

Effect of ethanol on the anthocyanin extraction from the purple rice of Vietnam

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Abstract: Anthocyanin is not only the common natural color, but also has potential applications such as the antioxidant component, and many other health benefits. This pigment has good application in the food industry. Purple rice (*Oryza sativa*) is a special cereal in Vietnam, and some other Asian countries. This rice contains high anthocyanin (~0.4%). The anthocyanin extraction was evaluated, and the best method of the extraction was determined. The experiment was conducted with the following variables: ethanol-to-water ratio as solvent (E/W, 1:9-6:4) (v/v), purple rice-to-water ratio (P/W, 1: 6-1:16) (w/v), solvent pH (pH, 2.0-3.0), solvent temperature (ST, 30-70 °C) and extraction time (ET, 60-180 min). The result showed that the best adequate extraction conditions were E/W, 2:8; P/W, 1:10; pH, 2.2; 60°C; and ET, 90min. At such condition, 116.7 ± 5.8 mg / 100g anthocyanin was extracted.

Keywords: *Oryza sativa*, Anthocyanin, Food Color, Ethanol, Pigment Extraction

1. Introduction

Anthocyanins are considered secondary metabolites; it is a food additive E163. It is a natural pigment used quite safely in food; create more attractive colors for food products. At present, there are more than 300 anthocyanins which were identified the structure, including 6 main anthocyanins, such as: pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin.

The purple rice (*Oryza sativa*) consists of two main anthocyanins: cyanidin and peonidin. Those anthocyanins react like the antioxidant agents, which reduce the density of lipoprotein and reduce the nitric oxide formation (Zawistowski et al., 2003). Cyanidin helps to reduce the atherosclerotic plaque formation that was developed by lipid forming plaque (Wang et al., 2001). Chen et al., (2006) determined that peonidin helps to inhibit the cancer cell of human.

The anthocyanin molecule is not altered under the effect of intestinal bacteria in the stomach system of human. Thus, the anthocyanin molecules are also not changed in plasma and urine (Gui, and Rodriguez-Saona, 1999, Jon and David, 1998,

Passamonti et al., 2003). The recent studies showed that the anthocyanins are absorbed only at low levels, from 0.016% to 0.11%, in human. Anthocyanins also protect the stomach lining against damage from oxidation, so delayed the early stages of stomach cancer and colon cancer (Halliwell, et al., 2000).

In this study, various anthocyanins extraction from the purple rice was investigated to achieve the highest extraction, for the selection of anthocyanin extraction methods to use in food industry.

The aim of this research was to focus on the optimum anthocyanin extraction by using ethanol from *purple rice*, which is grown in Vietnam.

2. Materials and Methods

Materials: The purple rice was collected from Soc Trang province, Vietnam. After collecting, the sample was storage in the dry place for use in the experiments.

The sample was ground into flour, and then the anthocyanin was extracted using ethanol solvent in suitable condition. The extracted solution was centrifuged (5000

rpm/min) for 10 min to remove the sediment. To extract anthocyanin, vacuum rotary evaporator was used to get the high color content of the received liquid. (Dam Sao Mai, Le Van Tan, 2013)

Optimization of anthocyanin extraction: The ground purple rice were treated with different ratio of water and ethanol (1:9, 2:8, 3:7, 4:6, 5:5, 6:4 (v/v)), different ratio of raw material and selected solvent (1:6, 1:8, 1:10, 1:12, 1:14, 1:16 (v/v)) with different pH (2.0, 2.2, 2.4, 2.6, 2.8, 3.0) at different temperature (30, 40, 50, 60, 70°C), and different extraction time (1.0, 1.5, 2.0, 2.5, 3.0 hrs.).

Analysis:

The quantity of anthocyanins was determined by the pH differential method as described by Huynh et al (2004). This was simple method that measures the absorption of extracted liquid at two light wavelength $\lambda = 520$ nm, and $\lambda = 700$ nm (the A_{\max} wavelength) at two different pH, (1.0 and 4.5). The results were then compared with standards anthocyanin measured under similar conditions.

