

# The effect of extrusion processing on the physicochemical and antioxidant properties of fermented and non-fermented Jabuticaba pomace

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## Abstract

Previous studies have proven that the flour obtained from the residue of Jabuticaba (Jab) juice and wine industries is source of bioactive compounds and an option for the production of food such as extrudates. The objective of this work was to produce extrudates with different concentrations (0, 5, 10, 15, 20%) of non-fermented and fermented Jab pomace flour, as well as to evaluate their physical, chemical and technological properties and the effect of the extrusion process on the antioxidant capacity. Results showcased that the extrudates have low content of resistant starch (0.26 g/100 g) and extrusion conditions decreased the content of polyphenols and antioxidant potential. The addition of 20% non-fermented Jab pomace reached an antioxidant activity of 2904 µg trolox/g in the DPPH method, and promoted rheological changes in the product, such as lower expansion index, higher density and hardness; while presenting higher phenolic content and antioxidant capacity.

**Keywords:** extrudates, *Myrciaria cauliflora*, peels, polyphenols, seeds.

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## 1. Introduction

Jabuticaba (Jab) is a highly perishable tropical fruit which is native to south-central Brazil. Amongst the known species of Jab are *Myrciaria cauliflora* (DC) Berg and *Myrciaria Jabuticaba* (Vell) Berg, whose ripe fruits showcase dark, thin and fragile peels, while the pulps present whitish color and slightly sour-sweet mouthfeel<sup>[1]</sup>.

The industrialization of Jab results in products such as juices, jellies, ice cream and fermented beverages. Regarding Jab wine production, its agroindustrial residue has a remarkably high volume due to the discard of half of the fruit content, which is mostly composed by peels and seeds<sup>[2]</sup>.

Jab pomace contains large amounts of phenolics such as anthocyanins when compared to the whole fruit<sup>[3]</sup>. Leite-Legatti et al.<sup>[4]</sup> conducted an experiment to assess the nutraceutical effect of adding freeze-dried Jab peel in a high fat diet, and their results evidenced that Jab peel consumption increased HDL-cholesterol, therefore suggesting a protective effect against cardiovascular disease and improved insulin resistance. Moreover, extracts prepared with Jab peel

showcased antimutagenic (*in vivo*) and antiproliferative (*in vitro*) effects against leukemia and prostate cancer<sup>[5]</sup>.

Extrusion is a processing technique that can be used in the development of new food products from food industry byproducts<sup>[6]</sup>. Besides, extrusion facilitates the inclusion of fibers in the starchy material and improves the sensorial and functional characteristics of the produced extrudates<sup>[7]</sup>.

In a previous study, Morales et al.<sup>[8]</sup> analyzed the fermented and non-fermented Jab residues and found that the pomaces are a source of bioactive compounds such as tocopherols, polyunsaturated fatty acids and phenolic compounds with high antioxidant potential.

Owing to the fact that Jab pomace may be considered a functional ingredient in the fabrication of human and livestock food, the objective of this work was to prepare extrudates with different concentrations of fermented and non-fermented Jab pomace flour, and evaluate the physical, chemical and technological properties of the developed products.

## 2. Materials and Methods

### 2.1 Sample preparation

This study used the industrial byproducts of Jab wine and juice production, whose industrial plant is located in the region of Nova Fatima, GO, Brazil. These residues were constituted by fermented (JF) and non-fermented (JNF) peels and seeds. The industrial byproducts were subjected to 24 hours dehydration in a forced-air drying oven (Imperial IV Microprocessor Oven, Lab-Line Instruments, Melrose Park, USA) at 50 °C, and were grounded thereafter in a knife mill (Wiley, Thomas Scientific, New Jersey, USA). After grounding, the material was sieved in a 100-mesh stainless steel tamis with a 0.15 mm aperture and thereafter, packed under vacuum in plastic bags.

The mixture of lentil and rice flour (70:30) (L/R) was used a complementary input for extrudate elaboration. The proportion was established by the content of starch, lipids and fibers, as well as their effect on the blend processability in the extruder. Furthermore, this mixture also provides a balanced composition of nutrients and is a gluten-free product.

The raw materials were weighed and mixed in the following proportions: non-fermented/fermented Jab flour (0, 5, 10, 15, 20%); salt (1.25%); sugar (5%) and L/R (70:30), resulting in 10 formulations. The research was developed at the Western Regional Research Center (WRRRC) of the United States, Department of Agriculture (USDA), Albany, California, USA.

### 2.2 Extrusion conditions

A single screw extruder was employed, and the following conditions were set to each zone ( $n = 1$  to  $6$ ): at first the material was water cooled ( $n = 1$ ), then the temperature was raised up to 60 °C ( $n = 2$ ). Thereafter, the temperature was once again raised, up to 80 °C ( $n = 3$ ), and then kept steady at 90 °C ( $n = 4$  and  $5$ ). At the final zone, the temperature reached 100 °C ( $n = 6$ ). The screw speed was 500 rpm; the screw diameter was 2.5 mm; the screw length was 200 mm; the feed rate was 50 kg/h and the feed moisture content was 21%.

