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Carbohydrate composition of peach palm (*Bactris gasipaes* Kunth) by-products flours

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ABSTRACT

The flours obtained from peach palm by-products are rich in dietary fiber (62–71%) and they can be used as food ingredients. The aim of this work was to investigate the carbohydrate composition of the flours processed from the residual parts (stem and median sheath) of a hearts-of-palm industry. The flours were fractionated, based on their solubility, whose monomeric compounds were determined. The fraction containing mostly cellulose (S5) was the most abundant (26–28%), followed by the sum of fractions (S2, 53, S4) extracted with alkaline solutions (21–22%). The S1 fraction contained the highest percentage of uronic acids, which characterizes the presence of pectin. Xylose and arabinose were found in high proportion in S2 and S3 fractions. The S4 and S5 fractions, rich in glucose, were the main portion of the cell wall material and correspond to the insoluble fraction of the dietary fiber.

1. Introduction

Brazil is one of the largest producers and consumers of heart-of-palm (*palmito*) of the world. Among the palm species, the peach palm (*Bactris gasipaes* Kunth) yields two food crops with commercial potential, the fruit and the heart-of-palm. The processing of heart-of-palm from *B. gasipaes* (locally known as *pupunha*) for the international market is expanding, because of the appreciated sensory characteristics of the product (Santos, Corrêa-Júnior, & Neves, 2008). However the canning process generates a large amount of by-products due to the non uniform diameter and variable texture of the harvested rod. The by-products generated during the processing of the food result in cost to dispose of the material and in an environmental problem, due to the high volumes of discarded material (Mateos-Aparicio, Redondo-Cuenca, & Villanueva-Suárez, 2010b). The median sheaths that cover the heart-of-palm and the stem part located below the edible portion are peach palm by-products that correspond to an average of 46% of the weight of the harvested rod. The flours produced from these parts are high in dietary fiber (62–71%), mainly insoluble fiber 59 to 69% of the total (Bolanho, Danesi, & Beléia, 2013, 2014).

The interest in ingredients rich in dietary fiber (DF) has increased and the importance of this food component has led to the development of a large market for fiber-rich products and ingredients (Pszczola, 2008). The intake of foods with high DF content has been related to several physiological and metabolic effects: increase of the fecal bulk, provide a favorable environment for beneficial intestinal microbiota multiplication, prevention and control of obesity, atherosclerosis, coronary heart diseases, colorectal cancer and diabetes (Vergara-Valencia et al., 2007).

The determination of the polysaccharides composition in the DF (pectin, cellulose and hemicellulose) is important to understand their physiological function, structure and organization in food products, and it allows a planned application in functional foods (Waldron, Parker, & Smith, 2003). Furthermore, studies on the characterization of neutral sugar composition of the major cell wall components are scarce. Therefore, the aim of this work was

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to fractionate the polysaccharides present in the peach palm by-product flours and investigate the monosaccharides composition of the fractions.

2. Materials and methods

2.1. Peach palm by-products flours

The peach palm flours were processed from the median sheaths and parts of the unused stem, harvested in a heart-of-palm farm in Mariluz, Paraná, Brazil, which were subjected to washing, sanitizing, cutting and drying in an oven with forced air circulation at 60 °C for 36 h (MA 035, Marconi, Piracicaba, Brazil). The dried material was ground in a knife mill type Willye (SL-031, Solab, Piracicaba, Brazil) and passed through a set of sieves with particle separation from 150 μm to 600 μm, subjected to vibration for 10 min. The flours from median sheath (MSF) and stem part (SF) of peach palm with particle size of 150 μm were used in the analysis. This fraction would have the most possibility of application being similar in size to flours used for breakfast cereals and baking.

