Chapter 9

Lutein-Enriched Emulsion-Based Delivery System: Impact of Casein-Phospholipid Emulsifiers on Chemical Stability

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Abstract

The health benefits of carotenoids in terms of their role in decreasing the risk of diseases, particularly certain cancers and eye disease, are limited by their chemical degradation. Emulsion delivery systems with water dispersions of a carotenoid enhance chemical stability and bioavailability to the host. An emulsified carotenoid delivery system can be based on carotenoid dissolution in lipid media and its stabilization by a surfactant mixture of milk proteins (the caseins) and phospholipids. The inclusion of lutein into an emulsified delivery system comprised of bovine casein or caprine casein in combination with phospholipids (soybean lecithin) enhanced the chemical stability of lutein during storage for 7 days at pH 7.0 at incubation temperatures of 5 and 15°C. The chemical stability of lutein in the corn oil-in-water emulsions stabilized by bovine and caprine caseins in combination with soybean lecithin was in the following order: caprine α_{s1}-II-casein/lecithin > caprine α_{s1}-I-casein/lecithin > bovine casein/lecithin. The results suggest that the chemical stability of lutein in oil-in-water emulsions can be enhanced by altering the thickness of the interfacial layer. Caprine casein/lecithin has the potential for use as an emulsifier in beverage emulsions.

Keywords: lutein, casein, phospholipids, emulsion, stability

1. Introduction

Among the polar oxygenated xanthophylls of the carotenoids, lutein has received attention for its potent antioxidant activity [1]. Lutein may protect the DNA of photoreceptive cells in

the retina from the harmful effects of strong light [2]. In the skin, lutein is believed to protect against UV radiation [3]. Lutein is naturally synthesized by plants, and is commercially available as a food supplement from Marigold flowers (*Tagetes erecta*) [4]. Another source of lutein, and one that is the most bioavailable of all, is the egg. A large egg yolk contains approximately 252 μg of lutein (and zeaxanthin); while there is not a tremendous amount of lutein in egg yolks, it is so bioavailable that it is taken into the bloodstream with great efficiency, giving a significant boost to the serum levels of this protective carotenoid [5]. Indeed, the inclusion of lutein in a lipophilic matrix (egg yolk phospholipids) leads to dramatic improvement in the absorption of lutein in humans [6]. However, the segment of the population that has been recommended to control dietary cholesterol intake avoids consumption of eggs. American adults have a poor supply of lutein and their intake of ~1–2 mg/day by eating fruits and vegetables may not be enough to attain health benefits [7].

Fortification of foods and beverages with fat-soluble bioactive components such as the carotenoids, especially β-carotene, is considered important in the elimination of acute deficiency symptoms, in optimizing health, and in providing protection from many chronic ailments on a long-term basis [8, 9]. The difficulties encountered in fortifying foods with the carotenoids are primarily due to their instability (air, light) and low water solubility [10]. Since carotenoids cannot be incorporated into aqueous-based foods, an emulsion-based delivery system provides a suitable means for dispersing the lipophilic carotenoids into the aqueous environments of foods [11]. Emulsion-based delivery systems also allow for improved absorption of non-polar or fat-soluble bioactive compounds such as the carotenoids [12]. The type of emulsion used should be considered when carotenoids are added to food and beverage products to inhibit the rate of their degradation [11].

Food emulsions are usually one of the simplest forms of oil-in-water emulsions, consisting of small oil droplets dispersed within an aqueous medium, with the oil droplets having mean diameters ranging from 10 to 100 nm (nanoemulsions) or 100 nm to 100 μm (conventional emulsions). The formation of successful emulsion-based food and beverage products requires emulsifiers [13]. Emulsifiers are surface-active (surfactants) substances that play a crucial role in the mixing of two immiscible liquids. These surface active substances normally have a polar (hydrophilic) and a non-polar (hydrophobic) ends that break the surface tension between different liquids thereby, facilitating the formation of the mix and maintaining the stability of the mix. Food manufacturers have traditionally used both synthetic and natural emulsifiers in food formulations; however, the clean label movement is creating a new trend toward the use of natural emulsifiers. In this context, the interest of the general public and the food industry professionals are toward identifying, characterizing, and utilizing naturally occurring substances such as proteins, polysaccharides, phospholipids, and saponins as emulsifiers in formulated foods. The desire is to find which naturally occurring compounds have the appropriate properties to efficiently form stabilized emulsions in foods with possible commercial applications.

