Article

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Isolation of pectin-enriched products from red beet (*Beta vulgaris* L. var. *conditiva*) wastes: composition and functional properties

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Abstract

The present work was dedicated to the development of an extraction process for red beet (*Beta vulgaris* L. var. *conditiva*) by-products that preserves the high molecular weight of the macromolecules with the primary aim of waste upgrading. Our study concerns the extraction of pectin-enriched products with potential thickening properties for their usage in food formulation, as well as with some healthy physiological effect, by using citrate buffer (pH = 5.2) either alone or with enzymes (hemicellulase or cellulase) active on cell wall polysaccharide networks. Considering that red beet tissue contains ferulic acid, which cross-links pectin macromolecules through arabinose residues to anchor them into the cell wall, an alkaline pretreatment was also evaluated in order to perform polysaccharide hydrolysis in the cell wall network to accomplish higher renderings. Chemical composition and yield, as well as the in vitro glucose retention exerted by the isolated fiber products were finally analyzed.

Keywords

Red beet, wastes, pectin, enzym, glucose retention

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INTRODUCTION

By-products of vegetable–food processing represent a major disposal problem for the industry (Laufenberg et al., 2003). The increasing demand by consumers for minimally processed (fresh-cut) fruits and vegetables has prompted the sale of precut red beet roots, as well as other vegetables in trays commercialized in markets or sold in bulk for institutions. This processing leads simultaneously to increasing vegetable wastes in addition to those coming from the canned industry.

Red beets are available all year-round in the market. Since they are roots, their tissue accumulates a considerable amount of sugars which confer sweet taste to the tissue, being at the same time a source of bioactive compounds (Gasztonyi et al., 2001). By-products of its industrialization are used for animal feeding or disposed as industrial wastes. Wastes from the food-processing industry have some common characteristics, such as large amounts of organic material: proteins, carbohydrates and lipids. Transformation of vegetable waste of the food industry into value added products, as fibers are, may contribute to diminish pollution and to recover valuable biomass and nutrients (Laufenberg et al., 2003). The isolation of pectin-enriched products from the food industry wastes of vegetable origin

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constitutes an interesting alternative, since pectins are widely used as texture modifiers in food and pharmaceutical industries and, at the same time, they are also recognized for their capacity to delay glucose (Glc) absorption and enzymatic degradation of starch. This kind of cell wall polysaccharides (CWP) has been reported to lower serum cholesterol levels, to bind heavy metals, and to have immuno-stimulating and anti-ulcer activities. Pectins constitute a family of complex polysaccharides present in all plant primary cell walls (CWs; Ridley et al., 2001). The composition, structure, molecular weight and physiological properties of pectin, characteristics that will determine its functionality, might be influenced by conditions of extraction as well as sources, location and many other environmental factors. Tartaric, malic, citric, lactic and acetic acids can be used for pectin extraction, but it can be observed a tendency to use the cheaper mineral acids such as sulphuric, hydrochloric and nitric acids (Kertesz, 1951), nonenvironmentally friendly reagents. Canteri-Schemin et al. (2005) 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. It has been demonstrated the presence of ester-linked ferulic acid in pectin fractions isolated from certain plants like red beet roots, which confers special mechanical resistance to thermal processing (Waldron et al., 1997a). Ferulic acids are attached to arabinosyl and galactosyl residues in the side chains of pectin macromolecules.

The objective of this research was to explore the feasibility of isolation of pectin-enriched products from wastes of red beet root industrialization by using citrate buffer (pH = 5.2) either alone or with enzymes (hemicellulase or cellulase) active for hydrolysis of CW networks. Considering that red beet tissue contains ferulic acid, which cross-links pectin macromolecules through arabinose (Ara) residues to anchor them into the CW network, an alkaline pretreatment was also evaluated in order to obtain adequate renderings in conjunction with valuable functional properties of the isolated products. Their chemical composition and in vitro Glc retardation effect were determined for characterization. Results of this study can help in the utilization of by-products, contributing to the efficiency of the process, to the sustainability of the environment and to add value to vegetable wastes.

