

Chemical Characterization of Pectic Heteropolysaccharide Extracted from Babassu Mesocarp Flour

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Abstract— Babassu mesocarp flour (BMF) is a product basically composed of starch, fibers, mineral salts, vitamins, tannins and proteins. Pectins are composed of a structurally heterogeneous polysaccharide chain widely distributed in the primary cell wall and in the middle lamella of plants. New sources of pectins are important for industry. In this sense, BMF, produced from the fruit of the Amazon region, rich in carbohydrates, becomes an attractive raw material for extracting this compound. In view of the above, the present study aimed to extract and characterize the material obtained from the BMF. An acid extraction was applied using BMF as raw material. Yield was checked. From the extracted sample moisture, ash and pH analyzes were carried out, titration quality evaluated, chemical composition by Nuclear Magnetic Resonance (NMR) and structural characterization by polarized light microscopy. With the results it was possible to observe an extraction yield of 24.13%. The material had 6.5% of moisture, pH 2.77 and 0.90% ash. Regarding the titrimetric quality, the parameters were compatible with values reported in the literature for high methoxylation pectins. To evaluate the characterization of the chemical composition of the material extracted from the BMF by NMR, it can be observed through the ¹H and ¹³C NMR spectra three distinct regions: the carboxylic region, the anomeric region and the methylic region, which are characteristic regions of pectic material. And through polarized light microscopy, it was possible to identify the presence of glucose. In the end, it is understood that the material extracted from FBM is characterized as a pectic heteropolysaccharide.

Keywords— High methoxylation pectins: New source of pectin: Pectic heteropolysaccharide extracted from babassu mesocarp flour.

I. INTRODUCTION

Babassu is a palm tree native to the Amazon region, of great economic and technological importance for the chemical, cosmetic and food industries Fertonani *et al.*, (2009). The different parts of the composition of the babassu fruit have been used, mainly, for the elaboration of products, such as flour and oil. Babassu is considered the world's largest source of wild oil for use domestic and in recent years, mesocarp flour, the object of this study, has leveraged food processing (Camili *et al.*, 2019; Cavalcante, 2012).

Polysaccharides are substances that are often soluble in water and have the ability to significantly alter the rheology of aqueous solutions in which they are added, even at relatively low concentrations. This property is essential for their functions, particularly as thickening and gelling agents, which explains their widespread use and value, especially in the food industry (Delattre, Fenoradosoa, and Michaud, 2011). As a result, alternative sources of polysaccharides are being extensively researched due to the growing demand and increasing interest in substances with various functional properties (Christiaens *et al.*, 2015).

The flour extracted from the fruit, called babassu mesocarp flour (BMF) is a 100% natural product, which represents 17 to 22% of the fruit, consisting of 60% starch, about 20% fiber, 8 to 15 % of moisture and 4 to 5% of various compounds, including mineral salts, vitamins, proteins and tannins, the latter being responsible for the brownish color of the BMF (Almeida *et al.*, 2011 and Couri & Giada, 2016).

Due to its starch-rich composition, it is interesting to investigate the composition of this fraction, especially in terms of pectin evaluation in this material. It should be noted that pectin is a very important additive for the food industry. It is applied in the prevention of flotation in fruit preparations, acts in the stabilization of bakery products, stabilization protein, improves texture, providing softness, increased volume and control syneresis (Voragen *et al.*, 2009).

According to Luo *et al.*, (2017) pectins are distributed in the primary wall and in the middle lamella of the plant cell wall. Commercially, pectin is commonly extracted from vegetable matrices such as apple pomace, beet pulp, sunflower heads and citrus albedo, those are the most used (Canteri *et al.*, 2012). Also, according to the authors, research with new sources of pectins is exceedingly important for the industry, elucidating unusual technological and/or structural aspects, enabling the

discovery of pectins with different structural properties and, consequently, different applications in food development (Canteri et al., 2012).

For this purpose, the extraction and characterization of pectins from new plant sources has become thought-provoking and challenging. Allied to this factor, there is a need to explore and characterize the great variety of plants of Amazonian origins. Thereupon, the highlight of this study is the babassu fruit which presents technological potentialities for the food industry and that its flour is still little known and chemically characterized. Therefore, this work aimed to characterize the pectic material extracted from BMF in terms of its chemical properties.

II. MATERIALS AND METHODS

2.1 Obtaining the raw material

The BMF pectic material was extracted following the methodology described by Oliveira (2019), adapted from Munhoz et al., (2010). The BMF was dissolved in an acidic solution (1:30 v/v). The extraction took place at constant temperature (90 °C), with the variants of the extraction process in the time of 90 minutes and citric acid concentration of 4.5%. After the acid extraction, the samples were subjected to cooling at 4°C for 120 minutes, centrifuged at 1500 g for 10 minutes, and were filtered under vacuum, discarding the supernatant. Ethyl alcohol (95%) was added to the filtrate containing the material in a 1:2 (v/v) ratio. It was left to rest for 60 min. The precipitation was separated from the solution by filtering with filter paper. The pectic material retained on the filter was dried in an oven at 55 °C until we constant weight.