2.2. Polysaccharides fractionation

The procedure of Shiga, Lajolo, and Filisetti (2004) was followed for the fractionation of the carbohydrates with some modifications. The soluble sugars present in MSF and SF were extracted with an aqueous ethanol solution (80%) for 20 min at 80 °C with eventual shaking. The material was centrifuged at 8000 × *g* (Shimadzu, Himac CFD2, São Paulo, Brazil) and the supernatant discarded. This procedure was repeated 8 times. The precipitate was dried in an oven with forced air circulation at 40 °C for 12 h (MA 035, Marconi, Piracicaba, Brazil) and weighed afterwards. The starch was removed from this material using 90% DMSO (dimethylsulfoxide) with shaking at 200 rpm (Marconi, MA 830/A) for 24 h. The solution was centrifuged and the precipitated was dried and weighed (Seibel & Beléia, 2008). The glucose content of alcoholic and DMSO fractions were determined after acid hydrolysis, using an enzymatic kit of glucose oxidase (Biotécnica, Varginha, Brazil). The conversion of glucose into starch was calculated multiplying by the factor 0.9.

The S1 extraction used 0.5% ammonium oxalate with agitation in a magnetic stirrer at 80 °C for 1 h and the material was centrifuged at 8000 × *g* for 15 min at 4 °C. This procedure was repeated three times. The supernatants, containing the S1 fraction, were combined and dialyzed against water (24 h) and distilled water (24 h). The precipitates of the soluble pectin fraction were washed three times with distilled water, freeze dried (Telstar, LyoQuest, São Paulo, Brazil) and weighed.

To the remaining solid material of S1 extraction, sodium chlorite (NaClO), glacial acetic acid, and deionized water were added for lignin removal. The mixture was placed in a water bath at 75 °C for 2 h, the suspension was centrifuged at 8000 × *g* for 15 min at 4 °C and the lignin in the supernatant fraction was dialyzed against water (48 h) and freeze dried.

The residue free of lignin was sequentially treated with 0.1, 1.0 and 4.0 M NaOH solutions containing 3 mg L^{-1} of $NaBH_4$ (sodium borohydrate) for 1 h at 25 °C with shaking at 200 rpm, originating the S2, S3 and S4 fractions, respectively. The supernatants were neutralized with acetic acid, dialyzed against water (24 h) and distilled water (24 h) and centrifuged at 8000 × *g* for 15 min at 4 °C. The final supernatants were freeze dried and weighed.

To the final precipitate, after S4 extraction, it was added the Updegraff reagent (5% nitric acid and 15% acetic acid). The mixture was placed in water bath at 100 °C for 90 min with shaking every 15 min, and afterwards, it was centrifuged at 8000 × *g* for 15 min at 4 °C. The supernatant was discarded and the precipitate was washed three times with distilled water, freeze dried and weighed, corresponding to the S5 fraction.

Table 1
Yield of polysaccharides fractions from flours of peach palm by-products.

Flour	Fraction (% of flour)				
	S1	S2	S3	S4	S5
SF	6.8 ± 0.8	8.1 ± 1.1	7.9 ± 1.0	5.4 ± 0.7	26.9 ± 3.3
MSF	6.5 ± 0.7	6.2 ± 0.8	9.5 ± 1.1	6.9 ± 0.9	28.5 ± 3.7

The means values are not significantly different ($p > 0.05$). SF (stem flour), MSF (median sheath flour). Soluble fractions in ammonium oxalate (S1); 0.1 M NaOH (S2); 1.0 M NaOH (S3); 4.0 M NaOH (S4); and S5 residual fraction.

2.3. Monosaccharides and uronic acid determination

The monosaccharides were determined according to Seibel and Beléia (2008) with some adaptations. The samples obtained from the fractionations were pre-hydrolyzed with 300 μL of 72% sulphuric acid in a water bath at 30 °C for 45 min. Distilled water (5.0 mL) was added to the samples and incubated in a water bath at 100 °C for 3 h. Afterwards the material was neutralized with sodium hydroxide (NaOH) and subjected to salts removal with anionic resins (Dowex 1 × 8 50–100 mesh, Cl Form, Sigma-Aldrich) and cationic resins (Dowex 50 w × 8 50–100 mesh, H Form, Sigma-Aldrich).

