

Studies on the Optimal Process to Extract Flavonoids from Red-raspberry Fruits

Yaqin Xu, Rui Zhang, Hong Fu

Science College, Northeast Agriculture University, Harbin, Heilongjiang 150030, China, Xu-yaqin@163.com

Abstract: Effects of single factors such as temperature, time, material ratio and concentration of ethanol solution on the contents of flavonoids were investigated. On this basis, employing the orthogonal design, the optimum extraction conditions for flavonoids from raspberry fruits were determined by colorimetric estimation. Experiment results: the amount of flavonoids extract reached its maxima when extracted for 3 h by using 95% ethanol solution at 80°C with the ratio of raw materials to ethanol solution of 1:10. [Nature and Science. 2005;3(2):43-46].

Key words: red-raspberry; flavonoids; extract; single-factor experiments; orthogonal experiments

1 Introduction

Red-raspberry, which belongs to genus *Rubus* L. of family to *Rasaceae*, is a kind of berry with tender and juicy fruits. Fruits of red-raspberry are rich in vitamins and minerals and especially abundant in cancer-fighting, anti-aging substances such as SOD and gallogen (Häkkinen, 2000; Puupponen-Pimiä, 2005), therefore famous as "Fruit of Life" in the US. Apart from those, raspberry fruits contain significant flavonoids. It was reported that flavonoids could remove the $O_2^- \cdot$ in human bodies, strengthen the natural disease-fighting system, improve blood circulation and lower blood pressure (Fang, 1998; Liu, 2002; Wang, 1996). At present, studies on the extraction of flavonoids from raspberry fruits have not been reported. In this study the optimum conditions to extract flavonoids from red-raspberry fruits were studied systematically in order to achieve scientific evidence for the processing and utilizing of raspberry fruits.

2 Materials and Methods

2.1 Materials

The raspberry fruits were collected from the Horticultural Center of Northeast Agricultural University and put into air-tight containers to store in a refrigerator after instantly frozen and minced.

2.2 Extracting process

At first the main factors of the extraction temperature, the concentration of the extracting agent, extraction time and the materials ratio (the weight of fruits: volume of the extracting agent), which affect the extraction of flavonoids were studied individually, and then the optimum extracting conditions of flavonoids from raspberry fruits were determined by adopting

$L_9(3^3)$ orthogonal experiments after making analysis of variance and significance evaluation diagram of extracting process was: materials → extract → filter → enrichment → extracted by using ethyl acetate → raw extract of flavonoids → confirm the volume by using 95% ethanol solution → measure.

2.3 Measuring of the contents of flavonoids

The contents of flavonoids was measured using colour comparing method of $NaNO_2-Al(NO_3)_3$ and calculated using the following formula (Xu, 2000):

Relative contents of flavonoids ($\mu g/g$) = $5YV/W$
 $Y = 75.944A - 0.3494$

A: absorbance

W: the weight of raspberry fruits precisely measured: g

V: the volume of extracting agent: mL

3 Results and Discussions

3.1 Effects of single factor for flavonoids extraction

3.1.1 The effect of the concentration of ethanol solution on the content of flavonoids extracted

The result of Figure 1 showed that the content of raw flavonoids extract increases with the concentration of ethanol solution and the contents increased slowly when the concentration was greater than 75%. However, raw flavonoids extract had better solubility and the solvent was easily vaporized and reused in 95% ethanol solution, 95% ethanol solution was used.

3.1.2 The effect of temperature on the contents of flavonoids extracted

Figure 2 was contents of raw flavonoids extracted at 50°C, 65°C, 80°C, 95°C under certain conditions.

The contents of raw flavonoids extract tended to increase gradually with the rise of temperature in range of 50°C~80°C. It may be probable that the greater speed

of the molecule movements in higher temperature so that flavonoids diffused more quickly from cell to extracting agent. But the flavonoids could be oxidized at temperature of surpassing 80° so that the contents of flavonoids extracted started to decrease gradually. 80°C was the optimum temperature.

