Carbohydrate Polymers 89 (2012) 230–235

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Effect of extraction time and temperature on the characteristics of loosely bound pectins from Japanese plum

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ARTICLE INFO

Article history:
Received 29 December 2011
Received in revised form 28 February 2012
Accepted 1 March 2012
Available online 7 March 2012

Keywords:
Japanese plum
Pectins
Water extraction
Flow behavior
Viscosity

ABSTRACT

The cell wall composition of Japanese plums (*Prunus salicina*) at six developmental stages was previously evaluated (Ponce et al., J. Agric. Food Chem. 2010, 58, 2562–2570). This fruit is an interesting source of pectins, polysaccharides of valuable functionality for pharmaceutical and food formulations. In the present work it was investigated how the different conditions for the aqueous extraction of pectins from Japanese plums affect the yield as well as their chemical and rheological characteristics. It has been determined that extraction with water at room temperature for periods longer than 2 h did not produce additional increment of yield (12%) but decreased the average molecular weights of the extracted pectins. Pectins with a degree of methylation ≈40% with high viscosity in water and with adequate molecular weights (≈72,000) were obtained. Conversely, utilization of boiling water for extraction increased considerably the yields (33–38%) but the extracted pectins showed significant lower viscosity in water in spite of their higher molecular weights. The poorer thickening ability was associated to the lower proportion of arabinose residues present in the hairy regions of the pectin macromolecules extracted by hot water, which led the polymers to interact more transiently in a 2% w/v water solution.

1. Introduction

Pectin is a high value functional pharmaceutical excipient (Beneke, Viljoen, & Hamman, 2009) and food ingredient widely used as a thickener, gelling agent and stabilizer. It is also an abundant, ubiquitous and multifunctional component of the cell walls of all land plants; thus, in a normal Western diet, around 4–5 g of pectin are consumed every day (Willats, Knox, & Mikkelsen, 2006). Pectins consist mostly of polymers rich in D-galacturonic acid (GalA), often containing significant amounts of L-rhamnose (Rha), L-arabinose (Ara) and D-galactose (Gal) as well as up to other 13 different monosaccharides, some of them in trace amounts (Vincken et al., 2003). It is widely believed that different pectic structures (classified as four types, namely, homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II) and/or xylogalacturonan) are covalently inter-linked to form pectin complex *in muro*, although the way by which different blocks of these pectic polysaccharides are positioned relative to one another in such macromolecular pectin complexes is still a matter of controversy (Yapo, 2011).

Functional properties of pectins involve their ability to hydrate (Jarvis, 2011) and to interact through non-covalent interactions (mainly hydrogen bonding, but also electrostatic and hydrophobic) leading to network development. These properties depend on macromolecular features such as molecular weight and its distribution, degree of methylation (DM) and acetylation (DA), distribution of HG chains (so-called smooth regions) in the polymer, as well as the frequency of rhamnose kinks that constitute the RG-I disordered regions of these biopolymers (Lapasin & Pricl, 1995; Willats et al., 2006; Yapo, 2011). Furthermore, the size and distribution of the blocks with non-methylated HG produce pectins with different rheological properties and calcium reactivity, even for pectins with the same DM (Guillotin et al., 2005; Luzio & Cameron, 2008). Hydrophilicity and macromolecular interactions through non-covalent bonds develop pectin networks which can be more or less transient and hence they might behave as thickeners in aqueous solutions or emulsions (Voragen, Coenen, Verhoef, & Schols, 2009), gels (Fissore, Rojas, & Gerschenson, 2012) or constitute films after dehydration (De'Nobili, Pérez, Navarro, Stortz, & Rojas, 2011). Pectins have high potential as hydrophilic polymeric materials for controlled release matrix drug delivery systems (Beneke et al., 2009).

