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ORIGINAL PAPER

Pectin Extraction from Lemon By-Product with Acidified Date Juice: Effect of Extraction Conditions on Chemical Composition of Pectins

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Abstract Mixtures of date and lemon pectins were extracted from lemon by-product with acidified date juice under different conditions of temperature, pH and time. Individual pectins from date and lemon, respectively, were also extracted using the same experimental conditions, then analysed and compared to pectin mixtures. It was found that the use of extreme conditions resulted in higher galacturonic acid content, lower degree of methylation, lower neutral sugar content, lower molecular weight and darker colour pectins. Examination of the individual neutral sugars showed that the main ones were galactose (1.6–5.4%), arabinose (1.6–4.2%) and rhamnose (0.5–0.8%). The Gal A/Rha molar ratios varied from ~53 to ~149. Moreover, mixture of pectin extracted at the optimal extraction conditions (84.34 °C, pH 2.8 during 3 h 34 min) had interesting properties, with a high galacturonic acid content (63.4%), low degree of methylation (~35%) and a mass molecular weight of about 243 kg/mol.

Keywords Pectin · Date · Lemon by-product · Mixture of pectins · Extraction conditions · Chemical composition

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Introduction

Pectins are complex polysaccharides from the cell walls of higher plants. They mainly consist of an α-1,4-linked D-galacturonic acid backbone (smooth regions) interrupted in places by residues of 2-O-linked-α-L-rhamnose (Yapo et al. 2007). In addition, other regions are present (called hairy regions), constituted by alternating rhamnose/galacturonic acid sequences (rhamnogalacturonan I), where neutral side chains (galactan, arabinan or arabinogalactans) are attached to rhamnose moeties (Pilnik and Voragen 1991; Voragen et al. 1995). The galacturonic acid residues could be partly methyl esterified at C-6 (Pilnik and Voragen 1970) and the hydroxyl groups partly acetyl-esterified at O-2 and/or O-3 (Rombouts and Thibault 1986).

These polysaccharides are obtained by aqueous extraction of raw material under mild, acidic or alkaline treatment. The basic extraction process leads to low methylated pectins, whereas high methylated pectins are recovered by acidic extraction (Joe and Luzio 2000). In the acid extraction process, pH from 1 to 3, temperatures between 70 and 95 °C are generally used during a sufficient time. Then, the extract is filtered and pectin is precipitated and washed by alcohol, dried and milled (Robert et al. 2006). Other authors used milder conditions for pectin extraction. Indeed, Joe and Luzio (2000) extracted pectin

from lemon peel at mild pH (3–3.3), while Pagan and Ibarz (1999) used temperatures ranging from 40 to 80 °C for pectin extraction from fresh peach pomace.

Pectin has been extracted from many plant by-products and fruits such as sugar beet pulp, peach pomace and especially citrus (orange and lemon) and apple pomace which are the main sources for commercial pectin production (May 1990; Fernandes et al. 2010).

Previous works reported that extraction conditions such as temperature, pH, time and the nature of the acid could have important effects not only on the extraction yield but also on the physico-chemical properties and thus the quality of the final extracted material (Mesbahi et al. 2005). In fact, degree of esterification (DE), molecular weight (Mw), attached chains of neutral sugars, acetylation and cross-linking of pectin can affect its ability to gel as well as the textural properties of the final gel (BeMiller 1986; May 1990).

Many authors have found that, under extreme conditions, pectin extracted from sugar beet pulp had higher galacturonic acid (Gal A) content, lower degree of methylation (DM) and lower molecular weight (Levigne et al. 2002; Yapo et al. 2007). Robert et al. (2006) found the same result when they extracted pectin from chicory roots. Moreover, Joe and Luzio (2000) reported that higher acid concentration and longer extraction time contribute to the de-esterification of lemon pectins.

However, compared to other sources of pectin, there are few scientific publications that study date pectin and are mainly limited to the determination of the pectin content or of some physico-chemical analysis such as the DE during maturation of dates (Mustapha et al. 1986; El-Zoghbi 1994; Myhara et al. 2000; Benchabane et al. 2006). No information is available on the characteristics of pectin dates as influenced by the experimental conditions.

In a previous work, we have extracted pectins from lemon using acidified date juice. The latter, enriched with lemon pectin and flavour, was selected as it will be used in a subsequent study for the formulation of date–lemon jelly. Optimal extraction conditions leading to the maximum yield of pectin were determined (Masmoudi et al. 2008).

In the present study, we tried to isolate first pectins from date (*Phoenix dactylifera* L.) and lemon (*Citrus limon* L.) by-products and then their pectin mixtures by extracting lemon pectin directly in acidified date juice using different extraction conditions. Mixtures of pectins are widely used in food applications to obtain products with better properties (Löfgren et al. 2002). Guillotin (2005) showed that commercial pectins, in general, are mixtures of several sources having various degrees of methylation. New objectives were fixed in the present paper. The first one was to study the influence of the extraction conditions on the chemical properties of lemon pectin, date pectin and their mixtures. Thus, pectin samples were characterised by analysis of their Gal A, neutral sugars, proteins and ash content as well as their degree of methylation, average molecular weights and colour. The second one was to isolate mixtures of date and lemon pectins having interesting properties, suitable for the preparation of fruit jellies.

