แอนโทไซยานินและสารประกอบที่เป็นประโยชน์และกิจกรรมทางชีววิทยาในรำข้าวนิล

Anthocyanin and Beneficial Compounds Contents and Biological Activities in Black rice Bran

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บทคัดย่อ

ประโยชน์ทางสุขภาพมีส่วนเกี่ยวข้องกับคุณสมบัติพิเศษของพฤษเคมี ในงานวิจัยนี้ได้ศึกษาสารประกอบฟีนอลิกและคุณสมบัติทางชีววิทยาของรำข้าวเหนียวนิลของข้าวที่เก็บรักษาที่อุณหภูมิ 25 องศาเซลเซียสและ 37 องศาเซลเซียสเป็นระยะเวลา 0-5 เดือน สารประกอบฟีนอลิกถูกวัดโดยใช้น้ำยาเคมี Folin-Ciocalteu รำข้าวนิลถูกสกัดโดยใช้เมทานอลเป็นสารละลายสกัดสารประกอบฟีนอลิกของรำข้าวนิลที่เก็บไว้เป็นเวลา 0-5 เดือน ที่อุณหภูมิ 25 องศาเซลเซียสมีปริมาณเท่ากับ 22.11 ถึง 44.61 มิลลิกรัมต่อกรัมในขณะที่สารประกอบฟีนอลิกของรำข้าวนิลที่เก็บรักษาที่อุณหภูมิ 37 องศาเซลเซียสมีปริมาณเท่ากับ 22.33 ถึง 44.61 มิลลิกรัมต่อกรัม ปริมาณสารประกอบฟลาโวนอยด์ของรำข้าวนิลที่เก็บไว้เป็นเวลา 0-5 เดือน ที่อุณหภูมิ 25 องศาเซลเซียสมีปริมาณเท่ากับ 1.20 ถึง 2.25 มิลลิกรัมต่อกรัมในขณะที่สารประกอบฟีนอลิกของรำข้าวนิลที่เก็บรักษาที่อุณหภูมิ 37 องศาเซลเซียสมีปริมาณเท่ากับ 1.32 ถึง 2.25 มิลลิกรัมต่อกรัม ปริมาณแอนโทไซยานินในรำข้าวนิลที่เก็บไว้เป็นเวลา 0-5 เดือน ที่อุณหภูมิ 25 องศาเซลเซียสและ 37 องศาเซลเซียสมีค่าอยู่ในช่วง 33.06- 40.74 มิลลิกรัมต่อกรัมและ 31.39 - 40.74 มิลลิกรัมต่อกรัม ตามลำดับ การต้านอนุมูลอิสระของสารละลายสกัดของรำข้าวนิลตรวจวัดโดยใช้ 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical เอสเซย์ รำข้าวที่ไม่ได้เก็บรักษามีการต้านอนุมูลอิสระและการยับยั้งกิจกรรมไทโรซิเนสสูงกว่ารำข้าวที่ผ่านการเก็บรักษา อุณหภูมิและระยะเวลาการเก็บรักษาจึงมีผลต่อปริมาณพฤษเคมีและกิจกรรมทางชีววิทยา ดังนั้นการทราบสภาวะที่มีผลต่อคุณสมบัติของสารประกอบพฤษเคมีของรำข้าวนิลจะมีประโยชนต่อการนำไปใช้ได้อย่างมีศักยภาพต่อสุขภาพต่อไป

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Abstract

Health benefits have been attributed in part to their unique plant phytochemicals. This study was carried out to investigate phenolic compounds and the biological activities of black glutinous rice brans of stored rice at 25 ºC and 37ºC for 0-5 month. Phenolic compounds were measured by using Folin-Ciocalteu's reagent.

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Black rice brans were extracted by using methanol as extraction solvent. Phenolic compounds of black ricebran stored at 0-5 month at 25 ºC were 22.11 to 44.61 mg/g while that of the bran stored at 0-5 month at 37 ºC were 22.33 to 44.61 mg/g. Flavonoid contents of black rice bran stored at 0-5 month at 25 ºC were 1.20 to 2.25 mg/g while that of the bran stored at 0-5 month at 37 ºC were 1.32 to 2.25 mg/g. Anthocyanin contents of black rice bran stored at 0-5 month at 25 ºC and 37ºC ranged from 33.06 to 40.74 mg/g and 31.39 to 40.74 mg/g, respectively. Antioxidant activities of the extracts of black rice bran was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay. The non-stored rice bran showed the higher antioxidant activity (EC_{50}) and tyrosinase inhibitory property (IC_{50}) than that of stored rice bran. Storage time and temperature affected to the phytochemical contents and biological activity. Knowing the effect on the phytochemical properties of black rice bran gives insights to its potential application to promote health.

