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An experimental design approach to the chemical characterisation of pectin polysaccharides extracted from *Cucumis melo* Inodorus

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ABSTRACT

Extracted pectins have been utilised in a number of applications in both the food and pharmaceutical industries where they are generally used as gelling agents, thickeners and stabilisers, although a number of pectins have been shown to be bioactive. These functional properties will depend upon extraction conditions.

A statistical experimental design approach was used to study the effects of extraction conditions pH, time and temperature on pectins extracted from *Cucumis melo* Inodorus.

The results show that the chemical composition is very sensitive to these conditions and that this has a great influence on for example the degree of branching. Higher temperatures, lower pHs and longer extraction times lead to a loss of the more acid labile arabinofuranose residues present on the pectin side chain. The fitting of regression equations relating yield and composition to extraction conditions can therefore lead to tailor-made pectins for specific properties and/or applications.

1. Introduction

Pectins are a family of heteropolysaccharides that constitute a large proportion of the primary cell walls of dicotyledons and play important roles in growth and development (Houben, Jolie, Fraeye, van Loey, & Hendrickx, 2011). Extracted pectins have been utilised in applications in both the food and pharmaceutical industries where they are generally used as gelling agents, thickeners and stabilisers, although a number of pectins have been shown to be bioactive (Adams et al., 2011; Grønhaug et al., 2010; Inngjerdingen et al., 2007, 2008; Simpson & Morris, 2014).

Pectic polysaccharides are made of several structural elements, the most important of which are the homogalacturonan (HG) and type I rhamnogalacturonan (RG-I) regions often described in simplified terms as the "smooth" and "hairy" regions respectively (Williams, Cucheval, Ström, & Ralet, 2009). The smooth regions consist of 1,4-linked α-D-galacturonic acid (GalA) units which may be methylated at the carboxyl group at position 6 (Pilnik & Voragen, 1970). Positions O-2 and O-3 can also be acetyl esterified though this is less common (Rombouts & Thibault, 1986). Interspersed along this GalA backbone are the hairy regions made up of alternating 1,4-linked α-D-GalA and 1,2-linked α-L-rhamnose (Rha) units, the latter having neutral sugar side chain substituents at position 4. These chains can be branched, of different lengths and mainly consist of galactose (Gal) and (Ara) though the presence of other sugars is possible (Fig. 1).

The changing nature of pectin composition between sources and even growing conditions makes it relevant to characterise extracts from a number of sources. Indeed, work has been conducted on pectins extracted from a variety of sources including Brazilian cupuassu (Vriesmann & Petkowicz, 2009), broccoli, tomatoes and carrots (Houben et al., 2011), pomelo (Burana-osot, Soonthornchareonnon, Chaidedgumjorn, Hosoyama, & Toida, 2010), peach pomace (Faravash & Ashtiani, 2008), sugar beet (Levigne, Ralet, & Thibault, 2002; Morris, Ralet, Bonin, Thibault, & Harding, 2010; Morris & Ralet, 2012a, 2012b), and quince (Thomas, Crépeau, Rumpunen, & Thibault, 2000; Thomas & Thibault, 2002) but few studies has been done to systematically work through the different members of a plant family (*Cucurbitaceae*).

Cucurbitaceae are a family that include pumpkins (Košťálova, Hromádková, & Ebringerová, 2013; Košťálova, Hromádková, Ebringerová, Polovka, et al., 2013), squashes (Noelia, Roberto, de Jesus, & Alberto, 2011), gourds (Huang, Tan, Tan, & Peng, 2011) and melons. This work aimed to investigate and characterise the components of the pectic polysaccharides extracted from Honeydew melon (*Cucumis melo* Inodorus or muskmelon). The components and characteristics studied were neutral sugar and uronic acid composition and degree of esterification (DE). A statistical experimental

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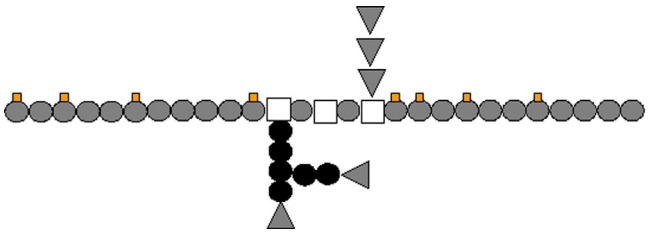

Fig. 1. Schematic structure for pectin: galacturonic acid ( ); galactose (●); arabinose ( ); rhamnose (□) and methyl groups ( ).
Adapted from Morris et al. (2008) and Perez, Rodríguez-Carvajal, and Doco (2003).

design approach was chosen to study the effects of extraction pH, time and temperature on pectins and any interactions they may have (Levigne et al., 2002) in order to potentially maximise desired characteristics *e.g.* yield, molar mass or degree of esterification.

