Fat content affects bioaccessibility and efficiency of enzymatic hydrolysis of lutein esters added to milk and yogurt

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ABSTRACT
Addition of lutein to dairy products is an alternative that widens the range of foods which could be lutein source. However, bioaccessibility is an essential aspect to be considered during the development of products with added bioactive substances. We evaluated the *in vitro* bioaccessibility of lutein esters added to milk and yogurt with different fat contents, and determined the efficiency of enzymatic hydrolysis of the esters during digestion. Bioaccessibility of lutein and efficiency of hydrolysis were significantly lower in skimmed products than semi-skimmed and whole products, indicating thata minimal amount of fat is required to allow micellization and hydrolysis. The efficiency of ester hydrolysis ranged between 12-35%, which was attributed to pancreatic lipase. Whole and semi-skimmed samples were shown to be good vehicles for the addition of lutein, since presented bioaccessibility indices (38.3-47.5%) similar to those found in natural food sources of xanthophylls.

Keywords: milk; yogurt; enzymatic hydrolysis; pancreatic lipase; functional food; carotenoids.

1. Introduction

The carotenoids are a group of natural pigments synthesized by higher plants, algae and bacteria, whose coloration varies from yellow to red. Although widely spread in Nature, with more than 700 carotenoids already isolated, only about 40 are found in the foods included in the human diet (Holden et al., 1999), and of these, only lutein and zeaxanthin accumulate in the human *macula lutea*, the area containing the highest concentration of cone cells responsible for central and high resolution vision. It appears that these xanthophylls play an important protective role in maintaining ocular health, probably due to their action in filtering blue light and deactivating reactive oxygen species (Li, Ahmed & Bernstein, 2010). Elevated lutein (about 6 mg/day) and zeaxanthin levels in diet and plasma have been related to a 43% decrease in the risk of occurrence of age-related macular degeneration (AMD), the main cause of irreversible blindness in elderly (Landrum, Bone, Joa, Kilburn, Moore & Sprague, 1997). Evidence for a role of the macular pigments in primary prevention of AMD, retardation or arrest of AMD progression, and finally improved vision is available from observation and intervention studies (Loane, Nolan & Beatty, 2010; Wong, Koo & Chan, 2010). In addition, presence of oxidation products of these xanthophylls in retina reinforces the hypothesis of an antioxidant mechanism of action (Bhosale, Serban & Bernstein, 2009).

Lutein together with zeaxanthin is among the most abundant carotenoids in our diet and several common food sources of this xanthophyll are available. High amounts are found in dark green leafy vegetables such as spinach (40 µg/g) and kale (50 µg/g), and in yellow foods such as corn (5 µg/g) and egg yolk (8 µg/g) (Rodriguez-Amaya, Kimura, Godoy & Amaya-Farfán, 2008; Perry, Rasmussen & Johnson, 2009). Consumption of these and other lutein sources accomplish an estimated daily intake of 1-4 mg (Goldbohm et al., 1998; Manzi et al., 2002; Lucarini et al., 2006) depending on the country.

Milk and milk products are sources of calcium, proteins and vitamins A and E, being related to healthy food habits and presenting great acceptance by consumers. This fact, combined with the growing market of functional foods, brings dairy products to light as potential vehicles to addition of beneficial compounds (Hayes, Pronczuk & Perlman, 2001; Bhat & Bhat, 2011). The consumption of foods enriched with bioactive substances has been encouraged with the objective of obtaining the biochemical effects expected from these substances without the need to ingest supplements or change food habits of individuals (Granado-Lorencio et al., 2010). There are some studies in literature concerning viability of adding lutein to dairy products (Jones, Aryana & Losso, 2005; Aryana, Barnes, Emmick, Mcgrew & Moser, 2006; Kubo, Maus, Xavier, Mercadante & Viotto, 2012; Domingos, Xavier, Jorge, Mercadante, Petenate & Viotto, 2014). However, data regarding bioavailability of lutein from these products are still scarce, although this is an essential aspect to consider during the development of fortified or supplemented products (Fernández-García, Carvajal-Lérida & Pérez-Gálvez, 2009). Additionally, it should be taken into account that in commercially available sources of lutein dye, xanthophyll is present in its native esterified form, and esterification means that several properties are affected regarding the free form, including stability and bioavailability (Pérez-Gálvez & Mínguez-Mosquera, 2005).