The samples were filtered (Millex-GV, PVDF hydrophilic membrane, 0.22 μm pore size; Millipore, Billerica, MA, USA) and analyzed by high performance anion exchange chromatography (HPAEC-PAD, model ICS 5000). Aliquots (10 μL) of the filtrate were injected automatically and the carbohydrates were separated by CarboPac® PA1 analytical column (Dionex Corporation, Sunnyvale, CA, EUA) preceded by a CarboPac® PA1 guard column. The flow rate was of 0.7 mL min^{-1} at 25 °C with gradient elution of 19 mM of NaOH for 17.25 min and 1 mM up to 22 min. After this first elution a washing step with 200 mM of NaOH for 10 min and a stabilization step with 19 mM of NaOH for 15 min were used before any other sampling. Chromatogram analysis was carried out using Chromeleon version 6.8 software (Dionex Corporation).

The standard curves were obtained with different concentrations of monosaccharides: glucose (0.7–23 μg mL^{-1}), galactose (0.8–7.0 μg mL^{-1}), mannose (0.1–8 μg mL^{-1}), arabinose (0.3–6.0 μg mL^{-1}) and fucose (0.1–1.5 μg mL^{-1}), all from Sigma-Aldrich (New Orleans, USA).

The uronic acid (UA) was determined in the hydrolysates by reaction with 0.15% *m*-hydroxydiphenyl dissolved in 0.5% sodium hydroxide. The absorbance was read in a spectrophotometer (700 Plus, Femto, São Paulo, Brazil) at 520 nm. A standard curve with different concentrations of galacturonic acid (10–60 μg mL^{-1}) was used for uronic acid content (Kintner &Van Buren, 1982).

2.4. Statistic analysis

The analyses were performed in triplicate and the results were expressed as mean ± standard deviation. ANOVA, Tukey's test and principal component analysis were performed using the Statistica software version 6.0 (StatSoft, Inc.).

3. Results and discussion

The ethanol fraction that contained the soluble sugars had 13.1 and 9.2% of glucose in SF and MSF, respectively. The starch content varied from 2.2 for the SF to 2.5% for the MSF. These values are in accordance to that found by Bolanho et al. (2013, 2014).

The polysaccharides fractions obtained from SF and MSF had similar yields ($p > 0.05$) (Table 1). Glucose and uronic acids were the

Table 2
Monomeric composition of polysaccharides fractions obtained from flours of peach palm by-products.

3Flour	Fraction	Neutral sugars (mg g^{-1})						UA (mg g^{-1})	Total (mg g^{-1})
		Fuc	Ara	Gal	Glu	Man	Xyl		
SF	S1	nd	2.3 ± 0.1^{f}	6.6 ± 0.3^{cd}	33.1 ± 2.7^{c}	2.5 ± 0.1^{c}	nd	124.3 ± 7.4^{a}	168.7
	S2	nd	14.3 ± 0.7^{a}	7.4 ± 0.3^{c}	6.4 ± 0.3^{d}	nd	39.4 ± 1.5^{c}	39.9 ± 1.9b^{c}	107.4
	S3	nd	5.2 ± 0.0^{c}	3.4 ± 0.1^{e}	6.9 ± 0.4d	nd	100.8 ± 8.9^{a}	36.3 ± 1.6^{c}	152.6
	S4	1.7 ± 0.1^{a}	3.6 ± 0.1^{de}	14.1 ± 0.2^{b}	68.4 ± 0.2^{c}	24.3 ± 0.2^{a}	47.5 ± 4.6^{c}	21.3 ± 0.7^{e}	180.9
	S5	nd	nd	nd	452.3 ± 22.5^{a}	nd	nd	23.3 ± 1.2^{de}	475.6
MSF	S1	nd	4.3 ± 0.4^{de}	16.1 ± 1.6^{a}	43.4 ± 4.9^{c}	19.5 ± 1.7^{b}	nd	131.4 ± 5.6^{a}	214.8
	S2	nd	6.1 ± 0.4^{b}	5.7 ± 0.5^{d}	3.5 ± 0.3^{d}	nd	17.2 ± 1.5^{d}	48.9 ± 1.6^{b}	81.5
	S3	nd	3.4 ± 0.3^{d}	3.5 ± 0.3^{e}	5.3 ± 0.4^{d}	nd	64.9 ± 0.3^{b}	30.7 ± 2.0^{cd}	107.9
	S4	1.2 ± 0.0^{b}	4.5 ± 0.3^{d}	7.4 ± 0.4^{c}	48.6 ± 4.8^{c}	20.7 ± 0.5^{b}	44.5 ± 4.3^{c}	34.9 ± 0.8^{c}	161.8
	S5	nd	nd	nd	356.3 ± 25.4^{b}	nd	nd	15.7 ± 1.4^{e}	371.9

