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Microencapsulation of purple Brazilian cherry juice in xanthan, tara gums and xanthan-tara hydrogel matrixes

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ABSTRACT

The purple Brazilian cherry (*Eugenia uniflora* L.) juice was encapsulated in xanthan, tara and xanthan-tara hydrogel matrixes. Encapsulation efficiency, Differential Scanning Calorimetry (DSC), X-ray diffractometry, release profile, stability of carotenoids, phenolic compounds and antioxidant activity of microparticles were evaluated. Encapsulation was confirmed. The highest encapsulation efficiency was obtained with xanthan gum and hydrogel was mostly indicated for the release of carotenoids in GFS and IFS medium. Phenolic compounds had the highest release rate but not in a gradually way, regardless of wall material and fluids under analysis. Stored microparticles at 4 and 25 °C, showed carotenoid degradation. Xanthan and hydrogel wall material provided the greatest stability to these compounds. The microparticles' anti-oxidant activity decreased during storage due to the degradation of carotenoids.

1. Introduction

The Brazilian cherry tree is native from Brazil, belongs to the Myrtaceae family and it is the main representative of the genus Eugenia. Its fruits, called 'pitanga' or 'Brazilian cherry' (*Eugenia uniflora* L.), are gummy berries, flat at the top and bottom, with approximately 7–10 longitudinal grooves, with an intense aroma and a sweet-acid taste, which have high rates of bioactive compounds such as phenolics and carotenoids (Lira Junior et al., 2007). Among the phenolic compounds present in purple Brazilian cherry juice, the gallic acid was the major compound, followed by the anthocyanins, catechin, epicatechin and quercetin. In relation to the carotenoids, the lycopene was found in higher quantities, followed by the β-carotene (Rutz, 2013).

The chemical structure of carotenoids, a group of pigments widely diffused in nature, consists of a variable number of conjugated double bonds provided with the ability to absorb light in different wave lengths, with colorings in the yellow to red bands, which cause pigmentation in a great number of fruits, leaves and flowers. Due to their capacity in receiving electrons of reactive species, they may neutralize free radicals and thus act as powerful natural antioxidants (Gonnet, Lethuaut, & Boury, 2010).

Phenolic compounds provide color, astringency, aroma and oxidative stability to many types of food. The group is formed by compounds featuring simple molecules up to those with a high degree of polymerization present in vegetables, either freely or linked to proteins and sugars (Angelo & Jorge, 2007). Their structure is provided with at least an aromatic ring bonded to one or more hydroxyl groups. Their antioxidant activity is related to redox capacity which makes the compounds act as reducing agents, hydrogen donors and suppressors of singlet oxygen, and they also may act as metal chelators (John & Shahidi, 2010).

However, carotenoids and phenolic compounds are unstable at high temperatures, in the presence of light and oxygen (Bagetti, 2009). Microencapsulation technique is an alternative method to increase the stability of these bioactive compounds in adverse environmental conditions, such as storage and processing, and to preserve their antioxidant activity. Microencapsulation is the packaging of solid, liquid or gaseous materials in extremely small capsules, prepared by different techniques. Besides protection for encapsulated material, microencapsulation provides the possibility of releasing it in a controlled way and under specific conditions (Favaro-Trindade, Pinho, & Rocha, 2008).

The materials used in the encapsulation must be food grade, with easy manipulation, low hygroscopicity, biodegradable and able to form a barrier between the internal and the external phase,

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in aim to protect the encapsulated material of the environmental conditions and maintain the stability during processing and storage (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011). Carbohydrates are the most used materials for encapsulation as a function of its ability to bind to a variety of compounds, in addition to its diversity and lower cost.

Xanthan gum is an extracellular polysaccharide produced by micro-organisms of the species *Xanthomonas*, that is commercially produced by *Xanthomonas campestris*. Structurally, this gum is a heteropolysaccharide, its primary structure is a chain composed by units of β-D-glucose linked by link 1-4, containing branching formed by β-D-mannose – 1,4-β-D-glucuronic acid – 1,2-α-D-mannose, may also contain pyruvic and acetic acid. It is considered an anionic polymer due to the presence of carboxylic groups in its structure (García-Ochoa, Santos, Casas, & Gómez, 2000). This gum has been employed as encapsulating material of enzymes (Liu, Nakagawa, Kato, Chaudhary, & Tade, 2011), micro-organisms (Jiménez-Pranteda et al., 2012), and phenolic compounds present in extracts of blackberry (Rosa, 2012).

Tara gum is a galactomannan extracted from the endosperm of the seed of *Caesalpinia spinosa*. It is a polysaccharide composed of a main chain formed by a units of β-D-mannose linked by glycosidic bonds of the type 1-4, with branching formed by units of β-D-galactose, which are linked at carbon 6 by bonds of the type α-1-6 (Fig. 11) (Pollard & Fischer, 2006). It is used in foods as a thickening agent, and like other galactomannans, is able to interact with various polysaccharides, such as xanthan gum, carrageenan and agar, thus becoming a gelling agent. These gums isolated are able to form solutions, but when mixed they form a thermoreversible and transparent gel (Lucyszyn, Quorin, Koehler, Reicher, & Sierakowski, 2006).

Formation of the hydrogel may strongly affect the properties of the polymer compared to single compounds such as solubility, rheology, conductivity, mechanical properties and permeability (Lee et al., 1999). Hydrogels formed by the mixture of xanthan and tara gums have not been used as encapsulants, however hydrogels xanthan gum and other galactomannans such as guar gum, have been used as hydrophilic matrix for controlled release theophylline (Vendruscolo, Andreazz, Ganter, Ferrero, & Bresolin, 2005) and as a matrix for formulations of ascorbic acid (Koop, Praes, Reicher, Petkowicz, & Silveira, 2009), which suggests that these types of hydrogels can be good encapsulating agents, because they have the characteristic of controlled-release.

The current assay aims at microencapsulating the purple Brazilian cherry juice by lyophilization as a drying method and using wall material, xanthan, tara gums and xanthan-tara hydrogel. Microparticles' morphology, encapsulation efficiency, thermal behavior, release and stability during storage was also investigated.

