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Xyloglucan from *Hymenaea courbaril* var. *courbaril* seeds as encapsulating agent of L-ascorbic acid

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ABSTRACT

This study evaluated the L-ascorbic acid (AA) encapsulation in microparticles of xyloglucan (XAA) extracted from *Hymenaea courbaril* seeds by spray drying (SD) and its application in tilapia fish burgers. The encapsulation efficiency was 96.34 ± 1.6% and the retention of the antioxidant activity was of 89.48 ± 0.88% after 60 days at 25 °C. SEM images showed microspheres with diameters ranging from 4.4 to 34.0 μm. FTIR spectrum confirmed the presence of AA in xyloglucan microparticles, which was corroborated by DSC and TGA. The release of ascorbic acid was found to be pH-dependent. The application of XAA in tilapia fish burger did not change the pH after heating and the ascorbic acid retention was greater compared to its free form. The results indicate that xyloglucan can be used to encapsulate AA by SD and suggest that XAA was able to reduce undesirable organoleptic changes in fish burgers.

1. Introduction

Over the last years, encapsulation studies with several polymeric materials from vegetal origin are attracting the attention of researchers because they are natural sources and cheaper than synthetic polymers [1]. Xyloglucan is a polysaccharide with specific functions in the plant and plays an important structural role, while also acting as a storage material in seeds of certain species such as *Hymenaea courbaril* var. *courbaril*, known as Jatoba, abundant native leguminous in the Brazilian forests, from North to South. Xyloglucan is composed by glucose (40%), xylose (34%), galactose (20%) and arabinose (6%) [2].

In pharmacology xyloglucan is used in drug carrier systems due to its solubility in water, being a natural biodegradable non-antigenic polymer [3,4]. In the food industry, for instance, xyloglucan is used as a thickener, stabilizer, anti-microbicide agent and crystallization inhibitor [5], there being no reports of its actuation as an encapsulating agent for additives.

Microencapsulation by spray drying (SD) is one of the standard methods for encapsulation, in which a liquid product is atomized and dried instantaneously by a hot air stream, resulting in a powder [6]. Microencapsulation is widely used in the protection of active components against factors that can cause degradation, such as chemical substances, environmental adverse reactions and loss of volatiles, improving the stability and contributing to a controlled release [7]. One of the active compounds that have been encapsulated with different polymers is ascorbic acid [8,9]. It is used by the food industry and is considered one of the best alternatives as antioxidant, protecting the sensory and nutritional characteristics of the food, while also increasing the content of vitamin C in products; however, this additive is highly unstable and reactive [10–12]. Also due to its acidic nature, ascorbic acid can interact with other food components [13]. This interaction may result in compounds such as furan, the formation of which will occur during the warm-up time [14]. Microencapsulation with controlled release of additives such as ascorbic acid for certain food formulations is

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interesting to maintain the appropriate characteristics throughout the processing and heating of the food product [15].

In the present study the xyloglucan from *Hymenaea courbaril* var. *courbaril* seeds was used as an ascorbic acid encapsulating agent by SD, and the microparticles obtained were characterized and applied to fish burgers for the controlled release of the active compound during the beneficiation phase and protection during baking.

2. Experimental

2.1. Materials

The seeds of *Hymenaea courbaril* var. *courbaril* were collected in September 2015 in the city of Carnaubal, Ibiapaba Sierra region in the Ceará State, Brazil. The L-ascorbic acid (99.7%), 2,6-dichlorophenolindophenol sodium salt dihydrate (DCIP; 97%, Vetec, Duque de Caxias, Brazil) and methyl alcohol (99.8%) were obtained from VETEC (Brazil). Absolute ethanol (96°GL) was obtained from Santa Cruz (Brazil), acetone PA from Dynamics (Brazil), oxalic acid from Cromoline Fine Chemicals Ltd. (Brazil) and 2.2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (Germany).

2.2. Extraction and purification of xyloglucan

The xyloglucan (X) from the seeds of *H. courbaril* was extracted with 0.1 M NaCl [5% (w/v)], precipitated with 46% ethanol [1:3 (v/v)] for 18 h, filtered on screen printing cloth (110 thread type), washed with 100% ethanol [1:3 (w/v)] for 30 min and two times with acetone PA [1:3 (w/v)] for 30 min [16]. The precipitated xyloglucan obtained was dried at 60 °C until constant weight, powdered and stored in amber glass bottles in dry place until further use.

2.3. Preparation of xyloglucan microparticle with L-ascorbic acid

The 1% (w/v) xyloglucan solution was prepared dissolving 10 g in 1000 mL of distilled water under magnetic stirring (200 rpm) at 25 °C for 24 h. Afterwards, were added 0.4 g of L-ascorbic acid (AA) and the solution was maintained under the same condition for more 1 h. After that, the solution was atomized in a spray dryer device (Buchi Mini Spray Dryer B-290) under the following operating conditions: inlet air temperature at 160 °C, outlet air temperature at 103–107 °C, an air flow (at standard temperature and pressure) of 538 L/h and pump speed of 233 mL/h [17]. The obtained microparticles of xyloglucan containing AA (XAA) and xyloglucan after the SD process (XSD) were collected and stored in amber glass bottles at room temperature (25 °C) for 60 days. The experiments were carried out in quintuplicate.

2.4. Morphology of microparticles

The surface micrographs of microparticles of XAA and XSD were carried out in a scanning electron microscope (SEM) model EVO/LS15 (Zeiss, USA) at an accelerating voltage of 10 kV under vacuum conditions. Samples were prepared on stubs with carbon double-sided tape (8 mm × 20 mm) and subjected to a gold coating in a Q150T ES coater (Quorum Technologies, UK).

2.5. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra were obtained with a Cary 630 FTIR spectrometer (Agilent Technologies, USA) within the range 4000–700 cm^{-1}. Powdered samples of XAA microparticles, X and AA were placed in contact with the previously clean crystal to then display the spectra [18].

