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Impact of legume protein type and location on lipid oxidation in fish oil-in-water emulsions: Lentil, pea, and faba bean proteins

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ABSTRACT

Emulsion-based delivery systems are being developed to incorporate ω-3 fatty acids into functional foods and beverages. There is interest in formulating these delivery systems from more sustainable and label-friendly ingredients. The aim of this study was therefore to examine the impact of plant-protein emulsifiers on the oxidative stability of 1 wt% fish oil-in-water emulsions. Fish oil emulsions stabilized by three types of legume protein (lentil, pea, and faba bean) were produced using a high-pressure microfluidizer. The formation of primary (peroxides) and secondary (TBARS) lipid oxidation products was measured when the emulsions were stored at 37 °C under accelerated (+ 100 μM iron sulfate) or non-accelerated (no added iron) conditions for 21 or 33 days, respectively. The particle size, charge and microstructure of the emulsions were monitored during storage using light scattering and microscopy to detect changes in physical stability. Emulsions stabilized by whey protein isolate, a commonly used animal-based protein, were utilized as a control. The emulsions formed using whey protein had smaller initial particle sizes, better physical stability, and slightly better stability to lipid oxidation than the ones formed using plant-based proteins. The impact of protein location (adsorbed *versus* non-adsorbed) on the oxidative stability of the emulsions was also investigated. The presence of non-adsorbed proteins inhibited lipid oxidation, presumably by binding transition metals and reducing their ability to interact with ω-3 fatty acids in the lipid droplets. Overall, these results have important implications for fabricating emulsion-based delivery systems for bioactive lipids, *e.g.*, they indicate that including high levels of non-adsorbed proteins could improve oxidative stability.

1. Introduction

Lipid oxidation is an important factor causing loss of product quality and nutrients in foods (Jacobsen, 2015; Jacobsen, Sorensen, & Nielsen, 2013). The primary products of lipid oxidation, such as lipid hydroperoxides, are odorless and tasteless, whereas the secondary products, such as hexanal and propanal, change the flavor of the product considerably (Elias, Kellerby, & Decker, 2008; McClements & Decker, 2000). This is especially important for food and beverage products containing ω-3 fatty acids as the sensory threshold for the detection of lipid oxidation products from these fatty acids is especially low (Barden & Decker, 2016). Moreover, potentially toxic reaction products, such as carcinogenic or inflammation-promoting substances, may be formed as a result of lipid oxidation of polyunsaturated lipids (Duthie, Campbell, Bestwick, Stephen, & Russell, 2013; Goicoechea, Brandon, Blokland, & Guillen, 2011; Kasai & Kawai, 2008; Matsuo, 1961; Rubio-Rodriguez et al., 2010). The oxidation rate of lipids increases with an increase in number of double bonds, because conjugation increases the ease of hydrogen abstraction and because there are more sites available for attack. Due to their high level of unsaturation, ω-3 polyunsaturated fatty acids (PUFAs) are subject to rapid oxidation when exposed to air, light and high temperature (Jacobsen, 2015; Jacobsen et al., 2013). Consequently, food manufacturers must develop effective strategies to manage lipid oxidation in functional food and beverage products enriched with these bioactive lipids.

Many approaches have been developed to manage lipid oxidation, including adding antioxidants, using chelating agents, controlling oxygen levels, engineering interfacial properties, and controlling storage conditions (such as light exposure, temperature, and water activity) (McClements & Decker, 2000). Antioxidant addition is one of the most widely used approaches because of its effectiveness, versatility, and simplicity (Laguerre, Lecomte, & Villeneuve, 2007). Numerous highly effective synthetic antioxidants can be used in foods, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ethylenediaminetetraacetic acid (EDTA), and propyl gallate (PG), but their use is declining because of consumer interest in more clean-label

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products (Duthie et al., 2013). Natural antioxidants, such as tocopherols and tocotrienols (vitamin E), can be used in foods (Barden & Decker, 2016), but their efficiency is often much less than that of synthetic antioxidants (Ragnarsson, Leick, & Labuza, 1977). Consequently, there is interest in the identification and evaluation of other natural food antioxidants, especially those from plant sources (Duthie et al., 2013; Embuscado, 2015; Sardarodiyan & Sani, 2016).

