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Role of oxygen scavengers in limiting oxygen permeation into emulsions and improving stability of encapsulated retinol

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ABSTRACT

The oxidation reactions significantly limit quality and shelf life of food products. The overall goal of this study was to evaluate the relative efficiency of selected oxygen scavengers in limiting oxygen permeation into the oil phase of the emulsion and its impact on stability of encapsulated model bioactive compound. Sodium sulfite (Na_2SO_3), glucose oxidase + catalase (GOx + catalase) and ascorbic acid (AA) were evaluated as water-soluble oxygen scavengers. The results show that GOx + catalase was approximately 6 times more effective in improving stability of retinol encapsulated in the oil phase of the emulsion than Na_2SO_3. Na_2SO_3 reduced the rate of permeation of oxygen into the oil phase of the emulsion by approximately 2.5 times as compared to the control emulsion but was not effective in limiting degradation of retinol encapsulated in the emulsion. AA was not effective in limiting oxygen permeation into the emulsion. This study demonstrates the potential of oxygen scavengers in limiting oxygen permeation in emulsion and their impact on improving oxidative stability of encapsulated bioactives.

1. Introduction

Oxidation reactions in food systems, especially oxidation of lipids or functional bioactives are a major cause of deterioration in food quality. Food quality changes induced by oxidation can influence various functional properties such as taste, shelf life, appearance and nutritional content of food products (Frankel, 1991). Oxidation reactions in food can be initiated upon exposure to oxygen, heat, natural and artificial light, transition metal ions and certain enzymes. In the case of lipids, above mentioned oxidation initiation processes result in removal of a hydrogen atom from the methylene group of a polyunsaturated fatty acid present in the oil. Once formed, these radicals react rapidly with dissolved oxygen present in the product to form lipid peroxide. The lipid peroxides can then further extract another hydrogen atom to form a hydroperoxide, which propagates the radical reaction (Halliwell et al., 1995). In the case of bioactive compounds such as polyphenols, the presence of oxygen can cause deterioration by direct reactions with the bioactive compound or by generating secondary reaction oxidation products (Patras et al., 2010). Thus, the presence of oxygen and its reactivity with various food components including lipids and bioactive compounds has a major impact on the shelf life of food products.

The presence of dissolved oxygen can accelerate oxidation reactions in various food products. The rate of oxidation in dispersed systems such as oil-in-water (O/W) emulsion is significantly enhanced compared to bulk food products such as oils (McClements and Decker, 2000). This increase in the oxidation rate is attributed to the large surface area and preferential localization of lipid hydroperoxides at the oil–water interface. To suppress oxidation reactions in emulsion-containing products, various approaches have been explored which include the use of sacrificial antioxidants such as butylated hydroxyanisole, hydroxytoluene, ascorbic acid and tocopherols (Banias et al., 1992), use of metal ion chelates such as EDTA (Mahoney and Graf, 1986) and the use of oxygen scavengers (Nagai et al., 2005). The sacrificial

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antioxidants can react both with oxygen and the secondary products of oxidation reactions thereby quenching radical initiation and propagation reactions. In contrast to sacrificial antioxidants, oxygen scavengers only react with free oxygen, thus suppressing the radical initiation step by scavenging free oxygen molecules. Typical oxygen scavengers include iron powder, sodium sulfite, ascorbic acid and enzymes. These oxygen scavengers can be included as a part of packaging material or included in the product itself, e.g. iron powders have been included in the packaging material (Han, 2005), while sodium sulfite, ascorbic acid and enzymes have been included in both products and packaging (Tian et al., 2013).

The oxidative stability of encapsulated bioactives in colloidal systems presents a significant challenge (McClements et al., 2007). O/W emulsions are commonly used for encapsulating hydrophobic bioactive molecules, but the shelf life of products in these emulsions is limited due to oxidation of the encapsulants. In the current literature, there are a number of studies that have evaluated the role of sacrificial antioxidants and metal chelates in improving the oxidative stability of lipids and encapsulated bioactives in O/W emulsions (Lin et al., 1993; Ruben and Larsson, 1985; Sims et al., 1979). In contrast, there is very limited evaluation of the role of oxygen scavengers in improving oxidative stability in emulsions especially for the lipid encapsulated bioactive molecules.

In this study, three oxygen scavenging systems were evaluated: sodium sulfite (Na_2SO_3), a combination of glucose oxidase and catalase (GOx + catalase) and ascorbic acid (AA). Na_2SO_3 is a small molecular weight oxygen scavenger; GOx + catalase is an enzyme based oxygen scavenger system while AA is an antioxidant. Sulfites such as Na_2SO_3 are widely used as anti-browning agents, preservative and antioxidant additives in the food and pharmaceutical industries (Challen, 1990; Taylor et al., 1986). GOx enzyme acts as a catalyst for the oxidation reaction of β-D-Glucose to δ-D-gluconolactone in the presence of dissolved oxygen (Meyer and Isaksen, 1995). Hydrogen peroxide is a byproduct of this reaction which is removed by reaction with the catalase enzyme. The ascorbate ion formed after dissolution of AA in water is a reducing agent and it can react with free oxygen as well as the radical species such as hydroxyl ions (Frankel, 1996). Tween-20 was selected as an emulsifier to stabilize the O/W emulsion in this study. Tween-20 is commonly used in food, pharmaceutical and cosmetic formulations and does not have significant endogenous antioxidant properties that could influence the oxidative stability of the encapsulated retinol (Pan et al., 2013). Retinol was used as a model hydrophobic bioactive compound encapsulated in the oil phase of the emulsion. Retinol was selected as it is highly susceptible to oxidation and it is widely used in food, pharmaceutical and cosmetic formulations.

