Ultrasonic-assisted extraction of pectin from *chaenomeles*

LI Xin-shi(李新社), LIU Zheng-yan(刘争艳), LU Bu-shi(陆步诗)

Department of Biology and Chemistry Engineering, Shaoyang University, Shaoyang 422000, China

© Central South University Press and Springer-Verlag Berlin Heidelberg 2014

Abstract: Ultrasound was applied for the extraction of pectin from *chaenomeles*. The content of pectin was evaluated by carbazole colorimetric method. Based on the results of the single factor experiment and the orthogonal tests, the optimum extraction parameters are as follows: the solid-to-liquid ratio is 1:4 g/mL, the ultrasonic power is 320 W, the ultrasonic-assisted extraction temperature is 60 °C, the ultrasonic-assisted extraction time is 50 min, and pH value is 2.0. Compared with acid hydrolyze methods, the extraction time of the present technique decreases from 70 min to 50 min, and the extraction yield increases from 1.39% to 2.61%. The results show that ultrasonic-assisted extraction is time-saving and highly efficient, so it provides a new way to extract pectin from *chaenomeles*.

Key words: *chaenomeles*; pectin; orthogonal experiments

1 Introduction

Papaya, also known as quince, is in the genus papaya of the family Rosaceae. It is widely distributed in China, especially in Anhui, Shandong, Shaanxi, Zhejiang, Yunnan, Guizhou, Sichuan and so on. Papaya is widely used as ornamental, edible and medicine. Modern medical science has confirmed that papaya contains more than 17 kinds of amino acids and a variety of nutrition elements. It is anti-bacterial and anti-inflammation. It can relax the muscles, stimulate the circulation of the blood, flood wind acetanilide detumescence, and prevent the synthesis of human carcinogen nitrosamine. Usually, the pectin content in papaya is 9.5%. Besides, the papaya pectin exhibits good properties and color. Consequently, papaya is a good resource for pectin extraction [1].

Pectin is a hydrophilic plant gum, and widely exists in the cell walls of the roots, stems leaves and fruits of the Embryophyte. The main ingredients of pectin are D-galacturonic acid. Some of these galacturonic acids are methyl esterified with relative molecular mass ranging from 2 to 400000 [2]. Pectin mainly exists as three forms in the plant tissue: original pectin, pectin fat acid and pectic acid, with the color of white, yellow and light yellow, respectively. They show special aroma. Pectin exhibits positive effects on emulsification, thickening, stability and gelling, and thus it is widely used in food, textile, printing and dyeing, tobacco, metallurgy and so on [3]. According to incomplete statistics, the consumption of pectin in China is more than 1500 t per year, in which the imported pectin contributes to about 80% [4]. The demand of pectin still shows a rapid-growth tendency. Therefore, the development of the abundant pectin resources in China, in order to produce high quality pectin and meet the demand of the markets, has been extremely urgent [5].

During recent years, the extraction of pectin has been widely reported [6–12]. Ultrasonic-assisted pectin-extraction process is a physical crushing process. It applies cavitation and mechanical vibration effects to burst the cell wall structure instantly, so that the diffusion of active ingredients of plant can be accelerated, and thus, can improve the extraction yield of pectin [13–15]. The raw material of papaya pectin is abundant, and the quality of papaya pectin is good. It has been reported to extract pectin from papaya [16–18], but the ultrasound-assisted extraction method has not been reported yet. Therefore, the aims of this work were to use ultrasonic-assisted extraction technology to extract pectin from papaya, find the optimum extraction parameters, and make a comparison with the traditional acid hydrolysis method, so as to provide references for the development and utilization of pectin in papaya.

2 Materials and methods

2.1 Raw materials and reagents

The raw material used in the present investigation was papayas, bought in supermarket.
Reagents of aladdin reagent, D-(-)-galacturonic acid, anhydrous ethanol, zinc powder, potassium hydroxide and sulfuric acid, hydrochloric acid, 99% ethanol, ethyl ether, and petroleum ether with commercial purity were used.

2.2 Instruments and equipments

DHG-9101-2S electro-thermostatic drying oven on forced convection (Shanghai SANFA Scientific Instruments Co., Ltd.), pH meter of PHSJ-4 type (INESA instrument Co., Ltd.), Waring blender (Jintan Jieruiier Electrical Equipment Co., Ltd.), JA2003N electronic balance (Beijing Sartorius Co., Ltd.), KDB KQ-400 high power numerical control ultrasonic cleaning device (Kunshan Ultrasonic Instrument Co., Ltd.), GM-0.33 II diaphragm vacuum pump (Tianjin Shanda Filtering Device Factory), HH-2 digital constant temperature water-bath water (Changzhou Guohua Electric Appliance Co., Ltd.), 722 visible spectrophotometer (Shanghai Yoke Instrument Co., Ltd.) Colorimetric tube, color plate, volumetric flask, measuring cylinder and several beaker, etc, were used.

2.3 Methods

2.3.1 Processing of raw materials

Uniform-sized and uniform-matured papayas were chosen. After weighing, the epicarp and seeds of the papayas were removed. Then, the papayas were mashed in the warring blender, followed by rinsing in boiling water for 5–7 min, in order to inactivate the pectin enzyme [19]. Afterwards, the papayas were cooled, and then dried in air at the temperatures lower than 50 °C. After drying, the papayas were weighed in a beaker, followed by adding 70% hot ethanol into the beaker, stirring in order to extract saccharides, and filtering. This step was repeated until no saccharide could be detected by MuLiXu Reaction [20]. The residue was washed by 99% ethanol, followed by diethyl ether washing to remove the lipid and pigment. After the volatilization of diethyl ether, the residue was dried and collected.

2.3.2 Extraction of pectin in papaya

A certain amount of the processed papaya powder was weighed and put into quantitative distilled water. The hydrochloric acid was used to adjust the pH value of the solution. Under the set circumstance, ultrasound was applied for the extraction, and the centrifugal filtration was used right after the extraction in order to get the filtrate, i.e., the pectin extract. In the end, certain amount of pectin was transferred into the volumetric flask. Distilled water was used for dilution in order to obtain samples.

2.3.3 Determination of pectin content

The content of pectin was determined using carbazole colorimetric method [21]. Eight 25 mL colorimetric tubes were used with 12.0 mL sulfuric acid in them. The tubes were bathed in ice-water. During cooling, 2 mL pectin standard solution with different concentrations of 0, 10, 20, 30, 40, 50, 60, 70 μg/mL was added into the eight tubes and mixed, respectively, followed by boiling water bath for 10 min. After cooling down to the room temperature, 0.15% ethanol solution carbazole (1 mL) was added into each tube, followed by shaking and reaction in a dark room for 30 min at room temperature. Tube 1 without pectin addition was used as a reference. The absorbance was determined at the wavelength of 530 nm. The regression equation between absorbance (A) and concentration (C) is

\[
A = 0.0051 - 0.0148C, \quad R^2 = 0.9941
\]

The extraction yield of pectin is calculated according to

\[
X = \frac{cVK}{10^6M} \times 100\%
\]

where \(X\) is the extraction yield of pectin, %; \(V\) is the volume of the extracted pectin solution, mL; \(K\) is the dilution ratio of the extraction agent; \(C\) is the pectin concentration obtained from the standard curve, μg/mL; \(M\) is the mass of sample, g.

3 Results and discussion

3.1 Single factor experiments

3.1.1 Influence of solid-to-liquid ratio

The 0.2 g processed pectin powder was extracted at 60 °C for 40 min. The hydrochloric acid (pH=2.0) was applied as the extraction agent. The ultrasonic power was fixed to be 280 W. Different solid-to-liquid ratios, i.e., 1:2, 1:3, 1:4, 1:5 and 1:6, were used to investigate their influence on the extraction yield of pectin, as shown in Fig. 1.
Figure 1 shows that the extraction yield increases with decreasing solid-to-liquid ratio until reaching 1:4. Higher solid-to-liquid ratios lead to poor dissolution of pectin into the extraction agent, resulting in high viscosity and low extraction yield. In contrast, when the solid-to-liquid ratio is lower than 1:4, less pectin could be obtained, because excessive liquid leads to low concentration of pectin, which lowers the filtering rate. As a consequence, the optimized solid-to-liquid ratio is determined as 1:4. The solid-to-liquid ratios, 1:3, 1:4 and 1:5, were selected for orthogonal tests.

