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ORIGINAL ARTICLE

Cow milk enriched with nanoencapsulated phenolic extract of jaboticaba (*Plinia peruviana*)

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Abstract This study evaluated the total phenolic content (TPC) and the antioxidant activity (AA) of cow's milk enriched with phenolic compounds extracted from jaboticaba peel, either by adding jaboticaba crude extract or a jaboticaba-loaded nanoemulsion. Three nanoemulsions with 5, 10 and 15% of jaboticaba extract were prepared. Average particle diameter (166.7–181.7 nm), polydispersity index (0.138–0.156) and zeta potential (ranging from − 35.30 to − 38.60 mV) were measured for the three different colloidal systems. The nanoemulsion with 15% of jaboticaba extract (J15-NE) was chosen for milk enrichment. J15-NE showed an encapsulation efficiency of 85.6% and remained stable for 60 days at 8 °C. Transmission electron microscopy of J15-NE displayed nanoparticles with a well-defined spherical shape. Reference milk, milk enriched with jaboticaba extract and milk enriched with J15-NE were characterised by a TPC of 93, 171 and 161 µg/ml GAE (gallic acid equivalent), respectively, and an AA of 0.04, 0.17 and 0.14 µg/ml TEAC (trolox equivalent antioxidant capacity), respectively. Thus, this study showed that nanoemulsion with jaboticaba peel extract could be exploited as an ingredient to enrich the properties of milk.

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Introduction

Interest is increasing in the incorporation of bioactive compounds in foods owing to their potential effect on human health and well-being (Hasler 1998; Pang et al. 2012). In particular, phenolic compounds (PCs) are considered of high value because of their antioxidant activity (AA), along with other beneficial health effects (Servili et al. 2009; Yilmaz 2006).

Phenolic compounds are secondary metabolites of the plant kingdom and are synthesized by plants during normal development and in response to physical injury, infection or other stress conditions (Beckman 2000). They constitute a group of about 8000 molecules, characterised by the presence of an aromatic ring with one or more hydroxyl substituents, and they range from simple phenolic molecules to highly polymerised compounds (Bravo 1998).

Phenolic compounds are widespread in the human diet (Manach et al. 2005). Main dietary sources of polyphenols are fruits, beverages (e.g., tea, coffee), and vegetables. The average daily intake of PCs is estimated to be around 1 g/day (Scalbert and Williamson 2000), but this value can vary considerably, depending on the dietary habits of different populations. For example, recent studies show an average consumption of 460, 820, 863 and 1193 mg/day for Brazilian, Spanish, Finnish and French populations, respectively (Corrêa et al. 2015; Ovaskainen et al. 2008; Pérez-Jiménez et al. 2011; Tresserra-Rimbau et al. 2013).

Epidemiological studies suggest that the long-term consumption of diets rich in these compounds can offer

protection against oxidative stress recognised as the main role in human aging and in the development of chronic and degenerative diseases, such as cancer, arthritis, and autoimmune disorders, as well as cardiovascular and neurodegenerative diseases (Pandey and Rizvi 2009; Ness and Powles 1997; Steinmetz and Potter 1996). This has driven efforts to design specific nutritional supplements and include these compounds to enrich foods with functional and health properties.

In food, polyphenols may contribute to bitterness, astringency, colour, flavour, odour and oxidative stability (Pandey and Rizvi 2009). The manufacture of food products enriched with phenolic compounds, either extracted or recovered from natural sources, such as fruits, vegetables and herbs, requires proper design and optimisation to comply with aspects related to both quality and sensory properties of the products, as well as bioavailability and health benefits.

Milk and dairy products are characterised by a high nutritional value based on the presence of essential macronutrients which, along with minor compounds (e.g. calcium, vitamins, immunoglobulins and peptides), contribute to their health-giving properties. Some studies have shown that milk contains a limited amount of phenolic compounds, most of them derived from feed (Hoikkala et al. 2007; King et al. 1998; Kuhnen et al. 2014; Mustonen et al. 2009), although a fraction may be the product of amino acid catabolism by bacteria and contamination with sanitising agents (O'Connell and Fox 2001). In addition, polyphenols of bovine origin can be formed by the action of gut bacterial flora on plant PCs, and secondary metabolites, such as equol, can be found in the milk of dairy cows (Mustonen et al. 2009). Thus, any variation of the phenolic profile of milk could mainly depend on feed formulation.