### 2.3 Dietary fiber and Total, available and resistant starch

Content of soluble and insoluble dietary fiber in JNF and JF was determined according to a standard enzymatic-gravimetric method<sup>[9]</sup>. The content of total, available and resistant starch was determined for both of the investigated flours (JNF, JF), mixes and extrudates, using the MEGAZYME Kit following the methods 2002.02<sup>[9]</sup> and 32-40.01<sup>[10]</sup>.

### 2.4 Total soluble polyphenols

Total soluble polyphenol content was determined in the mixtures and the extrudates using the methodology described by Swain and Hillis<sup>[11]</sup> with some modifications. Five grams of sample were homogenized with 20 mL of methanol using a shaker (Waring, Torrington, USA), wrapped in dry ice and shaken for one minute. The tubes were centrifuged (SA-600 rotor, 15600 rpm for 15 min at 4°C), and aliquots of 150 µL were withdrawn from the clear supernatant and diluted with 2400 µL of nanopure water.

Then, 150 µL of 0.25 N Folin-Ciocalteu reagent was added and the solution was incubated for three-minutes at room temperature. The reaction was quenched by the addition of 300 µL 1N Na<sub>2</sub>CO<sub>3</sub> and the mixture was incubated again for 25 min. Absorbance was measured at 725 nm using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). The blank was prepared using methanol and a standard curve (0-0.375 mg/mL) developed with gallic acid (GA) was used for the quantification of phenolics. The content was expressed as mg of GA equivalentes per g of sample.

### 2.5 Antioxidant capacity

The antioxidant capacity was determined for the mixes and extrudates using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method adapted from Brand-Williams et al.<sup>[12]</sup> with some modifications. The analysis used the same methanolic extract that was prepared for the phenolic compounds quantification. A 50 µL aliquot of the sample was reacted with 2950 µL of DPPH (103.2 µM in methanol, with an absorbance of ~1.2 at 515 nm). The reaction occurred at room temperature under stirring at 180 rpm x 22 hours on a horizontal shaker (HS 250 basic, IKA Labortechnik, Staufen, Germany)<sup>[13,14]</sup>. The spectrophotometer was calibrated with methanol and the absorbance was measured at 515 nm. A standard curve was assayed for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (0-750 µg/mL) and results were expressed as µg of Trolox equivalentes per g of sample.

### 2.6 Expansion index

The expansion index was calculated by the ratio of the extrudate diameter (mm) to the extruder diameter (mm) using stick-shaped samples of 6 cm length<sup>[15,16]</sup>.

### 2.7 Apparent density

A Syntron Vibra-Flow Feeder equipment (F-T01A, Syntron Co., Homer City, USA) was used to determine the apparent density (g/cm<sup>3</sup>) of the extrudates<sup>[17]</sup>. The bulk volume that was occupied by the extrusion product was calculated from the weight of the displaced beads and the density by the ratio between weight and bulk solution volume.

### 2.8 Pasting properties

The viscosity was determined for the mixes and extrudates using a RVA (Rapid Visco Analyzer)<sup>[18]</sup>. Therefore, 3 g of sample were weighed in RVA crucibles, followed by addition of deionized water, according to the moisture of each sample, so that all samples reached 10% The stirring was carried out to prevent the formation of lumps in the RVA. The mixture was initially held at 25 °C for 2 min, the temperature was gradually raised at a rate of 5.83 °C/min up to 95 °C. When the system reached 95 °C, the temperature was kept for 4 min. Cooling was also gradual at a rate of 11.25 °C/min until reaching a final temperature of 50 °C, which was kept for 2 min.

### 2.9 Texture analysis

The texture of the extrudates was determined with a TA-TX2 Texture Analyzer (Stable Micro Systems, Goldaming, Surrey, UK) using the Texture Expert software (Stable Micro

System version 1.22). The conditions were the following: pre-test speed of 2.5 mm/s; test speed of 2.00 mm/s; post-test speed of 10.0 mm/s; distance of 3.0 mm; compression force of 20 g; and trigger (Self-20g). The hardness (N) and crunchiness (N/mm) were determined using 15 randomly selected samples of 6 cm length, and the force peak (hardness) and the area under the curve (crunchiness) were chosen to represent the textural properties of the extrudates.

## 2.10 Color evaluation

The samples were grounded in a Cyclone Sample Mill (Udy Corporation, Fort Collins, USA) to obtain particles of 1 mm. The JF and JNF flours, mixes and extrudates were evaluated the instrumental parameters of color in the colorimeter (CM-3500d, Minolta, Tokyo, Japan)<sup>[19]</sup>. Color attributes were expressed according to the Cielab system with values of L\* (black-white component, luminosity), a\* (+red to -green component) and b\* (+yellow to -blue component). The following Equation 1 was used to obtain the total color difference ( $\Delta E^*$ ) between the extrudate and the mix.

$$\Delta E^* = \left( \Delta L^*{}^2 + \Delta a^*{}^2 + \Delta b^*{}^2 \right)^{1/2} \quad (1)$$

## 2.11 Scanning electron microscopy

A scanning electron microscope (Quanta-200, FEI Company, Netherlands, USA) was used to visualize the ultra-structural morphology of the materials. The extrudate flours were fixed in aluminum stubs using a double-sided adhesive tape, bathed in a thin gold film (10 nm) and examined under a 2 kV acceleration voltage. Micrographs were obtained at 30× magnification.

