Study on Ultrasonic-assisted Water Extraction of Polysaccharides from Sunflower Tray

Yingmin Fei¹, Xinci Liu², Gensheng Zhang² *

¹ Food science, Heilongjiang Vocational College for Nationalities 150066, Harbin, Heilongjiang
² Processing of agricultural products, Harbin University of Commerce, 150028, Harbin, Heilongjiang
E-mail: zhanggsh@hrbcu.edu.cn

Abstract. Sunflower tray has been widely studied in recent years because of its rich polysaccharide. In this study, sunflower tray powder was used as raw material to optimizing the process parameters of ultrasonic-assisted extraction of polysaccharides. Single-factor experiments were used to explore the effects of material-liquid ratio, ultrasonic temperature, ultrasonic time and ultrasonic power on the extraction rate of polysaccharides; response surface experiments were used to optimize the process parameters for the optimal polysaccharide extraction rate. The experimental results show that: the ratio of material to liquid is 1:37, the ultrasonic temperature is 52.50°C, and the ultrasonic time is 31 min, the ultrasonic power is 300W. Extracted twice under this condition, the yield of sunflower tray polysaccharide reached 10.15%, the polysaccharide extraction process is reproducible and stable.

1. Introduction
Sunflower is the main oil crop of the Chrysanthemum genus, its seeds can be eaten directly and are also an important raw material for the production of sunflower oil. Sunflower roots, stems, leaves and flowers are good medical material [1,2]. Sunflower tray is full of phenols, flavonoids and other substances such as chlorogenic acid, active polysaccharides, total flavonoids, terpenoids and other ingredients, which make the sunflower tray has edible and medicinal value [3,4], but they are only used as by-products of sunflower in industrial production. Therefore, the application scope range is narrow which limited to three major aspects of fermentation production of biogas, return to field treatment and feed treatment, and the utilization rate is low. Most sunflower trays are still treated by incineration, which not only pollutes the environment, but also wastes resources [5]. It can be seen that the efficient and stable extraction method of sunflower tray polysaccharide is beneficial to improve the utilization value of sunflower tray and reduce environmental pollution.

As one of the most abundant substances in nature, polysaccharides not only participate in the structural composition of plant cells, provide energy, but also serve as broad-spectrum immunomodulators to enhance immunity, sun protection and cancer prevention [6]. It can be combined with chemotherapy and play a great role in inhibiting the body's adverse reactions [7]. The polysaccharides in sunflower trays are mainly galacturonic acid and rhamnose [8-10]. The methods for extracting sunflower trays polysaccharides include hot water extraction, ethanol reflux extraction, ultrasonic assisted extraction, microwave assisted extraction, enzyme-assisted extraction, supercritical extraction and grinding-assisted extraction [11-14]. Hot water extraction takes a long time and operation
is complicated; microwave treatment temperature is difficult to control; enzymatic extraction is
difficult to operate and the type of enzyme and the optimum temperature will affect the extraction rate
of polysaccharides. By contrast, ultrasonic extraction does not require high temperature treatment and
it is efficient in a short time. Therefore, ultrasonic extraction method is convenient for large-scale
production due to its high activity, high degree of automation.

In this paper, ultrasonic-assisted water extraction was used to extract polysaccharides. Through
single factor experiment, the influence of the four factors of material-liquid ratio, ultrasonic
temperature, ultrasonic time and ultrasonic power on the yield of polysaccharides from sunflower tray
was studied. On this basis, the response surface experiment was used to screen the extraction process
parameters of polysaccharides. Besides, the optimal extraction times were determined according to the
response surface experiment results. Finally, the optimal process conditions for the extraction of
polysaccharides from sunflower trays by ultrasonic water extraction were obtained.

2. Materials and Methods

2.1. Materials

Anhydrous ethanol (analytical purity): Tianjin Tianli Chemical Reagent Co., Ltd.; glucose: Tianjin
Hengxing Chemical Reagent Manufacturing Co., Ltd.; Anthrone (analytical purity): Shanghai Hushi
Pharmaceutical Technology Co., Ltd.; sulfuric acid (analytical purity): Xilong Science Co., Ltd.

2.2. Glucose standard curve

Different concentrations were adjusted to 1 mL from 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL
and 0.7 mL, 4 mL of anthrone-sulfuric acid reagent was added and mixed. The mixed solution was
bathed in boiling water for 10 minutes and then passed in a cold water bath for 10 minutes. Set blank
group with 1mL distilled water and 4mL a throne-sulfuric acid reagent and measure absorbance value
at 620nm., the regression equation is: y=1.8214x+0.0028.

2.3. Polysaccharide extraction

The solution containing polysaccharides is obtained by centrifuging the slurry of 5.0g sunflower tray
powder mixed with distilled water in a certain proportion under certain conditions of ultrasonic power,
ultrasonic temperature and ultrasonic time. Put the extracted slurry in a centrifuge (500 r/min),
centrifuge for 10 minutes, and filter to obtain the supernatant. Add twice volume of concentrated
absolute ethanol to the supernatant obtained after filtration, put it in a refrigerator at 4 ℃ and alcohol
precipitation for 12 h, discard the supernatant to obtain a precipitate. Wash the precipitate with 80%
ethanol and anhydrous ethanol, filter with suction. The obtained precipitate will dried in a constant
temperature drying oven to a constant weight and weigh the crude polysaccharide.

