Carbohydrate Polymers 77 (2009) 72–79

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Polysaccharides from the pulp of cupuassu (*Theobroma grandiflorum*): Structural characterization of a pectic fraction

Lúcia Cristina Vriesmann, Carmen Lúcia de Oliveira Petkowicz *

Universidade Federal do Paraná, Departamento de Bioquímica e Biologia Molecular, CP 19046, CEP 81531-990, Curitiba-PR, Brazil

ARTICLE INFO

Article history:
Received 30 April 2008
Received in revised form 30 October 2008
Accepted 4 December 2008
Available online 24 December 2008

Keywords:
Cupuassu
Theobroma grandiflorum
Polysaccharides
Pectin
Starch

ABSTRACT

Cupuassu (*Theobroma grandiflorum*, Schumann) is a Brazilian Amazonian fruit whose pulp contains volatile compounds that have been extensively studied. In this work, the pulp from fruits of cupuassu was ground, treated with MeOH–H_2O, and defatted with *p*-Tol–EtOH. The residue (4% in relation to the fresh pulp) was submitted to sequential extractions with water, aqueous citric acid and aqueous NaOH, resulting in polysaccharide fractions with 0.3–15% yield. The main pectic fraction (7% yield) was obtained with water at 25 °C (W-1 fraction) and was chosen to be better characterized. Chemical and spectroscopic analyses showed that W-1 is composed mainly of a homogalacturonan highly esterified (DE 53%; DA 1.7%) with some rhamnogalacturonan insertions, carrying side chains containing galactose and arabinose.

1. Introduction

Theobroma grandiflorum, Schumann, family Sterculiaceae, is known locally as cupuassu, a fruit occurring in the Brazilian Amazon, and which belongs to the same genus as that of cocoa (*T. cacao*). The seeds of cupuassu are used to prepare a chocolate-like product (“cupulate”), and are surrounded by a yellowish white pulp with a strong fragrance (Cavalcante, 1991). Its volatile compounds have been extensively studied (Franco & Shibamoto, 2000; Quijano & Pino, 2007), among the major components identified being ethyl butanoate, ethyl hexanoate (Franco & Shibamoto, 2000; Quijano & Pino, 2007), and linalool (Quijano & Pino, 2007).

Beside its flavor, what makes cupuassu different from other tropical fruits native to the Amazon region is the excellent industrial yield. Many types of delicacies are made from the pulp and the hull can be used in several crafts (Ministério da Educação, 2007).

The pulp of cupuassu contains 0.025–0.035% vitamin C (Vieira, Teixeira, & Silva, 2000) and is highly appreciated for its pleasant acidic taste and intense fragrance. It is widely consumed fresh or processed into juice, ice cream, yogurt, desserts, liquor and candy, as well as in the domestic production of jams and jellies (Cavalcante, 1991), indicating the presence of gelling agents, which are probably cell wall polysaccharides.

Pectins are an important family of heterogeneous polysaccharides from the plant cell wall (Albersheim, Darvill, O’Neill, Schols, & Voragen, 1996), and are solubilized from various fruits during jam-making, giving the final product its characteristic gel-like texture. Pectins thus have a very long history of practical use in the domestic production of jams and jellies, their molecular interactions being manipulated unwittingly (Rolin, 1993).

The complex structures of pectins consist of homogalacturonan (HG) and rhamnogalacturonans (RG-I and RG-II). HG segments or ‘smooth regions’ have linear chains of (1 → 4)-linked α-D-galacturonic acid (GalA) residues. These can be partly methyl esterified at CO_2H-6, while the hydroxyl groups at position O-2 or O-3 can be acetylated (Pilnik & Voragen, 1970; Rolin, 1993).

These HG sequences may be interspersed with (1 → 2)-linked α-L-rhamnopyranosyl units, substituted at O-4 with neutral sugar chains, such as arabinans, galactans or arabinogalactans, known as RG-I segments or ‘hairy regions’. RG-II is less abundant and has a backbone of HG with complex side chains containing rare sugars such as, apiose, aceric acid (3-*C*-carboxy-5-deoxy-L-xylose), 2-*O*-methylfucose, 2-*O*-methylxylose, and Dha (3-deoxy-D-lyxo-2-heptulosaric acid) (Carpita & McCann, 2000).

In addition to these pectic polymers, arabinoxylan, xyloglucan and cellulose are considered to make up the six polysaccharide components of all plant cell walls. Depending on the type and function of the cell wall, other types of polymers may be present, having different structures, such as xylogalacturonan and apiogalacturonan, both being pectic polysaccharides (Albersheim et al., 1996).

As cupuassu is one of the most important native fruit to the Amazon region with a wide economic potential, it is essential to gain information on their compounds. In the first half of 2005

* Corresponding author. Tel.: +55 41 3361 1661; fax: +55 41 3266 2042.
E-mail address: clop@ufpr.br (C.L. de Oliveira Petkowicz).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.12.007

alone, Brazil exported 50 tons of pulp to other countries. The amount of pulp is important, being ~40% of the total weight of the fresh fruit (Ministério da Educação, 2007). The crescent demand for the fruit, especially for its pulp, shows the necessity of a better knowledge of its composition. No study on the polysaccharides from the fruit of cupuassu has yet been published, so we now describe the isolation and partial chemical characterization of polysaccharides present in its pulp and a more detailed one on its predominant pectic fraction.

2. Experimental

2.1. Materials

The fresh fruits of cupuassu (*T. grandiflorum*), with weights between 0.8 kg and 2 kg, were purchased in a market of Belém, State of Pará, Brazil, and their pulp was manually separated from the skin and seeds.

2.2. Extraction of polysaccharides from cupuassu pulp

The pulp fruit was ground in a blender and submitted to enzyme inactivation with methanol–H_2O (4:1, v/v) under reflux for 20 min, immediately cooled to room temperature, and centrifuged. Insoluble material was sedimented at 15,400*g* for 20 min, washed with ethanol, dried under vacuum, milled, and defatted with *p*-toluene–ethanol (2:1, v/v) in a Soxhlet. The residue was dried and used for polysaccharide extraction following the scheme in Fig. 1. Successive extractions were performed in a mechanical blender and after each one, centrifugation was carried out and the residue was submitted to the next extraction. Each extract was concentrated and treated with ethanol (2:1, v/v) in order to obtain precipitated polysaccharides, which were then washed three times with ethanol and dried under vacuum.

