

**BIOACTIVE COMPOUNDS IN JAMELÃO YOGURT (*Syzygium cumini* L)****COMPOSTOS BIOATIVOS EM IOGURTE DE JAMELÃO (*Syzygium cumini* L)**

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**ABSTRACT:** The jamelão fruit has been used in traditional Indian medicine and has recently attracted interest as a functional food, as it is rich in anthocyanins. Anthocyanins are of interest of the food industry due to their antioxidant power, attractive color and stability in acid-rich foods. This research used the gelation process with sodium alginate solution to obtain bioactive yogurt from the production of jamelão capsules added to natural yogurt. The proportion was 80% yogurt and 20% jellybean pulp capsules. The treatments were control yogurt (without the addition of jamelão capsules), jamelão capsules and bioactive yogurt (with the capsules). The objective was to study the antioxidant activity, physical-chemical, nutritional and microscopic stability of the product kept under refrigeration for 28 days at 4±1°C. The addition of jamelão capsules in the yogurt changed the product's physical properties (increased humidity and decreased Brix and ash). There was an increase in the amount of phenols and anthocyanins, in addition to the antioxidant potential at 28 days of storage. The interior of the microcapsules was composed of a mesh structure through which the encapsulated material was distributed, as the capsules can be added to yogurt, to improve the antioxidant and nutritional capacity, which proves to be a promising and viable alternative.

**KEYWORDS:** Antioxidants. Composition. Ionic gelation.

**CONTENTS**

Yogurt is a fermented dairy product obtained through the fermentation of lactic acid by the action of the bacteria *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*. The lactic acid produced interacts with milk proteins and promotes the characteristic texture and sensory properties of the product (SERAFEIMIDOU et al., 2013). However, the literature points out that dairy products have a limited content of bioactive compounds. Therefore, in order to overcome this limitation, some authors suggest the incorporation of plant-based or fruit additives to enrich yogurt (KARAASLAN et al., 2011).

There is a wide variety of commercial yogurts, which vary according to composition, texture and taste. The development of dairy products with new flavors and health benefits has a great potential in the market by increasing sales (ALLGEYER et al., 2010). It should be mentioned that there are some studies that were conducted to elaborate functional products supplemented with fruits, vegetable oil and nutrient fortification (PERINA et al., 2015; GHORBANZADE et al., 2017; BOSNEA et al., 2017).

Regular consumption of yogurt has positive effects on health. It is effective in reducing cholesterol levels, improving lactose digestion, avoiding intestinal syndromes and infections, such as diarrhea and colon cancer, also strengthening the immune defense mechanisms (VASILJEVIC;

SHAH, 2007). At the same time, natural antioxidants in fruits have attracted attention particularly from researchers and consumers (CHAN et al., 2010; MUNIANDY; SHORI; BABA, 2016). This is a consequence of bioactivity, and the relevant health attributes of fresh tropical fruits are an ever-increasing field of research (NOBREGA et al., 2014; BOSNEA et al., 2017).

Tropical fruits stand out for the great number of species and varieties, and they are excellent sources of polyphenols, carotenoids, vitamins and minerals. Among the Brazilian regions, the Northeast is notable for offering a range of fruits with different flavors, colors and odors, with an exotic taste that can be consumed in the fresh or processed form. Most exotic fruits contain a high amount of bioactive compounds, in addition to a high antioxidant potential (GONZALEZ-AGUILAR et al., 2010).

Jamelão (*Syzygium cumini* L.) is a native tree of tropical regions, mainly from India, Malaysia and Australia (AYYANAR; SUBASH-BABU, 2012). Although not originally from Brazil, it has been extensively cultivated in several Brazilian states. Jamelão is a tropical fruit rich in anthocyanins and has a natural phytochemical source with pharmacological properties (AYYANAR; SUBASH-BABU, 2009; AYYANAR; SUBASH-BABU; IGNACIMUTHU, 2013). Despite the great bioactive potential of jamelão, there is no

established market for this fruit's products in Brazil. Therefore, it is necessary to develop technological processes that allow the use of the fruit as a food ingredient.

