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Chemical characterization of pectin from green tea (*Camellia sinensis*)

Jean-Pierre Ele-Ekouna, Corinne Pau-Roblot*, Bernard Courtois, Josiane Courtois

Laboratoire des Polysaccharides Microbiens et Végétaux, Université de Picardie Jules Verne, IUT d'Amiens, dept GB, Avenue des Facultés, Le Bailly, 80025 Amiens cedex, France

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ABSTRACT

From green tea leaves, two distinct pectin fractions were obtained based on their solubility in water. Polyphenols were detected only in the easily water soluble fraction (P1). The estimated uronic acids/neutral sugars ratio was 1.7 in the easily water soluble pectin fraction (P1), and 1.0 in the less water soluble fraction (P2). Homogalacturonan sequences (HGAs) corresponded to about 62% of the P1 pectin fraction but only 47% of the P2 fraction. After degradation of the two pectin fractions by pectin lyase, chemical studies revealed rhamnogalacturonan RG I and RG II regions present in the P1 pectin fraction, whereas only RG I sequences were detected in the P2 pectin fraction. The degree of substitution was lower for HGAs of the P1 pectin fraction than P2. Different acetylation patterns for the two fractions were observed. Polyphenols extracted simultaneously with pectins were present only in HGA fractions from P1.

1. Introduction

Pectins are polysaccharides present in all plant primary cell walls and in the middle lamella of dicotyledons, where, the polymers are involved in plant growth and development (Ridley, O'Neill, & Mohnen, 2001). Numerous scientific reports, for more than 50 years, have described the composition of pectins obtained from various plants. However, the complete structure determination of these molecules remains difficult in view of their high molecular mass, the lack of homogeneity and absence of repeating units (Mohnen, 2008; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Three sequences are identified in all pectins. The first one corresponds to a linear chain of α-(1 → 4)-linked D-galacturonic acid residues partially substituted by methyl and acetyl groups. In this case, the degree of methylation and acetylation varies relative to the pectin origin (Bédouet, Denys, Courtois, & Courtois, 2006; Parrone et al., 2002; Ralet et al., 2005) and also throughout the plant development (Bédouet et al., 2006; Stewart, Iannetta, & Davies, 2001). This sequence noted smooth region or homogalacturonan (HGA) is occasionally interrupted by two hairy regions noted rhamnogalacturonans (RG): RG I and RG II (O'Neill, Albersheim, & Darvill, 1997). The RG I sequence is composed of alternating α-D-galacturonic acid and α-L-rhamnose with side chains linked to C-4 of rhamnose. These RG I side chains are composed mainly of arabinose and galactose and depending on the pectin origin, various concentrations of minor components such as xylose or glucose are observed (Duan, Wang, Dong, Fang, & Li, 2003). The RG II sequence corresponds to a main chain made of α-(1 → 4)-linked galacturonic acid residues, similar to a HGA sequence, associated with four different oligosaccharide side chains containing common monosaccharides such as Rha, Fuc, Ara, Xyl, Gal and GlcA and also rare ones as Kdo, Api and Dha (Herve du Penhoat, Gey, Pellerin, & Perez, 1999). The RG II structure is maintained whatever the pectin origin (O'Neill et al., 1996; Thomas, Darvill, & Albersheim, 1989). In addition to the three sequences present in all pectins, a fourth one corresponding to a xylogalacturonan has been detected in apple pectins (Schols, Bakx, Schipper, & Voragen, 1995).

Several properties are allotted to pectins as cementing agents in plant cell walls (Rees & Wight, 1969) or during fruit ripening (Knee, 1978), but they are mainly known for their roles in food processing (Rombouts & Pilnik, 1978) and as dietary fibre. When extracted from plants, these pectins are used as thickening agents in the food and in the pharmaceutical industries (Drusch, 2007; Kim & Fassihi, 1997; May, 1990). The quality of thickening depends on the presence of sucrose for high-methoxyl pectins or an adequate concentration of a divalent cation such as calcium for low-methoxyl pectins (Pilgim, Walter, & Oakenfull, 1991). In plant foods, pectins are often classified as indigestible dietary fibres; by increasing the viscosity of the chyme in the upper tract, they encourage regular bowel movement and take part in the absorption of nutriments such as glucose (Kim, 2005; Vervuert, Klein, & Coenen, 2009). Besides, pectins are considered as preventing agents against hyperlipidemia (Chau, Chen, & Wang, 2004; Sudheesh &

* Corresponding author. Tel.: +33 0322534092; fax: +33 0322534094.
E-mail address: Corinne.pau-roblot@u-picardie.fr (C. Pau-Roblot).

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Vijayalakshmi, 1999), and activities against bowel cancer have been proposed (Kritchevsky, 1995).

Numerous studies are available for pectins from fruits as citrus, apple or grape, but limited information is available for pectins present in tea infusions, the most consumed beverage in the world. Studies concerning the health benefit of tea have been reported since 1992 (Huang et al., 1992), and concern mainly polyphenols diffusing from the plant to the water (Chung, Wong, Wie, Huang, & Lin, 1998; Ferrara, Montesano, & Senatore, 2001; Huang et al., 1992; Kilmartin & Hsu, 2003; Perva-Uzunalić et al., 2006). In fact, anti-oxidant properties have been described for these polyphenols (Almajano, Carbó, López Jiménez, & Gordon, 2008; Frei & Higdon, 2003; Lu, Lee, Maud, & Lin, 2010), in addition to other activities on cancer and cardiovascular diseases (Khan & Mukhtar, 2007; Kris-Etherton & Keen, 2002). More recently, tea extracts have been proposed as natural preservatives in food due to their anti-oxidant and anti-microbial activities (Almajano et al., 2008). In contrast, few studies are available on tea pectins. The aim of this study was to characterize pectin molecules found in green tea beverages. The results obtained could serve as foundation for further research on the potential bioactivity of tea pectins in relation to the polymer structure.

