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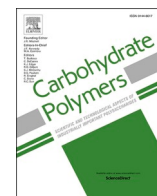
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Structure and emulsifying properties of unprecedented glucomannan oligo- and polysaccharides from Amazonia *Acrocomia aculeata* palm fruit

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ABSTRACT

Acrocomia aculeata fruit pulp contains oil (4.1–82.8 % fresh matter) and carbohydrates (6.6–98.0 % fresh matter). To date, only the oil fraction is valorized because very little is known about the nature of carbohydrates. This study explores new ways of adding value to this pulp by developing simple and efficient extraction processes for its carbohydrate components and characterizing their structure and physicochemical properties over two harvest periods. A water-soluble monosaccharide fraction F1 (solubility limit (SL): 98.5–99.3 g/L) (yield: 21 % dry pulp (DP)), a water-soluble polysaccharide fraction F2 (SL: 93.3–95.3 g/L) (yield: 26 % DP) and two additional water-insoluble polysaccharide fractions F3 and F4 (SL: <8 g/L) (yields: 10 and 19 % DP, respectively) were isolated. NMR structural characterizations of fraction F2 revealed it to be a linear glucomannan with β-(1 → 4) osidic linkages between D-Manp and D-Glcp residues. F2 is unique for its D-Manp/D-Glcp ratio of 3:1 and the position of its acetyl group (13–14 %, C-2 D-Manp). Finally, the polysaccharide showed a molecular weight (Mw) variation ranging from 8.2×10^4 to 1.1×10^3 Da over the two harvest periods, with remarkable emulsifying properties associated with a low Mw of F2 (stability >6 months, 1 % w/v in a water-in-oil emulsion).

1. Introduction

Acrocomia aculeata (Jacq.) Lodd. ex Mart., 1824 is a palm tree endemic to Latin America, which extends from southern Mexico to northern Argentina (Díaz et al., 2021). Its fruit, commonly called “moucaya”, “dendé” or “macaúba” in Guyanese, Guadeloupe creole and Brazilian (Portuguese) respectively, is a greenish globular drupe that can reach 2.5 to 5 cm in diameter (Coimbra & Jorge, 2011a, 2011b) (Fig. 1A & B). The fruit consists of an outer shell (epicarp), a pulp (mesocarp) and a seed composed of a kernel (albumen) and a shell (endocarp). The whole fruit is edible, and its pulp or kernel can be eaten fresh, sweetened, or cooked in ice creams, sweets, cookies, cakes or in a variety of recipes (Coimbra & Jorge, 2011a, 2011b). Regarding the phytochemical composition of the fruit (Table 1), the epicarp and endocarp are mainly composed of lignin-type phenolic compounds representing 18 to 35 % and 40 % of dry matter respectively (del Río et al., 2016; Rencoret et al., 2018). The other fruit compartments (mesocarp and albumen) are the richest chemically, consisting mainly of primary metabolites (lipids, carbohydrates, and proteins) and a minority of secondary metabolites

(phenolic compounds, sterols, carotenoids, triterpenic alcohols and vitamins) (Table 1). Of these two compartments, the pulp has the advantage of being the most abundant compartment within the fruit (40 % of the average total weight of a fresh fruit, compared with 7 % for the albumen) (García Cabrera et al., 2023). For several decades now, the pulp has been attracting the attention of researchers for its very high oil content (4.1 to 82.8 % fresh matter) (Sá et al., 2022), making it the second most important source of vegetable oil after palm oil (*Elaeis guineensis* (Jacq.)), with a production of 1.5 to 5 tons per hectare per year. This productivity could reach 6 tons per hectare per year if palms were domesticated (Navarro-Díaz et al., 2014). Nowadays, the main way to use this oil, which is rich in triglycerides consisting mainly of unsaturated fatty acids (oleic acid) and saturated fatty acids (palmitic acid), is for energy purposes, through the production of biofuels (Cardoso et al., 2017; Lieb et al., 2019; Navarro-Díaz et al., 2014). Although its oil composition is well known, very little is known about the polysaccharides present in the pulp of this fruit.

Regarding carbohydrates, Sá et al. (2022) reported that the fruit pulp contains 7 to 98 % of the total sugars of fresh material. This

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carbohydrate richness depends on the maturity stage of the fruit as with oils. Indeed, Montoya et al. (2016) reported that ripe fruits would be richer in oil unlike unripe fruits, which would be richer in carbohydrates. About the nature of the carbohydrates present in the pulp, Ramos et al. (2008) identified the presence of glucose (9.47 ± 2.48 % of fresh matter), sucrose (0.07 ± 0.17 % of fresh matter) and starch (12.56 ± 0.48 % of fresh matter). However, the values mentioned by Ramos et al. (2008) are questionable because they are based on colorimetric methods and mathematical models. For starch, Vianna et al. (2017) hypothesized that amylose is more likely present than amylopectin, which is not

common. However, this is only a hypothesis based on microscopic observations of starch grains in the *Acrocomia aculeata* fruit pulp, which have a spherical shape, typical of amylose-rich starches, according to the authors. But starches with less amylose can have a spherical shape (e.g., *Pisum sativum* contains 63 to 75 % amylose, but its starch grains are kidney shaped, whereas *Sorghum bicolor*, which contains 21 to 34 % amylose, has spherical and polyhedral starch grains) (Belitz et al., 2009), which somewhat skews this hypothesis. Apart from starch, the presence of cellulose in the form of nanocrystals and mucilaginous pectins has been reported by Corrêa et al. (2019) and Vianna et al. (2017)

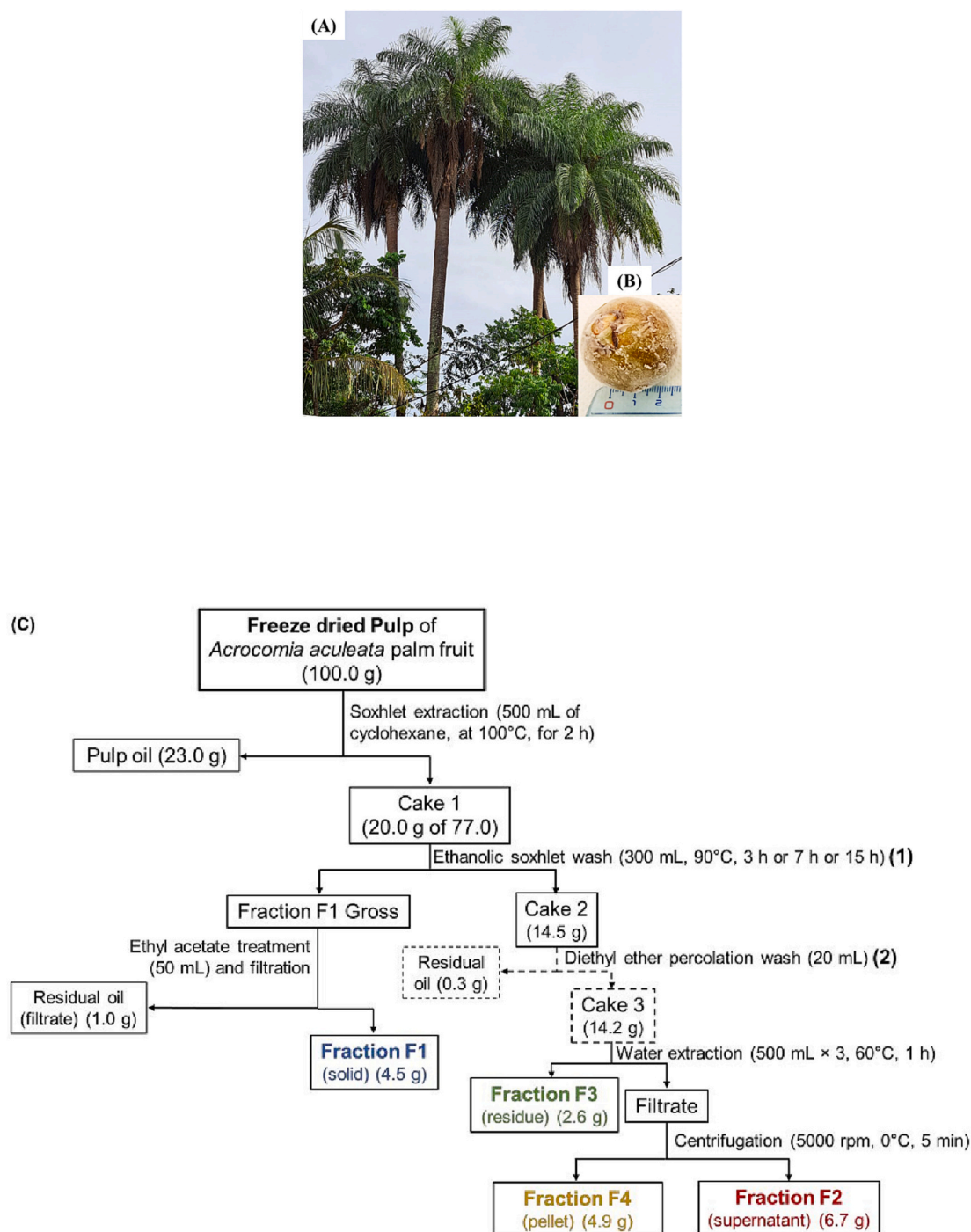


Fig. 1. *Acrocomia aculeata* (A) palms and (B) fruit in French Guiana. (C) Scheme of extraction process and isolation of mono- di- and polysaccharides from pulp of *Acrocomia aculeata* palm fruit. (1) 3 h for the first process version (PI), 7 h for the first optimization process (PII-1) and 15 h for the second optimization process (PII-2); (2) This step was removed during the optimization of the extraction process (PII-1 and PII-2).

respectively, but not quantified.

The only polysaccharide from the pulp of *Acrocomia aculeata* fruit known to date is galactoglucomannan (GGM). This hemicellulose extracted by da Silva et al. (2009) represented 0.0046 % of the fresh pulp and had immuno-adjuvant properties. Structurally, GGM consisted of D-galactose, D-glucose, and D-mannose residues in a molar ratio of 18:22:60, respectively. The polysaccharide has an estimated molar mass of 350 kDa and consists of a main chain of (1 → 4)-linked β-D-mannopyranosyl residues attached to an initial chain of (1 → 3)-linked β-D-galactopyranosyl residues and a terminal chain of (1 → 4)-linked α-D-glucopyranosyl residues (da Silva et al., 2009).