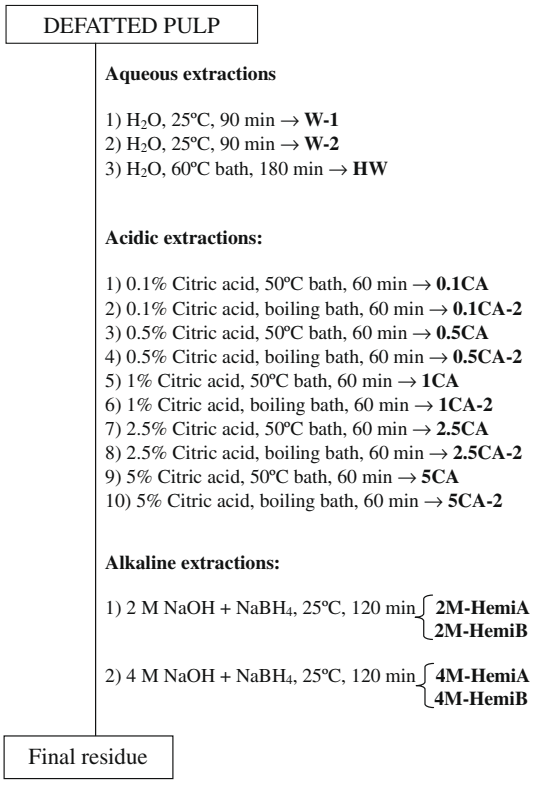

Fig. 1. Scheme for polysaccharide extraction of cupuassu pulp.

Aqueous extractions were performed at 25 °C (90 min, 2×) and at 60 °C (180 min). Successive acidic extractions were performed with increasing citric acid concentrations of 0.1%, 0.5%, 1%, 2.5% and 5%, using two temperatures of 50 °C and 100 °C at each concentration,. Alkaline extractions were performed with 2 M and then 4 M NaOH (25 °C, 120 min), in the presence of a trace of $NaBH_4$. Each extract was neutralized with aqueous 50% acetic acid, and the polysaccharides precipitated (HemiA) were isolated by centrifugation. The resulting supernatants were dialyzed, concentrated to a small volume and then precipitated with ethanol (2:1, v/v), to give HemiB fractions.

2.3. Determination of total sugar, protein and uronic acid contents

Total carbohydrate was measured by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using glucose as standard. Uronic acid was estimated by the sulfamate/3-phenylphenol colorimetric method (Filisetti-Cozzi & Carpita, 1991), using galacturonic acid as standard. Protein was determined according to Hartree (1972), using BSA as standard.

2.4. Monosaccharide composition

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (5 h, 100 °C), and after concentration to dryness, the residues were reduced with $NaBH_4$ (Wolfrom & Thompson, 1963b) and acetylated with pyridine–acetic anhydride (1:1, v/v, 16 h, at 25 °C) (Wolfrom & Thompson, 1963a).

The final residue was solubilized and partially hydrolyzed with 72% (w/w) H_2SO_4 for 1 h at 0–4 °C, diluted to 8% and kept at 100 °C for 15 h (Biermann, 1989). The hydrolysate was neutralized with $BaCO_3$ and the insoluble material removed by filtration. Monosaccharides were reduced and acetylated as above.

The resulting alditol acetates were analyzed by gas–liquid chromatography (GLC) using a model 5890 S II Hewlett–Packard gas chromatograph at 220 °C (flame ionization detector and injector temperature, 250 °C) with a DB-210 capillary column (0.25 mm internal diameter × 30 m), film thickness 0.25 μm, the carrier gas being nitrogen (2.0 ml/min).

2.5. HPSEC–MALLS analysis

High pressure size exclusion chromatography (HPSEC) was carried out using a multidetection equipment: a Waters 2410 differential refractometer (RI) and a Wyatt Technology Dawn F multi-angle laser light scattering (MALLS) detector. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection equipment. A 0.1 M $NaNO_2$ solution, containing NaN_3 (0.5 g/l), was used as eluent. Previously filtered samples (0.22 μm; Millipore) were analyzed at 1.5 mg/ml and the data were collected and processed by a Wyatt Technology ASTRA program.

2.6. Determination of degree of O-methyl esterification

Fourier transform-infrared (FT-IR) spectra were collected at the absorbance mode in the frequency range of 4000–400 cm^{-1} using a Bomem MB-100 spectrophotometer (Hartman & Braun, Canada), at 4 cm^{-1} resolution.

Spectroscopic grade KBr powder was used and discs were prepared using a 90:10 salt: sample proportion. Previous to FT-IR analysis, KBr was dehydrated at 120 °C for 24 h, and the pectin samples were desiccated under vacuum in an Abderhalden equipment containing P_2O_5.

Pectin standards with known degrees of esterification (DE) of 22% and 89% were obtained from SIGMA (Germany) and samples

with degrees of esterification of 35.4%, 44%, 55.5%, 66% and 75.6% were prepared by mixing appropriate amounts of each. From each standard two independent samples were taken and their FT-IR spectra recorded and the area of interest measured. The band areas were determined (using the Win-Bomem Easy software) at 1749 and 1630 cm^{-1}, corresponding to methyl esterified and free uronic acids, respectively. The average of the ratio of the peak area at 1749 cm^{-1} (COO-R) over the sum of the peak areas of 1749 cm^{-1} (COO-R) and 1630 cm^{-1} (COO^-) of the standard duplicates was calculated, and then plotted against their degrees of esterification. Thus, a calibration curve was constructed based on pectin standards and then used to determine the DE of cupuassu polysaccharides.

The DE of W-1 was also determined by ^1H nuclear magnetic resonance spectroscopy (Grasdalen, Bakøi, & Larsen, 1988) using a Bruker DRX 400 Avance spectrometer.

2.7. Nuclear magnetic resonance spectroscopy (NMR)

The ^{13}C NMR spectrum of fraction W-1 in D_2O at 70 °C was obtained using a Bruker DRX 400 Avance spectrometer incorporating Fourier transform. Chemical shifts are expressed in δ (ppm) relative to acetone (δ 30.2).

