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Nanofibers of Jussara Pulp: A Tool to Prevent the Loss of Thermal Stability and the Antioxidant Activity of Anthocyanins after Simulated Digestion

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Abstract: Electrospinning can produce a new composite for coating sensitive bioactive compounds, such as anthocyanins, and the product obtained from this process presents characteristics that potentialize the application of natural pigments in foodstuffs. The present work aimed to develop a new nanofiber composite with incorporated anthocyanins from jussara pulp using polyethylene oxide through electrospinning. A decay in the percentage of anthocyanins during digestion was observed. However, the polymeric solution and composites produced maintained the antioxidant activity, showing their protective effect on bioactive compounds; furthermore, both nanofibers and polymer solution improved the thermal stability of the anthocyanins. Thus, the results obtained potentiate electrospinning composites in processed food products since the nanofibers presented superior thermal stability and antioxidant activity, even after the digestion process in vitro.

Keywords: biodiversity; anthocyanins; bioaccessibility; bioactive compounds; nanotechnology

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1. Introduction

Jussara pulp, abundant in phenolic compounds, mainly anthocyanins, is the fruit from the palm tree *Euterpe edulis* Mart., belonging to the biodiversity of the Brazilian Atlantic Forest, being very similar to açaí berry (*E. oleracea*) in terms of composition [1,2]. In recent decades, the consumption of this fruit has been incentivized by government agencies and nonprofit organizations aiming to promote conscious and sustainable extraction, consequently benefiting local producers, while promoting the conservation of biodiversity [3,4].

The anthocyanin content in jussara pulp is attractive not only for its intense color, which can be potentially exploited to replace artificial dyes, but also for its beneficial effects on human health, highlighted by its antioxidant properties [1,2,5–9]. Nonetheless, the efficacy of those positive effects, as well as the potential for application as a natural pigment in foodstuffs, depends on the stability and bioavailability of the anthocyanins, which are highly sensitive to light, pH, oxygen, and temperature [7,10].

Furthermore, color is an important attribute related to the visual appeal and the quality of food products. Thus, an excessive loss of color during thermal processing compromises sensory acceptance and product commercialization. To evaluate the potential of jussara anthocyanins as a food ingredient providing natural pigments, kinetic parameters, such as the reaction order, reaction constant, and activation energy, can supply information on the change in the quality of any food colored with jussara that must undergo thermal processing [11].

The kinetics of color degradation have been often, but not exclusively, considered as following a first-order reaction. It is also crucial to determine the thermodynamic parameters because the industrial use of these compounds requires as much information as possible when considering the thermal degradation of color [12,13]. To date, although the thermostability of anthocyanins in solution has been studied, no report about the behavior of the thermal degradation of anthocyanins incorporated into nanofibers is available.

Additionally, the digestion process has an important influence on the absorption of bioactive compounds since constant changes in pH occur, causing modifications in the behavior of anthocyanins [14,15]. Therefore, several works have been carried out to assess the possibilities of protecting bioactive compounds, not only due to their application as ingredients, but also to evaluate their uptake behavior in order to ensure their effectiveness. One of the most recent and efficient alternatives is the use of nanotechnology, mainly the electrospinning technique [16–19].

The electrodynamic process of electrospinning uses the comprehension of nanotechnology to obtain nanomaterials with distinguishing characteristics and features. The polymeric solution containing anthocyanins, or any other bioactive compound, is ejected after using a high electric potential; this way, stable nanofibers are obtained with a uniform size and distribution. Nanofibers are defined as long polymeric filaments possessing a large surface area concerning volume. Depending on the polymer used to produce the nanofibers, they can present distinct characteristics regarding elasticity, porosity, and mechanical resistance [20]. Among the polymers reported in the literature, PEO is biodegradable and hydrophilic, has a high molecular weight, is certified as Generally Recognized as Safe (GRAS) (FDA UNII 16P9295IIL) [21], and has been largely used to incorporate bioactive compounds in nanostructures. There is also an interest in designing nanostructures to release the incorporated bioactive compound in the intestine, where it may be absorbed into the bloodstream.

The step prior to incorporation in electrospun nanostructures involves the preparation of the solution containing the bioactive compound and the polymer of choice. The polymeric solution itself can also be considered as an option to protect and maintain the thermal stability of the compound, even after the simulated digestive process [12,22,23].

Within this context, the present study aimed to verify the antioxidant activity of anthocyanins from jussara pulp (JP), the polymeric solution with pulp (JPP), and also nanostructures with the pulp (JN), as produced by the electrospinning technique after simulated digestion.