Materials and Methods

Materials

Lemon By-Product

One batch of 25 kg of lemon by-product (*Citrus limon* L.) was supplied by a fruit beverage industry (Zina, Sfax, Tunisia) using mixed lemon varieties from Nabeul region (Tunisia). After removal of the pips, the remaining matter (pulp and peel) was lyophilised, milled and sieved (60-mesh size screen). The obtained powder was stored at −20 °C.

Dates

Dates (*P. dactylifera* L.) of "Deglet Nour" variety were provided by the National Institute of Arid Zone (Degach, Tunisia). We used a batch of 50 kg of dates of second category (hard texture) collected at the "Tamr" stage (full ripeness). Dates were pitted, washed in running tap water and dried for 12 h in an atmospheric oven at 45 °C. Then, the collected pulp was milled to obtain date paste.

Date Juice Preparation

The date juice was prepared as described by Masmoudi et al. (2007) and stored at −20 °C until use.

Pectin Extraction

Mixture of Date/Lemon Pectins (Pdl1 to Pdl9) Pectin was extracted from lemon by-product using acidified date juice as described in a previous study, then precipitated and purified by washing three times with ethanol 50%, 75% and absolute ethanol, respectively (Masmoudi et al. 2008). The extractions were carried out using different conditions of temperature, pH and time. For each condition, extraction was conducted at least six times, and the obtained pectin was collected. These pectins are composed of a mixture of lemon and date pectins, originating respectively from the lemon by-product and the date juice. These pectin samples were designated by Pdl (pectins from dates and lemon—Pdl1 to Pdl9). Pdl9 represents pectins extracted using experimental conditions leading to the maximal extraction yield: 84.34 °C, pH 2.8 during 3 h 34 min. These conditions were determined in a previous study (Masmoudi

et al. 2008). Experimental conditions for each sample are shown in Table 1.

Lemon Pectins (Pl1 and Pl2) In order to obtain only lemon pectin (without date pectin), two distinct extractions were carried out using acidified water (instead of date juice). Lemon pectins are designed by Pl1 and Pl2. Extraction conditions were 80 °C, pH 2.8 during 4 and 1 h, respectively, for Pl1 and Pl2. Pectins were precipitated, purified and analysed.

Date Pectins (Pd, Pdc5 and Pdc8) Date pectin (Pd) was recovered directly from the date juice by adding ethanol 96% (1:3; v:v). The precipitated pectin was purified as described previously.

Other date pectin samples were recovered: 150 mL of date juice was poured in a glass flask (without lemon by-product) and agitated in a water bath at 40 °C, pH 2.8, 4 h and at 40 °C, pH 3.4, 1 h, respectively, for the samples designated as Pdc5 and Pdc8. The pH of date juice was adjusted with citric acid. The obtained date juice was added with ethanol to precipitate and purify pectin. The purified pectin samples were considered as controls. These samples were analysed only for their molecular weight in order to study the effect of temperature, pH and time on the molecular weight of date pectin.

Chemical Analysis

Moisture content was determined by drying pectin samples at 105 °C until constant weight (AOAC 1995). All values were calculated on a dry-weight basis.

Ash content was determined after incineration in a muffle furnace at 550 °C (AOAC 1995).

Table 1 Experimental conditions used for the extraction of pectin from lemon by-product with acidified date juice (pectin mixtures)

Pectin sample	Temperature (°C)	pH	Time (h)
Pdl1	80	2.8	4
Pdl2	80	2.8	1
Pdl3	80	3.4	4
Pdl4	80	3.4	1
Pdl5	40	2.8	4
Pdl6	40	2.8	1
Pdl7	40	3.4	4
Pdl8	40	3.4	1
Pdl9	84.34	2.8	3.57

Pdl represents mixtures of date and lemon pectins (samples from Pdl1 to Pdl9)

Galacturonic acid (Gal A) content of pectins was determined by high performance anion exchange chromatography with a pulsed amperometric detector as described in Garna et al. (2006). Pectin was hydrolysed with a commercial liquid enzymatic preparation VL 9 (Viscozyme L9, Novo Nordisk, Denmark). Twenty-five microlitres of samples was injected into the Dionex system (DX-500 Bio-LC, Dionex Corp., Sunnyvale, CA, USA) equipped with a CarboPac PA-100 column (250×4 mm) in combination with a guard CarboPac PA-100 column (50×4 mm). Elution was carried out with 0.1 M NaOH and 0.17 M CH_3COONa ($3H_2O$) at a constant temperature of 30 °C and a flow rate of 1 mL/min. The column was washed post-injection with 0.5 M NaOH over 17 min followed by 20 min re-equilibration before the next injection. Pure galacturonic acid monohydrate was used as an external standard.

Individual neutral sugars were released from pectin molecules by acid hydrolysis with H_2SO_4 (1 M) at 100 °C for 3 h and converted to alditol acetate according to the Blakney et al. (1983) method. Separation and quantification of alditol acetate derivatives were performed by gas chromatography using a high performance capillary column, HP-1 methylsiloxane (30 m L×0.32 mm ID, 0.25 μm film thickness, Scientific Glass Engineering, S.G.E. Pty, Ltd., Melbourne, Australia) method, on a Hewlett-Packard HP-6890 series GC system (Hewlett-Packard Co., Palo Alto, CA, USA). 2-Deoxy-D-glucose (purity >99.5%, Sigma Chemical Co., St. Louis, MO, USA) was used as an internal standard.