3.1.3 The effects of the material ratio on the contents of flavonoids extracted

The result of Figure 3 showed that the contents of flavonoids increased significantly with the rise of the material ratio in a range of 1:5~1:10 and changed unobviously when the ratio was greater than 1:10. This was because when the material ratio was between 1:5 and 1:10, flavonoids were extracted fully with the rise of volume the extracting agent so as to the contents of flavonoids increased. Otherwise, when the material ratio reached a certain level, the extract has well soluted in the solution lead to the contents of extract become steady and wouldn't increase significantly. The optimum material ratio was 1:10.

3.1.4 Effect of flavonoid extraction time

The result of Figure 4 showed contents of flavonoids extracted for 3 h reached its maxima and then decreased with prolonging extraction time. This may increase amount of impurities with the rise of time.

Therefore the optimum extraction time was 3 h.

3.2 Optimum conditions for flavonoid extraction

On the basis of the effects of single factor in above chapters, adopting extracting agent of 95% ethanol solution, the trials of tri- factor, tri-level Orthogonal design were conducted and the results were given in Table 1 and Table 2.

The results of orthogonal experiment were made an analysis of range, single-factor analysis of variance and the analysis of linear regression by SPSS software. Table 2, Table 3 and Table 4 showed the results.

The results of all kinds of analysis showed the degree of affecting the contents of flavonoid extract was C>B>A. The material ratio had the greatest effect on the test results, time of extraction rank second and the temperature came last. When extraction temperature and extraction time were at the 2nd level, the contents of extract reached its maxima. When the material ratio was at the 3rd level, the amount of extract reached its maxima. However, to save the solvent, it's not economical to adopt for the material ratio of 1:15 even it got the greatest contents of extract. Table 2 showed that the 2nd level was suitable for all three factors because of the greater contents of extract than the 1st level.