The anthocyanins are calculated as cyanidin-3-glucoside equivalents, mg/L, using the following equation:

$$\text{The measured anthocyanins} = \alpha = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \text{ (g)}$$

where

$$A = (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 1.0 - (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 4.5;$$

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu);

DF = dilution factor (1:10); l = path length in cm;
 ε (= 26900 molar extinction coefficient, in $\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, for cyd-3-glu;

10^3 = factor for conversion from g to mg.

$$\% \text{ total antocyanins} = \frac{a}{m(100-w) \cdot 10^{-2}} \times 100\%$$

where a= the measured anthocyanins (g); m= initial materials, w = the moisture contents of raw material (%)

Statistical analysis: All the analyses were done in triplicate and measurement was done using Modde 5 software to perform optimization. Analyses of Variance (ANOVA) were conducted by using SPSS Version 13.0 for Windows (SPSS). Turkey tests were performed to test the significant differences between the mean values for treatments ($p < 0.05$)

3. Results and Discussion

3.1. Effect of the Ratio between Water and Ethanol of the Extraction Solvent on the Amount of Anthocyanins

Anthocyanins consist of hydrocarbon, which is water resistant, but they also have the polyphenol compounds that are highly soluble in water and polarized solvent. That's why the suitable solvent for anthocyanins extraction should be chosen from the organic solvent and then mixes with water, which is safe for human health and cheap.

Table 1. The effect of water and ethanol ratio on the anthocyanin extraction

Sample	Ratio of water and ethanol	Amount of extracted anthocyanin (g)	Concentration of extracted anthocyanin from raw material (%)
M ₁	1:9	0.00705361 ^{bc}	0.078460 ^{bc}
M ₂	2:8	0.00750448 ^d	0.083475 ^d
M ₃	3:7	0.00728906 ^{cd}	0.081079 ^{cd}
M ₄	4:6	0.00702856 ^b	0.078182 ^b
M ₅	5:5	0.00638231 ^a	0.070993 ^a
M ₆	6:4	0.00617191 ^a	0.068653 ^a

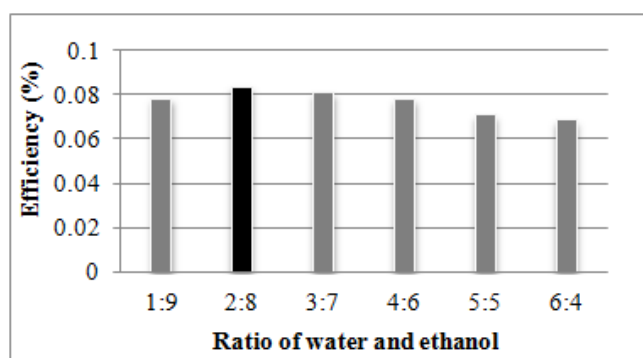


Fig. 1. The effect of water and ethanol ratio on the amount of anthocyanin extraction

This evaluation investigated the ratio of water and ethanol, which was used as extracting solvent. Data presented in Table 1 and Fig. 1 showed that, when unsuitable ratio of

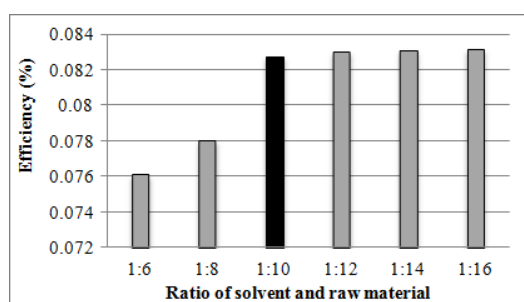
ethanol was used, the anthocyanins content in the extracted liquid was not high enough. When ratio of water: ethanol was: 1:9, 2:8, 3:7, and 4:6, the anthocyanins content in the extracted liquid was stable and nearly equivalent. Among this ratio of water and ethanol as 2: 8 ratios showed the best result. This result was used for the following study.