After food ingestion, only a part of the components present in foods are efficiently digested and assimilated and then, once absorbed, perform a positive function in the body. The term bioavailability has several working definitions, depending on the research area it applies to. From the nutritional point of view, bioavailability refers to the fraction of the nutrient or bioactive compound ingested available for use in physiologic functions or to be stored (Fairweather-Tait, 1993). This concept includes bioaccessibility, which is the entire sequence of events that take place during the digestive transformation of food into material that can be assimilated by the body, the absorption/assimilation into the cells of intestinal epithelium, and lastly, the presystemic metabolism (Fernández-García, Carvajal-Lérida & Pérez-Gálvez, 2009). Carotenoid

bioaccessibility is influenced by several factors, including its physicochemical properties, food matrix composition and processing level, interactions with other dietary components, nutritional status, gut health and genotype of the host (Zaripheh & Erdman, 2002). Efficiency of carotenoid assimilation from foods can be determined by different analytical approaches, either *in vivo* or *in vitro*. Analysis of the carotenoid concentration in postprandial quilomicra after a single dose of a carotenoid-rich meal is an affordable practice but the ratio among labor intensive and throughput is low. Development of several *in vitro* approaches to accomplish assessment of bioaccessibility has increased in last years, and with these methodologies considerable insights have been achieved regarding the multifactorial scenario that affects bioaccessibility of nutrients. *In vitro* procedures simulate physiologic conditions and the sequence of events that occur during digestion in human gastrointestinal tract. Garret, Failla & Sarama (1999) developed the first procedure to estimate digestibility and assimilation of carotenoids from meals and several alternative experimental procedures have been described since then (Granado-Lorencio, Olmedilla-Alonso, Herrero-Barbudo, Blanco-Navarro, Pérez-Sacristán & Blázquez-García, 2007; Werner & Böhm, 2011; Stinco, Fernández-Vázquez, Escudero-Gilete, Heredia, Meléndez-Martínez & Vicario, 2012). *In vitro* methods are a simple and low cost option to estimate the bioaccessibility of carotenoids, and they have been validated by comparing model-derived bioaccessibility data with studies where bioaccessibility was measured in humans (Reboul, Richelle, Perrot, Desmoulins-Malezet, Pirisi & Borel, 2006). In fact, a significant positive correlation was found among *in vitro* and *in vivo* approaches.

The present study evaluated the *in vitro* bioaccessibility of lutein from dairy products, whole, semi-skimmed and skimmed milk and their corresponding yogurts, formulated with a water-soluble mixture of lutein esters. The lutein concentration was added to provide an estimated intake of 1.2 mg of lutein by yogurt (considering 1 yogurt portion = 120 g), which correspond to 20% of concentration that showed positive effects in ARMD (Landrum, Bone, Joa, Kilburn, Moore & Sprague, 1997). Lutein bioaccessibility was measured with an *in vitro* digestion procedure considering the efficiency of enzymatic process that during digestion hydrolyzes lutein esters, to establish whether free lutein or their esters are the predominant available form in micelles.

2. Materials and Methods

2.1. Samples

UHT fluid milk and powder milk samples (Corporación Peñasanta Alimentaria S.A., Granada, Spain) were acquired in a local supermarket, including whole (fat content: 3.60% in fluid milk; 3.25% in powder milk, w/v), semi-skimmed (fat content: 1.55% in fluid form, w/v) and skimmed milks (fat content: 0.25% in fluid milk; 0.10% in powder milk, w/v). Milk powders were reconstituted using filtered water according to manufacturer instructions (10% w/v for skimmed milk and 12.5% w/v for whole milk). A freeze-dried mixed lactic culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YO-MIX 505 LYO 200) provided by Danisco (Cotia, Brazil), was used to prepare the skimmed, semi-skimmed and whole yogurts from the corresponding fluid milks and reconstituted dried milk powders. Dairy products were formulated with 0.3% Vegex Lutein WS® water-soluble lutein formulation for food purposes (Christian Hansen, Horsholm, Denmark) adding the necessary amount to reach a final lutein concentration of 10 μg/g product. Solution of lutein formulation diluted in water with the same final lutein concentration (10 μg/g) was prepared for comparative purpose.

