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Physicochemical properties of pectins from ambarella peels (*Spondias cytherea*) obtained using different extraction conditions

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Abstract

Extraction and use of pectins from ambarella peels could add value to the waste products arising from processing of the fruit. Dried alcohol-insoluble residues (AIR) of ambarella peels were treated separately with HCl, deionised water and oxalic acid/ammonium oxalate solutions, and the resulting pectin extracts analysed for some biochemical and physicochemical parameters. The results show that pectin yield (9–30% dry AIR), uronic acid (557–727 mg/g dry weight), neutral sugars (125–158 mg/g), degree of methylation (50–58%) and acetylation (4–6%), molar mass (263,000–303,000 g/mol) and intrinsic viscosity (179–480 ml/g) varied significantly ($p < 0.05$) with the various extraction methods used. Extraction with oxalic acid/ammonium oxalate solution gave the highest pectin yield, with high molar mass and degree of methylation, making the extracts suitable for use as additives in the food industry. The results compared well to lime pectin extracted under the same conditions, indicating their commercial significance.

Keywords: Ambarella peels; Pectins; Physicochemical properties; Extraction conditions

1. Introduction

Ambarella or golden apple (*Spondias cytherea*) is a tropical fruit belonging to the family of Anacardiaceae (Morton, 1987). In addition to its sweet and pleasant flavour (Graham, Wickham, & Mohammed, 2004; Jirovetz, Buchbauer, & Ngassoum, 1999), this fruit is a good source of minerals and vitamin C (Ishak, Ismail, Noor, & Ahmad, 2005). Ambarella fruit is quite delicious to eat when fresh and it is often used for making jelly, jams, pickles, relishes, soups, stews and juices (Morton, 1987) or may be canned in sucrose (Ramsundar, Comissiong, Badrie, Baccus-Taylor, & Spence, 2002). The peels represent about 19% of the total fruit weight and they constitute the main part of byproducts. Citrus peel and apple pomace are the major raw materials used for the production of commercially acceptable pectins (May, 1990). Pectin preparations from ambarella peels could serve as an alternative.

Pectins are a family of complex polysaccharides mainly present in the primary cell wall and intercellular regions of dicotyledons (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Structurally, they are generally described as an alternation of "smooth" (made of homogalacturonans, HGs) and "hairy" (made of type I rhamnogalacturonans, RGs-I) regions. HG is composed of (1 → 4)-linked α-D-galacturonic acid (Gal*p*A) residues that can be partly methylesterified at C_6 (Pilnik & Voragen, 1970) and possibly partly acetylesterified at O_2 or O_3 (Ralet, Bonnin, & Thibault, 2005).

Pectins have many applications in food science and nutrition, cosmetics and pharmacy. They are widely used

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as food additives for thickening, gelling and emulsifying jams, soft drinks, fish, meat products and milk products (Barrera, Ramírez, González-Cabrialezs, & Vázquez, 2002; May, 1997; Ralet et al., 2005). Commercially, pectins are extracted by treating the raw material with hot dilute mineral acids, mainly nitric acid (Rolin, 2002). The extraction conditions are in the range of pH 1.5–3, temperature 60–100 °C and time 0.5–6 h. Solid:liquid ratios of 1:17 for apple and 1:35 for citrus are often used (Ralet, Bonnin, & Thibault, 2002). These native pectins are generally highly methylated (DM > 50%) and slightly acetylated (Voragen et al., 1995). They gel at low pH (2.5–3.8) with a high sugar concentration (>55%) (Ralet, Crépeau, Bucholt, & Thibault, 2003; Rolin, Nielsen, & Glahn, 1998).

Relationships between pectin fine structure and functional properties do exist (Ralet & Thibault, 2002). Many studies have shown that the biochemical characteristics of pectins depend on the plant source and the extraction method used. Sugar beet pectins extracted by acid (HCl or HNO_3) at variable pH (1–3), temperatures (75–90 °C) and times (30–90 min) exhibited a galacturonic acid content and a degree of methylation varying from 295 to 528 mg/g (dry weight) and from 34 to 94%, respectively (Levigne, Ralet, & Thibault, 2002a). Pectins from *Ceni* mango peels extracted by deionised water exhibited a galacturonic acid content and a degree of methylation of 860 mg/g and 73%, respectively. When extraction was performed with HCl (pH 1.5/85°C/ 1 h), *Ceni* mango pectins exhibited a lower galacturonic acid content and a similar high degree of methylation (660 mg/g and 76%, respectively) (Kratchanova, Benemou, & Kratchanova, 1991). Hence the source of pectin and the extraction conditions used have a significant impact on pectin biochemical characteristics and their suitability for application. Presently, there is hardly any information on the extraction of ambarella pectin.

