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Nguyen Phuoc Minh
Tra Vinh University, Vietnam.

Correspondence:
Nguyen Phuoc Minh
Tra Vinh University, Vietnam.

Antioxidant enhancement of anthocyanin pigment in purple fragrant rice ST during wine fermentation

Nguyen Phuoc Minh

Abstract
Anthocyanins are of particular interest to the food colorant industry due to their ability to impart vibrant colors. They are the largest group of water-soluble pigments in the plants, which are responsible for the red, purple and blue hues**.** Anthocyanins have been incorporated into the human diet for centuries and have been used as traditional herbal medicines due to their diverse physiological abilities to treat conditions such as hypertension, pyrexia, liver disorders, dysentery and diarrhea, urinary problems and the common cold. Recently anthocyanin-containingmaterials are being incorporated into food products and such products require further research to demonstrate their physiological effects. Purple fragrant rice ST is a specific agriculture product in Mekong river delta, Vietnam containing a lot of nutritious elements such as amino acids, minerals and antioxidants. This purple fragrant rice contains about 75% of starch, with a very high mineral content, rich in amino acids that specifically contain large amounts of anthocyanins with the antioxidant effect good for the health of users. Purple fragrant rice wine is a traditional fermented product of ethnic minority people in Mekong river delta, Vietnam. However owing to poor knowledge of processing, this product can't maintain its specific anthocyanin pigment. So it's necessary to study the extraction method to enhance natural color of purple fragant rice. Our results show that temperature, ethanol concentration and extraction time have positive effect to pigment extraction. Specifically, optimal parameters of extraction include temperature 70 ^{o}C; ethanol 60% v/v. pH is nearly stable during wine preservation. Optical density of mixed wine has changed during preservation, high in the beginning and low after 1-2 weeks. Purple fragant rice wine mixed with the extracted pigment has the significantly improved appearance.

Keywords: Purple fragrant rice, anthocyanin pigment, extraction, wine.

1. Introduction

Anthocyanins (Greek *anthos*, flower and Greek *kyanose* blue) are the largest group of water-soluble pigments in the plant kingdom and belong to the family of compoundsknown as flavonoids which are part of an even larger group of compounds known as polyphenols (Konczak *et al.*, 2004; Mazza *et al.*, 2007) [17]. Anthocyanins are responsible for the red, purple and blue hues in fruits, vegetables, flowers and grains, but also play important roles in plant physiology such as attractants for insect pollinators and seed dispersal (Schemske *et al.*, 1999) [24]. Anthocyanins are widely distributed in the human diet and the estimated daily intake has been found to be 12.5 mg/d in the United States (Wu X *et al.*, 2006). Therefore, they can be incorporated as a functional food ingredient into our diet. Currently, anthocyanins in blue and purple corn are being used for the production of naturally colored blue tortillas. Radish and potato extracts have color characteristics very similar to those of Allura red (a red dye used in food applications) (Giusti *et al.*, 2003) and therefore have the potential to be incorporated as food colorants. Incorporating anthocyanins as food colorants is not only valuable for improving overall appearance but also is very beneficial to our health.

Studies have demonstrated that anthocyanin extracts can improve sight acuteness (Ghosh *et al.*, 2007; Jang *et al.*, 2005; Kalt *et al.*, 2008; Lee et al, 2005; Matsumoto *et al.*, 2006) display antioxidative and radical-scavenging activity (Abdel-Aal *et al.*, 2008; Astadi *et al.*, 2009; Bellido *et al.*, 2009; Fukumoto *et al.*, 2000; Kahkonen *et al.*, 2003; Sanchez-Moreno *et al.*, 2000) and to act as chemoprotective agents (Colditz *et al.*, 1985; Fimognari *et al.*, 2004; Galvano *et al.*, 2007; Hagiwara *et al.*, 2001; Kang *et al.*, 2003; Liu *et al.*, 2005). Anthocyanins have been suggested as promising dietary compounds with an important role in human health. Anthocyanins have been suggested as promising dietary compounds with an important role in human health (Jaclyn Shipp and El-Sayed M. Abdel-Aal, 2010).