Among the existing techniques, encapsulation is a method that follows the cellular model, in which a semipermeable membrane that protects it from the external environment and at the same time controls the entry and exit of substances from the cell, wraps the nucleus. The main purpose of encapsulation is to protect the core material from adverse conditions, such as unwanted light and oxygen effects, which contribute to increase shelf life, by slowing down changes that may result in loss of taste, color and nutritional value. In addition to this, it promotes controlled release of encapsulated matrix material. In this way, it is possible to increase the useful life of the product and the gradual release of the encapsulated compound (DEPYPERE, 2003; FANG; BHANDARI, 2010; NEDOVIC et al., 2011).

The process of producing capsules by ionic gelling is simple and inexpensive, and it occurs by binding a hydrocolloid with ions. The reaction occurs between an aqueous polymer solution containing the nutrients and an ionic solution in which the oppositely charged polyelectrolytes interact forming a complex; for example, between alginate or pectin with ions, such as calcium (MÜLLER; SANTOS; BRIGIDO, 2011).

It has been feasible to use the encapsulation technique to provide functional components such as nutraceuticals, antimicrobials, flavorings, pigments, acidulants, plant extracts, enzymes, probiotics and preservatives in edible capsules (LIN et al., 2015; GHORBANZADE et al., 2017). In this way, the bioactivation of products is accomplished by the inclusion of functional ingredients in other foods, enriching the final product. The objective of this research was to obtain capsules of the jamelão pulp by means of the ionic gelation process and to add them to the yogurt, in order to study the fruit's physical-chemical properties, to quantify the bioactive compounds and its antioxidant capacity.

Milk and jamelão (*Syzygium cumini*) were purchased from the market of Aracaju (SE). The pulp of jamelão was obtained by crushing in the laboratory of Food Analysis (LAA) of the Federal University of Sergipe (UFS). The commercial culture of yogurt (BV-Bela Vista, YOG-03, Brazil) consisted of *Lactobacillus ssp. bulgaricus* and *Streptococcus ssp. thermophilus*. The reactants, 2,20-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4, 6-Tri (2-pyridyl) -s-triazine (TPTZ), ferric chloride, ferrous sulphate, folin-Ciocalteu and gallic acid were

obtained from Sigma Aldrich. Sodium acetate, sodium carbonate, and potassium chloride were from Synth.

For the ionic gelling process, the dripping methodology with a Caviar Box® kit was used, making use of two solutions: (1) a solution composed of 2% homogenized jamelão pulp and sodium alginate (m/v) with the aid of a mixer and (2) an aqueous solution of 1% calcium chloride. Solution 1 was sucked into the Caviar Box® kit and dripped onto solution 2. The formed capsules were drained and immersed in a vessel with water, to remove the residue from solution 2. After washing, the capsules were packed in sterile 100 mL acrylic packs.

For the preparation of the starting culture, sterilized reconstituted skimmed powdered milk (12% w/v) with 0.1% culture were inoculated and incubated at 41°C, with pH 5.3. The natural yogurt was processed using 10 L of whole milk and 4% of skimmed powdered milk mixed after homogenization and heated to 90° C for 5 minutes for pasteurization of ingredients. It was then cooled to 43° C for the addition of the inoculum. After the addition of milk inoculum, it was aseptically transferred into the capsule containing the packages, and incubated at 41°C until the pH 4.6 was reached. Yogurt fermentation reached after cooling until 4°C. The product was sealed and stored at 4±1° C for 28 days. The proportion used was 80% yogurt and 20% jamelão pulp capsules in each container. The treatments were the yogurt control, the capsules of jamelão, and the bioactivated yogurt with the capsules.

Physico-chemical analyzes were performed to characterize the pulp and the capsule, as well as the final product (bioactive yogurt) during days 1, 14 and 28 of storage. For the bioactivated yogurt, the analyzes were carried out separately of the capsules and yogurt, due to the study of migration of compounds between the two components of the product.

The lipid content of the yogurt was determined by the Gerber method. The protein was calculated as a function of the total nitrogen contents determined by the micro Kjeldahl method, multiplied by the correction factor 6.38. The analysis of reducing sugars in lactose followed the manual of techniques described by the Instituto Adolfo Lutz (2005). Changes in pH during fermentation and storage were monitored using a digital pH meter (Ion, PHB-500-BI). The acid content of the yogurt was determined by ISO11869 (1997) and the jamelão pulp according to the AOAC method N° 22.058 (1996) and expressed as g of citric acid/100 g. For the determination of soluble solids, an Abbé bench refractometer was used and the result was expressed in °Brix. For the ash, the method described by the AOAC (1996) was used. The moisture content was determined by the direct oven drying method at 105°C (INSTITUTO ADOLFO LUTZ, 2005). The calcium content was expressed as mg/100 g of the sample according to Silva (1990).

