

# Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum

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## Abstract

The influences of protein concentration (0.2, 1, 2 wt%) and oil-phase volume fraction (5%, 20%, 40% v/v) on emulsion stability and rheological properties were investigated in whey protein isolate (WPI)-stabilized oil-in-water emulsions containing 0.2 wt% xanthan gum (XG). The data of droplet size, surface charge, creaming index, oxidative stability, and emulsion rheology were obtained. The results showed that increasing WPI concentration significantly affected droplet size, surface charge, and oxidative stability, but had little effect on creaming stability and emulsion rheology. At 0.2 wt% WPI, increasing oil-phase volume fraction greatly increased droplet size but no significant effect on surface charge. At 1 or 2 wt% WPI, increasing oil-phase volume fraction had less influence on droplet size but led to surface charge more negative. Increasing oil-phase volume fraction facilitated the inhibition of lipid oxidation. Meanwhile, oil-phase volume fraction played a dominant role in creaming stability and emulsion viscosity. The rheological data indicated the emulsions may undergo a behavior transition from an entropic polymer gel to an enthalpic particle gel when oil-phase volume fraction increased from 20% to 40% v/v.

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**Keywords:** WPI concentration; Oil-phase volume fraction; Xanthan gum; WPI bridging; Depletion flocculation; Droplet size; Surface charge; Creaming stability; Lipid oxidation; Emulsion rheology

## 1. Introduction

Protein and oil are two key ingredients in food emulsions. Oil dispersions in the form of small spherical droplets are stabilized in the aqueous phase by protein in an oil-in-water (O/W) emulsion. The surface-active protein is adsorbed at the interface between oil and the aqueous phase to lower surface tension and prevent oil droplets from coming close enough together to aggregate (Dickinson & Golding, 1997; Paraskevopoulou, Boskou, & Paraskevopoulou, 2007). Whey protein isolate (WPI) is widely used as a proteinaceous emulsifier in food emulsions. WPI contains both hydrophobic and hydrophilic regions and can be rapidly adsorbed on the oil–water interface in the form of a protective film, consequently providing structural support for oil droplets through a

combination of electrostatic and steric interactions (Djordjevic, Kim, McClements, & Decker, 2004; Gwartney, Larick, & Foegeding, 2004; Reiffers-Magnani, Cuq, & Watzke, 2000). WPI has been reported to possess the antioxidant activity (Hu, McClements, & Decker, 2003; Sun, Gunasekaran, & Richards, 2007; Tong, Sasaki, McClements, & Decker, 2000), which could be extremely beneficial to the systems containing oxidative labile components as the dispersed phase.

Besides emulsifiers, polysaccharides are often added to stabilize emulsions in an O/W emulsion (Diftis, Biliaderis, & Kiosseoglou, 2005; Vega, Dalgleish, & Goff, 2005). Xanthan gum (XG), an extracellular polysaccharide produced by *Xanthomonas campestris*, is an excellent stabilizer not only because of its ability to increase emulsion viscosity but also due to its antioxidant property we have previously reported (Gohtani, Sirendi, Yamamoto, Kajikawa, & Yamamoto, 1999; McClements & Decker, 2000a, 2000b; Shimada et al., 1994; Sun et al., 2007).

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Omega ( $\omega$ -3) polyunsaturated fatty acids (PUFAS) are essential for normal body development and provide active protection against coronary heart disease, hypertension, obesity, type 2 diabetes, other inflammatory and autoimmune disorders, and cancer (Simopoulos, 1999). However,  $\omega$ -3 PUFAS are highly unsaturated and very susceptible to lipid oxidation. Lipid oxidation is a major factor of quality deterioration in many natural and processed foods containing  $\omega$ -3 PUFAS (McClements & Decker, 2000a, 2000b). Therefore, successful incorporation of  $\omega$ -3 PUFAS into food emulsions is of great interest for food scientists and manufacturers to make the best use of beneficial functions to health if oxidative rancidity can be overcome.

Many studies have focused on improving stability of incorporated  $\omega$ -3 PUFAS by changing its environmental conditions, such as thermal processing, pH, ionic strength, and chelator addition (Alamed, McClements, & Decker, 2006; Gu, Regnier, & McClements, 2005; Hu, McClements, & Decker, 2004; Keowmaneechai & McClements, 2002; Kulmyrzaev & Schubert, 2004; McSweeney, Mulvihill, & O'Callaghan, 2004). Nevertheless, protein concentration and the oil volume fraction perform profound effects on the physicochemical and viscoelastic properties of the emulsions, such as droplet size distribution, creaming, oxidative stability, and rheology (Dickinson & Chen, 1999; Hemar, Tamehana, Munro, & Singh, 2001). Therefore, the objectives of this study were: (1) to evaluate the effect of WPI concentration and oil-phase volume fraction on emulsion stability and rheological properties of WPI-stabilized emulsions containing 0.2 wt% XG and (2) to develop the relationship between emulsion properties and emulsion stability.

