Macromolecular and Viscoelastic Properties of Low Methoxy Pectin from Cashew Apple Pomace

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Abstract The study on the yield and some structural and rheological properties of acid-extracted pectins from cashew (Anacardium occidentale L.) apple pomace (AOP) showed that about 25% of pectins could be produced under optimized acid conditions. AOP was mainly composed of galacturonic acid (84.5%) with some neutral sugars of which rhamnose (1.9%), arabinose (3.8%), and galactose (5.5%) were the main constituents. The degree of methylation (DM) of AOP was 41%, indicating extraction of low methoxy pectin (LMP). The intrinsic viscosity and average-molecular weight of AOP were 398 mL/g and 142 kDa, respectively. AOP exhibited a calcium-mediated gelling strength of 128 which appeared to be higher than that (104) of commercial citrus pectin with similar DM. AOP yielded firmer Ca²⁺-mediated LMP gels, thereby showing a high power of gelation. Viscoelastic analyses also substantiated good gelling behaviour of the produced AOP. Therefore, cashew apple pomace, which is an industrial cell wall residue from the production of cashew nut, appears to be a potentially viable source of production of marketable LMP without the need for enzymatic and/or chemical demethylation.

Keywords Cashew Apple Pomace, Low Methoxy Pectin, Extraction, Macromolecular Characteristics, Rheological Properties

1. Introduction

Among the three groups of polysaccharides present in the primary cell wall of higher plants, pectic substances are undoubtedly the most diversified, including at least 8 galacturonic acid-based polysaccharide types, of which homogalacturonan (RG-I) and type one rhamnogalacturonan (RG-I) are the two commonly purified.

Pectins are generally viewed as gelling polysaccharides, from miscellaneous plant byproducts, mainly composed of α-D-galactopyranosyluronic acid (α-D-GalpA) units (>65%) with some neutral sugars, and partially methyl-esterified at C-6 position (degree of methylation >10–95%). Various structural studies, with highly purified enzyme preparations, revealed that the different glycosyl units are likely concentrated in different regions, giving rise, generally, to two main building block copolymers, namely HG and RG-I. HG is an unbranched 1,4-α-D-GalpA polymer which is partially methyl-esterified at C-6 position and sometimes acetyl-esterified at O-2 and/or O-3 positions. RG-I is a →4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→jn polymer partly branched with diverse neutral sugar side chains. Common side chains of RG-I are 1,5-α-L-arabinan, 1,4-β-D-galactan, and arabinogalactan-I. They may, however, be ramified with more complex polysaccharide moieties, such as arabinogalactan-II and the rather scarce galactoarababin [1-2].

Commercial low methoxy pectins (LMP) are so far produced from high methoxy pectins (HMP) from citrus peel and apple pomace in western countries [3,4] by chemical or enzymatic procedures which may require highly purified pectin methyltransferases. This consequently adds to the final cost of commercial LMP products. However, the worldwide increasing demand requires the search for other sources which can yield pectins with good gelling characteristics.

Cashew plant (A. occidentale, Anacardiaceae) is mainly cultivated for its fruit nut, which is consumed as food and as ingredient in various cosmetic and pharmaceutical products. From an annual production of 300,000–350,000 tons over the 2008/2012 period [5], Côte d’Ivoire has become the second top producer of cashew nuts in the world, after India which has produced about 400,000 tons. Once the valuable nut has been extracted, the apple, which represents approximately 60–90% of the fruit fresh weight, is traditionally abandoned in plantations to rot, with no added value, because of its astringent taste due to high anacardic acid content [6]. This poses a serious problem of plant disease inoculum. It is, therefore, important to find a way of adding value to it thereby increasing the farmer’s income and at the same time solving the ecological problem it causes. This study reports on some macromolecular and rheological properties of acid-extracted pectins from cashew apple...
2. Materials and Methods

2.1. Alcohol Insoluble Material Preparation

Fresh cashew apple fruits were donated by factory-made producers and sellers of cashew nut (CAJOUCI, Korhogo, Côte d’Ivoire). Fruits were minced in a Kenwood mincer and immediately soaked in 3 volumes of boiling 80% (v/v) ethanol for 25 min and cooled to room temperature. Alcohol-insoluble material (AIM) was continuously washed with 70% (v/v) ethanol to remove free sugars, pigments, and other impurities as much as possible. The residue was then dried by solvent exchange (95% ethanol and acetone), placed in a fume hood for 5 h for the evaporation of residual acetone and oven-dried for 15–16 h. Dried AIM was ground in a hammer mill (Model 912, Winona Attrition Mill Co., Winona, MN) to pass through a 12 mm size sieve and was kept under moisture-free conditions until use.

