Physicochemical characterization and sensory evaluation of yogurts incorporated with beta-carotene-loaded solid lipid microparticles stabilized with hydrolyzed soy protein isolate

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Abstract Beta-carotene is an extremely interesting bioactive compound in the food industry due to its antioxidant capacity and pro-vitamin A activity. However, its incorporation into food products can be challenging, as it is highly hydrophobic and chemically unstable. The aim of this study was to evaluate the feasibility of incorporating beta-carotene-loaded solid lipid microparticles (SLMs) stabilized with a hydrolyzed soy protein isolate in yogurt. The SLMs were produced using palm stearin as the lipid phase. Microparticle dispersions containing only beta-carotene and both beta-carotene and alpha-tocopherol were incorporated into yogurts, comprising 5% of its total mass. This addition itself was efficient to provide color, and the presence of the lipid microparticles did not change the physicochemical or the rheological characteristics of the product. Based on the sensory evaluation, the panelists approved the yogurt, as average grades of global acceptance were around 8.0 (“liked it very much”) on the hedonic scale.

Keywords Microencapsulation · Carotenoid · Dairy product · Lipid particles · Yogurt

Introduction

Carotenoids are natural pigments of the isoprenoid family with several physiological benefits, such as antioxidant capacity and pro-vitamin A activity. Beta-carotene is especially interesting for the food industry as it is the most nutritionally active compound (Gonnet et al., 2010; Fraser and Bramley, 2004). In addition, there is much evidence in the literature demonstrating its action as an antioxidant, including its role in the prevention of cancer and cardiovascular diseases (Fraser and Bramley, 2004). This natural pigment is used to replace artificial dyes, to standardize the color of products, and to recover the color lost from foods during processing and storage (Priamo et al., 2010).

Although beta-carotene has several attractive benefits, its application in food has still several drawbacks because it is an extremely hydrophobic and a sensitive compound. Thus, it is completely insoluble in water, its isoprenoid chain results in high chemical instability, and its high lipophilicity decreases its bioavailability during digestion (Porter et al., 2007). One alternative to reduce these drawbacks, as well as to increase its bioavailability, is to encapsulate beta-carotene in lipid matrices such as lipid microparticles and nanoparticles (Brito-Oliveira et al., 2017; Hentschel et al., 2008; Mehrad et al., 2018).

Solid lipid microparticles (SLMs) are lipid carriers similar to oil-in-water emulsions, but their oily core is replaced by a solid lipid. These particles are commonly used to encapsulate and protect sensitive lipophilic compounds such as drugs and bioactive compounds with functional properties. The solid lipids normally show low toxicity and are fully biocompatible and biodegradable (Mehnert and Mäder, 2001). In addition, there are other advantages associated with the use of SLMs, such as the possibility of controlled release of bioactive compounds,

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the protection of sensitive substances, and their low cost (Müller et al., 2002). These lipid carriers can be produced using purified lipids, which can increase the chances of expelling the encapsulated compound during storage, and therefore, they can show a limited ability to incorporate bioactive compounds. The alternative to overcome the expulsion of the encapsulated compounds is to produce SLMs using a mixture of lipids. Particles produced with a mixture of lipids in their core have a disorganized structure and are less crystalline than particles produced with purified lipids, as well as they are capable of accommodating the encapsulated compounds, minimizing or even avoiding their expulsion (Pan et al., 2016; Tikekar and Nitin, 2011).

In the past few years, there has been an increasing interest in developing functional food products, and yogurts are among the most studied food in this category (Bertolino et al., 2015; Comunian et al., 2017; Karnopp et al., 2017; Toniazzi et al., 2014). Such interest is due to the fact that they are some of the most popular foodstuffs in the world, and their health benefits related to their nutritional profile and the presence of live microorganisms are widely known (Vital et al., 2015). Due to these benefits, consumers consider that yogurt can be a higher aggregate value food product, and, therefore, they can have a higher willingness to pay for it (Ares et al., 2010; Vecchio et al., 2016).