2.8. Degree of acetylation of fraction W-1

The degree of acetylation (DA) of W-1 was determined by the procedure of Bédouet, Courtois, and Courtois (2003) using ^1H NMR and the Hestrin colorimetric method (1949).

2.9. Carboxy-reduction of fraction W-1

Carboxyl groups of the uronic acid residues of W-1 were reduced by the carboxydiimide method, incorporating $NaBD_4$ reduction to give fraction RW-1 with corresponding 6,6-dideuterioglycosyl units (Stone & Anderson, 1985; Taylor & Conrad, 1972). Two successive carboxy-reduction cycles were done and the reduction of uronic acid residues was measured by colorimetric assay (Filisetti-Cozzi & Carpita, 1991).

2.10. Methylation analysis

Fraction RW-1 in DMSO was *O*-methylated using two cycles of NaOH–MeI (Ciucanu & Kerek, 1984). The per-*O*-methylated product was hydrolyzed with 45% (v/v) formic acid at 100 °C for 15 h. The hydrolyzate was evaporated to dryness and the residue then reduced with $NaBH_4$ and acetylated with Ac_2O to give a mixture of partially *O*-methylated alditol acetates, which was qualitatively and quantitatively analyzed by GC–MS, as follows.

2.11. Gas chromatography–mass spectrometry (GC–MS)

GC–MS was performed using a 3800 Varian gas chromatograph linked to a 2000 R-12 Varian Ion-Trap mass spectrometer, with helium as carrier gas (2 ml/min). A capillary column (30 m × 0.25 mm internal diameter) of DB-225 was held at 50 °C during injection and then programmed at 40 °C/min to 220 °C (constant temperature).

3. Results and discussion

3.1. Polysaccharide fractions from cupuassu pulp

The pulp of fresh cupuassu fruit was isolated, then ground and immediately treated with refluxing methanol–water in order to inactivate enzymes that could degrade polysaccharides of the cell wall. The material was defatted, yielding 4% of dry material based on fresh pulp. It was then submitted to sequential extractions of polysaccharides (Fig. 1). The yield, total sugar and protein contents of each fraction were determined (Table 1).

The extractions furnished seventeen polysaccharide fractions with yields ranging from 0.3% to 15% based on defatted dry pulp. In general, for the acidic extractions, the increase in the yield was directly related to the increase in temperature of extraction, when the same acid concentration was employed. The highest yield was obtained using 0.1% citric acid, for 60 min at 100 °C. The yields were comparable to those found for pectins of spent hops (Oosterveld, Voragen, & Schols, 2002), but lower than those obtained from lemon (Kravtchenko, Voragen, & Pilnik, 1992), mango (Iagher, Reicher, & Ganter, 2002), and apples (Kravtchenko et al., 1992).

The carbohydrate content of the fractions varied from 19% to 98%. All fractions contained protein in different proportions. Pectins from various sources have been reported to contain protein (Iagher et al., 2002; Oosterveld et al., 2002; Singthong, Ningsanond, Cui, & Goff, 2005; Yapo & Koffi, 2006; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). However, it is possible that the carbohydrate contents had an error incorrect due to the use glucose as standard since different monosaccharides give differing absorbances in the phenol–sulfuric assay (Dubois et al., 1956). On the other hand, the protein contents could be overestimated due to the interference of non-proteinaceous substances. Phenols and carbohydrates can also interfere with protein quantitation using the Folin-Ciocalteau reagent (Hartree, 1972).

Pectins are spatially localized in cell wall domains and their composition varies depending on the plant source, as well as the conditions employed during isolation and purification (Rolin, 1993). In general, pectic fractions contain predominant galacturonic acid residues of their main chains, as well as those of rhamnose, arabinose and galactose in side chains (Voragen, Pilnik, Thibault, Axelos, and Renard, 1995).

We now selected the main pectic fraction obtained with aqueous extraction at 25 °C (fraction W-1) for more detailed analysis. Its yield was 7%, higher than pectins from *Citrus depressa* (4.1%; Tamaki, Konishi, Fukuta, & Tako, 2008) and yellow passion fruit (2.9%; Yapo & Koffi, 2006).

Other fractions with considerable uronic acid contents and monosaccharides typical of pectins were W-2, HW and 5-CA (Table 2). The first two fractions had similar pectic monosaccharide com-

Table 1
Yield, % sugar and protein contents of fractions obtained from cupuassu pulp.

Fraction	Yield[a] (%)	Total sugar[b] (%)	Protein[c] (%)
W-1	7.0	61	6
W-2	1.0	58	13
HW	1.5	55	16
0.1CA	0.5	57	13
0.1CA-2	14.7	91	1
0.5CA	1.5	84	5
0.5CA-2	7.7	93	4
1CA	0.3	98	1
1CA-2	4.5	82	4
2.5CA	0.4	77	16
2.5CA-2	0.5	44	25
5CA	0.6	57	2
5CA-2	1.0	37	36
2 M-HemiA	3.4	19	56
2 M-HemiB	3.7	68	14
4 M-HemiA	1.3	77	tr
4 M-HemiB	0.4	77	13

tr, trace.
[a] Based on dry and defatted pulp.
[b] Determined by colorimetric method of Dubois et al. (1956).
[c] Determined by colorimetric method of Hartree (1972).

Table 2
Monosaccharide[a] composition and degree of esterification[b] of fractions obtained from cupuassu pulp.

Fraction	Rha (mol %)	Fuc (mol %)	Ara (mol %)	Xyl (mol %)	Man (mol %)	Gal (mol %)	Glc (mol %)	Uronic acid[c] (mol %)	DE[b] (%)
W-1	3	1	6	4	1	13	7	65	53
RW-1	4	tr	2	2	tr	89	3	–	–
HW	3	1	6	5	2	15	27	41	40
0.1CA	2	1	6	24	2	13	25	27	39
0.1CA-2	1	–	tr	tr	tr	1	91	7	nd
0.5CA	1	–	1	1	–	2	79	16	nd
0.5CA-2	1	–	1	1	tr	1	87	9	nd
1CA	tr	tr	1	1	2	3	82	11	nd
1CA-2	tr	–	tr	tr	tr	1	98	1	nd
2.5CA	1	–	2	tr	1	5	66	25	50
2.5CA-2	1	tr	3	1	1	14	66	14	nd
5CA	4	tr	5	2	2	23	19	45	52
5CA-2	tr	–	–	–	4	40	31	25	39
2 M-HemiA	1	–	–	–	17	26	56	–	nd
2 M-HemiB	2	–	2	41	14	13	26	2	nd
4 M-HemiA	1	tr	3	17	33	12	35	–	nd
4 M-HemiB	2	–	8	24	26	11	27	1	nd
Residue	2	tr	18	6	2	5	67	nd	nd

tr, trace; nd, not determined; RW-1, reduced W-1.
[a] Determined by GLC, mol %.
[b] Determined by FT-IR.
[c] Determined by colorimetric method, Filisetti-Cozzi and Carpita (1991).

positions, while the last contained a greater proportion of galactose. The galacturonic acid content found in these fractions (between 40 and 48 mol %) is similar to those found by Yapo et al. (2007) and Ptitchkina, Danilova, Doxastakis, Kasapis, and Morris (1994).