The preparation of extracts for the analysis of antioxidants and phenolic compounds proceed as it

follows: the samples were made by precipitating the yogurt proteins with 20% trichloroacetic acid (ATA) (ZULETA et al. 2009). We used a ratio of 1:1 (v/v) yogurt and 20% ATA in water. After stirring for 30 seconds, the samples were incubated in a heating bath at 42°C for 10 minutes, then centrifuged for 15 minutes at 25°C. The supernatant was used for the analysis of phenolic compounds, anthocyanins and antioxidant activity.

For the determination of the total phenolic compounds, the spectrophotometric method adapted from Swain and Hillis (1959) was used. From each extract, a 150 µL aliquot was mixed with 2.4 mL of distilled water and 150 µL of 0.25 N Folin-Ciocalteu, and kept at rest for 30 minutes at room temperature. Then, 3.0 mL of 1N Na<sub>2</sub>CO<sub>3</sub> was added. The samples were incubated in the dark at 23°C for 2 hours and the absorbance was measured at 725 nm using a spectrophotometer (Rayleigh UV-2601). The concentration of the phenolic compounds was calculated using a standard curve of gallic acid (GAE) and expressed as mg equivalent of gallic acid/100 g of sample.

Total monomeric anthocyanins were measured by the differential pH method (GIUSTI; WROLSTAD, 2003). The results were expressed as mg eq. Cyanidine 3-glycoside/100 g, according to the dilutions made at each determination.

The method of capture of the ABTS • + radical was carried out as described by Embrapa (2007). The preparation of the solution of the ABTS • + radical was carried out from the reaction of 5.0 mL of the ABTS aqueous solution (7 mM) with 88 µL of the potassium persulfate solution 140 (mM), protected from light and at room temperature by 16h. The ABTS • + radical was diluted in ethanol until absorbance of 0.700 ± 0.05 at wavelength equal to 734 nm was obtained. After addition of 30 µL of the extract to 3.0 mL of the ABTS • + solution, homogenization and rest of 6 minutes, the absorbance at 734 nm was measured in a spectrophotometer (Rayleigh UV-2601). A calibration curve was constructed using Trolox (0-2000 µmol/L).

The antioxidant capacity of the sample was calculated in relation to the activity of the Trolox antioxidant (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid), under the same conditions, and the results were expressed in antioxidant activity equivalent to Trolox (µmol Trolox/g sample).

The FRAP (Ferric Reducing Ability Power) assay was performed according to the methodology described by Benzie; Strain (1996) with modifications. The FRAP solution was prepared with the mixture of solutions of acetate buffer (300 mmol/L, pH 3.6), TPTZ (10.0 56 mmol/L) and FeCl<sub>3</sub> (20.0 mmol/L) in 10:1 ratio respectively, and then warmed under light to 37°C until use.

After that, 100 µL of the extract, 300 µL of distilled water and 3.0 mL of the FRAP reagent were incorporated and remained for 30 minutes at 37° C in the dark. The absorbance was measured in comparison with a white

tube at 593 nm. The calibration curve was obtained by aqueous solutions of known concentrations of Fe (II) in the range 0-2000 µmol/L (FeSO<sub>4</sub>·7H<sub>2</sub>O).

Ascorbic acid was determined according to AOAC (1984) method N° 43.065, modified by Benassi and Antunes (1988), in which it replaces the extraction solution of metaphosphoric acid with oxalic acid. The analyzes were repeated three times, each parameter was performed in triplicate and the results were expressed with the mean and standard deviation. The results obtained in the laboratory analyses were submitted to ANOVA, considering three treatments (control yogurt, capsules and bioactive yogurt) and five storage times (1, 7, 14, 21 and 28). A complete factorial scheme was represented using the comparative procedure multiple LSD from the SAS (Statistical Analysis System) 9.1 software package (SAS Institute Inc., Cary, NC, USA). The Tukey test was used to compare the averages, considering a significant difference at 5%.