2.2. Reagents

Pepsin from porcine gastric mucosa, porcine bile extract, and pancreatin and lipase from porcine pancreas were obtained from Sigma (St. Louis, USA). Acetone and tetrahydrofuran (HPLC quality) were provided by Romyl (Teknokroma, Barcelona,

Spain), and the purified water was obtained from a Milli-Q water purification system (Millipore, Milford, USA). All other reagents were of analytical grade.

2.3. Elaboration of yogurt products

To prepare the yogurts, the lactic culture was added to milk (containing 10 μg of lutein/g milk) at a concentration of 2.5% (w/v). The milk with added lactic culture was distributed in plastic screw-top flasks, and placed in an incubator at 45 ºC for fermentation. Fermentation was stopped when the pH reached 4.8 (after approximately 3 h incubation) by placing flasks in an ice bath. Yogurts were fermented directly in flasks and consequently, retention of lutein when milk was converted to yogurt was considered 100%. Two yogurts were prepared from each type of milk, and a composite sample obtained by mixing these two yogurts was used for analyses. The yogurts were maintained in a cold chamber at 4 ºC until analysis, which was accomplished within 2 days.

2.4. In vitro *digestion procedure*

The experimental conditions described by Garret, Failla & Sarama (1999) and Fernández-García, Rincón & Pérez-Gálvez (2008) were used with slight modifications. Briefly, milk or yogurt samples (2 g) were mixed with 20 mL of 0.05% pepsin solution in 0.1 M HCl (pH 2.2) and incubated for 2 h under magnetic stirring in a water bath at 37 ºC (gastric phase). At the end of incubation period, samples were cooled in water, pH adjusted to 7.0 with a 5% NaOH solution, and mixed with 30 mL of a 0.3% bile extract in saline solution (3 M NaCl and 75 mM $CaCl_2$, pH 6.2), and incubated at 37 ºC with magnetic stirring for 30 minutes. The samples were then cooled again and mixed with 40 mL of a saline solution (0.1 M $NaHCO_3$, pH 8.4) containing 0.4% pancreatin and 0.07% lipase and incubated for a third time at 37 ºC with magnetic stirring for 2 hours. The micellar fraction was isolated from digested sample by centrifugation (12000×*g*, 5 min, 4 ºC) in an Avanti™ J-25 centrifuge (Beckman Coulter™, Brea, USA) equipped with a Beckman model JA-25.50 rotor (Kildare, Ireland). The supernatant (micellar fraction) was collected and used for measurement of lutein content. Bioaccessibility of lutein from the water-soluble lutein formulation was determined, diluting an appropriate amount of mixture in water (10 μg/g of solution). The *in vitro* digestion procedure was carried out in triplicate for all products.

2.5. Measurement of total lutein content in micellar fraction

Lutein from micelles was extracted with diethyl ether and 10% NaCl aqueous solution. The mixture was gently shaken and spun at 3000×*g* for 5 min to facilitate separation of water and organic layers. The latter was collected and remaining lutein in water phase was recovered applying the same procedure. Combined organic extracts were dried in a rotary-evaporator and residue was dissolved in absolute ethanol. Absorbance of final solution was measured at 445 nm in an HP-8452A diode array spectrophotometer (Hewlett Packard, Palo Alto, USA). Data were managed with UV-visible ChemStation version A.02.05 software (Hewlett Packard, Palo Alto, USA). Lutein content in micellar fraction was calculated applying Beer´s law with the extinction coefficient of lutein in ethanol, $E^{1\%}_{1\ cm}$ = 2550 (Davies, 1976). Once the absorbance was measured, extract was dried again and re-dissolved in 400 μL of tetrahydrofuran/acetone (1:1), and stored in vials at -40 ºC for subsequent chromatographic analysis.

2.6. HPLC analysis of lutein and lutein esters in micelles

Lutein from water-soluble formulation and from micellar fraction extracts (from digestion of dairy products and water-soluble formulation) were analyzed using a Jasco HPLC (Easton, USA) equipped with quaternary pump (model PU-2089-plus), autosampler (model AS-2055-plus) and diode array detector (MD-2010-plus). Chromatographic data were acquired and managed using the Jasco ChromPass

Chromatography Data System software (version 1.8.6.1). Lutein and lutein esters were separated on a Luna (Phenomenex, Torrance, USA) C_{18} column (250 x 4 mm, 5 µm particle size), using a linear gradient of acetone/water, from 75:25 (v/v) to 95:5 in 5 min, hold 95:5 for 7 min and to 100:0 in 3 min, maintaining this proportion for 10 min, going back to 75:25 in 5 min. Flow rate was set at 1.5 mL/min and 80 µL of sample were injected. The UV-visible absorption spectra were acquired between 200 and 600 nm and the chromatograms processed at 450 nm. The lutein, lutein monoesters and lutein diesters were identified according to elution order on C_{18} column and characteristics of UV-visible spectrum (λ_{max}, spectral fine structure (% III/II), and peak *cis* intensity (% A_B/A_{II})), as compared to standards and data available in the literature (Britton, 1995).