The DM was determined after pectin saponification with NaOH 0.2 M at 4 °C for 2 h. Methoxy groups were separated and quantified by HPLC on an Aminex HPX-87 H ion exchange column (300 mm L×7.8 mm ID, Bio-Rad Labs, Hercules, CA, USA) according to the Voragen et al. (1986) method. Elution was carried out with 5 mM H_2SO_4 solution at 30 °C and at a flow rate of 0.6 mL/min. DM was expressed as the molar ratio of methanol to galacturonic acid.

Protein content (N×6.25) of pectin was determined by the Kjeldahl method (AOAC 1995).

Average Mw of the extracted pectins was determined by high performance size exclusion chromatography (HPSEC) method using a Waters 2690 HPLC system (Waters Inc., Milford, MA, USA), equipped with a TSK gel G4000 PWXL column (300 mm L×7.8 mm ID) and coupled on line with three detectors: a Waters 2410 differential refractometer (RI), a right angle laser light scattering (RALLS) detector and a differential viscometer (Model T-SDA, Viscotek, Houston, TX, USA). Pectin solutions (0.2%, w/w) were prepared and filtered through 20- and 0.45-μm membrane filters (Millipore Co., Milford, MA, USA), respectively. To calculate the extract pectin concentration of the solution, about 20 mL was dried to a constant weight at 106 °C. Samples were filtered once again through 0.45-μm membrane filters (GHP Acrodisc, Pall

Gelman Corp., East Hills, NY, USA). Fifty microlitres was injected to the column. Elution was carried out with sodium nitrate $NaNO_5$ solution (0.05 M) containing sodium azide (NaN_3, 0.05%) as a bactericide at 30 °C and a flow rate of 0.7 mL/min. RI detector was used to determine the specific refractive index increment (dn/dc) value for pectin samples. This index was used to determine the absolute Mw of pectins using the RALLS detector or the differential viscometer and a universal calibration curve constructed with dextran standards ($Mw = 5 \times 10^3 - 670 \times 10^3$ Da). Data from the RI and RALLS detectors were acquired and processed by Millennium V 2.1 Software (Waters) and those from the viscometer by TriSEC Software (Version 2.7, Viscotek).

Pectin Colour

The CieLab coordinates (L^*, a^*, b^*) of the pectin samples were directly read with a MS/Y-2500 spectrophotometer (HunterLab, Inc., Reston, VA, USA), calibrated with a white tile. In this coordinate system, the L^* value is a measure of lightness, ranging from 0 (black) to +100 (white). The a^* value ranges from −100 (green) to +100 (red) and the b^* value ranges from −100 (blue) to +100 (yellow). The hue angle (h^*_{ab}) and chroma or intensity (C^*) were calculated according to the following equations:

$$h^*_{ab} = \tan^{-1}(b^*/a^*)) \quad (1)$$

$$C^* = \left(\left(a^{*2} + b^{*2}\right)^{-2}\right) \quad (2)$$

Statistical Analysis

Analysis of pectin samples was performed in duplicate. Values of different parameters were expressed as the mean±standard deviation. Statistical analysis was performed using the Statistical Package for the Social Sciences "SPSS" (version 13). Duncan test was performed to evaluate the significance of differences between mean values at the level of $P<0.05$.

Results and Discussion

Chemical Composition of Pectins

The chemical composition of the extracted pectins is shown in Table 2.

Galacturonic Acid Content

The galacturonic acid (Gal A) content of the pectin mixture samples ranged from 41.5% to 74.5% (on a dry-weight

Table 2 Chemical composition (% w/w), degree of methylation (% mol) and average molecular weight (kg/mol) of pectins

	Pd	Pdl1	Pdl2	Pdl3	Pdl4	Pdl5	Pdl6	Pdl7	Pdl8	Pdl9	Pl1	Pl2
Moisture	11.0±0.3e	9.0±0.1d	20.3±0.9f	4.1±0.5b	8.4±0.3d	5.6±0.1c	1.3±0.4a	3.2±0.2b	3.2±0.4b	10.8±0.7e	10.8±0.5e	10.6±0.4e
Gal A	44.0±0.5b	69.5±0.5f	74.5±1.2 g	73.4±0.1 g	67.5±1.4f	60.7±0.1d	41.5±0.2a	46.2±2.2c	42.9±1.0ab	63.4±0.4e	61.1±0.8d	65.0±0.2e
DM	16.1±0.5a	31.1±0.0b	30.7±0.4b	29.3±0.5b	34.9±0.0c	34.5±0.1c	39.8±0.9d	39.9±0.1d	43.7±1.4e	34.9±1.0c	58.9±0.2f	62.8±1.6g
Mw	66.9±7.6a	132.0±10.4b	123.5±14.5b	113.8±12.3b	189.0±12.9c	214.8±11.4cd	350.8±12.6e	310.4±14.4e	330.0±13.1e	243.4±13.9d	328.9±8.7e	–
Protein	8.7±0.1g	2.8±0.2cd	1.9±0.1b	1.8±0.1b	2.9±0.1cd	3.3±0.1de	3.7±0.1e	3.6±0.1e	4.6±0.1f	2.8±0.1cd	2.6±0.1c	1.2±0.1a
Ash	14.0±0.9e	4.7±0.5b	6.2±0.2cd	6.0±0.1c	6.7±0.3cd	7.2±0.1d	7.3±0.4d	7.8±0.1d	7.5±0.2d	5.6±0.2c	0.9±0.2a	0.8±0.1a
TNS	5.6±1.4ab	6.7±0.5abc	8.3±0.4c	5.1±0.7a	–	–	–	–	10.9±0.4d	9.2±0.4d	7.1±0.1bc	–