The yield of the jamelão capsules was 76.13%, due to the losses of the product in the Caviar Box® kit, which retained the pulp. Also, because of the deformation of some capsules, they that had to be discarded as a result of their differences from what was established (standard spherical). Pagani et al. (2014), microencapsulated papaya through the ionic gelation method and observed a yield of 75.95% (*Carica papaya*), whereas Quispe-Condori et al. (2011) microencapsulated flaxseed oil and concluded that spray drying processes provided higher encapsulation efficiency (93.26%) than lyophilization (59.63%).

The chemical composition parameters evaluated are within the normal range as established for whole yogurts according to the Identity and Quality for Fermented Milks (Brasil, 2007). In relation to the composition of the formulated yogurts, there was a significant difference (p<0.05) only for salts, mainly due to the addition of jamelão pulp capsules in bioactive yogurt, increasing the number of salts in 19.24% (Table 1).

In relation to analyzes related to jamelão fruit products (pulp and capsules) there was a difference (p<0.05) between the studied variables. Moisture content for jamelão capsules was higher than that of the pulp and this increase is mainly due to the dilution of the pulp to obtain the ideal concentration of the alginate to form the capsules. The values for the jamelão pulp found by Mussi et al. (2015) and Tavares et al. (2016) were 85.70% and 87.08% respectively, and the results are close to those obtained in this study.

Regarding acidity and pH, the results presented values of 0.57 to 0.78 and 3.6 to 3.89 concomitantly for pulp and jamelão capsules. These values are in agreement with Morais et al. (2015),

who analyzed passion fruit microcapsules, obtaining acidity of 0.88 and 3.61 for pH. It is important to control pH, as extreme pH variations degrade the alginate polymer, resulting in loss of gel properties (PASIN; AZÓN; GARRIGA, 2012).

In relation to the ash content, the capsules presented a higher amount of ash (Table 1). Certainly, not only the alginate film formed during the ionic gelling, but also the calcium chloride incorporated ashes into the final product. This fact also justifies a greater amount of calcium in the capsules presenting similar behavior. The amount of

soluble solids in the pulp (11.53) was higher in relation to jamelão capsules (8.4). This fact has its bases on the correction of the ideal concentration of alginate in the pulp to encapsulate, due to a change in the chemical properties, with an increase of moisture and reduction of solids. According to Morais et al. (2015), the decrease of the soluble solids content during the ionic gelation process in fruits occurs as a result of leaching during the immersion in calcium chloride solution and then in the water, to remove the excess of calcium in the capsule.

**Table 1.** Physical-chemical characterization of control yogurt, bioactive yogurt, pulp and jamelão capsules.

Chemical composition	Control yogurt	Bioactive yogurt
Fat%	4.02 ± 0.11	4.03 ± 0.45
Protein%	4.64 ± 0.13	4.51 ± 0.09
Lactose%	4.11 ± 0.04	4.30 ± 0.13
Salts%	1.04 ± 0.02 <sup>b</sup>	1.24 ± 0.10 <sup>a</sup>
Calcium (mg / 100g)	109.37 ± 1.36	119.66 ± 1.07
	Jamelão pulp	Capsules of Jamelão
Moisture %	82.30 ± 0.97 <sup>b</sup>	87.10 ± 1.47 <sup>a</sup>
Acidity (g citric acid / 100g)	0.57 ± 0.01 <sup>b</sup>	0.78 ± 0.02 <sup>a</sup>
pH	3.60 ± 0.06 <sup>b</sup>	3.89 ± 0.28 <sup>a</sup>
Ashes (%)	0.34 ± 0.01 <sup>b</sup>	1.01 ± 0.05 <sup>a</sup>
Soluble Solids (°Brix)	11.53 ± 0.35 <sup>a</sup>	8.40 ± 0.10 <sup>b</sup>
Calcium (mg / 100g)	0.35 ± 0.01 <sup>b</sup>	124.98 ± 1.64 <sup>a</sup>

Means followed by the same letter in the row do not differ statistically from each other. The Tukey test was applied at a 5% probability level. Results are expressed as mean and standard deviation.