Pectins are industrially obtained mainly from citrus peel and apple pomace yielding essentially "pure" HG polymers after acid extraction, showing low (≈50%) or high (≈70%) DM (Voragen et al., 2009). However, other plant sources were studied as potential sources of pectins (Kulkarni & Vijayanand, 2010). Canteri et al. (2011) assayed the extraction of pectins from residues of the

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doi:10.1016/j.carbpol.2012.03.001

industrialization of yellow passion fruit by using diluted nitric acid. They found that the pectins with the higher apparent viscosity and higher molecular weight were obtained after 5 min of extraction at 80 °C with 50 mM nitric acid. In a previous report, Rovaris Pinheiro et al. (2008) extracted pectins from the same source but using citric acid. They observed that the DM was affected by the citric acid concentration and concluded that 0.086% w/v citric acid for 60 min was the optimal condition for extraction of high-ester pectins.

Room-temperature water extracts the pectin polysaccharides loosely attached to the cell wall material, since this condition preserves covalent bonds, and β-elimination does not occur (Fry, 1986). This step of extraction can vary in time: there are papers reporting 2 h (Femenia, Bestard, Sanjuan, Roselló, & Mulet, 2000; Ng & Waldron, 1997), 12 h (Manganaris, Vicente, Crisosto, & Labavitch, 2008), 16 h (Raffo, Ponce, Sozzi, Vicente, & Stortz, 2011; Sozzi, Greve, Prody, & Labavitch, 2002) or 24 h (Ponce, Ziegler, Stortz, & Sozzi, 2010). Some researchers avoid the water extraction, and use directly a calcium chelator (Brummell, Dal Cin, Crisosto, & Labavitch, 2004), thus joining loosely attached and calcium-attached pectins. In order to explore pectins with different macromolecular characteristics and potentiality, water at higher temperatures was also applied to the extraction of cell wall polymers: 4 h at 30 °C (Gross & Wallner, 1979) for the cell-wall material (CWM) isolated from red tomato, water at room temperature from the CWM of kiwifruit tissue (Schröder et al., 2001), water at 100 °C for 5 min from the alcohol insoluble residue (AIR) obtained from pretreated and thermally processed carrots (Sila, Doungla, Smout, van Loey, & Hendrickx, 2006). Houben, Jolie, Fraeye, Van Loey, and Hendrickx (2011) also applied the later procedure to extract the water soluble pectins from the CWM of broccoli, carrot and tomato. Increased liberation of pectin was achieved at 37 °C compared to 25 °C over a 24 h period (Donaghy & McKay, 1994). Yang et al. (2011) compared the pectin contents in different extraction media including hot water, from roots of *Arabidopsis*. An attempt to study the extraction of pectins at different temperatures, pHs, and extraction times was also carried out (Pagán, Ibarz, Llorca, Pagán, & Barboza-Cánovas, 2001), but using only acidic pHs and high (>60 °C) temperatures.

In some of those reports, the extracted pectins were chemically characterized but their rheological performance, which is related with their biological function and expected functionality, was seldom investigated. It is known that pectins constitute the matrix network of the cell walls since they swell in water and hence disturb the water flow, producing thickening or gelling (Zsivanovits, MacDougall, Smith, & Ring, 2004). High-methoxyl pectins gels are stabilized by hydrogen bonds and hydrophobic interactions (Lapasin & Pricl, 1995). Conversely, low methoxyl pectins can form gel networks through ionic cross links with divalent cations, usually calcium. These junction zones are also stabilized by hydrogen bonds and include highly retained water molecules (Braccini & Pérez, 2001). It is known that viscosity increases with pectin concentration and molecular weight (Srivastava & Malviya, 2011).

Plums are the most taxonomically diverse stone fruits (Okie, 2008). There are about 42 species of plums, but most commercial plums currently grown are classified into one of two groups: the hexaploid ($2n = 6x = 48$) European type (primarily *Prunus domestica* L.) and the diploid ($2n = 2x = 16$) Japanese or Asian type (*Prunus salicina* Lindl.). Climacteric Japanese plums are largely grown for fresh consumption and are regarded as the most common fresh market plums in the United States, being extremely popular in Japan where they are processed. Most of them are the result of extensive breeding. Genetic improvement of Japanese plums includes the achievement of high-quality fruit with long storage life (Srinivasan, Padilla, & Scorza, 2005). Ponce et al. (2010) studied the cell wall composition of Japanese plums at six developmental stages. Arabinose proved to be the principal neutral monosaccharide constituent in cell walls during growth and the most dynamic neutral sugar in pectic fractions. The aim of the present work was to investigate how the different conditions for the aqueous extraction of pectins from Japanese plums affect the chemical and rheological characteristics of the extracts.