Proteins contain a mixture of hydrophilic and hydrophobic amino acids along their polypeptide chains that render them naturally surface active agents. This characteristic enables most proteins to quickly absorb to oil-in-water interfaces and coat the oil droplets that are formed during mixing and homogenization. The negatively charged carboxylic groups ($–COO^-$) or the positively charged amino groups ($–NH_3^+$) of amino acids that make up proteins can stabilize

droplets from aggregation by generating electrostatic repulsions. Proteins are generally relatively small molecules (about 10–50 kDa) that rapidly absorb to droplet surfaces and form thin, electrostatically charged interfacial layers. Such layers are important in the formation of stabilized emulsions.

Currently, caseins and whey proteins from bovine origin are the most commonly used protein-based emulsifiers in the food industry. Caseins are amphiphilic proteins with flexible structures (α_{s1}-, α_{s2}-, β-, and κ-caseins) whereas whey proteins are globular proteins with fairly rigid structures [β-lactoglobulin, bovine serum albumin (BSA), and immunoglobulins]. Due to their structural flexibility, caseins rapidly undergo conformational changes, with the hydrophilic groups protruding into the water phase and the hydrophobic groups into oil phase.

Phospholipids, similar to proteins, are amphiphilic molecules (loving both water and oil) with hydrophobic fatty acid tail groups and phosphoric acid esterified with glycerol and other substitutes as the hydrophilic head groups. Phospholipids are generally referred to as lecithin, and they occur in nature in the cell membranes of animals, plants, and microbial species. Phospholipids are industrially extracted from soybeans, egg yolk, milk, and sunflower kernels for use in foods. Lecithin contains a mixture of different phospholipids, with the most common being phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. While lecithin ingredients are surface-active agents and facilitate the mixing of oil and water, they also are prone to coalescence because they form interfacial layers. Combining lecithin with proteins such as bovine caseins [14] can minimize this issue and help form stable emulsions.

Milks from different mammalian species present differences in their relative proportions and characteristics of caseins [15]. The degree of variability of caprine casein components from the individual milks of French-Alpine and Anglo-Nubian breeds of goats has been previously reported [16]. For both breeds the quantity of β- and κ-caseins are relatively constant while the content of α_s-caseins of these breeds vary significantly [16]. In bovine milk the constancy of the ratio of caseins results in a rather distribution of structure for casein micelles (with Ca^{2+}), which has been used for the delivery of fat-soluble bioactive compounds such as curcumin and vitamin D in aqueous solutions [17, 18]. Casein sub-micelles (without Ca^{2+}) from bovine and caprine milks have been used for the delivery of lutein in food emulsions [19, 20].

The aim of our study was to evaluate the effects of bovine and caprine caseins (sub-micelles) in combination with phospholipids (soy lecithin) as emulsifiers on the chemical stability of lutein in corn oil-in-water emulsions at pH 7.0 during storage at 5 and 15°C. The corn oil allows the carotenoids to be absorbed [21].

2. Materials and methods

2.1. Materials

A commercial preparation of lutein consisting of 20% (wt/wt) lutein dissolved in corn oil was a gift from Hoffman La Roche (Pleasanton, CA). Mazola corn oil was purchased from a local supermarket. A lutein standard for chromatographic analysis was purchased from

Extrasynthèse SA (Genay, France). Soybean lecithin Beakin LV3 was a gift from Archer Daniels Midland Co (Decatur, IL, USA). Ethanol, thimerosal, phenylmethanesulfonyl fluoride, and monobasic potassium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific (Pittsburg, PA, USA). Deionized water was prepared by passing distilled water over a mixed bed of cation-anion exchanger and was used throughout this study.

2.2. Source of caseins

Whole caseins were prepared by isoelectric precipitation of the individual skimmed milk samples obtained from a Jersey cow and French-Alpine goats. The precipitate was dissolved by the addition of NaOH to yield a solution of pH 7.0. The casein was re-precipitated, washed, and then re-suspended. The sodium caseinate was subsequently cooled to 4°C and centrifuged at 100,000 × g for 30 min to remove residual fat using a Beckman Optima XL-A (Beckman Instruments Inc., Palo Alto, CA, USA) analytical ultracentrifuge. Finally, the suspension was dialyzed against cold deionized water at 4°C for 72 h with three changes and then lyophilized. The integrity of the samples was confirmed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and the percentages of the various component caseins estimated by densitometry as previously described [22]. The casein composition of the samples is as follows: α_{s2}-casein (bovine casein 12.1%; caprine α_{s1}-I-casein 9.2%; caprine α_{s1}-II-casein 5.3%), α_{s1}-casein (type I): (bovine casein 0%; caprine α_{s1}-I-casein 4.0%; caprine α_{s1}-II-casein 0%); α_{s1}-casein (type II): (bovine casein 39.5%; caprine α_{s1}-I-casein 21.1%; caprine α_{s1}-II-casein 25.6%); β-casein: (bovine casein 37.2%; caprine α_{s1}-I-casein 51.6%; caprine α_{s1}-II-casein 60.6%); and κ-casein: (bovine casein 11.2%; caprine α_{s1}-I-casein 13.8%; caprine α_{s1}-II-casein 9.6%) [20].