In this study, we developed a simplified method for the extraction of monosaccharides and polysaccharides present in the pulp fruit of the *Acrocomia aculeata* palm, harvested at two close periods. The different fractions obtained were characterized in terms of osidic composition and a water-soluble polysaccharide fraction (F2) was structurally characterized by NMR spectroscopy. This revealed that it is a glucomannan (GM) polysaccharide, whose physicochemical properties have been evaluated to identify the likely pathways for its valorization. Surprisingly, promising emulsifying properties have been observed for low-molecular-weight GM oligosaccharides.

2. Experimental

2.1. Materials and reagents

The fruits of *Acrocomia aculeata* palm were picked on the ground in the city of Remire-Montjoly (GPS details: 52°16'44"W, 4°55'12"N, Altitude = −4 m) in French Guiana the 24th of March 2021 (first harvest period, named H1) and between 7th and 8th of April 2021 (second harvest period, named H2). The fruits were then pretreated with water and 2 % bleach and the pulp was lyophilized after manual separation of the rest remains fruit. The lyophilized samples were then stored at −80 °C.

The reagents used for the extraction process, characterization procedures and physicochemical studies were purchased from VWR (ethanol), Sigma-Aldrich (TFAA, acetic acid, diethyl ether, propionic acid, acetic anhydride, silver carbonate, pyridine, sodium acetate (NaOAc)), Carlo Erba (cyclohexane, ethyl acetate), Fluka (Myo-inositol) Supelco (3 N methanol/HCl solution, Sylon BFT Reagent (BSTFA + TMCS 99:1)), Biosolve (dichloromethane), Alfa Aesar (PentaErythritol), Fisher Bioblock (sodium hydroxide solution (NaOH) 46/48 %) and PUGET (olive oil).

Table 1

Proximate composition of *Acrocomia aculeata* palm fruit. n.d. = not determined. d.q.n.r. = detected but quantity not reported. M^I = primary metabolites. M^{II} = secondary metabolites. n.q. = negligible quantity. FM = Fresh Matter. RE = Retinol Equivalent. GAE = Gallic Acid Equivalent. *The presence of lignins (0.4 g/g dry matter) which are part of phenolic compounds has been reported. Superscript alphabetical letters (a to w) refer to references. a: Sá et al. (2022), b: Trentini et al. (2017), c: Silva (2007), d: Cardoso et al. (2017), e: Andrade et al. (2020), f: Toledo e Silva et al. (2022), g: del Rio et al. (2017), h: Bereau (2001), i: Schex et al. (2018), j: Trentini et al. (2016), k: Coimbra and Jorge (2011a, 2011b), l: Evaristo et al. (2016), m: Lescano et al. (2015), n: Machado et al. (2015), o: Alfaro-Solís et al. (2020), p: Oliveira et al. (2014), q: Montoya-Arroyo et al. (2021), r: Peres et al. (2013), s: Patient (2017), t: Correia et al. (2013), u: Rencoret et al. (2018), v: Duarte et al. (2022), w: Montoya et al. (2016).

Composition	Fruit compartments			
	Epicarp	Mesocarp	Endocarp	Albumen
Moisture (g/100 g FM)	39.5 ^l – 61.0 ^w	5.98 ^k – 66.4 ^o	15 ^l	3.2 ⁿ – 25.2 ^l
Ash (g/100 g FM)	n.d.	1.5 ^m –3.2 ⁿ	n.d.	1.3 ⁿ –2.1 ^k
M ^I				
Lipids (g/100 g FM)	n.d.	4.1 ^a –82.8 ^a	n.d.	0.7 ^b –95.2 ^a
Carbohydrates (g/100 g FM)	n.d.	6.6 ^{c,d} –98.0 ^a	n.d.	1.0 ^c –46.9 ^{c,d}
Proteins (g/100 g FM)	n.d.	0.8 ^a –10.5 ^a	d.q.n.r. ^g	2.8 ^a –37.7 ^f
Sterols (g/100 g oil)	n.d.	n.q. ^h –0.4 ^g	n.d.	n.q. ^h –0.1 ^g
Carotenoids (mg/100 g FM)	n.d.	0.1 ⁱ –124.2 ^j	n.d.	0.2 ^k –3.7 ⁱ
Phenolic compounds (mg GAE/g FM)	0.1 ^t –5.6 ^s	0.5 ^r –2.6 ^e	n.d. ^{*g,u}	2.0 ^k –4.0 ^v
Triterpene alcohols (g/100 g oil)	n.d.	1.1 ^g	n.d.	0.1 ^g
Vitamins				
Vit A (mg/100 g FM)	n.d.	0.6 RE ^p	n.d.	n.d.
Vit C (mg/100 g FM)	n.d.	34.0 ^k – 118.2 ^p	n.d.	n.d.
Vit E (mg/100 g oil)	n.d.	1.3 ^q – 21.3 ^k	n.d.	1.4 ^q – 2.3 ^k

2.2. Extraction and isolation

A first version of the carbohydrate extraction and isolation process was developed (named PI) before undergoing modifications to produce two variants (named PII-1 and PII-2). For the first version (PI), the pulp cake obtained during the oil extraction (cake 1) was dried at room temperature (25 °C) overnight and stored at −20 °C until use (Fig. 1C). It was then washed with ethanol at 90 °C for 3 h in a Soxhlet with a plant material/solvent ratio of 1/6.25. The crude ethanolic fraction (F1 crude) thus obtained was washed with ethyl acetate (50 mL) to separate the residual oils from the simple sugars (F1 fraction), after filtration on paper and solvent removal with a rotavapor. The ethanol wash cake (cake 2) was percolated with diethyl ether (20 mL). The interest of this treatment is to extract more oily materials. The resulting cake from this washing (cake 3) was left to dry at 40 °C for 30 min under ventilation in an oven, before undergoing an aqueous extraction (500 mL of distilled water) under stirring for 1 h at 60 °C. The mixture was then filtered through muslin cloth (1 × 1 mm) and the residue obtained (Fraction F3) was taken back in distilled water using the same procedure. The process was repeated three times in total. The filtrate from this aqueous extraction was centrifuged at 5000 rpm for 5 min at 0 °C, resulting in a pellet (Fraction F4) and a supernatant (Fraction F2) (Fig. 1C). All fractions (F1, F2, F3 and F4) were freeze-dried. For the PII-1 and PII-2 variants, the diethyl ether percolation washing step was omitted, and the ethanol washing time at Soxhlet was extended to 7 h for PII-1 and 15 h for PII-2. These modifications allowed to simplify the process, while maintaining the same efficiency for the removal of residual oily substance (Fig. 1C).

2.3. Determination of sugar composition

The analyses were subcontracted at CERMAV (Centre de recherches sur les macromolécules végétales, Grenoble, France). For polysaccharide fractions (F2, F3 and F4), their osidic compositions were determined according to Kamerling et al. (1975) method modified by Montreuil et al. (1986). The method consists in carrying out a methanolysis of the polymer to obtain only monomers which are analyzed by GC-FID after trimethylsilylation.

Each sample was solubilized in ultrapure water (1 g/L). Three 500 µL samples were placed in three hydrolysis tubes in the presence of 60 µL of a 1.5 g/L internal standard solution (inositol) and then lyophilized. The tubes were then placed in a dry bath in the presence of 500 µL of a methanol/3 N hydrochloric acid mixture for 4 h at 110 °C. After cooling to room temperature, the methanolysate was neutralized with silver

carbonate (its use prevents the elimination of methanolic HCl by evaporation but rather by neutralization with formation of an AgCl precipitate and a CO₂ gas. Indeed, it has been demonstrated that the evaporation of methanolic HCl leads to the partial degradation of the methylglycosides formed. The various studies have therefore suggested that the best way to carry out neutralization is to add an agent such as silver carbonate (Chambers & Clamp, 1971; Wulff, 1965)) and 20 µL of acetic anhydride was added to reacylate the osamines present. After 14 h in darkness at room temperature, the samples were centrifuged for 15 min at 3000 rpm and the supernatant was evaporated under nitrogen. The compounds were then dissolved in 70 µL pyridine and incubated for 14 h at room temperature with 70 µL sylon (BSTFA: TMCS, 99:1). After gentle evaporation of excess reagents under nitrogen, the trimethylsilylated methylglycosides were taken back by 650 µL of dichloromethane and then injected into gas chromatography (GC-6850 AGILENT system, in-column injection, FID detector: flame ionization). The carrier gas was hydrogen. The column type HP-5MS (30 m, 0.25 mm internal diameter) was apolar. The temperature rise programme was as follows: start at 120 °C and gradient from 3 °C/min to 180 °C followed by a gradient from 2 °C/min to 200 °C.

Each monosaccharide was identified by comparing its retention time relative to the internal standard with those of pure monosaccharides (ribose, fucose, rhamnose, arabinose, xylose, mannose, glucose, galactose, glucuronic acid, galacturonic acid, iduronic acid, guluronic acid, mannuronic acid, *N*-acetylated galactosamine, and *N*-acetylated glucosamine) treated under the same conditions. A response coefficient was calculated for each monosaccharide relative to the internal standard to define the proportion of each monosaccharide within the polysaccharide fractions analyzed. The analyses were performed in triplicate.

For the fraction consisting of simple sugars (F1), the analyses were carried out by ion chromatography. The lyophilized sample was dissolved in ultrapure water at 4 g/L. The solution was then diluted 10 times and filtered to 0.2 µm before being injected into anion exchange liquid chromatography on a DIONEX ICS6000 system (ThermoFisher) equipped with a pre-column and a CarboPac PA1 column (250 × 2 mm) and a PAD detector (pulsed amperometry). The column was thermostated at 28 °C and the volume injected was 5 µL. Elution was performed at 0.18 mL/min under the following conditions: T0 to T27 min (0.016 M NaOH), T27 to T29.1 min (0.016 M NaOH + 0 to 0.02 M NaOAc linear gradient), T29.1 to T37 min (0.016 M to 0.1 M and linear gradient in NaOAc from 0.02 M to 0.5 M) and T37 to T43.1 min (linear gradient in NaOH from 0.1 M to 0.2 M + 0.5 M NaOAc). A calibration curve of the reference sugars (mannitol, fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, sucrose, galacturonic acid and glucuronic acid) was performed under the same elution conditions.