2. Experimental

Fresh honeydew melon (*Cucumis melo* Inodorus) was purchased from the local supermarket; the seeds and any damaged skin were removed and the remaining skin and flesh grated. The pieces were soaked in ethanol followed by acetone for a total of 2 days then left to dry at 35 °C for 3 days to leave the alcohol insoluble residue (AIR).

2.1. Experimental design

A full factorial design was used establish all possible interactions of the studied parameters and was set out in a Yates matrix (Table 1) using Minitab version 15 (Minitab Inc., Philadelphia, U.S.A.). Two levels (high and low) for three extraction parameters (pH, temperature and time) were established, the lower level (−1) corresponding to pH 1, 60 °C and 2 h and the upper level (+1) corresponding to pH 3, 80 °C and 4 h. Samples A through H were repeated and a centre point (CP) chosen for comparison (pH 2, 70 °C, 3 h). HCl was chosen as the single extraction acid with the understanding that the type of acid had no effect (at least in the case of sugar beet extractions) on the pectin characteristics (Levigne et al., 2002).

2.2. Extractions

Extraction conditions were followed according to the factorial design (see Table 1). 1.5 g AIR in 45 mL hydrochloric acid (pH 1 or pH 3) heated on a hotplate with magnetic stirring. The final solution was then adjusted to pH 4.5 with 2 M NaOH. Centrifugation was carried out and the supernatant dialysed with 12,000 molecular weight cut-off tubing (Sigma, Gillingham, UK) against distilled water for 20 h with repeated changes prior freeze-drying.

Table 1
Full factorial design using levels −1 and 1 for pH 1 and 3, 60 °C and 80 °C and 2 and 4 h respectively. Level 0 has the center values of pH 2, 70 °C and 3 h.

Sample	pH	Temperature	Time
A	−1	−1	−1
B	1	−1	−1
C	−1	1	−1
D	1	1	−1
E	−1	−1	1
F	1	−1	1
G	−1	1	1
H	1	1	1
CP	0	0	0

2.2.1. FT-IR

Degree of esterification (DE) was calculated using infra-red spectroscopy. The characteristic peaks at approximately 1630 cm^{-1} and 1750 cm^{-1} relate to the vibration of esterified carbonyls and free carbonyls respectively, the ratio of these areas (esterified to total, obtained from solid samples on a Nicolet 380 FT-IR (Thermo Fisher, Loughborough, UK) were extrapolated using a calibration graph constructed from ratios of citrus pectins of known DE.

2.3. High performance anion exchange chromatography coupled with pulsed amperometric detection

Samples (2 mg) were hydrolysed with 4 M trifluroacetic acid (TFA) at 121 °C for 2 h and dried under N_2 at 65 °C then redissolved in 2 mL ultra pure water prior to neutral sugar and uronic acid composition analysis using a Dionex ICS-5000 HPAEC-PAD system and separated on a CarboPac PA20 (3 x 150 mm) column (Thermo Fisher, Loughborough, UK). A 0.5 mL/min flow rate was used the first 12 m at 10 mM NaOH followed by a 0.05 m step from 0 to 17% 1 M sodium acetate in 150 mM NaOH and the remainder of the run at the upper limit of this gradient to elute any uronic acids present. A pre-run equilibration step of 10 min using 200 mM NaOH followed by 20 min of 10 mM NaOH was used to regenerate the column prior to each injection.

2.4. Statistical analysis

Data from experiments were analysed using Minitab version 15 (Minitab Inc., Philadelphia, U.S.A.). Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1. Yield

Approximately 2.5 kg of fresh honeydew melon tissue and skin yielded 4.4% of AIR. After freeze-drying this yielded pectin in varying amounts between 2.2% and 7.9% of AIR (Table 2) and could be fitted by equation (1).

$$\begin{aligned}\text{Yield} = {} & 22.3 - 15.3\ \text{pH} - 0.097\ \text{Temp} - 2.96\ \text{time} \\ & + 2.68\ \text{pH}^2 + 0.0430\ \text{pH} \times \text{Temp} + 0.75\ \text{pH} \times \text{time} \\ & + 0.0478\ \text{Temp} \times \text{time} - 0.0127\ \text{pH} \times \text{Temp} \times \text{time}\end{aligned} \quad (1)$$

$$r^2 = 0.93$$

These yields are very low compared to those obtained by Levigne et al. (2002) from fresh sugar beet. Extractions conducted at pH 1 (A, C, E and G) gave significantly ($p \leq 0.05$) higher yields which is consistent with the findings of Levigne et al. (2002). Both pH and pH^2 (in Eq. (1)) had significant influences on yield, $p = 0.029$ and 0.014 respectively. However, neither temperature nor time had a significant influence ($p > 0.05$).