Means followed by the same letter within a line are not significantly different (α=0.05). All values given are means of two determinations

Gal A galacturonic acid, *DM* degree of methylation, *Mw* average molecular weight, *TNS* total neutral sugars

basis). These values were similar to those observed from sugar beet pulp (35.2–76.3%) (Yapo et al. 2007). The highest values were obtained when pectin was extracted at the extreme conditions: lower pH, more extraction time and especially higher temperature (74.5% versus 42.9% for Pdl2 and Pdl8, respectively). This suggests that extreme extraction conditions lead to higher amount of pectin (Masmoudi et al. 2008) with higher purity probably by release of neutral sugar side chains as a product of partial acid hydrolysis of pectin (Garna et al. 2004). The same effect was found by Robert et al. (2006) who extracted pectin from chicory roots.

The optimal extraction conditions adopted in a previous work (84.34 °C, pH 2.8 during 3 h 34 min) (Masmoudi et al. 2008) led to a pectin (Pdl9) with a high content in Gal A (63.4%) which is an acceptable level. In fact, industrial pectins contain about 65% of Gal A (w/w) (Food Chemical Codex 1996).

The Gal A content of the pectin was low (44%). This could be attributed to the extraction conditions. In fact, date pectin was recovered from the date juice. This pectin extract had a pH of about 5.62 (Masmoudi et al. 2007, 2008) which was not low enough to permit the extraction of a high amount of galacturonic acid.

Degree of Methylation

Generally, pectin is characterised by its degree of methylation which can determine the mechanism of formation of pectin gels, their conformation and rheological properties (Fishman et al. 1984; Diaz et al. 2009; Alvarez et al. 2010). Date pectin (Pd) had a low DM: 16.1%. Myhara et al. (2000) also extracted low methoxyl pectins from Khalas variety but with higher value (39%). This difference was probably due to date variety and method of extraction.

However, lemon pectin was high methylated (HM; 58.9% and 62.8% for Pl1 and Pl2, respectively). Similar values (59–75%) were obtained by Fishman et al. (2006) who extracted pectin from lime using microwave. Joe and Luzio (2000) also found high methoxyl pectins from lemon (64.8–78.9%).

The pectin mixture had a DM ranging between 29.3% and 43.7%. The DM was inferior to 50% in all samples, indicating that the mixture of the two pectins was low methylated (LM). The lowest DMs were obtained for the pectins extracted at the extreme extraction conditions probably because they increased the de-esterification of polygalacturonic chain (Mort et al. 1993). These results are consistent with those previously published by Joe and Luzio (2000) for lemon pectin as well as Levigne et al. (2002) and Yapo et al. (2007) for pectin extracted from sugar beet pulp. The low degree of methylation of the pectin mixture could be attributed to the coexistence of date and lemon pectins which are of low and high DM, respectively. This characteristic is interesting. In fact, contrary to HM pectins which require high sugar content (~65%) and pH of about 3, LM pectins may form gels in the presence of calcium over a wide range of pH with or without sugar, which allows it to have many applications in dietetic foods (Fu and Rao 2001).

Neutral Sugars

The total neutral sugar (TNS) content varied from 5.1% to 10.9% (Table 2). Experimental conditions seemed to have a little effect on the TNS content. Generally, use of more extreme conditions led to smaller amounts (6.7% versus 10.9% for Pdl1 and Pdl8, respectively). The same effect was reported by Robert et al. (2006) for pectin from chicory roots. The TNS content was comparable to that of commercial citrus pectins (Miyamoto and Chang 1992; Schols et al. 1998). However, examination of the individual neutral sugars (Table 3) showed that the main ones were galactose (1.6–5.4%), arabinose (1.6–4.2%) and rhamnose (0.5–0.8%). Small amounts of glucose, xylose and mannose were also identified and could originate from hemicellulose. Table 3 shows also that rhamnose content was slightly lower than that reported for commercial citrus pectin, whereas arabinose content was slightly higher (Miyamoto and Chang 1992).

The Gal A/Rha molar ratios ranging from ~53 to ~149 were higher than those obtained for chicory roots (Robert et al. 2006) or lemon (Ralet and Thibault 1994). This shows that the studied pectin samples contained probably a lower proportion of rhamnogalacturonic regions. This could be attributed to the pH values used for the pectin extraction (pH 3.4 and 2.8) which are not very low in comparison to that used at industrial scale (pH 2) (May 1990). In fact,

Table 3 Composition of the pectin sugars (%)

