Subcritical water extraction of low methoxyl pectin from pomelo (Citrus grandis (L.) Osbeck) peels

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A B S T R A C T

Low methoxyl (LM) pectin was extracted from pomelo peels using subcritical water in a dynamic mode. The effects of pressure and temperature were analyzed through a face-centred central composite design. Extraction yield and the rate of extraction were found to be predominantly influenced by temperature. Optimization of the subcritical water extraction (SWE) yielded an optimized operating condition of 120 °C and 30 bar with a predicted pectin yield of 18.8%. The corresponding experimental yield was 19.6%, which is in close agreement with the predicted data. The pectin obtained from the optimized condition was further analyzed for its physicochemical properties. The kinetics of the SWE was also evaluated whereby the one-site kinetic desorption model was found to be in good agreement with experimental data (R² > 0.94).

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1. Introduction

Pectin can be derived from fruit peels. It is an acidic hydrocolloid that is widely used as ingredients and excipients in food, pharmaceutical and packaging products. The primary structural feature of pectin is a linear chain of poly-α-(1→4)-D-galacturonic acid with varying degrees of esterification (DE). Conventional methods of extracting pectin generally require the use of harsh acid that commensurate with long extraction time. The recent techniques employed, such as ultrasonic and microwave, also require the use of acids. Acid is needed for the hydrolysis of protopectin; the parent substance from which pectin is derived. Acid is corrosive and hazardous; requiring equipment that are resistant to corrosion and necessitating further treatment to the liquid waste generated from the acid extraction process [1,2]. Therefore, the use of acid tends to increase production costs along with increasing wastewater treatment and maintenance costs. Hence, an approach to extracting pectin without acid can potentially reduce the cost of extraction [3].

The present paper looks into the use of subcritical water as an alternative medium for extracting pectin; the chief reason being that the pH of water drops as temperature increases [4,5] and thus, circumventing the need for acid solvents. Moreover, the viability of subcritical water as a medium for hydrolysis has been demonstrated by a number of authors [6–9]. The use of subcritical water has other added advantages including an increase in the quality and purity of the extract [10]. In addition, water is non-toxic, inexpensive, readily available and easily disposed [11]. A number of investigators had also successfully extracted various value-added products using subcritical water technology and their work can be found in the following references [12–18].

In the present paper, pectin is extracted from pomelo peels using subcritical water in a dynamic mode. An optimization study of process parameters, based on temperature and pressure, was also conducted for a maximum yield of the extracted pectin. The physicochemical properties of pectin and the kinetics of the extraction process were also studied.

2. Subcritical water extraction of pectin from pomelo peels

2.1. Apparatus and experimental method

A dynamic extractive method was utilized to extract pectin from pomelo (Citrus grandis (L.) Osbeck). A schematic diagram of the subcritical water extraction apparatus is shown in Fig. 1. Pomelo fruit at the same stage of ripeness (green-yellow skin, February harvest season) was obtained from Go Chin Pomelo Nature Park, Perak, Malaysia. The peels of the fruit were cut and dried in a hot air oven (600, Memmert) at 60 °C until a constant weight was attained. The peel was ground and sieved into a 250–500 μm mesh. The pomelo peel powder was stored in an air tight container and kept dry. Sodium nitrate, sodium azide, hydrochloric acid, isopropyl alcohol, ethanol of 95 v/v, and ethanol of 70% v/v used in the present study were purchased from R&M, Malaysia. α-Galacturonic acid (D-GaLA, >98%) and dextran (–95%) were purchased from Sigma-Aldrich, Germany. Ultrapure water (611UF, Sartorius) used in the extraction was degassed prior to use.