A number of food proteins are effective at inhibiting lipid oxidation, and can therefore be used as natural antioxidants (Clausen, Skibsted, & Stagsted, 2009; Elias et al., 2008; Kiokias, Gordon, & Oreopoulou, 2016; Sugiarto, Ye, Taylor, & Singh, 2010; Villiere, Viau, Bronnec, Moreau, & Genot, 2005). Under some circumstances, proteins may oxidize faster than unsaturated fatty acids, thereby delaying lipid oxidation and rancidity (Berton, Ropers, Guibert, Sole, & Genot, 2012; Elias et al., 2008). The preferential oxidation of proteins occurs if they are more susceptible to oxidation than the fatty acids in the system, or if they are physically located closer to the free radicals or reactive oxygen species (ROS) than the lipids (Elias et al., 2008; Elias & Decker, 2011). Proteins inhibit lipid oxidation by scavenging free radicals, inactivating ROS, chelating pro-oxidative transition metals (such as iron or copper), reducing hydroperoxide formation, and by altering the interfacial properties of foods so as to physically separate reactive species (Clausen et al., 2009; Elias et al., 2008; Villiere et al., 2005). Consequently, proteins can act as multifunctional antioxidants capable of inhibiting lipid oxidation through different mechanisms (Elias et al., 2008; Samaranayaka & Li-Chan, 2011; Sugiarto et al., 2010).

Emulsified food products, such as beverages, coffee creamers, desserts, dressings, and sauces, may be fortified with polyunsaturated fatty acids to deliver beneficial health properties (Berton-Carabin, Ropers, & Genot, 2014; Jacobsen, 2015; McClements & Decker, 2000). Transition metal-catalyzed decomposition of lipid hydroperoxides is the dominant oxidation pathway in emulsions (McClements & Decker, 2000; Walker, Decker, & McClements, 2015). Lipid hydroperoxides are surface active molecules and therefore tend to migrate to the lipid droplet surfaces after they are formed, where they are then decomposed by a metal-catalyzed pathway. Proteins can influence lipid oxidation in emulsions through a number of mechanisms: (i) non-adsorbed proteins may bind metal ions and prevent them from reaching the lipid droplet surfaces; (ii) adsorbed proteins may bind metal ions and bring them into close proximity with the droplet surfaces; (iii) adsorbed proteins with a positive charge may electrostatically repel cationic metal ions; (iv) adsorbed proteins may form a physical barrier that sterically hinders the ability of metal ions to interact with peroxides; and, (v) proteins have antioxidant side groups that can scavenge free radicals (Berton-Carabin et al., 2014; Elias et al., 2008; Genot et al., 2013). The relative importance of these different mechanisms depends on the type, concentration, and location of the proteins present in an emulsion.

There is a growing interest by consumers in products containing plant-based natural ingredients, rather than those of animal origin (such as milk, egg, fish, or meat proteins), and so the food industry is looking for effective plant-based protein emulsifiers (Liu, Gao, McClements, & Decker, 2016). Legumes are gaining popularity for this purpose due to their high natural abundance, sustainability, low cost, and functional attributes (Karaca, Low, & Nickerson, 2011). As well as being effective emulsifiers, many legume proteins are also effective antioxidants (Carbonaro, Virgili, & Carnovale, 1996; Cardador-Martinez, Loarca-Pina, & Oomah, 2002; Han & Baik, 2008). Previous studies have shown that incorporation of chickpea or lentil proteins into flaxseed oil-in-water emulsions inhibits lipid oxidation of the powdered product during storage (Can Karaca, Low, & Nickerson, 2013; Karaca, Nickerson, & Low, 2013). A recent study also showed that pea proteins were more effective than dairy proteins (casein or whey proteins) at inhibiting the degradation of polyunsaturated lipids (lycopene) in oil-in-water emulsions (Ho, Schroen, San Martin-Gonzalez, & Berton-Carabin, 2017).