Purple fragrant rice ST is an heirloom variety of rice that contains high levels of pigments, which make each grain appear dark purple to black in color. Consuming purple fragrant rice provides our body with a number of nutrients, including some compounds not found in white or brown rice. *Aspergillus* species have attracted attention for their role in fermentation of oriental food products or industrial application of hydrolytic enzymes. Within the range of carbohydrolases produced by this species, the majority has been considered as α -amylase and glucoamylase. Recently there were several researches to utilize antioxidant pigment (anthocyanin) in rice wine fermentation to enhance its value. Kanitha Tananuwong and Wanida Tewaruth (2010) investigated the extraction and application of antioxidants from black glutinous rice**.** This research aimed to determine optimum extraction condition of black glutinous rice crude extract and to determine its application as an antioxidant in fish oil enriched mayonnaise. Kim *et al.*, (2012) demonstrated antioxidant activity and fermentation characteristics of traditional black rice wine. In this study they examined the antioxidant activity and fermentation characteristics of black rice wine. The antioxidant activity of black rice wine was higher than the control, and DPPH radical scavenging activity showed a correlation with the anthocyanin content of rice wine. Yossaporn Plaitho *et al.*, (2013) showed the protective effect of Thai fermented pigmented rice on urethane induced somatic mutation and recombination in Drosophila melanogaster. This study was aimed to proof that fermentation could increase phenolic and anthocyanin contents, antioxidant activities and antimutagenicity of Thai pigmented rice.

Purpose of our research is to improve anthocyanin pigment in purple fragrant rice wine so that we can accelerate its functional, nutritional and commercial value.

2. Material & Method

2.1 Material

Purple fragrant rice ST is originated from Mekong river delta, Vietnam. Its specifications are as follows: maximum moisture 15%; broken ration 4%; aflatxin B1, B2, G1, G2 < 0.2 µg/kg; packed into 5 kg PP bags with PE inside. *Aspergillus oryzae* and *Sacharomyces cerevisae* are supplied from Pasteur Institute, HCM City, Vietnam.

Fig 1: Purple fragrant rice ST

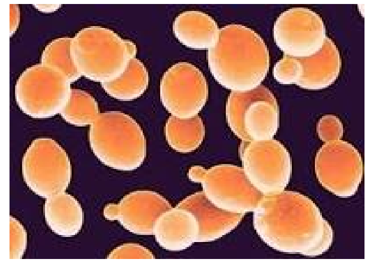

Fig 2: *Sacharomyces cerevisae*

Fig 3: *Aspergillus oryzae*

2.2 Research method

2.2.1. Experiment #1: Effect of temperature and ethanol concentration to anthocyanin extraction in cooked purple fragrant rice

Experiment is randomly arranged with 2 factors and 3 replications. Factor A: Extraction temperature (A1: 50ºC, A2: 60ºC, A3: 70ºC, A4: 80ºC, A5: 90ºC). Factor B: Ethanol concentration for extraction (B1= 50 %v/v, B2= 60 %v/v, B3= 70 %v/v, B4 = 80 %v/v). Total testings: 5 x 4 = 20.

Weigh 100 gram of raw material, soak in 30 minutes, well cook rice, cool to 30÷35 ºC. Pigment extraction is performed by taking 2 gram of cooked purple fragrant rice and dissolve with 30 ml solvent (ethanol 50, 60, 70, 80% v/v), extraction temperature 50, 60, 70, 80, 90 ºC, keep stable in water bath in 30 minutes. Analysis results include pH and otptical density of pigment.

2.2.2. Experiment #2: Effect of temperature and ethanol concentration to anthocyanin extraction in fermented wine waste

Experiment is randomly arranged with 2 factors and 3 replications. Factor A: Extraction temperature (A1: 50 ºC, A2: 60 ºC, A3: 70 ºC, A4: 80 ºC, A5: 90 ºC). Factor B: Ethanol concentration for extraction (B1= 50 %v/v, B2= 60 %v/v, B3= 70 %v/v, B4 = 80 %v/v). Total testings: 5x4= 20.

Weigh 200 gram of raw material, soak in 30 minutes, well cook rice, cool to 30÷35 ºC, add 2 gram of yeast-mold mixture, incubate 3 days, add 300 ml distilled water, ferment 4 days, filter to get wine waste. Weigh 2 gram of wine waste with 30 ml solvents (ethanol 50, 60, 70, 80%v/v), extract at temperature 50, 60, 70, 80 and 90 ºC, keep in water bath in 30 minutes. Analysis results include pH and optical density of pigment.

2.2.3. Experiment #3: Pigment improvement of mixture of extracted pigment and purple fragrant wine

Experiment is arranged in 1 factor and two replications. Factor C: kind of wine (C_0: Wine combined with stock wine and preserved in 2 months. C_1: Wine combined with extracted pigment from fermented wine waste. C_2: Wine combined with extracted pigment from the cooked rice. C_3: Wine control without mixing).