The dried pectic material samples were properly packed in plastic packages and stored under refrigeration until the time of analysis.

2.2 Evaluation of the chemical composition of pectic material extracted from BMF

Moisture, ash and pH concentrations of pectic material extracted from BMF were determined according to AOAC (2006).

2.3 Assessment of titrimetric quality

The characterization of the titrimetric quality of the pectic material extracted from the BMF was performed according to (Mohamed, 2016; Wang, Pagan and Shi, 2002). Approximately 250mg of the pectic material was moistened with 2 mL of PA ethyl alcohol and dissolved in 25 mL of deionized water under constant agitation for 30 minutes in a magnetic stirrer, after which the pH of the solution was determined.

The free carboxyls of the anhydrogalacturonic acids were neutralized with 0.1 N NaOH solution. The esterified carboxyls after saponification with 10 mL of 0.25 N NaOH solution for 30 minutes at room temperature were neutralized with 10 mL of 0.0 HCl solution. 25 N and again neutralized with 0.1 N NaOH solution, obtaining then the values of mEq of NaOH referring to the two types of carboxyls, free and esterified, respectively represented by mEq' and mEq".

With the data obtained, calculations were performed to determine the amount of mass per mEq (Equation 1), content of

galacturonic residues (Equation 2), content of methoxyls (Equation 3), percentage of polygalacturonic acids (Equation 4), percentage of sugars neutral (Equation 5) and degree of esterification (Equation 6).

$$Z = \frac{\text{Material mass}}{\text{mEqtotal}} \quad (1)$$

$$AUA(\%) = \frac{17600}{Z} \quad (2)$$

$$MeO(\%) = \frac{\text{mEq''} \times 31 \times 100}{\text{mass (mg)}} \quad (3)$$

$$\text{Acid fraction}(\%) = AUA + MeO \quad (4)$$

$$\text{Neutral fraction}(\%) = 100 - \text{Acid fraction} \quad (5)$$

$$DE(\%) = \frac{\text{mEq''}}{\text{mEqtotal}} \times 100 \quad (6)$$

Where:

Z = amount of mass per milli-equivalent-gram;

mEq = milli-gram-equivalent;

AUA = anhydrogalacturonic acid residue content;

MeO = methoxyl content;

DE = degree of esterification.

2.4 NMR evaluation of pectic material extracted from BMF

All NMR measurements were acquired in a Tesla 14.1 equipment (600 MHz for hydrogen frequency), using a temperature of +69.85°C. Dimethylsulfoxide (DMSO - d4) and an inverse detection probe except for ¹³C (broadband direct observation) were used as solvent.

For the ¹H spectrum with HDO pre-saturation signal in continuous wave, the analyzed parameters were: acquisition time (AQ = 3.27s); scan length (SWH = 10000 Hz); relaxation delay (d1 = 0.1s); the 90 ° pulse time (p1 = 9.8 μs) and the number of sweeps (ns = 64).

For ¹³C{¹H} the parameters were acquisition time (AQ = 0.52s); scan length (SWH = 31250 Hz); relaxation delay (d1 = 0.1s); the 90 ° pulse time (p1 = 9.5 μs) and the number of sweeps (ns = 4K).

For all 2D experiments for all heteronuclear correlation (HSQC-edit, HSQC-TOCSY) the SWHF1 10000 Hz and SWHF2 31250 Hz, d = 1.5s and number of experiments in F1 = 256, AQ in F2 = 0.27s were used. All data were processed using Top Spin software.

It should be noted that the samples were dissolved in dimethylsulfoxide (DMSO), at a temperature of 59.85°C and chemical shifts were expressed in δ ppm.

2.5 Evaluation by polarized light microscopy (PLM) of pectic material extracted from BMF.

For the determination of the Maltese cross aspects of the pectic heteropolysaccharide of BMF, the methodology described by Deladino et al., (2015). A LEICA ICC50 optical microscope was used, using a polarized light filter, with magnification magnitudes of 40×. For the determination of birefringence, the methodology of Almeida et al., (2019) and analyzed using the Leica LAS EZ software (Leica Microsystems, Switzerland, version 1.8.1).

III. RESULTS AND DISCUSSION

3.1 Yield of pectin extraction from BMF

The overall extraction yield of pectic material from BMF was $24.13 \pm 1.13\%$. Results close to those of Santos and Silva (2010) who found a yield of 21% and an esterification degree of 62.41% with acidic conditions, high temperatures and time, for the pectin extracted from the peel of the yellow passion fruit. Canteri-Schemin et al., (2005) obtained an average yield of 17.82% of apple pomace pectin. Similar extraction yield values were also obtained using gabiropa fruits (21%) (Santos et al., 2009) and beet pulp (6.3 to 23% depending on the extraction condition) (Li et al., 2015).

