunasekaran (E-mail: guna@wisc.edu).*

© 2009 Institute of Food Technologists®
doi: 10.1111/j.1750-3841.2009.01387.x
Further reproduction without permission is prohibited

may result in poor interfacial mechanical properties, which would be detrimental to long-term stability of emulsions (Kim and others 2005). Addition of XG may affect the denaturation and aggregation behaviors of WPI. Bryant and McClements (2000) observed phase separation in the mixed solution of XG and heat-denatured WPI due to thermodynamic incompatibility.

The objective of this study was to investigate droplet size, oxidative stability, and rheological properties of WPI-stabilized emulsions containing XG as a function of heating temperature and time in the presence of $CaCl_2$ and EDTA. This study would help understand the mechanism of interactions between heat-denatured WPI and XG in the emulsions and facilitate formulation of food emulsions with desirable texture and appearance.

Materials and Methods

Materials

Menhaden oil (eicosapentaenoic acid [EPA], 8% to 18%; docosahexaenoic acid [DHA], 7% to 18%; total ω-3 PUFAS, 20% to 26%; 200 THBQ and 1000 ppm mixed tocopherols) was obtained from Omega Protein, Inc. (Reedville, Va., U.S.A.). WPI (protein, 98 wt%; moisture, 4.4 wt%; ash, 1.8 wt%; fat, 0.3 wt%) was obtained from Davisco Foods Intl., Inc. (Eden Praire, Minn., U.S.A.). XG was purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis., U.S.A.). Cumene hydroperoxide was purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Para-anisidine, ammonium thiocyanate, ferrous sulfate, barium chloride, isooctane, isopropanol, butanol, and methanol were purchased from Fisher Scientific (Fair Lawn, N.J., U.S.A.). All other reagents were of analytical grade or purer and distilled water was used to prepare all the solutions.

Emulsion preparation

WPI solutions and XG solutions were prepared separately by dissolving measured quantities of WPI and XG powders into distilled water at room temperature, followed by stirring for 6 h to ensure complete dispersion. O/W emulsions were prepared by slowly mixing menhaden oil into WPI solutions, then adding XG solutions into the mixture of WPI solution/menhaden oil, finally emulsifying the mixtures of WPI solution/menhaden oil/XG solution 3 times with a PowerGen 125 homogenizer at 75-W output for 2 min (Fisher Scientific). To the WPI/menhaden oil/XG mixture, 0.11 wt% of $CaCl_2$ powder was added right before emulsification to obtain 10 mM $CaCl_2$ in the final emulsion. Certain volume of 10 mM EDTA solution was added right after emulsification to get 200 μM EDTA in the final emulsions. Sodium azide (1%, w/v) was added as an antimicrobial agent. The additions of $CaCl_2$ and EDTA changed the pH values of the emulsions so 0.1 N HCl and 0.1 N NaOH were mixed to adjust pH values of the final emulsions to 7. The final emulsions contained 20 v/v% menhaden oil, 2 wt% WPI, 0.2 wt% XG, 10 mM $CaCl_2$ and 200 μM EDTA, and 0.02% (w/v) sodium azide at neutral pH. All emulsions were prepared in duplicates.

Effect of heating temperature and heating time

Four 60-mL aliquots of emulsions were transferred to 125-mL Erlenmeyer flasks. One was kept at room temperature (25 °C) and other 3 in a water bath for 5 min at 55, 70, and 90 °C, respectively. Emulsions were heated for 5 min because prolonged heating at 90 °C would lead to the formation of elastic gels, which cannot be compared in parallel with the emulsions heated at other temperatures. Therefore, to determine the effect of heating time, only 1 temperature was used (70 °C) for different durations of 0 to 30 min. After the heat treatment, the emulsions were cooled down to room temperature by placing the Erlenmeyer flasks in a water bath at room temperature. All the emulsions were prepared in duplicates.

Droplet size characterization

A particle size analyzer (90Plus, Brookhaven Instruments Corp., N.Y., U.S.A.) was used to determine the surface-area-average diameter (d_{32}) of the emulsions. The d_{32} of the droplets was calculated as:

$$d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$$

where n_i is the number of droplets of diameter d_i. To prevent multiple scattering effects, the emulsions were diluted with distilled water or 10 mM $CaCl_2$ solution to keep the concentration typically between 10^{-5} and 10^{-2} volume fractions prior to particle size measurements. At such low concentration, the difference between oil droplet volume and liquid volume is insignificant, and the difference between volume fraction ϕ and mass concentration is often ignored. The refractive index of the emulsion droplets was 1.456.

Rheological measurements

Steady shear and dynamic shear measurements were performed using a dynamic rheometer (Bohlin CVOR, Malvern Instruments Inc., Southborough, Mass., U.S.A.). The 40-mm dia, 4° cone angle cone-and-plate geometry (CP 40/4°) was used for steady shear tests and 20-mm parallel plate (PP 20) geometry was used for dynamic shear tests. All measurements were performed within 24 h after emulsion preparation. Emulsion viscosity was measured at 25 ± 0.1 °C over a shear rate range of 0.0716 to 100 per second. Power-law model was used to analyze the flow curves: $\sigma = K \cdot (\dot{\gamma})^n$, where σ is shear stress, K is consistency index, $\dot{\gamma}$ is shear rate, and n is flow behavior index.

For dynamic shear tests, the emulsions were poured directly on the holding stage of the rheometer and covered with a thin layer of paraffin oil to prevent water evaporation during measurement. Strain sweep tests were performed at a fixed frequency of 1 Hz and 25 °C isothermal conditions. The amplitude of strain was swept from 0.01 to 10 with a logarithmic scale increment. Both elastic modulus G' and viscous modulus G'' were measured as a function of strain (γ). Strain sweep was performed first to determine the linear viscoelastic region. Frequency sweep tests in the range of 0.1 to 10 Hz were performed at constant strain (within the linear viscoelastic region). The frequency at the $G' - G''$ crossover point is called the characteristic frequency (f_c), which is related to the relaxation time of the system (Tadros 2004). Phase angle (δ) curves exhibit the relationship between G' and G'', that is, $\tan \delta = G''/G'$; so a crossover occurs at $\delta = 45°$.

