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Influence of an anionic polysaccharide on the physical and oxidative stability of omega-3 nanoemulsions: Antioxidant effects of alginate

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ABSTRACT

The aim of this study was to assess the impact of an anionic polysaccharide on the physical properties and chemical stability of omega-3 enriched nanoemulsions. Fish oil-in-water nanoemulsions stabilized with a non-ionic surfactant (Tween 80) were mixed with sodium alginate at several concentrations, and then their viscosity, creaming stability, particle size, microstructure, and oxidation were measured. The viscosity of the mixed systems (2.5% w/w of oil) increased with polysaccharide addition, and was primarily governed by the alginate rather than the oil droplets. Droplet flocculation was observed at sodium alginate concentrations exceeding 0.05% (w/w), which led to rapid creaming and an increase in droplet size due to coalescence. This effect was attributed to depletion flocculation arising from the exclusion of non-adsorbed polysaccharide molecules from the immediate vicinity of the droplet surfaces. Nevertheless, the addition of alginate (0.1% w/w) to the nanoemulsions decreased the rate and extent of lipid oxidation during storage. This effect was probably due to the ability of anionic groups on the alginate molecules to chelate pro-oxidant transition metals (such as iron) in the aqueous phase. This study highlights the potential of using alginate as a natural antioxidant in nanoemulsions; however, it also highlights the potential for this polysaccharide to promote physical instability. This information could be used to optimize the composition and structure of food matrices designed to improve the oxidative stability of polyunsaturated lipids.

1. Introduction

Fish oils are naturally rich in omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are long chain polyunsaturated fatty acids (PUFAs). Omega-3 fatty acids play an important role in human nutrition, since their consumption has been associated with a reduced risk of cardiovascular diseases and cancer, improved brain development, and prevention of inflammatory related pathologies (Adkins & Kelley, 2010; Lorente-Cebrian et al., 2013; Shahidi & Miraliakbari, 2004, 2005). Due to their reported health benefits, the food industry has an increasing interest in food fortification with fish oils (Ganesan, Brothersen, & McMahon, 2014). However, there are challenges associated with their incorporation into food formulations due to their low water-solubility and poor chemical stability. Omega-3 fatty acids are highly susceptible to lipid oxidation, which can negatively impact the shelf life, nutritional value, and flavor of foods (Arab-Tehrany et al., 2012; Sun, Wang, Chen, & Li, 2011). Lipid oxidation is the result of a complex reaction between unsaturated fatty acids and oxygen-reactive species, which can be divided into initiation, propagation, and termination phases (Waraho, McClements, & Decker, 2011).

In oil-in-water (O/W) emulsions, the oxidation of PUFAs is usually initiated by the interaction of lipid hydroperoxides located at the surface of the oil droplets with transition metals located in the surrounding aqueous phase (Berton-Carabin, Genot, Gaillard, Guibert, & Ropers, 2013; Berton-Carabin, Ropers, & Genot, 2014; McClements & Decker, 2000). Transition metals are able to break down unsaturated lipids into alkyl radicals, but this reaction occurs extremely slowly and is therefore not considered to be the main cause of lipid oxidation (Akoh & Min, 2008). The dominant reaction causing lipid oxidation is the decomposition of lipid hydroperoxides into peroxyl and alkoxyl radicals by transition metals, which are highly reactive species able to react with unsaturated lipids in the oil droplet surface. In turn, this reaction leads to the formation

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of lipid radicals that cause the propagation of the lipid oxidation reaction when they interact with other lipid molecules in their vicinity. The formation of lipid radicals eventually terminates when they react with other radicals due to the formation of non-radical compounds (McClements & Decker, 2000). The rate and extent of lipid oxidation in emulsion-based food products is affected by several factors, including temperature, pH, exposure to UV-light, and the ratio of antioxidant to pro-oxidant species present in the food matrix. Thus, there is a need to investigate the role of specific food constituents on lipid oxidation in food emulsions so as to develop more stable food products that are susceptible to lipid oxidation, such as those enriched with omega-3 fatty acids.

O/W nanoemulsions are a type of emulsion-based delivery system that consists of small (r < 100 nm) oil droplets dispersed within an aqueous continuous phase David Julian McClements and Rao (2011). Edible nanoemulsions are particularly suitable for the encapsulation, protection, and release of omega-3 fatty acids because of their good physical stability and high optical clarity (Walker, Decker, & McClements, 2015). In addition, recent *in vivo* studies have shown that nanoemulsions lead to higher intestinal absorption of omega-3 fatty acids compared to conventional emulsions (Dey, Ghosh, Ghosh, Koley, & Dhar, 2012). The incorporation of omega-3 fatty acids in nanoemulsions has been the focus of several research papers, confirming the feasibility to achieve highly stable delivery systems (Belhaj, Arab-Tehrany, & Linder, 2010; Gulotta, Saberi, Nicoli, & McClements, 2014). Nevertheless, there is currently a lack of information on the oxidative stability of omega-3 enrich nanoemulsions once they are incorporated into food systems. Therefore, there is a need to study the potential interactions between omega-3 nanoemulsions and other ingredients present in food formulations that might alter their physicochemical stability.

