Cinnamon Apple Pomace Pectins: Physicochemical Characteristics and Gel-Forming Properties

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Abstract

The physicochemical features and gelling capability of cinnamon apple pectins (CAP) were evaluated under different extraction conditions. The results showed that the pectin yield (2.8-10.9%) and sugar compositions, viz. galacturonic acid (GalA: 38.5-67.2%), rhamnose (Rha: 4.7-6.2%), arabinose (Ara: 12.3-24.8%) and galactose (Gal: 4-11.2%) were significantly influenced by the extraction conditions (P <0.05). Moreover, the degree of methoxylation (DM: 43-68), molecular weight (Mw: 41-89 kDa), and gelling ability (92-158) of the produced CAP were also significant affected by extraction conditions (P <0.05). Partial structural analyses, by enzymatic degradations, suggested that the rhamnogalacturonan-I (RG-I) regions of CAP were mainly branched with (galacto) arabino-side chains. Optimized extraction yielded about 11.0% high methoxy pectin (HMP) which fulfilled the required gelling grade (>150). Cinnamon apple pomace therefore appears to be a potentially viable source of marketable pectins.

ABBREVIATIONS

Ara: Arabinose; CAP: Cinnamon Apple Pectins; CWM: Cell Wall Material; DAc: Degree of acetyl-esterification; DM: Degree of Methyl-esterification (or Methoxylaton); DBr: Degree of Branching; DRM: Dried Raw Material; TNS: Total Neutral Sugar; Gal: Galactose; GalA: Galacturonic acid; HG: Homogalacturonan; HMP: High Methoxy Pectins; LMP: Low Methoxy Pectins; NAD(H): Nicotinamide Adenine Dinucleotide (Hydrogen); RG-I: Rhamnogalacturonan-I; Rha: Rhamnose

INTRODUCTION

Pectins are structurally complex polysaccharides from the cell wall of higher plants. They are basically composed of α-D-galactopyranosyluronic acid (α-D-GalP) and various neutral sugars, three of which: α-L-rhamnopyranose (α-L-Rhap), α-L-arabinofuranose, and β-D-galactopyranose are the typical neutral monosaccharides. These glycosyl residue constituents are believed to be distributed in no less than 8 block copolymers, two of which: homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) are commonly present within all (if not most of) the pectin sources hitherto investigated [1]. HG is a linear polymer of 1, 4-linked-α-D-GalP 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-GalP 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-GalP 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-GalP-1,2-α-L-Rhap-(1,4)n polymer with the α-L-Rhap 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[2,3].

The main functional property of pectins is the formation of gels under specified conditions [4]. It is now well-demonstrated that the pectin HG block copolymers are accountable for gelation, whereas the neutral sugar-branched RG-I block copolymers are likely to play mainly a gel-stabilizing role [5,6]. Pectins are therefore widely used for the manufacturing of various gelling products such as high calorie marmalades, jams, and preserves and low calorie jellies. The preparation of sugar-acid-mediated high calorie gels, with 55-65% sucrose content at pH 2.2-3.2,

requires high methoxy pectins (HMP; DM >50%), whereas the preparation of calcium-induced low calorie gels, with 0-30% sucrose content at pH 2.0–7.0, needs low methoxy pectins (LMP; DM 50%). The mechanism of gelation, the setting time and temperature and strength of the gel can all be affected by the physicochemical characteristics of pectin, namely the sugar composition (especially the molar ratio of GalA to neutral sugars), DM, and molecular weight [7,8]. Also, the chemical and macromolecular features of pectins are depended on the extraction conditions, such as the ratio of the raw material of pectins to extracting agent, temperature, time and pH, used for isolating them from the cell wall matrix. It is therefore necessary to optimize the pectin extraction process in order to obtain pectin products with good yield and quality characteristics for marketing possibility.

To compensate for the striking unbalance between high cost of import of industrial 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 the present study is to evaluate the pectin potential of cinnamon apple (Annona squamosa) pomace which is locally available in large quantities.