2.4. Determination of maximum water solubility of different fractions (F1, F2, F3 and F4)

The maximum water solubility of the four fractions of mono-, oligo-, and polysaccharides was determined according to the method previously used for starch by Mukerjee et al. (2007) and for sulfated polysaccharides by Zhang et al. (2015) with some adaptations. Each sample (50 mg) was suspended in 0.5 mL of ultrapure water in a 2 mL microcentrifuge tube. By dissolved at room temperature for 16 h with constant stirring (250 rpm, 20 °C), the solution was centrifuged at 10,000 rpm for 20 min. The undissolved residue was then separated from the supernatant and freeze dried. The maximum water solubility was determined by the following Eq. (1):

$$\text{Water solubility (g/L)} = \frac{(Mo - Mp)}{V} \quad (1)$$

where *Mo* is the mass of the original sample (mg), *Mp* is the mass of the precipitate after freeze dry (mg) and *V* is the volume of the water used to

dissolve the sample (mL).

2.5. Per-acetylation of polysaccharide F2

The polysaccharide F2 was per-acetylated according to the method described by Enomoto-Rogers et al. (2013) with some adaptations. Trifluoroacetic anhydride (TFAA) (2 mL) was mixed with acetic acid (2 mL) and heated to 50 °C for 20 min. Then, 70 mg of F2 was immediately added to the mixture. The solution was then left at 50 °C for 1 h under nitrogen. After returning to room temperature, the per-acetylated polysaccharide was isolated by precipitation in ethanol (200 mL). It was then filtered, washed with ethanol, and lyophilized.

2.6. Determination of acetylation degree and acetyl position of native polysaccharide F2 by NMR after per-propionylation of polysaccharide F2

For the NMR determination of the degree of acetylation (*D_{Ac}*) of the native polysaccharide F2, in a first step, the free hydroxyl groups were totally propionylated to make it soluble in chloroform-*d*. The per-propionyl form of F2 was obtained by reacting the polysaccharide (30 mg) (50 °C, 1 h) with the mixture TFAA (2 mL) and propionic acid (2 mL), previously heated (50 °C) for 20 min. The per-propionylated polysaccharide was then precipitated with ethanol (200 mL), filtered, washed, and lyophilized. After the acquisition of the 1D ¹H NMR spectrum of the per-propionyl product, the *D_{Ac}* of the native polysaccharide was determined from the ratio of the integrated areas of the methyl protons of the acetyl groups to the polysaccharide ring protons, according to Eq. (2). The position of the acetyl groups on the native polysaccharide F2 was determined using 2D HMBC (¹H–¹³C) NMR spectra.

$$D_{Ac} (\%) = \frac{[CH_3]/3}{[Polysaccharide \text{ proton ring}]/7} \times \left(\frac{100}{3}\right) \quad (2)$$

where *[CH₃]* represents the area of integration of the 3 protons of the acetyl group and *[Polysaccharide proton ring]*, the area of integration of the 7 protons of the polysaccharide unit (between 3.00 and 6.00 ppm). In **100/3**, **3** represent the total number of positions in which an acetyl group may be present and **100**, the percentage conversion.

2.7. Nuclear magnetic resonance (NMR) measurements

NMR spectra were recorded on a Bruker Avance III 400 spectrometer operating at 400.13 MHz for ¹H, equipped with a BBFO probe with a Z-gradient coil and a GREAT 1/10 gradient unit. All experiments were carried out at 25 °C, using chloroform-*d* as solvent. ¹H chemical shifts (δ) were given in ppm relative to the solvent residual peak and ¹³C chemical shifts were relative to the central peak of the solvent signal. The zg30 Bruker pulse program was used for 1D ¹H NMR, with a TD of 32,768, a relaxation delay d1 = 5 s and 88 scans. The spectrum width was set to 20 ppm. Fourier transform of the acquired FID was performed without any apodization in most of the case. 1D ¹³C NMR spectra were recorded at 100.61 MHz's. The zgpg30 Bruker pulse program was used with 6 k scans. TD is 65,536 and a relaxation delay d1 = 2 s. The spectrum width was set to 250 ppm. Fourier transform was performed after apodization with an exponential function using a LB of 0.6 Hz. 2D COSY (¹H–¹H) experiments were acquired using the “cosygpmfqr” Bruker pulse program (COSY with multiple quantum filter). Matrices consisting of 512 (F1) × 2048 (F2) complex data points were recorded; 8 scans were carried out per F2 increment with a 1.5 s recovery delay (d1) and an AQ time of 0.25 s (F2) to 0.12 s (F1). Processing was performed with a QSINE function in both dimensions (SSB = 0). 2D HSQC (¹H–¹³C) experiments were acquired using the “hsqcedetgspisp2.4” Bruker pulse program for high sensitivity, (HSQC with multiplicity editing during selection step, using shaped pulses for all 180-degree pulses on F2 – channel for matched sweep adiabatic pulses, with gradients in backinept and with

suppression of COSY type artefacts) with an AQ = 0.27 s, d1 = 1.5 s, ns = 64. TD is 512 and 3072, respectively for F1 and F2. Fourier transform was performed in both dimensions with a SINE function (SSB = 3). **2D HMBC** (^1H ^{13}C) experiments were performed with either the “*hmbcgp-impact*” (phase sensitive HMBC using Echo/Antiecho gradient selection with three-fold lowpass *J*-filter to suppress one-bond correlations, no decoupling during acquisition, using constant time for suppressing F1 skew and using ASAP mixing for suppressing F1 ridges) or “*hmbcgpplrnd*” Bruker pulse programs (HMBC optimized on long range couplings, with low-pass *J*-filter to suppress one-bond correlations). D1 was set to 0.25 s or 1.5 s, delay d6 for the evolution of long-range coupling was set to 65 ms, ns = 76 and the number of series was 128 (t1). All the data were processed with a SINE function in both dimensions (SSB = 3).

2.8. Size exclusion chromatography (SEC)

2.8.1. Molecular weight determination

Samples were dissolved at a concentration of 2 mg/mL in sodium nitrate 0.1 M. Samples were filtered at 0.22 μm before being injected into the Malvern A4000, Aq GPC/SEC column (300 $\times$ 8.0 mm, particle size: 10 μm L1670029) at a temperature of 25 $^{\circ}\text{C}$ (Dextran equivalent column exclusion limit: 10^6 Da). An eluent flow rate was 0.5 mL/min. The injected volume for each measurement was 100 μL . Detection was performed using a refractometer, a VE3580 VISCOTEK. The value refractive index increments dn/dc used for this analysis was 0.147 mL/g, the default value for polysaccharides. The polydispersity index was calculated as $I_p = M_w/M_n$.

2.8.2. Fractionation of polysaccharide F2-H1-PI for identification of between *D-Glcp* – *D-Glcp* and *D-Glcp* – *D-Manp* bonds by NMR spectroscopy

100 mg of polysaccharide F2-H1-PI were dissolved in 3 mL ultrapure water and the resulting solution was deposited on a pre-prepared Sephadex® G-25 column (17.5 $\times$ 1 cm). A first sub-fraction was obtained (SF1) as the dead volume. Then 5, 15, 10 and 50 mL of ultrapure water were successively added, and the sub-fractions SF2, SF3, SF4 and SF5 were collected respectively. The analysis focused on SF2 after freeze-drying.

2.9. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was carried out using a SDT Q600 V20.9 Build 20 instrument under nitrogen atmosphere (100 mL/min). Thermograms were acquired between 20 and 700 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C}/\text{min}$ between 20 and 110 $^{\circ}\text{C}$, and 20 $^{\circ}\text{C}/\text{min}$ between 110 and 700 $^{\circ}\text{C}$. The proportions of moisture, organic matter and inorganic matter are then determined as a function of sample mass loss (moisture: 0–100 $^{\circ}\text{C}$, organic matter: >100 $^{\circ}\text{C}$ and inorganic matter: the residue remaining at the end of the experiment).

2.10. Rheological test

Rheological measurements were performed using a HAAKE™ MARS™ III rheometer (Thermo Scientific™) cone-plate model. The sample (F2) was solubilized in ultrapure water at a concentration of 1 % (w/v) and the measurements were taken at 20 $^{\circ}\text{C}$.

2.11. Emulsifying test and characterization

2.11.1. Emulsifying test

The stability of water-in-olive oil (W/O: 25/75, w/w) and olive oil-in-water (O/W: 25/75, w/w) emulsions formed from polysaccharide F2 were studied. For each emulsion, polysaccharide (1 %, w/v) was dissolved in ultrapure water and olive oil was added. The mixture was then homogenized with the ULTRA TURRAX at 11000 rpm for 10 min. Finally, the stability of the emulsions formed was monitored over time.

2.11.2. Emulsion characterization by optical microscopy

Samples of stable emulsions (approx. 0.1 mL) were deposited on a glass slide topped with a thin slide before being viewed through the 40 $\times$ /0.65na C Plan Objective of a Leica DMLS Binocular optical microscope. The images observed were captured using the camera of an iPhone 11 (1.3 $\times$) and then contrasted in white/black.

2.11.3. Emulsion characterization by surface tension measurement

Surface tension of emulsion was determined using *du Noüy* ring method (Krüss tensiometer, software: *Krüss Laboratory Desktop*, program: *Surface and Interfacial Tension*, mode: “ring tear-off” (e.g., ASTM D971)) following the procedure described by Kanna et al. (2017) and Jankar et al. (2020). The method involves using a platinum ring to measure surface tension. The ring is completely immersed in the sample to be analyzed. It is then slowly removed from the sample until maximum force is reached. The studies were carried out at 25 $^{\circ}\text{C}$ and the results were expressed in millinewton/m (mN/m).

3. Results and discussion

3.1. Carbohydrate extraction and sugar composition of H1-PI (1st harvest period and initial version of extraction process)

The pulp of the *Acrocomia aculeata* palm fruit had 55 % relative humidity before freeze-drying, which was similar to the content reported by Evaristo et al. (2016) (46 %), although the moisture level was depending on the palm tree environment and the climatic factors.