The aim of this study was to evaluate the impact of different extraction conditions on the yield and some biochemical characteristics of ambarella peel pectins and their suitability as a source of industrial pectin.

2. Materials and methods

2.1. Sampling and sample treatment

Ripe ambarella (*S. cytherea*) and lime (*Citrus latifolia*) were harvested in an orchard near the city of Yaounde (Cameroon). The peels of the fruits were removed and chopped into pieces of 1 cm^2 using a stainless steel knife. The peels were then bleached in boiling water for 5 min and dried in an air convection oven at 50 °C. Alcohol-insoluble residue (AIR) was prepared by treating the dried peels four times with ethanol (85 vol.%) at 70 °C for 20 min.

2.2. Extraction of pectin

Three different extraction conditions were applied: hydrochloric acid (0.03 M; pH 1.5) at 85 °C for 1 h, deionised water at 75 °C for 1 h and oxalic acid/ammonium oxalate (0.25%), pH 4.6, at 85 °C for 1 h. About 100 g of AIR were stirred with 4 l of each of the above extracting solutions. The extracts were separated from the AIR residues by filtering through a nylon cloth, and the pectin was coagulated with 3 volumes of ethanol (96%). Pectin coagulates were washed several times with 70% ethanol and finally with 96% ethanol and acetone before vacuum-drying at 50 °C overnight.

2.3. Chemical characterisation

The uronic acid contents of the peels, AIR and extracted pectins were determined colorimetrically by the automated *m*-phenylphenol method (Thibault, 1979).

Neutral sugars were analysed as their alditol acetate derivatives by gas liquid chromatography (GLC) after acid hydrolysis. The pectin was hydrolysed with 1 M H_2SO_4 (3 h, 100 °C). The peels and AIR were prehydrolysed with 13 M H_2SO_4 (30 min, 25 °C), diluted to 1 M and heated (2 h, 100 °C). The individual neutral sugars obtained were reduced with $NaBH_4$, acetylated and analysed by GLC according to (Blakeney, Harris, Henry, & Stone, 1983). Inositol was used as internal standard.

Pectin methoxyl and acetyl contents were determined by HPLC. Pectins were treated with 1 N NaOH in the presence of $CuSO_4$, and the methoxyl and acetic acid released were quantified by HPLC on a C18 column, as previously described (Levigne, Thomas, Ralet, Quemener, & Thibault, 2002b). Isopropanol was used as internal standard. The degree of methylation (DM) and the degree of acetylation (DAc) were calculated as the molar ratio of methanol and acetic acid to galacturonic acid, respectively.

2.4. Determination of macromolecular parameters

Pectin average molar masses and intrinsic viscosities were determined using high-performance size-exclusion chromatography (HP-SEC), as described by Ralet, Bonnin, and Thibault (2001).

HP-SEC was performed at room temperature on a system constituted of one Shodex OH SB-G pre-column, followed by two columns in series (Shodex OH-Pack SB-804 HQ and OH-Pack SB-805 HQ, Shodex, Showa Denko KK, Miniato, Japan) eluted with 0.05 M $NaNO_3$ buffer, containing 0.02% NaN_3 as preservative, at a constant flow rate of 42 ml/h. Detectors used were a refractometer (RI) (ERC 7517 A), a differential viscosimeter (T-50A, Viscotek, Houston, TX) and a multiple angle laser light scattering device (MALLS) (Mini Dawn, Wyatt, Santa Barbara, CA) operating at three angles (41°, 90° and 138°). Pectins (~6 mg/ml) were solubilized in 0.05 M $NaNO_3$ containing 0.02% NaN_3, left overnight with tail-over-head continuous mixing, centrifuged and filtered (0.45 μm) before injection of 50 μl. Data for molar mass determinations were analyzed using Astra software (Wyatt, Santa Barbara, CA) taking a dn/dc of 0.146. Data for viscosimetry

determinations were analyzed using TriSEC software (Version 3.0, Viscotek, Houston, TX).

All analyses were performed in triplicate and the means of the results given on a dry weight basis. Results were compared using the Student's *t* test. Differences were determined by variance analysis ($p < 0.05$).

