Extraction of Novel Galactoarabinan-Rich Pectin with Gelling Capacity from Soursop Pomace

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Abstract

The effect of the strength of nitric acid-extractant (pH 1.2, 1.6, and 2.0) on the yield, sugar composition and molecular weight, and gelling ability of pectin from soursop (Annona muricata) was investigated to find out the optimum conditions for producing marketable pectin. The results showed that the yield (3.5-12.9%) of pectin (AMP) extracted from Annona muricata pomace was pH-dependent. Likewise, its galacturonic acid content (GalA: 41.3-72.8%), total neutral sugar content (TNS: 11.4-24.3%), methylesterification degree (DM: 64-82), viscosity-average-molecular weight (Mv: 62-105), and gelling capacity (GC: 98-163) were significantly influenced by the extraction pH (p<0.05).

Individual neutral sugar analysis revealed that arabinose was the most abundant, followed by galactose with a rather high presence of rhamnose. Partial structural analysis, by enzymatic degradations, suggested that AMP was mainly branched with an unusual galactoarabian side chain type rather than with arabinogalactan-I. As the other extraction parameters, namely dried raw material to solvent ratio, temperature, and time, were fixed to 1:25 (w/v), 75°C, and 90 min, respectively; pH 1.2 was found to be the optimum pH condition for isolating, from soursop pomace, high methoxyl pectin (DM>60%) with rather good yield (8.0%) and gelling power (>150).

Keywords: Annona muricata; Pomace; Pectin; Galactoarabinan; Gelatio

Introduction

The cell walls of higher plants are composed of three kinds of polysaccharides, namely, cellulose, non cellulosic cross-linking heteroglycans (ex. hemicelluloses) and pectic substances. The latter polymers are highly diversified that they are believed to form a family of at least eight polysaccharides, two of which (homogalacturan and rhamnogalacturan-I) are commonly found in all (if not most) of the plant cell walls so far investigated. Homogalacturan (HG) is an unbranched 1,4-α-D-GalpA polymer methyl-esterified at C-6 position and sometimes acetyl-esterified at O-2 and/or O-3 positions. Rhamnogalacturan-I (RG-I) is a [1,4]-α-D-GalpA-1,2-α-L-Rhap-[1,4] polymer branched with different types of neutral sugar for the inulin chains. Common branches of RG-I consist of 1,5-α-L-arabinan, 1,4-β-D-galactan, and arabinogalactan-I (AG-I). They may, however, be ramified with more complex polysaccharide moieties, such as arabinogalactan-II (AG-II), commonly found in arabinogalactan-protein (AGP) and unusual galactoarabinan (GA) [1]. No less than 17 monosaccharides have been detected in pectic polysaccharides [2]. However, typical acid (AS) and neutral (NS) sugar constituents of the pectin HG and RG-I block copolymers are of four sorts, viz., α-D-galactopranosyluronic acid (α-D-GalpA), α-L-rhamnopyranose (α-L-Rhap), α-L-arabinofuranose (α-L-Araf), and β-D-galactopyranose (β-D-Galp). As conveyed by the name per se, “pectin” [in reference to the Greek word ‘pektikos’, which means to “congeal, solidify or curdle” [3] is a naturally gelling polysaccharide mainly exploited for its functionality in the art of manufacturing various gelling products such as marmalades, jams, preserves, and low calorie jellies and confections.

To date, commercial pectins are produced from two pectin-rich sources (citrus peels and apple pomace) under specified extraction conditions kept secret by producers, but thought to be generally confined to the following: dry raw material to solvent weight ratio 1:35-1:15 (w/v), water acidified with HNO3 (or HCl) to pH 1-3, temperature 60-100°C, and time 30-180 min [4-6]. Diverse studies have shown that the yield, sugar composition (especially GalA content), esterification degree, and functional (gelling) properties of pectins are influenced by the conditions used for extraction [7,8] and therefore optimization is required for every new pectin source, which is being investigated for marketing purposes.