2.7. Calculations

In vitro bioaccessibility of lutein (%) was determined as the ratio of lutein content in micelles to lutein content added to sample. Efficiency of hydrolysis (%) was calculated from the HPLC data of free micellar lutein taking the total micellar lutein content (free lutein plus mono and diesters of lutein) as the reference.

2.8. Statistical analysis

The results of *in vitro* bioaccessibility of lutein were statistically analyzed using Statistica software (Statistica 5.5. StatSoft, Tulsa, OK, USA). Results are expressed as the mean ± standard deviation of three independent measurements, each one containing 3 replicates. Data were tested for normality by means of the Shapiro-Wilk test, and statistical comparison for significant differences for effects was performed by ANOVA, setting significance level at $p<0.05$. Means were compared using the Tukey´s test and significant at $p<0.05$.

3. Results and Discussion

3.1. In vitro bioaccessibility of lutein from dairy products

Among the factors influencing bioaccessibility of carotenoids are the structure of food and its fat content. The location and physical state of carotenoids in the food matrix and their interactions with other components of food determine their liberation from matrix, while presence of lipids facilitates dissolution of carotenoids in the fat droplets of gastric emulsion and also stimulates liberation of bile secretions and pancreatic lipase, favoring formation of micelles in the intestinal lumen (Hornero-Méndez & Mínguez-Mosquera, 2007). Figure 1 shows the results of *in vitro* bioaccessibility of lutein from skimmed, semi-skimmed and whole fluid milks and the values for their corresponding yogurts. Skimmed milk showed a significantly lower micellization level (19.7%) than the other milks, which, in turn, did not present significant differences among them (46.5% and 45.8% for whole and semi-skimmed milk, respectively). *In vivo* studies on interaction between presence of fat and carotenoid absorption show that a minimum quantity of fat would be required to assure the intestinal absorption of carotenoids and, once this amount is reached, further increments in the quantity of ingested fat does not suppose an increase on bioavailability (Roodenburg, Leenen, Van het Hof, Westsrate & Tijburg, 2000). In fact, *in vitro* bioaccessibility of lutein esters increased when the amount of fat increased in digesta by adding 100 µL of vegetable oil, but the addition of 300 µL neither increased nor decreased the efficiency of micellization (Fernández-García, Mínguez-Mosquera & Pérez-Gálvez, 2007). Our results support these conclusions. The amount of fat in semi-skimmed milk is enough to reach a significant bioaccessibility level, and once that level is reached an increase on the fat content does not caused an increase on micellization efficiency. Yogurts showed the same behavior as milks, since the whole and semi-skimmed yogurts present similar lutein bioaccessibility levels with no significant differences among them (47.5% and 38.3%, respectively), while the bioaccessibility of lutein from skimmed yogurt presented the lowest level (17.8%).

There are many studies in literature reporting *in vitro* bioaccessibility of xanthophylls in foods, and results vary widely. Values between 29% and 37% were found, respectively, for lutein micellization from a puree of cooked vegetables containing spinach, carrot and tomato (Garrett, Failla & Sarama, 2000) and from spinach (Reboul, Richelle, Perrot, Desmoulins-Malezet, Pirisi, & Borel, 2006). The bioaccessibility of lutein varied between 63% and 78% from cooked durum wheat pasta, whereas in cooked pasta containing eggs the *in vitro* bioaccessibility of lutein reached 58% (Werner & Böhm, 2011). For different pepper species, lutein micellization ranged from 36 to 106% (O'Sullivan, Jiwan, Daly, O'Brien & Aherne, 2010). Up to now, only one study evaluating the bioaccessibility of lutein dye added to a formulated product was found in the literature (Granado-Lorencio et al., 2010). In this study, lutein extracted from microalgae and dispersed in olive oil was added to Frankfurt type sausages and values between 29% and 35% for lutein micellization were found from the crude low fat sausages (2.5% of fat) and between 61% and 68% from the crude high fat sausages (15% of fat). The results found for bioaccessibility of lutein from semi-skimmed and whole milks and from whole yogurt were in the same range to those values already reported in the literature.

