Microwave-assisted Chelating Agent Extraction of Pectin from Hawthorn Wine Pomace

Xiaowen Chen and Chuanhe Zhu

Key Laboratory of Food Processing Technology and Quality Control in Shandong Province, College of Food Science and Engineering, Shandong Agricultural University, Taian 271018, China

*Corresponding author’s e-mail: chhzhu@sdau.edu.cn

Abstract. Pectin was extracted by microwave-assisted chelating agent (sodium hexametaphosphate) method from hawthorn wine pomace. Based on single factor experiments, the effect of chelating agent dosage, solid-liquid ratio, microwave power and microwave time on extraction rate of hawthorn pectin was evaluated by response surface methodology. The results showed that factors of effect on extraction rate in order from large to small were solid-liquid ratio, chelating agent dosage, microwave time and microwave power. The best process factors were: chelating agent dosage of 1.35 %, solid-liquid ratio of 1:9, microwave power of 440 W and microwave time of 80 s. Under these conditions the average extraction rate of hawthorn pectin was 72.89 ± 0.45 %, which was basically consistent with the predicted value. It was showed that this model was reliable.

1. Introduction

Hawthorn (Crataegus pinnatifida Bunge) belongs to the Crataegus L. of the family Rosaceae. And the hawthorn is one of the most frequently used plants in Chinese medicine and nutrition [1]. Hawthorn contains of pectin, organic acid, flavonoids, vitamin C and minerals such as calcium, phosphor, iron, etc [2-3]. Particularly, the pectin content in hawthorn can reach 6.4 % [4]. Hawthorn is usually processed into dried fruit, juice, wine, etc. Subsequently it produced a large number of scraps such as hawthorn pomace, which contains a large number of pectin and other functional components. So, it can be used as a good raw material of pectin extraction.

Pectin is a complex plant polysaccharide which mainly exists in the primary cell walls and middle cellular [5-6]. It is a non-toxic natural product, usually used as a thickener or stabilizer in food processing such as yogurt and jelly jam [7-8]. The chemical structure of pectin is closely related to plant origin, location, extraction method, etc., so it has different functional properties [9-10]. Many extraction methods such as acid extraction, enzyme-, chelating agent-, ultrasonic- or microwave-assisted extraction have been proven to be effective in extracting pectin from plants [11-12]. The chelating agent is associated with calcium pectate (the main form of natural pectin is calcium pectate and insoluble in water), which makes the pectin dissociate out and dissolve in water [13-14].

In this study, the best condition (chelating agent dosage, solid-liquid ratio, microwave power and microwave time) on the highest extraction rate of pectin from hawthorn wine pomace was obtained by response surface methodology. This work will provide a theoretical basis for the industrial production
of hawthorn pectin, and it has great significance to full use of hawthorn resources and environmental protection.

2. Materials and methods

2.1. Materials
Hawthorn (Crataegus pinnatifida Bunge) wine pomace were supplied by the College of Food Science and Engineering (Shandong Agricultural University, Taian, Shandong, China).

2.2. Preparation of pectin
The hawthorn wine pomace was used as raw material and the extracted solution of pectin was obtained by different conditions. The conditions were: chelating agent dosage (0.8 %, 1.2 % and 1.6 %), solid-liquid ratio (1:6, 1:8 and 1:10), microwave power (264 W, 440 W and 616 W) and microwave time (40 s, 60 s and 80 s). In the next step, the extracted solution of pectin was centrifuged at 4000 rpm for 20 min (TDZ5-WS, Xiangyi Centrifuge Instrument Co., Ltd., Changsha, Hunan, China) and the supernatant was obtained. Then anhydrous ethanol was added to it and made the ethanol concentration in the system to about 60 %. And it was keep at 4 ℃ for 12 h. Afterwards the pectin precipitation was obtained by centrifugation at 4000 rpm for 20 min. The pectin precipitation was washed with anhydrous ethanol at least twice. It was dissolved in deionized water and placed in a dialysis bag (3.5 kDa) at 4 ℃ for about two days. Freeze-dried and the hawthorn pectin was obtained (MH-HP). The extraction rate was calculated as following:

\[
\text{Extraction rate (\%)} = \left( \frac{M \times W}{W_0} \right) \times 100 \%
\]

Where M is the weight of pectin sample, W is the Gal A content of pectin sample, W₀ is the pectin content of hawthorn wine pomace, which is 1.7907.

2.3. Experimental design
The four factors and three levels experiments was designed by Design-Expert 8.0.6. Factors of chelating agent dosage (A), solid-liquid ratio (B), microwave power (C) and microwave time (D) on the extraction rate were studied. The factors and levels of the orthogonal experiments were shown in Table 1.

Table 1. Factors and Levels for response surface methodology.

| Levels | Factors |
|--------|---------|
|        | A (%)   | B | C (W) | D (s) |
| -1     | 0.8     | 1:6 | 264   | 40    |
| 0      | 1.2     | 1:8 | 440   | 60    |
| 1      | 1.6     | 1:10| 616   | 80    |

2.4. Galacturonic acid content
The Galacturonic acid content of hawthorn pectin was determined by the m-hydroxydiphenyl method [15]. 1mL of pectin solution (0.1 mg/mL) and 5ml of borax sulfuric acid solution (0.0125 M) were thoroughly mixed under ice water-bath. Then it was put in 80 ℃ water-bath for 6 min and cool down. Afterwards, 0.1 mL of m-hydroxybiphenyl solution (0.15 %) was add to the mixture and stand for 20 min. The absorbance was measured at 520 nm using a UV spectrophotometer (UV-755B, Shanghai Youke Instrument Co., Ltd, Shanghai, China). D-Gal A was used as a standard and the standard curve was: \( Y = 0.0148x - 0.0069 \), \( R^2 = 0.9998 \).

3. Results and discussion

3.1. Statistical analysis
Response surface methodology was used to evaluate effects chelating agent dosage (A), solid-liquid ratio (B), microwave power (C) and microwave time (D) on extraction rate of hawthorn pectin. The experimental design and results were shown in Table 2. And the variance analysis of regression model is shown in Table 3.

Table 2. Design matrix of BBD and results for pectin extraction using microwave-assisted chelating agent method.

|    | A (%) | B          | C (W) | D (s) | Extraction rate (%) |
|----|-------|------------|-------|-------|---------------------|
| 1  | -1 (0.8) | 1 (1:10)   | 0 (440) | 0 (60)  | 47.51               |
| 2  | 0 (1.2) | 0 (1:8)    | -1 (264) | -1 (40) | 60.15               |
| 3  | 0      | 0          | 0      | 0      | 69.24               |
| 4  | 0      | -1 (1:6)  | 1 (616) | 0      | 38.82               |
| 5  | -1     | 0          | 1      | 0      | 50.84               |
| 6  | -1     | 0          | 0      | -1     | 56.45               |
| 7  | 0      | 0          | 0      | 0      | 71.71               |
| 8  | 0      | 0          | 1      | -1     | 67.16               |
| 9  | -1     | 0          | 0      | 1 (80) | 47.40               |
| 10 | 0      | 1         | -1     | 0      | 51.15               |
| 11 | 0      | 0          | 0      | 0      | 73.61               |
| 12 | -1     | 0          | -1     | 0      | 49.6                |
| 13 | 1 (1.6)| 0          | -1     | 0      | 57.09               |
| 14 | 0      | -1        | 0      | -1     | 58.66               |
| 15 | 0      | 0          | 1      | 1      | 54.4                |
| 16 | 0      | 0          | -1     | 1      | 68.12               |
| 17 | 1      | 0          | 0      | 1      | 64.14               |
| 18 | 1      | 0          | 0      | -1     | 59.45               |
| 19 | 0      | -1        | -1     | 0      | 43.62               |
| 20 | 1      | -1        | 0      | 0      | 37.45               |
| 21 | -1     | -1        | 0      | 0      | 37.84               |
| 22 | 0      | 1         | 0      | 1      | 64.91               |
| 23 | 1      | 1         | 0      | 0      | 62.44               |
| 24 | 0      | 1         | 1      | 0      | 59.49               |
| 25 | 0      | 0         | 0      | 0      | 72.98               |
| 26 | 0      | -1        | 0      | 1      | 42.15               |
| 27 | 1      | 0         | 1      | 0      | 42.42               |
| 28 | 0      | 1         | 0      | -1     | 63.07               |
| 29 | 0      | 0         | 0      | 0      | 69.89               |

The quadratic models were expressed as follows:

Extraction rate (%) = 71.49 + 2.78A + 7.50B - 1.38C - 1.99D + 3.83AB - 3.98AC + 3.44AD + 3.29BC + 4.59BD - 5.18CD - 12.68A^2 - 13.37B^2 - 8.90C^2 - 1.00D^2