2.6. Thermal analysis

The thermal properties of xyloglucan after the SD process (XSD), without the process (X) and of the microparticles XAA were determined by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC measurements were carried out with a PerkinElmer DSC 6000 (PerkinElmer, Massachusetts, EUA) calibrated with Indium as standard. Approximately 3 mg of the sample was placed in aluminum DSC pans (Perkin-Elmer pans 30 μL BO143016) sealed with DSC covers (Perkin-Elmer Al cover BO143003). The measurements were taken following the temperature ramp, heating from 20 °C to 120 °C, then cooling to −45 °C and finally heating at 300 °C, at a rate of 10 °C min^{-1} under a nitrogen atmosphere. The melting enthalpy (ΔHm), melting peak (Tm) and glass transition temperature (Tg) were determined in the second heating scan. TGA was completed with a PerkinElmer TGA 4000 (PerkinElmer, Massachusetts, EUA). Samples were placed in the balance system and heated from 20 °C to 450 °C at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere.

2.7. Encapsulation efficiency of L-Ascorbic acid

Ascorbic acid content in the microparticles was determined according to Tillmans method based on the titration principle, as described previously [9]. Briefly, a volume of 10 mL of 0.05% (w/v) microparticle solution in 1% (w/v) oxalic acid was added in Erlenmeyer flask containing 50 mL of 1% (w/v) oxalic acid and titrated with 0.2% (w/v) DCIP solution until persistent pink color for 15 s. The amount of ascorbic acid was calculated according to the following equation [19]: AA (%) = (V x F)/A x100, where V = Volume spent in the titration; F = volume of DCIP in mL spent on standardization; A = mass (g) of the sample used in the titration. The encapsulation efficiency (EE) was calculated according to the equation [20]: EE(%) = (QD/QA) x 100, where QD is the total amount of ascorbic acid determined in the system and QA the amount of ascorbic acid added to the system. The encapsulation experiments were carried out in quintuplicate and for each one of the five samples, ascorbic acid was determined in triplicate. Consequently, ascorbic acid results are expressed as an average ± standard deviation of the 15 values found.

2.8. Evaluation of stability of XAA microparticles

The evaluation of the stability of the XAA microparticles, was carried out at time intervals of 0, 7, 15, 30 and 60 days, through ascorbic acid analysis mentioned in the section 2.7, taking the result of the total ascorbic acid at time 0 as the value of 100%. It was also measured the antioxidant activity using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical method [20], in which the methanolic solution of the XAA sample, with the concentration of 10 mg mL^{-1}, was initially subjected to ultrasonic bath (UNIQUE-Ultrasonic Cleaner) for solubilization and release of AA from the nucleus. From this solution different concentrations (from 0.1 to 7.5 mg mL^{-1}) were obtained with dilution with methanol. In parallel, a methanolic solution of DPPH (0.026 mg mL^{-1}) was prepared. The reaction media consisted of 0.1 mL of each one of the concentrations together with 3.9 mL of the free radical methanolic solution DPPH. As control, 0.1 mL of methanol were used with 3.9 mL of methanolic solution of DPPH. The analysis was carried out in triplicate and after 1 h the absorbance was measured in a spectrophotometer (HACH-DR 6000) at 515 nm. The inhibition percentage (PI%) of samples from each concentration was calculated by the equation PI (%) = [(Absorbance Control − Absorbance

Sample)/Absorbance Control] X 100. The PI% results of each concentration were plotted and through linear regression the 50% Inhibitory Concentration (IC50) of the DPPH radical was calculated in relation to the amount of antioxidant present in the polymer matrix.

2.9. Analysis of ascorbic acid release from microparticles

The ascorbic acid release profiles were obtained by dialysis [21]. Samples of 100 mg XXA (with 5 mg of AA) were solubilized in 5 mL of different buffers, according to their pK_a value (Clark and Lubs pH 2.0 and phosphate pH 6.5 and 7.0), and after that added to a dialysis membrane (MAP 33 mm, 1.3 in., Dialysis Pipe Cellulose membrane, Sigma-Aldrich, USA). The membrane was subsequently placed in 95 mL of each buffer solution with magnetic stirring. At certain time intervals (from 0 to 4320 min), 1.0 mL of samples were taken from the dialysis buffers and added to the system of 1.0 mL of fresh buffer to keep the volume of release medium constant. The amount of AA released from the microparticles was evaluated by measuring the absorbance at 260 nm [22] in a spectrophotometer (HACH-DR 6000, USA). All release tests were carried out in triplicate, and to calculate the results, ascorbic acid calibration curves were constructed in the concentration ranges from 20 to 200 μg/mL.

2.10. Application of XAA microparticles in fish burger

2.10.1. Formulation of fish burger

Fresh tilapia fillets (*Oreochromis niloticus*) were obtained from the local market of Sobral city (Ceará, Brazil) on the same day of slaughter and transported to the laboratory in polystyrene boxes along with an appropriate quantity of flaked ice. The fillets were then washed with water at 4 °C containing 5 ppm of chlorine for 15 min prior to processing. Fish burgers were processed as described previously with some modifications [23]. Four formulation of fish burger were produced. For each batch containing 1000 g of chopped tilapia, the following ingredients were incorporated (w/w): 1.5% of sodium chloride, 0.2% of monosodium glutamate, 0.1% of onion powder, 0.1% of garlic powder, 0.2% of coriander powder, 0.1% of white pepper powder, 5% of wheat flour, 10% of water at 4 °C, and the resultant mass was homogenized on a cutter (Metvisa, Brazil) and kept at low temperature (ice water bath) for partial formation of protein gel for 20 min. The mass obtained from each batch of 1000 g were divided into 4 portions of 250 g to create 4 formulations: A1 (control – without any addition of free AA, XAA or X); A2 (with free ascorbic acid addition – 0.02 g/100 g of formulation); A3 (with addition of 0.28 g of XAA containing 0.02 g AA/100 g of formulation) and A4 (with addition of 0.28 g of X/100 g of formulation). Three batches of each formulation were prepared resulting in five units of fish burger (50 g each) in each batch, which were molded with a manual hamburger mold with 7.6 cm diameter. The fifteen fish burgers of each formulation were individually packed in high density polyethylene bags and stored at 4 °C. All fish burgers were submitted immediately to physicochemical analyzes after preparation (raw samples) and after baking (baked samples) in a conventional electric oven (Cook, Suggar, Brazil) at 200 °C for 15 min.