Some studies have been carried out to investigate the intrinsic antioxidant activity (AA) and phenolic content of milk derivatives (e.g., cheese) in the absence of food supplements (Han et al. 2011; Hilario et al. 2010). In this case, phenolic compounds are present in low concentrations and seem to have limited AA. Most recently, the potential of improving the content of bioactive compounds in milk and dairy products has been investigated, and to this aim, two main strategies have been differently applied: (1) enrichment of the diet with feed fortified with specific phenolic compounds or with ingredients rich in these compounds (Branciari et al. 2014; Santos et al. 2014) and (2) post-milking addition (Rashidinejad et al. 2014; Servili et al. 2011).

Tropical fruits have been attracting the interest of researchers for the diversity and quantity of compounds they contain (Denardin et al. 2015; Rufino et al. 2010). Among these, jaboticaba (*Plinia peruviana*), a Brazilian native fruit, has been recognised as a promising source of phenolic compounds since the fresh whole fruit has a total phenolic content of about 440 mg GAE/100 g, much higher than many other fruits (Rufino et al. 2010; Fu et al. 2011). Jaboticaba fruit contains a large variety of PCs, such as cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, ellagic acid, gallic acid, rutin, quercetin, quercitrin, isoquercitrin, quercimeritrin, myricitrin, kaempferol, and tannins (Bailão et al. 2015; Borges et al. 2014). In addition to their antioxidant power, these compounds give jaboticaba anti-inflammatory, antibacterial, antifungal, antiproliferative, antimutagenic, hypoglycemic and hypolipidemic bioactive properties (Borges et al. 2014; Leite-Legatti et al. 2012). During extraction of jaboticaba juice, pomace (ca. 40%), a waste containing mainly skins and seeds, is produced. This waste product, much richer in phenolics and anthocyanins than the whole fruit (ca. 2.5 and 3.5 times, respectively), could constitute a cheap source of such molecules for use in various sectors, including the food and pharmaceutical industries (Gurak et al. 2014).

Phenolic compounds are rather unstable molecules, but their inclusion in milk and milk derivatives, rich in protein and calcium, could contribute to their protection and at the same time enrich the nutritional and health value of the products. However, phenolic compounds and tannins could bind onto various sites of proteins and also crosslink separate molecules affecting both physical and sensory properties of the food system. Furthermore, some phenolic compounds are characterised by low water solubility and poor bioavailability (Rashidinejad et al. 2014). To enhance the gastrointestinal absorption of phenolic compounds, improve their bioavailability and prevent their degradation caused by environmental stress agents, different colloidal systems have been developed (Joung et al. 2016), and emulsion-based delivery systems have been investigated to overcome these problems (Huang et al. 2010; McClements and Decker 2000). Nanoemulsions are kinetically stable submicron-sized emulsions wherein droplets of oil or water are finely dispersed in the opposite phase with the aid of an adequate emulsifier that stabilizes the colloidal dispersion (Sutradhar and Amin 2013). Nanoemulsions can offer several advantages compared to conventional emulsions, such as improving physical stability, absorption penetration, and bioavailability (Gupta et al. 2016).

Therefore, this study aimed to evaluate the total phenolic content and the antioxidant activity of cow's milk enriched with phenolic compounds extracted from jaboticaba peel, either by adding jaboticaba crude extract, or by adding a jaboticaba-loaded nanoemulsion. Colourimetry was also performed to evaluate any differences between the reference milk and the two differently enriched milks.