**MATERIALS AND METHODS**

**Preparation of cell wall material for pectin extraction**

The dried raw material (DRM), 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 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 [9]. 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 dried by solvent exchange (95% ethanol and acetone), and oven-dried at 40 °C for 15–16 h and weighed.

The different types of neutral sugar side chains of the RG-I block copolymers were discriminated by treating the pectins with 12 mol.L⁻¹ H₂SO₄ (23 °C, 1 h), followed by dilution to 1 mol.L⁻¹ H₂SO₄ (100 °C, 3 h) and purified pectins were directly hydrolyzed with 1 mol.L⁻¹ H₂SO₄ (100 °C, 3 h) as previously reported [9]. The GalA content of CWM and purified pectins was colorimetrically determined at 525 nm by a modified sulfamate-meta-hydroxydiphenyl assay using monoGalA standard [10]. Liberated neutral monosaccharides from the purified pectins, especially Ara, Gal, and Rha were spectrophotometrically quantified at 340 nm using Megazyme assay kits (Megazyme International Ldt., Bray, Co. Wicklow, Ireland). The neutral sugar assays were based on the quantitative oxidation of Ara/Gal and Rha to corresponding lactonic derivatives (D-galactono-{1,4}-lactone for α-L-Ara and β-D-Gal and L-rhamm-{1,4}-lactone for α-L-Rha) in the presence of corresponding dehydrogenases [β-Gal dehydrogenase plus Gal mutarotase for α-L-Ara and β-D-Gal, and L-Rha dehydrogenase for α-L-Rha] and the coenzyme NAD⁺, which was stoichiometrically reduced to NADH with absorbance maximum at 340 nm. D-Gal was quantitatively differentiated from L-Ara by reading absorbance at different reaction times, viz. after 6 min- and 12 min-reaction at room temperature, respectively. L-rhamnose was quantitatively determined after 1 h-reaction at room temperature [11]. Total neutral sugar (TNS) was measured by the tri-reagent colorimetric assay as previously reported [6].

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.

**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 carbohydrates 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.

**Analytical**

To quantify monosaccharide constituents, CWM was hydrolyzed after the two-stage Saeman method, viz. pretreatment with 12 mol.L⁻¹ H₂SO₄ (23 °C, 1 h), followed by dilution to 1 mol.L⁻¹ H₂SO₄ (100 °C, 3 h) and purified pectins were directly hydrolyzed with 1 mol.L⁻¹ H₂SO₄ (100 °C, 3 h) as previously reported [9]. The GaL content of CWM and purified pectins was colorimetrically determined at 525 nm by a modified sulfamate-meta-hydroxydiphenyl assay using monoGalA standard [10]. Liberated neutral monosaccharides from the purified pectins, especially Ara, Gal, and Rha were spectrophotometrically quantified at 340 nm using Megazyme assay kits (Megazyme International Ldt., Bray, Co. Wicklow, Ireland). The neutral sugar assays were based on the quantitative oxidation of Ara/Gal and Rha to corresponding lactonic derivatives (D-galactono-{1,4}-lactone for α-L-Ara and β-D-Gal and L-rhamm-{1,4}-lactone for α-L-Rha) in the presence of corresponding dehydrogenases [β-Gal dehydrogenase plus Gal mutarotase for α-L-Ara and β-D-Gal, and L-Rha dehydrogenase for α-L-Rha] and the coenzyme NAD⁺, which was stoichiometrically reduced to NADH with absorbance maximum at 340 nm. D-Gal was quantitatively differentiated from L-Ara by reading absorbance at different reaction times, viz. after 6 min- and 12 min-reaction at room temperature, respectively. L-rhamnose was quantitatively determined after 1 h-reaction at room temperature [11]. Total neutral sugar (TNS) was measured by the tri-reagent colorimetric assay as previously reported [6].