Keyword: anthocyanin, antioxidant, tyrosinase

Introduction

Rice bran, a by-product of the rice milling process constitutes about 10 wt % of rough rice grain. The bran layer contains 18 - 22% oil, making it the richest oil source from a grain by-product.[1] Rice bran contains high amounts of fiber and bioactive phytochemicals, such as tocopherols, tocotrienols, oryzanols, vitamin B complex, and phenolic compounds. Most of these phytochemicals are recognized as bioactive compounds improving human health and well-being. Their vital functions include scavenging free radicals, antioxidant activity, enhancement of immune systems, and reduction of the risk of developing cancer and heart disease.[2] Several studies have reported the phytochemical content and antioxidant activity of whole grain white rice also reported the phytochemicals and antioxidant activity of rice bran.[3] Anthocyanin pigments are responsible for the attractive red to purple to blue colors of many fruits and vegetables. Anthocyanins are relatively unstable and often undergo degradative reactions during processing and storage. Measurement of total anthocyanin pigment content along with indices for the degradation of these pigments are very useful in assessing the color quality of these foods. Interest in the anthocyanin content of foods and nutraceutical preparations has intensified because of their possible health benefits. They may play a role in reduction of coronary heart disease[4] and increased visual acuity[5] and also have antioxidant and anticancer properties.[6,7] Anthocyanins have also found considerable potential in the food industry as safe and effective food colorants;[8] interest in this application has increased in recent years. In 1980, the annual world production had been estimated as reaching 10,000 tons from grapes alone.[9] Quantitative and qualitative anthocyanin composition are important factors in determining the feasibility of the use of new plant materials as anthocyanin-based colorant sources.

Rice is a staple food being consumed by nearly half of the world population. Nutritional quality of black rice has received more attention.[10] Rice contains various types of phenolic compounds possessing antioxidant activity.[11] However, in rice, phenolics occur primarily in conjugated form with one or more sugar residues binding to the hydroxyl group[12] This condition lowers the

antioxidant activity since availability of free hydroxyl group on the phenolic structure is an important characteristic for the resonance stabilization of free radicals. Recent interest in food phenolics has increased greatly and the most studied effects are those focusing on health, because of their antioxidant and radical scavenging activities. Phenolics are able to donate hydrogen and to form relatively stable resonance hybrids of delocalized unpaired electrons allowing the molecule to act as reducing agents, singlet oxygen quenchers and free radical hydrogen donors. Their presumed role is the protection of cell constituents against oxidative damage and in addition, epidemiological studies tend to confirm the protective effects of polyphenols against cancers and cardiovascular diseases.[13] Milling of the brown rice to obtain rice bran removes bran layers that are rich in protein, fiber, oil, minerals, vitamins, and other phytochemicals.[14,15]

However, there is no report on changes in phytochemical content or antioxidant activity in black rice bran during storage. Therefore, the aims of this study were to determine total phenolic compound (TPC), flavonoid, anthocyanin conterits, antioxidant activities and inhibition of tyrosinase activity of black rice bran during storage at different temperatures.

Materials and Methods

Black rice

One hundred kilos of the paddy black rice cv. Khaw Klum were provided by the rice Research Center, Phathumthani province. The bags were place at 25°C. Black rice grains were air-dried and stored at 25°C and 37°C for 0-7 month. Then they were dehusked on a Satake Black rice Machine, black rice bran was obtained by 10% polishing using a Grainman polisher and the black rice bran were ground to pass through a 100-mesh sieve on a Ultracentrifilgal Mill.

Chemicals

Gallic acid, Folin-Ciocalteu's reagent, methanol, linoleic acid, quercetin, 1,1- diphenyl-2-picrylhydrazyl, butylated hydroxytoluene, sodium carbonate, sodium hydroxide, aluminium chloride hydrated, sodium nitrite, dibasic sodium phosphate and monobasic sodium phosphate were purchased from Fluka Chemie AG.

Extraction of black rice bran samples

A 5 g portion of each black rice **bran** samples stored at 25°C and 37°C for 0-5 month, was extracted with 75 ml of methanol at room temperature for 12 h (repeated three times) and was then filtered through Whatman No. 1 filter paper. The residue was evaporated at 50°C under vacuum.