Materials and methods

Materials

Jaboticaba fruits, collected in October 2015, were obtained from an orchard cultured in Guaxupé County (Minas Gerais, Brazil). The voucher specimen (FLOR 55902) was preserved at the FLOR herbarium (Department of Botany, Federal University of Santa Catarina, Florianopolis, Brazil). These fruits were produced by native jaboticaba trees located in the remaining Atlantic Forest (Mata Atlântica) territories and were not subjected to any kind of agrochemicals for the control of disease and pests. Peels were manually separated from pulp and seeds and stored at − 20 °C until use. All chemicals of analytical grade used in this study were purchased from Sigma-Aldrich (São Paulo, Brazil) and Vetec (São Paulo, Brazil). Miglyol 812 N was kindly donated by PIC Química (São Paulo, Brazil).

Methods

Extraction of phenolic compounds

Jaboticaba peels were first freeze dried in a freeze-drying system (FreeZone 6, Labconco, Missouri, US) at − 52 °C and at a pressure of 0.021 mbar for 24 h. After lyophilisation, dried peels were finely and uniformly milled (Cadence MDR301, Santa Catarina, Brazil). Phenolic compounds were extracted from powder using a high-pressure method (125 mPa, 3 h) in a 50% (v/v) ethanol solution acidified with hydrochloric acid at pH 3.6. This pH was chosen for extraction because most polyphenols are more stable against oxidation under acidic conditions (Janeiro and Oliveira-Brett 2004). Ethanol was removed by means of a rotary evaporator at 50 °C in dark conditions; then, the aqueous extract was filtered with paper with pore size = 14 µm (80 g/m^2, Unifil, Niederlenz, Switzerland).

Characterisation of jaboticaba extract

Determination of total phenolic compounds Total phenolic compounds (TPC) were determined according to the Folin–Ciocalteu method (Singleton and Rossi 1965). In this procedure, 1 ml diluted (1:10, v/v, in water) Folin reagent and 800 µl sodium carbonate aqueous solution (7.5%, w/v) were added to 200 µl of properly diluted extract. The mixture was kept protected from light at room temperature for 30 min before recording the absorbance at 760 nm (Kuhnen et al. 2014). An external standard curve for gallic acid (Sigma, 5–160 µg/ml, $r^2 = 0.999$) was constructed for the quantification. Results are expressed as gallic acid equivalents (GAE) in mg/ml and are the average of three replicates.

Determination of total anthocyanins Total anthocyanins (TA) were determined using the pH differential method (Wrolstad et al. 2005). An aliquot of jaboticaba extract was diluted in 0.025 M chloride buffer (pH 1) at a dilution factor ratio of 1:150, and another aliquot of the same extract was diluted in 0.4 M acetate buffer (pH 4.5) at the same ratio. The two solutions were separately mixed and incubated for 30 min at room temperature under dark conditions. Then, the absorbance of both solutions was measured at 510 nm and 700 nm. Cyanidin-3-glucoside (c3g), with a molar attenuation coefficient (ε) of 26900 and molar weight (MW) of 449.2, was used as the standard.

Total anthocyanins content in mg/l was calculated as

$$\mathrm{TA} = \frac{\mathrm{A} * \mathrm{MW} * \mathrm{D} * 1000}{\varepsilon} \quad (1)$$

where D is the dilution factor, and A is the absorbance calculated as

$$\mathrm{A} = (\mathrm{A}_{510\,\mathrm{nm}} - \mathrm{A}_{700\,\mathrm{nm}})_{\mathrm{pH}\,1} - (\mathrm{A}510\,\mathrm{nm} - \mathrm{A}700\,\mathrm{nm})_{\mathrm{pH}\,4.5} \quad (2)$$

Results are expressed as mg cyanidin-3-glucoside (c3g) for ml and are the average of three replicates.

Evaluation of in vitro antioxidant activity The evaluation of jaboticaba crude extract antioxidant activity (AA) was performed using the DPPH method (Sharma and Bhat 2009) based on the capture of the free radical DPPH (1,1-diphenyl-2-picrylidrazyl) by antioxidants. A 0.00316% DPPH solution (w/v) in 80% methanol was used. 2600 µl of DPPH solution and 400 µl of properly diluted extract were placed in a glass cuvette, gently shaken and incubated for 5 min at room temperature protected from light. The control assay was prepared according to the procedure described above, but adding 400 µl of 80% methanol instead of the extract. The decrease of absorbance was recorded at 530 nm, and the free radical scavenging activity was calculated as

$$\%\mathrm{Inhibition} = \left[\frac{(\mathrm{CA} - \mathrm{SA})}{\mathrm{CA}}\right] * 100 \quad (3)$$

where CA is the control assay absorbance, and SA is the sample absorbance.