2. Materials and methods

2.1. Plant material

Green tea (*Camellia sinensis*; white Monkey) was from China, Fujian province. It corresponds to tea leaves only submitted to steam, prior to drying.

2.2. Pectin extraction and fractionation

To remove lipids and pigments, green tea leaves (3 g) were first grinded to a powder and suspended during 5 min at 4 °C in 100 ml of sodium acetate buffer (50 mM, pH 5). After filtration through a Whatman filter (no. 3), the retained material was washed twice (30 min) with 100 ml of a CH_3OH–$CHCl_3$ (1:1, v/v) mixture. Then, a new filtration through a Whatman filter (no. 3) was performed and the retained material was suspended in a 60% (v/v) aqueous methanol solution (100 ml). After 35 min-stirring at ambient temperature, filtration through a Whatman filter (no. 3) was performed and the retained material, called the insoluble tea leaf fraction (TLF), was collected for subsequent pectin extraction.

Two extraction procedures were applied. For the first one, pectin was first obtained from TLF by distilled water (100 ml) upon a 30 min-stirring at 70 °C. The suspension was then cooled at room temperature and centrifuged (10 min, 12,000 × *g*, 15 °C). The supernatant was recovered, supplemented with three volumes of a 95% (v/v) aqueous ethanol solution, and finally the mixture was stored at 4 °C overnight. The precipitate formed called F was recovered by centrifugation (10 min, 10,000 × *g*, 4 °C), dried in an oven (40 °C) and weighted. The dried extract was then suspended in water (10 ml), and the solution was stirred at ambient temperature. After a 3 h-stirring, the precipitate was not completely dissolved, so, the solution was centrifuged (10,000 × *g*, 4 °C, 10 min) in order to collect the supernatant called F1 representing the fraction easily dissolved in water. The pellet called F2 was suspended in water (10 ml), which was stirred during 10 h for complete dissolution. F1 and F2 fractions were dried by lyophilisation and weighted.

A second procedure was applied: 100 ml of HCl (50 mM) was added to the TLF material instead of distilled water. After 30 min-stirring at 70 °C as above, the preparation was cooled, and the pH was adjusted to 5 with 1 M NH_4OH. The preparation was centrifuged, and the supernatant was supplemented with ethanol as above. A precipitate called P was obtained, dried as described previously for F and weighted. The P product was suspended in water, and the preparation was stirred as for F. After a 3 h-stirring the dissolution was incomplete, the solution was centrifuged as for F, the supernatant was called P1 and a complete dissolution of the pellet called P2 was obtained after a 10 h-stirring as for F2. Fractions P1 and P2 were dried and weighted as F1 and F2.

2.3. Quantitative determination of protein and polyphenol contents

The protein content of pectin extracts was estimated according the method described by Bradford (Bradford, 1976) using bovine serum albumin (BSA) as a standard.

For polyphenol assays, 4 ml of pectin extracts (5 g/l) were added to 5 ml of a ferrous tartrate tetrahydrate solution in water (ferrous sulphate and potassium sodium tartrate tetrahydrate 0.1% and 0.5% (w/v), respectively), and then 15 ml of potassium phosphate buffer (0.067 M, pH 7.5) was added (Ruan & Li, 1983; Song et al., 1987). The mixture was maintained at ambient temperature during 10 min. The polyphenol content of the mixtures was determined by comparison of its absorbance at 540 nm to the absorbance from a calibration curve obtained with quercetin (Sigma–Aldrich Chimie S.a.r.l., Saint Quentin Fallavier, France) solutions from 0.1 to 1.0 mg/ml. Analyses were carried out in duplicate for three different samples.