Furthermore, industrial pectins are hitherto mainly produced in Europe and the United States of America. Thus, pectin import in developing countries such as Côte d’Ivoire and neighboring countries to satisfy the interior and regional demands represents an expensive enterprise with a low added value to domestically manufactured gelling products and confectioneries. As a consequence, importation of ready-to-eat preserves and confectioneries are prioritized at the expense of some home-made gelling products by food firms, thereby resulting in bankrupct of small- and medium-size local gelling food plants and jobless increase.

To partially remedy to this “abnormal situation”, various unutilized agricultural byproducts, available in large quantities, are selectively screened for possible production of marketable pectins. This paper presents the results of our investigation on the yield, quality characteristics, and gelling ability of pectins extracted from soursop pomace. The pomace is a byproduct of medium-size juice plants. Daily drink of soursop fruit juice (as well as its leaves infusion) is gaining more and more attraction in Ivoirian society, because these beverages are thought to possess some “miracle health-benefit effects” such as anti-insomnia, anti-stressing, anti-depressant, anti-microbial and even anti-tumor properties.

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Material and Methods

Alcohol insoluble material (AIM) preparation

Dried pomace of soursop was a gift from a medium-size factory of domestic juice producer (ATOU, Abidjan, Côte d'Ivoire). Alcohol insoluble material (AIM) was prepared from the raw material, by heating pomace in boiling 80% (v/v) ethanol for 25 min and washing the residue with 70% (v/v) ethanol, to remove free sugars, pigments and other impurities until the filtrate gave a negative response with the phenol-sulfuric acid test [9]. The residue was then dried by solvent exchange (95% ethanol and absolute acetone), placed in a fume hood for 5 h for residual acetone evaporation and oven-dried overnight. 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.

Pectin extraction

Prior to extracting pectins by water acidified with 1 N HNO₃ to different pH (1.2, 1.6, and 2.0), AIM was successively treated with heat stable aamylase (Termamyl, Novozymes, Bagsvaerd, Denmark), protease (Sigma Chemical Co., St. Louis, MO), and amyloglucosidase (Sigma Chemical Co., St. Louis, MO) as previously described [10]. On the basis of our previous work on various pectin sources [6,8], pectins were extracted from destarched and deproteinated AIM (DDAIM) using a relatively simple experimental design for optimization. Only the pH of solvent was varied from 1.2 to 2.0 per 0.4 unit intervals (1.2, 1.6, and 2.0), the other extraction parameters, namely solid to liquid extractant (S/L) ratio, temperature (°C), and time (t), being fixed to 1:25 (w/v), 75°C and 90 min, respectively. Two successive extractions were carried out before discarding any remaining insoluble cell wall fraction. At the end of every extraction, slurry was clarified and pectin extract was rapidly brought to pH 4 for stability. The first and second extracts were combined, 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 kept for a while under a fume extractor (for residual acetone evaporation), and finally oven-dried at 45°C overnight and weighed. Extraction of pectins was performed in three independent runs for each selected pH value. Dried pectin flakes were finely ground to powders passing through 60- mesh (40.25 mm) size sifters, canned in plastic containers and store at room temperature under airless and moisture-free conditions until use.

Pectin characterization

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 until the filtrate gave a negative response for chloride ions with silver nitrate. This treatment aimed at removing free sugars and pigments and converting all the carboxyl groups of pectin to the free acid (-COOH) form for titration with 1 N NaOH solution. Pectins were characterized for the glycosyl residue and linkage compositions, esterification degree, molecular weight, and gelling capacity.

