

Pectic substances from babassu mesocarp flour: extraction and characterization

Substâncias pécicas da farinha do mesocarpo de babaçu: extração e caracterização

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Abstract

The Amazon region contains distinct fruit species, such as the babassu, which are still little known, but may have great scientific and technological potential. Oil and flour are extracted from babassu fruits. The oil is used industrially and is listed in the Codex Alimentarius as a commodity. Babassu mesocarp flour is consumed in the Amazon region but is little known technologically. For this purpose, the present work aimed to use babassu mesocarp flour to extract pectin, an important additive for the food industry. The yield was quantified and the titration quality of the extracted material was evaluated. In order to determine the best extraction conditions, were tested different extraction time (30, 60 and 90 min) and concentration of citric acid (3, 4.5 and 6%) at constant temperature (90°C). The best results of quality were found for Experiment 5. Yield was

approximately 25% when operating conditions were 60 min and 4.5% citric acid. Titrimetric quality of pectic material extracted in these conditions (experiment 5) was better than the others, with highest concentration of galacturonic acids (50%) and the highest degree of esterification (71.4%). Results of this study showed that mesocarp of a fruit from the amazon region (babassu) proved to be a great alternative source for pectic substances extraction. Technological quality of its pectic substances still needs to be better evaluated.

Keywords: Degree of esterification. Amazon fruit. Galacturonic acid. Yield.

Resumo

A região amazônica contém espécies frutíferas distintas, como o babaçu, que ainda são pouco conhecidas, mas podem ter grande potencial científico e tecnológico. Dos frutos do babaçu, são extraídos o óleo e a farinha. O óleo já é utilizado industrialmente, está listado no Codex Alimentarius como commodity. Já a farinha de mesocarpo de babaçu, é consumida na região amazônica mas, pouco conhecida tecnologicamente. Neste intuito, o presente trabalho teve como objetivo utilizar farinha do mesocarpo de babaçu, para extrair pectina, aditivo importante para a indústria de alimentos. Foi quantificado o rendimento e avaliada a qualidade titulométrica do material extraído. Para determinar as melhores condições de extração, foram testados diferentes tempos de extração (30, 60 e 90 min) e concentrações de ácido cítrico (3, 4,5 e 6%) em temperatura constante (90°C). Os melhores resultados de qualidade foram encontrados no experimento 5. O rendimento foi de aproximadamente 25% quando as condições de operação foram 60 min e 4,5% de ácido cítrico. A qualidade titulométrica do material pectico extraído nestas condições (experimento 5) foi melhor que a dos demais, pois apresentou a maior concentração de ácidos galacturônicos (50%) e o maior grau de esterificação (71,4%). Os resultados deste estudo mostraram que o mesocarpo de um fruto da região amazônica (babaçu) mostrou-se uma ótima fonte alternativa para extração de substâncias pecticas. A qualidade tecnológica dessas substâncias pecticas ainda precisa ser melhor avaliada.

Palavras-chave: Grau de esterificação. Fruta amazônica. Ácido galacturônico. Rendimento.

1. Introduction

In the Amazon region, there is a wide diversity of fruit with great technological, nutritional and economic potential which has been little explored (Pires, 2016). These fruit exhibit unusual sensory quality and draw interest to their composition aiming at some technological use. Fruit from the Amazon region are becoming increasingly attractive to the food industry and represent new options for consumers.

Babassu (*Attalea speciosa* Mart.), shown in Figure 1, is one of the best-known native fruit from the Amazon region. Soler *et al.* (2007) state that babassu coconut is composed of approximately 11% epicarp, 23% mesocarp, 59% endocarp and 7% almond.