2. Materials and methods

2.1. Materials

Purple Brazilian cherry was obtained in Pelotas, RS, Brazil, during the 2011–2012 harvest. Xanthan (X) (Sigma–Aldrich) and Tara (T) (Gastronomy Lab) gums were the encapsulating agents and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich) was used to determine the antioxidant activity. The other PA degree reagents were employed to perform the spectrometry analyses.

2.2. Purple Brazilian cherry juice extraction

Purple Brazilian cherry juice (J) was extracted with a fruit centrifuge (Britânia BRCT 800), and conditioned in a polyethylene terephthalate bottle and frozen in an ultrafreezer at −80 °C until characterization analyses and encapsulation process.

2.3. Xanthan-tara hydrogel (H) preparation

Xanthan-tara hydrogel was obtained according to method by Vendruscolo et al. (2005) and Koop et al. (2009), with few modifications. Solutions with the two gums were prepared in a 5% concentration. Gums were slowly dissolved in water at a temperature ranging between 20 and 25 °C, with constant stirring, until complete dissolution. Solutions were mixed at proportion of 1:1 and stirred during 10 min. The hydrogel was then frozen at −80 °C and submitted to lyophilization (LIOBRAS L101).

2.4. Preparation of microparticles

The microencapsulation of purple Brazilian cherry juice with X, T and H as wall material was performed by lyophilization technique, following method described by Pralhad and Rajendrakumar (2004) and Laine, Kylli, Heinonen, and Jouppila (2008), with few modifications. Microparticles were prepared by first dissolving the wall materials and then adding juice at 1:1 proportion for juice solid rates. The mixture was stirred for 3 h and then frozen at −80 °C and lyophilized in LIOBRAS L101.

2.5. Preparation of physical mixtures (PM)

Physical mixtures were performed by homogenizing the lyophilized juice with the respective wall material (X, T and H) by mortar and pestle at 1:1 proportion.

2.6. Characterization of microparticles

2.6.1. Encapsulation efficiency (EE)

Since the juice has lipo- and hydro-soluble bioactive compounds, the encapsulation efficiency took into consideration these two compound classes. Carotenoid and phenolic compounds rates were evaluated to represent both classes.

Efficiency encapsulation of carotenoid followed method described by Sutter, Buera, and Elizalde (2007). Carotenoids on the microparticles' surface were quantified by adding 0.1 g of the sample and 5 mL hexane in a test tube, stirred by vortex stirrer for 10 s, centrifuged at 3420 × *g* for 10 min, after which the supernatant was separated. Total carotenoids within and outside the microparticles were quantified by the dispersion of microparticles in 5 mL hexane, stirred strongly for total removal of carotenoids and filtered with cotton for a 10 mL. The residue was washed in hexane. The two fractions were evaluated by spectrophotometer (JENWAY 6705 UV/Vis.) in 470 nm, with hexane as control. Total carotenoid content was determined by Eq. (1) and results given in μg Lycopene/g of sample.

$$\text{Carotenoid contents} = \frac{\text{Absorbance} \times 50\,\text{mL} \times 1,000,000}{3450 \times 100 \times \text{g of sample}} \quad (1)$$

Result of encapsulation efficiency was given in percentage of encapsulated carotenoids, calculated by Eq. (2).

$$\%EE = \frac{\text{total carotenoids} - \text{surface carotenoids}}{\text{total carotenoids}} \times 100 \quad (2)$$

Encapsulation efficiency of phenolic compounds was performed following method described by Deladino, Anbinder, Navarro, and Martino (2008) and Laine et al. (2008), with few modification. Quantification of compounds on the surface was obtained by weighing 0.1 g of microparticles, adding 5 mL methanol, stirred in a vortex stirrer for 10 s and centrifuged at 3420 × *g* for 10 min. The methanol fraction was then removed. Total compounds within and

outside microparticles were quantified by weighing 0.1 g of the microparticles, adding 5 mL water when gums were used (Laine et al., 2008) and 5 mL hydrochloric acid 0.1 M for chitosan (Kosaraju, D'ath, & Lawrence, 2006) to break up the particles. The two collected fractions were evaluated for total phenolic compound rates following the Folin–Ciocalteau method (Swain & Hillis, 1959). The method consisted of adding 4 mL of distilled water, 250 μL of extract and 250 μL of Folin–Ciocalteau 0.25 M solution in a Falcon tube, stirred and left to react for 3 min. Further, 500 μL of sodium carbonate 1 M were added, left to react for 2 h and read in spectrophotometer (JENWAY 6705 UV/Vis.) at 725 nm. Quantification of phenolic compounds was done by standard curve for gallic acid and results were given in mg gallic acid/100 g of sample.

Result for encapsulation efficiency was given in percentage of encapsulated phenolic compounds by Eq. (3).

$$\%EE = \frac{\text{total phenolic compounds} - \text{surface phenolic compounds}}{\text{total phenolic compounds}} \times 100 \quad (3)$$

2.6.2. Antioxidant activity

Antioxidant activity was determined by the sample compounds' capacity to quench the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl). Therefore, 0.1 g of the sample was dissolved in 10 mL water; 300 μL of the extract were collected and added to 3.7 mL of DPPH solution. Spectrophotometer (JENWAY 6705 UV/Vis.) readings at 517 nm wave-length were provided after 30 min. Antioxidant activity was given in percentage of radical DPPH inhibition per 1 mg of sample, by Eq. (4).

$$\%\ \text{Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \quad (4)$$

2.6.3. Differential Scanning Calorimetry (DSC)

DSC analysis of J, X, T and H, physical mixtures of PMJX, PMJT and PMJH and of the microparticles of JX, JT and JH, were performed by DSC Q20 TA Instruments. Further, 10 mg of each sample were warmed in aluminum containers at a rate of 10 °C min^{-1}, between 25 and 280 °C, with a 40 mL min^{-1} nitrogen flow.

2.6.4. X-ray diffractometry

Microstructural profile of J, X, T and H, physical mixtures of PMJX, PMJT and PMJH and microparticles of JX, JT and JH, were characterized by X-ray diffractometry (X'pert PRO Multi-Purpose, PanAnalytical) in which X-ray source was radiation Cu Kα (λ = 1.5418 Å), with 45 kV and 40 mA, measured at angle 2θ, ranging between 10° and 100°.