2.2. Pectin Production

Pectins were extracted from AIM by water acidified with 1 N HNO₃ to three different extractant strengths (pH 1.0, 1.5, and 2.0), while the other extraction parameters, namely, solid to liquid extractant ratio, temperature, and time, were invariably kept to 1:25 (w/v), 75 °C, and 90 min, respectively. Two successive extractions were performed before discarding any remaining insoluble cell wall residue. At the end of each extraction, the slurry was clarified and the pectin extract was rapidly brought to pH 4 for stability. The first and second extracts were pooled and treated with 0.5 M imidazole buffer (pH 7) and extensively dialyzed against distilled water in 12000 molecular weight cut-off tubing to readily and completely remove Ca²⁺-imidazole complexes. The retentate was then concentrated to the desired solution quantity and precipitated in 3 volumes of 95% ethanol, washed two-times with 70% (v/v) ethanol, followed by 95% ethanol and acetone, and kept for a while under a fume extractor (for residual acetone evaporation), and finally oven-dried at 40–45 °C for 15–16 h and weighed. The extraction of pectins was carried out in three independent runs for each selected pH value. Dried pectin flakes were finely ground to pass through 60-mesh (US) sieves and stored at room temperature under airless and moisture-free conditions pending analysis.

A purified (sucrose-free) commercial citrus low methoxy pectin from Genupectin LM12CG (PLM12 CG; DM = 34%) (Hercules, Copenhagen, Denmark) was used for comparison purposes.

2.3. Characterization of Pectins

The pectin samples were first treated with a mixture of 1% (v/v) HCl/60% (v/v) ethanol (three times), and insolubles were exhaustively washed with 60% (v/v) ethanol to totally remove free sugars. This treatment indeed aimed at simultaneously removing free sugars and salts and converting all the carboxyl groups of pectin macromolecules to the free acid (-COOH) form, prior to correctly titrating them by 1 N NaOH solution. Pectins were analyzed for their glycosyl residue composition, esterification degree, molecular weight, and gel-forming capability.

2.3.1. Proximate Analyses

The protein content of the pectin extracts was colorimetrically determined at 750 nm by a Folin-phenol reagent assay [7] using bovin serum albumin standard. Calcium element was analyzed by flame atomic absorption spectrometry at 422.7 nm using an Aanalyst 300 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT).

2.3.2. Analyses of the Glycosyl Residue Composition

To quantify the monosaccharide constituents of the different samples, AIM was hydrolyzed with 1 mol.L⁻¹ H₂SO₄ (100 °C, 3 h) after pretreatment with 12 mol.L⁻¹ H₂SO₄ (23 °C, 1 h) and purified pectins were directly hydrolyzed with 1 mol.L⁻¹ H₂SO₄ (100 °C, 3 h) as previously reported [8].

The GalA content of AIM and purified pectins was colorimetrically quantified at 525 nm by a modified sulfamate-meta-hydroxydiphenyl assay using monoGalA standard [9].

The neutral sugars, which were liberated from the purified pectins, especially galactose, arabinose, and rhamnose [10] were spectrophotometrically quantified at 340 nm using Megazyme assay kits (Megazyme International Ireland Ldt., Bray, Co. Wicklow, Ireland). The neutral sugars assays were based on the quantitative oxidation of galactose/arabinose and rhamnose to corresponding lactonic derivatives (D-galactono-(1,4)-lactone for α-L-arabinose and β-D-galactose and L-rhamno-(1,4)-lactone for α-L-rhamnose) in the presence of corresponding dehydrogenases [β-galactose dehydrogenase plus galactose mutarotase for α-L-arabinose and β-D-galactose, and L-rhamnose dehydrogenase for α-L-rhamnose] and the coenzyme NAD⁺, which is stoichiometrically reduced to NADH with absorbance maximum at 340 nm. D-Gal was quantitatively differentiated from D-Gal by reading absorbances at different reaction times, namely after 6 min- and 12 min-reaction at room temperature, respectively. L-rhamnose was quantitatively determined after 1 h-reaction at room temperature.

