ผลของสภาวะที่ใช้ในการสกัดรำข้าวที่มีสีต่อปริมาณแอนโทไซยานินและความสามารถในการต้านออกซิเดชั่นและความคงตัวในระหว่างการเก็บรักษา

Effects of Extracting Conditions of Pigmented Rice Bran on Anthocyanin and Antioxidant Activity and Its Storage Stability

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บทคัดย่อ

งานวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของสายพันธุ์รำข้าวที่มีสี (PRB) และสภาวะที่เหมาะสมในการสกัดปริมาณสารประกอบฟีนอลิก (TPC), ปริมาณแอนโทไซยานิน (TAC), ความสามารถในการต้านออกซิเดชั่น (DPPH) และศึกษาความคงตัวในระหว่างการเก็บรักษา โดยในการทดลองจะศึกษาสายพันธุ์ของข้าว 3 สายพันธุ์, ขนาดอนุภาคของรำข้าว 3 ขนาด, สภาวะในการสกัดแบบเขย่าและไม่เขย่า, ตัวทำละลายในการสกัด 6 รูปแบบ, เวลาและอุณหภูมิในการสกัด 4 แบบ จากผลการทดลองพบว่า สายพันธุ์ที่เหมาะสมที่สุดคือ ข้าวหอมนิลที่มีขนาดอนุภาค 60 เมช สกัดในสภาวะที่มีการเขย่า ด้วยตัวทำละลาย 2%ของสารละลายซิตริก โดยทำการสกัดเป็นเวลา 3 ชั่วโมง ที่อุณหภูมิ 40 องศาเซลเซียส โดยมีค่า TPC, TAC และ ความสามารถในการต้านออกซิเดชั่น ด้วยวิธี DPPH มีค่าเท่ากับ 320 มิลลิกรัมแกลลิค (GAE)/100กรัม, 238.02 มิลลิกรัมไซยานิดิน /100กรัม และ 87.09% ตามลำดับ นอกจากนั้นในการศึกษาความคงตัวในระหว่างการเก็บรักษาที่ 3 อุณหภูมิ ในช่วงเวลา 0-63 วัน โดยเก็บในสภาวะที่มีแสงและไม่มีแสงจากการทดลองพบว่า ปริมาณ TPC, TAC และกิจกรรมการต้านอนุมูลอิสระด้วยวิธี DPPH จะมีค่าลดลงเมื่อเวลาในการเก็บนานขึ้นและเก็บในสภาวะที่มีแสง ดังนั้น ในการสกัดแอนโทไซยานินจากรำข้าวที่มีสีสามารถนำมาใช้เป็นสารออกฤทธิ์ชีวภาพในอาหารและในอุตสาหกรรมเครื่องดื่มได้ แต่อย่างไรก็ตาม ควรเก็บสารสกัดไว้ในสภาวะที่เหมาะสมก่อนนำไปใช้เพื่อให้มีความคงตัวและเกิดประโยชน์สูงสุด

คำสำคัญ: รำข้าวที่มีสี, การสกัด, แอนโทไซยานิน, สายพันธุ์ข้าว, ความคงตัว

สาขาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะเทคโนโลยีการเกษตรและอุตสาหกรรมเกษตร มหาวิทยาลัยเทคโนโลยีราชมงคลสุวรรณภูมิ พระนครศรีอยุธยา 13000
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ABSTRACT

The objectives of this study were to determine the effects of pigmented rice bran (PRB) cultivars and anthocyanin extraction conditions on total phenolic content (TPC), total anthocyanin content (TAC) and DPPH scavenging activity (DPPH) and then to assess conditions for the stability of the optimal anthocyanin. We tested 3 cultivars, 3 particle sizes, shake vs. no shake, 6 solvents, 4 extraction times and 4 temperatures. The results showed that the appropriate cultivar and extraction parameters were Homnil cultivar, 60 mesh particle size, shaking, 2% (w/v) citric acid and extraction for 3 h at 40°C. The TPC, TAC and DPPH scavenging activity were 320 mg gallic acid equivalent (GAE)/100g, 238.02 mg cyanidin /100g and 87.09% respectively. We investigated stability at 3 storage temperatures ranging from 0-63 days and with the presence and absence of light. The results showed that the TPC, TAC and DPPH activity decreased with increasing storage temperature, time and light exposure. Therefore, anthocyanin from pigmented rice bran extract is useful as a bioactive food or beverage ingredient; however, it should be kept under appropriate conditions for stability.

Key words: pigmented rice bran, extraction, anthocyanin, rice cultivars, stability.

INTRODUCTION

Pigmented rice (*Oryza sativa* L.) has been consumed for a long time in Asia, especially China, Japan, Korea and many countries in Southeast Asia. Several varieties of pigmented rice, particularly red and black rice, are cultivated in Thailand. The healthy properties of pigmented rice were reported to include the capability of preventing atherosclerosis in a mouse model and human study (Kannan *et al*., 2010). These results may in part be attributed to the presence of natural antioxidants (Oki *et al*., 2002). Moreover, pigmented rice was reported to have a greater antioxidant capacity than white rice (Ahuja *et al*., 2007). Pigmented rice is an economically important rice species and derives its name from its rich natural anthocyanin compounds, such as cyanidin 3-glucoside and peonidin 3-glucoside, which possess anti-oxidative and anti-inflammatory activities. Previous investigators have shown that dietary supplementation with black rice pigment significantly inhibited atherosclerotic plaque formation in rabbits (Maier *et al*., 2009).

