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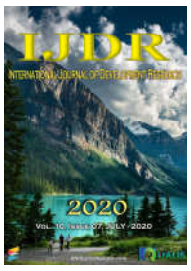

RESEARCH ARTICLE OPEN ACCESS

CHARACTERIZATION OF DIETARY FIBER, INCLUDING ASSOCIATED POLYPHENOLS, IN RESIDUES OF THREE TROPICAL FRUITS SUBJECTED TO DIFFERENT INDUSTRIAL PROCESSING

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ABSTRACT

In this work, the main characteristics of natural products rich in antioxidant food fiber and associated compounds obtained in the industry from the bagasse of assai, cashew apple and guava are described. The components of the total dietary fiber and its soluble and insoluble fractions from the industrial process of pulping and refining were evaluated. The method used to determine the dietary fiber contents was the indigestible fraction. The total amount of dietary fiber in the guava (85% pulped and 82% refined), assai (98% pulped and 85% refined) and cashew apple (85% pulped and 78% refined), showed that they can be considered excellent sources of Dietary fiber, presenting a new food alternative to the industrial sector, and also it can be used in the elaboration of new products.

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INTRODUCTION

Despite the current concern about healthy eating and general health, the consumption of dietary fiber by the Brazilian population has greatly decreased in the last three decades. Data shows that the consumption of staple food, in which constitutes significant fiber content, was reduced between the 70's and 90's in the common Brazilian diet and it was replaced by other types of food (Bernaud *et al*., 2013). This is a reflection of the change in social and economic factors, such as the transfer of domestic food production to the market system and the increase in the standard of living, which influenced the standard diet of the population. Factors such as the increase and the great diversity of industrialized food, which are generally rich in fats, refined carbohydrates and have high energy value, directly affected the total intake of dietary fiber by the population (Contreras, 2011). However, the increase in industrialization can be used to reverse this for enriched and/or fortified food. Regarding to processed food, nutrients are added in order to strengthen or supplement their content at a higher level than normal (Velozzo *et al*., 2010). Thus, the fiber contained in source food can be used for this purpose, contributing to the increase in the consumption of dietary fiber.

Research into the beneficial effects of dietary fiber on preventing the risk of chronic degenerative diseases and weight loss has led to the development of products enriched with this substance. To these products, which are often traditional foods, different amounts of dietary fiber are added from a variety of sources, Ice cream with linseed (Lamounier *et al.,* 2012), from guava (Roberto 2012), pineapple (Fonseca *et al*., 2011). According to Saura-Calixto (2017), the consumption of dietary fiber in Western countries counts only about one-third of the substrates required for the renewal of intestinal cells through fermentation by bacteria in the bowel colon. During fermentation, there is a formation of gases that provide energy for cell's renewal, synthesis of vitamins and the decrease of pH of the colon, collaborating with the balance of the intestinal microflora, thus forming a medium that hinders the growth of pathogenic bacteria. In the literature, investigations in tropical fruits report the high dietary fiber content as natural sources of this nutrient (Camacho *et al*., 2018). Despite the existing studies on fiber in tropical fruits, the information is still incipient regarding the total dietary fiber content, regarding the method used in agro-industry residues. The aim of this work was to ascertain the DF and total phenolic contents in the residues originating from the pulping and refining processes of assai, cashew apple and guava fruits, and their antioxidant capacity. Based

on these results, it would be possible to evaluate the application of the waste of these tropical fruits as antioxidants and sources of DF in functional foods or as naturally antioxidant dietary supplements.

MATERIAL AND METHODS

Samples Information: Industrial residues of fruits of assai (Euterpe oleracea), cashew apple (Anacardium occidentale) and guava (Psidium guajava) originated from the steps of pulping and refining processes. The assai and cashew apple fruit residues were collected at the Brazilian Beverage and Food Company - EBBA, in the city of Aracati - CE, and the guava fruit residue at the Pulp Orchard Company, Fortaleza - CE. In the processing of the cashew apple it was used a pulp with a screen of 1.5 mm of diameter for pulping and of 0.6 mm for the refinement. The assai fruits were pulped in a 1.5 mm diameter screen and refined in 1.0 mm diameter. The guava fruits were pulped with 0.8 mm diameter sieve and refined in 0.6 mm diameter. These residues are heterogeneous because they are derived from fruits of different areas of cultivation and maturation point. The samples were collected in a single time, being composed of 5 kg of the agro-industry residue of each frozen fruit conditioned in plastic bags and transported in isothermal boxes to the Laboratory of Physiology and Postharvest Technology of Embrapa Tropical Agroindustry in Fortaleza, Where they were stored in a freezer at -20°C. Then, the samples were homogenized and separated in about 400 g of each residue, which were lyophilized in LIOTOP L202 equipment and went though an analytical Grinding mill IKA A11 Basic to standardize the particle size in (<0.5 mm). After that, they were stored in a freezer at -20°C until the analytical tests were performed.

