

Characterization of Acid-Extracted Pectin-Enriched Products Obtained from Red Beet (*Beta vulgaris* L. var. *conditiva*) and Butternut (*Cucurbita moschata* Duch ex Poiret)

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Chemical and rheological characteristics of fractions enriched in soluble dietary fiber are reported. These fractions were obtained through acid hydrolysis of butternut (*Cucurbita moschata* Duch ex Poiret) and red beet (*Beta vulgaris* L. var. *conditiva*) cell wall enriched powders. Hydrolysis was performed using citric acid at different pH values and reaction times (2 and 3 h). Yields obtained for butternut fractions were between 21 and 28 g/100 g; for red beet, yields were 24 and 31 g/100 g for pH 1.5 and 11 and 17 g/100 g for pH 2.0 for previously mentioned times; in general, the increase of the yield was directly correlated with the decrease of pH and the increase of reaction time. Products enriched in low methoxyl pectins were obtained in all cases. At the lowest pH assayed, pectins were essentially constituted by homogalacturonan; a significant content of neutral sugars was determined at the higher extraction pH. Neutral sugars were constituted mainly by arabinose, galactose, rhamnose, and glucose in different proportions for each fraction; in general, butternut fractions showed high glucose contents. Flow behavior for 2.00% (w/v) aqueous systems of the different products was evaluated. Data obtained for fractions isolated at pH 1.5 fit to Herschel–Bulkley and Cross models while those isolated at pH 2.0 fit to Ostwald and Cross models. All samples showed low viscosity and, hence, poor thickening properties.

KEYWORDS: Butternut; red beet; pectin; citric acid; hydrolysis

INTRODUCTION

Pectic substances are a large family of structural elements of primary cell walls and intercellular regions of many land plants where they function as hydrating agent and cementing material of the cellulose network. The highest concentration of pectins in the cell wall occurs in the middle lamella, with a gradual decrease from the primary cell wall toward the plasma membrane (1). The major residue found in pectins is D-galacturonic acid (GalA), partly esterified with methanol; sugars commonly present are L-rhamnose (Rha), L-arabinose (Ara), D-galactose (Gal), and, to a lesser extent, D-xylose (Xyl) and other different monosaccharides. There is broad agreement that the main structural elements are homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (2–4).

The composition, structure, and physiological properties of pectin might be influenced by conditions of extraction as well as sources, location and many other environmental factors. The pectic network must be disrupted to enable extraction; therefore, extraction using chemical agents such as sodium hydroxide, hydrochloric acid as well as enzymes have generally been used for its obtention (2, 5, 6). Although various alternative processes

have been patented in recent years, commercially, pectin is extracted by treating the raw material with hot dilute mineral acid at pH ≈ 2. The precise length of time varies with raw material, with the type of pectin desired, and from one manufacturer to another (7, 8). Levigne et al. (9) have shown that extractive conditions (pH, temperature, time, acid) have important effects on the features of the extracted pectins. Faravash and Ashtiani (10) studied the effects of acid volume, acid-washing time and pH variation on the extraction of pectin from peach pomace and concluded that the stability of pectin molecules in an aqueous solution depends on the temperature and acidity. Tartaric, malic, citric, lactic, acetic and phosphoric acids can be used for pectin extraction, but a tendency to use cheaper mineral acids such as sulfuric, hydrochloric and nitric acids can be observed (11). Canteri-Schemin et al. (12) studied the extraction of pectin from apple pomace using a variety of organic and mineral acids and concluded that both nitric and citric acids showed the highest yields but citric acid is economical and better from an environmental point of view. As it is known, nitric acid is an oxidant and, as such, it can alter the original pectin composition.

The use assigned to the pectin-enriched product isolated will be a function of its chemical composition, structure and molecular weight, properties that will determine its functionality (13). Although pectins are widely used as texture modifiers, they are

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also recognized for their capacity to delay glucose absorption (14) and enzymatic degradation of starch (15).

Butternut (*Cucurbita moschata* Duchesne ex Poiret) and red beet (*Beta vulgaris* L. var. *conditiva*) are raw materials used by the food industry. Byproducts of their industrialization are used for animal feeding or disposed as industrial wastes. Most research on beet pectins has been performed on sugar beet (*Beta vulgaris* var. *altissima*) and on the byproduct of the extraction of sugar. Red beet or table beet is mostly used for salads and purees, and also to extract a natural pigment which confers its characteristic red color to a variety of food products (16). Red beets are available all year round in the market. Since they are roots, their tissue accumulates a considerable amount of sugars which confer on them a sweet taste. Their caloric content is moderate, and they are a good source of fiber; in addition, they can be consumed either fresh or cooked. Butternut (*Cucurbita moschata* Duch ex Poiret), popularly known as “Calabacita criolla” or “Anco”, is widely grown and consumed in Argentina. It is rich in carotene, pectin, mineral salts, vitamins and other substances beneficial to health, like fibers, resulting in the development of salty or sweet food products (5).

The aim of this work was to characterize the chemical composition and functional properties of pectin-enriched products extracted from *Cucurbita moschata* Duch ex Poiret and *Beta vulgaris* L. var. *conditiva* through citric acid extraction, with the aim of adding value to leftover of their industrialization.

MATERIALS AND METHODS

Sample Preparation. Butternut (*Cucurbita moschata* Duch ex Poiret) and red beet (*Beta vulgaris* L. var. *conditiva*) bought in a local market were used in this work. Each tissue was separated and juice was removed from mesocarp using a juice extractor. The residue was washed twice with distilled water, dried (85 °C, 2 h) in a convection oven (air rate: 0.508 m s⁻¹), milled (Wemir E909, Wemir SA, Buenos Aires, Argentina) and sieved for obtaining butternut and beet powders with particles in the range 420–710 μm. The moisture content of powders obtained was 3.84 g/100 g (dry basis, db) and 10.33 g/100 g (db), respectively.

The experimental design used considered two variables: pH at three levels (1.5, 2 and 3) and time at two levels (2 and 3 h); since the extraction at pH 3 gave very low yields, this pH condition was, finally, discarded. Citric acid was selected for extraction considering environmental factors (12). Both powders were treated under stirring (10 rad s⁻¹) in citric acid solution of pH previously mentioned for 2 or 3 h at 85 °C (8). Insolubles obtained after acid extractions were separated through filtration with glass fiber filter (Schleicher and Schuell, Germany), under vacuum, and cell wall polysaccharides (CWP) were finally precipitated from each supernatant through ethanol addition (2.5 volumes). These CWPs were collected through filtration using a nylon mesh (pore: 0.06 mm²) and dehydrated by freeze-drying.