The different types of neutral sugar side chains of the RG-I block copolymers were distinguished by treating AOP with purified enzymatic solutions of α-L-arabinanase, α-L-arabinosidase, β-D-galactanase, and β-D-galactosidase individually and in combination. The enzymatically-treated pectin samples were extensively dialyzed against 0.05 M acetate buffer (pH 4.8) in 12000 molecular weight cut-off tubing, precipitated in 3 volumes of 95% ethanol, washed
three times with 70% ethanol, followed by dry acetone, and finally oven-dried at 40 °C for 15–16 h. The dried pectic oligosaccharide materials were milled to pass through 60-mesh (# 0.25 mm) size sifters, canned in plastic containers, and kept at room temperature under airless and moisture-free conditions pending analysis.

The overall esterification degree of pectic samples was potentiometrically determined as previously described [11]. The acetylation degree (DAc) was colorimetrically determined as previously described [11]. The acetylesterification degree (DAc) was colorimetrically determined as previously described [11]. The intrinsic viscosities ([η]) of the samples were finally estimated by plotting the reduced viscosities ([η]/C) versus concentration (C) and extrapolating to zero polysaccharide concentration. For each sample analyzed, experiments were carried out five times and the average values were taken for plotting.

\[ \eta_{sp} = (\eta - \eta_s)/\eta_s \]  

The molecular weight of the pectins was analyzed by gel-filtration chromatography on a high resolution Superdex-200 HR 10/30 column (Amersham Biosciences Corp., NJ). The same solvent specified above (90 mM sodium chloride + 10 mM sodium fluoride + 1 mM Na2EDTA at pH 6.5) was used as eluent and the polysaccharide concentrations in the eluate were monitored using a differential refractometer or refractive index detector (Waters Corp., Milford, MA). A molecular weight kit of pullulan standards (\( M_w \sim 6.0, 10.0, 21.7, 48.8, 113.0, 210.0, 393.0, \) and 805.0 kDa; \( M_w / M_n \sim 1.0–1.2; \) American Polymer Standards Corp., Mentor, OH) and purified homogenous HG standards (\( M_w \sim 60 \) and 100 kDa, \( M_w / M_n \sim 1.0–1.2 \)) [13], with known intrinsic viscosity ([η]) and \( M_w \) values, were used for calibration. To better estimate the \( M_w \) of the pectins, the so-called universal calibration technique was used by plotting log ([η], \( M_w \)) versus the elution volume of standards. Analyses were done in triplicates.

2.4. Gelling and Viscoelastic Properties

The gelling capability of pectins was evaluated according to the “adapted SAG-method” of Food Chemical Codex (FCC) to LMP. The final composition of gels was 1.0% pectin material for AOP (and PLM12CG and 1.4% for LM12CG in order to have similar GalA contents) and 30% sucrose at pH 3.0 and 23.8–31.6 mg Ca2+/g pectin. The calcium effect was excluded by using the stoichiometric ratio of binding (\( R = 2 ([\text{Ca}^{2+}]/[\text{COO}^-]) \), which described the relationship between the molar concentrations of Ca2+ ions and ionisable carboxyl groups of polygalacturionate on the basis of the pectin de-methylesterification degree (100 – DM) [11]. Pectin dispersions (1.0–1.4%), containing calcium ions which concentration was varied according to the R value and 30% sucrose, were prepared as follows:

Briefly, mixtures of weighed amounts of pectin powder and a half amount of sucrose were dissolved in 100 mM sodium chloride solution, under gentle stirring, at room temperature for 15–16 h. The pH of the solutions obtained was fined-tuned to 3.0 using few drops of 0.25 M citric acid or sodium citrate buffer if necessary. The mixtures were then heated to boiling point under stirring and an appropriate amount of a pre-heated calcium chloride dihydrate solution, prepared in 100 mM sodium chloride, was slowly added under vigorous stirring until the desired calcium content was reached. To prevent pre-gelation, when adding the calcium ion solution to mixtures containing sucrose, the other half amount of sucrose was dissolved in the amount of CaCl2·2H2O solution to be added. The pH of the mixtures was controlled and kept constant during gelation.