2. Materials and Methods

2.1. Jussara Pulp

The producers who are linked to the Jussara Project from Ubatuba, São Paulo, Brazil, provided the jussara pulp (JP) used in the present work. The frozen pulp was transported in coolers, freeze-dried, and stored in hermetically sealed packaging in a freezer (−40 °C) until the analysis. Before preparing the polymeric solution, acetate buffer (pH 4.5) was utilized to reconstitute the lyophilized jussara pulp; after that, it was filtered.

2.2. Production of PEO-Jussara Composites Using Electrospinning

The polymeric solution (JPP) was constituted by adding 7.7% (*w*/*v*) of PEO (900,000 g·mol·L^{-1}, Sigma Aldrich, St. Louis, MO, USA), 0.36% (*w*/*v*) of NaCl, and reconstituted JP and acetate buffer. A magnetic stirrer was utilized to homogenize this mixture at room temperature (20 °C). Then, the electrospinning process was conducted on a laboratory scale (FLUIDNATEK LE-10, BIOINICIA, Valencia, Spain) following these parameters: a steel needle of 0.6 mm diameter, a flow rate of 3000 µL·h^{-1}, 24 kV of voltage, and a length of 10 cm from tip to collector [18,24]. The JPP was utilized to produce nanostructures, quantify anthocyanins, and determine antioxidant activity. Also, after the JN samples were produced, a characterization of the material was performed [24] using field emission scanning electron

microscopy (FE-SEM Supra 35 VP- equipment, Carl Zeiss, Aalen, Germany) to obtain the micrographic images of the samples (Figure 1).

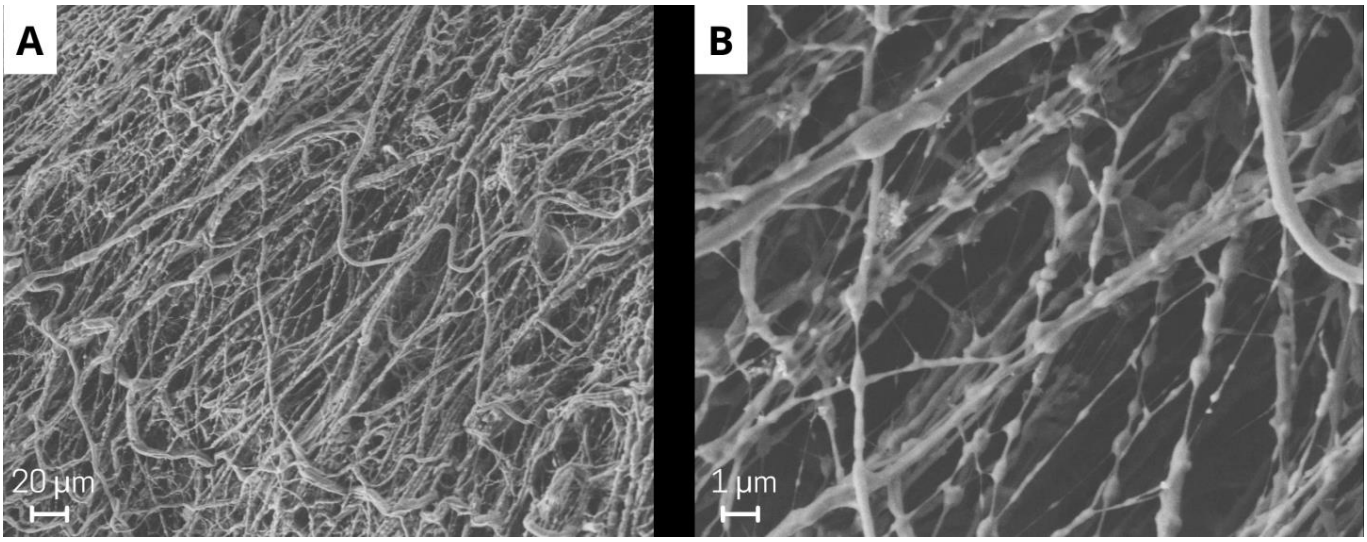

Figure 1. FE-SEM images of electrospinning jussara nanofibers, scale bars 20 µm (**A**) and 1 µm (**B**).