2.7. Releasing profile of the encapsulated compounds

The released profile of the encapsulated compounds were evaluated by *in vitro* assay simulating gastric and intestinal fluids (Chiu et al., 2007; Paramera, Konteles, & Karathanos, 2011; Zheng, Ding, Zhang, & Sun, 2011) and in distilled water (Belščak-Cvitanović et al., 2011). Solutions of citric acid 0.1 M and di-sodium phosphate were mixed in adequate proportions for solutions with final pH at 2.00 and 8.00 (Chiu et al., 2007). Solution at pH 2.00 (gastric fluid simulation – GFS) comprised 0.3% pepsin enzyme, whereas solution at pH 8.00 (intestinal fluid simulation – IFS) comprised 0.1% pancreatic enzyme (Paramera et al., 2011).

Microparticles weighing 0.1 g were added to 20 mL of the solutions and incubated at 37 °C while stirring; 1.5 mL aliquots were retrieved at 0, 20, 40, 60, 120, 180 and 240 min and then centrifuged at 3420 g for 15 min. Supernatant was used for assays in the microparticles for total phenolic compounds and carotenoids content (Zheng et al., 2011). Sample amount retrieved for analyses was replaced by the same quantity of the respective solutions and distilled water (Belščak-Cvitanović et al., 2011).

Table 1
Encapsulation efficiency of microparticles.

Microparticles	Encapsulation efficiency (%)	
	Phenolic compounds	Carotenoids
JX	76.37 ± 0.38 aB	91.52 ± 1.03 aA
JH	70.27 ± 0.64 bB	74.06 ± 0.17 bA
JT	70.22 ± 1.58 bB	92.67 ± 0.64 aA

Means followed by the same small letter in the column did not differ at 5% significance. Means followed by the same capital letters on the line did not differ at 5% significance.

2.8. Analysis of stability

2.8.1. Analysis of light stability

Some microparticles, conditioned in glass vials, were stored in the dark and others exposed to a 100 W lamp as an artificial light source, placed perpendicularly and suspended 50 cm above the samples, following procedure by Matioli and Rodriguez-Amaya (2002). Light stability test was performed every 7 days for 28 days, at a temperature ranging between 20 and 25 °C. Total rates of phenolic compounds, total carotenoids and antioxidant activity were evaluated.

2.8.2. Analysis of temperature stability

Analysis of temperature stability was performed with microparticles conditioned in glass vials in a fridge at 4 °C and in a room conditioned at 25 °C (Sansone, Mencherini, et al., 2011). Stability test was performed during 12 weeks with evaluations at 0, 2, 4, 8, 12 weeks during which total rates of phenolic compounds, total carotenoids and antioxidant activity were evaluated.

2.9. Statistical analysis

Results were given in means of assays done in triplicates and submitted to analysis of variance.

Tukey's test ($p < 0.05$) was used to evaluate the encapsulation efficiency, the stability of encapsulated compounds stored in the light and in the dark, and the stability of encapsulated compounds stored at 4 °C and 25 °C, for the comparison of wall materials; and the profile of the release of encapsulated compounds to compare wall materials and fluids.

Test t ($p < 0.05$) was used to evaluate encapsulation efficiency by comparing groups of encapsulated compounds; the stability of encapsulated compounds by comparing storage with and without light; and the stability of encapsulated compounds by comparing storage at 4 °C and 25 °C.

3. Results and discussion

3.1. Encapsulation efficiency (EE)

Encapsulation efficiency of the purple Brazilian cherry juice was affected by wall materials and by the group of encapsulated compounds, with the highest rates for carotenoid encapsulation (Table 1). Xanthan and tara gums as wall materials showed similar encapsulation efficiency and significantly higher than that of hydrogel formed by the association of the two gums.

Xanthan gum in wall materials in the encapsulation of phenolic compounds was significantly more efficient than tara gum and hydrogel formed by both gums, which did not differ significantly between them at 5% significance.

It is suggested that during the encapsulation process may have occurred hydrogen bonding and dipole–dipole interactions between encapsulating materials and phenolic compounds, mainly due to the presence of free hydroxyl groups in the major phenolic

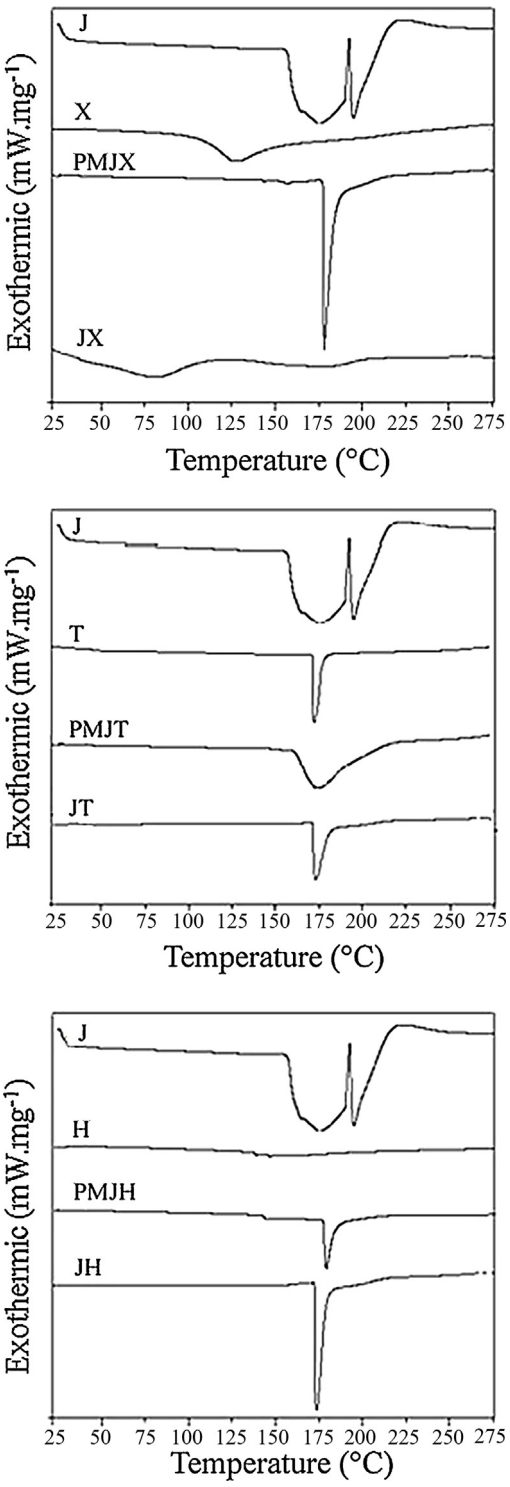

Fig. 1. DSC thermograms of the purple Brazilian cherry juice, polymers used in encapsulation, physical mixtures and microparticles.