Lipid oxidation measurements

The effects of temperature and heating time on oxidative stability of WPI-stabilized emulsions were evaluated during 2 wk of storage at room temperature. Emulsion samples (40 mL) were taken in sealed 150-mL Erlenmeyer flasks and covered with aluminum foil to avoid exposure to light, which is an oxidation initiator. Peroxide value (PV) and anisidine value (AV) were measured to evaluate oxidative stability of oil droplets in the heated emulsions.

The PV was determined by adding 0.3 mL emulsion to 1.5 mL of isooctane/isopropanol (3 : 2, v/v), followed by vortexing 3 times for 10 s each. After centrifuging for 2 min at 10000 rpm, 0.2 mL of the clear upper solvent layer was collected and mixed

with 2.8 mL of methanol/1-butanol (2 : 1, v/v) and 30 μL of thiocyanate/Fe^{2+} solution and then vortexed. The thiocyanate/Fe^{2+} solution was made by mixing 1 part 3.94 M thiocyanate solution with 1 part 0.072 M Fe^{2+} solution (obtained from the supernatant of a mixture of 1 part 0.144 M $FeSO_4$ and 1 part 0.132 M $BaCl_2$ in 0.4 M HCl). Absorbance was measured at 510 nm after 20 min incubation at room temperature. Lipid hydroperoxide concentrations (measured as PV) were determined using a cumene hydroperoxide standard curve (Richards and others 2002). The induction period is the time when the PV sharply increase.

The AV test was modified according to the British Standard Method (Rossell 1986): 0.5 to 4 mL emulsion was added into a 25-mL volumetric flask and made up to the mark with isooctane. The test tubes were vortexed twice for 10 s each. After centrifugation for 10 min at 5000 rpm, the absorbance (A_1) of the samples was measured at 350 nm against a pure isooctane blank using spectrophotometer (UV-1601PC, Shimadzu Corp., Columbia, Md., U.S.A.). Sample (5 mL aliquot) or 5 mL isooctane (as blank) was then transferred to 10-mL test tubes and 1-mL para-anisidine solution (0.25% [w/v] solution in glacial acetic acid) was added. After vortexing for 10 s and standing for 10 min, its absorbance (A_2) was measured at 350 nm against the isooctane blank containing para-anisidine. The AV were calculated using the following equation:

$$\mathrm{AV} = \frac{25 \times (1.2 \times A_2 - A_1)}{\text{Sample mass}}$$

Statistical analysis

All experiments were performed in duplicates. Analysis of variance (ANOVA) was used to determine if the means of responses were significant ($P < 0.05$) (Microsoft Excel 2003, Microsoft Corp., Redmond, Wash., U.S.A.).

Results and Discussion

Effects of heating temperature and time on droplet size (d_{32})

The effects of heating temperature and dilution agent on droplet size are shown in Figure 1. The data show that heating temperature had no significant effect on droplet size when the measurements were taken upon the dilution of distilled water. However, when the emulsions were diluted by 10 mM $CaCl_2$, droplet size first reached a maximum at 70 °C and then decreased at 90 °C. The effect of heating time at 70 °C on droplet size is shown in Figure 2. Upon water dilution, droplet size was independent of heating time. However, upon $CaCl_2$ dilution, d_{32} increased initially to 3 μm for 10 min of heating treatment but it subsequently decreased to 1.5 μm for 30 min. The effect of heating time at 70 °C on droplet size shows a trend somewhat similar as heating temperature. Apparently, the emulsion droplet aggregate via depletion flocculation in the initial stage of heating and further heating causes the aggregates to fall apart. Such a change in droplet size upon $CaCl_2$ dilution has been reported in whey protein-stabilized O/W emulsions without XG (Demetriades and others 1997; Sliwinski and others 2003). These results indicate that nonadsorbing XG does not complex with heat-denatured WPI at the droplet surfaces. A significant fraction of 2 wt% of WPI left unadsorbed in the aqueous phase after sufficient amount of WPI is adsorbed at the droplet surfaces to adequately stabilize the emulsions droplets (Sun and Gunasekaran 2009). During heating, both XG and unadsorbed WPI in the aqueous phase contribute to the enhancement of droplet aggregation via depletion flocculation.

The differences between the results of water dilution and $CaCl_2$ dilution suggest that the change in droplet size is due to droplet flocculation rather than droplet coalescence. Dickinson (2001) reported that the stability of emulsions containing whey proteins was very sensitive to the ionic strength. Water dilution lowers the ionic strength around the droplets and changes their effective charges and subsequently strengthens electrostatic repulsion and steric stabilization between emulsion droplets. WPI molecules adsorbed at the droplet surfaces can extend several nanometers into the aqueous phase (Le Bon and others 1999), and prevent the droplets from getting close to each other. Therefore, little changes were observed in droplet size upon water dilution measurements.

On the other hand, $CaCl_2$ dilution does not change the ionic strength of the system and hence can obtain the real properties of droplets such as surface charge and droplet size. The initial increase and subsequent decrease in droplet size during heating can be explained by droplet aggregation and the extent of aggregation. Droplet aggregation may be due to the combined contribution of both the effect of heating on increased hydrophobic attractions and the influence of $CaCl_2$ on decreased electrostatic repulsions (Keowmaneechai and McClements 2006). Holding the emulsions at 70 °C

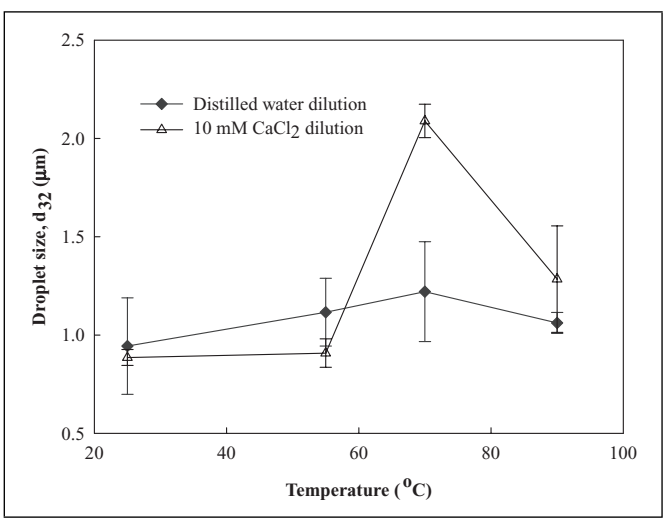

Figure 1—Effect of heating temperature (°C) on droplet size (d_{32}) of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil.