In this context, the objective of this study was to evaluate the feasibility of incorporating the beta-carotene-loaded SLMs into yogurts by carrying out their physicochemical characterization and sensory evaluation. The method (high shear mixing) used for the production of the beta-carotene-loaded SLMs would be relatively simple to scale up to an industrial scale. Therefore, the SLMs obtained here could be considered as an inexpensive ingredient for incorporation into food products, and the present study intends to make a contribution to make possible the effective use of this encapsulation technique in food products.

Materials and methods

Chemicals and reagents

Palm stearin was obtained from Agropalma (Belém, PA, Brazil), and soy protein isolate (SPI, Supro[®] 780, 81% protein) was obtained from The Solae Company (St. Louis, MO, USA). Beta-carotene (BC) and alpha-tocopherol (TOC) were obtained from Sigma (St. Louis, MO, USA). Xanthan gum (GRINDSTED[®] Xanthan 80) was donated by Danisco (Cotia, Brazil). All other chemicals were of reagent grade. Ultrapure water (Direct Q3, Millipore, Billerica, MA, USA) was used throughout the experiments.

The yogurt was produced using whole pasteurized milk (3% fat), sugar (Itaquerê, Nova Europa, SP, Brazil), freeze-dried lactic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Chr. Hansen, YC-X11, Horsholm, Denmark), dehydrated carrot (Lio-técnica, Embu das Artes, Brazil), honey, and orange flavors (Symrise, Cotia, SP, Brazil).

Production and determination of the average particle size of SLMs

The SLMs were produced as described by Brito-Oliveira et al. (2017). These microparticles were chosen due to their stability (in terms of average size and BC preservation) that is long enough to be considered for incorporation into yogurts. First, an alkali-thermal treatment was used to solubilize the SPI. It was hydrated with ultrapure water and stirred magnetically for 30 min. Then, the pH of the dispersion was adjusted to 12, and it was heated to 85 °C for 30 min, producing a dispersion of hydrolyzed soy protein isolate (HSPI).

SLMs were prepared by dispersing the aqueous phase (containing HSPI, 1% w/w) in the palm stearin (5% w/w) using ultra-agitation (Ultra-Turrax IKA T25, IKA, Staufen, Germany) at 15,000 rpm for 5 min. After the ultra-agitation step, the xanthan gum (0.15% w/w), used as a thickener, was added slowly, under magnetic stirring, while the temperature of the lipid microparticle dispersions was decreased to room temperature. The samples were produced in triplicate and stored at a controlled temperature of 10 °C. Sodium benzoate (0.02% m/m) was added to the samples to avoid microbiological contamination. The SLMs were produced in three formulations as follows:

- SLM-1: encapsulating BC (0.03% w/w), no alpha-tocopherol;
- SLM-2: encapsulating BC (0.03% w/w) and alpha-tocopherol (0.015% w/w);
- SLM-3: encapsulating BC (0.03% w/w) and alpha-tocopherol (0.03% w/w).

The average particle size was obtained using a laser diffraction analyzer (SALD-201 V, Shimadzu, Kyoto, Japan).

Production of the yogurts

The yogurts were produced using four formulations according to Table 1. The “white” yogurt was produced by fermentation of a mixture containing whole milk, refined sugar, and bacterial culture (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*, YC-X11 freeze-dried 50U, CHR. Hansen, Horsholm, Denmark) at 42 °C for 3 h. After production, the yogurts were stored in high-density

Table 1 Composition of the yogurts produced with different formulations (% in mass)

Ingredient	Control	Yogurt 1	Yogurt 2	Yogurt 3
“White” yogurt	10 L	10 L	10 L	10 L
Beta-carotene-loaded lipid microparticles without alpha-tocopherol	–	500 mL**	–	–
Beta-carotene-loaded lipid microparticles (2:1 BC:TOC)*	–	–	500 mL**	–
Beta-carotene-loaded lipid microparticles (1:1 BC:TOC)*	–	–	–	500 mL**
Honey flavor	–	0.05%	0.05%	0.05%
Orange flavor	–	0.015%	0.015%	0.015%
Dehydrated carrot	–	0.2%	0.2%	0.2%

*BC beta-carotene, TOC alpha-tocopherol

**Approximately 25 g of SLMs

polyethylene flasks sealed with plastic lids at 5 °C, in a BOD incubator, for 25 days (Fig. 1).

