Macrothink Institute™

Journal of Food Studies
ISSN 2166-1073
2014, Vol. 3, No. 1

Cinnamon Apple Pectins: Structural and Rheological Properties

Veronique BESSON

Food Research and Technology Division, Cargill West Africa, Abidjan, Côte d'Ivoire

E-mail: vbesson2000@gmail.com

Beda Marcel YAPO (Corresponding author)

Subunit of Pedagogy in Biochemistry and Microbiology

Unit of Training and Research in Agroforestry, University of Jean Lorougnon Guédé

P.O. Box 150 Daloa, Côte d'Ivoire

E-mail: bmyapo@gmail.com

Grah Avit Maxwell BEUGRE

Subunit of Pedagogy in Biochemistry and Microbiology

Unit of Training and Research in Agroforestry, University of Jean Lorougnon Guédé

P.O. Box 150 Daloa, Côte d'Ivoire

E-mail: maxwellfrb@yahoo.fr

Kouassi KOFFI

Food Research and Technology Division, Cargill West Africa, Abidjan, Côte d'Ivoire

E-mail: kofalyx@yahoo.fr

Dago GNAKRI

Subunit of Pedagogy in Biochemistry and Microbiology

Unit of Training and Research in Agroforestry, University of Jean Lorougnon Guédé

P.O. Box 150 Daloa, Côte d'Ivoire

E-mail: gnakri_dago@yahoo.fr

Received: Dec. 7, 2013 Accepted: January 24, 2014 Published: December 1, 2014

doi:10.5296/jfs.v3i1.4646 URL: http://dx.doi.org/10.5296/jfs.v3i1.4646

Abstract

The structural and viscoelastic characteristics of pectins (CAP) extracted from cinnamon apple pomace, under different acid conditions, were investigated. The yield of pectins ranged from 2.8 to 10.9% and was significantly (P <0.05) affected by the solvent strength. The optimum yield (11.0%) was recorded at pH 1.2 as the other extraction parameters, viz. temperature, time, and solid to liquid ratio were kept constant to 75 °C, 90 min, and 1:25 (w/v), respectively. The main monosaccharide constituents of CAP were galacturonic acid, arabinose, galactose, and rhamnose; thus suggesting that the pectic polymers were built of linear homogalacturonan and non-linear rhamnogalacturonan block copolymers branched with arabinose- and/or galactose-containing side chains. Enzymatic degradation of CAP indicated that the neutral sugar side chains were mainly galactoarabinans with some arabinogalactans. However, the β-Galactosyl Yariv assay, specific to 1,3-β-galactan, was found to be negative, indicating that detected arabinogalactans might “exclusively” be of type I. The degree of methylesterification was in the range of 43–68%, showing extraction of both low (HMP) and high methoxy (LMP) pectins. The trends of sugar-acid-mediated gelation of the purified CAP were significantly different from one another (P <0.05), as judged by the conventional SAG method (gel strength) and by small amplitude oscillatory shear tests (viscoelastic properties). Depending on the extraction conditions, potentially good LMP and HMP can be produced from cinnamon apple pomace.

Keywords: Cinnamon apple pomace; Pectins; Structural features; Viscoelastic properties

Abbreviations Used: AG-II: arabinogalactan-II; AG-I: arabinogalactan-I; **Ara**: Arabinose; **CAP:** Cinnamon Apple pomace Pectins; **CWM:** Cell Wall Material; **DAc**: Degree of Acetyl-esterification; **DM**: Degree of Methyl-esterification (or methoxylation); **Gal**: Galactose; **GalA**: Galacturonic Acid; **HG:** Homogalacturonan; **HMP:** High Methoxy Pectins; **LMP:** Low Methoxy Pectins; LVR: Linear Viscoelastic Region; **RG-I:** Rhamnogalacturonan-I; **Rha**: Rhamnose; **SAG**: Sugar-Acid-Gels; **SAOS**: Small Amplitude Oscillatory Shear