A two phase mechanism for the extraction of pectin from fresh peach pomace has previously been proposed; the first step being acid solubilisation of the pectin followed by a second hydrolysis reaction where the pectin is degraded, lowering the yield at longer extraction times, and once all the "extractable" pectin has been exhausted the hydrolysis predominates (Pagan & Ibarz, 1999).

3.2. Degree of esterification (DE)

Degree of esterification calculated from solid samples using IR spectroscopy and stretching bands centred around 1630 cm^{-1} and 1750 cm^{-1} (Manrique & Lajolo, 2002) gave DE values between 40%

Table 2
Experimental values for yield, degree of esterification (DE), neutral sugar (Rha, Ara, Gal, Glc and Man) and galacturonic acid (GalA) composition of pectins extracted according to a full factorial design.

Pectin	Yield (% AIR)	DE (%)	Sugar content (mol% of AIR)					
			GalA	Rha	Ara	Gal	Glc	Man
A	6.3 ± 0.2^{a}	$54 \pm 7^{a,b}$	24 ± 3^{a}	5 ± 2^{a}	21 ± 1^{a}	32 ± 1^{a}	12 ± 2^{a}	5 ± 2^{a}
B	2.4 ± 0.2^{b}	$59 \pm 2^{a,b}$	28 ± 2^{a}	4 ± 1^{a}	14 ± 1^{b}	21 ± 1^{b}	17 ± 1^{b}	12 ± 1^{b}
C	6.7 ± 1.4^{a}	$56 \pm 2^{a,b}$	$29 \pm 10^{a,b}$	9 ± 2^{b}	$8 \pm 11^{b,c,d}$	$36 \pm 3^{a,d}$	11 ± 1^{a}	4 ± 1^{a}
D	3.4 ± 0.2^{c}	$68 \pm 2^{a,b}$	43 ± 1^{b}	4 ± 1^{a}	12 ± 1^{c}	16 ± 1^{c}	12 ± 1^{a}	8 ± 1^{b}
E	6.1 ± 0.4^{a}	40 ± 6^{a}	31 ± 1^{a}	10 ± 1^{b}	1 ± 1^{d}	40 ± 1^{d}	8 ± 1^{c}	4 ± 1^{a}
F	2.2 ± 0.4^{b}	75 ± 16^{b}	28 ± 1^{a}	4 ± 1^{a}	15 ± 1^{b}	21 ± 1^{b}	16 ± 1^{b}	12 ± 1^{b}
G	7.9 ± 1.8^{a}	47 ± 11^{a}	43 ± 1^{b}	9 ± 2^{b}	1 ± 1^{d}	31 ± 2^{a}	7 ± 1^{c}	3 ± 1^{a}
H	3.6 ± 0.1^{c}	70 ± 4^{b}	46 ± 5^{b}	4 ± 1^{a}	11 ± 1^{c}	$18 \pm 2^{b,c}$	10 ± 1^{a}	$6 \pm 2^{a,b}$
CP	2.1^{b}	$57^{a,b}$	28^{a}	4^{a}	16^{b}	21^{b}	16^{b}	12^{b}

Means of each attribute followed by different letters in the same column (a–d) are significantly different ($p \leq 0.05$).

(E) and 75% (F) with the majority being high DE (>50%) (Table 2) and could be fitted by Eq. (2).

$$\begin{aligned} DE = {} & 137 - 57.7\ \ pH - 0.86\ \ Temp - 37.4\ \ time \\ & + 1.25\ \ pH^2 + 0.662\ \ pH \times Temp + 21.9\ \ pH \\ & \times time + 0.375\ \ Temp \times time - 0.237\ \ pH \times Temp \times time \end{aligned} \quad (2)$$

$$r^2 = 0.80$$

Although Eq. (2) fits the data reasonably well, none of the terms included were significant at the level $p < 0.05$, however the most important effects are time ($p = 0.265$), pH × time ($p = 0.320$) and pH ($p = 0.323$). It is worth noting that Levigne et al. (2002) found this pH–time interaction to be the most significant factor when building the regression equation for degree of methyl esterification (DM). Nonetheless, we can reasonably say that lower pH (A, C, E and G) results in a lower degree of esterification and this correlates with a higher yield (Fig. 2). The most likely explanation is that lower pH during the extraction process contributes to de-esterification (Joye & Luzio, 2000).