Physicochemical characterization of the yogurts

The yogurts were characterized in terms of total lipid content, protein content, moisture, pH, titratable acidity, density, instrumental colorimetry, and rheology. The changes in pH during storage were monitored using a glass electrode pH meter. The lipid content was determined by the Gerber method, with a 10 × dilution in distilled water. The protein content and moisture content were determined as described by the AOAC (2006) methods 920-87 with N × 6.38 and 925-09, respectively. Titratable acidity and density were determined as described by Adolfo Lutz (2008). All analyses were carried out in triplicate.

The color of the yogurts was measured using instrumental colorimetry (Miniscan XE, Hunterlab, Reston, VA, USA). The parameters of the color system ($L^*a^*b^*$) were obtained by reflectance, an illuminator D65 with the observer at 10°, and the total color difference (TCD), chroma (C^*), and the hue angle (h°) parameters were calculated. The yogurts were placed in a transparent, flat-faced cuvette, and the measurements were obtained after the colorimeter had been pressed against the cuvette surface. All measurements were repeated three times.

Table 2 Characteristics of the yogurts produced with and without beta-carotene-loaded solid lipid microparticles

Yogurt	Density (g/mL)	Total lipid (%)	Protein (%)	Moisture (%)
1	1.046 ^a ± 0.005	2.75 ^a ± 0.35	4.47 ^a ± 0.02	81.1 ^a ± 0.011
2	1.047 ^a ± 0.006	2.75 ^a ± 0.00	4.30 ^a ± 0.04	80.2 ^a ± 0.009
3	1.036 ^b ± 0.006	2.63 ^a ± 0.18	4.43 ^{a,b} ± 0.35	80.9 ^a ± 0.002
Control	1.029 ^c ± 0.009	2.38 ^b ± 0.18	4.52 ^b ± 0.16	80.2 ^a ± 0.005

Average ± SD of 3 yogurt samples. Same letters in a column indicate no significant difference according to Tukey's test ($p > 0.05$)

The viscosity of the yogurts was determined using a rheometer (AR2000 Advanced Rheometer, TA Instruments, New Castle, DE, USA) with concentric cylinders (internal radius = 14 mm, external radius = 15 mm, height = 42 mm, and gap = 5920 μm). All samples (≈ 12 mL) were analyzed under a controlled temperature of 10 ± 1 °C, which was controlled by a Peltier temperature system. The relaxation period of the samples before beginning the process was 2 min. The flow curves were obtained by registering shear stress when the shear rate was increased from 0.01 to 300 s⁻¹ and decreased from 300 to 0.01 s⁻¹. The experimental data were fitted to a Herschel–Bulkley model according to Eq. (1). The results were analyzed using the Rheology Advantage Data Analysis V.5.3.1 software (TA Instruments, New Castle, DE, USA).

$$\sigma - \sigma_0 = K\dot{\gamma}^n \quad (1)$$

where σ is the shear stress (Pa), K is the consistency index (Pa sⁿ), n is the flow index, and $\dot{\gamma}$ is the shear rate (s⁻¹).