After selecting the optimal parameters in experiment #1 & #2, we take 50 gram of material (waste + cooked rice), dissolve with 150 ml of ethanol (60% v/v), extract pigment at 70oC in 30 minutes. Then we mix 100 ml of fermented rice wine with 27 ml of extracted pigment (fermented and cooked). By the way, we also take 100 ml of the mixed wine to combine with 43 ml of stock wine (18% v/v). Keep the mixed wine in preservation by time. Analysis result includes sensory evaluation of wine (both the mixed wine and the control wine).

2.2.4. Experiment #4: Change of optical density and pH during preservation

Experiment is arranged in 2 factors and three replications. Factor C: Kind of wine (C1: Wine combined with extracted pigment from fermented wine waste. C2: Wine combined with extracted pigment from cooked rice. C3: Wine combined with stock wine). Factor T: Preservation time (T0: Immediately after miing. T1: After 1 week of preservation. T2: After 2 weeks of preservation).

Experiment #4 is conducted on basis of experiment #3 after finding the optimal parameters in experiment #1 & #2. We take 50 gram of material (fermented waste + cooked rice), dissolve with 150 ml of ethanol (60% v/v), and extract pigment at 70°C in 30 minutes. Then we mix 100 ml of fermented rice wine with 27 ml of extracted pigment (fermented and cooked). By the way, we also take 100 ml of the mixed wine to combine with 43 ml of stock wine (18% v/v). Keep the mixed wine in preservation by time. Analysis results include pH and otptical density of wine during preservation.

2.3 Statistical analysis

All data are processeed by Excel 2003 and ANOVA (Startgraphics) to check the significant difference via LSD.

3. Result & Discussion

3.1 Effect of temperature, ethanol concentration to anthocyanin pigment extraction in the cooked purple fragrant rice

3.1.1 Effect of temperature, ethanol concentration to pH of the cooked purple fragrant rice liquid.

Table 1. Effect of temperature, ethanol concentration to pH of the cooked purple fragrant rice liquid

Extraction temperature (°C)	Ethanol concentration (%v/v)				Average
	50	60	70	80	
50	8.00	8.24	8.59	8.71	8.39[b]
60	8.03	8.30	8.59	8.72	8.41[b]
70	8.21	8.79	8.48	8.33	8.45[b]
80	7.96	8.08	8.41	8.75	8.30[b]
90	7.96	7.69	7.71	8.14	7.88[a]
Average	8.03[a]	8.22[ab]	8.36[ab]	8.53[b]	8.29

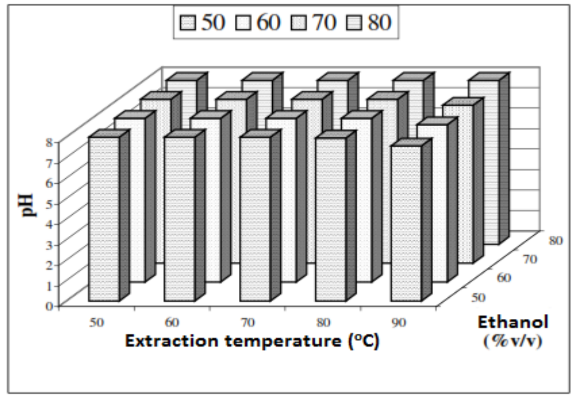

Fig 4: Effect of temperature, ethanol concentration to pH of the cooked purple fragrant rice liquid

From figure 4, there is not high fluctuation of pH. From table 2, there is not statistically significant difference of pH by temperature and ethanol concentration for anthocyanin extraction. So method of optical density surveillance is accepted for anthocyanin determination.

3.1.2. Effect of temperature, ethanol concentration to optical density of the cooked purple fragrant rice liquid

Table 2: Effect of extraction temperature, ethanol concentration to optical density of the cooked purple fragrant rice liquid measure at λ max= 530 nm

Extraction temperature (°C)	Ethanol concentration (%v/v)				Average
	50	60	70	80	
50	0.22	0.26	0.17	0.08	0.18[a]
60	0.32	0.32	0.23	0.12	0.25[b]
70	0.40	0.40	0.32	0.17	0.32[c]
80	0.48	0.54	0.42	0.31	0.44[d]
90	0.83	1.00	0.84	0.68	0.84[e]
Average	0.45[c]	0.50[d]	0.40[b]	0.27[a]	0.41

From table 2, extraction temperature and ethanol concentration affect to optical density of the extracted liquid. At 90°C and ethanol concentration 60% v/v we get the highest optical density. However when combining with sensory evaluation, we decide to choose 70°C, 60%v/v for anthocyanin extraction.

