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Study on optimization of extraction process of anthocyanin from cherry wine lees

Abstract

This paper took cherry wine lees as the main raw material, and used three extraction methods (ultrasound-assisted enzymatic method, microwave extraction method, condensation reflux extraction method) to extract anthocyanin. The result showed that the extraction number of anthocyanins by ultrasonic-assisted enzymatic method was the highest. On the basis of single factor experiment, the response surface method was used for optimization. The impacts of experimental factors on extraction amount were successively the enzymolysis time, enzymolysis temperature, pH value, ultrasonic power, amount of enzyme added and solid-liquid ratio. Through polynomial regression analysis, the regression model of anthocyanin extraction rate was established, and according to the actual production condition, the following optimal process parameters were determined: enzyme addition: 1.5%; solid-liquid ratio: 1:32; pH value: 4.30; enzymolysis temperature: 54 °C; enzymolysis time: 55 min, and the ultrasonic power: 300 W. Under this condition, the extraction amount of anthocyanin from Cherry Wine lees was 4.19 mg/g.

Keywords: cherry wine lees, anthocyanin, extraction, response surface

Volume 9 Issue 1 - 2019

Chaoshuang Jia,[1] Fengmin Han,[2] Xiaoyu Miao,[1] Qiyue Zhang,[1] Aonan Yan,[1] Peng Wu[1]

[1]College of Food Science and Engineering, Shandong Agricultural University, China

[2]College of Mechanical and Electrical Engineering, Shandong Agricultural University, China

Correspondence: Chaoshuang Jia, College of Food Science and Engineering, Shandong Agricultural University, No.61 daizong street, taishan district, Tai'an City, shandong, China, Tel +86 13325216202, Email 1912363172@qq.com

Received: February 03, 2019 | **Published:** February 15, 2019

Introduction

Many plants contain biologically active compounds that have been shown to be effective in the treatment of a variety of conditions, especially those related to oxidative stress.[1,2] In this regard, the daily intake of total polyphenols is inversely proportional to the risk of cardiovascular disease, all-cause mortality and cardiovascular cancer in high-risk populations.[3] Many studies have investigated the anthocyanin content and antioxidant activity of plant extracts, including blueberry, berry and grape extracts.[1–4] In daily life, Sweet cherries are not only widely used in fresh food, but also in the production of jams, jellies, syrups and a variety of soft drink products.[5] The limited life span does not exceed 21days, which greatly limits its shelf life.[6] Cherry wine is becoming more and more popular in China.[5–7] Among them, the cherry is made into cherry wine, which not only prolongs its shelf life, but also greatly preserves the active ingredients of the fruit, thereby fully developing the cherry resources. In addition, cherry wine has a unique flavor and health function due to its rich antioxidants.[8,9]

China's cherries are rich in resources, and a large amount of cherries lees is produced during the brewing process. Direct discharge of the cherries not only pollutes the environment but also wastes resources. At present, with the expansion of cherry planting area, the output of cherry wine has increased sharply. However, a large amount of wine lees is generally used as feed or fertilizer and is not fully utilized. Because cherry liqueur is rich in anthocyanins, soluble dietary fiber and other substances, and the winemaking process has little effect on scavenging free radicals and antioxidants, if the anthocyanins are extracted from them, and this resource can be comprehensively utilized, it can be greatly improved the application and economic value of cherries. At present, there are many reports about the extraction of anthocyanins at home and abroad. The main extraction methods are microwave method, condensation reflux method, enzymatic method, ultrasonic method and soon.[10] Microwave extraction has the advantages of short time and easy operation, so it is widely used in the extraction of active ingredients.[11]

The condensing reflux extraction method is widely used in the industrial production of natural products, and has the advantages of convenient operation and low energy consumption, but also has the disadvantages of long extraction time and low efficiency. Enzymatic extraction has the advantages of mildness, rapidity and high efficiency. The ultrasonic method utilizes the action of ultrasonic waves to destroy the structure of the cell membrane and the cell wall, so that the anthocyanin can be better eluted, and has the advantages of pure extract, high efficiency, and short time.[12] Because many researches mainly focus on the separation and extraction of anthocyanins from cherry fruit, but there are few reports on the extraction technology of anthocyanins from cherry wine lees, this experiment uses cherry wine lees as raw material, using three extraction methods (ultrasound-assisted enzymatic extraction, microwave extraction, condensation reflux extraction) to extract anthocyanins. It provides guidance for the basic research of the bioactive components in cherry lees and the industrial production of the products.

Materials and methods

Materials, reagents and instruments

Cherry wine lees: Cherry wine lees separated from fermented Qinlin Cherry Wine from the Food College of Shandong Agricultural University was dried in a blast drying oven at a temperature of 40°C for about 12-18h. Then it was crushed by a multi-functional crusher and stored in a black sealed bag at 4°C until analysis. Methanol, Shandong Yuwang industrial co., LTD. Chemical branch; anhydrous sodium acetate, Shanghai Guangnuo Chemical technology co., LTD.; glacial acetic acid, Tianjin Yongda Chemical reagent co., LTD.; acetone, potassium chloride, anhydrous ethanol, Tianjin Kaitong Chemical reagent co., LTD. All of the above reagents except methanol were analytically pure, and others were of analytical grade. pH meter, METTLER TOLEDO Instrument co., LTD.; 800Y multifunctional grinder, Yong Kang Platinum Metal Products co., LTD.; FA2004 electronic analytical balance, Shanghai Jing Tian electronic instrument factory co., LTD. Fcd-2000 intelligent electric thermostatic air dryer,

Shanghai Lang Gan experimental equipment co., LTD. SHB - type multi-purpose III circulating water vacuum pump, Zhengzhou Great Wall industry &trade co., LTD. G80F20CN2L-B8 (R0) Galanz microwave oven, Foshan Shunde Galanz microwave oven electric appliance co., LTD. TGL-20bR low temperature and high speed table centrifuge, Shanghai anting scientific instrument factory; HH-4 digital constant temperature water bath, Guo Hua electric appliance co., LTD. UV-8000uv-visible spectrophotometer, Shanghai Yuan Xi instrument co., LTD. KQ-500DE CNC ultrasonic cleaner, Kun Shan ultrasonic instrument co. LTD.