3.3. Constituent sugar composition

Neutral sugar and uronic acid constituents were a quantified using HPAEC-PAD, the major components were arabinose (Ara), galactose (Gal), glucose (Glc), mannose (Man), rhamnose (Rha) and galacturonic acid (Table 2). Glucuronic acid, fucose, xylose and glucosamine were also indentified but were only present in trace amounts (≤1 mol%). The sugar composition is similar to different extracts of melon previously reported (Dos-Santos, Jiménez-Araujo, Rodríguez-Arcos, & Fernandez-Trujillo, 2011; Rose, Hadfield, Labavitch, & Bennett, 1998).

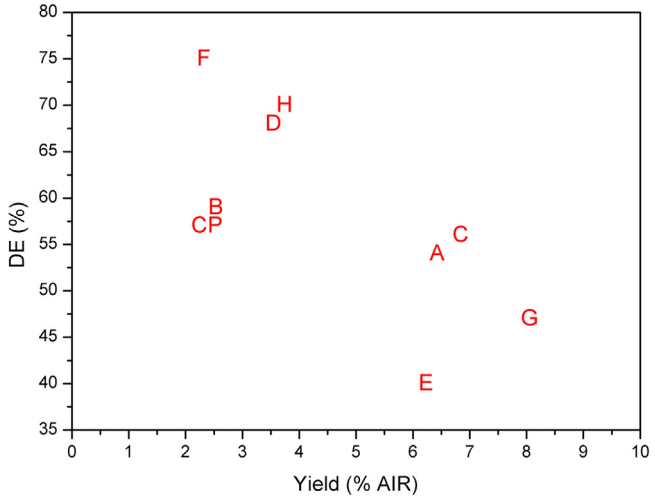

Fig. 2. The inter-relationship between yield and degree of esterification (DE) for pectins extracted from *Cucumis melo* Inodorus. Letters represent the samples described in Table 1.

The galacturonic acid content varied from 24 to 46 mol% of the AIR (Table 2) and could be fitted by Eq. (3). Again none of the terms in Eq. (3) were significant at the level $p < 0.05$, however the most important effects are pH^2 ($p = 0.240$) and pH ($p = 0.262$) and there is no correlation between Gal A content and the yield as has been previously reported (Levigne et al., 2002).

$$\begin{aligned} Gal\,A = {} & 51.5 - 36.9\ \ pH - 0.397\ \ Temp - 6.6\ \ time \\ & + 5.76\ \ pH^2 + 0.330\ \ pH \times Temp + 0.79\ \ pH \\ & \times time + 0.203 Temp \times time - 0.044\ \ pH \times Temp \times time \end{aligned} \quad (3)$$

$$r^2 = 0.87$$

Rhamnose contents varied from 4 to 10 mol% of the AIR and a number of terms in Eq. (4) were found to be significant including time ($p = 0.018$), temperature ($p = 0.021$) and temperature × time ($p = 0.034$).

$$\begin{aligned} Rha = {} & -36.3 + 7.43\ \ pH + 0.660\ \ Temp + 15.3\ \ time \\ & + 2.16\ \ pH^2 - 0.225\ \ pH \times Temp - 5.28\ \ pH \times time \\ & - 0.186\ \ Temp \times time + 0.0638\ \ pH \times Temp \times time \end{aligned} \quad (4)$$

$$r^2 = 0.89$$

However we can see that on average the rhamnose content is a factor of 2 higher at pH 1 than at pH 3. This is indicative of different populations of pectins being extracted under different conditions. This has been proposed previously in the extraction of sugar beet pectin (Levigne et al., 2002) and is demonstrated in Fig. 3.

Therefore pectins extracted at pH 1 (A, C, E and G) are richer in rhamnogalacturonan whilst pectins extracted at pH 2 and 3 are richer in homogalacturonan.

Arabinose and galactose contents varied from 1 to 21 mol% and 16 to 40 mol% respectively and were fitted by Eqs. (5) and (6). The most significant extraction parameters in the fitting of arabinose are time ($p = 0.018$), temperature ($p = 0.024$), pH × time ($p = 0.044$) and temperature × time ($p = 0.049$) and in the case of galactose all parameters except pH ($p = 0.306$) are significant.