2.3. Emulsion preparation

A corn oil-in-water emulsion was prepared as follows: an organic phase was prepared by diluting 2.5% (wt/wt) of the commercial lutein in corn oil. Soybean lecithin 0.5% (wt/wt) was dissolved in the corn oil. An aqueous phase was prepared by dispersing 1.0% (wt/wt) of lyophilized bovine casein or caprine casein into the aqueous buffer solution (5 mM phosphate, pH 7.0). A coarse emulsion of oil-in-water was prepared by mixing the organic phase (10%, wt/wt) and the aqueous phase (90%, wt/wt) using a hand-held homogenizer (Biospec Products, Inc., Bartlesville, OK, USA) at low speed. The coarse emulsion was then homogenized five times at 82.74 MPa (12,000 psi) through a high-pressure TC5 homogenizer (Stansted Fluid Power, Harlow, UK). The fine emulsion produced was then diluted (1:1, v/v) with buffer solution containing an antimicrobial agent [5 mM phosphate buffer at pH 7.0 and 1 mM (wt/v) thimerosal]. The final diluted emulsions that were used for the stability studies contained 5% (wt/wt) oil phase and 250 mg per liter of lutein. Resulting emulsions were stored in the dark at 5 and 15°C for 7 days.

2.4. Physical characterization of the emulsion

The particle size of the oil droplets in the lutein-enriched emulsions was measured at day 0 and day 7 after homogenization with a SALD-2101 laser diffraction particle analyzer (Shimadzu Corporation, Columbia, MD, USA). The charge of the oil droplets in the lutein-enriched

emulsion (zeta potential, ζ, mV) was measured at day 0 with a Zetasizer nano ZS (Malvern Instruments, Worcestershire, UK). Samples were diluted 100 times in 5 mM phosphate buffer at pH 7.0. All measurements were carried out in triplicate at 21 ± 1°C.

2.5. Chemical stability of lutein in the emulsion

The chemical stability of lutein was assessed by measuring the concentration of lutein in the lutein-enriched emulsions during storage at 5 and 15°C and analyzed right after production (0 day) and at 1, 2, 4, 6, and 7 days. The concentration of lutein was determined from absorbance measurements at 460 nm using a Beckman UV/visible model DU-530 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). To prepare the samples for the spectrophotometric measurements, the lutein-enriched emulsions were diluted 100 times in DMSO (50 μl of emulsion was diluted into 4.95 ml of DMSO). The DMSO was used because it dissolves lutein, oil, lecithin, and protein to form transparent solutions suitable for UV/visible analysis. The emulsion without lutein was used as a blank. A calibration curve was constructed by dissolving lutein standards in DMSO within a range from 0.5 to 5 mg/ml.

2.6. Statistical analysis

In each experiment, the results of triplicate analyses were used to test experimental variables. The data were analyzed by ANOVA using PRO GLM procedure of SAS (version 8.2, SAS Institute, Cary, NC, USA). The least significant test was used to determine significant differences among means at $p < 0.05$.

3. Results and discussion

3.1. Relationship between casein composition and emulsion interfacial properties

Caprine caseins have markedly higher content of β-casein than bovine casein. The specific oxidative stability found in emulsified lipids has been explained by the formation of a highly protective interface produced from β-casein as an emulsifier in the emulsions [23, 24]. Likewise, emulsions exert good protective effects on the carotenoids. Thus, it has been reported that the multilayer emulsions around the oil droplets can potentially reduce the amount of light reaching the carotenoid [19, 20]. A lutein dispersion was achieved using bovine casein or caprine caseins (caprine α_{s1}-I-casein and caprine α_{s1}-II-casein) as emulsifier in an emulsion beverage [19, 20]. The caprine casein emulsifier, in particular caprine α_{s1}-II-casein, in combination with arabinogalactan, a water-soluble polysaccharide, is noteworthy because this lutein dispersion system remains stable after light exposure during storage [20].