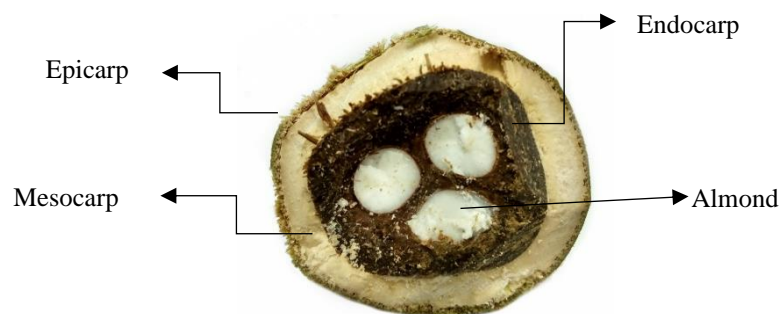


Figure 1 - Babassu fruit (Authors, 2024)

The most harnessed part of the fruit is almond, which is used for oil production (Carrazza *et al.*, 2012). Other parts of the fruit are discarded. In a few cases, regionally, the mesocarp it is used as flour in food supplements (Silva, 2011; Pires, 2016). Demand for flour has recently increased and the fruit has been more used. Nevertheless, it is not known in the whole country and, consequently, it is little commercialized (Pires, 2016).

Babassu mesocarp flour (BMF) has starchy composition. Pavlak (2007) points out that the fruit mesocarp is majority constituted of starch (52%) and fiber (10%). Borges *et al.*, (2023) demonstrated, through previous studies, that this raw material is strongly starchy and presents notable variations in chemical composition. Fibers dietary are constituted of different interconnected polysaccharides and classified as soluble and insoluble fibers (Li and Komarek, 2017). Pectin increasing solubility along the polymer (Nassato *et al.*, 2015; Gill *et al.*, 2021). Maniglia *et al.* (2017) and Soares *et al.* (2020) analyzed BMF reported a content of 11.11% and 17.9%, respectively of dietary fiber.

In view of the great technological capacity found in BMF and lack of data on its characterization in the literature, more studies are needed, research has to be developed and its effect on food application should be analyzed (Morales, 2012). Based on this information, studies of the chemical composition of flour carbohydrates may lead to alternative uses of this raw material.

Starch dominates the food industry worldwide but other polysaccharides, such as pectin, with its gelling properties, progress too in the food industry (Canteri *et al.*, 2012). Pectin is a group of structurally heterogeneous polysaccharides widely distributed in primary cell walls and middle lamella of plants (Luo *et al.*, 2017). Commercial pectins are usually extracted from apple pomace and citrus peels, such as lemon and orange. However, alternative sources are mango peel (Guandalini *et al.*, 2019), passion fruit peel (Freitas *et al.*, 2020), guava pulp (Spiller *et al.*, 2018) and others (Ciriminna *et al.*, 2015; Moura *et al.*, 2017).

Pectin extraction is influenced by several factors, such as temperature, pH, solid:liquid ratio, solvent type and extraction time, which must be minimally controlled. Variation in the parameters depends on the type of fruit species while extraction methods directly influence pectin yield and properties (Pagán *et al.*, 2001; Campos *et al.*, 2022).

Thus, pectin extraction from Amazon fruit byproducts is important because: a) it can increase commercial value of the fruit, which is still little explored; b) it can provide scientific data on its chemical composition to the literature; and c) it can increase the diversity of pectin available for technological applications to the food industry, especially as an emulsifying, stabilizing and thickening agent. Therefore, the general objective of this study was to extract and characterize pectin from BMF.

2. Material and methods

2.1 Acquisition and characterization of raw material

BMF was obtained from local producers in Ariquemes, Rondônia (RO) state, Brazil, in 2019. BMF flour was packed in polymeric packaging and was stored under refrigeration until extraction. Analyses were performed in the Laboratory of Food Science and Technology at the Federal University of Rondônia (UNIR), Ariquemes, RO.

BMF was characterized, in moisture, protein, lipid, ash, pH, titratable acidity (TA) and carbohydrate by difference (IAL, 2008). Total fiber content was determined by the enzymatic-gravimetric method (AOAC-985.29, 2010).

2.2 Pectin extraction

The procedure of pectin extraction from BMF was performed by acid, as described by Munhoz *et al.* (2010), with modifications. Flour samples were dissolved in acidic solution (1:30 w/v) and submitted to constant temperature (90 °C). The procedure of the extraction was evaluated at different time periods (30, 60 and 90 min) and citric acid concentrations (3, 4.5 and 6%). Choice of parameters was based on previous studies found in the literature (Munhoz *et al.*, 2010; Siqueira *et*

al., 2012; Liew *et al.*, 2014).

