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Oxidative stability of yogurt with added lutein dye

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ABSTRACT

This study evaluated the effect of adding lutein dye on the oxidative stability of yogurt during 35 d of refrigerated storage, in the presence and absence of light. Yogurts manufactured without and with the equivalent of 1.5 mg of lutein in 120 g of the final product were characterized for their total carotenoid and riboflavin contents, and the behaviors of both riboflavin and lutein were monitored during storage. A decrease in riboflavin content occurred, with concurrent appearance of its derived-oxidation products in the yogurts without added lutein and exposed to light during storage. The yogurts with added lutein dye showed constant lutein and riboflavin contents throughout storage both for the samples stored under light and for those stored in the dark. Yogurts (120 g) with the addition of 0.5, 1.5, and 2.5 mg of lutein dye were evaluated for their sensory acceptance, and the statistical analysis showed no differences between the samples for the attributes of aroma and flavor. These results indicate that the added lutein remained stable throughout the storage period and conferred protection for the riboflavin against photooxidation, preserving the quality of the yogurts.
Key words: fermented milk, carotenoid, riboflavin, photooxidation

INTRODUCTION

Carotenoids are natural pigments known for their biological functions and dyeing properties. They are synthesized by higher plants, algae, bacteria, and certain fungi, and their presence in animals is attributed to the ingestion of foods. Lutein is the second-most-prevalent carotenoid in the human body (Khachik et al., 1997; Calvo, 2005), and accumulates mainly in the macula, in the central region of the human retina, which is responsible for visual acuity and where the greatest number of the photoreceptors is concentrated (Landrum et al., 1999). The richest sources of lutein include green vegetables such as spinach (40 μg/g) and kale (50 μg/g), and lutein also can be found in yellow vegetables such as corn (5 μg/g), and egg yolk (8 μg/g) (Rodriguez-Amaya et al., 2008; Perry et al., 2009).

It is believed that lutein exerts 2 main protective functions in the eyes: (1) as a filter of blue light and (2) as scavenger of reactive oxygen species (**ROS**). Blue light is the form of visible light with the greatest energy (wavelength of approximately 450 nm), and is known to induce photooxidative damage by generating ROS. Lutein shows a maximum absorption peak at a wavelength of 446 nm and, for this reason, is capable of absorbing blue light, decreasing the intensity of the light that reaches the retina, and probably reducing the formation of ROS (Krinsky, 1989; Kijlstra et al., 2012). The consumption of lutein has been associated with a decrease and prevention of the occurrence of cataracts and age-related macular degeneration, and hence research on the properties of this carotenoid has been increasing in recent decades (Landrum et al., 1997; Bhosale et al., 2009). Age-related macular degeneration is the main cause of irreversible blindness in the elderly and affects about 50 million people over 75 yr of age throughout the world, including more than 10 million in the United States and more than 190,000 in the United Kingdom (Evans et al., 2004; Klein et al., 2004). The ingestion of 6 mg of lutein per day has been related to a decrease of more than 43% in the risk of age-related macular degeneration (Seddon et al., 1994). Lutein can also be an important ally in reducing oxidative stress and damage to DNA, which may contribute to the development of cancerous cells in the organism (Serpeloni et al., 2010, 2012).

The application of lutein as a functional ingredient in dairy products is a convenient option, considering that, in general, the population is searching more and more for foods that provide health benefits. Nevertheless, it is important that the carotenoid does not degrade during storage, being available at the moment of consumption. Few studies concerning the addition of lutein to dairy products were found up to the present. Jones et al. (2005) evaluated the addition of different concentrations of lutein to Cheddar cheese, and showed that the carotenoid did not degrade during the 24 wk of cheese

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ripening. On the other hand, different concentrations of lutein added to yogurt showed an approximately 10% reduction after 5 wk of refrigerated storage (Aryana et al., 2006).

Despite its antioxidant properties, lutein is sensitive to the action of light due to the presence of conjugated double bonds, as these degrade mainly at wavelengths between 200 and 400 nm and at 463 nm, as observed in a model beverage with added carotenoid filled into transparent packages (Kline et al., 2011).