1. Introduction

The cell wall of all higher plants encompasses three classes of polysaccharides, namely cellulose, cross-linking heteroglycans (ex. hemicelluloses) and pectic substances. The latter are extremely diversified that they are usually viewed as a heterogeneous family of polysaccharides with various functional properties. To date, no less than 8 polymer types may be included in this family. However, only two out of the eight, viz. homogalacturonan (HG) and rhamnogalacturonan-I (RG-I), are commonly present in pectin extracts from various cell wall materials (Yapo, 2011). HG is a linear polymer of 1,4-linked-α-D-Gal*p*A residues, which are partially methyl-esterified at *C*-6 position and sometimes acetyl-esterified at *O*-2 and/or *O*-3 positions. The degree of methyl-esterification (DM), also known as degree of methoxylation (or degree of methylation), is the number of (carboxyl groups of) α-D-Gal*p*A residues out of 100 residues, esterified with methyl alcohol in pectin chain. The degree of acetylation (DAc) is the number of (hydroxyl groups of) α-D-Gal*p*A residues out of 100 residues, esterified with acetic acid in pectin chain, assuming one acetylation per each esterified residue. RG-I is a [1,4)-α-D-Gal*p*A-1,2-α-L-Rha*p*-(1,4]n polymer with the α-L-Rha*p* partly branched at O-3/O-4 with 1,5-α-L-arabinan, 1,4-β-D-galactan, arabinogalactan-I, more scarcely arabinogalactan-II and galactoarabinan (Tharanathan et al., 1994; Øbro et al., 2004). Pectins are usually considered to be gelling polysaccharides with a galacturonic acid content of no less than 65% (w/w) (May, 1990), and which structure is mainly composed of unbranched HG block copolymers (or “smooth regions”) and some neutral sugar-branched RG-I block copolymers (or “hairy regions”). Depending on the DM of the (HG domain of) pectin polymers, high methoxy pectins (HMP; DM > 50%), which form sugar-acid-induced gels (SAG), and low methoxy pectins (LMP; DM ≤ 50%), which form calcium (Ca^{2+})-mediated gels, can be distinguished (Voragen et al., 1995). In either case, the gelling behavior and the gel formed strength are dependent upon a variety of intrinsic factors such as the pectin galacturonic acid content, neutral sugar content, HG average length and relative proportion, and average-molecular weight (Voragen et al., 1995; Yapo, 2009; Yapo & Koffi, 2013). These intrinsic characteristics are contingent upon the extraction conditions (pH, temperature, time, and solid to liquid ratio) of pectin polymers from the cell wall matrix. Therefore, the extraction process needs to be optimized in order to produce pectins with good quality characteristics for marketing possibility.

Cinnamon apple pomace is a local industrial product, available in large quantities, to which no value has been added hitherto. To compensate for the striking unbalance between high cost of import of commercial citrus and/or apple pectins, from western countries to developing countries such as Côte d’Ivoire, and low added values to domestically manufactured gelling

products, new sources of marketable pectins from local agricultural byproducts are being sought for. The scope of this study was to evaluate, by structural and rheological studies, the pectin potential of cinnamon apple (*Annona squamosa*) pomace for possible production of commercial grade products. The results of the macromolecular and viscoelastic analyses of conventionally extracted pectins, from cinnamon apple pomace, showed that this raw material could be used for the production of marketable HMP and LMP.

2. Materials and methods:

2.1. Preparation of cell wall material for pectin extraction

The dried raw material, namely cinnamon apple pomace, was a gift from a medium-size factory of the local juice industry (ATOU, Abidjan, Côte d'Ivoire). Prior to extracting pectin polymers from the cell wall matrix, DRM was successively treated with heat stable α-amylase (Termamyl, Novozymes, Bagsvaerd, Denmark), protease, and amyloglucosidase (Sigma Chemical Co., St. Louis, MO) to extensively remove proteins and starch as previously reported (Yapo & Koffi, 2008). The resulting insoluble solids were boiled in 80% (v/v) ethanol for 25 min, followed by two washings with 70% (v/v) ethanol to remove free sugars, pigments, and other impurities as much as possible. The residue left was further dried by solvent exchange (95% ethanol and acetone), and finally oven-dried at 35 °C for 15–16 h and weighed. The dried cell wall material (CWM) 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 pending analysis and utilization for pectin production.

2.2. Extraction of pectins

Pectins were extracted from the prepared CWM by water acidified with 1 N HNO_3 to different extractant strengths (pH 1.2, 1.6, and 2.0), while the other extraction parameters, namely the ratio of CWM to extractant, temperature, and time, were fixed to 1:25 (w/v), 75 °C, and 90 min, respectively, on the basis of our previous studies on diverse pectin sources. Two successive extractions were performed before discarding any remaining insoluble cell wall residue. At the end of each extraction, the slurry was clarified and pectin extract was rapidly brought to pH 4 for stability. The first and second extracts were pooled, concentrated, and precipitated in 3 volumes of 95% ethanol at 5 °C for 2 h. Pectin precipitates were washed two-times with 70% ethanol, followed by 95% ethanol and acetone, and oven-dried at 40 °C for 15–16 h and weighed. The dried pectin flakes 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. Extraction of pectins was carried out in three independent runs for each selected pH value.

2.3. Characterization of pectins

The crude pectin extracts were further purified before analysis as follows. Aqueous dispersions of pectin extracts (1% w/v) were treated with a mixture of 1% (v/v) HCl/60% (v/v) ethanol (three times), and acidified-ethanol insolubles were exhaustively washed with 60% (v/v) ethanol until the filtrate gave a negative response for chloride ions with silver nitrate. This treatment allowed the removal of all low-molecular weight sugars and salts

initially present in extracts and protonation of all the carboxylate groups of pectin chains prior to correctly titrating them by 1 N NaOH solution. Pectins were characterized for their monosaccharide composition, degrees of (methyl- and -acetyl) esterification (DM and DAc), molecular weight, and gel-forming ability.