Samples obtained were the following:

- R12: sample extracted from beet powder at pH 1.5 for 2 h
 - R13: sample extracted from beet powder at pH 1.5 for 3 h
 - R22: sample extracted from beet powder at pH 2 for 2 h
 - R23: sample extracted from beet powder at pH 2 for 3 h
 - B12: sample extracted from butternut powder at pH 1.5 for 2 h
 - B13: sample extracted from butternut powder at pH 1.5 for 3 h
 - B22: sample extracted from butternut powder at pH 2 for 2 h
 - B23: sample extracted from butternut powder at pH 2 for 3 h
- For naming them, the first letter of “red beet”, R, or “butternut”, B, was used according to their origin. Either letter was followed by 1 or 2 for treatment pH 1.5 or 2.0, respectively, and then by 2 or 3, for treatment times of 2 h or 3 h, respectively.

In each case, the yield was calculated as grams of product obtained per 100 g of powder submitted to extraction.

Obtention of Alcohol-Insoluble Residue (AIR). The AIR was obtained after boiling the powders in 85% (v/v) ethanol as it was described by Martin-Cabrejas et al. (17). Residue obtained after treatment was freeze-dried and milled.

Chemical Analyses. Deionized (Milli-Q; Millipore, Billerica, MA) water was used for preparation of all reagents used for chemical analyses.

Analyses performed on each sample obtained were as follows:

- uronic acids through the spectrophotometric method of Filisetti-Cozzi and Carpita (18), using D-galacturonic acid as standard;
- total carbohydrates by the phenol–sulfuric acid photometric method of Dubois et al. (19) using D-galacturonic acid as standard; in the case of AIR, D-glucose was used as standard;
- starch using an enzymatic method involving α-amylase, amyloglucosidase and o-dianisidine (20) using a kit provided by SIGMA (St. Louis, MO);
- degree of esterification by the saponification and spectrophotometric methanol estimation method of Wood and Siddiqui (21);
- acetyl groups according to the method of Naumenko and Phillipov (22);
- degree of methylation (DM) was calculated as the molar percent ratio between methanol and GalA contents in a given sample, whereas degree of acetylation (DA) was calculated as the molar percent ratio between acetyl group and total carbohydrate content in each sample;
- 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo) and 2-keto-3-deoxy-D-lyxo-heptulosaric acid (Dha) were simultaneously determined (23) through a modified thiobarbituric acid spectrophotometric assay (24, 25);
- protein content of pectin-enriched fractions was determined by Lowry et al. (26, 27) assay.

For each AIR obtained, protein content was determined by the Kjeldhal–Arnold–Gunning method (28). Carbohydrates and lignin were determined according to de Escalada Pla et al. (29). The content of neutral sugars was also determined for AIR.

All these determinations were performed three times on each sample.

Sugar Analysis. Proportions of neutral monosaccharides constituting the CWP were determined after hydrolysis with 2000 mol m⁻³ CF₃COOH (TFA, trifluoroacetic acid) for 2 h at 121 °C. Every hydrolysis step was carried out in duplicate.

Hydrolysates were derivatized to the alditol acetates according to Albersheim et al. (30) and analyzed by gas–liquid chromatography (GLC) coupled to flame ionization detection for quantitative determinations or to electron impact mass spectrometric detection when qualitative characterization was required. The first GLC method used a capillary column on a HP-5890 gas chromatograph (U.S.A.) equipped with a flame ionization detector (FID), and nitrogen was used as the carrier gas. The GLC–mass spectrometry (MS) analyses of the alditol acetates were carried out to confirm structures of unusual sugars present in RG-II in a Shimadzu QP 5050 A GC/MS apparatus (Japan) working at 70 eV and using He as gas carrier. Chromatographic runs were isothermally performed at 220 °C.

Carboxyl Reduction of the Sample with a Soluble Carbodiimide. One millimole of *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) was added to 0.5 μm³ of methanol–water solution containing enough sample to give 100 μequiv of carboxylic acid, taking into account the uronic acid content previously determined for each sample by the spectrophotometric method of Filisetti-Cozzi and Carpita (18). This solution was stirred for 2 h at constant pH (4.7–5.0). Around 1.5 mg of NaBD₄ was then added while pH was maintained at 7.0–7.5. This system was stirred for 3 h and finally dialyzed for 2 days against tap water and an additional day against distilled water. The dialyzed material was finally freeze-dried. The proportions of neutral monosaccharides (galactose from polygalacturonic or HG chains) obtained after GalA reduction were again determined after hydrolysis with 2000 mol m⁻³ CF₃COOH (TFA, trifluoroacetic acid) for 2 h at 121 °C using inositol as internal standard. Hydrolysates were derivatized to the alditol acetates according to Albersheim et al. (30) and analyzed by GC as previously indicated.

Methylation Analysis. The fraction R22 (after dialysis) and its carboxyl-reduced counterpart were methylated according to Ciucanu and Kerek (31). Shortly, the samples (ca. 7 mg) were suspended in anhydrous dimethyl sulfoxide (0.5 mL) with the help of warming (40 °C) and sonication. To the slurries, finally powdered NaOH was added (100 mg) and left to react with stirring at room temperature for 30 min. Then, methyl

iodide (0.5 mL) was added, and left stirring for another 30 min (two additions). The reaction mixtures were quenched with water (3 mL), and were dialyzed and freeze-dried. The permethylated polysaccharides were hydrolyzed and derivatized to the alditol acetates as described above. The partially methylated alditol acetates were separated, identified and quantitated using the above-mentioned GC and GC-MS systems, but using the programmed run on the SP-2330 column as described by Shea and Carpita (32).

Nuclear Magnetic Resonance Spectroscopy. The spectra of fraction R22 were obtained on a Bruker Avance II 500 spectrometer, provided with a 5 mm probe, at 500.13 (^1H) and 125.77 (^{13}C) MHz and at room temperature using ca. 15 mg polysaccharide in 0.7 mL of D_2O . The 2D spectra were obtained using standard Bruker software. Chemical shifts were referred to TMS, using acetone as internal standard (31.1 ppm in ^{13}C , 2.22 ppm in ^1H).

Rheological Characterization under Flow. Around 0.0200 g of each isolated fiber was suspended in $1\ \mu\text{m}^3$ of deionized water and vortexed until complete hydration in order to get a 2% (w/v) final concentration. Then, systems were stored at 25 °C for 18 h to attain swelling equilibria before measurement.

Rheological characterization was performed using a controlled stress rheometer (RheoStress RS600, Haake, Germany) equipped with a serrated parallel plate (PP35) geometry (35 mm diameter). A gap size of 500 μm was set, and data points were recorded at steady state.

Flow curves were determined at a constant temperature of 25 °C (Haake DC 50 water bath, Germany) in the 0.05–100 Pa shear stress range. Models such as Ostwald, Herschel–Bulkley, Cross and Carreau were used to evaluate the experimental data (33).

Statistical Analyses. Statistical analyses of results were performed through ANOVA (level of significance, α : 0.05) followed by pairwise multiple comparisons using Tukey's significant difference test (34) using Statgraphics package (version 2.6, STSC, 1987, Rockville, MD).