MATERIALS AND METHODS

Sample preparation

Red beet (*Beta vulgaris* L. var. *conditiva*) harvested in Argentina was bought in a local market. Roots were carefully washed and peeled and were used to emulate the residues obtained in the manufacture of ready-to-eat or minimally processed vegetables. Juice was removed by means of a domestic 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), milled (Wemir E909, Wemir, Buenos Aires, Argentina) and sieved for obtaining a powder with particle size in the range 420–710 μm. The moisture content of powder obtained was 10.33 g/100 g (dry basis). This product (10 g), enriched in cell wall material (CWM), was treated with the enzymes below indicated, either with or without a pretreatment with 2 mol/L sodium hydroxide (NaOH) solution (500 mL). In the case of application of this pretreatment, it was performed at 25 °C for 30 min under constant agitation and then partial neutralization with citric acid (solid) and filtration under vacuum using glass fiber filter (Schleicher and Schuell, Dassel, Germany) were performed. Either the residue obtained from the alkaline pretreatment or, directly, the beet root powder obtained as firstly indicated, were poured into a beaker containing 1000 mL of 0.05 mol/L sodium citrate buffer solution with 0.01 g/100 g of sodium azide (final concentration). The pH was adjusted to 5.20 with citric acid and the system was then submitted to digestion under stirring (10 rad/s), either without or with a CW-degrading enzyme, according to Fissore et al. (2007). Enzymes used were:

- Hemicellulase H2125 (SIGMA, St. Louis, USA): hemicellulase from *Aspergillus niger* with side cellulose activity. One unit will produce a relative fluidity change of 1 per 5 min, using locust bean gum as substrate at pH 4.5 at 40 °C.
- Cellulase C9422 (SIGMA, St. Louis, USA): cellulase from *Trichoderma viride*. One unit will liberate 1.0 micromole of Glc from cellulose in 1 h at pH 5.0 at 37 °C (2 h incubation time).

Both enzymes were obtained from Sigma (St. Louis, USA) and the assay was performed at 30 °C for 20 h (Fissore et al., 2007). The enzyme/substrate ratios herein used were chosen based on a previous work that was done with butternut tissue (Fissore et al., 2007) for the purpose of comparing results obtained. Since pectin yields obtained when using red beet were very low, it was decided to modify the method of extraction by adding an alkaline pretreatment to break the diferulic bonds present in this tissue. The following products or fractions were obtained:

- BARESE (with alkaline pretreatment) or RESE (without alkaline pretreatment): product obtained from the treatment of 10 g of red beet residue with 1000 mL of citrate buffer (no enzyme addition),

Table 1. Chemical composition of pectin-enriched fractions isolated from red beet either through citrate-buffer pH 5.20 (RESE) or after enzymatic hydrolysis with hemicellulase (REH1, REH2) or cellulase (REC1, REC2) in citrate-buffer pH 5.20

	Pectin-enriched fractions (mean ± SD, $n = 3$)				
Composition	RESE	REH1	REH2	REC1	REC2
Total carbohydrates (as galacturonic acid g/kg)	597 ± 9	546 ± 8	998 ± 15	276 ± 4	613 ± 21
Uronic acids (g/kg)	555 ± 3	213 ± 9	198 ± 12	210 ± 7	493 ± 28
Methanol (g/kg)	36 ± 2	12 ± 2	12.4 ± 0.5	13 ± 1	32.2 ± 0.9
Protein (g/kg)	73 ± 3	63 ± 4	29 ± 1	76 ± 4	23 ± 4
DM (%)[1]	36	30	35	34	36
Yield (g/kg)	11	19	1	9	8

[1]Degree of methylation (DM) was calculated as a percent ratio between moles of methanol and moles of Uronic acids per 100 g of sample.

- BAREH1 (with alkaline pretreatment) or REH1 (without pretreatment): product obtained from the treatment of 10 g red beet residue with 0.25 g hemicellulase and 1000 mL of citrate buffer,
- BAREH2 (with alkaline pretreatment) or REH2 (without pretreatment): product obtained from the treatment of 10 g of red beet residue with 0.75 g hemicellulase and 1000 mL of citrate buffer,
- BAREC1(with alkaline pretreatment) or REC1 (without pretreatment): product obtained from the treatment of 10 g of red beet residue with 0.05 g cellulase and 1000 mL of citrate buffer,
- BAREC2 (with alkaline pretreatment) or REC2 (without pretreatment): product obtained from the treatment of 10 g of red beet residue with 0.15 g cellulase and 1000 mL of citrate buffer.

Deionized (Milli-Q™, USA) water was used for all treatments. Insoluble obtained after enzymatic digestions were separated through filtration under vacuum, with glass fiber filter paper (Schleicher and Schuell, Dassel, Germany), and CWP were finally precipitated from each supernatant through ethanol addition (2 volumes). These CWPs were collected through filtration under vacuum using glass fiber filter paper, washed and, finally, freeze-dried.

In each case, yield was calculated as grams of product obtained per kilogram of powder enriched in CWM used (Tables 1 and 2).