2.3. Bioaccessibility

The JP, JPP, and JN samples were submitted to an in vitro digestion process according to the method proposed by Chitchumroonchokchai and Failla [25]. The simulated digestion began with the homogenization of 2 g of each sample with 10 mL of salt solution (NaCl: 120 mol·L^{-1}, $CaCl_2$ 6 mmol·L^{-1}, and KCl 5 mmol·L^{-1}) and 6 mL of artificial saliva solution containing α-amylase (10^6 U·mL^{-1}) (Sigma® A3176). The oral phase ended with incubation in an orbital shaker at 150 rpm, 37 °C, for 10 min. Next, the gastric phase was initialized with the pH adjusted to 2.5 with HCl 1 M, 2 mL of pepsin (Sigma® P7000; 50,000 units·mL^{-1} in HCl 100 mM) were added, and then the volume was completed to reach 40 mL, and it was incubated at 37 °C, at 150 rpm, for 1 h. For the intestinal and final phase, the pH was changed to 6.0 with 1 M $NaHCO_3$, and then porcine and ovine bile solution (3 mL; Sigma® B8381; 40 mg·mL^{-1} in 100 mM $NaHCO_3$), 4000 U·mL^{-1} of porcine pancreatin (Sigma® P1750), and 1000 U·mL^{-1} of lipase from porcine pancreas (Sigma® L3126) were added to the samples, adjusting the pH to 6.5. The volume was completed until 50 mL before incubation at 37 °C, at 150 rpm, for 2 h. To obtain the supernatant with the bioaccessible anthocyanins, the last step was to centrifugate the samples for one h at 6000 rpm and 4 °C. Samples from each stage of digestion were separated to quantify the anthocyanins and evaluate the antioxidant activity.

2.4. HPLC Analysis of the Anthocyanins

Anthocyanins were extracted from the JP, JPP, and JN at each stage of the simulated digestion phases (oral, gastric, and intestinal). Using 75 mL of acidified methanol (0.5% HCl) and an ultrasonic probe at 80 W of potency for 3 min, a vacuum pump filtered the mixture, and a rotary evaporator was used to concentrate it at 38 °C. The extracts were diluted in water containing 5% formic acid/methanol (85:15, *v*/*v*) before the HPLC analysis since this blend is the mobile phase gradient. The anthocyanin separation and identification were conducted as presented by De Rosso and Mercadante [26]. The anthocyanins from all samples, in triplicate, were quantified by HPLC-DAD configured with the optimized conditions of chromatography with a C18 Shim Pack column at 28 °C, using seven-point analytical curves of cyanidin 3-glucoside (5–125 µg/mL) and cyanidin 3-rutinoside (10–200 µg/mL), r^2 = 0.998; the limit of detection was 0.05 mg·mL^{-1}, and the limit of quantification was 0.1 mg·mL^{-1}. The concentration was expressed in µg of cyanidin 3-glucoside/mL and/or µg of cyanidin 3-rutinoside/mL. The percentage of anthocyanin

content relative to the results found before in vitro digestion, called remainder (%), was calculated considering the final and initial values.

2.5. Antioxidant Activity

The preparation of the extracts, considering the same samples used to quantify the anthocyanins (JP, JPP, and JN), was conducted by adding 30 mL of 80% cold acetone, agitation in a magnetic stirrer for 15 min; filtering the mixture, repeating it twice, and then, concentrating it in a rotary evaporator at 40 °C. The antioxidant activity against the peroxyl radical ($ROO^{\bullet}$) was determined by an ORAC assay, which is based on monitoring the fluorescence decay through the effect of the hydrophilic extract or standard (Trolox), on those results from $ROO^{\bullet}$ induced oxidation of fluorescein [27]. This assay was performed in a 96-well microplate with fluorescein (61 µM) prepared in phosphate buffer 75 mM, pH 7.4, AAPH solution (19 mM) in phosphate buffer, hydrophilic extract, or Trolox (50 µM) in phosphate buffer. The microplate was preincubated for 10 min before adding AAPH; then, the fluorescence signal was monitored each minute via the reader (excitation: 485 ± 20 nm; emission: 538 ± 20 nm) for 1 h. The data presented as µmol of Trolox equivalent/g of sample. The antioxidant activity (AA) against the $ABTS^{+}$ radical was determined by reading the absorbance at 734 nm of the extract homogenized with a diluted solution of $ABTS^{+}$ (7 mM), and it was compared with a known Trolox standard curve [28]. The results were expressed as µmol of Trolox equivalent/g of the sample, and the percentage of antioxidant activity relative to the results found before in vitro digestion, called remainder AA (%), was calculated considering the final and initial values.