2.10.2. Physicochemical analysis of fish burger

2.10.2.1. pH. The pH was measured in a suspension resulting from the homogenization of 10 g of each sample of fish burger with 100 mL of distilled water for 2 min, using a pH meter (PHS-3E, Inolab®, Brazil) previously calibrated [19]. The determinations were carried out in triplicate.

2.10.2.2. Ascorbic acid. The amount of ascorbic acid in the fish burgers before and after baking was determined according to the Tillmans method [19] with modifications [24]. Samples were ground in a cutter (Metvisa, Brasil). Then, 5 g of sample was added to an Erlenmeyer flask, and 50 mL of oxalic acid solution (1 g/100 mL) was added. The mixture was kept under mild stirring for 5 min and then placed in an ultrasound bath for 2 min. Samples were titrated with DCFI solution (0.2 g/100 mL). Determinations were carried out in triplicate.

2.10.2.3. Color. The surface color of fish burger samples, raw and cooked, was determined with a portable colorimeter (Colorium, Delta color, Brazil), previously calibrated. The parameters determined were L^* (L^* = 0 [black] and L^* = 100 [white]), a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness), $C^* = (a^{*2} + b^{*2})^{0.5}$) and hue angle [$h^o$ = arctg (b^*/a^*)]. These parameters are the ones recommended by the International Commission on Illumination. Four readings were made on randomly chosen reading positions for each sample in triplicate.

2.11. Statistical analyzes

Statistical analysis was carried out using the analysis of variance (ANOVA). The comparison between samples was analyzed using the *Tukey* test. The statistical significance was established at $p < 0.05$ (GraphPad Prism, version 6, 2012, USA).

3. Results and discussion

Xyloglucan was efficiently extracted with the same extraction yield (72 ± 5%), monosaccharide composition and structure as shown in the NMR spectra reported by Arruda et al [2].

3.1. Morphology of microparticles

The SEM micrograph of the surface morphology of the microparticles of xyloglucan (XSD) and xyloglucan samples containing L-ascorbic acid (XAA), spray dried, presented imperfect spherical shapes and smooth surfaces.

The xyloglucan microparticles (Fig. 1A) exhibited a collapsed appearance, i.e., highly crimped, kneaded, while in the xyloglucan microparticles containing L-ascorbic acid (Fig. 1B) more defined spherical shapes of different sizes were observed. In this case, the values varied from 4.4 to 34.0 μm due to the presence of L-ascorbic acid in the interior of the microparticles, leading to filled nuclei. The absence of crystals of cubic form characteristic of ascorbic acid [2] could be related to the efficient antioxidant encapsulation.

These spherical structures are typical of materials subjected to SD and dented surfaces with remarkable indentation is commonly observed in encapsulation agents consisting of polysaccharides [25–27], which can be attributed to the effects of the drying rate on the structure of the polysaccharide, leading to a rapid solidification of the wall and, therefore, indentation shape [28].

3.2. Fourier transform infrared (FTIR) spectroscopy

Fig. 2 shows the infrared spectrum of L-ascorbic acid samples, XAA microparticles and xyloglucan. The L-ascorbic acid displayed a FITR spectrum similar to the result obtained other authors [29,30]. In xyloglucan sample are very similar peaks with those reported in the literature [31,32], as the 3280 cm^{-1} peak that shows the corresponding functional groups to hydroxyl (OH) and the stretch C—H (2852–2922 cm^{-1}). The 1637 cm^{-1} peak can be attributed to the effect of water absorbed, and the peak of 1374 cm^{-1} for the designation C—H. At the peak of 1016 cm^{-1} was observed similarity with xylans and 870 cm^{-1} is attributed to the β-glycosidic linkages ($1 \rightarrow 4$) between xylose units in hemicelluloses [33].

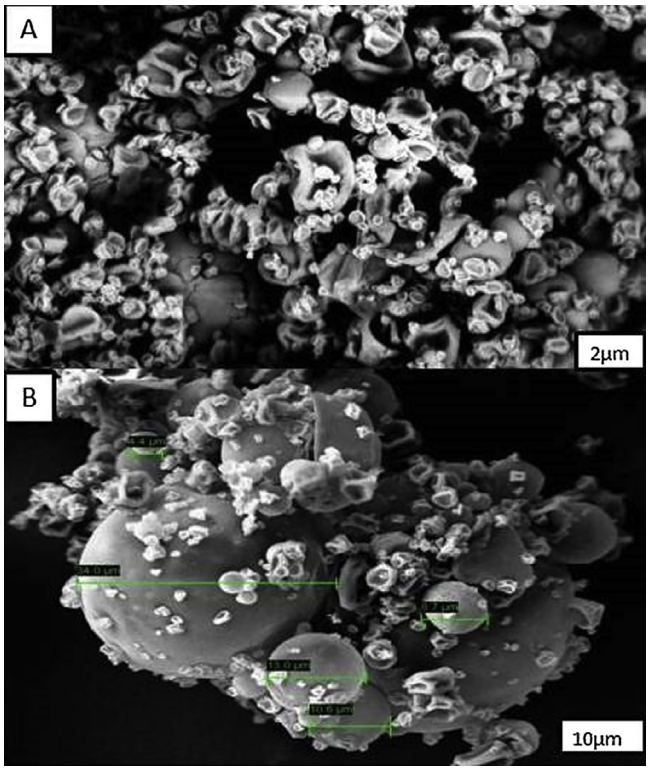

Fig. 1. SEM micrographs of XDS (A) and XAA (B).

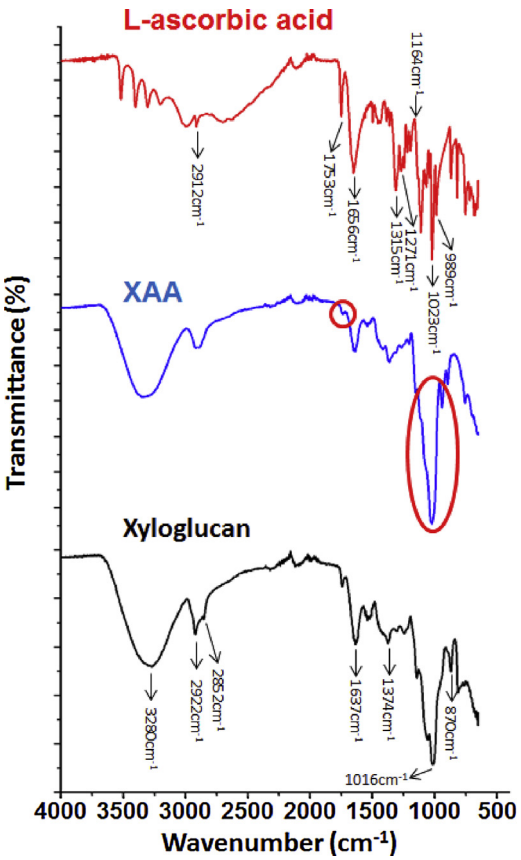

Fig. 2. FTIR of L-ascorbic acid (AA), XAA microparticles and xyloglucan.