Dietary Fiber Determination: The DF was measured based on the procedure described by Saura-Calixto *et al.,* (2000) with some modifications by Rufino *et al*., 2010. This method combines enzymatic treatments and separation of digestible compounds by dialysis using physiological conditions (temperature and pHs), obtaining the fraction of food that is not digested and reaches the large intestine where it is susceptible to fermentation by colonic microbiota. Nine samples of each fruit residue (n1- n9) were incubated with digestive enzymes simulating digestion in the intestine. After enzymatic treatment under controlled physiological conditions (temperature and pH), the fractions resistant to digestion were obtained in the human small intestine: soluble indigestible fraction (supernatant) and insoluble indigestible fraction (residue). For this, 0.5 g of lyophilized sample was weighed, 10 ml of 0.08 M HCl-KCl buffer solution at pH 1.5 and incubated with 0.2 ml of the enzyme pepsin (300 mg / ml) at 40 ° C for 1 h. Followed by 4.5 mL of 0.1 M phosphate buffer solution at pH 7.5, incubated with 1 mL of the enzyme pancreatin (5 mg / mL) at 37° C for 6 h. 9 mL of 0.1 M tris-maleate buffer solution at pH 6.9 and 1 mL α-amylase (120 mg / mL) at 37° C for 16 h was added. Subsequently, the samples were centrifuged for 15 min at 3000 rpm and then the supernatant was separated from the residue.

To the supernatants (n1 to n9) it was added 10 ml of 0.4 M sodium acetate buffer, pH 4.75 and incubated with 100 µL of the amyloglucosidase enzyme at 60° C for 45 min. It was then transferred to a dialysis bag (25.4 mm, 12-14000 daltons, INLAB, Brazil) and placed in a container with water for 48 h in a temperature range from 25 to 28 ºC with the aid of a peristaltic pump (Watson Marlow brand) Of flow 7 L / h. After the dialysis was complete, the acid hydrolysis was performed with 1 mL of sulfuric acid at 100 ° C for 90 min. The extracts were submitted to the analysis of non-starch polysaccharides (NSP), monosaccharides, uronic acids and polyphenols, thus it was obtained the indigestible fraction of soluble dietary fiber (SDF). The residues of samples n3, n6 and n9 were washed twice with 5 mL of distilled water and put in the oven for 12 h at 105 ° C to quantify proteins and ash content. In samples n1, n4 and n7 acid hydrolysis was performed with 3 mL of 12 M sulfuric acid at 30 ºC for 1 h, to determine the NSP, monosaccharides, uronic acids, lignin klason and polyphenols, after which the indigestible fraction of the fiber Diet (IDF).

Nonstarch Polysaccharides: Determined by DNS method (3,5-dinitro-salicylic acid) and glucose as standard. The readings were performed using a Shimadzu spectrophotometer (UV-1800 model) at 530 nm and the results were expressed as a percentage (Southgate 1969). Monosaccharides were determined by the Antrone method, using glucose as standard. The readings were performed in Shimadzu spectrophotometer (Model UV - 1800) at 620 nm. The results were expressed as percentage (Loewus 1952).

Uronic Acids: For its determination, galacturonic acid standard was used and the results were done in Shimadzu spectrophotometer (Model UV - 1800) at 448 nm. The results were expressed as percentage (Scott 1979).

Klason Lignin (KL): Determined in the insoluble DF, gravimetrically from the residue resulting from the acid hydrolysis, expressed as a percentage (Southgate 1969).

Resistant Protein (RP): Quantified in the insoluble FD through the micro Kjeldahl, from three steps: (Model TE-040/25), distillation in nitrogen distillation (model TE-036/1) and titration using the factor 6.25 for conversion of nitrogen into protein (AOAC 1997), with results Expressed as a percentage.