2.6. Thermal Stability

The degradation kinetics of JP, JPP, and JN were studied in triplicate in covered flasks at a constant temperature. The temperatures of 60 °C and 90 °C were studied to obtain the degradation rate constant (K_d) for each proposed condition. Aliquots were removed periodically until half of the initial absorbance of the samples was reached. The values for K_d obtained at each temperature were used to determine the half-life of each condition [12].

The K_d (min^{-1}) was estimated by regression of the experimental data on time. Assuming first-order reaction kinetics, K_d was determined according to Equation (1):

$$\frac{dAbs}{dt} = -K_d Abs \quad (1)$$

where Abs is the absorbance at 520 nm, t is time (s), and K_d is the degradation rate constant (min^{-1}).

The half-life values, $t_{1/2}$ (min), for the first-order degradation kinetic model, were given by Equation (2):

$$t_{1/2} = \frac{ln2}{K_d} \quad (2)$$

The Arrhenius equation relates the temperature to the constant for the speed of elementary reactions and allows for determining the activation energy and the frequency factor of degradation reactions, as expressed by Equation (3):

$$K_d = A_e^{-\frac{E_a}{RT}} \quad (3)$$

where K_d is a degradation rate constant (min^{-1}), A is the frequency factor (min^{-1}), E_a is the activation energy degradation reaction ($kJ \cdot mol^{-1}$), T is the temperature (K), and R is the gas constant (8.31 $JK^{-1} \cdot mol^{-1}$).

2.7. Statistical Analysis

All of the analyses were realized in triplicate samples; the results were expressed as the mean ± standard deviation (SD); to enable comparisons, ANOVA was utilized to detect differences between the samples followed by a Tukey test, and the differences were

considered to be significant at $p < 0.05$. Statistica 14.0 software was used to process the data analyses.

3. Results

3.1. Anthocyanin Bioaccessibility

To assess the beneficial properties to human health from the consumption of foods rich in phenolic compounds, especially anthocyanins, it is essential to verify the bioaccessibility of this natural pigment to understand its metabolism and then to plan future actions to improve its positive effects. Therefore, as mentioned before, the present work evaluated the bioaccessibility of jussara pulp anthocyanins using three different preparations (JP, JPP, and JN) after the in vitro digestive process. It is important to highlight that the analyses with the polymeric solution and during the digestive process are rarely assessed in the literature, so little is known about the behavior of this compound under these circumstances.

FE-SEM images from JN were obtained (Figure 1) to measure the diameters (nm), and the results confirmed that nanofibers were obtained since the average JN diameter was 120 ± 80 nm, which are in accordance with the results obtained by Ramos et al. [24] This also ensures that the electrospinning process is reproducible.

The JN was submitted to in vitro digestion, and Figure 2 shows the chromatograms of the anthocyanin profile of JP in its initial form and in each digestion stage: oral, gastric, and intestinal. It is essential to emphasize that the chromatograms of JPP and JN showed the same peak profiles. In the figure, it is possible to identify two main peaks: peak 1 refers to cyanidin 3-rutinoside, and peak 2 refers to cyanidin 3-glucoside, the major anthocyanins present in jussara pulp, as detected by da Silva et al. [3].

