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Lutein-enriched emulsion-based delivery systems: Influence of emulsifiers and antioxidants on physical and chemical stability

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ABSTRACT

The impact of emulsifier type (quillaja saponin, Tween 80, whey protein and casein) and antioxidant type (EDTA, ascorbic acid, catechin, alpha tocopherol, ascorbic acid palmitate) on the physical and chemical stability of lutein-fortified emulsions was investigated. Quillaja saponin produced emulsions with the best overall stability to droplet aggregation, creaming, and colour fading during storage at 45 °C for ten days. The impact of antioxidant type on the stability of lutein-fortified emulsions prepared using quillaja saponin was therefore investigated further. The extent of droplet aggregation and creaming was largely independent of antioxidant type. Surprisingly, most of the antioxidants promoted lutein degradation. Only ascorbic acid showed some ability to inhibit colour fading during storage, although EDTA had some inhibitory effects in the early stages of storage. This study suggests that lutein-enriched emulsions prepared using quillaja saponin as an emulsifier and ascorbic acid as an antioxidant may be the most suitable as delivery systems.

1. Introduction

Lutein is a natural pigment found in certain animal products, fruits and vegetables, including egg yolk, tomatoes, corn and marigold flowers, and is mainly responsible for their characteristic yellow, orange and red colours (Abdel-Aal, Akhtar, Zaheer, & Ali, 2013; Boon, McClements, Weiss, & Decker, 2010; Sajilata, Singhal, & Kamat, 2008). Lutein is an oxygenated carotenoid (xanthophyll) that appears yellow at low concentrations, but red at high concentrations (Sajilata et al., 2008). In the food industry, lutein is mainly isolated from marigold flowers (*Tagetes erecta*) (Lin, Lee, & Chang, 2015). Market research indicates that the sales of lutein are growing faster than those of other carotenoids, with a market value of around US$233 million in 2010, which is predicted to rise to around US$309 million in 2018 (Berman et al., 2014).

The pigmented region of the human eye (the macula) normally contains carotenoids, and studies have shown that accumulation of lutein within this region of the eye may reduce the risk of age-related macular degeneration and cataracts (Abdel-Aal et al., 2013; Boon et al., 2010; Sajilata et al., 2008). Lutein protects the macula by absorbing light waves that enter the eye, thereby reducing their potential to cause UV-visible damage (Krinsky, Landrum, & Bone, 2003; Roberts, Green, & Lewis, 2009). Moreover, carotenoids may protect other cells from oxidative stress by acting as antioxidants, e.g., by scavenging free radicals or by quenching singlet oxygen (Boon et al., 2010). Lutein must be obtained directly through the diet, because the human body cannot synthesize carotenoids (Khalil et al., 2012; Nagao, 2014; Sajilata et al., 2008). The Joint FAO/WHO Expert Committee on Food Additives recommends a daily intake of 0–2 mg lutein/kg body weight (JECFA, 2005). Other researchers have suggested that an intake of 10 mg lutein/day may provide protection against diseases, such as age related macular degeneration and cataracts (Frede et al., 2014). Eye examinations of individuals taking relatively high doses of lutein (40 mg/day) suggested that there were no adverse health effects, such as formation of lutein crystals within the eye. Thus, lutein can be considered to be a safe natural colorant that may also have beneficial health effects (Berman et al., 2014).

Despite the potential benefits of using lutein as a colorant or nutraceutical there are some limitations to its incorporation into commercial food and beverage products. Lutein is a highly hydrophobic molecule that has a very low water-solubility, which limits its direct incorporation into aqueous-based products (Boon et al., 2010). Moreover, lutein is highly susceptible to chemical degradation due to oxidation, which is promoted by pro-oxidants, elevated temperatures, light exposure, and acidic environments associated with certain types of foods (Maiani et al., 2009). The chemical degradation of lutein leads to undesirable colour fading and loss of bioactivity (Alam, Ushiyama, & Aramaki, 2009; Sajilata et al., 2008). Another potential

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problem for the development of functional foods containing lutein is that only a small fraction of this nutraceutical may actually be absorbed by the human body due to its low bioaccessibility in gastrointestinal fluids (Frede et al., 2014; Khalil et al., 2012; Nagao, 2014; Sajilata et al., 2008). Emulsion-based delivery systems have therefore been developed to encapsulate, protect, and deliver lutein so as to improve its utilization and efficacy in foods and beverages (Barnes & Roberts, 2005; McClements, 2015b).

The two most common emulsion-based delivery systems for lipophilic bioactive agents are oil-in-water emulsions ($r > 100$ nm) and nanoemulsions ($r < 100$ nm) (McClements & Rao, 2011; McClements & Xiao, 2012). Emulsion-based delivery systems can be designed to improve the water-dispersion, increase the bioaccessibility, and inhibit the chemical degradation of carotenoids (Boon et al., 2010). A successful delivery system intended for commercial applications must be physically and chemically stable under the conditions that food products experience during their manufacture, transport, storage, and utilization, such as exposure to light, thermal processing, ingredient interactions, and pH changes (Davidov-Pardo, Gumus, & McClements, 2016; McClements, 2015b).