RESULTS AND DISCUSSION

Chemical Composition. The juice extraction performed on butternut (*Cucurbita moschata* Duch ex Poirlet) and red beet (*Beta vulgaris* L. var. *conditiva*) tissues produced the concentration of the cell wall material (CWM). Pulp was submitted to water-washing in order to eliminate starch (≈ 0.2 g/100 g of raw butternut and 0.03 g/100 g of raw red beet) and cytoplasmic water-soluble components (globular proteins, aminoacids, mono and oligosaccharides) that could be present.

Pulp dehydration rendered 1.9 or 2.4 g of powder per 100 g of fresh tissue for butternut or red beet, respectively. Powders obtained after drying at 85 °C were mainly constituted by cell wall polymers, as it is indicated by their high proportion of alcohol insoluble residues (AIR): 53.4 or 69.7 g per 100 g of powder for butternut or red beet, respectively. It has to be mentioned that starch content was 9.5 g/100 g of powder for butternut whereas red beet powder had a lower content of 1.4 g/100 g (Table 1).

AIRs were essentially carbohydrates ($\approx 100\%$), with minimal contents of lignin (2.8 g/100 g for butternut and 6.5 g/100 g for red beet) and protein (5.4 and 7.1 g/100 g, respectively) as can be observed in Table 1. Total carbohydrate contents included starch (14.2 g/100 g of butternut AIR and 1.2 g/100 g of red beet AIR), cellulose (46 and 55 g/100 g, respectively), and uronic acids (12.5 and 13.7 g/100 g, respectively) from HG and RGs (3, 4). It can be concluded from the difference between total carbohydrate and uronic acid contents that an amount of ≈ 30 –37 g/100 g of neutral sugars (for butternut and red beet, respectively) were present as constituents of the pectic macromolecules (RG-I and RG-II). All pectic polysaccharides had a high degree of methylation (DM $\approx 90\%$).

Carbodiimide assays showed that uronic acids present in butternut powder and AIR were constituted by D-galacturonic acid (GalA). In the case of red beet, a small proportion of glucuronic acid (GlcA) was also found. Neutral sugar (NS) composition of AIRs is also reported in Table 1 and will be commented on later.

Butternut. Pectin-enriched fractions B12, B13, B22, B23 were obtained with yields of 27, 28, 21, 21 g/100 g respectively

Table 1. Composition of Butternut and Red Beet AIRs (g/100 g). Starch Content of Their Powders (g/100 g)

	butternut		red beet	
	powder ^a	AIR ^a	powder ^a	AIR ^a
uronic acids		12.5 ± 0.5		13.7 ± 0.9
total carbohydrates (D-glucose)		103 ± 3		107 ± 5
lignin		2.8 ± 0.2		6.5 ± 0.5
cellulose		46 ± 3		55 ± 3
protein		5.4 ± 0.2		7.1 ± 0.1
total starch	9.5 ± 0.3	14.2 ± 0.7	1.4 ± 0.2	1.2 ± 0.1
nonresistant starch	9.4 ± 0.1	13.6 ± 0.3	0.8 ± 0.3	0.7 ± 0.1
degree of methylation ^b (% molar)		91		93
degree of acetylation ^b (% molar)		34		90
	AIR sugar content (g/100 g)			
	butternut		red beet	
Rha ^c	3.6		8.3	
Fuc	0.4		0.6	
Ara	3.3		51.7	
Xyl	3.7		4.1	
Man	2.2		1.9	
Gal	4.9		14.9	
Glc	68.2		4.9	

^a Mean and standard deviations are shown ($n = 3$). Results are expressed in dry basis. ^b DM and DA were calculated as a percent ratio between moles of methanol or acetyl group and moles of GalA or total carbohydrates per 100 g of sample, respectively. ^c Rha: rhamnose. Fuc: fucose. Ara: arabinose. Xyl: xylose. Man: mannose. Gal: galactose. Glc: glucose.

(Table 2). The increase of the yield was directly correlated with the decrease in pH. Faravash and Ashtiani (10) extracted pectin from peach with HCl solutions at different pHs and times and obtained the highest yield (10%) with the highest time and lowest pH.

In Table 2, it can be observed that total carbohydrates showed a minimum for B13 (≈ 41 g/100 g of fraction) and a maximum for B22 (≈ 93 g/100 g of fraction). The protein content of the fractions was low with a value of 2.2 to 3.4 g/100 g of fraction, probably because denaturalization diminished protein solubilization in acid, leading to a lower recovery of the protein fractions associated with the cell wall during ethanol precipitation. Low-methoxyl pectins were isolated in all cases as can be observed from DM ranging from ≈ 17 to 39 mol/100 mol, while DA varied between 1.7 and 3.0 mol/100 mol. The fractions B12, B22 and B23 showed the highest DM and DA. Galacturonic acid (GalA) content varied from 23 to 41 g/100 g, and its proportion was not related to extraction yields. An increase in the time of extraction produced an increase in the uronic acid/total carbohydrate ratios (Table 2) trend that can be ascribed to the hydrolysis of neutral sugar side chains, remaining the homogalacturonan backbone. Carbodiimide reduction showed that uronics were exclusively constituted by GalA. Yapo et al. (35) observed that pH was the main parameter influencing the galacturonic acid content when they studied pectin extraction from sugar beet pulp using sulfuric acid (pH 1.5 or 2.0) and times of 1 and 4 h; the pectins extracted at pH 1.5 contained more galacturonic acid than those at pH 2.0.

Table 3 shows the sugar composition of the isolated fractions. It can be observed that shorter extraction times and the higher pHs (B22) led to a better recovery of the pectin RG-I unit, as indicated by the Rha, Gal and Ara contents, suggesting the presence of arabinan and galactan side chains in the "hairy regions". Yapo et al. (35) observed that pH and time affected

Table 2. Chemical Composition of Fiber Enriched Products Isolated from Butternut and Red Beet^a

sample	Butternut			
	B12	B13	B22	B23
total CH ^{b,c}	52.4 ± 4.8	41.3 ± 0.6	92.9 ± 2.3	61.1 ± 9.3
GalA ^b (g/100 g)	23.0 ± 0.4	41.0 ± 4.8	33.3 ± 3.8	38.9 ± 0.3
protein ^b (g/100 g)	2.2 ± 0.5	2.3 ± 0.4	3.4 ± 0.6	2.7 ± 0.3
starch ^b (g/100 g)	22.9 ± 0.1	12.4 ± 0.1	28.3 ± 0.1	37.4 ± 1.1
DM ^d (mol/100 mol)	37.3	16.8	39.3	35.6
DA ^d (mol/100 mol)	3.1	1.7	3.0	2.7
yield ^e (g/100 g)	27	28	21	21

sample	Red Beet			
	R12	R13	R22	R23
total CH ^{b,c}	42.5 ± 1.9	45.0 ± 2.5	79.4 ± 4.6	75.9 ± 7.9
UA ^b (g/100 g)	41.7 ± 1.9	44.1 ± 2.5	30.2 ± 1.4	27.3 ± 1.4
protein ^b (g/100 g)	4.6 ± 0.6	4.1 ± 0.2	8.3 ± 0.2	7.7 ± 0.8
starch ^b (g/100 g)	0.9 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	0.6 ± 0.1
DM ^d (mol/100 mol)	20.3	17.2	43.0	45.4
DA ^d (mol/100 mol)	3.3	3.1	19.8	21.7
yield ^e (g/100 g)	24	31	11	17

