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Gum arabic microcapsules as protectors of the photoinduced degradation of riboflavin in whole milk

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ABSTRACT

Microcapsules (MC) made with gum arabic (GA) as shell material without and with β-carotene (βc) as core material were prepared by the spray-drying technique. The effect of these MC on the photodegradation of riboflavin (Rf) in whole milk by fluorescent daylight lamp irradiation was evaluated at a storage temperature of 4°C. The additions of 1.37 mg/mL of MC without βc (MC-GA) and with 0.54 μg/mL of βc (MC-βc-GA) decreased the apparent first-order rate constant of Rf photodegradation by approximately 26 and 30%, respectively. A systematic kinetic and mechanistic analysis of the results indicates that the global protective effect of the MC is mainly due to the combination of quenching of the electronically excited triplet state of Rf and scavenging of the photogenerated reactive oxygen species, such as singlet molecular oxygen, superoxide radical anion and hydroxyl radical. A minor contribution to the photoprotective effect can be also associated with the inner-filter effect exerted by the MC, which partially blocks the direct excitation of Rf. These results allow us to conclude that photodegradation of Rf in milk can be considerably reduced by the addition of small amounts of MC, avoiding large losses in the nutritional value of milk.
Key words: riboflavin photodegradation, β-carotene, gum arabic, reactive oxygen species

INTRODUCTION

The oxidative stability of milk and its products is of great importance for the dairy industry. It is well known that some of the photoinduced degradation process in milk occurs mainly because of the presence of vitamin B_2 [riboflavin (**Rf**)], which acts as a photosensitizer, absorbing environmental light to generate electronically excited states of the flavin, in particular the triplet excited state (^{3}**Rf***; Montenegro et al., 2007). In the presence of triplet molecular oxygen ($^3O_2$) or electron donors, or both, the ^{3}Rf* is able to generate several reactive oxygen species (**ROS**), such as superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($HO^{\bullet}$), and singlet molecular oxygen ($^1O_2$) either by electrotransfer (type I mechanism) or energy-transfer (type II mechanism) reactions (Massad et al., 2004; Skibsted, 2010). These photogenerated ROS have a deleterious effect in milk, as they have a large degree of reactivity and may cause oxidation of proteins, vitamins, and lipids, with the collateral production of low-molecular-weight volatile compounds, responsible for off-flavor and nutritional quality loss in milk (Bradley, 1980; Mortensen et al., 2003; Mestdagh et al., 2011). In turn, the oxidative stability of milk depends on a delicate balance between the anti- and prooxidants processes (Halliwell and Gutteridge, 1999), which are influenced by several factors, such as the unsaturation degree of FA, content of transition metals, and antioxidant molecules (**AOx**; Kristensen et al., 2004). Adding AOx to scavenge harmful ROS species or molecules with the capability of quenching ^{3}Rf*, avoiding the formation of ROS, seem to be suitable strategies to avoid the undesired photoinduced off-flavor of milk.

Currently, natural AOx are preferred to synthetic ones to be added to milk to avoid or lessen toxicological side effects. Among these types of molecules, the lipid-soluble carotenoids (**CAR**) show exceptional nutritional and health-promoting properties, such as provitamin A activity and scavenging activity against ROS, in particular very efficient quenching of $^1O_2$, promoting the prevention and (or) reduction of human diseases associated with oxidative stress (Burton and Ingold, 1984; Liebler, 1993).

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However, the highly nonpolar properties of CAR preclude their direct utilization in aqueous media, where they aggregate and precipitate with the complete loss of their antioxidant properties. Among the several approaches for CAR vehiculization and controlled delivery in aqueous food matrices, spray-dried microencapsulation with edible biopolymers as coating material is a suitable and economical feasible method (Rodriguez-Huezo et al., 2004; Barbosa et al., 2005; Gharsallaoui et al., 2007). In this process, the biopolymer wall acts as a physical permeable barrier to diffusion of oxygen and other molecules (Edge et al., 1997; Bustos et al., 2003) and allows the stabilization, transport, and controlled delivery of CAR into the aqueous media (Rodríguez-Huezo et al., 2004; Barbosa et al., 2005).