2.3.1. Analytical

Monosaccharide constituents

The monosaccharide constituents were quantified after complete hydrolysis of CWM polysaccharides (12 mol.L^{-1} H_2SO_4/23 °C/1 h and 1 mol.L^{-1} H_2SO_4 /100 °C/3 h) and purified pectins (1 mol.L^{-1} H_2SO_4/100 °C/ 3 h). The GalA content of CWM and purified pectins was colorimetrically determined at 525 nm by a modified sulfamate-meta-hydroxydiphenyl assay using monoGalA standard (Yapo, 2010). Liberated neutral monosaccharides from the purified pectins, especially Ara, Gal, and Rha were spectrophotometrically quantified at 340 nm using Megazyme assay kits (Megazyme International Ireland Ldt., Bray, Co. Wicklow, Ireland). The total neutral sugar content was estimated by the tri-reagent colorimetric assay as previously reported (Yapo & Koffi, 2013).

Enzymatic probing of the neutral sugar side chains of extracted pectins

The different types of neutral sugar side chains of the RG-I block copolymers were discriminated by treating the pectins with highly purified solutions of α-L-arabinanase, α-L-arabinosidase, β-D-galactanase, and β-D-galactosidase. 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 Yariv reagent assay for the detection of pectin arabinogalactan-II (AG-II) side chains

Assay of Yariv reactivity was performed with β-D-Galactosyl Yariv reagent (Cat. No. 100-8; Biosupplies Australia Pty Ltd., La Trobe Uni, Bundoora, Victoria, Australia) as described elsewhere (Kitazawa et al., 2013). Briefly, Yariv reactivity was determined by radial gel diffusion assay. The pectic sample was applied to a gel plate containing 0.004% (w/v) β-D-Gal-Yariv, 75 mM NaCl, 0.01% (w/v) sodium azide, and 1% (w/v) agarose. The relative reactivity was quantified based on the halo area using gum arabic (Sigma Chemical Co., St. Louis, MO) as the standard, and expressed based on equal sugar amount.

The overall degree of esterification of pectins was potentiometrically determined as previously described (Yapo, 2009). The DAc was colorimetrically measured at 510 nm by the hydroxamic acid assay using glucose pentaacetate standard and the DM was differentially assessed (Yapo & Koffi, 2013). All the measurements were performed in triplicates.

2.3.2. Macromolecular characteristics

The intrinsic viscosities of the samples were determined by capillary viscometric measurements as follows. Briefly, the viscosities (η) of the samples prepared at different

solution concentrations were first estimated by capillary viscometric experiments using an Ubbelohde capillary viscometer (capillary no.: I; ID: 0.63 mm; Schott-Geräte GmbH, Mainz, Germany) with a constant K of 0.01, immersed in a water bath thermostated at 25 °C. The solutions of the samples were prepared at seven different concentrations (C) (0.01, 0.03, 0.05, 0.08, 0.10, 0.15, and 0.20 g / 100 ml) in aqueous solvent containing 90 mM sodium chloride, 10 mM sodium fluoride, and 1 mM Na_2EDTA at pH 6.5. The sample solutions and solvent were filtered using 0.45 µm membrane filters (Millipore Corp., Bedford, MA) before measurements. The solutions (15 mL) were pipetted into the capillary viscometer and flow times were recorded with a stopwatch with a precision of ± 0.1 s. The densities of the sample solutions were measured by a 25 mL-Gay-Lussac pycnometer (Boeco, Boeckel & Co (GmbH & Co), Hambourg, Germany). The viscosities of the sample solution (η) and solvent (η_s) were calculated from the measured flow times and densities of solutions, followed by calculations of the specific viscosities (η_{sp}) using equation 1. The intrinsic viscosities ([η]) of the samples were finally estimated by plotting the reduced viscosities (η_{sp}/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.

$$\eta_{sp} = (\eta - \eta_s)/\eta_s \quad (1)$$

The molecular weights of the pectins were analyzed by gel-filtration chromatography on a high resolution Superdex-200 HR 10/30 column (Amersham Biosciences Corp., NJ). The polysaccharide distributions were monitored using a differential refractometer or refractive index detector (Waters Corp., Milford, MA). A molecular weight kit of pullulan standards ($\overline{M_w}$ ~ 6.0, 10.0, 21.7, 48.8, 113.0, 210.0, 393.0, and 805.0 kDa; $\overline{M_w}/\overline{M_n}$ ~ 1.0–1.2; American Polymer Standards Corp., Mentor, OH) and purified homogenous HG standards ($\overline{M_w}$ ~ 60 and 100 kDa, $\overline{M_w}/\overline{M_n}$ ~ 1.0–1.2), with known intrinsic viscosities ([η]) and molecular weights were used for column calibration. The molecular weights of the pectins were assessed according to the universal calibration technique by plotting log ([η].$\overline{M_w}$) against the elution volume (Ve) of standards. Analyses were performed in triplicates.