## 2. Materials and methods

### 2.1. Materials

Menhaden oil (eicosapentaenoic acid (EPA), 8–18%; docosahexaenoic acid (DHA), 7–18%; total  $\omega$ -3 PUFAS,

20–26%; consisting of 2000 ppm mixed tocopherols) was obtained from Omega Protein, Inc. (Reedville, VA). WPI (protein, 98 wt %; moisture, 4.4 wt%; ash, 1.8 wt%; fat, 0.3 wt%) was obtained from Davisco Foods International, Inc. (Eden Prairie, MN). XG was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Cumene hydroperoxide was purchased from Sigma Chemical Company (St. Louis, MO). Para-anisidine, ammonium thiocyanate, ferrous sulfate, barium chloride, isooctane, isopropanol, butanol, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). All other reagents were of analytical grade or purer. Distilled water was used to prepare all the solutions.

### 2.2. Emulsion preparation

Certain concentrations (Table 1) of WPI 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. The O/W emulsions were prepared by slowly mixing menhaden oil into WPI solution, then adding XG solution; the WPI solution/menhaden oil/XG solution mixture was finally emulsified with a PowerGen 125 homogenizer (Fisher Scientific, Fair Lawn, NJ) three times at 75 W output for 2 min. No pH adjustments were made to the final emulsions because both WPI concentration and oil-phase volume fraction had little effect on pH. Sodium azide (1% w/v) was added to the final emulsions as an antimicrobial agent. Nine formulations of the above O/W emulsions were randomly prepared by following the run order presented in Table 1. The final composition of these emulsions were 5–40% v/v menhaden oil, 0.2–2 wt% WPI, 0.2 wt% XG, 0.02% w/v sodium azide, and pH 6.7–7 (Table 1).

### 2.3. Particle size and zeta-potential measurements

A particle size analyzer (90Plus, Brookhaven Instruments Corporation, New York, USA) was used to

Table 1  
3<sup>2</sup> factorial design for investigating the effect of the compositions on the physicochemical properties of O/W emulsions<sup>a</sup>

Run order	Conc. of WPI solution (wt%)	WPI (mL)	Conc. of XG solution (wt%)	XG (mL)	Menhaden oil (mL)	final composition in the emulsions				Marked in Fig. 3
						WPI conc. (wt%)	Menhaden oil (%v/v)	XG conc. (wt%)	pH	
1	0.42	38	0.42	38	4	0.2	5	0.2	6.78	A
7	2.1	38	0.42	38	4	1	5	0.2	6.91	G
3	4.2	38	0.42	38	4	2	5	0.2	7.04	C
9	0.5	32	0.5	32	16	0.2	20	0.2	6.94	I
8	2.5	32	0.5	32	16	1	20	0.2	6.83	H
5	5.0	32	0.5	32	16	2	20	0.2	7.01	E
6	0.67	24	0.67	24	32	0.2	40	0.2	6.91	F
4	3.3	24	0.67	24	32	1	40	0.2	6.93	D
2	6.7	24	0.67	24	32	2	40	0.2	6.87	B

<sup>a</sup>On basis of total 80 mL.

determine the surface-area-average diameter ( $d_{32}$ ) of the emulsion droplets, which 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 effect, the emulsions were diluted, prior to particle size measurement, with distilled water to keep the concentration typically between  $10^{-5}$  and  $10^{-2}$  volume fraction. At such low concentration the difference between oil droplet volume and liquid volume is insignificant, and the difference between volume fraction  $\phi$  and mass concentration is often ignored. The refractive index of the emulsion droplets was 1.456.

Zeta potential is not a direct measurement of surface charges, but it reflects the net charge of the droplets inside the shear plane. The prepared emulsions were diluted with distilled water to keep the droplet concentration typically between  $10^{-5}$  and  $10^{-2}$  volume fraction prior to zeta-potential measurement. Diluted emulsions ( $\sim 1.6$  mL) were then poured into the sample cell and the zeta potential was measured using the ZetaPlus instrument (Brookhaven Instruments Corporation, New York, USA).

#### 2.4. Rheological characterization

Steady shear viscosity and small-amplitude oscillatory shear (SAOS) tests were conducted using a dynamic rheometer (Bohlin CVOR, Malvern Instruments Inc., Southborough, MA). Emulsion viscosity was measured at  $25 \pm 0.1$  °C over a shear rate range of  $0.0716$ – $100$  s $^{-1}$  with cone-plate geometry (CP 40/4°). All measurements were performed within 24 h of emulsion preparation. Power-law model was used to analyze the emulsion flow curves

$$\sigma = K\dot{\gamma}^n,$$

where  $\sigma$  is shear stress,  $K$  is consistency coefficient,  $\dot{\gamma}$  is shear rate, and  $n$  is flow behavior index.

SAOS tests were performed by pouring emulsion samples directly on the holding stage and the samples were covered with a thin layer of paraffin oil to prevent water evaporation during the measurement. A 4°/40 mm cone-and-plate geometry was used in the dynamic rheometer to test strain amplitude sweep at a fixed frequency of 1 Hz and 25 °C isothermal conditions. The amplitude of strain was swept from 0.01 to 10 with the increment of a logarithmic scale. During strain amplitude sweep, elastic modulus  $G'$  was measured as a function of strain ( $\gamma$ ).