As we can see from Table 3, the model was extremely significant (P < 0.0001 < 0.01) and the lack-of-fit tests were not significant (P = 0.1097 > 0.05). The R-Squared was 0.9579. The result was indicated that the models had a high fitting degree and could better predict the extraction rate of pectin for any combination of independent factors within the range of this paper. In addition, the low coefficient of variation (C.V. = 5.73) also showed that the regression model had high reliability and its error was small. According to the F value and P value, the influence of factors on extraction rate of pectin can be determined. The larger the F-value (or the smaller the P-value), the stronger of the effect on extraction rate was. Analysis of the data showed that the effect of each single factor on the extraction rate of pectin was: solid-liquid ratio > chelating agent dosage > microwave time > microwave power. Among them, the effect of solid-liquid ratio and chelating agent dosage on pectin extraction rate was significant (P < 0.05), while microwave time and microwave power had no
significant effect on pectin extraction rate (P > 0.05). What's more, quadratic term coefficients AB, AC, BD, CD, A$^2$, B$^2$, and C$^2$ were all significantly affected the extraction rate of pectin (P < 0.05).

| Table 3. Analysis of variance (ANOVA) for regression model of pectin extraction rate. |
|---------------------------------------------------------------|
| Sum of squares | DF | Mean square | F-Value | p-Value | Significant |
| Model          | 3351.90 | 14 | 239.42 | 22.76 | < 0.0001 | ** |
| A              | 92.69 | 1 | 92.69 | 8.81 | 0.0102 | * |
| B              | 675.45 | 1 | 675.45 | 64.22 | < 0.0001 | ** |
| C              | 22.96 | 1 | 22.96 | 2.18 | 0.1617 | |
| D              | 47.28 | 1 | 47.28 | 4.50 | 0.0523 | |
| AB             | 58.68 | 1 | 58.68 | 5.58 | 0.0332 | * |
| AC             | 63.28 | 1 | 63.28 | 6.02 | 0.0279 | * |
| AD             | 47.20 | 1 | 47.20 | 4.49 | 0.0525 | |
| BC             | 43.16 | 1 | 43.16 | 4.10 | 0.0623 | |
| BD             | 84.18 | 1 | 84.18 | 8.00 | 0.0134 | * |
| CD             | 107.43 | 1 | 107.43 | 10.21 | 0.0065 | ** |
| A$^2$          | 1042.58 | 1 | 1042.58 | 99.12 | < 0.0001 | ** |
| B$^2$          | 1159.16 | 1 | 1159.16 | 110.20 | < 0.0001 | ** |
| C$^2$          | 513.71 | 1 | 513.71 | 48.84 | < 0.0001 | ** |
| D$^2$          | 6.48 | 1 | 6.48 | 0.62 | 0.4457 | |
| Residual       | 147.26 | 14 | 10.52 | 3.69 | 0.1097 | |

3.2. Response surface plot
The 3D response surface represented the interactions between the two factors, in which the other factors were fixed at zero level. The response surface plot was shown in Fig. 1. Fig. 1a displays the interrelations between the solid-liquid ratio and chelating agent dosage on extraction rate of pectin. The response surface was steep, which indicated that the solid-liquid ratio and chelating agent dosage have a significant effect on the extraction rate of pectin. From the steep degree of Fig.1b and Fig.1d, the effect of solid-liquid ratio on the extraction rate of pectin was more significant than chelating agent dosage. In addition, similar result could be obtained from Fig.1c and Fig.1e. From Fig.1f, the response surface was gentle compared to fig.1a. This phenomenon showed that the effect of microwave time and microwave power on the extraction rate was weak.

3.3. Optimization of pectin extraction conditions
Based on the regression model, the optimal extraction conditions were as follows: chelating agent dosage of 1.35 %, solid-liquid ratio of 1:8.92, microwave power of 375.39 W and microwave time of 80.00 s. Under these conditions, the predicted extraction rate was 73.65 %. According to the operability of this experiment, the test conditions were set as follows: chelating agent dosage of 1.35 %, solid-liquid ratio of 1:9, microwave power of 440 W and microwave time of 80 s. Three experiments were repeated under these conditions and the average extraction rate was 72.89 ± 0.45 %, which was basically consistent with the predicted value. This was indicated that the model was reliable.

3.4. Galacturonic acid content
Hawthorn pectin was extracted by the optimal extraction conditions described above. And the galacturonic acid content was 68.94 ± 1.10 %.
Figure 1. Response surface plots.

4. Conclusions
In this work, microwave-assisted chelating agent method was successfully employed to extract pectin from hawthorn wine pomace. The extraction conditions obtained by response surface methodology were chelating agent dosage of 1.35 %, solid-liquid ratio of 1:9, microwave power of 440 W and microwave time of 80 s. Under these conditions, the extraction rate of pectin was 72.89 ± 0.45 % the galacturonic acid content was 68.94 ± 1.10 %. The results showed that microwave-assisted chelating agent method was an effective method for pectin extraction.

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