Analytical: To quantify monosaccharide constituents, DDAIM was first 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 [10]. The GaLA content of DDAIM and purified pectins was colorimetrically determined by the method of Dubois et al. [11] after acid hydrolysis at 100°C for 1 h. The GalA content of DDAIM and purified pectins was colorimetrically determined by the method of Dubois et al. [11]. The liberated monosaccharides (NS) from purified pectins, especially galactose/arabinose (Megazyme procedure) and rhamnose [12] were quantified spectrophotometrically at 340 nm using Megazyme assay kits (Megazyme International Ireland Ltdt., Bray, Co. Wicklow, Ireland). The NS 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-rhamono-(1,4)-lactone for α-L-rhamnose) in the presence of corresponding dehydrogenases [β-galactose dehydrogenase (β-GalDH) plus galactose mutarotase (GalM) for α-L-arabinose and β-D-galactose, and L-rhamnose dehydrogenase (RhaDH) for α-L-rhamnose] and the coenzyme NAD⁺, which is stoichiometrically reduced to NADH with maximum absorbance at 340 nm. D-galactose was quantitatively differentiated from L-arabinose by reading absorbance 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. Total neutral sugar (TNS) was calculated as the sum of individual amounts of NS (Ara, Gal, and Rha) determined. Alternatively, TNS was estimated by the tri-reagent (anthrone, orcinol, and MHDHP) colorimetric-H₂SO₄ assay as reported previously [13]. The glycosyl linkage was determined by methylation analysis as previously reported [6].

The molar ratio of HG to RG-I block copolymer was roughly estimated using relation 1 [1,14].

\[ \text{HG/RG-I} = 100 \times \frac{\text{GalA} - \text{Rha}}{2 \times \text{Rha} + \text{Ara} + \text{Gal}} \]  

The degree of branching (DBr) of pectins rhamnolysin residues with NS side chains was roughly estimated, by equation 2, as previously reported [15].

\[ \text{DBr} = \frac{\text{Rha}}{\text{Ara} + \text{Gal}} \]

The types of NS side chains branching the RG-I block copolymer were discriminated by treating pectin samples with highly purified α-L-arabinanase (+α-L-arabinosidase and/or β-D-galactanase (+β-D-galactosidase). Each of the enzymatic preparations had no measurable activity other than the activity which it was specified for. The overall esterification degree (DE) of pectic samples was potentiometrically determined as previously described [13]. The acetyl esterification degree (DAC) was colorimetrically measured at 510 nm by the hydroxamic acid assay using glucose pentacetate standard [16] and the monosaccharide content (DM) was determined by the phenol-sulfuric acid test. The data were statistically evaluated by the global test of 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 significantly different at p<0.05.

Results and Discussion

Extraction yield of Annona muricata pectins (AMP)

The “pectin content” of DDAIM, which accounted for about 70.2 ± 3.1% (three measurements) of the dried raw material (DRM), was 24.5 ± 1.3% on a GaA basis. Thus, the pectin content of DRM was estimated at approximately 17.2 ± 1.9%. This showed that dried sour sop pomace was a pectin-rich source, which deserved to be explored for possible mass production of marketable pectin. The yields of extracted Annona muricata pectins (AMP) are shown in Table 1. The yields of pH 1.2-AMP, pH 1.6-AMP, and pH 2.0-AMP isolates were 8.2, 12.9, and 3.5%, respectively. These pectin yields were significantly different from one another (p<0.05), showing that the pectin yield was greatly influenced by the extraction pH. It appeared that increasing the acid-extractant strength resulted in increase of the pectin yield. However, the fact that the yield significantly decreased was 12.9 to 8.2%, as the strength of extractant was increased from pH 1.6 to pH 1.2, indicated that solubilized pectin macromolecules were rather substantially degraded in the HG and/or RG-I (block copolymer) domains.