Figure 2 shows the results of *in vitro* bioaccessibility of lutein from the whole and skimmed milks obtained from reconstituted dried milk powders and the corresponding values for yogurts produced from those milks. Lower *in vitro* bioaccessibility values were observed in milk and yogurts produced from the dried material as compared to fluid milk, although significant differences were only denoted in whole milks. Moreover, the amount of lutein incorporated into mixed micelles was 33.3% lower from digestion of reconstituted whole milk ($p<0.05$, Tukey test) than the fluid whole milk. A similar decrease was revealed in case of skimmed milks, since reconstituted milk showed lutein bioaccessibility 34.6% lower than fluid milk, although this difference was not statistically significant. The same trend was observed for yogurt products with decreases of 19.4% and 6.3% for whole and skimmed products, respectively. Bettler, Zimmer, Neuringer & DeRusso (2010) measured the lutein concentration in sera of infants fed with human milk or formulas with added lutein. The amount of lutein in breastfed infants was significantly higher than that in infants who consumed formula, and to reach similar serum lutein concentrations among breastfed and formula fed infants, the content of lutein in fortified formula should be four times higher than the lutein concentration in breast milk. Most probably this fact occurred due to differences in lutein bioaccessibility from milk formula and breast milk, which corroborates our findings. Processing conditions applied to milk during different steps for obtaining dry milk powders (pre-heating, evaporation, homogenization, spray-drying) change chemical and physical interactions among fat, proteins, carbohydrates and water-based food components, yielding a different microstructure in the final product (Le et al., 2010). Regarding fat functionality, heating modifies the composition of milk fat globule membrane so that emulsifying properties are totally different among products that have been processed with diverse temperature profiles (Kanno, 1989), and differences in emulsifying properties may cause different bioaccessibility efficiency.

In vitro bioaccessibility of lutein from lutein formulation diluted in water was 59.0%, about 1.5 fold the value of lutein bioaccessibility from fluid whole milk and its corresponding yogurt, and two times the bioaccessibility from reconstituted whole milk and its respective yogurt. Unlike dairy products, higher bioaccessibility of lutein from aqueous solution of lutein formulation is not related to the sample fat content. Since formulation is an emulsion designed to be water-dispersible, emulsifiers contained in this formulation probably facilitated solubilization of lutein in the digesta and its incorporation into micelles. Moreover, water provided a simplest medium as compared to dairy products, without interferences of large molecules such as proteins and carbohydrates, thus allowing lutein to be more accessible to the bile salts and digestive enzymes.

3.2. Efficiency of hydrolysis

As only free carotenoid forms but not esters are found in human serum and peripheral tissues, bioaccessibility of esterified xantophylls depends on the efficiency of the enzymatic hydrolysis during digestion (Pérez-Gálvez & Mínguez-Mosquera, 2005). Figure 3 shows the HPLC profiles of the lutein formulation used in the study (Figure 3A) and of lutein in micelles obtained after the *in vitro* digestion applied to whole fluid milk (Figure 3B). The lutein formulation contains only lutein esters and after *in vitro* digestion of whole milk and yogurt samples, a decrease in the intensity of lutein ester peaks and the appearance of a new peak corresponding to free lutein was observed in the chromatograms. The same carotenoid profile was found after digestion of semi-skimmed and skimmed milks and yogurts (data not shown). Considering the total peak area, the percentage of the different forms of lutein (free, mono and diesters) in micelles of different digested dairy products and aqueous solution of lutein formulation is shown in Table 1. Micelles from lutein formulation presented the lowest content of free lutein (37.5%) and the highest content of lutein diesters (55.3%), whilst the dairy products showed 53.8-77.0% of free lutein and 13.3-44.3% of lutein diesters.

Regarding the total amount of lutein added to the dairy products (10 μg/g), the efficiency of hydrolysis was in the range of 12-35%, with the lowest values observed in the digestion of skimmed products (12.5% and 12.3% for milk and yogurt, respectively (Table 1). Although lutein hydrolysis from semi-skimmed and whole milks and yogurt was not statistically different, a tendency of the highest efficiency of hydrolysis was observed in the whole products. This fact could be explained considering that pancreatic lipase exerts its action in the lipid-water interface of micellar substrates (Breithaupt, Bamedi & Wirt, 2002), and a minimum amount of fat is needed to provide the hydrophobic medium where carotenoids are solubilized.