#### 2.5. Creaming stability

Creaming stability was investigated to evaluate the relative stability of the emulsions immediately after they were prepared. The emulsion samples were poured in 20 mm diameter, 60 mm high glass test tubes, and tubes were sealed to prevent evaporation. They were kept at ambient temperature and the movement of any creaming boundary

was tracked visually with time over several days. The emulsions separated into a top cream layer and a bottom serum layer. The total emulsion height ( $H_T$ ) and serum layer height ( $H_S$ ) were measured. A creaming index (CI) was calculated as (Keowmaneechai & McClements, 2002)

$$CI (\%) = 100 \frac{H_S}{H_T}.$$

CI can provide indirect information about the extent of droplet aggregation in an emulsion: the more the aggregation, the larger the flocs and the faster the creaming.

#### 2.6. Oxidative stability

The effects of WPI concentration and oil-phase volume fraction on oxidative stability of WPI-stabilized emulsions at room temperature were investigated during 1–2 wk of storage. Emulsion samples, 40 mL each, were taken in 150 mL sealed Erlenmeyer flask and were covered with aluminum foil to avoid exposure to light, an initiator of oxidation. Peroxide value (PV) and anisidine value (AV) were measured to evaluate oxidative stability.

PV value was determined by adding 0.3 mL emulsion to 1.5 mL of isooctane/isopropanol (3:2, v/v), followed by vortexing three times for 10 s each. After centrifuging for 2 min at 10,000 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  $\mu$ L of thiocyanate/ $Fe^{2+}$  solution and then vortexed. The thiocyanate/ $Fe^{2+}$  solution was made by mixing one part 3.94 M thiocyanate solution with one part 0.072 M  $Fe^{2+}$  solution (obtained from the supernatant of a mixture of one part 0.144 M  $FeSO_4$  and one 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, Chaiyasit, McClements, & Decker, 2002).

The test for AV was modified according to the British Standard Method (Rossell, 1986): 0.5–4 mL emulsion was added into a 25 mL volumetric flask and made up to the mark with isooctane. The test tubes were vortexed two times 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 Corporation). Five milliliter aliquots 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 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 values were calculated using the following equation:

$$AV = \frac{25(1.2 \times A_2 - A_1)}{\text{Sample mass}}. \quad (4)$$

## 2.7. Statistical analysis

All experiments were performed in duplicate. Statease DE 6 software (Stat-Ease, Inc., Minneapolis, MN) was used to design the experiments and randomize the run order. Analysis of variance (ANOVA) was used to determine if the means of responses were significant ( $P < 0.05$ ) (Microsoft Excel 2003, Microsoft Corporation, Redmond, WA).

## 3. Results and discussion

### 3.1. Droplet size and zeta potential

Droplet size ( $d_{32}$ ) profile of all the emulsions studied is shown in Fig. 1. The emulsion with 0.2 wt% WPI and 40% v/v oil had the largest average  $d_{32}$  (~1.65  $\mu\text{m}$ ), and the emulsion with 2 wt% WPI and 5% v/v oil the smallest (~0.60  $\mu\text{m}$ ). In general, droplet size decreased with increasing WPI concentration or decreasing oil-phase volume fraction; ANOVA indicated that there was no significant interaction between WPI concentration and oil-phase volume fraction (Table 2).

An increase in WPI concentration enhanced WPI adsorption and surface coverage of oil droplets, which effectively inhibited droplet aggregation or coalescence. The high  $d_{32}$  values of the emulsions containing 0.2 wt% WPI and 20% or 40% v/v oil suggested that WPI content may not be enough to cover the oil droplets and form sufficiently dense adsorption layer. As a result, WPI acted as bridges among the oil droplets and led to droplets flocculation (Dickinson & Galazka, 1991; McClements, Monahan, & Kinsella 1993). Bridging flocculation is expected to be irreversible and resists disruption by water

Table 2

Sets of  $P$ -values from the analysis of variance (ANOVA) for the droplet size and zeta potential of O/W emulsions containing 0.2 wt% XG

Factors	Droplet size ( $d_{32}$ )	Zeta potential
WPI concentration	0.0096	0.0002
Oil-phase volume fraction	0.0000	0.2156
Interaction	0.1615	0.0017

dilution prior to particle size measurement (Ye & Singh, 2000). Larger droplets were expected for the emulsions containing higher oil content and lower WPI concentration because of less available amount of WPI and more droplet flocculation via WPI bridging. Therefore, as for the emulsions containing 0.2 wt% WPI and 20% or 40% v/v oil, almost all the WPI were expected to be adsorbed at the droplet surface and the introduction of more WPI would allow the formation of smaller droplets with larger interfacial areas. In addition, increasing oil-phase volume fraction from 20% to 40% enhanced collision frequency among oil droplets and subsequently the rate of flocculation. Thus, on the basis of our results it may be concluded that emulsion droplet size is likely to increase with oil volume fraction ( $\phi$ ) and decrease with initial protein concentration ( $C_0$ ).