Before extraction of the polysaccharides from the fruit pulp, the oil present (23 % of dry pulp) (Fig. 1C) was removed with a Soxhlet for a relatively shorter time (2 h) than usual to avoid possible degradation of the sugars (Belitz et al., 2009).

The resulting cake 1 (77 % dry pulp) was first treated with ethanol, a process known for its efficiency in extracting simple sugars (fraction F1, 21 % dry pulp) (Fig. 1C). The choice to perform the extraction of sugars from 20 g of cake 1 was based on the fact that the product tended to form a very viscous paste in distilled water (500 mL) beyond 20 g. In addition, the ethanol treatment allowed to remove some of the residual oils from cake 1 (Fig. 1C). Following this treatment, cake 2 (56 % dry pulp) was washed with diethyl ether to eliminate the trapped ethanol and thus eliminate the residual oils present (1 % dry pulp). Finally, by successive mild aqueous extractions (60 $^{\circ}\text{C}$), three polysaccharide fractions were obtained from cake 3 (55 % dry pulp). A water-soluble fraction F2 (26% dry pulp), a water-insoluble fraction F3 (10% dry pulp) and a very slightly water-soluble fraction F4 (19% dry pulp) (Fig. 1C). Of the three polysaccharide fractions (F2, F3 and F4), the F2 fraction was the most important from a quantitative viewpoint (26 % of dry pulp against 10 and 19 % for F3 and F4, respectively).

Analysis of the sugar composition of the fraction F1 revealed that it contained an average of 68 % simple sugars (Table 1). We therefore assumed that the remaining 32 % of F1 should be oil that was not completely removed with ethyl acetate (Fig. 1C) or unreferenced simple sugars or other compounds. For the first time in this work, we have identified the presence of minority monosaccharides: *D*-mannose (1 % of F1 or 0.2 % of dry pulp), *D*-xylose, *D*-galactose and *L*-arabinose (whose mass proportions were <1 % of F1), in addition to the known majority ones: sucrose (34 % F1 or 6 % dry pulp), *D*-fructose (18 % F1 or 3 % dry pulp) and *D*-glucose (13 % F1 or 2 % dry pulp) (Table 2).

We obtained a sucrose content (2.65 % fresh pulp) 37 times higher than that reported by Ramos et al. (2008) (0.07 % fresh pulp) and a *D*-glucose content (1.02 % fresh pulp) 9 times lower than that reported by the same authors (9.47 % fresh pulp). These differences could be due to the palm trees environment, the maturity stage of the fruit and the different processing and characterization methods used.

However, it is worth noting that the sugar composition within a plant matrix is not fixed because, as reported by Montoya et al., 2016, there is a relationship between the bioavailability of sugars and oils within the

Table 2

Sugar composition of fractions F1, F2, F3 and F4 of H1-PI (% of dry fraction). Standard Deviation ≤ 0.1 for fraction F1

Sugar composition (% of dry fraction)					
Composition	F1	Composition	F2	F3	F4
Sucrose	34.2	D-glucose	10.2 $\pm$ 0.5	18.5 $\pm$ 5.5	31.6 $\pm$ 2.1
D-fructose	18.3	D-mannose	31.2 $\pm$ 1.7	3.3 $\pm$ 0.8	2.6 $\pm$ 0.3
D-glucose	13.1	D-xylose	1.0 $\pm$ 0.1	28.0 $\pm$ 5.0	14.2 $\pm$ 3.1
D-mannose	1.2	L-arabinose	0.3 $\pm$ 0.0	2.9 $\pm$ 0.6	2.5 $\pm$ 0.2
D-xylose	0.5	D-galactose	0.7 $\pm$ 0.0	2.4 $\pm$ 0.5	2.5 $\pm$ 0.2
D-galactose	0.3	L-rhamnose	0.0 $\pm$ 0.0	0.7 $\pm$ 0.1	0.0 $\pm$ 0.0
L-arabinose	0.2	D-galacturonic acid	0.0 $\pm$ 0.0	3.0 $\pm$ 0.6	3.3 $\pm$ 0.5
Other	32.2	Total	43.5 $\pm$ 2.4	58.8 $\pm$ 13.0	56.6 $\pm$ 6.4
Total	100				

fruit pulp of *Acrocomia aculeata* and their maturity stage. Indeed, ripe fruits would be richer in oils (4–83 % fresh matter) and usually poorer in sugars (7–80 % fresh matter), while unripe fruits would be richer in sugars (35–98 % fresh matter) and poorer in oils (<0.1 %) (Montoya et al. (2016), Sá et al. (2022), Silva (2007), Cardoso et al. (2017)). Logically, Sá et al. (2022) reported that sucrose, D-glucose, and D-fructose contents (72.46 to 3.70 %, 48.38 to 0.27 % and 45.33 to 0.49 % of fresh pulp, respectively), depended on fruit maturity stage and palm location, covering very wide ranges. The highest sucrose, D-glucose and D-fructose contents obtained by Sá et al. (2022) came mostly from relatively unripe fruits (age: between 8 and 48 weeks after anthesis). For the lowest contents, they are from ripe fruits (age: 60 weeks after anthesis). Considering that our sampling strategy was to collect fruits directly from the ground (ripe fruits, approximate age: 60 to 62 weeks after anthesis), we can therefore estimate that our sucrose, D-glucose and D-fructose contents (2.65, 1.02 and 1.42 % of fresh pulp, respectively) were in agreement with the values obtained by Sá et al. (2022) for ripe fruit (age: 60 weeks after anthesis) (3.70, 0.27 and 0.49 % of fresh pulp, for sucrose, D-glucose and D-fructose, respectively).

Concerning the polysaccharide fractions F2, F3 and F4, analysis of their osidic composition revealed that they consisted respectively of 43, 59 and 57 % sugars (Table 2). However, solubility problems were encountered during the studies, thus affecting the percentage of hydrolysis of the samples. For fractions F3 and F4, the analyses revealed that they were mainly composed of D-glucose (18 and 32 % dry fractions, respectively) and D-xylose (28 and 14 % dry fractions, respectively) with other minority residues (between 0.7 and 3.3 % dry fractions) but not negligible (Table 2). Regarding the polysaccharide nature of F3 and F4 fractions, it is possible that these samples contain galactoglucomannan previously isolated by da Silva et al. (2009) from fruit pulp. Starch (Sá et al., 2022) and/or glucan-type polysaccharides could also be present. Moreover, the presence of rhamnose in the F3 fraction could suggest the presence of pectins (Vianna et al., 2017).

Finally, concerning the F2 fraction, the results revealed that it was composed of two major residues (D-mannose and D-glucose) in a 3:1 mass ratio, respectively. Other residues present (D-xylose, L-arabinose, and D-galactose) were almost negligible (<1 % of dry fraction) (Table 2). This suggested that the F2 fraction could be a glucomannan polysaccharide present in significant quantity in the pulp (26 % dry pulp).

3.2. Water solubility of carbohydrate fractions (F1, F2, F3 and F4)

Water solubility tests were carried out on the various mono- and polysaccharide fractions and they revealed, unsurprisingly, a very high solubility limit (93.3–95.3 g/L) for the initially water-soluble polysaccharide fraction F2 and for the monosaccharide fraction F1 (98.5–99.3 g/L) (Table 3). For the polysaccharide fractions F3 and F4, low solubility limits were observed (2.5–4.6 and 5.6–7.9 g/L,

Table 3

Water solubility (g/L) of different fractions of mono-, and polysaccharides (room temperature). Average of 3 repetitions. n.d. = not determined.

Fraction	H1-PI	H2-PI	H2-PII-1	H2-PII-2
F1	99.3 $\pm$ 0.4	99.3 $\pm$ 0.3	99.3 $\pm$ 0.6	98.5 $\pm$ 1.5
F2	95.3 $\pm$ 0.9	93.3 $\pm$ 3.8	n.d.	94.1 $\pm$ 2.8
F3	4.1 $\pm$ 0.9	3.2 $\pm$ 0.4	2.5 $\pm$ 0.5	4.6 $\pm$ 0.5
F4	7.4 $\pm$ 1.4	7.9 $\pm$ 0.6	7.3 $\pm$ 1.8	5.6 $\pm$ 1.2

respectively) (Table 3). Thus, the water-solubility of the F2 polysaccharide fraction, combined with its simple osidic composition (mannose and glucose) and the high yield for its extraction (26 % dry pulp), make it a priority for structural characterization.

3.3. Structural characterization by NMR of polysaccharide F2-H1-PI in triacetate form

Per-acetylation of the polysaccharide F2-H1-PI enabled much higher resolution NMR spectra to be obtained, making structural characterization simpler and more complete, in contrast to characterization of the native polysaccharide in D₂O. Thus, all peaks in the ¹H and ¹³C NMR spectra of triacetate polysaccharide F2-H1-PI were carefully assigned, based on the 2D NMR spectra (COSY, HSQC and HMBC), as shown in Figs. 2 and 3.

The previous NMR studies of polysaccharide of the mannoglycans family of konjac glucomannan revealed that the anomeric carbon (C1) of D-Manp unit was observed upfield from that of the D-Glcp unit (Enomoto-Rogers et al., 2013). In this work, we observed two major peaks in the ¹³C NMR spectra at 98 and 100 ppm, which were assigned respectively to the C1 of the D-Manp (M) and D-Glcp (G) units. The chemical shifts of the anomeric carbons (C1) thus obtained, were traced back to their respective anomeric protons (H1) from the 2D HSQC (¹H–¹³C) NMR spectra (Fig. 2B).