Pigmented rice bran has a higher content of bioactive compound as compared to white rice bran (Shao *et al*., 2014). The antioxidant activity of pigmented rice bran extract is pH dependent; its antioxidant activity decreases as pH increases from pH 2 to 7 (Sukhapat *et al*., 2004). However, there is little information regarding the extraction of anthocyanin or the effects of other extraction or storage conditions on the antioxidant properties of pigmented rice bran extract from different cultivars in Thailand. Therefore, the objectives of this study were to investigate optimum anthocyanin extracting conditions among three rice cultivars and then to investigate conditions to achieve higher TPC content, TAC and DPPH by using the Homnil cultivar. Finally, we investigated the effect of storage time, temperature and lighting on the shelf-life and stability of the extracted anthocyanin.

MATERIALS AND METHODS

Materials and chemicals

Rice bran samples of pigmented rice (*Oryza sativa* L.) from 3 cultivars (Homnil (RB1) from Surin province, Rice berry (RB2) from Kampangsan province, and Hommali dang (RB3) from Phattalung province) were obtained by milling rice grains in a local mill and the obtained rice bran was passed through a 60 mesh screen sieve. The content of moisture on pigmented rice bran was 8.75%. The rice

bran samples were kept at -18°C for extraction and Folin Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and sodium carbonate were purchased from Sigma Chemical Co., Ltd (St. Louis, USA). Other chemical reagents were analytical grade purchased from Sigma-Aldrich Co., Ltd (Steinheim, Germany).

Factors affecting anthocyanin extraction

Rice cultivars

Five grams of each cultivar was extracted with 20 ml of 95 % ethanol: 1.5N HCl 85:15(v/v) in a shaker (SWB25, New Brunswick Scientific, USA) at 150 rpm at room temperature for 3 h. Samples were then filtered through Whatman No.1 filter paper and the solvent was removed with a rotary evaporator (BUCHI R-3, Germany) at 50°C under vacuum. The obtained concentrated extracts (with moisture content of 70 % (wet basis)) were weighed for % yield (wet matter basis, wb) calculation and then stored at -18°C until use for determination of the yields, total phenolic content (TPC), total anthocyanin content (TAC), and DPPH-scavenging activity (DPPH). We used the best performing cultivar in all subsequent studies. The experiment involved as a completely randomized design (CRD).

Shaking and particle size

Five grams of selected pigmented rice bran particle sizes 40, 60 and 80 mesh was extracted with 20 mL of 95% ethanol:1.5N HCl, 85:15(v/v) in a shaker at 150 rpm at room temperature for 3 h under shaking compared with non-shaking conditions. The samples were filtered through Whatman No.1 filter paper and stored at -18°C until used for the determination of TPC, TAC, and DPPH. The experiment employed a completely randomized design (CRD).

Type of solvents

Five grams of selected pigmented rice bran particle sizes from the effect of shaking and particle sizes study was extracted with 20 ml of one of 6 solvent treatments (distilled water (control); 95 % ethanol:1.5N HCl, 85:15 (v/v) and 0.5, 1, 1.5 and 2% (w/v) citric acid) under conditions in the effect of shaking and particle sizes study and shaking at 150 rpm at room temperature for 3 h. The samples were filtered through Whatman No.1 filter paper and stored at -18°C until used for the determination of TPC, TAC and DPPH. The experiment utilized a completely randomized design (CRD).

Temperature and time

Five grams of selected pigmented rice bran was extracted with 20 ml of selected solvent from the effect of type of solvents study by varying the extraction time (1, 2, 3 and 4 h) and temperature (room temperature (RT), 40, 50, 60 and 70 °C). Samples were then filtered through Whatman No.1 filter paper. The sample were stored at -18°C for the determination of TPC, TAC, and DPPH. The experiment used a completely randomized design (CRD).

Effect of storage conditions on anthocyanin stability

The pigmented rice bran extract was stored at 3 temperatures (4°C, 15 °C and RT), time (0-63 days) and in the presence and absence of light. The TPC, TAC and DPPH scavenging activity of the samples were determined (see below).

Analysis of pigmented rice bran extract

Determination of the pigmented rice bran extraction yield

The obtained extracts were weighed for % yield (wet basis, wb) calculated using the following equation:

$$\text{Yield (\% wet basis)} = W1/W2 \times 100$$

Where: W1 = weight of pigmented rice bran extract after evaporation
W2 = weight of pigmented rice bran before extract

Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu reagent according to the method of Masuda *et al*. (1999). The reaction mixture contained 100 µL of the diluted pigmented rice bran extract, 500 µL of freshly prepared diluted Folin Ciocalteu reagent, and 400 µL of 7.5% sodium carbonate. Mixtures were kept in the dark for 2 h at room temperature to complete the reaction. The absorbance at 750 nm was measured with a UV-Vis spectrophotometer (Shimadzu Corp., Bara Scientific Co., Ltd.). Gallic acid was used as a standard and the results were expressed as mg gallic acid (GAE)/100g rice bran extract.

Determination of total anthocyanin content (TAC)

The total anthocyanin content in the pigmented rice bran was measured by a pH-differential method (Giusti and Wrolstad, 2001). Anthocyanin from the pigmented rice bran extracted by 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) was measured at 510 and 700 nm, respectively. The content of total anthocyanin was calculated using the following formula:

$$\text{anthocyanin contents} = (A \times MW \times DF \times 1000)/\varepsilon$$

where MW represents molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor (20), ε is molar absorptivity of cyanidin-3-glucoside (26,900 l/mol cm) and calculated from the following equation:

$$A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$$

Note that A_{700} was measured and subtracted off in order to eliminate the effect of haze or sediments in the sample.