3.1.2 Influence of ultrasonic power

The solid-to-liquid ratio, pH value of the extraction agent, the ultrasonic-assisted extraction temperature and time were set as 1:4, 2.0, 60 °C and 40 min, respectively. Different ultrasonic powers, i.e., 200 W, 280 W, 320 W and 360 W, were used for the investigation of their influence on the extraction yield of pectin, as presented in Fig. 2.

As presented in Fig. 2, when the ultrasonic power increases from 200 W to 320 W, the extraction yield of pectin increases. With increasing ultrasonic power, the cavitation effect becomes more significant, leading to higher medium velocity and acceleration. The higher diffusion rate contributes to the crushing effect on cell walls, resulting in enhanced pectin dissolution. However, when ultrasonic power exceeds 320 W, the decomposition of pectin might become significant. Consequently, the optimized ultrasonic power is determined to be 320 W. The ultrasonic-assisted extraction times of 40 min, 50 min and 60 min were applied for orthogonal tests.

3.1.3 Influence of ultrasonic-assisted extraction temperature

The solid-to-liquid ratio, ultrasound power, pH value of the extraction agent and extraction time were set as 1:4, 320 W, 2.0 and 40 min, respectively. Different ultrasonic-assisted extraction temperatures, i.e., 40 °C, 50 °C, 60 °C, 70 °C and 80 °C, were applied in order to investigate their influence on the extraction yield of pectin, as shown in Fig. 3.

As presented in Fig. 3, higher ultrasonic-assisted extraction temperature increases the extraction yield of pectin, because higher temperature enhances the hydrolysis of insoluble pectin into soluble pectin. However, when the extraction temperature exceeds 70 °C, the hydrolysis of the pectin in peel is too severe to maintain its structure, leading to lower extraction yield of pectin. Consequently, the optimum extraction temperature is determined to be 70 °C. The ultrasonic-assisted extraction temperatures of 60 °C, 70 °C and 80 °C were selected for orthogonal tests.

3.1.4 Influence of ultrasonic-assisted extraction time

The solid-to-liquid ratio, ultrasound power, pH value of the extraction agent and ultrasonic-assisted extraction temperature were set as 1:4, 320 W, 2.0 and 70 °C, respectively. Different ultrasonic-assisted extraction times, i.e., 30 min, 40 min, 50 min, 60 min and 70 min, were used in order to investigate their influence on the extraction yield of pectin, as shown in Fig. 4.

As shown in Fig. 4, with increasing extraction time until reaching 50 min, the extraction yield of pectin increases, owing to higher amount of dissolved pectin from chaenomeles. However, ultrasonic-assisted extraction for longer time may decompose pectin, leading to lower extraction yield of pectin. Consequently, the optimized ultrasonic-assisted extraction time is determined to be 50 min. The ultrasonic-assisted extraction time of 40 min, 50 min and 60 min were applied for orthogonal tests.

3.1.5 Influence of pH value

The solid-to-liquid ratio, ultrasound power, ultrasonic-assisted extraction temperature and time were
set as 1:4, 320 W, 70 °C and 50 min, respectively. Different pH values, i.e., 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0, were used in order to investigate their influence on the extraction yield of pectin, as shown in Fig. 5.

Figure 5 shows that with increasing pH value from 0.5 to 2.0, the extraction yield of pectin increases. When pH value is too low, high hydrolysis effect of the extraction agent leads to the decomposition of soluble pectin into monosaccharides and the decomposition of some cellulose and hemicelluloses into hexose and pentose, so the extraction yield of pectin is low in such a highly acidic environment. In contrast, for pH value higher than 2.0, the low amount of H\(^+\) decreases the extraction yield of pectin. Consequently, the optimum pH value is determined to be 2.0.

### 3.2 Orthogonal tests

According to Refs. [22–24], L\(_{9}(3^{4})\) orthogonal tests, based on single factor experiments, were applied to determine the influence of solid-to-liquid ratio, ultrasonic power, ultrasonic-assisted extraction temperature and time on the pectin extraction. Tables 1–3 present the

<table>
<thead>
<tr>
<th>Group</th>
<th>A: Solid-liquid ratio/((\text{g·mL}^{-1}))</th>
<th>B: Ultrasonic power/W</th>
<th>C: Ultrasonic temperature/°C</th>
<th>D: Ultrasonic time/min</th>
<th>Yield/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2.360</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2.364</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2.528</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2.496</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2.635</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2.763</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2.453</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2.587</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2.489</td>
</tr>
<tr>
<td>K1</td>
<td>7.252</td>
<td>7.309</td>
<td>7.710</td>
<td>7.484</td>
<td></td>
</tr>
<tr>
<td>K2</td>
<td>7.894</td>
<td>7.586</td>
<td>7.349</td>
<td>7.611</td>
<td></td>
</tr>
<tr>
<td>K3</td>
<td>7.529</td>
<td>7.780</td>
<td>7.616</td>
<td>7.580</td>
<td></td>
</tr>
<tr>
<td>K1(K2/3)</td>
<td>2.417</td>
<td>2.436</td>
<td>2.570</td>
<td>2.495</td>
<td></td>
</tr>
<tr>
<td>K2(K2/3)</td>
<td>2.631</td>
<td>2.529</td>
<td>2.449</td>
<td>2.537</td>
<td></td>
</tr>
<tr>
<td>K3(K3/3)</td>
<td>2.510</td>
<td>2.593</td>
<td>2.539</td>
<td>2.527</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>0.214</td>
<td>0.157</td>
<td>0.121</td>
<td>0.042</td>
<td></td>
</tr>
</tbody>
</table>
3.3 Comparison of ultrasonic-assisted method and acid hydrolysis method

The optimized parameters obtained from orthogonal tests were applied for the extraction of pectin from Chaenomeles. Parallel tests were repeated for three times. Table 4 shows the comparison of ultrasonic-assisted method and acid hydrolysis method.

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Solid-liquid ratio/(g·mL⁻¹)</th>
<th>Ultrasonic temperature/°C</th>
<th>Ultrasonic time/min</th>
<th>Yield/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid hydrolysis</td>
<td>1:4</td>
<td>60</td>
<td>70</td>
<td>1.39</td>
</tr>
<tr>
<td>Ultrasound-assisted</td>
<td>1:4</td>
<td>60</td>
<td>50</td>
<td>2.61</td>
</tr>
</tbody>
</table>

As presented in Table 4, compared with acid hydrolysis method, the extraction of ultrasonic-assisted method decreases from 70 min to 50 min, and the extraction yield of pectin increases from 1.39% to 2.61%. Such a result reveals that the ultrasonic-assisted extraction is time-saving and highly efficient.

4 Conclusions

1) Single factor experiment reveals that the optimum parameters are as follows: the solid-to-liquid is 1:4, ultrasonic power is 320 W, ultrasonic-assisted extraction temperature is 70 °C, ultrasonic-assisted extraction time is 50 min, and pH value is 2.0. In this case, the extraction of pectin is better.

2) Orthogonal experiment shows that the optimum conditions are as follows: the solid-to-liquid ratio is 1:4, ultrasonic power is 320 W, ultrasonic-assisted extraction temperature is 60 °C, ultrasonic-assisted extraction time is 50 min, and pH value is 2.0. Under the optimum conditions, pectin yield can be more than 2.6%.

3) Compared with the acid hydrolysis method, the ultrasonic-assisted method can decrease the extraction time from 70 min to 50 min, and increase the extraction yield from 1.39% to 2.61%. Consequently, it is revealed that the ultrasonic-assisted extraction method can reduce cost and resource consumption, owing to its time-saving and highly efficient nature.

References


Edited by YANG Bing