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**Effects of Xanthan-Locust Bean Gum Mixtures on the Physicochemical  
Properties of Oil-In-Water Emulsions**

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## **Abstract**

The effects of xanthan gum (XG)-locust bean gum (LBG) mixtures (0.05, 0.1, 0.15, 0.2 and 0.5 wt%) on the physicochemical properties of whey protein isolate (WPI) stabilized oil-in-water (O/W) emulsions containing 20% v/v menhaden oil was investigated. The apparent viscosity of the emulsions containing XG/LBG mixtures was significantly higher ( $p < 0.05$ ) than the emulsions containing either XG or LBG alone at higher concentrations of XG/LBG mixtures. Locust bean gum showed the greatest phase separation, followed by XG. Microstructure images showed depletion flocculation at lower biopolymer concentrations, and thus led to an increase in creaming instability and apparent viscosity of the emulsions. Addition of 0.15, 0.2 and 0.5 wt% XG/LBG mixtures greatly decreased the creaming of the emulsions. The rate of lipid oxidation for 8-week storage was significantly lower ( $p < 0.05$ ) in emulsions containing XG/LBG mixtures than in emulsions containing either of the biopolymer alone.

**Keywords:** Menhaden oil, xanthan gum, locust bean gum, emulsion, oxidative stability.

## 1. Introduction

The benefits of consuming long chain omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFA) have been widely reported (Ruxton, Reed, Simpson, & Millington, 2004; Siddiqui, et al., 2004; Shahidi & Miraliakbari, 2004; Sahena et al., 2009). Dietary intake of  $\omega$ -3 PUFA decreases risk of heart disease (Kris-Etherton, Harris, & Appel, 2002), provides an inhibitory effect on the growth of prostate and breast cancer (Pandalai, Pilat, Yamazaki, Naik, & Pienta, 1996), delays the loss of immunological functions (Fernandes, 1995), and is required for normal fetal brain and visual development (Neuringer, Anderson, & Conner, 1998). However, PUFA are highly susceptible to oxidation during storage, which creates various aldehydes and ketones products that render unacceptable odor and flavor in PUFA containing foods (McClements & Decker, 2008). In addition, lipid oxidation products, such as lipid peroxides, unsaturated aldehydes and malonaldehydes, and several cholesterol oxidation products (Sangeetha, Das, Koratkar, & Suryaprabha, 1990; Esterbauer, Schaur, & Zollner, 1990; Addis, 1986) have a negative impact on human health due to their cytotoxic and genotoxic effects (Kanner, 2007; Fang, Vaca, Valsta, & Mutanen, 1996). The rate of oxidation of PUFA can be ~~retarded~~ decreased by the addition of many synthetic or natural antioxidants. However, consumer health consciousness has led to a demand for natural alternatives to synthetic antioxidants (Huber, Rupasinghe, & Shahidi, 2009).

Oil-in-water emulsions in which the lipid portion is dispersed as miniscule droplets within an aqueous continuous phase can be effective vehicles for delivering  $\omega$ -3 PUFA into food systems as they can be easily incorporated into many food matrix types. One approach to inhibit lipid oxidation in oil-in-water emulsions is to use food biopolymers such as proteins and polysaccharides that can bind transition metals and partition them away from the emulsion droplet surface (Chen, McClements, & Decker, 2010).

Several studies have demonstrated that proteins dispersed in the continuous phase of oil-in-water emulsions can inhibit deleterious oxidation reactions by a combination of metal chelation and free radical scavenging (Elias, McClements, & Decker, 2005; Elias, McClements, & Decker, 2007). Whey protein isolate (WPI) is a surface-active globular protein and can be adsorbed to oil droplet surfaces in the form of a monolayer. They contain cysteyle residues, disulfide bonds and thiol functional groups, which can scavenge free radicals to inhibit lipid oxidation. Therefore, WPI-stabilized emulsions may act as an antioxidant system (Sun, Sundaram, & Mark, 2007).