The objective of the current study was to compare the efficacy of a number of legume-based proteins (pea, lentil, and faba bean) at forming and stabilizing oil-in-water emulsions, with particular emphasis on their ability to inhibit lipid oxidation during storage. The results obtained for the legume proteins were compared to those obtained for whey protein isolate, since this animal-based protein is widely used as an emulsifier in the food industry. We hypothesized that there would be appreciable differences in the ability of the legume proteins to act as antioxidants depending on their type and location within the system (adsorbed *versus* non-adsorbed). The results of this study would provide valuable information that could be used to form plant-based functional foods and beverages fortified with polyunsaturated fatty acids.

2. Materials and methods

2.1. Materials

Pea, lentil, and faba bean protein concentrates (Vitessence Pulse 1550, 2550 and 3600, respectively) were donated by Ingredion, Inc. (Bridgewater, NJ). Detailed information about their composition and properties has been given in an earlier manuscript using the same ingredients (Gumus, Decker, & McClements, 2017). Whey protein isolate (BiPro JE 011-4-420) was donated by Davisco Foods International, Inc. (Le Sueur, MN). Fish oil was donated by DSM, Inc. (Columbia, MD), which was reported to contain at least 550 mg/g total omega-3 fatty acids, with 300 mg/g from DHA and 150 mg/g from EPA. An analysis of the same oil in a previous study reported that it initially contained 1.6 mmol lipid hydroperoxide/kg oil and 0.5 mmol TBARS/kg oil (Johnson, Gisder, Lew, Goddard, & Decker, 2016). All other chemicals were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Double distilled water (DDW) was used for all experiments.

2.2. Protein purification

The pulse protein concentrates provided by the ingredient suppliers only contained around 55 to 60% of protein by weight. Therefore, a protein isolation and purification procedure was carried out as described previously (Joshi et al., 2012), with some slight modifications. Briefly, the pulse protein concentrates were dispersed in sodium phosphate buffer (10 mM, pH 7) at a concentration of 20% (w/w) for 1 h using a magnetic stirrer followed by centrifugation (Sorvall Lynx 4000 Centrifuge, Thermo Scientific, Agawam, MA) for 30 min at 15,000*g* at 10 °C. The supernatant was collected and centrifuged again using the same conditions to remove any starch, fiber, and insoluble compounds. The protein extract was then adjusted to pH 4.5 using hydrochloric acid (HCl) solution to precipitate the protein. The protein precipitate was recovered by centrifugation, washed with distilled water and dispersed in pH 7.0 sodium phosphate buffer by stirring at room temperature for an hour. The solution was then readjusted to pH 7.0 using sodium hydroxide (NaOH) solution and the system was stirred overnight at 5 °C to ensure complete protein dispersion. The protein solution was brought to room temperature with continuous stirring for 30 min and then centrifuged to remove any insoluble protein. The protein content of the resulting supernatant were determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951), using a standard curve prepared with bovine serum albumin (R^2 = 0.992). The protein solutions were diluted to 20 mg/mL protein content using buffer solutions prior to utilization.

2.3. Blocking of protein sulfhydryl groups

The importance of sulfhydryl groups for the antioxidant activity of the proteins was determined by using *N*-ethylmaleimide (NEM), which is a chemical known to block sulfhydryl groups (Friedman, 1973; Tong, Sasaki, McClements, & Decker, 2000). Protein solutions (20 mg/mL) were stirred with NEM (3.45 mmol/g protein) in a water bath at 25 °C