$$\begin{aligned} Ara = {} & 152 - 30.6\ \ pH - 1.91\ \ Temp - 45.4\ \ time - 5.41\ \ pH^2 \\ & + 0.641\ \ pH \times Temp + 16.4\ \ pH \times time \\ & + 0.506\ \ Temp \times time - 0.187\ \ pH \times Temp \times time \end{aligned} \quad (5)$$

$$r^2 = 0.85$$

$$\begin{aligned} Gal = {} & -42.0 + 10.3\ \ pH + 1.43\ \ Temperature + 36.1\ \ time \\ & + 6.11\ \ pH^2 - 0.601\ \ pH \times Temp - 13.1\ \ pH \times time \\ & - 0.503\ \ Temp \times time + 0.186\ \ pH \times Temp \times time \end{aligned} \quad (6)$$

$$r^2 = 0.99$$

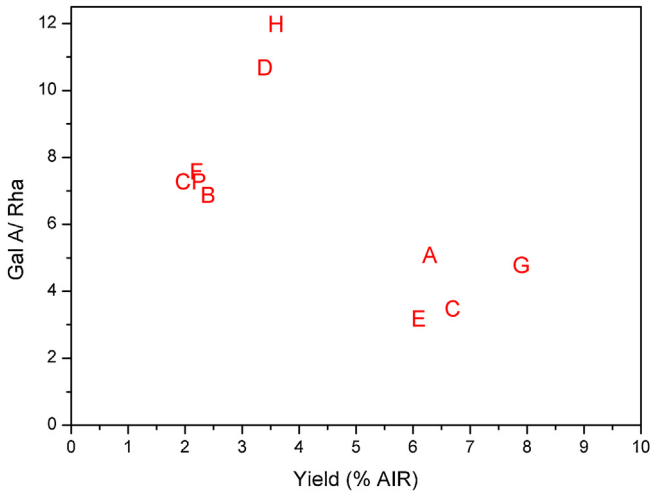

Fig. 3. The relationship between galacturonic acid (GalA) to rhamnose (Rha) ratio and the yield for pectins extracted from *Cucumis melo* Inodorus. Letters represent the samples described in Table 1.

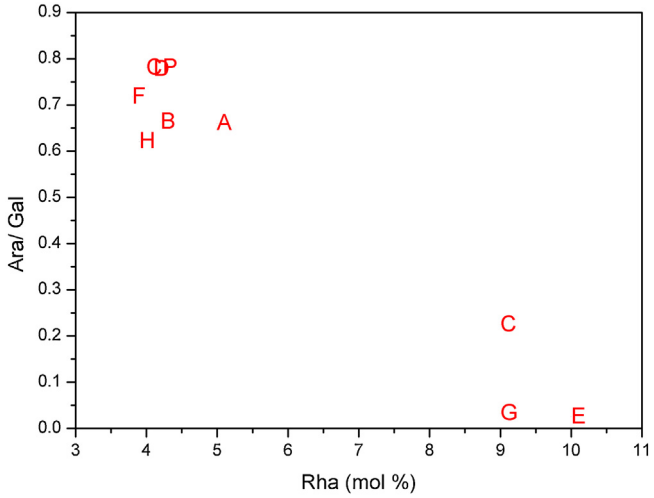

Fig. 4. The arabinose: galactose ratio as function of rhamnose content for pectins extracted from *Cucumis melo* Inodorus. Letters represent the samples described in Table 1.

The ratio of arabinose: galactose can yield some important information about the side chains in the rhamnogalacturonan region (Fig. 4).

As we can see in Fig. 4 as the process conditions become more harsh *e.g.* low pH coupled with higher temperature and/or longer extraction times leads to the loss of arabinose side chains most probably due to their acid lability (BeMiller, 1967; Levigne et al., 2002; Morris et al., 2010) and furanose structure (Wrolstad, 2012, chap. 4).

The data in Table 2 was used to generate an overall principal component score for each of the samples (Fig. 5). It can be seen clearly that the first component is clearly driven by pH, whereas it is extraction temperature which is the main driver in second component.

Several sugar ratios can be calculated from the monosaccharide composition (Houben et al., 2011) to reveal information regarding the pectin structure (Table 3). The first is the ratio of the pectin backbone sugar Gal A to the neutral sugars involved in side chains and is therefore a useful tool to estimate the linearity of pectin (ratio 1 in Table 3). Higher values of this ratio are indicative of more linear pectins and although there is not a great variation in this ratio we can see that pectins extracted at 60 °C (A, B, E and F) are generally less linear than those extracted at 70 and 80 °C. Interestingly our values for this ratio are similar to those for the water soluble pectins from broccoli florets of 0.64 but very different to other pectins extracted under the same aqueous conditions, having values of 3.1–4.8 (Houben et al., 2011). Ratio 2 is defined as the proportion of Rha to Gal A and is therefore indicative of the contribution of RG to the entire pectin population. Lower values of this ratio are indicative of less branched pectins and again the results suggest heavily branched structures very similar to those of the highly branched water soluble pectin extracted from broccoli florets (Houben et al., 2011).