Polysaccharides have been widely used as thickening, stabilizing, and gelling agents in the food industry for the control of microstructure, texture, flavor and shelf-life. The function of polysaccharides as emulsion stabilizers is not only attributable to their ability to increase the viscosity of the continuous phase and inhibit coalescence (Chen et al., 2010), but also to their effect on protein adsorption at the oil/water interface. Polysaccharides can induce continuous water phase and promote encapsulation of oil/water emulsion droplets by the protein-rich phase (Tolstoguzov, 1997). The antioxidant ability of polysaccharides in emulsions has also been reported (Chen et al., 2010; Kishk & Al-Sayed, 2007; Paraskevopoulou, Boskou, & Parakesvopoulou, 2007).

A synergistic interaction occurs between xanthan gum and galactomannans such as guar and locust bean gum (LBG) in solutions to result in enhanced viscosity or gelation (Dea, Morris, Rees, & Welsh, 1997; Tako, 1993; Tako & Nakamura, 1984, 1989; Goycoolea, Richardson, & Morris, 1995; Khouryieh H. A., Herald, Aramouni, & Alavi, 2006; Khouryieh H. A., Herald, Aramouni, Bean, & Alavi, 2007a, 2007b; Long et al., 2013). Despite the great number of studies dealing with milk protein– polysaccharide emulsions, relatively limited studies have been

performed on comparison of the influence of ionic and neutral hydrocolloids in protein–hydrocolloid emulsions. In addition, the effects of the synergistic interaction between xanthan gum and locust bean gum (LBG) combined with a protein on emulsion stability have never been investigated. This research focused on the effect of the synergistic interaction between xanthan gum (XG) and locust bean gum (LBG) on the stability of menhaden oil-in-water emulsions. The objectives of this study were to investigate the optimum levels of XG/LBG mixtures that allow incorporation of the  $\omega$ -3 PUFAs into emulsions and enhance the oxidative stability of  $\omega$ -3 PUFAs and to characterize the stability and microstructural properties of the emulsions.

## **2. Materials and Methods (any references for methodologies? Where did they come from?)**

### *2.1. Materials and chemicals*

Menhaden oil (14:0 myristic acid 6-9%, 16:0 palmitic acid 15-20%, 16:1 palmitoleic acid 9-14%, 18:0 stearic acid 3-4%, 18:1 oleic acid 5-12%, 18:2 linoleic acid < 3%, 18:3 linolenic acid < 3%, 20:4 arachidonic acid < 3% 18:4 octadecatetraenoic 2-4%, 20:5 eicosapentaenoic 10-15% and 22:6 docosahexaenoic 8-15%), xanthan gum, locust bean gum, iron(II) chloride tetrahydrate, xylenol orange disodium salt, 2-thiobarbituric acid, iron(III) chloride, methanol, 1-butanol, 1,1,3,3-tetraethoxypropane were purchased from Sigma Aldrich, Co. (St.Louis, MO, USA). Whey protein isolate was obtained from Davisco Foods International, Inc. (Le Sueur, MN). Hydrogen peroxide was obtained from Fischer Scientific (Fair Lawn, NJ, USA). All other chemicals and solvents were of analytical grade. Deionized water was used to prepare all the emulsions.

## 2.2. *Emulsion preparation*

Stock solutions WPI (10%, w/v), XG (1%, w/v) and LBG (1%, w/v) were prepared separately by dissolving the required amount of WPI, XG and LBG powders into deionized water at room temperature, followed by continuous stirring with a magnetic stirrer for 6 h to ensure complete dispersion. The stock solutions of XG and LBG were heated in a water bath at 80 °C for 30 min and 0.02% (v/v) sodium azide was added to prevent microbial growth.