After this period, samples were cooled at 4 °C for 120 min, centrifuged at 1500 g for 10 min and vacuum-filtered. Ethyl alcohol (95%) was added to the filtrate containing pectin in the ratio of 1:2 (v/v). After 60 min, pectin separated as a precipitate, which was separated from the solution by a filtration process. The precipitate (pectin) was washed with alcohol and acetone to remove impurities, such as pigments, soluble solids and other constituents found in pectin (Voragen *et al.*, 2009). Resulting pectin was oven dried at 55 °C until constant weight.

The experiments were performed in triplicate. The results were submitted to analysis of variance. (ANOVA) and Tukey test ($p < 0.05$) for comparison of means.

2.3 Pectin characterization

Pectin extraction yield was calculated by the ratio between the mass of the end product and BMF mass submitted to extraction; it was expressed as g pectin 100 g⁻¹ BMF. Titrimetric quality of extracted pectin was performed in agreement with Wang *et al.*, (2002) and Mohamed (2016). Extracted pectin (250 mg) was solubilized in ethyl alcohol and deionized water under constant agitation for 30 min. Free carboxyls of the anhydro galacturonic acid, before and after sample saponification, were neutralized with NaOH 0,1 N. In the saponification step, 10 mL NaOH solution 0.25 N was added at room temperature for 30 min. Then, it was neutralized with 10 mL HCl solution 0.25 N. At the end, NaOH mEq values were obtained for two types of carboxyls – free and esterified ones – represented by mEq' and mEq'', respectively. Titrimetric quality of pectin from BMF was assessed to determine the amount of pectin mass per mEq (Equation 1), anhydrouronic acid content (AUA) (Equation 2), methoxy content (MeO) (Equation 3), percentage of acid fraction (Equation 4), percentage of neutral fraction (Equation 5) and degree of esterification (DE) (Equation 6). Analyses were performed in triplicate.

$$z = \frac{\text{Weight of sample}}{\text{meq}' + \text{meq}} \quad (1)$$

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

$$\text{MeO} (\%) = \frac{\text{meq}'' \times 31 \times 100}{\text{Weight of sample}} \quad (3)$$

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

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

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

3. Results and discussions

3.1 Pectin characterization

Extraction yield: Results of acid pectin extraction from BMF (Table 1) evidenced that the time variable positively influenced pectin extraction yield. When the acid concentration was constant (experiments 1 and 3) and only the time variable was modified (30 and 90 min, respectively), a positive effect was observed since pectin yield increased approximately 20%. Raji *et al.* (2017) stated that increase in yield at longer extraction times is favored by hydrolysis of glycosidic bonds in cellulose and hemicellulose chains.

Table 1 - Experimental conditions from pectin BMF extraction and the yield results

Experiments	Extraction Time (min)	Acid concentration (%)	Yield (g pectin 100 g ⁻¹ BMF)*
1	30	6	16.42 ^c
2	30	3	3.63 ^d
3	90	6	40.45 ^a
4	90	3	21.25 ^{bc}
5	60	4.5	25.01±1.51 ^b

*Means followed by the same letter in same column do not differ by the Tukey test at 5% probability of error

Experiment 3 exhibited the highest pectin extraction yield (40.45 g pectin per 100 g⁻¹ BMF); it is almost the double of the extraction yield obtained by experiment 4. The difference between both is the acid concentration, 6% and 3%, respectively. Thus, it should be emphasized that the combination of longer time and acid concentration was determinant to obtain higher yield. Low pH, the hydrogen ion concentration of the solution is increased, ionization of the carboxylate groups is repressed, i.e., the highly hydrated carboxylate group is converted into hydrated carboxylic acid groups. The lost of carboxylate groups is able to reduce the repulsion of the polysaccharide molecules which promotes the gelation properties of pectin giving more precipitated pectin at lower pH (Bemiller *et al.*, 1986). This fact agrees with Liew *et al.*, (2014), who highlighted that pH is considered a crucial parameter which affects the amount and properties of extracted pectin. Studies of pectin extracted from other sources, such as beet pulp and banana peel, also showed that pectin yield significantly increases at decreasing pH (Yapo *et al.*, 2007).