## 2.12 Statistical analysis

The Minitab® 16 software was used for statistical analysis. For comparison of fiber contents a t-Student test was performed. The data of total, available and resistant starch of the raw materials (JF, JNF, L/R) was analyzed with one way Anova. For the analysis of polyphenols, color parameters and antioxidant capacity of mix and extrudates was performed Anova with 3 factors, being the factors the type of jab flour (JF or JNF), the % of Jab flour (0, 5, 10, 15 and 20%) and the type of sample (Mix or ext). For the analysis of density, expansion index and texture Anova was made with 2 factors being the factors Jab flour type in the extrudate (JF or JNF), and the percentage of Jab flour in the extrudate (0, 5, 10, 15, and 20%). Tukey's test was used for comparing means.

The results were considered significant whenever  $p \leq 0.05$ . The data was expressed as mean  $\pm$  standard deviation and reported as means of triplicates (except expansion index, texture analysis and pasting properties which were means of ten replicates).

## 3. Results and Discussions

### 3.1 Dietary fiber and total, available and resistant starch

The content of dietary fiber was determined only for JNF and JF flours in order to verify its effect on the

extrusion process. For JNF, 26.3 g/100 g of insoluble fiber, 12.1 g/100 g of soluble fiber and 38.4 g/100 g of total fiber were quantified. The JF obtained a higher total fiber content (53.9 g/100 g) which was distributed in 42.4 g/100 g of insoluble and 11.5 g/100 g of soluble fiber.

The available starch content of the raw materials (JF, JNF) was lower than the contained in L/R (Table 1), in contrast, resistant starch presented higher values in JNF ( $p < 0.05$ ). A comparison of the mixes pointed out that the addition of either 20% JF or JNF increased the content of resistant starch ( $p < 0.0053$ ).

After the extrusion, we observed that the available starch in the extrudates decreased and Berrios et al.<sup>[20]</sup> observed a decrease in the total carbohydrate content after extrusion, suggesting that the cause would be the degradation of the starch into low molecular weight derivatives which were involved in Maillard and caramelization reactions.

Starch has a great importance in the extrusion process, influencing the texture and the expansion of the extrudates. The mixes showcased significant contents ( $p < 0.0002$ ) of available starch (58.47 to 71.92 g/100 g); however, the resistant starch contents detected in the extrudates were low (0.06 to 0.26 g/100 g).

Morales et al.<sup>[21]</sup> revealed that the total fiber content decreased after the extrusion of lentil flour, which may also have occurred in the extrudate with 20% Jab flour, because the resistant starch has properties similar to fibers.

### 3.2 Total soluble polyphenols (TSP) and antioxidant activity

Table 2 shows the content of TSP and antioxidant activity in mixes and extrudates. It was observed that the values increased with the addition of Jab flour. The comparison of the mixtures and their respective extrusions showed that the extrusion process decreased the phenolic content ( $p < 0.011$ ). However, the loss was greater in JF extrudates (about half in relation to their respective mixture). This fact is probably related to the degradation of the polyphenol molecules after the yeast action during the fermentation, making these compounds fragile to the extrusion conditions<sup>[22]</sup>.

Lohani and Muthukumarappan<sup>[23]</sup> reported that the extrusion process alters the phenolic content due to the polymerization or degradation of these compounds upon incidence of heat.

The addition of Jab flour increased the antioxidant activity ( $p < 0.05$ ). Regarding the extrudate without jabuticaba flour, antioxidant activity was observed even when phenolic compounds were not detected and this could be due to the presence of other compounds with antioxidant action, such as tocopherols and organic acids.

Table 2 shows that after extrusion, the antioxidant capacity decreased in formulations containing JF and JNF, with the exception of JNF 20% which remained stable ( $p < 0.05$ ). A possible explanation for the stability of the antioxidant activity with 20% JNF could be the presence of sugars, gums and mucilages in the raw material, which crystallize and encapsulate antioxidant compounds, thus protecting them from the effects of temperature and pressure during extrusion. The decline of the antioxidant capacity may be