2. Materials and methods

2.1. Materials

Japanese plum samples ('Roysum') were picked from trees located in the Río Negro Upper Valley, Argentina (39°01′00″ S, 67°40′00″ W, 242 m above sea level). Fully ripe fruit were harvested, and the whole fruit firmness determined in puncture tests by measuring the force required to penetrate each plum, with the skin removed, to a depth of 8 mm. The samples with a firmness between 5 and 10 N were pooled and used for further studies. All chemical reagents were of analytical grade from Sigma–Aldrich or Merck.

2.2. Cell wall preparation and extraction

Japanese plums were sliced into pieces, frozen immediately in liquid N_2 and stored at −50 °C until used. Cell wall preparation was performed as previously described (Ponce et al., 2010) with slight modifications: 20 g were dropped into 80 mL of ice-cold 80% EtOH and homogenized in a Warring blender and an Omni Mixer homogenizer. The homogenate was immediately boiled for 30 min, then allowed to cool down, and filtered through glass filter paper (Whatman GF/C). The retentate was thoroughly washed with 95% EtOH. The solids were then resuspended in 100 mL of $CHCl_3$:MeOH (1:1), stirred during 15 min and filtered. The retentate was washed with 40 mL of the same solvent mixture. The insoluble material was washed with acetone until decolorized, yielding the crude cell wall extract (alcohol insoluble residue, AIR). The AIR was air-dried in a hood and in a vacuum desiccator overnight and then weighed.

The extractions were carried out in duplicate as follows: 300 mg of AIR were stirred for different periods (0.5 h, 2 h, 8 h and 24 h) at room temperature with 30 mL of 0.02% [w/v] thimerosal aqueous solution and filtered. The supernatants were recovered after centrifugation at 13,100 × *g*, dialyzed (M_w cut-off 6000–8000) exhaustively against tap water for 2 d and against distilled water for another day at 4 °C, and recovered by lyophilization, yielding fractions **Ps-0.5**, **Ps-2**, **Ps-8** and **Ps-24**, respectively. A separate AIR fraction was extracted in the same manner, but using water at 95 °C for 2 h. The product (recovered as mentioned above) was named **Ps-HW**. Besides, the residue of the **Ps-2** extraction was re-extracted with water at 95 °C for 2 h, yielding fraction **Ps-2-RHW**.

2.3. General methods

Uronic acids were quantified using the *m*-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991) using GalA as the standard, and expressed as anhydro units. Total carbohydrates were determined by the phenol–H_2SO_4 method (Dubois, Gilles, Hamilton, Robers, & Smith, 1956) using Glc as the standard. The proportion of neutral sugars was determined after subtracting the uronic acid content from that of total carbohydrates. For this purpose, the phenol–H_2SO_4 reaction was also carried out with a GalA standard, which showed an absorbance ratio of 0.28 against the same Glc weight. The degree of methylation was calculated as the molar ratio between methanol (determined by the method of Wood & Siddiqui, 1971) and uronic acids. In order to determine the monosaccharide

Table 1
Chemical composition and molecular weights of loosely bound pectin fractions extracted from the cell walls of Japanese plum tissue using water.[a]

