EXTRACTION, PURIFICATION OF CASHEW POLYSACCHARIDE AND CHARACTERIZATION BY GC-MS, FTIR, NMR, TG/DTG

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ABSTRACT
The cashew polysaccharide was obtained from A. occidentale trees by alcohol precipitation, purified in a Sephacryl S-400 HR chromatographic column, and characterized by GC-MS, GPC, FTIR, NMR, and TG/DTG, for use in foods. The comparison of CG-MS results showed that the purified cashew polysaccharide had the same neutral monosaccharides, but in different amounts. The GPC results showed that the purified cashew polysaccharide had a low molecular mass and little polydispersivity. The FTIR spectra showed bands characteristic of O-H, C-O-C, and C-O groups that are present in the structure of polysaccharides. The ¹H NMR spectra of the samples were similar. Thermal analysis revealed a decomposition temperature around of 240 °C (Tonset) for both the samples; however, the purified cashew polysaccharide had fewer residues at 800 °C.

Keywords: Cashew, polysaccharide, characterization.

1. INTRODUCTION
Anacardium occidentale L., a tree species from the Anacardiaceae family, is native to Brazil [1]. It grows mostly in the northeastern region of Brazil that has around of 770,000 ha of planted area [2]. Currently, it also grows in several other countries, such as Vietnam, India, Nigeria, the Ivory Coast, Indonesia, the Philippines, and Tanzania [3]. The properties and composition of the cashew root, stem, leaves, flower, and fruit have been investigated and described. The pseudo fruit, known as cashew apple, is a good source of vitamin C [4]. The bark is rich in tannins and alkaloids with intense antimicrobial activity. The leaves are rich in tannins, flavonoids, and glucose [1]. The cashew nut contains proteins, lipids, carbohydrates, vitamins, and minerals [5]. Cashew gum is a polysaccharide that is obtained through the exudates from A. occidentale L trees. Polysaccharides have been widely investigated and various studies have reported on their capacity to stimulate the defense mechanism of organisms. The pharmacological properties have generated interest in research for their use in the production of vaccines, antitumor, antiviral, anticlotting, and healing agents [1].

The hydrolysis of the cashew polysaccharide results in a higher galactose content and other constituents such as arabinose, glucose, rhamnose, mannose, xylose, and glucuronic acid [1,6-7]. The structural analysis of cashew polysaccharide from India revealed a branched polysaccharide consisting of chains of β-(1→3)-linked D-galactose residues interspersed with β-(1→6) linkages. Arabinose is present as end-groups or in short (1→2)-linked chains up to five units long. Glucose, rhamnose, mannose, xylose, and uronic acid are all present as end-groups [8].

Due to its emulsifying, adhesive, stabilizing [9], antitumor [10], and antihypertensive [11] properties, cashew polysaccharide has high application potential in the food and pharmaceutical industries.

The aim of the present work was the extraction, purification and hydrolysis of the polysaccharides from cashew tree exudates and their characterization by Gas Chromatography-Mass Spectrometry (GC-MS), Gel Permeation Chromatography (GPC), Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Thermogravimetry/Derivative Thermogravimetry (TG/DTG), for use in functional foods.

2. EXPERIMENTAL
2.1 Extraction and Purification of the cashew exudate
Crude cashew tree exudate (Figure 1a) was collected from A. occidentale trees in the CNPAT-EMBRAPA, State of Ceará, Brazil. Exudate free of bark was treated by trituration, solubilization, centrifugation, alcohol precipitation, and vacuum drying [12] (Figure 1b).

The extracted cashew polysaccharide (100 mg) was dissolved in 0.2 M ammonium bicarbonate (pH 7.0) and stirred for 5 min at room temperature. Next, the solution was centrifuged (3000 rpm, 15 min) and the supernatant was separated. The supernatant was filtered through a 0.22 μm PVDF filter, and injected into the gel filtration column using Sephacryl S-400 HR (200 cm x 1.5 cm), equilibrated with 0.2 M ammonium bicarbonate (pH 7.0) for 48 h and a flow of 0.28 mL/min. Fractions of 3 mL were collected and analyzed for the presence of total sugars by the phenol-sulphuric acid method at 490 nm [13] in a Ultrospec 3100 pro UV/Visible spectrophotometer, Amersham Biosciences.
After the determination of the elution profile, the fractions from the peak (figure 2) were combined and lyophilized. The lyophilized sample was dissolved in distilled water and dialyzed in a dialysis cellulose membrane (MWCO 1,000 Da) against distilled water at room temperature for 48 h. The dialyzed sample was lyophilized and designated purified cashew polysaccharide (Figure 1c). The presence of uronic acids was confirmed by the carbazole method at 525 nm [14].