Means values in the same column followed by the same letter are not significantly different ($p > 0.05$). SF (stem flour), MSF (median sheath flour), Fuc (fucose), Ara (arabinose), Gal (galactose), Glu (glucose), Man (manose), Xyl (xylose), UA (uronic acids), nd (not detected). Soluble fractions in ammonium oxalate (S1); 0.1 M NaOH (S2); 1.0 M NaOH (S3); 4.0 M NaOH (S4); and S5 residual fraction.

major monomers of the polysaccharides fractions (Table 2). Fucose was only found in the S4 fraction while rhamnose was not detected in the materials analyzed. Fig. 1 shows a representative elution pattern of S4 fraction in the HPAEC-PAD. Simas et al. (2010) observed that xylose and glucose were the main components of king palm flour.

The S1 fraction was extracted with ammonium oxalate in order to cause minimal degradation and to retain maximum chelating power of Ca and Mg ions that are complexed with the galacturonic acids (Selvendran & O'Neill, 1987). The values obtained for S1 fraction (6.5–6.8%) were lower than that found by Seibel and Beléia (2008) for the cell wall of soy fiber (13.2–15.4%). Pectin is the main component of this fraction, and it can be present in other fractions because the highly ramified pectic molecules can be found associated with cellulose (Mateos-Aparicio, Mateos-Peinado, Jiménez-Escrig, & Rupérez, 2010a). The pectin is a soluble fiber fraction that can delay glucose and lipid uptake into the blood stream and may reduce the serum cholesterol level. The fermentation of pectin can also help to prevent some diseases or disorders such as intestinal infections (Lattimer & Haub, 2010).

Pectin is mainly composed by chains of galacturonic acid with side chains of neutral sugars in different amounts depending on the pectin source (Mudgil & Barak, 2013). Thus, the S1 fraction of SF and MSF had galacturonic acid as the main component (61–74%), which is consistent with the presence of homogalacturonan. Mateos-Aparicio et al. (2010b) also found UA as the major monomer of S1 fraction of legume by-products (okara, pea pod and broad bean pod), with values ranging from 12.4 to 71.4%. According to the authors, in this fraction of pea pod and bean pod, residues of rhamnose and fucose were not detected, as observed in the flours produced from peach palm by-products. The levels of mannose (2–9%), galactose (4–8%) and arabinose (1–2%) may indicate the presence of mannans, galactans, arabinans and arabinogalactans, whose chains may contain glucose molecules.

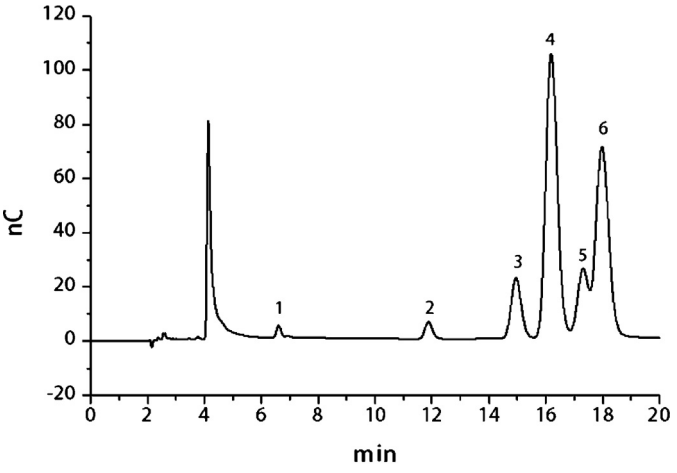

Fig. 1. Separation of sugars in flour of peach palm by-products using high-performance anion-exchange chromatography associated with pulsed-amperometric detection (HPAEC-PAD). Monosaccharides fucose (1), arabinose (2), galactose (3), glucose (4), mannose (5) and xylose (6) are shown with their respective retention times.