***Ash*:** Evaluated in the insoluble FD, using the muffle equipment (Quimis, model 318.2), in which the contents of the fiber were incinerated at 550 °C (IAL 2004). The results were expressed as resistant gray g/100 g dry mass.

Extractable Polyphenols (EP): The extraction was carried out in the insoluble and soluble DF samples using methanol/water extracting solution (50:50, v/v) acidified with HCl and acetone / water (70:30, v/v), measuring the flask with a (50% acidified methanol + 70% acetone in 50:50 ratio) according to Rufino *et al*. (2006). The determination was by the method Folin-Ciocalteau according to Larrauri et al. (1997), using spectrophotometric and standard curve of gallic acid with results expressed in percentage.

Calculation of dietary fiber: For the soluble DF result, the sum of NSP + AU + TEP was performed. The insoluble DF was calculated by adding the NSP + AU + LK + PR + CZ + TEP. The total dietary fiber (total DF) result was obtained by the sum of the insoluble DF + soluble DF.

Statistic Analysis: A completely randomized design was used, with three replications, in a 3x2 factorial arrangement, with three fruit residues analyzed and two types of processes. The analysis of variance (ANOVA) and Tukey test were used at the 5% probability level for comparison between means, by the statistical program SISVAR 5.1. Results were expressed as mean ± standard deviation.

RESULTS

The content and composition of DF in assai, cashew apple and guava, including neutral sugars, uronic acids, Klason lignin, resistant protein, ash and polyphenols are presented in Table 1. It is observed that in the skimming process the residue had a higher fiber content than the refining step. In this work the carbohydrate content found in the residues from the pulped and refining stages was: guava - 25.54% and 31.64%, cashew apple - 35.44% and 29.47% and assai - 30.0% and 27.36%, respectively. Which demonstrates the eminent role of carbohydrates in fiber composition. In this research, the klason lignin results found in the pulping and refining stages were: guava - 49.94% and 40.98%; Cashew apple - 30.57% and 33.01%; Assai - 53.71% and 47.75%. The resistant protein contents found in this research were: guava 6.46% (pulped) and 7.32% (refining), cashew apple 15.81% (pulped) and 12.93% (refining), assai 12.69% (pulped) and 6.22% (refining). As regards the ash content present in dietary fiber, this research found more prominent values, such as the cashew that obtained 62% of the mineral fraction in the pulping process. The levels of phenolic compounds found in the residues at the pulped

Table 1. Characterization of DF (g / 100g dm) in the industrial processing residues of three different tropical fruits

	Assai		Cashew apple		Guava	
	Pulped	Refining	Pulped	Refining	Pulped	Refining
Soluble DF						
RS	3.26 ± 0.13[a]	3.28±0.05[a]	n.d.	n.d.	3.18 ± 0.16[a]	3.12 ± 0.13[a]
UA	0.82 ± 0.02[a]	0.53±0.05[a]	2.20 ± 0.14[a]	0.77 ± 0.06[b]	1.21 ± 0.04[a]	1.01 ± 0.05[a]
Polyphenols	0.10±0.003[a]	0.08±0.002[a]	0.19 ± 0.01[a]	0.08±0.003[b]	0.11 ± 0.01[a]	0.13 ± 0.01[a]
TC	4.28 ± 0.13	3.89 ± 0.07	2.39 ± 0.14	0.85 ± 0.06	4.50 ± 0.16	4.26 ± 0.14
Insoluble DF						
RS	18.39±0.71[a]	17.45±0.72[a]	16.02±1.13[a]	15.22±1.21[a]	13.75±1.38[a]	16.38±1.56[a]
UA	1.84 ± 0.12[a]	2.00 ± 0.14[a]	2.99 ± 0.21[a]	2.00 ± 0.05[a]	2.33 ± 0.20[a]	4.23 ± 0.14[a]
KL	53.71±0.39[a]	47.75±1.88[a]	30.57±1.84[a]	33.01±2.26[a]	49.94±1.30[a]	42.13±1.61[b]
EP	0.06±0.0008[a]	0.02±0.001[b]	0.14 ± 0.01[a]	0.04±0.005[a]	0.02±0.002[a]	0.03±0.002[a]
HP	0.09 ± 0.002[a]	0.08±0.004[a]	0.12 ± 0.01[a]	0.10 ± 0.01[a]	0.11±0.001[a]	0.13±0.001[a]
NEPA	3.19 ± 0.01[a]	1.91 ± 0.25[b]	0.77 ± 0.01[a]	0.61 ± 0.04[a]	0.70 ± 0.01[a]	1.19 ± 0.04[a]
RP	12.69 ± 0.86[a]	6.22 ± 0.31[b]	15.81±0.98[a]	12.93±0.12[a]	6.46 ± 0.62[a]	7.32 ± 0.43[a]
Ash	2.37 ± 0.05[a]	1.18 ± 0.08[b]	3.62 ± 0.23[a]	2.86 ± 0.15[a]	1.44 ± 0.08[a]	1.90 ± 0.07[a]
TC	92.34 ± 1.19	76.61± 2.06	70.04± 2.39	66.77± 2.57	74.75± 2.01	72.31± 2.29
TDF	96.62 ± 1.20	80.50± 2.06	72.43± 2.39	67.62± 2.57	79.25± 2.01	76.57± 2.29