Different dairy products (milk and yogurt) with the same fat contents showed similar hydrolysis efficiency, indicating that only fat content but not food structure influenced the enzymatic hydrolysis. Moreover, solution of lutein formulation showed an intermediate rate of hydrolysis (22.2%), similar to all fluid milks and yogurts. Granado-Lorencio, Olmedilla-Alonso, Herrero-Barbudo, Blanco-Navarro, Pérez-Sacristán, & Blázquez-García (2007) found that *in vitro* hydrolysis level in digesta of orange products was 20% for orange segments, while 36% for orange juice, and the mean hydrolysis efficiency reached 32% for extracted loquat and 23% for homogenized canned. In that study the cholesterol esterase was used , considering that this enzyme was the most active towards hydrolytic activity among the different lipases tested (Breithaupt, Bamedi & Wirt, 2002). The levels of hydrolysis of xanthophyll esters from fruits (red pepper, squash and wolfberry) mediated by cholesterol esterase were similar to those described above (Chitchumroonchokchai & Failla, 2006). During the assessment of *in vitro* bioaccessibility of β-cryptoxanthin esters from citrus juices, porcine bile extract and pancreatin, without addition of cholesterol esterase, was used and hydrolysis efficiency values for de-esterification of β-cryptoxanthin from citrus juices was found in similar range of 11-44% (Dhuique-Mayer, Borel, Reboul, Caporiccio, Besancon & Amiot, 2007). The authors pointed that the pancreatic lipase was the enzyme responsible for hydrolysis of xanthophyll esters, since it accepts the same types of substrate as does cholesterol esterase (Lindstrom, Sternby & Borgström, 1988).

Therefore, either with the use of cholesterol esterase or with the use of pancreatic lipase, hydrolysis of xanthophylls esters is incomplete and according to the data in literature and to our results the efficiency lays within a similar range (12-35%) for different food sources (fresh fruits, juices and dairy products). Indeed, the use of cholesterol esterase increased the hydrolysis efficiency of xanthophylls esters from loquat and papaya oleoresins, while the hydrolysis efficiency was lower than 10% from paprika and marigold oleoresins, although for the latter these values were very similar to those achieved with pancreatic lipase (Breithaupt, Bamedi & Wirt, 2002).

Furthermore, it seems that other critical factor(s) should be involved on the digestion of xanthophylls esters that may explain differences and similarities described for enzymatic hydrolysis. One of the factors to be considered is the length of acyl chains and the number and place of double bonds that may produce differences in site of the enzymatic hydrolysis (Carriere, Withers-Martinez, Van Tilbeurgh, Roussel, Cambillau & Verger, 1998). The results obtained in the present study suggest that the de-esterification of lutein esters was carried out by pancreatic lipase, which was the main lipolytic enzyme in the *in vitro* digestion model employed. Cholesterol esterase is produced by the pancreas and by the mammary glands in higher mammals, and if present in milk or yogurt could take part in the hydrolysis of esters (Hui & Howles, 2002). Nevertheless, it is unlikely that considerable concentrations of this enzyme would be present in milk or yogurt used in this study, since the commercial sterilization processing (UHT) of milk would inactivate this enzyme (Nilsson, Bläckberg, Carlsson, Enerbäck, Hernell & Bjurssel, 1990).

4. Conclusions

In summary, whole and semi-skimmed milks and their corresponding yogurts were shown to be good vehicles for the addition of lutein, according to the conditions of *in vitro* digestion protocol. These matrices presented lutein bioaccessibility indices similar to those found in different food sources of xanthophylls. Moreover, there was a tendency to lower lutein bioaccessibility from reconstituted products (milk and yogurt) in comparison with fluid milks and their corresponding yogurts. In the current study, pancreatic lipase was capable to carry out enzymatic hydrolysis of lutein esters at different extension, depending on the fat content.

The information provided by this research could be useful for planning and development of new products with added lutein, assuring that in addition to providing color to the food and protection against its oxidation (Domingos, Xavier, Jorge, Mercadante, Petenate & Viotto, 2014), lutein is also available in reasonable concentrations for use by the human organism.

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