	Ps-0.5	Ps-2	Ps-8	Ps-24	Ps-HW	Ps-2-RHW
Yield (% w/w)	9.2 ± 2.1	12.2 ± 0.1	12.7 ± 0.8	12.8 ± 1.3	33.6 ± 2.4	38.3 ± 0.6
Protein (% w/w)	11	12	13	9	7	7
Neutral sugars (% w/w)	22 ± 2	40 ± 1	23 ± 3	27 ± 6	36 ± 4	27 ± 6
Uronic acids (% w/w)	60 ± 8	56 ± 2	59 ± 1	53 ± 3	50 ± 4	73 ± 1
DM[b] (%)	35	39	34	32	54	28
Molecular weight (kDa)	75.6	71.4	59.9	42.2	89.2	143.5
Neutral sugar composition (mol/100 moles)						
Rhamnose	14 ± 2	8 ± 2	7 ± 1	9 ± 2	11 ± 1	10 ± 1
Fucose	–	–	–	–	tr.	1 ± 0
Arabinose	39 ± 4	29 ± 1	45 ± 6	35 ± 3	25 ± 5	21 ± 2
Xylose	7 ± 1	5 ± 1	7 ± 0	6 ± 1	3 ± 0	2 ± 0
Mannose	1 ± 1	4 ± 1	3 ± 1	4 ± 1	4 ± 1	3 ± 2
Galactose	33 ± 3	46 ± 2	32 ± 3	36 ± 4	51 ± 5	54 ± 1
Glucose	6 ± 3	8 ± 1	6 ± 1	10 ± 3	6 ± 2	9 ± 3

[a] Mean and standard deviations are shown (n = 2–4).
[b] The DM (degree of methylation) was calculated as a percent molar ratio between methanol and uronic acids.

composition, each fraction (ca. 3 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 1 mL) for 90 min at 120 °C in closed-cap vials. The TFA was eliminated by evaporation, and the resulting monosaccharides were reduced to alditols using $NaBH_4$ and converted to alditol acetates, which were analyzed using a Hewlett Packard 5890 gas chromatograph fitted with a capillary column 30 m × 0.25 mm i.d. 0.20 μm, SP-2330 (Supelco) and equipped with a FID operated at 240 °C. The injector temperature was 240 °C and the oven temperature was kept isothermally at 220 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. Aliquots were injected with a split ratio of ca. 80:1. *Myo*-inositol was used as the internal standard, and the different alditol acetates were identified by comparison with authentical standards. The percentage of the different monosaccharides was calculated by considering that the FID responses are proportional to the molecular weight of the alditol acetates.

2.4. Size-exclusion chromatography (SEC)

To obtain the size distributions of polymers and their average molecular weights, 3 mg of each extract were dissolved in 0.8 mL of 0.4 mg/mL imidazole to which 0.2 mL of 1 M NH_4AcO (pH 5) were added. Solutions were cleaned up by centrifugation, and chromatographed on a low-pressure SEC by applying it to a 300 mm × 9 mm i.d. Sepharose CL-2B column eluted at room temperature with 0.2 M NH_4AcO, pH 5 (Brummell et al., 2004). Fractions were collected and assayed for total carbohydrates. The column was calibrated with dextrans with molecular weights 40,000, 80,700 and 500,000.

2.5. Flow assays

Flow curves were determined in triplicate at a constant temperature of 20 °C with a rheometer (Paar Physica MCR300, Austria) in the 0.001–1 s^{-1} shear rate ($\dot{\gamma}$) range, after steady state was reached before recording each data point. A viscosity curve recorded over a wide range of shear rate ($\dot{\gamma}$) values is essential to test the fitting of the data points to different viscosity models. In this paper Cross (1) model was considered:

$$\eta_{app} = \eta_\infty + \frac{(\eta_0 - \eta_\infty)}{1 + (\tau\dot{\gamma})^m} \quad (1)$$

wherein η_0 represents the zero-shear rate viscosity or Newtonian viscosity, τ is the time constant corresponding to the Cross model, and m is a dimensionless constant.