	Pd	Pdl1	Pdl2	Pdl3	Pdl8	Pdl9	Pl1
Rhamnose	0.6±0.1a	0.5±0.0a	0.5±0.0a	0.6±0.1a	0.8±0.0b	0.8±0.1b	0.5±0.0a
Arabinose	1.6±0.2a	3.2±0.2b	3.2±0.2b	2.0±0.2a	3.9±0.1c	4.2±0.2d	3.2±0.1b
Xylose	0.4±0.1b	0.2±0.0a	0.2±0.0a	0.3±0.0ab	0.3±0.0ab	0.4±0.0a	0.2±0.0a
Mannose	0.2±0.0a	0.1±0.0a	ND	0.2±0.0a	0.1±0.0a	0.1±0.0a	0.1±0.0a
Glucose	0.7±0.2b	0.4±0.0a	0.3±0.0a	0.4±0.0a	0.4±0.0a	0.2±0.0a	0.3±0.0a
Galactose	2.1±0.4ab	2.3±0.2ab	4.1±0.1c	1.6±0.2a	5.4±0.2d	3.5±0.1e	2.8±0.0b

Means followed by the same letter within a line are not significantly different (α=0.05). All values given are means of two determinations

ND not detectable

Levigne et al. (2002) as well as Garna et al. (2007) reported that extraction at lower pH values led to higher amounts of pectins with more rhamnogalacturonan regions. In fact, linkages between uronic acids or between uronic acids and rhamnose were the most resistant, whereas arabinofuranosyl linkages are the most labile to acid hydrolysis (Renard 1989).

Protein and Ash Content

The analysis of the protein content of the different pectin samples showed that the highest value was that of the date pectin (8.7%), whereas lemon pectin had lower amounts (1.2% and 2.6% for Pl2 and Pl1, respectively) (Table 2). These values were close to that found by Mesbahi et al. (2005) who reported a protein content of 1.1% for commercial citrus pectin. The protein content of the mixture date–lemon pectins ranged from 1.8% to 4.6%. The lowest values of proteins were obtained at extreme extraction conditions, confirming the higher purity levels of pectins extracted under these conditions. Kravtchenko et al. (1992) as well as Garna et al. (2007) reported that proteins can be linked to pectin molecules or exist in free form. In this study, the extraction of lemon pectin with the acidified date juice under severe conditions as well as its recovery and purification by ethanol precipitation and washing was probably selective. It seemed that, under such severe extraction conditions, pectins and proteins had less interaction between each other.

Ash content of the lemon pectin was very low (0.9% for Pl1). This value was similar to that found by Mesbahi et al. (2005) who reported that ash content of commercial citrus pectin was 0.55%. Pectin mixture had higher content of ash ranging from 4.7% to 7.8%. No significant effect was found for each individual experimental parameter (pH, temperature or time) on the ash content. However, pectins extracted at the more extreme conditions seemed to have the lowest values (4.7% and 5.6% for Pdl1 and Pdl9, respectively). Similar ash content was found by Miyamoto and Chang (1992) who reported a content of 5.22% for high methylated commercial pectin. Date pectin had high content in ash (~14%). This could be attributed to the high pH of the date juice from which pectin was recovered (pH=5.62) (Masmoudi et al. 2007). In fact, Lin et al. (1976), who analysed sunflower pectin, reported that ash content was mainly pH dependent.

Average Molecular Weight of Pectins

The average Mw of the different pectin samples are shown in Table 2. Date pectin (Pd) had a low Mw of about 67 kg/mol. This value was much smaller than that of lemon (Pl1)

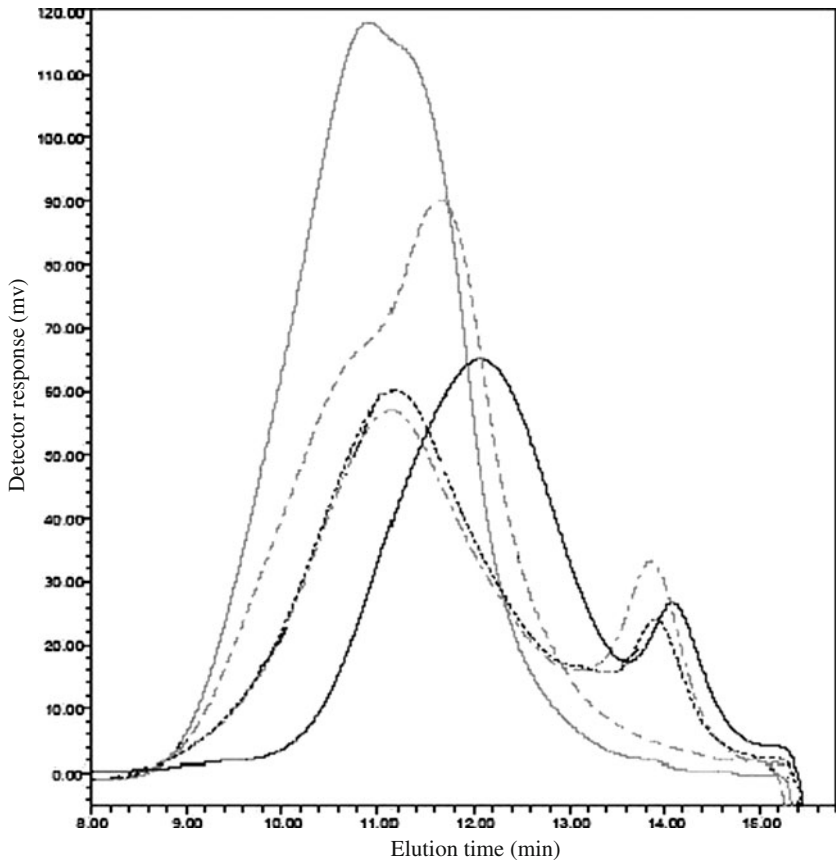

Fig. 1 HPSEC elution profiles of date, lemon and mixture pectins (— Pd, — Pl1, – – – Pdl1, ----- Pdl7, — – — Pdl8)