Many of the chemical bonds shown in XAA microparticles have similarities not only for xyloglucan but also for L-ascorbic acid, with only minor shifts in the peaks of the chemical bonds that have been reported for both biomolecules.

The presence of the peaks of the lactone ring (1020–989 cm^{-1}) confirm the presence of ascorbic acid in the microparticles. A decrease in the intensity of the C—O stretch (1753 cm^{-1}) was observed in XAA in comparison with L-ascorbic acid, suggesting that this carbonyl group is covalently interacting with the free —OH of xyloglucan.

3.3. Thermal analysis

In order to thermally stabilize the sample and remove any moisture, preliminarily heating was carried out until the temperature of 120 °C. Afterwards, the experimental temperature was increased up to 300 °C. According to peaks observed in the thermogram (Fig. 3), the glassy temperature (*Tg*) and crystallization temperature were not observed, but only the melting point (*Tm*) and melting enthalpy (ΔH). The hemicelluloses usually have lower thermal stability than the cellulose, due to lack of crystallinity [34]. For the xyloglucan sample subjected to the atomization process (XSD), the melt peak occurred at 176.47 °C with ΔH of 116.91 J/g. For xyloglucan which was not subjected to atomization process (X) occurred at 173.64 °C with ΔH of 115.99 J/g. Other authors observed in the DSC thermal event the low-melting point (78 °C) [35] and at temperatures above 200 °C have been reported in the literature for xyloglucan from another species [32,34], thus demonstrating that the peaks of the XSD and X showed thermal average intensity, despite to be amorphous polymers. The XAA endothermic peak was 164.55 °C associated with the melting point and ΔH of 125.55 J/g, thus demonstrating that the presence of L-ascorbic acid decreased the *Tm* and increased the ΔH in relation with the controls. Furthermore, with the absence of the strong endothermic peak (190 °C) of L-ascorbic acid [30] in the thermogram of XAA, it should be considered, the change from the crystalline state to the soluble when it was incorporated into the polysaccharide, suggesting so the incorporation of the active compound by xyloglucan.

In the samples subjected to TGA they were found two weight loss events (Fig. 4). In xyloglucan control the first and second mass loss event occurred at temperatures of 75.33 ± 4.5 °C (ΔY = 8.20%) and 297 ± 6.22 °C (ΔY = 56.45%), respectively, and in xyloglucan subjected to SD, at 63.06 ± 1.03 °C (ΔY = 8.56%) and 289.66 ± 1.38 °C (ΔY = 63.97%), respectively. For the microparticles the first peak of

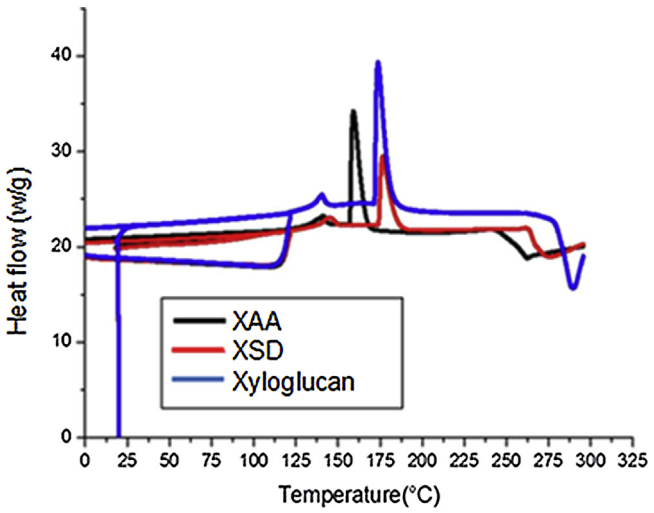

Fig. 3. Differential scanning calorimetry (DSC) analysis of XAA, XSD and xyloglucan samples.

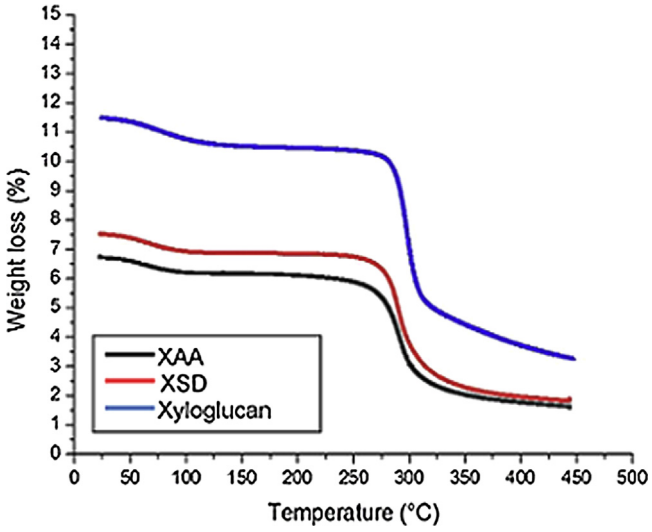

Fig. 4. Thermogravimetric analysis (TGA) of XAA, XSD and xyloglucan samples. Each data point is an average of 3 determinations.

temperature was at 60.09 °C ± 6.1 (ΔY = 8.05%) and the second at 289.41 ± 2.53 °C (ΔY = 61.54%).