3. Results and discussion

3.1. Chemical analyses

The cell wall polymers were obtained from Japanese plums (mesocarp and exocarp tissues) as the alcohol insoluble residue (AIR). It is known that room temperature-water extracts pectins loosely attached to the cell walls of vegetable tissues (Fry, 1986). Parallel extraction at different times led to the isolation of four pectin fractions. The results of the chemical analyses performed on these fractions are summarized in Table 1. At room temperature, water was able to extract 9% of the cell wall polymers by stirring for 30 min (**Ps-0.5** fraction), yield that tended to increase to ca. 12.5% after stirring for either 2 (**Ps-2**), 8 (**Ps-8**) or 24 (**Ps-24**) hours. Significantly ($p < 0.05$) higher yields (33 and 38%), were respectively obtained by extraction of the AIR (**Ps-HW** fraction) or **Ps-2** fraction (called **Ps-2-RHW**) for 2 h with boiling water. The polymeric fractions extracted from the cell wall of Japanese plum were constituted by a small amount of proteins (≈10%) and mostly by pectic polysaccharides (Table 1), as inferred from their contents of uronic acids (≈62%) and neutral sugars (≈28%). Taking into account molar ratios, the monosaccharide composition (Table 1) reveals essentially pectin polymers with an (Ara + Gal)/Rha ratio that increased significantly ($p < 0.05$) from 5 to ≈9 for pectins extracted at room temperature for more than 30 min. This result is indicating that the less-substituted rhamnogalacturonans are extracted first, in agreement with previous reports, which suggested that a higher substitution in RG-I domains contributes to pectin anchoring (Peña & Carpita, 2004; Vincken et al., 2003) in the cell wall matrix. Pectins extracted with boiling water showed (Ara + Gal)/Rha molar ratios of 7 with a significant loss of arabinose (Ara/Rha ≈ 2) from the hairy regions or RG-I domains (Table 1). Xylose presence may be ascribed to the xylogalacturonan domains of pectin polymers (Pérez, Rodríguez-Carvajal, & Doco, 2003), whereas the detectable proportion of fucose in the pectin fractions extracted by boiling water can be probably ascribed to vestiges of RG-II (Mazeau & Perez, 1998; Pérez et al., 2003). On the other hand, all polysaccharides extracted by water for longer than 30 min presented significant proportions of mannose, as previously determined by Ponce et al. (2010). Pereira da Silva, de Medeiros Silva, and Paz Parente (2009) extracted galactoglucomannans (GGM) from the fresh mesocarp of *Acrocomia aculeata* with hot water at 80 °C under stirring for 1 hour. On the other hand, Schröder et al. (2001) characterized the GGM component of the cell wall polymers of kiwifruit tissue by a previous extraction with 4 M KOH, whose supernatant contained the xyloglucans (hemicellulose) and GGM, accompanied by remaining pectins which embedded the hemicellulose–cellulose network. In the skin and underlying flesh of fruits like ripe tomato and plum

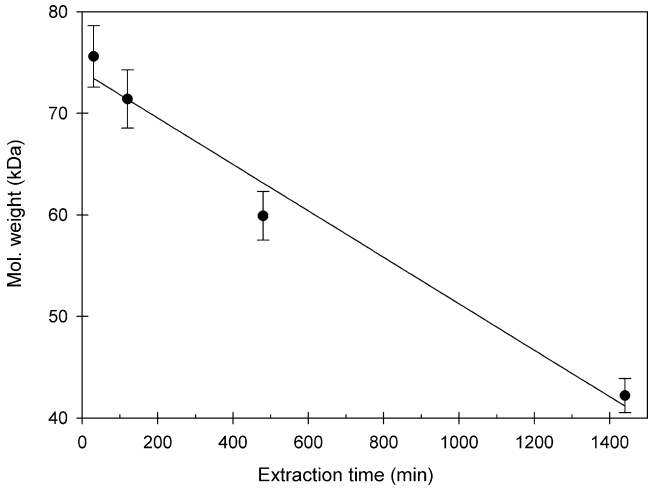

Fig. 1. Relationship (correlation coefficient = −0.988) between the average molecular weight of pectins and the stirring time applied for their extraction by water at room temperature (**Ps-0.5**, **Ps-2**, **Ps-8**, **Ps-24**) from the cell walls (AIR) of Japanese plum tissue. The error bars correspond to the SD ($n = 3$).