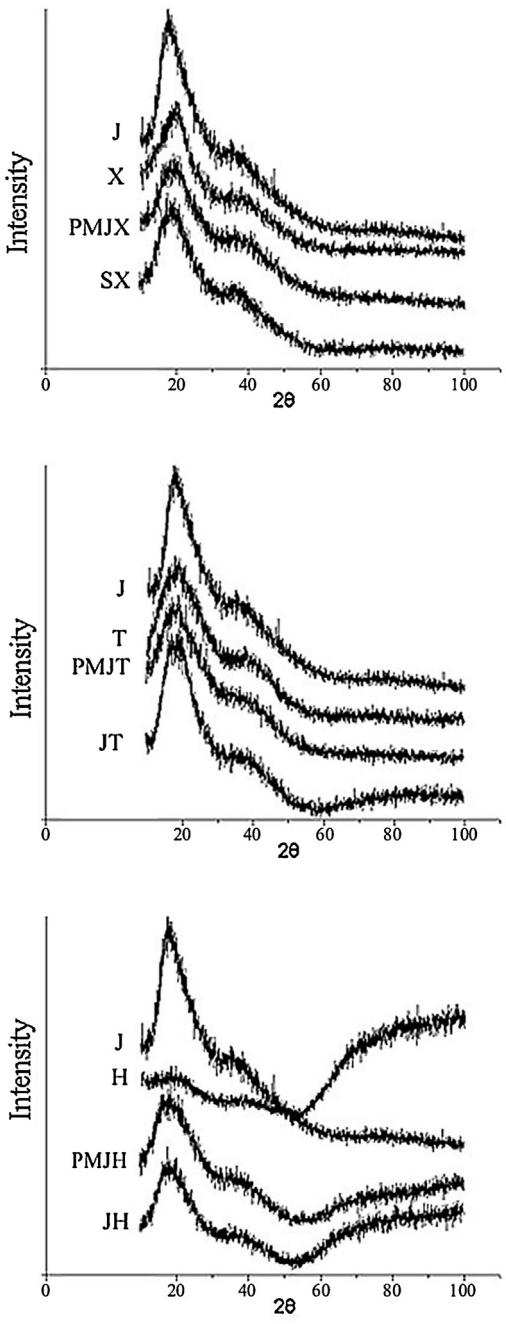

Fig. 2. X-ray diffractograms of the purple Brazilian cherry juice, polymers used in encapsulation, physical mixtures and microparticles.

compounds present in the juice and also in polymers xanthan gum, tara and hydrogel. In relation to the carotenoid is more likely to have occurred induced dipole–dipole interactions between the methyl groups of the major carotenoids of the juice with the methyl groups of the polymers. Moreover, because of xanthan gum to be considered a compound anionic, may also have occurred ion-permanent dipole interactions with the phenolic compounds and ion-induced dipole with carotenoids.

In encapsulation efficiency depended on wall material and on the encapsulation process. The encapsulation efficiency of carotenoids-rich extracts encapsuled with differents wall material ranged between 85% and 97% (Kha, Nguyen, & Roach, 2010; Laos, Lõugas, Mändmets, & Vokk, 2007). In encapsulation of phenolics compounds, Rosa et al. (2013) and Merlin, Prasad, Shibli, and Sebeela (2012) using xanthan gum and poly-D,L-lactide-co-glycolide as wall material, respectively, had results very similar to the encapsulation efficiency for purple Brazilian cherry juice.

3.2. Thermal behavior

Fig. 1 shows DSC thermograms of the purple Brazilian cherry juice, of the polymers used in encapsulation, and of the physical mixtures and microparticles.

