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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.

**Keywords:** Experimental design; extraction conditions; chemical characterisation; pectin; melon
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 and 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 (Inngjerdingen, et al., 2007; Inngjerdingen, et al., 2008; Grønhaug, et al., 2010; Adams, et al., 2011; Simpson and 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 and 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 and Voragen, 1970). Positions O-2 and O-3 can also be acetyl esterified though this is less common (Rombouts, and 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 (Figure 1).

Figure 1 near here

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 and Petkowicz, 2009), broccoli, tomatoes and carrots (Houben, et al., 2011), pomelo (Burana-osot, Soonthornchareonnon, Chaidedgumjorn, Hosoyama and Toida, 2010), peach pomace (Faravash and Ashtiani, 2008), sugar beet (Levigne, Ralet and Thibault, 2002; Morris, Ralet, Bonin, Thibault and Harding, 2010; Morris and Ralet, 2012a,b), and quince (Thomas, Crépeau, Rumpunen and Thibault, 2000; Thomas and 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 and Ebringerová, 2013; Košťálova, Hromádková Ebringerová, Polovka, Michaelsen and Paulsen, 2013), squashes (Noelia, Roberto, de Jesus and Alberto, 2011), gourds (Huang, Tan, Tan and 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 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 two days then left to dry at 35 °C for three 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 hours and the upper level (+1) corresponding to pH 3, 80 °C and 4 hours. Samples A through H were repeated and a centre point (CP) chosen for comparison (pH 2, 70 °C, 3 hours). 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).

Table 1 near here
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 hours with repeated changes prior freeze-drying.

2.3. 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.

2.4. High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection
Samples (2 mg) were hydrolysed with 4 M trifluoroacetic acid (TFA) at 121 °C for 2 hours 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 (Thermo Fisher, Loughborough, UK). A 0.5 mL/min flow rate was used the first 12 minutes at 10 mM NaOH followed by a 0.05 minute step from 0-17 % 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 minutes using 200 mM NaOH followed by 20 minutes of 10 mM NaOH was used to regenerate the column prior to each injection.

2.5. Statistical analysis
Data from experiments were analyzed 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).

\[
Yield = 22.3 - 15.3 \, \text{pH} - 0.097 \, \text{Temp} - 2.96 \, \text{time} + 2.68 \, \text{pH}^2 + 0.0430 \, \text{pH} \times \text{Temp} + 0.75 \, \text{pH} \times \text{time} + 0.0478 \, \text{Temp} \times \text{time} - 0.0127 \, \text{pH} \times \text{Temp} \times \text{time} \quad (1) \\
\]

\[r^2 = 0.93\]

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 ≤ 0.05) higher yields which is consistent with the findings of Levigne, et al. (2002). Both pH and pH^2 (in equation 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 and 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 and Lajolo, 2002) gave DE values between 40 % (E) and 75 % (F) with the majority being high DE (> 50 %) (Table 2) and could be fitted by equation (2).

\[
DE = 137 - 57.7 \, \text{pH} - 0.86 \, \text{Temp} - 37.4 \, \text{time} + 1.25 \, \text{pH}^2 + 0.662 \, \text{pH} \times \text{Temp} + 21.9 \, \text{pH} \times \text{time} + 0.375 \, \text{Temp} \times \text{time} - 0.237 \, \text{pH} \times \text{Temp} \times \text{time} \quad (2) \\
\]

\[r^2 = 0.80\]

Although equation (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 (Figure 2). The most likely explanation is that lower pH during the extraction process contributes to de-esterification (Joye and Luzio, 2000).

**Figure 2 near here**

### 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 (Rose, Hadfield, Labavitch and Bennett, 1998; Dos-Santos, Jiménez-Araujo, Rodriguez-Arcos and Pablo Fernández-Trujillo, 2011).

The galacturonic acid content varied from 24 – 46 mol% of the AIR (Table 2) and could be fitted by equation (3). Again none of the terms in equation 3 were significant at the level p < 0.05, however the most important effects are pH² (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).