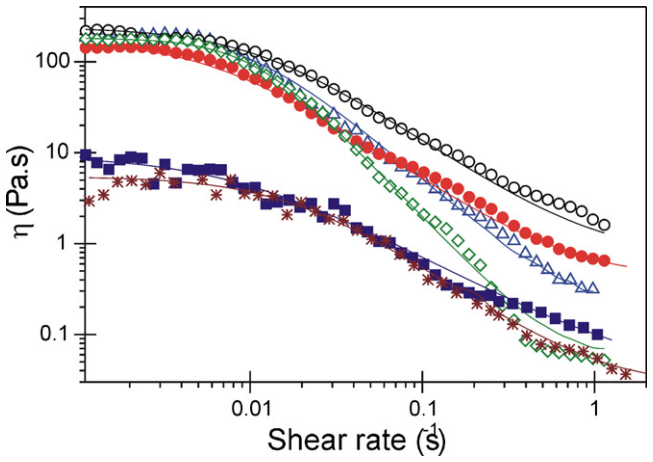

Fig. 2. Flow curves recorded from the pectin enriched fractions extracted from Japanese plum either by water at room temperature (**Ps-0.5** ●, **Ps-2** ○, **Ps-8** △, **Ps-24** ◇) or by boiling water (**Ps-HW** ✱, **Ps-2-RHW** ■); the continuous lines corresponds to the Cross' model fitted.

(*P. domestica*), Schröder, Wegrzyn, Bolitho, and Redgwell (2004) found some level of mannan transglycosylase activity specific for mannan-based plant polysaccharides like GGM, galactomannan, glucomannan and plain mannan.

A slight though significant (Fisher's $F = 80.49$; $n = 3$; $p < 0.05$) decrease of the number-average molecular weight of the isolated pectin fraction is observed as the time involved in the extractive procedure is increased (Fig. 1). A significant linear correlation (coefficient = −0.988) relates both parameters. Thus, pectins with very similar chemical composition (Table 1) but molecular weights decreasing from 75,600 to 42,200 Da were obtained through extraction with water at room temperature. The degree of methylation also showed a slight decrease (from ≈40% to ≈35–31%) as time of extraction in water at room temperature increased from 2 to 24 h. The **Ps-HW** pectin-enriched fraction extracted from AIR with boiling water for 2 h showed a slightly higher molecular weight (89,200 Da) than the **Ps-2** pectin isolated with cold water for the same period. The **Ps-2-RHW** fraction extracted with boiling water from the residue developed after extraction of **Ps-2** pectins from the AIR, shows an even higher average molecular weight (143 kDa), for a fraction very rich in uronic acids (Table 1). However, this fraction showed low degree of methylation (28%) whereas **Ps-HW** pectins showed the highest degree of methylation (Table 1). Pectins extracted through boiling water (**Ps-HW**, **Ps-2-RHW**) have suffered some significant ($p < 0.05$) loss of arabinose, probably indicating peeling of the hairy regions (RG-I domains): heating might have produced partial hydrolysis of side-chain glycosidic linkages (mainly furanosidic) and some β-elimination (Brett & Waldron, 1996; Fry, 1986). After the pectins are peeled, their extraction might be facilitated, hence leading to higher yields (Table 1). An important part of the newly extracted pectins are larger, according to their average molecular weight.

3.2. Rheological performance of the isolated cell wall polymers

In order to determine if the chemical differences observed in the monosaccharide composition (Table 1) and molecular weights (Fig. 1) lead to differences in their rheological behavior, several flow assays were carried out. Pectins constitute the matrix of the cell walls necessary for the viscous drag of the elastic xyloglucan–cellulose network (Carpita & Gibeaut, 1993). In the cell walls, pectins swell in the presence of water altering the flow behavior which is a biological characteristic of these macromolecules (Jarvis, 2011; Zykwinska, Ralet, Garnier, & Thibault, 2005). Pectin polymers self interact in water through non-covalent bonds (hydrogen bonds, hydrophobic and electrostatic interactions). Hence, the isolated pectin-enriched fractions (Table 1) were evaluated from their physiological expectable behavior by this rheological study.