Results are expressed in TEAC (Trolox Equivalent Antioxidant Capacity).

Preparation of jaboticaba nanoemulsions

An important part of this study involved the production of stable nanoemulsions containing as much encapsulated jaboticaba extract as possible so that milk could be enriched using smaller amounts of nanoemulsion. To accomplish this, different colloidal systems were prepared and added with 5, 10 and 15% of jaboticaba crude extract. Nanoemulsions were obtained by high-pressure (HP) homogenisation and were made of 1% polysorbate 80 (Tween 80, Vetec), 5% medium chain triglycerides (MCT, Miglyol 812 N) and 94% aqueous phase, according to the procedure previously described by Mazzarino et al. (2017). The oil phase (MCT) was previously mixed with Tween 80, the aqueous phase was added, and then the mixture was homogenised for 15 min by magnetic stirring. Next, the pre-emulsion was transferred to a high-pressure homogeniser (Homolab, FBF Italy, Parma, Italy) and homogenised at 500 bar for 3 cycles. First, a reference nanoemulsion, consisting of Tween 80, TCM and ultrapure water (ref-NE), was prepared. Then, by using the same procedure, three jaboticaba-loaded nanoemulsions were prepared and added to the aqueous phase with different concentrations of jaboticaba peel extract: 5% (J5-NE), 10% (J10-NE), and 15% (J15-NE) (w/v). Extract quantities higher than 15% were added and tested, but the present experimental conditions did not allow us to obtain homogeneous pre-emulsions owing to the formation of a precipitate. Therefore, we did not transfer such extracts to high-pressure homogenisation.

Characteristics of jaboticaba nanoemulsions

Particle size and zeta potential Mean particle size, polydispersity index (PDI) and zeta potential were determined by dynamic light scattering (DLS) and laser-doppler anemometry (LDA), respectively, using a Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK). The measurements were performed at 25 °C after appropriate dilution (1:30) in ultrapure water. The particle size analyses were performed at a fixed angle of 90°. For the measurements of zeta potential, samples were placed in electrophoretic cells, followed by applying a potential of ± 150 mV. Each datum reported is the average of at least three measurements.

As the goal of this study was to enrich milk with a stable colloidal system, without excessively diluting the product, the experiments of milk enrichment were carried out only with the most concentrated nanoemulsion (J15-NE).

pH Nanoemulsion pH was measured using a pH-meter SP1800 equipped with an electrode SC09 (Sensoglass, São Paulo, SP, Brazil).

Encapsulation efficiency The encapsulation efficiency (%) of nanoemulsions containing 15% of jaboticaba extract was determined using the ultrafiltration/centrifugation technique. Nanoemulsions were placed in Amicon Centrifugal Filter Devices with Ultracel-100 membrane (100 kDa, Millipore Corp., Billerica, MA) and centrifuged (2000*g*, 30 min) to separate the free compounds in the supernatant from the compounds loaded in the colloidal dispersion. Encapsulation efficiency was calculated as the difference between the total phenolic content of the suspensions of nanoparticles and the TPC present in the supernatant. TPC contents were determined spectrophotometrically using the Folin–Ciocalteu assay (Singleton and Rossi 1965).

Morphological evaluation The morphology of J15-NE was evaluated by transmission electron microscopy (TEM) using Jeol JEM-1011 equipment (Jeol, Tokyo, Japan). Droplets of nanoparticle suspensions were deposited on carbon-coated grids and stained with 5% uranyl acetate.

Nanoemulsion colloidal stability J15-NE stability was evaluated by monitoring particle size, polydispersity index, ζ-potential and pH for 60 days at 8 °C and at 20 °C. The samples were stored in amber glass bottles under dark conditions. Tests were performed at 7, 14, 21, 30, and 60 days after preparation.