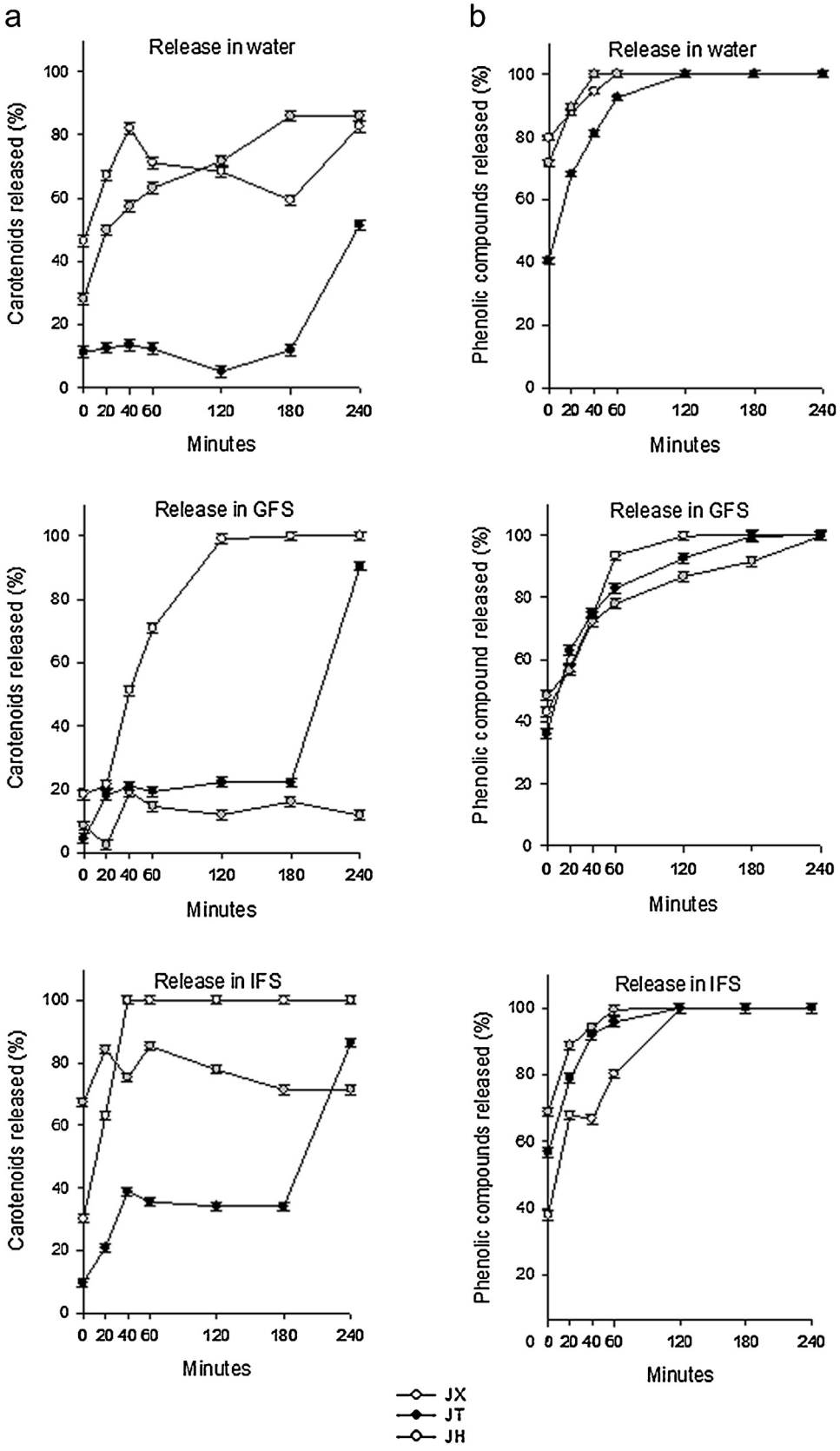

Fig. 3. Release profiles of carotenoids (a) and phenolic compounds (b) from microparticles in water and in fluids that simulated gastric and intestines conditions.

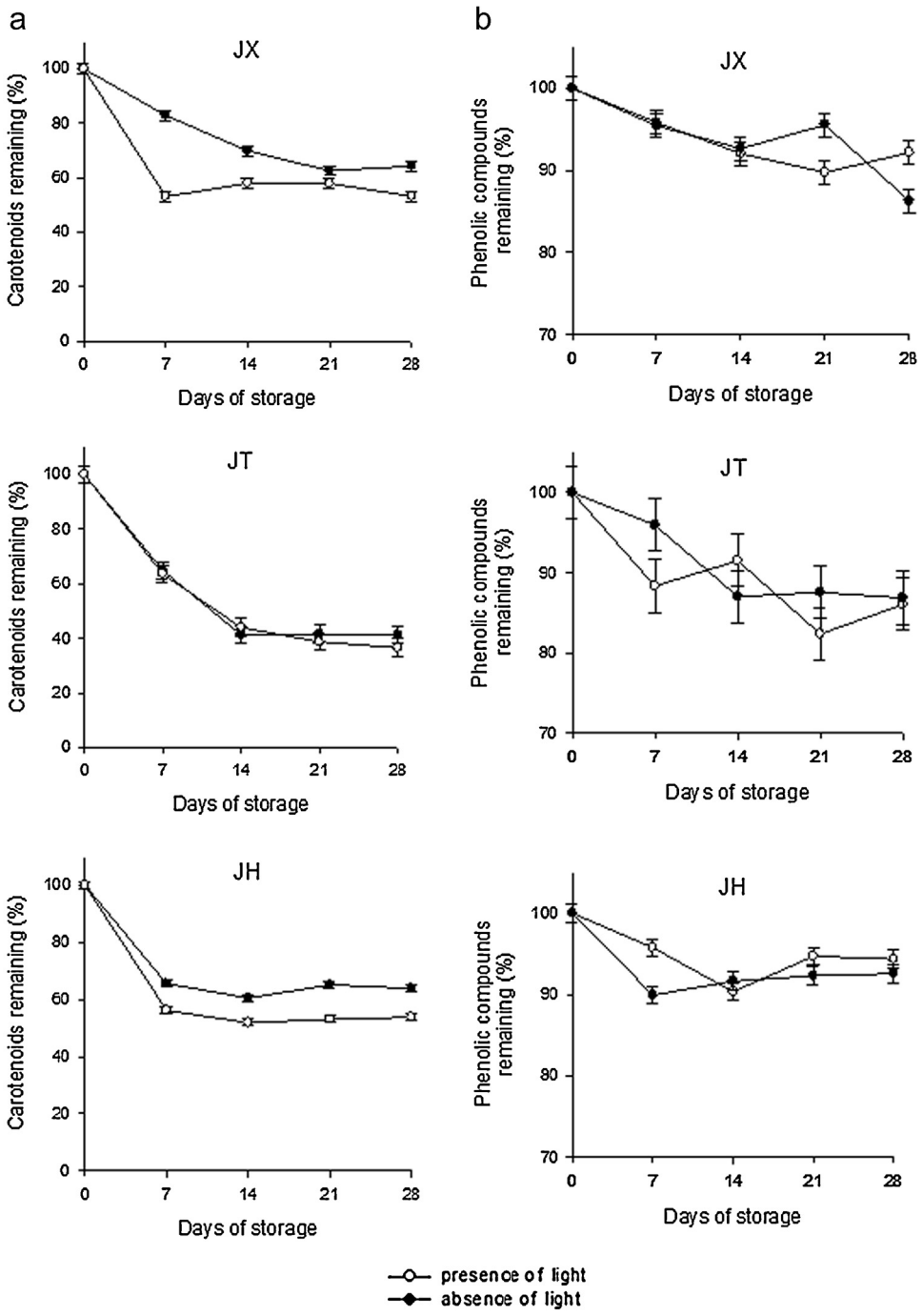

Fig. 4. Microparticles stored in presence and absence of light for 28 days. Stability of carotenoids (a) and phenolic compounds (b).

DSC thermogram of lyophilized juice revealed two endothermal events at 175.03 °C and 195 °C, with regard to the juice's different contents. Endothermal peaks at 129.01 °C and 175.44 °C were obtained in polymer endothermal transitions for xanthan and tara gums, respectively. No endothermal events occurred for hydrogel composed of xanthan and tara gums.

The characteristic curve of a physical mixture is generally composed by peaks related to the polymer used as encapsulating material and to the encapsulated compound. However, these events were not detected in the thermograms referring to physical mixtures of juice and polymers. According to Wu et al. (2008), the above behavior is due to the interaction of the compounds during heating.