The similarity between these works is that the increase in yield is related to the increase in temperature, extraction time and pH reduction. point out that release of soluble pectin from a plant cell wall occurs at high temperatures when an acidic solution is added, due to the occurrence of several hydrolytic reactions. According to Kliemann (2005) the pectin extraction profile influences the yield and chemical structure of the final product.

Oliveira (2019) employing greater time and acid concentration in the extraction of pectin from BMF found the highest yield, 40.45%. Studies by Voragen et al., (2009) point out that due to the cleavage of strong bonds between protopectins and cell wall materials at high temperatures and low pH, the yield of extracted pectin may be higher. Furthermore, Fertoni et al., (2006) explain that extractions of pectin from plant material with a high acidic extracting solution and a temperature close to boiling can lead to the dragging of other compounds, which may also occur due to the binding of the extracting acid to the pectic structure precipitable in ethanol, overestimating the yield quantification and material characteristics.

3.2 Evaluation of the chemical composition of pectic material extracted from BMF

It was observed that the moisture content of the pectic material extracted from the BMF was low, $6.5 \pm 0.99\%$, a value lower than those reported in the literature for pectins extracted from Ginger lemon (12.94%), Cardamom lemon (14.16%) and Chinese lemon (10.43%) (Ahmed and Sikder, 2019), dragon fruit (11%) (Ismail et al., 2012) and carrot pectin (9.8%) (Fernandes et al., 2014). On the other hand, pectin extracted from apple presented a value close to that found in this study (6.8%) (Almeida et al., 2017).

There are no reports of the optimal moisture value for pectin, however, Mohamadzadehv et al., (2010) state that high moisture content can increase the growth of microorganisms and produce pectinase enzymes that can affect pectin quality. In addition, during storage, pectin can undergo depolymerization and demethylation, in a process called autohydrolysis, especially if it is in acid form and the moisture content is above 5% (Sakai et al., 1993).

The ash content of the pectic material extracted from the BMF was $0.90 \pm 0.14\%$. Oliveira (2019) reports that the ash content of a sample is related to the existence of minerals. Quantification of the ash content extracted from the pectin of cardamom lemon peel ($2.61 \pm 0.37\%$), ginger lemon ($3.10 \pm 0.37\%$) and china lemon ($4.15 \pm 0.56\%$) were higher than those found in this study (Ahmed and Sikder, 2019).

Almeida et al. (2017) reported an ash content of 0.61% for pectin extracted from apples, while Pinheiro (2007) found a value of 1.1% in pectin from the peel of yellow passion fruit (*Passiflora edulis flavicarpa*). According to Ahmmed et al. (2017) the amount of pectin yield decreases as the ash content increases. Ismail et al. (2012) reports that the ash content is a parameter of good requirement for gel formation.

The pH of the pectic material extracted from the BMF was 2.77 ± 0.01 . Kamal et al., (2021) in their studies on the extraction and characterization of pectin extracted from sweet orange peel (*Citrus sinensis*) had a pH of 1.45. On the other hand, Aina et al., (2012) extracted pectin from lemon (*Citrus limon*) where they reported a pH of 3.41. Pectin extracted from orange pomace had a pH very close to the value found in this study (2.68 ± 0.04) (Filho and Castro, 2020).

Pectin gels with low pH showed a positive influence on the process of gelation (Jiang et al., 2012). However, a very acidic pH causes loss of gel elasticity (Ruano et al., 2020) and must be controlled during processing.

3.3 Assessment of titrimetric quality

The values of the evaluation of the titrimetric quality of the pectic material extracted from BMF are shown in Table 1.

TABLE 1. Titrimetric quality of pectic material extracted from BMF.

Parameters	Results
Z* (mg/mL)	227.74 ± 14.6
AUA* (%)	77.44 ± 4.98
MeO*(%)	11.16 ± 0.88
Acid fraction (%)	88.60 ± 5.85
Neutral fraction (%)	11.40 ± 5.85
DE* (%)	81.78 ± 1.2

BMF (Bassu mesocarp flour); Z* (amount of mass per milli-equivalent-gram); *AUA (content of galacturonic residues); *MeO (methoxyl content); *DE (degree of esterification).

According to Yadav et al., (2015), the variable Z (amount of mass per milliequivalent-gram) can vary due to a wide range of factors and depends on the method and composition of the raw material used to extract the pectin. Azad (2014) pointed out that the Z value found in the extraction of pectin from lemon pomace increased with decreasing extraction time, temperature and pH increase. The higher this parameter, the lower the concentration of pectin galacturonic acid (Mohamed, 2016).