Chemical analyses

Deionized (Milli-Q™, USA) water was used for preparation of all reagents used. The following analyses were performed to each sample obtained:

a. *Uronic acids* through the spectrophotometric method reported by Filisetti-Cozzi and Carpita (1991).
b. *Total carbohydrates* by the phenol–sulfuric acid colorimetric method of Dubois et al. (1956).
c. *Methanol* by the spectrophotometric method of Wood and Siddiqui (1971).
d. *Acetyl groups* according to the method of Naumenko and Phillipov (1992).
e. *Degree of methylation* (DM) was calculated as the percentage ratio between moles of methanol and moles of galacturonic acid (GalA) in the analyzed sample, whereas degree of acetylation (DA) was calculated as the percentage ratio between moles of acetyl group and moles of total carbohydrates in the samples.
f. 2-keto-3-deoxi-D-*manno*-octulosonic acid (*Kdo*) and 2-keto-3-deoxi-D-*lyxo*-heptulosaric acid (*Dha*) were simultaneously determined through a modified thiobarbituric acid spectrophotometric assay (Karkhanis et al., 1978; York et al., 1985).
g. *Protein content* was determined into each sample by Lowry et al. (1951) assay using bovine serum albumin as standard (Sigma, St. Louis, USA).
h. *Starch content* was determined according to American Association of Cereal Chemists (AACC, 1995), using α-amylase and amyloglucosidase (Sigma starch kit, St. Louis, USA).

All these determinations were performed three times on each sample.

(i) *Neutral sugar analysis*: The proportions of neutral monosaccharides constituting the CWP were determined after hydrolysis with fresh 2 mol/L trifluoroacetic acid (TFA) solution for 2 h at 121 °C. Every hydrolysis step was carried out in duplicate. It is important to remark that hydrolysis conditions used were not able to hydrolyze cellulose.

Hydrolysates were derivatized to the alditol acetates according to Albersheim et al. (1967) and analyzed by

Table 2. Chemical composition of pectin-enriched fractions isolated from red beet after alkaline pretreatment followed either by citrate-buffer pH 5.20 (BARESE) or by enzymatic hydrolysis with hemicellulase (BAREH1, BAREH2) or cellulase (BAREC1, BAREC2) in citrate-buffer pH 5.20

	Pectin-enriched fractions (mean ± SD, $n = 3$)				
Composition	BARESE	BAREH1	BAREH2	BAREC1	BAREC2
Total carbohydrates (as galacturonic acid g/kg)	827 ± 7	940 ± 27	842 ± 20	877 ± 13	904 ± 20
Uronic acids (g/kg)	455 ± 22	471 ± 19	522 ± 37	541 ± 24	553 ± 34
Methanol (g/kg)	2.6 ± 0.2	2.0 ± 0.2	2.3 ± 0.2	2.0 ± 0.2	2.6 ± 0.5
Protein (g/kg)	12 ± 2	20 ± 3	5.8 ± 0.8	58 ± 4	26 ± 2
DM (%)[1]	3.14	2.33	2.42	2.04	2.59
Acetyl groups (g/kg)	1.30 ± 0.08	1.65 ± 0.04	1.59 ± 0.04	2.4 ± 0.2	1.84 ± 0.08
DA (%)[2]	1.21	1.43	1.25	1.78	1.37
Starch (g/kg)	8.6 ± 4.3	103 ± 6	152.0 ± 0.4	8.9 ± 0.6	10.7 ± 1.3
Yield (g/kg)[3]	99	96	82	207	152

DM: degree of methylation, DA: degree of acetylation, GalA: galacturonic acid.
[1]DM and [2]DA were calculated as a percent ratio between moles of methanol or acetyl groups and moles of GalA (uronic acids) or total carbohydrates per 100 g of sample, respectively.
[3]Grams per kg of powder submitted to the extractive procedure.

gas–liquid chromatography (GLC) coupled to flame ionization detection for quantitative determination or to electron impact–mass spectrometric detection, when qualitative characterization was required. The first GLC method used a capillary column on a HP-5890^{TM} gas chromatograph (USA) 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 was carried out to confirm structures of unusual sugars present in rhamnogalacturonan II (RG-II), through a Shimadzu QP 5050 A GC/MS apparatus (Japan), working at 70 eV and using helium (He) as carrier. Chromatographic runs were isothermally performed at 220 °C.

(j) *Treatment of samples with carbodiimide to confirm the type of uronic acids present:* A total of 1 mmol of carbodiimide was added to 0.5 mL of methanol–water solution containing enough sample material to give 100 microequivalents of carboxylic acid, taking into account the uronic acid content previously determined for each sample by the spectrophotometric method of Filisetti-Cozzi et al. (1991). This solution was stirred for 2 h at constant pH (4.7–5.0). Around 1.5 mg of sodium tetradeuteroborate ($NaBD_4$) 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 proportion of neutral monosaccharides (galactose (Gal) from polygalacturonic or homogalacturonan (HG) chains) obtained after GalA reduction were again determined after hydrolysis with 2 mol/L TFA solution for 2 h at 121 °C using inositol as internal standard. Hydrolysates were derivatized to the alditol acetates according to Albersheim et al. (1967) and analyzed by GC, as previously indicated.

