Effect of storage time and temperature on antioxidant components and properties of milled rice

Pimpaporn Thanajiruschaya[1], *Wathinee Doksaku*[1]
Phanee Rattanachaisit[1] *and Jirasak Kongkiattikajorn*[2*]

Abstract

The objective of this study was to determine the phenolic, flavonoid contents and antioxidant properties in the milled rice cv. Khaw Dawk Mali 105 during storage for 0-7 months at different temperature. The total phenolics content of non-stored rice was 17.02 mg/g and 7-month stored rice at 25°C was 6.07 ± 0.01 mg/g while storage for 7 months at 37°C, the phenolics contents was 7.29 ± 0.35 mg/g. The total flavonoids contents of non-stored rice was 13.26 ± 0.01 mg/g and 7 month stored rice at 25°C was 6.74 ± 0.01 mg/g while storage for 7 months at 37°C, the flavonoid content was 6.45 ± 0.12 mg/g. The antioxidant property determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical expressed in the value of $EC_{50\ (DPPH)}$ of non-stored rice was 0.27 mg/ml and 7- month stored rice at 25°C was 12.15 mg/ml while storage for 7 months at 37°C, the antioxidant property was 6.34 mg/ml. The antioxidant property determined by lipid peroxidation expressed in the value of $EC_{50\ (lipid\ peroxidation)}$ of non-stored rice was 0.12 mg/ml and 7 month stored rice at 25°C was 1.18 mg/ml while storage for 7- months at 37°C, the antioxidant property was 1.10 mg/ml. The results showed that storage time at 25°C and 37°C caused decrease of extractable phenolic and flavonoid contents and antioxidant activities of the milled rice however 7- month stored milled rice at 37°C contained phenolic contents and antioxidant activities higher than stored milled rice at 25°C.

Keywords: antioxidant, phenolic, flavonoid, rice, storage

[1] *Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok, Thailand.*
[2] *Department of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand.*
* *Corresponding author, e-mail: jirasak.kon@kmutt.ac.th*

Introduction

Rice is a staple food being consumed by nearly half of the world population. Nutritional quality of rice has received more attention (Bouis et al., 2003). Rice contains various types of phenolic compounds possessing antioxidant activity (Sosulski et al., 1982). However, in rice, phenolics occur primarily in conjugated form with one or more sugar residues binding to the hydroxyl group (Bravo, 1998). 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 (Arts et al., 2001). Milling of the brown rice to obtain milled rice removes bran layers that are rich in protein, fiber, oil, minerals, vitamins, and other phytochemicals (Orthoefer and Eastman, 2004; Yokoyama, 2004).

The objectives of this research were to determine the contents of antioxidant components in the milled rice. The antioxidant properties also determined and the influence of storage temperature and time were evaluated on contents of antioxidant components and the antioxidant properties of the milled rice.

Materials and Methods

One hundred kilos of the paddy rice Khao Dawk Mali 105 were provided by the Rice Research Center, Phathumthani province. The bags were place at 25°C. Rice grains were air-dried and stored at 25°C and 37°C for 0-7 months. Then they were dehusked on a Satake Rice Machine (Satake Co., Japan), milled rice was obtained by polishing using a Grainman polisher model 60-115-60-2AT (Douglas International Corp, Coral Gables, FL) for 90% milling and the milled rice were ground to pass through a 100-mesh sieve on a Ultracentrifilgal Mill (UDY Corporation, Fort Collins, Colorado, USA). Gallic acid, Folin-Ciocalteu's reagent, methanol, linoleic acid, quercetin, 1,1- diphenyl-2-picrylhydrazyl, butylated hydroxyanisole (BHA), sodium carbonate, sodium hydroxide, aluminium chloride hydrated, sodium nitrite, dibasic sodium phosphate and monobasic sodium phosphate were purchased from Fluka Chemie AG (Buchs, Switzerland).

1. Extraction of milled rice samples

A 5 g portion of each of the milled rice samples, with stored at 25°C and 37°C for 0-7 months, was extracted with 75 ml of methanol at room temperature for 12 h (repeated three times) and then was filtered through Whatman No. 1 filter paper (Whatman Internation Ltd., Maidstone, England). The residue was evaporated at 50°C reduced pressure.