Consumer acceptance of the yogurts

The sensory evaluation of the yogurts was performed using affective tests to evaluate consumer acceptance. The tests were evaluated using a structured, 9-point hedonic scale (1 = dislike extremely to 9 = like extremely), and the panelists evaluated the attributes of taste, texture, color,

Table 3 Physico-chemical and color parameters obtained for the yogurt with added beta-carotene-loaded lipid microparticles stabilized with hydrolyzed soy protein isolate

Parameter	Fresh yogurt			Day 15				
				Control	1	2	3	Control
	1	2	3					
pH	4.63 ^a ± 0.05	4.66 ^a ± 0.01	4.64 ^a ± 0.04	4.68 ^a ± 0.01	4.41 ^{b,c} ± 0.05	4.39 ^{c,d} ± 0.04	4.38 ^{c,d} ± 0.04	4.51 ^b ± 0.01
Titration acidity (as lactic acid)	0.96 ^{a,b} ± 0.03	0.94 ^b ± 0.02	1.00 ^{a,b,c,d} ± 0.02	0.98 ^{a,b,c} ± 0.02	1.02 ^{a,b,c,d} ± 0.01	1.01 ^{a,b,c,d} ± 0.04	1.04 ^{a,c,d} ± 0.03	1.05 ^{c,d} ± 0.01
L*	85.9 ^a ± 0.09	86.6 ^b ± 0.07	85.4 ^c ± 0.07	—	86.5 ^d ± 0.01	86.3 ^e ± 0.04	85.3 ^c ± 0.04	—
a*	4.80 ^{a,b} ± 0.23	5.20 ^a ± 0.16	6.83 ^c ± 0.14	—	4.34 ^{b,d} ± 0.01	5.37 ^a ± 0.06	6.87 ^c ± 0.04	—
b*	24.3 ^a ± 0.05	26.1 ^b ± 0.12	30.3 ^c ± 0.13	—	24.6 ^a ± 0.01	26.1 ^b ± 0.10	31.8 ^d ± 0.12	—
Hue angle	79.1 ^{a,b} ± 0.25	78.8 ^b ± 0.30	77.3 ^{c,d} ± 0.31	—	80.0 ^{a,f} ± 0.01	78.4 ^{b,c,e} ± 0.09	77.8 ^{c,d} ± 0.03	—
Chroma	24.8 ^a ± 0.02	26.6 ^b ± 0.21	31.1 ^c ± 0.13	—	25.0 ^a ± 0.01	26.6 ^b ± 0.11	32.5 ^d ± 0.13	—
TCD (total color difference)	—	—	—	—	—	—	—	—
Parameter	Day 25			Control				
				2	3			Control
	1							
pH	4.27 ^e ± 0.06			4.29 ^{d,e} ± 0.01		4.29 ^{d,e} ± 0.01		4.32 ^{c,d,e} ± 0.01
Titration acidity (as lactic acid)	1.06 ^{c,d} ± 0.02			1.05 ^{c,d} ± 0.03		1.04 ^{a,c,d} ± 0.02		1.07 ^d ± 0.02
L*	86.9 ^f ± 0.01			86.9 ^f ± 0.04		85.6 ^g ± 0.06		—
a*	4.12 ^d ± 0.13			5.05 ^a ± 0.01		7.06 ^c ± 0.02		—
b*	26.6 ^e ± 0.03			26.2 ^b ± 0.08		30.6 ^c ± 0.01		—
Hue angle	80.1 ^f ± 0.27			79.1 ^{a,b} ± 0.01		77.0 ^d ± 0.04		—
Chroma	23.9 ^e ± 0.10			26.6 ^b ± 0.08		31.4 ^c ± 0.01		—
TCD (total color difference)	1.82 ^a ± 0.90			0.27 ^b ± 0.03		0.45 ^b ± 0.05		—

Average ± SD of 3 yogurt samples. Same letters in a row indicate no significant difference according to Tukey's test ($p > 0.05$)

Fig. 1 Fluxogram of the production process of yogurts

aroma, and global quality of the three yogurt formulations produced with beta-carotene-loaded SLMs (yogurts 1, 2, and 3 shown in Table 1). A total of 60 panelists participated in the test, including both genders and representatives from different age groups. The yogurts were served in single plastic cups in volumes of 20 mL.