For the remaining protons (H2, H3, H4, H5 and H6), their chemical shifts were obtained using the COSY (¹H–¹H) 2D NMR spectra starting from the anomeric protons (H1) (Fig. 3A). Indeed, the observation of the correlations between the protons of the D-Glcp and D-Manp units of the polysaccharide in COSY (¹H–¹H) allowed the identification of the chemical shifts of H2 from H1, H3 from H2, H4 from H3, H5 from H4 and finally H6 from H5. Once all the protons have been assigned, their respective carbons (C2, C3, C4, C5 and C6) could be easily traced back to the 2D HSQC (¹H–¹³C) NMR spectra (Fig. 2B). Finally, analysis of the 2D HMBC (¹H–¹³C) NMR spectra revealed the polysaccharide constituent units (D-Manp–D-Manp, D-Manp–D-Glcp, D-Glcp–D-Manp or D-Glcp–D-Glcp), the types of bonds and the absence or presence of branches (Fig. 3B). Thus, on the HMBC spectra (Fig. 3B) a strong correlation was observed between C1 (D-Manp) (98.0 ppm) and H4 (D-Manp) (3.85 ppm) testifying to the abundant presence of D-Manp – D-Manp sequences in the polysaccharide. In addition, a low intensity correlation was observed between C1 (D-Manp) (98.0 ppm) and H4 (D-Glcp) (3.75 ppm) indicating low presence of D-Manp – D-Glcp sequences in the polysaccharide. At this stage, we did not identify the presence of D-Glcp – D-Manp or D-Glcp – D-Glcp sequences in the polysaccharide.

In order to verify the presence or absence of D-Glcp – D-Manp and D-Glcp – D-Glcp sequences within the polysaccharide F2-H1-PI, NMR analysis of SF2 sub-fraction in its triacetate form showed the presence of the desired sequences. Thus, on the HMBC spectra (¹H–¹³C) (Fig. 4) we observed a correlation between C1 (G) (100.6 ppm) and a H4 (G and M) (3.75 ppm) corresponding to D-Glcp – D-Manp and D-Glcp – D-Glcp sequences. Given the low intensity of the correlation and the difficulty in identifying the latter two sequences, it was assumed that the polysaccharide F2-H1-PI should contain a very small minority of them.

Finally, about the α or β configuration of the osidic identified bonds, the chemical shifts obtained were both compared to those obtained from a commercial konjac glucomannan (Supplementary Material, S1) and to

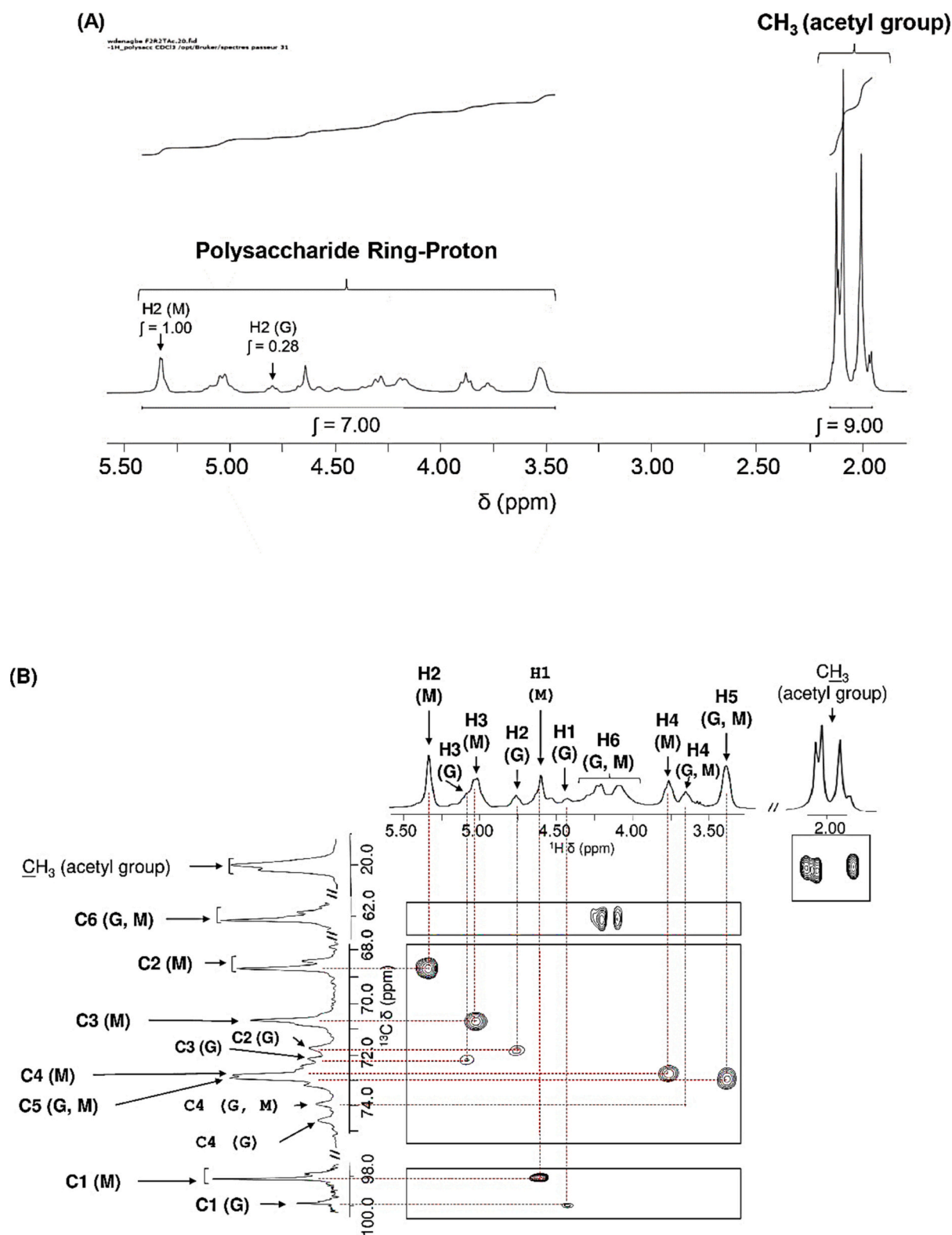


Fig. 2. (A) 1D ^1H and (B) 2D HSQC (^1H – ^{13}C) NMR spectra of polysaccharide F2-H1-PI triacetate in CDCl_3 (with G for D-Glcp and M for D-Manp).

those reported in the literature (Enomoto-Rogers et al., 2013) (Table 4). The similarity of chemical shifts allowed us to confirm that the polysaccharide F2-H1-PI was a linear glucomannan with β -(1 $\rightarrow$ 4) osidic bonds.

3.4. Acetylation degree and acetyl positions of polysaccharide F2-H1-PI

Glucomannans, generally of the konjac type, have acetyl groups in the C6 position of the D-Manp units at an average rate between 0 and 5 % (Nishinari et al., 1992). To highlight them within the glucomannan of the fruit pulp of the *Acrocomia aculeata* (AA) palm (fraction F2), we

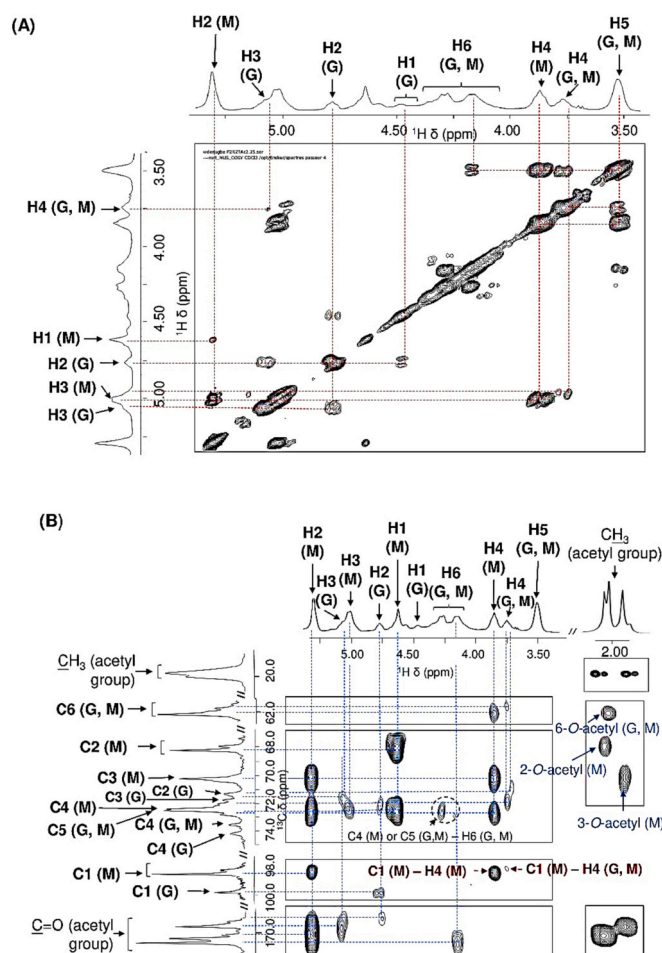


Fig. 3. (A) 2D COSY (^1H – ^1H) and (B) 2D HMBC (^1H – ^{13}C) NMR spectra of polysaccharide F2-H1-PI triacetate in CDCl_3 (with G for D-Glcp and M for D-Manp).

carried out a *per*-propionylation of the polysaccharide. This guaranteed the solubility of the product in the same study solvent (CDCl_3) and at the same time allowed to obtain the chemical shifts of the protons of the acetyl groups (if there are any naturally) offset from the protons of the grafted propionyl groups (Enomoto-Rogers et al., 2013). The analysis of the proton NMR spectrum of the polysaccharide F2-H1-PI in its *per*-propionyl form showed the presence of protons of acetyl group at characteristic chemical shifts (1.94 to 2.15 ppm) (Fig. 5). The integration area of these protons demonstrated that our polysaccharide F2-H1-PI had a degree of acetylation of 13 to 14 %, 3 times higher than that of konjac glucomannan in general (0 to 5 %). (Nishinari et al., 1992).

Regarding the identification of the acetyl group positions within the polysaccharide F2-H1-PI, the 2D HMBC (^1H – ^{13}C) NMR spectra revealed that the acetyl group would be in the C2 position of the D-Manp units (Fig. 6A), which was totally different from konjac glucomannan (C6 (D-Manp)) (Nishinari et al., 1992). The characterization results thus obtained from the polysaccharide F2-H1-PI allowed us to establish its chemical structure as shown in Fig. 6B. This was the first time that such a structure has been demonstrated in AA fruit pulp.