As can be seen in Fig. 2, oil-phase volume fraction did not significantly affect the surface charge of droplets; however, the WPI concentration had a significant ( $P < 0.05$ ) effect. ANOVA showed a significant interaction effect ( $P < 0.05$ ) between WPI concentration and oil-phase volume fraction on surface charges of emulsion droplets (Table 2). In other words, the effect of oil-phase volume on surface charge depended on WPI concentration because negatively charged WPI can be adsorbed on the droplet surfaces and higher oil-phase volume fraction needs more WPI to lower surface tension. Adsorption of more WPI on oil droplets contributed to more negative values of zeta potential. Increasing WPI concentration enhanced WPI adsorption to oil droplets because of the availability of more WPI to form the interfacial WPI monolayer, and even the multilayer, around emulsion droplets, which contributed to more negative charges around droplet surfaces. The multilayer adsorption reportedly contained a significant fraction of protein aggregates, and the first monolayer functioned as a substrate for the adsorption of the aggregates (Tcholakova, Denkov, Sidzhakova, Ivanov, & Campbell, 2003). The protein adsorption in the first monolayer is practically irreversible due to the large adsorption energy per protein molecule, but the protein molecules adsorbed in the multilayers may be bound less strongly to oil droplets and are partially reversible (Tcholakova, Denkov, Ivanov, & Campbell, 2002).

Another possible explanation for the results of zeta potential was that more negative surface charge may be attributed to the changes in iron binding to the droplet surfaces. Higher WPI concentration in the emulsions led to more unabsorbed WPI in the aqueous phase, which could

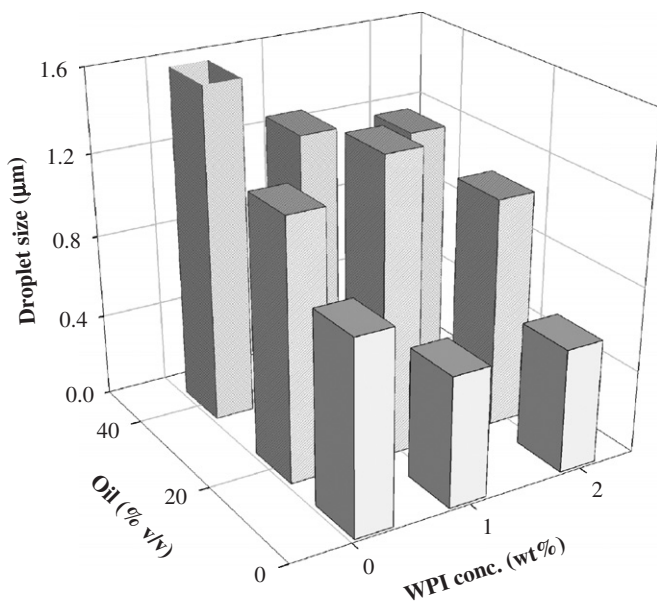


Fig. 1. Effects of WPI concentration (wt%) and oil phase volume fraction (% v/v) on droplet size of O/W emulsions containing 0.2 wt% XG.

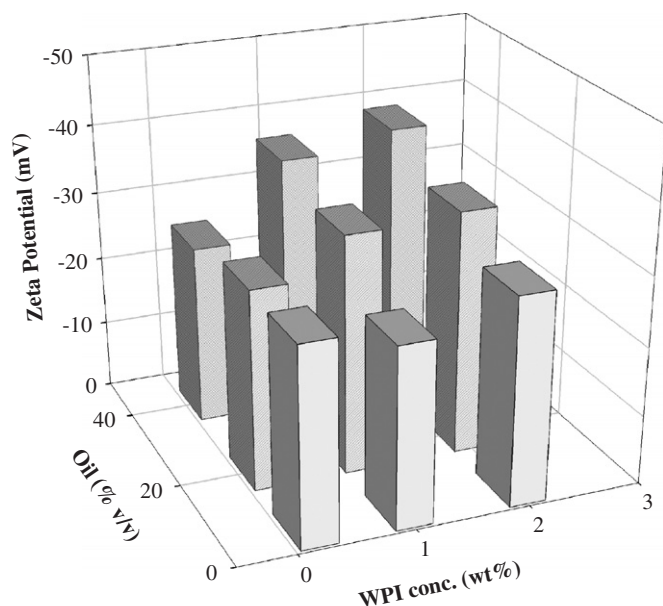


Fig. 2. Effects of compositions on zeta potential of O/W emulsions containing 0.2 wt% XG.

bind iron and remove it from the droplet surface, consequently causing the surface charge of droplets more negative.

### 3.2. Creaming stability

The creaming profile and the plots of creaming index vs. storage time are shown in Figs. 3 and 4 as functions of WPI concentration and oil-phase volume fraction. Increasing WPI concentration slightly decreased the creaming index of 5% v/v O/W emulsions. As for 20% v/v O/W emulsions, increasing WPI concentration from 0.2 to 2 wt% lowered the creaming index, and delayed the lag period prior to visible creaming from 1 to 5 d (Fig. 3). The 40% v/v O/W emulsions did not show any creaming regardless of WPI concentration (Figs. 3 and 4). These results indicated that oil-phase volume fraction played a dominant role in the creaming of emulsions. Emulsion stability improved with increasing oil-phase volume fraction because of the increase in packing fraction of oil droplets (Dickinson & Golding, 1997), which enhanced emulsion viscosity and lowered the creaming rate.