The first peak is associated with the loss of water adsorbed by the molecules that should be between 35 and 100 °C [35] and presented lower values in the XSD and XAA in comparison with xyloglucan, being due to the fact that the first two ones were already with a lesser amount of water, because they were subjected to an atomization process, differing statistically from the control at the level of 0.05%. The second mass loss event occurred as a result of sample degradation and are similar in XSD and XAA, not differing between them and the control at the level of 0.05%, showing so that the L-ascorbic acid present in the XAA sample did not affected the sample degradation. The second peak of temperature of xyloglucan is consistent with the literature [35,36]. The weight loss of xyloglucan occurs at the maximum point of 250 °C, ending the total degradation at 300 °C [34].

3.4. Encapsulation efficiency of ascorbic acid

The encapsulation efficiency (EE) was found to be 96.34 ± 1.6%, indicating that the xyloglucan and the conditions used in SD process did not cause significant damage to the active compound. The EE value found here is similar to the other polysaccharides used for the encapsulation of ascorbic acid, such as galactomannan that varied from 95.40 to 97.92% in different environmental conditions [20] and arabic gum that presented 100.80% [9].

3.5. Evaluation of the stability of ascorbic acid in the microparticles

The retention of ascorbic acid and antioxidant activity of the xyloglucan microparticles containing ascorbic acid over 60 days are shown in Table 1. A slight decay of about 9.00% occurred on the seventh day, remaining practically constant, and on the sixtieth day of the experimental period was found to be 89.48 ± 0.88% of retention (Table 1). Various microencapsulation studies of ascorbic acid in different polysaccharides by SD have shown high retentions, among them the one obtained 85.20% retention stability of rice starch microparticles and 100% with arabic gum for 60 days [37] and using chitosan as the encapsulating of ascorbic acid in which they found about 92% after 20 days of the study [8].

The activity of ascorbic acid in XAA expressed as IC50%, which is the concentration in mg mL^{-1} of microparticles necessary to inhibit 50% of the DPPH radicals [38], is shown also in Table 1. It was observed that there was no statistically significant difference ($p > 0.05$) of the antioxidant power of the microparticles containing ascorbic acid, over 60 days, which allows to state that the xyloglucan microparticles obtained by SD have proved to be efficient in the encapsulation of ascorbic acid.

Table 1
Retention and Inhibitory Concentration (IC50) of ascorbic acid of XAA.

Time (Days)	AA Retention (%)	IC50% (mg mL^{-1})
0	100.00^{a}	4.92 ± 0.66 ^{a}
7	91.01 ± 0.95 ^{b}	4.72 ± 0.60 ^{a}
14	90.83 ± 0.33 ^{b}	4.50 ± 0.88 ^{a}
21	90.46 ± 0.92 ^{b}	4.19 ± 0.54 ^{a}
30	90.46 ± 0.13 ^{b}	4.01 ± 0.70 ^{a}
60	89.48 ± 0.88 ^{b}	4.05 ± 0.16 ^{a}

Equal superscript letters in the same column indicate the absence of statistically significant differences ($p > 0.05$).

3.6. Ascorbic acid release from xyloglucan microparticles

The release standard of a compound may be pH dependent [39]. In Fig. 5 it can be seen that the ascorbic acid release from xyloglucan microparticles is characterized by different phases in the experiments. At pH 2.0 an increasing of AA release was observed from 0 to 180 min. After that, began a decline and at the end of 1440 min (24 h) totalized about 93.68% of AA released during the analysis time.

At pH 6.5 condition, a rapid release was firstly observed in the first 10 min, which can be described as a bursting effect, and thereafter became constant until the end of analysis time, totalizing a release of 37.17%. Finally, at pH 7.0, the release was lower than the other two conditions, that is, AA was released at more controlled rate, mainly after 120 min, a phase that is characterized as a latency time, totalizing only 11.08% of ascorbic acid released in relation to the value added to the system. These initial burst effects followed by reduction in the rate of ascorbic acid release, approaching a steady state, was similar to that observed by another author [22] in gelatin microcapsules with oxidized corn starch tested at pH 7.4. The effect of rupture may be due to the volume expansion of the polymer when immersed in liquid environment [21]. The extended release period is possibly due to the existence of diffusion pathways, where the active ingredient is released by this effect or by pre-existing pores in the polymer [40,41]. Both the rupture and latency mechanisms were observed in studies with different polysaccharides [21,39,42,43]. Controlled release by microcapsule

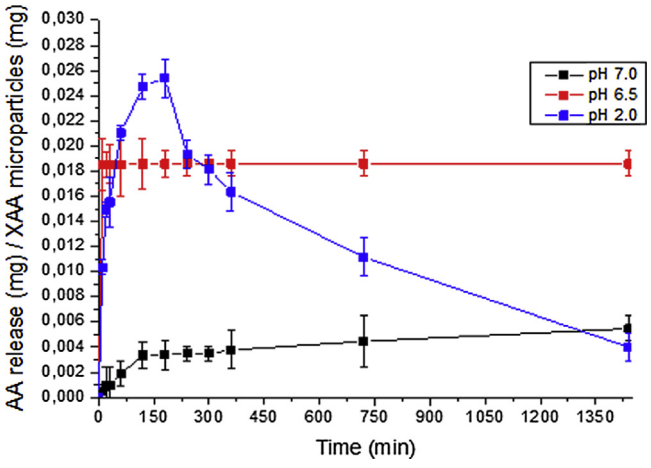

Fig. 5. AA release profiles from XAA at pH 7.0, 6.5 and 2.0. Each data point is an average of 3 determinations.

Table 2
pH and ascorbic acid analyzes in fish burgers.

Sample type	pH		AA Amount g/100 g fish burger
	Raw	Baked	Baked
A1	6.16 ± 0.05^{aB}	6.33 ± 0.11^{aA}	0.00
A2	6.16 ± 0.05^{aA}	6.30 ± 0.10^{aA}	0.0086 ± 0.0005^{b}
A3	6.16 ± 0.05^{aA}	6.26 ± 0.11^{aA}	0.0120 ± 0.0017^{a}
A4	6.16 ± 0.05^{aA}	6.26 ± 0.05^{aA}	0.00

The results are averages ± standard deviation. The same lowercase letters in the same column and same uppercase letters in the same line indicate the absence of significant differences ($p > 0.05$). A1 (control – without any addition of free AA, XAA or X); A2 (with free ascorbic acid addition – 0.02 g/100 g of formulation); A3 (with addition of 0.28 g of XAA containing 0.02 g AA/100 g of formulation) and A4 (with addition of 0.28 g of X/100 g of formulation).

is used to increase the efficiency of many additives that are introduced into food and release the compound under the influence of a specific stimulus [44]. Depending on the application, a slower release rate may be required, and for ascorbic acid to be used as a feed additive it must be present throughout the entire shelf-life of the food, so the importance of controlled release would be the reduction of additives loss during processing, storage and cooking [45].