2.2 Obtainment of monosaccharides by hydrolysis and characterization by GC-MS
The samples b and c (5 mg) were hydrolyzed with 600 µL of 2 M trifluoroacetic acid (TFA) at 100 °C in a sealed tube for 8 h. The excess acid was removed by co-distillation with MeOH by vacuum distillation. The hydrolyzed samples were reduced with NaBH₄ and acetylated with acetic anhydride (200 µL) and pyridine (300 µL) for 30 min at 100 °C under reflux. The alditol acetates were analyzed by GC-MS using a HP model 6890 Series gas chromatograph coupled to a HP 5973 mass selective detector equipped with a DB-225ms capillary column (30 m x 0.25 mm). Helium was used as the carrier gas (1.0 mL/min), injector temperature was kept at 220 °C, split mode (50:1), injection volume of 1.0 µL, and detector temperature at 230 °C. The initial temperature was 50 °C and raised at 20 °C/min to 230 °C, followed by an isotherm period of 21 min. Galactose, glucose, mannose, xylose, arabinose, and rhamnose were used as standards and inositol was used as an internal standard. Identifications were based on peak retention times and by comparison of mass spectra. The quantification was carried out from peak areas, using response factors.

2.3 GPC characterization
The homogeneity and molecular weight of the samples were determined by GPC using an apparatus equipped with a HP1047A differential refractometer detector and three Waters-Shodex (SB-802.5 HK, k-804, and k-805) columns. The mobile phase was 0.3 M NaCl containing 0.1% (w/v) NaN₃ at a flow rate of 1 mL/min. The sample solutions were prepared using the same eluent and filtered through 0.22 μm PVDF filters before each injection. Dextran standards with different molecular weights were used for the calibration curve.

2.4 FTIR characterization
FTIR spectra were recorded on a FTIR spectrometer, Perkin Elmer Co., Norwalk, CT, USA, with attenuated total reflectance (ATR) and operating between 4000 and 600 cm⁻¹ for b and c samples.

2.5 ¹H NMR characterization
¹H NMR spectra were recorded on a Bruker DRX 800 MHz spectrometer with a triple resonance probe. The samples (b and c) with 10 mg were dissolved in D₂O 99.9% (0.5 mL).

2.6 Thermal Analysis characterization
Thermogravimetry/Derivative Thermogravimetry (TG/DTG) curves were obtained on a TA Instruments, model SDT 2960, under nitrogen atmosphere. The b and c samples (around 10 mg) were heated with heating rate of 10 °C/min from room temperature to 800 °C.
3. RESULTS AND DISCUSSION

The cashew exudate was extracted by alcohol precipitation, and it was purified using gel filtration chromatography. Figure 2 shows the elution profile of the cashew polysaccharide in Sephacryl S-400 HR. A single peak was detected by the sulphuric-phenol method at 490 nm, and its fractions were combined, lyophilized, dialyzed, and lyophilized. The yield of the purified cashew polysaccharide was 70 % (w/w) based on the weight of cashew polysaccharide.

![Elution profile of the cashew polysaccharide](image)

Figure 2. Elution profile of the cashew polysaccharide (b) by Sephacryl S-400 HR column eluted with 0.2 M ammonium bicarbonate (pH 7.0) at 0.28 mL/min.

The GC-MS analysis was used to identify and compare the neutral monosaccharides present in the samples. It is important to emphasize that the result is a comparison between the cashew polysaccharide and its fraction, and it is not a final composition, because the uronic acids were not quantified. The results showed that the samples contain the same neutral monosaccharides, however, in different amounts (Table 1). The purified cashew polysaccharide has a lower amount of galactose and greater amounts of arabinose, glucose, and rhamnose. These neutral monosaccharides were reported in previous work for cashew tree exudate of similar origin [15-16].