The delignification before the alkaline extraction yielded an estimated value of 10.1% in MSF to 15.7% in SF, however these values are super estimated due to the solubility of some carbohydrate (5–10%), presence of phenolic compounds and glycoproteins extracted by the sodium chlorite solution (Seibel & Beléia, 2008).

The saponification with 0.1 (S2), 1.0 (S3) and 4.0 M (S4) of NaOH released hemicellulose chains, that constitute a significant part of whole cell wall polymers, totalizing 21.4% in SF and 22.5% in MSF. Similarly Gáspár, Juhász, Szengyel, and Réczey (2005) studying the effect of alkaline extraction in corn fiber obtained a total yield of 20.9% for hemicellulose. The increasing concentrations of alkali extracted first the most soluble polysaccharide fraction (6.2–8.1%), the second extracted the low molecular weight polymers (7.9–9.5%), and the third the higher molecular weight hemicelluloses (5.4–6.9%) (Selvendran & O'Neill, 1987). To ensure that the fractions do not have other saccharides the dialysis steps are important. Among these fractions, the S3 was the major fraction in the flours studied, whose concentration was similar to the values found by Seibel and Beléia (2008) in fibers of soybean cotyledons (7.8–8.1%). Hemicelluloses are important for intestinal regulation, help to increase the number of beneficial bacteria in the gut and directly bind cholesterol, preventing its absorption from the intestine (Mudgil, Barak, & Khatkar, 2012).

The 0.1 M NaOH solution (S2 fraction) solubilized a mixture of polymers with a high concentration of xylose (21–37%), and a lower concentration of glucose (4–6%) demonstrating the occurrence of xylans, possibly along with xyloglucans, the most widespread neutral hemicellulose. Arabinose (8–13%), galactose (7–8%) and UA (37–60%) content of this fraction suggests a mixture of pectin and hemicellulose polymers. The uronic acids could be galacturonic or glucuronic acids, the first generally forms pectin molecules strongly bound or wrapped firmly in cellulose/hemicellulose network, while the second would probably be a hemicellulose substituent (Huisman, Schols, & Voragen, 1998). The level of these monomers