Ratios 3 and 4 compare the amount of side-chain sugars to Rha as an estimate of the length of RG-I branching. These ratios are again typical of highly branched structures which are easily solubilised from the cell wall even at the relatively mild conditions studied here. This fits in well with the relatively high degrees of esterification (Houben et al., 2011). Ratio 4 differentiates itself in that it helps to establish the length of the galactose side chains present in the RG region, higher values being consistent with a longer sugar chains. Ratio 5 compares the amount of typical pectin components

Table 3
Composition ratios and pectin region % based on the mol% quantifiable neutral sugars and galacturonic acid.

Pectin	Ratio 1[a]	Ratio 2[b]	Ratio 3[c]	Ratio 4[d]	Ratio 5[e]	Ratio 6[f]	Ratio 7[f]	Ratio 8[f]	% HG	% RG-I	HG:RG-I
	Linearity of pectin	Contribution of RG		Branching of RG	Co-extractants	Severity of extraction			GalA – Rha	2Rha + Ara + Gal	
	$\frac{GalA}{Rha+Ara+Gal}$	$\frac{Rha}{GalA}$	$\frac{Ara+Gal}{Rha}$	$\frac{Gal}{Rha}$	$\frac{Rha+Ara+Gal+GalA}{Glc+Man}$	$\frac{GalA}{Ara}$	$\frac{Gal}{Ara}$	$\frac{Rha}{Ara}$			
A	0.4	0.21	10.6	6.3	4.8	1.1	1.5	0.2	19	63	0.3
B	0.7	0.14	8.8	5.3	2.3	2.0	1.5	0.3	24	43	0.6
C	0.5	0.31	4.9	4.0	5.5	3.6	4.5	1.1	20	62	0.3
D	1.3	0.09	7.0	4.0	3.8	3.6	1.3	0.3	39	36	1.1
E	0.6	0.32	4.1	4.0	6.9	31.0	40.0	10.0	21	61	0.3
F	0.7	0.14	9.0	5.3	2.5	1.9	1.4	0.3	24	44	0.5
G	1.0	0.21	3.6	3.4	8.4	43.0	31.0	9.0	34	50	0.7
H	1.4	0.09	7.3	4.5	4.9	4.2	1.6	0.4	42	37	1.1
CP	0.7	0.14	9.3	5.3	2.5	1.8	1.3	0.3	24	45	0.5

[a] A larger value is indicative of more linear/less branched pectins.
[b] A smaller value is indicative of more linear/less branched pectins.
[c] A larger value is indicative of larger average size of the branching side chains.
[d] A larger value is indicative of larger average size of the branching side chains excluding arabinose.
[e] A larger value is indicative of a "more pure" pectin extract.
[f] A larger value is indicative of more severe extraction conditions and loss of arabinofuranoside (Araf) residues.

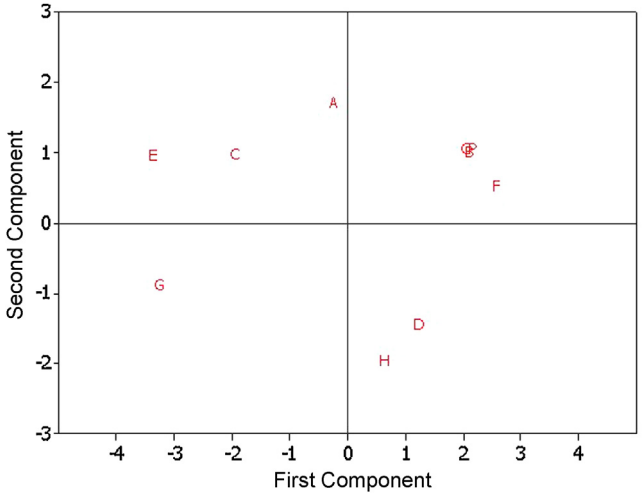

Fig. 5. Principal component scores for pectin extracted from *Cucumis melo* Inodorus. Letters represent the samples described in Table 1. The first component is positively correlated with degree of esterification, arabinose, glucose and mannose, whereas the second component is positively correlated with yield, rhamnose, arabinose, galactose, glucose and mannose.

to any co-extractants, in this case glucose and mannose, and as we can see from Fig. 6 when harsher extractions conditions (C, E and G) are used the extract becomes more enriched in pectin.