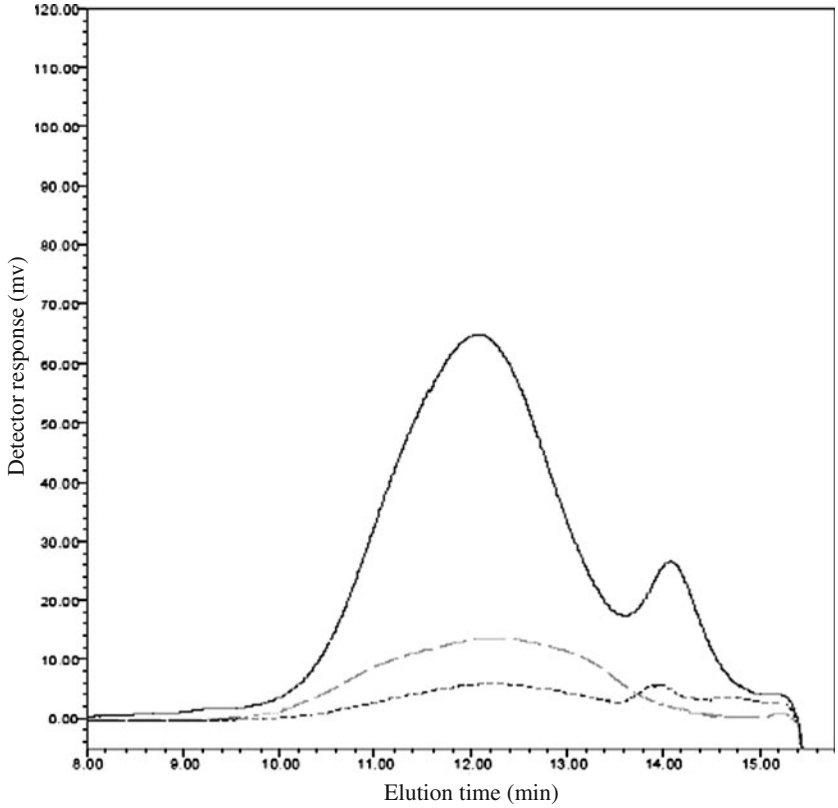

Fig. 2 HPSEC elution profiles of the dates pectins (— Pd , ------ Pdc5, – – – Pdc8)

which was 328.9 kg/mol. Similar results were found by Fishman et al. (2006) who obtained an Mw of 335 kg/mol for lime albedo pectin using microwave at pH 2. However, a lower value (158 kg/mol) was obtained by Joe and Luzio (2000) who extracted pectin from lemon peel at 70 °C, pH 2.5 and during 3 h. This could be due to differences in the extraction conditions (lower pH, use of nitric acid) or to the lemon variety.

Average molecular weight of pectin mixtures ranged from 113.8 to 350.8 kg/mol. Figure 1 shows the molar mass distribution for five pectin samples. Generally, two peaks can be observed: the first is big and corresponds to pectin substances rich in galacturonic acids, and the second one is small and represents probably neutral sugars and other components (ions, acids, etc.), which were eluted later. This could be confirmed by the galacturonic acid content of these samples. In fact, the second peak was not visible in the case of samples having high Gal A content, showing the presence of less amounts of small compounds (the second peak was missing, but existed respectively for Pdl1 and Pdl8 for example).

Pectins extracted at extreme conditions (higher temperature, lower pH and longer time) had lower molecular weights than those extracted at milder conditions (~132 and ~330 kg/mol for Pdl1 and Pdl8, respectively). This was shown by the displacement of the first peaks from high molecular weights toward lower molecular weights (Fig. 1). Pectin was probably partially degraded into smaller molecules by the extreme extraction conditions. Similar elution patterns were observed by Garna et al. (2004, 2007) who found that, under severe extraction conditions, the Mw of pectin decreased and attributed this to degradation of the side sugars chains and the hydrolysis of galacturonic acid chains.

Table 4 CieLab coordinates (L^*, a^*, b^*, h^*_{ab}, C^*_{ab}) of pectins

CieLab coordinates	Pd	Pdl1	Pdl2	Pdl3	Pdl4	Pdl5	Pdl6	Pdl7	Pdl8
L^*	51.6±0.0a	55.1±0.1c	59.5±0.0b	53.7±0.0d	62.9±0.1e	60.3±0.1g	64.6±0.1f	58.2±0.0i	65.8±0.0h
a^*	4.6±0.0a	6.0±0.0c	4.0±0.0b	5.9±0.0d	3.2±0.0e	4.3±0.0g	3.5±0.0f	4.0±0.0b	2.4±0.0h
b^*	11.1±0.0a	17.4±0.1c	15.6±0.0b	17.1±0.0d	14.3±0.1e	16.3±0.0g	14.7±0.0f	16.0±0.0h	11.6±0.0a
h^*_{ab}	67.4±0.1a	70.8±0.0c	75.5±0.0b	70.9±0.1c	77.2±0.1d	75.1±0.0f	76.5±0.1e	75.7±0.0b	78.0±0.1g
C^*_{ab}	12.0±0.0a	18.4±0.1c	16.1±0.0b	17.1±0.0d	14.7±0.1e	16.6±0.3g	15.1±0.1f	16.5±0.0g	11.8±0.0a