The relative molar proportions of HG to RG-I block copolymers were roughly estimated using equation 1.

\[
\text{HG/\text{RG-I}} \times 100 = \left( \frac{\text{GalA(mol%) - Rha(mol%)}}{2 \times \text{Rha(mol%) + Ara(mol%) + Gal(mol%)}} \right) \\
\text{(1)}
\]

The degree of branching (DBr) of the rhamnosyl residues of pectin chains with neutral sugar side chains was roughly estimated by equation 2.

\[
\text{DBr} \times 100 = \left( \frac{\text{Rha(mol%)}}{\text{Ara(mol%) + Gal(mol%)}} \right) \\
\text{(2)}
\]

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 acetic buffer (pH 4.8) in 12,000 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 degree of esterification of pectins was potentiometrically determined as previously described [6]. The DAc was colorimetrically measured at 510 nm by the hydroxamic acid assay using glucose pentaacetate standard [12] and the DM was differentially assessed. All the measurements were performed in triplicates.

Macromolecular characteristics

The intrinsic viscosities of the samples were determined by capillary viscometric measurements as described previously [13]. 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., Milford, MA). A molecular weight kit of pullulan standards (\( M_\theta ~\sim~ 6.0, 10.0, 21.7, 48.8, 113.0, 210.0, 393.0, \) and 805.0 kDa; \( M_v / M_\theta ~\sim~ 1.0–1.2; \) American Polymer Standards Corp., Mentor, OH) and purified homogenous HG standards (\( M_\theta \sim 60 \) and 100 kDa, \( M_v / M_\theta ~\sim~ 1.0–1.2), \) with known intrinsic viscosities [(\( \eta_s \))] 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 [(\( \eta_s \), \( M_v \))] against the elution volume (Ve) of standards [8]. Analyses were performed in triplicates.

Gelling properties

The gelling capability of pectins was appraised, according to the standardized SAG method, by the determination of the strength of molded gels containing 65.0% sucrose, 0.70 wt% pectins at pH 2.3. The gel preparation and firmness measurements were performed as described previously [8].

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.

RESULTS

The yield of cinnamon apple pectin (CAP)

The cinnamon apple pomace contained 20.8 ± 3.4% anhydrogalacturonic acid on a dry-weight basis (average of three independent measurements), showing that it was a pectin-rich source, which might be utilized for the production of marketable pectins. The different yields of isolated pectins are shown in (Table 1). The yields of CAP ranged from 2.8 to 10.9% and were significantly different from one another (\( P < 0.05 \)). The pectin yield increased with increasing extractant strength. The highest yield (10.9%) was obtained as the extractant strength was increased up to pH 1.2. This indicated that more severe acid conditions were necessary for isolating CAP with reasonably good yield (>10%), as most pectic substances might initially be firmly anchored within the cell wall matrix. However, further extraction at pH 1.0 resulted in significant decrease of the yield from 10.9 to 5.8% (data not shown), indicating concomitant degradation of the solubilized pectin polymers under these conditions. Therefore, the conditions using pH 1.2 appeared to be the optimum conditions for producing high amount pectins from cinnamon apple pomace.

Structural features of isolated CAP

Monosaccharide composition: (Table 1) illustrates the sugar composition of isolated CAP. The GalA content varied from 38.5 to 67.2%. The amounts of GalA of the three purified CAP were significantly different from one another (\( P < 0.05 \)). The GalA content (38.5%) of the pH 2.0-isolate was rather low, which strengthened the idea that the bulk of pectin chains might be tightly bound within the cell wall matrix and therefore would require higher extractant strength to be released. It could indeed be seen that the GalA content of CAP considerably increased from 38.5 to 67.2% with increasing strength of extractant from pH 2.0 to 1.2. Furthermore, only the pH 1.2-isolate had a GalA content >65%, one of the recommended quality characteristics for marketing possibility. On the whole, the GalA content of CAP was moderately high (38.0–67.0%),-presaging absence of neutral sugar constituents.

Table 1: Glycosyl residue composition, macromolecular features, and gelling capability of acid-extracted pectins from cinnamon apple pomace.