Morales-Contreras *et al.* (2020) report that the highest yields of pectin substances are generally obtained at high temperatures and low pH values. It may happen due to the ease of cleaving strong bonds between protopectin and other cell wall material under these conditions.

In this study, both variables (time and citric acid concentration) in the extraction process increased pectin yield of BMF. Li *et al.* (2015) observed similar behavior of (extraction time and citric acid concentration) in the extraction of beet pulp pectin.

The high extraction yield achieved by this study (40.45%) in experimental conditions (6% citric acid for 90 min at 90 °C) highlights the fact that the use of BMF for pectin extraction is promising. It is important to mention that there is no data on pectin extraction from babassu mesocarp in the literature to be compared with this study. Morales-Contreras *et al.*, (2020) emphasize that search for new sources for pectin, such as agro-industrial byproducts or underutilized fruit as raw material, is essential to contribute to a more sustainable and global supply.

However, the evaluation of pectin extraction yield reached by other studies that used unconventional raw material showed that lower pectin concentrations were found. Vriesmann (2008) obtained 7% pectin yield by aqueous extraction from cupuassu pulp (*Theobroma grandiflorum*). Munhoz *et al.* (2010) studied pectin extraction from dehydrated pulp flour and guava peel, with citric acid, and achieved yields above 11% in both types of flour. Banerjee *et al.* (2017) performed pectin extraction from watermelon rind and found yield between 19% and 21%.

On the other hand, Siqueira *et al.* (2012) extracted pectin from pequi shell and obtained pectin yields between 14% and 55% (experimental conditions were 1% citric acid, 120 min and 83°C and

8% citric acid, 156 min and 75°C, respectively). Liew *et al.* (2018) evaluated the method of pectin extraction from pomelo peels and reported yield of 39.72%, in the following conditions: 141 min, 88°C and pH 1.8. Oliveira *et al.* (2018) reported 32.13% yield of pectin extracted from Ubá mango peel; extraction conditions were 97°C, pH 2 and 60 min. In these studies, high yield concentrations were obtained in conditions of high acidity and long extraction times. Besides, pequi shell flour showed about 42% of total dietary fiber (10% corresponds to soluble fibers) (Leão *et al.*, 2017). Pomelo peel is reported with 3.75% fibers (9.21% cellulose, 10.5% hemicellulose, 0.84% lignin and 42.5% pectin) (Darah *et al.*, 2013). Ubá mango peel flour showed 17.4% of soluble fiber (Oliveira *et al.*, 2018).

These observations show that pectin yield may be higher than the dietary fiber content of raw material. Some authors report that polysaccharides with degree of polymerization between 3 and 9 monosaccharide units, such as oligosaccharide, hemicellulose, gum, lignin, pectin and other associated secondary substances, are considered dietary fibers, which, in most cases, are not precipitated by alcohol (Zielinski *et al.*, 2013; Dai and Chau 2017). Thus, considering results of the dietary fiber content found in BMF (19.88%) and pectin yield in experiment 3 (40.45 g pectin 100 g⁻¹ BMF), two situations must have occurred simultaneously: i) the dietary fiber content was underestimated due to limitations in the methodology and; ii) the pectin yield was overestimated by the dragging of other substances.

Fertonani *et al.* (2006) evaluated the influence of acid concentration on the extraction process and on the quality of apple pomace pectin. They recorded 36% of yield, but they stated that pectin extracted at high concentrations of citric acid and temperature close to boiling can either contain other compounds or bond to citric acid. In addition, Siqueira *et al.*, (2012) and Oliveira *et al.* (2018) stated that severe extraction conditions increased yield and can also lead to polymer degradation.

Thus, yield obtained by the experiment 5 – average yield of 25% – was assumed to be more consistent with data on pectin extraction found in the literature.

Titrimetric quality of pectin extracted from BMF: Titrimetric quality of pectin extracted from BMF is shown in Table 2. According to Fertonani *et al.* (2006), characteristics of titrimetric quality comprise relative levels of galacturonic acid (AUA) and methoxyl (MeO), which define the acid fraction of pectin (polygalacturonic acids). The neutral fraction, however, corresponds to the rest of the composition, and neutral sugars, such as arabinose and galactose attached to the rhamnose in the main chain, may be present as polymers (Willats *et al.*, 2006). High concentrations of these sugars in pectin may signal that there has been hydrolysis and release of other polymers from the cell wall (Moura *et al.*, 2017).