Since thermogram of xanthan microparticles shows the disappearance of endothermal events related to juice, the wall material protection and interaction with the nucleus have been suggested. Similar results were achieved by Kalogeropoulos, Yannakopoulou, Gioxari, Chiou, and Makris (2010). The above result corroborates with those obtained for encapsulation efficiency in which xanthan gum is considered the best encapsulating material for the two compound classes.

The thermogram of tara gum microparticles showed only the polymer's thermal profile, what also suggested the encapsulation of the juice. Similar result was reported by Sansone, Mencherini, et al. (2011).

A low intensity peak related to non-encapsulated juice, which is shifted, also suggesting encapsulation, may be seen in the thermal curves of the xanthan-tara hydrogel microparticles. The lowest efficiency rate, especially with regard to carotenoids, was

obtained when related to efficiency results by using this wall material.

3.3. Microstructural profile

Fig. 2 shows X-ray diffractograms of the purple Brazilian cherry juice, of the polymers used in encapsulation, and of the physical mixtures and microparticles.

A diffused halo, which is characteristic of amorphous compounds, may be seen in all diffractograms. However, peak intensities in the microparticles were lower to the intensity of the juice.

According to Takahashi (2009), complex formation may be evaluated by comparing the peak size characteristic of the host molecule with the size in the complex. Decrease in peaks may reveal partial complexation.

The calculation of relative intensity, which took into account the peak height of the encapsulated related to the juice peak, showed that microparticles formed by xanthan gum, tara gum and hydrogel formed by the two gums had a relative intensity of 65.55%, 82.03% and 57.60%.

3.4. Releasing profile of the encapsulated compounds

Among the steps involved in the release of encapsulated compounds are the absorption of the solvent by the microparticles, dissolution of the wall material, dissolution of the encapsuled material, permeation of the encapsuled material by wall and diffusion of the encapsuled material by solvent, therefore, the solubility of the wall material and of the encapsulated compounds in the fluid release is main responsible for the controlled release of the core (Rocha, 2009).

Releasing profiles of carotenoids and phenolic compounds from encapsulated purple Brazilian cherry juice were evaluated in water and in fluids that simulated gastric (GFS) and intestinal (IFS) conditions (Fig. 3).

3.4.1. Releasing in water

The releasing of carotenoids from microparticles depended on the polymer used in encapsulation. Microparticles coated with xanthan-tara hydrogel gradually released the carotenoids in the water, with a maximum releasing of 86% in 180 min. Xanthan gum-coated particles revealed a different behavior, with 46% released during dissolution, achieving a maximum of 82% in 40 min. Tara gum-coated microparticles provided a low 12% release up to 180 min and 51% in 240 min.

Behavior in the release of phenolic compounds from microparticles was similar to that reported with xanthan- and xanthan-tara hydrogel-coated microparticles, or rather, they had high release percentages at dissolution, ranging between 71 and 79%, with total content release between 40 and 60 min. Tara gum microparticles provided an initially low release of 40%, with 100% only at 120 min.

Activation by water is one of the most common forms in the release of encapsulated compounds since most wall materials are hydrosoluble. Controlled release of bioactive compounds is highly important so that the application of encapsulated microparticles in food products may be viable owing to the avoidance of compound losses during processing (Belščak-Cvitanović et al., 2011). Consequently, wall materials in current study are rather recommended for the protection of carotenoids since most wall materials trigger a fast release of phenolic compounds in water.

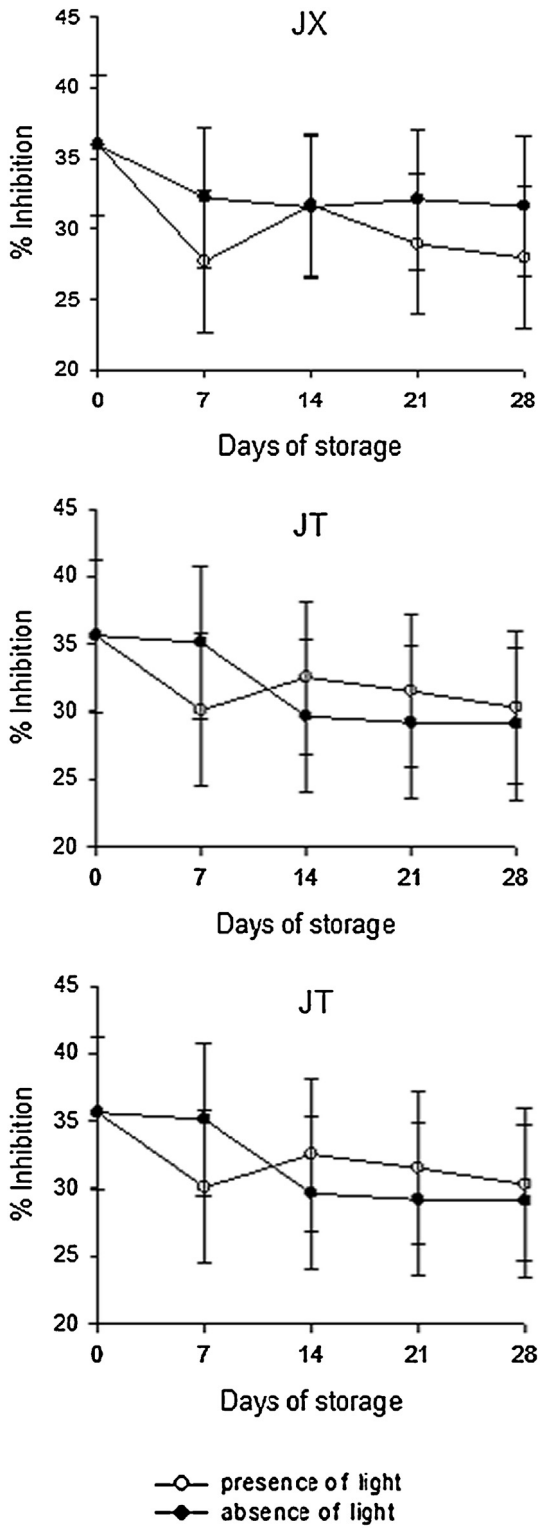

Fig. 5. Antioxidant activity of microparticles stored in presence and absence of light for 28 days.