Chemical features of extracted pectins

Sugar composition and block copolymers: The sugar composition of extracted pectins is shown in Table 1. The galacturonic acid (GaA) content of pectins ranged from 41.3-72.8% as the extraction pH was varied from pH 1.2 to pH 2.0. The amounts of GaA in extracted pectins were significantly different from one another (p<0.05), showing that this important constituent of pectin was affected by the extraction pH. The pH 1.2-AMP isolate had the highest GaA content, which was >65%, one of the required quality characteristics for possible mass production. However, as can be seen above, the yield of pH 1.2-AMP isolate was lower than the yield of pH 1.6-AMP isolate, suggesting that more extensive degradation of solubilized pectin polymers at pH 1.2 might mainly occurred in the NS-containing RG-I regions rather than in the HG regions. The three typical NS of pectic substances, namely rhamnose, arabinose, and galactose were detected in all the purified samples, but in different relative amounts from one sample to another. Arabinose (5.4-13.2%) was the major NS, followed by galactose (3.8-6.5%) and rhamnose (2.2-4.6%). These results suggested that the sugar composition from sour sop pomace were profusely branched, in their RG-I domains, with arabinose-containing side chains. This observation is in accord with the hypothesis that cold water-insoluble pectin, contained in AIM of corossol pulp, might be less linear than cold-water soluble pectin from the same AIM, due to high recurrence of rhamnose in the backbone bearing mainly arabinose-containing side chains [17].

To discriminate the types of NS-side chains RG-I polymers were branched with, highly purified arabinox- and galactan-degrading enzymatic preparations were used to treat the AMP isolate which had the highest NS content, notably the isolate obtained at pH 1.6. It was observed that α-L-arabinanase was very active, removing more 80% of the arabinose initially present, whereas α-L-arabinosidase was inhibited by this pectin isolate. However, the mixture [α-L-arabinanase+α-L-arabinosidase] resulted in more than 95% removal of arabinose residue and the mixture [α-L-arabinosidase+βD-galactosidase] led to about 10% removal of arabinosyl residues. These results, taken together, strongly suggested that the bulk of arabinosyl residues were not in chain-terminating position, but in relatively long (and scarcely ramified) arabinans. Furthermore, β-D-galactanase was found to be inactive against the pectin isolate, whereas β-D-galactosidase was considerably active, removing no less than 98% of the galactose initially present with the sample. This indicated that most galactosyl residues were in chain-terminating position. On the other hand, glycosyl linkage analysis substantiated that most arabinosyl residues were 1,3-linked Ara residues with some 1,3-linked Ara residues, and galactosyl residues were chiefly terminal Gal residues with some 1,4-linked Gal residues (results not shown). In the light of all these data, we believed that the RG-I regions of AMP were primarily branched with unusual type of NS polymers, namely galactoarabinans (GaA), rather than by type one arabinogalactans (AG-I), commonly found in pectins. Pectic fractions from other plant sources such as sugar beet pulp, potato tubers, and blackgram native and fermented products have also been reported to be branched with GaA [1,18-20].

The TNS contents of pH 1.2-AMP, pH 1.6-AMP, and pH 2.0-AMP isolates, determined by calculating the sum of rhamnose, arabinose, and galactose were 11.4, 24.3, and 15.7%, respectively (Table 1). These values were similar to those (12.2, 25.1; 20.4%, respectively) measured by the tri-reagent method, except for the pH 2.0-AMP isolate where underestimation occurred with the former method. Hence, the monosaccharides contained in all the AMP isolates, but the pH 2.0-isolate, were all from pectin macromolecules, thereby indicating that the other two were highly purified pectin samples. This discrepancy could be ascribed to contaminating glucose-containing oligosaccharides from residual degraded starchy polysaccharides present in DDAIM. Corossol fruit pulp, like apple fruit pulp, has indeed been reported to be rich in starchy polysaccharides [17].

The amounts of TNS in all the AMP isolates were significantly different from one another (p<0.05). The TNS content of the pH 1.2-isolate was the lowest, probably due to more substantial degradation of GaA-branched RG-I regions. This was likely supported by the observation that this pectin isolate had much lower Rha to GaA molar ratio (3.6%), compared with the other two samples (7.8-9.2%). In contrast, the degree of branching (DBr: 20.0-23.2%) was not significantly affected, suggesting that arabinose, galactose, and rhamnose might be degraded at similar rates in the course of pectin polymers solubilisation in acid-extractant from sour sop pomace.