2. Total phenolic content

The total phenolic content was determined using a modified Folin–Ciocalteu method (Singleton and Rossi, 1965). 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 (Fluka) 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 using a spectrophotometer (Lambda 35/FIAS 300, Perkin Elmer, USA.). A calibration curve was constructed using 50, 100, 150, 200 and 250 mg/l gallic acid (Fluka) as a standard. The total phenolic content is expressed as milligrams of gallic acid per gram.

3. Total flavonoid content

The total flavonoid content was determined using a modified version of the method described by Zhishen et al. (1999). 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 tubes were vortexed again and then allowed to stand for 5 min at room temperature. The absorbance of the solution was measured against a blank at 510 nm using a spectrophotometer (Lambda 35/ FIAS 300, Perkin Elmer, USA.). A calibration curve was constructed using 0.125, 0.25, 0.5 and 1.0 g/l quercetin (Fluka) as a standard. The total flavonoid content is expressed as milligrams of quercetin per gram of dry extract. All measurements in this study were made in triplicate and the data are reported as the mean value ± one standard deviation.

4. 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. (1992). 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 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)} × 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.

5. Lipid peroxidation

The antioxidant activities of sample of each fraction were measured based on the method of Osawa and Namiki (1985) with some modifications. 50, 250, 500, 750 and 1,000 mg/l of sample was dissolved in 4.0 ml of 0.2 M phosphate buffer (pH 7.0), and added 200 µl of linoleic acid (10 mM) and left to stand at 37°C in the dark for 15 h. Each sample was added with ethanol (60%) and the absorbance was then measured at 234 nm against a blank.

Results and Discussion

1. Total phenolics, flavonoid contents and antioxidant capacity

Stored rices at 25°C and non-stored rice, total phenolic content ranged from 6.076 ± 0.009 to 17.170 ± 0.061 mg/g, with the lower values coming from the 7-month stored rice, while the higher values were from non-stored rice (Table 1, Figure. 1). The results showed that stored rice had lower total phenolic contents than non-stored rice. There were narrow range of variations in the total flavonoids in stored rice grain at 25°C.

Flavonoid contents in all the stored rice ranged from 6.651± 0.056 to 7.142 ± 0.028 mg/g while the mean flavonoid contents among the non-stored rice were 13.263 ± 0.010 mg/g. The total antioxidant capacity was measured using the DPPH assay and converted to EC_{50}. It was EC_{50} 0.267, among the non-stored rice. Among the stored rice, the mean EC_{50} was 10.153, ranging from 8.120 to 12.152. The 7-month stored rice sample had antioxidant capacity of 12.152, around 45.445 times less than of that of the non-stored rice (Table 1).

Among stored rices at 37°C and non-stored rice, total phenolic content ranged from 7.297 ± 0.353 to 17.170 ± 0.061 mg/g, with the lower values coming from the 7- month stored rice at 37°C, while the higher values were from non-stored rice.

Table 1. Variations in phenolic, flavonoid contents, antioxidant capacity and lipid peroxidation among stored rice at 25°C.

Storage time (month)	Flavonoid[a] (mg/g)	Phenolic[a] (mg/g)	Antioxidant capacity[b]	Lipid peroxidation[b]
0	13.264 ± 0.010a	17.170 ± 0.061a	0.267	0.118
1	6.914 ± 0.066b	13.606 ± 0.087b	8.120	1.005
2	6.737 ± 0.029c	10.964 ± 0.221c	9.540	0.869
3	6.665 ± 0.046c	9.824 ± 0.343c	9.492	1.016
4	6.651 ± 0.056c	9.045 ± 0.084d	9.315	0.853
5	6.735 ± 0.039c	8.053 ± 0.049e	10.532	0.980
6	7.142 ± 0.027d	7.823 ± 0.208e	11.923	1.179
7	6.740 ± 0.014c	6.076 ± 0.009f	12.152	1.182

[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 ($P < 0.05$).

[b] EC_{50} value, the effective concentration at which the antioxidant activity was 50%; the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals or lipid peroxidation were scavenged by 50%; and linoleic acid autoxidation were inhibited by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis.

Table 2. Variations in phenolic, flavonoid contents, antioxidant capacity and lipid peroxidation among stored rice at 37°C.