Lactic products are susceptible to photooxidation due to the presence of riboflavin (**RBF**; vitamin B_2), which is a sensitizing molecule. Riboflavin is capable of absorbing luminous energy and passing from its fundamental singlet state to an excited triplet state (^{3}RBF*), which can follow 2 reaction mechanisms: react directly with a biological molecule, producing free radicals and radical ions, or transfer its energy to oxygen molecules, forming singlet oxygen, which can also degrade biological molecules. These biological molecules can be the proteins, FA, or vitamins present in milk, and hence these reactions can lead to nutritional losses and sensory alterations, representing an indicator of the occurrence of photooxidation in dairy products (Borle et al., 2001).

Riboflavin is characterized by a ring structure with conjugated double bonds and nitrogen bases, with a maximum range of light absorption at 225, 175, 370, and 450 nm (Drössler et al., 2003), the latter being the most critical one with respect to the photooxidation of dairy products, as it matches with the visible light region emitted by the fluorescent light present in the majority of commercial establishments. The distribution of the ROS formed due to the sensitization of RBF depends on the availability of oxygen, RBF concentration, and the presence of other oxidant or antioxidant substances (Choe et al., 2005). Lutein can act as an antioxidant in photooxidation, principally through physical quenching (that is, transference of energy from the singlet oxygen or from the ^{3}RBF* to the carotenoid), resulting in the excited carotenoid plus the oxygen or sensitizer in their fundamental state. The carotenoid rapidly dissipates this energy and returns to its fundamental state, being capable of again scavenging other species (Di Mascio et al., 1989).

Riboflavin is highly fluorescent (with maximum emission around 525 nm) as are also the products generated by its photodegradation, lumichrome and lumiflavin, which show maximum emission in the region from 444 to 479 mm and from 516 to 522 nm, respectively (Fox and Thayer, 1998). Thus, one of the ways of evaluating photooxidation in dairy products is by detecting RBF and its degradation products using fluorescence spectroscopy (Miquel Becker et al., 2003; Andersen et al., 2005; Wold et al., 2006; Zandomeneghi et al., 2007).

The present study evaluated the influence of adding lutein dye on the oxidative stability of yogurt in the presence and absence of light, by monitoring the RBF and lutein contents. At the end of the experiments, a sensory acceptance test was carried out to verify the influence of different concentrations of lutein on consumer preference.

MATERIALS AND METHODS

Materials

Powdered skim milk (Molico; Nestlé, Araçatuba, Brazil) was acquired, all from the same batch and in an amount sufficient for the entire research project. The freeze-dried mixed lactic culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YO-MIX 505 LYO 200) was provided by Danisco Brasil Ltda. (Cotia, Brazil). The 0.3% lutein formulation used was the water-dispersible Vegex Lutein WS natural lutein dye for food purposes from Chr. Hansen A/S (Hørsholm, Denmark). According to the manufacturer, this formulation contains lutein extracted from marigold flower, water, modified starch, maltodextrin, sunflower oil, ascorbic acid, ascorbyl palmitate, α-tocopherol, and sodium benzoate. In addition, more than 90% of the lutein was found to be esterified with FA (Xavier et al., 2012). The water used in the experiments was initially bidistilled and then deionized. The yogurt was filled into opaque polypropylene cups provided by Dixie Toga SA (Votorantim, Brazil).

Yogurt Preparation

The yogurts were made from powdered skim milk, reconstituted to 10% TS, plus a mixed freeze-dried lactic culture. Lutein dye was added to half of the total volume before inoculating with the lactic culture. The lutein dye was added such that the final concentration in the product was equivalent to 1.5 mg of lutein/120 g of yogurt. This amount was added considering that the yogurt with lutein would be a complementary source of lutein in food. The culture was added to the milk and distributed in the polypropylene cups, which were sealed by heat induction. The milk was fermented in a Marconi model MA 415/S incubator (Marconi Equipamentos para Laboratórios Ltda., Piracicaba, Brazil) at 45°C and was interrupted by cooling the cups in an ice bath when the pH value reached 4.8. Yogurt with and without lutein were equally distributed in biochemical oxygen demand (BOD) incubators (Marconi model MA 415; Marconi Equipamentos para Laboratórios Ltda.),