Milk enrichment

Commercial fresh pasteurised milk was differently enriched, either by adding the jaboticaba crude extract (10.5% dry matter), or by adding the J15-NE. The jaboticaba extract was previously diluted with ultrapure water to a concentration of 15%. The diluted extract, or J15-NE, was added to milk in the same quantity (10%). Milk was then thoroughly mixed for 15 min with a magnetic stirrer at 4 °C and kept at this temperature until analysis. All analyses were carried out within a maximum of 6 h from the preparation of the enriched milks.

Milk characteristics

Colour measurement The colour analysis of the milk samples was performed using a Chroma Meter CR-400 (Konica Minolta, Tokyo, Japan) adjusted to operate with a D65 illuminator and 10° observation angle and previously

calibrated. The CIELab scale was used to determine the values of the parameters L*, a* and b*, where L* represents the luminosity ranging from black (0) to white (100), a* represents the variation of the color from red (+) to green (−), and b* represents the variation of the color from yellow (+) to blue (−). For each milk sample, the results were expressed as the mean of three measurements.

Total phenolic compounds and in vitro antioxidant activity Milk samples were first deproteinized according to the following procedure (Kuhnen et al. 2014). Briefly, a volume of 15 ml methanol was added to 5 ml of milk in a test tube with a screw cap. After shaking for 30 s, the mixture was incubated for 40 min at room temperature and centrifuged (15 min, 4000×*g*); then the supernatant was removed for analysis. Reference milk (no addition) and differently enriched milks were analysed for total phenolic compounds (TPC) and antioxidant activity (DPPH method), as described above, and the correlation between TPC and AA was determined.

Statistical analysis

All analyses were performed in triplicate, and the data are presented as mean ± standard deviation (SD). Significant differences were evaluated by analysis of variance (ANOVA) using SPSS Statistics, v 20, software (IBM, New York, USA).

Results and discussion

Characteristics of jaboticaba peel extract

The yield of the jaboticaba aqueous extract (%) was 10.54 ± 0.02. The values of pH, total phenolic compounds (TPC), total anthocyanins (TA) and antioxidant activity (AA) are shown in Table 1. Similar values were reported by other authors (Gurak et al. 2014; Leite-Legatti et al. 2012; Silva et al. 2010; Souza et al. 2017).

Jaboticaba whole fruit was shown to contain a greater amount of total phenolic compounds compared to different blueberry species (Dai et al. 2009; Reynertson 2007). Among the different classes of polyphenols, flavonoids and, in particular, anthocyanins represent the major compounds in jaboticaba fruit (Reynertson et al. 2006). A study carried out by Abe et al. (2012) showed that the major contribution to the total phenolic content of the whole fruit came from jaboticaba peel.

Table 1 pH, total phenolic compounds (TPC), total anthocyanins (TA) and antioxidant activity (AA) of the Jaboticaba aqueous extract

pH	3.33 ± 0.01
TPC (mg GAE/ml)	18.51 ± 0.52
TA (mg c3g/ml)	2.10 ± 0.02
AA (μg TEAC/ml)	36.75 ± 0.70

These results confirm the high total phenolic and anthocyanin content of the jaboticaba fruit and its high antioxidant power, in turn validating this fruit and, more particularly, its skin as an excellent source of these bioactive compounds. This also highlights the importance of their use in the pharmaceutical industry (Shahidi 2009) and, more recently, in food additives (Ayala-Zavala et al. 2010).

Characterisation of jaboticaba nanoemulsions

The nanoemulsions containing both different jaboticaba extract and reference were characterised for their colloidal properties, and in Table 2, the results of zeta average diameter, polydispersity index and zeta potential are reported. The addition of the jaboticaba extract affected the average diameter of the nanoparticles such that it decreased with increasing concentration of the extract. This result could be attributed to a surfactant effect of some bioactives and phenolics present in the extract that could locate at the oil/water interface during emulsification, thus affecting interfacial tension (Richards et al. 2002; Frankel et al. 1996). Some studies have highlighted this ability of plant phenolics, such as that found in olive and olive leaves, to play a major role in the stabilisation of food colloids owing to their amphiphilic nature (Di Mattia et al. 2009, 2011; Polychniatou and Tzia 2016).