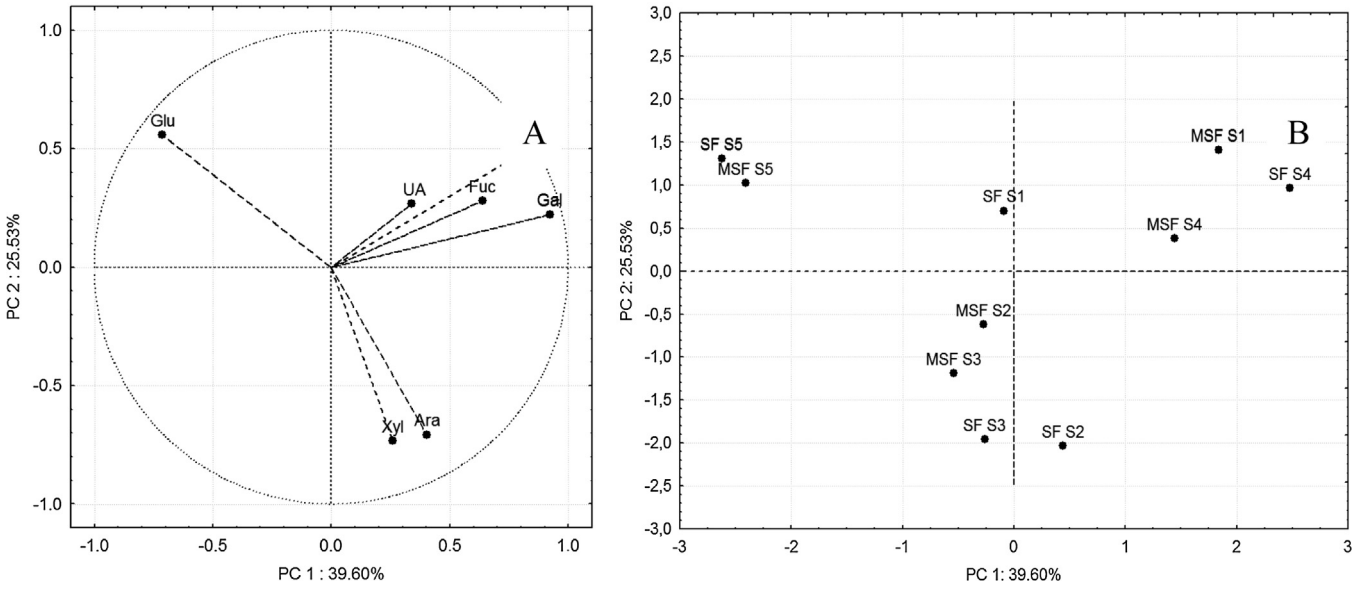

Fig. 2. Principal component analysis of the monomer components of flours from peach palm by-products: projections of the chemical components (A) and samples (B). SF S1 (soluble fraction in ammonium oxalate of stem flour), SF S2 (soluble fraction in 0.1 M NaOH of stem flour), SF S3 (soluble fraction in 1.0 M NaOH of stem flour), SF S4 (soluble fraction in 4.0 M NaOH of stem flour), SF S5 (residual fraction of stem flour), MSF S1 (soluble fraction in ammonium oxalate of median sheath flour), MSF S2 (soluble fraction in 0.1 M NaOH of median sheath flour), MSF S3 (soluble fraction in 1.0 M NaOH of median sheath flour), MSF S4 (soluble fraction in 4.0 M NaOH of median sheath flour), MSF S5 (residual fraction of median sheath flour).

also indicates the presence of arabinans, galactans and arabinogalactans.

The alkali concentration of 1.0 M originated S3 fraction that had higher xylose concentration (60–66% in both flours) than S2 fraction, inferring a hemicellulose polymer rich in xylans. It also had UA (24–28%), glucose (4–5%), galactose (2–3%) and arabinose (3%), demonstrating the possibility of some pectin inclusion. The complex architecture of the cell wall, probably due to a high degree of ramification of the polysaccharides implies insolubility and association with other polymers (Ng, Parr, Ingham, Rigby, & Waldron, 1998). Mateos-Aparicio et al. (2010a,b) found values of 5.0 and 21.9% for arabinose, 5.8 and 37.4% for galactose, 3.6 and 5.0% for glucose and 4.4 and 16.2% of uronic acids, respectively, in 1 M KOH soluble fraction of soybean by-products.

The S4 fraction (extracted with 4 M NaOH) probably has a lower concentration of xylans than the S3 fraction due to the level of xylose (26–28%) found. The xylose chains of this fraction may have fucose in its composition (1–2%), and the content of mannose (12–13%) and galactose (5–8%) may indicate the presence of galactomannans. Some pentosans containing arabinose and xylose and/or polymers of galactose, mannose and glucose may also participate in the S4 fraction. The high content of glucose in S4 (30–38%) demonstrates the potential existence of glucose-rich neutral hemicellulose and/or some acid hemicellulose with glucuronic acid, due the presence of UA (12–22%). According to Redgwell et al. (2011), strong alkali can solubilize hemicelluloses or other polysaccharides which are strongly hydrogen bonded to the cellulose fibrils.