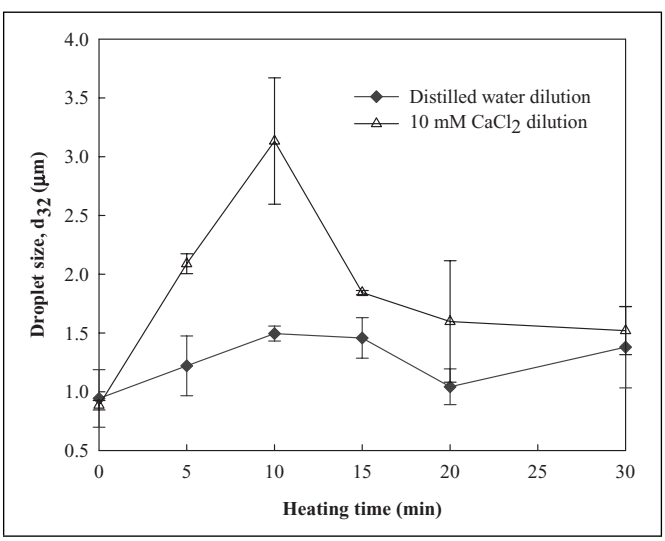

Figure 2—Effect of heating time on droplet size (d_{32}) of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil at 70 °C.

for 10 min caused droplet size to significantly increase, which indicates that hydrophobic attraction may be strong enough to overcome electrostatic repulsion and thus induced extensive droplet aggregation. With further heating, substantial unfolding and denaturation increases surface hydrophobicity and accumulates more WPI at the droplet surfaces. Meanwhile, thermodynamic incompatibility between XG and unadsorbed WPI in the aqueous phase is more pronounced during heating. As a result, more WPI are adsorbed at the droplet surfaces and less unadsorbed WPI are left in the aqueous phase. Euston and others (2002) reported that the removal of unadsorbed protein in the aqueous phase significantly reduced the aggregation rate. Consequently, the rate of droplet aggregation decreases and some aggregates may fall apart, leading to smaller droplets in the emulsions under thermal treatment at 90 °C or at 70 °C for 15 min or longer.

The competition between inter- and intra-droplet interactions (Dickinson and Matsumura 1991; Demetriades and others 1997) may also help explain the reduction in droplet size at 90 °C or for prolonged heating at 70 °C. When the emulsions are heated at 90 °C or for over 10 min at 70 °C, one or more WPI molecules are adsorbed at the same droplet and intra-droplet interactions are favored, so electrostatic repulsion and steric stabilization are strengthened; the degree of aggregation is reduced and a viscoelastic WPI layer is likely formed around an emulsion droplet. When the emulsions are heated for shorter time (<10 min) at 70 °C, one or more WPI molecules are adsorbed at different droplets and inter-droplet interactions are favored, so emulsion droplets tend to aggregate. Thus, inter-droplet interactions lead to an increase in droplet aggregation while intra-droplet interactions lead to an increase in droplet viscoelasticity.

Effect of heating temperature and time on oxidative stability

Temperature had no significant effect on the oxidation of oil droplets in the emulsions up to 70 °C during 2 wk of storage, but oxidative stability improved significantly ($P < 0.05$) as for the emulsions with 90 °C of thermal treatment (Figure 3A and 3B). Lower peroxide and anisidine values are indicators of better stability against oxidation. The initial peroxide and anisidine values of menhaden oil in the emulsions were 2.67 mmol/kg oil and 0.03, respectively. After 2 wk of storage, the decreased PV and increased AV suggest that the 1st oxidation step (formation of hydroperoxides) might be almost inhibited and the secondary oxidation step (decomposition of hydroperoxides) slowly proceeded. The inhibition may be attributed to the effects of EDTA and heat treatment. Alamed and others (2006) stated that 2.5 μM EDTA can completely inhibit oxidation and calcium has a lower tendency to bind with EDTA than does iron. Our data indicate that 200 μM EDTA might be enough to inhibit oxidation of oil droplet in the emulsions in the presence of 10 mM $CaCl_2$. Lower peroxide values at 90 °C support that heating contributes to better protection against oxidation as well. Higher denaturation rate upon substantial thermal treatment (90 °C) leads to an increase in the adsorbed amount of WPI and facilitates WPI–WPI interactions at droplet surfaces, which results in the formation of viscoelastic WPI layers around the droplet. These thick layers become barriers to prooxidants in the aqueous phase and protect emulsion droplets against oxidation (Kiokias and others 2007). PV decreased with heating time, a trend similar to that with heating temperature (Figure 4). Lower PV and negligible change in AV at higher temperature or longer time of heating indicate that lipid oxidation could be effectively reduced or even completely inhibited in WPI/XG-stabilized system. Better protection against oxidation at 90 °C or for over 10 min of heating at