2.4. Determination of sugar composition

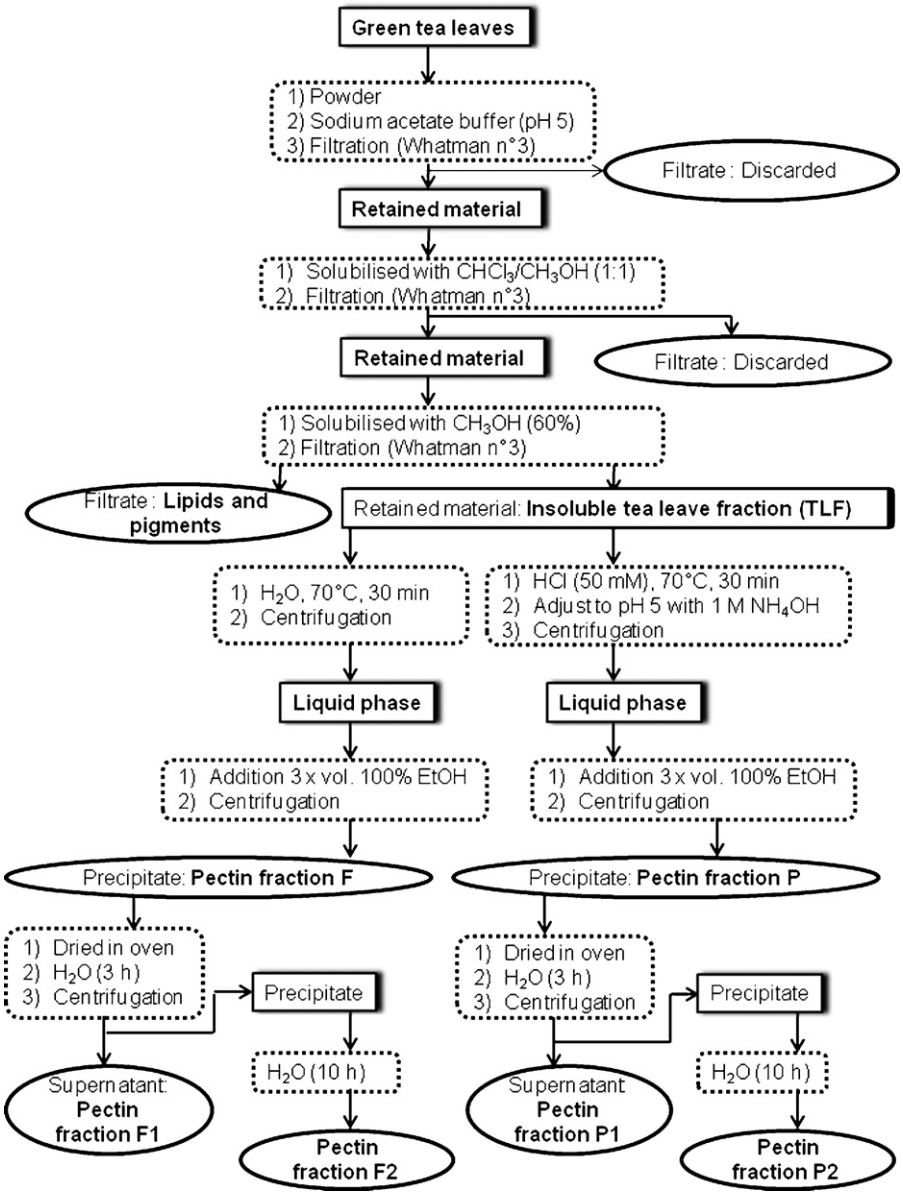
The uronic acid (UA) and neutral sugar (NS) content of the pectin extracts was determined using, respectively, *meta*-hydroxybiphenyl (*m*-HBP) (Thibault, 1979; Van den Hoogen et al., 1998) and resorcinol (Monsigny, Petit, & Roche, 1998) on microtitration plates (nunc, Maxisorp, VWR international S.A.S., Fontenay-sous-bois, France) further analysed with a microplate spectrophotometer (Opsis MR; Dynex technologies, VA, USA). L-Arabinose and D-galacturonic acid (Sigma–Aldrich Chimie S.a.r.l., Saint Quentin Fallavier, France) were used as standards. The UA content was directly determined with the *m*-HBP test. The NS quantification was calculated after correction of the interference due to uronic acid for the resorcinol assay. All analyses were performed in triplicate.

2.5. Polysaccharide hydrolysis and trimethylsilylation of sugars

Pectins and pectin oligomers (5 mg/ml) were methanolysed with 0.5 M HCl in methanol (2.5 ml), under argon, at 80 °C during 20 h, and then the methanolysates were dried under argon. *O*-methylglycosides were dissolved in 400 μl of a pyridine/BSTFA [*N*,*O*-bis (trimethylsilyl)-trifluoroacetamide with trimethylchlorosilane] mixture (1:1, v/v) provided by Pierce Thermo Scientific Inc., Rockford, IL. The preparation was incubated at 4 °C for 10 h before GC–MS analysis.

2.6. GC–MS analysis

The sugar composition was determined by gas chromatography (GC) (HP 6890) coupled with a mass spectrometer (MS) (Agilent Technologies, Massy, France) operating in EI-MS mode. The column was a HP1 polydimethylsiloxane capillary column (25 m × 200 μm). The GC temperature program was 120 °C for 10 min, linear temperature gradient of 5 °C/min to 240 °C, and hold at 240 °C for 10 min. The carrier gas was helium at flow rate of 0.9 ml/min (inlet 250 °C, transfer line 280 °C, source 220 °C, electron impact ionization (EI) 70 eV).



Scheme 1. Extraction procedure of pectin from green tea leaf.

2.7. NMR analysis

^{1}H NMR spectra of pectin extracts dissolved in D_2O (99.9% D, Aldrich) (10 mg/ml) were recorded at 80 °C on a Bruker Avance 300 spectrometer (Bruker BioSpin S.A., Wissembourg, France) with pre-saturation of the residual water signal. ^{1}H spectra were obtained with a spectral window of 3000 Hz for 16 k data points with a pulse length of 7 μs, an acquisition time of 2.74 s, and a relaxation delay of 1 s. ^{1}H chemical shifts were expressed in parts per million (ppm) relative to internal 3-trimethylsilylpropionate-d_4 (TSP-d_4). All NMR experiments were carried out using the pulse sequences provided by Bruker.