Total phenolic content

The TPC was determined using a modified Folin–Ciocalteu method.[16] Each test sample (250 μl) was added to a test tube that contained 6.0 ml of distilled water. After vortexing the tubes, 500 μl of Folin–Ciocalteu's phenol reagent was added to each tube. The tubes were vortexed and 2 min later, 2.0 ml of 15% Na_2CO_3 was added to each tube. 1.25 ml of distilled water was added to each tube. The tubes were vortexed again and then allowed to stand for 2 h at room temperature. Thereafter, the absorbance of each sample was measured against a blank at 750 nm. A calibration curve was constructed using 50, 100, 150, 200 and 250 mg/l gallic acid as a standard. The TPC is expressed as milligrams of gallic acid per gram.

Total flavonoid content

The total flavonoid content was determined using a modified version of the method described by Zhishen et al.[17] Each test sample (250 μl) and

1.25 ml of distilled water were added then 75 µl of 5% $NaNO_2$, 150 µl of 10% $AlCl_3$ was added. After 6 min 0.5 ml of 1 M NaOH was added. The absorbance of the solution was measured against a blank at 510 nm using a spectrophotometer. A calibration curve was constructed using 0.125, 0.25, 0.5 and 1.0 g/l quercetin as a standard. The total flavonoid content is expressed as milligrams of quercetin per gram of dry extract.

Free-radical-scavenging activity

Antioxidant activity was measured based on the scavenging of the stable free radical, 1,1-diphenyl-2-picrylhydrzyl (DPPH). A sample of each fraction (2.0 ml), in methanol, was added to 2.0 of a solution that contained DPPH. After 30 min, the absorbance was measured at a wavelength of 517 nm using UV-visible spectrophotometer.

DPPH assay

The free radical scavenging activity of different fractions was measured by the DPPH (1-1 Diphenyl-2-picryl hydrazyl) scavenging method proposed by Shimada, et al.[18] 2.5 x 10^{-4} M solution of DPPH in methanol was prepared and 2.0 ml of this solution was added to 2.0 ml of different black rice extracts obtained in different storage conditions. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank. The DPPH radical-scavenging activity was calculated according to the following: % of DPPH scavenging activity = {1- (AbS/AbC)} x 100, where AbC was the absorbance of the control and AbS was the absorbance in the presence of the test compound. EC_{50} is the effective concentration in mg extract/ml which inhibits the DPPH activity by 50%. BHA was used for comparison.

Anthocyanin analysis

Monomeric anthocyanin content was determined using the pH-differential method.[19] A pigment content was calculated as cyanidin-3-glucoside and expressed as milligrams per 100 g of fresh weight, using an extinction coefficient of 26,900 $Lcm^{-1}mol^{-1}$and molecular weight of 449.2 $gmol^{-1}$.

Inhibition of tyrosinase activity

Potassium phosphate buffer (0.07 mL, 50 mM) at pH 6.5, 0.03 mL tyrosinase (350 units/mL) and 2 µL of the tested compounds (0.5–500 µM), dissolved in absolute ethanol were inserted into 96-well plates. After 5 min incubation at room temperature, 0.1mL L-tyrosine (2 mM) or 12 mM L-DOPA were added and incubated for additional 20 min. The optical density of the samples at 492 nm were measured (Elisa SLT Lab instruments Co. A-5082) relative to control containing ethanol (2 µL) and without inhibitor, demonstrating a linear colour change with time during the 20 min of the experiment.

Results and Discussion

Total phenolics, flavonoid contents and antioxidant capacity

Stored black rice brans at 25°C and non-stored black rice bran, TPC ranged from 22.11 ± 0.14 to 44.61 ± 0.88 mg/g, with the lower values coming from the 5 month stored black rice, while the higher values were from non-stored black rice bran (Table 1). The results showed stored black rice brans had lower TPC than non-stored black rice bran. There were narrow range of variations in the total flavonoids in stored black rice grain at 25°C. Flavonoid contents in all the stored black rice bran ranged from 1.20 ± 0.05 to 2.18 ± 0.01 mg/g while the mean flavonoid contents among the non-stored black rice bran were 2.25 ± 0.01

mg/g. Anthocyanin contents in all the stored black rice ranged from 33.06 ± 0.06 to 39.73 ± 0.12 mg/g while the mean anthocyanin contents among was 0.35, ranging from 0.11 to 0.73. The 5 months stored black rice samples had

Among stored black rice brans at 37°C and non-stored black rice bran, TPC ranged from 22.33 ± 0.05 to 44.61 ± 0.88 mg/g, with the lower values coming from the 5 month stored black rice at 37°C, while the higher values were from non-stored black rice bran. The results also showed stored black rice brans at 37°C had lower TPC than non-stored black rice bran. Flavonoid contents in all the stored black rice brans ranged from 1.20 ± 0.05 to 2.18 ± 0.01 mg/g while the mean flavonoid contents among the non-stored black rice bran were 2.25 ± 0.01 mg/g. Anthocyanin contents in all the stored black rice ranged from 31.39 ± 0.04 to 37.73 ± 0.14 mg/g while the mean anthocyanin contents among the non-stored black rice bran were 40.74 ± 0.07 mg/g.