At low oil-phase volume fraction (5% v/v), creaming was rapid (Fig. 3) because the weakly flocculated network simply collapsed under its own weight. However, higher WPI slightly lowered creaming rate possibly due to the unadsorbed WPI in the aqueous phase. Papalamprou, Makri, Kiosseoglou, & Doxastakis (2005) stated that the presence of XG increased the amount of protein adsorbed at the interface due to the incompatibility between WPI and XG. Hence, higher WPI concentration may facilitate the adsorption of WPI to the interface of oil droplets, slightly increase the density of oil droplets, and consequently decrease the creaming rate.

At medium oil-phase volume fraction (20% v/v), lower WPI concentration (0.2 wt%) was certainly expected to induce enhanced creaming due to WPI bridging and droplet flocculation. However, the presence of 0.2 wt% XG prevented the rapid creaming by forming the emulsion network induced by extensive depletion flocculation (Parker, Gunning, Ng, & Robins, 1995; Sun et al., 2007). Increasing WPI concentration enhanced the surface coverage of oil droplets against flocculation and reduced the scope for WPI bridging, leading to decreased creaming.

### 3.3. Oxidative stability

Fig. 5 shows that in the 5% v/v O/W emulsions increasing WPI concentration from 0.2 to 2 wt% significantly decreased the formation of hydroperoxide during 8 d storage. As discussed previously, 0.2 wt% WPI was sufficient to cover oil droplet surfaces in 5% v/v O/W emulsions; the addition of 1 and 2 wt% WPI provided more unadsorbed WPI in the aqueous phase, which acted as an antioxidant to deactivate the iron (Sun et al., 2007). Additionally, the excessive WPI may also form the WPI multilayer around the oil droplets and decrease the contact between oil droplets and iron at the interfaces. Hence, both the adsorbed WPI multilayer at the droplet surface and the unadsorbed WPI in the aqueous-phase contributed to further decrease in the formation of hydroperoxide.

Peroxide values were also significantly affected by oil-phase volume fraction (Fig. 5). The 5% v/v O/W emulsions had appreciably higher amounts of hydroperoxides over the storage period compared with the 20% and 40% v/v O/W emulsions. We believed that it was mainly attributed to rapid creaming in the 5% v/v O/W emulsions. Meanwhile, the amount of pro-oxidant iron per gram of oil would decrease when oil-phase volume fraction increased. The higher PV values of O/W emulsions containing lower oil fraction had been previously reported in safflower oil (Sims, Fioriti, & Trumbetas, 1979) and canola oil (Osborn & Akoh, 2004) emulsions. One explanation for the higher PV was that the number of radicals produced per oil droplet probably increased at lower oil fraction concentration (Osborn & Akoh, 2004); at higher oil fraction more unsaturated fatty acids may have moved into the interior of the oil droplet, and thus these fatty acids became less accessible to direct interaction with the pro-oxidants at the interface (McClements & Decker, 2000a, 2000b).

In 20% and 40% v/v O/W emulsions, increasing WPI concentration from 1 to 2 wt% had no significant effect on PV values (Fig. 5). However, 40% v/v O/W emulsions had slightly higher PV values than 20% v/v O/W emulsions in the 0.2 wt% WPI-stabilized emulsions. There were three possible explanations. First, some hydroperoxides formed in 20% v/v O/W emulsions had decomposed into secondary oxidation products, leading to lower PV and higher AV values. Second, 40% v/v O/W emulsions stabilized by 0.2 wt% WPI left more oil droplets without