2.4. Single factor experiment

Admixturing 5.0g sunflower tray powder with distilled water in different proportions, ultrasound the
mixture at different temperatures, time and different power for polysaccharides extracted.

2.5. Response surface experiment design

In order to obtain the best process for the extraction of polysaccharides from sunflower tray, factors
and levels of material-liquid ratio (A), ultrasonic temperature (B) and ultrasonic time (C) were
screened out by single factor experiment in Table 1.

| Level | Material ratio (g/mL) | Ultrasonic temperature (°C) | Ultrasonic time (min) |
|-------|-----------------|-----------------|------------------|
| -1    | 1:50            | 40              | 20               |
| 0     | 1:40            | 50              | 30               |
| 1     | 1:30            | 60              | 40               |

Table 1 Level of response surface experiment
2.6. Number of extractions
The extraction times of sunflower tray polysaccharides are determined based on the optimal extraction conditions obtained by response surface experiments. Comparing the polysaccharide yields in different extraction times of 1, 2, 3 to determine the optimal extraction times.

2.7. Polysaccharide content determination
Pipetting 0.1 mL of the filtrate after centrifugal filtration in 2.3 into a beaker bottle and mix it with 15 mL of distilled water, pipette 0.5 mL of the solution and dilute to 10 mL, measure the absorbance as in 2.2. Using the regression equation to calculate polysaccharide content, the content is brought into the following formula to calculate the polysaccharide yield.

\[ \text{Polysaccharide yield(%)=CVN/m×100%} \]  

In the formula above, \( C \) means the mass concentration of polysaccharide obtained from the standard curve (%), \( V \) means the total volume after constant volume (mL), \( N \) means the dilution multiple (mL) and \( m \) means the sunflower powder quality (g).

3. Results and discussion

3.1. Single factor experiment results

Fig. 1 shows the change of sunflower tray polysaccharide yield under different influencing factors. As shown in Fig. 1a, the surface contact area of polysaccharide and water was expanded by the material-liquid ratio increased, which improves the solubility of polysaccharide and get the polysaccharide yield enlarged. When the material-liquid ratio exceeds 1:40, it causes other impurities in the sunflower tray extracted and led the yield goes down \([15]\). Temperature can also increase the solubility of polysaccharides in water (Fig. 1b). With the temperature increases, the diffusion coefficient of polysaccharides in water, solubility increases and dissolution of sunflower tray polysaccharides are all increases \([16]\). When the ultrasonic temperature exceeds 50 °C, the thermal effect causes the structure of the polysaccharide in sunflower tray destroyed. Simultaneously, the destruction speed of the
polysaccharide is faster than the dissolution rate result a significant decrease in the yield of the polysaccharide. When the temperature reaches 60 °C~70 °C, the polysaccharide is and deploy merization at high temperature reconciliation which made the rate further decrease [17]. The reason is that with the temperature continue rising, the structure of polysaccharide become stable and difficult to change after oxidation, led the polysaccharide yield increasing. Similarly, the solubility of polysaccharides is also improved when the ultrasound time is less than 30 minutes (Fig.1c). Once the ultrasound time exceeds 30 minutes, the thermal effect caused by ultrasound and ultrasound destroys the structure of polysaccharides, resulting in a significant decrease in the yield of polysaccharides. Different from the first three influencing factors, the ultrasonic power changes the yield of polysaccharides by affecting the structure of sunflower tray cells [18]. When the ultrasonic power is between 200W~300W, although the cavitation phenomenon is aggravated and the rate of sunflower tray cell wall breaking increases, the ultrasonic power After more than 300W, the structure of the dissolved polysaccharide is destroyed [19], resulting in a decrease in the yield of polysaccharide.

3.2. Response surface experiment results

| Std | Factor | A | B | C | Yield% |
|-----|--------|---|---|---|--------|
| 1   | -1     | -1| 0 |  | 7.49   |
| 2   | 1      | -1| 0 |  | 7.52   |
| 3   | -1     | 1 | 0 |  | 7.51   |
| 4   | 1      | 1 | 0 |  | 7.93   |
| 5   | -1     | 0 | -1|  | 7.09   |
| 6   | 1      | 0 | -1|  | 7.43   |
| 7   | -1     | 0 | 1 |  | 7.46   |
| 8   | 1      | 0 | 1 |  | 7.49   |
| 9   | 0      | -1| -1|  | 7.19   |
| 10  | 0      | 1 | -1|  | 8.06   |
| 11  | 0      | -1| 1 |  | 8.18   |
| 12  | 0      | 1 | 1 |  | 8.11   |
| 13  | 0      | 0 | 0 |  | 8.69   |
| 14  | 0      | 0 | 0 |  | 8.50   |
| 15  | 0      | 0 | 0 |  | 8.70   |
| 16  | 0      | 0 | 0 |  | 8.77   |
| 17  | 0      | 0 | 0 |  | 8.67   |