Statistical analyses

The experiments were performed in triplicate, and the average of the results and the standard deviations were obtained. The mean values of the treatments were compared using Tukey's tests with a 5% ($p < 0.05$) significance level on the SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

Results and discussion

Physicochemical characterization of yogurts

The SLMs showed high stability, with average diameters of about 1.20 μm , which remained highly stable after 45 days of storage under refrigeration. A complete characterization of these SLMs has already been published (Brito-Oliveira et al., 2017), and their characteristics led to the conclusion that they could be incorporated into foodstuffs, which has been already carried out with vanilla ice creams (Lima et al., 2016). It is important to note that the addition of pure BC in the yogurts was not possible, as the carotenoid was so hydrophobic that it did not mix with the product.

Regarding the produced yogurts, they were analyzed in terms of fat percentage, density, protein, and moisture, and the data obtained are shown in Table 2. The densities, as well as lipid, protein, and moisture contents, of the formulations containing the beta-carotene-loaded lipid microparticles and those of the control yogurt ("white" yogurt) were quite similar. Therefore, it can be concluded that the addition of SLMs did not significantly affect the proximate composition of the dairy product. Such fact is relevant as the physicochemical characteristics of the enriched product should be similar to those of the control.

Regarding the stability of the yogurts over the storage period (25 days), various parameters important to the product quality were evaluated and are shown in Table 3. The addition of the SLMs did not change the pH and the values of titratable acidity. Despite the fact that the dispersions were produced at pH 12, the buffering capacity of the yogurts was enough to avoid drastic changes in their pH values. In addition, the pH values remained above 4, indicating that the enrichment of the yogurt with SLMs did not contribute to the syneresis of the product, which is extremely relevant to maintain its quality and consumer acceptance (Robinson et al., 2006).

The colorimetric parameters of the yogurts produced with lipid microparticles as the only colorant were an indirect measurement of the preservation of the encapsulated BC in the product. It is important to emphasize that the encapsulated BC is the only colorant present in the yogurts. The values of the hue angle of the yogurts were in the range 75°–80°, or in the region of yellow as assessed by the consumers, which is required for a yogurt with orange, honey, and carrot flavor. It is also important to note the behavior of parameter a^* , which is directly proportional to the red color. In the case of our yogurts, the red color is only due to the presence of BC. Brito-Oliveira et al. (2017) found that BC decay was more intense for SLMs produced without alpha-tocopherol. The same occurred in the present study, as the value of parameter a^* decayed faster for yogurt 1 (from 4.80 to 4.12), incorporated with beta-carotene-loaded SLMs without alpha-tocopherol. In the case of yogurts 2 and 3, incorporated with beta-carotene-loaded SLMs with alpha-tocopherol, no significant changes in red color (parameter a^*) were detected.

Regarding the chroma value, according to Pathare et al. (2013), it is a quantitative measurement of colorfulness, and it indicates the color intensity perceived by consumers. In the case of yogurt 1 (incorporated with SLMs without alpha-tocopherol), the chroma values changed significantly on day 25 of storage, which could be another consequence of BC degradation. Compared to the chroma values obtained for yogurts 2 and 3, the latter were higher, probably due to the presence of higher amounts of preserved encapsulated BC in SLM-2 and SLM-3. In addition,

Fig. 2 Flow curves of the yogurts (control and incorporated with beta-carotene-loaded lipid microparticles), at different storage times: (A) fresh yogurts; (B) day 15 of storage; (C) day 25 of storage. Legend: (open diamond) yogurt 1, (open square) yogurt 2, (filled triangle) yogurt 3 and (open circle) control yogurt (curves are representative of 3 samples of yogurts)

the chroma values did not change along the storage period for yogurts 2 and 3, indicating that there would be no changes in color perception by consumers.