Molecular weight profiles

Samples were dialyzed against 0.5 mol/L imidazole buffer (pH 7.0) at 5 °C during 48 h, by using a cellulose dialysis membrane with a molecular weight cutoff of 12 000 (N° 4465-A2A2, Erovne S.A., Buenos Aires, Argentina) before freeze-drying after immersion in liquid nitrogen. The solid obtained was then dissolved in the 0.5 mol/L imidazole buffer, as recommended by Mort et al. (1991). Molecular weight profiles were determined by gel filtration analysis using a Fast Protein Liquid Chromatograph (FPLC, Pharmacia, Sweden) with a Superose 12HR 10/30 column (Amersham Biosciences-GE Healthcare, Buenos Aires, Argentina), eluted with 0.5 mol/L imidazole buffer (pH 7.0) at 0.5 mL/min (Wessels et al., 1998). Dextrans of 65 000 and 40 210 molecular weights and blue dextran (Sigma, St. Louis, USA), $CoCl_2$ and sucrose (Merck, Argentina) were used as standards for column calibration.

Total carbohydrate content was determined into each collected fraction through the phenol – sulfuric acid spectrophotometric method (Dubois et al., 1956).

In vitro Glc retardation effect

Previous to the Glucose Dialysis Retardation Index (GDRI) assay and, in order to remove free Glc from the material, samples were dialyzed (48 h, 5 °C) against deionized water using the dialysis membranes like in molecular weight determination, and finally freeze-dried. Semipermeable membrane permeation method of Chau et al. (2004) was used with slight modifications. After inserting ≈0.1000 g of red beet dietary fiber sample into the cellulose dialyzing bag, 5000 μL of a 0.22 mol/L Glc solution was added. This bag was closed and put into a 250 mL glass beaker containing 160 mL of deionized water under continuous magnetic stirring (10 rad/s) for 180 min to perform the permeation experiment. The same procedure was followed but without addition of dietary fiber for the control sample. In all cases, the system was maintained at 37 °C.

At constant intervals, 100 μL of dialyzed solution were taken and the GDRI was determined as a function of time through measurement of free Glc content, by the phenol and 4-aminophenazone colorimetric reaction with previous enzyme-specific oxidation of Glc by glucose oxidase and peroxidase (Wiener Lab^{TM} glycemia kit, Rosario, Argentina). The retardation effect was calculated according to the following equation:

$$\mathrm{GDRI} = 100 - \left[\left(\frac{\mathrm{Glc_{sample}}}{\mathrm{Glc_{control}}}\right).100\right] \qquad (1)$$

Wherein GDRI is reported as percentage (%). Assay was carried out in triplicate.

Statistical analyses

Nonlinear fittings were performed through Prism 5 Statistical Software for Windows (GraphPad, USA), as well as through the Solver function of the Excel program for Windows XP (Microsoft, USA). Statistical analyses for result comparison was carried out through ANOVA (level of significance, $\alpha = 0.05$) followed by pairwise multiple comparisons using Tukey's significant difference test (Sokal and Rohlf, 2000).

RESULTS AND DISCUSSION

The juice extraction performed on red beet tissue produced the concentration of the CWM in the residue, which was then twice submitted to water washing to eliminate starch and residual water-soluble components belonging to the cytoplasmic medium (globular proteins, aminoacids, mono and di/oligosaccharides, salts and organic acids), probably contaminating the solid residue of extraction. Total as well as nonresistant starch contents of red beet powder were low (1.4 ± 0.2 and 0.8 ± 0.3 g/100 g of powder, respectively), as previously determined by Fissore et al. (2010). Some water-soluble pectins, which are loosely bound in the CW matrix (Fry, 1986) may also be in part eliminated during washing.

Yield and chemical composition of fractions obtained

Red beet powder obtained after drying at 85 °C was submitted to enzymic digestion in sodium citrate buffer of pH 5.2. Buffers are useful for control of pH which affects β-elimination (Kravtchenko et al., 1992). On the other hand, they are active substances as cross-link cleaving agents of CWP because of the salts that they contain (Fry, 1986); consequently, the same digestion was repeated without enzymes with the aim of evaluating the characteristics of pectin extracted from the CWM by the buffer itself (fraction called RESE; Table 1). Noncovalent cross-links like ionic bonds between CWP are disrupted by salt effect, as well as by the acid pH (5.2), mainly when stirring is performed at temperatures higher than 18–20 °C (Brett and Waldron, 1996), which are herein needed for enzyme activity. In addition, other noncovalent cross-links like Ca^{2+}-bridges can be broken specifically by citrate used in this buffer because of its chelating effect.