According to the Food Chemical Codex (1996) the AUA values indicate the purity of the pectin. FAO (1994) establishes the minimum percentage of 65% of galacturonic acid for commercial pectins. The value for the AUA content of the BMF pectic material was 77.44%. Ahmed and Sikder (2019) reported values of AUA content found in cardamom lemon was 85.45%, ginger lemon 82.12% and Chinese lemon 74.06%. For pomelo pectin (*Citrus maxima*) the average value was 85.57% (Roy et al., 2018).

On the other hand, lower values were reported for pectin extracted from sweet orange peel powder 40.36% (Kamal et al., 2021) and for pectin from apple pomace it was 61.72% (Ismail et al., 2012). These authors reported that the content of galacturonic acid is dependent on the extraction parameters, pH time and temperature. And yet, the low values of AUA may

mean that the extracted pectin presents an amount of protein (Singthong et al., 2005; Yapo et al., 2007).

Depolymerization can occur by hydrolysis, during acid or enzymatic extraction, or by β -elimination reactions, where the hydrogen in C-5 becomes acid due to the methyl ester group, it is acted upon by the hydroxide ion, which results in the rupture of the bond glycosidic, due to the presence of carboxyl (Singthong et al., 2004). According to this authors β – elimination when undergoing reactions of pectin lyases and pectatolyases can cause the direct depolymerization of pectins.

Fertonani et al., (2009) and Voragen et al., (1995) state that the solvent used in the extraction of pectic substances can influence the chemical structure of the molecule, through bonds and for this reason the connection between the raw material and the solvent used in the extraction process must be adjusted in relation to the separation of solid and liquid phases, filterability and cost of water evaporation, making it possible to control pectin extraction and optimize its potential.

According to Thibault et al., (1991) protocols and pectin extraction agents are one of the factors that are related to difficulties in studying pectic molecules. Along with the polymeric character, presence of electrical charges and the heteropolymeric polysaccharide structure.

The value for the methoxyl content (MeO) of the BMF pectic material was 11.16%. MeO is an important factor in the application of pectin in gel production, to define the time and capacity of pectin to form gel (Constenla and Lozano, 2003).

The results were higher than the methoxyl content found in the pectins of guava pulp flour (4.45%) and guava pulp flour (4.95%) (Munhoz et al., 2010). Filho and Castro (2020) found a methoxyl content of (0.7%) in an experiment with pectin extracted from orange mesocarp flour, and pointed out that the content obtained is below those reported in other studies with pectins. Ahmed and Sikder (2019) reported mean MeO values for pectin extracted from cardamom lemon (9.35%), ginger lemon (3.97%) and Chinese lemon (2.86%).

Kamal et al., (2021) pointed out that the methoxyl content in his research on pectin extracted from sweet orange peel was 5.27%. Values that corroborate with Kulkarni and Vijayanand (2010) who indicated the methoxyl content of 5.11%. The authors found that the methoxyl content of pectin can vary based on the origin and extraction condition used and that it depends on the maturity of the raw materials, since during ripening, the sugar content of the fruits increases and the methoxyl content decreases.

The value for the acidic fraction of the BMF pectic material was 88.60%. This parameter comprises the relative contents of anhydrogalacturonic acid (AUA) and methoxyls (MeO), whose sum represents the percentage of polygalacturonic acid (Fertonani et al., 2006). The values obtained were similar to those found for pectin from carrot flour (81.47%) (Fernandes et al., 2014).

The acid fraction values were higher than those found by Munhoz et al. (2010) for pectin extracted from dehydrated guava flour, with pectin extracted from pulp flour (73.81%) and pulp flour with peel (69.33%). Filho and Castro (2020) reported a value of 45.4% for orange pectin obtained from the response surface experiment by Box-Behnken and also the values

obtained by Fertonani (2006) for pectin extracted from apple pomace (58.57%).

The value for the neutral fraction of the BMF pectic material was 11.40%. The neutral fraction of the sample represents the non-acidic components. Pectin extracted from carrot flour showed a neutral concentration of 18.52% (Fernandes et al., 2014).

The degree of esterification of the pectic material extracted from the BMF was 81.78%. According to Kumar and Chauhan (2010) the degree of esterification of pectin induces gelling properties. The values obtained are similar to those reported for pomelo pectin (*Citrus maxima*) of 76.6% (Sotanaphun et al. 2012). The value found in this work is higher than those found by Ahmed and Sidker (2019) where they obtained the degree of esterification of cardamom lemon pectins (62.12%), ginger lemon (27.38%) and Chinese lemon (21.96 %). Kamal et al. (2021) reported the DE value for pectin extracted from sweet orange (75.41%). The authors pointed out that the ED decreased significantly with the advancement of the extraction time. The degree of esterification (DE) of pectins obtained from lemon pomace was 79.51% (Azad, 2014) and passion fruit peel, 67.31% (Liew et al., 2014) both classified with a high degree of esterification.