Methods

Cherry wine lees anthocyanin extraction process

Cherry wine lees ➡ Weighing (1.00±0.05 g) ➡Adding appropriate amount of extraction solvent ➡ Extraction ➡ Centrifugation ➡ Taking the supernatant ➡ Measuring the absorbance value.

Determination of anthocyanin extraction amount of cherry wine lees[13]

Accurately measured 1 mL of the extract and diluted with KCL buffer (pH=1.0) and CH_3COONa buffer (pH=4.5), and then diluted to 10mL, mixed and balanced the extract in darkness for 1 h, meanwhile, distilled water was used as a blank, and the absorbance was measured at 520 nm and 700 nm, respectively, and calculated:

$$A= (A_{520}-A_{700})\ pH_{1.0}- (A_{520}-A_{700})\ pH_{4.5}$$

C= (A X MW X n X V/e X m)

C= anthocyanin mass concentration, mg/g; MW = 449.2; e = 26900; n = dilution ratio; V= total extract volume, mL; m = quality of cherry wine lees, g.

Comparison of anthocyanins extracted from cherry wine lees by different extraction methods

Microwave extraction Accurately weighed 1.00±0.05g cherry wine lees in a centrifuge tube. According to the method optimized by orthogonal test, the ethanol concentration was 60%, the ratio of material to liquid was 1:30 (g/mL), the microwave time was 40s, and the microwave power was 400W. in triplicate, and extracted three times. Cooled, centrifuged at 8000r /min for 5min, took 1mL supernatant, measured the absorbance according to the method of 2.2.2 section and calculated the amount of anthocyanin. Condensation reflux extraction Accurately weighed 1.00±0.05g cherry wine lees in a centrifuge tube, according to the orthogonal experiment optimization method, the solid-liquid ratio was 1:30 (g/mL), added 10 mL of 80 % ethanol solution (pH = 2.5), it was leached in a water bath at 70°C for 80min,[14] in triplicate, and leached three times. Cooled, centrifuged at 8000r/min for 5min, took 1 mL supernatant, and the measurement method was the same as in 2.2.3.1 section.

Ultrasonic assisted enzymatic method

Accurately weighed 1.00±0.05g cherry wine lees in a conical flask, according to the orthogonal experiment optimization method, the solid-liquid ratio was 1:30, added 30 mL of 60% ethanol solution (pH = 4.0), the hemicellulose addition amount was 1%, the enzymolysis temperature was 50℃, the ultrasonic power was 300W, and the enzymolysis time was 50min. Cooled, centrifuged at 8000r /min for 5min, and took 1mL of supernatant, and the measurement method was the same as in 2.2.3.1 section.

Single-factor test

Accurately weighed 1.00±0.05g cherry wine lees in a centrifuge tube, respectively studied when under the condition of the extraction solvent was 60% ethanol, pH = 4.0, the solid-liquid ratio was 1:30, enzyme addition was 0.4%, enzymolysis time was 50min, enzymolysis temperature was 50°C and the ultrasonic power was 600W, in this case, studied on the effect of single factor on extraction, the single factors were as follows: the types of added enzymes were respectively cellulase, pectinase hemicellulose and composite enzyme (cellulose: pectinase: hemicellulase = 1:1:1), the amount of adding enzymes was respectively 0.2%, 0.6%, 1.0%, 1.4%, 1.8%, the solid-liquid ratio was respectively 1:10, 1:20, 1:30, 1:40, 1:50, the pH value was respectively 1, 2, 3, 4, 5, the enzymolysis temperature was respectively 30, 40, 50, 60, 70°C, the ultrasonic power was respectively 200, 300, 400, 500W and the enzymolysis time was respectively 30, 40, 50, 60, 70 min, repeated 3 times in parallel, averaged.

Response surface test design

Under the condition of hemicellulase as the fixed enzyme type, response surface analysis was carried out with the addition of enzymes (A), solid-liquid ratio (B), pH value (C), enzymolysis temperature (D), enzymolysis time (E) and ultrasonic power (F) as independent variables respectively, and the test factor level was shown in Table 1.

Table 1 Factors and levels used in response surface methodology

	Factors					
level	*A*	*B*	*C*	*D*	*E*	*F*
	Enzyme dosage/%	Feed and liquid ratio/g·mL^{-1}	pH value	Enzymolysis temperature/°C	Enzymolysis time/min	Ultrasonic power/W
-1	0.6	1:20	2	40	40	200
0	1.0	1:30	3	50	50	300
1	1.4	1:40	4	60	60	400

Results and analysis

Comparison of different extraction methods

The anthocyanin in cherry wine lees was extracted respectively by microwave extraction, condensing reflux extraction and ultrasound-assisted enzymatic extraction, as shown in Figure 1. It can be seen from Figure 1 that among the three extraction methods, the highest amount of anthocyanin was extracted by ultrasound-assisted enzymatic method, followed by microwave extraction, and the lowest was condensation and reflux method. This maybe because of the damage of microwave on anthocyanin while crushing the cherry wine lees cells, thus the extraction of anthocyanin was reduced. Although the extraction conditions of condensation reflux extraction are mild,

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the extraction time is long and the efficiency is low. Extraction of anthocyanin by ultrasound-assisted enzymatic method is characterized by short time, high efficiency and low temperature.[15,16] Therefore, in this study, the anthocyanin in cherry wine lees was extracted by ultrasound-assisted enzymatic method.