Means followed by the same letter within a line are not significantly different (α=0.05). All values given are means of two determinations

On the other hand, in the elution pattern of the pectin mixture samples, in addition to the last peak referring to low molecular size compounds, we expected to find two other peaks referring respectively to date and lemon pectins. But only one additional peak was observed corresponding probably to lemon pectin fraction. This could be explained by hydrolysis of date pectin (Pd) fraction, which had initially a low molecular weight (~67 kg/mol), into smaller Mw fractions.

In order to visualise this hydrolysis more clearly, date juice was subjected to the same experimental conditions of temperature, pH and time, without lemon by-product. The elution profile of the initial date pectin (Pd) and two control pectin samples is shown in Fig. 2. Peaks of the controls (Pdc5 and Pdc8) were slightly displaced to longer elution times compared to Pd and could be considered as smaller pectin fractions of different molecular weights. This could confirm Pd hydrolysis under the experimental conditions.

Colour of Pectins

Colour parameters of powders of the different pectin samples are shown in Table 4. L^* values, representing the lightness of the lemon/date mixture, decreased when extraction temperature increased at the same pH and time (60.3 versus 55.1 for pectin extracted at a pH of 2.8 and during 4 h at 40 and 80 °C, respectively). The same effect was observed when the extraction time was longer (59.5 versus 55.1 for pectin extracted in a pH of 2.8 and at 80 °C during 1 and 4 h, respectively). This difference in L^* may be attributed to Maillard reactions which happened during the extraction process. These browning reactions were accentuated by higher temperature and time. The same change in colour was observed for apple pectin when Constenla et al. (2002) dried apple pomace at higher temperature or for longer time.

Conclusion

In this investigation, mixtures of date and lemon by-product pectins were isolated by extracting lemon pectin with acidified date juice.

It was shown that chemical composition of the pectin samples were affected by the extraction conditions. Use of extreme conditions led to pectins of higher amount of Gal A, lower degree of esterification, lower neutral sugar content and lower Mw.

These pectin mixtures had interesting properties, particularly pectin extracted at optimal conditions (Pdl9) (Masmoudi et al. 2008). This pectin was low methylated (~35%), had high Gal A content (63.4%), high molecular weight (~243 kg/mol) and low neutral sugar content (9.2%). These properties allow it to have high potential for commercial food applications.

Further investigations should be done to look for the gelling properties of these pectins by means of rheological studies which will be the subject of a future paper.

References

Alvarez, M. D., Fernandez, C., & Canet, W. (2010). Oscillatory rheological properties of fresh and frozen/thawed mashed potatoes as modified by different cryoprotectants. *Food and Bioprocess Technology, 3*, 55–70.

AOAC (Association of Official Analytical Chemists) (1995) Official methods of analysis, 15th ed. Washington, DC, USA

BeMiller, J. N. (1986). An introduction to pectins: structure and properties. In: J. Fishman (Eds.), *Chemistry and function of pectins*, (pp. 2–12). American Chemical Society. ACS Symposium Series 310, Washington, USA

Benchabane, A., Abbeddous, S., Bellal, M. M., & Thibault, J.-F. (2006). Evolution des pectines et des activités polygalacturonases au cours de la maturation de la datte Deglet-Nour. *Science des Aliments, 26*, 233–246.

Blakney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research, 113*, 291–299.

Constenla, D., Ponce, A. G., & Lozano, J. E. (2002). Effect of pomace drying on apple pectin. *Lebensmittel-Wissenschaft und - Technologie, 35*, 216–221.

Diaz, E. L., Giannuzzi, L., & Giner, S. A. (2009). Apple pectic gel produced by dehydration. *Food and Bioprocess Technology, 2*, 194–207.

El-Zoghbi, M. (1994). Biochemical changes in some tropical fruits during ripening. *Food Chemistry, 49*, 33–37.

Fernandes, F. A. N., Rodrigues, S., Law, C. L., & Mujumdar, A. S. (2010). Drying of exotic tropical fruits: a comprehensive review. *Food and Bioprocess Technology*. doi:10.1007/s11947-010-0323-7.

Fishman, M. L., Pfeffer, P. E., Baford, R. A., & Doner, L. W. (1984). Studies of pectin solution properties by high performance size exclusion chromatography. *Journal of Agricultural and Food Chemistry, 32*, 372–378.

Fishman, M. L., Chau, H. K., Hoagland, P. D., & Hotchkiss, A. T. (2006). Microwave-assisted extraction of lime pectin. *Food Hydrocolloids, 20*, 1170–1177.

Food Chemical Codex. (1996). *IV monographs* (p. 283). Washington DC: National Academy Press.

Fu, J.-T., & Rao, M. A. (2001). Rheology and structure development during gelation of low-methoxyl pectin gels: the effect of sucrose. *Food Hydrocolloids, 15*, 93–100.