Antioxidant activity analyses were determined every seven days. The results during storage are presented in Table 2. There was significant interaction ( $p < 0.05$ ) for antioxidant activity and the compounds analyzed. Trolox equivalent antioxidant capacity was determined by the capture of the ABTS • + radicals and iron reduction (FRAP) of the bioactive yogurt increased during storage, presenting results equal to 289.37 at the initial time, and 754.68  $\mu\text{mol}$  Equiv. trolox/100g at 28 days, while  $\text{Fe}_2\text{SO}_4/100\text{g}$  increased from 97.69 to 722.10 at the 28<sup>th</sup> day, which means a statistical difference between them ( $p < 0.05$ ).

Farvin et al. (2010) reported that the high oxidative stability of yogurt is associated with antioxidant peptides released during milk fermentation by lactic acid bacteria. In relation to the bioactive yogurt, the capsules of jamelão were incorporated. Through the migration of the bioactive compounds, they presented greater activity in comparison with the control, and were able to increase

by 156.96% the sequestering ability of the ABTS • + radical and 639.17% the iron reduction potential at the 28<sup>th</sup> day of analysis (Table 2). A study by Chouchouli et al. (2013) also reported increasing antioxidant activity of yogurt supplemented with grape seed extract during storage.

**Table 2.** Characterization of formulated yogurt extracts in relation to antioxidant activity (ABTS and FRAP) during storage.

Parameters	Treatment	Storage period (days)					p-Value		
		1	7	14	21	28	T	S	T*S
ABTS $\mu\text{mol}$ TE/100g	Control	289.37 $\pm$ 0.18 <sup>cc</sup>	291.60 $\pm$ 0.97 <sup>cc</sup>	298.12 $\pm$ 0.66 <sup>ac</sup>	293.98 $\pm$ 0.81 <sup>bc</sup>	290.76 $\pm$ 0.71 <sup>cc</sup>	<.0001	<.0001	<.0001
	Capsules	753.63 $\pm$ 0.22 <sup>ba</sup>	723.66 $\pm$ 0.35 <sup>da</sup>	740.78 $\pm$ 0.90 <sup>ca</sup>	493.34 $\pm$ 1.03 <sup>cb</sup>	756.22 $\pm$ 0.95 <sup>aa</sup>			
	Bioactive	293.69 $\pm$ 1.15 <sup>cb</sup>	519.78 $\pm$ 0.52 <sup>cb</sup>	517.47 $\pm$ 1.03 <sup>db</sup>	729.95 $\pm$ 0.63 <sup>ba</sup>	754.68 $\pm$ 0.42 <sup>ab</sup>			
FRAP $\mu\text{mol}$ Fe <sub>2</sub> SO <sub>4</sub> /100g	Control	99.51 $\pm$ 0.22 <sup>bb</sup>	76.08 $\pm$ 1.06 <sup>cc</sup>	127.36 $\pm$ 0.69 <sup>ac</sup>	82.17 $\pm$ 0.22 <sup>dc</sup>	90.19 $\pm$ 0.73 <sup>cc</sup>	<.0001	<.0001	<.0001
	Capsules	749.33 $\pm$ 0.45 <sup>ba</sup>	665.21 $\pm$ 0.95 <sup>ca</sup>	687.71 $\pm$ 0.67 <sup>db</sup>	770.87 $\pm$ 0.86 <sup>ab</sup>	701.06 $\pm$ 1.12 <sup>cb</sup>			
	Bioactive	97.69 $\pm$ 0.45 <sup>cb</sup>	468.61 $\pm$ 0.87 <sup>db</sup>	798.63 $\pm$ 0.86 <sup>aa</sup>	783.82 $\pm$ 1.03 <sup>ba</sup>	722.10 $\pm$ 1.30 <sup>ca</sup>			

Means with different lowercase letters on the same row differ statistically ( $p < 0.05$ ). Means with different capital letters in the same column differ statistically ( $p < 0.05$ ). The Tukey test was applied at 5%. Results are expressed as mean and standard deviation. T- treatment, S- Storage period (days).

The antioxidant capacity of the bioactive yogurt increased linearly from the seventh day of storage, demonstrating the excellent antioxidant capacity of jamelão, especially in longer storage periods, as it can be seen on the 28<sup>th</sup> day, when it obtained greater expressiveness.