The oil-in-water emulsions were prepared first by slowly mixing the required amount of menhaden oil into WPI solution; to this, either XG, LBG, or XG-LBG mixtures solution was added. The mixed solutions were first emulsified using a lab scale power homogenizer (PowerGen 500, Fischer Scientific, Fair Lawn, NJ, USA) for 5 min at 300 W output power. The emulsions were then sonicated in ultrasonic water bath (B 1500A-MT, VWR, San Francisco, CA, USA) for 1 min at high speed. The final composition of the oil-in-water emulsions was 20% v/v menhaden oil, 2 wt% WPI, and either 0-0.5 wt% XG, 0-0.5 wt% LBG or 0-0.5 wt% XG/LBG mixtures. XG and LBG solutions were blended in 50:50 synergistic ratios for the XG/LBG mixtures. Emulsions were stored in closed 20 mL bottles at room temperature (23 °C) in the dark (explain why i.e. why do we want to prevent light induced oxidation) for 8 weeks for the measurements of lipid oxidation.

## 2.3. *Viscosity Measurements*

Emulsion viscosity was measured using a Brookfield DV-II + Pro Viscometer (Brookfield Engineering, Middleboro, MA, USA). Readings were taken at a speed of 100 rpm using spindle No. 2 at 22 °C within 24 h of the emulsion preparation. Viscosities were determined at day 1 of emulsion preparation, making sure that all samples were measured within the same timeframe.

#### 2.4. *Creaming index*

Creaming index (CI) provides indirect information about the extent of the droplet aggregation in an emulsion. The more the aggregation, the larger the flocs and the faster is the creaming. Immediately after preparation emulsions were transferred to 21 mm diameter, 70 mm high glass test tubes and were sealed to prevent evaporation. They were kept at ambient temperature and the movement of any creaming boundary was tracked for a period of 15 d. Emulsions separated into a top cream layer and a bottom serum layer ~~was monitored~~. The total emulsion height ( $H_T$ ) and serum layer height ( $H_S$ ) were measured. The creaming index was reported as  $CI (\%) = 100(H_S / H_T)$ .

#### 2.5. *Microstructure*

Microstructure of the emulsions was studied using a deconvolution microscopy (Zeiss Axioplan IIe imaging, Carl Zeiss microscopy LLC, Thornwood, NY, USA) equipped with an attached camera. A 10x objective magnification was used to visualize the images at 50 mm below the cover slip. ~~The~~ A 50  $\mu$ L droplet of freshly made emulsion was taken on a 1.2 mm thickness of glass slide. A coverslip was placed on the top of the droplet ensuring no air gap or bubbles are seen. The images were processed using the software axiovision. (company name, address...)

#### 2.6. *Lipid Oxidation Measurements*

The effect of the gums concentration on stability of WPI-stabilized emulsions at room temperature was investigated during 8-week storage time in sealed 2 mL vials. Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were measured to evaluate the oxidative stability of the emulsions.

##### 2.6.1. *Peroxide value*



The PV was determined using the method described in detail by Shantha and Decker (1994). A 100 mg sample of emulsion was added to 9.9 mL of 7:3 chloroform/methanol solutions and the sample was vortexed for 2-4 sec. A 50  $\mu$ L of 10 mM xylenol orange was added to the sample and vortexed 2-4 sec and then 50  $\mu$ L iron (II) chloride was added and vortexed again. After 5 min of incubation at room temperature the absorbance of the sample was determined at 560 nm using a spectrophotometer UV-1201 (Shimadzu, Kyoto, Japan). A standard curve of  $\text{Fe}^{+3}$  concentration vs Absorbance was constructed using a standard solution of an iron(III) chloride (10  $\mu\text{gFe/ml}$ ). PV values were determined by matching absorbance of samples with the standard curve.

#### 2.6.2. TBARS value

TBARS ~~was~~ were determined using the method described by Ronald, Decker, Schwartz, and Sporns (2005). ~~The TBARS was determined by~~ A 100 mg sample of emulsion was dissolved in 25 mL of 1-butanol. A 5 mL of sample solution was transferred to dry screw cap glass test tube. To the sample solution, 5 mL of 0.2% thiobarbituric acid was added and glass tubes were vortexed. After 2 h of incubation in a water bath at 95 °C the tubes were cooled under water for 10 min. After cooling, the absorbance was measured at 532 nm using a spectrophotometer UV-1201 (Shimadzu, Kyoto, Japan). TBA values were determined using a standard curve made from 1,1,3,3-tetraethoxypropane(TEP). TBARS were expressed in  $\mu\text{mol}$  of malonaldehyde per/g of emulsion.