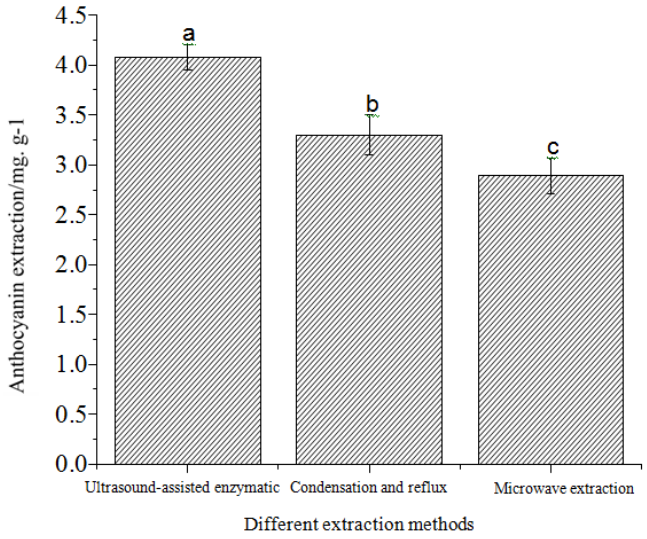

Figure 1 Effect of different extraction methods on the extraction of anthocyanin.

Single factor test results and analysis

Effect of enzyme type on the extraction of anthocyanin

As can be seen from Figure 2, anthocyanin in cherry wine lees was extracted with different enzymes, and the results were significantly different (P<0.05). Among them, hemicellulase has the best extraction effect, reaching 2.3040 mg/g, followed by the compound enzyme (1:1:1), and pectinase was the worst, which maybe because hemicellulase can decompose cellulose and hemicellulose, made plant cell wall dissolved, and released more of intracellular solutes. Therefore, hemicellulase was selected to extract anthocyanin from cherry wine lees.

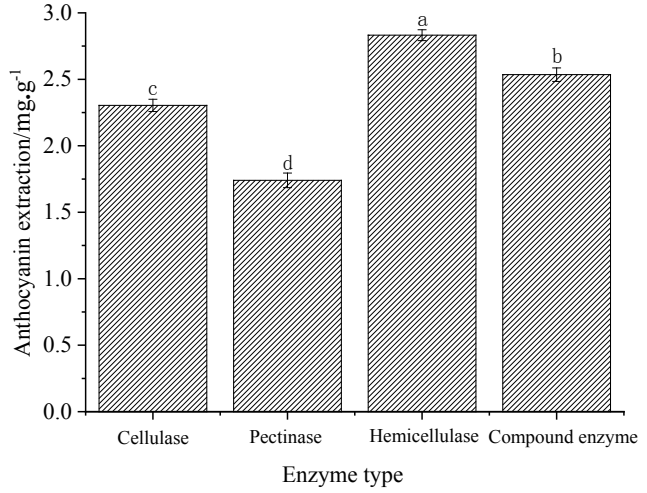

Figure 2 Effect of types of enzymes on the extraction of anthocyanin.

Effects of enzyme dosage on anthocyanin extraction

It can be seen from Figure 3 that the amount of hemicellulase has a significant impact on the extraction amount of anthocyanin (P<0.05). When the enzyme dosage reached 1.0%, anthocyanin in the extract reached the highest. The amount of anthocyanin in the solution decreased as the enzyme dosage continued to increase. It may because the dosage of enzyme added at the initial stage was small and the enzymolysis reaction was incomplete. When the enzyme is excessive, the substrate concentration cannot meet the saturation state of the enzyme, thus inhibiting the enzymolysis reaction.[17] Therefore, the optimal amount of added hemicellulase was determined to be 1.0%.

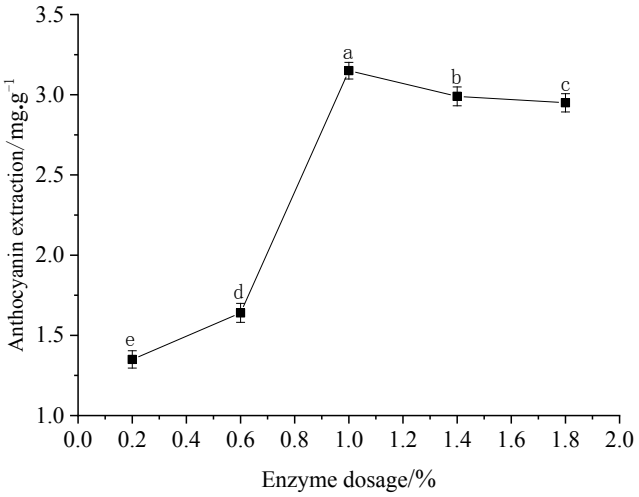

Figure 3 Effect of enzyme dosage on the extraction of anthocyanins.

Effect of ratio of material to liquid on anthocyanin extraction

As can be seen from Figure 4, anthocyanin extraction increased significantly with the increase of solid-liquid ratio (p<0.05), and reached the highest value at 1:40, then decreased. It may because with the increase of the amount of solvent, anthocyanin would be conducive to the dissolution, so that the extraction of its increased; When the solid-liquid ratio increases to a certain value, the concentration of hemicellulase in the extract decreases and the enzyme activity decreases, which affects the dissolution of anthocyanin.[18,19] When the solid-liquid ratio was 1:30 and 1:40, the extraction effect was not much different. Considering the extraction efficiency and cost, the solid-liquid ratio of 1:30 will be selected for testing in the later stage.

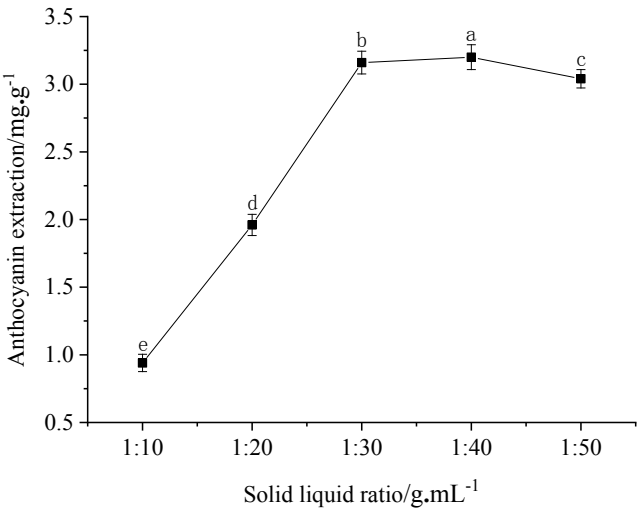

Figure 4 Effect of solid liquid ratio on the extraction of anthocyanin.

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Effect of pH value of enzymolysis on anthocyanin extraction

It can be seen from Figure 5 that the extraction amount of anthocyanin from cherry wine lees increases significantly with the increase of pH value ($p<0.05$). When pH value was 3.0, the extraction amount of anthocyanins reached the maximum, followed by a downward trend. This is because too low pH of the extract will cause hydrolysis of anthocyanin glycosylation and decrease stability, thus reducing the amount of anthocyanin extraction. If the pH value is too high, hemicellulase structure will be damaged and enzyme activity will be decreased, which will affect anthocyanin dissolution and reduce extraction effect.[20] Therefore, the optimal pH value of hemicellulase enzymolysis was 4.0.

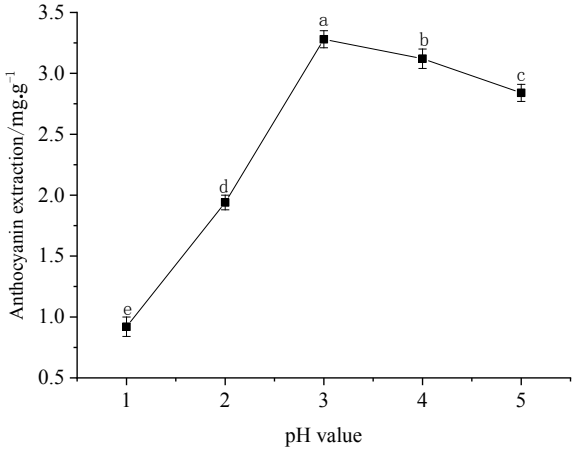

Figure 5 Effect of enzymolysis pH value on the extraction of anthocyanin.

Effect of enzymolysis temperature on anthocyanin extraction

The Figure 6 shows that the extraction amount of anthocyanins from cherry wine lees increases significantly with the increase of enzymolysis temperature at the first ($p < 0.05$), when the temperature reached 50℃, the highest amount of anthocyanins was extracted, then continue to raise the temperature, the extraction volume declines. This maybe because the temperature is so low that lowers the activity of the enzyme. On the one hand, raising the temperature can destroy the cell and increase the permeability of the cell membrane, which is conducive to the solvent diffusion into the cells. On the other hand, the solubility and diffusion capacity of anthocyanin increase with the increase of temperature. But excessive temperature will cause anthocyanins to be decomposed and the extraction amount to decrease. So 50°C was the best enzyme solution temperature.[21]

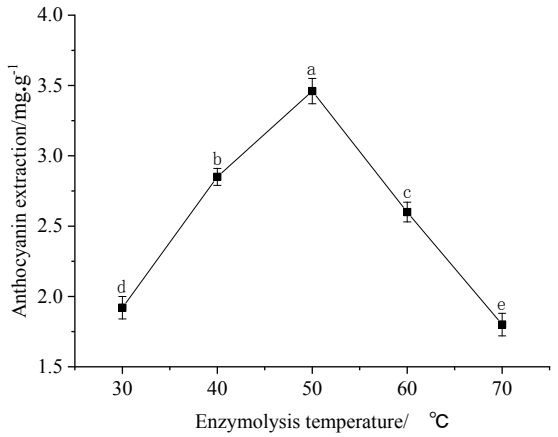

Figure 6 Effect of enzymolysis temperature on the extraction of anthocyanins.

Effect of enzymolysis time on anthocyanin extraction

It can be seen from Figure 7 that the enzymolysis time has a significant impact on the extraction effect of anthocyanin from cherry wine lees ($p<0.05$). At the initial stage, with the increase of enzymolysis time, the extraction amount of anthocyanins first rose and then decreased. When the enzymolysis time was 50min, the anthocyanin extraction amount reached to the peak, and then increased the enzymolysis time, the extraction effect decreased instead. Theoretically, because the raw material has more contact with the extraction solvent, with the increase of extraction time, the extraction effect should be better. However, because the dissolved anthocyanin will undergo the oxidative decomposition with the increase of time, the extraction rate will reduce. Thus, the best enzymolysis time should be 50min.[22]

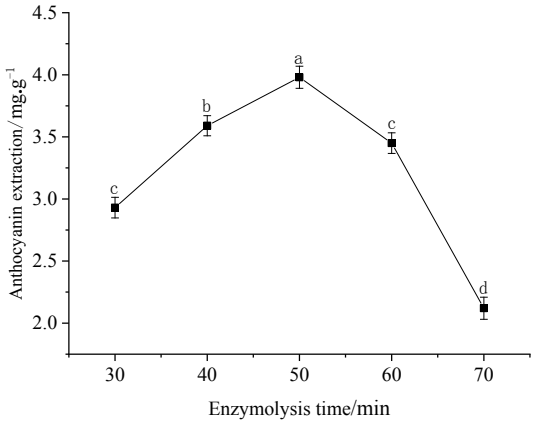

Figure 7 Effect of enzymolysis time on the extraction of anthocyanin.

Effect of ultrasound power on anthocyanin extraction

Figure 8 shows that under the condition of ice bag and thermometer monitoring, the extraction effect of anthocyanin from cherry wine lees is significantly affected by the ultrasonic power ($p<0.05$). The initial extraction rate increased with the increase of power, and reached the peak when the power was 300W, and then the extraction amount of anthocyanin decreased instead. This may because ultrasound has cavitation effect and stirring effect, etc. which can destroy the cell structure of cherry wine lees, thus increasing anthocyanin dissolution. However, when the ultrasonic power is too high, the stability of anthocyanin will be destroyed, and the anthocyanin was hydrolyzed to reduce the extraction amount.[23,24] Therefore, the best ultrasound power was 300 W.

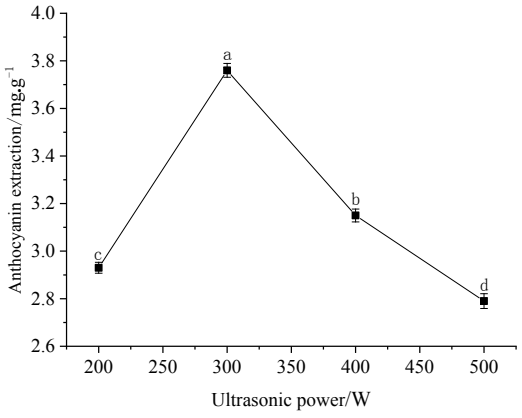

Figure 8 Effect of ultrasonic power on the extraction of anthocyanins.

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Response surface test

Response surface analysis

According to the results of single factor analysis, the response surface software was used to optimize the process parameters. Taking the anthocyanin extraction amount as the objective, the effects of the amount of anthocyanin addition, the ratio of material to liquid, pH value, enzymolysis time, enzymolysis temperature and ultrasonic power on the extraction amount of anthocyanin were studied. The analysis results are shown in Table 2. By fitting and analyzing the data in Table 2 with the design-expert 8.0 software, the regression model equation of the extraction rate of anthocyanin for enzyme dosage (A), solid-liquid ratio (B), pH value (C), enzymolysis time (D), enzymolysis temperature (E) and ultrasonic power (F) was obtained as follows: Anthocyanins extracted amount/mg·g^{-1}=4.37+0.053A+0.051B+0.059C+0.059D+0.055E+0.065F-5×10^{-3}AB+3×10-3AC+3×10^{-}AD+8×10^{-3}AE+8×10^{-3}AF+0.016BC-2×10^{-3}BD-3×10^{-3}BE+0.018BF-8×10^{-3}CD+0.026CE+8×10^{3}CF+0.016DE+0.029DF+0.013EF-0.11A^2-0.13B^2-0.14C^2-0.11D^2-0.12E^2-0.11F^2

Table 2 Design and results of response surface tests

Number	*A* amount of anthocyanin addition/%	*B* ratio of material to liquid/mL g^{-1}	*C* pH value	*D* enzymolysis time /℃	*E* enzymolysis temperature / min	*F* ultrasonic power/W	extraction amount of anthocyanin / mg g^{-1}
1	1.0	20	2	50	40	300	3.86
2	1.0	40	2	50	40	300	3.91
3	1.0	20	4	50	40	300	3.88
4	1.0	40	4	50	40	300	4.01
5	1.0	20	2	50	60	300	3.91
6	1.0	40	2	50	60	300	3.99
7	1.0	20	4	50	60	300	4.03
8	1.0	40	4	50	60	300	4.18
9	1.0	30	2	40	50	200	3.84
10	1.0	30	4	40	50	200	3.96
11	1.0	30	2	60	50	200	3.92
12	1.0	30	4	60	50	200	4.02
13	1.0	30	2	40	50	400	3.91
14	1.0	30	4	40	50	400	4.07
15	1.0	30	2	60	50	400	4.12
16	1.0	30	4	60	50	400	4.23
17	0.6	30	3	40	40	300	3.89
18	0.6	30	3	60	40	300	3.90
19	0.6	30	3	40	60	300	3.98
20	0.6	30	3	60	60	300	4.08
21	1.4	30	3	40	40	300	3.96
22	1.4	30	3	60	40	300	4.05
23	1.4	30	3	40	60	300	4.08
24	1.4	30	3	60	60	300	4.21
25	1.0	20	3	50	40	200	3.82
26	1.0	40	3	50	40	200	4.00
27	1.0	20	3	50	60	200	3.94
28	1.0	40	3	50	60	200	4.01
29	1.0	20	3	50	40	400	3.92
30	1.0	40	3	50	40	400	4.10

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Table Continued....

Number	*A* amount of anthocyanin addition/%	*B* ratio of material to liquid/mL g^{-1}	*C* pH value	*D* enzymolysis time /℃	*E* enzymolysis temperature / min	*F* ultrasonic power/W	extraction amount of anthocyanin / mg g^{-1}
31	1.0	20	3	50	60	400	3.99
32	1.0	40	3	50	60	400	4.19
33	0.6	30	2	50	50	200	3.85
34	0.6	30	4	50	50	200	3.95
35	0.6	30	2	50	50	400	3.94
36	0.6	30	4	50	50	400	4.06
37	1.4	30	2	50	50	200	3.96
38	1.4	30	4	50	50	200	4.05
39	1.4	30	2	50	50	400	4.06
40	1.4	30	4	50	50	400	4.26
41	0.6	20	3	40	50	300	3.81
42	0.6	40	3	40	50	300	3.93
43	0.6	20	3	60	50	300	3.96
44	0.6	40	3	60	50	300	4.04
45	1.4	20	3	40	50	300	3.98
46	1.4	40	3	40	50	300	4.05
47	1.4	20	3	60	50	300	4.10
48	1.4	40	3	60	50	300	4.22
49	1.0	30	3	50	50	300	4.36
50	1.0	30	3	50	50	300	4.36
51	1.0	30	3	50	50	300	4.37
52	1.0	30	3	50	50	300	4.36
53	1.0	30	3	50	50	300	4.39
54	1.0	30	3	50	50	300	4.35

Table 3 shows that the regression model of anthocyanin extraction test is highly significant (P<0.0001). R2=0.9832 indicated that only about 1.68% could not be explained by the model, with no significant dissimulation error (P=0.2239>0.05), indicating that the fitting degree was good and the experimental error was small. This model could be used to analyze and predict the technical parameters of the ultrasonic assisted extraction of anthocyanin from cherry wine lees.

Table 3 Variance analysis of regression model

Source	Degree of freedom	Quadratic sum	Mean square	*F* value	Level of significance	Significance
	DF				*Prob<F*	
model	27	1.2	0.044	128.35	<0.0001	**
A	1	0.068	0.068	197.12	< 0.0001	**
B	1	0.063	0.063	182.02	< 0.0001	**
C	1	0.083	0.083	239.2	< 0.0001	**
D	1	0.084	0.084	242.6	< 0.0001	**
E	1	0.1	0.1	292.8	< 0.0001	**
F	1	0.073	0.073	209.64	< 0.0001	**

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Table Continued....

Source	Degree of freedom	Quadratic sum	Mean square	*F* value	Level of significance	Significance
	DF				*Prob<F*	
model	27	1.2	0.044	128.35	<0.0001	**
AB	1	2×10^{-4}	2×10^{-4}	0.58	0.4541	
AC	1	1×10^{-4}	1×10^{-4}	0.32	0.5736	
AD	1	1×10^{-4}	1×10^{-4}	0.45	0.5077	
AF	1	6×10^{-4}	6×10^{-4}	1.77	0.1951	
AE	1	6×10^{-4}	6×10^{-4}	1.77	0.1951	
BC	1	2×10^{-3}	2×10^{-3}	6.1	0.0204	*
BD	1	5×10^{-5}	5×10^{-5}	0.14	0.7071	
BF	1	1×10^{-4}	1×10^{-4}	0.45	0.5077	
BE	1	2×10^{-3}	2×10^{-3}	7.07	0.0132	*
CD	1	1×10^{-3}	6×10^{-4}	1.77	0.1951	
CF	1	5×10^{-3}	5×10^{-3}	15.92	0.0005	**
CE	1	2×10^{-3}	1×10^{-3}	3.54	0.0713	
DF	1	1×10^{-3}	2×10^{-3}	6.1	0.0204	*
DE	1	6×10^{-3}	6×10^{-3}	19.09	0.0002	**
EF	1	6×10^{-4}	1×10^{-3}	3.61	0.0686	
A^2	1	0.13	0.13	366.67	< 0.0001	**
B^2	1	0.18	0.18	523.62	< 0.0001	**
C^2	1	0.19	0.19	557	< 0.0001	**
D^2	1	0.13	0.13	383.36	< 0.0001	**
E^2	1	0.11	0.11	331.79	< 0.0001	**
F^2	1	0.15	0.15	438.65	< 0.0001	**
Residual	26	9×10^{-3}	3×10^{-4}			
Misalignment	21	8×10^{-3}	3×10^{-4}	2.02	0.2239	
Pure error	5	9×10^{-4}	1×10^{-4}			
Total error	53	1.21				

Note: * indicates significant difference (P<0.05); ** means highly significant (P<0.01).

The significance of the regression equation shows that the first term A (P<0.0001) is highly significant. B (P<0.0001) is highly significant; C (P<0.0001) significant; D (P<0.0001) is highly significant; E (P<0.0001) is highly significant; F (P<0.0001) is highly significant. It can be seen from the F value that the influence of various single factors on the extraction amount of anthocyanin is strong or weak. The influence of six factors is the enzymolysis time (E) > enzymolysis temperature (D) > pH value (C) > ultrasonic power (F) > plus enzyme dosage (A) > ratio of material to liquid (B). Secondary terms A^2 (P<0.0001), B^2 (P<0.0001), C^2 (P<0.0001), D^2 (P<0.0001), E^2 (P<0.0001) and F^2 (P<0.0001) indicated that the effect of 6 experimental factors on extraction rate was not a simple linear relationship, and the surface effect was significant. From the interaction terms CF value (P=0.0005<0.01), DE value (P=0.0002<0.01), BC value (P=0.0204<0.05), BE value (P=0.0132<0.05) and DF value (P=0.0204<0.05), it can be seen that the interaction between pH value and ultrasonic power, enzymolysis temperature and enzymolysis time, ratio of material to liquid and pH value, ratio of material to liquid and enzymolysis time, enzymolysis temperature and ultrasonic power is obvious.

Response surface analysis and optimization

The response surface can reflect the degree of interaction and relationship between factors.[25,26] When the contour line is round, the interaction effect is not significant, and when it is oval, the interaction effect is significant. By observing the change of response surface and the sparseness of the contour line, the relationship between the two factors can be reflected more clearly and intuitively. The data were regression fitted by Design Expert software, and the response surface and its contour lines were obtained as shown in Figure 9-13.

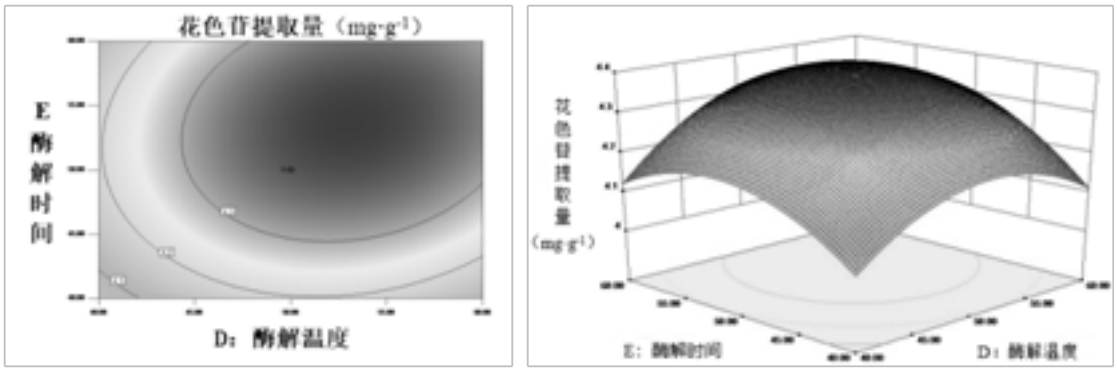

Figure 9 Response of contour plots and surface plots of the extraction yield under the interaction of enzymolysis time and enzymolysis temperature.

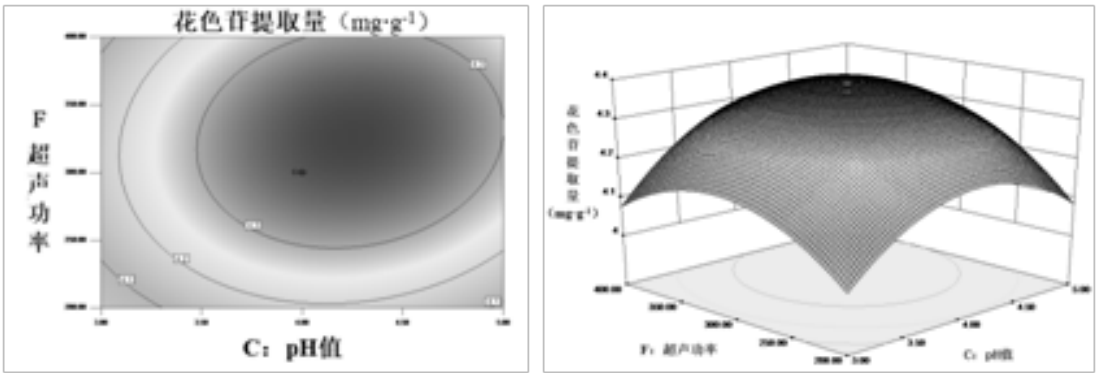

Figure 10 Response of contour plots and surface plots of the extraction yield under the interaction of ultrasonic power and pH value.

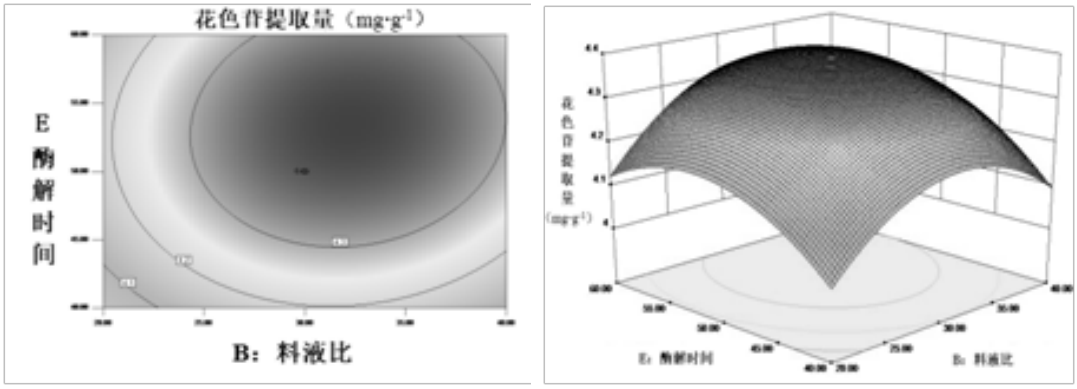

Figure 11 Response of contour plots and surface plots of the extraction yield under the interaction of enzymolysis time and solid liquid.

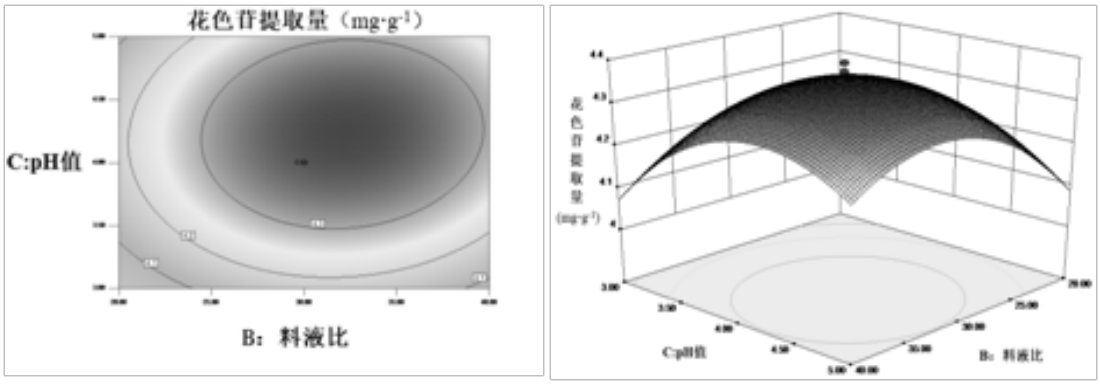

Figure 12 Response of contour plots and surface plots of the extraction yield under the interaction of pH value and solid liquid.

