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Lutein-enriched emulsion-based delivery systems: Influence of pH and temperature on physical and chemical stability

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ABSTRACT

Lutein may be utilized in foods as a natural pigment or nutraceutical ingredient to improve eye health. Nevertheless, its use is limited by its poor water-solubility and chemical instability. We evaluated the effect of storage temperature and pH on the physical and chemical stability of lutein-enriched emulsions prepared using caseinate. The emulsions (initial droplet diameter = 232 nm) remained physically stable at all incubation temperatures (5–70 °C); however the chemical degradation of lutein increased with increasing temperature (activation energy = 38 kJ/mol). Solution pH had a major impact on the physical stability of the emulsions, causing droplet aggregation at pH 4 and 5. Conversely, the chemical stability of lutein was largely independent of the pH, with only a slight decrease in degradation at pH 8. This work provides important information for the rational design of emulsion-based delivery systems for a lipophilic natural dye and nutraceutical.

1. Introduction

An important trend in the modern food industry is for products that are manufactured "without artificial additives" 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 natural pigment that has been shown to exhibit a range of potentially beneficial biological effects, and it is therefore an interesting food ingredient for replacing artificial dyes and for creating functional foods. 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., 2015).

Like other carotenoids, lutein is one of the major pigments in fruits and vegetables that lead to their characteristic yellow, red and orange colors. These carotenoids are found in appreciable levels in green leafy vegetables such as kale, spinach, lettuce, broccoli, peas, Brussel sprouts, and parsley, as well as in egg yolks, tomatoes, corn, and marigold flowers (Abdel-Aal el, Akhtar, Zaheer, & Ali, 2013; Boon, McClements, Weiss, & Decker, 2010; Krinsky, Landrum, & Bone, 2003; Sajilata, Singhal, & Kamat, 2008). Lutein belongs to the xanthophyll class of carotenoids, which are oxygenated carotenes (Sajilata et al., 2008).

Lutein, as well as other xanthophylls, may decrease the risk of age-related macular degeneration and cataracts (Abdel-Aal el et al., 2013; Boon et al., 2010; Sajilata et al., 2008). Xanthophylls accumulate in the pigmented region of the human eye, which is called the macul, and since they have high absorptivity within a specific wavelength range, they absorb the blue light that reaches the eye. Moreover, they can act as antioxidants by scavenging free radicals or quenching singlet oxygen (Krinsky et al., 2003; Nagao, 2014; Sajilata et al., 2008), thus decreasing oxidative stress in the retina. Since carotenoids, including lutein, cannot be synthesized in the human body, it is essential that they be consumed as part of the daily diet (Khalil et al., 2012; Nagao, 2014; Sajilata et al., 2008). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the acceptable daily intake (ADI) for lutein and zeaxanthin is 0–2 mg/kg body weight (JECFA, 2005). In addition, 10 mg/day has been reported to be an effective dose for providing protection against diseases such as age-related macular degeneration and cataracts (Frede et al., 2014). Dosages of up to 40 mg/day in humans showed no adverse effects after eye

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examinations. The presence of lutein crystals that could cause retinal damage was also not observed. The only adverse effect was carotenedermia, which is a reversible and harmless cutaneous hyperpigmentation (Alves-Rodrigues & Shao, 2004). Eggs are one of the major natural sources for carotenoids and they also contain them in a very bioavailable form. However, there are some concerns about the consumption of eggs leading to increased serum cholesterol levels.

Another concern is that lutein is sensitive to the thermal processing and storage process and thus can degrade in foods that are naturally rich or enhanced with lutein (Alam, Ushiyama, & Aramaki, 2009). Carotenoid oxidation can be enhanced by photodegradation, thermal degradation, acid exposure, autoxidation, and singlet oxygen; these different pathways can cause bioactivity and quality (color loss and rancidity) loss in food products fortified with carotenoids (Boon et al., 2010; Sajilata et al., 2008). Therefore, it is crucial to understand the degradation process of lutein in order to develop better protection systems for them in foods (Boon et al., 2010).