Five grams (5 g) of dried pomelo peel powder was placed together with 4 ± 1 mm glass beads (R&M, Selangor, Malaysia) in a 50 mL
of the dynamic extraction. The pressure in the system was maintained by the syringe pump and measured with a pressure transducer (PDCR 4030, Druck) coupled with a digital pressure indicator (DPI 280, Druck). After equilibration, extraction commenced. All valves were opened and the flow rate of the solution was maintained at 1 ml/min with the aid of a needle valve (NV). When the extract solution exited the oven, it was allowed to cool by passing the solution through a coil immersed in an ice bath. The extracted solution was collected in 20-min fractions. Fifty milliliter (50 mL) of 95% (v/v) ethanol was added into each fraction of the extract collected to precipitate out pectin. The precipitates would then float onto the surface of the solution. Extraction stopped once precipitation of pectin was no longer observed. The samples were then stored in the dark at room temperature for 24 h. The pectin in the sample was subsequently separated by filtration and washed in duplicates using 70% (v/v) ethanol to remove low molecular weight sugars, amino acids, organic acids and inorganic salts [13]. The resulting wet pectin was dried in a hot air oven (Memmert 600, Schwabach) at 65 °C until a constant weight was attained.

### 2.2. Design of experiment

The general mechanism of an extraction process involves a solvent diffusing into the matrix of a substrate followed by the extract dissolving into the solvent before being carried out of the substrate together with the solvent. Both the diffusivity and solubility components are affected by pressure and temperature. Hence, an examination of the effect of temperature and pressure on subcritical water extraction is necessary. Generally, subcritical water extraction temperatures may extend beyond 200 °C [10] as long as the liquid state is maintained. The extraction temperature selected for the present study is ranged between 90 and 120 °C. Extraction of pectin was not conducted beyond 120 °C as pectin floatation was not observed at 130 °C and above. The experimental pressure was ranged between 30 and 100 bar.

A response surface methodology (RSM) was used to design the experiment and to determine an adequate model for SWE of pectin. A two-factor, three-level (-1, 0, 1) face-centred central composite design was chosen to investigate and optimize the effect of process variables on pectin yield. The variables considered were extraction pressure (P) and temperature (T). A total of 13 sets of test conditions including 5 replicates for the centre point were conducted for theoretical modeling. The experimental data were fitted to a second-order polynomial equation to establish the relationship between independent variables and responses. The generalized form of the equation is:

\[
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2
\]  

(1)

where \(Y\) represents the response variable while \(X_1\) and \(X_2\) are the uncoded values of the independent variables of extraction pressure (bar) and temperature (°C), respectively. \(\beta_0\), \(\beta_1\), \(\beta_2\), \(\beta_{11}\), \(\beta_{22}\), and \(\beta_{12}\) are constant coefficients where \(\beta_0\) is a constant, \(\beta_1\) and \(\beta_2\) are coefficients for linear terms, \(\beta_{11}\) and \(\beta_{22}\) are coefficients for quadratic terms, and \(\beta_{12}\) is the coefficient for interaction terms. The effects of process variables were analyzed statistically using an analysis of variance (ANOVA). The adequacy of the model equation for predicting the optimum response values was validated with experimental results.

### 2.3. Physicochemical analyses

#### 2.3.1. Pectin yield

Pectin yield was calculated as a ratio of the dry weight of pectin to the dry weight of pomelo powder. The yield of pectin extracted was calculated based on Eq. (2).

\[
\text{Pectin yield (％)} = \frac{m_w}{m_i} \times 100
\]  

(2)
where \( m_0 \) is the weight of dried pectin (g) and \( m \) is the weight of dried pomelo peel powder (g).

2.3.2. Degree of esterification

The method to determine the Degree of Esterification (DE) of pectin is briefly described here. A detailed description of the method is reported in Liew, Ngoh, Yusoff and Teoh [19]. The FT-IR spectra of the samples were recorded with a Bruker Tensor 27 FTIR spectrometer from 600 to 4000 cm\(^{-1}\) with 32 scans. The measuring resolution was 2 cm\(^{-1}\). DE is the ratio of the esterified carboxyl group to the number of total carboxyl groups present. DE was calculated based on the absorbance intensities at 1630 cm\(^{-1}\) and 1745 cm\(^{-1}\) that corresponded with the non-methyl-esterified carboxyl groups and the methyl-esterified carboxyl groups. The sum of the bands absorbance intensities at 1630 cm\(^{-1}\) and 1745 cm\(^{-1}\) corresponding to the total carboxyl groups was measured by an OPUS software. The degree of esterification is calculated based on the following Eq. (3) [20].

\[
DE(\%) = \frac{A_{1745}}{A_{1745} + A_{1630}} \times 100
\]

where \( A_{1745} \) and \( A_{1630} \) are the intensity of non-methyl-esterified carboxyl group and the intensity of methyl-esterified carboxyl group, respectively.