^a Pectin-enriched product extracted with different conditions: B12, pH 1.5, time 2 h; B13, pH 1.5, time 3 h; B22, pH 2, time 2 h; B23, pH 2, time 3 h; R12, pH 1.5, time 2 h; R13, pH 1.5, time 3 h; R22, pH 2, time 2 h; R23, pH 2, time 3 h. B: from butternut powder. R: from beetroot powder. CH: carbohydrates. Gal A: galacturonic acid. UA: uronic acids. DM: degree of methylation. DA: degree of acetylation. ^b Mean and standard deviations are shown ($n = 3$). ^c Total carbohydrates are calculated as GalA (g/100 g). ^d DM and DA were calculated as a percent ratio between moles of methanol or acetyl group and moles of GalA or total carbohydrates per 100 g of sample, respectively. ^e Yield is expressed as g of fraction/100 g of powder.

neutral sugar content of sugar beet; the highest neutral sugar content was obtained after treatment at pH 2.0, for 1 h; the neutral sugar content of the pectins extracted at pH 2.0 was higher than those at pH 1.5 indicating that, at pH 1.5, some degradation of pectin side chains occurred. In the present research, the poor content of side chain substituents in RG-I due to acid hydrolysis of glycosidic linkages is evident. The ratio Ara/Gal decreased with time increase at each pH, showing a faster hydrolysis of arabinosyl units, as expected from their furanosyl linkages. Only B22 showed the presence of some xylose. As butternut AIR contained 3.7 g of xylose per 100 g (Table 1), the absence of Xyl in its fractions confirms the strong effect of low pHs and long times on the structure of products obtained. The B13 pectin fraction was only constituted by HG; the other pectin fractions showed a high content of glucose which can be, at least partially, attributed to the presence of starch which took values of 23–37 g/100 g of the product for B12, B22 and B23 (Table 2). Only traces of RG-II were detected, as inferred from the negligible contents of 2-*O*-methyl Xyl, 2-*O*-methyl Fuc, Kdo and Dha.

Red Beet. Fractions were obtained with yields of 24 g/100 g of powder (time: 2 h) and 31 g/100 g of powder (time: 3 h) and with carbohydrate contents of ≈43 g/100 g when the pH used for extraction was 1.5. These carbohydrates were constituted in ≈98% by uronic acids (Table 2). Therefore, the pectin isolated from red beet powder at pH 1.5 was essentially a homogalacturonan. As can be observed in Table 3, the low quantity of neutral sugars present (around 0.8–0.9 g/100 g of fraction) were mainly constituted by Rha, Gal, Ara and Glc. DM was lower than 21 mol/100 mol for these samples, and DA was slightly greater than 3 mol/100 mol; it is known that acidification produces de-esterification. The general absence of Xyl confirms the strong effect of low pH on the structure of products obtained. Protein content was higher (4.1–4.6 g/100 g) than the one for butternut

Table 3. Sugar Composition (g/100 g) of Fiber Enriched Products Isolated from Butternut and Red Beet^a

	Butternut			
	B12	B13	B22	B23
2- <i>O</i> -Me Fuc	0	tr	0	0
2- <i>O</i> -Me Xyl	tr	tr	tr	tr
Dha + Kdo	0.006	0.0001	0.03	0.009
Rha	0.53	0.02	1.1	0.41
Fuc	0	0	0	0
Ara	0.73	0	1.5	0.37
Xyl	0	0	0.5	0
Man	0	0	0	0
Gal	0.88	0.04	1.8	0.67
Glc	27.3	0.3	54.7	20.8

	Red Beet			
	R12	R13	R22	R23
2- <i>O</i> -Me Fuc	tr	tr	0	0
2- <i>O</i> -Me Xyl	tr	0	tr	tr
Dha + Kdo	0.001	0.001	0.064	0.073
Rha	0.21	0.18	4.98	6.37
Fuc	0	0	0	tr
Ara	0.26	0.32	25.04	21.95
Xyl	0	0	0	tr
Man	0	0	0	tr
Gal	0.26	0.29	6.01	8.07
Glc	0.11	0.11	13.11	11.83

^a Pectin-enriched product extracted with different conditions: B12, pH 1.5, time 2 h; B13, pH 1.5, time 3 h; B22, pH 2, time 2 h; B23, pH 2, time 3 h; R12, pH 1.5, time 2 h; R13, pH 1.5, time 3 h; R22, pH 2, time 2 h; R23, pH 2, time 3 h. B: from butternut powder. R: from red beet powder. 2-*O*-Me Fuc: 2-*O*-methyl L-fucose. 2-*O*-Me Xyl: 2-*O*-methyl D-xylose. Dha: 2-keto-3-deoxy-D-lyxo-heptulosaric acid. Kdo: 2-keto-3-deoxy-D-manno-octulosonic acid. Rha: rhamnose. Fuc: fucose. Ara: arabinose. Xyl: xylose. Man: mannose. Gal: galactose. Glc: glucose.

fractions and starch content was very low (0.5–0.9 g/100 g). On the other hand, Levigne et al. (9) studied the optimization of pectin extraction from sugar beet using HCl, pH 1–3 in the temperature range 75–95 °C and during 30–90 min; they observed that, at the lower pH, they obtained the higher Rha contents, the extracts being richer in RG at pH 1.0 and richer in HG at pH 3.0.

For fractions isolated at pH 2.0, yields were lower: 11 g/100 g for 2 h and 17 g/100 g for 3 h. Total carbohydrates took values of 76–79 g/100 g while uronic acid content was 27–30 g/100 g. Increasing the pH to 2.0 (R22 and R23) increased the proportion of extracted neutral sugars to ≈63.0% of the total carbohydrate content (Table 2), and this trend is coincident with those reported by Yapó et al. (35). Neutral sugars were mainly constituted by Glc, Ara, Rha, and Gal (Table 3). It is important to remark that products R22 and R23 showed a very high content of Ara (Ara/Rha = 5.0 and 3.7, respectively), showing that weaker acid conditions precluded hydrolysis to occur to a high degree (36). Unlike the Ara/Rha ratio, the ratio Gal/Rha (≈1.2) did not change with increasing treatment time at pH 2.0. Evidently, red beet pectins resist better the hydrolysis at pH 2.0 than those from butternut, at the RG-I level. The only explanation could be the high content of arabinan side chains, replacing RG-I of red beet pectins, that might anchor these pectins to the cell wall by entanglements with cellulose, protecting them better from hydrolysis. Glucose concentration was around 12 g/100 g (Table 3); as starch concentration in these fractions is very low (Table 2), probably Glc originated from other polysaccharides present in these fractions. DM and DA were higher than the ones observed

for fractions obtained at pH 1.5: DM was around 44 mol/100 mol, and DA was around 20 mol/100 mol. As expected, a lower de-esterification was produced by hydrolysis at pH 2.0 (9). As can be concluded, low methoxyl pectins were obtained in this research, in all cases, the lowest DM (16.8) for sample obtained from butter-nut being observed at pH 1.5 after 3 h of treatment, and the highest one (DM: 45.4) for sample obtained from beetroot being observed at pH 2.0 after 3 h of treatment. Happi Emaga et al. (37) extracted pectin from dried peels of banana at pH 1.5 and 2.0 and observed that the values of DM increased with increasing pH; temperature had a higher effect on DA than pH and time. In the present research, only traces of RG-II were detected on red beet fractions and protein content of fractions obtained at pH 2.0, doubled those of fractions extracted at pH 1.5 (Table 3).