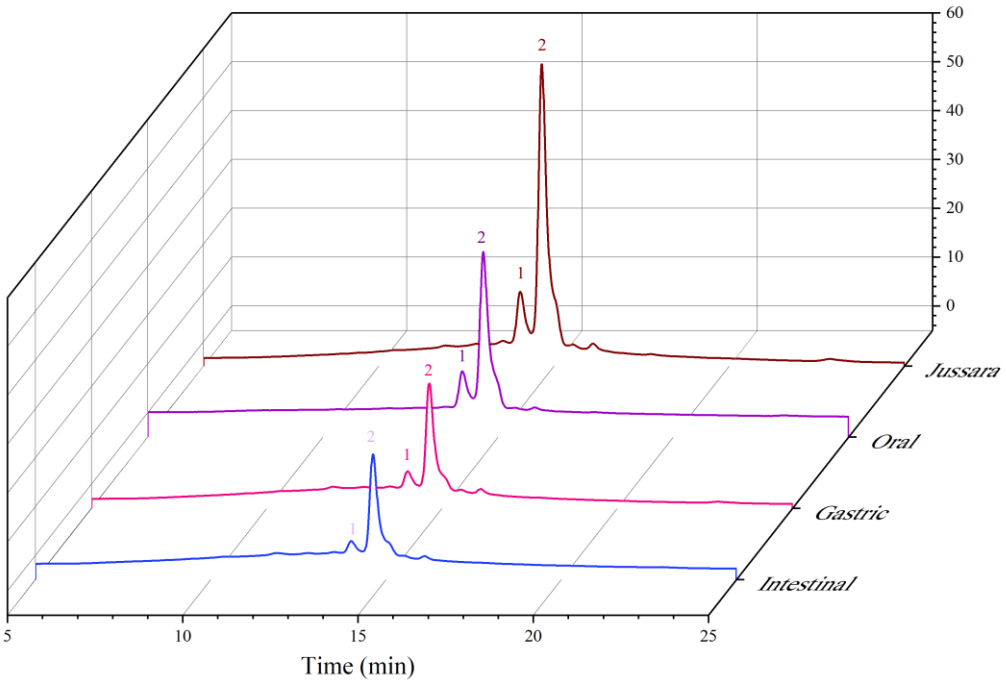

Figure 2. Chromatograms represent the anthocyanin profiles of the jussara pulp, initially and during the in vitro digestive process (oral, gastric, and intestinal). Peak 1 refers to cyanidin 3-rutinoside, and peak 2 refers to cyanidin 3-glucoside.

Based on the chromatograms, it is possible to observe a decrease in anthocyanin content throughout the simulated digestive process. Table 1 shows the anthocyanin content of each sample before, during, and after the bioaccessibility assay. Regarding the remaining percentage, it is possible to observe better maintenance of this compound in JN, with 17.9%, as compared to JP and JPP. These results indicate the potential protective effect of the nanostructure with jussara pulp. The oppositive is true for JPP, where it is not possible to

identify this potential behavior in the protection of this specific compound. On the other hand, in the following results for antioxidant activity, the polymeric solution was also able to maintain this activity, which contributes to the hypothesis of anthocyanin bioconversion.

Table 1. Determination of anthocyanins during the simulated digestion process (in vitro) of jussara pulp (JP), jussara pulp solution and PEO (JPP), and jussara pulp nanofibers (JN).

Digestion Steps	Anthocyanins (mg/100 g of Sample)					
	JP	Remain (%)	JPP	Remain (%)	JN	Remain (%)
Initial	215.20 ^{a} ± 10.89	100.0	5.97 ^{a} ± 0.05	100.0	4.92 ^{a} ± 0.21	100.0
Oral	58.44 ^{b} ± 4.75	27.2	1.16 ^{b} ± 0.01	19.5	1.64 ^{b} ± 0.21	33.4
Gastric	39.29 ^{c} ± 13.97	18.3	0.87 ^{c} ± 0.03	14.6	1.04 ^{c} ± 0.19	21.2
Intestinal	30.18 ^{d} ± 2.65	14.0	0.61 ^{d} ± 0.01	10.2	0.88 ^{c} ± 0.30	17.9

Different superscript letters on the same column represent values different from each other ($p < 0.05$).

3.2. Antioxidant Activity

As previously described, the antioxidant activity determination of the simulated digestion phases (oral, gastric, and intestinal) for JP, JPP, and JN had the purpose of monitoring the behavior of anthocyanin effects during this process, which involved several pH variations and enzymatic actions, thus enabling the comparison of the polymer at the end of digestion concerning the behavior of the jussara pulp. The results of both samples are shown in Table 2.

Table 2. Determination of antioxidant activity (AA) during the simulated digestion process (in vitro) of JP: jussara pulp; JPP: the solution of jussara pulp and PEO (PEO 7.7% and 0.36% NaCl); and JN: jussara nanofibers (PEO 7.7% and 0.36% NaCl).

Samples		Digestion Steps				
		Initial	Oral	Gastric	Intestinal	Remain AA (%)
ABTS (μM TE/g)	**JP**	121.5 ^{a,A} ± 8.1	86.0 ^{a,C} ± 5.7	104.7 ^{a,B} ± 4.3	72.3 ^{a,D} ± 2.9	59.5 ^{b}
	JPP	79.6 ^{b,A} ± 2.8	46.9 ^{b,D} ± 6.6	71.2 ^{b,B} ± 0.9	66.1 ^{b,C} ± 3.7	82.9 ^{a}
	JN	84.73 ^{b,A} ± 3.4	79.5 ^{a,A,B} ± 14.1	77.5 ^{b,B,C} ± 12.3	72.2 ^{a,b,C} ± 10.1	85.3 ^{a}
ORAC (μM TE/g)	**JP**	204.9 ^{a,B} ± 34.6	248.6 ^{a,A} ± 20.8	209.7 ^{a,B} ± 46.9	111.8 ^{a,C} ± 27.0	54.6 ^{b}
	JPP	199.8 ^{a,A} ± 29.2	210.1 ^{b,A} ± 28.5	189.7 ^{b,A} ± 30.1	135.7 ^{a,B} ± 30.1	64.4 ^{a}
	JN	201.0 ^{a,A} ± 28.5	153.6 ^{c,C} ± 22.8	182.7 ^{b,B} ± 12.9	151.0 ^{a,C} ± 25.9	75.1 ^{a}