Among the neutral sugars usually found in the side chains of pectins, galactose was the predominant monosaccharide of all pectic fractions obtained from cupuassu pulp, along with a lower proportion of arabinose. These results are similar to those obtained for pectins of quince (Forni, Penci, & Pollesello, 1994), spent hops (Oosterveld et al., 2002), sugar beet pulp (Yapo et al., 2007), and butter squash fruit (O'Donoghue and Somerfield, 2008). However, they differ from those for pectins of sunflower (Miyamoto & Chang, 1992), apple (Schols, Vierhuis, Bakx, & Voragen, 1995), lemon albedo (Ros, Schols, & Voragen, 1998), and prickly pear fruit skin (Habibi, Heyraud, Mahrouz, & Vignon, 2004), while the arabinose is greater than the galactose content. Thus, the side chains in pectic fractions from cupuassu pulp are mainly galactans or arabinogalactans.

FT-IR spectroscopy can determine the degree of esterification (DE) of pectins. Compared with other methods, it has the advantages of being faster and non-destructive (Chatjigakis et al., 1998).

The pectins FT-IR spectra contain intense bands at 1760–1730 cm^{-1}, arising from ester carbonyl groups and 1630–1600 cm^{-1}, from the carboxyl ion stretching band. The intensity of the absorbance of the ester carbonyl groups increases with the proportion of DE, in contrast with the decrease of that of carboxyl stretching. Similarly, the intensity of the absorbance of free carboxyl groups increases with decrease of DE. These observations established the basis for the quantitative analysis of DE of pectins by FT-IR (Chatjigakis et al., 1998).

Fig. 2 depicted the calibration curve established from the ratio of A1749/(A1749 + A1630) to DE standard values. Based on this curve, the DE of cupuassu pulp fractions with uronic acid contents greater than 20% was determined (Table 2). Pectins with a degree of esterification greater than 50% (HM pectins) and less than 50% (LM pectins) were obtained. The majority of fractions were LM pectins, with the DE ranging from 39% to 53%.

As seen in Table 2, glucose was present as the main monosaccharide in many pulp fractions, mainly those using citric acid extraction. This suggested the presence of starch, which was confirmed by a Lugol iodine test. Hot dilute acids have been used in many investigations to extract pectins which are tightly anchored in the cell walls (Rolin, 1993). However, extractions with 0.1% citric acid at boiling bath (0.1CA-2 fraction) to 2.5% citric acid at boiling bath (2.5CA-2 fraction) afforded mainly starch with minor amounts of pectins. The extractions with 5% citric acid gave rise to pectic fractions with lower content of starch, but in a low yield (0.6% and 1%). O'Donoghue and Somerfield (2008) also obtained starch-rich fractions from buttercup squash fruit using aqueous HCl, HNO_3 and citric acid for extraction of pectins. According to Zobel and Stephen (1995), certain fruits contain 30–85% starch on a dry weight basis.

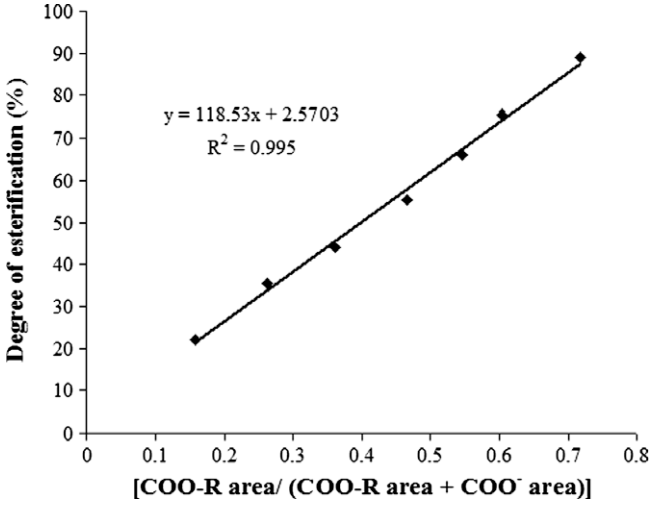

Fig. 2. Calibration curve of the FT-IR spectra obtained at the absorbance mode: ratio of the peak area at 1749 cm^{-1} (COO-R) over the sum of the peak areas of 1749 cm^{-1} (COO-R) and 1630 cm^{-1} (COO^-) versus degree of esterification of standard pectins.

Fraction 5CA showed monosaccharide composition close to that of fractions W-2 and HW, with a higher proportion of galactose. Its DE was similar to that of fraction W-1, suggesting that the extraction procedure did not favor a drastic degradation of the material.

Hemicelluloses, more recently called cross-linking glycans (Carpita & McCann, 2000), were now extracted with 2 M and 4 M NaOH. The fractions had yields from 0.4% to 3.7%, lower values than those of alkali-soluble fractions obtained from fruit pulp of

Argania spinosa (8% and 9%) (Aboughe-Angone et al., 2008) and fruits of *Limonia acidissima* (7.5% and 17.8%) (Mondal, Ray, Thibault, & Ghosal, 2002). The majority of the hemicellulosic polysaccharides of cupuassu were extracted with the lowest NaOH concentration (2 M). The alkaline fractions obtained from the pulp of cupuassu were mainly composed of xylose, mannose, galactose, and glucose, monosaccharides typically found in hemicelluloses.