With regard to PDI, no statistically significant differences between the different preparations were determined. A PDI value lower than 0.2 means that the particles in the nanoemulsion are monodisperse (Izquierdo et al. 2005). A lower, i.e., negative, zeta potential value was observed in the ref-NE and could be attributed to the stabilisation of droplets of triglycerides by non-ionic surfactant (Mazzarino et al. 2017). The addition of the fruit extract in the nanoemulsion caused a decrease of zeta potential (Table 2). This result could confirm the effect of minor amphyphilic compounds of fruit extracts on the colloidal properties of nanoemulsions. The increased negative charge of the jaboticaba-enriched nanoemulsions could also contribute to their physical stabilisation by increased charge repulsion (Patel and Agrawal 2011).

Nanoemulsions made with 15% jaboticaba showed a pH of 3.53, a TPC equal to ca. 2.8 mg GAE/ml , and an encapsulation efficiency of 85.6%. In previous studies focused on the preparation of nanoemulsions containing at most 10% of jaboticaba peel extract, Mazzarino et al.

Table 2 Average particle diameter (Z-mean diameter), polydispersity index (PDI) and zeta potential of the nanoemulsions, without jaboticaba extract (reference, ref-NE) and with different concentrations of extract: 5% (J5-NE), 10% (J10-NE), and 15% (J15-NE)

Sample	Z-mean diameter (nm)	PDI	Zeta potential (mV)
Ref-NE	186.6 ± 0.75^{a}	0.149 ± 0.022^{a}	-17.70 ± 0.40^{a}
J5-NE	181.7 ± 1.85^{b}	0.156 ± 0.08^{a}	-35.30 ± 1.48^{b}
J10-NE	175.6 ± 1.11^{c}	0.172 ± 0.08^{a}	-40.03 ± 2.06^{c}
J15-NE	166.7 ± 0.41^{d}	0.138 ± 0.027^{a}	-38.60 ± 0.26^{c}

[a,b,c,d]Values with different letters in the same column differ significantly ($p < 0.05$)

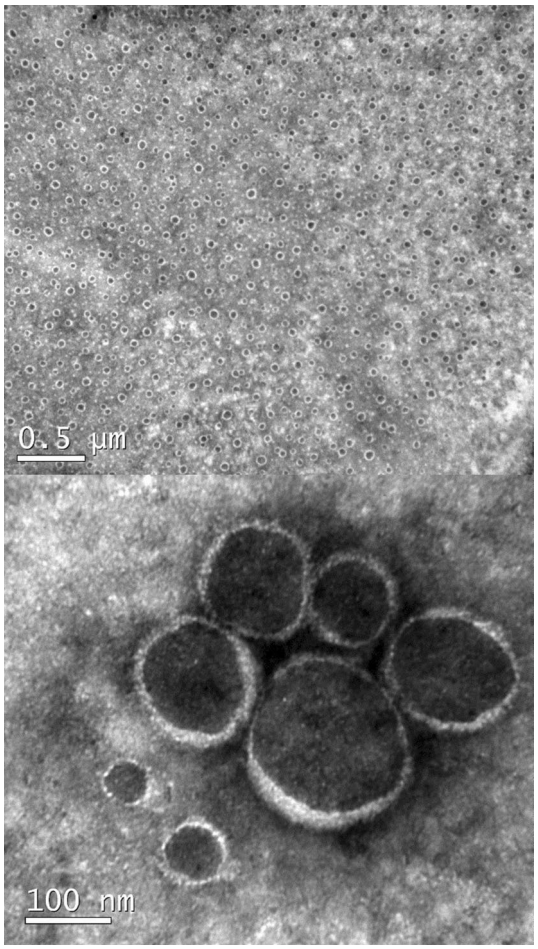

Fig. 1 TEM micrographs of J15-NE at two different magnifications

(2017) found that an encapsulation efficiency of more than 90% was achieved.