Direct treatment of the red beet CWM with hemicellulase or cellulase, gave origin to water-soluble products with an important content of total carbohydrates (Table 1). It can be observed that extracted RESE fraction was HG, since its total carbohydrate content was uronic acids (Table 1). It was herein determined that GalA and ≈6 g/100 g of glucuronic acid constituted the total uronic acid content in red beet root pectins, as previously reported by Strasser and Amadó (2002). The REC1 and REC2 fractions separated with increasing cellulase activity in buffer solution, also showed a main proportion of uronic acids (≈80 g/100 g total carbohydrates). Buffer activity seemed to prevail in the extraction of pectin domains in the REC1 and REC2 products, showing similar yields and composition than RESE. Thus, in these fractions, calcium complexation by citrate may have produced the main extraction of HG side chains of rhamnogalacturonan I (RG I) core of pectin macromolecules, more probably belonging to the middle lamella regions and cell corners (Vincken et al., 2003). On the other hand, the use of increasing concentrations of hemicellulase produced pectin fractions (REH1 and REH2) constituted by decreasing HG contents (39–20 g/100 g total carbohydrates) and higher proportion of neutral sugars (NS), as could be determined from the difference between the total carbohydrate and uronic acid contents (Table 1).

Hemicellulase are a group of enzymes that are defined and classified according to their substrate, hemicellulose. They represent an array of enzymes such as xylanases, mannases, arabinases (both endo and exo kinds) and their corresponding glycosidases (Ghose and Bisaria, 1987). It is important to remark that, according to providers, the hemicellulase herein used has side cellulase activity, which can result in a certain degree of cellulose framework disruption as well.

All pectin-enriched isolated fractions were of low DM (<50%) (Table 1). However, it was determined a high DM (≈87%) in the red beet powder used in the present work. Hence, some proportion of methyl-ester hydrolysis occurred during extraction because of the acidic medium and temperature. Protein contents were between 23 and 76 g/kg and may come in part from CW due to buffer activity on pectin-in-extension entanglements. It has to be kept in mind that temperatures higher than 18–20 °C can lead to removal and cleavage of some pectic backbones regardless of pH (Fry, 1986), promoting some degree of β-elimination (Brett and Waldron, 1996).

Renderings of pectin-enriched fractions were between 1 and 19 g/kg (Table 1). Low renderings obtained after direct treatments either with or without enzymes in buffer citrate solution could be ascribed to the presence of ferulate dehydrodimers cross-linking pectic polysaccharides in the CWM of red beet, which would not allow obtaining the desirable pectin-enriched products. It has been reported that the content of Ara as well as of dehydrodimers of ferulic acid in pectic polysaccharides of sugar beet (Oosterveld et al., 2000) and red beet (Waldron et al., 1997a, 1997b). Levigne et al. (2004) found a dehydrodiferuloylated oligoarabinan in sugar beet CWs. Two dimers of α-(1→5)-linked Ara units esterified by a central 8-O-4' ferulic dimer were determined by the authors. The in vivo oxidative coupling to form dehydrodimers, covalently cross-links the polysaccharide they esterify. Such coupling may contribute to wall assembly, promote tissue cohesion and restrict cell expansion (Waldron et al., 1997a). Evidently, acidic treatment (pH 5.2) by citrate buffer solution at 30 °C, coupled or not to enzymic treatment, as applied in the present work, was not enough to accomplish significant hydrolysis of diferulate–Ara esters, as observed from the renderings obtained (Table 1). These low yields led to think that enzymatic treatments were by itself not adequate for efficient extraction of macromolecules with potentially valuable functional properties from red beet CWM, as it was previously carried out from butternut (Fissore et al., 2007). Fissore et al. (2010) in a previous work, used longer periods of treatment with citrate buffer, at similar temperature conditions, for red beet, obtaining fractions with low HG content and poor functionality as thickening agents, though high yields (17–31%) were obtained.

In the present research, it was decided to perform an alkaline hydrolysis (30 min; 25 °C) to the red beet powder herein obtained, in order to saponify the esterified ferulic dimers present (Waldron et al., 1997a), previous to buffer or buffer/enzyme treatment. This procedure led to significantly higher yields (>80 g/kg); after cellulase attack, renderings attained values of 150–200 g/kg, as can be observed in Table 2.