2.8. Pectin esterification analysis

The degree of methylation (DM) and the degree of acetylation (DA) were determined by quantification of methanol and acetate after saponification of pectin extracts. Pectins were dissolved in D_2O (10 mg/ml), and a first ^{1}H NMR experiment at 80 °C was performed in order to check the absence of free methanol and acetate. Then, 100 μl of NaOD (1 M) in D_2O was added in the NMR tube, and 256 scans were further recorded 10 min after the addition of NaOD. Under the conditions applied, saponification was complete, and no methanol evaporation was observed. The DM and DA values were then calculated as described previously (Bédouet, Courtois, & Courtois, 2003) and expressed in percent per residue.

2.9. Enzymatic digestion and oligosaccharides purification

The endo pectin lyase (PL) (EC 4.2.2.10) from *Aspergillus niger* (Sigma–Aldrich Chimie S.a.r.l., Saint-Quentin Fallavier, France) was used to cleave pectins. Oligomers produced by enzymatic hydrolysis contained a terminal non-reducing residue with a 4,5-double bond due to a β-elimination cleavage mechanism. PL (0.02 U/mg of pectin) was added to pectin extracts and dissolved in a sodium phosphate buffer (50 mM, pH 5). After 8 h of incubation at 37 °C, the degradation was stopped by heating at 80 °C for 10 min; then, the mixture was cooled to 4 °C and centrifuged (10,000 × *g*, 4 °C, 5 min). The supernatant containing pectin oligomers was recovered, desalted over cation-exchange resins (Dowex 50Wx8

Table 1
Yield and composition of pectin fractions extracted from green tea leaves with hot water (F1 and F2) and acidic (P1 and P2) extraction. Values were obtained from five distinct analyses.

	F1	F2	P1	P2
Yield (%)[a]	0.050 ± 0.010	0.015 ± 0.010	2.000 ± 0.100	1.200 ± 0.050
Protein (%)[b]	Trace[c]	Trace[c]	0.13 ± 0.01	Trace[c]
Polyphenol (%)[b]	13.0 ± 0.1	nd	11.0 ± 0.1	nd
Sugar (%)[b]	87.0 ± 0.2	12.8 ± 0.1	88.9 ± 0.2	11.0 ± 0.1
UA (%)[d]	62.6 ± 0.2	50.2 ± 0.2	62.1 ± 0.2	49.5 ± 0.2
NS (%)[d]	36.9 ± 0.2	49.7 ± 0.2	36.8 ± 0.2	50.1 ± 0.2

nd: not detected.
[a] Calculated as weight % of initial material.
[b] Calculated as weight % of extracted fractions.
[c] Present at trace levels and not quantified.
[d] Uronic acid (UA) and neutral sugar (NS) contained in the sugar fraction.

miniature columns, Sigma–Aldrich Chimie S.a.r.l., Saint-Quentin Fallavier, France) and dried by lyophilisation.

Size fractionation of the degraded pectins was carried out in a column (21.5 × 300 mm) filled with TSK G 2000 SW gel (Tosoh Bioscience GmbH, Stuttgart, Germany). The eluent was 50 mM ammonium acetate buffer pH 5 operating at a flow rate of 6 ml/min, and the injected samples were 2 ml of pectin solutions in the elution buffer (5 mg/ml). The detector was a RID10A refractometer (Shimadzu, Champs-sur-Marne, France).

3. Results and discussion

3.1. Characterization of pectin fractions

The pectin extraction procedure from green tea leaves is described in Scheme 1. The hot water pectin extract produce fraction called F which was dried and weighted. From five independent extractions, fraction F represented only 0.065 ± 0.010% (w/w) of the total tea leaf fraction (TLF). Two sub-fractions were obtained from F: the first one called F1 was dissolved in water more quickly (3 h) and more easily than the second one called F2 (10 h). These fractions represented, respectively, 77% and 23% of the F extract (w/w). In the two sub-fractions from F, protein was detected as trace, whereas polyphenols were present only in F1 (Table 1). The sugar composition of the two F sub-fractions revealed to be different: in F1, the UA/NS ratio was 1.69 ± 0.20 (Table 1) compared to 1.01 ± 0.20 in F2. These results indicate the presence of two distinct polysaccharides in green tea leaf extracts. Our results were compared to those described in the literature in order to determine if the presence of the two polysaccharides was due to the existence of two distinct macromolecules in a same organ or to leaves collected on plants at different steps of their development. Until now, only the sugar content in pectins, but also the content of alkaloids and catechins in flax or in tea leaves, have been described to change along the development of the plant (Bédouet et al., 2006; Perva-Uzunalić et al., 2006). So, the presence of two sub-fractions in F may correspond to two distinct macromolecules extracted from leaves harvested on plants at different steps of development, such as nascent leaves in buds and mature ones. However, at this level of experimentation, it was not possible to explain the presence of polyphenols in F1. In fact, macromolecules in F1 contaminated by polyphenols were collected after precipitation with ethanol, but, normally, under the conditions applied, polyphenols should be soluble in this solvent. Further analysis of the polysaccharide structure would require more plant extracts which was not feasible with the hot water extraction procedure used in this study. The polysaccharide yield was too low. Another extraction methodology was considered to increase the yield. An acid extraction methodology, HCl (50 mM) at 70 °C for 30 min, was used and shown not to affect the sugar content and substitution of pectin extracts (Bédouet, Courtois, & Courtois, 2005; Bédouet et al., 2006). A fraction called P was extracted, which represented 3.20 ± 0.15% of the TLF (w/w). The P extract was composed of two sub-fractions differing by their water solubility as observed previously for the F extract. The sub-fraction quickly and easily dissolved in water (3 h) was called P1 and represented 62.5 ± 0.2% of the P extract (w/w). The second sub-fraction, P2, dissolved in water after a 10 h-stirring and corresponded to 37.5 ± 0.1% of the P extract (w/w) (Table 1). Proteins were present as traces in P as in F sub-fractions. Polyphenols were detected only in P1. In sub-fraction P1, the ratio UA/NS was 1.69 ± 0.20, as in F1 and 0.99 ± 0.20 both for F2 and P2 (Table 1). Extraction yield and composition for P1 and P2 after HCl (50 mM) extraction and for F1 and F2 after hot water extraction revealed to be similar except for the yields (Table 1) where the ratio P1/P2 was lower than that of F1/F2. Due to the small amount of material derived from F, the characterization of the two distinct fractions obtained from green tea was further developed for P1 and P2.