Despite the lower encapsulation efficiency, J15-NE was selected for milk enrichment as it contains a higher quantity of encapsulated phenolic compounds by volume. Figure 1 shows TEM images of J15-NE at two different magnifications. Micrographs highlight the spherical shape of the nanoparticles and their nanoscale size on the same order as that determined by scattering dynamic light. At higher magnification, it is also possible to clearly distinguish the structure of the nanoparticles, the surfactant interface of which surrounds the lipid phase containing the jaboticaba extract.

The physicochemical stability of the J15-NE system was evaluated over storage time at two different temperatures, and the change in colloidal properties is summarised in Table 3. After 60 days of storage at 8 °C, a slight, but significant, increase of nanoparticles size was observed, along with an insignificant increase of the polydispersity index that remained below 0.2. No visual phase separation was observed. Neither pH nor zeta potential changed over the selected storage time, and, overall, these results confirm the relative colloidal stability of J15-NE at 8 °C. On the contrary, at 20 °C, J15-NE showed a significant change in the physicochemical properties of the nanoemulsion after 21 days of storage, and for longer storage time, a loss of monodispersity occurred.

Milk characteristics

Reference milk (milk-R), milk enriched with jaboticaba diluted extract (milk-E) and milk enriched with J15-NE (milk-N) were characterised by a total phenolic content of 93, 171 and 161 µg GAE/ml, respectively, and an antioxidant activity of 0.04, 0.17 and 0.14 TEAC, respectively (Table 4). These results show that enrichment arising from extract and nanoemulsions increased both phenolic content and in vitro antioxidant activity of the reference milk. Several studies have been performed with the aim of evaluating the characteristics of milk and milk-based products enriched with phenolic compounds, or their derivatives (Branciari et al. 2014; Han et al. 2011; Rashidinejad et al. 2014; Santos et al. 2014; Servili et al. 2011). Phenolic compounds and phenolic derivatives found in milk can be modified, either by supplementing the diet of the lactating animal or by directly adding phenolic compounds to milk. Post-milking enrichment has been carried out in different ways, e.g., by adding single phenolic compounds, plant extracts, microencapsulated phenolic compounds or phenolic compounds incorporated in nanoliposomes. The use of encapsulated phenolic

Table 3 J15-NE physicochemical stability test at 8 °C and 20 °C

J15-NE	Z-mean d. (nm)		PdI		Zeta potential (mV)		pH	
	8 °C	20 °C	8 °C	20 °C	8 °C	20 °C	8 °C	20 °C
T0	167.5 ± 3.81[a]	166.7 ± 0.42[a]	0.173 ± 0.011[a]	0.168 ± 0.027[a]	− 38.4 ± 1.16[a]	− 38.6 ± 0.26[a]	3.5 ± 0.015	3.5 ± 0.006
T7	181.7 ± 1.50[b]	187.9 ± 5.69[c]	0.179 ± 0.007[a]	0.200 ± 0.003[a]	− 37.2 ± 1.08[a]	− 38.5 ± 0.25[a]	3.6 ± 0.010	3.5 ± 0.015
T14	183.1 ± 1.42[b]	226.1 ± 3.47[d]	0.180 ± 0.005[a]	0.248 ± 0.003[b]	− 38.3 ± 0.81[a]	− 38.5 ± 0.40[a]	3.6 ± 0.020	3.4 ± 0.015
T21	184.1 ± 1.63[b]	322.4 ± 1.78[e]	0.181 ± 0.003[a]	0.365 ± 0.040[c]	− 38.4 ± 0.21[a]	− 34.5 ± 1.00[b]	3.7 ± 0.010	3.4 ± 0.006
T30	184.5 ± 3.43[b]	716.6 ± 90.23[f]	0.182 ± 0.009[a]	0.744 ± 0.241[d]	− 38.3 ± 1.76[a]	− 42.4 ± 0.90[c]	3.7 ± 0.006	3.3 ± 0.015
T60	188.9 ± 0.14[c]	–	0.199 ± 0.011[a]	–	− 37.7 ± 0.25[a]	–	3.6 ± 0.010	–