**Table 1.** Total, available and resistant starch (g/100 g) of the flours, mixes and extrudates.

		Available starch		Resistant starch		Total starch	
	L/R	57.91±0.12 <sup>a</sup>		0.27±0.03 <sup>c</sup>		58.19±0.11 <sup>a</sup>	
	JF	2.40±0.16 <sup>c</sup>		1.78±0.11 <sup>b</sup>		4.17±0.27 <sup>c</sup>	
	JNF	6.88±0.25 <sup>b</sup>		3.46±0.09 <sup>a</sup>		10.34±0.33 <sup>b</sup>	
		Mix	Ext.	Mix	Ext.	Mix	Ext.
(%)	0	71.92±0.23 <sup>aA</sup>	70.35±0.59 <sup>aB</sup>	0.35±0.006 <sup>cA</sup>	0.07±0.01 <sup>bB</sup>	73.31±0.22 <sup>aA</sup>	70.40±0.31 <sup>aB</sup>
JF	20	58.12±0.43 <sup>dA</sup>	58.47±0.29 <sup>dA</sup>	1.05±0.02 <sup>bA</sup>	0.25±0.006 <sup>aB</sup>	58.46±0.29 <sup>dA</sup>	58.86±0.82 <sup>dA</sup>
	0	68.12±0.30 <sup>bA</sup>	68.57±0.43 <sup>bA</sup>	0.39±0.01 <sup>cA</sup>	0.06±0.006 <sup>bB</sup>	69.17±0.45 <sup>bA</sup>	68.64±0.59 <sup>bA</sup>
JNF	20	63.86±0.82 <sup>cA</sup>	61.86±0.85 <sup>cB</sup>	1.39±0.02 <sup>aA</sup>	0.26±0.01 <sup>aB</sup>	64.11±0.44 <sup>cA</sup>	62.12±0.85 <sup>cB</sup>

Mean and standard deviation of three replicates. For the Tukey test in raw materials, means with different lower case letters in the same column are significantly different ( $p \leq 0.05$ ). For the Tukey test in mix and extrudates, means with different lower case letters in the same column and upper case letters in the same line are significantly different ( $p \leq 0.05$ ). Ext: extrudates; L/R: lentil and rice flour (30:70). Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF).

**Table 2.** Total soluble polyphenols (TSP) and antioxidant capacity (AC) in the mixes and extrudates (Ext). Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF).

		Total soluble polyphenols (mg of GA equivalentes per g of sample)		Antioxidant capacity ( $\mu$ g of Trolox equivalentes per g of sample)	
		Mix	Ext	Mix	Ext
(%)	0	0.03 ± 0.01 <sup>d</sup>	-	247.00 ± 16.8 <sup>dA</sup>	189.26 ± 8.32 <sup>eB</sup>
	5	0.17 ± 0.03 <sup>cA</sup>	0.06 ± 0.005 <sup>dB</sup>	758.84 ± 25.31 <sup>cA</sup>	323.26 ± 22.8 <sup>dB</sup>
	10	0.37 ± 0.01 <sup>bA</sup>	0.15 ± 0.004 <sup>eB</sup>	1779.9 ± 7.12 <sup>bA</sup>	721.21 ± 11.9 <sup>eB</sup>
JF	15	0.72 ± 0.01 <sup>aA</sup>	0.26 ± 0.006 <sup>bB</sup>	2976.3 ± 0.67 <sup>aA</sup>	1071.9 ± 26.8 <sup>bB</sup>
	20	0.79 ± 0.07 <sup>aA</sup>	0.43 ± 0.004 <sup>aB</sup>	2979.6 ± 0.42 <sup>aA</sup>	1772.5 ± 32.7 <sup>aB</sup>
	0	0.02 ± 0.007 <sup>c</sup>	-	252.14 ± 6.76 <sup>dA</sup>	181.36 ± 4.59 <sup>eA</sup>
	5	0.15 ± 0.009 <sup>dA</sup>	0.10 ± 0.003 <sup>dA</sup>	708.40 ± 14.88 <sup>cA</sup>	487.17 ± 18.7 <sup>dB</sup>
JNF	10	0.35 ± 0.004 <sup>cA</sup>	0.27 ± 0.005 <sup>cA</sup>	1887.8 ± 16.46 <sup>bA</sup>	1244.6 ± 27.3 <sup>eB</sup>
	15	0.66 ± 0.008 <sup>bA</sup>	0.38 ± 0.029 <sup>bB</sup>	2971.2 ± 2.30 <sup>aA</sup>	1859.8 ± 72.2 <sup>bB</sup>
	20	0.82 ± 0.033 <sup>aA</sup>	0.64 ± 0.052 <sup>aB</sup>	2981.7 ± 1.72 <sup>aA</sup>	2898.1 ± 36.7 <sup>aA</sup>

Mean and standard deviation of three replicates. For the Tukey test, means with different lower case letters in the same column and upper case letters in the same line are significantly different ( $p \leq 0.05$ ). Ext: extrudates, Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF).

related to the decrease of phenolic compounds, organic acids and tocopherols, which are present in Jab's flour<sup>[8]</sup>, as a consequence of the extrusion conditions of temperature and shear. The same behavior was observed in lentil flour after extrusion<sup>[21]</sup>.

Carbohydrates are the most frequently used compounds for sample encapsulation as exemplified by Azeredo<sup>[24]</sup>. This protection of antioxidant compounds through encapsulation does not occur in JF, because sugars and polysaccharides are consumed and hydrolyzed during fermentation.