2.4. Gelling and viscoelastic analyses

The preparation of gels was carried out as described elsewhere (Da Silva et al., 1995) with slight modifications (Yapo, 2009). Briefly, purified pectins were first dispersed in 0.1 mol.L^{-1} citrate buffer (pH 3.0) at room temperature for 15–16 h and then clarified after centrifugation (30000g) for 10 min. The pectin dispersions were then adjusted to pH 2.3 using 0.25 mol.L^{-1} citric acid and heated at 100 °C in an oil bath. The required amount of sucrose was added to the dispersions, under stirring, and heated until the desired weight and sucrose content of the "pre-gelling systems" were reached. The hot pre-gelling systems were then molded for one-point non-breaking measurements by the conventional SAG method with the help of a Ridgelimeter (Bulmer Food Co., UK) after being cured (aged) for 24 h at ambient temperature or were directly transferred to a rheometer plate at the desired test temperature. Final pre-gelling systems contained 0.70 wt% pectins and 65.0% (w/w) sucrose at pH 2.3.

The viscoelastic properties, viz. the trends of viscous (G'') and elastic (G') moduli, were determined by small amplitude oscillatory shear (SAOS) tests 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, single frequency (1 Hz) experiments were performed throughout testing. The viscoelastic behavior of pectin dispersions was analyzed 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 (LVR) of pectin gels. The prepared hot pectin dispersions were directly applied to a pre-heated rheometer at 95 °C after the desired weight was reached. A thin layer of low viscosity paraffin oil was used to cover the exposed surface of 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 obtained 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). The means of different treatments were considered to be significantly different at *P-value <0.05*.

3. Results and Discussion

3.1. The extraction yield of cinnamon apple pectin (CAP)

The yield of pectins extracted from cinnamon apple pomace is shown in Table 1.

Table 1. Effect of the acid solvent strength of the extraction yield of cinnamon apple pomace pectins

	Cinnamon apple pectins (CAP)			
	pH 1.0	pH 1.2	pH 1.6	pH 2.0
Yield (g/100 g dried weight)	5.8 ± 0.7a	10.9 ± 2.1b	6.4 ± 0.9ac	2.8 ± 0.3d

Data are expressed as mean ± SD (n = 3). The mean values in the same line with different letters (a, b, c, and d) are significantly different (P <0.05).

It can be seen that the yield of pectins varied from 2.8 to 10.9% and was significantly affected by the solvent strength (P <0.05). Generally, the yield increased with decreasing pH of the solvent (i.e., with increasing strength of the extracting agent). The highest yield (10.9%) was obtained at pH 1.2, suggesting that severe acid conditions were required for the extraction, from cinnamon apple pomace, of an amount (>10.0%) of pectins considered to be commercially viable (Mohamed & Hasan, 1995). This result also suggests that most pectic polymers might initially be firmly anchored within the cell wall matrix. However, increasing further the acid solvent strength to pH 1.0 resulted in significant decrease of the yield from

10.9 to 5.8% ($P < 0.05$), indicating that solubilisation of pectin polymers under the latter conditions was concomitantly followed by notable degradation of part of them into shorter oligomers that were removed during the subsequent purification step. Hence, the extracting conditions using pH 1.2 was the optimum conditions for isolating reasonably high amount (≈10.0%) of pectin polymers from cinnamon apple pomace.

3.2. Structural features of isolated CAP

3.2.1. Sugar content, block copolymers and degree of esterification

The results of analyses of sugar composition are presented in Table 2.

Table 2. Glycosyl residue composition and degree of esterification of acid-extracted pectins from cinnamon apple pomace

	Cinnamon apple pectins (CAP)		
	pH 1.2	pH 1.6	pH 2.0
GalA (% w/w)	67.2 ± 4.2a	53.6 ± 3.1b	38.5 ± 2.9c
Rha (% w/w)	4.7 ± 0.9	6.2 ± 1.3	5.5 ± 0.7
Total neutral sugar (% w/w)	25.1 ± 2.8a	35.4 ± 2.7b	44.6 ± 3.1c
Rha/GalA molar ratio	8.4: 100a	13.8:100b	17.1:100c
DM	68 ± 2a	54 ± 3b	43 ± 2c
DAc	3 ± 1	5 ± 2	8 ± 2

Data are expressed as mean ± SD (n = 3). The mean values in the same line with different letters (a, b, and c) are significantly different (P <0.05).