Table 2 - Titrimetric quality and degree esterification of BMF pectin

Experiments	AUA (%)*	MeO (%)*	Acid fraction (%)	Neutral fraction (%)	DE (%)*
1	27.10±0.01 ^c	4.03±0.4 ^d	31.13±0.01 ^d	68.87±0.01 ^a	68.42±0.0 ^d
2	33.97±1.24 ^b	5.74±0.22 ^b	39.70±1.46 ^{bc}	60.30±1.46 ^{bc}	90.24±0.3 ^a
3	34.03±1.42 ^b	4.55±0.18 ^c	38.57±1.6 ^c	61.43±1.60 ^b	55.70±0.3 ^e
4	36.37±1.02 ^b	5.79±0.19 ^b	42.16±1.2 ^b	57.84±1.2 ^c	78.86±0.5 ^b
5	50.57±0.42 ^a	7.68±0.06 ^a	58.25±0.48 ^a	41.75±0.48 ^d	71.48±0.4 ^c

*AUA = galacturonic acid content; MeO = methoxy content; DE = degree of esterification. Means followed by the same letter in each column do not differ by the Tukey test at 5% probability of error

The highest AUA and MeO concentrations were found at the experiment 5. Consequently, the higher the acid fraction (AUA + MeO), the lower the neutral fraction. Results of the experiment 5 differed statistically from the other experiments. This fact corroborates the hypothesis that experiment 3 may not have been effective in extracting only pectin. The experiment 5 exhibited pectin with better titrimetric quality.

The value of AUA found by this study, experiment 5 (average 50.57%) was comparable to passion fruit peel (51.30%) (Moura *et al.*, 2017) and dry pomace (average value of 50%) (Sato *et al.*, 2011). MeO is an important factor in the application of pectin to gel production, to set setting time and ability of pectin to form gel (Constenla and Lozano, 2003). Results of this study (7.68%) are similar to the ones of white grapefruit (7.54%) (Mohamed, 2016).

The degree of esterification (DE) increase as acid concentration increases (Table 2). When the effect of time was verified (experiments 1 and 3), this parameter also exerted influence on the DE (reduction in degree of esterification of pectin molecule, with increasing time). According to Yapo *et al.* (2007), process conditions, such as pH, temperature and extraction time, influence the degree of esterification of extracted pectin.

Interactive effects between pH and temperature contributed to pectin extraction and the degree of esterification of BMF. When pH increases, the amount of free H⁺ ions is insufficient to de-esterify pectin during extraction. With increasing extraction time and with thermal conditions, hydrolysis of the ester bonds occurs and increases de-esterification, thereby reducing the DE of pectin (Adetunji *et al.*, 2017).

All experiments exhibited degrees of esterification above 50% (Table 2). DE values at the experiment 5 was >70%. According to this characteristic, pectin of BMF could be classified into high methoxyl content (HMC). Gel formation in pectin of the HMC occurs by chemical bonds among galacturonic acid, hydrogen bond and hydrophobic forces of methyl groups. pH reduction (from 2.5 to 3.3) and high sugar concentration (> 50%) made this process easier (Yapo *et al.*, 2007).

4. Future Perspectives

To expand the applications of pectic substances from BMF, future research directions should be addressed to expand the possibilities of using the raw material and, consequently, its valorization. 1) evaluate and standardize the extraction method, avoid extraction of other non-pectic substances; 2) more specific analyzes should be explored, including NMR, polarized light microscopy, rheological profile, galacturonic acid content by MHDP.

5. Conclusions

BMF proved to be an alternative source for pectic substances extraction, comparable to other commercial sources. The best extraction yield result was experiment 3, however, the titration quality was not suitable for classification as pure pectin (AUA of 34.03%). On the other hand, the experiment 5, exhibited better results of titrimetric quality (AUA > 50%). This study demonstrated that a byproduct of a fruit from the Amazon region can be used to extract pectic substances with excellent yield, however, more analysis needs to be carried out assess its quality.

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