### 3.3 Apparent density, expansion index and texture analysis

The density of the extrudate products varied from 0.08 to 0.34 g/cm<sup>3</sup> (Table 3). The extrudates with 20% JNF and JF were denser to other extrudates ( $p < 0.014$ ). In addition, the extrudates with JNF and JF presented significantly different densities between them ( $p < 0.014$ ). JF flour presented lower apparent density due to the fermentation process that allows microorganisms to convert the nutritional compositions of the substrate into organic acid, causing this reduction in density<sup>[25,26]</sup>.

As showcased in Table 3, increasing concentrations of Jab flour lead to a decrease of the expansion index, thus reaching the lowest value (1.6) with 20% of JNF ( $p < 0.05$ ). This possibly occurs because Jab flour has high fiber content.

For JNF 38.4 g/100 g of total fiber were quantified and for JF obtained fiber content (53.9 g/100 g). It is reported that insoluble fiber decreases the proportion of starchy material and reduces the viscosity for expansion. The fiber also binds to water during extrusion, therefore reducing their availability for expansion<sup>[27,28]</sup>.

The highest expansion index of 0% Jab flour in the extrudates may be attributed to the starch content, which influences the elastic characteristics of the mass due to the hygroscopic nature of this polysaccharide and also contributes to increase the expansion<sup>[7]</sup>.

The hardness values varied from 495.0 to 1868.2 N. The 20% Jab flour extrudates presented the highest hardness values in addition to the lowest expansion rates and the highest densities ( $p < 0.05$ ). The reduction in the expansion and increase in hardness are characteristic of products with high fiber content, whose action decreases the elasticity of the molten material upon exiting the extruder<sup>[29]</sup>. The extrudates with 15 and 20% JF showed hardness values statistically higher ( $p < 0.05$ ) than JNF extrudates, possibly due to the higher fiber content in JF flour (53.9 g/100 g), compared to JNF (38.4 g/100 g) ( $p < 0.05$ ).

The crunchiness presented values from 0.22 to 0.66 N/mm, the least crunchy extrudates were the ones with 20% Jab flour addition (Table 3). As the % of Jab flour increased, the fiber content also increased and the crunchiness decreased. The incorporation of fibers in extrudates limits their expansion



**Table 3.** Apparent density, expansion index and instrumental texture analysis of the extrudates. Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF).

	% flour				
	0	5	10	15	20
<b>Apparent density (g/cm<sup>3</sup>)</b>					
JF	0.09 ± 0.001 <sup>aD</sup>	0.09 ± 0.001 <sup>bD</sup>	0.12 ± 0.006 <sup>bC</sup>	0.19 ± 0.003 <sup>bB</sup>	0.24 ± 0.005 <sup>bA</sup>
JNF	0.08 ± 0.003 <sup>aE</sup>	0.12 ± 0.002 <sup>aD</sup>	0.14 ± 0.003 <sup>aC</sup>	0.28 ± 0.006 <sup>aB</sup>	0.34 ± 0.010 <sup>aA</sup>
<b>Expansion index</b>					
JF	3.5 ± 0.31 <sup>aA</sup>	3.2 ± 0.22 <sup>aB</sup>	2.9 ± 0.32 <sup>aBC</sup>	2.8 ± 0.25 <sup>aC</sup>	1.8 ± 0.16 <sup>aD</sup>
JNF	3.5 ± 0.12 <sup>aA</sup>	3.0 ± 0.18 <sup>aB</sup>	2.8 ± 0.20 <sup>aBC</sup>	2.7 ± 0.14 <sup>aC</sup>	1.6 ± 0.18 <sup>aD</sup>
<b>Hardness (N)</b>					
JF	495.0 ± 47.1 <sup>aC</sup>	602.7 ± 27.9 <sup>aC</sup>	691.1 ± 67.7 <sup>aC</sup>	1599.5 ± 27 <sup>aB</sup>	1868.2 ± 88 <sup>aA</sup>
JNF	536.9 ± 41 <sup>aC</sup>	553.6 ± 40.5 <sup>aC</sup>	590.0 ± 74.6 <sup>aC</sup>	848.4 ± 81.6 <sup>bB</sup>	1324.7 ± 72 <sup>bA</sup>
<b>Crunchiness (N/mm)</b>					
JF	0.61 ± 0.17 <sup>aA</sup>	0.50 ± 0.26 <sup>aAB</sup>	0.45 ± 0.24 <sup>aAB</sup>	0.28 ± 0.05 <sup>aB</sup>	0.28 ± 0.10 <sup>aB</sup>
JNF	0.78 ± 0.47 <sup>aA</sup>	0.64 ± 0.31 <sup>aAB</sup>	0.44 ± 0.24 <sup>aBC</sup>	0.31 ± 0.16 <sup>aC</sup>	0.20 ± 0.06 <sup>aC</sup>

Mean and standard deviation of three replicates. For the Tukey test, means with different lower case letters in the same column and upper case letters in the same line are significantly different ( $p \leq 0.05$ ). Ext: extrudates, Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF).

and reduces their crunchiness<sup>[30]</sup>. Both the hardness and the crunchiness of the extrudates are associated to the expansion and cellular structure of the product<sup>[28]</sup>. Therefore, an extrudate with low expansion results in a product with reduced crunchiness and high hardness.