Fractions obtained after alkaline pretreatment were essentially constituted by water-soluble polysaccharides (≈100 g/100 g), which showed balanced ratios between uronic acids (50–62 g/100 g total carbohydrates) and NS (38–50 g/100 g total carbohydrates) contents (Table 2). This suggested that a more complete extraction of pectin macromolecules than without alkaline pretreatment (Table 1) was performed. Fractions were characterized by an almost total demethylation (DM ≈ 2.5%, molar ratio) as expectable, after saponification suffered by the methyl esters at the C6-carboxylic groups of GalA monomers, during the alkaline pretreatment. The methyl esterification of HG, in particular, has drawn the attention of many research groups, because it determines to a large extent the industrial applicability of pectin. Not only the amount of methyl-esterification is important, but also the distribution of methyl groups on the HG backbone (Willats et al., 2001). Blocks of more than 10–15 unesterified GalA residues generally yield pectin molecules, which are sensitive to Ca^{2+} cross-linking (Guillotin et al., 2005). Low–acetylation degree (≈1%, molar ratio) was also found after alkaline-pretreatment as well as in the original red beet powder herein used, though CW (alcohol-insoluble material, AIR) extracted in a previous work (Fissore et al., 2010) showed a 90% DA. Thus, drying temperature (85 °C) of the red beet residue seems to be specifically detrimental for acetylation of GalA and NS monomers of pectins. It was not observed as an important difference in the NS composition of the pectin polysaccharides isolated from the different fractions (Table 3), even though a higher proportion of starch was found contaminating BAREH1 and BAREH2 fractions (Glc monosaccharide content: 10.44 and 3.12 mol%). On the other hand, some detectable content of sugars like 2-O-Me-fucose, 2-O-Me-xylose, Dha and Kdo, revealed the presence in all fractions of some residual proportion of RG-II (Strasser and Amadó, 2002), though it seems to be more abundant in fractions BAREH1, BAREH2 and BAREC2. It was also determined, in general, a lower content of protein (5.8–58 g/kg) in the isolated products (Table 2), probably as a consequence of a more selective extraction of CWP.

Table 3. Sugar composition of fiber-enriched products isolated from red beet after alkaline pretreatment followed either by citrate-buffer pH 5.20 (BARESE) or by enzymatic hydrolysis with hemicellulase (BAREH1, BAREH2) or cellulase (BAREC1, BAREC2) in citrate-buffer pH 5.20

	Fiber-enriched products				
	BARESE	BAREH1	BAREH2	BAREC1	BAREC2
2-O-Me Fuc (g/kg)[1]	tr	0	3.7	tr	tr
2-O-Me Xyl (g/kg)[1]	tr	4.4	4.1	0	4.2
Dha+Kdo (g/kg)[1]	0.5	0.7	0.2	0.1	0.3
Rhamnose (g/kg)	22	24	17	19	18
Fucose (g/kg)	tr	2	0	0	0
Arabinose (g/kg)	320	300	246	298	303
Xylose (g/kg)	0	0	0	0	0
Mannose (g/kg)	0	0	0	0	0
Galactose (g/kg)	29	32	22	20	22
Glucose (g/kg)	0	106	28	0	4

[1]2-O-Me Fuc: 2-O-methyl L-fucose, 2-O-Me Xyl: 2-O-methyl D-xylose, Dha: 2-keto-3-deoxi-D-*lyxo*-heptulosaric acid, Kdo: 2-keto-3-deoxi-D-*manno*-octulosonic acid.

Sugar composition (Table 3) of isolated fractions showed that they were highly enriched in pectins. They were mainly constituted by HG side chains attached to RG-I core, which are regions determined by the existence of some kinks of alternating rhamnose (Rha) monomers (Vincken et al., 2003). In the latter domain, rhamnosyl residues were highly substituted by α-(1→5)-linked arabinan side chains (Levigne et al., 2004) remaining in the polymer after alkaline and enzymatic treatments, as suggested by the high content of Ara (Table 3). In this sense, high molar ratios of Ara/Rha varying between 13 (BAREH1), 15 (BARESE) and 18 (after cellulase treatment) were calculated. Consequently, it can be hypothesized that these arabinan-side chains of the removed pectins were mainly related by the covalent cross-links of diferulic bridges in the original red beet powder, and were liberated after the alkaline pretreatment applied. Since very low contents of Gal were found in the isolated fractions, only a few of the Ara residues could be covalently attached to the remaining Gal monomers of the galactan backbone, for constituting the arabinogalactan type I (AG-I; Vincken et al., 2003). Hence, the hairy regions on RG-I of the isolated fractions seemed to be essentially constituted by arabinan and, only in a very low proportion, by AG-I. It has to be mentioned that the alkaline pretreatment of red beet powder (mainly CWM) decisively affected glycoside linkages between Gal of galactans (AG-I) and RG-I core, since an important proportion of Gal was found in the original alcohol-insoluble residue, AIR (molar ratio: Gal/Rha = 1.6), while the relation in these pectin fractions takes a value of 1. The high Ara content for all fractions is in accordance with the research of Strasser and Amadò (2001) on red beet, who observed high concentrations of Ara among NS. They stated that RG-I backbone is highly ramified, since two-thirds of the Rha residues are branched. These authors indicated that side chains consist of arabinans, galactans and type-II arabinogalactans (AG-II), being the latter, the most complex side chain within red beet RG-I pectins. They also observed the existence of some arabinans linked directly to the pectic backbone. However, Vincken et al. (2003) indicated that AG-II is mainly associated with proteins (arabinogalactan proteins or AGPs), and it is still unclear whether this polysaccharide is part of the pectin complex. Pectin and AG-II often seem to coextract and are subsequently difficult to separate from each other, suggesting that they are covalently linked. Strasser and Amadó (2002) stated that, in the case of RG-II of red beet, Ara residues are only β-glycosidically linked.