n.d., not detected. Different superscript letters indicate significant differences ($p < 0.05$) between processes, after applying-student's T test for paired samples. Reducing sugars (RS); Uronic acids (US); Total contente (TC); Klason Lignin (KL); Extractable Polyphenols (EP); Hydrolysable Polyphenols (HP); Non-Extractable Proanthocyanidins NEPA); Resistant protein (RP); Total dietary fiber (TDF).

Table 2. Summary of the analysis of variance for soluble, insoluble and total dietary fiber from the pulp and refining processes of guava, assai and cashew apple fruits

		Medium Square		
FV	GL	DF soluble	DF insoluble	DF total
Residue	1	11.51	231.66	347.37
Fruits	2	4.36	214.18	226.42
Res. x Frut.	2	6.27*	62.69*	51.15*
Erro	12	0.03	10.63	10.39
Média		5.24	81.27	86.53
CV (%)		3.34	4.01	3.72

*Significant at the 5% probability level

Table 3. Soluble, insoluble and total dietary fiber content of residues resulting from the skimming and refining processes of guava, açaiseiro and cashew fruit, expressed as % dry mass

	DF soluble		
	Guava	**Cashew apple**	**Assai**
Pulped	6.20±0.16Aa	6.33±0.19Aa	5.59±0.13Ba
Refining	5.89±0.15Aa	2.37±0.10Ca	5.06±0.09Ba
	DF insoluble		
	Guava	Cashew apple	Assai
Pulped	79.61±2.01Ba	79.44±2.43Ba	95.94±1.18Aa
Refining	76.13±2.31Aa	76.06±2.60Aa	80.85±2.05Aa
	DF total		
	Guava	Cashew apple	Assai
Pulped	85.81±2.01Ba	85.80±2.43Ba	98.93±1.18Aa
Refining	82.03±2.31Aba	78.40±2.66Ba	85.94±2.05Aa

Averages followed by letters, upper and lower case vertical, do not differ statistically from each other, at the level of 5% by the Tukey test.

Table 4. Antioxidant capacity associated with DF in industrial processing residues of different types of three tropical fruits

	Assai		Cashew apple		Guava	
	Pulped	**Refining**	**Pulped**	**Refining**	**Pulped**	**Refining**
ABTS•+ (µM Trolox/ g dm)*						
IDF-NEPA	38.78 ± 2.72	42.67 ± 0.11	n.d.	37.86 ± 1.05	n.d.	n.d.
DPPH• (EC_{50},g dm/g)						
SDF-EP	24.06 ± 0.70	29.02 ± 1.60	n.d.	35.09 ± 2.6[a]	45.08 ± 2.10[a]	27.15 ± 1.50[b]
IDF-EP	71.6 ± 3.4[a]	187.5 ± 5.7[b]	42.06 ± 2.3[a]	84.4 ± 2.0[b]	203.95 ± 3.8[a]	227.94 ± 1.10[b]
IDF-HP	82.7 ± 3.7[a]	102.8 ± 11.6[a]	31.10 ± 0.30[a]	23.0 ± 0.5[a]	42.14 ± 2.1[a]	36.05 ± 1.3[b]
FRAP (µM $FeSO_4$ /g dm)						
SDF-EP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IDF-EP	34.77 ± 0.88[a]	17.57 ± 0.68[b]	n.d.	16.18 ± 1.02	n.d.	15.00 ± 15.56
IDF-HP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not detected. Different superscript letters indicate significant differences ($P< 0.05$) between processes, after applying-student's T test for paired samples.
[1]DPPH and FRAP assay cannot be applied to NEPA.
* ABTS method was applied to the other polyphenol fractions, but values were below the limit of detection