**Table 1. Comparison of the neutral monosaccharides present in the samples**

<table>
<thead>
<tr>
<th>Monosaccharide (% w/w)</th>
<th>cashew polysaccharide</th>
<th>Purified polysaccharide</th>
<th>cashew polysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>80.0</td>
<td>56.7</td>
<td>56.7</td>
</tr>
<tr>
<td>Arabinose</td>
<td>5.8</td>
<td>10.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.5</td>
<td>19.7</td>
<td>19.7</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>3.7</td>
<td>13.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Figure 3 shows the GPC curves for the polysaccharides. It can be seen that the curves have a similar behavior, but the fractionated cashew polysaccharide showed a smaller mass dispersion. The cashew polysaccharide had a weight-average molecular weight (M_w) of 4.8 x 10^4, a number-average molecular weight (M_n) of 2.6 x 10^4, and a polydispersity index (M_w/M_n) of 1.84. The purified cashew polysaccharide had M_w = 4.3 x 10^4, M_n = 2.8 x 10^4, and a polydispersity index of 1.53. Similar values was reported by Botelho et al. [17] for the purified cashew polysaccharide that found one M_w = 1.5 x 10^4 and polydispersity index of 1.49 and de Paula et al [6] with values in the range of 10^4.
Figure 3. GPC curves for cashew polysaccharide (CG) and purified cashew polysaccharide (FCG) eluted with 0.3 M NaCl containing 0.1% (w/v) NaN₃ at a flow rate of 1 mL/min.

The FTIR spectra are shown in Figure 4. It can be seen that the fractionated cashew polysaccharide showed more intense absorption bands. The broader band in the region between 3500 and 3100 cm⁻¹ corresponds to O-H stretching and refers to the polysaccharide. The band of the cashew polysaccharide appears at 3295 cm⁻¹ and band of the fractionated cashew polysaccharide appears at 3310 cm⁻¹. The bands at 2945 and 2891 cm⁻¹ are due to C-H stretching. The bands at 1071 and 1070 cm⁻¹ are due to C-O bonds. The bands at 1152-1149 cm⁻¹ are dominated by the glycosidic linkage (C-O-C) of the polysaccharides [18-19].

Figure 4. FTIR spectra for cashew polysaccharide (CG) and for cashew polysaccharide purified (FCG).

The ¹H NMR spectra of the samples also were similar and only the spectrum of the fractionated cashew polysaccharide is shown (Figure 5). The chemical shifts were similar to previous works. H-1 signals corresponding to the L-Araf were detected at 5.08 ppm [20-21]. The signal at 4.52 ppm corresponding to β-D-GlcPA [22]. The H-1 signal of the α-L-Rhap was assigned to signal at 4.74 ppm, and the H-6 signal was assigned to signal at 1.25 ppm [21]. The signals at 4.68 ppm and 4.4-4.45 ppm were attributed to β-galp [20-22]. These residues were previously reported for cashew polysaccharide [15, 23].
Figure 5. $^1$H NMR spectrum of fractionated cashew polysaccharide in $D_2O$ (99.9 %).

Figure 6a shows a comparison of the TG curves for the polysaccharides. It can be observed that the cashew polysaccharide has a slightly higher thermal stability than the purified cashew polysaccharide. The main decomposition stage of both samples started at around 240 °C (Tonset). In the first stage, 11 % of mass loss occurred between 50 and 100 °C for the cashew polysaccharide. This mass loss could be attributed to moisture evaporation. The second stage, between 240 °C and 330 °C, is attributed to decomposition of polysaccharides with mass loss of 57 %. It was observed a residue of 13 % at 800 °C. The purified cashew polysaccharide had a mass loss of 12.5 % in the first stage. Between 240 and 330 °C, it occurred a mass loss of 64 %, and it was observed a residue of 5 % at 800 °C. Three stages were evidenced in the DTG curves (Figure 6b). For the cashew polysaccharide it exhibited peaks at 60 °C, 260 °C and 310 °C. For the purified cashew polysaccharide it exhibited peaks at 50 °C, 260 °C and 310 °C.
4. CONCLUSION
In this present study, the cashew polysaccharide was obtained from A. occidentale trees by alcohol precipitation and purified by Sephacryl S-400 HR column. The CG-MS analysis showed that the purified cashew polysaccharide had a lower amount of galactose and greater amounts of arabinose, glucose, and rhamnose. The purified cashew polysaccharide showed a smaller mass dispersion. The FTIR spectra of the samples showed characteristic bands of polysaccharide structures with bands at 1071-1070 cm\(^{-1}\) referent to C-O bonds and bands at 1152-1149 cm\(^{-1}\) referent to the glycosidic linkage (C-O-C). The \(^1\)H NMR spectra of the samples were similar with H-1 signals identified of L-Araf, \(\beta\)-D-Glc\(p\)A, \(\alpha\)-L-Rhap, and \(\beta\)-galp. Thermal analysis showed that the main decomposition stage started at around 240 °C (Tonset) for both samples; however, the fractionated cashew polysaccharide had fewer residues at 800 °C. The DTG curves showed three stages with maximum temperature at 310 °C.
5. ACKNOWLEDGEMENTS
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6. REFERENCES