Ratios 6–8 assess the severity of the extraction conditions by comparing the presence of labile arabinose to galacturonic acid, galactose and rhamnose, respectively. These three ratios are consistent with Fig. 4 in that low pH (pH = 1) coupled with higher temperature and/or longer extraction times (*e.g.* E and G) results in the hydrolysis of the arabinan side chains which is perhaps consistent with the two phase mechanism for the extraction of pectin proposed by Pagan and Ibarz (1999) the first step being acid solubilisation of the pectin followed by a second hydrolysis reaction where the pectin is degraded.

The mol% of neutral sugars and GalA can further be used to calculate the % of key pectin regions HG and RG-1 (Table 3). The ratio of HG:RG-I gives us some information which is complementary to Fig. 3, where we can see that pectins extracted at pH 2 and 3 (B, D, F, H and CP) are richer in HG pectins, although their RG-I regions are in some cases more branched. The only exception is pectin G

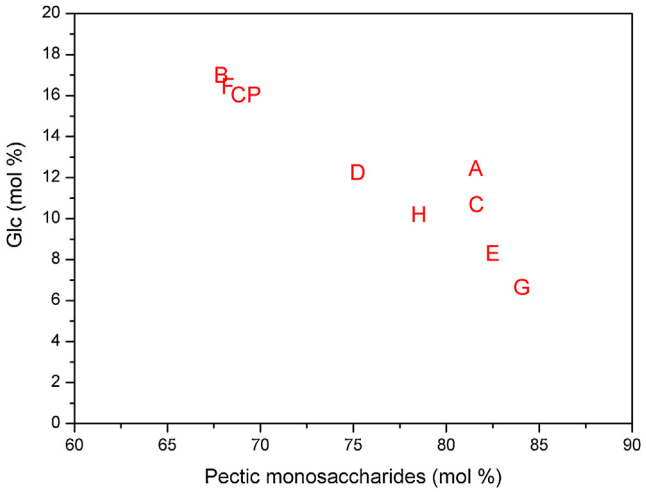

Fig. 6. The effect of extraction conditions on the "purity" of the pectins extracted from *Cucumis melo* Inodorus. Letters represent the samples described in Table 1.

which appears to also be rich in HG regions, but it is expected that this is due to complete lack of arabinose.

4. Conclusions

Pectins from melon (*Cucumis melo* Inodorus) were extracted under a number of experimentally designed extraction conditions. The results show that the chemical composition is very sensitive to these conditions and that this has a great influence on for example the degree of branching. Higher temperature, lower pH and longer extraction time lead to a loss of the more acid labile arabinofuranose residues present on the pectin side chain. The fitting of regression equations relating yield and composition to extraction conditions can therefore lead to tailor-made pectins for specific properties and/or applications. It is also expected that these extraction conditions will also have an influence on molar mass (M_w), intrinsic viscosity [η] and conformation and any potential bioactivity.

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References

Adams, G. G., Imran, S., Wang, S., Mohammad, A., Kök, M. S., Gray, D. A., et al. (2011). The hypoglycaemic effect of pumpkins as anti-diabetic and functional medicines. *Food Research International*, *44*, 862–867.

BeMiller, J. N. (1967). Acid-catalyzed hydrolysis of glycosides. *Advances in Carbohydrate Chemistry*, *22*, 25–108.

Burana-osot, J., Soonthornchareonnon, N., Chaidedgumjorn, A., Hosoyama, S., & Toida, T. (2010). Determination of galacturonic acid from pomelo pectin in term of galactose by HPAEC with fluorescence detection. *Carbohydrate Polymers*, *81*, 461–465.

Dos-Santos, N., Jiménez-Araujo, A., Rodríguez-Arcos, R., & Fernandez-Trujillo, J. P. (2011). Cell wall polysaccharides of near-isogenic lines of melon (*Cucumis melo* L.) and their inbred parentals which show differential flesh firmness or physiological behavior. *Journal of Agriculture and Food Chemistry*, *59*, 7773–7784.

Faravash, R. S., & Ashtiani, F. Z. (2008). The influence of acid volume, ethanol-to-extract ratio and acid-washing time on the yield of pectic substances extraction from peach pomace. *Food Hydrocolloids*, *22*, 196–202.

Grønhaug, T. E., Ghildyal, P., Barsett, H., Michaelsen, T. E., Morris, G., Diallo, D., et al. (2010). Bioactive arabinogalactans from the leaves of *Opilia celtidifolia* Endl. ex Walp (Opiliaceae). *Glycobiology*, *20*, 1654–1664.

Huang, G., Tan, J., Tan, X., & Peng, D. (2011). Preparation of polysaccharides from wax gourd. *International Journal of Food Sciences and Nutrition*, *62*, 480–483.

Houben, K., Jolie, R. P., Fraeye, I., Van Loey, A., & Hendrickx, M. (2011). Comparative study of the cell wall composition of broccoli, carrot, and tomato: Structural characterization of the extractable pectins and hemicelluloses. *Carbohydrate Research*, *346*, 1105–1111.