3.7. Analysis of pH, ascorbic acid and color of fish burgers

Regarding pH, it is observed (Table 2) that there is no significant difference at the 5% level among the samples when the hamburger is raw. Soon after baking there was a discrete increase, but only the control sample presented a significant difference. Similar values were observed in the production of fish burgers of tilapia [23] and *Catla catla* [46].

The remaining ascorbic acid values in the fish burger samples containing free ascorbic acid (A2) and ascorbic acid encapsulated in xyloglucan (A3) after the baking are presented in Table 2. It can be seen that A2 samples containing free ascorbic acid showed a retention of ascorbic acid of 43% and A3 samples containing ascorbic acid encapsulated in xyloglucan a retention of 60%, i.e., A3 was able to reduce the loss of ascorbic acid by 17%. This result demonstrates the efficiency of microencapsulation by SD in preservation of active substance content. Similar results were obtained by another author [9] which applied microparticles of gum arabic with ascorbic acid in biscuits and found that microencapsulation by SD protected the active substance during cooking, with a reduction of about half of the loss observed for the free active substance after baking. Therefore, the microencapsulation showed to be effective in the release of ascorbic acid reducing its reactivity and consequently the formation of furan during heating [15].

The color evaluation of fish burgers, before and after baking process, is an important parameter and should be explored, especially due to the occurrence of changes during heating, which may mask undesirable color changes in the final product induced by the formulation [47]. Table 3 shows the color measurements of the raw and baked samples, showing that there are no significant differences ($p > 0.05$) among the raw samples for the L* parameter; however, for baked samples (A2, A3 and A4) the behavior was different in comparison with control (A1). It is also possible to observe significant differences between A2 and A3, whose brightness was statistically lower for A3. In relation to the a* parameter and among raw samples, only A3 did not present statistical difference in relation to the control (A1) and that, the addition of free ascorbic acid (A2) and of xyloglucan without ascorbic acid (A4), led the raw samples with less redness appearance ($p > 0.05$) in comparison with control (A1).

After the baking process, all samples presented statistical difference in relation to the control (A1) but, A2 and A4 showed the same pattern of decrease in the appearance of redness in comparison with the control (A1). All samples presented positive values for the b* parameter, i.e., the yellowing appearance is evident for both raw and baked samples. It is important to point out that the addition of free ascorbic acid (A2) decreased the value of b* parameter compared to other raw samples. After the baking process, there was no significant difference among the samples in comparison with control (A1). It is also possible to observe that the intensity increase in the b* parameter among raw and baked samples was more evident in A2 sample. This suggests that AA encapsulation was able to reduce undesirable reactions in fish burger. The values of C* and h* confirmed the yellowing tendency for the raw and baked samples however, the baking process increased the color intensity (higher chroma and higher hue angle) for all samples in comparison with control (A1).

These results are in accordance with the appearance of the formulations observed in Fig. 6. Similar behavior for the color parameters were reported by another author [23] for tilapia fish hamburgers containing hydrated textured soy protein (TSP). These authors reported that the raw hamburger was brighter (higher L* value), had a less intense yellow color (lower b* and lower chroma) and closer to the red axis (lower hue angle) than baked fish hamburgers. This behavior may be due to the various reactions occurring during heating, including the Maillard reaction, which are responsible for the color change on the surface of the fish burgers.

4. Conclusion

Atomization by spray drying led to the production of xyloglucan microparticles, extracted from *Hymenaea courbaril* var. *Courbaril* seeds, with great encapsulation capacity of L-ascorbic acid (AA),

Table 3
Color measurements for raw and baked fish burgers.

Formulations	L*	a*	b*	C*	h*
Raw					
A1	49.35 ± 1.12^{a}	4.60 ± 0.12^{a}	5.18 ± 0.31^{a}	6.93 ± 0.32^{a}	48.35 ± 0.96^{c}
A2	50.07 ± 0.19^{a}	3.35 ± 0.82^{b}	3.85 ± 1.02^{b}	5.10 ± 1.31^{b}	$48.93 \pm 0{,}81^{c}$
A3	49.89 ± 1.32^{a}	4.74 ± 0.36^{a}	5.72 ± 0.63^{a}	7.43 ± 0.71^{a}	50.22 ± 1.04^{b}
A4	50.43 ± 0.60^{a}	1.51 ± 0.02^{c}	5.20 ± 0.00^{a}	5.41 ± 0.00^{b}	73.70 ± 0.22^{a}
Baked					
A1	40.83 ± 0.67^{c}	2.74 ± 0.24^{a}	8.62 ± 0.67^{ab}	9.05 ± 0.71^{ab}	$72.34 \pm 0{,}63^{b}$
A2	42.11 ± 0.30^{a}	2.12 ± 0.09^{c}	8.76 ± 0.97^{a}	9.01 ± 0.97^{ab}	76.26 ± 0.93^{a}
A3	41.31 ± 0.09^{b}	2.38 ± 0.18^{b}	9.57 ± 1.27^{a}	9.85 ± 1.29^{a}	76.15 ± 0.63^{a}
A4	41.66 ± 0.25^{ab}	1.95 ± 0.07^{c}	8.33 ± 0.42^{b}	8.56 ± 0.43^{b}	76.79 ± 0.26^{a}

The results are averages ± standard deviation. The same lowercase letters in the same column indicate the absence of significant differences ($p > 0.05$). A1 (control – without any addition of free AA, XAA or X); A2 (with free ascorbic acid addition – 0.02 g/100 g of formulation); A3 (with addition of 0.28 g of XAA containing 0.02 g AA/100 g of formulation) and A4 (with addition of 0.28 g of X/100 g of formulation).