The determination of the antioxidant activity of fruits is the result of the cumulative, and often synergistic, action of all the antioxidants present in the fruit extract. However, antioxidant activity is not related to the concentration of phenolic compounds, but to the type of such phenolic compounds, that is, specific phenolic compounds present at higher concentrations in a sample are not necessarily those with the highest antioxidant potential.

The number of phenolic compounds decreased with storage time for the control treatment, however with the addition of the capsules in the bioactive yogurt it increased by 55.20% GAE/100g at 28 days of storage. In contrast, Vital et al. (2015) found lower values when adding 0%, 0.5%, 1.0%, 1.5% and 2% concentrations of *Pleurotus ostreatus* to yogurt and observed that, at 28 days of storage, the levels of polyphenols ranged from 2.04 to 4.36 mg GAE/100 g, and as the amount of substrate increased, the number of polyphenols also increased, but did not change during storage. Bezerra et al. (2015) studied frozen goat yogurt produced with pulp and jamelão powder and verified that the addition of pulp had a higher content of phenolics and anthocyanins compared to powder.

**Table 3.** Characterization of formulated yogurt extracts in relation to phenolic compounds, anthocyanins and vitamin C during storage.

Parameters	Treatment	Storage period (days)					p-Value		
		1	7	14	21	28	T	S	T*S
Phenolic compounds mgGAE/100g	Control	3.19 $\pm$ 0.18 <sup>ac</sup>	2.55 $\pm$ 0.11 <sup>ac</sup>	2.64 $\pm$ 0.46 <sup>ac</sup>	1.83 $\pm$ 0.28 <sup>ac</sup>	1.95 $\pm$ 0.63 <sup>ac</sup>	<.0001	<.0001	<.0001
	Capsules	23.78 $\pm$ 0.66 <sup>aa</sup>	21.36 $\pm$ 0.54 <sup>ba</sup>	15.96 $\pm$ 0.96 <sup>ca</sup>	12.83 $\pm$ 0.85 <sup>db</sup>	22.27 $\pm$ 0.92 <sup>aba</sup>			
	Bioactive	12.11 $\pm$ 0.73 <sup>cb</sup>	11.95 $\pm$ 0.42 <sup>cb</sup>	13.10 $\pm$ 0.57 <sup>cb</sup>	17.13 $\pm$ 0.83 <sup>ba</sup>	19.68 $\pm$ 0.24 <sup>ab</sup>			
Anthocyanin cyanidin mg/100g	Control	0.07 $\pm$ 0.03 <sup>ab</sup>	0.13 $\pm$ 0.02 <sup>ac</sup>	0.10 $\pm$ 0.07 <sup>ac</sup>	0.30 $\pm$ 0.38 <sup>ab</sup>	0.53 $\pm$ 0.18 <sup>ab</sup>	<.0001	<.0001	<.0001
	Capsules	26.08 $\pm$ 0.52 <sup>aa</sup>	26.60 $\pm$ 0.75 <sup>aa</sup>	9.50 $\pm$ 0.89 <sup>ba</sup>	10.46 $\pm$ 0.28 <sup>ba</sup>	9.70 $\pm$ 0.08 <sup>ba</sup>			
	Bioactive	0.12 $\pm$ 0.01 <sup>db</sup>	6.29 $\pm$ 0.60 <sup>cb</sup>	8.00 $\pm$ 0.68 <sup>bb</sup>	11.61 $\pm$ 0.75 <sup>aa</sup>	10.53 $\pm$ 0.21 <sup>aa</sup>			
Vitamin C mgAa/100g	Control	13.61 $\pm$ 0.33 <sup>ab</sup>	11.30 $\pm$ 1.08 <sup>bc</sup>	11.57 $\pm$ 0.43 <sup>abc</sup>	10.75 $\pm$ 0.32 <sup>bc</sup>	10.02 $\pm$ 0.02 <sup>bc</sup>	<.0001	<.0001	<.0001
	Capsules	36.30 $\pm$ 0.65 <sup>aa</sup>	29.38 $\pm$ 1.10 <sup>ba</sup>	27.76 $\pm$ 0.47 <sup>bca</sup>	26.16 $\pm$ 0.82 <sup>cb</sup>	27.52 $\pm$ 1.10 <sup>bcB</sup>			
	Bioactive	13.81 $\pm$ 0.41 <sup>cb</sup>	20.02 $\pm$ 0.57 <sup>bb</sup>	32.00 $\pm$ 0.99 <sup>aa</sup>	32.70 $\pm$ 1.19 <sup>aa</sup>	33.63 $\pm$ 0.82 <sup>aa</sup>			

Means with different lowercase letters on the same row differ statistically ( $p < 0.05$ ). Means with different capital letters in the same column differ statistically ( $p < 0.05$ ). The Tukey test was applied at 5%. Results are expressed as mean and standard deviation. T- treatment, S- Storage period (days).

