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Reduction of omega-3 oil oxidation in stable emulsion of caseinate-omega-3 oil-oat beta-glucan☆

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ABSTRACT

Lipid oxidation of ω-3 fatty acids has posed a serious challenge for incorporating heart-healthy oil products into healthful foods and beverages. In this study, plant and marine based ω-3 oils were dispersed into sodium caseinate dispersions and then mixed with purified heart-healthy oat gum comprised of 97% β-glucan in a homogenizer under room temperature. The stable emulsions underwent shelf life tests to assess their stabilities and oxidative activities. Various preparations of ω-3 oil-in-water emulsions with 10mL oil/100mLstabilized with sodium caseinate ranging from 0 to 3g caseinate/100g sample and β-glucan ranging from 0 to 0.3g β-glucan/100g sample were formed. The physical properties such as creaming index, particle size, and viscosity of the emulsions were measured. The lipid oxidation was measured as lipid hydroperoxide concentration and reduction of lipid oxidation was observed under shelf-life stress tests at 28 °C. The fatty acids of the oils were also measured using gas chromatography. Omega-3 fatty acids compositional changes were observed over the shelf life tests. It was found that caseinate helped reduce the oxidation of the oils in general and there is no significant impact of β-glucan on oxidation. Addition of caseinate and β-glucan in the emulsions slightly increased both particle size and viscosity.

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1. Introduction

Omega-3 fatty acids, which are present in many species of fishes and plants and have been known to have cardiovascular health effects, are increasingly incorporated into a variety of functional food products. For example, it has been reported that β-glucan rich oat products combined with ground omega-3 oil rich chia seeds could be used as food ingredients in functional food applications with good nutritional and texture qualities (Inglett, Chen, Liu, & Lee, 2014; Inglett, Chen, & Liu, 2014). There were reported studies of using milk proteins to reduce fish oil oxidation in energy bars (Nielson & Jacobsen, 2009) and in oil-in-water emulsions (Djordjevic, McClements, & Decker, 2013). Commercial omega-3 fatty acid containing healthful beverages, however, are relatively uncommon in the market. This is in part due to the issue of oil oxidation in the types of emulsions found in many heart-healthy beverages and emulsion stability of this category of beverages. Polyunsaturated omega-3 oil fatty acids in beverage emulsions are vulnerable to oxidation, resulting in loss of nutritional quality and development of objectionable off-flavors. Beverage emulsions belong to a class of very dilute dispersion systems containing a stable oil phase that could last as long as 12 months (Given 2009). The beverage industry has used emulsions as carriers for flavors that are mostly hydrophobic in many diverse beverages ranging from soda to energy drinks. Hydrocolloids such as modified starch, gum acacia, milk proteins, and other emulsifiers are used to stabilize the emulsions. Some of these hydrocolloids, noticeably milk proteins, are ideal carrier media for bioactive compounds (Livney, 2010). Milk proteins, a relatively inexpensive food materials, have unique physicochemical properties that facilitate the binding, emulsifications, and in some cases, protection of bioactive compounds. Milk proteins, such as caseinates and β-lactoglobulins, are known to render oxidative protection to lipids (Liu, Chen, & Mao, 2007; Nielson & Jacobsen, 2009). In addition to ω-3 oil, there has been growing interests in incorporating soluble fiber such as β-glucan into foods and beverages (Inglett et al., 2014; Singh, Kim, & Liu, 2012; Temelli, Bansema, & Stobbe, 2004). Beta-Glucan, a

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soluble dietary fiber commonly derived from oat and barley products, has been widely studied for its beneficial health effects on coronary heart disease prevention by reducing serum cholesterol and post-prandial serum glucose level (Klopfenstein, 1988). In fact, both US Food and Drug Administration and Health Canada now allow foods containing oats to carry heart health claim in reference to soluble fibers, primarily β-glucan (Health Canada, 2010; U.S. FDA, 1997). It is therefore advantageous to incorporate both ω-3 oil and β-glucan into functional food products as beverages are excellent carriers for delivering heart-healthy bioactive compounds to diets of nutritionally challenged consumers (Temelli et al., 2004). In the present work, we developed a prototype of heart-healthy beverage emulsion concentrate and evaluated its oxidative stability and physicochemical properties under room temperature for extended period.

2. Materials and methods

2.1. Materials

Flaxseed oil and marine (fish) oil from the blend of anchovies and sardines (Nordic Naturals, Watsonville, CA, USA) were purchased from commercial sources in Peoria, IL, USA and used as-is. Oils used in this study were frozen at −8 °C until needed. Sodium caseinate (89 g/100 g purity) was obtained from the American Casein Company (Burlington, NJ, USA). Oat β-glucan (70 g/100 g purity) was obtained from Garuda Intl., Inc. (Lemon Cove, CA, USA). All other materials used were reagent grade or higher.

