Influence of milling time on the nutritional composition and antioxidant content of Thai rice bran

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Abstract

Four regular rice strains (KDML105, PT1, SPT and RD31) and one pigmented rice strain (DSK) were assessed regarding their composition and antioxidant content. Phenolic compounds and γ-oryzanol were the dominant antioxidants in all rice varieties. The highest total phenolic content was found in the black glutinous rice DSK with 1.04 mg GAE/g. Anthocyanin was also found at a high concentration in DSK (1.13 mg/g). Brown rice samples were abrasively milled for 0-60 seconds to make a degree of milling (DOM) curve. A non-linear relationship between milling time and DOM was observed in all samples. The curve demonstrated that 30 sec of milling removed almost allof the bran fraction. The brans collected after 10, 20 and 30 sec of milling were assessed regarding their compositions and antioxidant content. Longer milling time decreased the content of protein, fiber and ash in the brans, while carbohydrates were increased. The total phenolic content in rice bran was reduced with longer milling time. On average, bran after 10 sec of milling contained 8.9% and 21.5% more total phenolics than the rice bran after 20 and 30 sec of milling, respectively. The γ-oryzanol and α-tocopherol contents of rice bran were in the range of 1.40-2.60 mg/g and1.24-21.56 μg/g, respectively. The DPPH radical scavenging activity of DSK bran after 10 sec of milling was highest, and was approximately three-fold higher than the other bran samples. However, the DPPH radical scavenging activity of all extracts was lower than that of BHT, a synthetic antioxidant.

Keywords: Rice bran, degree of milling, oryzanol, anthocyanin, DPPH radical scavenging activity

1. Introduction

In the last decade, there has been increasing interest in antioxidants and functional foods. Several epidemiological studies have shown that dietary intake of foods rich in natural antioxidants correlates with a reduced risk of coronary heart disease, cancer, inflammatory and aging-related disorders (Garewal, 1997). The demand for natural antioxidants is widespread and the healthy food product market has expanded. Consequently, new sources of natural antioxidants are desirable for customers and the food industry.

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Rice is the second-largest crop of the world. Thailand produces 33 million tons of paddy rice a year (Thai Rice Exporters Association, 2010). A milling process is necessary to produce white rice, which is preferred by the consumer. This process consists of removing the embryo and bran layer from brown rice after the hull is removed from paddy rice. The degree of milling (DOM) indicates the amount of bran left on kernels after milling. It is well-known that the major portion of the nutrients and antioxidants in rice are located in outer layer of the caryopsis. Therefore, rice bran is a cheap and valuable by-product that contains high contents of nutritional components. Moreover, rice bran is known to be a source of many antioxidants such as phenolics, γ-oryzanol, tocopherols, and tocotrienols. Rice phenolics have antioxidant, antimutagenic and anticancer properties, and play a role in maintaining health (Birosova et al., 2005; Gomes et al., 2003). γ-Oryzanol has the ability to lower human serum cholesterol levels. It has greater antioxidant activity in cholesterol than vitamin E, possibly due to the similarity in the structures of γ-oryzanol and cholesterol (Xu et al., 2001). Additionally, γ-oryzanol also decreases hepatic cholesterol biosynthesis and possesses the ability to reduce cholesterol absorption (Rong et al., 1997). Pigmented rice varieties have been developed in many countries, including Thailand, because they are rich in antioxidants. The pigments found in black rice are anthocyanins which possess high antioxidant activity and many health benefits. Black rice has been reported for its health benefits against chronic diseases such as cancer and cardiovascular disease (Zhao et al., 2004; Chen et al., 2006). For food industries, synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) have been applied in many food products to extend shelf-life. However, in the last decade, there has been an enormous demand for natural antioxidants because of adverse toxicological reports on many synthetic antioxidants (Madhavi et al., 1996).

The DOM influences the nutrient content and antioxidant properties of rice. Lambert et al. (2007) reported that the protein and mineral contents of rice decreased as a function of DOM, while the starch content increased. It was indicated that some nutrients were not homogenously distributed in the rice grain. Many physical properties of rice grains were reported to be influenced by DOM such as length, width, thickness, aspect ratio, volume and bulk density (Liu et al., 2009). Mohapatra and Bal (2006) found that the removal of bran and an increased DOM increased the cooking index. The effect of milling time on the antioxidant content of rice bran was reported by Rohrer and Siebenmorgen (2004). They found that the highest concentration of vitamin E was found in rice bran after the second round of 10 sec of milling, whereas the highest co ncentration

of γ-oryzanol was in the rice bran from the first round of 10 sec of milling. However, relatively little research on antioxidant compounds in bran obtained with different milling times has been reported, especially regarding antioxidant activities.

Rice breeders have generated many diverse Thai rice varieties. Since 2010, there have been 118 rice varieties certified by the Bureau of Rice Research and Development (Thailand). The important rice varieties produced in Thailand are Jasmine rice (KDML105), Phatumthani 1 (PT1), San Patong (SPT), Gor Kor31 (RD31) and the glutinous black rice, KumDoiSaket (DSK). The objectives of this study were to determine the nutritional and antioxidant composition of five Thai rice varieties and to evaluate the effect of milling time on their bran nutritional and antioxidant composition.

2. Materials and Methods

2.1 Rice samples and milling process

Paddy rice samples (KDML105, PT1, SPT, RD31 and DSK; Table 1) were obtained from the Chiang-Mai Rice Research Center (2012 harvest). After dehulling, the samples (250g, <14% moisture content) were abrasively milled for 0-60 sec to make a DOM curve. Three replicates of rice milling were performed with a McGill type rice miller (Sinthavee Garage, Thailand) to obtain rice with different DOM. Broken rice kernels were removed using a TGR rice length grader (Satake, Australia). DOM was calculated from the weight of rice before and after the milling (Wadsworth, 1994). For the study of the effect of milling time on the nutrient and antioxidant content in rice bran, each dehulled rice sample (250g) was milled for 10, 20 and 30 sec. Broken rice kernels were removed from the bran as described above and then immediately kept at -18^{o}C until further analysis.