stage followed the increasing order: 0.34% (cashew apple)> 0.16% (assai)> 0.14% (guava). In the refining process, the results followed the order: 0.17% (guava)> 0.13% (cashew apple)> 0.11% (assai). These values demonstrate that polyphenols may be associated with both the soluble and insoluble fiber matrix. The analysis of variance for soluble, insoluble and total FD variables showed a significant difference at the 5% probability level ($p\leq0.05$) in the interaction between the residues and the analyzed fruits. This difference is presented in the comparative test between means. The coefficients of variation (CV), obtained in the studied variables of soluble DF 3.34%, insoluble 4.01% and total 3.72%, demonstrate a satisfactory reproducibility of the data, considering the lack of classification of cultivars and stage of maturation from which fruits (Table 2). The comparative test between averages showed that guava residue (6.19%

skim and 5.87% refining) and cashew (6.32% skimpy) had the highest polyphenol content in soluble DF (Table 3). In the insoluble DF there was a difference between the fruits evaluated only in the refining process, where the assai residue reached the highest average (95.53% pulped). The assai was distinguished by obtaining the highest total DF content (98.93% pulped and 82.62% refining). These values corroborate with the research carried out by Rufino *et al*. (2011) with assai which was 76.68%. Table 4 shows the analysis of variance for the antioxidant capacity determined by the DPPH •, ABTS • + and FRAP methods demonstrating a significant difference at the 5% probability level ($p \leq 0.05$) in the interaction between the residues and the fruits analyzed. This difference is presented in the comparative test between means. No antioxidant activity was detected in soluble DF by the FRAP method. However, in the insoluble DF activity was verified by the same method, where the assai residue was highlighted by the high content of 34.77 μM FeSO4 / g in the pulped stage and 17.57 μM FeSO4 / g in the refining step.

DISCUSSION

The carbohydrate quantification determined by the analyzes of monosaccharides, non-starch polysaccharides and uronic acids represents the non-digestible portion of carbohydrates that reach the colon, serving as a substrate for fermentation by intestinal bacteria (Rufino *et al.,* 2010). Jiménez-Escrig *et al*. (2001), determining the total DF in guava, obtained a carbohydrate fraction of 30.0% in the barking and 30.99% in the pulping. Works developed by Rufino *et al*. (2010) analyzing the cashew apple obtained in total DF values of 5.75% in carbohydrates. Rufino *et al*. (2011) analyzing the total DF in assai fruits found 29.47% for this same analysis. The klason lignin is an insoluble residue remaining after acid hydrolysis, through the procedure commonly used to determine polysaccharides, which releases and determines gravimetrically (Saliba *et al.,* 2001).Salgado *et al.* (1999) in a study on dietary fiber content in fruit pulp, point out that the high fiber content in guava fruits is directly related to the lignin content of this fruit evidenced, therefore, in this research. During determination of dietary fiber, part of the protein is solubilized and another portion remains the same, being denominated resistant protein because it is not digestible by proteolytic enzymes. Some of the samples had a resistant protein content greater than 10%, which is an undesirable characteristic. In this case, specific processes can be developed to reduce their content.