Citation: Jia C, Han F, Miao X, et al. Study on optimization of extraction process of anthocyanin from cherry wine lees. *J Nutr Health Food Eng.* 2019;9(1):18–27. DOI: 10.15406/jnhfe.2019.09.00321

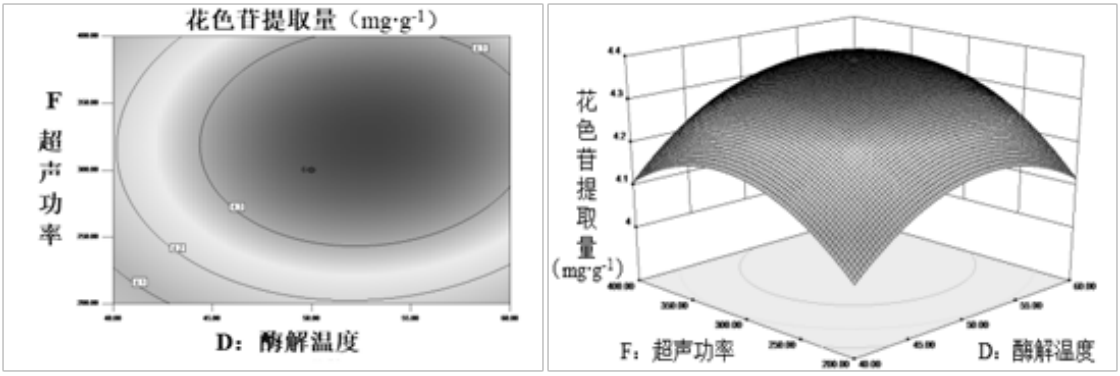

Figure 13 Response of contour plots and surface plots of the extraction yield under the interaction of enzymolysis temperature and ultrasonic power.

It can be seen from Figure 9 that the variation surface of enzymolysis time is steeper than that of enzymolysis temperature, indicating that the effect of enzymolysis time on the extraction quantity is more significant, which is consistent with the results of variance analysis. It can be seen from Figure 10 that the variation surface of pH value is steep, by contract, the variation surface of ultrasonic power is gentle, indicating that pH value has a significant impact on the extraction amount compared with ultrasonic power, which is consistent with the result of variance analysis. It can be seen from Figure 11 that the change surface of enzymolysis time is steeper than that of solid-liquid ratio, indicating that the effect of enzymolysis time on extraction effect is more significant, which is consistent with the result of variance analysis. It can be seen from Figure 12 that the change surface of pH value is steeper than the change surface of solid-liquid ratio, indicating that pH value has a significant impact on the extraction amount compared with solid-liquid ratio, which is consistent with the result of variance analysis. It can be seen from Figure 13 that the variation surface of enzymolysis temperature is relatively steep, while the variation surface of ultrasonic power is relatively gentle, indicating that the effect of enzymolysis temperature on the extraction amount is significantly higher than that of ultrasonic power, which is consistent with the result of variance analysis. The contour plots in Figures 8-12 are all clearly elliptical, indicating that the interaction between enzymolysis time and enzymolysis temperature, pH value and ultrasound power, enzymolysis time and solid-liquid ratio, pH value and solid-liquid ratio, enzymolysis temperature and ultrasound power is relatively significant, which has a great impact on the extraction rate.

Determination of optimal conditions and model validation

The optimum conditions for extracting anthocyanins from cherry wine residue by response surface method were as follows: enzyme dosage 1.5%, the solid-liquid ratio 1:32.09, pH value of 4.28, enzymolysis temperature is 53.48℃, enzymolysis time 54.56min, ultrasonic power 334.50W. At this time, the anthocyanin extraction amount was 4.31mg/g. The actual adjustment is slightly: enzyme dosage 1.5%, the solid-liquid ratio 1:32, pH value of 4.30, enzymolysis temperature 54℃, enzymolysis time 55min, ultrasonic power 300 W, extraction under this conditions for three times for parallel experiments, with a mean of 4.19 mg/g.

Conclusion

In this study, cherry wine lees were used as major raw materials to compare the effects of three different extraction methods on anthocyanin content. The results showed that the ultrasonic assisted enzyme extraction was the best. Using the Box-Behnken principle and design-expert 8.0 software, response surface analysis was carried out on the basis of single factors, and the enzyme dosage, solid-liquid ratio, pH value, enzymolysis temperature, enzymolysis time and ultrasonic power were affected the extraction amount of anthocyanins. The regression model affected by the extraction amount has R^2=0.9832 and the out-of-fitting term P=0.2239>0.05, indicating that the model was well fitted to the actual situation. The influence of factors on the extraction amount was followed by enzymolysis time, enzymolysis temperature, ultrasonic power, pH value and enzyme dosage, solid-liquid ratio. The optimal extraction conditions were adjusted according to the actual conditions: enzymatic dosage 1.5%, the solid-liquid ratio 1:32, pH value 4.30, enzymolysis temperature 54℃, enzymolysis time 55min, ultrasonic power 300W. At this time, the extraction amount was 4.19mg/g which was basically consistent with the theoretical prediction. This study is of great significance for improving the economic value of cherry wine by-products and promoting the development of the cherry industry.

Acknowledgments

None.

Conflicts of interest

The authors declare that there is no conflicts of interest.

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