3.4 NMR evaluation of pectic material extracted from BMF

By analyzing the frequency of hydrogenanomeric, shown in Figure 1, it was possible to observe the points of δ 5.02; 5.04; 5.07 and 5.10 ppm, characteristic signs of the presence of monosaccharides. These data demonstrate that there is, possibly, the presence of at least two types of sugars present in the composition of the pectic material extracted from the BMF.

These results are close to those found by Nascimento et al. (2015) and show that with the constant value of anomeric hydrogen coupling in the δ 5.65 ppm signal, it pointed to the presence of a glucose unit in the β configuration. Corroborating Freitas, Lima and Silveira (2007), corroborate by pointing out that in the anomeric hydrogen signal at δ 5.88 ppm, there is the presence of a β -glycosidic unit.

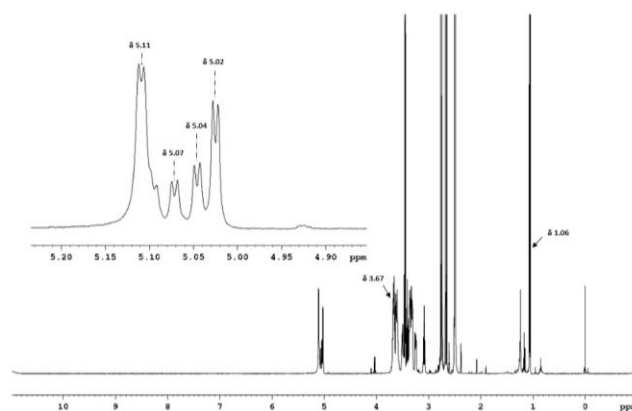


Figure 1. ¹H NMR spectrum for pectic material extracted from FMB in DMSO-d₄ at 59.85°C.

On the other hand, when checking the ¹³C NMR spectrum, it was possible to verify three distinct regions, namely, the carboxylic region, the anomeric region and the methylic region, as can be seen in Figure 2.

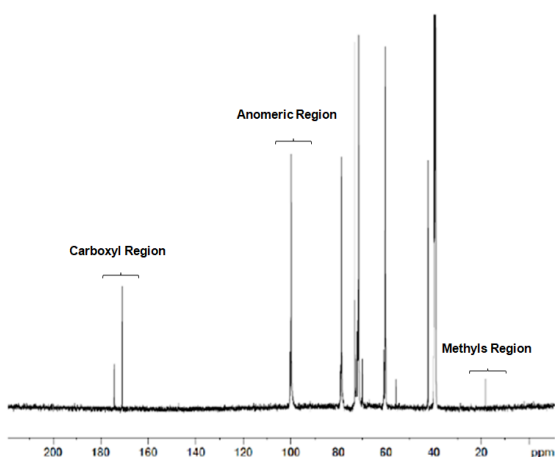


Figure 2. Regions of the ¹³C NMR spectrum

The carboxyl region, identified at a frequency of approximately δ 170 ppm, is the characteristic carboxyl region, carbonyl of uronic acid. According to Provin (2012), the chemical shift patterns observed in the spectra of the Caryocar brasiliense Cambess husks samples demonstrate that the 174.8 ppm peak is a characteristic sign of non-esterified units, that is, uronic acid, confirming the presence of pectin in the husks of these fruits.

In the methyl region, region close to 20 ppm, indicates the presence of rhamnose, as it is a methylated polysaccharide. Provin (2012) identified characteristic signs of rhamnose unit at peak 16, ppm, possibly referring to carbon C-6. It should be noted that rhamnose is an attribute of pectins, being a branching point of side chains and formed by arabinan units (Voragen et al., 1995).

The anomeric region of the spectrum (Figure 2), due to overlapping, the image was enlarged in this region, making it possible to observe four signals, assigned at frequencies δ 99.84; 100.08; 100.24 and 100.49 ppm (Figure 3). According to Marson, Ascêncio and Baggio (2012), an analysis of the anomeric region of the ¹³C NMR spectrum shows intense signals when compared to characteristic signals described in the literature. Such results corroborate the ones proposed by Gorin and Mazurek (1975) for methyl-b-Dgalactopyranoside and methyl- α L galactopyranoside patterns.

Figure 3 also shows two additional very low-field signals (δ 170.86 and 174.22 ppm) corresponding to the carboxylic groups of methyl-esterified and non-esterified galacturonic acid, respectively. The signs reported in the present study are in line with those reported by Miranda et al., (2020) applying the technique for analyzing pectic material extracted from jaboticaba bark. It is also noteworthy that the authors pointed out the presence of uronic acid (related to α -D-Galactose units) in the non-esterified region at δ 173.7.