Galacturonic acid (GalA) was the major monosaccharide detected (38.5–67.2%, w/w), thereby confirming the pectic character of the different extracts. It can be seen that the three extracts contained significantly different GalA quantities (P <0.05), showing that this basic constituent of pectins was also affected by the solvent strength. The fact that the pectin isolate obtained under milder acid conditions (at pH 2.0) had the lowest GalA content supports the idea that most pectin chains might be initially tightly bound in the cell wall matrix, and therefore would require more drastic extraction conditions to be released. The neutral sugar content of the isolated pectins was rather high, ranging from 44.6 to 25.1%. High amounts of neutral sugars have also been reported in pectins from other cell wall residues such potato tubers (Øbro et al., 2004) and sugar beet pulps.

The pectin extract obtained at pH 2.0 was remarkably rich in neutral sugars (44.6%), which suggested abundance of RG-I block copolymers. This result further substantiated that more severe acid conditions were indeed required for solubilizing HG-rich pectin polymers from the cell wall matrix of cinnamon apple pomace. However, the molar ratio of Rha to GalA indicated that the HG block copolymers might be dominant over the RG-I ones irrespective of the extract type.

The DM of isolated CAP ranged from 43 to 68 (Table 2), which showed extraction of both HMP and LMP. The fact that the latter pectins were recovered under relatively milder acid extraction conditions (pH 2.0) suggested that they may be originally present within the cell

wall matrix as the result of *in muro* (partial) deesterification by pectin methylesterases during fruit maturation. LMP have also been reported from some other cell wall residues such as olive fruit pomace (Cardoso et al., 2003), sunflower head residues (Iglesias & Lozano, 2004) and yellow passion fruit rind (Kliemann et al., 2009). On the whole, the pectin products were weakly acetyl-esterified (DAc < 10%)

3.2.2. Determination of the types of neutral sugar side chains of isolated pectins

The different types of neutral sugar-side chains (of the RG-I regions) of CAP were determined using highly purified arabinan- and galactan-degrading enzymes to treat the pectin product obtained at pH 2.0, which appeared to be particularly rich in neutral sugars. The results are shown in Table 3. As it can be seen, arabinanase removed more than 93% of the arabinose initially present in the pectic chains, whereas arabinosidase was inactive. Furthermore, about 98% of arabinose was degraded using the enzymatic combination of arabinanase and arabinosidase. These results indicated that most arabinosyl residues were incorporated in relatively long arabinan side-chains. Also, galactanase was almost completely inhibited by the pectic preparation, whilst galactosidase removed more than 99% of the galactose initially present within the pectic material and the combination of the two enzymes did not significantly increase this amount. This indicated that most galactosyl residues were at terminal positions in the side chains. All these results substantiated that the RG-I regions of CAP carried mainly galactoarabinan side chains, as has also been reported for pectic polysaccharides from some other CWM such potato tubers and blackgram native and fermented products (Tharanathan et al., 1994; Øbro et al., 2004). Some arabinogalactan side chains were also likely present, as judged by the observed slight removal of arabinose and/or galactose by either of the two enzymatic combinations. However, the assay of Yariv reactivity for specific binding to 1,3-β-D-galactan (Kitazawa et al., 2013) was negative, indicating that no 1,3-β-D-galactan-containing arabinogalactan-II (AG-II) side chains were present. Hence, the detected arabinogalactan side chains could exclusively be of type I (AG-I).

Table 3. Changes in relative molar composition of the neutral sugar constituents of CAP after enzymatic degradations with arabinanase, arabinosidase, galactanase and galactosidase

	Relative compostion (mol%)		
	Rha	Ara	Gal
Without enzymes	100.00	100.0	100.0
Arabinanase	99.12 ± 0.58	6.25 ± 0.49	98.21 ± 0.41
Arabinosidase	99.94 ± 0.24	98.77 ± 0.52	92.83 ± 3.14
Arabinanase + Arabinosidase	97.18 ± 0.45	1.46 ± 0.09	87.69 ± 2.14
Galactanase	99.75 ± 0.24	95.21 ± 3.47	99.77 ± 1.06
Galactosidase	99.53 ± 0.09	97.34 ± 2.06	0.86 ± 0.02
Galactanase + Galactosidase	98.75 ± 1.02	93.62 ± 4.28	0.81 ± 0.07

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

3.3. Physicochemical and gelling capability

The intrinsic viscosity ([η]) and viscosity-average molecular weight ($\overline{M_v}$) of CAP were in the range of 179–346 mL/g and 41–89 kDa, respectively (Table 4). The $\overline{M_v}$ increased with increasing [η], in accordance with the Mark-Houwink relationship between the two parameters for pectin polymers with molecular weights in the range of 10–100 kDa. The pH 1.2-isolate had the highest [η] (346.0 ml/g) and $\overline{M_v}$ (89.0 kDa) which fulfilled the required molecular weight value (≥ 80 kDa).

