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Lutein-enriched emulsion-based delivery systems: Impact of Maillard conjugation on physicochemical stability and gastrointestinal fate

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ABSTRACT

The utilization of lutein as a natural colorant or nutraceutical in many foods, supplements, and other commercial products is currently limited because of its low water-solubility and chemical instability. The purpose of this study was to evaluate the effect of Maillard conjugates on the physical and chemical stability of lutein-enriched emulsions exposed to different temperatures and pH values, as well as on their potential gastrointestinal fate. Oil-in-water emulsions were prepared using either casein or casein-dextran conjugates as emulsifiers. Both types of emulsions showed a slight increase in particle aggregation at temperatures exceeding 37 °C, and became more prone to color fading (lutein degradation) as the temperature was increased. Casein-coated oil droplets were highly unstable to flocculation near their isoelectric point (pH 4–5) due to the reduction in electrostatic repulsion. However, casein-dextran-coated droplets were stable from pH 3 to 7, which was attributed to strong steric repulsion by the dextran moiety. The casein-coated droplets were unstable to aggregation in the gastric phase of the simulated GIT, whereas the casein-dextrin-coated ones were stable, which was again attributed to increased steric repulsion. Emulsifier type did not strongly influence lutein bioaccessibility. This work shows that Maillard conjugates can improve the physical stability of lutein-enriched emulsions without adversely affecting the bioaccessibility of the bioactive agent.

1. Introduction

Lutein is a natural colorant found in a variety of biological materials, such as yellow corn, egg yolk and marigold flowers. Lutein belongs to the xanthophyll class of carotenoids, which are oxygenated carotenes (Sajilata, Singhal, & Kamat, 2008). As with other xanthophylls lutein has an intense yellow color when present at low concentrations but a reddish color when present at high concentrations. Its characteristic color is due to selective absorption of electromagnetic radiation in the visible region by conjugated double bonds in its backbone (Sajilata et al., 2008). Lutein is known to accumulate in the pigmented region of the human eye, which is called the macula. The accumulation of lutein in the macula has been associated with a decrease in the risk of age-related macular degeneration and cataracts (Abdel-Aal el, Akhtar, Zaheer, & Ali, 2013; Boon, McClements, Weiss, & Decker, 2010; Sajilata et al., 2008). One of the proposed mechanisms for the protection of the macula by carotenoids is the absorbance of damaging light waves (Krinsky, Landrum, & Bone, 2003). Moreover, they can act as antioxidants by scavenging free radicals or quenching singlet oxygen, which protects cells including the ones in the macula from oxidative stress. Lutein cannot be synthetized by the human body and must therefore be ingested through the diet (Khalil et al., 2012; Nagao, 2014; Sajilata et al., 2008). The acceptable daily intake for lutein approved by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 0–2 mg/kg body weight (JECFA, 2005, pp.157). Moreover, the effective dose of lutein to provide protection against diseases such as age-related macular degeneration and cataracts has been reported to be about 10 mg/day (Frede et al., 2014).

Due to the beneficial effects of lutein on human health it can be considered to be a nutraceutical ingredient to create functional foods and beverages. Moreover, its yellow-red color and its hydrophobicity make lutein a natural lipid-soluble colorant that can

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be used to replace artificial ones. An important trend in the modern food industry is towards products that are manufactured "without artificial additives" such as preservatives, flavorings, and colorings (Sloan, 2015). In addition, consumers are tending to purchase more functional food products that claim to provide additional health benefits beyond their normal nutritional effects (Sloan, 2015). Lutein is a promising ingredient to fulfill these market trends, indeed it has recently been reported that lutein, which is mainly extracted from Marigold flowers (*Tagetes erecta*), has the fastest growing market among the carotenoids with a market value of around US$233 million in 2010, projected to grow to US$309 million by 2018 (Berman et al., 2014).

Nevertheless, the use of lutein in the food industry presents challenges related to its poor chemical stability, water-solubility, and bioaccessibility characteristics. In common with other carotenoids, lutein is sensitive to heat and acidic environments, which in the presence of oxygen enhance its degradation through autoxidation (Boon et al., 2010). The degradation of lutein causes a reduction in its bioactivity, as well as a change in its desirable quality attributes due to color fading and formation of rancid off-flavors (Boon et al., 2010; Sajilata et al., 2008). The poor oral bioaccessibility of lutein can be attributed to its low water-solubility, high melting point, and poor chemical stability (Frede et al., 2014; McClements & Li, 2010). As with other lipophilic compounds, lutein has to be solubilized within the mixed micelle phase formed in the small intestine before it can be absorbed by the epithelial cells, packaged into lipoproteins, and transported to the blood stream (Abdel-Aal el et al., 2013). The efficacy of solubilization in the mixed micelle phase therefore plays a major role in determining the overall bioavailability of lutein (Nagao, 2014).