[a,b,c]Values with different letters in the same column differ significantly ($p < 0.01$)

Table 4 TPC and AA of the milk samples in the presence and absence of Jaboticaba peel as nanoemulsion or crude extract

Sample	TPC (μg GAE/ml)	AA (μg TEAC/ml)
Reference milk	93 ± 2.42[a]	0.04 ± 0.00052[a]
Milk enriched with the crude extract	171 ± 1.90[b]	0.17 ± 0.00002[b]
Milk enriched with the J15-NE	161 ± 1.68[c]	0.14 ± 0.00482[c]

[a,b,c]Values with different letters in the same column differ significantly ($p < 0.01$)

compounds in the form of nanoemulsions or liposomes presents the merits of protecting the compounds from degradation and improving their bioavailability (Huang et al. 2010). In the present study, the small, but significant, differences in total phenolic content and antioxidant activity between milk-E and milk-N can be explained by the encapsulation of phenolic compounds inside the droplets of nanoemulsion, thereby avoiding contact with the reagent. This result was also observed by Mazzarino et al. (2017). The antioxidant activity of the milk increases proportionally with the content of phenolic compounds with a significant correlation (r = 0.997).

Table 5 shows the mean values of the main colorimetric parameters, as well as Yellow Index (YI) and a*/b* chromatic index of the reference and enriched milk samples. In general, both milk-E and milk-N presented lower L* (lightness), higher a* (redness), lower b* (yellowness), and lower YI (yellow index) when compared to the reference milk based on the addition of the jaboticaba fruit peel extract, which is characterised by an intense and dark purple colour. The mode of adding extract to milk affected the colourimetric and chromatic properties since milk-N, compared to Milk-E, had a stronger degree of lightness, lower redness, as well as a stronger degree of yellowness and YI. These differences result from the different colour of J15-NE compared to the crude extract, and, in particular, the chromatic changes of a solution undergoing emulsification. Colourimetric and visual properties of a colloidal system are affected by various factors, such as particle size, concentration, refractive index and spatial distribution of the droplets, as well as the presence of any coloured component (McClements 2002). Thus, the use of nanoemulsions containing jaboticaba extract, instead of crude extract, resulted in less change in the chromatic properties of the milk, while significantly improving the content of bioactives and their in vitro antioxidant activity. In Brazil, the addition of 10% of jaboticaba extract in milk could be sold as a milk-based product because it does not comply with the legislative requirements regarding colour. Therefore, a milk-based product enriched with phenolic compounds would comply with nutritional characteristics as set forth by Brazilian legislation. However, future studies are needed to optimise milk enrichment and evaluate additional quality and nutritional properties of milk

Table 5 Colour analysis of the milk samples

Sample	L*	a*	b*	YI	a*/b*
Reference milk	80.43 ± 0.323[a]	− 3.03 ± 0.023[a]	7.70 ± 0.072[a]	13.73 ± 0.048[a]	− 0.39 ± 0.001[a]
Milk enriched with crude extract	62.70 ± 0.156[b]	4.17 ± 0.061[b]	0.16 ± 0.032[b]	0.33 ± 0.012[b]	26.16 ± 4.980[b]
Milk enriched with J15-NE	66.69 ± 0.299[c]	2.23 ± 0.032[c]	1.76 ± 0.031[c]	3.75 ± 0.056[c]	1.26 ± 0.029[c]

[a,b,c]Values with different letters in the same column differ significantly ($p < 0.01$)

enriched with jaboticaba-loaded nanoemulsion, including digestibility and bioavailability of the added bioactive compounds.

Conclusion

This study confirmed the feasibility of obtaining nanoemulsions enriched with jaboticaba fruit peel extract at a relatively high extract concentration (15%) to be exploited as an ingredient to improve the antioxidant properties of milk. Milk enriched in this way resulted in more phenolic compounds and more antioxidant activity compared to reference milk, while, at the same time, limiting chromatic changes to milk with the addition of crude jaboticaba peel extract.

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