The results of gelling assays of CAP are also shown in Table 4. The three pectin isolates exhibited significantly different ($P < 0.05$) gel-forming abilities. The strengths of the sugar-acid gels prepared with the pH 1.2- and pH 1.6-pectin isolates were 158 and 92 °sag, respectively. Moreover, only the pH 1.2-pectin isolate fulfilled the required gelling grade (>150) for marketing possibility as HMP. By contrast, the pH 2.0-pectin isolate was unable to form sugar-acid-gels, yielding only highly viscous dispersions. This behavior was likely due to its RG-I-like characters with a moderately high GalA content (39.0%, w/w) together with extremely high neutral sugar content (45.0%, w/w) (see Table 2). The rather low DM (43.0%) and $\overline{M_v}$ (41.0 kDa) (see Tables 2 and 4) of this pectin isolate might also contribute, to some extent, to the expressed non-gelling behavior.

Table 4. Macromolecular features and sugar-acid-gel-forming capability of acid-extracted pectins from cinnamon apple pomace

	Cinnamon apple pectins (CAP)		
	pH 1.2	pH 1.6	pH 2.0
[η] (mL/g)	346 ± 4a	258 ± 5b	179 ± 3c
$\overline{M_v}$ (kDa)	89 ± 7a	72 ± 5b	41 ± 8c
Gel strength (°sag)	158 ± 4a	92 ± 1b	Non-gelling

Data are expressed as mean ± SD (n = 3). The mean values in the same line with different letters (a, b, and c) are significantly different (P <0.05).

3.4. Rheological properties

Small amplitude oscillatory shear (SAOS) tests were performed to appraise the temperature dependence of the viscoelastic characters of the sugar-acid-gels prepared with the different pectin products (Figure 1). It can be seen, during the cooling scan from 95 to 5 °C, that both the viscous (G'') and elastic (G') moduli increased with temperature decreasing whatever the pectin-sucrose system. For the gels prepared with both the pH 1.2- and pH 1.6-pectin isolates, a cross-over of the graphic traces of G'' and G' occurred at a characteristic temperature known as the “gel point (Tg)” (Figure 1a–b).