**Table 2 near here**

\[
\text{GalA} = 51.5 - 36.9 \text{pH} - 0.397 \text{Temp} - 6.6 \text{time} + 5.76 \text{pH}^2 + 0.330 \text{pH*Temp} + 0.79 \text{pH*time} + 0.203 \text{Temp*time} - 0.044 \text{pH*Temp*time} \\
\]

\( r^2 = 0.87 \)  

Rhamnose contents varied from 4 – 10 mol% of the AIR and a number of terms in equation (4) were found to be significant including time (p = 0.018), temperature (p = 0.021) and temperature*time (p = 0.034).
\[ Rha = -36.3 + 7.43 \text{ pH} + 0.660 \text{ Temp} + 15.3 \text{ time} + 2.16 \text{ pH}^2 - 0.225 \text{ pH} \times \text{Temp} - 5.28 \text{ pH} \times \text{time} - 0.186 \text{ Temp} \times \text{time} + 0.0638 \text{ pH} \times \text{Temp} \times \text{time} \] 
\[ r^2 = 0.89 \]

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 Figure 3.

**Figure 3 near here**

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 – 21 mol\% and 16 – 40 mol\% respectively and were fitted by equations 5 and 6. The most significant extraction parameters in the fitting of arabinose are time (\( p = 0.018 \)), temperature (\( p = 0.024 \)), \( \text{pH} \times \text{time} \) (\( p = 0.044 \)) and \( \text{temperature} \times \text{time} \) (\( p = 0.049 \)) and in the case of galactose all parameters except \( \text{pH} \) (\( p = 0.306 \)) are significant.

\[ \text{Ara} = 152 - 30.6 \text{ pH} - 1.91 \text{ Temp} - 45.4 \text{ time} - 5.41 \text{ pH}^2 + 0.641 \text{ pH} \times \text{Temp} + 16.4 \text{ pH} \times \text{time} + 0.506 \text{ Temp} \times \text{time} - 0.187 \text{ pH} \times \text{Temp} \times \text{time} \] 
\[ r^2 = 0.85 \]

\[ \text{Gal} = -42.0 + 10.3 \text{ pH} + 1.43 \text{ Temperature} + 36.1 \text{ time} + 6.11 \text{ pH}^2 - 0.601 \text{ pH} \times \text{Temp} - 13.1 \text{ pH} \times \text{time} - 0.503 \text{ Temp} \times \text{time} + 0.186 \text{ pH} \times \text{Temp} \times \text{time} \] 
\[ r^2 = 0.99 \]

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

**Figure 4 near here**
As we can see in Figure 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).

The data in Table 2 was used to generate an overall principal component score for each of the samples (Figure 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.

Figure 5 near here

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

**Figure 6 near here**

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 Figure 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.

**Table 3 near here**

The mol % of neutral sugars and GalA can further be used to calculate the % of key pectin regions HG and RG-I (Table 3). The ratio of HG:RG-I gives us some information which is complementary to Figure 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 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, $[\eta]$ and conformation and any potential bioactivity.
5. Acknowledgements
The authors would like to thank the University of Huddersfield for funding this project.

6. References


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

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>C</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>E</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>-1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CP</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
**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.