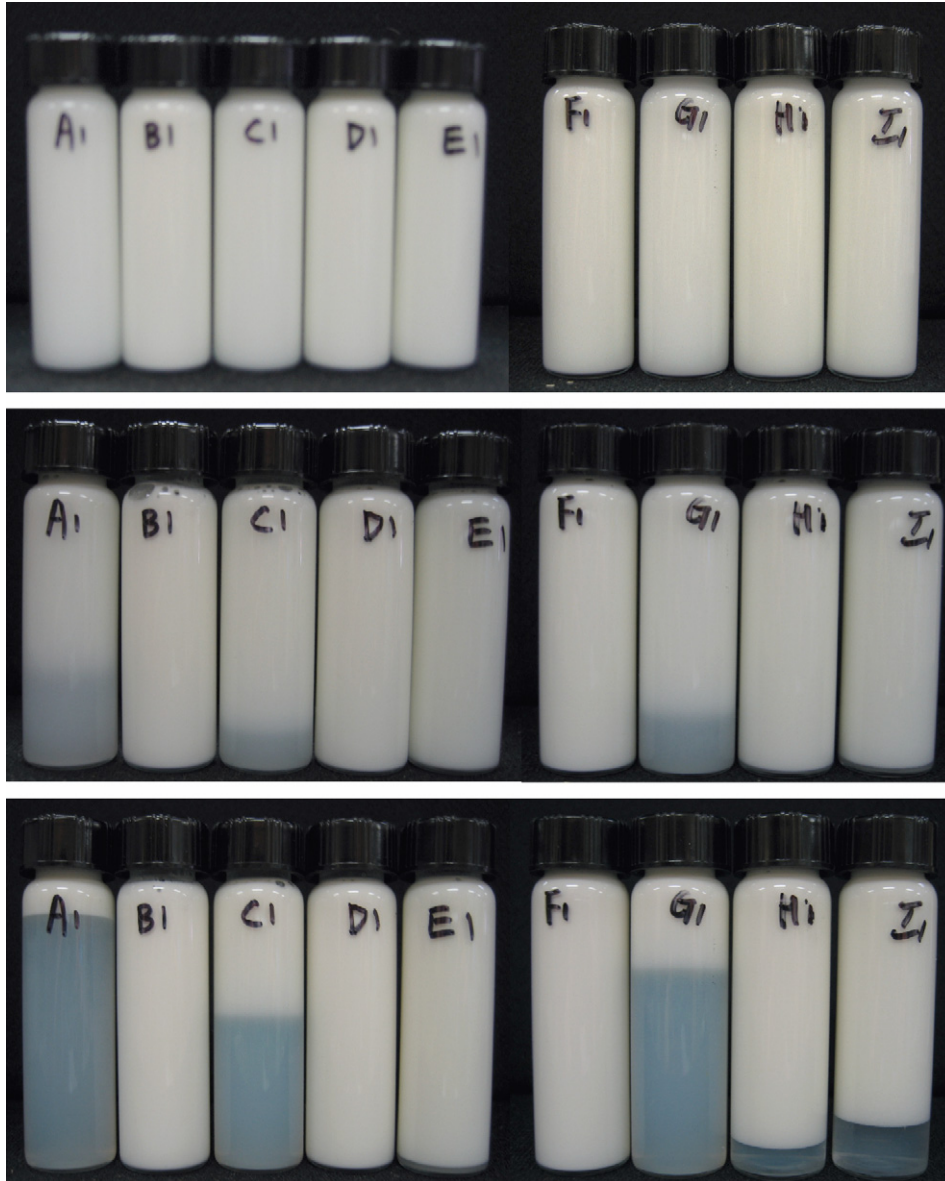


Fig. 3. Effect of compositions and storage time on creaming profile of the emulsions (The letter on the bottle (A–I) represents each formulation and refers to Table 1. Top: 1st-day profile; middle: 3rd-day profile; and bottom: 6th-day profile).

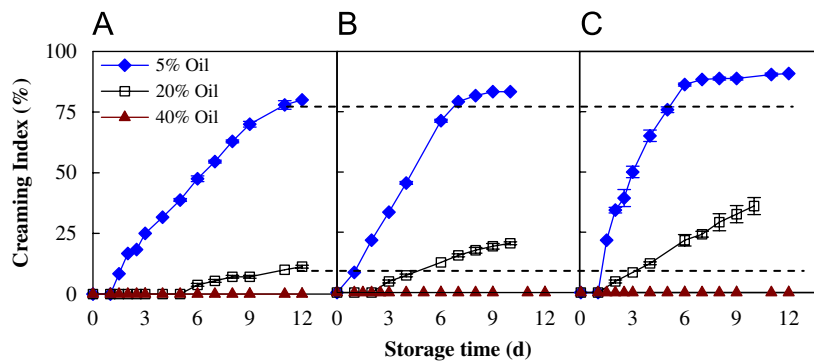


Fig. 4. Effects of compositions on creaming index of O/W emulsions containing 0.2 wt% XG: (A) 2 wt% WPI; (B) 1 wt% WPI, and (C) 0.2 wt% WPI.

WPI coating and enhanced the possibility of the contact between oil and the pro-oxidants. Third, some amount of hydroperoxides was available in the system (At 0 d,

PV ~25 mmol/kg oil) and they may have been present at the droplet surface in the O/W emulsion systems studied.

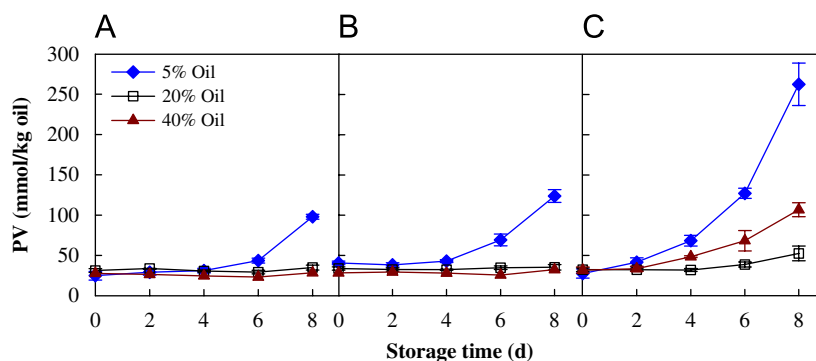


Fig. 5. Effects of WPI concentration, oil volume fraction, and storage time on peroxide value (PV) of O/W emulsions containing 0.2 wt% XG.