Close values were found by Rufino *et al*. (2010) in cashews (4.12%) and 5.60% in assai fruits (Rufino *et al*., 2011). Grape is well studied, including dietary fiber content, so Lobera and Cañellas 2007) analyzing the content of dietary fiber in fruits to produce vine obtained a resistant protein content of 12% in their bagasse. Saura-Calixto *et al*. (2000) state that the minerals resulting from the enzymatic treatment are strongly bound to the plant, cell's wall; nevertheless, it constitutes a small fraction of less analytical importance. These same authors obtained in fruits, such as banana, contents of 1.70%, apple 0.43% and orange 0.39% of minerals. Polyphenols can be found separated or associated with dietary fiber. Most polyphenols in food research refer to free polyphenols, with fiber-associated polyphenols being ignored, although they are also bioactive compounds that have health benefits. Free polyphenols during the digestive process are susceptible to absorption in the small intestine. However, fiber-associated polyphenols are not bioavailable, reach the colon along with dietary fiber serving as a substrate for bacterial microflora, producing absorbable metabolites such as phenylacetic, phenylpropionic and phenylbutyric acids. These polyphenols can also contribute to a healthy environment, due to antioxidant action, eliminating free radicals and counterbalancing the effects of the pro-oxidant diet (Saura-Calixto 2010). Saura-Calixto (2010), evaluating some fruits individually, found averages of polyphenols associated with dietary fiber of 0.34% in plum, 0.56% in strawberry and 0.36% in apple with peel, stating that, generally in fruits one can find the highest content of these compounds. Gõni *et al*. (2009) evaluated polyphenols associated with fiber in different types of foods, obtaining fruits (1.53%) higher than in plants (0.43%) and cereals (0.24%), evidencing that fruits are considered the best source of polyphenols.

Figueiredo *et al*. (2009), soluble DF is characterized by dissolving in water forming viscous gels and being easily fermented by the microflora of the large intestine. The insoluble DF is not dissolved in water and undergoes partial fermentation in most of the food. The insoluble DF corresponds to about two thirds of the total fibers found in foods, a higher proportion than soluble solids which equals only one third. The information on the soluble and insoluble fractions of dietary fiber are important due to the effects provided by each of them, since in the food intake the two fractions will be ingested, but the effects on the digestive and metabolic processes will depend on the predominance of a fraction In relation to another, of its composition and structural organization (Monteiro 2005). Its quantification is also important for information on diet composition and food labeling. Although there is generally a predominance of insoluble fiber, the lower the relationship between soluble and insoluble fiber in a food, the more balanced the diet will be. In general, the residues of the analyzed fruits had a high content of total DF, since the results of the analyzes reached a percentage above 70% of dry matter (Saura-Calixto 2010; Rufino *et al.,* 2011). The evaluated residues have fiber content comparable to other tropical fruits such as apple, banana, orange (Saura-Calixto *et al*., 2000) and guava (Jiménez-Escrig *et al.,* 2001) that are considered sources of dietary fiber. Rufino *et al*. (2010) analyzing the extractable polyphenols content associated with cashew fiber did not detect for soluble DF, however, they obtained a content of 0.39% in the insoluble FD higher than that found in this research. Subsequently, Rufino *et al*. (2011) evaluating the extractable polyphenols associated with assai fiber, detected about 1% in the soluble DF and 0.4% in the insoluble DF.

In the evaluation of the extractable polyphenols, the antioxidant activity was not detected by the ABTS • + method in any of the fiber fractions. However, Saura-Calixto (2010) presents results of high antioxidant activity associated with dietary fiber in assai fruit (78.2 μM trolox / g) and guava in bark (226.3 μM trolox / g) by the method ABTS • +. In the DPPH method, the soluble DF of the residual fruit residue (24.06 g ms / g DPPH • pulped and 29.02 g ms / g DPPH refining) and guava (27.15 g ms / g DPPH refining) stood out for reaching the highest antioxidant activity. Rufino *et al*. (2011), analyzing soluble DF in fruits of assai, found a mean of 39.43 g m.s./g DPPH •, being lower than the results of this research. In insoluble DF the cashew apple residue (42.06 g m.s./g DPPH • pulped and 84.4 g m.s./g DPPH • refining) had the highest antioxidant activity, however this activity was lower than that obtained by Rufino *et al*. (2010) in cashew apple 16.62 g m.s./g DPPH •, considering that this method is inversely proportional.

CONCLUSION

All samples had a high total fiber content, greater than 65% and, in seven of the eight samples, the results were higher than 70%. The fiber is extremely insoluble, with a soluble fiber content of less than 10% in all cases, being able to make combinations with other products with higher content of soluble fiber in order to obtain a fiber of better nutritional quality. All samples presented polyphenols associated with fiber, again indicating the role of these compounds as constituents of the fiber. Specifically, the samples have between 1% and 3% of fiber-associated polyphenols. The most sensitive and indicated method to determine the antioxidant capacity associated with fiber matrix was DPPH.

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