<table>
<thead>
<tr>
<th>Cinnamon apple pectins (CAP)</th>
<th>pH 1.2</th>
<th>pH 1.6</th>
<th>pH 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (g/100 g DRM)</td>
<td>10.9 ± 2.1a</td>
<td>6.4 ± 0.9b</td>
<td>2.8 ± 0.3c</td>
</tr>
<tr>
<td>GalA (% w/w)</td>
<td>67.2 ± 4.2a</td>
<td>53.6 ± 3.1b</td>
<td>38.5 ± 2.9c</td>
</tr>
<tr>
<td>Ara (% w/w)</td>
<td>4.7 ± 0.9a</td>
<td>6.2 ± 1.3a</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>Gal (% w/w)</td>
<td>12.3 ± 1.4a</td>
<td>17.7 ± 2.2a</td>
<td>24.8 ± 2.6c</td>
</tr>
<tr>
<td>TNS (% w/w)</td>
<td>6.4 ± 0.5a</td>
<td>9.3 ± 0.8ab</td>
<td>11.2 ± 1.7b</td>
</tr>
<tr>
<td>Rha/GalA</td>
<td>8.4: 100a</td>
<td>13.8:100b</td>
<td>17:1100c</td>
</tr>
<tr>
<td>HG (mol%)</td>
<td>24.1 ± 0.9a</td>
<td>22.0 ± 1.2a</td>
<td>14.6 ± 0.5c</td>
</tr>
<tr>
<td>RG-I (mol%)</td>
<td>64.1 ± 2.1a</td>
<td>48.7 ± 3.1b</td>
<td>35.3 ± 1.7c</td>
</tr>
<tr>
<td>HG/RG-I (%)</td>
<td>3.5 ± 1.3a</td>
<td>51.3 ± 1.9a</td>
<td>64.7 ± 2.5c</td>
</tr>
<tr>
<td>Rδ (%)</td>
<td>1.8 ± 0.9a</td>
<td>1.0 ± 0.2ab</td>
<td>0.6 ± 0.1b</td>
</tr>
<tr>
<td>DM</td>
<td>68 ± 2a</td>
<td>54 ± 3b</td>
<td>43 ± 2c</td>
</tr>
<tr>
<td>DAc</td>
<td>3 ± 1</td>
<td>5 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>(nL/mL/g)</td>
<td>346 ± 4a</td>
<td>258 ± 5b</td>
<td>179 ± 3c</td>
</tr>
<tr>
<td>η (kDa)</td>
<td>89 ± 7a</td>
<td>72 ± 5b</td>
<td>41 ± 8c</td>
</tr>
<tr>
<td>Gel strength (<em>sag</em>)</td>
<td>158 ± 4a</td>
<td>92 ± 1b</td>
<td>Non-gelling</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n = 3). Mean values in the same line with different letters are significantly different (\( P < 0.05 \)).
amounts (P < 0.05) from one sample to another. Ara was the major neutral monosaccharide, followed by Gal and finally Rha. The TNS content ranged from 25.1 to 44.6% and was in line with the overall amount of the three neutral monosaccharides (Ara + Gal + Rha: 23.4–41.5%). This indicated that the CAP isolates were highly purified products.

Nevertheless, the profusion of neutral sugars in the pH 2.0-isolate suggested that it might correspond to RG-I-like polysaccharides rather than to pectin polymers encompassing both HG and RG-I regions. The latter result further substantiated that more severe acid conditions were indeed required for solubilizing pectin polymers from the cell wall matrix of cinnamon apple pomace.

**Enzymatic probing of the CAP neutral sugar side chains:** To identify the different types of neutral sugar-side chains of the RG-I regions of CAP, highly purified arabinan- and galactan-degrading enzymes were used to treat the pH 2.0-isolate in which neutral sugars were particularly abundant. The results are presented in (Table 2). Arabinanase removed more than 93% of arabinose initially present within the pectic fraction, whereas arabinosidase was inactive. Further, about 98% arabinose was degraded using the enzymic combination [arabinanase + arabinosidase]. These results indicated that most arabinosyl residues were present in the form of relatively long arabinoxylan side-chains. Galactanase was inhibited by the pectic preparation, whilst galactosidase removed more than 99% of galactose initially present within the pectic material and the combination of the two did not increase further this amount. This indicated that most galactosyl residues were at terminal positions. All these results substantiated that the RG-I regions of CAP could carry arabinan and/or the rather scarce galactoarabinan side chains as also reported for pectic polysaccharides from some other cell wall materials such potato tubers and blackgram native and fermented products [2,3].

