# Response Surface Optimization of Ultrasound-assisted Aqueous Two-phase Extraction of Sweet Potato Leaf Polysaccharides

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**Abstract** [**Objectives**] The ultrasound-assisted aqueous two-phase extraction of sweet potato leaf polysaccharides was studied. [**Methods**] With the yield of sweet potato leaf polysaccharides as the index, the aqueous two-phase extraction system was determined, and the optimal extraction conditions were optimized by single-factor experiments and response surface methodology. [**Results**] The optimal parameters were ethanol concentration 25.68%, liquid-to-material ratio 55.83, and ultrasonic treatment time 38.33 min. Under these conditions, the yield of sweet potato leaf polysaccharides could reach 20.646 mg/g. [**Conclusions**] The ethanol/ammonium sulfate aqueous system is a rapid and efficient method for extracting sweet potato leaf polysaccharides, which is of great significance for the application of sweet potato leaf extract as a natural food additive.

**Key words** Aqueous two-phase system; Ultrasound assistance; Response surface optimization; Sweet potato leaf polysaccharides

Sweet potato belongs to *Ipomoea* of Convolvulaceae. Traditional Chinese medicine records that the stems and leaves of sweet potato are used for medicinal purposes, and have characteristics such as sweetness, astringency, slight coolness, and non-toxicity. They have the effects of evacuating pus and expelling toxin, ulcerating carbuncle, and dredging the mammaryduct. According to domestic and international data, sweet potato leaves can improve the body's disease resistance, enhance immune function, and strengthen health functions such as platelets. Meanwhile, sweet potato leaves also have the characteristics of green and natural fruit and vegetables including tolerance to barren soil, low pollution, cold tolerance, and wide adaptability, and are increasingly being valued by people. There are also various trace components in sweet potato leaves, among which polysaccharides account for 6% – 7%[1].

At present, the extraction of polysaccharides mainly includes several processes such as hot water method, water-extraction and alcohol-precipitation method, enzyme-assisted method, and fermentation method. The hot water method and water-extraction and alcohol-precipitation method have drawbacks such as cumbersome experimental steps and long time consumption. The enzyme-assisted and fermentation methods have problems such as poor stability and high consumption of organic solvents. Compared with traditional extraction and separation techniques, the aqueous two-phase extraction technology has the characteristics of simple operation, mild conditions, and environmental friendliness. The aqueous two-phase system could be adjusted in the composition and proportion according to the properties of extracted substances to ensure the activity of the extracted substances while achieving the highest extraction efficiency. It can be widely used in the separation and purification of active ingredients in natural products, purification and separation of proteins and nucleic acids in biological samples, and enrichment and separation of heavy metal ions in samples. Ultrasonic extraction is the latest and more mature method applied in the extraction and separation of effective ingredients in Chinese herbal medicines in recent years. Ultrasonic extraction is applicable to the extraction of effective ingredients in traditional Chinese medicine, and it has following prominent features: (1) there is no need for high temperature, and it does not damage some thermally-unstable, easily-hydrolyzed, and easily-oxidized medicinal ingredients in traditional Chinese medicine. (2) It is a normal pressure extraction method which has good safety, simple operation, and convenient maintenance. (3) High extraction efficiency: Ultrasound-assisted extraction for 20 – 40 min can achieve the best extraction rate[2].

In this study, ultrasound-assisted aqueous two-phase extraction was adopted to extract polysaccharides from sweet potato leaves, and a fast and efficient ultrasound-assisted aqueous two-phase extraction process was established through response surface methodology optimization. This study provides a theoretical basis for the application of aqueous two-phase extraction in plant polysaccharide extraction and further promotes the utilization and development of polysaccharides, which is of great significance for the application of sweet potato leaf extract as a natural food additive.

## Materials and Methods

### Materials and reagents

Sweet potato leaves were collected from the campus of Qilu University of Technology. After picking, the leaves that had turned

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yellow, decayed, or damaged by pests and diseases were removed, and the rest were sieved with an 80-mesh sieve for later use.

Anhydrous ethanol (analytically pure, Sinopharm Chemical Reagent Co., Ltd.); anhydrous glucose standard (analytically pure, Tianjin Kemiou Chemical Reagent Co., Ltd.); polyethylene glycol (1 000), potassium hydrogen phosphate, ammonium sulfate (analytically pure, Tianjin Dengfeng Chemical Reagent Co., Ltd.); acetone, concentrated sulfuric acid (analytically pure, Sinopharm Chemical Reagent Co., Ltd.).

**Instruments and equipment**

Digital food industry blast drying oven (CX881-8): Wujiang Guanzhuo Electric Heating Equipment Co., Ltd; Household single-phase universal pulverizer (XFB-800): Jishou Zhongcheng Pharmaceutical Machinery Factory; 304 stainless steel 80-mesh standard mesh screen: Han Neng Electromechanical; electronic hydrostatic balance (1%) (ES-B): Tianjin D&T Transducer Technology Co., Ltd.; CNC ultrasonic cleaner (KQ-400DB): Kunshan Shumei Ultrasonic Instrument Co., Ltd; desktop room-temperature high-speed centrifuge (TG16G): Hunan Kaida Scientific Instrument Co., Ltd.; UV-VIS spectrophotometer UV-2900: Shanghai Sunny Hengping Scientific Instrument Co., Ltd.; vortex mixer (K-1 XH-C): Wuxi Woxin Instrument Manufacturing Co., Ltd.