It has to be mentioned that carbodiimide reaction showed that for red beet AIR, approximately 94.0% of uronic acids was GalA and the remaining 6.0% was GlcA. Strasser and Amadó (38) reported the presence of GlcA in red beet tissue.

Structural Analysis of R22. Fraction R22, rich in side chain sugars, was chosen for a detailed structural analysis. In order to remove the remaining citric acid, the sample was exhaustively dialyzed. During this process, a large proportion of glucose disappeared, indicating that this sugar is probably part of small oligosaccharides related to starch. The molar composition of the dialyzed sample was Rha 16.3%, Ara 58.8%, Xyl 2.0%, Gal 20.6% and Glc 1.7%, plus traces of Fuc (fucose). The glycosyl linkages inferred from the methylation of this fraction are shown in Table 4. Undermethylation (shown by the presence of nonmethylated sugars) was low, although more relevant for the less common sugars. It is worth noting that the sum of partly methylated sugars arising from each monosaccharide agreed very well with the original monosaccharide composition. For arabinose, the derivatives found are compatible with chains with a 1→5 backbone, one-third of which are substituted in C-3 (and much less in C-2) by terminal Araf single stubs. The similarity of proportions of terminal Araf and 3,5-disubstituted branching points of the same sugar suggests that most of the terminal sugar appears on homoarabinan side chains, and not attached to galactose, as expected to occur in RG-I (39). Strasser and Amadó (40) found on enzyme-treated red beet samples the same proportions of linked arabinose, but more than double of the terminal sugar. Probably in our acid-treated sample, the arabinose side chains linked to galactose were cleaved. Rhamnose derivatives indicate mostly substitution in position 2, about half also being substituted in position 4, as expected from a RG-I backbone (39). The presence of small amounts of other rhamnose derivatives (as that of terminal rhamnose) appears to be related to the small amount of RG-II present in the sample. Galactose appears with different linkages (Table 4). However, most appears to be 4-linked and terminal units, suggesting that the 4-linked β-D-galactopyranosyl side chains are much more common than those with mixed 1,3- and 1,6-linkages. The presence of significant amounts of terminal galactose are compatible with the above-mentioned loss of terminal arabinose in branched arabinogalactan chains. The derivatives of glucose present indicate the presence of low-branching starchlike products. Methylation analysis of carboxyl-reduced fraction R22 showed the same general pattern (Table 4), although mass spectra of the deuterated derivatives allowed recognition that all the GalA products appear 4-linked, a small amount also being substituted on C-2. This is compatible with the presence of RG-I. On the other hand, the GlcA present in the sample appears only as terminal. Strasser and Amadó (40) also indicated that most of this uronic acid is terminal, although they found other minor derivatives.

Table 4. Glycosyl-Linkage Composition (mol %) of the Red Beet Fraction R22 and Its Carboxyl-Reduced Counterpart

glycosyl residue	dialyzed R22	reduced R22
T-Rhap	0.2	0.4
T-Rhaf	0.5	
2-Rhap	6.0	5.8
4-Rhap		0.8
3,4-Rhap	0.6	0.6
2,3-Rhap	0.7	0.6
2,4-Rhap	6.9	4.7
2,3,4-Rhap ^a	2.9	2.2
T-Araf	10.8	9.7
T-Arap	0.5	
3-Araf	2.2	2.0
5-Araf	19.1	17.6
2,5-Araf	2.8	2.2
3,5-Araf	12.0	10.8
2,3,5-Araf ^a	2.9	3.4
T-Xylp	0.8	0.7
4-Xylp		0.7
2,3,4-Xylp ^a	0.7	
T-Galp	4.5	4.4
2-Galp	0.6	0.9
4-Galp	8.9	4.7
6-Galp	2.5	2.3
3-Galp	2.1	0.9
3,4-Galp	1.5	2.9
2,4-Galp	0.6	0.5
4,6-Galp	2.5	1.6
3,6-Galp	1.7	0.9
2,6-Galp	0.1	
2,3,4-Galp ^a	0.2	
3,4,6-Galp ^a	0.3	
2,4,6- + 2,3,6-Galp ^a	0.3	
2,3,4,6-Galp ^a	0.7	1.2
4-Manp		1.1
2,3,4,6-Manp ^a	0.9	2.1
T-Glcp		0.4
4-Glcp	1.6	2.0
4,6-Glcp	0.2	
2,3,4,6-Glcp ^a	1.2	2.1
4-GalpA		7.0
2,4-GalpA		1.3
T-GlcpA		1.5

^a Possibly arises from undermethylation of the sample.

In order to deepen the structural study of fraction R22, a HSQC (2D) NMR spectrum was obtained (Figure 1). A quite simple spectrum was attained, in spite of the expected complexity. The signals corresponding to the core GalA and Rha units do not appear, given the low mobility of their residues. Even those corresponding to the anomeric carbons/protons cannot be found, in spite of being expected to appear in a clean region. On the other hand, the signals belonging to terminal Araf units and to 5-linked Araf units have been identified completely, as they showed chemical shifts identical to those appearing in the literature (41). Furthermore, they appear in proportions similar to those shown by methylation analysis (Table 4). On the other hand, the less mobile 3,5-linked Araf units are not observed (the signal of the more mobile C5/H5 may appear partly overlapping the same atoms in the monosubstituted unit). Minor signals corresponding to the atoms of 4-linked β-linked galactose units (including the anomeric peak) are also observed; they match literature data (41, 42); on the other hand, only one signal (at 69.3/3.90 ppm) can be assigned to C4/H4 of a terminal galactose unit, as some other signals overlap with the other galactose unit. Besides, a peak at 53.7/3.78 ppm, corresponding to the methoxyl group esterifying