3. Results and discussion

3.1. Peels and AIRs uronic acid and neutral sugars contents

The uronic acid and neutral sugars contents of ambarella and lime peels and their corresponding AIRs are summarised in Table 1. Uronic acid (108 mg/g dry weight) and total neutral sugar contents (347 mg/g) of ambarella peels were found to be lower than those of their corresponding AIR (140 and 403 mg/g, respectively). Glucose (probably of cellulosic origin) was the main neutral sugar; the other neutral sugars were galactose, arabinose, mannose, xylose and rhamnose. Ambarella AIRs exhibited an overall distribution of individual sugars similar to that of raw peels. Compared to ambarella peels and AIR, lime peels and AIR exhibited higher amounts of uronic acid (192–248 mg/g) and total neutral sugars (347–484 mg/g) (Table 1). Compared to the raw peels, AIRs were richer in uronic acids and neutral sugars, most likely because some ethanol-soluble compounds, e.g., lipids and pigments, were removed by the ethanolic treatment (Selvendran & Du Pont, 1980). Values of uronic acid found in the literature show that mango AIRs are richer in uronic acids (213 mg/g) than are ambarella AIR (Kratchanova et al., 1991).

3.2. Yield, extractability and biochemical characteristics of ambarella peels pectins

3.2.1. Extraction yields

The yield of ambarella pectin extraction varied from 16% to 22% dry weight of AIR, depending on the extraction condition used. The highest yields were obtained with oxalic acid/ammonium oxalate (OAAO) and the lowest with water (Table 2). In comparison, yields for lime (9–30%) were lower under water extraction conditions while similar results (17%) were found for *Tommy Atkins* mango pectins extracted with H_2SO_4 (pH 1.5) (Berardini et al., 2005). Other results showed high extraction yields using HCl (pH 1.5) for *Ceni* mango pectin (24.5 dry weight% AIR) (Kratchanova et al., 1991).

Table 1
Individual neutral sugars and uronic acid contents (mg/g dry weight) of peels and their alcohol-insoluble residues (AIRs) from ambarella and lime

Characteristics		Samples			
		Ambarella		Lime	
		Peel	AIR	Peel	AIR
Individual neutral sugars (mg/g)	Rha	6.9 ± 0.2^{c}	7.2 ± 1.6^{c}	15.1 ± 0.1^{b}	19.6 ± 0.1^{a}
	Ara	55.4 ± 3.7^{c}	98.7 ± 1^{b}	97.2 ± 0.6^{b}	135.7 ± 0.5^{a}
	Xyl	18.2 ± 1.9^{c}	22 ± 3.3^{c}	26 ± 0.07^{b}	32.1 ± 0.1^{a}
	Man	21.8 ± 2.6^{b}	15.9 ± 1.3^{c}	22.1 ± 0.1^{b}	26.7 ± 0.1^{a}
	Gal	33.1 ± 2.8^{d}	75.2 ± 6.7^{a}	43.9 ± 0.1^{c}	55.7 ± 0.2^{b}
	Glc	208 ± 4^{b}	179 ± 13^{c}	207.3 ± 0.7^{b}	214.2 ± 0.8^{a}
Total neutral sugars (mg/g)		347 ± 16^{c}	403 ± 10^{b}	412 ± 6^{b}	484 ± 4^{a}
Uronic acids (mg/g)		108 ± 8^{d}	140 ± 6^{c}	192 ± 14^{b}	248 ± 10^{a}

Mean values from triplicate measurements ± standard deviation. Values in the same line followed by different superscripts are significantly different ($p < 0.05$).

3.2.2. Uronic acid and neutral sugars contents

Ambarella pectins had uronic acid (557–727 mg/g dry weight) and total neutral sugars (125–158 mg/g) contents that varied widely, depending on the extraction method used. Water-extracted pectins were particularly rich in uronic acids while OAAO-extracted pectins were rich in total neutral sugars. HCl-extracted pectins were poorest in uronic acid. Lime pectins extracted with HCl and OAAO were rich in uronic acid and poor in neutral sugars content (Table 2). Previous results showed that *Ceni* mango pectins extracted using HCl (pH 1.5) had high levels of uronic acids (660 mg/g) and total neutral sugars (320 mg/g) (Kratchanova et al., 1991).

HCl-extracted ambarella pectins were low in arabinose and rhamnose, and high in galactose and glucose. However, with the other extraction conditions, both arabinose and galactose contents were high (Table 2). There were only small amounts of xylose and mannose. The distribution patterns of individual neutral sugars for water-extracted ambarella pectins were similar to those observed with water-extracted lime pectins, and significantly different ($p < 0.05$) from those obtained under the other extraction conditions (Fig. 1). Other authors have found that galactose, arabinose and glucose are the three main neutral sugars of pectins and their proportions depend on the source of pectin and the extraction process used (Iagher, Reicher, & Ganter, 2002; Ralet & Thibault, 1994).