#### 2.7. Statistical Analysis

All data were analyzed to detect for significant differences using one- or two-way analysis of variance (ANOVA) procedure, as appropriate, with bonferroni's post-hoc comparisons tests. Level of significance was set at  $p < 0.05$ . All experiments were carried out at

least in duplicate. The results were reported as the mean  $\pm$  standard deviation (SD) of these measurements. Statistical analyses were conducted using the SAS (version 9.1.3) software (SAS Institute Inc., Carry, NC, USA).

### **3. Results and Discussions**

#### *3.1. Effect on viscosity of the emulsions*

Viscosity values increased with the increase in the concentration of gums in the emulsions (Fig. 1). The apparent viscosity values in WPI-stabilized oil-in-water emulsions containing LBG alone were significantly lower ( $p < 0.05$ ) than those with either XG or XG/LBG. This is because of weak polymer intermolecular interaction of neutral polysaccharide with the emulsion (Dea et al., 1997). Emulsions containing XG showed improved viscosity when compared to the emulsions containing LBG. The emulsion viscosity gradually increased with the increased concentration of XG and XG/LBG mixtures from 0 to 0.15 wt% and sharply increased when concentration exceeded 0.15 wt%. These results are in agreement with Sun et al. (2007) who reported sharp increase in the emulsion viscosity when XG concentration exceeded 0.2 wt%. This indicates that the viscosity of an emulsion is directly proportional to the viscosity of the continuous phase, any alteration in the rheological properties of the continuous phase has a corresponding influence on the rheology of the whole emulsion (McClements, 1999). The viscosity of the emulsions containing XG/LBG mixtures was significantly higher ( $p < 0.05$ ) than the emulsions containing either XG or LBG alone. The viscosity was sharply enhanced at higher concentrations of XG/LBG mixtures, which explains the synergistic interaction between anionic XG and nonionic LBG gum mixtures. This is due to the intermolecular binding that occurs between side chains of the xanthan and locust bean gum or due to cooperative interactions between ordered segments of XG and unsubstituted zones of the LBG mannan backbone or

could be interactions between disordered segments of xanthan molecules and LBG (Renou, Odile, Malhiac, & Grisel, 2013).

### 3.2. *Effect on microstructure of the emulsions*

Microstructure of the emulsions with LBG, XG and XG/LBG mixtures at various concentrations is shown in Fig. 2. Emulsions containing LBG appear to be flocculated at all of the concentration levels. In the presence of  $\geq 0.05$  wt% XG (Fig. 2B), oil droplets appear partially flocculated with more empty spaces between the droplets and the extent of flocculation increases slightly with increasing XG concentration up to 0.15 wt%. At 0.2 wt% concentration, oil droplets show considerable flocculation, but with smaller flocs. In the presence of 0.5 wt%, little or no flocculation was observed. In contrast, emulsion containing a concentration of 0.5 wt% appears with little or no flocculation. This is probably due to increased viscosity of the high concentration, which immobilizes the oil droplets and prevents them from coming close together. Similar results were reported by Sun et al. (2007). In the emulsions containing XG/LBG mixtures (Fig. 2C), the flocculation was much lower at  $\leq 0.1$  wt% when compared to either of the gum alone. At  $> 0.15$  wt% XG/LBG concentration, oil droplets appears to show little or no flocculation. This could be explained by the kinetic energy barriers associated with the restricted movement through highly viscous or networks of XG/LBG mixtures, which in turn influenced the rate and extent of phase separation in creaming.