Oil in water (O/W) nanoemulsions are a promising platform for creating delivery systems to incorporate lipophilic compounds into food products and increase their bioavailability (McClements, Decker, & Weiss, 2007). O/W nanoemulsions are thermodynamically unstable colloidal systems in which oil is dispersed in water in the form of small spheres ($r < 100$ nm) (McClements & Rao, 2011; McClements & Xiao, 2012). The functional performance of nanoemulsions can be tailored to specific applications by controlling their compositions or structures. A particularly promising approach to improving nanoemulsion performance is to use novel emulsifiers formed by covalently linking proteins and polysaccharides together using the Maillard reaction (Markman & Livney, 2012; Yang et al., 2015; Zhou et al., 2012). The protein part helps the emulsifiers rapidly adsorb to oil droplet surfaces, whereas the polysaccharide part helps prevent the oil droplets from aggregating by generating a strong steric repulsive interaction. For example, studies have shown that protein-polysaccharide emulsifiers formed by the Maillard reaction can improve the physical stability of emulsions, and alter their gastrointestinal fate (Lesmes & McClements, 2012; Yang et al., 2015).

The Maillard reaction is a non-enzymatic reaction that involves the condensation of the carbonyl group of a reducing carbohydrate with a free amino group of a protein (such as a lysine or a arginine residue or an N-terminal amino group). In the initial stages of the reaction an aldimine (Schiff base) is formed. The Schiff base subsequently undergoes an Amadori rearrangement when aldoses are involved or a Heyns rearrangement in the case of ketoses (Ames, 1990; Oliver, Melton, & Stanley, 2006). It is often important to prevent the later stages of the Maillard reaction from occurring when preparing protein-polysaccharide conjugates since they lead to the degradation of the Amadori products and the formation of a wide variety of undesirable reaction products (Van Lancker, Adams, & De Kimpe, 2011).

The aim of this work was to establish the impact of casein-dextran Maillard conjugates on the physicochemical stability and gastrointestinal fate of lutein-enriched nanoemulsions. In particular, this study examined to determine if these conjugates could improve the stability of the nanoemulsions, without adversely affecting the bioaccessibility of lutein. A source of long chain triacylglycerols (corn oil) was used as the lipid phase since this type of lipid has previously been shown to increase the bioaccessibility of carotenoids (Rao, Decker, Xiao, & McClements, 2013; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013).

2. Materials and methods

2.1. Materials

MariLut (20% lutein in corn oil) was kindly donated by PIVEG (San Diego, CA). Mazola corn oil was purchased from a local store. Spray dried sodium caseinate was purchased from the American Casein Company (Burlington, NJ). A lutein standard for HPLC analysis was purchased from Extrasynthese (Genay, France). Sodium azide, calcium chloride, sodium phosphate mono- and dibasic, dextran 37 kDa, porcine bile extract, pepsin and lipase were purchased from Sigma–Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO), potassium persulfate, ethanol, and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Fisher Scientific (Waltham, MA).

2.2. Maillard conjugates formation

Sodium caseinate (2.0 w/v%) and dextran (3.5 w/v%) were individually solubilized overnight at 5 °C in water. The completely solubilized and hydrated samples were subsequently mixed in a one-to-one ratio, which led to final sodium caseinate and dextran concentrations of 1.00 and 1.75 w/v%, respectively. The mixture was spray-dried using a mini spray drier (Buchi B-290, Switzerland) with an inlet temperature of 150 °C, a feed rate of 7.5 mL/min, a compressed air pressure of 600 kPa, and an air flow rate of 35 m^3/h (Shah, Davidson, & Zhong, 2012). Maillard conjugation reactions were performed by incubating the spray-dried mixture at 76% relative humidity (using a saturated KBr solution in a desiccator) and 60 °C (in an incubator) for 48 h (Markman & Livney, 2012; Pan, Mu, Hu, Yao, & Jiang, 2006). After conjugation, the samples were allowed to cool to room temperature and ground using a mortar and pestle. The samples were subsequently stored in a desiccator prior to use.

2.3. Maillard conjugates characterization

2.3.1. Conjugation efficiency

The conjugation efficiency was determined by measuring the reduction in free amino groups using the OPA assay (Pan et al., 2006). The OPA reagent was prepared according to Pan et al. (2006). In short, 40 mg OPA (dissolved in 1.0 mL 95% ethanol), 25 mL 0.10 M sodium tetraborate buffer (pH 9.5), 2.5 mL 20% SDS solution, and 0.10 mL 2-mercaptoethanol were mixed together and brought to a final volume of 50 mL. The OPA reagent was prepared freshly before use. After dispersing the conjugates, 0.10 mL of the dispersion was mixed with 2.70 mL of OPA reagent and incubated for 1 min at room temperature, the absorbance at 340 nm was measured immediately using an UV–visible spectrophotometer Ultrospec 3000 pro (Biochrom Ltd., Cambridge, England). A calibration curve was constructed using L-leucine (0,2–5 mM) as a standard amino group-containing compound (Markman & Livney, 2012; Pan et al., 2006). The conjugation efficiency was defined as follows: