Optimization of Pectin Extraction from Passion Fruit Peel using Response Surface Methodology

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Abstract

Pectin from passion fruit peels was extracted using citric acid extraction. The effects of citric acid concentration from 0.05 to 0.21 % (wt/wt), extraction time from 30 to 120 minutes and extraction temperatures from 35 to 85 °C on pectin yield and the degree of esterification (DE) were investigated. Using the central composite design (CCD), the optimized pectin yield was 6.44 % and degree of esterification was 75.72 % at optimum conditions of citric acid concentration 0.13 % (wt/wt) for 102 minutes of extraction time at 75 °C.

Keywords
Pectin, Citric Acid, Fruit Peel, Response Surface Methodology

1. Introduction

Pectin, a carbohydrate polymer contained in the primary cell walls of terrestrial plants is a widely used functional food ingredient as gelling agent and stabilizer. Pectin is used for the production of jams, fruit jellies, bakery fillings, and for various productions in the pharmaceutical and biotechnology industries. Pectin is generally extracted from fruit peels. The main raw-materials for pectin production are dried citrus peel [1] or apple pomace [2]. Other sources of pectin include the sugar beet [3], dragon fruit [4], sunflower head residue [5], cocoa husk [6], soy hull [7], mango peels [8] and banana peels [9].

Passion fruit is round to oval shape, either yellow or dark purple at maturity, with a soft to firm, juicy interior filled with numerous seeds. The fruit is eaten or juiced. The passion fruit juice is often added to other fruit juices to enhance the aroma. Passion fruits are easily found in Southeast Asia especially Malaysia. The disposal of its fruit peel has become a burden to the industry as well to the environment. Hence, pectin extraction from fruit peels might provide an alternative way to reduce expenses for waste disposal.

Researches on extraction methods, extraction agent and characterization of fruit pectin have been reported elsewhere but extraction by using citric acid is not much reported so far [10], [11], [12]. Citric acid was used for pectin extraction in this study because it is an organic based acid which warrants safety for the environment and food processing. In previous work by Erika Kliemann et al. [13] and Eloísa Rovaris Pinheiro et al. [14], they have extracted pectin from yellow passion fruit. Erika Kliemann et al. who have used hydrochloric and nitric acids stated that the citric acid gave the optimal pectin yield extraction from passion fruit. In Eloísa Rovaris Pinheiro’s work, although citric acid concentration and extraction time were studied, the extraction temperature, an important aspect of the extraction process was not studied. Extraction temperature may be affecting the type of pectin extracted, its yield and the degree of esterification.

The objective of this research was to improve pectin extraction techniques from passion fruit skin, to chemically characterize the pectin obtained in terms of low methoxyl pectin (LM) or high methoxyl pectin (HM) and to find the
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optimized conditions for pectin extraction using different citric acid concentration at different extraction time and temperature.

2. Materials and methods

2.1 Materials

Yellow passion fruit (Passiflora edulis var. flavicarpa) at same ripening stage and with similar peel color were selected from a same harvest from the Multi-Rich Pitaya Orchard, Selangor (Malaysia). All the chemical reagents used were analytical grade supplied by Systerm Sdn Bhd, Selangor (Malaysia).

2.2 Sample preparation

The fruit was washed twice with distilled water and the flesh was separated from the fruit peel. The fruit peel was removed. The peels were dried in a Memmert Universal Oven (UNB 100, Memmert, Germany) at 55 °C until a constant weight was achieved. The dried peels were then milled into powder of about 500 µm mesh size using an electronic miller (DFT-150, Dickson, China). The powder particle size was measured using a particle size analyzer (Mastersizer 2000, Malvern, United Kingdom). The fruit peel flour was packaged in a polyethylene bag and stored at -15 °C in freezer (ACF15F, Acsion, Malaysia) until extraction exercise.

2.3 Citric acid extraction method

10 g of fruit peel powder was blended with 250 mL distilled water using a stirrer. It was then mixed with different volumes of 0.1 N citric acid, 20, 65, and 110 mL which the concentration citric acid in the mixture was 0.05, 0.14, and 0.21 % (wt/wt) respectively. The mixture was then stirred until all the fruit peel powder was evenly wetted by the distilled water in homogenous form. The pectin extraction procedure continued with treating the samples for a different period of time (30, 75, 120 min) at different extraction temperature (35, 60, 85 °C) in a shaking water bath (Lab Companion 37L, Jeio Tech, Korea). The mixture solution was kept at room temperature for 24 hours. Precipitated pectin was recovered by centrifuging (Mikro 22R, Hettich, Germany) at 6000 rpm for 10 minutes. Water bath heat treated samples were then filtered and 95 % ethanol (solute:solvent=1:25) was added in to allow pectin precipitation. The samples stored in dark condition at 25 °C for 24 hours in order to allow pectin flotation. The floating pectin was separated by filtration and subsequently washed twice with 70 % ethanol. Acetone was then added in drop wise manner to remove unwanted color of pectin. Acetone was added until the top liquid phase was completely clear. The resulted pectin substance was dried in a convection oven at 65 °C until a constant weight. The percentage yield of the dried passion fruit pectin was determined as gram of product obtained per 10 g of fruit peel powder used in each citric acid extraction, see Equation (1) [14].

\[
Yield (\%) = \frac{\text{Product obtained (g)}}{10 (g) \text{ Fruit peel powder}} \times 100 \%
\]  

(1)

2.4 Potentiometric titration method

Pectin can have different levels of esterification. The degree of esterification is defined as the ratio of esterified galacturonic acid groups to the galacturonic acid groups present [15]. The degree of esterification (DE) is an important property which determines the gelling nature of pectin. The DE values for commercial HM pectin range from 60-75 % and LM pectin range from 20-40 %. The DE of fruit pectin was determined using the potentiometric titration method by Bochek et al. [16]. Dried pectin (0.2 g) was placed in a conical flask for titration and wetted with ethanol. Distilled water (20 mL) was mix with pectin. Then the mixture was shaking automatically by water bath at 45 °C until the pectin dissolved completely. The resulted solution was titrated with 0.1 N NaOH and a few drops of phenolphthalein. The titration volume was recorded as the initial titrate (It). Then, another 10 mL of 0.1 NaOH solutions was added to neutralize polygalacturonic acid sample after determination of the free carboxy groups. The conical flask was plugged with a stopper and the mixture was stirred at room temperature for 2 hours to de-esterify the pectin. Then, 0.1 N HCl (10 mL) was added to neutralize NaOH. The mixture was further titrated with 0.1 N NaOH in the present of phenolphthalein. The number of the esterified carboxy groups was calculated from the
volume of 0.1 N NaOH solution spent for titration. The DE was calculated using the equation below, see Equation (2):

\[ DE \% = \left( \frac{F_I}{I_t + F_t} \right) \times 100 \% \]  

(2)

Where,

DE = degree of esterification, %

\( F_t \) = final titration volume, mL

\( I_t \) = initial titration volume, mL

3. Results and Discussion

3.1 Effect of citric acid concentration, extraction time and extraction temperature on pectin yield and degree of esterification

Table 1 shows the result percentage of pectin yield and degree of esterification for 20 different treatments designed using central composite design. The yield of pectin and degree of esterification of pectin extracted in citric acid method was typically 3.76-7.71 % and 33.33-78.57 %, respectively. A high citric acid concentration, longer extraction time and moderate extraction temperature contributed to a larger percentage of pectin yield while a lower citric acid concentration, longer extraction time and higher extraction temperature within the levels studied contributed to a higher percentage of the degree of esterification.

The \( p \) values for each response are summarized in Table 2. It was found that among the three investigated factors, extraction temperature \((p<0.05)\) gave significant effect to pectin yield, while citric acid concentration and extraction time have less significant effect to pectin yield \((p>0.05)\). The degree of esterification was most significantly affected by citric acid concentration \((p<0.05)\) while extraction time and extraction temperature have less significant effect on degree of esterification \((p>0.5)\).

Citric acid concentration is particularly important for pectin yield. It was found that increasing in citric acid concentration will increase the pectin yield. As reported by Yapo \textit{et al.} [17], it is recognized that an increase in acid strength plays an important role in increasing the content of galacturonic acid. Pectin is a polymer of galacturonic acid [18]. As the content of galacturonic acid increases, pectin yield will increase as well. Extraction times also an important factor in pectin extraction. Yujaroen \textit{et al.} [19] stated that the longer the extraction time, the higher the percentage of pectin derived. Results show that 120 minutes of extraction time gave the highest pectin yield 7.71 %. The reason of this is because carbohydrate polymer needs certain temperature to soften its structure for extracting pectin and a longer extraction time allows more reaction opportunity. For temperature, there seems to be an optimal temperature for extraction at 60 °C. Too high or too low extraction temperatures gave low pectin yields. With high temperature, pectin will decompose. At too low temperature, the heat might not be enough to assist the dissolution of pectin.