Garna, H., Mabon, N., Nott, K., Wathelet, B., & Paquot, M. (2004). New method for a two-step hydrolysis and chromatographic analysis of pectin neutral sugar chains. *Journal of Agricultural and Food Chemistry, 52*, 4652–4659.

Garna, H., Mabon, N., Nott, K., Wathelet, B., & Paquot, M. (2006). Kinetic of the hydrolysis of pectin galacturonic acid chains and quantification by ionic chromatography. *Food Chemistry, 96*, 477–484.

Garna, H., Mabon, N., Robert, C., Cornet, C., Nott, K., Legros, H., et al. (2007). Effect of extraction conditions on the yield and purity of apple pomace pectin precipitated but not washed by alcohol. *Journal of Food Science, 72*, C001–C009.

Guillotin SE (2005) Studies on the intra- and intermolecular distributions of substituents in commercial pectins. Ph.D. thesis, Wageningen University, The Netherlands

Joe, D. D., & Luzio, G. A. (2000). Process for selective extraction of pectin from plant material by differential pH. *Carbohydrate Polymers, 43*, 337–342.

Kravtchenko, T. P., Berth, G., Voragen, A. G. J., & Pilnik, W. (1992). Studies of the intermolecular distribution industrial pectins by means of preparative size exclusion chromatography. *Carbohydrate Polymers, 18*, 253–263.

Levigne, S., Ralet, M. C., & Thibault, J. F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydrate Polymers, 49*, 145–153.

Lin, M. J. Y., Humbert, E. S., & Sosulski, F. W. (1976). Extraction of pectins from sunflower heads. *Canadian Institute of Food Science and Technology Journal, 9*, 70.

Löfgren, C., Walkenstrom, P., & Hermansson, A.-M. (2002). Microstructure and rheological behavior of pure and mixed pectin gels. *Biomacromolecules, 3*, 1144–1153.

Masmoudi, M., Besbes, S., Blecker, C., & Attia, H. (2007). Preparation and characterization of osmodehydrated fruits from lemon and date by-products. *Food Science and Technology International, 13*, 405–412.

Masmoudi, M., Besbes, S., Chaabouni, M., Robert, C., Paquot, M., Blecker, C., et al. (2008). Optimization of pectin extraction from lemon by-product with acidified date juice using response surface methodology. *Carbohydrate Polymers, 74*, 185–192.

May, C. (1990). Industrial pectins: sources, production and applications. *Carbohydrate Polymers, 12*, 79–99.

Mayhara, R. M., Al-Alawi, A., Karakarlas, J., & Taylor, M. S. (2000). Sensory and textural changes in maturing Omani dates. *Journal of the Science of Food and Agriculture, 80*, 2181–2185.

Mesbahi, G., Jamalian, J., & Farahnaky, A. (2005). A comparative study on functional properties of beet and citrus pectins on food systems. *Food Hydrocolloids, 19*, 731–738.

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

Mort, A. J., Qui, F., & Maness, N. O. (1993). Determination of the pattern of methyl esterification in pectin. Distribution of contiguous nonesterified residues. *Carbohydrate Research, 247*, 21–35.

Mustapha, A. B., Harper, P. D., & Johnston, D. E. (1986). Biochemical changes during ripening of some Sudanese date varieties. *Journal of the Science of Food and Agriculture, 37*, 43–53.

Pagan, J., & Ibarz, A. (1999). Extraction and rheological properties of pectin from fresh peach pomace. *Journal of Food Engineering, 39*, 193–201.

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

Pilnik, W., & Voragen, A. G. J. (1991). In: P. F. Fox (Ed.), *Food enzymology*, (pp 303–306). London: Elsevier

Ralet, M.-C., & Thibault, J.-F. (1994). Extraction and characterisation of very highly methylated pectins from lemon cell walls. *Carbohydrate Research, 260*, 283–296.

Renard, C. M. G. C. (1989). Etude des polysaccharides pariétaux de la pomme. Extraction et caractérisation par des méthodes chimiques et enzymatiques. Ph.D. thesis, Université de Nantes, France

Robert, C., Devillers, T., Whathelet, B., Van Herck, J.-C., & Paquot, M. (2006). Use of a Plackett–Burman experimental design to examine the impact of extraction parameters on yields and compositions of pectins extracted from chicory roots (*Chicorium intybus* L.). *Journal of Agricultural and Food Chemistry, 54*, 7167–7174.

Rombouts, F. M., & Thibault, J.-F. (1986). Sugar beet pectins: chemical structure and gelation through oxidative coupling. In J. Fishman (Ed.), *Chemistry and function of pectins* (pp. 49–60). Washington, DC: American Chemical Society.

Schols, H. A., Ros, J. M., Daas, P. J. H., Bakx, E. J., & Voragen, A. G. J. (1998). *Gums and stabilisers for the food industry 9. Structural features of native and commercially extracted pectins* (pp. 3–15). London: The Royal Society of Chemistry.

Voragen, A. G. J., Schols, H. A., & Pilnik, W. (1986). Determination of the degree of methylation and acetylation of pectins by HPLC. *Food Hydrocolloids, 1*, 65–70.

Voragen, A. G. J., Pilnik, W., Thibault, J.-F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In: A. M. Stephen (Ed.), *Food polysaccharides and their applications*, (pp. 287–339). New York: Taylor and Francis

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