Xylans were extracted mainly with 2 M NaOH, being isolated in HemiB fraction (41 mol % of Xyl). Xylose also was the most abundant sugar of the first hemicellulosic fraction obtained from olive fruit (Vierhuis, Schols, Beldman, & Voragen, 2000) and of the two alkali-soluble fractions obtained from *A. spinosa* fruit (Aboughe-Angone et al., 2008).

In general, the other hemicellulosic fractions of cupuassu pulp showed a monosaccharide composition profile close to that of papaya fruit (Glc > Man > Xyl > Gal > Ara) (Manrique & Lajolo, 2004). The proportion of mannosyl, galactosyl and glucosyl of fractions 2 M-HemiA and 4 M-HemiA suggests the presence of galactomannans, galactoglucomannans, and glucomannans. Due to the low proportion of arabinose, the presence of arabinogalactans in alkaline fractions cannot be excluded.

In addition, the uronic acids in HemiB fractions suggest the presence of acidic xylans, although it could also come from remaining pectins as indicated by the presence of Rha, Ara and Gal. According to Aboughe-Angone et al. (2008), residual pectic compounds may also be present in the alkali fractions from *A. spinosa* fruit.

The residue obtained finally on the series of extractions of cupuassu pulp was composed mainly of glucose, with minor amounts of other monosaccharides, as found for papaya fruit (Manrique & Lajolo, 2004), apricot, peach and pumpkins (Kurz, Carle, & Schieber, 2008), indicating a predominance of cellulose.

In addition to monosaccharide composition, the HPSEC–MALLS/RI analysis confirmed that all polysaccharide fractions extracted from the cupuassu pulp are heterogeneous when both detectors (MALLS and RI) are used (data not shown).

3.2. Characterization of fraction W-1

3.2.1. Chemical composition

Extraction of cupuassu pulp using water at 25 °C gave rise to fraction W-1 (7% yield). It consisted of 61% carbohydrate and 6% protein. These values are similar to those obtained for spent hop (Oosterveld et al., 2002) and sugar beet pulp pectins (Yapo et al., 2007).

The chemical composition of W-1 (Table 2) indicated that the fraction consists of 65 mol % of uronic acid with degrees of methyl esterification of 53% and acetylation (DA) of 1.7%. Its DA is slightly lower than that reported for LM pectin from yellow passion fruit rind (1.9%) (Yapo & Koffi, 2006) and slightly greater than HM commercial lemon pectin (1.5%) (Kravtchenko et al., 1992), although both pectins had a higher uronic acid content (76.3% and 72.0%, respectively).

As shown in Table 2, galactose was the predominant neutral monosaccharide of W-1 (13%). Others typical of pectins were rhamnose (3%) and arabinose (6%). The data suggest that the structure of this pectic fraction consisted mainly of a highly methyl esterified homogalacturonan (HG) and a type I rhamnogalacturonan (RG-I) with galactan or arabinogalactan side chains.

In the 'hairy regions' of pectins, xylogalacturonans have been reported. These consist of a galacturonan backbone, with single xylosyl units substituting O-3 of the GalA residues. This type of subunit was found in pectins from apples (Schols, Bakx, Schipper, & Voragen, 1995) and peas (Le Goff, Renard, Bonnin, & Thibault, 2001), and could also be present in our fraction W-1, whose xylose content was 4 mol %, although it also could arise due to the co-extraction of hemicelluloses.

Besides the carbohydrates typical of pectins, W-1 also contains minor amounts of mannosyl and glucosyl units. Mannose was present in different proportions in pectins from various sources (Iagher et al., 2002; Koubala et al., 2008; Miyamoto & Chang, 1992; O'Donoghue and Somerfield, 2008; Oosterveld et al., 2002; Ros et al., 1998; Yapo et al., 2007).

The presence of glucose suggested that starch could be present in a lower proportion in W-1, which was confirmed by a Lugol iodine test. Its presence has also been described for pectins of apple pomace (Bringand, Denis, Grall, & Lecacheux, 1990), mango (Iagher et al., 2002) and buttercup squash fruit (O'Donoghue and Somerfield, 2008).

3.2.2. HPSEC–MALLS analysis

Fig. 3 illustrates the elution profile of W-1 obtained by HPSEC–MALLS/RI as a function of elution time. RI gives a signal proportional to the concentration, whereas the MALLS response increases with the molar mass.

W-1 gave rise to a heterogeneous profile, probably arising from a mixture of acidic polysaccharides and starch. It is also possible to observe a peak eluted after ~38 min, detected with high intensity by light scattering, which coincides with very small RI intensity. Thus a high molar mass component was present in a very low concentration, and could be due to starch. An intense RI peak, eluted after ~50 min, coincides with that with a minimal light scattering intensity, indicating a predominant pectin with lower molar mass.

Iagher et al. (2002) isolated a pectic fraction from mango pulp which also displayed a heterogeneous profile by HPSEC–MALLS/RI. The purified fraction from mango pulp contained starch as contaminant, but in a higher proportion than W-1.

3.2.3. Carboxy-reduction and methylation analyses

Two cycles of carbodiimide-activated reduction of the carboxyl groups in W-1 incorporating $NaBD_4$ resulted in the formation of fraction RW-1. The complete reduction was confirmed by colorimetric assay (Filisetti-Cozzi & Carpita, 1991) and FT-IR analyses. This procedure made it possible to identify the uronic acid units using GC–MS. Formed following successive hydrolysis, $NaBH_4$ reduction, and acetylation was a mixture of acetylated alditol acetates, one of corresponding to a galactitol derivative according to its retention time and EI fragmentation pattern. The latter contained ions with *m/z* 147, 189, 219, 261 and 291 arising from –CD_2OAc groups at C-6 from the carboxyl groups of typical, pectin galacturonic acid residues in W-1 (Table 2). After carboxy-reduction of W-1, the majority of monosaccharide contents in RW-1 decreased proportionally, one exception being rhamnose, which content increased. This can be explained by the greater lability to acid of the glycosidic linkage between Gal–Rha, when compared with that of GalA–Rha (Yapo et al., 2007).