### 3.3. *Effect on creaming stability of the emulsions*

Creaming stability was investigated to evaluate the relative stability of O/W emulsions. The creaming profile of the emulsions with different gum concentrations is shown in Fig.3. The creaming index of the emulsions as a function of time was plotted in Fig.4. The visual examination of the emulsions over period time shows slight phase boundary differentiation in

few of them and there was no separation in few. The emulsions with different concentrations of LBG showed serum separation even at high concentrations. The serum phase remained clear throughout the storage in these emulsions. The emulsion containing all LBG concentrations showed rapid cream separation for first 3 h except 0.5 wt% and then gradually slowed after 24 h and reached close to the plateau value for the emulsion stabilized by WPI alone (control). Around 60% creaming was observed at 24 h for 0.05 wt% of emulsion containing LBG. Similar creaming behaviors of different concentrations of XG were explained in Sun et al. (2007). The creaming was extremely rapid at 0.05 wt% of XG alone at 24 h with the creaming of 66%. Creaming rate was ended as 24.53% and 19.7% at 24 h for emulsions containing 0.1 wt% and 0.15 wt% of XG respectively. Addition of 0.2 wt% XG along with WPI significantly decreased the creaming of the emulsion compared with lower concentrations and serum separation was observed after the 96 h storage. The emulsions with 0.5 wt% XG did not show any phase separation during the storage period of 15 d. This is due to sharp increase in viscosity at 0.5 wt%, which prevented the fluid from moving there by decreases creaming rate. In the presence of XG/LBG mixtures, the emulsions containing 0.05 wt% showed a small phase separation at 18 h of storage time, whereas 0.1 wt% showed a phase separation after 168 h. At 0.15 wt% or higher, no distinct serum separation was observed during the 15 d storage time. There was significant ( $p < 0.05$ ) difference between XG and XG/LBG emulsions for all concentrations except for 0.2 wt% and 0.5 wt%.

Depletion flocculation and thermodynamic incompatibility are the two main mechanisms that could lead to phase separation in protein-polysaccharide mixtures. In the beginning there was no clear boundary separation between the cream layer and serum layer, but as the time progress there was sharper separation due to the larger droplets moved to the top cream layer and

small ones to the bottom in the emulsions containing all the concentrations of LBG and XG concentrations with  $< 0.5$  wt%. The creaming behaviors can be explained by flocculation from Fig. 2. From the images the flocculation was much lower in emulsions containing XG/LBG mixtures ( $\geq 0.1$  wt%) when compared to either XG or LBG emulsions because of the enhanced viscosity, which immobilizes the oil droplets. At higher concentration of XG/LBG mixtures ( $\geq 0.1$  wt%) and XG ( $\geq 0.2$  wt%), lighter depletion flocculation was observed (Long et al., 2013). In this case, larger droplets did not cream faster than small ones as they were to form a weak gel like network throughout the system (Dickinson, 2003). Because of weak polymer intermolecular interaction of neutral polysaccharide with the emulsion, the creaming index was much faster for LBG emulsions when compared to XG or XG/LBG emulsions even with high concentrations of LBG in the emulsion. Inhibition of creaming at high concentrations ( $\geq 0.1$  wt%) of XG/LBG mixtures is probably due to the synergistic interaction between XG and LBG, which resulted in the enhanced viscosity and form a weak gel like network. The excellent physical stability in the emulsions containing polysaccharides is likely due to the ability of the polysaccharide to increase the viscosity of the continuous phase, which decreases droplet collisions, thus decreasing flocculation and coalescence. In addition, viscosity enhancement by the polysaccharides would decrease creaming rates (McClements, 2004).