Degree of esterification of more than 50 % can be considered as high methyl ester (HM) pectin. Therefore, pectin extracted were HM pectin. The result of DE in this study shows the passion fruit contains high methyl ester group which is similar to the result found by Erika Kliemann \textit{et al.} and Elo'isa Rovaris Pinheiro \textit{et al.} HM pectin is advantageous to make high sugar products like jelly and jam because its pectin molecule allows additional links of sugar molecule to form gel network [20]. In this experiment, highest degree of esterification was obtained by sample treated for 0.09 % (wt/wt) citric acid concentration, 102 minutes extraction time at 75 °C. From experiment results (Table 1), citric acid concentration at 0.09 % (wt/wt) was the optimum citric acid concentration for extraction of pectin with high percentage of degree of esterification (59.18- 78.57 %). Result also indicated that degree of esterification was closely related to the duration of heat treatment. When extraction time prolong, degree of esterification was found to increase at the same time. Therefore, it could be assumed that extraction time at 102 minutes gave high degree of esterification. It is interesting to note that citric acid concentration and extraction time were the most significant interactive effect on degree of esterification, which is similar to that reported by Woo \textit{et al.}
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[21], Happi Emaga et al. [22] and Wong et al. [23] who studied extraction of condition on pectin from dragon fruit peel, banana peel and durian rind. The degree of esterification was not much related to the extraction temperature. High degree of esterification was obtained at both high and low extraction temperature.

Table 1: Experimental design and response of pectin yield and degree of esterification based on citric acid mixture concentration, extraction time and extraction temperature

<table>
<thead>
<tr>
<th>Citric Acid Mixture Concentration (wt/wt)</th>
<th>Extraction Time (min)</th>
<th>Extraction Temperature (˚C)</th>
<th>Pectin Yield (%)</th>
<th>Degree of Esterification (%)</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>75</td>
<td>60</td>
<td>7.51±0.12</td>
<td>33.33</td>
<td>LM</td>
</tr>
<tr>
<td>0.09</td>
<td>48</td>
<td>45</td>
<td>5.91±0.11</td>
<td>78.26</td>
<td>HM</td>
</tr>
<tr>
<td>0.09</td>
<td>102</td>
<td>45</td>
<td>3.76±0.06</td>
<td>72.34</td>
<td>HM</td>
</tr>
<tr>
<td>0.09</td>
<td>102</td>
<td>75</td>
<td>6.62±0.06</td>
<td>78.57</td>
<td>HM</td>
</tr>
<tr>
<td>0.09</td>
<td>48</td>
<td>75</td>
<td>5.55±0.08</td>
<td>59.18</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>60</td>
<td>4.25±0.05</td>
<td>77.78</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>60</td>
<td>5.27±0.05</td>
<td>72.00</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>60</td>
<td>3.91±0.02</td>
<td>71.11</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>60</td>
<td>4.04±0.04</td>
<td>70.91</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>60</td>
<td>4.43±0.07</td>
<td>73.33</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>35</td>
<td>3.68±0.03</td>
<td>77.08</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>60</td>
<td>4.18±0.03</td>
<td>72.50</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>75</td>
<td>85</td>
<td>5.76±0.04</td>
<td>78.43</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>120</td>
<td>60</td>
<td>7.71±0.13</td>
<td>60.38</td>
<td>HM</td>
</tr>
<tr>
<td>0.14</td>
<td>30</td>
<td>60</td>
<td>4.02±0.06</td>
<td>68.75</td>
<td>HM</td>
</tr>
<tr>
<td>0.19</td>
<td>48</td>
<td>45</td>
<td>3.84±0.03</td>
<td>67.21</td>
<td>HM</td>
</tr>
<tr>
<td>0.19</td>
<td>48</td>
<td>75</td>
<td>6.12±0.11</td>
<td>78.38</td>
<td>HM</td>
</tr>
<tr>
<td>0.19</td>
<td>102</td>
<td>75</td>
<td>6.58±0.12</td>
<td>67.69</td>
<td>HM</td>
</tr>
<tr>
<td>0.19</td>
<td>102</td>
<td>45</td>
<td>5.05±0.12</td>
<td>62.50</td>
<td>HM</td>
</tr>
<tr>
<td>0.21</td>
<td>75</td>
<td>60</td>
<td>4.62±0.08</td>
<td>58.33</td>
<td>HM</td>
</tr>
</tbody>
</table>