3.2 Effect of extraction temperature, ethanol concentration to anthocyanin pigment extraction in the fermented wine waste discarded from purple fragrant rice wine

3.2.1 Effect of extraction temperature, ethanol concentration to pH in the fermented wine waste discarded from the purple fragrant rice wine

Table 3: Effect of extraction temperature, ethanol concentration to pH in the fermented wine waste discarded from the purple fragrant rice wine

Extraction temperature (°C)	Ethanol concentration (%v/v)				Average
	50	60	70	80	
50	4.97	5.66	5.66	5.67	5.49[ab]
60	5.89	6.07	5.62	6.17	5.94[c]
70	5.82	6.63	6.36	5.65	6.12[c]

80	6.32	5.80	5.43	5.71	5.82[bc]
90	5.05	5.73	5.24	5.50	5.38[a]
Average	5.61[a]	5.98[a]	5.66[a]	5.74[a]	5.75

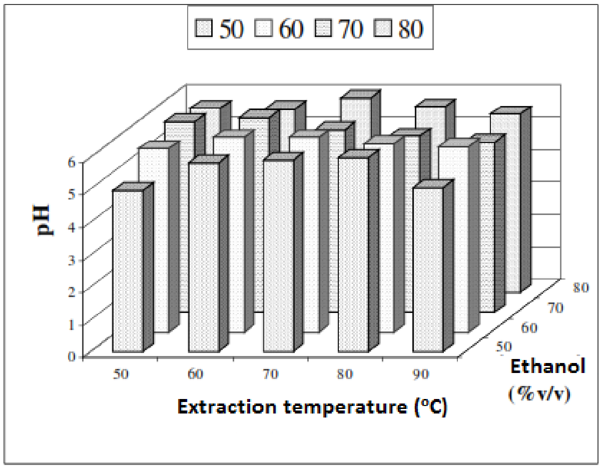

Figure 5: Effect of extraction temperature, ethanol concentration to pH in the fermented wine waste discarded from the purple fragrant rice wine

From table 3, ethanol concentration doesn't significantly affect to pH in the fermented waste discarded from the purple fragrant rice wine. Optical density measurement is applicable to determine anthocyanin concentration.

3.2.2. Effect of extraction temperature, ethanol concentration to change of optical density in the fermented wine waste discarded from the purple fragrant rice wine

Table 4: Effect of extraction temperature, ethanol concentration to change of optical density in the fermented wine waste discarded from the purple fragrant rice wine measured at λmax = 530 nm

Extraction temperature (°C)	Ethanol concentration (%v/v)				Average
	50	60	70	80	
50	0.10	0.10	0.12	0.10	0.11[a]
60	0.21	0.25	0.25	0.19	0.22[b]
70	0.12	0.13	0.13	0.16	0.14[a]
80	0.26	0.31	0.28	0.28	0.29[b]
90	1.32	2.06	2.51	2.40	2.07[c]
Average	0.40[a]	0.57[b]	0.66[b]	0.63[b]	0.57

From table 4, extraction temperature and ethanol concentratuion affect to optical density of the fermented fluid. Extraction at 90°C and ethanol 60-80% v/v show the highest optical density.

3.3 Sensory evaluation of different kinds of purple fragrant rice wine

Table 5: Pigment improvement of different kinds of purple fragrant rice wine during preservation by descriptive scale

Kind of purple fragrant rice wine	Preservation time (week)			Average
	0	1	2	
Wine combined with stock wine preserved in 2 months	1.35	1.20	1.00	1.83[a]
Wine combined with stock wine	3.15	2.60	2.20	2.65[b]
Wine combined with extracted pigment from cooked rice	4.20	3.90	3.40	3.83[c]
Wine combined with extracted pigment from fermented waste	3.30	4.15	4.45	3.97[c]
Average	3.00[b]	2.96[ab]	2.76[a]	2.91

3.4 Optical density of different kind of rice wine by preservation time

3.4.1 Change of optical density of different kinds of rice wine by preservation time measured at λmax = 530nm

Table 6. Change of optical density of different kinds of rice wine by preservation time measured at λmax = 530nm

Preservation time (week)	Kind of rice wine			Average
	Wine combined with stock wine	Wine combined with extracted pigment from fermented waste	Wine combined with extracted pigment from cooked rice	
0	0.32	0.54	0.49	0.45[b]
1	0.11	0.15	0.21	0.16[a]
2	0.11	0.14	0.20	0.15[a]
Average	0.18[a]	0.28[b]	0.30[c]	0.25