3.2. Evaluation of the Amount of Solvent Requirements for the Anthocyanins Extraction

This study used the best solvent ratio to extract anthocyanins from the purple rice. Data presented in Table 2 and Fig. 2 showed that, when the amount of solvent was too low, the anthocyanins were not extracted. The raw material and solvent ratio 1:10 and 1:16 gave the best results, and a non-significant difference was observed between the mean values for treatments. From the economic viewpoint, raw material and solvent ratio 1:10 was found more economical.

Table 2. The effect of raw material and solvent ratio on the anthocyanin extraction

Sample	Ratio of raw material and solvent	Amount of received anthocyanin (g)	Concentration of received anthocyanin from raw material (%)
M ₁	1:6	0.006837 ^a	0.076058 ^a
M ₂	1:8	0.007013 ^a	0.078014 ^a
M ₃	1:10	0.007438 ^b	0.082733 ^b
M ₄	1:12	0.007462 ^b	0.082999 ^b
M ₅	1:14	0.007468 ^b	0.083067 ^b
M ₆	1:16	0.007474 ^b	0.083142 ^b

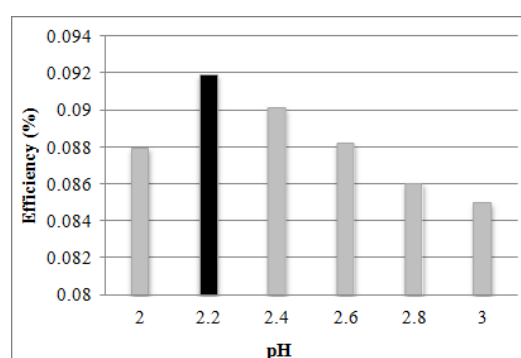
**Fig. 2.** The effect of ratio of raw material and solvent on capacity of the anthocyanin extraction

3.3. Evaluation of the Solvent Optimal pH on Anthocyanins Extraction

The pH of the solvent plays an important role during anthocyanin extraction from the purple rice with best solvent raw materials ratio. Data showed in Table 3 and Fig. 3 revealed that when the pH was high, the anthocyanin might be destroyed, so the extracted amounts were very low. The highest amount of anthocyanin (0.035938%) was obtained at pH of 2.2. The results showed that a pH of < 2.2 and > 2.4 would be best to achieve high anthocyanin. This result is in agreement with other reported research results.

Table 3. The effect of the extraction pH on the anthocyanin extraction

Sample	Extraction pH	Amount of received anthocyanin (g)	Concentration of received anthocyanin from raw material (%)
M ₇	2.0	0.00790859 ^a	0.087971 ^a
M ₈	2.2	0.00826261 ^d	0.0919089 ^d
M ₉	2.4	0.0081023 ^c	0.0901257 ^c
M ₁₀	2.6	0.00792863 ^a	0.0881939 ^a
M ₁₁	2.8	0.00773492 ^b	0.0860392 ^b
M ₁₂	3.0	0.00764141 ^b	0.084999 ^b

**Fig. 3.** The effect of the extraction pH on the capacity of the anthocyanin extraction

3.4. Evaluation of the Solvent Optimal Temperature for Anthocyanins Extraction

The extraction temperature might have influence on the ability to extract anthocyanins from samples. It was observed that increasing temperature was increased the ability of anthocyanins extraction by reducing the viscosity of the solution, while increasing the speed of diffusion of solutes from the material to the solvent. The highest concentration of anthocyanins was achieved at 60°C. At 70°C temperature the quality of anthocyanins reduced rapidly, because the anthocyanins were susceptible denaturated by higher temperature. hence, the optimal temperature for the extraction was 60°C. (Table 4, Fig. 4).