3.2. Polymers composition

^{1}H NMR spectra from P1 and P2 (data not shown) presented a high similarity except for signals corresponding to protons of methyl and acetyl residues. From these spectra, the degree of methylation was estimated to be about 17% higher in P2 than in P1, whereas the degree of acetylation of P2 molecules was about 11% lower than that for P1 (Table 2). Sugar analysis revealed that both fractions were rich in galactose, arabinose, rhamnose, xylose and fucose in decreasing order. The ratio of galacturonic acid residues was about 25% lower in P2 than in P1 (Table 2), but the proportion of the two deoxyoses, rhamnose and fucose, was higher in the

Table 2
Degree of substitution by methyl groups (DM) and acetyl groups (DA) and monosaccharide composition (mol%) for pectin fractions obtained from green tea leaves under acidic extraction. Values were obtained from five distinct analyses.

	P1	P2
DM[a]	52.0 ± 0.2	61.0 ± 0.2
DA[a]	36.0 ± 0.2	32.0 ± 0.2
GalA	60.70 ± 0.20	48.40 ± 0.10
Ara	14.50 ± 0.05	17.50 ± 0.05
Gal	14.10 ± 0.05	18.90 ± 0.05
Rha	3.40 ± 0.02	7.30 ± 0.02
Xyl	0.60 ± 0.01	2.50 ± 0.01
GlcA	1.20 ± 0.01	nd
Fuc	0.40 ± 0.01	1.40 ± 0.01
Glc	0.80 ± 0.01	nd
Api	Trace	nd

nd: not detected.
[a] Calculated as % of sugars fraction methylated or acetylated.

less water soluble pectin fraction (P2). In addition, the presence of glucuronic acid and glucose as well as traces of apiose in P1 was detected. The presence of such carbohydrates led to suspect the presence of RG II sequences in the P1 pectin extract, in addition to the RG I sequences generally found in all pectin extracts. So, the pectin fraction easily dissolved in water (P1) contained high amounts of HGA, some RG I and traces of RG II. In P2, RG II seemed to be absent, and the proportion of HGA was lower than that of RG I. The presence of xylose in RG sequences from the two distinct pectin fractions may be due to xylogalacturonan sequences in tea leaf pectin as described for apple pectins (Schols et al., 1995). Moreover, the methyl esterification of carboxylic groups from galacturonic residues was higher in P2 than in P1. The differences of the sugar composition and the substitution degrees for P1 and P2 pectin fractions could explain their different water solubility.

3.3. Characterization of fractions derived from pectin degradation

In order to characterize the P1 and P2 pectin fractions obtained from green tea leaves, the analyses of RG and oligogalacturonan (OGA) sequences in the two extracts were considered. A partial degradation of the pectin fractions was carried out using an endo-pectin lyase. The degradation products were then fractionated by size-exclusion chromatography on a TSK SW 2000 column (Fig. 1). The two elution profiles from P1 and P2 pectin fractions were similar, only the ratios between the different fractions varied. The three peaks obtained were noted A, B, and C. Sub-fractions A from P1 and P2 eluted between 7 and 12 min that is in the column void volume, so the molecular weight of molecules contained in sub-fraction A is likely to be higher than 3×10^4 according to the column characteristics, and sub-fractions B and C were classified as oligomers. Sub-fractions A, B and C from P1 and P2 were collected, lyophilised and weighted.

Monosaccharide sugars composition analysis (Table 3) revealed that arabinose and galactose were the main components of the high-molecular-weight fractions P1A and P2A, suggesting that these fractions consist mainly of RG I sequences (Fig. 2). However, the detection of apiose and glucuronic acid residues in P1A led us to consider the presence of RG II sequences only in the P1 easily water soluble tea pectin extract. The presence of low amounts

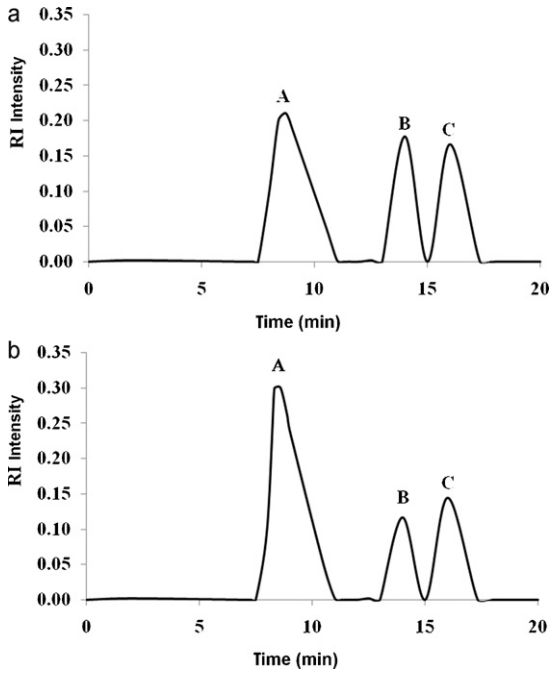


Fig. 1. Size-exclusion chromatography on a TSK Gel G2000 SW column of pectin fractions obtained upon pectin-lyase degradation of P1 (a) and P2 (b) (5 mg/ml in 50 mM CH_3COONH_4 buffer pH 5). The eluent was 50 mM CH_3COONH_4 buffer pH 5. The flow rate was 6 ml/min. The detector was a refractometer. A, B and C corresponded to the collected fractions.