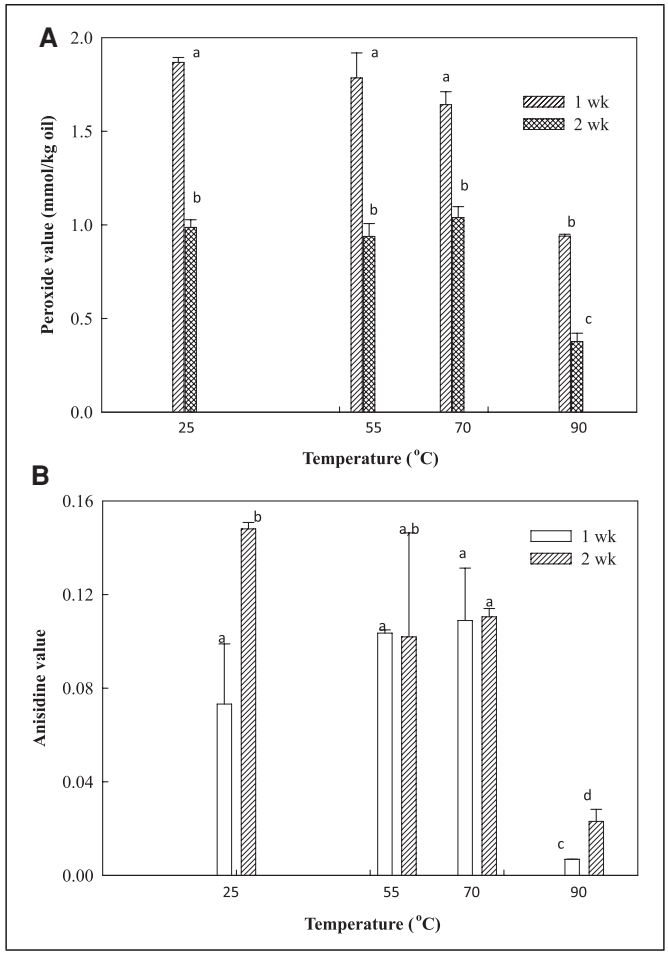

Figure 3—(A) Effect of heating temperature (°C) on primary lipid oxidation (PV) of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil. Means of PV followed by the different letters (a, b, c) are significantly ($P < 0.05$) different. (B) Effect of heating temperature (°C) on secondary lipid oxidation as measured by anisidine value of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil. Means of AV followed by the different letters (a, b, c, d) are significantly ($P < 0.05$) different.

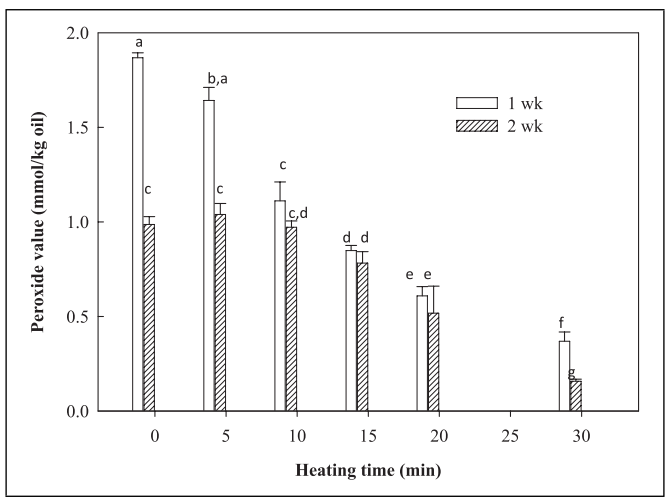

Figure 4—Effect of heating time on primary lipid oxidation as measured by peroxide value of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil at 70 °C. Means of PV followed by the different letters (a, b, c, d, e, f, g) are significantly ($P < 0.05$) different.

70 °C supports the suggested mechanisms of droplet aggregation and protein adsorption as well. At less than 10 min of heating, heat-denatured WPI was favored to be available for droplet aggregation while at 90 °C or longer time of heating, more heat-denatured WPI was available for adsorption at the droplet surfaces (Sliwinski and others 2003). Addition of incompatible XG enhanced both droplet aggregation and additional WPI adsorption in the heated emulsions since thermodynamic incompatibility between WPI and XG in the aqueous phase was more pronounced upon heating (Bryant and McClements 2000). As a result, increased amount of WPI is expected to be adsorbed at droplet surfaces, which renders better protection against oxidation since WPI can act as an effective antioxidant in the emulsions (Sun and others 2007).

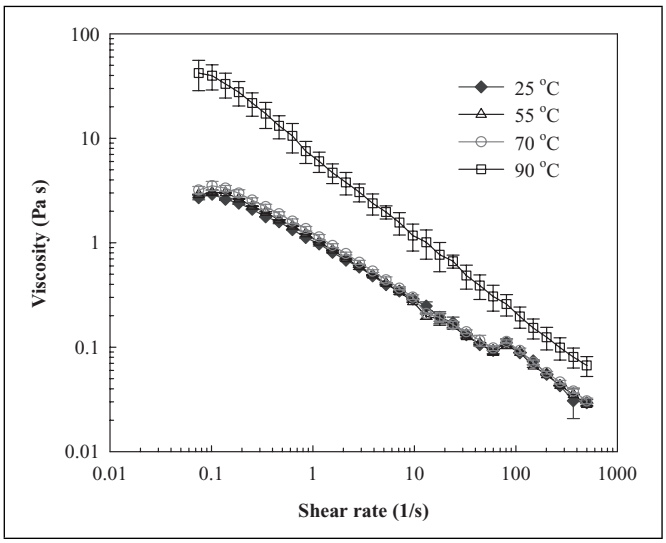

Figure 5—Effect of heating temperature on flow curves of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil.

Effect of heating temperature on rheological properties

Flow curves of 2 wt% WPI-stabilized emulsions, as a function of heating temperature, are shown in Figure 5. Addition of XG rendered all the emulsions strongly shear-thinning. Heating at 90 °C significantly shifted flow curves up; this increase in emulsion viscosity is attributed to the adsorption of increased amounts of WPI at the droplet surface and the formation of viscoelastic layers around the droplet. A small shift up in viscosity profile of emulsions at 70 and 55 °C is due to droplet aggregation but this change is not significant compared to the viscosity profile of unheated emulsion (at 25 °C).