Depending on the species and variety, branched konjac *O*-acetylglucomannans with acetyl groups in the C2, C3 and C2 + C3 positions were reported. (Shi et al., 2020). In the literature, the existence of glucomannan polysaccharide with acetyl group in the C2 position (D-Manp and D-Glcp) from *Dendrobium officinale* stem was reported by Hua et al. (2004). However, the 2-*O*-acetylglucomannan described by these authors differs completely from our polysaccharide from a structural point of view. In addition to possessing acetyl group at both C2 (D-Manp and D-Glcp) of its main chain, it has branches connected by β -(1 $\rightarrow$ 6) bonds to the main chain. These branches consist of β -(1 $\rightarrow$ 3) bonds between D-Manp and D-Glcp residues, with D-Manp and arabinofuranosyl residues in terminal positions (Hua et al., 2004). With regard to glucomannans from *Dendrobium officinale*, Xing et al. (2014) and Xing et al. (2015) later revised the structure, reporting that they had obtained linear *O*-acetylglucomannans. According to their studies, these glucomannans would have acetyl group only on mannose residues in positions C2 or C3 (majority), in positions C2 and C3 (minority), in position C6 (very minority), in positions C2 and C6 (very minority) and in positions C3 and C6 (very minority) (Xing et al., 2014; Xing et al., 2015). Other plant sources such as: *Aloe barbadensis*, *Aloe plicatilis*, *Bletilla formosana*,

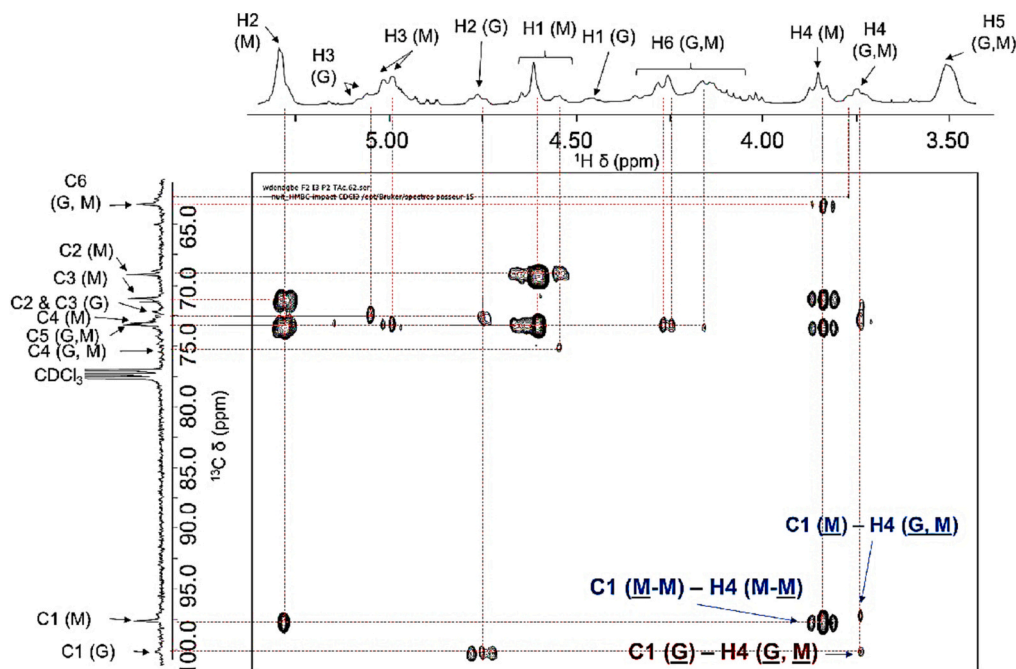
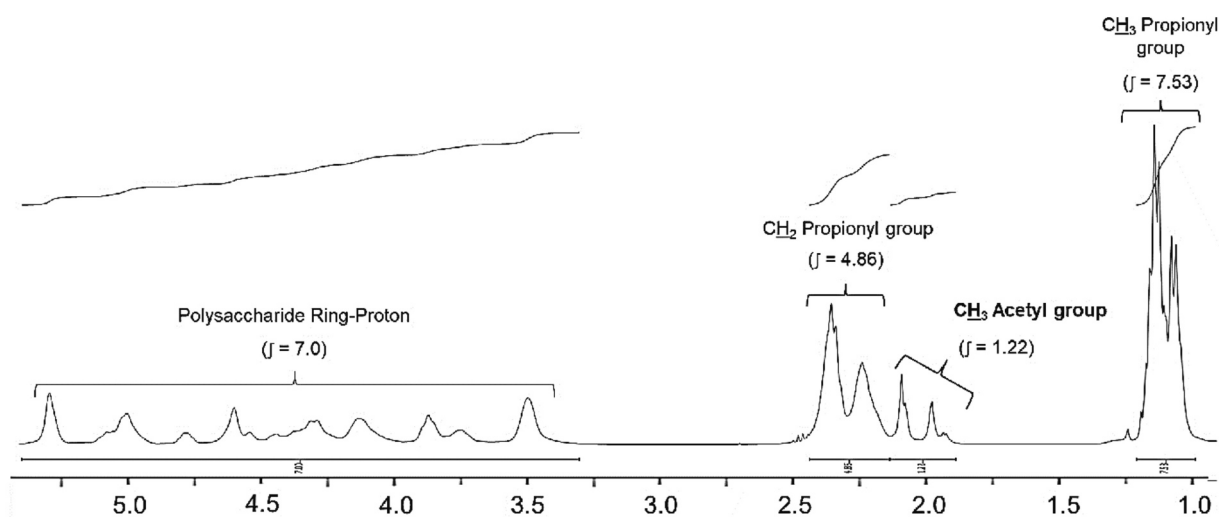
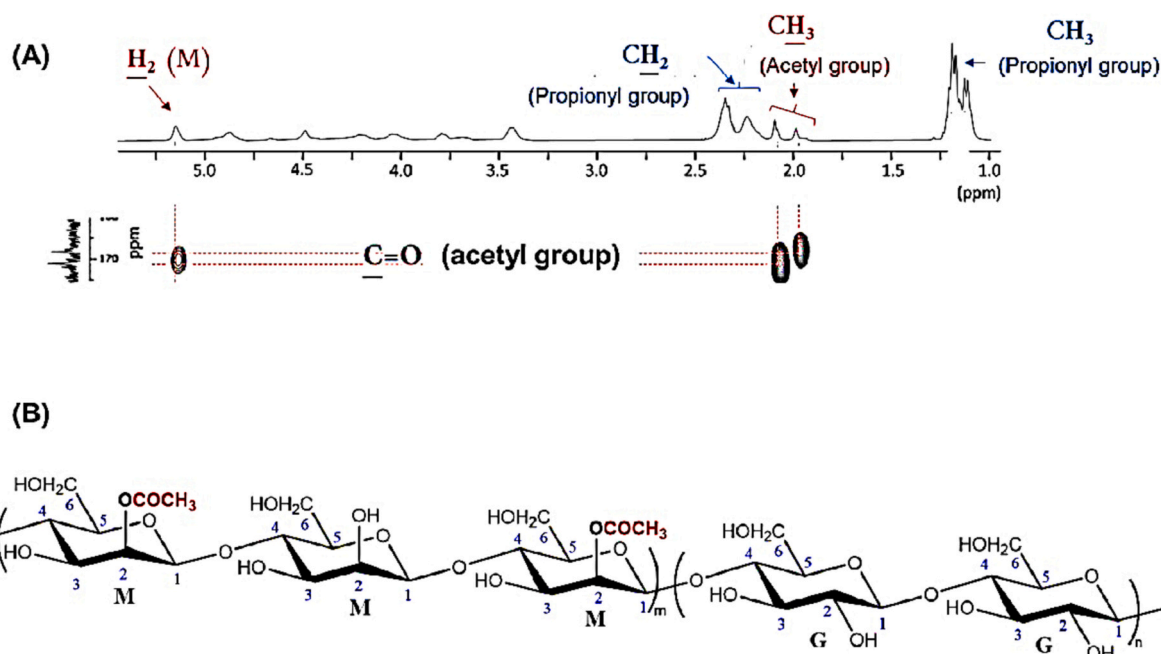


Fig. 4. HMBC spectra (^1H – ^{13}C); of polysaccharide F2-H1-PI (sub-fraction SF2 triacetate) in CDCl_3 after SEC (with G for D-Glcp and M for D-Manp).

Table 4NMR Chemical shifts (ppm) (^1H , ^{13}C) in CDCl_3 of polysaccharide F2-H1-PI triacetate and konjac glucomannan triacetate (commercial & literature).

Atom no.	Polysaccharide F2 triacetate (This study)				Konjac glucomannan triacetate (This study, Supplementary Material, S1)				Konjac glucomannan triacetate (Enomoto-Rogers et al., 2013)			
	$\beta\text{-D-Glcp}$		$\beta\text{-D-Manp}$		$\beta\text{-D-Glcp}$		$\beta\text{-D-Manp}$		$\beta\text{-D-Glcp}$		$\beta\text{-D-Manp}$	
	$\delta\ ^1\text{H}$	$\delta\ ^{13}\text{C}$	$\delta\ ^1\text{H}$	$\delta\ ^{13}\text{C}$	$\delta\ ^1\text{H}$	$\delta\ ^{13}\text{C}$	$\delta\ ^1\text{H}$	$\delta\ ^{13}\text{C}$	$\delta\ ^1\text{H}$	$\delta\ ^{13}\text{C}$	$\delta\ ^1\text{H}$	$\delta\ ^{13}\text{C}$
1	4.46	100.2, 100.6	4.56, 4.62	97.4–98.0	4.37, 4.48	100.4, 100.6	4.56, 4.64	97.6–98.0	4.44, 4.51	100.3, 100.5	4.59, 4.68	97.3–97.9
2	4.77	71.8	5.30	68.4, 68.7	4.79	72.0	5.32	68.5, 68.7	4.81	71.6, 71.8	5.34	68.3, 68.5, 68.6
3	5.06, 5.09	72.2	5.01	70.7, 70.8	5.08	72.4	5.01	71.0	5.10	72.0, 72.1	5.04	70.6, 70.8
4	3.75	74.5	3.75, 3.85	72.8, 73.9	3.77	74.5, 76.0	3.77, 3.87	72.9, 74.5	3.79	74.4, 75.9	3.79, 3.89	72.8, 73.8
5	3.51	72.9	3.51	72.9	3.52	72.9	3.52	72.9	3.55	72.8, 73.1	3.55	72.8, 73.1
6	4.15–4.34	62.3, 62.8	4.15–4.34	62.3, 62.8	4.03–4.34	62.4, 62.7	4.03–4.34	62.4, 62.7	4.07–4.38	62.2, 62.6	4.07–4.38	62.2, 62.6

**Fig. 5.** NMR ^1H spectra (in CDCl_3) of polysaccharide F2-H1-PI in per-propionylated form.**Fig. 6.** (A) NMR 2D HMBC (^1H – ^{13}C) spectra (in CDCl_3) of *per*-propionylated polysaccharide F2-H1-PI. (with M for D-Manp). (B) Molecular structure of glucomannan polysaccharide from the pulp of the amazonian palm fruit *Acrocomia aculeata* (F2-H1-PI) (with M for D-Manp and G for D-Glcp).