### 3.4 Pasting properties

Figure 1 shows the paste viscosity profiles of the mixes and extrudates as a function of time and temperature. The mixes showcased similar behavior regarding the time to achieve the maximum and final viscosity. The highest values of maximum and final viscosity were obtained with the mix without Jab and the lowest at 20% Jab.

The addition of flours with high fiber content can contribute to the reduction of the viscosity values, by the fact of reducing the total accessible starch content that will gelatinize during the analysis<sup>[31]</sup>. This fact was observed with the addition of jabuticaba flour that presents high fiber content as previously mentioned.

The mix without Jab showed a breakdown of 522.5 cP and a setback of 1649.5 cP, differing from both JNF (20%, breakdown: 230.5 cP) and JF (20%, setback: 829.5cP). This means that the mix without Jab presented lower stability at higher temperatures under agitation (higher breakdown value) and a greater tendency to retrograde (high setback value).

The analysis of the extrudates (Figure 1c and 1d) revealed that the initial viscosities showcased the highest values reached during the RVA analysis. After 3 min, was observed a viscosity drop. The minimum viscosity was reached between 8 and 10 min at 95 °C. From there, a slight increase of viscosity was observed until the final viscosity was recorded.

During the extrusion of the formulations the same conditions of temperature, moisture and rotation speed were maintained and because they remained constant, the different values of paste properties were attributed completely to the jab flour.

The absence of maximum peak viscosity demonstrates that heat treatment during extrusion may have destroyed

the crystal structure of the starch granules resulting in low viscosity values during heating at RVA<sup>[27,31]</sup>.

The viscosity profiles of the extrudates demonstrate that the starch was gelatinized in the extrusion process, because it showed the ability to increase the viscosity of the solution at room temperature and showed no tendency to retrograde. These findings therefore suggest the use of this extrudates flour in instant foods.

### 3.5 Color evaluation

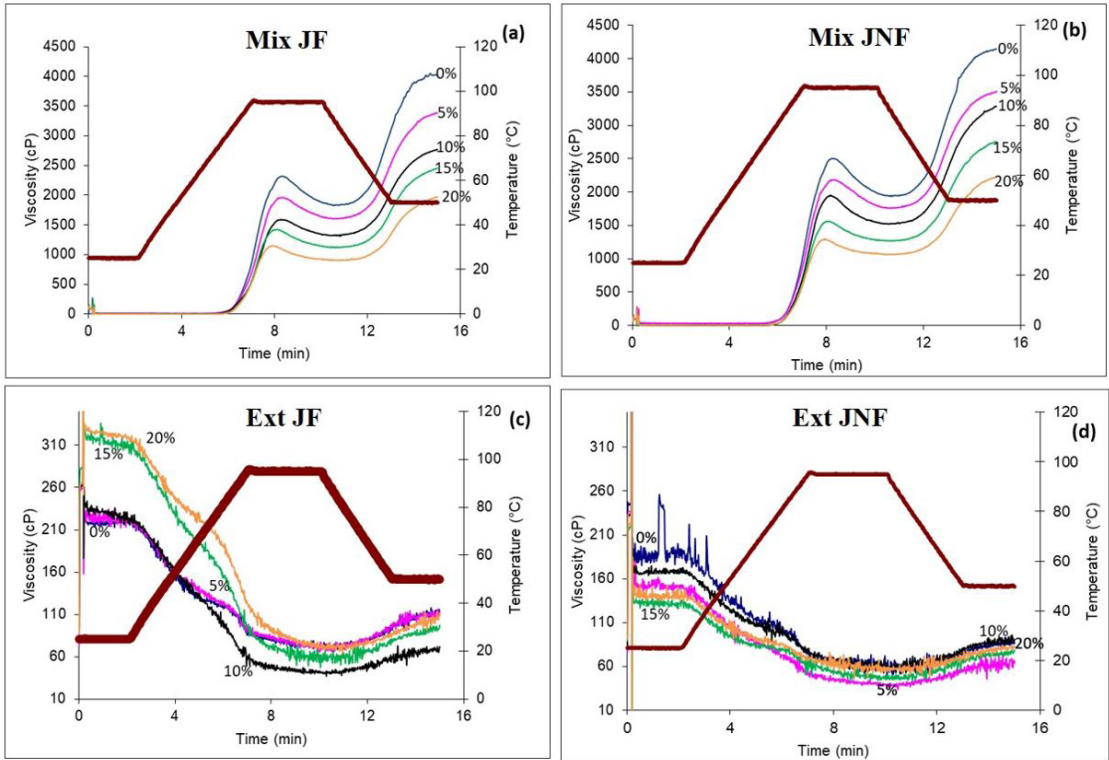
The results of the color analysis are presented in Table 4. Regarding luminosity (L\*), JNF flour is darker than JF flour ( $p < 0.001$ ). JF flour was obtained from the residue of wine production, probably during fermentation there was release of pigments such as anthocyanins to the must, making JF flour lighter<sup>[27]</sup>.

As the Jab flour content increased, the mixtures became darker (decreasing the L\* value), which was also observed in the extrudates. The mixes without Jab were lighter than the other samples ( $p < 0.001$ ).