The flow behavior of the extracted fractions was determined through the viscosity curves recorded over a range of shear rates (10^{-3}–$2\ s^{-1}$) from each 2.0% w/v aqueous solution. An initial Newtonian plateau was observed at the lowest shear rates up to $\approx 2.3 \times 10^{-3}\ s^{-1}$ for all pectin solutions (Fig. 2a), from which an initial viscosity (η_0) of ≈200 Pa s was determined from the curves recorded from the solutions of pectins extracted at room temperature. The Newtonian viscosity of the pectins extracted through boiling water (**Ps-HW** and **Ps-2-RHW**) was one order of magnitude lower, and then significantly different ($p < 0.001$) than the other pectin solutions, more precisely 5.5 and 9 Pa s, respectively. Hence, the physical networks developed as a consequence of the macromolecular interactions in the **Ps-HW** or **Ps-2-RHW** aqueous solutions were more transient and less structured. Following the Newtonian plateau, the viscosity continuously decreased with the increment in shear rate for all aqueous systems (Fig. 2a). Thus, a shear thinning or pseudoplastic behavior was clearly observed: its onset occurs when the rate of the externally imposed motion becomes progressively greater than the rate of formation of new entanglements between the hydrated pectin macromolecules. Therefore the "cross-link density" of the network was depleted and thus, viscosity was reduced (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). Consequently, the timescale of intermolecular network rearrangement should be related to intramolecular relaxation times of the macromolecules or, at least, of the whole steady-network. The upper Newtonian plateau of the third zone at the highest shear rate, which corresponds to the residual or infinite viscosity (η_∞) was experimentally inaccessible, as usually occurs (Ross-Murphy, 1994).

For a better comparison between the flow curves, experimental points were fitted through the Cross model (eq. (1)) in order to obtain rheological parameters (Table 2). The adjusted model is showed as a continuous line for each curve in Fig. 2. The Newtonian viscosity (η_0) obtained from fitting of curves according to the Cross model revealed that all the pectins extracted through water at room temperature were able to self interact through overlapping and entanglements. Hence, these pectins constituted sufficiently structured physical networks. They were less transient than those

Table 2
Cross model-parameters[a] calculated after fitting of experimental data obtained in flow assays at 20 °C from 2% w/v aqueous solutions of the pectin fractions extracted from Japanese plum either by water at room temperature or by boiling water.

Sample	η_∞ (Pa s)	η_0 (Pa s)	τ (s)	m	R^2
Ps-0.5	0.4 ± 0.1	174 ± 32	162 ± 37	1.3	0.997
Ps-2	0.7 ± 0.2	238 ± 25	92 ± 6	1.3	0.998
Ps-8	0.15 ± 0.3	215 ± 3	98 ± 2	1.6	0.998
Ps-24	0.05 ± 0.1	182 ± 2	97 ± 2	1.8	0.994
Ps-HW	0.025 ± 0.05	5.5 ± 0.3	57 ± 8	1.3	0.976
Ps-2-RHW	0.035 ± 0.07	9 ± 1	116 ± 33	1.0	0.970

η_0 represents the Newtonian viscosity or the viscosity at zero shear rate; η_∞ represents the infinite or residual viscosity, τ is the time constant and m is a dimensionless parameter.

[a] Mean and standard errors are shown ($n = 3$). R^2: goodness of fit.

developed from pectins extracted through boiling water, beyond their molecular weight differences. There was no dependence of η_0 on the extraction time (Fig. 2). Higher values of structural relaxation time (τ) along the shear thinning zone (Table 2) were in general observed for the most structured systems (**Ps-0.5**, **Ps-2**, **Ps-8**, and **Ps-24**). The **Ps-0.5** pectin fraction showed some lower destructuring rate ($1/\tau$) than **Ps-2**, **Ps-8**, and **Ps-24**, whereas $1/\tau$ for the latter pectins was not significant different from the destructuring velocity of **Ps-2-RHW** pectins (Table 2) extracted from the residue of Ps-2 through boiling water. Specifically, the **Ps-2-RHW** fraction showed the highest molecular weight (143 kDa), the highest content of uronic acids and uronic acids/Rha ratio (≈80), as well as the lower DM (≈27%). Hence, this pectin fraction presented a predominant proportion of HG (semi-flexible coil) over RG-I (random coil) domains, which may contribute to the different rheological behavior (Morris, Ralet, Bonnin, Thibault, & Harding, 2010) and performance into the cell wall matrix. Ralet et al. (2008) determined that HG was over fourfold more rigid than RG-I, so that specific reduction of the number of HG blocks leads to an increased flexibility of pectins. The η_∞ values of the polymer solutions derived from fitting to the Cross model were usually higher than the water viscosity (0.01 Pa s) (Table 2). The degree of thickening provided by a polysaccharide is dependent on its chemical composition and of its concentration. In solution, viscosity is directly related to fundamental molecular properties, i.e. molecular conformation, molecular weight and molecular weight distribution, intramolecular and intermolecular interactions (Lefebvre & Doublier, 2005). An adequate molar ratio of HG to RG-I, and distribution pattern of HG between RG-I cores (Pérez et al., 2003; Vincken et al., 2003) are needed to interact with water and thicken. The 10% of cell wall proteins extracted with the pectin polysaccharides in the present work may also contribute to the rheological performance of the isolated polymers (Nuñez, Fishman, Fortis, Cooke & Hotchkiss, 2009; Siew & Williams, 2008).