2.3.3. Proximate analyses

The moisture, ash, protein and fat of pectin were verified through the approved AOAC methods (1995). The pH of pectin was determined with a pH meter (827 pH lab, Metrohm) by mixing 0.5 g of sample with 50 mL of distilled water. Galacturonic acid (GlaA) content was ascertained through a colorimetric method [21,22], with D-galacturonic acid being used as a standard.

2.3.4. Molecular weight determination

The molecular weight of the extracted pectin was determined using a high performance size exclusion chromatography (HPSEC) coupled with a Wyatt Technology Dawn Helios multiangle laser light-scattering (MALLS) detector and an OptilabreX differential refractometer. The HPSEC-MALLS conditions and mobile phase composition were as described by Muhammad, Mohd. Zahari, Gannasin, Mohd. Adzahan and Bakar [23]. Mono dispersed dextrans were used as the standard and Astra software was used to collect the data.

2.3.5. Pectin solubility

The solubility of the extracted pectin in water was measured by mixing 0.5 g of sample in 50 mL of distilled water. The mixture was then stirred and incubated at 40 °C for 30 min. Subsequently, the solution was centrifuged at 25 °C and 4200 rpm for 20 min. The supernatant was transferred to a beaker and allowed to evaporate in a water bath at 90 °C, followed by drying in an oven at 105 °C until a constant weight was attained [24]. The solubility of the extracted pectin was calculated based on Eq. (4)

\[
\text{Solubility(\%)} = \frac{m_1}{m_2} \times 100
\]

where \( m_1 \) and \( m_2 \) are the constant weight of the dry supernatant (g) and the weight of the extracted pectin sample (g), respectively.

2.3.6. Colorimetry

The CIELab coordinates \((L^*, a^*, b^*)\) of the pectin samples were directly read with a colorimeter (WF30, iWAVE). The \( L^* \) value represents the lightness of color, ranging from 0 (black) to +100 (white) whereas the \( a^* \) value ranged from \(-100\) (green) to +100 (red) and the \( b^* \) value ranged from \(-100\) (blue) to +100 (yellow). The hue angle \((H_h^a_b)\) and chroma \((C^*)\) were calculated according to the following equations:

\[
H_h^a_b = \arctan \left( \frac{b^*}{a^*} \right)
\]

\[
C^* = \left( a^{2*} + b^{2*} \right)^{1/2}
\]

3. Results and discussion

3.1. Process optimization

The yields of the extracted pectin at various temperatures and pressures are presented in Table 1. An analysis of variance (ANOVA) was employed to determine the significant level of the main parameters and the interaction effects between the parameters. The ANOVA results are listed in Table 2. A low p-value (0.0003) was obtained for the regression model; demonstrating that the developed model is significant and well fitted. The lack of fit for the quadratic model with a p-value at

![Fig. 1. Schematic diagram of the dynamic subcritical water extraction system.](image-url)
The high value of \( R^2 \) (0.94) and adj-\( R^2 \) (0.90) further signify that the response and independent variables were well correlated. Based on the quadratic model, an optimum pectin yield of 18.8% and DE of 40.5% was predicted at an extraction temperature of 120 °C and extraction pressure of 30 bar. Validation of the predicted optimum extraction condition was conducted in triplicate with an average pectin yield of 19.6 ± 0.9% and a DE value of 40.1 ± 2.1%; the results further establishing that the quadratic model was well-fitted to the experimental data. The difference between the predicted optimum yield and the validated experimental value is 0.8%, which is well within the relative standard deviation (RSD) of the centre point (RSD centre point = 7.3%). The relationship between the responses and the experimental variables are illustrated graphically in a three-dimensional response surface plot shown in Fig. 2.

The results obtained from ANOVA showed that the effect of temperature on pectin yield is significant \((p < 0.0001)\). The linear and quadratic terms of pressure were found to be insignificant within the range considered in the present study. The quadratic term of temperature and the interaction between temperature and pressure were also found to be insignificant. The obtained quadratic model can be reduced to a linear equation by taking out all insignificant terms \((p > 0.05)\), giving Eq. (8).

\[
Pectin \ yield \ (\%) = 10.63 + 0.27X_1 + 7.00X_2 + 0.55X_1^2 + 0.59X_2^2 - 0.28X_{12} \tag{7}\]

A second order polynomial equation was first suggested to determine the optimum conditions that maximize the extraction yield of pectin. The quadratic model is given by Eq. (7).