The raw materials JNF and JF presented a red tone and yellow subtones (positive values for a\* and b\* color coordinates, respectively). While for the mixes, yellow tones and red subtones were observed, as well as for the extrudates (values of b\* stood out from the values of a\*). JF appeared to be more reddish than JNF ( $p < 0.05$ ), probably due to the detachment of the mesocarp during fermentation<sup>[27]</sup>.

For  $\Delta E^*$ , the lowest values were found for the samples without Jab flour ( $p < 0.05$ ), indicating that the extrusion had little effect on the color parameters when the formulations did not present Jab flour. With the addition of Jab residues, the color difference between mix and extrudates was significant, possibly due to Maillard reaction that occurred during extrusion and also the degradation of anthocyanins present in jabuticaba flour.

The extrusion allows a greater interaction between sugars and proteins, triggering non-enzymatic browning (caramelization and Maillard reaction)<sup>[7]</sup> and the degradation of anthocyanins also contributes to the darkening of the extrudates, as reported for purple potato and pea flour extrudates<sup>[32]</sup>.



**Figure 1.** Paste viscosity of the mixes (a-b) and extrudates (Ext) (c-d). Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF). Lines in dark blue (0%); pink (5%); black (10%); green (15%); orange (20%) and brown (temperature).

**Table 4.** Color attributes of the flours, mixes and extrudates.

	L*		a*		b*		ΔE*	
JF	48.85±0.75		7.39±0.09		6.03±0.25		-	
JNF	53.37±0.67		8.32±0.14		6.75±0.28		-	
	Mix	Ext	Mix	Ext	Mix	Ext		
JF	0	90.21±0.5 <sup>aA</sup>	85.00±0.48 <sup>aB</sup>	-0.42±0.1 <sup>bB</sup>	-0.29±0.01 <sup>bA</sup>	9.24±0.52 <sup>aB</sup>	14.70±0.02 <sup>aA</sup>	7.55±0.99 <sup>b</sup>
	5	84.47±1.29 <sup>bA</sup>	63.76±1.33 <sup>bB</sup>	1.21±0.28 <sup>dB</sup>	3.64±0.1 <sup>aA</sup>	5.56±0.45 <sup>bB</sup>	8.38±0.09 <sup>bA</sup>	21.05±2.34 <sup>a</sup>
	10	79.16±0.62 <sup>cA</sup>	59.10±0.65 <sup>cB</sup>	2.10±0.21 <sup>cB</sup>	3.71±0.1 <sup>aA</sup>	5.36±0.82 <sup>bB</sup>	7.79±0.06 <sup>bA</sup>	20.29±1.17 <sup>a</sup>
	15	74.58±1.04 <sup>dA</sup>	56.91±0.19 <sup>cB</sup>	3.22±0.27 <sup>bB</sup>	3.67±0.1 <sup>aA</sup>	5.43±0.43 <sup>bA</sup>	5.80±0.08 <sup>cA</sup>	17.70±1.05 <sup>a</sup>
	20	73.58±0.31 <sup>dA</sup>	54.37±1.91 <sup>dB</sup>	3.52±0.03 <sup>aB</sup>	4.07±0.1 <sup>aA</sup>	5.38±0.12 <sup>bA</sup>	5.56±0.21 <sup>cA</sup>	19.23±1.91 <sup>a</sup>
JNF	0	90.48±0.35 <sup>aA</sup>	85.61±0.12 <sup>aB</sup>	-0.38±0.01 <sup>dB</sup>	-0.32±0.02 <sup>cA</sup>	9.34±0.22 <sup>aB</sup>	13.91±0.04 <sup>aA</sup>	6.68±0.26 <sup>c</sup>
	5	83.03±0.25 <sup>bA</sup>	64.64±0.49 <sup>bB</sup>	1.96±0.05 <sup>cB</sup>	3.65±0.11 <sup>dA</sup>	6.28±0.22 <sup>bB</sup>	10.27±0.06 <sup>bA</sup>	18.89±0.37 <sup>a</sup>
	10	78.23±0.22 <sup>cA</sup>	60.62±0.48 <sup>cB</sup>	3.32±0.04 <sup>bB</sup>	4.43±0.16 <sup>cA</sup>	5.85±0.16 <sup>cB</sup>	9.36±0.21 <sup>cA</sup>	17.99±0.50 <sup>a</sup>
	15	75.21±0.36 <sup>dA</sup>	57.64±0.19 <sup>dB</sup>	4.15±0.03 <sup>aB</sup>	4.86±0.11 <sup>bA</sup>	6.25±0.11 <sup>bB</sup>	8.77±0.06 <sup>dA</sup>	17.76±0.54 <sup>a</sup>
	20	72.43±0.89 <sup>eA</sup>	56.59±0.59 <sup>dB</sup>	4.45±0.09 <sup>aB</sup>	5.40±0.15 <sup>aA</sup>	6.22±0.11 <sup>bB</sup>	7.29±0.18 <sup>cA</sup>	15.91±1.44 <sup>b</sup>

Mean and standard deviation of three replicates. For the Tukey test, means with different lower case letters in the same column and upper case letters in the same line are significantly different ( $p \leq 0.05$ ). Ext: extrudates, Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF).