No other significant changes were detected in the other colorimetric parameters at the end of the storage period for any of the three yogurt formulations produced. The value of TCD was slightly higher for yogurt 1 (produced with lipid microparticles without alpha-tocopherol), indicating

that, as observed for the SLMs alone, the presence of the antioxidant was also important to preserve the color of the yogurt (Brito-Oliveira et al., 2017). However, such loss of color was not detected by the panelists in the sensorial evaluation. All these results are shown in Table 3.

Rheological characterization of yogurts

The flow curves shown in Fig. 2 indicated that all the produced yogurts exhibited pseudoplastic and thixotropic behavior (Purwandari et al., 2007). The Herschel–Bulkley model fitted well with the rheological data shown in Table 4, with correlation coefficients in the range of 0.895–0.972. The fitting to this model is important because it takes into consideration the yield stress, an important parameter in yogurt processing. All values of n (flow index) were less than 1.0, confirming the pseudoplastic behavior of the yogurts. The consistency indexes (k) were higher for the control (without SLMs), which was expected as the SLM dispersions comprised 5% of the total mass of the yogurt. As the SLMs were composed of 94% (w/w) water, the yogurts incorporated with encapsulated BC had approximately 5% more water than the control. The addition of this water mass was reflected in slightly lower values of k . However, the values of the apparent viscosity did not show any statistically significant differences among the samples or with storage time. The panelists did not seem to have realized this small difference in the rheological characteristics.

Thixotropy can be considered as a qualitative comparison among different yogurts; the energy required to partially destroy their structure is proportional to the area of the hysteresis (Steffe, 1996). The areas of the hysteresis loops, shown in Table 4 and Fig. 2, were smaller at the end of the storage period, indicating that the fresh yogurts tended to have higher structural reversibility (Purwandari et al., 2007); however, this decreasing tendency was the same for the control and the yogurts with lipid microparticles. Therefore, this destabilization of the structure was not caused by the addition of the SLMs.

Consumer acceptance of yogurts

The sensory evaluation data are shown in Fig. 3. The yogurts were graded by 60 panelists who evaluated the color, consistency, flavor, and global quality of the products. The results can be considered very satisfactory because most of the panelists indicated that the attributes corresponded to the expected values for yogurts with orange, honey, or carrot flavors. The addition of beta-carotene-loaded SLMs can be considered as successful because it provided the color expected by the panelists for this flavor of yogurt (honey + orange + carrot) and did

Table 4 Rheological parameters of the yogurts: τ_0 (yield stress), k (consistency index) and n (flow index), fitted with the Herschel-Bulkley model, and values of apparent viscosity and thixotropy

Yogurt	τ_0 (Pa)	k (Pa s ⁿ)	n	Apparent viscosity (Pa s)	Thixotropy (area of hysteresis loop)
Fresh					
1	1.450 ± 0.139 ^a	2.771 ± 0.168 ^b	0.542 ± 0.007 ^c	1.15 ± 0.02 ^a	4879 ± 348 ^{abc}
2	1.358 ± 0.026 ^a	3.336 ± 0.155 ^b	0.525 ± 0.007 ^{cd}	1.26 ± 0.04 ^a	5693 ± 105 ^{ab}
3	1.543 ± 0.260 ^a	3.232 ± 0.610 ^b	0.522 ± 0.014 ^{cd}	1.20 ± 0.04 ^a	5610 ± 798 ^{ab}
Control	1.418 ± 0.421 ^a	4.056 ± 0.513 ^b	0.518 ± 0.006 ^{cd}	1.30 ± 0.02 ^a	6893 ± 626 ^a
Day 15					
1	1.680 ± 0.543 ^a	2.194 ± 0.846 ^b	0.501 ± 0.002 ^{cd}	0.89 ± 0.32 ^a	3034 ± 1189 ^c
2	1.616 ± 0.419 ^a	3.679 ± 0.499 ^b	0.469 ± 0.024 ^{cd}	1.26 ± 0.09 ^a	4395 ± 73.5 ^{bc}
3	1.430 ± 0.338 ^a	4.000 ± 0.010 ^b	0.451 ± 0.007 ^{cd}	1.28 ± 0.04 ^a	4633 ± 374 ^{bc}
Control	0.823 ± 0.067 ^a	4.729 ± 0.255 ^b	0.448 ± 0.004 ^{cd}	1.33 ± 0.05 ^a	5670 ± 138 ^{ab}
Day 25					
1	1.255 ± 0.631 ^a	3.544 ± 1.636 ^b	0.469 ± 0.060 ^{cd}	1.18 ± 0.28 ^a	3787 ± 782 ^{bc}
2	1.379 ± 0.129 ^a	4.084 ± 0.413 ^b	0.446 ± 0.013 ^{cd}	1.32 ± 0.05 ^a	4233 ± 297 ^{bc}
3	1.926 ± 0.599 ^a	4.536 ± 1.150 ^b	0.433 ± 0.036 ^d	1.30 ± 0.01 ^a	4310 ± 38 ^{bc}
Control	1.619 ± 0.841 ^a	4.594 ± 0.028 ^b	0.446 ± 0.028 ^{cd}	1.40 ± 0.01 ^a	5131 ± 186 ^{abc}