**3.1.1. The temperature effect on extraction yield**

Extraction temperature is an important factor as it affects the physicochemical properties of water such as viscosity, surface tension and dielectric constant [26]. Moreover, temperature also affects the sublimation pressures of solutes, thereby affecting the solubility of a solute in a solvent [11] and its subsequent extractability. As water temperature increases, the strength of the hydrogen bonds in water reduces. The weakened hydrogen bond leads to a decrease in the dielectric constant value followed by a reduction in water polarity; subsequently, reducing the energy needed for division in solute-matrix interactions and increasing extraction efficiency [27]. Hence, an increase in temperature generally commensurate with an increase in extraction yields. The results of the present study (Table 1) show that yields obtained at 120 °C were at least, three times higher than the yields at 90 °C.

The maximum temperature used in the present study is 120 °C as pectin floatation was not observed at 130 °C. A further examination at 150 °C also yielded similar result. One possible explanation for the observation is the degradation of pectin due to high temperature and/or prolonged exposure to subcritical conditions. The degradation could be indicated from the appearance of the extract, as the color of extracts turned dark brown at 130 °C and 150 °C. The dark brown solution can be contrasted to a typical observed extract solution which is light brown.

### Table 1

<table>
<thead>
<tr>
<th>Run</th>
<th>Temperature (°C)</th>
<th>Pressure (bar)</th>
<th>Pectin yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>105</td>
<td>100</td>
<td>13.1</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>65</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>105</td>
<td>65</td>
<td>11.4</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>30</td>
<td>4.3</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>30</td>
<td>20.4</td>
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<tr>
<td>6</td>
<td>105</td>
<td>65</td>
<td>10.9</td>
</tr>
<tr>
<td>7</td>
<td>105</td>
<td>30</td>
<td>9.2</td>
</tr>
<tr>
<td>8</td>
<td>105</td>
<td>65</td>
<td>9.7</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>100</td>
<td>3.7</td>
</tr>
<tr>
<td>10</td>
<td>120</td>
<td>65</td>
<td>16.6</td>
</tr>
<tr>
<td>11</td>
<td>105</td>
<td>65</td>
<td>10.3</td>
</tr>
<tr>
<td>12</td>
<td>105</td>
<td>65</td>
<td>10.8</td>
</tr>
<tr>
<td>13</td>
<td>120</td>
<td>100</td>
<td>18.7</td>
</tr>
</tbody>
</table>

---

**Fig. 2.** A response surface plot showing the effect of process variables on pectin yield.

---

**Table 2**

ANOVA for the regression model of pectin yield.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>297.95</td>
<td>5</td>
<td>59.59</td>
<td>23.40</td>
<td>0.0003</td>
</tr>
<tr>
<td>(X_1)-pressure</td>
<td>0.42</td>
<td>1</td>
<td>0.42</td>
<td>0.17</td>
<td>0.6960</td>
</tr>
<tr>
<td>(X_2)-temperature</td>
<td>294.28</td>
<td>1</td>
<td>294.28</td>
<td>115.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(X_1^2)</td>
<td>0.83</td>
<td>1</td>
<td>0.83</td>
<td>0.33</td>
<td>0.5844</td>
</tr>
<tr>
<td>(X_2^2)</td>
<td>0.98</td>
<td>1</td>
<td>0.98</td>
<td>0.38</td>
<td>0.5547</td>
</tr>
<tr>
<td>(X_{12})</td>
<td>0.32</td>
<td>1</td>
<td>0.32</td>
<td>0.13</td>
<td>0.7334</td>
</tr>
<tr>
<td>Residual</td>
<td>17.78</td>
<td>7</td>
<td>2.54</td>
<td>13.54</td>
<td>0.0146</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>16.19</td>
<td>3</td>
<td>5.40</td>
<td>13.54</td>
<td>0.0146</td>
</tr>
<tr>
<td>Pure error</td>
<td>1.59</td>
<td>4</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor total</td>
<td>315.73</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R^2)</td>
<td>0.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adj(R^2)</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Additionally, at an extraction temperature of 150 °C, a burning odor was noticed in the extract solution. For the pectin extracts obtained at, and below 120 °C, the odor was found to be pleasant and recognizable while at 130 °C, the odor from the solution was rather unpleasant. Similar observations (in terms of solution color and odor) were made in a number of studies [18,28]. Moreover, a number of authors have found that an increase in temperature to 120 °C increased SWE yield [12,13]. However, as they continued to increase the extraction temperature, a decline in extraction yields was observed; also pointing to a possible degradation in their materials above 120 °C.