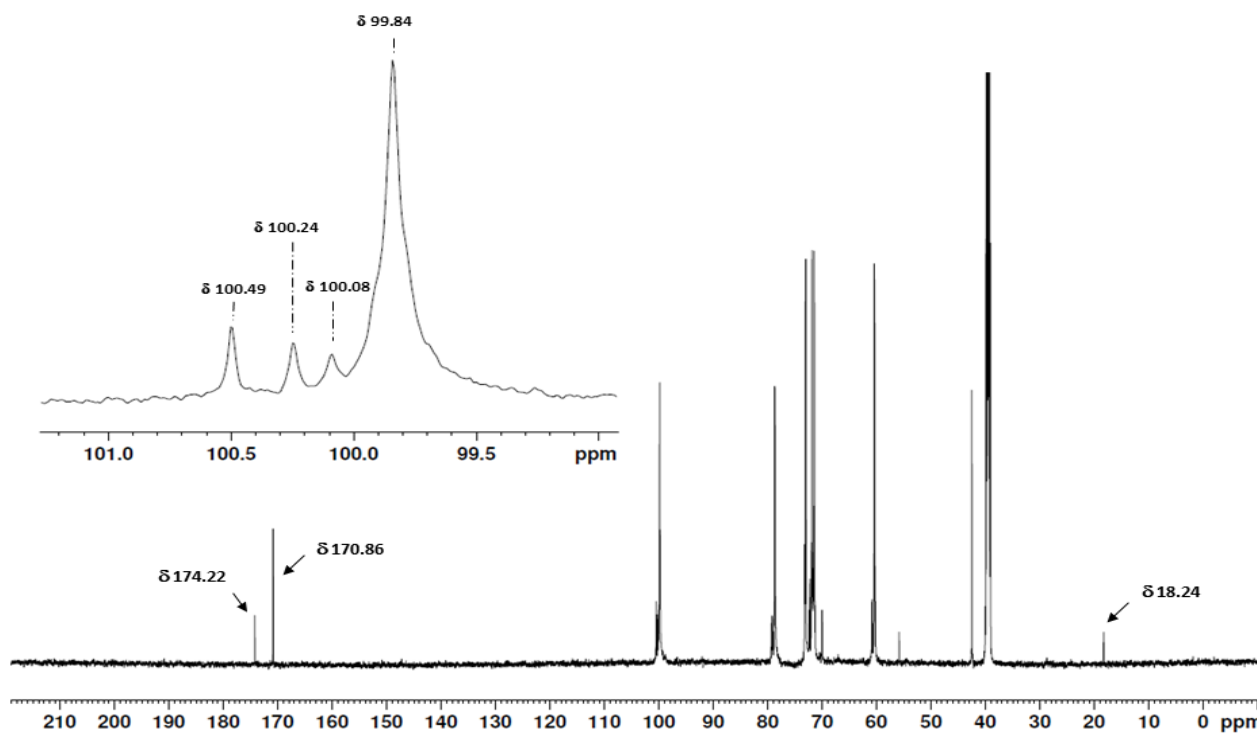


Figure 3. ¹³C NMR spectrum of pectic material extracted from FMB in DMSO-d₄ at 59.85°C

According to the studies by Muhidinov et al., (2021) in the low-field region of the ¹³C NMR spectrum of apple pectin obtained by different purification methods, two intense resonance signals were observed at δ 173.5 and 184.7 ppm,

respectively, from C-6 of the carboxyl group of galacturonic acid units in esterified and ionized forms, respectively. The chemical modification of pectin from caipira melon (*Cucumis melo* Var. *Acidolos*) was evaluated by ¹³C NMR spectrum. It

was observed that acid extraction caused a chemical shift in the anomeric region of α -D-galacturonic acid, demonstrated in the signals δ 100.8 and δ 100.5 respectively, corresponding to the non-esterified and esterified units (Souza et al., 2017).

Ribeiro (2017) elucidates in his work on the isolation and characterization of cashew pectin chemical shifts equivalent to the anomeric region of α -D-galacturonic acid, confirmed by the signals δ 96.92, δ 99.81, δ 104.01 and δ 108.18 ppm, corresponding to C-1 of esterified and non-esterified units. In addition to a very low field signal at δ 171.96 ppm equivalent to the C-6 of the esterified and free methyl carboxylic groups. According to Nascimento et al. (2015), the chemical shifts are related to carbons C-2, C-3, C-5 and C-4 of galacturonic acid. All these studies corroborate the signs found in the NMR analysis of the material extracted from the BMF and point out that pectic substances are present in its composition.

In order to investigate and confirm the presence of H and C present in the chemical structure of the study material, the HSQC correlation map (Simple Quantum Heteronuclear Correlation) was evaluated, which can be seen in Figure 4 (A). Observing the signals, it is possible to identify two carbons in correlation with two anomeric hydrogens δ 99.84/5.10 and 100.49/5.02 ppm. This indicates two nuclei involved, that is, it refers to the possible presence of two sugars in the material.