3.2. Physical stability of lutein emulsions prepared with bovine casein/lecithin and caprine casein/lecithin as emulsifiers

In order to understand the effects of bovine casein or caprine casein in combination with phospholipids i.e., soybean lecithin on the chemical degradation of lutein, three sets of emulsions with corn oil were made. The three sets were stabilized by bovine casein/lecithin,

caprine α_{s1}-I-casein/lecithin, or caprine α_{s1}-II-casein/lecithin. The mean particle diameters of oil droplets in the lutein-enriched emulsions stabilized by bovine casein/lecithin or caprine casein/lecithin ranged from 205.6 ± 2.3 to 208.9 ± 2.5 nm at pH 7.0 (**Table 1**). We observed no significant differences ($p > 0.05$) in the particle sizes of oil droplets in lutein-enriched emulsions with either bovine casein or caprine caseins during 0 and 7 days of storage at 21 ± 1°C.

The zeta potentials of the casein/lecithin-coated oil droplets in the lutein-enriched emulsions were negative at pH 7.0 (**Table 2**). The zeta potentials of the oil droplets in the lutein-enriched emulsions prepared with the two caprine casein/lecithin emulsifiers were not different ($p > 0.05$), showing mean values of −36.8 ± 1.8 mV for caprine α_{s1}-I-casein/lecithin and −35.9 ± 1.9 mV for caprine α_{s1}-II-casein/lecithin, respectively. The lower zeta potentials of the oil droplets in the lutein-enriched emulsions stabilized by the two caprine casein/lecithin emulsifiers compared to the lutein-enriched emulsions stabilized by bovine casein/lecithin emulsifier can imply a decreased 'net' negative charge [25]. Differences in the amino acids of side chains of κ-, α_s- and β-casein fractions in caprine whole caseins, where the less charged β-casein quantitatively dominates, could lead to less repulsive charge-charge interactions and this phenomenon can possibly explains why caprine caseins have lower zeta potentials (**Table 2**). On the basis of these findings, we can conclude that the lutein-enriched emulsions prepared with bovine casein/lecithin emulsifier and the two caprine casein/lecithin emulsifiers exhibited relatively good physical stability [13].

3.3. Chemical stability of lutein emulsions prepared with bovine casein /lecithin and caprine casein/lecithin as emulsifiers

Lutein degradation in the emulsions that were prepared with different emulsifiers was in the following order: bovine casein/lecithin > caprine α_{s1}-I-casein/lecithin > caprine α_{s1}-II-casein/

Particle size (nm)[1]			
Storage (d)	Bovine casein/lecithin	Caprine (α_{s1}-I)-casein/lecithin	Caprine (α_{s1}-II)-casein/lecithin
0	207.8 ± 2.0	208.9 ± 2.5	208.0 ± 2.1
7	205.6 ± 2.3	206.1 ± 2.4	205.7 ± 2.2

[1]Mean value ± Standard error.

Table 1. Particle size of oil droplets in lutein-enriched emulsions stabilized by bovine casein/lecithin or caprine casein/lecithin in phosphate buffer at pH 7.0 and 21 ± 1°C.

Emulsifier	**Zeta potential (mV)[1]**
Bovine casein/lecithin	−39.7 ± 1.8[a]
Caprine (α_{s1}-I)-casein/lecithin	−36.8 ± 1.8[b]
Caprine (α_{s1}-II)-casein/lecithin	−35.9 ± 1.9[b]

Means with different lowercase superscripts are significantly different ($p < 0.05$).
[1]Mean value ± Standard error.

Table 2. Zeta potential of oil droplets in lutein-enriched emulsions stabilized by bovine casein/lecithin or caprine casein/lecithin in phosphate buffer at pH 7 and 21 ± 1°C.