To study the viscoelastic properties, a dynamic small amplitude oscillatory shear (SAOS) test was performed in a Bohlin CVO50 controlled-stress rheometer (Bohlin Instruments Ltd., London, UK) using a cone-plate geometry (40 mm plate diameter, 4° cone angle, 150 µm gap). A frequency sweep carried out on selected samples showed non-significant frequency-dependence within the selected interval of frequency (0.1-10 Hz). Therefore, a single frequency (1 Hz) experiment was performed throughout testing. The viscoelastic behavior of pectin dispersion was studied on a temperature sweep during a cooling scan from 95 to 5°C at the rate of 3°C/min. Dynamic measurements were performed for a 1% strain amplitude. First of all, an
amplitude sweep was performed to assure that the selected strain amplitude matches the linear viscoelastic region of pectin gels. The prepared hot pectin dispersion was directly applied to a pre-heated rheometer at 95 °C after the desired weight was reached.

To evaluate the gelling strength, some prepared gels were molded and allowed to cool to room temperature, and were finally rested for 24 h at 4 °C. The strength (or firmness) of the molded gels were measured with the help of a Ridgelimeter (Bulmer Food Co., UK).

A thin layer of low viscosity paraffin oil was used to cover the exposed surface of the gels in order to minimize weight loss by water evaporation.

Experiments were carried out in three times for each pectin sample analyzed.

2.5. Statistical Analysis

All the data were statistically appraised by a single-factor analysis of variance (ANOVA), followed by the Bonferroni’s posthoc test for multiple comparisons, whenever applicable, using a GraphPad Prism V.3 software (GraphPad software Inc., San Diego, CA). Means of different treatments were considered to be significantly different at \( P\)-value <0.05.

3. Results and Discussion

3.1. The Yield of Pectins Extracted from Cashew Apple Pomace

The yield of AOP extracted under different acid solvent strengths ranged from 10.7 to 25.3% (Table 1). The pectin yield was significantly affected by the extractant strength (\( P <0.05\)). The highest (25.3%) and lowest (10.7%) yields were recorded at pH 1.5 and pH 1.0, respectively. This indicated that the best conditions for extracting high amounts of pectins were those using pH 1.5 and that, at lower pH (1.0), part of the solubilized pectin polymers were likely degraded into oligomers that were removed by the subsequent process of purification. Nevertheless, the pectin yield was >10% whatever the extraction conditions used, suggesting that cashew apple pomace was a potentially viable source of marketable pectins.

| pH   | Yield (g/100 dried weight) |
|------|---------------------------|
| 1.0  | 10.7 ± 0.9a                |
| 1.5  | 25.3 ± 4.4b                |
| 2.0  | 16.4 ± 2.1c                |

Table 1. Effect of the acid solvent strength of the extraction yield of A. occidentale pectins

| AOP  | pH 1.5  | pH 1.5  | pH 1.0  |
|------|---------|---------|---------|
| Yield (g/100 dried weight) | 10.7 ± 0.9a | 25.3 ± 4.4b | 16.4 ± 2.1c |

Data are expressed as mean ± SD (n = 3). Mean values in the same line with different letters are significantly different (\( P <0.05\)). AOP: A. occidentale pectins

The yield of pectin obtained, in this study, was rather lower than to the one (38.7%) reported by extraction of pectins from cashew apple with sodium hexametaphosphate (SHMP), followed by precipitation with acidified acetone [6]. It should, however, be underlined that the SHMP-extracted cashew apple pectin has been found to contain high amounts of impurities, mainly from the extracting agent (SHMP).