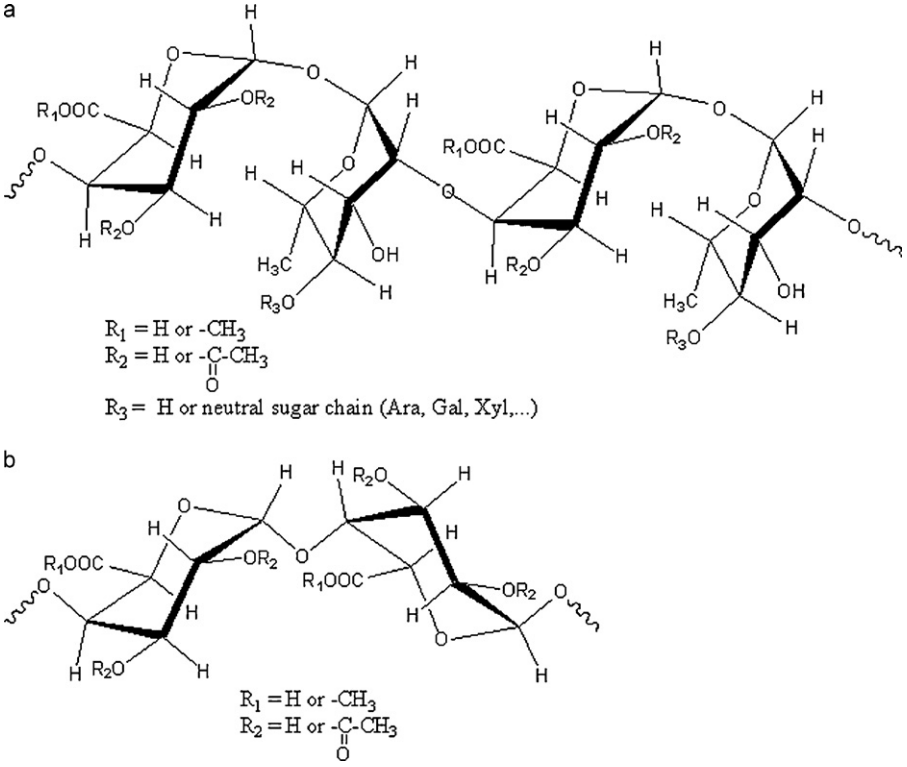


Fig. 2. Chemical structure of tea pectin fractions: (a) structure for P1A and P2A: RG I sequence and (b) structure for P1B, P2B, P1C and P2C: HGA sequence.

Table 3
Yield and composition of sub-fractions (A, B, C) obtained from P1 and P2 pectin fractions degraded by a pectin lyase. Values were obtained from five distinct analysis.

	A		B		C	
	P1	P2	P1	P2	P1	P2
Yield (%)[a]	38.0 ± 0.2	54.0 ± 0.2	32.0 ± 0.2	21.0 ± 0.1	30.0 ± 0.2	26 ± 0.1
Polyphenol[b]	–	–	+	–	+	–
DM[c]	22.1 ± 0.2	24.4 ± 0.3	23.8 ± 0.2	56.0 ± 0.5	46.0 ± 0.5	73.1 ± 1.0
DA[c]	26.3 ± 0.2	10.3 ± 0.1	47.5 ± 0.5	63.9 ± 0.8	41.5 ± 0.5	63.9 ± 1.0
GalA[d]	7.20 ± 0.20	13.90 ± 0.30	97.00 ± 2.00	97.00 ± 2.00	98.00 ± 2.00	96.00 ± 2.00
Ara[d]	43.20 ± 1.00	37.30 ± 1.00	2.00 ± 0.05	2.00 ± 0.05	1.00 ± 0.05	1.70 ± 0.05
Gal[d]	39.10 ± 1.00	34.00 ± 1.00	1.30 ± 0.05	1.50 ± 0.05	1.20 ± 0.05	2.30 ± 0.05
Rha[d]	4.90 ± 0.10	8.90 ± 0.20	nd	nd	nd	nd
Xyl[d]	1.20 ± 0.05	4.00 ± 0.10	nd	nd	nd	nd
GlcA[d]	1.40 ± 0.05	nd	nd	nd	nd	nd
Fuc[d]	0.80 ± 0.05	1.90 ± 0.05	nd	nd	nd	nd
Glc[d]	1.60 ± 0.05	nd	nd	nd	nd	nd
Api[d]	0.70 ± 0.05	nd	nd	nd	nd	nd

nd: not detected.
[a] Calculated as weight % of P1 and P2 fractions.
[b] +: presence of polyphenols in fraction.
[c] Degree of substitution by methyl residues (DM) and acetyl residues (DA) calculated as % of sugars methylated or acetylated.
[d] Monosaccharide composition (mol%).

of xylose and fucose in P1A as in P2A indicated that these sugars were components of the tea pectin or a contamination of pectin extracts by hemicelluloses. Rhamnose was not detected in B and C sub-fractions from P1 and P2, where galacturonic acid was the main component; so, we concluded that they contained essentially HGA sequences (Fig. 2). Polyphenols, which were detected in the pectin fraction easily dissolved in water (P1B and P2B), were already present in the derived HGA sequences.