Cyrtopodium andersonii, *Cyrtopodium cardiochilum*, *Dendrobium nobile*, *Dendrobium devonianum*, He et al. (2022) *Eremurus iae*, *Eremurus zangezurus* and *Lilium davidii*, contain O-acetylglucomannans. However, these glucomannans present major structural differences (presence or absence of branching and position of acetyl group) both between themselves and between them and our polysaccharide F2-H1-PI. Indeed, some of them had a linear structure but possessed acetyl group in the C2 position of both D-Manp and D-Glcp residues (*Cyrtopodium cardiochilum*), or in the C2 and/or C3 position of D-Manp residues (*Bletilla formosana*, *Dendrobium nobile*, *Dendrobium devonianum*, *Eremurus iae* and *Eremurus zangezurus*), or in the C2, C3 and C6 position at the same time, or C2 & C3 at the same time, or C2 or C3 of D-Manp and D-Glcp residues (*Aloe plicatilis*). Others had linear structures but with mixtures of β -(1→4) and β -(1→3) osidic bonds between D-Glcp and D-Manp residues and included acetyl group in the C2 or C3 or C6 or C2 and C3 or C2 and C6 or C3 and C6 positions (*Aloe barbadensis*). Additional polysaccharides were found to have a branched structure in the C2, C3 and C6 or C2 and/or C3 positions and acetyl groups in the C2 or C3 position of D-Manp residues (*Cyrtopodium andersonii* and *Lilium davidii*). (Barreto & Parente, 2006; Gu et al., 2022; Hui et al., 2019; Parente et al., 2014; Paulsen et al., 1978; Shi et al., 2018; Smirnova et al., 2001; Zhang et al., 2020). Clearly, our polysaccharide has an original structure. Its D-Manp/D-Glcp ratio (3:1) obtained by GC-FID was also verified by NMR investigations by integrating an isolated proton (H2) from each residue (Fig. 2). This method gives a precise ratio of 3.6:1, compared with 1.5:1 for commercial konjac glucomannan (Supplementary Material, S1).

3.5. Comparison of the characteristics of fractions F2 obtained according to the harvesting period (H1 and H2) and the extraction process (PI, PII-1 and PII-2)

Comparing the structural characteristics of F2 (from the same extraction process, PI) over the two harvest periods H1 and H2, the degree of acetylation of the polysaccharides F2-H1-PI (13–14 %) (Fig. 5) and F2-H2-PI (12–13 %) remains virtually unchanged. Also, the determination of the D-Manp/D-Glcp ratio by NMR did not reveal any significant variation between F2-H1-PI (3.6:1) and F2-H2-PI (3.2:1) (Supplementary Material, S2). Nevertheless, a highly significant variation in molecular weight (Mw) was observed. Within 2 weeks, we go from polysaccharides (F2-H1-PI, Mw = 8.2×10^4 Da) to oligosaccharides (F2-H2-PI, Mw = 1.1×10^3 Da) (Table 5). This important variation in Mw could be associated with the fruit stage of maturity and even with the palm environmental conditions. Indeed, since glycolysis within the pulp is linked to lipogenesis (Montoya et al., 2016), we believe that depending on the needs of the fruit, its maturity stage and/or the palm environmental conditions, the starting polysaccharide is hydrolyzed, leading to the production of carbohydrates with a low Mw.

Optimization of the extraction process on samples from the second harvest period (H2) led to the exclusive isolation of oligosaccharides F2-H2-PII-1 (D_{Ac} = 13–14 %, D-Manp/D-Glcp ratio of 3.0:1, Mw = 2.4×10^3 Da) and F2-H2-PII-2 (D_{Ac} = 13–14 %, D-Manp/D-Glcp ratio 3.2:1, Mw = 1.2×10^3 Da) (Table 5) (Supplementary Material, S2) with

structural features virtually unchanged from F2-H2-PI (D_{Ac} = 12–13 %, D-Manp/D-Glcp ratio 3.2:1, Mw = 1.1×10^3 Da). The optimized versions of the extraction process therefore do not alter the characteristics of the glucomannans in the fruit pulp and our glucomannans had a low Mw compared to commercial konjac glucomannan (KGM, Mw = 7.6×10^5) (Table 5).

3.6. Thermal studies

The thermal stability of commercial konjac glucomannan (KGM) and oligo- and polysaccharide F2 were analyzed as shown in Fig. 8A and B. Analysis of the thermograms (Fig. 8A) revealed that all samples had similar decomposition patterns. However, the derived spectra (Fig. 8B) reported some differences. Three zones of maximum peaks were observed for all samples: a first zone between 51 and 121 °C, a second zone between 153 and 216 °C and a third zone between 261 and 320 °C (Fig. 8B). The first zone of peaks was attributed to the evaporation of water molecules weakly bound to the samples, the second one to water molecules strongly bound to the samples and the third one to the pyrolysis of organic matter (Kurt & Kahyaoglu, 2017; Neto et al., 2005; Nokhodchi et al., 1996; Xiao et al., 2000). Thus, we note that samples F2-H1-PI, F2-H2-PI and F2-H2-PII-2 contain many water molecules strongly bound to their structure, unlike F2-H2-PII-1, which contains very few. As for KGM, no peak is observed in the region under consideration (153 to 216 °C), but we believe that its interaction with water molecules is much stronger, so that evaporation must have taken place at a much higher temperature (>244 °C). This is evidenced by the non-Gaussian shape of the peak at 320 °C and the presence of two overlapping peaks (Fig. 8B). To support this result, we suggest that glucomannan oligo- and polysaccharides exhibit a certain 3D organization depending on molecular weight, which may or may not favor strong interactions with water. Their thermal stability was 320, 294, 261, 295 and 299 °C for KGM, F2-H1-PI, F2-H2-PI, F2-H2-PII-1 and F2-H2-PII-2 respectively. By processing the information obtained from these results, we were able to determine the humidity, organic and inorganic content of the samples analyzed (Table 6). These observations are in line with those reported in the literature. Indeed, large hydration layers are often observed on polysaccharides with hydrophobic groups (e.g., acetyl group) where the evaporation of these water layers can vary considerably and reach around 200 °C (Zohuriaan & Shokrolahi, 2004).

3.7. Rheological properties

Generally, Konjac glucomannans exhibit exceptional rheological properties associated with their high molecular weights (Onishi et al., 2005; Zhou et al., 2022). Our oligo- and polysaccharides F2 having a rather low average molecular weight, it was not expected to present a viscosity in water that could challenge the konjac polysaccharide. Thus, the rheological studies carried out from oligo- and polysaccharides F2 (1%, w/v in water) revealed that the latter only had a maximum viscosity of 11.7 mPa.s (at 20 °C) (F2-H1-PI). Indeed, a viscosity much lower than that of commercial konjac glucomannan (1430 mPa.s) was obtained in the same conditions (Table 5). However, as reported by Liu et al. (2015), the mannose richness and the very low viscosity of F2 due to their low molecular weight could confer biological properties (e.g.,

Table 5

Molecular weights (Da) of glucomannan carbohydrates (systematic errors: ± 10 %) and rheological studies at 20 °C of carbohydrate (1 % w/v in water).

Carbohydrate	KGM	F2-H1-PI	F2-H2-PI	F2-H2-PII-1	F2-H2-PII-2
Mw (Da)	7.6×10^5	8.2×10^4	1.1×10^3	2.4×10^3	1.2×10^3
Mn (Da)	5.8×10^5	2.2×10^4	0.6×10^3	0.9×10^3	0.7×10^3
Dispersity (Mw/Mn)	1.3	3.7	1.6	2.7	1.7
Viscosity (mPa.s)	1430 $\pm$ 1	11 $\pm$ 1	5 $\pm$ 0	3 $\pm$ 0	3 $\pm$ 0

Table 6

Humidity, organic and inorganic carbohydrate content (% mass) of commercial KGM and oligo- and polysaccharides F2.

Carbohydrate	KGM	F2-H1-PI	F2-H2-PI	F2-H2-PII-1	F2-H2-PII-2
Humidity (%)	10	9	10	15	9
Organic matter (%)	69	68	68	62	67
Inorganic matter (%)	21	23	22	23	24
Total (%)	100	100	100	100	100

antioxidant activities, osteoprotective effects, cryoprotectant, prebiotic activity or immunomodulatory effects) (Huang et al., 2018; Jiang et al., 2018; Liu et al., 2015; Walayat et al., 2022).

3.8. Emulsifying properties

Glucomannans F2 were evaluated for their emulsifying properties in olive oil-in-water (25/75, v/v) (O/W) and water-in-olive oil (25/75, v/v) (W/O) systems. Firstly, given the hydrophilic nature of polysaccharides, we would expect to obtain stable O/W emulsions, as reported by Dickinson (2003). Indeed, the hydrophilic/lipophilic balance (HLB) of our glucomannans is certainly high, since they possess fewer lipophilic groups (acetyl group 12–14 %), which should make them suitable for stabilizing emulsion systems with a predominantly aqueous phase (Dickinson, 2003). Yet, no emulsion stability was observed for any samples, except for konjac glucomannan, for which the emulsion remained stable for 15 min (Table 6). In the case of the W/O system, the formation of emulsions was observed for all samples.