Storage time (month)	Flavonoid[a] (mg/g)	Phenolic[a] (mg/g)	Antioxidant capacity[b]	Lipid peroxidation[b]
0	13.263 ± 0.010a	17.170 ± 0.061a	0.267	0.118
1	11.408 ± 0.099b	14.017 ± 0.001b	2.067	0.528
2	9.289 ± 0.052c	12.604 ± 0.958c	2.835	0.547
3	9.251± 0.019c	11.070 ± 0.012d	4.960	0.772
4	9.194 ± 0.074c	10.704 ± 0.534d	5.483	0.738
5	7.536 ± 0.149d	9.033 ± 0.004e	5.993	0.817
6	7.779 ± 0.106c	8.909 ± 0.179e	6.329	0.966
7	6.446 ± 0.029e	7.297 ± 0.353f	6.335	1.103

[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 ($P < 0.05$).

[b] EC_{50} value, the effective concentration at which the antioxidant activity was 50%; the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals or lipid peroxidation were scavenged by 50%; and linoleic acid autoxidation were inhibited by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis.

The results also showed stored rice at 37°C had lower total phenolic contents than non-stored rice. Flavonoid contents in all the stored rice ranged from 6.446 ± 0.028 to 11.408 ± 0.099 mg/g while the mean flavonoid contents among the non-stored rice were 13.263 ± 0.010 mg/g.

The total antioxidant capacity was measured using the DPPH assay and converted to EC_{50}. It was EC_{50} 0.267, among the non-stored rice (Table 1). Among the stored rice at 37°C, the mean EC_{50} was 4.8573, ranging from 2.067 to 6.335. The 7-month stored rice sample had antioxidant capacity of 6.335, around 24 times less than of that of the non-stored rice (Table 2).

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

Contrary to the scavenging abilities on DPPH radicals, at high temperature might impart certain components with better scavenging ability on DPPH radicals. The high scavenging ability of non-stored rice extracts might be attributed to the presence of isoflavones (Lee et al., 2004; Lee et al., 2005). It seems that phenolic complexs were formed during storage, which contributed to this free radical scavenging ability. Peroxidation of fatty acids can cause deleterious effects in foods by forming complex mixture of secondary breakdown products of lipid peroxides. Further intake of these foods can cause a number of adverse effects including toxicity to mammalian cells. Therefore, the milled rice extracts fractions were further characterized for their antioxidant activity by assessing their ability to protect linoleic acid against oxidation. Lipid peroxidation is thought to proceed via radical mediated abstraction of hydrogen atoms from methylene carbons in polyunsaturated fatty acids (Rajapakse et al., 2005). The antioxidant properties of milled rice extracts, determined using the inhibition of linoleic acid autoxidation method, were compared with those of BHT. As shown in Figure. 3, the oxidation of linoleic acid was markedly inhibited by the addition of milled rice extracts. Among the non-stored rice and stored rice extracts, the highest antioxidant activity was found in non-stored rice extracts, which exhibited a significant ($P < 0.05$) inhibition of linoleic acid peroxidation. The non-stored rice extract exhibited an inhibition of linoleic acid peroxidation ($EC_{50\ (lidpid\ peroxidation)}$ 0.1182). The results showed that the antioxidant properties both of DPPH (1-1 Diphenyl-2-picryl hydrazyl) scavenging and lipid peroxidation of the stored rice at 37°C were higher than that of the stored rice at 25°C. Many natural antioxidants are less potent than synthetic antioxidants, but they can be used at higher concentrations than the synthetic ones, due to the very restrictive toxicological parameters of these latter. In addition, the incorporation of protein hydrolysate to foods could confer desirable nutritional and functional properties (Kim et al., 2007).

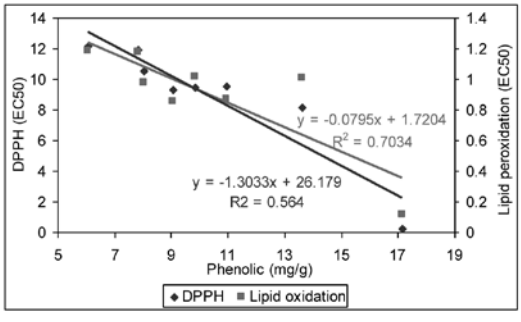
3. Free-radical-scavenging by milled rice stored for 0-7 months

The scavenging of free radicals by the methanol extract of milled rice stored for 0-7 months, was tested by measuring scavenging of the stable free radical, DPPH. The non-stored milled rice sample exhibited the highest free-radical-scavenging activity. The free-radical-scavenging ability of the 0.267 mg/ml milled rice methanol extract decreased monotonically as the storage time decreased, from 45 folds and 24 folds after storage for 7 months at 25°C and 37°C, respectively (Table 1). These results suggest that milled rice, at a non-storage, is a better source of antioxidants, substances that may inhibit cancer.

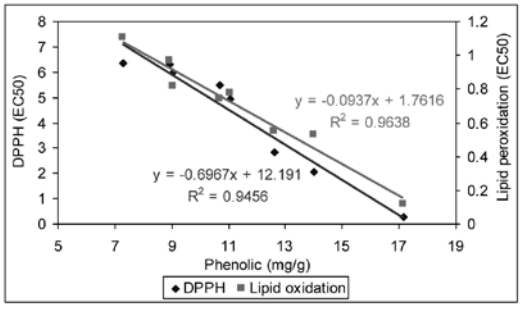
4. Correlation of free-radical-scavenging activity with phenolic and flavonoid contents

The correlation coefficients for free-radical-scavenging activities versus the total phenolic and flavonoid contents of milled rice during storage are shown in Figure. 1 and 2 and the correlation coefficient values are listed in Tables 3 and 4. The total phenolic and flavonoid contents of the samples decreased monotonically,

respectively, with the storage time. The phenolic content of milled rice decreased from 17.17 to 6.076 mg gallic acid/g, and the flavonoid content decreased from13.263 to 6.74 mg/g during storage at 25°C for 0-7 months. The phenolic content of milled rice decreased from 17.17 to 7.297 mg gallic acid/g, and the flavonoid content decreased from 13.263 to 6.446 mg/g during storage at 37°C for 0-7 months. The correlation coefficients for the correlation of free radical-scavenging with the phenolic and flavonoid contents are listed in Table 4.



(a)

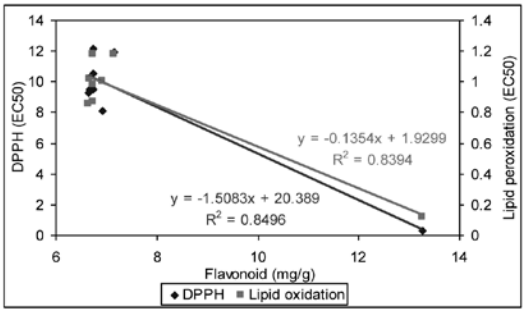


(b)

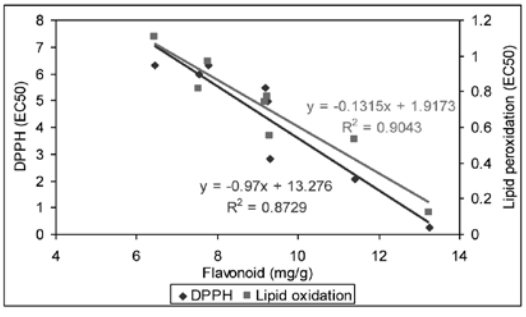
Figure 1. Relationship between phenolic content and free-radical-scavenging activities and lipid peoxidation of rice during storage at 25°C (a) and at 37°C (b).

The correlation coefficients were 0.564 and 0.9456 for the total phenolic content versus free-radical-scavenging activity in milled rice samples during storage at 25°C and 37°C, respectively, while the flavonoid contents versus free-radical-scavenging activity (Table 4), which suggest that there are strong correlation (0.8496, 0.8729) between free-radical-scavenging activity and the total flavonoid content during storage at 25°C and 37°C, respectively.