The main component of both flours was the S5 fraction (26.9–28.5%). This insoluble residue fraction also corresponds to approximately 49% of total cell wall polymers extracted, which is in accordance to the values found by Mateos-Aparicio et al. (2010b) (21.9–71.0%) when analyzing the by-products okara, pea pod and broad bean pod. This fraction is mainly composed by cellulose. Cellulose forms about one third of dietary fiber in vegetables and its insolubility in water helps to increase of fecal volume promoting regular bowel movements (Mudgil & Barak, 2013).

The fraction solubilized in the 4 M NaOH solution together with the final residue (S5) obtained after the acid hydrolysis reached the highest number of monomers. This indicates that the complex structure of the cell wall of peach palm by-products prevents the total accessibility of the selected reagents for complete extraction of the various fractions. The cellulose-rich residue (S5) of the flours was formed mostly by glucose (95–96%) and a low amount of uronic acids (4–5%). Kosmala et al. (2013) found similar glucose content (333 mg g^{-1}) in cellulosic residue of plum pomace and glucose was the main component of the final residue obtained by Favaro, Beléia, Fonseca-Junior, and Waldron (2008), Mateos-Aparicio et al. (2010a) and Shiga and Lajolo (2006), but these authors also found lower amounts of other sugars. The absence of additional monosaccharides may be the result of Updegraff reagent, used to isolate this fraction. However, as described by Ramirez-Truquea, Esquivela, and Carleb (2011) bonds between the cellulose and mannose or xylose may be extremely stable and difficult to cleave even by treatment with mineral acids, indicating that the S5 composition is characteristic of each material.

S4 and S5 fractions are predominantly considered insoluble DF, due to their composition and low solubility (Mateos-Aparicio et al., 2010a). The high yield of these fractions and the high content of monomers allow us to conclude that the insoluble DF is the major component of the flours studied as also observed by Bolanho et al. (2014), that found values between 59 and 69% of insoluble fibers in flours of peach palm by-products using the enzymatic–gravimetric method. Insoluble fibers are characterized by their porosity, low density and by the ability to increase fecal bulk.

Fiber-rich by-products may be incorporated into food products as inexpensive, non-caloric bulking agents for partial replacement of flour, fat or sugar, as enhancers of water and oil retention and to improve emulsion or oxidative stability (Elleuch, Bedigian, Roiseux, Besbes, Blecker 2011). Furthermore, the market of functional foods is increasing, and there are few types of flours commercially available that can be added to food products to increase their fiber content at low cost. Thus, the flours produced have a promising

market, especially for being very light in color, which facilitate their incorporation to food products.

The principal components analysis (PCA) helps to understand the monomers distribution in the fractions, the PC1 explained 65% of the total variance and it was correlated to the concentration of glucose, mannose, fucose and galactose, whereas PC 2 was characterized by uronic acid, arabinose and xylose content (Fig. 2A). Fig. 2B illustrates that the S5 fraction of both flours studied, was allocated to the left of the vertical line and above the horizontal line of the graph due to its high glucose content. The S2 and S3 fractions of SF and MSF are closer (below in the graph) because of the xylose and arabinose level. S4 fractions of both flours are located to the right and above of the horizontal line due to the high percentage of mannose, fucose and galactose. The high UA content of the S1 fractions situates it above the line in the graph, but S1 from SF is at the left side due to the high glucose content, and the same fraction from MSF is at right because of the high levels of mannose and galactose. Therefore, each fraction can be characterized by its main components and its profile is similar in the two flours analyzed.

This study contributes to the knowledge of cell wall polysaccharides composition of peach palm by-products, which have an environmental impact as waste for the food industries and that have a great potential as a fiber-rich ingredient. Furthermore, it is important to increase information and knowledge about this food crop genuinely national.

4. Conclusion

The flours processed from peach palm by-products have high level of non starch polysaccharides and consequently they are important as a source of dietary fiber for inclusion in other foods, such as breakfast cereals or bakery products. The high yield of S4 and S5 fractions indicated that the flours are predominantly formed by insoluble fiber, which can promote health benefits. The monomer characterization of the fractions allowed describing the composition of the different fractions extracted.

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