Fig. 6. Appearance of the raw and baked fish burger: A1 (control – without any addition of free AA, XAA or X); A2 (with free ascorbic acid addition – 0.02 g/100 g of formulation); A3 (with addition of 0.28 g of XAA containing 0.02 g AA/100 g of formulation) and A4 (with addition of 0.28 g of X/100 g of formulation).

good morphological characteristic and stability. The incorporation of xyloglucan microparticles containing ascorbic acid in the tilapia fish burger resulted in the retention of a greater amount of ascorbic acid, leading to a lower variation in the color parameter b* between raw and baked samples, when compared to samples containing free ascorbic acid. The results suggest that the incorporation of xyloglucan microparticles containing ascorbic acid in tilapia fish burger formulation appears to be a viable alternative to avoid undesirable organoleptic changes after baking.

Conflicts of interest

The authors declare no competing financial interests.

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References

[1] T. Coviello, P. Matricardi, C. Marianecci, F. Alhaique, Polysaccharide hydrogels for modified release formulations, J. Control. Release. 119 (2007) 5–24.
[2] I.R.S. Arruda, P.B.S. Albuquerque, G.R.C. Santos, A.G. Silva, P.A.S. Mourão, M.T.S. Correia, A.A. Vicente, M.G. Carneiro da Cunha, Structure and rheological properties of a xyloglucan extracted from Hymenaea courbaril var. courbaril seeds, Int. J. Biol. Macromol. 76 (2015) 31–38.
[3] Y. Cao, Y. Gu, H. Ma, J. Bai, L. Liu, P. Zhao, H. He, Self-assembled nanoparticle drug delivery systems from galactosylated polysaccharide-doxorubicin conjugate loaded doxorubicin, Int. J. Biol. Macromol. 46 (2010) 245–249.
[4] T.A. Jó, D.F.S. Petri, L.M. Beltramini, N. Lucyszyn, M.R. Sierakowski, Xyloglucan nano-aggregates: physico-chemical characterisation in buffer solution and potential application as a carrier for camptothecin an anti-cancer drug, Carbohyd. Polym. 82 (2010) 355–362.
[5] C.S. Kumar, S. Bhattacharya, Tamarind Seed: properties, processing and utilization, Crit. Rev. Food Sci. 48 (2008) 1–20.
[6] S.H. Peighambardoust, A.G. Taftia, J. Hesari, Application of spray drying for preservation of lactic acid starter cultures: a review, Trends Food Sci. Tech. 22 (2011) 215–224.
[7] N. Agnihotri, R. Mishra, C. Goda, M. Arora, Microencapsulation –A novel approach in drug delivery: a review, J. Pharm Sci. 2 (2012) 1–20.
[8] A. Alishahi, A. Mirvaghefi, M.R. Tehrani, H. Farahmand, S.A. Shojaosadati, F.A. Dorkoosh, M.Z. Elsabee, Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles, Food Chem. 126 (2011) 935–940.
[9] I.D. Alvim, M.A. Stein, I.P. Koury, F.B.H. Dantas, C.L.C.V. Cruz, Comparison between the spray drying and spray chilling microparticles contain ascorbic acid in a baked product application, Food Sci. Technol. 65 (2016) 689–694.
[10] T.A. Comunian, M. Thomazini, A.J.G. Alves, F.E. Matos Junior, J.C.C. Balieiro, C.S. Favaro-Trindade, Microencapsulation of ascorbic acid by complex coacervation: protection and controlled release, Food Res. Int. 52 (2013) 373–379.
[11] F.E. Matos-Jr, T.A. Comunian, M. Thomazini, C.S. Favaro-Trindade, Effect of feed preparation on the properties and stability of ascorbic acid microparticles produced by spray chilling, LWT – Food Sci. Technol. 75 (2017) 251–260.
[12] T. Sartori, L. Consoli, M.D. Hubinger, F.C. Menegalli, Ascorbic acid microencapsulation by spray chilling: production and Characterization, LWT – Food Sci. Technol. 63 (2015) 353–360.
[13] T.A. Comunian, A. Abbaspourrad, C.S. Favaro-Trindade, D.A. Weitz, Fabrication of solid lipid microcapsules containing ascorbic acid using a microfluidic technique, Food Chem. 152 (2014) 271–275.
[14] M. Shen, F. Zhang, T. Hong, J. Xie, Y. Wang, S. Nie, M. Xie, Comparative study of the effects of antioxidants on furan formation during thermal processing in model systems, LWT – Food Sci. Technol. 75 (2017) 286–292.
[15] K.S. Özdemir, V. Gökmen, Effect of microencapsulation on the reactivity of ascorbic acid, sodium chloride and vanillin during heating, J. Food Eng. 167 (2015) 204–209.
[16] P.B.S. Albuquerque, W. Barros Jr., G.R.C. Santos, M.T.S. Correia, P.A.S. Mourão, J.A. Teixeira, M.G. Carneiro-da-Cunha, Characterization and rheological study of the galactomannan extracted from seeds of Cassia grandis, Carbohyd. Polym. 104 (2014) 127–134.
[17] F.C.A. Buriti, S.C. Freitas, A.S. Egito, K.M.O. dos Santos, Effects of tropical fruit pulps and partially hydrolysed galactomannan from Caesalpinia pulcherrima seeds on the dietary fibre content probiotic viability, texture and sensory features of goat dairy beverages, LWT – Food Science and Technology 59 (2014) 196–203.
[18] A. Shah, A. Gani, M. Ahmad, B.A. Ashwar, F.A. Masoodi, β-Glucan as an encapsulating agent: effect on probiotic survival in simulated gastrointestinal tract, Int. J. Biol. Macromol. 82 (2016) 217–222.
[19] Analytical Norms of the Adolfo Lutz Institute, Métodos físico-químicos para análise de alimentos: normas analíticas do Instituto Adolfo Lutz. Instituto Adolfo Lutz, São Paulo, 2008; 4ª ed, [1ª ed. digital].
[20] C.A.G. Souza, S.M.C. Siqueira, A.F.V. Amorim, S.M. Morais, T. Gonçalves, R.N. Gomes, A.P. Cunha, N.M.P.S. Ricardo, Encapsulation of L-ascorbic acid in natural biopolymer galactomannan by spray drying: preparation, characterization and antioxidant activity, Quim. Nova. 38 (2015) 877–883.
[21] S. Beirão-da-Costa, C. Duarte, A.I. Bourbon, A.C. Pinheiro, M.I.N. Januário, A.A. Vicente, I. Delgadillo, Inulin potential for encapsulation and controlled delivery of oregano essential oil, Food Hydrocoll. 33 (2013) 199–206.
[22] X. Dang, M. Yang, Z. Shah, S. Mansouri, B.K. May, X. Chen, H. Chen, M.W. Woo, On spray drying of oxidized corn starch cross-linked gelatin microcapsules for drug release, Mat. Sci. Eng. C 74 (2017) 493–500.
[23] E.M. Bainy, L.C. Bertan, M.L. Corazza, M.K. Lenzi, Effect of grilling and baking on physicochemical and textural properties of tilapia (Oreochromis niloticus) fish burger, J. Food Sci. Tech. 52 (8) (2015) 5111–5119.
[24] M.D.T. Benassi, A.J. Antunes, A comparison of metaphosphoric and oxalic acids as extractants solutions for the determination of vitamin C in selected vegetables, Arq. de Bio. e Tec. 31 (1988) 507–513.
[25] H.S. Mahajan, S.A. Gundare, Preparation, characterization and pulmonary pharmacokinetics of xyloglucan microspheres as dry powder inhalation, Carbohyd. Polym. 102 (2014) 529–536.