3.2.3. Pectin extractability

Pectin extractability was expressed as the amount of uronic acid extracted from the AIR [uronic acid (g) of the extracted pectin × yield per 100 g of uronic acid initially present in the AIR] (Table 2). Pectin extractability from ambarella was very high with OAAO extraction (98% of the uronic acids initially present in the AIR). Fairly high values were observed with HCl and water extractions (77–81%). As for lime, we obtained similar values with HCl and OAAO, while a very low value of extractability (14%) was found under water-extraction conditions. The lowest value obtained for lime is explained by the fact that the extractability is based on the yield and uronic acid content of the extracted pectins. Uronic acids, which are the main components of pectin, are well extracted under OAAO conditions.

3.2.4. Degree of methylation (DM) and acetylation (DAc)

Results in Table 3 show that OAAO- and water-extracted ambarella pectins were more methylated (DM

Table 2
Neutral sugar contents, uronic acid contents, yields and extractabilities of pectins obtained under different extraction conditions from ambarella and lime

Pectin characteristics		Extraction process and raw material used					
		HCl (pH 1.5)		Water		Oxalic acid/ammonium oxalate	
		Ambarella	Lime	Ambarella	Lime	Ambarella	Lime
Uronic acids (mg/g)		557 ± 13^{e}	776 ± 3.7^{b}	727 ± 4.5^{c}	493 ± 14^{f}	631 ± 7^{d}	831 ± 16^{a}
Total neutral sugars (mg/g)		125 ± 3^{c}	88 ± 1^{e}	130 ± 7^{bc}	136 ± 0.4^{b}	158 ± 2.7^{a}	97 ± 0.6^{d}
Individual neutral sugars (mg/g)	Rha	17	7.8	13.8	13	19	6.9
	Ara	19	4.4	51	51	63	5.7
	Xyl	3.2	0.9	1.9	0.8	2	0.8
	Man	4.3	2.8	1.9	1.5	1.6	1.5
	Gal	35	29	31	26	41	25
	Glc	46	3	28	28	31	4
Yield (mg/g)	Dry AIR	19.4 ± 0.6^{d}	26.9 ± 0.3^{b}	15.6 ± 0.5^{e}	8.8 ± 0.6^{f}	22 ± 0.3^{c}	29.7 ± 1.2^{a}
	Dry Peel	11.8 ± 0.4^{d}	19.8 ± 0.2^{b}	9.5 ± 0.3^{e}	6.7 ± 0.5^{f}	13.4 ± 0.2^{c}	22.6 ± 0.9^{a}
Extractability (%)		77 ± 4^{b}	82 ± 3^{b}	81 ± 2^{b}	14 ± 1^{c}	98 ± 2^{a}	97 ± 3^{a}

Mean values from triplicate measurements ± standard deviation. Values in the same line followed by different superscripts are significantly different ($p < 0.05$).

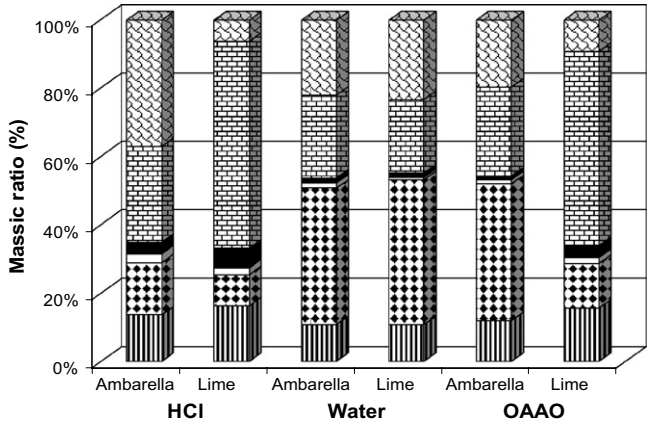

Fig. 1. Distribution patterns of individual neutral sugars of pectins extracted with HCl, water and oxalic acid/ammonium oxalate (OAAO) from ambarella and lime peels. (▥) Rhamnose; (▦) Arabinose; (□) Xylose; (■) Mannose; (▤) Galactose; (▨) Glucose.