The total antioxidant capacity was measured using the DPPH assay and converted to EC_{50} (Table 2). It was EC_{50} 0.72, among the non-stored black rice bran. Among the stored black rice, the mean EC_{50} was 3.11, ranging from 1.20 to 5.53. The 5 months stored black rice samples had antioxidant capacity of 5.53, around 7.66 times less than of that of the non-stored black rice bran. The antityrosinase activity was measured and converted to IC_{50}. It was IC_{50} 0.063, among the non-stored black rice bran. The total antioxidant capacity was measured using the DPPH assay and converted to EC_{50}. It was EC_{50} 0.72, among the non-stored black rice bran (Table 2). Among the stored black rice brans at 37°C, the mean EC_{50} was 3.45, ranging from 1.43 to 6.185. The 5 months stored black rice samples had antioxidant

the non-stored black rice bran were 40.74 ± 0.07 mg/g. Among the stored black rice, the mean IC_{50}

antityrosinase activity of 0.73, around 11.62 times less than of that of the non-stored black rice bran.

capacity of 6.19, around 8.58 times less than of that of the non-stored black rice bran. The antityrosinase activity was measured and converted to IC_{50}. It was IC_{50} 0.06, among the non-stored black rice bran. Among the stored black rice, the mean IC_{50} was 0.46, ranging from 0.15 to 1.04. The 5 months stored black rice samples had antityrosinase activity of 1.04, around 16.53 times less than of that of the non-stored black rice bran.

Scavenging ability on 1,1-diphenyl–2-picrylhydrazyl radicals

Contrary to the scavenging abilities on DPPH radicals, high temperature might impart certain components with better scavenging ability on DPPH radicals. The high scavenging ability of non-stored black rice bran extracts might be attributed to the presence of isoflavones.[20,21] It seems that phenolic complexs formed during storage, which contribute to this free radical scavenging ability. The antioxidant properties of black rice bran extracts, determined using the inhibition of linoleic acid autoxidation method, were compared with those of BHT. Among the non-stored black rice bran and stored black rice extracts, the highest antioxidant activity was found in non-stored black rice bran extracts, which exhibited a significant (P < 0.05) inhibition of linoleic acid peroxidation. The results showed that the antioxidant properties both of DPPH (1-1 diphenyl-2-picryl hydrazyl) scavenging of the stored black rice bran at 25°C were higher than that of the stored black rice at 37°C.

Table 1 Variations in TPC, flavonoid, anthocyanin contents, among stored black rice bran at 25°C and 37°C

Storage time (month)	TPC[a] (mg/g)		Flavonoid[a] (mg/g)		Anthocyanin[a] (mg/g)	
	25ºC	37ºC	25ºC	37ºC	25ºC	37ºC
0	44.61 ± 0.88[a]	44.61 ± 0.88[a]	2.25 ± 0.01[a]	2.25 ± 0.01[a]	40.74 ± 0.07[a]	40.74 ± 0.03[a]
1	39.63 ± 0.23[b]	43.27 ± 0.35[b]	2.12 ± 0.01[b]	2.23 ± 0.01[a]	39.73 ± 0.12[b]	37.73 ± 0.14[b]
2	31.15 ± 0.41[c]	38.32 ± 0.05[c]	2.01 ± 0.02[c]	2.16 ± 0.06[a]	39.07 ± 0.04[c]	35.39 ± 0.07[c]
3	25.31 ± 0.48[d]	29.12 ± 0.07[c]	1.69 ± 0.02[d]	1.86 ± 0.02[b]	38.56 ± 0.07[d]	34.39 ± 0.07[d]
4	24.02 ± 0.22[e]	27.17 ± 0.61[d]	1.32 ± 0.03[e]	1.63 ± 0.02[c]	36.06 ± 0.12[e]	33.39 ± 0.12[e]
5	22.11 ± 0.14[f]	22.33 ± 0.05[e]	1.20 ± 0.05[f]	1.32 ± 0.01[d]	33.06 ± 0.06[f]	31.39 ± 0.04[f]

[a] Each value is expressed as mean ± SE (n = 3). Means with different small letters within a column at a specific antioxidant attribute are significantly different ($P < 0.05$).