Table 2: The associated probability (p value) for each response (pectin yield and DE) by acid method

<table>
<thead>
<tr>
<th>Factors</th>
<th>Pectin yield</th>
<th>Degree of esterification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid concentration</td>
<td>0.217*</td>
<td>0.352*</td>
</tr>
<tr>
<td>Extraction time</td>
<td>0.106*</td>
<td>0.607</td>
</tr>
<tr>
<td>Extraction temperature</td>
<td>0.025**</td>
<td>0.852</td>
</tr>
</tbody>
</table>

**p<0.05, *p<0.5, denoting different significant effect**
3.2 Response optimization

Central composite design in response surface methodology with three independent factors was used to determine the optimum condition for pectin extraction from passion fruit peel powder. The optimal extraction conditions obtained for two responses using the Design Expert 8 software was 0.13 % (w/w) of citric acid concentration, 102 minutes of extraction time and extraction temperature of 75 °C which give the highest predicted pectin yield 6.44 % and highest predicted degree of esterification 75.72 %.

Three surface plots (Fig. 1a-f) were generated from each response at hold values of 0.14 % (wt/wt) citric acid concentration, 75 minutes of extraction time, and 60 °C extraction temperature. Within the levels studied, at constant extraction temperature, longer extraction time with lower citric acid concentration produced higher pectin yield (Fig. 1d). In the case of constant citric acid concentration, higher extraction time with higher extraction temperature caused a higher pectin yield and higher degree of esterification (Fig. 1e). While for constant extraction time, higher extraction temperature with lower citric acid concentration gave higher pectin yield (Fig. 1c) while moderate citric acid concentration with higher extraction temperature gave higher degree of esterification (Fig. 1f). In response surface methodology, the higher the percentage of desirability, the closer the predicted result given by the optimum condition to the target result set in the design expert software will be. The desirability of the optimization condition for two responses was 80.11 % and it meant that the predicted result was close to the setting target. To verify the model, the experimental value was compared with the predicted results from the optimized model by calculating the percentage error [24] to determine the adequacy of the response surface models. The percentage error which is lower than 10 % indicated a good fit. The experiment value of pectin yield and degree of esterification found was 6.78 % and 72.56 % respectively by using the optimized condition. The percentage error for the pectin yield and degree of esterification was 5.01 % and 4.36 % which indicated that there were no significant differences between the predicted and experimental values and it was a good fit to the model.
Figure 1: Effect of a citric acid concentration and extraction time, b extraction time and extraction temperature, c extraction temperature and citric acid concentration on pectin yield; effect of d extraction time and citric acid concentration, e extraction time and extraction temperature, f citric acid concentration and extraction temperature on degree of esterification.

4. Conclusion

The extraction of pectin from passion fruit peels using citric acid gave satisfying results in terms of yield and degree of esterification. Passion fruit peel has a good potential as source of pectin extraction. The highest pectin yield was 7.71% with degree of esterification 60.38% obtained at 0.14% (wt/wt) citric acid concentration, 120 minutes of extraction time and 60 °C extraction temperatures while the highest degree of esterification 78.57% with pectin yield 6.62% obtained at 0.09% (wt/wt) citric acid concentration, 102 minutes of extraction time and 75 °C extraction temperatures. The optimum extraction condition suggested by the software was 0.13% (wt/wt) of citric acid concentration and 102 minutes of extraction time at 75 °C which give the highest predicted pectin yield of 6.44% and highest predicted degree of esterification 75.72%. Response surface methodology was effective tool for estimating the effect or significance of the three factors and the desirability for this treatment was 80.11%. Among the three investigated factor, extraction temperature gave higher significant effect on pectin yield and citric acid concentration gave the higher significant effect on degree of esterification.
5. References