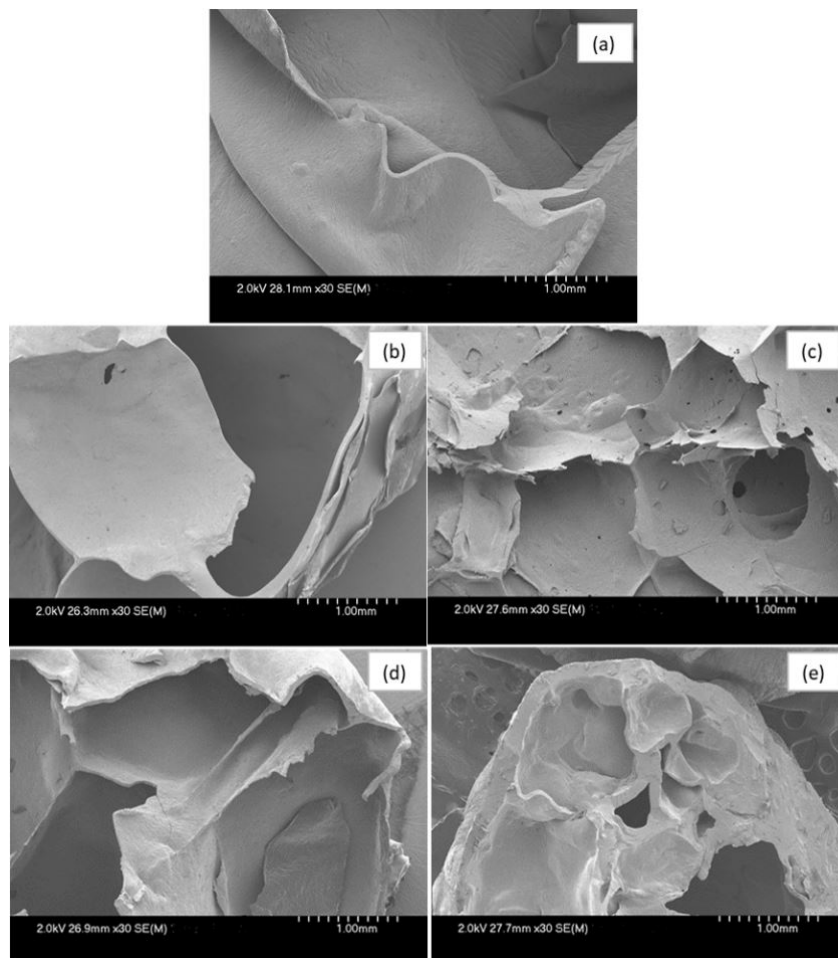
### 3.6 Scanning electron microscopy

The micrographs showcased differences in the structural characteristics of the extrudates (Figure 2). Differentiated and irregular cell sizes were observed. Figure 2a illustrates the complete gelatinization of the starch. The extrudate has the appearance of a homogeneous amorphous mass in which it is not possible to distinguish starch granules.

The extrudate with 10% JF (Figure 2b) showed large holes, which were formed by the expansion of the product at the extruder outlet, while the extrudate with 10% JNF

(Figure 2c) exhibited a larger number of air bubbles, thinner walls and was brittle and less hard, as well.

The extrudate with 20% JNF had the lowest expansion index. Figure 2e displays a compact, thick-walled structure, with highest density, unlike the extrudate with 20% JF (Figure 2d), probably because this sample had undergone fermentation. As previously mentioned, the fermentation process allows microorganisms to convert the nutrient compositions of the substrate into organic acid, causing this reduction in density<sup>[26]</sup>.



**Figure 2.** Micrographs of the extrudates (Ext). Non-fermented flour of Jab (JNF) and fermented flour of Jab (JF). JNF 0% (a); JF 10% (b); JNF 10% (c); JF 20% (d); JNF 20% (e).

#### 4. Conclusion

Fermented and non-fermented Jab pomace flours are industrial byproducts that aggregate nutrients to the extrudates but increasing their concentration in the extrudate products decreases the expansion index, increases the density and renders darker and reddish tones. The content of polyphenols and the antioxidant activity decreased after extrusion, although the products with 20% of non-fermented Jab pomace flour showcased stable antioxidant activity, thence emphasizing that the flours behaved differently post-extrusion.

The results showed that JNF is the flour with the best potential for the elaboration of extrudates, for presenting polyphenols and other antioxidant compounds that have shown to be resistant, to a certain extent, to the conditions studied in this work for the extrusion process. It is necessary to deepen this study looking for a way to encapsulate the antioxidant compounds to minimize their losses during the extrusion, as well as to expand the complements that can be used (in this work was used the rice and lentil flours). This work showed that the jaboticaba residues flour can be applied in order to enrich extrudates as snacks that have wide commercialization, as well as other food products that need enrichment with antioxidant compounds.

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