Different superscript lowercase letters on the same line represent values different from each other ($p < 0.05$). Different superscript uppercase letters on the same column represent values different from each other ($p < 0.05$).

3.3. Thermal Stability

Researchers have studied the application of the first-order model to describe the degradation of bioactive compounds [12,29]. Using Equations (1) and (2), the K_d and $t_{1/2}$ values were obtained for the different temperatures and are presented in Table 3.

Considering the evaluated conditions (JP, JPP, and JN), the half-life values decreased when the temperatures increased, which was expected since the color of the bioactive compounds present in the jussara, particularly anthocyanins, are sensitive to heat [30]. The same behavior was observed by Braga et al. (2016), who evaluated another heat-sensitive pigment, C-phycocyanin, in solution and nanofiber formats with PEO at different temperatures (55, 60, 65, 70, and 75 °C). The aim of this analysis was to investigate the temperatures to which foodstuff products are submitted in the food industry, so as to evaluate the potential use of nanostructures to prevent antioxidant activity loss during processes such as pasteurization.

Table 3. Degradation rate constants (K_d) and the respective correlation coefficients (R^2) and half-life values ($t_{1/2}$) of the jussara pulp, jussara pulp and PEO solution, and jussara nanofibers for each temperature studied.

Samples	Parameters	Temperature (°C)	
		60	90
Jussara pulp	K_d **(min^{-1})**	0.000008	0.00008
	R^2	1	1
	$t_{1/2}$ **(min)**	86,643.4	8664.3
Jussara pulp and PEO solution	K_d **(min^{-1})**	0.000004	0.000038
	R^2	1	1
	$t_{1/2}$ **(min)**	173,286.8	18,240.7
Jussara nanofibers			

4. Discussion

Figure 3 presents a summary of the acquired information that will be discussed. Considering the composites obtained, the diameters (nm) were measured, and nanofibers were obtained since the average JN diameter was 120 ± 80 nm and are therefore in accordance with the results obtained by Ramos et al. [24] This also ensures that the electrospinning process is reproducible, as can be seen in Figure 1. It was also possible to observe the formation of nodes or beans as already reported in the literature. The molecular weight and concentration of the solution used in electrospinning interfere with the stretching of the fibers during the process, and the formation of a continuous jet is important for uniformity in the morphology of the fibers. However, the stability of the jet can be affected during the process, either with the formation of small air bubbles in the solution or even with small oscillations in the applied voltage, causing the formation of beads. In addition, the molecular weight and concentration of PEO used were high, and the entanglement of the polymer chains also led to the formation of these nodes.