58%) than were HCl-extracted pectins (DM 50%). Compared to those of ambarella pectins, the DMs of lime pectins were significantly higher (58–82%) ($p < 0.05$). Previous studies on acid- (HCl or H_2SO_4, pH 1.5) extracted pectins from *Ceni*, *Alphonso* and *Tommy Atkins* mango peels showed that high degrees of methylation (66% and 76%) could be obtained (Berardini et al., 2005; Kratchanova et al., 1991; Srirangarajan & Shrikhande, 1979). The enhancing influence of the HCl extraction on methylation degree was also shown on soy hull (Kalapathy & Proctor, 2001) and sugar beet pectins extracted at various HCl concentrations (Levigne et al., 2002a).

Ambarella pectins exhibited low degrees of acetylation (4.2–5.5%) for all three methods of extraction. Lowest values of DAc were found for lime pectins (1.2–1.6%) following HCl and OAAO extractions. The DAc values of ambarella extracted pectins were similar ($p < 0.05$) to that of lime pectin extracted with water (6.0%). Previous studies on acid-extracted apple and lemon pectins reported DAc values of 5.0 and 1.4%, respectively (Kravtchenko, Voragen, & Pilnik, 1992).

3.3. Macromolecular parameters

The weight average molar mass ($\langle M_w \rangle$) of ambarella pectins varied from 263,000 to 303,000 g/mol and their corresponding intrinsic viscosities ([η]) from 179 to 480 ml/g. The molar masses were the same (at $p < 0.05$) using water and OAAO extraction processes. The same was true for intrinsic viscosities of OAAO- and water-extracted pectins which had the highest values. Compared to ambarella

Table 3
Degrees of methylation (DM) and acetylation (DAc), weight average molar mass $\langle M_w \rangle$ and intrinsic viscosity ([η]) of pectins extracted under different conditions from ambarella and lime peels AIRs

Extraction process	Raw material	DM (%)	DAc (%)	$\langle M_w \rangle$ (g/mol)	[η] (ml/g)
HCl (pH 1.5)	Ambarella	50 ± 1.4^{d}	4.2 ± 0.1^{b}	$303{,}000 \pm 8^{a}$	179 ± 7^{e}
	Lime	75 ± 1.9^{b}	1.6 ± 0.01^{c}	$112{,}000 \pm 5^{e}$	337 ± 8^{d}
Water	Ambarella	58 ± 0.8^{c}	5.5 ± 0.3^{a}	$263{,}000 \pm 22^{b}$	480 ± 7^{b}
	Lime	82 ± 2^{a}	6 ± 0.2^{a}	$252{,}000 \pm 18^{d}$	414 ± 9^{c}
Oxalic acid/ammonium oxalate	Ambarella	58 ± 0.7^{c}	5.5 ± 0.3^{a}	$263{,}000 \pm 20^{b}$	480 ± 4^{b}
	Lime	58 ± 1.4^{c}	1.2 ± 0.02^{d}	$255{,}000 \pm 10^{c}$	757 ± 8^{a}

Mean values from triplicate measurements ± standard deviation. Values in the same column followed by different superscripts are significantly different ($p < 0.05$).

pectins, the molar masses of HCl-extracted lime pectins (112,000) were lower. HCl- and water-extracted lime pectins exhibited intermediate intrinsic viscosities (337–414 ml g^{-1}) while OAAO-extraction gave correspondingly higher values (Table 3). These results show the important variability of molar mass and intrinsic viscosity of pectins according to the extraction conditions used. Macromolecular parameter values depend, not only on the extraction process used, but also on the plant material used. *Ceni* mango pectins extracted with the same extractant (HCl, pH 1.5) had low molar mass (83,000 g/mol) (Kratchanova et al., 1991) but sugar beet pectin extracted at pH 2 (HCl) had high molar mass (377,000) (Levigne et al., 2002a). These differences of molar masses could also be due to the type of method used for the determination of molecular parameter. According to Ralet et al. (2002), the water (in)-solubility and the aggregation of pectin could influence molar mass measurement.

4. Conclusion

The results showed that ambarella peels are a source of highly methylated pectins with high molecular weight. The extraction conditions had a significant impact on the biochemical characteristics of the extracted pectins. Oxalic acid/ammonium oxalate (OAAO) allowed high extraction yields, the recovered pectins being of high degree of methylation, high molar mass and high intrinsic viscosity. HCl extraction gave low yields of pectins with low uronic acids and DM. Water extraction gave the lowest yields of pectins with high degrees of methylation and high intrinsic viscosity. On the whole, OAAO-extraction of pectins from ambarella provides good yields, which are biochemically interesting due to their high molar mass. Since molar mass and degree of methylation are important parameters in gel-breaking strength (Voragen et al., 1995), OAAO ambarella pectins could be useful as food additives. Further studies are being conducted to investigate the rheological and gelling properties of ambarella peel pectins.

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