Determination of DPPH radical scavenging activity

Antioxidant capacity was determined by the DPPH (1, 1- diphenyl-2-picrylhydrazyl) assay according to the method of Masuda *et al.* (1999) with some modifications. Each sample was diluted 10-fold in methanol and then a 2 mL subsample was mixed with 2 mL of freshly prepared methanolic solution containing 0.1 mM of DPPH solution. The mixture was shaken vigorously and left to stand for 30 min in the dark. Vitamin C was used as a positive control. The absorbance was then measured at 517 nm. The DPPH scavenging activity was calculated as follows:

%DPPH scavenging activity = [1 - (absorbance of sample/absorbance of blank)] × 100.

Statistical analysis

Three sample replications were performed for each treatment combination. Data were analyzed by ANOVA and least significant difference procedures were used to separate means. Differences were reported to be significant at $p<0.05$, using a standard statistical software package.

RESULTS AND DISCUSSION

Factors affecting anthocyanin extraction

Rice cultivars

The effect of rice cultivars on the yields, total phenolic content (TPC), total anthocyanin content (TAC), and DPPH radical scavenging activity (DPPH) are shown in Table 1. The results showed that the yields, TPC, TAC, and DPPH of the RB1 (Homnil) cultivar (purple rice) were the highest with values of 6.90% (w/w by wet basis), 320.21 g gallic acid equivalent (GAE)/100g, 240.12 mg cyanidin/100g, and 87.09%, respectively. Yoshida *et al*. (2010) also reported that the DPPH radical scavenging activity of black rice was greater than that of red rice. Additionally, different environmental conditions could also alter the biosynthesis of phenolic compounds in grapes (Song *et al.*, 2015).

Table 1 Yield, total phenolic content (TPC), total anthocyanin content (TAC), and DPPH radical scavenging activity (DPPH) of different cultivars of pigmented rice bran extracts.

Varieties	Yield (%)	TPC (mg GAE/100g)	TAC (mg Cyn/100 g)	DPPH (%)
RB1	6.90 ± 1.03^{a}	320.21 ± 1.03^{a}	240.12 ± 0.11^{a}	87.09 ± 0.99^{a}
RB2	6.63 ± 1.06^{a}	309.71 ± 1.15^{b}	231.23 ± 0.22^{b}	76.09 ± 0.43^{b}
RB3	4.75 ± 1.12^{b}	265.45 ± 0.93^{c}	194.87 ± 0.87^{c}	67.45 ± 0.12^{c}

Means (standard deviation, n= 3) in the same column with different letters (a-c) are significantly different (p<0.05).

Rice bran particle sizes and shaking

The effect of particle sizes and shaking on the total phenolic content (TPC), total anthocyanin content (TAC), and radical scavenging activity (DPPH) from RB1 are displayed in Figure 1**(**A-B**).** When the particle size decreased from the 40 to 60 mesh, the TPC, TAC and DPPH of the extracts significantly increased. However, TPC, TAC and DPPH of the extracts from particle size of 60 mesh were not significantly different from those of 80 mesh. The results showed that the highest TPC and TAC levels were observed with particle size of 60 mesh and shaking at 150 rpm with values of 300 mg gallic acid (GAE)/100g rice bran extract, 235.01 mg cyanidin/100g and 80% DPPH activity, respectively. Hiba *et al*. (2014) reported that the smaller particle size of grape by products increased the extraction of total phenolic compounds, flavonoids and anthocyanins. Moreover, Maisuthisakul and Changchub (2014) also found significant differences in antioxidant activities among some Thai rice samples that were attributed to several complex factors including cultivar, growing environment, and extraction conditions. Particle size is one of the most significant factors affecting the efficiency of extraction because the particle size controls the kinetics of mass transfer and the access of the solvent to soluble compounds. An increase in the extraction rate of total polyphenols was observed with the decrease of particle size. The influence is predictable since the contact surface and the pore diffusion path increase with decreasing particle size, and leads to an easier permeability or diffusivity of the solvent into the material (Patrauanu *et al*., 2019) .