Table 3 Variance for sunflower tray polysaccharide extraction

| Sum ofSquares | df | Mean Square | F Value | P-value Prob > F |
|---------------|----|-------------|---------|------------------|
| Model         | 5.21 | 9 | 0.58 | 38.77 | <0.0001*** |
| A             | 0.084 | 1 | 0.084 | 5.63 | 0.0494* |
| B             | 0.19 | 1 | 0.19 | 12.66 | 0.0092** |
| C             | 0.27 | 1 | 0.27 | 18.09 | 0.0038** |
| AB            | 0.0038 | 1 | 0.0038 | 2.55 | 0.1546 |
| AC            | 0.024 | 1 | 0.024 | 1.61 | 0.2452 |
| BC            | 0.22 | 1 | 0.22 | 14.79 | 0.0063** |
| A²            | 2.60 | 1 | 2.60 | 173.95 | <0.0001*** |
| B²            | 0.30 | 1 | 0.30 | 20.25 | 0.0028** |
| C²            | 1.11 | 1 | 1.11 | 74.19 | <0.0001*** |
| Residual      | 0.10 | 7 | 0.015 |       |       |
| Lack of fit   | 0.064 | 3 | 0.021 | 2.14 | 0.2378 |
| Pure          | 0.040 | 4 | 0.010 |       |       |
| Error          | Cor Total |      |
|---------------|-----------|------|
|               |           | 5.32 |
|               | R²        | 0.9803 |

P<0.001: the difference is extremely significant**; P<0.01: the difference is highly significant**; P<0.05: the difference is significant*

The experiment results were fitted multiple times to obtain the quadratic regression equation model: 
\[ Y=8.67+0.10A+0.15B+0.18C+0.098AB-0.077AC-0.24BC-0.79A^2-0.27B^2-0.51C^2. \]

\( R^2 \) value (0.9803) showed high variability, meaning that the model is well fitted greater than 98% of the data is captured by the predicted model. The analysis of variance showed that the ratio of material to liquid has a significant effect on the yield of polysaccharides, and the temperature and time of ultrasound have extremely significant effects on the yield of polysaccharides. The absolute value of the coefficients of the first term in the regression equation is compared to determine the degree of influence of various factors on the yield of polysaccharides in descending order is: ultrasonic time, ultrasonic temperature, material-liquid ratio. According to the software prediction model, the highest polysaccharide yield is: material-liquid ratio 1:37, ultrasonic temperature 52.50 °C, ultrasonic time 31.16 min. Under this condition, the extraction rate of polysaccharide is 8.70.

3.3. Predicted vs. Actual analysis graph

Fig.2. Predicted vs. Actual for polysaccharides extraction

Fig.2 shows that the distribution of the corresponding relationship between the residual error and the predicted value of the equation is scattered and irregular, and there is a reasonable agreement between the predicted value and the experimental value.

3.4. Interaction analysis
It can be seen from the contour lines and response surface in Fig.3 that the slope of factor BC is steep and its contour is elliptical, while the slope of factor AB is slightly flat, and the AC contour is round, indicating that the interaction of the three factors of A, B, and C, ultrasound interaction between temperature and ultrasound time is large, which is consistent with the significant difference of P<0.05 in the analysis of variance.

3.5. Determination of the extraction times of polysaccharides from sunflower tray

Combined with actual operation and response surface prediction results, select the material-liquid ratio 1:37, ultrasonic temperature 52.50 °C, ultrasonic time 31 min, ultrasonic power 300 W. As it shown in Fig.4, the yield of polysaccharides reached the highest when the number of extractions was 3 times, but compared with extraction twice, the margin of increase in yield was reduced. In order to save energy and extract efficiently, select 2 as the best extraction times. At this extraction time, the extraction rate of polysaccharide can reach 10.18%. Compared with the 9.73% polysaccharide yield in the water extraction [20], ultrasonic-assisted water extraction increased polysaccharide yield of sunflower tray, the extraction method of sunflower tray polysaccharides has been optimized. Three verification experiments were carried out under this process parameter, and the average polysaccharide yield was 10.15%.
4. Conclusion

The best process parameters for the ultrasonic-assisted water extraction of sunflower tray polysaccharides are: material-liquid ratio 1:37, ultrasonic temperature 52.50 ℃, ultrasonic time 31 min, ultrasonic power 300 W, and this extraction need performed twice. The yield of sunflower tray polysaccharide is stable around 10.15%. The ultrasonic-assisted water extraction of polysaccharide from sunflower tray is efficient, the yield of polysaccharide is high and easy to reproduce. Based on the above characteristics, ultrasonic-assisted water extraction is suitable for large-scale extraction of sunflower tray polysaccharide.

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