Methylation analysis of fraction RW-1 showed that its GalA residues were 4-*O*-substituted. The main derivative obtained (78.2%) was 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl galactitol, having a characteristic fragmentation at *m*/z 235. The presence of 1,2,4,5-tetra-*O*-acetyl-6-deoxy-3-*O*-methyl rhamnitol (4.1%) indicated that the rhamnosyl units were 2,4-di-*O*-substituted. The chemical data thus confirmed that the core of the pectin from fraction W-1 is composed mainly of a homogalacturonan with some rhamnogalacturonan insertions. Usually, neutral side chains are substituents on O-4 of rhamnose residues. Among the partially *O*-methylated alditol acetates we also find some derivatives typical of galactans and arabinans, like 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl galactitol, 1,3,4,5-tetra-*O*-acetyl-2-*O*-methyl arabinitol and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl arabinitol.

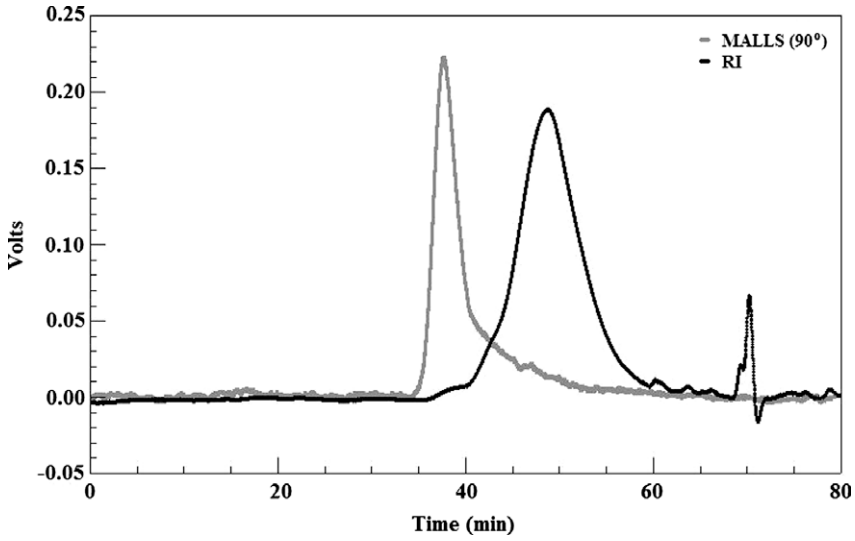

Fig. 3. Elution profile of fraction W-1 obtained by HPSEC–MALLS/RI.

3.2.4. ^{13}C NMR analysis

The chemical structure of W-1 was studied by ^{13}C NMR spectroscopy (Fig. 4). Signals at 100.1 ppm and 99.3 ppm were attributed to C-1 of esterified and non-esterified units of α-galacturonic acid. Two respective high-frequency C-6 signals were at 170.4 ppm and δ 174.6 ppm. A signal of $-OCH_3$ groups was at 52.8 ppm. These values are similar to those found by Westereng, Michaelsen, Samuelsen, and Knutsen (2008) for a pectic fraction (DE 56%) from white cabbage. They found the occurrence of the sharp 53.5 ppm resonance as diagnostic for methyl esterified Gal*p*A. The matching resonances at 100.8 and 99.7 ppm were assigned to C-1 of esterified and unesterified α-Gal*p*A, respectively. The corresponding resonances of C-6 of the carboxyl groups in Gal*p*A occurred in two regions (∼171.3 and ∼175.5 ppm, respectively).

Tamaki et al. (2008), when analyzing the ^{13}C NMR spectrum of pectin from endocarp of *C. depressa*, observed a signal at 55.7 ppm, which was assigned to methyl groups binding to carboxyl groups of GalA, and a signal at 173 ppm which was attributed to carboxyl groups bound by methyl groups. After de-esterification of the polysaccharide, the signal at 55.7 ppm disappeared and the signal at

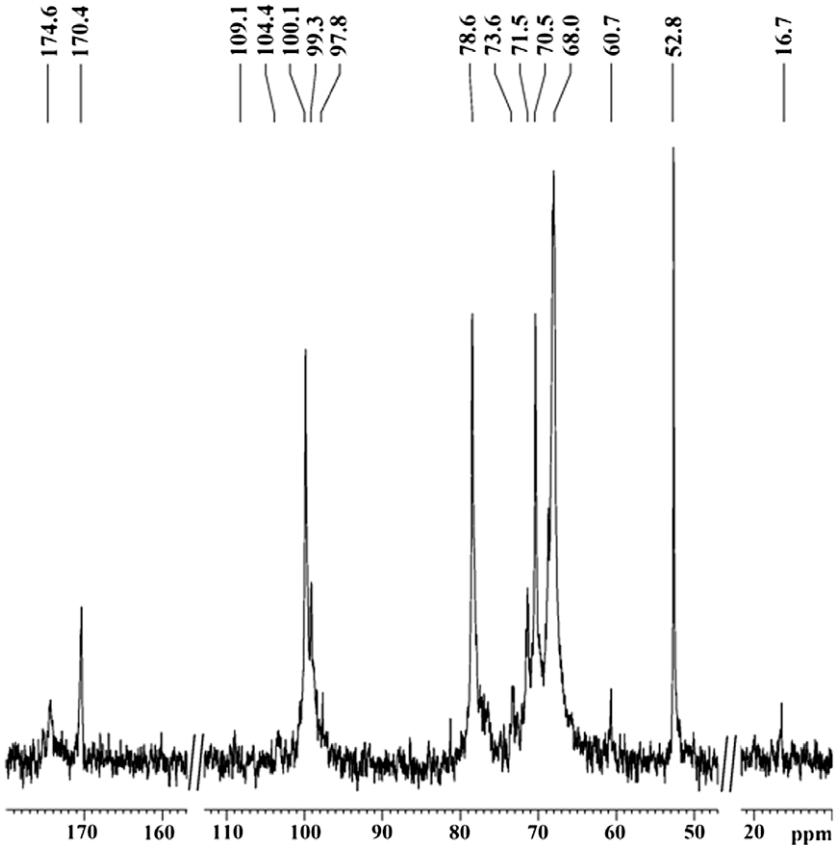

Fig. 4. ^{13}C NMR spectrum of fraction W-1 isolated from cupuassu pulp: solvent D_2O at 70 °C.

173 ppm was shifted to 177 ppm at which the signal was derived from ionic carboxyl groups.