Average values (of 3 yogurt samples) ± SD. Values followed by the same lowercase letter in the same column (on each day) were not significantly different ($p > 0.05$) by Tukey's test

Fig. 3 Average grades assigned by panelists in the affective sensory tests for the different formulations of yogurts: (grey square) yogurt 1; (open square) yogurt 2; (filled square) yogurt 3

not change the consistency of the product. The mean global acceptance grade of the yogurts was 7.4, indicating that a value of “liked very much” was given by the panelists. In addition, the incorporation of the SLMs did not lead to undesirable perception of the consistency of the product, which could have occurred as 5% of the mass of water was added (if compared with the yogurt without SLMs–“control”).

Therefore, it can be concluded that the results of physicochemical analyses were similar for all the yogurts obtained, indicating that the analyzed characteristics were not drastically affected by the incorporation of SLMs. Moreover, the colorimetric evaluations of the yogurts confirmed the importance of alpha-tocopherol coencapsulation in the SLMs to obtain a product with a longer stability. Sensory evaluation of the yogurts incorporated with BC SLMs also indicated that it is a technology with good potential to be used in nutritional fortification and/or replacement of artificial dyes in this type of dairy product.

Some suggestions for further studies include (1) incorporation of beta-carotene-loaded SLMs produced with other types of lipids; (2) evaluation of the average particle size in the physicochemical characteristics, shelf life, and sensory perception of the yogurts, and (3) evaluation of the feasibility of incorporating the beta-carotene-loaded lipid microparticles into yogurts produced with other flavors.