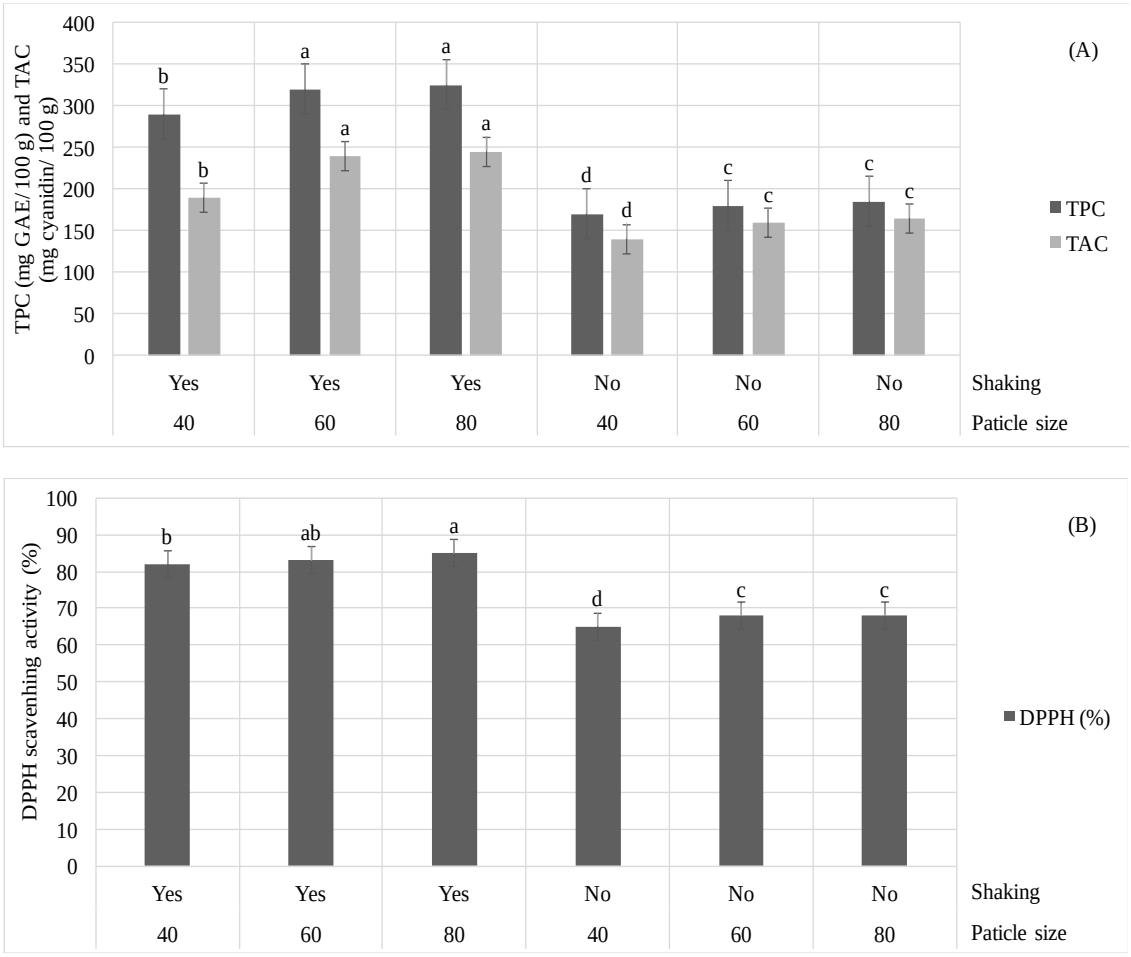


Figure 1 Effect of shaking on total phenolic content (TPC) and total anthocyanin content (TAC)(A) and DPPH assay (B) in Homnil rice bran extract. Error bars indicate the standard error of the mean (n=3). Mean bars with different letters (a-d) are significantly different (p<0.05).

Solvent types

The effect of solvent types on the total phenolic content (TPC), total anthocyanin content (TAC), and DPPH radical activity of Homnil rice bran extract are shown in Figure 2(A-B). The results indicated that the highest TPC and TAC were observed, using 2% citric acid (with values of 310 mg/100g and 238.31 mg/100g, respectively) and 95 % ethanol:1.5N HCl 85:15(v/v). However, when Homnil rice bran was extracted by citric acid at concentrations higher than 2% (at 2.5 %), the TPC and TAC were not significantly different from that extracted by 2% citric acid (data not shown). Extraction using distilled water (as a control) yields the lowest TPC and TAC. It is also known that the use of acid is necessary to obtain the flavylium cation form, which is red and stable in an acid medium. However, low pH levels or high concentrations of citric acid resulting in partial hydrolysis and anthocyanin decomposition (Ahuja *et al*., 2007). Fera *et al*. (2013) also showed that the highest yield of anthocyanin extraction from buni fruits was obtained by acidification of extraction solvent with citric acid, since citric acid could maintain the low pH that favors the formation of flavylium chloride. The 95% ethanol:1.5N HCl 85:15(v/v) solvent was reported as one of the good solvents for extraction of phenolic compounds from natural sources. However, 2% citric acid solution was selected to be used for extraction of anthocyanin from pigmented rice bran in the remaining experiments in this study

since it is simple, low cost and safer than the 95% ethanol:1.5N HCl 85:15(v/v) solvent. DPPH results from Figure 2B are presented.