The capsules of jamelão present a high value of antioxidant activity, mainly due to the amount of flavonoids and phenolic acids present in the fruit. This activity can be verified under cooling conditions, with the preservation of the active compounds even after being processed. Anthocyanin is the highest phenolic compound, and fruit pulp presented  $113.30 \pm 0.85$  mg of Cyanidin 3-glycoside/100 g. This high value is responsible for the intense blue-purple coloring and it is

responsible for the change in the yogurt color during refrigerated storage.

After the capsules of jamelão were processed, there was a reduction of the anthocyanins (76.98%). This decrease happened at the moment of the spherification process to form the capsules, since in this stage there is loss of the pigments in the solutions of calcium chloride and washing in water. However, there was migration of this flavonoid to the bioactive yogurt the 28<sup>th</sup> day of

storage as observed in (Table 2). Some authors report several factors that interfere with the stability of anthocyanins. Conditions such as pH, chemical structure, storage temperature, exposure to oxygen and light, proteins, solvents, enzymes, and metal ions can affect the stability of these pigments, thus they easily degrade during food processing and storage (CASTAÑEDA-OVANDO et al. 2009). Kuck and Noreña (2016) observed a reduction in the phenolic compounds content of the extracts using spray dried and lyophilized grapefruit.

Modesto Junior et al. (2016) found higher values of anthocyanins when making Greek yogurt from buffalo milk with different concentrations of *Eugenia uniflora* syrup. The concentration of anthocyanins increased significantly with the increase of the pulp concentration of the added syrups with respective values of 39.39 mg/100g, 49.80 mg/100g and 53.77 mg/100g at concentrations of 10, 20 and 30%. Arrazola, Herazo and Alvis (2014), on the other hand, when working with microencapsulation of eggplant anthocyanins (*Solanum melongena L.*) by atomized drying with maltodextrin, concluded that it was efficient to retain the bioactive compound in isotonic beverages and beverages with aloe vera stored for 40 days at 4°C.

According to the results for the ascorbic acid content, there was a significant difference ( $p < 0.05$ ). The control treatment and the capsules decreased during storage, however, the bioactive yogurt increased. There was migration of 7mg/100g of ascorbic acid from the capsules of jamelão to the bioactive yogurt on the seventh day of analysis, increasing by 143.51% at the 28<sup>th</sup> day. Modesto Junior et al. (2016) verified an increase of 453% of ascorbic acid in the formulation with higher concentration of pulp in relation to the control formulation.

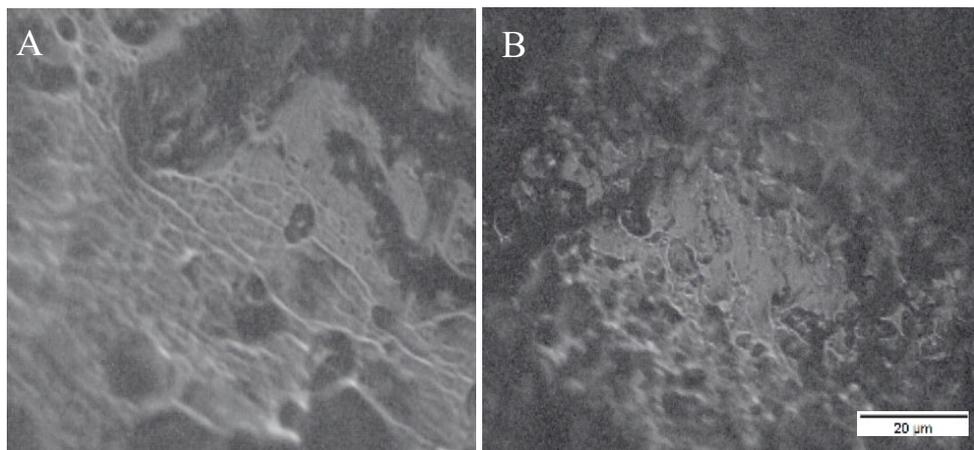
Santa Rosa (2015), when producing yogurt of buffalo milk with fruit pulps from the Amazon, found a variation during storage of 66.27 to 34.43 mg/100g of