On the other hand, pectins isolated after alkaline treatment followed by cellulase hydrolysis provided the highest yielding as well as HG proportion (Table 2). The mechanism of cellulase hydrolysis might involve physical disruption of insoluble cellulose in addition to endo- and exo-acting enzymes. BAREC1 and BAREC2 fractions seemed to be more complete pectin macromolecules than the other fractions (Tables 2 and 3) and it is suggested that they are belonging to the second and following layers of pectin deposited in the primary CW. As it was suggested by Vincken et al. (2003), there is some evidence that mainly the pectin molecules highly substituted with side chains associate in one way or another with cellulose (or cellulose/xyloglucan). For this reason, high-strength alkaline solutions in combination with a

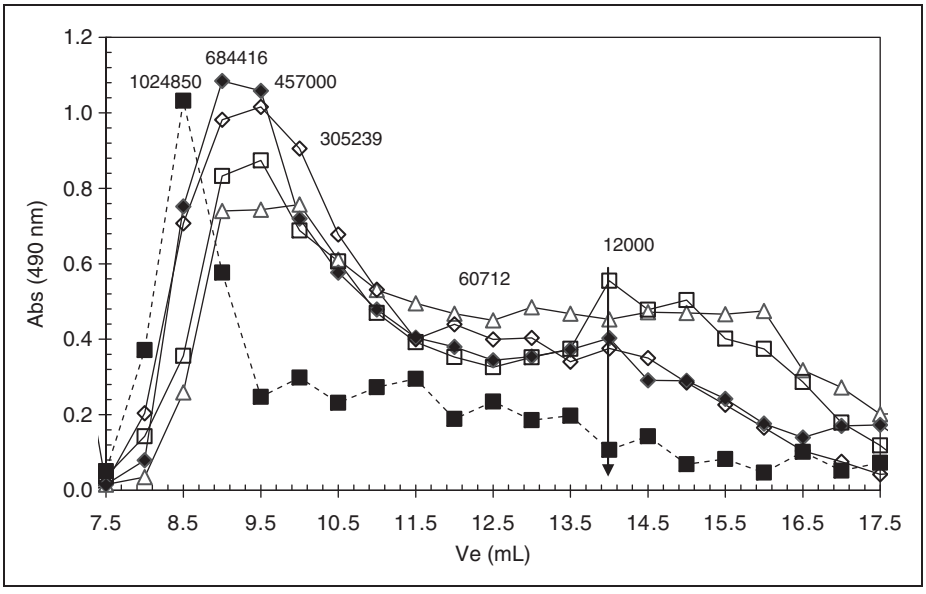

Figure 1. Molecular weight profiles of pectin fractions isolated from red beet. The absorbance (Abs) measured through the colorimetric reaction of total carbohydrates, was plotted against elution volume (Ve). Some molecular weights are indicated and the arrow points out the molecular weight cutoff of the membrane used. (△) BAREC1; (◆) BAREC2; (■) BARESE; (□) BAREH1; (◇) BAREH2.

Table 4. Glucose Dialysis Retardation Index of pectin-enriched fractions obtained after alkaline pretreatment followed either by citrate-buffer pH 5.20 (BARESE) or by enzymatic hydrolysis with hemicellulase (BAREH1, BAREH2) or cellulase (BAREC1, BAREC2) in citrate-buffer pH 5.20

Percentage of Glucose Dialysis Retardation Index (mean ± SD)					
Time (min)	BARESE	BAREH1	BAREH2	BAREC1	BAREC2
10	—	8.5 ± 0.7	53.7 ± 0.4	—	—
30	8.3 ± 0.5	13.3 ± 0.7	22 ± 7	—	—
60	13 ± 4	14 ± 5	19 ± 8	15 ± 5	—
90	8 ± 1	11 ± 2	12 ± 2	18 ± 3	9 ± 1
120	3.1 ± 0.8	4.3 ± 0.7	7.4 ± 0.7	11 ± 1	1.01 ± 0.02
150	4.9 ± 0.8	—	—	10 ± 1	2.1 ± 0.8
180	—	—	—	—	—

subsequent enzyme treatment were required to extract these polysaccharides. It has been also reported that the pectin molecules with low Ara content of a mutant wild tobacco line are poorly retained in the wall (Iwai et al., 2001). These results indicate a role for arabinan in anchoring pectin in the wall. Moreover, according to Waldron et al. (1997a), arabinan side chains from red beet CWP are cross-linked by ferulate dimers needing of alkaline hydrolyzing pretreatment for their removal from the cellulose network constraint, as above mentioned. On the other hand, galactans may play a more predominant role in controlling the pore size of the wall (Vincken et al., 2003).