In a previous study, we evaluated the effect of lycopene microencapsulation by spray drying with a gum arabic (**GA**)-sucrose (8:2) mixture in the Rf-mediated photosensitized degradation of vitamins A and D_3 in skim milk, using white fluorescence lamps as a light source, as the visible absorption band of Rf overlaps with the blue-shifted emission of the fluorescent light (Montenegro et al., 2007). The results indicated that the addition of 6.5 mg/mL of this microencapsulated skim milk produced a reduction of approximately 45% of the photosensitized degradation rate of both vitamins.

In a more recent study, we evaluated the $^1O_2$-quenching capacity of microcapsules (**MC**) of GA (**MC-GA**) or matodextrin containing natural AOx molecules such as CAR or tocopherol derivatives (Faria et al., 2010). The results indicated that the $^1O_2$-quenching efficiency by the AOx in MC was strongly dependent on the lipophilicity degree of the AOx, being more efficient in the polar ones due to compartmentalization effects of the AOx in the core of the MC that modified the accessibility of $^1O_2$. Additionally, it was demonstrated that empty MC of GA were efficient quenchers of $^1O_2$, due to the interaction with amino acid residues (Trp, His, and Met, among others) of the protein moiety present in this glycoprotein (Mahendran et al., 2008). Later, for the same series of microencapsulated AOx studied before, Rodrigues et al. (2012) analyzed the antioxidant activity against both reactive oxygen and nitrogen species, such as peroxyl radical ($ROO^{\bullet}$), hydrogen peroxide (H_2O_2), $HO^{\bullet}$, and peroxynitrite anion ($ONOO^-$). They found that the scavenging capacities were influenced by the wall material and by the type of antioxidant molecule, being in all cases higher for the microcapsules with GA than with maltodextrin.

Due to the relevance of Rf photochemistry in milk, the objective of this work was the evaluation of the efficiency of MC-GA itself and of MC-GA containing the provitamin A precursor β-carotene (**βc**) as scavengers of ROS generated by fluorescent light photosensitization of Rf in whole milk.

MATERIALS AND METHODS

Materials and Chemicals

β-Carotene (98% purity), Rf (≥98%), sodium azide (**NaN_3**), superoxide dismutase (**SOD**), nitro blue tetrazolium (**NBT**), 2-deoxy-D-ribose, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox (**TX**); 99.5% purity], and SDS were supplied by Sigma-Aldrich (St. Louis, MO). Food-grade GA (molecular weight = 3.5×10^5 g/mol) was purchased from Colloides Naturels Brasil (São Paulo, Brazil). Acetate buffer was from Merck Química Argentina S.a.i.c. (Buenos Aires, Argentina); $FeCl_3$, H_2O_2, KH_2PO_4, K_2HPO_4, ascorbic acid, thiobarbituric acid (**TBA**), TCA, hydroxylamine hydrochloride (**HAHC**), all analytical grade, were obtained from Biopack Productos Químicos (Buenos Aires, Argentina); and HPLC-grade solvents: methanol, hexane, 1-butanol, ultrapure water, and glacial acetic acid were from Merck KGaA (Darmstadt, Germany; LiChrosolv). The milk samples were prepared from whole-milk powder of a recognized Argentinean trademark.

Preparation of MC

The MC were prepared with GA as shell material (MC-GA) and with β-carotene (MC-βc-GA) by using a laboratory-scale spray-dryer system (Labplant SD-04; Labplant UK Ltd., Huddersfield, 123 UK), under the following working conditions: aspersion nozzle diameter of 0.7 mm, air pressure of 5 kgf/cm^2, and air flow rate of 30 mL/min, entrance and exit air temperatures of 170 and 110°C, respectively. Gum arabic solutions [30% (wt/vol) of soluble solid] were prepared in water at 45°C and kept under continuous stirring until the temperature reached 30°C. β-Carotene was dissolved in dichloromethane and a small aliquot was added to the GA aqueous solution. The mixture was stirred at 7,000 rpm for 30 min to obtain an emulsion. Afterward, the emulsion was diluted with water to obtain a 20% (wt/vol) GA solution. The emulsion was placed in the spray-dryer chamber, maintaining slow agitation during the spray-drying process. The microcapsules obtained were immediately stored in a glass bottle under N_2 atmosphere at −18°C to avoid βc degradation.

Quenching of ^{3}Rf* by MC and Laser Flash Photolysis Experiments

Generation and detection of ^{3}Rf* were performed by laser flash photolysis (LFP) experiments using the third