Signals at 68.0, 70.5, 78.6 and 71.5 ppm were assigned to C-2, C-3, C-4 and C-5 of GalA units, respectively. The C-1 signal at 97.8 ppm was from C-1 of α-L-rhamnosyl units and their CH_3-6 was found detected at a lower frequency, 16.7 ppm. The C-1 NMR region also contained signals at δ 109.1 and 104.4 from α-L-Ara*f* and β-D-Gal units, respectively. All above assignments were based on literature values (Mukhiddinov, Khalikov, Abdusamiev, & Avloev, 2000; Westereng et al., 2008). These data are in agreement with the presence of a HM homogalacturonan with some RG-I insertions.

The chemical data of fraction W-1, together with the determination of the Rha to GalA ratio (as inferred by Table 2), suggests that its pectins could consist mainly of polygalacturonic acid-rich 'smooth' regions, typically found in those from middle lamella (McCann & Roberts, 1991). The molar ratio of Rha/(Ara + Gal) also indicates the degree of side chain branching and, in this case, W-1 seems to be lightly branched.

The results are in agreement with results of Schols and Voragen (1996) and Albersheim et al. (1996), who commented that pectins present in the edible parts of fruit and vegetables can only consist of HG regions and RG-I-like segments with strictly alternating Rha–GalA sequences. These structures in the pulp of the cupuassu fruit should improve their edible and functional properties.

Acknowledgements

The authors wish to thank Sr. Ari Zugmann for supplying the fruits of cupuassu and the Brazilian agencies, CNPq and Fundação Araucária-PRONEX for financial support. Special thanks are due to Dr. Philip A.J. Gorin for providing suggestions to improve the English in this paper.

References

Aboughe-Angone, S., Nguema-Ona, E., Ghosh, P., Lerouge, P., Ishii, T., Ray, B., et al. (2008). Cell wall carbohydrates from fruit pulp of *Argania spinosa*: Structural analysis of pectin and xyloglucan polysaccharides. *Carbohydrate Research, 343*, 67–72.

Albersheim, P., Darvill, A. G., O'Neill, M. A., Schols, H. A., & Voragen, A. G. J. (1996). An hypothesis: The same six polysaccharides are components of the primary cell walls higher plants. In J. Visser & A. G. J. Voragen (Eds.). *Pectins and pectinases* (Vol. 14, pp. 47–55). Amsterdam: Elsevier Science.

Bédouet, L., Courtois, B., & Courtois, J. (2003). Rapid quantification of *O*-acetyl and *O*-methyl residues in pectin extracts. *Carbohydrate Research, 338*, 379–383.

Biermann, C. J. (1989). Hydrolysis and other cleavage of glycosidic linkages. In C. J. Biermann & G. D. McGinnis (Eds.), *Analysis of carbohydrates by GLC and MS* (pp. 27–41). Florida: CRC Press.

Bringand, G., Denis, A., Grall, M., & Lecacheux, D. (1990). Insight into the structure of pectin by high performance chromatographic methods. *Carbohydrate Polymers, 12*, 61–77.

Carpita, N., & McCann, M. (2000). The cell wall. In B. B. Buchanan, W. Gruissem, & R. L. Jones (Eds.), *Biochemistry and molecular biology of plants* (pp. 52–108). Maryland: American Society of Plant Physiologists.

Cavalcante, P. B. (1991). *Frutas comestíveis da Amazônia* (5th ed., pp. 90–91). Belém: Edições CEJUP, 1991: CNPq: Museu Paraense Emílio Goeldi.

Chatjigakis, A. K., Pappas, C., Proxenia, N., Kalantzi, O., Rodis, P., & Polissiou, M. (1998). FT-IR spectroscopic determination of the degree of esterification of cell wall pectins from stored peaches and correlation to textural changes. *Carbohydrate Polymers, 37*, 395–408.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research, 131*, 209–217.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Biochemistry, 28*, 350–356.

Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry, 197*, 157–162.

Forni, E., Penci, M., & Pollesello, A. (1994). A preliminary characterization of some pectins from quince fruit (*Cydonia oblonga* Mill.) and prickly pear (*Opuntia ficus indica*) peel. *Carbohydrate Polymers, 23*, 231–234.

Franco, M. R. B., & Shibamoto, T. (2000). Volatile composition of some Brazilian fruits: umbu-caja (*Spondias citherea*), camu-camu (*Myrciaria dubia*), araçá-boi (*Eugenia stipitata*), and copoazu (*Theobroma grandiflorum*). *Journal of Agricultural and Food Chemistry, 48*, 1263–1265.

Grasdalen, H., Bakøi, O. E., & Larsen, B. (1988). Determination of the degree of esterification and the distribution of methylated and free carboxyl groups in pectins by ^{1}H NMR spectroscopy. *Carbohydrate Research, 184*, 183–191.

Habibi, Y., Heyraud, A., Mahrouz, M., & Vignon, M. R. (2004). Structural features of pectic polysaccharides from the skin of *Opuntia ficus indica* prickly pear fruits. *Carbohydrate Research, 339*, 1119–1127.

Hartree, E. F. (1972). Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry, 48*, 422–427.

Hestrin, S. (1949). The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. *Journal of Biological Chemistry, 180*, 249–261.

Iagher, F., Reicher, F., & Ganter, J. L. M. (2002). Structural and rheological properties of polysaccharides from mango (*Mangifera indica* L.) pulp. *International Journal of Biological Macromolecules, 31*, 9–17.

Koubala, B. B., Mbone, L. I., Kansci, G., Tchouanguep-Mbiapo, F., Crepeau, M.-J., Thibault, J.-F., et al. (2008). Physicochemical properties of pectins from ambarella peels (*Spondia cytherea*) obtained using different extraction conditions. *Food Chemistry, 106*, 1202–1207.

Kravtchenko, T. P., Voragen, A. G. J., & Pilnik, W. (1992). Analytical comparison of three industrial pectin preparations. *Carbohydrate Polymers, 18*, 17–25.

Kurz, C., Carle, R., & Schieber, A. (2008). Characterisation of cell wall polysaccharide profiles of apricots (*Prunus armeniaca* L.), peaches (*Prunus persica* L.), and pumpkins (*Cucurbita* sp.) for the evaluation of fruit product authenticity. *Food Chemistry, 106*, 421–430.