Table 4. The effect of the extraction temperature on the anthocyanin extraction

Sample	Extraction temperature (°C)	Amount of received anthocyanin (g)	Concentration of received anthocyanin from raw material (%)
M ₇	30	0.00823589 ^a	0.0916117 ^a
M ₈	40	0.00880699 ^b	0.0979643 ^b
M ₉	50	0.00978555 ^c	0.108849 ^c
M ₁₀	60	0.0111749 ^d	0.124304 ^d
M ₁₁	70	0.00963526 ^c	0.107177 ^c

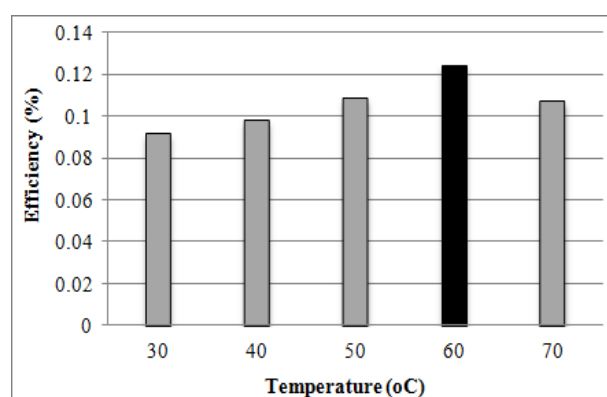


Fig. 4. The effect of the extraction temperature on the capacity of the anthocyanin extraction

3.5. Evaluation of the Optimal Extraction Time on the Anthocyanin Extraction

The extraction time affected to the ability of the anthocyanins extraction. If the extraction time (lower than 1.5 hrs) was too short, the anthocyanins did not come to the extracted liquid. on the other hand, if the extraction time was too long, anthocyanins got oxidized and the quality and quantity of anthocyanins reduced quickly. Table 5 showed that, when the sample was extracted in longer than 2.5 hrs, the amount of anthocyanins was reduced. The quantity of anthocyanins increased, and gave the best result from 1.5 to 2.5 hrs of extraction; the extracted amount of anthocyanin showed non-significant differences between the mean values of treatment times. From the economic viewpoint, extraction time 1.5 hrs was found sustainable (Table 5, Fig. 5).

Table 5. The effect of the extraction time on the anthocyanin extraction

Sample	Extraction time (hrs)	Amount of received anthocyanin (g)	Concentration of received anthocyanin from raw material (%)
M ₂₇	1.0	0.0101162 ^a	0.112527 ^a
M ₂₈	1.5	0.0116725 ^b	0.129839 ^b
M ₂₉	2.0	0.0116759 ^b	0.129876 ^b
M ₃₀	2.5	0.0116425 ^b	0.129505 ^b
M ₃₁	3.0	0.0103333 ^a	0.114942 ^a

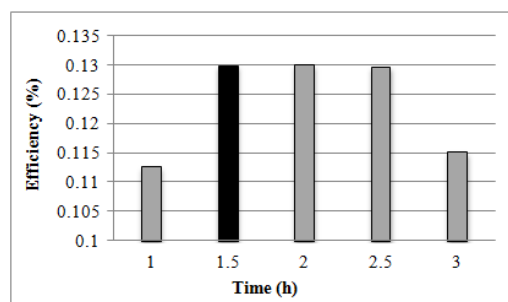


Fig. 5. The effect of the extraction time on the capacity of the anthocyanin extraction

4. Conclusion

This study results demonstrated that anthocyanin extraction is better when solvent (water: ethanol) ratio 2:8, raw material and solvent ratio 1:10, extraction time 1.5 hrs, pH 2.2 and temperature 60°C. This extraction condition was able to extract 0.1298% of anthocyanin from purple rice. The anthocyanin is one of the good components for human health, therefore, this extraction method could be applicable in food processing industries.

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