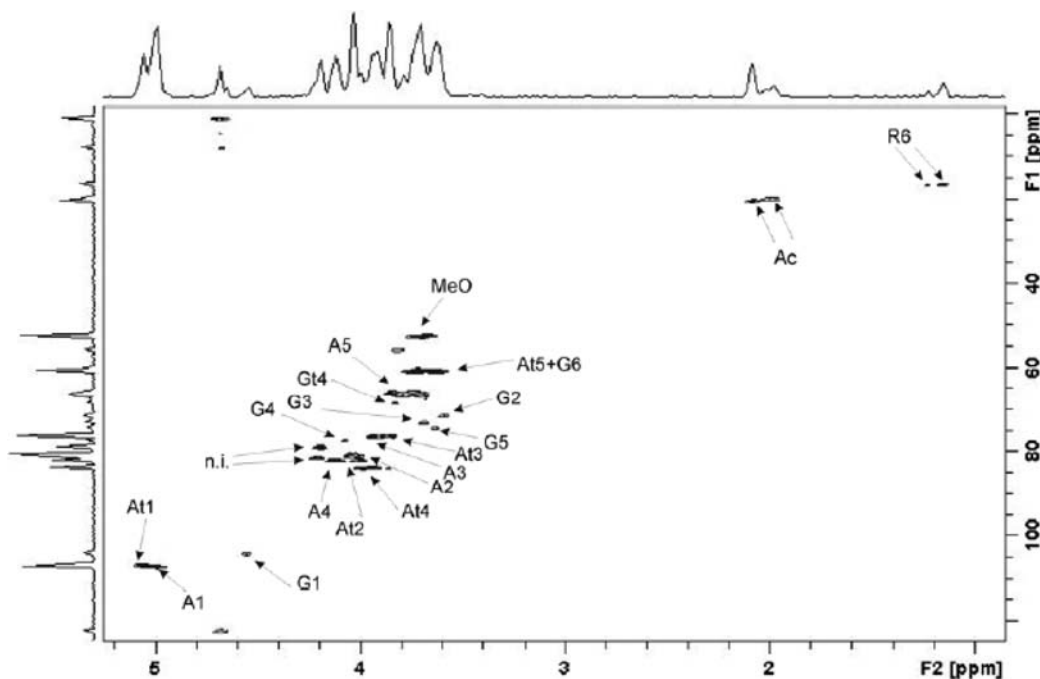


Figure 1. HSQC spectrum of fraction R22, at 500 MHz. Key: R = Rha, A = 5-linked Ara, At = terminal Ara, G = 4-linked Gal, Gt = terminal Gal, MeO = methoxy group, Ac = acetyl group, n.i. = not identified. The number following the acronym letter corresponds to the carbon/proton number.

Table 5. Apparent Viscosity and Herschel–Bulkley Model Parameters Calculated after Fitting Flow Experimental Data (25 °C) of 2.00% (w/v) Aqueous Systems Containing Different Pectin-Enriched Products^a

product	apparent viscosity at 20 s ⁻¹ (Pa s)	τ_0^b (Pa)	k^b (Pa s ^{<i>n</i>})	n^b	R^2
B12	0.014 ± 0.001	0.17 ± 0.04	0.07 ± 0.03	0.48 ± 0.06	0.8187
B13	0.016 ± 0.002	0.15 ± 0.05	0.04 ± 0.02	0.7 ± 0.1	0.9465
R12	0.016 ± 0.002	0.20 ± 0.02	0.04 ± 0.01	0.67 ± 0.06	0.9429
R13	0.018 ± 0.001	0.14 ± 0.03	0.06 ± 0.02	0.61 ± 0.07	0.9261

^a Pectin-enriched product extracted with different conditions: B12, pH 1.5, time 2 h; B13, pH 1.5, time 3 h; R12, pH 1.5, time 2 h; R13, pH 1.5, time 3 h. B: from butternut powder. R: from red beet powder. τ_0 : yield stress. k : consistency index. n : exponential index. R^2 : goodness of fitting (α : 0.05). ^b Mean and standard error are shown.

the GalA units, was identified. Two different peaks are recognized at 20.9/2.04 ppm and 21.4/2.15 ppm; they correspond to *O*-acetyl groups attached to different positions of the GalA units. According to the literature (43), the former corresponds to attachment to C2 and the latter to C3; by far, the latter is more important, indicating that most of the *O*-acetyl groups are attached to position 3 of the GalA units. C6 of rhamnose units is also observed at 17.4/1.22 ppm (assigned to 2-linked rhamnose units), with a minor signal at 17.7/1.30 ppm which corresponds to 2,4-linked rhamnose (43). The peaks corresponding to C6 of Rha units, methoxyl and acetyl groups on GalA units appear because the atoms to which they correspond are exocyclic, and thus have more mobility than the remaining ones appearing in the core. A couple of unidentified signals are observed at 79.9/4.25 ppm and 82.4/4.28 ppm. They might correspond to deshielded galactose units with other substitution patterns.

Detailed analysis of R 22 confirmed that RG-I represents its major structure.

Rheological Behavior under Flow. For this characterization there were constituted 2.00% (w/v) aqueous systems of the different products enriched in pectins obtained. They were found to be completely soluble.

Flow behavior was fitted to different models, and results obtained are shown in **Tables 5, 6** and **7**. Samples B12, B13, R12 and R13 fit adequately to the Herschel–Bulkley model (**Table 5**) showing yield stresses (τ_0) ranging from 0.14 to 0.20 Pa and low apparent viscosities (η_a) ranging from 14 to 18 mPa s,

Table 6. Apparent Viscosity and Ostwald Model Parameters Calculated after Fitting Flow Experimental Data (25 °C) of 2.00% (w/v) Aqueous Systems Containing Different Pectin-Enriched Products^a

product	apparent viscosity at 20 s ⁻¹ (Pa s)	k^b (Pa s ^{<i>n</i>})	n_o^b	R^2
B22	0.072 ± 0.008	0.16 ± 0.02	0.73 ± 0.02	0.9867
B23	0.035 ± 0.002	0.090 ± 0.004	0.69 ± 0.01	0.9953
R22	0.027 ± 0.009	0.04 ± 0.01	0.85 ± 0.06	0.9042
R23	0.018 ± 0.005	0.04 ± 0.01	0.73 ± 0.05	0.9189

^a Pectin-enriched product extracted with different conditions: B22, pH 2, time 2 h; B23, pH 2, time 3 h; R22, pH 2, time 2 h; R23, pH 2, time 3 h. B: from butternut powder. R: from red beet powder. k : consistency index. n_o : Ostwald exponent. R^2 : goodness of fitting (α : 0.05). ^b Mean and standard error are shown.

at shear rates of 20 s⁻¹. Butternut sample B12 was the one with lower exponential index (n : 0.48) showing the higher shear thinning behavior after surpassing τ_0 .

Fractions extracted at pH 2.0 fit adequately to the Ostwald model (**Table 6**), showing no τ_0 , and η_a values (shear rate: 20 s⁻¹) ranging from 18 to 72 mPa s. B22 showed the highest η_a . Samples extracted at pH 2.0 showed weaker pseudoplastic behavior with Ostwald exponents (n_o) ranging from 0.69 to 0.85 (**Table 6**). Fissore et al. (5) showed that fractions enriched in pectins isolated from butternut using citrate buffer (pH ≈ 5.2) or buffer/hemicellulase or buffer/cellulase showed pseudoplastic behavior and no τ_0 , showing, in general, higher values of apparent viscosity at 20 s⁻¹ (54–1330 mPa s) than B22 or B23.

