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Effect of xanthan/enzyme-modified guar gum mixtures on the stability of whey protein isolate stabilized fish oil-in-water emulsions

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ABSTRACT

The effect of xanthan gum (XG) and enzyme-modified guar (EMG) gum mixtures on the physicochemical properties and oxidative stability of 2 wt% whey protein isolate (WPI) stabilized oil-in-water (O/W) emulsions containing 20% v/v fish oil was investigated. EMG was obtained by hydrolyzing native guar gum using α-galactosidase enzyme. At higher gum concentrations (0.2 and 0.3 wt%), the viscosity of the emulsions containing XG/EMG gum mixtures was significantly higher ($P < 0.05$) of all emulsions. Increasing concentrations (0–0.3 wt%) of XG/EMG gum mixtures did not affect the droplet size of emulsions. Microstructure images revealed decreased flocculation at higher concentrations. Primary and secondary lipid oxidation measurements indicated a slower rate of oxidation in emulsions containing XG/EMG gum mixtures, compared to XG, guar (GG), and XG/GG gum mixtures. These results indicate that XG/EMG gum mixtures can be used in O/W emulsions to increase physical and oxidative stabilities of polyunsaturated fatty acids in foods.

1. Introduction

Due to the health benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA) there has been an increasing industrial interest using fish oils in foods during the past decade (Sorensen et al., 2008). However, the use of fish oils in foods is limited owing to their susceptibility to oxidation. In recent decades, much attention is given to the use of natural antioxidants, as alternatives of synthetic food additives, to minimize oxidation (Sorensen et al., 2008). In oil-in-water emulsions, the interfacial membrane formed by the emulsifiers can have a significant effect on the rate of lipid oxidation by affecting the location and reactivity of pro-oxidative transition metals, lipid hydroperoxides, free radical scavengers and metal chelators (Yi, Zhu, McClements, & Decker, 2014). Besides the oxidative deterioration, oil-in-water emulsions also suffer from physical instability. Because oil-in-water emulsions are thermodynamically unstable systems, they have propensity to breakdown overtime and separate into a layer of oil on top of a layer of water (McClements, 2015).

Proteins and polysaccharides are safe food additives that can form physically stable emulsions while altering the properties of the emulsion droplet interface in a way that increases oxidative stability of oil-in-water emulsions (Hu, McClements, & Decker, 2003). Research studies have shown (Guzey & McClements, 2007; Khouryieh, Puli, Williams, & Aramouni, 2015) that oil droplets coated by protein-polysaccharide complexes have better physical and oxidative stability than that those coated by proteins alone because of the difference in interfacial charge, structure, and thickness of layer. The stability of oil-in-water emulsions containing protein-coated droplets can be further increased by adding a polysaccharide that adsorbs onto the droplet surface and forms a protective layer (Ercelebi & Ibanoğlu, 2007; Guzey & McClements, 2007; Koupantsis & Kiosseoglou, 2009).

Whey proteins are surface-active proteins that adsorb on the surfaces of oil droplets and prevent the droplets from coming close enough to aggregate (Sun, Gunasekaran, & Richards, 2007). They increase the stability of formed droplets from aggregation by increasing the repulsive colloidal interactions between them (Surh, Ward, & McClements, 2006). Whey proteins can also inhibit lipid oxidation in emulsions when they are either at the surface of the emulsion droplet or in the aqueous phase (Hu et al., 2003).

Xanthan gum (XG) is a heteropolysaccharide and widely used in oil-in-water emulsions to increase their physical stability. Guar gum (GG) is a high molecular weight, neutral polysaccharide that is obtained from the guar plant *Cyamopsis tetragonolobus*

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(Chudzikowski, 1971). The chemical structure of guar is composed of a straight chain of D-Mannose units, linked together by β (1 → 4) glycoside linkages, and having on approximately every alternate mannose a single D-galactose unit, joined to it by an α (1 → 6) glycoside linkage (Chudzikowski, 1971). The synergistic interaction of xanthan gum with galactomannans such as locust bean gum and guar gum is of great interest because of the resulting distinctive rheological properties and formation of a mixed gel. Our previous research has showed increased physical and oxidative stabilities of whey protein-stabilized emulsions containing xanthan and locust bean gum mixtures (Khouryieh et al., 2015). The mannose to galactose ratio of galactomannans has been found to be an important factor for the interaction with xanthan gum. Guar gum, which has a mannose to galactose ratio of around 2:1, shows weak synergism, while locust bean gum, with a ratio of 4:1, interacts more strongly with xanthan gum. To make roughly guar structure identical to locust bean gum skeleton, some of the side-chain (1 → 6)-α-linked D-galactosyl residues of guar gum can be removed without notable cleavage of the backbone using an enzyme-mediated reaction. The suitable enzyme for this purpose is α-galactosidase (Bulpin, Gidley, Jeffcoat, & Underwood, 1990). Enzyme-modified guar (EMG) galactomannans containing similar mannose/galactose ratios to locust bean gum were found to exhibit similar physical properties, such as co-gelation with xanthan gum. Therefore, it has been demonstrated that α-galactosidase action on guar gum generates galactomannans with decreased galactose to mannose ratio and increased functional value (Bulpin et al., 1990).