2.2. Methods

2.2.1. Preparation of emulsions

A 6 g/100 g stock caseinate solution was made by dissolving sodium caseinate in water while stirring overnight. A 2 g oat β-glucan/100 g stock solution was made by first purifying the β-glucan to 97 g/100 g via a procedure described by Singh et al. (Singh et al., 2012). First, the β-glucan was dissolved in water and heated at 80 °C for 30 min. The solution was then centrifuged at 1000 g for 30 min, after which the supernatant was removed and used as the stock solution. The emulsion preparation procedure was modeled after work done by Matalanis, Decker, and McClements (Matalanis, Decker, & McClements, 2012) involving the encapsulation of emulsion droplets in hydrogel microspheres. Prior to emulsion preparation, the oil was removed from the freezer and gradually defrosted under cool running water. Samples containing oil, caseinate, and oat β-glucan were coarsely homogenized using a Polytron homogenizer (Kinematica AG, Luzern, Switzerland) for 2 min at 14,000 RPM. Samples were then introduced to a Panda 2K 2-stage homogenizer (1st stage: 80 mPa, 2nd stage: 8 mPa) (GEA Process Engineering, Columbia, MO, USA) and collected after two passes. During homogenization, the samples were placed on ice to reduce exposure to heat. After homogenization, 0.04 g sodium azide/100 g sample was added to each sample to inhibit bacterial growth during storage. The emulsions were then transferred to their respective storage containers and held at 28 °C.

For the purpose of developing a beverage emulsion concentrate, a concentration of 10 mL ω-3 oil/100 g sample, a concentration of 0, 1, and 3 g sodium caseinate/100 g sample, and a concentration of 0 and 0.3 g β-glucan/100 g sample was chosen. The concentration of β-glucan was kept low in the emulsion out of concern that it might cause phase separation or gelation when mixing with sodium caseinate in high concentrations (Lazaridou & Biliaderis, 2009).

2.2.2. Evaluation of the physical properties

2.2.2.1. Particle size. Particle size distributions were measured using a Horiba LB-550 dynamic light scattering instrument (Horiba Scientific, Kyoto, Japan). The dispersant (water) refractive index was set at 1.333 cP and the dispersant viscosity was temperature corrected for each sample via a temperature sensor that was placed in the sample during the measurement. Refractive indices of 1.479 and 1.481 were measured using ATAGO Abbe Benchtop Refractometer (Fischer Scientific, Waltham, MA, USA) and used for the flaxseed and fish oil samples, respectively. Particle sizes were reported as both the volume averages and number averaged mean diameter.

2.2.2.2. Creaming index. After emulsion preparations, 8 mL of the sample emulsion were dispensed into 15 mL polypropylene graduated centrifuge tubes. The samples were then placed in darkness and stored at 28 °C for the duration of the study. Samples were periodically examined and any separation or sedimentation was noted. Creaming was recorded when a complete separation of the cream layer (opaque) and serum layer (transparent) had occurred. The creaming index for each sample was determined using Equation (1):

$$\text{Creaming Index } (CI) = 100 \times (H_{serem}/H_{total}) \quad (1)$$

where H_{tot} is the total height of the emulsion sample and H_{ser} is the height of the serum layer. Creaming index values were reported as a percentage.

2.2.2.3. Viscosity. The viscosity measurement was designed to monitor the change in viscosity of emulsions βover time with different combinations of oil type, concentrations of β-glucan and caseinate, which will have implications in beverage handling and fluid transportation, and consumer acceptance in terms of mouth feel. Sample viscosity was measured using a Brookfield viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA, USA). Viscosity data were collected as the emulsions were prepared and at the conclusion of the study. A small volume spindle and jacket (spindle # 00) were used and an RCF of 0.12 g was used for all samples. The sample viscosity was recorded when the displayed viscosity remained constant for 5 s. Resulting viscosities at the aforementioned temperature were reported in mPa s.

2.2.3. Oxidative analysis

2.2.3.1. Lipid extraction from emulsion samples. The lipid fraction of the emulsion samples was extracted and used for both the peroxide value measurement and fatty acid analysis. Emulsion samples were briefly shaken and 1 mL of the emulsion was added to a capped glass test tube containing 7.5 mL of a 2:1 chloroform/methanol solvent. The test tubes were then vortexed vigorously for 12 min, with a 1–2 min resting period every 4 min. The test tubes were then immersed in a sonicator for 10–20 s and transferred to a centrifuge. Samples were centrifuged for 5 min at 1300× *g*. Once centrifugation was completed, samples had separated into two layers. The bottom organic layer was carefully removed and added to a small screw top vessel containing a small amount of magnesium sulfate (for drying) and agitated for roughly 10 s. Extracted samples were kept in a refrigerator until needed for oxidative analysis.

2.2.3.2. Peroxide values. The peroxide value measurement technique used in this study was based on a standard method developed by the International Dairy Federation and modified by Shantha and Decker (1994), Shantha and Decker (1994) for fats and oils. The current method relates the amount of ferric ions (Fe^{3+})

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