Inngjerdingen, K. T., Patel, T. R., Chen, X., Kenne, L., Allen, S., Morris, G. A., et al. (2007). Immunological and structural properties of a pectic polymer from *Glinus oppositifolius*. *Glycobiology*, *17*, 1299–1310.

Inngjerdingen, M., Inngjerdingen, K. T., Patel, T. R., Allen, S., Chen, X. Y., Rolstad, B., et al. (2008). Pectic polysaccharides from *Biophytum petersianum* Klotzsch, and their activation of macrophages and dendritic cells. *Glycobiology*, *18*, 1074–1084.

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

Košťálova, Z., Hromádková, Z., & Ebringerová, A. (2013). Structural diversity of pectins isolated from the Styrian oil-pumpkin (*Cucurbita pepo* var. styriaca) fruit. *Carbohydrate Polymers*, *93*, 163–171.

Košťálova, Z., Hromádková, Z., Ebringerová, A., Polovka, M., Michaelsen, T. E., & Paulsen, B. S. (2013). Polysaccharides from the Styrian oil-pumpkin with antioxidant and complement-fixing activity. *Industrial Crops and Products*, *41*, 127–133.

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.

Manrique, G. D., & Lajolo, F. M. (2002). FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology*, *25*, 99–107.

Morris, G. A., Garcìa de la Torre, J., Ortega, A., Castille, J., Smith, A., & Harding, S. E. (2008). Molecular flexibility of citrus pectins by combined sedimentation and viscosity analysis. *Food Hydrocolloids*, *22*, 1435–1442.

Morris, G. A., & Ralet, M.-C. (2012a). A copolymer analysis approach to estimate the neutral sugar distribution of sugar beet pectin using size exclusion chromatography. *Carbohydrate Polymers*, *87*, 1139–1143.

Morris, G. A., & Ralet, M.-C. (2012b). The effect of neutral sugar distribution on the dilute solution conformation of sugar beet pectin. *Carbohydrate Polymers*, *88*, 1488–1491.

Morris, G. A., Ralet, M.-C., Bonnin, E., Thibault, J.-F., & Harding, S. E. (2010). Physical characterisation of the rhamnogalacturonan and homogalacturonan fractions of sugar beet (*Beta vulgaris*) pectin. *Carbohydrate Polymers*, *82*, 1161–1167.

Noelia, J., Roberto, M. M., de Jesus, Z. J., & Alberto, G. J. (2011). Chemical and physicochemical characterization of winter squash (*Cucurbita moschata* D). *Notulae Botanicae Horti Agrobotanici Cluj*, *39*, 34–40.

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

Perez, S., Rodríguez-Carvajal, M. A., & Doco, T. (2003). A complex plant cell wall polysaccharide: Rhamnogalacturonan II. A structure in quest of a function. *Biochimie*, *85*, 109–121.

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

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

Rose, J. K. C., Hadfield, K. A., Labavitch, J., & Bennett, A. B. (1998). Temporal sequence of cell wall disassembly in ripening melon fruit. *Plant Physiology*, *117*, 345–361.

Simpson, R., & Morris, G. A. (2014). The anti-diabetic potential of polysaccharides extracted from members of the cucurbit family: A review. *Bioactive Carbohydrates and Dietary Fibre*, *3*, 106–114.

Thomas, M., Crépeau, M. J. C., Rumpunen, K., & Thibault, J.-F. (2000). Dietary fibre and cell-wall polysaccharides in the fruits of Japanese quince (*Chaenomeles japonica*). *Lebensmittel-Wissenschaft and Technologie*, *33*, 124–131.

Thomas, M., & Thibault, J.-F. (2002). Cell-wall polysaccharides in the fruits of Japanese quince (*Chaenomeles japonica*): Extraction and preliminary characterisation. *Carbohydrate Polymers*, *49*, 345–355.

Vriesmann, L. C., & Petkowicz, C. L. O. (2009). Polysaccharides from the pulp of cupuassu (Theobroma grandiflorum): Structural characterization of a pectic fraction. *Carbohydrate Polymers*, *77*, 72–79.

Williams, M. A. K., Cucheval, A., Ström, A., & Ralet, M.-C. (2009). Electrophoretic behaviour of co-polymeric galacturonans including comments on the information content of the intermolecular charge distribution. *Biomacromolecules*, *10*, 1523–1531.

Wrolstad, R. E. (2012). Food carbohydrate chemistry. In *Reactions of sugars*. Chicago, USA: IFT Press Books.