As can be observed on Table 2, the salt effect of buffer by itself could remove pectins (BARESE fraction) from the remaining cellulose microfibril network of the red beet powder after only alkaline pretreatment, because of Ca^{2+}-complexation. Lower renderings obtained for BAREH1 and BAREH2 showed that, degradation of hemicelluloses (xyloglucans, xylans, glucuronoxylans, arabinoxylans and glucomannans, depending on monocots or dicots), which are not covalently related with cellulose, was insufficient for efficient removal of pectin materials from red beet powder after alkaline pretreatment. And also that cellulase side activity was probably scarce in the hemicellulase used.

Hence, in BAREH1 and BAREH2 obtention, the buffer effect by itself seemed to dominate after alkaline pretreatment giving origin to similar renderings and chemical polymer composition for those fractions and for BARESE (Table 2). Anyhow, hemicellulase seemed to enhance the removal of starch (Table 1).

Molecular weight profiles

Molecular weight patterns obtained after elution with imidazole buffer at pH 7.00, at which pectin macromolecules are expanded due to some carboxylate anion repulsion, were very similar. Molecular weights between 685 and 300 kDa were mainly showed by all pectin-enriched fractions excepting BARESE, which presented a sharp elution peak corresponding to a MW $\approx$ 1000 kDa, as well as a less significant tailing toward lower elution volumes than the rest of the extracted fractions (Figure 1). Although pectins of high molecular weights were mainly isolated after each specific chemical procedure performed, some degree of polysaccharide hydrolysis evidently occurred after alkaline/enzymatic treatment as revealed by the tailing in Figure 1, with the exception of BARESE fraction. The most important distribution of molecular weights occurred around a broad (685 000–300 000) peak at 457 000 Da.

Glucose Dialysis Retardation Index

Dietary fiber has the capacity of binding different substances like bile salts and Glc, which have implications in cholesterol metabolism and control of diabetes, respectively. Many researchers have studied hypoglycemic properties of dietary fiber in vivo with animals (Oguido et al., 1998) and diabetic patients (Biolley et al., 1998). Also, in vitro techniques were developed and assayed (Ou et al., 2001).

The retardation in Glc diffusion exerted by the fractions isolated through alkaline treatment-enzymatic hydrolysis was expressed by the values of GDRI at different times (Table 4). The GDRI is a useful in vitro reported index to predict the effect of a fiber on the delay in Glc sorption in the gastrointestinal tract (Chau et al., 2004).

Compared with the control, the fractions BAREH1, BAREH2 and BAREC1 were the only isolated enriched pectin fractions that could significantly ($p < 0.05$) decrease the amount of diffused Glc in dyalisate up to 90 min. Moreover, BAREH2 product showed an important retention during the first 30 min of the assay (Table 4). The Glc retention by BAREC1 and BAREC2 is reported from 60 min of assay since there was no significant data before this time.

These values were significantly lower than Glc retardation showed by pectin-enriched products isolated from butternut (*Cucumis moschata* Duch ex Poiret) in a previous work (Fissore et al., 2007) as well as to retardation observed by Chau et al. (2004) for fibers isolated from *Averrhoa carambola* (GDRI: 20–35%), but higher than those of cellulose (GDRI: 3–10%). Goñi et al. (2002) informed that chondrus (*Chondrus crispus*), an edible seaweed, had a GDRI of 29%, similar to the one of citrus pectin. Gourgue et al. (1992) informed that dietary fiber isolated from mango had GDRI of 21–33% after 30 min of assay and of 19–28% after 60 min, while guar gum showed values of 44% for both times.

CONCLUSIONS

The extractive process developed on red beet (*Beta vulgaris* L. var. *conditiva*) involving an alkaline pretreatment followed by hemicellulase or cellulase hydrolysis into citrate buffer (pH = 5.2), permitted the extraction of products enriched in low methoxyl pectins with high yields, especially when cellulase was used. These water-soluble fiber products with important molecular weights have promising chemical characteristics in relation to their use as potential thickeners in food and pharmaceutical formulations, and also showed interesting in vitro physiological effects. The results obtained contribute to the conversion of residues of red beet into value added products and its adaptation for bioconversion of other agroindustrial residues can be performed tending to environmental protection, noncontaminating industrial procedures and optimum raw materials utilization.

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