3.4. ^{1}H NMR analysis

Proton NMR spectra (Figs. 3 and 4) revealed that molecules in sub-fractions A, B and C from P1 were structurally different from P2. In addition, molecules present in sub-fractions A from P1 and P2 were also different than molecules from B and C sub-fractions. Proton NMR spectra obtained from P1A and P2A revealed the presence of signals at 5.33 and 5.10 ppm, corresponding to anomeric protons from α-L-Ara and α-D-GalA, respectively (Zhao et al., 2006). By comparing the 5.10 ppm signal of P1A and P2A spectra, we concluded that the GalA content was higher in P2A molecules.

On the spectra of P1B and P1C (Fig. 3), signals in the regions 2.5–3.2 ppm, 6.0–6.2 ppm and 6.9–8.2 ppm were observed but not on spectra from P1A (Fig. 3A), P2A, P2B and P2C (Fig. 4). By comparison to ^{1}H NMR spectra of polyphenols extracted from tea (data not shown), we attributed these peaks to polyphenol residues. The presence of polyphenols in the P1B and P1C samples affected the spectra analysis with the overlapping of polyphenols and pectins signals. The assignment of all peaks was not feasible.

Proton NMR spectra from P2B and P2C, revealed signals at 5.10 ppm, 4.41 ppm and 4.77 ppm, attributed, respectively, to H-1, H-4 and H-5 of α-D-GalA residues. Other peaks in the region 5.10–5.30 ppm were attributed to H-1 of esterified α-D-GalA residues (Tamaki, Konishi, Fukuta, & Tako, 2007). The signal at 6.12 ppm observed on the spectra of P2B and P2C as on the spectra of P1B and P1C was assigned to H-4 in an unsaturated GalA residue (Δ-GalA) at the non-reducing end (Bédouet et al., 2003, 2005). This signal was markedly higher for the spectra of P1B and P1C than for P2. From ^{1}H NMR spectra, after Gaussian deconvolution of signals at 6.12 ppm and at 5.10 ppm corresponding to H-4 in unsaturated GalA residues and H-1 in GalA residues, respectively, the degree of polymerisation (DP) of HGA sequences was calculated as described previously for the DP determination in oligoglucuronan preparations (Dantas et al., 1994). The average DP calculated for HGA in P2B and P2C was 13 and 7, respectively. However, due to the presence of signals from polyphenols on the spectra of P1B and P1C, it was not possible to determine the DP of these HGA fractions.

Other differences concerned the degree of substitution by methyl (DM) and acetyl (DA) residues (Table 3). The DM was higher

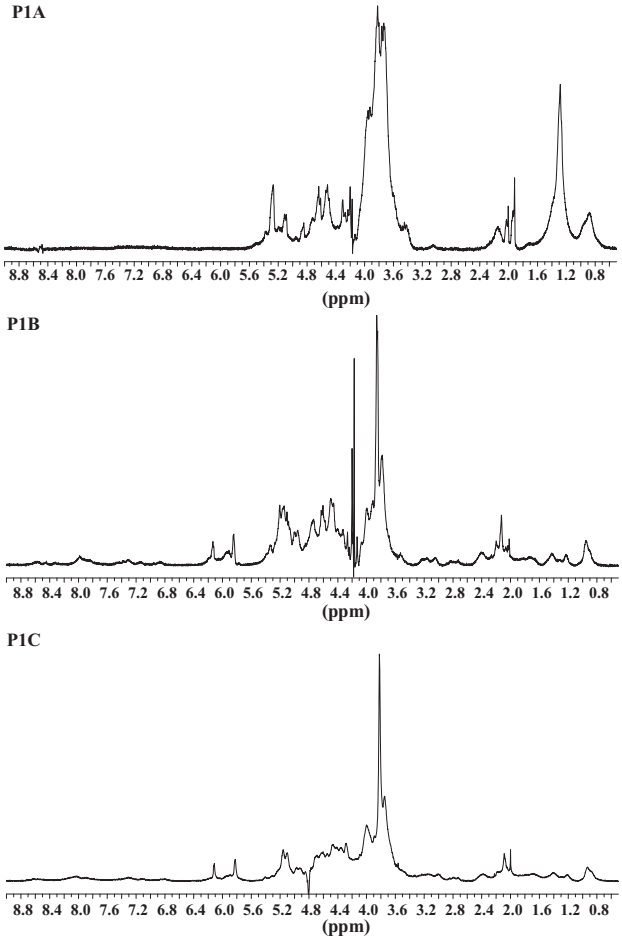


Fig. 3. ^{1}H NMR spectra (300 MHz, 80 °C) of the P1 A, B and C fractions in D_2O (10 mg/ml).