Emulsions obtained from oligosaccharides (F2-H2-PI, F2-H2-P-II-1 and F2-H2-P-II-2, stability >1 h30) showed greater stability than polysaccharides (KGM and F2-H1-PI, stability <1 h) (Table 6). The superior stability of oligosaccharides may be associated with their low Mw. Indeed, according to Dickinson (2003) a low Mw enables oligosaccharides to solubilize efficiently in the aqueous phase and thus favors their effective position at the water-oil interface, unlike polysaccharides whose complexity could prevent a total solubilization and a positioning at the water-oil interface.

In contrast, oligosaccharide F2-H2-P-II-1 ($M_w = 2.4 \times 10^3$ Da) showed much greater stability (> 6 months) than the other two oligosaccharides F2-H2-PI ($M_w = 1.1 \times 10^3$ Da, stability <3 h) and F2-H2-P-II-2 ($M_w = 1.2 \times 10^3$ Da, stability <3 h). This difference in stability is consistent with the TGA observations (Fig. 7B). Indeed, Biely (2012) reported that acetylated polysaccharides possess a significant hydration layer due to the methyl of their acetyl group. This is particularly pronounced in the F2-H2-PI and F2-H2-P-II-2 oligosaccharide samples, preventing them from positioning themselves effectively at the W/O interface, which explains their difference in stability. To break these hydration layers, we proceeded as Enomoto-Rogers et al. (2013) by heating the samples dissolved in the aqueous phase at 65 °C for 1 h. The aqueous solutions were then left to cool for 20 min at room temperature before new W/O emulsions were formed.

This operation enabled the oligosaccharides F2-H2-PI and F2-H2-P-II-2 to position themselves easily at the water-oil interface, arranging stability identical to F2-H2-P-II-1 for the W/O system (stability >6 months) (Table 7, Fig. 8A). These results were confirmed by the low surface tensions observed for oligosaccharides (3–10 mN/m) in contrast to polysaccharides (28–31 mN/m) (Table 7). Morphological

Table 7

Stability of water-in-olive oil (25/75, v/v) and olive oil-in-water (25/75, v/v) emulsions in the presence of 1 % (w/v) carbohydrate dissolved in the aqueous phase, before and after heating the aqueous phase. (0): No emulsion, (–): stability ≤1 h, (+): stability between ≥1 h 30 ≤ 3 h, (+++): stability >6 months and (n.d.): not determined. Surface tension (mN/m) of water-in-olive oil emulsions (25/75, v/v) before and after heating.

Carbohydrate	Emulsion stability			Emulsion surface tension (mN/m)	
	W/O (25/75, v/v) before heating	W/O (25/75, v/v) after heating	O/W (25/75, v/v)	W/O (25/75, v/v) before heating	W/O (25/75, v/v) after heating
Control	–	–	0	31 ± 0	31 ± 0
KGM	–	–	–	31 ± 0	29 ± 0
F2-H1-PI	–	–	0	30 ± 0	28 ± 0
F2-H2-PI	+	+++	0	10 ± 1	5 ± 1
F2-H2-P-II-1	+++	n.d.	0	n.d.	n.d.
F2-H2-P-II-2	+	+++	0	7 ± 2	3 ± 1

characterization of F2-H2-PI and F2-H2-P-II-2 W/O emulsions under the light microscope (Fig. 8B) showed that only simple emulsions were formed, with spherical droplets having diameters of <200 µm.

Note that this rather surprising behavior of these acetylated glucomannan to stabilize W/O emulsion systems while having a high HLB was also reported by Bhattarai et al. (2019) from low molecular weight ($M_w = 12 \times 10^3$ Da) acetylated galactoglucomannan at 1 % in a W/O system (water/rapeseed oil, mass ratio 0.4). In this case, the stability was <3 months (or 9 weeks). According to the authors, this was due to the formation of a Pickering emulsion in which these low-Mw polysaccharides organized themselves into solid aggregates to adsorb at the interface.

Plant-derived polysaccharides possess low toxicity (Yang et al., 2015), good food safety, excellent stability and are preferred over other emulsifiers for food applications (Shao et al., 2020). As a result, glucomannan oligosaccharides from *Acrocomia aculeata* fruit pulp can be used as emulsifiers in W/O systems, which are highly sought after by the cosmetic, dermatological and food industries (Colucci et al., 2020; Zhu et al., 2019). However, further toxicological evaluation of these products could be carried out beforehand, bearing in mind that they are derived from fruits that are already edible on the South American market (Coimbra & Jorge, 2011a, 2011b) and that toxicity studies have revealed that pulp oil is not toxic (Traesel et al., 2014).

4. Conclusion

New glucomannan oligosaccharides and polysaccharides were extracted aqueously from the fruit pulp of the amazonian palm

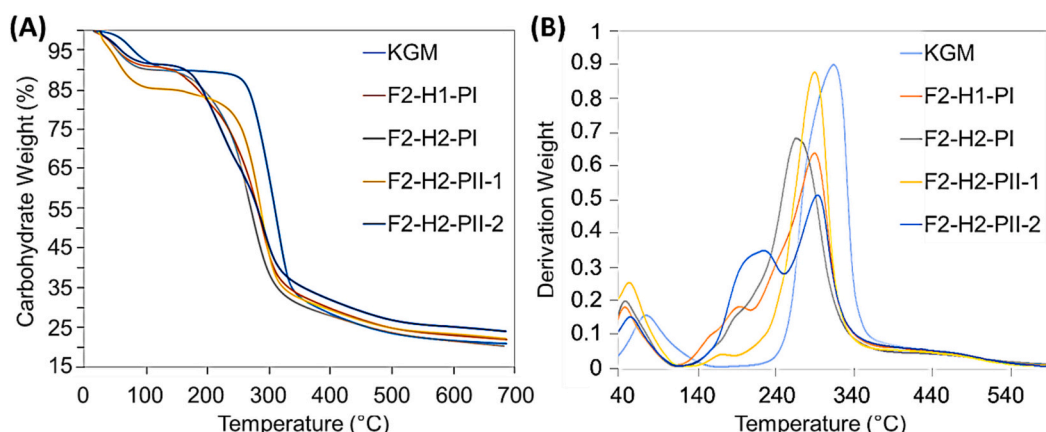


Fig. 7. TGA thermogram of glucomannan carbohydrates. (A) weight (%) by temperature (°C) (B) derivation weight by temperature.

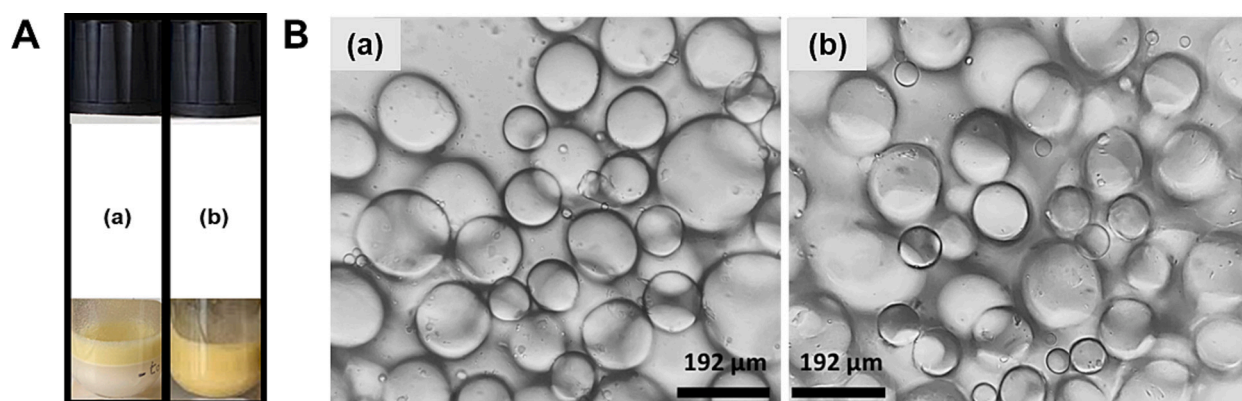


Fig. 8. A: Emulsions image. (a) Olive oil-in-water (O/W) (25/75, v/v) and (b) water-in-oil (W/O) (25/75, v/v) emulsions of F2-H2-PII-2 at 1 % (w/v) after heating. B: Optical microscopy of water-in-oil (W/O) emulsions (a) F2-H2-PI 1 % (w/v) (b) F2-H2-PII-2 1 % (w/v) after heating. Bar = 192 μ m, 52 $\times$ magnification.

Acrocomia aculeata. Their structural characterization by NMR spectroscopy in their triacetate form revealed them to be linear glucomannans whose residues are randomly linked together by β -(1 $\rightarrow$ 4) osidic bonds in a 3:1 general D-Manp/D-Glcp ratio. The carbohydrates exceptionally displayed the acetyl group in C2 position (D-Manp) with a substitution degree between 12 and 14 %. Due to their low molecular weight compared to konjac glucomannan in general, these new D-Manp-rich carbohydrates do not alter the rheological properties of water, which could enable their biological properties to be studied. Their 3-fold higher degree of acetylation compared with konjac glucomannan, combined with their low Mw, have enabled oligosaccharides to have very promising emulsifying properties (stability >6 months, to date). These oligosaccharides could be used for applications in cosmetics, dermatology, or food industry. The results of this study open a new way of adding value to *Acrocomia aculeata* fruit pulp. Further structural characterizations of the water-insoluble fractions also isolated using this simple extraction process, will be next investigated in order to have a complete knowledge of the carbohydrates present in the pulp of this fruit.

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CRediT authorship contribution statement

Wilfried Denagbe: Investigation, Writing – Original draft, Visualisation. **Rudy Covis:** Conceptualization, Investigation, Writing – Reviewing and Editing, Supervision. **Jean-Paul Guegan:** Investigation, Validation. **Jean-Charles Robinson:** Supervision, Funding acquisition. **Didier Bereau:** Writing – Review and Editing, Supervision, Funding acquisition. **Thierry Benvegnu:** Conceptualization, Investigation, Validation, Writing – Original draft, Writing – Review and Editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carbpol.2023.121510>.

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