The key objective of this study was to evaluate the role of oxygen scavengers in limiting oxygen permeation into the lipid core of emulsion and their impact on improving oxidative stability of an encapsulated model bioactive compound. The specific aims of this study were to compare the influence of various oxygen scavengers present in the aqueous phase of an O/W emulsion on the rate of oxygen transport to the lipid phase and to determine the efficacy of selected oxygen scavengers in limiting oxidation of a model bioactive compound encapsulated in the lipid phase of the emulsion.

2. Materials and methods

2.1. Materials

Tween-20, oxygen sensitive hydrophilic fluorescent dye tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate ($\lambda_{excitation}$ = 452 nm and $\lambda_{emission}$ = 620 nm), oxygen sensitive hydrophobic fluorescent dye ruthenium-tris(4,7-diphenyl-1,10-phenanthroline) dichloride ($\lambda_{excitation}$ = 455 nm and $\lambda_{emission}$ = 613 nm), sodium sulfite (Na_2SO_3), glucose oxidase (GOx), catalase, L-ascorbic acid (AA), retinol and reagent grade methanol were obtained from Sigma–Aldrich (St. Louis, Mo, USA). Canola oil was obtained from Spectrum (Boulder, CO). Ultrapure water (16 MΩ cm) was obtained from in-house water filtration system and used as is for all the experiments. Reagent grade chloroform and D-glucose were obtained from Thermo Fisher Chemicals (Pittsburgh, PA).

2.2. Emulsion preparation

All experiments were carried out at room temperature. Tween-20 (2% (w/w)) was dissolved in ultrapure water. Stock solutions of hydrophilic and hydrophobic oxygen sensitive dyes were prepared in water (10 mg/mL) and chloroform (1 mg/mL) respectively. Hydrophilic and hydrophobic dye stock solutions were mixed with tween-20 solution or canola oil to achieve a final dye concentration of 20 μg/g of tween-20 solution or 20 μg/g of oil respectively. Coarse emulsion was prepared by dispersing oil (4% (w/w)) in tween-20 solution using a hand-held disperser (Ultra-Turraxmodel T25, IKA Works, Wilmington, NC) set at 24,000 rpm for 2 min. The coarse emulsion was subsequently homogenized (30 s; amplitude = 50%) by a probe sonicator (Q55, QSonica, Newtown, CT, USA). The emulsion pH was adjusted to 6.5 using 0.1 M NaOH and 0.1 M HCl.

After preparation of emulsion, oxygen scavengers were mixed with the emulsions. Oxygen scavengers were mixed with the emulsions at following concentrations: Na_2SO_3 – 3 mM and 10 mM, 25 U/mL GOx, 25 U/mL GOx + 250 U/mL catalase, 250 U/mL catalase and AA – 3 mM and 10 mM. D-glucose (1% (w/v)) was also added to the emulsions with GOx and/or catalase. Hydrophobic oxygen sensitive dye was included in the oil phase (20 μg/g of oil) of the emulsion before addition of oxygen scavengers. For monitoring the stability of encapsulated retinol, 0.6% retinol (w/w of oil) was dissolved in the oil phase before emulsion preparation.

2.3. Average droplet diameter measurement

Hydrodynamic diameter of emulsion droplets was measured using a particle size analyzer (Model: Malvern Nano Series; Malvern Instruments, Inc., Westborough, MA). The analyzer was set to following specifications: material type – oil, droplet refractive index = 1.45, dispersant type – water, dispersant refractive index = 1.33, temperature = 25 °C. Average droplet diameter measurements were analyzed based on the number average droplet diameter distribution.

2.4. Fluorescence measurement

Before the fluorescence measurements, the emulsions were purged with nitrogen for 2 h to remove traces of oxygen. For each experiment, an emulsion without any oxygen scavenger was prepared with the dye present either in water or oil phase. This emulsion was not purged with nitrogen and henceforth referred as "non-purged emulsion". Fluorescence measurements were carried out using a plate-reader (Model: Synergy 2, BioTek Inc., Winooski, VT). Samples were pipetted into 96 well black plates with a transparent flat bottom (Corning, NY) optimized for fluorescence measurements. Fluorescence measurements were made using light excitation from the bottom of the plate. The wavelength settings for the plate-reader were – $\lambda_{excitation}$ = 485/20 nm, $\lambda_{emission}$ = 590/35 nm. Changes in fluorescence intensity as a function of time were recorded at a regular interval of 1 min. The normalized