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Complexes of lutein with bovine and caprine caseins and their impact on lutein chemical stability in emulsion systems: Effect of arabinogalactan

A. Mora-Gutierrez,[1] R. Attaie, M. T. Núñez de González, Y. Jung, S. Woldesenbet, and S. A. Marquez
Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TX 77446

ABSTRACT

Lutein is an important xanthophyll carotenoid with many benefits to human health. Factors affecting the application of lutein as a functional ingredient in low-fat dairy-like beverages (pH 6.0–7.0) are not well understood. The interactions of bovine and caprine caseins with hydrophobic lutein were studied using UV/visible spectroscopy as well as fluorescence. Our studies confirmed that the aqueous solubility of lutein is improved after binding with bovine and caprine caseins. The rates of lutein solubilization by the binding to bovine and caprine caseins were as follows: caprine α_{S1}-II-casein 34%, caprine α_{S1}-I-casein 10%, and bovine casein 7% at 100 μM lutein. Fluorescence of the protein was quenched on binding supporting complex formation. The fluorescence experiments showed that the binding involves tryptophan residues and some nonspecific interactions. Scatchard plots of lutein binding to the caseins demonstrated competitive binding between the caseins and their sites of interaction with lutein. Competition experiments suggest that caprine α_{S1}-II casein will bind a larger number of lutein molecules with higher affinity than other caseins. The chemical stability of lutein was largely dependent on casein type and significant increases occurred in the chemical stability of lutein with the following pattern: caprine α_{S1}-II-casein $>$ caprine α_{S1}-I-casein $>$ bovine casein. Addition of arabinogalactan to lutein-enriched emulsions increases the chemical stability of lutein-casein complexes during storage under accelerated photo-oxidation conditions at 25°C. Therefore, caprine α_{S1}-II-casein alone and in combination with arabinogalactan can have important applications in the beverage industry as carrier of this xanthophyll carotenoid (lutein).

Key words: casein, bovine, caprine, lutein, arabinogalactan

INTRODUCTION

Carotenoids are primarily symmetrical, C-40, polyisoprenoid structures with an extensive conjugated double-bond system. The C-40 carotenoids can be divided into carotenes, which are hydrocarbons (e.g., β-carotene), and their oxygenated derivatives, the xanthophylls (e.g., lutein, astaxanthin, zeaxanthin; Sajilata et al., 2008). Lutein, which is mainly extracted from Marigold flowers (*Tagetes erecta*), has recently come into the limelight because of animal and epidemiological studies that support its eye health benefits (Sasaki et al., 2012). The eye is susceptible to photosensitized damage and adequate intake of lutein can provide protection against photo-injury (Krinsky et al., 2003). The role of lutein is filtration of blue light and functions as an antioxidant (Eisenhauer et al., 2017). As the lutein levels increase in the macula of the eye, a significant decrease will occur in the amount of harmful light rays that reach the retinal cells that produce vision. Therefore, lutein reduces the risk of macular degeneration, an age-related disease of the retina that is a common cause of loss of vision in the elderly. Lutein acts as antioxidant by scavenging free radicals or quenching singlet oxygen, thereby decreasing oxidative stress in the retina (Eisenhauer et al., 2017).

Since humans cannot synthesize carotenoids, it is essential that they eat green leafy vegetables such as kale and spinach as part of their daily diet (Eisenhauer et al., 2017). To achieve a lifetime of excellent vision, a dosage of 10 mg of lutein per day is required (Frede et al., 2014). Although green leafy foods are good sources of the carotenoid lutein, enzymatic degradation of this carotenoid occurs immediately when the leaves are cut. Another source of lutein, and one that is the most bioavailable of all, is the egg (Eisenhauer et al., 2017). However, the segment of the population that has been recommended to control dietary cholesterol intake avoids consumption of eggs. Generally, older consumers are more interested in functional foods than the younger generation to improve health and extend life expectancy. Most functional foods that have been developed are beverages, but several challenges in formulating lutein-rich beverages still exist.

