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Research Article

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Optimization of ultrasonic -microwave synergistic extraction of anthocyanins from *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem

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ABSTRACT

To improve the extraction rate of Acanthopanax sessiliflorus (Rupr. et Maxim.) Seem anthocyanins, the method of ultrasonic-microwave synergistic extraction (UMSE) was used. Response surface methodology (RSM) using a central composite design (CCD) was employed to optimize the conditions of ultrasonic-microwave synergistic extraction (UMSE), and the mathematical model was constructed. The results show that ultrasonic power 120W, microwave power 180W, and extraction time 6.7min, the anthocyanins extraction rate of Acanthopanax sessiliflorus (Rupr. et Maxim.) Seem is 83.42%.

Key words: ultrasonic; microwave; anthocyanins; *Acanthopanax sessiliflorus (Rupr. et Maxim.)* Seem

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INTRODUCTION

Acanthopanax sessiliflorus (Rupr. et Maxim.) Seem belongs to the Araliaceae, Acanthopanax, and grown in northeast China and Japan, Korea, Russia [1]. It has the efficacy of immunomodulatory, anti-tumor, anti-aging, anti-radiation and anti-fatigue [2-3]. The berry is dark purple and rich in anthocyanins, contains all essential amino acids and essential trace elements such as Fe, Se, Cu, Mn, etc. *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins are water-soluble and safe non – toxic natural pigments. New research shows that it has broad application prospects with the physiological activity of scavenge free radicals, improve liver metabolic, hypolipidemic, anti-cell mutation, anti-aging, prevent atherosclerosis, improve vision, etc [4-5].

In recent years, ultrasonic-assisted extraction has been applied extensively in food and medical industries due to its advantages of short extraction time, simple operation, good reproducibility and relatively high efficiency, etc [6-7]. Microwave-assisted extraction has been widely used as a sample preparation technique in different analytical fields such as food and agricultural [8-9]. It requires less solvent consumption and lower energy consumption, higher selectivity [10-11]. Extracting plant polysaccharides and other biologically active substances have been reported by ultrasound - microwave synergistic extraction (UMSE) [12]. However response surface methodology (RSM) using a central composite design (CCD) was employed to optimize the conditions of ultrasound - microwave synergistic extraction (UMSE) anthocyanins from *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem by for the first time.

EXPERIMENTAL SECTION

MATERIALS

The *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem was picked from Changbai mountain (Tonghua, China). 95% ethanol, concentrated hydrochloric acid, all of analytical grade, purchased from Changchun Chemical Reagent Factory (Changchun, China).

THE EXTRACTION OF ANTHOCYANINS

The *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem berrys were washed, dried at 50 ℃ and crushed. *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem powder was added to solid-liquid ratio 1:10 pH2, 40% ethanol solution. The anthocyanins were extracted by 50℃ temperature at different extracting time, ultrasonic power and microwave power by SL-SM50 ultrasound - microwave reaction systems synergistic extraction (Nanjing Shunliu instrument Co., Ltd.). The extracts were diluted, constanted volume, standed, filtred, centrifuged at 4000 rpm for 10 min, and then measured the absorbance at wavelength of 525nm by TV-1901 UV-visible spectrophotometer (Beijing PERSEE Co., Ltd.). The extraction rate of *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins was calculated, the process conditions were optimized [13-14].

DETERMINATION OF ANTHOCYANINS CONTENT

1ml anthocyanins extract was diluted respectively to 10ml pH1.0 and pH4.5 buffer solution, and then measured the absorbance at 525nm. Anthocyanins content was calculated by method of pH differential method.

$C(mg/g)= (A_0-A_1)\times V\times n\times M/\varepsilon\times m$

A_0-the absorbance at wavelength of 525nm of pH1.0 anthocyanins, A_1- the absorbance at wavelength of 525nm of pH4.5 anthocyanins, V- the total volume of extract(ml), n-dilution factor, M- the relative molecular mass of cyanidin-3- glucoside(449), ε- the extinction coefficient(29600), m- the sample weight (g).

DETERMINATION OF ANTHOCYANINS EXTRACTION RATE

The percentage anthocyanins extraction rate (%) is calculated as the anthocyanins content of extract divided by the total anthocyanins content.

EXPERIMENTAL DESIGN

On the basis of single factor test, the effect of three independent variables ultrasonic power (X_1), microwave power (X_2), extraction time (X_3) on extraction rate (Y) was investigated using a three-factor CCD-RSM experimental, the factors and levels are shown in Table 1.

Table 1: Response surface analysis factors and levels

Levels	Factors		
	X_1 ultrasonic power /W	X_2 microwave power /W	X_3 extraction time / min
-1	75	100	5
0	100	150	8
1	125	200	11

RESULTS AND DISCUSSION

MATHEMATICAL MODELS AND ANALYSIS OF SIGNIFICANT

Response surface analysis results was shown in Table 2, *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins extraction rate(Y) for coding arguments X_1, X_2, and X_3 secondary multiple regression equation is obtained by quadratic multiple regression fitting of Design Expert 8.0.6 software.

The significance test and variance analysis of regression equation at the significance level α = 0.05, analysis of variance for response surface quadratic model was shown in Table 3. F =211.04, $P<0.0001$,regression models extremely significant. The regression coefficient test shows that the coefficient of determination R^2=0.9916 , and 99.16% of the test data can be explained by the model. Lack of fit P=0.1822＞0.05 and not significant showed that the predictive value and the actual value of model is high fitting. In addition, the analysis of variance of the regression equation showed that the effect of X_1, , X_2 , X_3, X_1X_3, X_1^2 , X_2^2 , X_3^2 on anthocyanins extraction rate is extremely significant($P<0.01$), the effect of X_2X_3 is significant($P<0.05$), and the effect of various factors on *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins extraction rate is not a simple linear relationship. The greatest impact of anthocyanins extraction rate is microwave power and ultrasonic power, extraction time secondly. Removing items not significant of α= 0.05, gets the following regression equation: $Y=83.30+1.13X_1+1.22X_2-0.32X_3-0.65X_1X_3+0.18X_2X_3-1.04X_1^2-0.94X_2^2-0.75X_3^2$。The simplified regression equation R=0.9957 showed the fit of regression models are better.

Table 2: Response surface analysis results

Run	Factors			
	X_1 ultrasonic power /W	X_2 microwave power /W	X_3 extraction time / min	extraction rate /%
1	-1	0	-1	80.19
2	0	-1	1	79.96
3	0	1	-1	82.88
4	0	-1	-1	80.78
5	-1	-1	0	79.05
6	-1	1	0	81.22
7	0	0	0	83.22
8	0	0	0	83.25
9	1	1	0	83.84
10	0	1	1	82.79
11	-1	0	1	80.67
12	1	-1	0	81.16
13	0	0	0	83.24
14	0	0	0	83.48
15	1	0	-1	83.64
16	0	0	0	83.31
17	1	0	1	81.52

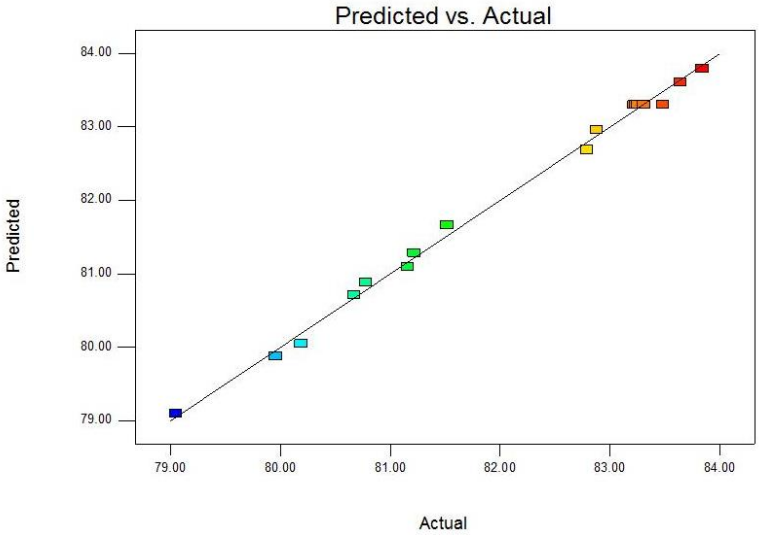

Fig..1: predicted and actual

Table 3: Analysis of variance for response surface quadratic model

Source	Squares	df	Square	Value	Prob > F	significance
Model	36.77	9	4.09	211.04	< 0.0001	**
X_1	10.19	1	10.19	526.46	< 0.0001	**
X_2	11.96	1	11.96	617.54	< 0.0001	**
X_3	0.81	1	0.81	41.98	0.0003	**
$X_1 X_2$	0.07	1	0.07	3.36	0.1095	
$X_1 X_3$	1.69	1	1.69	87.29	< 0.0001	**
X_2X_3	0.13	1	0.13	6.88	0.0342	*
X_1^2-	4.55	1	4.55	235.22	< 0.0001	**
X_2^2	3.74	1	3.74	193.19	< 0.0001	**
X_3^2	2.40	1	2.40	123.97	< 0.0001	**
Residual	0.14	7	0.019			
Lack of Fit	0.091	3	0.030	2.68	0.1822	not significant
Pure Error	0.045	4	0.011			
Cor Total	36.91	16				

***.P <0.01,extremely significant difference, *.P <0.05,significant difference.*

RESPONSE SURFACE ANALYSIS

Analysis of the data by design Expert 8.0.6 software in Table 2, the response surfaces and contour plots of Figures 2 show that anthocyanins extraction rate increased gradually with the increase of ultrasonic power under the conditions of extraction time 5min and fixed microwave power. Anthocyanins extraction rate increased insignificantly with the increase of ultrasonic power after reaching the maximum. Comprehensive analysis shows that interaction of ultrasonic power and microwave power influence on anthocyanins extraction rate is not

significant.

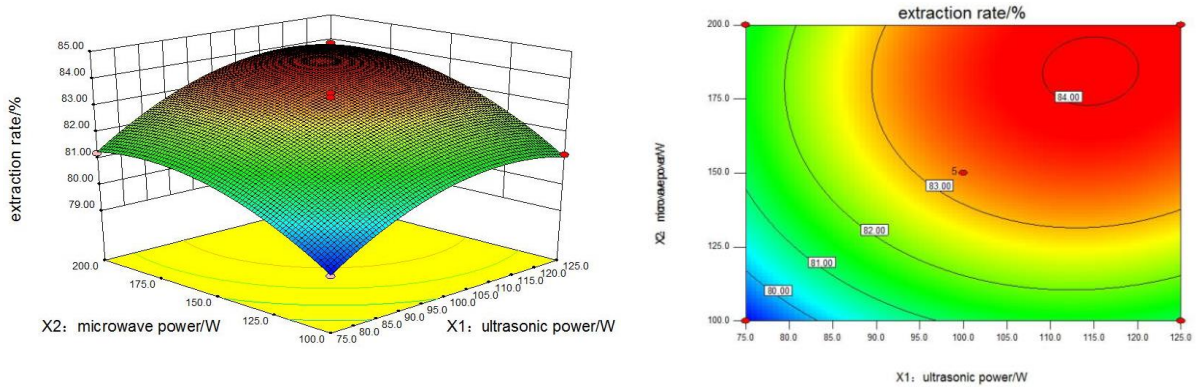

Fig.2 Response surfaces and contour plot showing the effects of ultrasonic power and microwave power on extraction rate

The response surfaces and contour plots of Figures 3 show that anthocyanins extraction rate increased gradually with the increase of ultrasonic power under the conditions of microwave power 150W and extraction time 5-7min. Anthocyanins extraction rate decreased significantly with the increase of ultrasonic power after reaching the maximum under the conditions of extraction time 7-11min. Comprehensive analysis shows that interaction of ultrasonic power and extraction time influence on anthocyanins extraction rate is extremely significant.

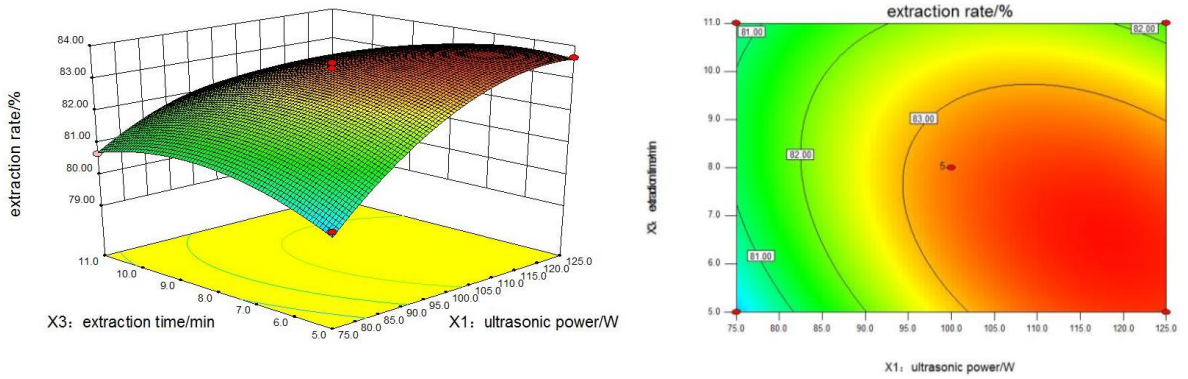

Fig.3 Response surfaces and contour plot showing the effects of ultrasonic power and extraction time on extraction rate

The response surfaces and contour plots of Figures 4 show that anthocyanins extraction rate increased gradually with the increase of microwave power under the conditions of ultrasonic power 100W and fixed extraction time. However, anthocyanins extraction rate significantly decreased with the increase of microwave power under the conditions of extraction for a long time. Comprehensive analysis shows that interaction of microwave power and extraction time influence on anthocyanins extraction rate is significant.

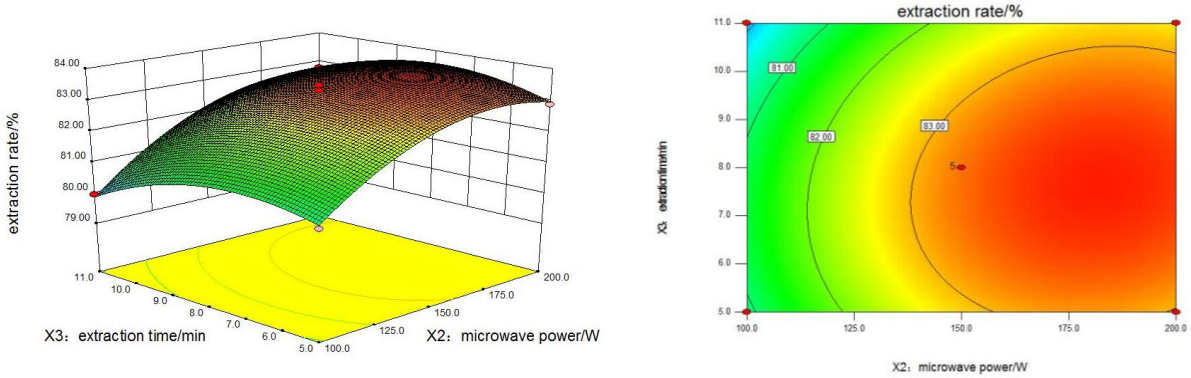

Fig.4 Response surfaces and contour plot showing the effects of microwave power and extraction time on extraction rate

Comprehensive analysis of the interaction between the various factors, the effect of ultrasonic power (X_1) and extraction time (X_3) interaction on *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins extraction rate is extremely significant($P<0.0001$), the effect of microwave power (X_2) and extraction time (X_3) interaction on *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins extraction rate is significant($P<0.0001$ 和

P=0.0342), the effect of ultrasonic Power (X_1) and microwave power (X_2) interaction on *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins extraction rate is extremely significant($P<0.0001$), the effect of microwave Power (X_2) and extraction time (X_3) interaction on *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins extraction rate is not significant. The effect of anthocyanins extraction rate in descending is microwave power, ultrasonic power and extraction time.

Optimization of UMSE of anthocyanins from *Acanthopanax.Acanthopanax sessiliflorus (Rupr. et Maxim.)* Seem is obtained by regression model mathematical analysis and software predicted: ultrasonic power 118W, microwave power 182.8W, and extraction time 6.7min. Predictive value of anthocyanins extraction rate is 84.18%.

VERIFICATION OF RESULTS

To verify the reliability of the model and the actual operation, the optimum conditions are revised ultrasonic power 120W, microwave power 180W, and extraction time 6.7min, the anthocyanins extraction rate of *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins is 83.42%, the theoretical prediction values error is only 0.9%. The results of analysis confirmed that using response surface methodology to optimize the results reliable and accurate.

CONCLUSION

In this study, response surface methodology (RSM) using a central composite design (CCD) was employed to optimize the conditions for extraction of antioxidants from *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem by ultrasound - microwave synergistic extraction (UMSE). Optimum conditions are ultrasonic power 120W, microwave power 180W, and extraction time 6.7min and *Acanthopanax sessiliflorus* (Rupr. et Maxim.) Seem anthocyanins extraction rate is 83.42%. Compared with traditional hot water extraction, the extraction rate has been greatly improved.

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