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Ability of whey protein isolate and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion

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ABSTRACT

The effect of pH on the capability of whey protein isolate (WPI) and fish gelatin (FG), alone and in conjugation, to form and stabilize fish oil-in-water emulsions was examined. Using layer-by-layer interfacial deposition technique for WPI–FG conjugate, a total of 1% protein was used to prepare 10% fish oil emulsions. The droplets size distributions and electrical charge, surface protein concentration, flow and dynamic rheological properties and physiochemical stability of emulsions were characterize at two different pH of 3.4 and 6.8 which were selected based on the ranges of citrus and milk beverages pHs, respectively. Emulsions prepared with WPI–FG conjugate had superior physiochemical stability compare to the emulsions prepared with individual proteins. Higher rate of coalescence was associated with reduction in net charge and consequent decrease of the repulsion between coated oil droplets due to the proximity of pH to the isoelectric point of proteins. The noteworthy shear thinning viscosity, as an indication of flocculation onset, was associated with whey protein stabilized fish oil emulsion prepared at pH of 3.4 and gelatin stabilized fish oil emulsion made at pH of 6.8. At pH 3.4, it appeared that lower surface charge and higher surface area of WPI stabilized emulsions promoted lipid oxidation and production of hexanal.

1. Introduction

Among the functional ingredients ω-3 and ω-6 fatty acids in fish oil which contain eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) has been claimed for their health benefits. These benefits include reduced susceptibility to mental illness, protection against heart disease, and improved brain and eye function in infants (Krutulyte et al., 2008; Ritter-Gooder, Lewis, Barber-Heidal, & Waltz-Hill, 2008; Siró, Kápolna, Kápolna, & Lugasi, 2008). As a result, food products containing these polyunsaturated fatty acids which positively affecting human health can be classified as so-called functional food (Kolanowski, Świderski, & Berger, 1999).

However, the ω-3 and ω-6 fatty acids are subject to rapid and/or extensive oxidation and other chemical changes by exposure to air, light or heat during processing (Jacobsen, Bruni Let, Nielsen, & Meyer, 2008; Medina, Cascante, Torres, & Pazos, 2008). The outcomes are production of aldehydes, ketones, alcohols and hydrocarbons (Coupland & McClements, 1996) that render unacceptable colours, odours and flavours in polyunsaturated fatty acid (PUFA) containing foods and nutraceutical products. In addition, products of lipid oxidation, such as hexanal, propanal, acrolein and malonaldehyde, among others, possess adverse health effects due to their cytotoxic and genotoxic effects (Giroux, St-Amant, Fustier, Chapuzet, & Britt, 2008; Huber, Vasantha Rupasinghe, & Shahidi, 2009).

Therefore, successful incorporation of ω-3 fatty acids into processed foods would most likely be in the form of lipid dispersions which are referred to as oil-in-water emulsions (Dalgleish, 2006). Small spherical oil droplets, in an oil-in-water emulsion, could be stabilized in the aqueous phase by surface-active hydrocolloids such as proteins, arabic gum and modified starch (Sun & Gunasekaran, 2009; Taherian, Fustier, Britten, & Ramaswamy, 2008). The surface-active hydrocolloid is adsorbed at the interface between oil and the aqueous phase to lower surface tension, increase force of repulsion and prevent oil droplets from aggregation. Proteins extracted from a variety of natural sources can be used as emulsifiers in foods because of their ability to facilitate the formation, improve the stability, and produce desirable physicochemical properties in oil-in-water emulsions (Surh, Decker, & McClements, 2006; Surh, Ward, & McClements, 2006).

Owing to its hydrophobic and hydrophilic regions, whey protein isolate has been widely used as an emulsifier for its ability to adsorb rapidly at the oil–water interface and provide protection for oil

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droplets through a combination of electrostatic and steric interactions (Matsumiya, Takahashi, Inoue, & Matsumura, 2010; Sun & Gunasekaran, 2009). Such adsorbed layers around the surface of oil droplets are responsible for stabilizing the vast majority of food emulsions against flocculation and coalescence. The unfolding of protein molecules at the oil–water interface leads to changes in secondary and tertiary structure, and to the exposure of residues which would normally be buried within the native globular structure (Dickinson & Matsumura, 1991).

Gelatin, a derivative of animal collagen, is a relatively high molecular weight protein which is prepared by sweltering animal tissues in the presence of either acid (Type "A", p$I \sim 7$–9) or alkaline (Type "B" p$I \sim 5$). The relatively high isoelectric point (p$I \geq 7.0$) of Type "A" gelatin allows the creation of oil-in-water emulsions with positively charged droplets. As a result, Type A gelatin may be suitable for preparing oil-in-water food emulsions with high oxidative stability since it could repel iron ions from oil droplet surfaces over most of the pH range typically found in foods (Surh, Decker, et al., 2006; Surh, Ward, et al., 2006). Gelatin as an emulsifier has been subject of several studies (Cheng, Lim, Chow, Chong, & Chang, 2008; Lobo, 2002; Ries, Ye, Haisman, & Singh, 2010; Surh, Gu, Decker, & McClements, 2005).