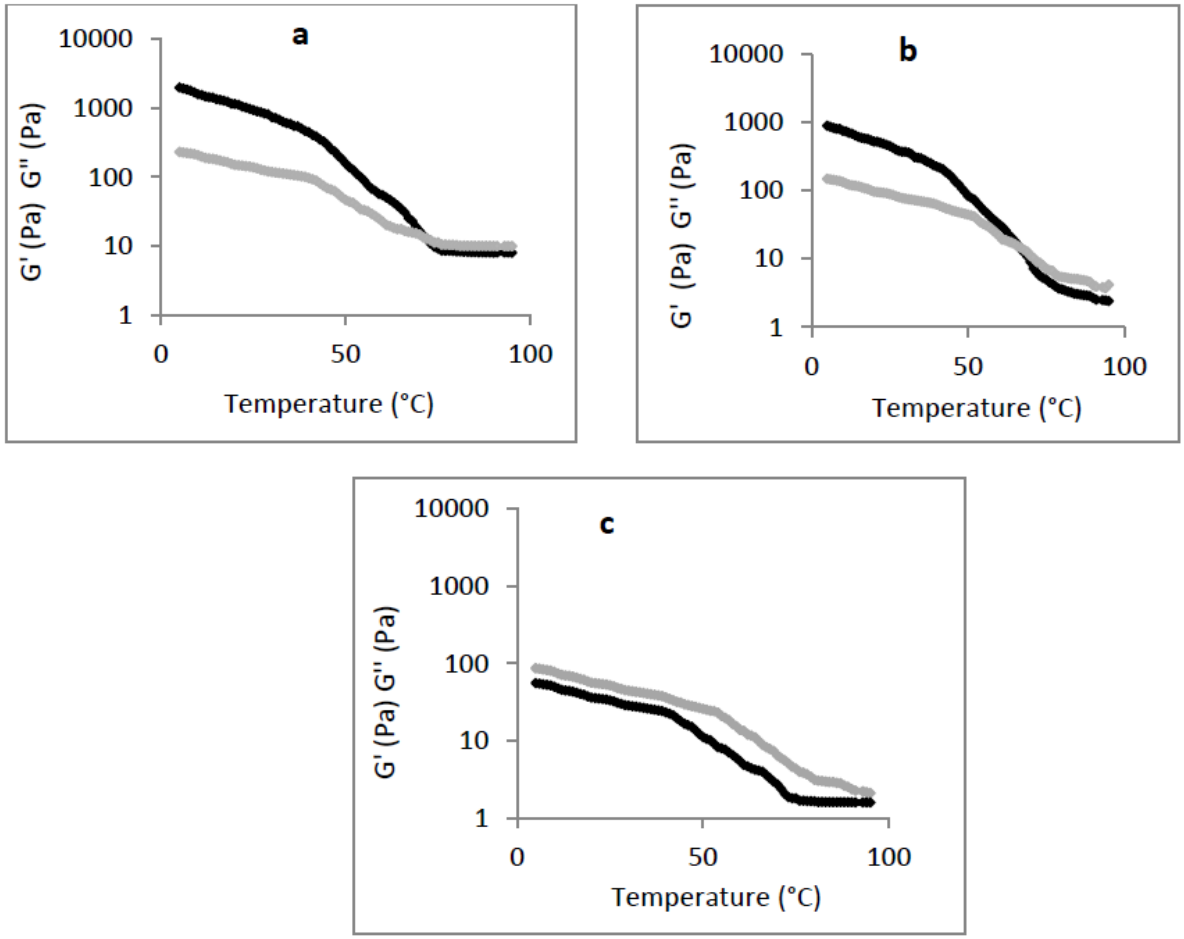

Figure 1. Temperature dependence of the viscous (G'') and elastic (G', black traces) moduli of cinnamon apple pectin (CAP)-sucrose gels: pH 1.2-CAP isolate (**a**); pH 1.6-CAP isolate (**b**); and pH 2.0-CAP isolate (**c**): Preparations contained 0.7%wt pectin and 65% (w/w) sucrose at pH 2.3.

This indicated the onset of formation of a tridimensional gelling (junction zones) network. Above T*g* (Tg < T), the magnitude of G'' was slightly higher than that of G' and the pectin-sucrose system was liquid-like. Below T*g* (T < T*g*), by contrast, the magnitude of G' appeared to be increasingly higher than that of G'', which indicated a reinforcement of the gelling network, a result of which was an increase in the firmness of the gel formed. Furthermore, the magnitude of G' (related to the strength of the gel formed), of the pH 1.2-pectin isolate gel was significantly greater (P <0.05), compared with that of the pH 1.6-pectin isolate over the whole range of testing temperature. This corroborates the results obtained with the one-point (non-breaking) measurements by the conventional SAG method, thereby confirming that the pH 1.2-pectin isolate was superior, in terms of gelling characteristics. On the other hand, the magnitude of G' was lower than that of G'', throughout the testing temperature range, for the pH 2.0-pectin isolate-sucrose system, and therefore no cross-over was observed (Figure 1c), which confirmed the non-gelling character of this pectin product.

Figure 2 illustrates the behavior of G' on an isothermal time sweep over a period of 24 h of

aging for pre-cooled gels at 23 °C.

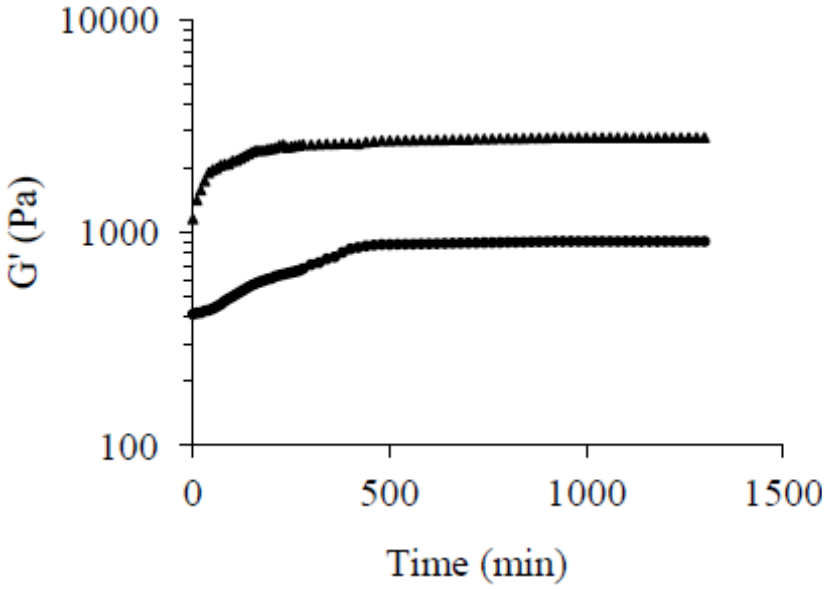

Figure 2. Evolution of the elastic modulus (G') of pectin-sucrose gels as a function of aging time at room temperature. pH 1.2-CAP isolate (▲) and pH 1.6-CAP isolate (●): Preparations contained 0.7%wt pectin and 65% (w/w) sucrose at pH 2.3.