**Experimental methods**

**Selection of aqueous two-phase system** An aqueous two-phase system was prepared according to its quality, while maintaining a total mass of 20 g. In specific, 20% of different inorganic salts ($K_2HPO_4$, ammonium sulfate) were weighed, mixed with three solvents (ethanol, acetone, polyethylene glycol 1 000) and a certain amount of distilled water evenly in a vortex mixer, and stood to allow complete dissolution. After the aqueous two-phase system was separated, 0.5 g of sieved sweet potato leaf powder was added to perform extraction for 10 min in an ultrasound-assisted extractor at a temperature of 40 ℃ and an ultrasonic power of 100 w. Next, the system was transferred to a centrifuge tube to perform centrifugation at 10 000 r/min for 10 min. The experiment was repeated three times, and the data of the upper and lower two-phase solutions of the two aqueous phases are shown as below[9].

$$K = Ct/Cb \quad (1)$$

$$R = Vt/V1 \quad (2)$$

$$Y1(\%) = R \times K/(1 + R \times K) \times 100 \quad (3)$$

$$Y2 = mt/ms \quad (4)$$

Ct is the concentration of sweet potato leaf polysaccharides in the upper phase of aqueous two-phase system (mg/ml); Cb is the concentration of sweet potato leaf polysaccharides in the lower phase of aqueous two-phase system (mg/ml); mt is the mass of polysaccharide in the aqueous two-phase system (mg); ms is the total mass of sweet potato leaf powder added (g); Vt is the volume of the upper phase in aqueous two-phase system (ml); Vb is the volume of the lower phase volume of aqueous two-phase system (ml); R is the volume ratio of the lower and upper phases in the aqueous two-phase system; K is the distribution coefficient of polysaccharides in the aqueous two-phase system, the lower phase to the upper phase; Y1 is the yield of polysaccharides in the lower phase (%); and Y2 is the yield of polysaccharides in the lower phase (mg/g).

**Determination of polysaccharide content in sweet potato leaves**

(1) Preparation of 5% phenol solution: Five grams of phenol standard sample was weighed, and dissolved and diluted in a beaker. The obtained solution was transferred to a 100 ml volumetric flask and diluted to constant volume[3].

(2) Drawing of glucose standard curve: First, 25 mg of glucose standard was accurately weighed into a beaker, and added with water to dissolve it completely. The obtained solution was then transferred to a 250 ml volumetric flask and diluted with water to constant volume to obtain a solution with a concentration of 0.1 mg/ml. Next, 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the solution were added, respectively, into a test tube, which was added with 1 ml of 5% phenol solution and 5 ml of concentrated sulfuric acid. Each solution was shaken well and cooled to room temperature. With distilled water as the blank, the absorbance value A was measured using a UV spectrophotometer at 488 nm. Based on the experimental data, a standard curve was drawn to calculate a linear regression equation. The experiments were all repeated three times.

(3) Determination of polysaccharide content in the sample[10]: First, 1.0 ml of the upper and lower phases of the aqueous two-phase system were added into a test tube, which was added with 1 ml of 5% phenol solution and 5 ml of concentrated sulfuric acid. Each solution was shaken well and stood to room temperature. The absorbance values were measured according to the method the same as that of the glucose standard curve, and the concentrations in the upper and lower phases of the aqueous two-phase system were calculated according to the formula.

**Drawing of phase diagram for ammonium sulfate/ethanol aqueous two-phase system** A 40% ammonium sulfate solution was prepared. In specific, 3 g of ammonium sulfate was weighed into a conical flask, into which ethanol was dropped slowly with continuous shaking for mixing thoroughly. The clarity of the liquid was observed until the liquid in the test tube became turbid, and the mass of ethanol added was recorded. Next, 1 g of distilled water was added. When the solution became clear, ethanol was added into the test tube again while mixing well continuously until the liquid in the test tube became turbid. The operation was repeated like this. The mass fractions of ethanol and ammonium sulfate in the total amount of the system were calculated each time when turbidity was reached. With the mass fraction of ammonium sulfate as the abscissa and the mass fraction of ethanol as the ordinate, a two-phase phase diagram was drawn. The experiments were all repeated three times in parallel.

**Single-factor optimization experiment** According to the extraction conditions of sweet potato leaves under "Selection of aqueous two-phase system" as fixed reaction conditions, one of the different reaction conditions was changed while keeping other

conditions unchanged, as below:

① Mass concentration of ammonium sulfate (17%, 19%, 21%, 23%, 25%); ② Ethanol mass concentration (19%, 23%, 27%, 31%, 35%); ③ Different liquid to material ratios (20, 30, 40, 50, 60 g/ml); ④ Ultrasonic treatment time (15, 20, 25, 30, 35 min); ⑤ Ultrasonic treatment temperature (25, 30, 35, 40, 45 ℃).

Different ultrasound-assisted ethanol/ammonium sulfate aqueous two-phase extraction experiments were conducted, with the yield and distribution coefficient of sweet potato leaf polysaccharides as reference values. The effects of different reaction conditions on the extraction efficiency of polysaccharides from sweet potato leaves were observed. The experiments were all repeated three times in parallel.

**Response surface optimization experiment**

The extraction process of sweet potato leaf polysaccharides was further optimized through response surface methodology with the results of single-factor experiments as a data reference. With the three main factors that affected the yield of sweet potato leaf polysaccharides (ethanol mass percent, ultrasonic treatment time, liquid-to-material ratio) as variables and the polysaccharide yield as the response value, the Box Behnken experimental design method in curved surface response was adopted to optimize the process parameters of ultrasound-assisted extraction of sweet potato leaf polysaccharides. The experimental factors and levels of response surface are shown in Table 1[4-5].

**Table 1 Table of experimental factors and levels of response surface**

| Factor | -1 | 0 | 1 |
|---|---|---|---|
| Ethanol concentration | 23 | 25 | 27 |
| Liquid-to-material ratio | 45 | 50 | 55 |
| Ultrasonic treatment time | 30 | 35 | 40 |

Data analysis: Response surface experiment design and data analysis were conducted using the experimental assistant Design Expert 6.0.8, and all experiments were repeated three times in parallel.

## Results and Analysis

**Analysis of standard glucose curve**

The regression curve equation was $A = 67.073C - 0.0042$ ($R^2 = 0.993$, where A represents absorbance and C represents concentration (mg/ml)).

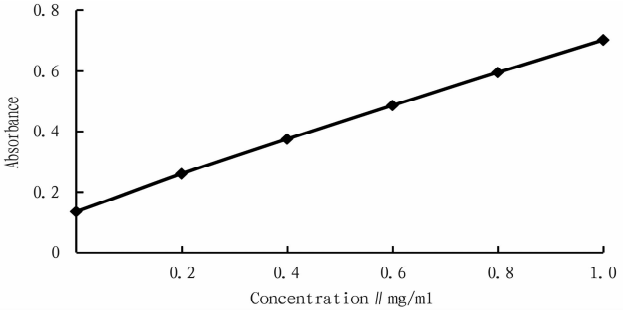


**Fig. 1 Glucose standard curve**

**Selection of aqueous two-phase system**

The inorganic salt solution competed with the solvent for water molecules to form associating hydrates, forming an aqueous two-phase system. The distribution of sweet potato leaf polysaccharides in the organic reagent/inorganic salt aqueous two-phase system is shown in Table 2. Sweet potato leaf polysaccharides were enriched in the lower phase of the aqueous two-phase system, and the experimental aqueous two-phase systems all yielded a good extraction rate. However, there were significant differences in the yield of polysaccharides in the lower phase under different systems, indicating significant differences in the distribution coefficient among different systems. In different systems, the ammonium sulfate/ethanol system had the best extraction effect, with a yield up to 19.584 mg/g. In six different systems, using the same inorganic salt, it could be seen from the experimental data chart that the extraction capacities of different organic solvents to sweet potato leaf polysaccharides ranked as ethanol > acetone > polyethylene glycol. For inorganic salts in the systems, $(NH_4)_2SO_4$ showed the best polysaccharide extraction capacity, followed by $K_2HPO_4$. In summary, the ethanol/ammonium sulfate aqueous two-phase system was selected, and the yield of sweet potato leaf polysaccharides in the lower phase of the system and the distribution coefficient in the experimental data were used as main indexes for further exploration[6].

**Table 2 Selection of aqueous two-phase system**

| | Ethanol | Acetone | Polyethylene glycol |
|---|---|---|---|
| $NH_4SO_4$ | | | |
| R | 0.75 | 1.022 | 1.25 |
| K | 2.542 | 1.7155 | 1.142 |
| Y1 (%) | 65.59 | 63.637 | 58.805 |
| Y2 (mg/g) | 19.584 | 17.578 | 13.77 |
| $K_2HKO_4$ | | | |
| R | 0.379 | 0.533 | 0.733 |
| K | 3.547 | 2.172 | 1.610 |
| Y1 (%) | 57.344 | 53.65 | 54.131 |
| Y2 (mg/g) | 16.85 | 16.70 | 16.368 |

**Analysis of ethanol/ammonium sulfate aqueous phase diagram**

The phase diagram obtained from the ethanol/ammonium sulfate aqueous two-phase system is shown in Fig. 2. The line divides the phase diagram into two parts, of which the upper part is a two-phase zone, and the lower part is a single-phase zone, without phase separation; and in the two-phase zone, the upper phase is rich in ethanol, and the lower phase is rich in ammonium sulfate solution. It could be concluded from the figure that the higher the mass fraction of ammonium sulfate in the system, the lower the volume fraction of ethanol required for phase separation. Furthermore, the larger the amount of ethanol added, the smaller the amount of salt needed for phase separation. Meanwhile, as shown in Fig. 2, when the mass fraction of ammonium sulfate is a certain value, only when the mass fraction of ethanol is higher than the corresponding minimum value, *i.e.*, the phase diagram line, can phase separation be ensured. For example, when the mass fraction

of ammonium sulfate is 20%, the mass fraction of ethanol in the entire system must reach over 20% to form a stable aqueous two-phase system. Therefore, the experimental points for aqueous two-phase extraction should be selected above the curve, and the mass fractions of ethanol and ammonium sulfate needed to be coordinated with each other[7].

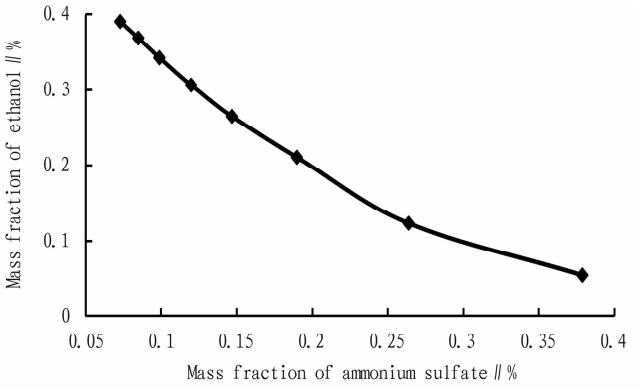


**Fig. 2 Ethanol/ammonium sulfate aqueous phase diagram analysis**

## Single-factor experiment on extracting polysaccharides from sweet potato leaves

**Effects of ammonium sulfate concentration on polysaccharide extraction** Fig. 3 shows that when the mass concentration of ammonium sulfate was within 18% -23%, the yield and distribution coefficient of sweet potato leaf polysaccharides also increased with the increase of ammonium sulfate mass concentration. However, when it exceeded 20%, the yield and distribution coefficient of sweet potato leaf polysaccharides decreased rather than increase. It might be because as the mass concentration of ammonium sulfate increased, the associative ability of the lower phase increased, leading to an indirect increase in the concentration of sweet potato leaf polysaccharides in the lower phase, making it easier for sweet potato leaf polysaccharides to dissolve in the lower phase. However, under the condition that the total volume of the system remained unchanged, the mass of the lower phase increased, and the amount of water added decreased, the volume of the lower phase decreased, and the dissolved polysaccharides decreased. The yield and distribution coefficient of polysaccharides first increased and then decreased. Therefore, it was determined that a mass fraction of 20% for ammonium sulfate yielded the optimal extraction rate.

**Effects of ethanol concentration on polysaccharide extraction** As shown in Fig. 4, with the increase of ethanol concentration, the yield of sweet potato leaf polysaccharides increased, and the distribution coefficient first increased and then decreased. The reason might be that as the concentration of ethanol increased, the mass of added water decreased, and the competitiveness of ethanol increased, and the concentration of ammonium sulfate increased, making it easier for polysaccharides to be extracted by ammonium sulfate solution in the next stage. As a result, the yield and distribution coefficient of polysaccharides in sweet potato leaves increased. However, as the concentration of ethanol increased, the system became unstable, and a small amount of polysaccharide crystals precipitated in the ammonium sulfate solution in the next stage. Thus, the yield of polysaccharides did not significantly increase, and the distribution coefficient also decreased. Therefore, an ethanol mass fraction of 25% was chosen to achieve the optimal extraction rate.

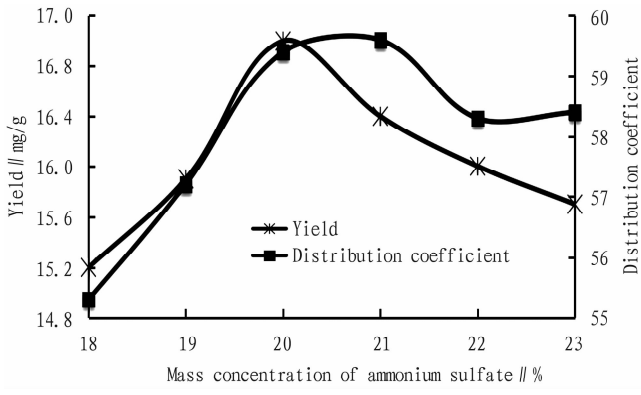


**Fig. 3 Effect of ammonium sulfate mass concentration on polysaccharide extraction**

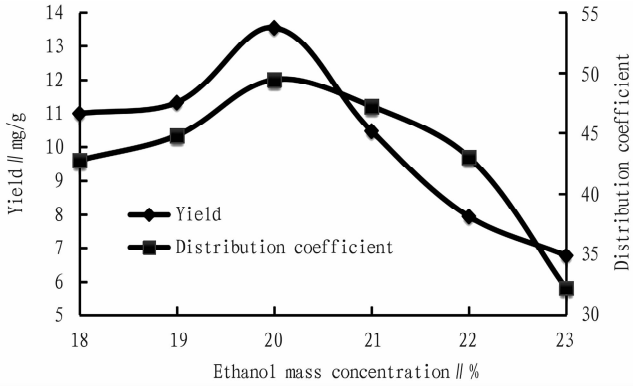


**Fig. 4 Effects of ethanol mass concentration on polysaccharide extraction**

**Effects of material-to-liquid ratio on polysaccharide extraction** As shown in Fig. 5, as the liquid-to-material ratio increased, the yield of polysaccharides in sweet potato leaves showed a continuous increasing trend, but after the liquid-to-material ratio reached 50 mg/g, the yield curve gradually tended to be gentle, while the distribution coefficient showed a trend of first increasing and then decreasing, and reached its maximum value at the liquid-to-material ratio of 50 mg/g. From the experimental chart curve, it could be seen that after the liquid-to-material ratio reached 50 mg/g, the yield of sweet potato leaf polysaccharides increased slowly, and the distribution coefficient curve in the lower phase decreased rapidly. Therefore, considering cost savings, a liquid-to-material ratio of 50 mg/g could achieve maximum efficiency.

**Effects of ultrasonic treatment time on polysaccharide extraction** Fig. 6 shows that the yield of sweet potato leaf polysaccharides generally exhibited a trend of first increasing and then decreasing with the prolongation of ultrasound time, and the distribution coefficient shows a rough distribution of first increasing, then decreasing, and then increasing with the prolongation of ultrasound time. At the ultrasonic treatment time of 35 min, the

polysaccharide yield reached its peak, while the distribution coefficient reached its maximum values at 35 and 50 min. However, comparison of the two points showed that the distribution coefficient was the highest at 35 min. Based on the above analysis, it could be seen that the ultrasonic extraction time was the best at 35 min, which took into account both the yield and the maximum distribution coefficient.

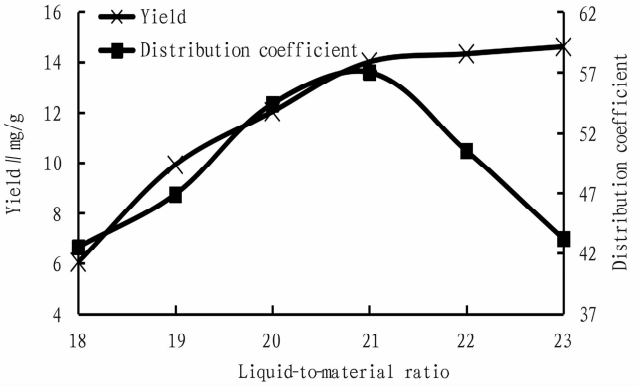


**Fig. 5 Effect of liquid-to-material ratio on the extraction of sweet potato leaf polysaccharides**

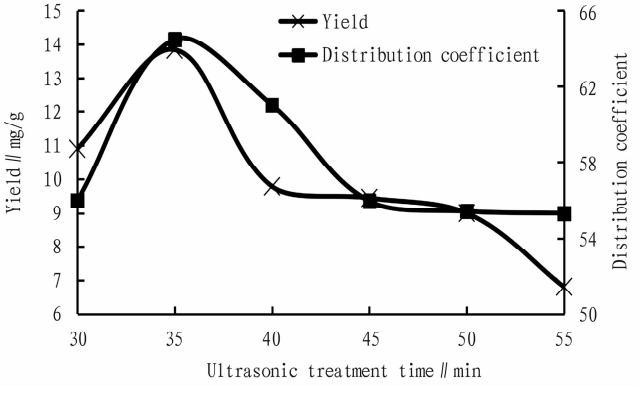


**Fig. 6 Effects of ultrasonic treatment time on extraction of aqueous polysaccharides**

**Effects of ultrasonic treatment temperature on polysaccharide extraction** Fig. 7 shows that in the range of ultrasonic extraction temperature from 40 to 65 ℃, as the extraction temperature increased, the yield and distribution coefficient of sweet potato leaf polysaccharides gradually increased. However, after exceeding 50 ℃, as the temperature continued to increase, the yield and distribution coefficient of polysaccharides no longer increased but began to decrease. The reason might be that when the ultrasonic extraction temperature was low, the polysaccharides from sweet potato leaves could not be completely dissolved, and as the temperature increased, the mass transfer rate accelerated, and the diffusion coefficient increased, and the viscosity of the extraction solution decreased, leading to more complete extraction. However, as the temperature exceeded 50 ℃, the entire system gradually became unstable, which was not conducive to the dissolution of polysaccharides, leading to a decrease in polysaccharide yield and distribution coefficient. Therefore, considering the yield and distribution coefficient, it was determined that the optimal ultrasonic treatment temperature should be 47 ℃.

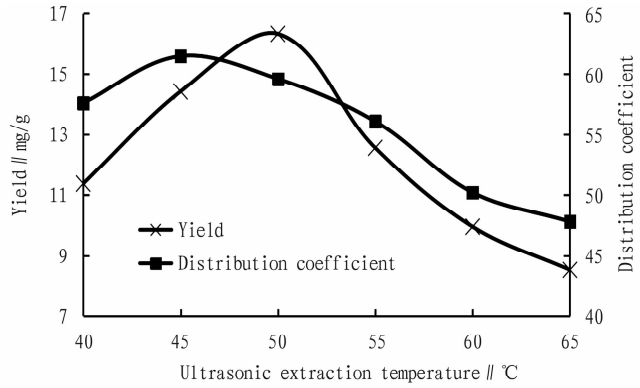


**Fig. 7 Effects of ultrasonic extraction temperature on polysaccharide extraction**

**Response surface experimental design and results**

On the basis of single-factor experiments, a three-factor three-level response surface experiment was carried out, with ethanol concentration, liquid-to-material ratio, and ultrasonic treatment time as independent variables, and the yield of sweet potato leaf polysaccharides Y2 as the response value. According to the box-behnken model, it was found that there was a significant impact on the ultrasound-assisted extraction rate of polysaccharides in the aqueous two-phase system[9-10]. The experimental design and results are shown in Table 3.

**Table 3 Response surface experimental design table**

| Run | A: Ethanol // % | B: Liquid-to-material ratio | C: Time // min | Y2 // mg/g |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 19.02 |
| 2 | 0 | 1 | -1 | 18.46 |
| 3 | -1 | 0 | 1 | 16.53 |
| 4 | 0 | -1 | -1 | 17.54 |
| 5 | 0 | -1 | 1 | 18.69 |
| 6 | 0 | 0 | 0 | 19.04 |
| 7 | 1 | 0 | 1 | 18.45 |
| 8 | 1 | 1 | 0 | 19.82 |
| 9 | 0 | 0 | 0 | 18.78 |
| 10 | 0 | 1 | 1 | 20.50 |
| 11 | 1 | 0 | -1 | 16.79 |
| 12 | -1 | 1 | 0 | 17.70 |
| 13 | 0 | 0 | 0 | 19.16 |
| 14 | -1 | 0 | -1 | 15.67 |
| 15 | 1 | -1 | 0 | 18.21 |
| 16 | -1 | -1 | 0 | 17.12 |
| 17 | 0 | 0 | 0 | 19.06 |

**Analysis of response surface experiment results and regression equations**

The regression equation was Y2 = +19.01 +0.7 * A +0.6 * B +0.7 * C +0.26 * A * B +0.20 * A * C +0.22 * B * C -1.37 * A2 +0.57 * B2 -0.78 * C2 (Analysis software: Design expert 8.0.6).

Design Expert V8.0.6.1 software was employed to perform

analysis of variance on the response surface data in Table 4, as shown in Table 4. The $R^2$ of the regression equation was 0.983 7, indicating that the model could explain a response value change of 98.37%, and there was a good linear relationship between various factors and polysaccharide extraction rate. The $P$ value of lack of fit was 0.317 5, indicating no significant difference and a good degree of fitting. $P<0.01$ indicated a significant difference. Therefore, the extraction rate of sweet potato leaf polysaccharides could be analyzed and predicted[11-12].

**Table 4 Variance analysis results of response surface optimization model**

| | Sum of squares Prob > $F$ | $df$ | Average source | $F$ | $P$ |
|---|---|---|---|---|---|
| Model | 24.44 | 9 | 2.72 | 108.58 | <0.000 1 * * |
| A-ethanol | 4.88 | 1 | 4.88 | 195.25 | <0.000 1 * * |
| B-liquid-to-material ratio | 3.03 | 1 | 3.03 | 120.99 | <0.000 1 * * |
| C-time | 4.08 | 1 | 4.08 | 162.97 | <0.000 1 * * |
| AB | 0.27 | 1 | 0.27 | 10.61 | 0.013 9 * |
| AC | 0.16 | 1 | 0.16 | 6.40 | 0.039 3 * |
| BC | 0.20 | 1 | 0.20 | 7.92 | 0.026 0 * |
| A2 | 7.89 | 1 | 7.89 | 315.32 | <0.000 1 * * |
| B2 | 1.36 | 1 | 1.36 | 54.51 | 0.000 2 * * |
| C2 | 2.58 | 1 | 2.58 | 103.36 | <0.000 1 * * |
| Error term | 0.18 | 7 | 0.025 | | |
| Lack of fit | 0.096 | 3 | 0.032 | 1.63 | 0.317 5 |
| Pure error | 0.079 | 4 | 0.020 | | |
| All terms | 24.61 | 16 | | | |

### Analysis of response surface experiment results and regression equation

The experimental results were subjected to response surface analysis by Design Expert 8.0.6 software. The response surface diagrams of the interaction between different two factors on the yield of sweet potato leaf polysaccharides are shown in Fig. 8 – Fig. 10.

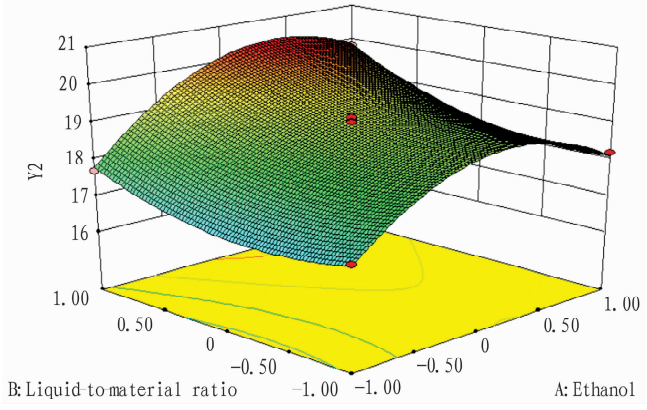


**Fig. 8 Response diagram of liquid-to-material ratio and ethanol**

Fig. 8 – Fig. 10 shows that among the selected factors, ethanol concentration and liquid-to-material ratio had the greatest impact on the extraction rate of sweet potato leaf polysaccharides, which was also consistent with the regression analysis results in Table 4. The $P$ values corresponding to ethanol concentration and liquid-to-material ratio were both less than 0.01, reaching an extremely significant level. The optimal process parameters for ultrasound-assisted aqueous two-phase extraction of sweet potato leaf polysaccharides were determined by analyzing the response surface regression equations to be an ethanol concentration of 25.68%, a liquid-to-material ratio of 54.83, and ultrasonic treatment time of 38.33 minutes, with which the optimal yield was 26.646%[13-14].

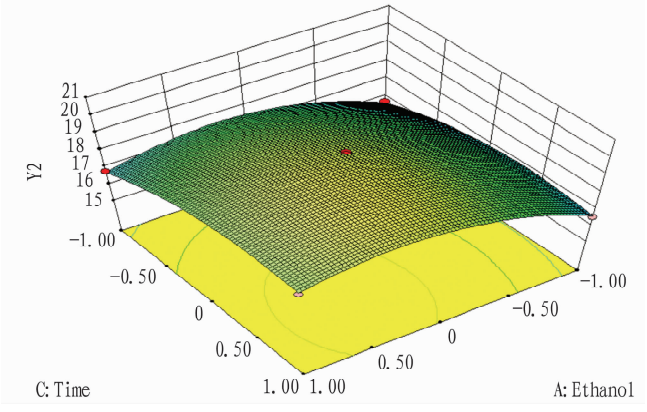


**Fig. 9 Response diagram of ethanol and time**

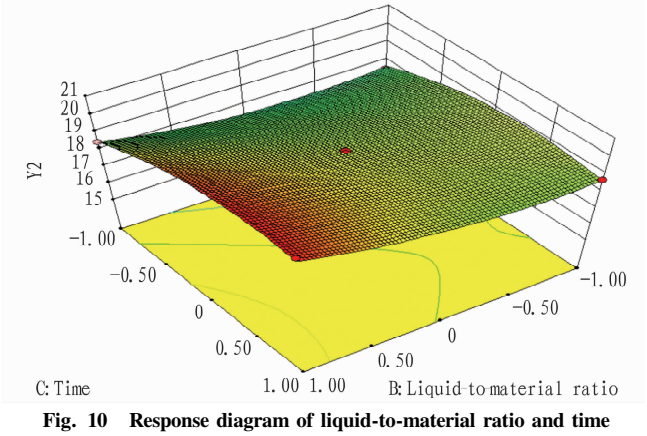


**Fig. 10 Response diagram of liquid-to-material ratio and time**

## Conclusions

In this study, ultrasound-assisted aqueous two-phase extraction was adopted to extract sweet potato leaf polysaccharides, in order to improve the extraction efficiency of sweet potato leaf polysaccharides. The main conclusions were as follows:

In the selection of the aqueous two-phase system, six groups of organic reagent/inorganic salt aqueous two-phase systems were used to extract polysaccharides from sweet potato leaves to test their capacities to extract polysaccharides. From the experimental data, it could be seen that the extraction rates of sweet potato leaf polysaccharides ranked as ethanol > acetone > polyethylene glycol. In terms of the polysaccharide extraction capacities of inorganic salts in the system, ammonium sulfate was the best, followed by $K_2HPO_4$. In summary, the ethanol/ammonium sulfate aqueous two-phase system was the best.

In order to further improve the extraction rate of polysaccharides from sweet potato leaves, ammonium sulfate mass concentration, ethanol mass concentration, liquid-to-material ratio, ultrasonic

treatment time and ultrasonic treatment temperature were selected to conduct single-factor experiments. Through data and line chart, it was determined that the best parameters were ammonium sulfate mass fraction 20% , ethanol mass fraction 25% , liquid-to-material ratio 50, ultrasonic extraction time 35 min, and ultrasonic extraction temperature 47 ℃.

On the basis of single-factor experiments, the surface response method was used to further optimize the extraction conditions, and the effects of ethanol concentration, liquid-to-material ratio and ultrasonic treatment time on the extraction efficiency of sweet potato leaf polysaccharides were studied. The experimental data results showed that the optimal parameters were ethanol concentration 25.68% , liquid-to-material ratio 55.83, and ultrasonic treatment time 38.33 min. Under these conditions, the yield of sweet potato leaf polysaccharides could reach 20.646 mg/g.

## *References*


[1] SHEN MR, LI YL, YU YL, *et al.* Ultrasonic-assisted aqueous two-phase extraction of flavonoids from tartary buckwheat seed[J]. Science and Technology of Food Industry, 2016, 37(15): 243 – 248, 275. (in Chinese).

[2] ZHAO QZ, MENG W, LIU YT, *et al.* Optimization for extraction process of ferulic acid from fermented grains of sesame-flavor Baijiu by response surface methodology [J]. China Brewing, 2022, 41(11): 174 – 179. (in Chinese).

[3] PANG TC, ZHONG QP, XIONG Z, *et al.* Extraction process optimization of *Suaeda salsa* polysaccharides by response surface methodology and analysis of its antioxidation activity[J]. Guangxi Agricultural Sciences, 2015, 46(7): 1280 – 1286. (in Chinese).

[4] LI J, GOU LT, CHEN J, *et al.* Primary study on extraction of seed protein from *Prunus humilis* by aqueous two-phase system[J]. Seed, 2014, 33(3): 82 – 86. (in Chinese).

[5] ZHU XT, HOU YQ, NIAN MM, *et al.* Extraction and antioxidant activities of polysaccharides from Phellinus linteus[J]. Natural Product Research and Development, 2016, 28(5): 761 – 765, 744. (in Chinese).

[6] LI P, XUE H, XIAO M, *et al.* Ultrasonic-assisted aqueous two-phase extraction and properties of water-soluble polysaccharides from *Malus hupehensis*[J]. Molecules. 2021, 12, 26(8): 2213

[7] ZHANG LF, LIU Y, WANG Y, *et al.* Optimization of ultrasonic assisted enzyme method extraction technique on shaddock peel polysaccharides by response surface methodology[J]. Science and Technology of Food Industry, 2018, 39(5): 146 – 150. (in Chinese).

[8] SONG LF, LIU CQ, LI MS, *et al.* Optimization of two-phase aqueous extraction for Astragali Radix by central composite design-response surface method[J]. Chinese Traditional Patent Medicine, 2017, 39(1): 70 – 75. (in Chinese).

[9] DU PY, WANG LH, LIU DJ, *et al.* Response surface methodology for optimizing extraction of polysaccharides from *Ganoderma applanatum*[J]. Journal of Anhui Agricultural Sciences, 2018, 46(34): 148 – 151, 168. (in Chinese).

[10] LI XL, MA CC, DENG HS. Optimization of extraction process of total flavonoids from *Senecio scandens* by response surface methodology[J]. Shaanxi Journal of Agricultural Sciences, 2018, 64(7): 61 – 65, 95. (in Chinese).

[11] WANG XX, TAN F, YAO S, *et al.* Studies on ultrasound-assisted extraction process of total flavonoids in *Vaccinium bracteatum* Thunb. leaves[J]. China Pharmacist, 2015, 18(1): 66 – 68. (in Chinese).

[12] LIU YX, LI S. Optimization of microcrystalline cellulose extraction from apple pomace using response surface methodology[J]. Feed and Animal Husbandry, 2018(11): 56 – 60. (in Chinese).


Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

(Continued from page 105)


[10] Selenium Industry Technology and Health China Innovation Platform Union. Blue book on the development of selenium rich agriculture in China (2017 edition)[M]. Beijing: China Agricultural University Press, 2019. (in Chinese).

[11] YU J. Combined transcriptome and metabolomics analysis of the effects of exogenous inorganic selenium on flavone genes CHS, FLS, PAL and FOMT in ginkgo leaves[D]. Wuhan: Wuhan Polytechnic University, 2018. (in Chinese).

[12] TIAN M, XU XY, LIU FX, *et al.* Untargeted metabolomics reveals predominant alterations in primary metabolites of broccoli sprouts in response to pre-harvest selenium treatment[J]. Food Research International, 2018(111): 205 – 211.

[13] HAN WN, LI XG, LIU H, *et al.* Comparative analysis of health-benefiting components in selenium-enriched and non-selenium-enriched *Amaranthus caudatus* L.[J]. Food Science, 2021, 42(22): 208 – 215. (in Chinese).

[14] LUO ZZ. Non-targeted metabolomics study on effects of yeast selenium on plasma metabolites during labor stress in dairy cows[D]. Ya'an: Sichuan Agricultural University, 2018. (in Chinese).

[15] WU T. Metabolomics study on selenium deficiency in *Procapra przewalskii*[D]. Mianyang: Southwest University of Science and Technology, 2020. (in Chinese).

[16] JIN Z. Effects of different levels of sodium selenite and oil treatment on growth performance, lipid composition, and metabolism of Rex rabbits [D]. Changchun: Jilin University, 2021. (in Chinese).

[17] ZHANG K, HAN YS, ZHAO QY, *et al.* Targeted metabolomics analysis reveals that dietary supranutritional selenium regulates sugar and acylcarnitine metabolism homeostasis in pig liver[J]. Journal of Nutrition, 2020, 150(4): 704 – 711.

[18] HU ZY, CHENG YX, NORIYUKI SUZUKI, *et al.* Speciation of selenium in brown rice fertilized with selenite and effects of selenium fertilization on rice proteins[J]. International Journal of Molecular Sciences, 2018(19): 3494.

[19] YANG JC, PENG SB, GU SL, *et al.* Changes in zeatin and zeatin riboside content in rice grains and roots during grain filling and the relationship to grain plumpness[J]. Acta Agronomica Sinica., 2001(1): 35 – 42. (in Chinese).


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