Le Goff, A., Renard, C. M. G. C., Bonnin, E., & Thibault, J. F. (2001). Extraction, purification and chemical characterisation of xylogalacturonans from pea hulls. *Carbohydrate Polymers, 45*, 325–334.

Manrique, G. D., & Lajolo, F. M. (2004). Cell-wall polysaccharide modifications during postharvest ripening of papaya fruit (*Carica papaya*). *Postharvest Biology and Technology, 33*, 11–26.

McCann, M. C., & Roberts, A. (1991). Architecture of the primary cell wall. In C. W. Lloyd (Ed.), *The cytoskeletal basis of plant growth and form* (pp. 109–129). New York: Academic Press.

Ministério da Educação, *Secretaria de Educação Profissional e Tecnológica*. (2007). Cupuaçu. *Thematic Pamphlets*, Brasília, 28p. <http://portal.mec.gov.br/setec/arquivos/pdf3/publica_setec_cupuacu.pdf/> Accessed 20.10.08.

Miyamoto, A., & Chang, K. C. (1992). Extraction and physicochemical characterization of pectin from sunflower head residues. *Journal of Food Science, 57*, 1439–1443.

Mondal, S. K., Ray, B., Thibault, J. F., & Ghosal, P. K. (2002). Cell-wall polysaccharides from the fruits of *Limonia acidissima*: Isolation, purification and chemical investigation. *Carbohydrate Polymers, 48*, 209–212.

Mukhiddinov, Z. K., Khalikov, D. K., Abdusamiev, F. T., & Avloev, C. C. (2000). Isolation and structural characterization of pectin homo and rhamnogalacturonan. *Talanta, 53*, 171–176.

O'Donoghue, E. M., & Somerfield, S. D. (2008). Biochemical and rheological properties of gelling pectic isolates from buttercup squash fruit. *Food Hydrocolloids, 22*, 1326–1336.

Oosterveld, A., Voragen, A. G. J., & Schols, H. A. (2002). Characterization of hop pectins shows the presence of an arabinogalactan–protein. *Carbohydrate Polymers, 49*, 407–413.

Pilnik, W., & Voragen, A. G. J. (1970). Pectin substances and other uronides. In A. C. Hulme (Ed.), *The biochemistry of fruits and their products* (pp. 53–87). New York: Academic Press.

Ptitchkina, N. M., Danilova, I. A., Doxastakis, G., Kasapis, S., & Morris, E. R. (1994). Pumpkin pectin: Gel formation at unusually low concentration. *Carbohydrate Polymers, 23*, 265–273.

Quijano, C. E., & Pino, J. A. (2007). Volatile compounds of copoazú (*Theobroma grandiflorum* Schumann) fruit. *Food Chemistry, 104*, 1123–1126.

Rolin, C. (1993). Pectins. In R. L. Whistler & J. N. BeMiller (Eds.), *Industrial gums: Polysaccharides and their derivatives* (3rd ed., pp. 257–293). San Diego: Academic Press.

Ros, J. M., Schols, H. A., & Voragen, A. G. J. (1998). Lemon albedo cell walls contain distinct populations of pectic hairy regions. *Carbohydrate Polymers, 37*, 159–166.

Schols, H. A., Bakx, E. J., Schipper, D., & Voragen, A. G. J. (1995). A xylogalacturonan subunit present in the modified hairy regions of apple pectin. *Carbohydrate Research, 279*, 265–279.

Schols, H. A., Vierhuis, E., Bakx, E. J., & Voragen, A. G. J. (1995). Different populations of pectic hairy regions occur in apple cell walls. *Carbohydrate Research, 275*, 343–360.

Schols, H. A., & Voragen, A. G. J. (1996). Complex pectins: Structure elucidation using enzymes. In J. Visser & A. G. J. Voragen (Eds.), *Progress in biotechnology 14: Pectins and pectinases* (pp. 3–19). Amsterdam: Elsevier.

Singthong, J., Ningsanond, S., Cui, S. W., & Goff, H. D. (2005). Extraction and physicochemical characterization of Krueo Ma Noy pectin. *Food Hydrocolloids, 19*, 793–801.

Stone, B. A., & Anderson, M. A. (1985). A radiochemical approach to the determination of carboxylic acid groups in polysaccharides. *Carbohydrate Polymers, 5*, 115–129.

Tamaki, Y., Konishi, T., Fukuta, M., & Tako, M. (2008). Isolation and structural characterization of pectin from endocarp of *Citrus depressa*. *Food Chemistry, 107*, 352–361.

Taylor, R. L., & Conrad, H. E. (1972). Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following

reduction of their carbodiimide-activated carboxyl groups. *Biochemistry, 11*, 1383–1389.

Vieira, M. C., Teixeira, A. A., & Silva, C. L. M. (2000). Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (*Theobroma grandiflorum*) nectar. *Journal of Food Engineering, 43*, 1–7.

Vierhuis, E., Schols, H. A., Beldman, G., & Voragen, A. G. J. (2000). Isolation and characterisation of cell wall material from olive fruit (*Olea europaea* cv koroneiki) at different ripening stages. *Carbohydrate Polymers, 43*, 11–21.

Voragen, A. G. J., Pilnik, W., Thibault, J.-F., Axelos, M. A. V., & Renard, M. G. C. (1995). Pectins. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 288–310). New York: Marcel Dekker, Inc.

Westereng, B., Michaelsen, T. E., Samuelsen, A. B., & Knutsen, S. H. (2008). Effects of extraction conditions on the chemical structure and biological activity of white cabbage pectin. *Carbohydrate Polymers, 72*, 32–42.

Wolfrom, M. L., & Thompson, A. (1963a). Acetylation. *Methods in Carbohydrate Chemistry, 2*, 21.

Wolfrom, M. L., & Thompson, A. (1963b). Reduction with sodium borohydride. *Methods in Carbohydrate Chemistry, 2*, 65.

Yapo, B. M., & Koffi, K. L. (2006). Yellow passion fruit rind – a potential source of low-methoxyl pectin. *Journal of Agricultural and Food Chemistry, 54*, 2738–2744.

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chemistry, 100*, 1356–1364.

Zobel, H. F., & Stephen, A. M. (1995). Starch: Structure, analysis, and application. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 19–66). New York: Marcel Dekker, Inc.