ascorbic acid for yogurt with cupuaçu (*Theobroma grandiflorum*) + camu-camu (*Myrciaria dubious*) and 17.27 to 14.30 mg/100g of ascorbic acid for yogurt with cupuaçu.

The total loss of ascorbic acid during storage for the control yogurt was 73.62% at 28 days of storage. This value of loss is close to the percentage reported by Maeda et al. (2007), and it can reach up to 80%. Moreover, factors such as pH, temperature and storage time influence the final product. According to Sun et al. (2002), vitamin C is the major contributor to antioxidant activity in fruits.

Optical microscope images of the microcapsules are shown in Figure 1. The morphology of the microcapsules presented variations in terms of size and shape, but the size was set in 4.66 mm for the capsules that had spherical shape with a smooth surface, to apply to the yogurt. This smooth and spherical structure of microencapsulates has also been observed by some authors like Chun, Kim and Cho (2014), when they were studying the microencapsulate *Lactobacillus plantarum* DKL 109 by ionic gelation, and Caparino et al. (2012), when they were investigating microencapsulation by spray dryer using maltodextrins in mango extracts. According to Lee et al. (2004), the alginate microparticles generally have a heterogeneous structure with a dense surface layer and a loose nucleus due to the heterogeneous gelation mechanism.

The interior of the microcapsules was composed of a mesh structure of wall materials through which the encapsulated material is distributed (Fig. 1A). Differences in structures were observed during storage, in which, at low temperature, there are retrograde sugar molecules that tend to get closer, expelling the contents of their envelope to the external environment. (Fig. 1B). This fact can also be justified by the migration of compounds.



**Figure 1.** Surface differences, in relation to the roughness, of the microcapsules: A- image on the first day; B- image at the 28<sup>th</sup> day. Presented by micrographs with magnification of 100x, in optic microscope.

Adding jamelão capsules to the yogurt altered the physical properties of the product and it was possible to increase the number of phenolic compounds, as well as

the antioxidant potential at 28 days of storage. The addition of jamelão pulp capsules in yogurts proves to be a promising and viable alternative, since the bioactive

compounds present in the fruit can migrate to the product in a way to make it more nutritious.

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**RESUMO:** A fruta Jamelão tem sido usada na medicina tradicional indiana e recentemente atrai interesse como alimento funcional, por ser rica em antocianinas. As antocianinas são de interesse da indústria de alimentos devido ao seu poder antioxidante, cor atraente e estabilidade em alimentos ácidos. Esta pesquisa utilizou o processo de gelificação com solução de alginato de sódio para obter iogurte bioativo, a partir da produção de cápsulas de jamelão adicionadas em iogurte natural. A proporção utilizada foi de 80% de iogurte e 20% de cápsulas de polpa de jamelão. Os tratamentos foram iogurte controle (sem adição de cápsulas de jamelão), cápsulas de jamelão e iogurte bioativo (contendo as cápsulas). O objetivo foi estudar a atividade antioxidante, a estabilidade físico-química, nutricional e microscópica do produto mantido sob refrigeração por 28 dias a  $4 \pm 1$  °C. A adição de cápsulas de jamelão no iogurte alterou as propriedades físicas do produto (aumento de umidade, diminuição de Brix e cinzas). Houve aumento na quantidade de fenóis e antocianinas, além do potencial antioxidante aos 28 dias de armazenamento. O interior das cápsulas era composto por uma estrutura em malha através da qual o material encapsulado foi distribuído. Desta forma, as cápsulas podem ser um ingrediente adicionado ao iogurte, melhorando a capacidade antioxidante e nutricional, provando ser uma alternativa promissora e viável.

**PALAVRAS- CHAVE:** Antioxidantes. Composição. Gelificação iônica.

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