3.1.2. The pressure effect on extraction yield

The insignificant influence of pressure on SWE yield was found to be in agreement with various authors [18,29]. For each level of temperature investigated in the present study, a distinct trend was not observed between pressure changes and yield. Hence, in optimizing the SWE of pectin, it is desirable that the extraction pressure is kept low provided that an increase in temperature to 120 °C increased SWE yield [12,13]. Moreover, a number of authors have found that an increase in temperature to 120 °C increased SWE yield [12,13]. However, as they continued to increase the extraction temperature, a decline in extraction yields was observed; also pointing to a possible degradation in their materials above 120 °C.

3.2. The effects of subcritical water extraction conditions on DE values

The values of DE obtained in the present study ranged between 29.7% and 45.5%, shown in Table 3. All DE values obtained were found to be lower than 50%; indicating that the extracted pectin is a type of LM pectin. LM pectin is often used to produce low-sugar or sugar-free jellies.

It is of interest to note that the present dynamic subcritical water extraction of pomelo produces LM pectin whereas conventional heating and sequential ultrasound-microwave assisted acid extraction (UMAE) methods were found to produce high methoxyl (HM) pectin [30,31]. One possible explanation for the marked difference is the absence of acid in the present work. For the conventional heating, ultrasound and microwave methods, the use of acid was essential to the extraction process. When acid was added during the extraction process, non-methyl-esterified carboxyl groups in pectin were converted to methyl-esterified carboxyl groups; thus, increasing DE value and, thereby, producing HM pectin. The HM pectin produced is then converted to LM pectin through the de-esterification of HM pectin using acids, alkali and enzyme [32,33].

In order to test the hypothesis that a lack of acid hindered the production of HM pectin, the precipitates extracted from dynamic SWE at 30 bar and 120 °C, were washed with two different solutions; one with ethanol and the other with 5% v/v hydrochloric acid (HCl) in 60% isopropyl alcohol (IPA). The DE of pectin was found to increase from 40.1% (LM grade) with ethanol washing to 61.1% (HM grade) with acid washing. The corresponding FTIR analyses for both washing conditions are shown in Fig. 3. When acid washing was applied, the corresponding FTIR spectrum showed an increase in the intensity of the peak at around 1745 cm⁻¹ and a decrease in the intensity of the peak at around 1630 cm⁻¹, relative to the FTIR spectrum with ethanol washing. The DE value is generally reflected in proportion to the 1745 cm⁻¹ peak. Hence, the FTIR spectra lead to an inference that the washing of the precipitated pectin extract with acid increases the DE value. In addition, the outcome of the present dynamic SWE with acid washing can be juxtaposed with the batch SWE conducted by Wang, Chen and Liu [12]. Pectin was extracted from citrus peel by the authors in a 5-min batch SWE. The authors subsequently washed the precipitates with HCl in IPA; resulting in HM pectin being produced. Hence, it can be concluded that the use of acid can aid in the production of HM pectin and a lack thereof, lowers the DE values.

Another possible explanation for the ensuing production of the LM pectin under dynamic SWE is a prolonged exposure to pressure. In a high hydrostatic pressure assisted enzymatic treatment of HM pectin to LM pectin, Zhao, Guo, Pang, Gao, Liao and Wu [32] found that the value of DE decreased as they increased the pressure holding time in the de-esterification process. In the present study, the total extraction time for one SWE generally took up to 140 min for completion. Hence, a prolonged exposure to pressure may aid in the conversion of pectin to the LM type. Therefore, the production of LM pectin in the present study, as oppose to other studies, can be due to a direct influence of prolonged exposure to high pressure and the absence of acid in the process.