In addition, the strong correlation coefficients were calculated based on the data of total phenolic and flavonoid contents versus lipid peroxidation during storage at 25°C and 37°C for 0-7 months (Table 4). It has been reported that the antioxidant activity of plant materials strongly correlates with their content of the phenolic compounds (Velioglu et al., 1998). In the present study, the flavonoid content was lowest in the methanol extract of milled rice stored at 37°C for 7-month stored samples (6.446 mg/g), whilst the total phenolic content was lowest in the methanol extract of milled rice stored at 25°C for 5 month stored samples 6.076 mg/g) (Table 3). Therefore, the higher free-radical-scavenging activity of the methanol extract of non-stored milled rice may be due to the higher amounts of phenolic compounds in those samples, it was assumed that phenolic compounds played an important role in the increased antioxidant activity. In conclusion, we found that the methanol extract of non-stored milled rice exhibited the highest degrees of free-radical-scavenging activity. In addition, the total phenolic and flavonoid contents decreased during storage progressed. These results suggest that non-stored milled rice extracts possess antioxidative activity, that are attributable to phenolic and flavonoid.



(a)



(b)

Figure 2. Relationship between flavonoid content and free-radical-scavenging activities and lipid peoxidation of rice during storage at 25°C (a) and at 37°C (b).

Table 3. Correlation coefficients (R^2) for free-radical-scavenging activities versus the total phenolic and flavonoid contents of methanol extracts of milled rice storage at 25°C and 37°C.

Temperature	Phenolic	Flavonoid
25°C	0.564	0.8496
37°C	0.9456	0.8729

Table 4. Correlation coefficients (R^2) for lipid peroxidation versus the total phenolic and flavonoid contents of methanol extracts of milled rice storage at 25°C and 37°C.

Temperature	Phenolic	Flavonoid
25°C	0.7034	0.8394
37°C	0.9638	0.9043

5. Storage effects

There was a consistent decrease in phenolic acid content in milled rice following storage. Furthermore, the decline in phenolic acids was greater at 37°C than at 25°C. These changes were significant ($P < 0.05$) and were consistent with previous observations (Mod et al., 1983) regarding oxidation of ferulate esters of hemicellulose, causing a reduction in phenolic acids. Given the contribution of phenolic acids to the total acid content, it is not surprising that similar trends were observed for total phenolic acids during storage. In contrast, the phenolic acids increased significantly ($P < 0.05$) during storage of milled rice, presumably as a result of enzymatic and non-enzymatic release of bound phenolic acids. It is notable that the linoleic acid content of the free-lipid fraction of the three rice cultivars decreased during storage (Zhou et al., 2003). However, the magnitude of the changes in lipid content was relatively small and it was not possible to establish a relationship between the change in free-lipids and free phenolic acids.

In the past few decades, it indicates that rice also contains a large spectrum of phenolic compounds, including derivatives of benzoic and cinnamic acids mainly ferulic acid and diferulates, besides anthocyanins, anthocyanidins and polymeric proanthocyanidins, also know as condensed tannins, that have received attention for their multiple biological activities. Ferulic acid, an important phytochemical which is concentrated in the aleurone layer of mature rice grains, also distributed in the pericarp layer, testa layer, endosperm (milled rice) and germ (Tian et al., 2004; Liu, 2004; Soobrattee et al., 2005). One of the difficulties in studying phenolic compounds in grains is the diversity of structures ranging from simple phenolic structures to highly polymerize compounds. They can be divided in extractable (free and conjugated), or non-extractable, insoluble forms, the later ones mostly bound to polysaccharides of the cell wall (Naczk and Shahidi, 2004).

According to Adom and Liu (2002), bound phenolics account for the major part of polyphenols in whole grains and suggest that contents may be underestimated in studies dealing with corn, oats, wheat and rice due to the difficulty in estimating their amounts. Esterified and conjugated phenolic, tannin-like compounds seem to be the main components of the soluble fraction, as the contribution of free phenolics seems to be less significant. However, it still poses doubts about the bioavailability of polymers of flavonoids with variable degrees of polymerization due to their high molecular weights (Scalbert et al., 2002).

Conclusions

In conclusion, this study found the phenolics, flavonoid contents and antioxidant capacity in the milled rice grain. These data provide opportunities to further maintain the content of phenolics, flavonoids and antioxidant capacity in stored rice, especially in white rice. Their relationships between phenolics, flavonoids and antioxidant capacity and lipid peroxidation could serve as indexes to indirectly maintain high in phenolics, flavonoids and antioxidant capacity. The results could provide commercial rice producers, with opportunities to promote the production of rice with maintain levels of the phytochemicals of postharvest rice.

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