The strain sweep measurement data (Figure 6) show similar trends when the emulsions are heated at 25, 55, and 70 °C, but the trend is much different at 90 °C. When the emulsions are heated below 70 °C, the G' value starts out being close to but lower than G'' value before G' value substantially drops off. However, at 90 °C the G' value starts out being greater than the G'' value and subsequently crosses over G'' curve. These results indicate that the emulsions undergo a transition from a viscous to an elastic state when heated from 70 to 90 °C. The critical strain (γ_c) is independent of heat treatment when the emulsions are heated below 70 °C. However, when the heating temperature is increased from 70 to 90 °C, γ_c dramatically decreases from 0.484 to 0.046, which indicates that the emulsions are more elastic at 90 °C and an emulsion gel is formed.

The mechanical spectra of the emulsions are shown in Figure 7. Heating emulsions below 70 °C has no significant effect on G' and G'' but both values increased with frequency with a distinct crossover point. The n values for each curve are summarized in Table 1. The data show that emulsions heated below 70 °C are close to being classically viscoelastic, that is, $G'(\omega) \propto \omega^2$ and $G''(\omega) \propto \omega$ (Tadros 1994), with deviations from these ideal conditions occurring as temperature increased. However, for emulsions heated at 90 °C, the G' value is significantly greater than the G'' value over the entire frequency range, and both G' and G'' values are nearly independent of frequency, with slopes of 0.28 and 0.21,

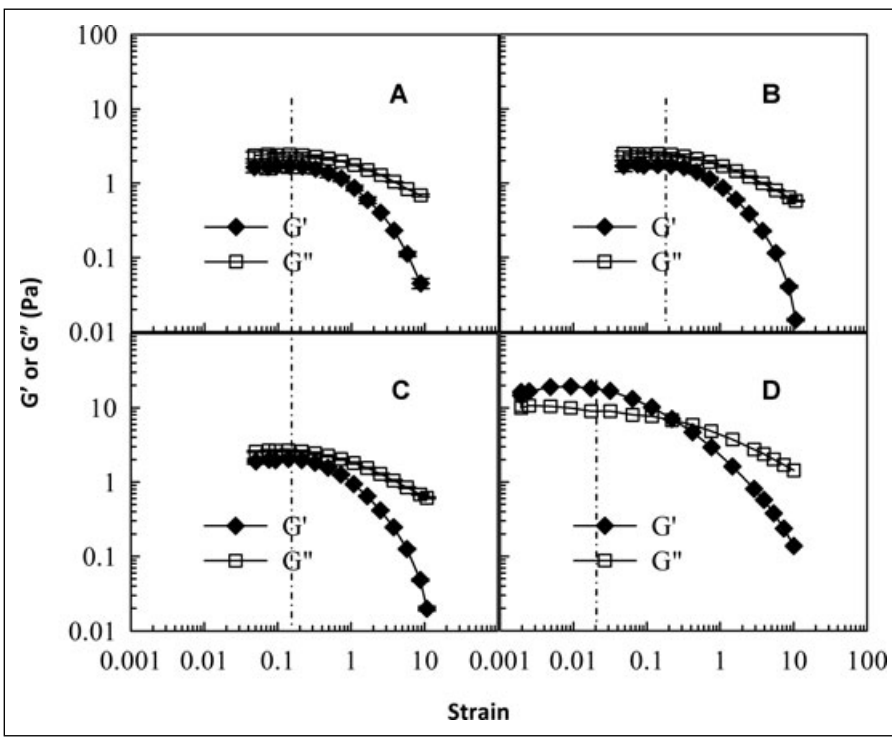

Figure 6—Effect of heating temperature on strain sweep of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil on strain amplitude measurements: (A) 25 °C, (B) 55 °C, (C) 70 °C, (D) 90 °C. The crossover point between each dotted line and *X*-axis represented the value of critical strain for each curve.

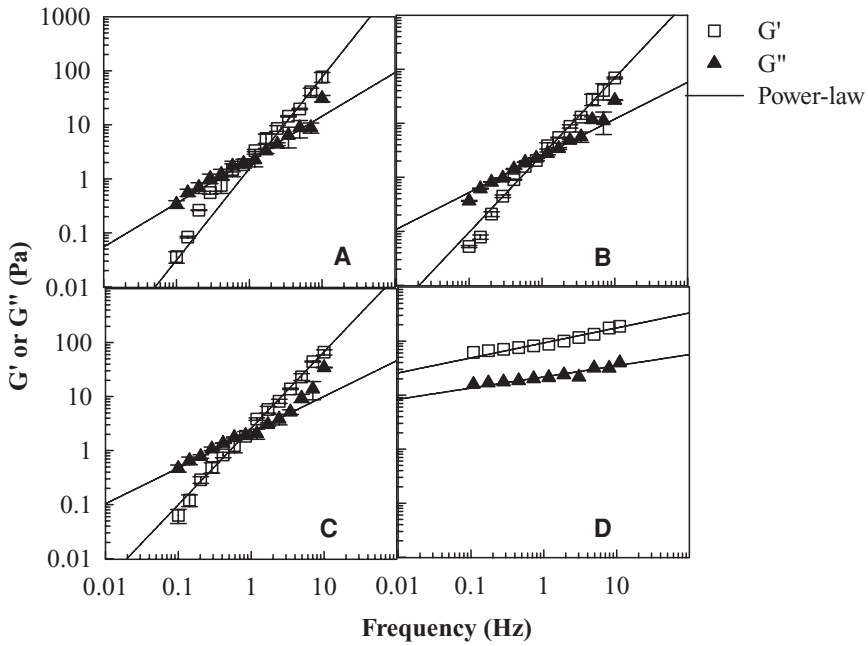

Figure 7 — Effect of heating temperature on frequency sweep of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil: (A) 25 °C, (B) 55 °C, (C) 70 °C, (D) 90 °C.