The objective of the present study was to develop stable all-natural oil-in-water emulsions that could be used to encapsulate, protect, and deliver lutein. These emulsion-based delivery systems could then be utilized by the food industry in applications where lutein was needed as a natural colorant or nutraceutical. Two of the most important stabilizers in this kind of emulsion are emulsifiers and antioxidants, and therefore the impact of emulsifier type (quillaja saponins, Tween 80, whey protein isolate, and casein) and antioxidant type (EDTA, ascorbic acid, catechin, alpha tocopherol, and ascorbic acid palmitate) on the physical and chemical stability of lutein-loaded emulsions was examined. Corn oil was used as a source of long-chain triacylglycerols because it is a commonly used edible oil that has previously been shown to increase the bioaccessibility of carotenoids (Rao, Decker, Xiao, & McClements, 2013; Salvia-Trujillo, Qian, Martín-Belloso, & McClements, 2013).

2. Materials and methods

2.1. Materials

A commercial lutein suspension, consisting of 20% of lutein dispersed in corn oil (MariLut Lutein Oil) was kindly provided by PIVEG (San Diego, CA, USA). Corn oil was purchased from a commercial supplier (Mazola, ACH Food Companies, Cordova, TN). Quillaja saponin (Q-Naturale® 200, 14% (w/w) active saponins) was provided by Ingredion Inc. (Westchester, IL, USA). Spray dried sodium caseinate was purchased from the American Casein Company (Burlington, NJ, USA). Whey protein isolate (WPI) was purchased from Le Sueur Food Ingredient Company (Le Sueur, MN, USA). Mono and dibasic sodium phosphate, L-ascorbic acid, ascorbic acid 6-palmitate, catechin, alpha tocopherol, Tween® 80 and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Double distilled water was used to prepare all solutions and emulsions.

2.2. Emulsion preparation

2.2.1. Emulsifier study

An oil phase was prepared by dispersing 2.5% (w/w) of lutein suspension in corn oil. An aqueous phase was prepared by dispersing 0.25% (w/w) emulsifier (quillaja saponin, Tween® 80, whey protein isolate, and sodium caseinate) in aqueous buffer solution (10 mM phosphate, pH 7.0).

2.2.2. Antioxidant study

Only quillaja saponin was used in this study because it was the most effective at forming physically and chemically stable emulsions of all the emulsifiers tested. The composition of the emulsions was the same as described in Section 2.2.1, except for the addition of antioxidants. Ethylenediaminetetraacetic acid (EDTA), L-ascorbic acid, and catechin were added to the aqueous phase, while alpha tocopherol and ascorbic acid 6-palmitate were added to the organic phase. A fixed level (15 μmol) of each antioxidant was solubilized in the appropriate phase used to form the emulsions prior to homogenization.

2.2.3. Homogenization procedure

Coarse oil-in-water emulsions containing 5% (w/w) organic phase and 95% (w/w) aqueous phase were formed using a high-shear mixer (M133/1281-0, Biospec Products, Inc. Bartelsville, OK, USA) operated for 2 min at 10,000 rpm. These emulsions were then passed through an air-driven microfluidizer (M110-L, Microfluidics, Newton, MA, USA) for 3 passes at 12,000 psi to form the final emulsions. The final emulsions contained 2.5% of lutein suspension (20% lutein in corn oil) dispersed in corn oil (which made up 5% of the total emulsion), and so the final amount of lutein present in the emulsions was 250 mg/l.

2.3. Emulsion stability study

The emulsions were adjusted to pH 7.0 using 0.1 and 1.0 N of hydrochloric acid and/or sodium hydroxide solutions. The emulsions were then stored for 10 days at 45 °C in the dark under quiescent (non-stirred) conditions.

2.3.1. Chemical stability

The change in colour of the emulsions during storage was used to determine the chemical stability of lutein. The colour was measured using an instrumental colorimeter (ColorFlex EZ, HunterLab Reston, VA, USA). Previous studies have shown that there is a good correlation between carotenoid degradation in emulsions and colour changes (Qian, Decker, Xiao, & McClements, 2012). An aliquot (10 ml) of emulsion was pipetted into a petri dish to perform the analysis. The colour measurement took place against a black background at room temperature.

The colorimeter measured the colour coordinates of the emulsions according to the CIE tristimulus system (L∗a∗b∗). Here, the L∗value represents the lightness, while the a∗ and b∗ values represent the colour: L∗ varies from black = 0 to white = 100; a∗ varies from red (positive) to green (negative); and, b∗ varies from yellow (positive) to blue (negative). The total colour difference (ΔE) was calculated from the tristimulus colour coordinates using the following expression:

$$\Delta E = \sqrt{(L^*-L_i^*)^2 + (a^*-a_i^*)^2 + (b^*-b_i^*)^2} \quad (1)$$

Here, L_i*, a_i*, b_i* are the initial values of the CIE L∗a∗b∗ colour coordinates measured immediately after emulsion preparation, and L∗, a∗, b∗ are the colour coordinates measured after a specific storage time. Additionally, the difference in chroma (ΔC∗) value, which represents the colour intensity of a sample, was calculated using the following expression (McGuire, 1992):

$$\Delta C^* = \sqrt{(a^*-a_i^*)^2 + (b^*-b_i^*)^2} \quad (2)$$

2.3.2. Physical stability

The physical stability of the emulsions was quantified by measuring the change in mean droplet diameter and ζ-potential during storage. The ζ-potential was measured at the beginning and the end of the experiment using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, Malvern, England). An aliquot (5 μl) of emulsion was diluted in 5 ml of buffer solution (10 mM phosphate buffer at pH 7.0) prior to measurement to avoid multiple scattering effects. The mean droplet diameter was determined using a laser diffraction instrument (LS 13320, Beckman Coulter, Brea, CA, USA). All measurements were made at 25 °C.

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