<table>
<thead>
<tr>
<th>Pectin</th>
<th>Yield (% AIR)</th>
<th>DE (%)</th>
<th>Sugar content (mol % of AIR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GalA</td>
</tr>
<tr>
<td>A</td>
<td>6.3 ± 0.2\textsuperscript{a}</td>
<td>54 ± 7\textsuperscript{a,b}</td>
<td>24 ± 3\textsuperscript{a}</td>
</tr>
<tr>
<td>B</td>
<td>2.4 ± 0.2\textsuperscript{b}</td>
<td>59 ± 2\textsuperscript{a,b}</td>
<td>28 ± 2\textsuperscript{a}</td>
</tr>
<tr>
<td>C</td>
<td>6.7 ± 1.4\textsuperscript{a}</td>
<td>56 ± 2\textsuperscript{a,b}</td>
<td>29 ± 10\textsuperscript{a,b}</td>
</tr>
<tr>
<td>D</td>
<td>3.4 ± 0.2\textsuperscript{c}</td>
<td>68 ± 2\textsuperscript{a,b}</td>
<td>43 ± 1\textsuperscript{b}</td>
</tr>
<tr>
<td>E</td>
<td>6.1 ± 0.4\textsuperscript{a}</td>
<td>40 ± 6\textsuperscript{a}</td>
<td>31 ± 1\textsuperscript{a}</td>
</tr>
<tr>
<td>F</td>
<td>2.2 ± 0.4\textsuperscript{b}</td>
<td>75 ± 16\textsuperscript{b}</td>
<td>28 ± 1\textsuperscript{a}</td>
</tr>
<tr>
<td>G</td>
<td>7.9 ± 1.8\textsuperscript{a}</td>
<td>47 ± 11\textsuperscript{a}</td>
<td>43 ± 1\textsuperscript{b}</td>
</tr>
<tr>
<td>H</td>
<td>3.6 ± 0.1\textsuperscript{c}</td>
<td>70 ± 4\textsuperscript{b}</td>
<td>46 ± 5\textsuperscript{b}</td>
</tr>
<tr>
<td>CP</td>
<td>2.1\textsuperscript{b}</td>
<td>57\textsuperscript{a,b}</td>
<td>28\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Means of each attribute followed by different letters in the same column (a-d) are significantly different (p ≤ 0.05).
Table 3. Composition ratios and pectin region % based on the mol % quantifiable neutral sugars and galacturonic acid.

<table>
<thead>
<tr>
<th>Pectin</th>
<th>¹Ratio 1</th>
<th>²Ratio 2</th>
<th>³Ratio 3</th>
<th>⁴Ratio 4</th>
<th>⁵Ratio 5</th>
<th>⁶Ratio 6</th>
<th>⁶Ratio 7</th>
<th>⁶Ratio 8</th>
<th>% HG</th>
<th>% RG-I</th>
<th>HG:RG-I</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Linearity of pectin</td>
<td>Contribution of RG</td>
<td>Branching of RG</td>
<td>Co-extractants</td>
<td>Severity of extraction</td>
<td>GalA−Rha</td>
<td>2Rha−Ara+Gal</td>
<td>Glc−GalA</td>
<td>% RG-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.4</td>
<td>0.21</td>
<td>10.6</td>
<td>6.3</td>
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<td>19</td>
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<td>8.8</td>
<td>5.3</td>
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<td>2.0</td>
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<td>4.0</td>
<td>6.9</td>
<td>31.0</td>
<td>40.0</td>
<td>10.0</td>
<td>21</td>
<td>61</td>
<td>0.3</td>
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<tr>
<td>F</td>
<td>0.7</td>
<td>0.14</td>
<td>9.0</td>
<td>5.3</td>
<td>2.5</td>
<td>1.9</td>
<td>1.4</td>
<td>0.3</td>
<td>24</td>
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<td>3.4</td>
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<td>0.7</td>
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<td>7.3</td>
<td>4.5</td>
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<td>37</td>
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</tr>
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<td>1.8</td>
<td>1.3</td>
<td>0.3</td>
<td>24</td>
<td>45</td>
<td>0.5</td>
</tr>
</tbody>
</table>

¹A larger value is indicative of more linear/less branched pectins
²A smaller value is indicative of more linear/less branched pectins
³A larger value is indicative of larger average size of the branching side chains
⁴A larger value is indicative of larger average size of the branching side chains excluding arabinose
⁵A larger value is indicative of a “more pure” pectin extract
⁶A larger value is indicative of more severe extraction conditions and loss of arabinofuranoside (Araf) residues
Legends to Figures

**Figure 1.** Schematic structure for pectin: galacturonic acid (●); galactose (●); arabinose (▼); rhamnose (▼). Adapted from Perez, Rodriguez-Caravajal and Doco (2003) and Morris, Garcia de la Torre, Ortega, Castile, Smith and Harding (2008).
Figure 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.
Figure 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.
Figure 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.
Figure 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.
Figure 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.