One of the major challenges to utilizing lutein as a functional food ingredient is its relatively low and variable oral bioavailability (Khalil et al., 2012; Nagao, 2014; Sajilata et al., 2008). The poor bioavailability profile 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 a result of these challenges, carotenoids cannot usually be directly incorporated into aqueous-based foods. Instead, a colloidal delivery system, such as an oil-in-water emulsion, is often required to overcome these limitations (Boon et al., 2010). An oil-in-water emulsion consists of small lipid droplets (containing the lipophilic bioactive) suspended in an aqueous medium. This type of emulsion-based delivery system provides a suitable means of dispersing a lipophilic bioactive into the aqueous environments found in many commercial food products. In addition, the lipid phase breaks down within the human gastrointestinal tract to form colloidal structures (mixed micelles) that are capable of solubilizing and transporting the bioactive agents, thereby increasing their bioavailability (Nagao, 2014). Furthermore, emulsion-based delivery systems may also be designed to inhibit the rate of carotenoid degradation (Boon et al., 2010).

For commercial applications, it is important that any delivery system should remain physically and chemically stable when exposed to the different pH and temperature environments during its processing, storage, and transportation (McClements, 2005). The aim of this work was therefore to study the effect of temperature and pH on the physical and chemical stability of lutein-enriched emulsions. A natural protein-based emulsifier (caseinate) was used to stabilize the emulsions, and 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 et al., 2013; Salvia-Trujillo et al., 2013).

2. Materials and methods

2.1. Materials

MariLut Lutein, consisting of 20% (w/w) of lutein dissolved 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 chromatography analysis was purchased from Extrasynthese (Genay, France). Sodium azide and mono- and dibasic sodium phosphate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (Waltham, MA, USA).

2.2. Emulsion preparation

An organic phase was prepared by diluting 2.5% (w/w) of the commercial lutein in corn oil. An aqueous phase was prepared by dispersing 1% (w/w) powdered sodium caseinate into aqueous buffer solution (5 mM phosphate, pH 7.0). A coarse oil-in-water emulsion was prepared by mixing the organic phase (10%, w/w) and the aqueous phase (90%, w/w) using a M133/1281-0 high-speed mixer (Biospec Products, Inc. Bartlesville, OK, USA) for 2 min at 10,000 rpm. The resulting coarse emulsion was then passed through a M-110L high-pressure microfluidizer for five passes at 12,000 psi (Microfluidics, Newton, MA, USA). The fine emulsion produced was then diluted (1:1, v/v) with buffer solution containing an antimicrobial agent (5 mM phosphate buffer, pH 7.0, 0.04% (w/v) sodium azide). The final diluted emulsions that were used for the stability studies contained 5% (w/w) oil phase and 250 mg/L lutein.

2.3. Stability study

The emulsions were stored for 7 and 14 days at different temperatures (5, 20, 37, 55 and 70 °C) and different pH values (2–8), respectively. The pH values were adjusted to the desired values using 0.1 and 1.0 N of hydrochloric acid and/or sodium hydroxide solutions. The emulsions with pH values of 2, 3, 4, 5, 6, 7 and 8 and were stored at 20 °C, while samples at pH 7 were stored at 5, 20, 37, 55 and 70 °C.

2.3.1. Chemical stability

The chemical stability of lutein was assessed by measuring the change in color and lutein concentration in the emulsions during storage. The color was monitored using a ColorFlex EZ colorimeter (HunterLab Reston, VA, USA). For the color analysis, 10 mL of emulsion was pipetted onto a plastic petri dish and the readings were performed against a black background. The concentration of lutein was determined from absorbance measurements (460 nm) made using a Cary 100 UV–visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). To prepare the samples for the spectrophotometric measurements, the emulsions were diluted 100 times in DMSO (50 μL of emulsion was diluted in 4.95 mL of DMSO). DMSO was used because it could dissolve lutein, oil, and protein to form transparent solutions suitable for UV–visible analysis. The emulsion without lutein was used as blank. A calibration curve was made by dissolving the lutein standard in DMSO in a range from 0.5 to 5 mg/L (r^2 = 0.9992).

2.3.2. Physical stability

The physical stability of the emulsions was assessed by measuring the change in mean droplet diameter and ζ-potential of the emulsions after 7 or 14 days of storage. The mean droplet diameters, particle size distributions, and ζ-potential were measured using a Zetasizer Nano ZS dynamic light scattering/micro-electro phoresis instrument (Malvern Instruments, Malvern, England). Samples were diluted 100 times in buffer solution (5 mM phosphate) at the same pH as the sample to obtain an attenuator value between 6 and 9 and therefore avoid multiple scattering. All measurements were made at 25 °C.

2.4. Data analysis

All experiments were performed in triplicate and the results are given as mean values ± standard deviation. Differences among the treatments were determined using an analysis of variance (ANOVA) and a post hoc Tukey test with a confidence level of 95%. The analyses were made using SPSS software (IBM Corporation, Armonk, NY, USA).