Although many studies have dealt with xanthan and galactomannan interactions (Khouryieh et al., 2015; Khouryieh, Herald, Aramouni, Bean, & Alavi, 2007a,b, 2006; Bresolin, Milas, Rinaudo, & Ganter, 1998; Rinaudo, Milas, Bresolin, & Ganter, 1999), no study, to our knowledge, has ever been conducted on the use of combined XG and EMG gum mixtures in oil-in-water emulsions. Therefore, the objective of this study was to investigate the effect of XG/EMG gum mixtures (ranged from 0 to 0.3 wt%) on the physical and oxidative stabilities of WPI-stabilized fish oil-in-water emulsions, and determine the optimum concentrations of XG/EMG gum mixtures that are capable of producing thermodynamically stable emulsions. To effectively assess the stability of oil-in-water emulsions, emulsions with XG/EMG gum mixtures were compared to emulsions containing XG/GG gum mixtures, XG, and GG. This research will provide better understanding of how the synergistic interaction between XG and EMG can affect the stability of oil-in-water emulsions, and determine if XG/EMG gum mixtures would provide better stability to fish oil-in-water emulsions than using either polysaccharide alone.

2. Materials and methods

2.1. Materials and chemicals

Menhaden oil without any added antioxidants (14:0 Myristic acid 6–9%, 16:0 Palmitic acid 15–20%, 16:1 palmitoelic acid 9–14%, 18:1 oleic acid 5–12%, 18:2 linoleic acid <3, 20:4 arachidonic acid <3%, 18:4 octadecatetraenoic acid 2–4%, 20:5 eocosapentanoic acid 10–15% and 22:6 docosahexaenoic acid 8–15%), xanthan gum, guar gum, isooctane, ammonium thiocyanate, barium chloride, ferrous sulfate, trichloroacetic acid, thiobarbituric acid, cumene hydroperoxide, and 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich Co. St Louis, MO, USA. Whey protein isolate was obtained from Davisco Foods Int'l, Inc.; xanthan gum and guar gum were purchased from Sigma-Aldrich Co.; α-Galactosidase enzyme was purchased from Megazyme International, Ireland. All other reagents and chemicals used were of analytical grade. Deionized water was used to prepare the emulsions.

2.2. Enzymatic degradation of guar gum

The method described by Pai and Khan (2002) was used for the enzymatic hydrolysis of guar gum. The hydrolysis was carried out at a constant temperature of 35 °C using α-galactosidase from guar seed. One percent (w/w) guar solutions were incubated with 0.4 U/mL of α-galactosidase. Incubation time was set at 5 h to obtain a modified guar sample. The enzyme was deactivated at the end of the incubation time by heating the solution at 85 °C for 10 min. The solution was then centrifuged at 10,000 rpm for 20 min. After the centrifugation, the clear, supernatant solution was discarded and the precipitate containing modified guar gum was lyophilized for 24 h using a freeze dryer. After 24 h, the freeze dried sample was collected and pulverized using a mortar and pestle to obtain a fine modified guar gum powder.

2.3. Emulsion preparation

Whey protein isolate (WPI) (20% w/v), xanthan gum (1%), guar gum (1%) and enzymatic modified guar gum (1%) stock solutions were prepared by stirring the solutions on a magnetic stirrer for 10 h. After 10 h, xanthan gum was heated at 70 °C in a water bath for 60 min to ensure homogeneous dispersion. To the stock solutions, 0.05% sodium azide was added to prevent microbial growth.

The oil-in-water emulsions were prepared by first emulsifying deionized water with WPI for 2 min using a Fisher Scientific PowerGen 500 homogenizer at 30,000 rpm. To the WPI solution, required amount of menhaden oil was slowly added and homogenized for 5 min. Finally, required quantities of gums were added and homogenized for 3 more min. The prepared emulsions were then sonicated in an ultrasonic water bath for 1 min at high speed using a VWR sonicator. Six different concentrations (0, 0.05, 0.1, 0.15, 0.2, and 0.3 wt%) of xanthan (XG), guar (GG), xanthan/guar (XG/GG), and xanthan/enzyme-modified guar (XG/EMG) gum mixtures were prepared in the emulsions. The final composition of the emulsions was 20% (v/v) menhaden oil, 2% (w/v) WPI, and 0–0.3% (w/v) of gums. The pH of the final emulsions was around 6.6. For emulsions with XG/GG and XG/EMG mixtures, the gums were blended in a 1:1 ratio.

2.4. Particle size measurements

The droplet size of the emulsions was determined using the Malvern Mastersizer hydro 2000MU (Malvern Instruments, Ltd., Worcestershire, UK). This instrument measures the angular dependence of the scattered light intensity when a laser beam passes through a dilute emulsion. The intensity vs angle profile is then converted to a droplet distribution by the instrument. Emulsions were diluted with 800 mL of deionized water to prevent multiple scattering effects. Emulsion sample was added until the obscuration is in 10–20% range. The refractive indices used for oil droplet and deionized water were 1.46 and 1.33, respectively. The droplet size of all emulsions samples were measured in duplicate. The surface weighted mean, D [3, 2] of oil droplets was calculated. The particle size is reported as a volume equivalent sphere diameter.

2.5. Viscosity measurements

Emulsion viscosity against shear rate was measured using a Discovery Hybrid Rheometer, TA Instruments, with the following settings: Geometry: 40 mm parallel plate; Temperature: ~24 °C; stop head gap: 1000.0 μm; geometry gap: 1000.0 μm; trim gap: 1000 μm; loading gap: 26500.0 μm; time gap offset: 50.0 μm; shear rate: 2.0–100.0 1/s; Velocity 0.0–5.0 rad/s; torque 10.0–1000.0 μN m. One mL of emulsion sample was taken and the