3.2. Chemical Composition and Structural Features of Extracted Pectins

3.2.1. Chemical Composition

The pectin extract with the highest yield (25.3%) was further analyzed for its glycosyl residue composition (Table 2). Galacturonic acid was the major monosaccharide (84.5%), followed by galactose (5.5%), arabinose (3.8%), and rhamnose (1.9%), suggesting that the pectin structure might mainly encompass HG regions with some RG-I regions bearing arabinose- and/or galactose-containing side chains.

|          | AOP* | PLM12CG |
|----------|------|---------|
| pH 1.5   |      | ----    |
| Galacturonic acid (% w/w) | 84.5 ± 4.2a | 75.8 ± 1.5b |
| Rhamnose (% w/w) | 1.9 ± 0.3a | 1.1 ± 0.1b |
| Arabinose (% w/w) | 3.8 ± 0.4a | 0.4 ± 0.1b |
| Galactose (% w/w) | 5.5 ± 0.3a | 3.9 ± 0.2b |
| Glucose (% w/w) | ND | 0.7 ± 0.1 |
| Rhamnose/Galacturonic acid molar ratio | 2.7:100 | 1.7:100 |
| DM       | 41 ± 7 | 31 ± 1  |
| DAc      | 3 ± 1  | Trace   |
| Protein  | 4.1 ± 0.5 |        |
| Calcium (µmol/g) | 55.6 ± 1.9a | 20.1 ± 1.1b |
| [η] (mL/g) | 398 ± 7 | ND      |
| \( M_w \) (kDa) | 142 ± 11 | ND      |
| Gel strength (*FCC) | 128 ± 1a | 104b    |

Data are expressed as mean ± SD (n = 3). Mean values in the same line with different letters are significantly different (\( P <0.05\)). AOP*: A. occidentale pectin [pH 1.5; 75 °C, 90 min, S/L: 1:25 (w/v)]; PLM12CG: Purified (sucrose-free) commercial citrus low methoxy pectin; ND: Not determined.

Small quantities of a proteinaceous fraction (4.1%) and elemental calcium (55.6 µmol/g) were also detected in the pectin isolates. The rather high yield of AOP obtained, coupled with its high galacturonic acid content, and enabled one to infer that cashew apple pomace could be a commercially viable source of pectins [6].

3.2.2. Enzymatic Probing of the AOP Neutral Sugar Side Chains
To identify the type(s) of neutral sugar-side chains of the RG-I regions of AOP, highly purified arabinan- and galactan-degrading enzymes were used for treatment. The results obtained are briefly shown in Table 3. Arabinanase was almost inactive, whilst arabinosidase removed more than 90% of the arabinose initially present in the pectic polysaccharide fraction. Furthermore, the combination of arabinanase and arabinosidase removed about 97% of the initial arabinose. This indicated that most arabinosyl residues were likely present as chain-terminating residues rather than as relatively long arabinan side chains.

**Table 3.** Changes in relative molar composition of the neutral sugar constituents of AOP after enzymatic degradation

| Relative molar composition (%) | Rha | Ara | Gal |
|-------------------------------|-----|-----|-----|
| Without enzymes               | 100.0 | 100.0 | 100.0 |
| Arabinanase                   | 99.8 ± 0.4 | 97.3 ± 0.2 | 98.9 ± 0.8 |
| Arabinosidase                 | 99.7 ± 0.2 | 95.1 ± 1.2 | 95.8 ± 0.6 |
| Arabinanase + Arabinosidase   | 98.4 ± 0.6 | 3.1 ± 0.7 | 94.2 ± 1.1 |
| Galactanase                   | 98.9 ± 0.7 | 11.6 ± 1.4 | 2.8 ± 0.9 |
| Galactosidase                 | 100.9 ± 0.1 | 98.9 ± 0.7 | 99.5 ± 0.4 |
| Galactanase + Galactosidase   | 98.4 ± 0.4 | 5.4 ± 1.3 | 1.0 ± 0.3 |

Data are expressed as mean ± SD (n = 3).