3.4.2. Release in gastric (GFS) and intestinal (IFS) fluid

Microparticles from xanthan-tara hydrogel showed low carotenoid release in GFS (gastric fluid simulation), with a maximum of 19% in 180 min. Such behavior was different from that reported for microparticles coated with individual polymers. Xanthan gum-coated microparticles released total contents in 180 min, whereas the tara gum-coated ones had low percentage

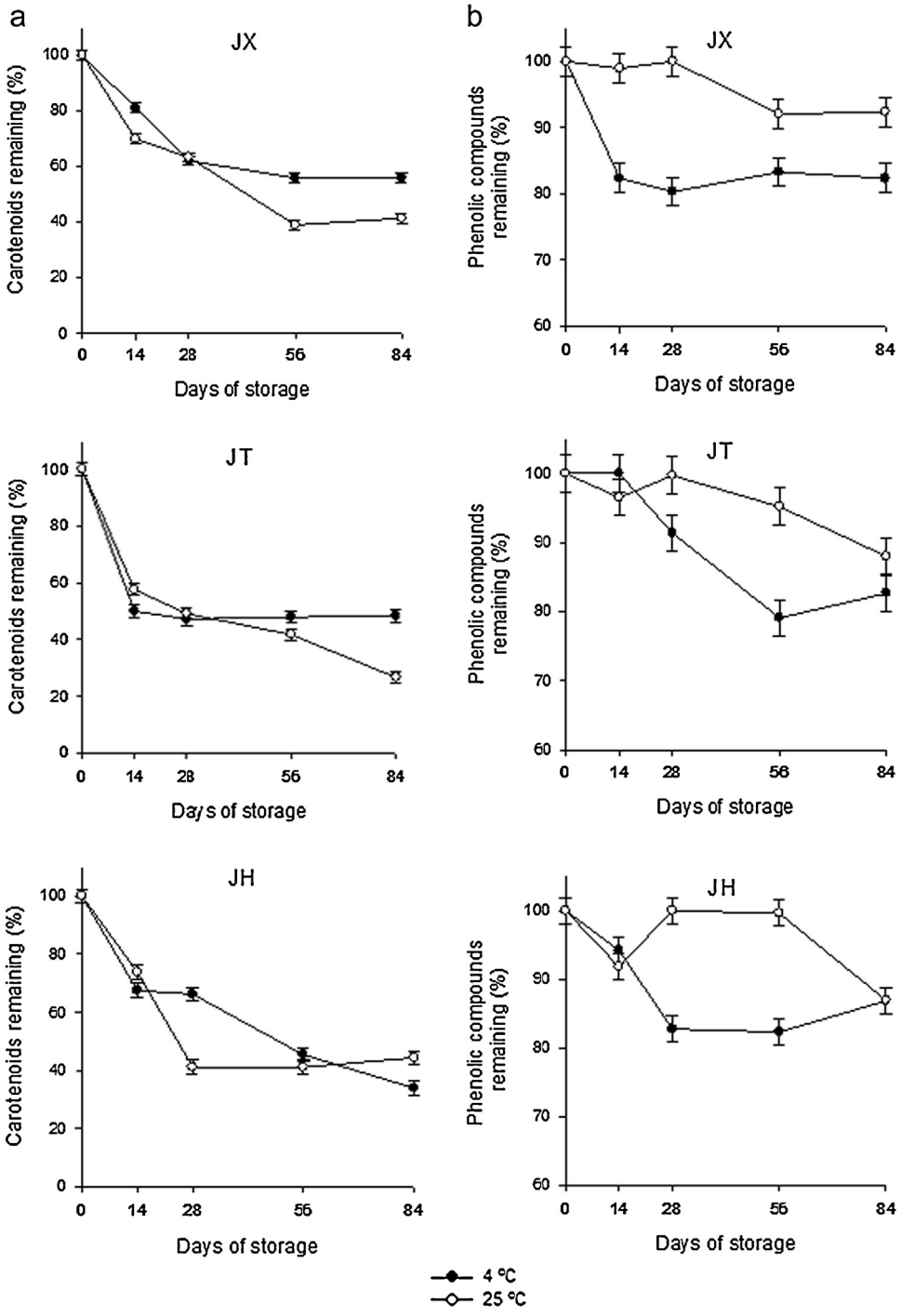

Fig. 6. Microparticles stored at 4 °C and 25 °C for 84 days. Stability of carotenoids (a) and phenolic compounds (b).

release (close to 22%) up to 180 min. However, they released 90% of contents from this time onwards.

In IFS (intestinal fluid simulation) fluid, microparticles coated with xanthan-tara hydrogel released higher carotenoid rates than those in GFS, or rather, 85% in 60 min. Tara gum-coated microparticles released carotenoids in a gradually way, with a maximum of 86% of contents in 240 min. On the other hand, xanthan gum-coated microparticles released 100% of contents in only 40 min.

When the release of phenolic compounds in GFS and IFS fluids were taken into consideration, microparticles provided high release percentages, with over 67% of contents after 20 min and 100% in 120 min.

It was expected that wall materials employed in encapsulation were able to protect the encapsulated compounds, releasing only small quantities in gastric conditions. However, they should have gradually release of the total contents in the intestinal fluid since most nutrients and vitamins are better absorbed in the intestine (Somchue, Sermsri, Shiowatana, & Siripinyanond, 2009). This characteristic has been observed in hydrogel-coated microparticles but only those with carotenoid encapsulation. Bonding between

molecules occurred during the formation of hydrogel and strongly affected isolated polymers. This fact might have produced a greater resistance in the release of encapsulated compounds in the gastric fluid.

3.5. Stability

3.5.1. Stability to light

The protection of carotenoids by the encapsulation technique with regard to light exposure depended on the wall material polymer (Fig. 4). Tara gum wall material provided the best protection to carotenoids (63.6%) up to 7 days storage, under light; after this period, the compounds gradually degraded throughout the following 28 days. However, the degradation of carotenoids in the microparticles in the absence of light was higher during the first 14 days of storage.

Microparticles prepared with xanthan gum and xanthan-tara hydrogel provided greater stability to carotenoids after 7 days of storage, with or without light. Carotenoid rate remained constant with such wall materials as from the second week of storage, with an arrest of approximately 53% of carotenoids at the end of microparticle storage under light and of approximately 64% in the dark.