Redgwell et al. (1997) determined a viscosity varying between 0.040 and 0.725 Pa s for a 1% w/v aqueous solution of cell wall polymers obtained from unripe kiwifruit and tomato, values that increased to 5.07 and 5.78 Pa s, respectively, for ripe kiwifruit and tomato. Cosgrove (1989) reported a viscosity of 0.328 Pa s for a buffered aqueous solution of 2% w/w citrus pectin. Boonrod, Reanma, and Niamsup (2006) extracted pectins at boiling temperature from the skin of *Carica papaya* using different concentrations of HCl followed by ethanolic precipitation. They determined viscosities from 0.015 to 0.025 Pa s for 1% w/v aqueous solutions at pH 1.0 measured at shear rates between 25 and 225 s^{-1}. In those works, the viscosity was measured in the pseudoplastic range, outside the Newtonian region observed in Fig. 2 (i.e. where the viscosity is dependent on the shear rate of measurement). Therefore, the viscosity values of 2% w/v pectin solutions recorded at shear rates of 1 and 23 s^{-1} are also indicated in Table 3 for comparison, showing that their magnitudes are similar to those reported by other authors.

Table 3
Apparent viscosity (η_{app}) determined at constant shear rates of 1 and 23 s^{-1} (20 °C) from the flow curves recorded from 2% w/v aqueous solutions of isolated pectin fractions.

Pectin sample in a 2% w/v solution	η_{app} (Pa s)	
	1 s^{-1}	23 s^{-1}
Ps-0.5	0.770	0.397
Ps-2	1.471	0.682
Ps-8	0.617	0.105
Ps-24	0.106	0.071
Ps-HW	0.082	0.026
Ps-2-RHW	0.14	0.044

4. Conclusions

All polymer fractions extracted were constituted by physiologically active pectins, that is, pectins with a thickening effect as expected to occur in the cell wall matrix. Water at room temperature extracts pectins (DM ≈ 40%) with high viscosity in water and with adequate molecular weights (≈72000) from cell walls of Japanese plums by stirring for 2 h (**Ps-2**), showing a yield of ca. 12%. Stirring for longer times did neither increase the yield nor modified substantially the chemical composition and viscosity performance in water, but decreased slightly the molecular weights of the isolated pectins (**Ps-8**, **Ps-24**). On the other hand, utilization of boiling water for extraction (**Ps-HW** and **Ps-2-RHW**) increased the yields (33–38%) and molecular weights, but produced pectins with significantly lower viscosity in water. Besides, they showed different analytical characteristics, such as lower proportion of arabinose residues at the RG-I hairy (disordered) regions of the pectin macromolecules (**Ps-HW** and **Ps-2-RHW**). An adequate molar ratio of HG to RG-I, as well as an adequate distribution pattern of HG between RG-I cores may be needed to interact with water and thicken.

The pectin fractions isolated by water at room temperature from Japanese plums have a rheologically behavior useful for pharmaceutical and food formulation, upgrading wastes of this fruit commercialization and processing.

Acknowledgements

This work was supported by grants from University of Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica de la República Argentina (ANPCyT) and CONICET. M.F.B. is a recipient of a Fellowship from CONICET, whereas N.M.A.P., A.M.R. and C.A.S. are Research Members of the same Institution. The authors are indebted to Ing. Agr. María D. Raffo for the collection of the plums.

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