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References

- AOAC. Official Methods of Analysis of AOAC International, 18th ed. (2006).
- Ares G, Gimenez A, Deliza R. Influence of three non-sensory factors on consumer choice of functional yogurts over regular ones. *Food Qual. Pref.* 21:351–367 (2010).
- Bertolino M, Belviso, S, Dal Bello, B, Ghirardello D, Giordano M, Rolle L, Gerbi V, Zeppa G. Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt. *LWT – Food Sci. Tech.* 63:1145–1154 (2015).
- Brito-Oliveira TC, Molina CV, Netto FM, Pinho SC Encapsulation of beta-carotene in lipid microparticles stabilized with hydrolyzed soy protein isolate: production parameters, alpha-tocopherol coencapsulation and stability under stress conditions. *J. Food Sci.* 82:659–669 (2017).
- Comunian T, Chaves IE, Thomazini M, Moraes ICF, Ferro-Furtado R, Castro IA, Favaro-Trindade CS. Development of functional yogurt containing free and encapsulated echium oil, phytosterol and sinapic acid. *Food Chem.* 237: 948–956 (2017).
- Fraser PD, Bramley PM. The biosynthesis and nutritional uses of carotenoids. *Prog. Lip. Res.* 43: 228–265 (2004).
- Gonnet M, Lethuaut L, Boury F. New trends in encapsulation of liposoluble vitamins. *J. Contr. Rel.*, 146:276–290 (2010).
- Hentschel A, Gramdorf S, Muller RH, Kurz T. β -Carotene-loaded nanostructured lipid carriers. *J. Food Sci.* 73:1–6 (2008).
- Instituto Adolfo Lutz. Physicochemical methods for food analyses. São Paulo (2008) (in Portuguese)
- Karnopp AR, Oliveira KG, Andrade EF, Postinger BM, Granato D. Optimization of an organic yogurt based on sensorial, nutritional, and functional perspectives. *Food Chem.*, 233: 401–411 (2017).
- Lima JG, Brito-Oliveira TC, Pinho SC. Characterization and evaluation of sensory acceptability of ce creams incorporated with beta-carotene. *Food Sci. Tech.* 36:664–671 (2016).
- Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. *Adv. Drug Del. Rev.* 47: 165–196 (2001).
- Mehrad, B, Ravanfar R, Licker J, Regenstein JM, Abbaspourrad A. Enhancing the physicochemical stability of β -carotene solid lipid nanoparticle (SLNP) using whey protein isolate. *Food Res. Int.* 105:962–969 (2018).
- Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv. Drug Del. Rev.* 54:S131–S155 (2002).
- Pan Y, Tikekar RN, Nitin N Distribution of a model bioactive within solid lipid nanoparticles and nanostructured lipid carriers influences its loading efficiency and oxidative stability. *Int. J. Pharm.* 511:322–30 (2016).
- Pathare PB, Opara UL, Al-Said FA. Colour measurement and analysis in fresh and processed foods: a review. *Food Bioproc. Tech.* 6:36–60 (2013).
- Porter CJH, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev.: Drug Disc.* 6:231–248 (2007).
- Priamo WL, Cezaro AM, Ferreira SRS, Oliveira JV. Precipitation and encapsulation of β -carotene in PHBV using carbon dioxide as anti-solvent. *J. Sup. Fluids* 54:0103–109 (2010).
- Purwandari U, Shah NP, Vasiljevic T. Effects of exopolysaccharide-producing strains of *Streptococcus thermophiles* on technological and rheological properties of set-type yoghurt. *Int. Dairy Sci.* 17:1344–1352 (2007).
- Robinson RK, Lucey JA, Tamime AY. In: Tamime AY (Ed.), *Fermented milks*. Oxford: Blackwell Science (2006).
- Steffe JF. Rheological methods in food process engineering. Freeman Press, Michigan (1996).
- Tikekar RV, Nitin N. Effect of physical state (solid vs. liquid) of lipid core on the rate of transport of oxygen and free radicals in solid lipid nanoparticles and emulsion. *Soft Matter*, 7: 8149–8157 (2011).
- Toniazzo T, Berbel IF, Cho S, Favaro-Trindade CS, Moraes ICF, Pinho SC. β -carotene-loaded liposome dispersions stabilized with xanthan and guar gums: Physico-chemical stability and feasibility of application in yogurt. *LWT - Food Sci. Tech* 59:1265–1273 (2014).
- Vecchio R, Van Loo EJ, Annunziata A. Consumers’ willingness to pay for conventional, organic and functional yogurt: evidence from experimental auctions. *J Cons Stu* 40:368–378 (2016).
- Vital ACP, Goto PA, Hanai LN, Gomes-da-Costa SM, Abreu Filho BA, Nakamura CV, Matumoto-Pinto PT. Microbiological, functional and rheological properties of low fat yogurt supplemented with *Pleurotus ostreatus* aqueous extract. *LWT – Food Sci Tech* 64:1028–1035 (2015).