Table 1 — Fitting of power-law model (G' or $G'' = K * f^n$) to frequency sweep of 2 wt% WPI-stabilized emulsions containing 0.2 wt% XG and 20% (v/v) menhaden oil as a function of heating temperature.

Heating temperature (°C)	n^A in G' vs f	R^2	n^A in G'' vs f	R^2
25	1.69 ± 0.10^a	0.980	0.81 ± 0.09^a	0.952
55	1.43 ± 0.08^a	0.983	0.68 ± 0.04^a	0.988
70	1.42 ± 0.05^a	0.993	0.66 ± 0.04^a	0.986
90	0.28 ± 0.02^b	0.994	0.21 ± 0.03^b	0.990

[A]Means within the same column, followed by the different letters (a, b) are significantly different ($P < 0.05$).

respectively. These are further evidences that elastic emulsion gels are formed when the emulsions are heated at 90 °C. Heating the emulsions below 70 °C did not significantly affect the relaxation time and droplet–droplet interactions (Figure 7A to 7C) since f_c was independent of temperature. However, there is no crossover point at 90 °C (Figure 7D) over the tested frequency range (0.1 to 10 Hz) because the emulsions became elastic. These rheological data support the idea that elastic WPI layers are formed around droplets and the emulsion gels are eventually developed at 90 °C.

Effect of heating time on rheological properties

As heating time increased, so did the emulsion viscosity over the shear rate range tested, and all the emulsions showed similar strongly shear-thinning behavior due to the presence of XG. As observed at shear rate of 50 per second in Figure 8, there is a slight increase in emulsion viscosity from 0 to 10 min, but a substantial increase after 10 min of heating. The initial small increase in emulsion viscosity is attributed to the aggregation of emulsion droplets, and the subsequent increase to increased WPI–WPI interactions and formation of denser protein layers around the droplets. These results indicate that 10 min heating at 70 °C probably signifies a transition of the emulsion from liquid-like to gel-like system. Power-law model constants K and n for flow curves are summarized in Table 2. Increasing heating time from 0 to 10 min at 70 °C resulted in emulsions with slightly increased K values but statistically same n values. However, over 10 min of heating at 70 °C resulted in emulsions with substantially higher K values and significantly lower n values due to the state transition.

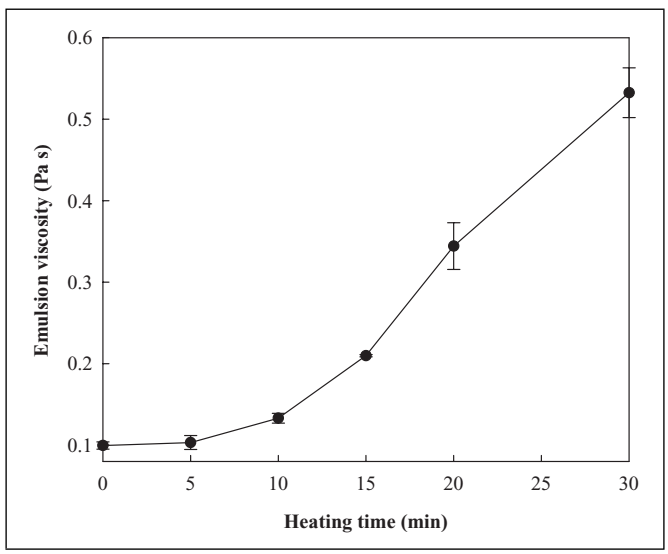

Figure 8 — Effect of heating time at 70 °C on the viscosity of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil under the shear rate of 50 per second.

Table 2 — Fitting of power-law model to flow curve of 2 wt% WPI-stabilized emulsions containing 0.2 wt% XG and 20% (v/v) menhaden oil as a function of heating time.

Heating time (min)	K (Pa.s^n)^A	n^A	R^2
0	0.74 ± 0.01^a	0.40 ± 0.01^a	0.996
5	1.18 ± 0.12^b	0.42 ± 0.02^a	0.988
10	1.57 ± 0.09^c	0.41 ± 0.01^a	0.996
15	2.52 ± 0.36^d	0.37 ± 0.03^b	0.970
20	5.37 ± 0.24^e	0.31 ± 0.01^c	0.996
30	12.6 ± 0.3^f	0.20 ± 0.005^d	0.997

[A]Means within the same column, followed by the different letters (a, b, c, d, e, f) are significantly different ($P < 0.05$).

Strain amplitude sweep data (Figure 9) show that heating the emulsions at 70 °C for less than 10 min hardly has any effect on G'

and γ_c but continued heating leads to a sharp increase in G' and a significant decrease in γ_c. These results can be attributed to different mechanisms of interactions among the emulsion droplets involved. During shorter heating time, noncovalent interactions are responsible for the aggregation of emulsion droplets, including hydrogen bonding, and hydrophobic and electrostatic interactions. Longer time of heating involves intermolecular disulfide bonds in polymerization and formation of protein network (Dalgleish 1997).

The frequency sweep curves show that heating time greatly enhances the G' values (Figure 10). Slopes of the lines were determined by fitting power-law model ($R^2 > 0.97$) and the values were 1.46, 0.97, 0.91, 0.41, and 0.30 corresponding to 5, 10, 15, 20, and 30 min of heating at 70 °C, respectively. The G' values were a strong function of frequency for less than 15 min of heating.

When the emulsions are heated for less than 20 min at 70 °C, phase angle decreases with tested frequency and passes through 45°, which represents the existence of a crossover point (Figure 11). When the emulsions are heated at 70 °C for 30 min, phase angle is always less than 45° and independent of frequency, which suggests that the emulsion is more elastic with smaller γ_c. The relaxation time data (Figure 12) show that heating the emulsions below 10 min at 70 °C has no significant effect on the droplet–droplet interactions, but further heating greatly increases the relaxation time. This demonstrates a significant increase in the interactions among emulsion droplets, which results from the transition of interaction contribution from weak noncovalent interactions to strong intermolecular bonds.