The aims of this work were first to find the evidence for preferential adsorbtion of the WPI over FG using deposition technique and characterize the physiochemical properties of the omega-3 fish oil emulsions as an influence of pH and understand the factor that determine the efficiencies of WPI and FG, alone and conjugated, for providing the steric and electrostatic stabilization against coalescence and flocculation.

2. Material and methods

2.1. Materials

Fish oil (OmegaPure, Houston, TX) containing 32–37% omega-3 fatty acid was kindly donated by NEX-XUS (Montreal, PQ). Based on the claim by OmegaPure the fish oil contains 35.2% omega-3 fatty acids and fatty acid profile was as follow:

Fatty acid	Area% of total fatty acid
Myristic, C14:0	8.2
Palmitic, C16:0	19.1
Palmitoleic, C16:1	11.7
Stearic, C18:0	3.0
Oleic, C18:1	13.2
Linoleic, C18:2 (n-6)	2.2
Alpha Linoleic, C18:3 (n3)	1.6
Stearidonic, C18:4 (n-3)	3.5
Arachidonic, C20:4 (n-3)	1.7
Eicosapentaenoic, C20:5 (n-3)	13.8
Docosapentaenoic, C22:5 (n-3)	2.2
Docosahexaenoic, C22:6 (n-3)	11.8
Other	7.0

Right after receiving the fish oil, 36×30 g fish oil was weighed in 36 screw cap bottles and store at -18 °C. Fish gelatin (275 FG 30) and whey protein isolate (Hilmar™ 9400) were respectively provided by Rousselot Inc (Wisconsin, WI) and Hilmar Ingredients (Hilmar, CA). Food grade citric acid and disodium phosphate dehydrate (donated by Canada Colors and Chemicals Limited, Brampton, ON) were used to adjust the acidity and 0.02% sodium azide to reduce the risk of contamination in prepared emulsions.

2.2. Preparation of stock solutions

Buffer solutions were prepared based on the method by Colowich and Kaplan (1995). The pHs of buffer solutions were adjusted at 3.4 (juice beverage) and 6.8 (milk beverage) using citric acid (0.1 M) and dibasic sodium phosphate (0.2 M) solutions mixed in appropriate ratios.

2.3. Preparation of emulsions

Prior to preparation of emulsions, fish oil was thawed in a refrigerator at 4 °C for 12 h. Pure protein emulsions were then prepared by slow addition of 3 g WPI or FG to 267 g buffer solution in a pre-homogenize vessel and successive blending at high speed for 2 min using a commercial blender (Waring, ON, Canada). Protein solutions were then placed in a screw cap bottle and kept overnight at 4 °C (WPI) or room temperature (fish FG) for complete hydration. Fish gelatin was stored at room temperature to prevent low temperature gelation. Following day, pre-weighed 30 g fish oil was slowly added into a 500 ml beaker containing hydrated WPI or FG solution while blending at low speed. A coarse emulsion (300 mL total volume) was then made by blending fish oil and protein solution for 3 min at high speed. Oil droplets size was further reduced with the aid of high pressure homogenizer (Emulsiflex-C5, Avestin, ON, Canada) at 4000 psi for 3 passes. Final protein and fat content in the emulsions were respectively 1 and 3%. The prepared emulsions were transferred into screw cap glasses bottle and tested right after preparation. The rest of emulsion was loaded with 0.02% sodium azide and stored at 4 °C before conducting the second series of test.

For the preparation of WPI–FG conjugate emulsions, the method of Aoki et al. (2005) was adopted with slight modifications. WPI (1.5 g) and FG (1.5 g) were separately hydrated in 133.5 g buffer solutions. Fish oil (30 g) was first added into the hydrated whey protein, while agitating, and blended for 3 min at high speed. The coarse emulsion was homogenized at 4000 psi for 3 passes to prepare primary emulsion. The primary emulsion was then diluted in hydrated FG following by blending for 3 min at high speed and high pressure homogenization at 4000 psi for 3 passes. Prepared emulsions were tested right after preparation and within 3 weeks for assessment of size growth kinetics.

2.4. Electrical charge and oil droplet size

The electrical charge (ζ-potential) and mean diameter of emulsion droplets were determined using a commercial instrument capable of electrophoresis and dynamic light scattering measurements (Zetasizer Nano-ZS, Malvern Instruments, Worcs., UK). Prior to conducting the measurements, emulsions were diluted 1: 250 (using double distilled water) in order to prevent multiple scattering effects in size measurement. Viscosity of diluted emulsion was measured at constant shear rate of 0.1 s^{-1} and 25 °C for 1 min to consider viscosity effect in ζ-potential assessment. Each individual ζ-potential data point was calculated from the average of at least 6 readings made on the duplicate samples.

2.5. Assessment of emulsion protein load: effect of protein concentration

Emulsions were first prepared at 4 level of protein concentrations (0.2, 0.6, 1, and 1.5 wt%) using the identical preparation methods provided earlier. The concentration of adsorbed and free protein at the interface in the emulsions was then determined by centrifugation of emulsions at $25{,}200 \times g$ during 60 min at 5 °C using Beckman model J2.21 and rotor model JA-20.1 (Beckman Centrifuge, USA) to