Table 4

| Physicochemical composition of the pomelo pectin extracted at 120 °C and 30 bar. |
|---------------------------------|-----------------|
| Yield (%)<sup>a,b</sup>         | 19.6 ± 0.9      |
| Degree of esterification (%)<sup>a,b</sup> | 40.1 ± 2.1      |
| Galacturonic acid (%)<sup>a,b</sup> | 76.62 ± 2.98    |
| Moisture (%)<sup>a,b</sup>      | 16.30 ± 0.34    |
| Ash (%)<sup>a,b</sup>           | 2.96 ± 0.11     |
| Protein (%)<sup>b</sup>         | 4.51            |
| Fat (%)<sup>b</sup>             | n/d             |
| pH<sup>b</sup>                  | 4.72 ± 0.02     |
| Molecular weight, Mw (Da)<sup>b</sup> | 6.26 × 10⁴     |
| Solubility (%)<sup>a,b</sup>    | 79.80 ± 0.50    |
| CieLab coordinates<sup>ab</sup> |                |
| L*                              | 12.56 ± 0.33    |
| a*                              | 2.37 ± 0.07     |
| b*                              | 14.36 ± 0.33    |
| H<sub>ab</sub>                  | 80.62 ± 0.47    |
| C<sub>ab</sub>                  | 14.56 ± 0.31    |

<sup>a</sup> Data are expressed as means ± standard deviations of triplicate.
<sup>b</sup> Dried pectin.
<sup>ab</sup> Wet pectin.

Fig. 3. FT-IR spectra of pomelo pectin using different washing solutions.
3.3. Physicochemical composition of the extracted pectin

In the present work, analyses were carried out on the physicochemical composition of the extracted pectin. The analyses were conducted on pectin obtained from the optimized extraction conditions described in Section 3.1. The extracted pectin was analyzed based on the following characteristics: yield, degree of esterification, molecular weight, solubility, colorimetry and proximate analyses. The characterization results are shown in Table 4.

The GalA content was found to be 76.62%, indicating that the SWE pectin is of good quality and within the acceptable limit (GalA ≥ 65%) stipulated by the US Food and Agriculture Organization (FAO). The pathways of synthesis, and the extraction conditions used to extract pectin, can affect its molecular weight; thereby, affecting its gelling and rheological properties [34]. The molecular weight of the subcritical water extracted pomelo pectin (6.26 × 10^4 Da) was found to be slightly lower than that of the conventional extraction technique (9.05 × 10^4 Da) and ultrasound-microwave-assisted acid extraction technique (9.30 × 10^4 Da) obtained through the methods described in reference [35]. It is also lower than those conventionally extracted from mango (2.45 × 10^3 Da) and lime (1.23 × 10^3 Da) [36] but higher than that from Akebia trifoliata var. australis peel (4.54 × 10^4 Da) and sunflower pectin (4.80 × 10^4 Da) [37]. The pectin extracted from SWE is of light brown color with the lightness, L* higher than the pectin extracted using conventional acid extraction. Lighter colored pectin is generally preferable for industry use. One possible reason for the lighter color in SWE pectin is a shorter exposure to the heat treatment compared to a conventional heating extraction set-up.

The yield and DE of pomelo pectin obtained through various extraction methods are shown in Table 5. While the yield of pectin obtained via SWE is comparatively lower, the pectin obtained is of the LM-type rather than the HM-type obtained through conventional heating and UMAE. The direct production of LM pectin using subcritical water is advantageous as it eliminates the need to de-esterify HM to LM pectin. Hence, the dynamic SWE of pectin removes the need for a de-esterification stage and eliminates the added costs for chemicals or enzymes typically required in the de-esterification stage [32].

4. Kinetic modeling

One-site and two-site kinetic models were applied in the present kinetic study. The one-site kinetic desorption model proposed by Kubátová, Jansen, Vaudoisot and Hawthorne [38] and Hawthorne, Poppendieck, Grabanski and Loehr [39], describes an intra-particle diffusion-controlled extraction. The intra-particle diffusion-controlled extraction can only be used as an extraction model on solute concentration that is below its thermodynamic limit. A further assumption of the one-site model is that the solute is uniformly distributed within the matrix. The one-site kinetic desorption model is given by Eq. (9)

\[
\frac{C}{C_0} = 1 - e^{-kt}
\]

where \(C\) (g/g) is the mass of analyte removed after time, \(t\) (min), \(C_0\) (g/g) is the initial mass and \(k\) (min^-1) is the desorption rate constant. The limitation of the one-site kinetic desorption model is that it only considers the overall rate of desorption of solute from the matrix of the source material.