The signal of the 100.49/5.02 ppm spectrum in the H1/C1 anomeric region may correspond to the galactose unit (Gla). Muhidinov et al. (2021) identified the same region in analysis of pectins obtained by different purification methods (intervals 100.6 – 103.2/ 4.902 – 5.364 ppm) and associated the presence of galactose. Miranda et al., (2020) pointed to the identification

of the glucose molecule by NMR in the correlation map in the $^1\text{H}/^{13}\text{C}$ anomeric region at 99.7/4.50 ppm, very close to that reported in this study (99.84/5.10 ppm). This fact corroborates the hypothesis that only pectin was not extracted from the BMF (24.13%) and possibly some other monosaccharide, such as glucose.

Figure 4 (B) represents an HMBC (Heteronuclear Multiple Bond Correlation) experiment that indicates the correlations between all the nuclei involved that are distant by more than one chemical bond, that is, all the bonds of carbons and hydrogens present in the material. From the present data, it is understood that it is a sugar molecule. However, as galactose and glucose are epimeric, it becomes difficult to discriminate which one is in greater quantity.

Still, in relation to the NMR analysis it was possible to observe a semi-quantitative approach of the method that reported that approximately 62.43% of the carbonyls of the sample of the pectic heteropolysaccharide of BMF are esterified, while 37.56% are in the acid form. When comparing this result with the values obtained by the titrimetric method (81%), it can be seen that the result was overestimated by the traditional method, corroborating the assumption that acidic substances are carried away during extraction (Fertonani et al., 2006). That is, of the 80% of DE found, 62% are uronic acids. Indicating that the sample has a reasonable amount of pectic material.

The values found are close to those of Muhidinov et al., (2021) where the authors point out that the semi-quantitative integration indicates that 63% of the carbonyls can be presented as esters and 37% as acids.

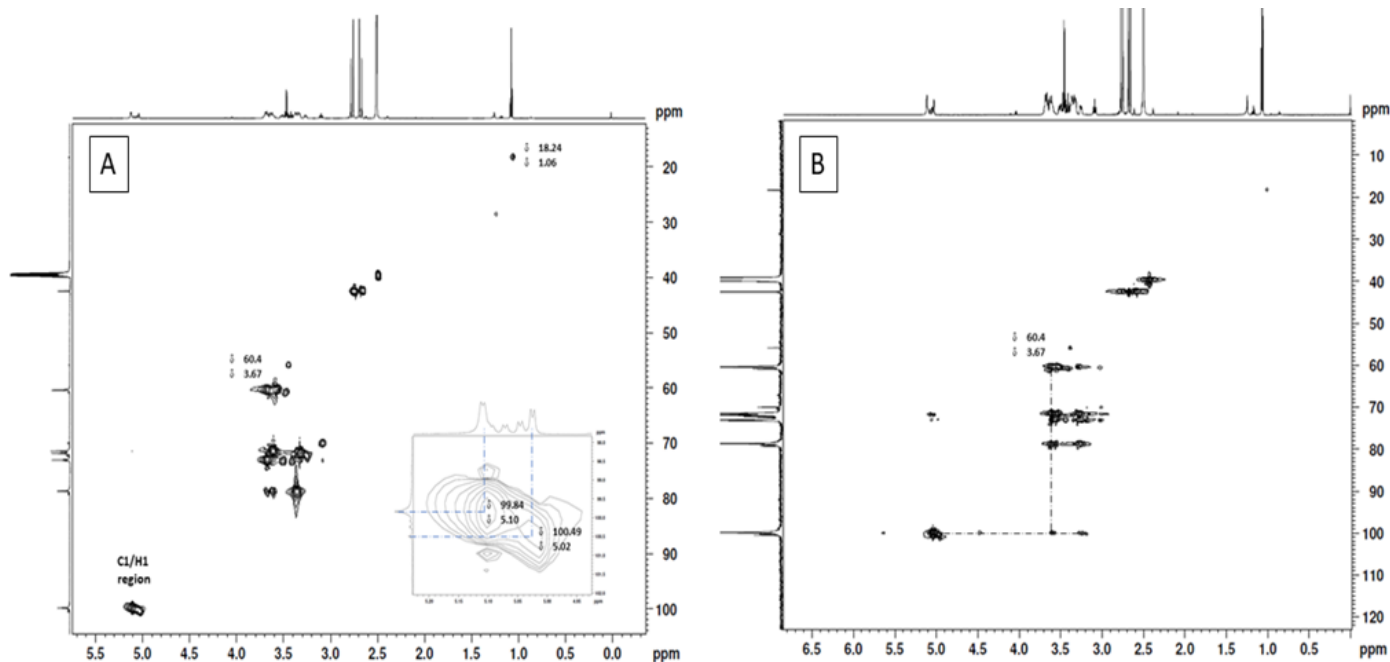


Figure 4. A) Anomeric and non-anomeric $^1\text{H}/^{13}\text{C}$ HSQC NMR correlation map. B) $^1\text{H}/^{13}\text{C}$ HMBC correlation map (Multiple Bond Heteronuclear Correlation).