According to the literature, protection of the bioactive nucleus provided by encapsulation technique depended on the encapsulated compound, wall material and conditions. All polymers employed as wall material protected the phenolic compounds when stored in the light and in the dark (Fig. 4b). All microparticles had high retention percentages, over 86%, in the light and in the dark, at the end of the 28 storage days. Zheng et al. (2011) reported a similar behavior to that obtained with microparticles of purple Brazilian cherry juice.

The antioxidant activity of xanthan-tara hydrogel microparticles (Fig. 5) greatly decreased during the first 7 days of storage, whereas the other microparticles coated with isolated polymers were gradually reduced during the storage period. Antioxidant activity at the end of the storage period did not vary significantly among microparticles coated with the same wall material when stored in the light and in the dark.

3.5.2. Stability to temperature

Results show carotenoid degradation throughout the storage period regardless of wall material and temperature applied to the microparticles (Fig. 6). The microparticles coated with xanthan gum and with xanthan-tara hydrogel provided a better protection to carotenoids during the entire storage period, at 25 °C, with 41.4% and 44.3% retention, respectively. Higher retention rate of carotenoids may be observed at 4 °C, especially for xanthan gum-coated microparticles which preserved 55.8% of the compounds during 84-day storage.

Regardless of the polymer used in the wall material, phenolic compounds were best preserved in microparticles stored at 25 °C, with high preservation percentages by the end of the storage period (Fig. 6). Microparticles coated with xanthan-tara hydrogel retained 99.6% of the compounds up to 84 days storage. Since low temperatures provided greater stability to phenolic compounds, higher preservation was expected for microparticles stored at 4 °C. However, the opposite occurred, perhaps due to other variables which were not evaluated, such as the environment's relative humidity.

In fact, Bakowska-Barczak and Kolodziejczyk (2011) reported differentiated behavior for temperature. The above researchers registered greater preservation of phenolic compounds at temperatures ranging between 8 and 10 °C. However, rates were similar to those obtained in present study.

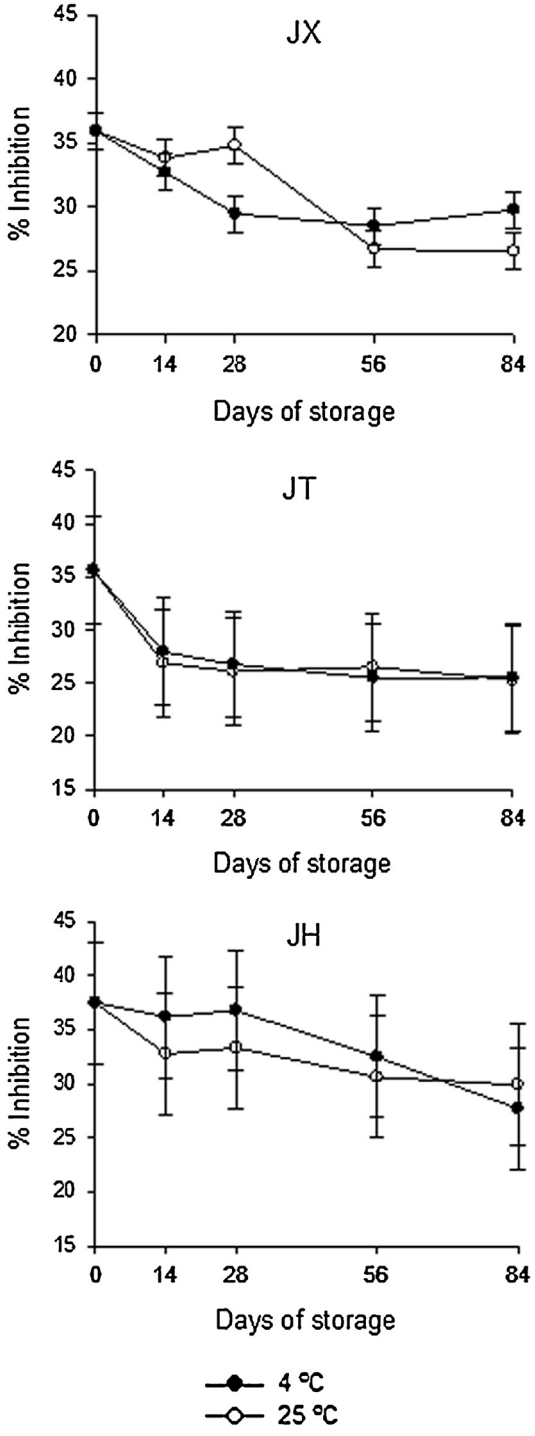

Fig. 7. Antioxidant activity of microparticles stored at 4 °C and 25 °C for 84 days.

Fig. 7 shows that there was a slight decrease in antioxidant activity in all microparticles during 84 days storage at both temperatures. Highest decrease occurred for tara gum-coated microparticles. In fact, they had a greater decrease in antioxidant activity during the first 14 days of storage at both temperatures. The antioxidant activity of xanthan gum microparticles, stored at 25 °C was lower as from the 56th day, whereas in the case of microparticles coated with xanthan-tara hydrogel, the antioxidant activity decreased gradually throughout the 84-day storage, regardless of temperature.

At the end of the storage period, the antioxidant activity did not vary significantly for microparticles coated with the same wall material and stored at both temperatures. The only exception was xanthan-coated microparticles with a lower antioxidant activity when stored at 25 °C.

Bakowska-Barczak and Kolodziejczyk (2011), Sansone, Picerno, et al. (2011) and Sansone, Mencherini, et al. (2011) evaluated the antioxidant activity of microencapsulated bioactive compounds and reported unchanged activity or approximately 10% decrease during a 6–12 month storage period.

4. Conclusion

Encapsulation was confirmed. Results were affected by wall material and encapsulated compound group. Higher efficiency in encapsulation occurred with xanthan gum.

Hydrogel was the most adequate for the release of carotenoids in GFS and IFS, with a gradual release of the compounds in water. Although phenolic compounds registered the highest release rates, it was not gradual and did not depend on wall material and fluids analyzed.

Stored microparticles, in the light and in the dark, at 4 and 25 °C, revealed carotenoid degradation. Xanthan gum and hydrogel wall materials provided more stability to the compounds. Phenolic compounds were stable regardless of the wall material employed. The microparticles' antioxidant activity decreased during storage due to the degradation of carotenoids.

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