By contrast, galactanase was very effective on the pectic sample, removing about 88% of the initial arabinose and more than 97% of the initial galactose, whereas galactosidase was inhibited by the pectic material and the combination of the two enzymes further increased up to 95% and to 99% the removal of arabinose and galactose, respectively. This indicated that most galactosyl residues were included in relatively long galactan chains which were likely terminated by short arabinosyl residues. From all these results, it could be inferred that the RG-I regions of AOP carried mainly galactan and/or arabinogalactan side chains.

The relative proportion of HG to RG-I block copolymers, as judged by the molar ratio (2.7/100) of rhamnosyl to galacturonosyl residues, suggested that the HG building blocks were dominant over the RG-I ones in AOP and in PLM12CG. However, AOP generally appears to be slightly more branched with neutral sugars than PLM12CG.

### 3.3. Degree of Esterification

The DM of AOP was 41 (Table 2), indicating extraction of LMP. Such pectins with a low methylesterification level have also been extracted from some other pectin sources, such as olive fruit pomace [14], sunflower head residues [15], and yellow passion fruit rind [16] and are generally believed to result from nascent HMP following the activity of plant pectin methylesterases. The DAc was rather low in all the extracted pectins (<10%).

### 3.4. Macromolecular Features

The [η] and $\overline{M}_v$ of AOP were 398 mL/g and 142 kDa, respectively (Table 1), indicating isolation of pectin polymers with a high average-molecular weight. This value is in line with that (100–300 kDa) reported for LMP and HMP from citrus peels and apple pomace, the two main sources of commercial pectins [3,4].

### 3.5. Gelling Capability

The results of the gelling assays of AOP are given in Table 1. The strength (128 °FCC) of the gels prepared was slightly higher with AOP than with PLM12CG (104 °C), showing that highly gelling pectin product could be obtained from cashew apple pomace. The highly interesting gelling capability of AOP could be attributed to its substantially high GalA content (85%), with a greater number of nonesterified galacturonate block sequences, and $\overline{M}_v$ (142 kDa); two intrinsic factors known to significantly effect on Ca²⁺-mediated gelation of LMP [4]. These results showed that the LMP extracted from cashew apple pomace, under specified conditions, can fulfill the required gelling grade (>100) for marketing possibility. Moreover, AOP was likely to form firmer gels than commercial citrus LMP with similar DM.

### 3.6. Viscoelastic Properties

The viscoelastic properties the gels prepared with AOP and PLM12CG comparatively were studied by following the evolution of the viscous (or loss) modulus ($G''$) and the elastic (or storage) modulus ($G'$) as a function of the temperature of testing during a cooling scan. Figure 1 illustrates the results of the viscoelastic studies. The temperature dependence of the viscoelastic moduli ($G'$ and $G''$) showed that both moduli increased with decreasing temperature. For either AOP or PLM12CG gel, a crossover between the graphic traces of the viscous and elastic moduli occurred at a characteristic temperature, known as the “gel point” temperature ($T_g$). It can be observed that the magnitude of the elastic modulus ($G'$), related to the strength of the gel, was much higher than that of the viscous modulus above $T_g$ as the temperature was further decreased.

![Figure 1](image-url). Temperature dependence of the viscous $G''$ (open symbols) and elastic $G'$ (filled symbols) moduli of the gels prepared with pectins from cashew apple pomace (AOP, lozenge) and citrus peel (PLM12CG, circle).
Furthermore, the magnitude of the elastic modulus ($G'$) of the AOP gel appeared to be higher than that of PLM12CG. Indeed, around 5 °C of testing temperature, the values of $G'$ for AOP and PLM12CG gels were approximately 665 and 415 Pa, respectively. These results substantiated that the gelling capability of AOP was greater, compared with citrus LMP.

4. Conclusion

With a galacturonic acid content of approximately 35% on a dry weight basis, cashew apple pomace is a pectin-rich industrial byproduct. Under optimized acid extraction conditions, 25% of low methoxy pectin ($\text{degree of methylation } \approx 40\%$) with a high galacturonic acid content ($>80\%$) and good gelling capacity and viscoelastic properties can be produced without the need for base or enzymatic deesterification. Therefore, the cashew apple pomace appears to be a commercially viable raw material for possible production of pectins required for manufacturing low calorie gelling food products.

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