Table 7. Cross Model Parameters Calculated after Fitting Flow Experimental Data (25 °C) of 2.00% (w/v) Aqueous Systems Containing Different Pectin-Enriched Products^a

product	η_{∞}^b (Pa s)	η_0^b (Pa s)	τ (s) ^b	m^b	R^2
B13		17.3 ± 0.9	36.4 ± 5.0	1.76 ± 0.34	0.8787
B22		4 ± 0	27.1 ± 0.6	6.5 ± 0.8	0.9722
B23	0.0155 ± 0.0007	0.060 ± 0.002	0.049 ± 0.005	1.5 ± 0.2	0.9337
R12		73.9 ± 3.1	182.2 ± 16.9	1.31 ± 0.09	0.9794
R13		22.1 ± 1.4	43.5 ± 7.4	1.79 ± 0.44	0.8211
R22		9.6 ± 0.2	45.2 ± 1.6	2.50 ± 0.20	0.9846
R23		12.4 ± 0.5	49.1 ± 4.7	2.29 ± 0.43	0.9111

^a Pectin-enriched product extracted with different conditions: B13, pH 1.5, time 3 h; B22, pH 2, time 2 h; B23, pH 2, time 3 h; R12, pH 1.5, time 2 h; R13, pH 1.5, time 3 h; R22, pH 2, time 2 h; R23, pH 2, time 3 h. B: from butternut powder. R: from red beet powder. η_0 represents the zero shear rate viscosity, η_{∞} represents the infinite shear rate viscosity, τ is the time constant corresponding to the Cross model, and m is a dimensionless parameter. R^2 : goodness of fitting (α : 0.05). ^b Mean and standard error are shown.

Experimental data for aqueous systems did not fit adequately to the Carreau model. Concerning the Cross model, only the B12 aqueous system did not fit and **Table 7** shows results obtained. It can be observed that the structural relaxation time (τ) derived from data fitting attained the highest value for the R12 product and decreased in one or more orders of magnitude for other fractions, being always higher for pectin-enriched products derived from red beet. A more stable network constituted by the hydrated polysaccharides needs more time for relaxation. As it can be observed, the upper Newtonian plateau was, as usual, inaccessible. The high-shear-rate viscosity (η_{∞}) determined was nonsignificantly different from zero with the exception of the one calculated from B23 experimental data. Butternut samples herein studied showed higher values for Cross parameters (η_0 , τ , m) than the ones observed for samples isolated from the same tissue by Fissore et al. (5) using enzymes.

Pagan and Ibarz (44) extracted pectin from peach pomace at different pH values (1.2–2.5), different temperatures (40, 60, 80 °C) and different times (10–80 min). They observed a decrease in yield values when the pH increased at constant temperatures and times. The highest yield was 12.4% at 80 °C, pH 1.4, 70 min. Gels prepared with the fraction of the highest yield showed a thixotropic behavior and also a lack of minimum shear stress; when thixotropy was eliminated, the behavior observed was Newtonian (viscosity at 25 °C \approx 70 mPa s). Mesbahi et al. (45) extracted pectin from sugar beet pulp with HCl solutions at different times and temperatures using two pH levels (1.0 and 1.5) and determined a good performance of extracted fractions as thickening agents.

It can be concluded that, when 2.00% (w/v) aqueous systems of the different products were evaluated at flow, they showed low viscosity and hence poor thickening properties. Samples B22 and B23 showed the highest η_a at a shear rate of 20 s⁻¹. Samples extracted at pH 1.5 showed yield stress (τ_0), and sample B12 showed the most marked pseudoplastic behavior after attaining τ_0 . The Cross model showed that pectin-enriched products derived from red beet had higher structural relaxation times than products derived from butternut.

LITERATURE CITED

- Goycoolea, F. M.; Cárdenas, A. Pectins from *Opuntia* spp.: A short review. *J. Prof. Assoc. Cactus* **2003**, *5*, 17–29.
- MacDougall, A. J.; Ring, S. G. Pectic polysaccharides. In *Chemical and Functional Properties of Food Saccharides*; Tomasik, P., Ed.; CRC Press LLC: Boca Raton, FL, 2004; Vol. 4, Chapter 12.
- Vincken, J. P.; Schols, H. A.; Oomen, R. J. F. J.; Beldman, G.; Visser, R. G. F.; Voragen, A. G. J. Pectin- the hairy thing: evidence that