Conclusions

Upon heating menhaden O/W emulsions up to 90 °C, thermodynamic incompatibility between XG and WPI is more pronounced and contributes to the enhancement of both aggregation rate and increased WPI adsorption at the droplet surfaces. The rheological properties of the emulsions suggest the formation of WPI

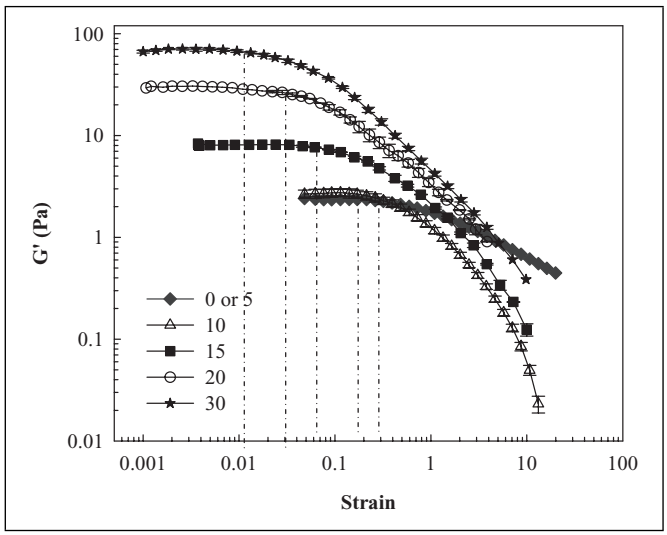

Figure 9—Effect of heating time (min) on the critical strain of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil at 70 °C. The crossover point between each dotted line and *X*-axis represented the value of critical strain for each curve.

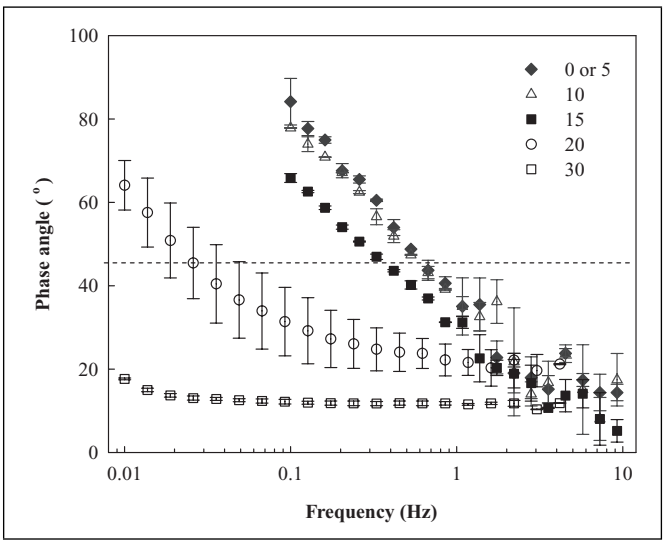

Figure 11—Effect of heating time (min) on phase angle of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil at 70 °C.

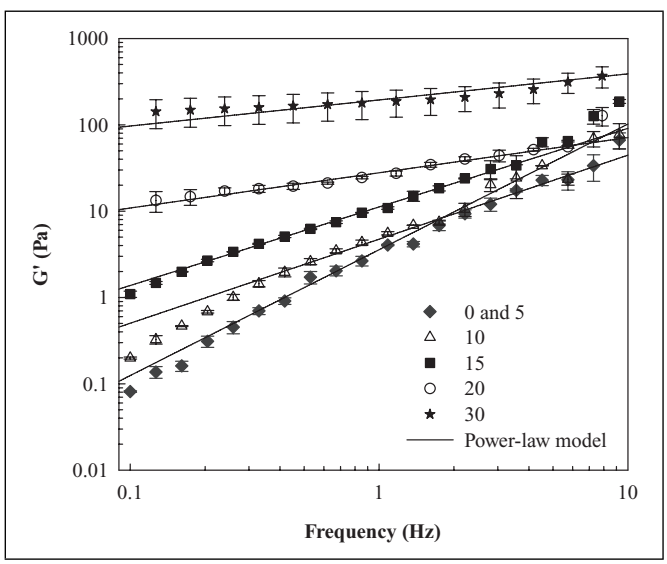

Figure 10—Effect of heating time (min) on frequency sweep of 2 wt% WPI-stabilized emulsion containing 0.2 wt% XG and 20% (v/v) menhaden oil at 70 °C.

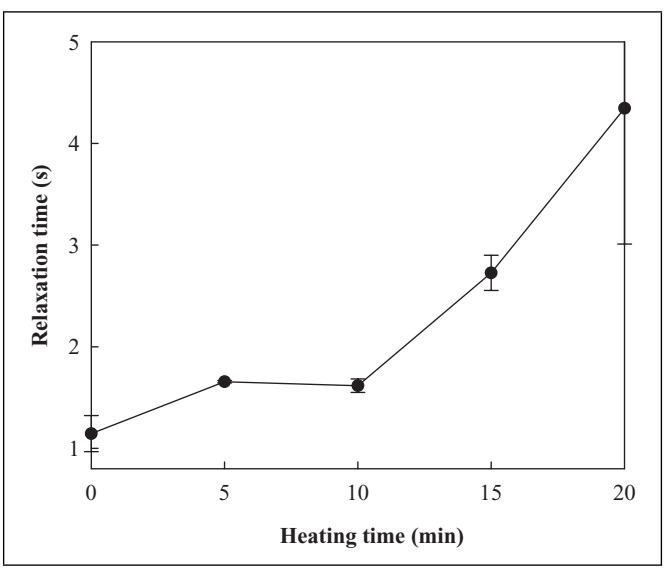

Figure 12—Relaxation time of 2 wt% WPI-stabilized emulsions containing 0.2 wt% XG and 20% (v/v) menhaden oil as a function of heating time (min) at 70 °C.

matrix around the droplet during heating and contribute positively to improved oxidative stability of menhaden oil in the emulsions. Higher temperature (90 °C) and longer time (>10 min) of heating at 70 °C slightly increase emulsion droplet size, but significantly improve both lipid oxidation and emulsion rheology. Emulsions undergo a viscous to the elastic state transition upon heating from 70 to 90 °C or over 10 min at 70 °C.