Table 2 Variations in antioxidant capacity and tyrosinase inhibitory activity (antityrosinase) among stored black rice bran at 25°C and 37°C

Storage time (month)	Antioxidant capacity[b] (mg/ml)		Antityrosinase[c] (mg/ml)	
	25ºC	37ºC	25ºC	37ºC
0	0.72	0.72	0.06	0.06
1	1.20	1.43	0.11	0.15
2	1.61	1.73	0.18	0.22
3	3.22	3.45	0.27	0.34
4	4.02	4.47	0.44	0.56
5	5.53	6.19	0.73	1.04

[b] EC_{50} value, the effective concentration at which the antioxidant activity was 50%; the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was scavenged by 50%. EC_{50} value was obtained by interpolation from linear regression analysis.

[c] IC_{50} value, the effective concentration at which the tyrosinase activity was 50. IC_{50} value was obtained by interpolation from linear regression analysis.

The scavenging of free radicals by the methanol extract of 0-5 month stored black rice bran, was tested by measuring scavenging of the stable free radical, DPPH. The non-stored black rice bran sample exhibited the highest free-radical-scavenging activity. The free-radical-scavenging ability of the 0.72 mg/ml black rice bran methanol extract decreased monotonically as the storage time decreased, from 7.66 folds and 8.58 folds after storage for 5 month at 25°C and 37°C, respectively (Table 2). These results suggest that black rice bran, at a non-storage, is a better source of antioxidants, substances that may inhibit cancer. It has been reported that the antioxidant activity of plant materials strongly correlates with their content of the phenolic compounds.[22] In the present study, the flavonoid content was lowest in the methanol extract of black rice bran stored at 25°C for 5 month samples (1.20 mg/g), whilst the TPC was lowest in the methanol extract of black rice bran stored at 25°C and 37°C for 5 month. Therefore, the higher free-radical-scavenging activity and tyrosinase inhibitory activity of the methanol extract of non-stored black rice bran may be due to the higher amounts of TPC, flavonoid and anthocyanin in those samples, it was assumed that these compounds produced played an important role in the increased antioxidant activity and inhibition tyrosinase activity. In conclusion, we found that the methanol extract of non-stored black rice bran exhibited the highest degrees of free-radical-scavenging activity. In addition, TPC, flavonoid and anthocyanin contents decreased during storage progressed. These results suggest that non-stored black rice bran extracts possess antioxidative activity and inhibition tyrosinase activity, that are attributable to phenolic, flavonoid and anthocyanin.

The correlation coefficients for anthocyanin in 0-5 month stored black rice bran at 25°C versus TPC, flavonoid, antioxidant capacity and tyrosinase inhibitory activity of black rice bran during storage are investigated and the correlation coefficient values are 0.68, 0.89, 0.93 and 0.99, respectively. The correlation coefficients for anthocyanin in 0-5 month stored black rice bran at 37°C versus TPC, flavonoid, antioxidant capacity and tyrosinase inhibitory activity of black rice bran during storage are investigated and the correlation coefficient values are 0.88, 0.79, 0.86 and 0.76, respectively.

Storage effects

There was a consistent decrease in flavonoid content in black rice bran following storage. Furthermore, the decline in TPC was greater at 25°C than at 37°C. These changes were significant ($P < 0.05$) and are consistent with previous observations[23] regarding oxidation of ferulate esters of hemicellulose, causing a reduction in flavonoids. Given the contribution of flavonoids, it is not surprising that similar trends were observed for total flavonoids during storage. In contrast, TPC increased significantly ($P < 0.05$) during storage of black rice, presumably as a result of enzymatic and non-enzymatic release of bound phenolic acids.

Conclusions

This study were to investigate physicochemicals and antioxidant activities of black glutinous rice bran by comparing non-stored rice bran and stored rice bran at 25 ºC and 37 ºC for 0-5 month. The nutritional constituents, including phenolic compounds, flavonoid, and anthocyanin were significantly different between stored rice brans. However, the non-stored rice bran showed the highest phenolic, flavonoid and anthocyanin contents. The non-stored rice bran also showed

higher scavenging activities against DPPH and tyrosinase inhibitory activity. The results also showed that storage time and temperature affected physicochemical and physiological properties and quality of black rice bran. These results suggest that glutinous rice bran would be considered to be functional compound due to its anti-oxidative effect and high nutrition. In conclusion, the results of this study demonstrated that glutinous rice bran methanol extract had potent DPPH radical scavenging activities as well as tyrosinase inhibitory. Total phenolic compound was also high in this extract. Therefore, our results suggest that black glutinous rice bran may be utilized as an effective tyrosinase inhibitory activity compound and antioxidant source due to its radical scavenging activities and phenolic compounds.

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