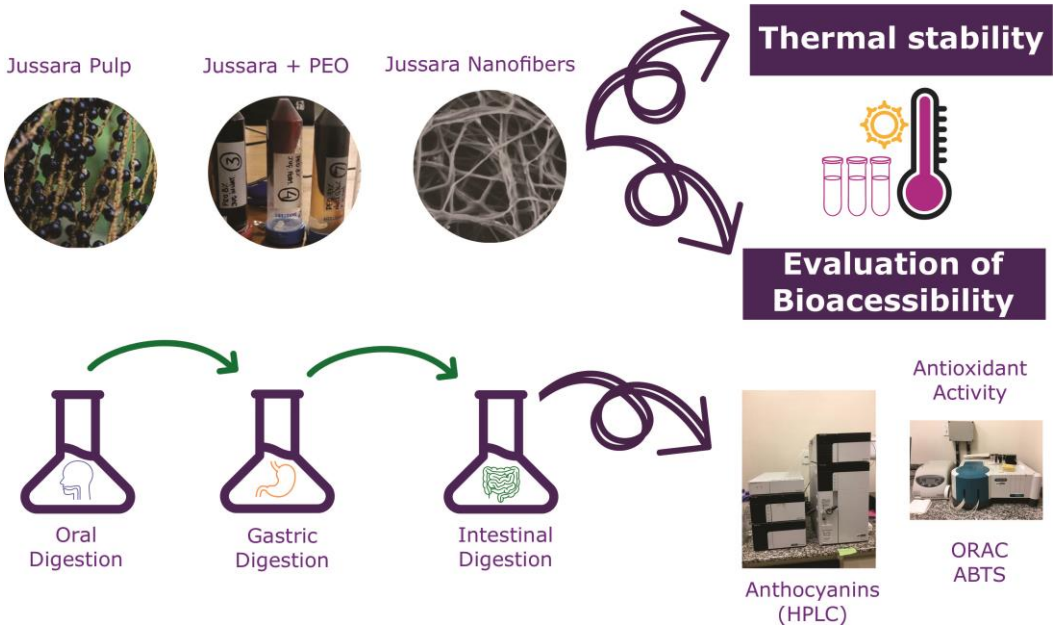

Figure 3. Schematical summary of the acquired data.

Regarding the chromatograms (Figure 2), it is possible to observe a decrease in anthocyanin content throughout the simulated digestive process. This decrease indicates a potential degradation of the anthocyanins, which was already expected, especially for the initial form, considering the well-established literature on the behavior of anthocyanins in the face of changes in pH and enzymatic activity throughout the digestive process, or

even a possible bioconversion of this compound into lower-molecular-weight phenolic compounds [4,5].

As already known, anthocyanin metabolism involves the cleavage of glycosylated bonds, the degradation of heterocyclic anthocyanidins, and further metabolization that can promote the bioconversion of anthocyanins into smaller phenolic compounds in the colon, such as protocatechuic acid, gallic acid, and p-coumaric acid, among others, which are produced after the enzymatic action of gut microbiota bacteria on this compound [5,6,31,32].

As organic compounds, these molecules, the components of the jussara nanofibers, j and the polymer possess a significant number of oxygen atoms and especially hydrogen atoms, which promote hydrogen-bridge type interactions, besides the fact that the polymer has a hydrophobic character and thus increases polar interactions and nonpolar repulsions with lipophilic molecules. Thus, interactions between JP and PEO can occur, mainly due to the complex matrix of the fruit that includes a considerable amount of lipids and fibers and the interactions between the other components of the fruit [33], besides it being a fruit rich in anthocyanins, a low-molecular-weight compound, as has already been described in the literature [34]. Additionally, the high molecular weight of PEO results in a high viscosity, even more so, considering the percentage used in the present study, which can influence the extraction and quantification of anthocyanins. From another perspective, this viscous property is advantageous in the formation of nanofibers as it helps in the conformation and homogeneity of the fibers, providing greater protection to the compound involved [17,24,35], and in this case of nanofiber conformation, the polymeric solution was evaporated, being in its solid-state, which corroborates with the extraction and consequently with the quantification of anthocyanins.

The effect of digestion on the anthocyanin content of Aronia nanoencapsulated into potato amylopectin was explored by Tong et al. [36], who had also observed the decay of anthocyanins during simulated gastrointestinal digestion. Despite this, they obtained promising results since, at the end of intestinal digestion, 9.12% were retained without protection, whereas 29.21% of the nanoencapsulated anthocyanins were retained. The same positive results were found by Isik et al. [37] with nanoencapsulated cherries, which supports the effectiveness of this technology in protecting bioactive compounds.

During and after the simulated digestion process, it was possible to observe the potential anthocyanin degradation or even conversion into other compounds in both samples, considering their decay of antioxidant activity, which probably occurred due to the variation in pH values [38], as well as the action of the different enzymes that convert this compound into its derivative chemical structures [5]. This result was expected and was also described by other authors, such as Schulz et al. [31], who studied the same fruit as the present work, and Lucas-Gonzalez et al. [39], where maqui berry was analyzed, which is very similar to jussara pulp.

Despite that, the JPP sample showed a better remaining value (82.9% and 64.4%) for the ABTS and ORAC methods, respectively) than JP (59.5% and 54.6% for the ABTS and ORAC assays, respectively), indicating a possible effect of the polymer in protecting bioactive compounds, probably due to its high molecular weight and viscosity [18], which may promote interaction between polymer and bioactive compounds, regarding their low molecular weight, protecting them against adverse conditions. Moreover, the comparison between the protection provided by the polymer before and after nanostructure production was also presented [12,40].

The positive results are promising and highlight the benefit of using PEO in a solution form to protect the beneficial effects of bioactive compounds from the jussara pulp. This finding may be advantageous for the food, pharmaceutical, and cosmetics industries since it can save the nanoencapsulation phase, depending on the purpose involved, which requires time, trained personnel, and a high economic value [41].