- homogalacturonan is a side chain of rhamnogalacturonan I. In *Advances in Pectin and Pectinase Research*; Voragen, A. G. J., Schols, H., Visser, R., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2003; pp 47–59.
- Vincken, J. P.; Schols, H. A.; Oomen, R. J. F. J.; McCann, M. C.; Ulvskov, P.; Voragen, A. G. J.; Visser, R. G. F. If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiol.* **2003**, *132*, 1781–1789.
 - Fissore, E. N.; Ponce, N. M.; Stortz, C. A.; Rojas, A. M.; Gerschenson, L. N. Characterization of fibre obtained from pumpkin (*Cucumis moschata* Duch) mesocarp through enzymatic treatment. *Food Sci. Technol. Int.* **2007**, *16*, 1–7.
 - Iglesias, M. T.; Lozano, J. E. Extraction and characterization of sunflower pectin. *J. Food Eng.* **2004**, *62*, 215–223.
 - May, C. D. Pectins. In *Handbook of Hydrocolloids*; Phillips, G. O., Williams, P. A., Eds.; Woodhead Publishing Limited: Cambridge, England, 2000; Chapter 10.
 - May, C. D. Industrial pectins: Sources, production, and applications. *Carbohydr. Polym.* **1990**, *12*, 79–99.
 - Levigne, S.; Ralet, M. C.; Thibault, J. F. Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydr. Polym.* **2002**, *49*, 145–153.
 - Faravash, R. S.; Ashtiani, F. Z. The influence of acid volume, ethanol-to-extract ratio and acid-washing time on the yield of pectic substances extraction from peach pomace. *Food Hydrocolloids* **2008**, *22*, 196–202.
 - Kertesz, Z. I. In *The Pectic Substances*; Interscience Publishers: New York, NY, 1951; pp 438–525.
 - Canteri-Schemin, M. H.; Ramos Feretonani, H. C.; Waszczynskij, N.; Wosiacki, G. Extraction of pectin from apple pomace. *Braz. Arch. Biol. Technol.* **2005**, *48*, 259–266.
 - Wang, Q.; Cui, S. W. Understanding the physical properties of food polysaccharides. In *Food Carbohydrates: chemistry, physical properties, and applications*; Cui, S. W., Ed.; Taylor and Francis: Boca Raton, FL, 2005; Chapter 4.
 - Gourgue, C. M. P.; Champ, M. M. J.; Lozano, Y.; Delort-Laval, J. Dietary fiber from mango byproducts: Characterization and hypoglycemic effects determined by in vitro methods. *J. Agric. Food Chem.* **1992**, *40*, 1864–1868.
 - Chau, C. F.; Huang, Y. L.; Lee, M. H. In vitro hypoglycemic effects of different insoluble fiber-rich fractions prepared from the peel of *Citrus Sinensis* L. cv. Liucheng. *J. Agric. Food Chem.* **2003**, *51*, 6623–6626.
 - Gasztanyi, M. N.; Daood, H.; Takács Hájos, M.; Biacs, P. Comparison of red beet (*Beta vulgaris* var *conditiva*) varieties on the basis of their pigment components. *J. Sci. Food Agric.* **2001**, *81*, 932–933.
 - Martin-Cabrejas, M. A. M.; Waldron, K. W.; Selvendran, R. R. Cell wall changes in Spanish pear during ripening. *J. Plant Physiol.* **1994**, *144*, 541–548.
 - Filiseti-Cozzi, T. M. C. C.; Carpita, N. C. Measurement of uronic acids without interference from neutral sugars. *Anal. Biochem.* **1991**, *197*, 157–162.
 - Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Robers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.
 - Karkalas, J. J. An improved enzymic method for the determination of native and modified starch. *J. Sci. Food Agric.* **1985**, *36*, 1019–1027.
 - Wood, P. J.; Siddiqui, I. R. Determination of methanol and its application for measurement of pectin ester content and pectin methyl esterase activity. *Anal. Biochem.* **1971**, *39*, 418–428.
 - Naumenko, I. V.; Phillipov, M. P. Colorimetric method for determination of acetyl groups in pectic substances. *Akad. Nauk Repub. Moldova Biol. Khim. Nauk.* **1992**, *1*, 56–59 (in Russian).
 - Stevenson, T. T.; Darvill, A. G.; Albersheim, P. 3-deoxy-D-lyxo-2-heptulosaric acid, a component of the plant cell-wall polysaccharide rhamnogalacturonan II. *Carbohydr. Res.* **1988**, *179*, 269–288.
 - Karkhanis, Y. D.; Zeltner, J. Y.; Jackson, J. J.; Carlo, D. J. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of Gram-negative bacteria. *Anal. Biochem.* **1978**, *85*, 595–601.

- (25) York, W. S.; Darvill, A. G.; McNeil, M.; Albersheim, P. 3-deoxy-D-manno-2-octulosonic acid (KDO) is a component of rhamnogalacturonan II, a pectic polysaccharide in the primary cell walls of plants. *Carbohydr. Res.* **1985**, *138*, 109–126.
- (26) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
- (27) Mattoo, R.; Ishaq, M.; Aleemuddin, M. Protein assay by Coomassie Brilliant Blue G-250 binding method is unsuitable for plant tissues rich in phenols and phenolases. *Anal. Biochem.* **1987**, *163*, 376–384.
- (28) AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; AOAC Inc.: USA, 1990; Volume 2; Inc. Method 928.08.
- (29) de Escalada Pla, M.; Ponce, N. M.; Stortz, C. A.; Gerschenson, L. N.; Rojas, A. M. Composition and functional properties of enriched fiber products obtained from pumpkin (*Cucurbita moschata* Duchesne ex Poiret). *Lebensm.-Wiss. Technol.* **2007**, *40*, 1176–1185.
- (30) Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydr. Res.* **1967**, *5*, 340–345.
- (31) Ciucanu, I.; Kerek, F. A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.* **1984**, *131*, 209–217.
- (32) Shea, E. M.; Carpita, N. C. Separation of partially methylated alditol acetates on SP-2330 and HP-1 vitreous silica capillary columns. *J. Chromatogr., A* **1988**, *445*, 424–428.
- (33) Wan Nik, W. B.; Ani, F. N.; Masjuki, H. H.; Eng Giap, S. G. Rheology of bio-edible oils according to several rheological models and its potential as hydraulic fluid. *Ind. Crops Prod.* **2005**, *22*, 249–255.
- (34) Sokal, R. R.; Rohlf, J. B. *Biometry. The principles and practice of statistics in biological research*; WH Freeman and Company: San Francisco, 2000; pp 253–380.
- (35) Yapo, B. M.; Robert, C.; Etienne, I.; Wathelet, B.; Paquot, M. Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chem.* **2007**, *100*, 1356–1364.
- (36) Oosterveld, A.; Beldman, G.; Schols, A.; Voragen, A. G. J. Characterization of arabinose and ferulic acid rich pectic polysaccharides and hemicelluloses from sugar beet pulp. *Carbohydr. Res.* **2000**, *328*, 185–197.
- (37) Happi Emaga, T.; Ronkart, S. N.; Robert, C.; Wathelet, B.; Paquot, M. Characterisation of pectins extracted from banana peels (*Musa AAA*) under different conditions using an experimental design. *Food Chem.* **2008**, *108*, 463–471.
- (38) Strasser, G. R.; Amadó, R. Pectic substances from red beet (*Beta vulgaris* L. var. conditiva). Part II. Structural characterization of rhamnogalacturonan II. *Carbohydr. Polym.* **2002**, *48*, 263–269.
- (39) Mohnen, D. Pectin structure and biosynthesis. *Curr. Opin. Plant Biol.* **2008**, *11*, 266–277.
- (40) Strasser, G. R.; Amadó, R. Pectic substances from red beet (*Beta vulgaris* conditiva). Part I. Structural analysis of rhamnogalacturonan I using enzymic degradation and methylation analysis. *Carbohydr. Polym.* **2001**, *44*, 63–70.
- (41) Navarro, D. A.; Cerezo, A. S.; Stortz, C. A. NMR spectroscopy and chemical studies of an arabinan-rich system from the endosperm of the seed of *Gleditsia triacanthos*. *Carbohydr. Res.* **2002**, *337*, 253–263.
- (42) Fransen, C. T. M.; Haseley, S. R.; Huisman, M. M. H.; Schols, H. A.; Voragen, A. G. J.; Kamerling, J. P.; Vliegthart, J. F. G. Studies on the structure of a lithium-treated soybean pectin: characteristics of the fragments and determination of the carbohydrate substituents of galacturonic acid. *Carbohydr. Res.* **2000**, *328*, 539–547.
- (43) Tamaki, Y.; Konishi, T.; Fukuta, M.; Kato, M. Isolation and structural characterisation of pectin from endocarp of *Citrus depressa*. *Food Chem.* **2008**, *107*, 352–361.
- (44) Pagán, J.; Ibarz, A. Extraction and rheological properties of pectin from fresh peach pomace. *J. Food Eng.* **1999**, *39*, 193–201.
- (45) Mesbahi, G.; Jamalian, J.; Farahnaky, A. A comparative study on functional properties of beet and citrus pectins in food systems. *Food Hydrocolloids* **2005**, *19*, 731–738.

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