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[1]Corresponding author: admora@pvamu.edu

Stabilizing beverages poses a unique challenge to formulators because of their very dilute nature. Formulators have to find a way of stabilizing sensitive ingredients such as colors, flavors, and micronutrients, many of which may not even be soluble in water, in microgram levels in liters of liquid. A liquid dietary food rich in lutein is difficult to design due to its low oral bioavailability. The poor oral bioavailability of lutein has been a challenge and was ascribed to its low water solubility and chemical stability (Mitri et al., 2011). Emulsion delivery systems have enabled the incorporation of lutein at a high dose with minimal formulation issues (Davidov-Pardo et al., 2016). The use of bovine casein assisted in the emulsion delivery process (Davidov-Pardo et al., 2016). However, caprine casein, due to its different composition (i.e., lower α_{S1}-CN and higher β-CN), has been shown to protect algae oil with added carotenoids against oxidation at oil-in-water interfaces (Mora-Gutierrez et al., 2010). The β-CN fraction of caprine whole casein absorbed to the oil-water interface of emulsions formed a dense interfacial layer surrounding oil droplets, thereby participating in the protective effect against lipid oxidation (Mora-Gutierrez et al., 2010). In the dispersion systems of carotenoids, which are lipophilic molecules with near zero inherent aqueous solubility, there is little understanding about the interactions between caseins from different commercially important ruminant species and the lipid-water interfaces.

A recent study suggests that the amount of exopolysaccharide (**EPS**), which has beneficial effects on human health produced by cocultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, is positively influenced by the presence of caprine α_{S1}-I-CN peptides and caprine α_{S1}-II-CN peptides in the culture medium (Mora-Gutierrez, 2016). This report of increased production of EPS by dairy strains of lactic acid bacteria grown in lactobacilli de Man, Rogosa, and Sharpe broth supplemented with caprine α_{S1}-I-CN peptides and caprine α_{S1}-II-CN peptides made these peptides and their parent proteins appealing as possible food ingredients with a potential prebiotic character. Bioactive peptides can be released from the parent protein during gastrointestinal digestion and may interact with *Bifidobacterium longum*, which is a species of bacteria commonly found in the human intestine. Levels of *Bifidobacterium* decline with age, therefore, a food ingredient that on ingestion has the ability to increase levels of bifidobacteria (Fiedorowicz et al., 2016) and production of EPS (Salazar et al., 2009) is a potential food ingredient in the prevention of digestive problems such as constipation and abdominal discomfort.

In this work, the interactions between lutein and the caseins isolated from bovine and caprine milks were studied by turbidity measurements and fluorescence spectroscopy. Furthermore, the effects of lutein-casein complexes on lutein's chemical stability in lutein-enriched emulsions during storage under conditions for accelerated photo-oxidation were also compared. Moreover, due to the importance of enhancing lutein water solubility and chemical stability in low-fat dairy-like beverages, we used a water-soluble polysaccharide (i.e., arabinogalactan). Arabinogalactan, a highly branched polysaccharide polymer composed of galactose and arabinose in a 6:1 ratio, has been shown to enhance the solubility and photo-stability of lutein in aqueous solutions (Polyakov and Kispert, 2015). Additionally, the effects of caprine α_{S1}-I-CN and caprine α_{S1}-II-CN on the chemical stability of lutein in lutein-enriched emulsions were also examined.

MATERIALS AND METHODS

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 chromatography analysis was purchased from Extrasynthèse SA (Genay, France). Arabinogalactan, L-tryptophan, ethanol, thimerosal, phenylmethanesulfonyl fluoride, and monobasic potassium phosphate were purchased from Sigma-Aldrich (St. Louis, MO). Dimethyl sulfoxide (**DMSO**) was purchased from Fisher Scientific (Pittsburgh, PA). Deionized water, prepared by passing distilled water over a mixed bed of cation-anion exchanger, was used throughout this study.

Preparation of Bovine and Caprine Caseins

Caseins were prepared from the milk of a Jersey cow and French-Alpine goats. The samples of caprine milk were collected from individual French-Alpine animals that were raised at the International Goat Research Center in Prairie View A&M University, Texas. The caprine milk caseins were selected based on yielding high levels of α_{S1}-CN as determined by reversed-phase HPLC (Mora-Gutierrez et al., 1991). The equipment for HPLC consisted of a Waters 600 multi-solvent delivery system, a 481 variable wavelength LC spectrophotometer and a 740 data module (Waters Corporation, Milford, MA).

Caseins were isolated from 2 L of fresh, uncooled milk to which phenylmethanesulfonyl fluoride (0.1 g/L) was added immediately to retard proteolysis. The milk was centrifuged at 4,000 × g for 10 min at room tem-