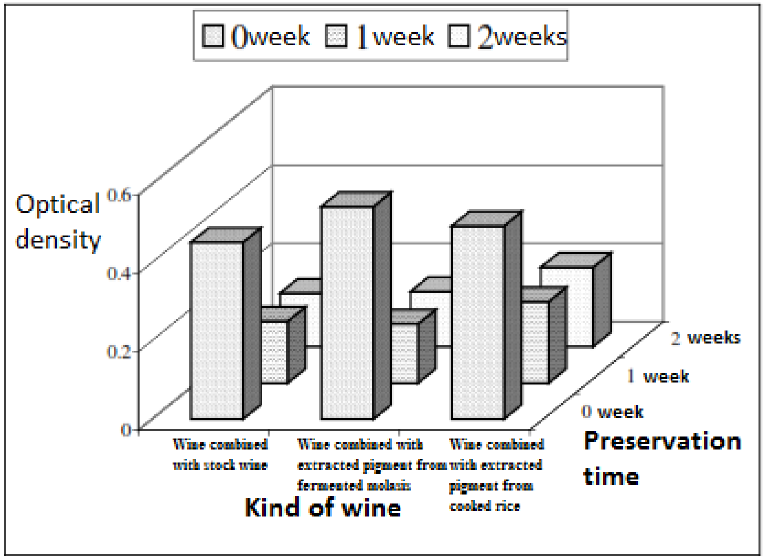

Fig 6: Change of optical density by preservation time of different kinds of rice wine

From table 6, extracted pigment mixed with rice wine has affected to optical density of the mixed wine during preservation. The wine combined with extracted pigment from fermented waste has the highest optical density. Meanwhile the wine combined with stock wine has the lowest optical density.

From table 6, preservation time has affected to the wine optical density. At initial point, the wine combined with extracted pigment from fermented waste has the highest optical density. After 1-2 weeks of preservation, this optical density will be decreased significantly owing to deposit by preservation time.

3.4.2 Change of pH by preservation time of different kinds of rice wine

Table 7: Change of pH by preservation time of different kinds of rice wine

Preservation time (week)	Kind of rice wine			Average
	Wine combined with stock wine	Wine combined with extracted pigment from fermented waste	Wine combined with extracted pigment from cooked rice	
0	4.26	4.26	4.27	4.26[a]
1	4.23	4.23	4.23	4.23[a]
2	4.31	4.35	4.32	4.33[a]
Average	4.27[a]	4.28[a]	4.27[a]	4.27

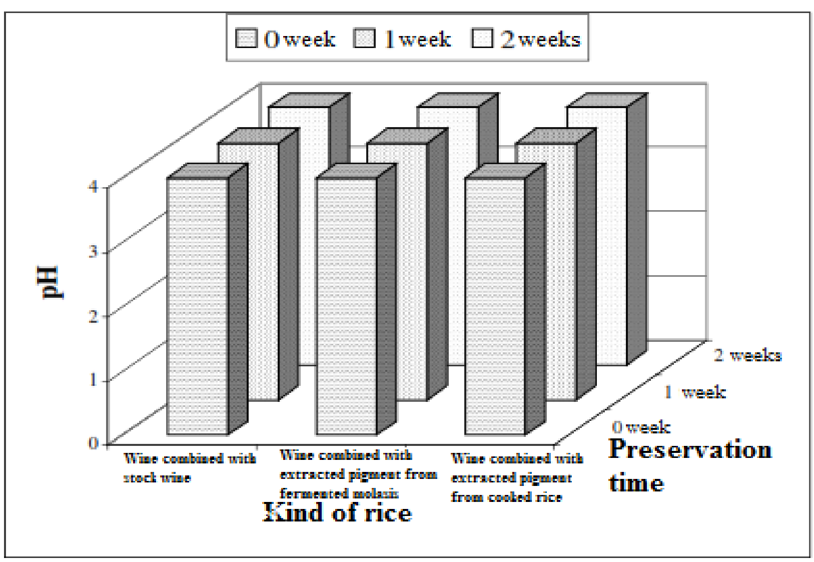

Fig 7: Change of pH by preservation time of different kinds of rice wine

From table 7 and figure 7, we can see the stable pH during wine preservation.

4. Conclusion

We have successfully exploit nutrient elements inside purple fragrant rice ST, contributing to its value-added enhancement during rice wine fermentation. Anthocyanin pigment is extracted from the purple fragrant rice (both the fermented waste and cooked rice) by ethanol 60% v/v at temperature 70^{o}C. Finished purple fragrant rice wine has the improved pigment appearance. From this approach, we can help ethnic minority people living in the Mekong river delta, Vietnam in improving value of their agricultural product.

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