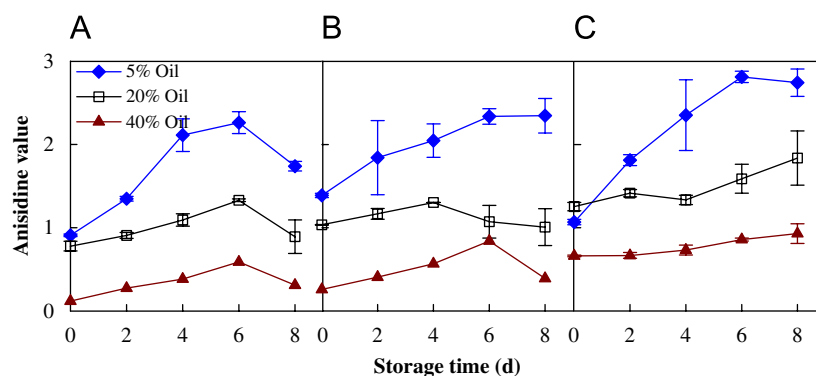


Fig. 6. Effects of WPI concentration, oil volume fraction, and storage time on anisidine value (AV) of O/W emulsions containing 0.2 wt% XG.

According to the above analysis on PV, the secondary lipid oxidation products should be investigated to evaluate oxidative stability of emulsion systems. As can be seen in Fig. 6, oil-phase volume fraction had a significant effect on AV over the storage period. The 5% v/v O/W emulsions had relatively higher AV over those days, which was in agreement with PV results we obtained. Lipid hydroperoxides were quickly produced in the 5% v/v O/W emulsions but were delayed in the emulsions containing higher oil-phase volume fraction (20% and 40% v/v). Those lipid hydroperoxides must first be formed before they decompose into secondary products. In the 0.2 wt% WPI-stabilized emulsions, 20% v/v O/W emulsions developed more aldehydes and had higher AV values than the 40% v/v emulsions, which confirmed our first explanation for lower PV values in the 20% v/v emulsions stabilized by 0.2 wt% WPI.

In emulsions containing 20% and 40% v/v emulsions, increasing WPI concentration from 0.2 to 2 wt% decreased the decomposition of hydroperoxides and AV changed negligibly at higher WPI concentrations (1 and 2 wt%, Fig. 6). These results indicated that WPI was an effective antioxidant to prevent the development of secondary oxidation products.

Lipid oxidation can be accelerated by reactions that take place at the surface of emulsion droplets (McClements & Decker, 2000a, 2000b). Therefore, the rate of lipid oxidation should increase as the droplet size decreases because

smaller droplets may expose larger surface area per unit volume to the pro-oxidants at the interface (McClements & Decker, 2000a, 2000b; Osborn & Akoh, 2004). Compared Fig. 1 with Fig. 5, the 5% v/v O/W emulsions stabilized by 2 wt% WPI had the smallest droplets but did not exhibit the fastest rate of lipid oxidation. Meanwhile, 40% v/v O/W emulsions stabilized by 0.2 wt% WPI had the largest droplets but did not show the slowest rate of lipid oxidation. Therefore, particle size did not directly affect lipid oxidation in our studies.

### 3.4. Rheological properties

Flow curves of the emulsions studied are shown in Fig. 7 as functions of WPI concentration and oil-phase volume fraction. All the emulsions studied exhibited pronounced shear thinning over the shear rate range tested in the presence of XG. As oil-phase volume fraction increased, low-shear viscosity increased and flow curves shifted upwards to higher viscosities due to the increase in the packing fraction of oil droplets. WPI concentration had no significant effect on the flow curves, which suggested that emulsion viscosity was insensitive to the amount of unadsorbed WPI in the aqueous phase.

Power-law model was applied to fit shear stress–shear rate plots of 2 wt% WPI-stabilized emulsions as a function of oil-phase volume fraction (Table 3); the  $K$  value increased with increasing oil volume fraction, which

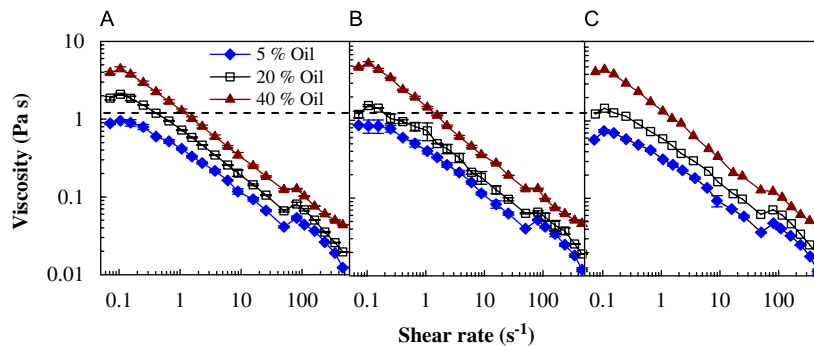


Fig. 7. Effects of compositions on the flow curves of O/W emulsions containing 0.2 wt% XG: (A) 2 wt% WPI; (B) 1 wt% WPI, and (C) 0.2 wt% WPI.