Acknowledgment

Omega Protein, Inc. and Davisco Foods Intl., Inc. are gratefully acknowledged for donating menhaden oil and WPI used in this study, respectively.

References

Alamed J, McClements DJ, Decker EA. 2006. Influence of heat processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in oil-in-water emulsions containing omega-3 fatty acids. Food Chem 95:585–90.

Bryant CM, McClements DJ. 2000. Influence of xanthan gum on physical characteristics of heat-denatured whey protein solutions and gels. Food Hydrocolloids 14:383–90.

Dalgleish DG. 1997. Absorption of protein and the stability of emulsions. Trends Food Sci Technol 8:1–8.

Demetriades K, Coupland JN, McClements DJ. 1997. Physicochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength. J Food Sci 62:462–7.

Dickinson E. 2001. Milk protein interfacial layers and the relationship to emulsion stability and rheology. Colloids Surf B: Biointerface 20:197–210.

Dickinson E, Golding M. 1998. Influence of calcium ions on creaming and rheology of emulsions containing sodium caseinate. Colloids Surf A-Physicochem Engr Asp 144:167–77.

Dickinson E, Matsumura Y. 1991. Time-dependent polymerization of beta-lactoglobulin through disulfide bonds at the oil-water interface in emulsions. Int J Biol Macromol 13:26–30.

Dickinson E, McClements DJ. 1995. Advances in food colloids. Glasgow, Scotland: Blackie Academic and Professional. 81 p.

Dickinson E, Radford SJ, Golding M. 2003. Stability and rheology of emulsions containing sodium caseinate: combined effects of ionic calcium and non-ionic surfactant. Food Hydrocolloids 17:211–20.

Djordjevic D, Kim HJ; McClements DJ, Decker EA. 2004. Physical stability of whey protein-stabilized oil-in-water emulsions at pH 3: potential omega-3 fatty acid delivery systems (Part A). J Food Sci 69:C351–5.

Euston SR, Finnigan SR, Hirst RL. 2002. Kinetics of droplet aggregation in heated whey protein-stabilized emulsions: effect of polysaccharides. Food Hydrocolloids 16:499–505.

Gwartney EA, Larick DK, Foegeding EA. 2004. Sensory texture and mechanical properties of stranded and particulate whey protein emulsion gels. J Food Sci 69:S333–9.

Kato A, Osako Y, Matsudomi N, Kobayashi K. 1983. Changes in the emulsifying and foaming properties of proteins during heat denaturation. Agric Biological Chem 47:33–7.

Keowmaneechai E, McClements DJ. 2002. Influence of EDTA and citrate on physicochemical properties of whey protein-stabilized oil-in-water emulsions containing $CaCl_2$. J Agric Food Chem 50:7145–53.

Keowmaneechai E, McClements DJ. 2006. Influence of EDTA and citrate on thermal stability of whey protein-stabilized oil-in-water emulsions containing calcium chloride. Food Res Int 39:230–9.

Kim DA, Cornec M, Narsimhan G. 2005. Effect of thermal treatment on interfacial properties of beta-lactoglobulin. J Colloid Interface Sci 285:100–9.

Kiokias S, Dimakou C, Oreopoulou V. 2007. Effect of heat treatment and droplet size on the oxidative stability of whey protein emulsions. Food Chem 105:94–100.

Le Bon C, Nicolai T, Durand D. 1999. Growth and structure of aggregates of heat-denatured beta-lactoglobulin. Int J Food Sci Technol 34:451–65.

McClements DJ, Decker EA. 2000. Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food systems [Reviews]. Journal Food Sc 65:1270–82.

Miller DD. 1996. Minerals. In: Fennema OR, editor. Food chemistry. New York: Marcel Dekker. p 617–49.

Reiffers-Magnani CK, Cuq JL, Watzke HJ. 2000. Depletion flocculation and thermodynamic incompatibility in whey protein stabilized O/W emulsions. Food Hydrocolloids 14:521–30.

Richards MP, Chaiyasit W, McClements DJ, Decker EA. 2002. Ability of surfactant micelles to alter the partitioning of phenolic antioxidants in oil-in-water emulsions. J Agric Food Chem 50:1254–59.

Rossell JB. 1986. Classical analysis of oils and fats. In: Hamilton RJ, Rossell JB, editors. Analysis of oils and fats. New York: Elsevier Applied Science Publishers. p 1–90.

Sliwinski EL, Roubos PJ, Zoet FD, van Boekel MAJS, Wouters JTM. 2003. Effects of heat on physicochemical properties of whey protein-stabilised emulsions. Colloids Surf B-Biointerfaces 31:231–42.

Sun C. 2006. Effects of pH and environmental conditions on the physicochemical properties of o/w emulsions [Ph.D. thesis]. Madison, Wis.: Univ. of Wisconsin-Madison. 152 p.

Sun C, Gunasekaran S. 2009. Effects of protein concentration and oil-phase volume fraction on stability and rheology of menhaden oil-in-water emulsions stabilized whey protein isolate with xanthan gum. Food Hydrocolloids 23:165–74.

Sun C, Gunasekaran S, Richards MP. 2007. Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. Food Hydrocolloids 21:555–64.

Tadros TF. 1994. Fundamental principles of emulsion rheology and their applications. Colloids Surf A- Physicochem Engr Asp 91:39–55.

Tadros TF. 2004. Application of rheology for assessment and prediction of the long-term physical stability of emulsions. Adv Colloids Interface Sci 108–9:227–58.

Verheul M, Roefs SPFM. 1996. Structural properties of heat-set whey protein gels. In: Dickinson E, Bergenstahl B, editors. Food colloids-proteins, lipids, and polysaccharides. Cambridge, U.K.: The Royal Society of Chemistry. 356 p.