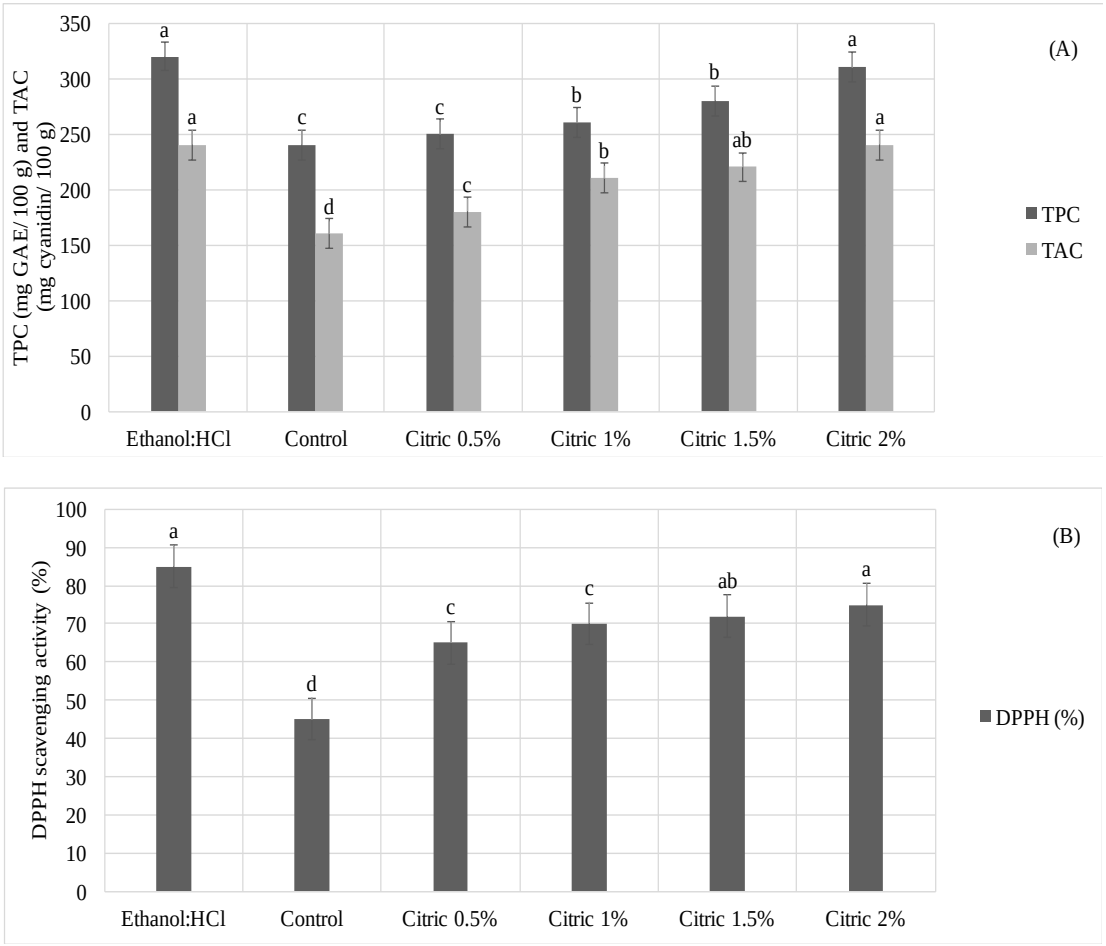


Figure 2 Effect of type of solvent on the total phenolic content (TPC) and total anthocyanin content (TAC)(A), and DPPH assay(B) of Homnil rice bran extract.
Error bars indicate the standard error of the mean (n=3). Mean bars in the same response variable with different letters (a-c) are significantly different ($p<0.05$).

Temperature and time

The effect of temperature and time of extraction of the RB1 cultivar on the TPC, TAC and DPPH assay are shown in Figure 3A-C. The extraction temperature and time affected the extractability of anthocyanins and phenolic compounds. The results revealed that the TAC of Homnil rice bran extracted at 40 ^{o}C or 50 ^{o}C, for 3 to 4 h were the highest ($p<0.05$) of all the treatment conditions. Similarity, the TPC and DPPH of Homnil rice bran extracted at 40 or 50 ^{o}C, for 3-4 h were also the highest. However, at temperatures above 60 ^{o}C, rates of anthocyanin decomposition were high. Jing and Giusti (2007) also found that temperatures higher than 70 °C can cause rapid degradation and discoloration of anthocyanins in purple corn.

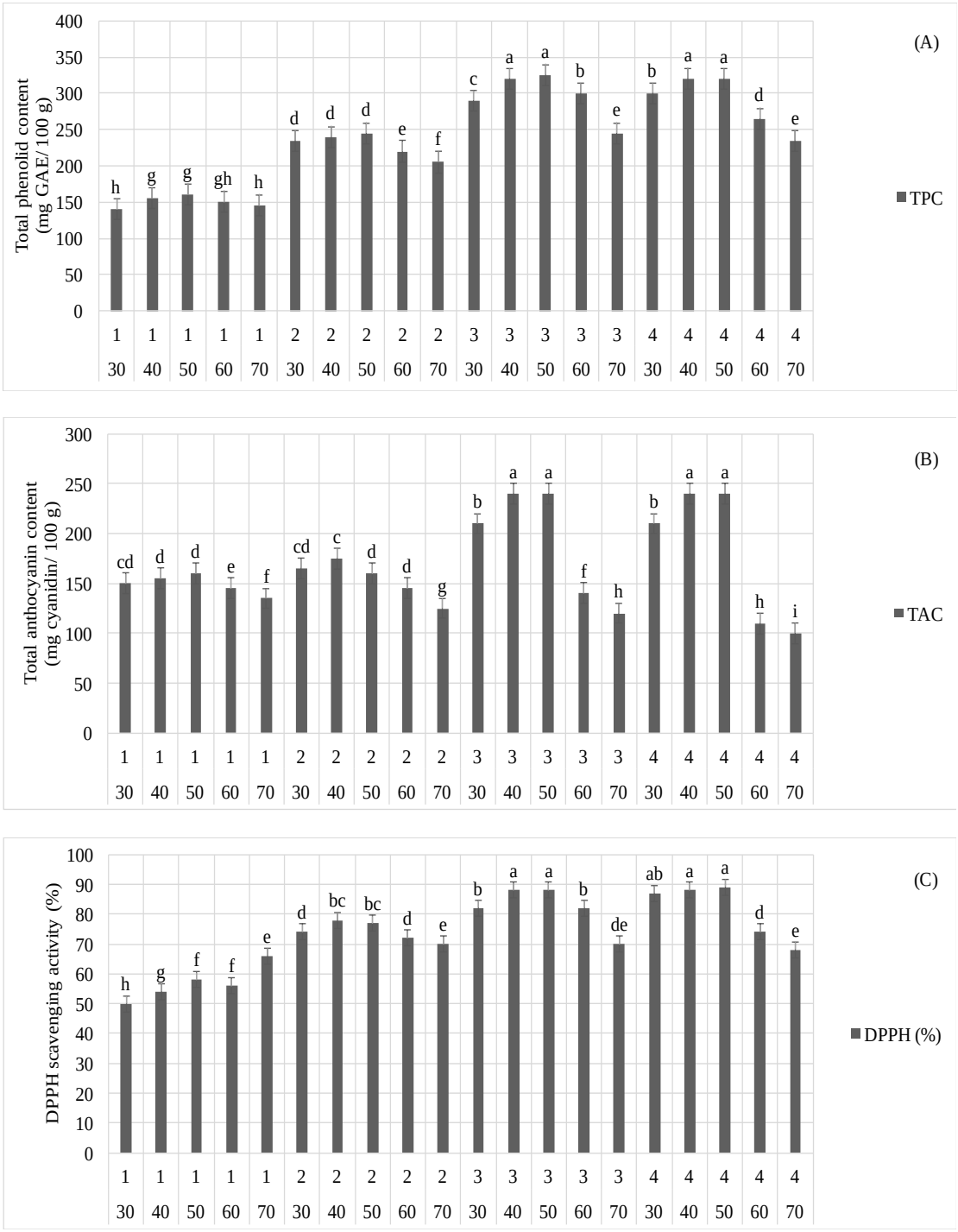


Figure 3 Effect of extraction temperature and time on the total phenolic content (TPC)(A), total anthocyanin content (TAC)(B) and DPPH assay(C) of Homnil rice bran extract. Error bars indicate the standard error of the mean (n=3). Mean bars with different letters (a-i) are significantly different (p<0.05).

Effect of storage conditions on anthocyanin stability

Effect of storage temperature

The TPC, TAC and DPPH assay results for Homnil rice bran extract during storage at 4 ºC, 15℃ and room temperature (30±2°C) for up to 63 days are given in Figure 4A-C. The results revealed that storage temperature had a significant effect on the stability of anthocyanin (p<0.05) with less degradation of TAC, TPC and DPPH over time at 4°C than the other

temperature treatments. Laleh *et al*. (2006) also found that higher storage temperatures accelerated the destruction of anthocyanins in *Berberis* (a genus of shrub). An increase of temperature shifted an anthocyanin equilibrium towards the chalaone form (Ahuja *et al*., 2007) Giusti and Wrolstad (2000) reported that the speedy destruction of anthocyanin in higher temperatures could be due to hydrolyzation of 3-glycoside structure. Moreover, the hydrolyzation of the pyrilium ring resulted in production of chalcone, which was responsible for brown color development of anthocyanin in food. Therefore, low temperature storage is crucial to maintaining the benefits of these anthocyanins.