[26] P.M. Outuki, L.M.B. Francisco, J. Hoscheid, K.L. Bonifacio, D.S. Barbosa, M.L.C. Cardoso, Development of arabic and xanthan gum microparticles loaded with an extract of Eschweilera nana Miers leaves with antioxidant capacity, Colloids Surf. A 499 (2016) 103–112.
[27] I. Peres, S. Rocha, J. Gomes, S. Morais, M.C. Pereira, M. Coelho, Preservation of catechin antioxidant properties loaded in carbohydrate nanoparticles, Carbohyd. Polym. 86 (2011) 147–153.
[28] T.-Y. Sheu, M. Rosenberg, Microstructure of microcapsules consisting of whey proteins and carbohydrates, J. Food Sci. 63 (3) (1998) 491–494.
[29] K.G.H. Desai, H.J. Park, Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying, J. Microencapsul. 22 (2005) 179–192.
[30] C. Garnero, M. Longhi, Study of ascorbic acid interaction with hydroxypropyl-beta-cyclodextrin and triethanolamine, separately and in combination, J. Pharmaceut. Biomed. 45 (2007) 536–545.
[31] S. Sonawane, M. Bhalekar, S. Shimpi, Preparation and evaluation of microspheres of xyloglucan and its thiolated xyloglucan derivative, Int. J. Biol. Macromol. 69 (2014) 499–505.
[32] A. Mishra, A.V. Malhotra, Graft copolymers of xyloglucan and methyl methacrylate, Carbohyd. Polym. 87 (2012) 1899–1904.
[33] X.F. Sun, R.C. Sun, P. Fowler, M.S. Baird, Extraction and characterization of original lignin and hemicelluloses from wheat straw, J. Agr. Food Chem. 53 (2005) 860–870.
[34] E.M. Bergstrom, L. Salmén, J. Kochumalayil, L. Berglund, Plasticized xyloglucan for improved toughness-thermal and mechanical behaviour, Carbohyd. Polym. 87 (2012) 2532–2537.
[35] C.K. Simi, T.E. Abraham, Physico chemical properties of aminated tamarind xyloglucan, Colloid. Surface B. 81 (2010) 513–520.
[36] K. Werner, L. Pommer, M. Brostrom, Thermal decomposition of hemicelluloses, J. Anal. Appl. Pyrol. 110 (2014) 130–137.
[37] M.A. Trindade, C.R.F. Grosso, The stability of ascorbic acid microencapsulated in granules of rice stach and in gum arabic, J. Microencapsul. 17 (2) (2000) 169–176.
[38] S.E. Lee, H.J. Hwangb, J.-S. Ha, H.-S. Jeongc, J.H. Kim, Screening of medicinal plant extracts for antioxidant activity, Life Sci. 73 (2003) 167–179.
[39] A.C. Pinheiro, A.I. Bourbon, M.A. Cerqueira, É. Maricato, C. Nunes, M.A. Coimbra, A.A. Vicente, Chitosan/fucoidan multilayer nanocapsules as a vehicle for controlled release of bioactive compounds, Carbohyd. Polym. 115 (2015) 1–9.
[40] L. Brannon-Peppas, Controlled release in the food and cosmetics industries, in: M.A. El-Nokaly, D.M. Piatt, B.A. Charpentier (Eds.), Polymeric Delivery Systems: Properties and Applications, ACS Symposium Series, American Chemical Society Washington, DC, 1993 42–52, 1993.
[41] N. Faisant, J. Siepmann, J. Richard, J.P. Benoit, Mathematical modeling of drug release from bioerodible microparticles: effect of gamma-irradiation, Eur. J. Pharm. Biopharm. 56 (2003) 271–279.
[42] K.C. Gupta, M.N.V. Ravi Kumar, Drug release behavior of beads and microgranules of chitosan, Biomaterials 21 (2000) 1115–1119.
[43] A. Ilie, C. Ghiţulică, E. Andronescu, A. Cucuruz, A. Ficai, New composite materials based on alginate and hydroxyapatite as potential carriers for ascorbic acid, Int. J. Pharm. 510 (2) (2016) 501–507.
[44] U.R. Pothakamury, G.V. Barbosa-Canovas, Fundamental aspects of controlled release in foods, Trends Food Sci. Tech. 6 (1995) 397–406.
[45] S. Abbas, C.D. Wei, K. Hayat, Z. Xiaoming, Ascorbic acid: microencapsulation techniques and trends – a review, Food Rev Int. 28 (4) (2012) 343–374.
[46] M. Vanitha, K. Dhanapal, G.V.S. Reddy, Quality changes in fish burger from Catla (*Catla catla*) during refrigerated storage, J. Food Sci. Tech. 52 (3) (2015) 1766–1771.
[47] V.C. Bochi, J. Weber, C.P. Ribeiro, A. de, M. Victório, T. Emanuelli, Fishburgers with silver catfish (Rhamdia quelen) filleting residue, Bioresour. Technol. 99 (2008) 8844–8849.