The relatively high sensitivity and lability of those neutral sugars, especially arabino- or galacturonic residue to acid are well demonstrated in the literature [7,8,21]. The amount of HG was above 50% (62.8-80.9%), indicating that this block copolymer was dominant over RG-I with the pectin structure assuming that isolates contained free pectic stretches. The pH 1.2-AMP isolate consisted of more HG block copolymers than the remainder, which was reflected in the HG RG-I ratio, ranging from 1.5 to 4.2 (Table 1). The values of the HG/RG-I ratio suggested that 2 to 4 HG per RG-I block copolymers might be present within AMP isolates.

Degree of esterification

The degree of methylesterification (DM) of extracted pectins ranged from 64-82 (Table 1), indicating that sour sop cell wall pectins are highly methylesterified. The highest value of DM was obtained at pH 2.0 and the lowest at pH 1.2, probably due to degradation of pectin ester-groups under more severe extraction conditions. The degree of acetylation (DAC) was relatively low (≤10%). On the basis the results obtained, the extraction conditions of S/L (1:25 w/v), T (75°C), t (90 min), and pH 1.2 appeared to be the optimum conditions for the
isolation of pectin with high GalA content (>65%) and DM (>60%), from soursop pomace, two of the quality characteristics that needed to be fulfilled for possible mass production and marketing.

Macromolecular features

The intrinsic viscosity ([η]) and molecular weight of AMP ranged from 307-408 mL/g and from 62-105 kDa, respectively (Table 1). The intrinsic viscosity of pH 1.6-AMP isolate was the smallest, followed by the intrinsic viscosity of pH 2.0-AMP isolate and finally by that of pH 1.2-AMP isolate. By contrast, the molecular weight of pH 1.6-AMP isolate was the highest, followed by the molecular weight of pH 1.2-AMP isolate and finally by the molecular weight of pH 2.0-AMP. These results suggested that the former pectin very likely had an overall compact sphere-like conformation, while the latter may have a rather extended rod-like conformation. This somewhat inverse relationship which was observed between the pectin Mv and [η] could be explained by higher degree of branching (DBr) of pH 1.6-AMP isolate with amply flexible GA side chains, thereby fostering a roll-up of the macromolecule over on itself, a result of which was an overall sphere-like compact macrostructure with a shorter hydrodynamic size [1,10,22].

Gelling capacity

The strength of AMP gels ranged from 98-163 (Table 1). The pH 1.2-AMP isolate had a much greater gelling ability (>150), one of the international quality characteristics required for possible marketing. This could be accounted for by high GalA content (>65%), DM (>60%), and Mv (>80 kDa), three parameters known to substantially influence sugar-acid-mediated gelation of pectin [5,6]. The gelling ability of pH 2.0-AMP was considerably low, and therefore this sample may be more suitable for the preparation of calcium-mediated gels rather than for sugar-acid-mediated gels. This rather weak gelling power might be caused by the relatively low GalA content and Mv, but probably also by the presence in this sample of contaminating starchy oligosaccharides, which acted as destabilizing agent of pectin gel networks [6].

The gelling capacity of pectin from a plant source is (one of the most determining factors for possible industrial production. Our results showed that pectin with rather good yield, quality characteristics and gelling capacity could be extracted from soursop pomace under specified conditions.

Conclusion

Investigation on the pectin content of soursop pomace showed that this domestically available byproduct is a pectin-rich source. Soursop pomace contains approximately 17% pectic substances. Under optimized extraction conditions about 8% pectin, which fulfills required quality characteristics (galacturonic acid amount >65%, degree of methoxylolation >60%, average-molecular weight >70-80 kDa and gelling ability >150) for possible mass production, has been purified from this raw material. Soursop pomace then appeared to be a potentially new source of industrial pectin able to partially substitute for benchmark citrus and apple pectins.

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