Table 3  
Fitting Power-law model to flow curve of 2 wt% WPI-stabilized emulsions containing 0.2 wt% XG as a function of oil-phase volume fraction

Volume fraction (% v/v)	$K^*$	$n^*$	$R^2$
5	$0.43 \pm 0.007^a$	$0.42 \pm 0.01^a$	0.995
20	$0.74 \pm 0.01^b$	$0.40 \pm 0.01^a$	0.996
40	$1.22 \pm 0.00^c$	$0.44 \pm 0.00^a$	1

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

indicated that increasing oil-phase volume fraction enhanced droplet interactions and the emulsions were more structured. However, increasing oil-phase volume fraction has no significant effect on  $n$ , the flow behavior index, which suggested that shear thinning behavior was mainly due to the presence of XG.

The presence of XG-induced depletion flocculation in the emulsions. Entrapment of a certain amount of continuous phase in the flocculated structure caused an increase in the effective volume fraction of hydrodynamically interacting entities, which in turn increased the viscosity of aggregated emulsion systems according to the Dougherty–Krieger equation (McClements, 1999). However, the flocculated structure broke up under high shear rate, and some trapped continuous phase was released, consequently lowering the viscosity (Dickinson & Golding, 1997); a region of lower Newtonian viscosity was expected at very high shear rate indicative of complete disruption of flocculated structure (Fig. 7).

Dynamic strain sweep data of the O/W emulsions studied are shown in Fig. 8. The WPI concentration had no significant effect on the linear range of storage modulus,  $G'$ . As was expected, higher oil-phase volume fraction contributed to an increase in  $G'$  due to the close packing of oil droplet. Regardless of WPI concentration, 5% v/v O/W emulsions did not show any linear range; 20% v/v oil fraction emulsions exhibited linear limit of  $\sim 0.76$  shear strain; the linear limit of  $G'$  of 40% v/v oil fraction emulsions was lower,  $\sim 0.21$  strain. These results indicated the extent of the linear region decreased with increasing oil-phase volume fraction from 20% to 40% v/v. Dickinson

and Chen (1999) suggested O/W emulsions may undergo a behavior transition from predominantly entropic behavior to predominantly enthalpic behavior with increasing oil-phase volume fraction. So the 20% v/v O/W emulsions behaved as polymer gels (strain hardening, long linear region, and large rupture strain), while the 40% v/v O/W emulsions behaved as particle gels (strain weakening, short linear region, small rupture strain).

#### 4. Conclusions

Physical properties (droplet size, surface charge, and viscosity) of the emulsions affected their creaming and oxidative stability. Increasing WPI concentration decreased droplet size and led to additional negative charges on droplet surfaces, which consequently contributed to the enhancement of steric stabilization and electrostatic repulsion against droplets flocculation. Increasing WPI concentration slightly decreased creaming due to the increase in the extent of droplet flocculation by the excessive unadsorbed WPI in the aqueous phase, and increased oxidative stability possibly due to the formation of WPI layers at droplet surfaces and antioxidant activity of the unadsorbed WPI in the aqueous phase. Oil-phase volume fraction played a dominant role in affecting creaming and lipid oxidation. Increasing oil-phase volume fraction had no significant effect on droplet size when there was sufficient WPI (1 or 2 wt%) in the emulsions to adequately stabilize the droplets, but appreciably increased low-stress viscosity and the strength of emulsion networks, and greatly increased the creaming stability, and significantly decreased the formation and decomposition of hydroperoxides and certainly enhanced oxidative stability of the O/W emulsions. The influence of oil-phase volume fraction on the physicochemical properties of the O/W emulsions mainly resulted from the effect of packing fraction of oil droplets within the emulsions. The study on creaming and rheological properties provided information on the mechanism of droplet flocculation and colloidal interactions between droplets. The rheological data qualitatively supported the experimental results on the creaming and oxidation. Therefore, the results of this study had



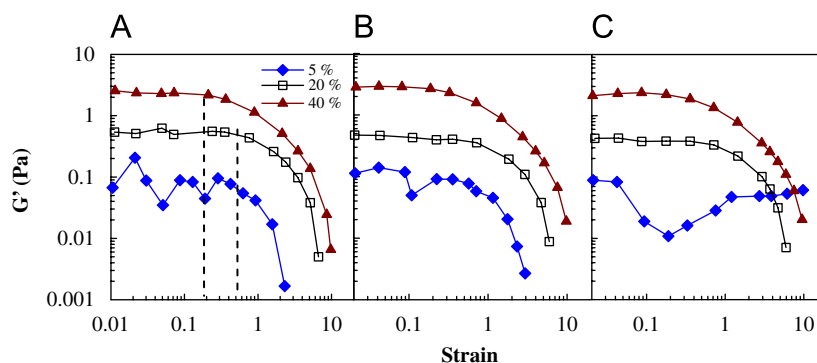


Fig. 8. Effect of compositions on the critical strain ( $\gamma_{cr}$ ) of O/W emulsions containing 0.2 wt% XG: (A) 2 wt% WPI; (B) 1 wt% WPI, and (C) 0.2 wt% WPI.

implications for the stability of WPI-based food emulsion formulations.

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