In the present study, Microsoft EXCEL solver was used to fit the experimental data with the kinetic models to obtain the values of \(k\). The kinetic models were evaluated based on the cumulative yields of pectin in five fraction-periods and are shown in Table 6. Each data is expressed as the mean of three replicates. The experimental extraction yield and the corresponding kinetic curves for the extraction of pectin using SWE are presented in Fig. 4 for various extraction conditions. The rate of extraction was found to be higher for the first two fractions, accounting for the first 40 min of the extraction. Consequently, the extraction rate for each subsequent fraction continued to decrease. In general, it can be observed that at all extraction conditions, the first 80% of the extraction yields collected were obtained within the first four fractions.

The theoretical model curves calculated based on Eq. (9) are illustrated in Fig. 5 together with the experimental data. The desorption rate constants (\(k\)) and the \(R^2\) values obtained using the one-site kinetic model are summarized in Table 7. During data fitting, the value of \(C_0\) was set equal to the maximum achievable extraction yield (\(C_0 = 0.2044\) g/g) as the actual \(C_0\) value is unknown [14]. In fact, the desorption rate constants (\(k\)) are dependent on \(C_0\). Therefore, \(k\) value is compared only relative to each of the data. It is worth noting that the trend of \(k\) remains similar with varying \(C_0\), which does not affect subsequent discussion. Based on Table 7, it can be observed that the desorption coefficients (\(k\)) for the one-site kinetic model are dependent on the extraction temperature. An increase in the extraction temperature causes an increase in the desorption rate constant (\(k\)). The observation is consistent with the findings of Islam, Jo, Jung and Park [40] and Langenfeld, Hawthorne, Miller and Pawliszyn [41].

In addition to the one-site kinetic model, the present authors also tried to fit the experimental data into the two-site kinetic desorption model. The two-site model considers a “fast” and a “slow” extraction period relevant to two different solute fractions [14,42]. However, the optimization result leads back to the one-site model, implying that the
“fast” and “slow” kinetics are not distinct in the present work and that the one site kinetic model is more suitable. By considering the relative errors ($R^2 > 0.94$), the one-site kinetic desorption model showed good agreement between model prediction and experimental data.

5. Conclusion

LM pectin was extracted from pomelo peels using a dynamic SWE method. The optimum extraction condition was determined to be at a pressure of 30 bar and 120 °C with a pectin yield of 19.6%. Temperature plays a significant role in SWE. The absence of acid and a prolonged exposure to pressure under dynamic SWE conditions facilitate the formation of LM pectin rather than HM pectin. The production of LM pectin from pomelo peels can now be obtained in a one-step extraction process rather than the conventional two-step extraction—de-esterification process. The experimental data and the one-site kinetic desorption model were found to be in good agreement.

Table 7
The desorption rate constants and the $R^2$ values obtained using Eq. (9) for pectin extracted under subcritical water conditions.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>30 bar</th>
<th>65 bar</th>
<th>100 bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>k (min$^{-1}$)</td>
<td>$R^2$</td>
<td>k (min$^{-1}$)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>90</td>
<td>0.002487</td>
<td>0.9519</td>
<td>0.003435</td>
</tr>
<tr>
<td>105</td>
<td>0.005399</td>
<td>0.9749</td>
<td>0.005778</td>
</tr>
<tr>
<td>120</td>
<td>0.023123</td>
<td>0.9406</td>
<td>0.014929</td>
</tr>
</tbody>
</table>

Conflict of interest
The authors declare no conflict of interest.

Acknowledgements
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Fig. 4. Cumulative pectin yield versus extraction time based on the first five fractions, shown together with the standard deviations, at various temperatures and pressures.

Fig. 5. Theoretical curves calculated using Eq. (9) for the SWE of pectin at various temperatures and pressures.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2018.05.013.

References