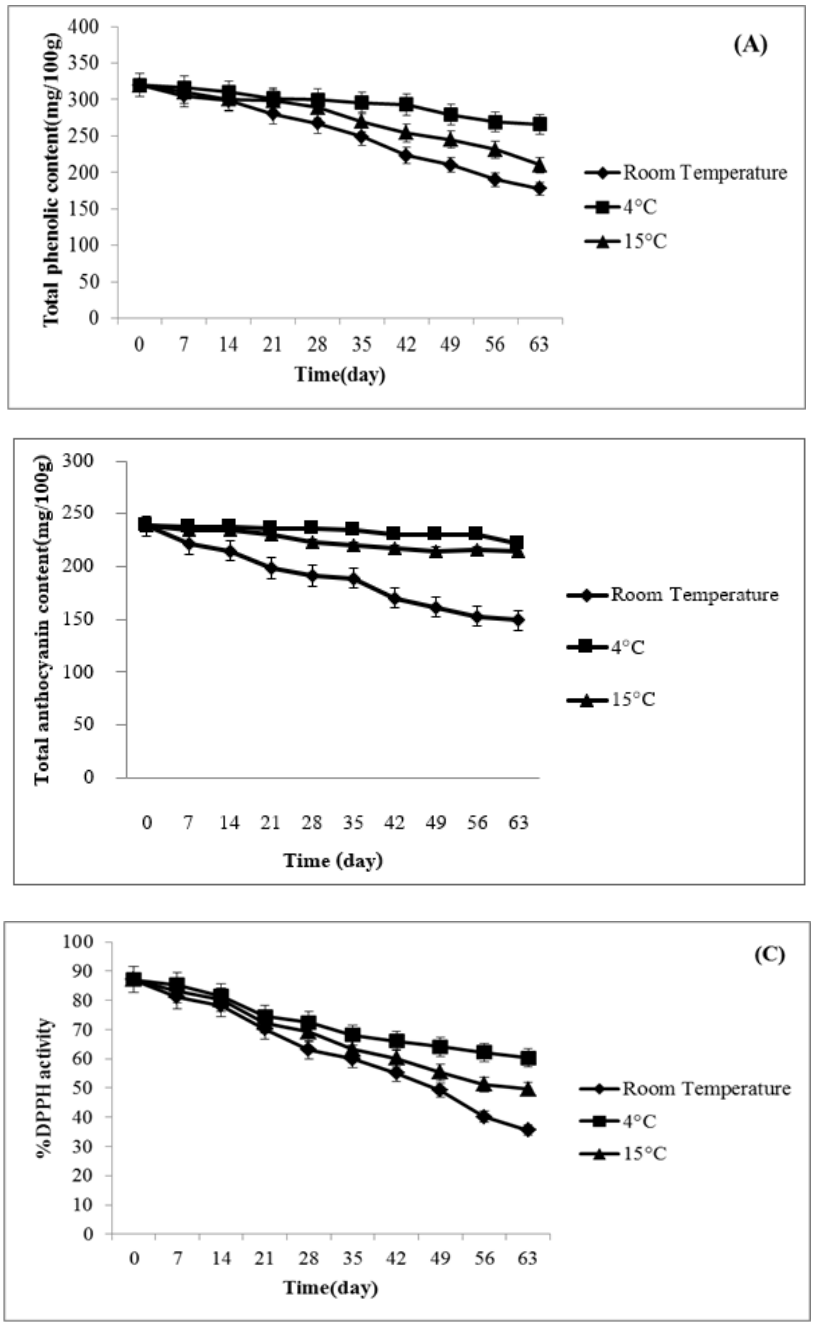
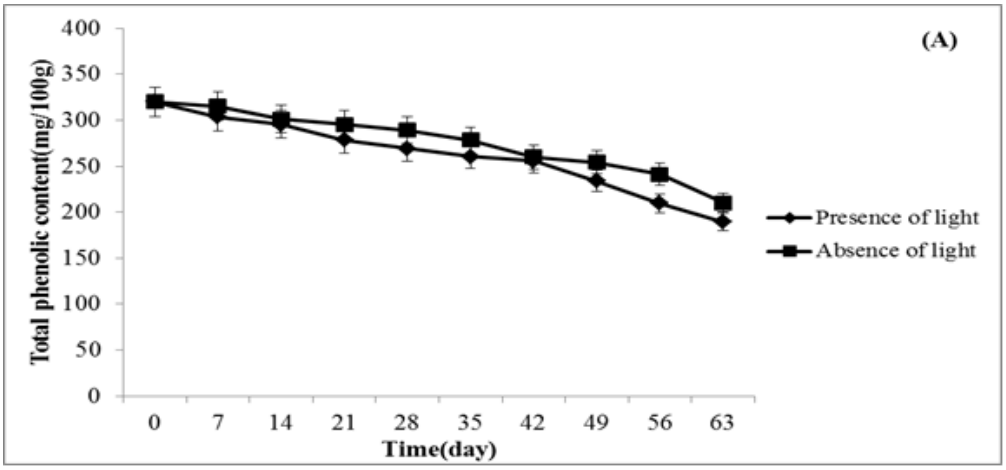
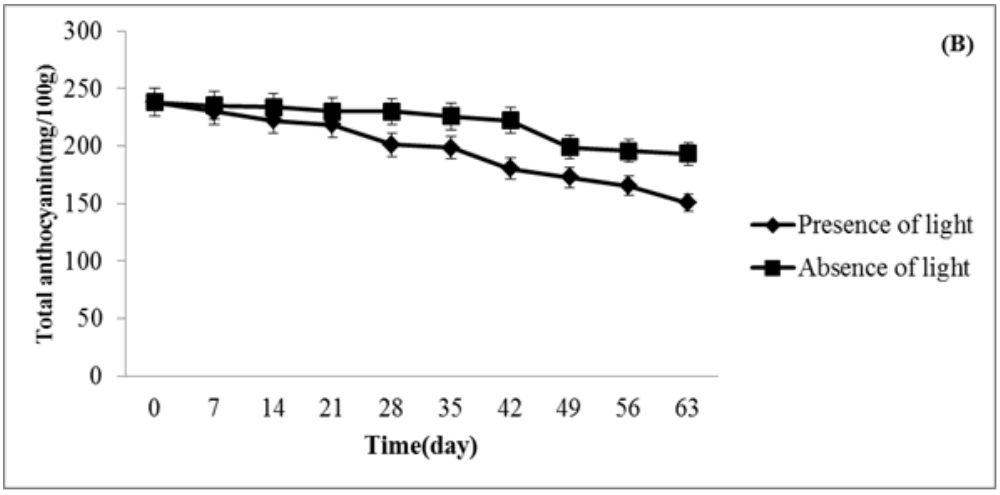


Figure 4 The effect of storage temperatures and times on total phenolic content (TPC)(A), total anthocyanin content (TAC)(B) and DPPH assay(C) of Homnil rice bran extract. Error bars indicate the standard error of the mean (n=3).

Effect of light exposure

The TPC, TAC and DPPH assay in Homnil rice bran extract during storage at room temperature (30±2°C) in the presence and absence of light for 63 days are shown in Figure 5 A-C. The results indicated that the TPC, TAC and DPPH assay of extracts stored in the absence of light were higher than in the presence of light (p<0.05). Light affected TAC and DPPH much more than TPC. The anthocyanin of Homnil rice bran extract lost only 16% when kept in the absence of light, and the most anthocyanin loss (37%) was observed in presence of light. Maier *et al*. (2009) showed that light considerably decreased the phenolic and anthocyanin content of pectin and gelatin gels enriched with grape pomace extracts during storage. Palamidis and Markakis (1975) also reported that grape anthocyanins in beverage had a half-life of 416 days in dark against only 197 days in daylight at 20 °C.





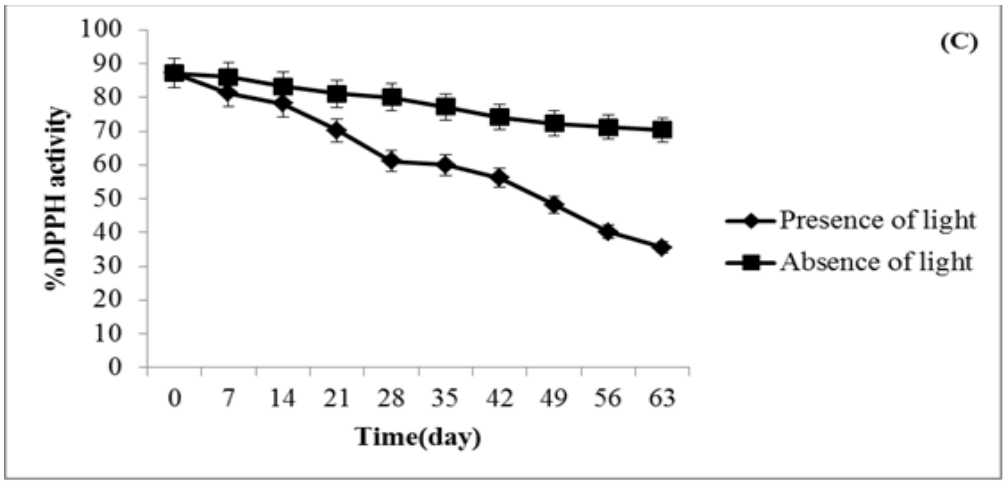


Figure 5 The effect of light exposure on total phenolic content (TPC)(A) and total anthocyanin content (TAC)(B) and DPPH assay(C) of Homnil rice bran extract during storage at room temperature (30 ±2°C) for 63days
Error line indicate the standard error of the mean (n=3).

CONCLUSION

The TPC, TAC and DPPH scavenging activity of Homnil rice bran extract were higher than those of Rice berry and Hommali dang rice bran extracts. The ideal extraction parameters for anthocyanin extraction from Homnil rice bran were particle size at 60 mesh, shaking, 2% (w/v) citric acid and extraction for 3 h at 40°C. The TPC, TAC and DPPH scavenging activity in Homnil rice bran extract decreased with increasing storage temperature, time and light. Therefore, pigmented rice bran extract could be potentially extracted for use as food or beverages ingredients; however, it should be stored under appropriate conditions and used as soon as possible.

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REFERENCES

Ahuja, U., Ahuja, S.C., Chaudhary, N. and Thakrar, R. 2007. Red Rices-Past, Present, and Future. **Asian Agri-History** 11: 291-304.

Fera, A., Galih, N.A., Arini, M., Alia, N.F., Sisca, U. and Mimiek, M. 2013. Extraction and stability test of anthocyanin from Buni fruits (*Antidesma bunius* L) as an alternative natural and safe food colorants. **Journal of Food and Pharmaceutical Science** 1: 49-53.

Giusti, M.M. and Wrolstad, R.E. 2001. Characterization and measurement of anthocyanins by UV-Vis spectroscopy, pp.26-29. *In* Wiley. **Current protocols in Food Analytical Chemistry Unit F1.2.** John Wiley & Sons, Inc., United States.

Hiba, N.R., Nada, El, D., Zeina H., Nadia, B., Eugene, V., Richard, G.M. and Nicolas, L. 2014. Extraction of total phenolic compounds, flavonoids, anthocyanins and tannins from grape byproducts by response surface methodology. Influence of solid-liquid ratio, particle size, time, temperature and solvent mixtures on the optimization process. **Food and Nutrition Sciences** 5: 397-409.

Jing, P. and Giusti, M.M. 2007. Effects of extraction conditions on improving the yield and quality of an anthocyanin-rich purple corn (*Zea mays* L.) color extract. **Journal of Food Science** 72(7): 363-368.

Kannan, A., Hettiarachchy, N.S., Lay, J.O. and R. Liyanage. 2010. Human cancer cell proliferation inhibition by a pentapeptide isolated and characterized from rice bran. **Peptides** 31: 1629-1634.

Laleh, G.H., Frydoonfar, H., Heidary, R., Jameei, R. and Zare, S. 2006. The effect of light, temperature, pH and species on stability of anthocyanin pigments in four *Berberis* species. **Pakistan Journal of Nutrition** 5: 90-92.

Maier, T., Fromm, M., Schieber, A., Kammerer, D.R. and Carle, R. 2009. Process and storage stability of anthocyanins and non-anthocyanin phenolics in pectin and gelatin gels enriched with grape pomace extracts. **European Food Research and Technology** 229: 949-960.

Maisuthisakul, P. and Changchub, L. 2014. Effect of extraction on phenolic antioxidant of different Thai rice (*Oryza sativa* L.) genotypes. **International Journal Food Properties** 17(4): 855-865.

Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y., Tanaka, T. and Andoh, T. 1999. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. **Journal of Agricultural and Food Chem** 47: 1749-175.

Oki, T., Masuda, M., Kobayash, M., Nishiba, Y., Furuta, S., Suda, I and Sato, T. 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. **Journal of Agricultural and Food Chemistry** 50(26): 7524-7529.

Patrauanu, O.A., Lazer, L., Popa, V.I. and Volf, I. 2019. Influence of particle size and size distribution on kinetic mechanism of spruce bark polyphenols extraction. **Cellulose Chemistry and Technology** 53: 71-78.

Palamidis, N and Markakis, P. 1975. Stability of grape anthocyanin in a carbonated beverage. **Journal of Food Science** 40: 1047-1048.

Shao, Y., Xu, F., Sun, X., Bao, J. and Beta, T. 2014. Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). **Journal of Cereal Science** 59:211-218.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A. and Qian, M.C. 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. **Food Chemistry** 173: 424-431.

Sukhapat, N., Ungphaiboon, S., Itharat, A., Puripattanavong, J. and Pinsuwan, S. 2004. Influence of pH on antioxidant activity of roselle (*Hibiscus sabdariffa* L.) extract in aqueous solution, p184. *In* **The 10th World Congress on Clinical Nutrition: Nutrition in the Next Decade: Nutraceutical/ Functional Food: Product Performance in Health, Disease and Safety, 30 Nov-3 Dec., 2004.** Organized by PSU, INC and BIOTEC, Phuket.

Yoshida, H., Tomiyama, Y. and Mizushina, Y. 2010. Lipid components, fatty acids and triacylglycerol molecular species of black and red rice. **Journal of Food Chemistry** 123: 210-215.