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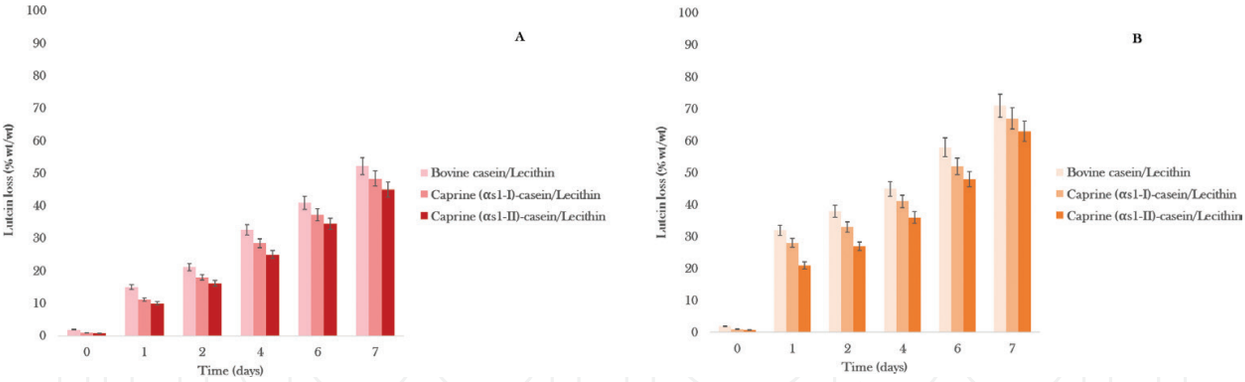

Figure 1. Lutein degradation in corn oil-in-water emulsions (pH 7.0) stabilized by bovine casein/lecithin, caprine (α_{s1}-I)-casein/lecithin or caprine (α_{s1}-II)-casein/lecithin at 5°C (A) and 15°C (B) as a function of storage time. Values represent the mean of three trials.

lecithin (**Figure 1**). The bovine casein/lecithin means on the bars are significantly different than the two caprine casein/lecithin means ($p < 0.05$) until four days of storage at 5°C (**Figure 1A**); the same tendency in lutein loss was observed during storage at 15°C (**Figure 1B**). Comparing the results of the two caprine casein/lecithin emulsifiers, the caprine α_{s1}-II/lecithin emulsifier overall showed lower means than caprine α_{s1}-I/lecithin emulsifier in both storage temperatures. In general, lutein degradation was faster in emulsions prepared with bovine casein/lecithin emulsifier than with the other two emulsifiers within 7 days of storage at 5 and 15°C (**Figure 1A** and **B**, respectively). The fact that lutein degradation was faster in the emulsions prepared with bovine casein/lecithin emulsifier compared to the emulsions prepared with caprine α_{s1}-I-casein /lecithin emulsifier or caprine α_{s1}-II-casein/lecithin emulsifier suggests that the interfacial layer formed by bovine casein/lecithin emulsifier was less efficient in protecting emulsified lutein against chemical degradation during storage at 5 and 15°C. The combination of caprine α_{s1}-I-casein or caprine α_{s1}-II-casein with soybean lecithin as emulsifiers resulted in a more favorable thickness of the interfacial layer thereby, slowing down the lutein degradation in these emulsions. This confirms that caprine caseins, which are 'rich' in their content of β-casein, formed a denser interfacial layer surrounding oil droplets and the possible role that the thickness of the interfacial layer played in the degradation of emulsified lutein. Furthermore, soybean lecithin, which is comprised of charged phospholipids such as phosphatidylinositol and phosphatidic acid [26], is more soluble in water and therefore, more easily absorbed at the oil-in-water interface thereby, producing a thicker interfacial layer protecting lutein.

4. Conclusions

The stabilizing role of phospholipids in emulsions is important in the absorption of lutein by the host. From the nutritional point of view consumption of a lutein-enriched beverage emulsion stabilized by bovine casein/lecithin emulsifier or caprine casein/lecithin emulsifier will be a valuable means to counterbalance the deficiency in consumption of fruits and vegetables; this approach will reduce the negative effects of lower ingestion of health-promoting carotenoids. Overall, these results indicate that a high chemical stability of lutein in corn oil-in-water emulsions can be achieved by altering the physical properties of the emulsion droplet

interface by the addition of emulsifiers such as caprine α_{s1}-I-casein/lecithin and caprine α_{s1}-II-casein/lecithin. The different effects of the bovine casein/lecithin and the caprine casein/lecithin emulsifiers on the stability of lutein is expected to be derived from the difference in the composition of bovine and caprine caseins, in particular the β-casein and its role at the interface of emulsion for the protection of emulsified carotenoids, specifically the lutein of the xanthophyll group. The charged phospholipids, phosphatidyl inositol and phosphatidic acid, in soybean lecithin may also decrease the degradation of lutein in oil-in-water emulsions by producing a thicker interfacial layer.

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Conflict of interest

The authors declare that they have no conflict of interest.

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