**Proportions of block copolymers in CAP:** The molar ratio of HG to RG-I ranged from 0.6 to 1.8 (Table 1), showing that relative proportions of HG to RG-I block copolymers were significantly different from one sample to another (P < 0.05). Moreover, only the pH 1.2-isolate had a HG amount >50% (64.1%) (Table 1). This indicated that HG to RG-I ranged from 0.6 to 1.8 (Table 1), showing that relative proportions of HG to RG-I block copolymers were significantly different from one sample to another (P < 0.05). Moreover, only the pH 1.2-isolate had a HG amount >50% (64.1%) (Table 1). This indicated that HG to RG-I regions of CAP could carry arabinan and/or the rather scarce galactoarabinan side chains as also reported for pectic polysaccharides from some other cell wall materials such potato tubers and blackgram native and fermented products [2,3].

**Degree of esterification of CAP:** The DM of isolated CAP ranged from 43–68 (Table 1), showing extraction of both high (HMP) and low (LMP) methoxy pectins. The highest DM was obtained at pH 1.2 and the lowest at pH 2.0, suggesting that both HMP and LMP might initially be present within the cell wall matrix of cinnamon apple pomace. Nascent pectin polymers are generally believed to be HMP. However, LMP have also been extracted from various pectin sources, such as olive fruit pomace [14], sunflower head residues [15] and yellow passion fruit rind [13, 16], and are believed to result from the activity of plant pectin-methyl esterases within the cell wall during fruit maturation. On the whole, the DAc was rather low (8%).

**DISCUSSION** To date, commercial (or industrial) pectins are naturally gelling polysaccharides (food additives) which are conventionally extracted from the cell wall matrix of citrus (lime, lemon, orange and/or grapefruit) peels and apple pomace under, acid conditions (pH 1.0–3.0), by water acidified with mineral acids, preferentially HNO₃ or HCl [4,5]. Therefore, pectins are incorporated in the formulation of various gelling food and non food products. However, the import of industrial citrus and apple pectins is an expensive enterprise with low added values to domestically manufactured gelling products in developing countries. As an attempt to remedy to this problem, cinnamon apple pomace, a locally available industrial byproduct, was investigated for marketable pectin potential. The results showed that cinnamon apple pomace was composed of approximately 21.0% pectin substances on a GalA basis, which was comparable to that of citrus peel (15–30%) and higher than that of apple pomace (10–15%) [4,5]. Extraction of pectins under different acid conditions (pH 1.2–2.0) revealed that the yield of extracted pectins increased with decrease in pH of the solvent. This indicated that extractability of the pectin polymers from the cell wall matrix was dependent upon extraction conditions, in agreement with previous studies [7]. About 11.0% HMP, which
fulfilled the required quality characteristics and standardized gel-grade (>150), was obtained under optimized acid conditions (at pH 1.2), thereby showing the potential of cinnamon apple for producing marketable pectins. A pectin source with a yield above 10%, coupled with good jelly grade is, indeed, usually considered commercially viable [18]. The good gelling capability of the pH 1.2-isolate could be accounted for by its high GalA content (>65%), DM (>60%), and $M_v$ (>80 kDa), three intrinsic conditions previously reported to positively influence sugar-acid-mediated gelation of HMP [4,5]. By contrast, the non gelling character of the pH 2.0-isolate might be due to its rather low [η] (and $M_v$), possibly caused by a roll-up of the polymer chains over on themselves and fostered by flexibility of the neutral sugar branches, thereby resulting in an overall sphere-like compact macromolecules with shorter hydrodynamic size [19].

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 form sugar-acid-mediated gels with the required grade (>150), could be produced. Cinnamon apple pomace could therefore stand as a new source of production of commercial-grade pectins.

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CONFLICT OF INTEREST

The authors declare that neither financial interest nor conflict of interest exists.

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