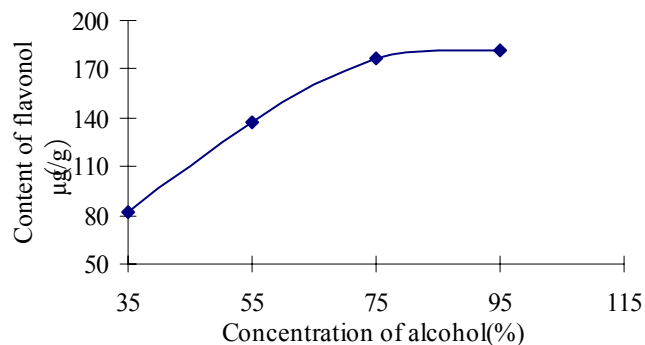


Fig.1 Effect of different concentration of alcohol

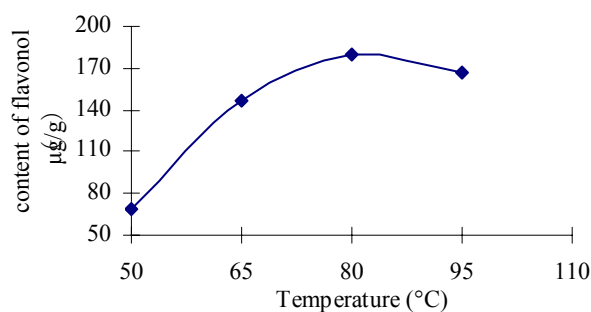


Figure 2. Effect of different temperature

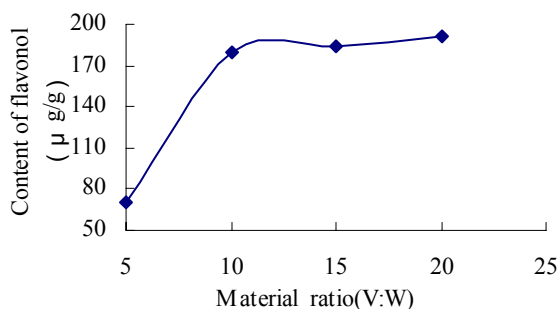


Fig3 Effect of the material ratio

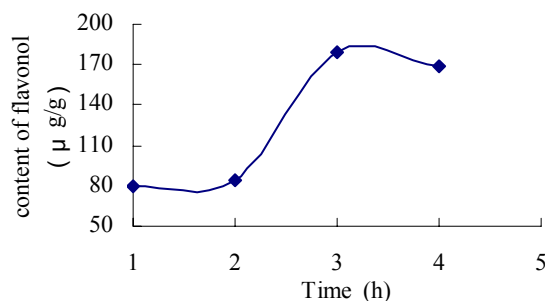


Fig4 Effect of the different extraction time

Table 1. Factors and levels

Level	Factor		
	A. Temperature (°C)	B. Extraction time (min)	C. Ratio of solid to liquid (W:V)
1	65	120	1: 5
2	80	180	1: 10
3	95	240	1: 15

Table 2. Experiment results and range analysis

Item	A	B	C	Flavonoids (μ g/g)
1	1	1	1	80.2
2	1	2	2	157.4
3	1	3	3	164.2
4	2	1	3	143.3
5	2	2	1	107.2
6	2	3	2	179.8
7	3	1	2	142.2
8	3	2	3	182.8
9	3	3	1	100.0
K ₁	401.8	365.7	287.5	
K ₂	430.3	447.4	479.3	
K ₃	425.0	444.0	490.3	
k ₁	133.9	121.9	95.8	
k ₂	143.4	149.1	159.8	
k ₃	141.7	148.0	163.4	
R	9.5	27.2	67.6	

Table 3. Single- factor analysis of variance

	Sum of Squares	df	Mean Square	F	P
A	153.3	2	76.6	0.044	F _{0.01} (2,2) =99
B	1423.0	2	711.5	0.467	F _{0.05} (2,2) =19
C	8670.9	2	4335.4	13.788	F _{0.1} (2,2) =9
Total	10247.2	6			F _{0.25} (2,2) =3

Table 4. Analysis of variance in linear regression

Model		Sum of Squares	df	Mean Square	F
1	Regression	7965.613	3	2655.204	5.122
	Residual	2591.897	5	518.379	
	Total	10557.510	8		

4 Conclusion

To sum up, the Optimum process to extract Flavonoids from raspberry fruits were obtained, namely Extracted for 3 h by using 95% ethanol solution at 80°C with the material ratio of 1:10 (W:V).

Correspondence to:

Yaqin Xu
59 Wood Street
Gongbin Road, Xifang District
Harbin, Heilongjiang 150030, China
Telephone: 01186-451-5519-0732
Cellular phone: 01186-130-7453-5378
E-mail: xu-yaqin@163.com

References

- [1] Fang P. Biological Effects of Flavones. *Journal of Dali medical college* 1998;7(4):52~54.
- [2] Häkkinen SH, Kärenlampi SO, Heinonen IM, Törrönen AR. Ellagic Acid Content in Berries: Influence of Domestic Processing and Storage. *European Food Research and Technology* 2000;212(1):75~80.
- [3] Liu M, Li X. Antioxidant and Antiprolife -rative Activities of Raspberries. *J Agric Food Chem* 2002;50:2926~2930.
- [4] Puupponen-Pimiä R, Nohynek L, Alakomi HL, Oksman-Caldentey KM. Bioactive Berry Compounds-novel Tools against Human Pathogens. *Applied Micro-biology and Biotechnology*. 2005;67(1):8~18.
- [5] Xu Y, Sun Y, Fu H. Studies on the Optimal extraction Conditions of Flavonoid in Leaves of Black Currant. *Journal of Northeast Agriculture University* 2000;7(2):144~146.
- [6] Wang H, Cao G, Prior R. Total antioxidant capacity of fruits. *J Agric Food Chem* 1996;44:701~705.