3.5 Characterization by optical microscopy of heteropolysaccharide extracted from BMF

Figures 5A and 5B show that the polysaccharide granules observed microscopically under polarized light showed a

Maltose cross centered on the hilum, which is an expected characteristic for a starchy polysaccharide. This is due to the crystallinity of its amylopectin structure which allows light to pass through. Buléon et al., (1998) indicates that amylopectin chains are formed by arranged structures, being responsible for its crystallinity.

According to Lajolo and Menezes (2006), polysaccharide granules when observed in optical microscopy under polarized light present a birefringence due to the high degree of internal molecular orientation, resulting in the formation of the Maltose cross, characterized by the radial orientation of the macromolecules. Figuero et al., (2018) points out that birefringence points to the orientation and alignment of the amylopectin structure inside the granule.

The vast majority of the polysaccharide is in the form of ovoid. According to He and Wei (2020), the shape and position of the hilum identified by microscopy are related to the biological, biochemical and physiological origin of the extracted source.

Roy (2018) observed similar and expected characteristics in a photomicrograph taken under the same condition of polarized light in the polysaccharide of the lyophilized avocado seed, having the formation of a Maltose cross in an ellipsoidal shape and a hilum at one end of the granule.

After observing these results, it was possible to understand that the material extracted from the BMF was not just pectic material. The presence of starch was confirmed with the images and, therefore, it is a pectic heteropolysaccharide material extracted from the BMF.

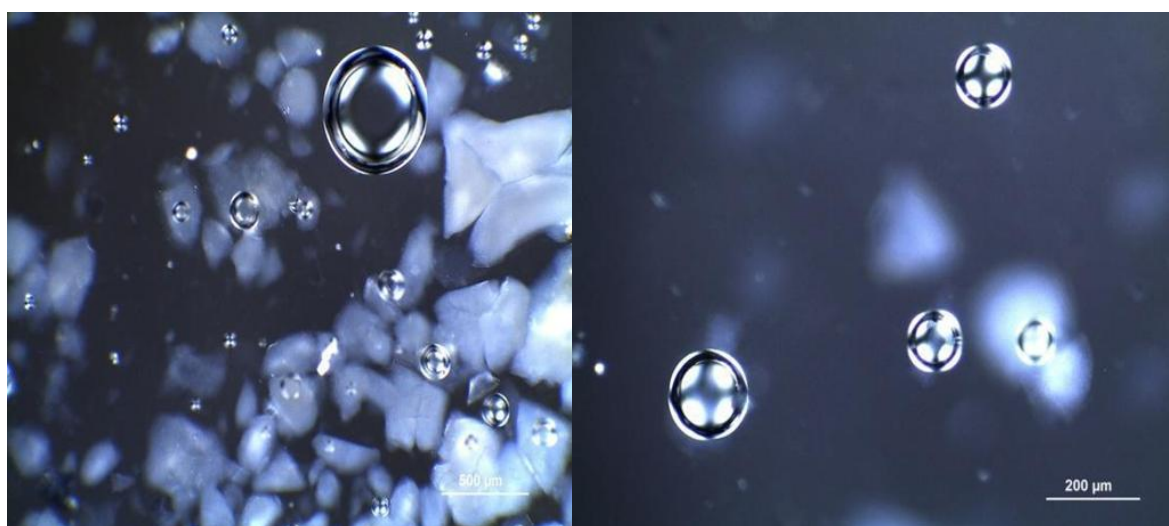


Figure 5. Polysaccharide granule represented at 200 (A) and 500 (B) micrometers

IV. CONCLUSION

The pectic material extraction process applied in this work presented an overall yield above 24%. The chemical composition of the extracted material presented humidity of 6.5%, pH of 2.77 and 0.90% of ash.

Considering the parameters and results observed by the Nuclear Magnetic Resonance (NMR) analyses, it is concluded that the compound found in the FBM is characterized as a quality pectic material, but there is also the presence of another monosaccharide in the sample. The results of polarized light microscopy confirmed the presence of glucose in the structure of the study material. Concluding that the material extracted from the FBM is characterized as a pectic heteropolysaccharide.

Ultimately, it is understood that it is necessary to implement a purification process, to obtain a purer sample of the pectic material from the FMB. Rheological characteristics should be analyzed. However, until now, it has been noticed that it is an innovative study because the pectic material was extracted from a fruit from the Amazon region and presents promising chemical parameters for technological applicability, in particular, for the food industry.

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Cleber do Amaral Mafessoni Liviz: investigation, methodology, writing—original draft; Jaine Oliveira: investigation, methodology; Gisele Teixeira de Souza Sora: writing—review and editing. Ladyslène Christyns de Paula: writing—review and editing; Maria Carolina: investigation, methodology; Gabrieli Oliveira-Folador: Writing—original draft; writing—review and editing, methodology, supervision, project administration.

Conflicts of Interest

The authors have no conflict of interest to disclose.

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