Polysaccharides are commonly used as functional ingredients in the food industry to formulate emulsion-based foods, *e.g.*, as stabilizing, thickening, or gelling agents (Phillips & Williams, 2000). The addition of sufficiently high levels of polysaccharide to the aqueous phase of an emulsion inhibits creaming by thickening or gelling the aqueous phase. However, over a certain concentration range polysaccharides may actually promote physical instability in emulsions by promoting droplet flocculation through a bridging or depletion mechanism (Dickinson, 2003; McClements, 2015). Consequently, the amount of polysaccharide added to an emulsion as a functional ingredient must be carefully controlled to produce the desired textural properties and physical stability. Certain types of polysaccharides may also play other important roles in stabilizing emulsion-based foods. For example, studies have shown that certain types of anionic polysaccharide can inhibit the oxidation of emulsified lipids due to their ability to chelate metallic ions (Kishk & Al-Sayed, 2007).

The specific aim of the present work was to study the influence of polysaccharide addition on the rheology, physical stability, and oxidative stability of omega-3 nanoemulsions. Nanoemulsions were prepared from an oil phase that consisted of a blend of fish oil and lemon oil. The fish oil was the source of omega-3 fatty acids, whereas the lemon oil was used to facilitate nanoemulsion formation by lowering the interfacial tension and dispersed phase viscosity (McClements and Rao, 2011). The impact of sodium alginate on the rheology and physical stability of the emulsions was examined, whereas the impact of three polysaccharides with different charge characteristics was used in the oxidation studies: sodium alginate (anionic), chitosan (cationic) and methylcellulose (non ionic). Overall, the objective of this research was to provide information about the potential beneficial or detrimental effects of polysaccharide addition on the properties and stability of omega-3 fatty acid enriched nanoemulsions.

2. Material and methods

2.1. Materials

Fish oil (Ropufa 30 $n-3$ food oil) was obtained from DSM Nutritional Products Ltd. (Basel, Switzerland). The oil contained 101 mg of EPA/g of oil, 148 mg of DHA/g oil, and a total of omega-3 PUFA of 312 mg/g of oil. Lemon oil (SC 020207, Lot: 2894308) used in this work was kindly donated by International Flavors and Fragrances (Union Beach, N.J.). Iso-octane, 2-propanol, methanol, buthanol and ethanol were obtained from Fischer Scientific (MA, USA). Fe_2SO_4, ethylenediaminetetraacetic acid (EDTA), thiocyanate, trichloroacetic acid, thiobarbituric acid, butylated hydroxytoluene (BHT), ethanol, 1,1,3,3-tetraethoxypropane (TEP), Tween 80, sodium acetate, acetic acid, sodium alginate, chitosan, methylcellulose, and Nile Red dye were purchased at Sigma–Aldrich (St. Louis, MO). All aqueous solutions were prepared using purified water from a Milli-Q filtration system.

2.2. Methods

2.2.1. Nanoemulsion formation

The lipid phase of nanoemulsions consisted of a mixture of fish oil and lemon oil (50:50 w/w). Both oils were mixed and stirred for 5 min at room temperature. The aqueous phase of the nanoemulsions consisted of 100 mM acetic-acetate buffer at pH 3.0. A coarse emulsion was formed by mixing lipid phase (10% w/w), Tween 80 (1% w/w), and aqueous phase (89% w/w) with a high-sheer blender for 2 min at 20,000 rpm. The resulting coarse emulsion was then passed three times through a microfluidizer (model M110-P, Microfluidics, Newton, MA) working at 15,000 psi to form a nanoemulsion.

2.2.2. Mixing experiments

A sodium alginate stock solution was prepared by stirring the powdered polysaccharide ingredient (2% w/w) into acetic-acetate buffer (100 mM, pH 3.0) until complete dissolution had occurred. Sodium alginate solution was used immediately without any further storage. A series of nanoemulsions containing different alginate concentrations (0, 0.01, 0.02, 0.05, 0.1, 0.25, 0.5, 0.75, 1% w/w) but the same oil (2.5% w/w) and surfactant (0.25% w/w) content were prepared by mixing sodium alginate stock solution (2% w/w), buffer solution (100 mM acetic-acetate, pH 3.0) and nanoemulsions together in different ratios. The systems were stirred for 5 min before physicochemical characterization.

2.2.3. Rheological properties

The rheological properties of the mixtures containing sodium alginate and nanoemulsions were measured using a dynamic shear rheometer (Kinexus, Malvern Instruments Ltd, Worcestershire, UK). In addition, sodium alginate solutions with the same polysaccharide concentration range as the mixed systems were also measured. A cup-and-bob measurement cell was used for all measurements: the bob had a diameter of 25 mm and the cup had a diameter of 27.5 mm. The shear stress was measured as a function of shear rate from 0.1 to 100 s^{-1} at 25 °C. The apparent shear viscosity is also reported at 10 s^{-1}, a rate that is similar to that experience by foods during mastication of semi-solid foods (Shama & Sherman, 1973). The measurements were programmed and recorded using the instrument software (Kinexsus rSpace, Malvern Instruments Ltd., MA, USA).

2.2.4. Creaming index

Creaming of mixed systems (10 mL) was monitored at room temperature by visual observation and measurement with a ruler