#### *3.4. Effect on oxidative stability of the emulsions*

Lipid hydroperoxides are primary oxidation products that have a shorter half-life than secondary oxidation products. The PV of the emulsions as a function of time is shown in Fig. 5. The PV was dramatically increased in all samples containing LBG or XG alone during the 8-week storage time. The PV for emulsions containing LBG or XG decreased with the increase in the concentration, but the decrease is not so prominent (Fig. 5A, B). However, XG was slightly

more effective at inhibiting lipid oxidation than LBG. Emulsions with XG may suppress the lipid oxidation by chelation of iron between two side chains with a pyruvate residue. Several authors (Mei, McClements, Wu, & Decker, 1998a; Donnelly, Decker, & McClements, 1998; Sun et al., 2007; Faraji, McClements, & Decker, 2004) reported the inhibition of the lipid oxidation by chelation of iron and therefore inactivating the peroxy radicals. Emulsions containing XG/LBG mixtures (Fig. 5C) had significantly ( $p < 0.05$ ) lower PV at higher concentrations ( $\geq 0.15$ ) than in that of emulsions containing either LBG or XG alone. At higher concentrations, the XG/LBG emulsions decreased the levels of primary hydroperoxides. This is probably due to the enhanced viscosity from the synergistic interactions between XG and LBG, which resulted in the slow diffusion of oxidants to oil droplet surface area and hence the decrease in the rate of lipid oxidation. The differences in the oxidative behavior depend on the interaction of the XG with LBG and concentration. It may also depend on the surface charge of emulsion droplets, in the decreasing order of anionic, nonionic and cationic emulsifier (Yoshida & Niki, 1992).

TBARS values are an indication of secondary oxidation products formed from the breakdown of oxidized PUFAs. TBARS values of WPI-stabilized emulsions in the presence of LBG, XG and XG/LBG mixtures at different concentrations are shown in Fig. 6. The TBARS values of the emulsions containing LBG (Fig. 6A) rapidly increased during the storage period, but the oxidation rate in the emulsion without LBG was faster than that in the emulsions containing LBG. The TBARS decreased with the increase in the concentrations for emulsions containing LBG or XG (Fig. 6A, B). However, the decrease in the TBARS is not so noticeable for the emulsion containing LBG or XG. The emulsion containing XG was slightly more efficient in reducing lipid oxidation than LBG. The TBARS values for emulsions containing XG/LBG mixtures (Fig. 6C) were significantly lower than in emulsions containing either XG or

LBG alone. Significant differences ( $p < 0.05$ ) were observed between the LBG and XG/LBG emulsions at all concentration levels. When compared with XG, emulsions containing XG/LBG mixtures had significantly ( $p < 0.05$ ) lower TBARS values only at higher concentrations ( $\geq 0.15$  wt%). At higher gum emulsion concentration, the packing of surfactant molecules at the oil-in-water interface is tighter; hence, the membrane acts an efficient barrier to the diffusion of lipid oxidation initiators into oil droplets (Coupland & McClements, 1996). The lipid oxidation was retarded more effectively at  $\geq 0.15$  wt% in the XG/LBG emulsions, which shows ability to inhibit the inactive peroxy radicals in the emulsified oil by the combination. TBARS measurements showed that the emulsions made with  $\geq 0.15$ wt% XG/LBG mixtures offered better protection against lipid oxidation when compared to 0.5 wt% XG emulsions or against all the concentrations of emulsion containing LBG.

#### **4. Conclusion**

The physicochemical properties and oxidative stability of the oil-in-water emulsions were strongly influenced by the biopolymer type and concentration level. The results revealed that emulsions containing XG/LBG mixtures had better creaming and oxidative stability than emulsions containing either XG or LBG alone. The XG/LBG mixtures in oil-in-water emulsions significantly increased apparent viscosity, reduced creaming, decreased depletion flocculation, and improved oxidative stability. This study has helped to identify the optimum levels of XG/LBG mixtures that allow incorporation of the  $\omega$ -3 PUFAs into emulsions and suggests that mixtures of XG and LBG could be used to as antioxidants for stabilizing  $\omega$ -3 PUFA-containing foods. Because protein-stabilized emulsions are particularly sensitive to pH and ionic strength effects, further research will be conducted to investigate the effects of XG/LBG mixtures on the

emulsions with a wide range of different pH values and ionic strengths.

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