The JN form also showed better results in comparison with JP. The antioxidant activity determined via the ABTS assay was 85.3%, and through the ORAC it was 75.1%, showing the nanofiber's protective effect on jussara compounds. It is possible to observe this behavior

using PEO in the studies conducted by Locilento et al. [42] and Aceituno-Medina et al. [43], who nanoencapsulated the extract of grape skin and phenolic compounds, respectively. Both measured the antioxidant activity after in vitro digestion of the nanofiber, and in both cases, this capacity was maintained.

Moreover, Tong et al. [36] also investigated the antioxidant activity of anthocyanins and found promising results after in vitro digestion, compared to the free extract, with nanoencapsulated *Aronia* anthocyanins in potato amylopectin. Following this, Jeong et al. [44], who nanoencapsulated various fruit combinations, and de Dicastillo et al. [45], who nanoencapsulated açaí extract in zein; both obtained promising results in relation to the maintenance of antioxidant activity after in vitro digestion. Given the above, it is possible to realize the promising effect of nanotechnology and the polymer PEO with bioactive compounds to protect their biological effects against adverse conditions.

These results suggest the existence of low-molecular-weight metabolic agents, as mentioned before, since the determination of antioxidant activity indicated the maintenance of their antioxidant capacity after bioaccessibility, so even with the decrease observed in the anthocyanins chromatograms, the samples are still conferring the desired beneficial actions on human health.

The jussara nanofibers were verified to have higher $t_{1/2}$ at both temperatures studied, as compared to jussara pulp, a solution of jussara pulp, and PEO. These results corroborate the hypothesis that incorporating bioactive compounds into nanostructures enhances their thermal stability [16]. Li et al. [46] and Ma and Jing [47] have shown the same protective effect when evaluating the thermal stability of silk fibroin (SF) nanocomposite and nanofibrils with anthocyanins at 80 °C and 90 °C. These authors, including Braga et al. (2016), also obtained higher $t_{1/2}$ for nanocomposite and nanofibril formats than for free pigment. Beyond that, it is essential to emphasize the significant decay time of anthocyanins under all conditions studied in this work.

The activation energy degradation reaction (Ea) was calculated based on linear regression analysis of natural logarithms of rate constants at different conditions evaluated against reciprocal absolute temperature, 1/T in K. The Ea values were 77.2, 85.4, and 114.0 kJ·mol^{-1} for the JP, JPP, and JN, respectively. The higher Ea implies that the jussara nanofibers require more energy to deactivate than do the jussara pulp, the solution of jussara pulp, and PEO. Consequently, the jussara nanofibers can be considered as being more thermostable than the other conditions evaluated in the present work for the temperature range studied. These findings highlight the premise of the protective effect of nanotechnology applied to bioactive compounds to preserve their beneficial impact on human health.

People across the world are becoming increasingly concerned about health and nutrition, and as already mentioned, anthocyanins have strong antioxidant potential, protecting the cell from free radical damage by neutralizing and scavenging them [6,8,9,15]. Several research publications reported the health-promoting effects of anthocyanins, including being anti-inflammatory, anticancer, antidiabetic, antiaging, neuroprotective, and preventing of cardiovascular diseases [5,9]. Jussara anthocyanins can be extensively exploited as a unique ingredient for producing value-added food products due to their positive qualities, thus attending to the consumers' request for more natural ingredients, particularly when the thermal stabilization of bioactive compounds is reached, and the results presented are a valuable contribution in this direction.

5. Conclusions

The antioxidant activity of anthocyanins from jussara pulp, polymer solution, and nanostructures produced with PEO and jussara pulp were partially maintained after in vitro digestion, even with a decay of anthocyanins during digestion. However, the results of the antioxidant activity were promising since the polymeric solution and nanofibers showed a protective effect on bioactive compounds, ensuring greater antioxidant action than the lyophilized pulp, which enables the hypothesis of bioconversion of anthocyanins into phe-

nolic compounds of lower molecular weight. In summary, the composites synthesized via electrospinning exhibited antioxidant activity even after the digestion process. Additionally, thermal degradation data provided evidence of the potential to apply the polymeric composites in foodstuffs to prevent the loss of the biological effects of jussara anthocyanins. Thus, we can consider the polymer solution and nanofibers' uses as an alternative to preserving the biological effects of anthocyanins even after the in vitro digestion process.

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