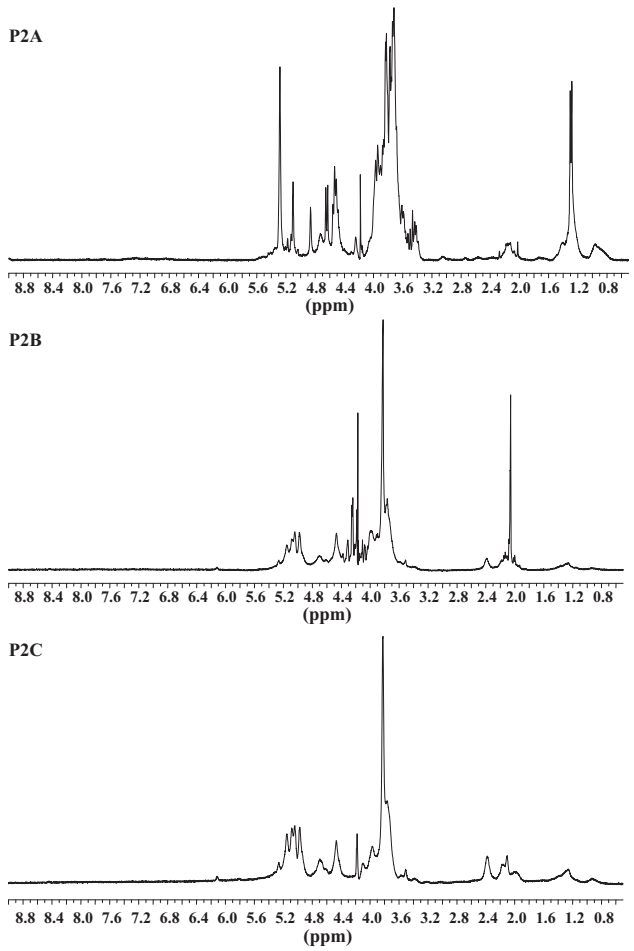


Fig. 4. ^{1}H NMR spectra (300 MHz, 80 °C) of the P2 A, B and C fractions in D_2O (10 mg/ml).

in galacturonan sub-fractions P2B and P2C and more especially in P2C. Moreover, the DA of P1A was at least double than that of P2A and the DA of P2B and P2C was at least 1.5 times higher than that of P1B and P1C.

For the ^{1}H NMR spectra of sub-fractions A, B and C from P1 and P2 (Figs. 3 and 4), signals at 2.14, 2.07, 2.09 and 2.01 ppm were attributed, respectively, to methyl of acetyl group at C-2 in a 2-*O*-acetyl residue, at C-3 in a 3-*O* acetyl residue, at C-2 and at C-3 of a 2,3-di-*O*-acetyl residue by comparison to NMR spectra of polysaccharide, previously reported in the literature (Courtois et al., 1994; Parrone et al., 2002; Uhrinova, Petrakova, Ruppeldt, & Uhrin, 1990). The difference in intensity observed for these signals indicated that galacturonic residues in P1 and P2 possessed different acetylation patterns. The P1B fraction obtained from the easily water-soluble pectin contained mainly residues monoacetylated at C-2 (2.14 ppm), whereas for the P1C extract, diacetylated residues were dominant (2.09 and 2.01 ppm). Regarding the P2B fraction derived from the less water-soluble pectin extract, monoacetylated residues at C-3 (2.07 ppm) were predominant, whereas in P2C diacetylated residues were dominant (2.09 and 2.01 ppm). So, the highest DM and DA (Table 3) were found in HGA sequences P2B and P2C of the less water-soluble pectin whereas HGA represented only 47% of the pectin sequence; while HGA represented 62% in the more water soluble pectin. Such differences have probably influenced the water solubility of the green tea leaves pectin extracts.

In summary, four different HGA sequences were characterized from green tea leaf pectin: one HGA sequence with GalA residues mainly monoacetylated at the C-2 position with a low DM (23.8%), a second HGA sequence with GalA residues mainly acetylated at the C-3 position with a DM value of 56%, a third HGA sequence with GalA residues mainly diacetylated presented DM and DA, respectively, of 46% and 41.5% and fourth HGA sequence with residues mainly diacetylated and high DM and DA.

4. Conclusion

In conclusion, two distinct pectin fractions according to their relative solubility were extracted from tea leaves by water or HCl aqueous solutions. The chemical characterization showed that the fraction easily soluble in water (P1) contained RG I and RG II sequences, whereas no RG II typical sugars were detected in the second pectin extract less water soluble (P2). In the easily water soluble pectin fraction from tea leaves (P1), the proportion of HGA was higher than the less water-soluble pectin fraction (P2). Polyphenols were present in the pectin easily soluble fraction (F1 and P1) and surprisingly these polyphenols remained in the galacturonan sequences obtained after pectin degradation with a pectin lyase, followed by a SEC chromatography. In addition, different DA and DM as well as acetylation pattern were deduced from NMR studies for the two pectin extracts obtained from tea leaves; the DA and DM were higher in HGA sequences obtained from the less water soluble fraction (P2) than in the easily water soluble fraction (P1).

As polyphenols were present only in oligogalacturonan sequences (OGA) with specific substitution patterns, we assumed that interactions between pectins and polyphenols may be present, as previously described for polyphenols and apples cell wall (Renard, Baron, Guyot, & Drilleau, 2001). Further studies on the interactions of polyphenols with OGAs presenting specific esterification patterns, as those observed on the OGAs from the tea leaf pectins will be completed. Such interactions will be considered due to the biological properties of polyphenols and the important consumption of tea beverage (Muktar & Ahmad, 2000).

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