The time dependence of the viscoelastic characters showed that the magnitude of *G*' increased rapidly, with a sharp slope over a relatively short period of aging, probably due to a slower formation and rearrangement of the gelling network (Da Silva & Gonçalves, 1995) and then started to be steady when an apparent “pseudo-plateau” was reached, reflecting a state close to pseudo-equilibrium, a typical behavior of HMP/sucrose gels also reported elsewhere (Da Silva & Gonçalves, 1995).

5. Conclusion

With a 20% anhydrogalacturonic acid content, cinnamon apple pomace appeared to be a pectin-rich source. Optimized extraction showed that about 11.0% of high methoxy pectin, able to yield good sugar-acid-gels with the required grade (>150), could be produced. Moreover, the pectin product obtained, under optimized conditions, exhibited interesting viscoelastic properties. Cinnamon apple pomace may therefore be a commercially viable source of production of at least high methoxy slow set pectins.

Acknowledgments

The authors thank Cosmivoire and Cargill West Africa for some financial support.

References

Cardoso, S.M., Coimbra, M.A., Lopes da Silva, J.A. (2003). Calcium-mediated gelation of an olive pomace pectic extract. *Carbohydr. Polym., 52,* 125–133. http://dx.doi.org/10.1016/S0144-8617(02)00299-0

Da Silva, J.A.L., Gonçalves, M.P., & Rao, M.A. (1995). Kinetics and thermal behaviour of the structure formation process in HMP/sucrose gelation. *Int. J. Biol. Macromol., 17*, 25-32. http://dx.doi.org/10.1016/0141-8130(95)93514-X

Iglesias, M.T., & Lozano, J.E. (2004). Extraction and characterization of sunflower pectin. *J. Food Eng., 62,* 215–223. http://dx.doi.org/10.1016/S0260-8774(03)00234-6

Kliemann, E., Nunes de Simas, K., Amante, E.R., Prudêncio, E.S, Teófilo, R.F., Ferreira, M.M.C., & Amboni R.D.M.C. (2009). Optimisation of pectin acid extraction from passion fruit peel (*Passiflora edulis flavicarpa*) using response surface methodology. *Int. J. Food Sci.Technol., 44,* 476–483. http://dx.doi.org/10.1111/j.1365-2621.2008.01753.x

Kitazawa, K., Tryfona, T., Yoshimi, Y., Hayashi, Y., Kawauchi, S., Antonov, L., et al. (2013). β-Galactosyl Yariv reagent binds to the β-1,3-galactan of arabinogalactan-proteins. *Plant Physiol. Prev*. http://dx.doi.org/10.1104/pp.112.211722

May, C.D. (1990). Industrial pectins: sources, production and applications. *Carbohydr. Polym., 12,* 79–99. http://dx.doi.org/10.1016/0144-8617(90)90105-2

Mohamed, S., & Hasan Z. (1995). Extraction and characterisation of pectin from various tropical agrowastes. *ASEAN Food J., 10,* 43–50.

Øbro, J., Harholt, J., Scheller, H.V., & Orfila, C. (2004). Rhamnogalacturonan-I in Solanum tuberosum tubers contains complex arabinogalactan structures. *Phytochemistry, 65,* 1429–1438. http://dx.doi.org/10.1016/j.phytochem.2004.05.002

Tharanathan, R.N., Reddy, G.C., Muralikrishna, G., & Susheelamma, N.S. (1994). Structure of a galactoarabinan-rich pectic polysaccharide of native and fermented blackgram (*Phaseolus mungo*). *Carbohydr. Polym*., 23, 121–127. http://dx.doi.org/10.1016/0144-8617(94)90036-1

Voragen, A.G.J., Pilnik, W., Thibault, J.F., Axelos, M.A.V., & Renard, C.M.G.C. (1995). Pectins. In A.M Stephen (Ed.), *Food polysaccharides and their applications*, (pp. 287-339). New York: Marcel Dekker.

Yapo, B.M. (2009). Biochemical characteristics and gelling capacity of pectin from yellow passion fruit rind as affected by acid extractant nature. *Journal of Agricultural and Food Chemistry, 57*, 1572–1578. http://dx.doi.org/10.1021/jf802969m

Yapo, B.M. (2010). Improvement of the compositional quality of monocot pectin extracts contaminated with glucuronic acid-containing components using a step-wise purification procedure. *Food. Bioprod. Process., 88,* 283–290. http://dx.doi.org/10.1016/j.fbp.2009.07.001

Yapo, B.M. (2011). Rhamnogalacturonan-I: A structurally puzzling and functionally versatile polysaccharide from plant cell walls and mucilages. *Polym. Rev., 51,* 391–413. http://dx.doi.org/10.1080/15583724.2011.615962

Yapo, B.M., & Koffi, K.L. (2013). Utilisation of model pectins reveals the effect of

demethylated block size frequency on calcium gel formation. *Carbohydr. Polym. 92,* 1–10. http://dx.doi.org/10.1016/j.carbpol.2012.09.010