Table 1.Description of rice samples.

Rice variety	Description
KDML105	Long grain, low amylose content rice (14.2% amylose)
PT1	Long grain, low amylose content rice (16.1% amylose)
SPT	Long grain, glutinous rice (<5% amylose)
RD31	Long grain, high amylose content rice (29.4% amylose)
DSK	Long grain, black glutinous rice (<5% amylose)

2.2 Determination of nutritional composition

Dehulled rice samples from the five varieties were assessed in triplicate for protein, fat, crude fiber, ash and carbohydrate levels according the AOAC Official Method (AOAC, 2000). The protein content was determined using an automated combustion protein analysis (FP-528, LECO). The brans, after milling at 10, 20 and 30 sec, were also assessed in triplicate for their contents of protein, fat, crude fiber and ash (AOAC, 2000).

2.3 Determination of antioxidant contents

2.3.1 Total phenolic content

The total phenolic content of dehulled rice and bran samples was determined using the Folin–Ciocalteu reagent according to the method reported by Goffman and Berman (2004) with some modification. Briefly, 200 mg of the sample was extracted with 5 mL of methanol (99.9%) overnight (vortexed twice, on first and final) and was centrifuged at 3822 g for 5 minutes. Four milliliters of the supernatant were filtered through a 1 µm syringe filter. The extracts were diluted with deionized water. Folin–Ciocalteu reagent (500 µL) and ethanolamine (1 mL, 0.5M) were added to 1.2 mL of the diluted solution, mixed and allow to stand at room temperature for 30 minutes; the absorbance at 600 nm was measured using a UV-vis spectrophotometer (Shimadzu, model UV-160). The results are expressed as mg gallic acid equivalents (GAE) per g of sample.

2.3.2 Determination of the α-tocopherol and γ-oryzanol content

α-Tocopherol and γ-oryzanol levels were determined in dehulled and rice bran samples according to the method reported by Aguilar-Garcia et al. (2007), with some modifications. Samples (100 mg) were extracted twice with 6 mL of methanol and centrifuged at 825 g for 10 minutes. The supernatants were combined and then evaporated to 4 mL, then made up to exactly 5.0 mL with HPLC-grade methanol in a volumetric flask. This solution was filtered through a 0.45 µm syringe filter before being subjected to HPLC analysis.

α-Tocopherol and γ-oryzanol were analyzed by HPLC using a Shimadzu LC-10AT apparatus equipped with fluorescent and UV/vis detectors. A C18 column (Inertsil ODS-3, 5 µm, 250x4.6 mm) was used to separate these compounds. The mobile phase was a mixture of methanol and acetonitrile (15:85 v/v) at a flow rate of 2 mL/min in isocratic mode. The sample loop was set at 20 µl. The UV/vis detector was set at 325 nm for γ-oryzanol and the fluorescent detector was set at 285 nm (excitation wavelength) and 325 nm (emission wavelength) for α-

tocopherol. A preliminary identification of the peaks was done by comparison with the retention times of pure standards. Both γ-oryzanol and α-tocopherol were quantified against standard curves, which related the known quantity of total γ-oryzanol and α-tocopherol to the total peak area of the absorbance.

2.3.3 Total anthocyanin content

The total anthocyanin content of the rice samples was determined by the pH differential method (Giusti and Wrolstad, 2005). The rice was extracted twice with 2% citric acid solution (rice: acid solution = 1:10) for 3 hours with constant stirring at room temperature. The solution was filtered through Whatman No.1 paper. The filtrate was evaporated at 40°C using a vacuum rotary evaporator. This crude extract was then diluted in potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). The absorbance at 510 nm and 700 nm was measured using a UV spectrophotometer (BioMate5, Thermo Spectronic). The content of anthocyanin was calculated and expressed as cyanidin-3-glucoside by the following equation:

$$\text{Total anthocyanin content (mg/L)} = (A_{diff} \times MW \times DF \times 1000)/\varepsilon$$

$$A_{diff} = (A_{510}-A_{700})_{pH1.0} - (A_{510}-A_{700})_{pH4.5}$$

where A_{diff} is the difference in absorbance at various pH levels, MW is the molecular weight (g/mol) of cyanidin-3-glucoside and molar absorptivity (ε) is 26,900 L/mol.cm

2.4 Determination of antioxidant activities

2.4.1 Extraction

Dehulled rice and rice bran samples were finely ground and defatted twice with hexane (1:20 w/v) for 30 minutes. The defatted rice fraction was extracted twice with 99.9% methanol (1:20 w/v) in an electrical shaker overnight at room temperature and then filtered through Whatman No.1 filter paper. The extracts were evaporated by vacuum rotary evaporator at 50°C to dryness. The evaporator flask was eluted with methanol and the volume was made to 100 mL in a volumetric flask. The extracts were stored in the freezer at -18°C until used for further analysis. All analyses were performed within two weeks of extraction.

2.4.2 DPPH radical scavenging activity

The free radical scavenging capacity of selected antioxidants and rice fraction sample extracts was estimated following a previously reported procedure using the stable 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) (Brand-Williams et al., 1995). Briefly, different dilutions of the extracts were prepared. An aliquot of the solution or diluted extract (1.0 mL) was vigorously mixed with 1.0 mL of freshly prepared 0.004% DPPH in methanol and held in the dark for 30 minutes at room temperature. The absorbance was then read at 517 nm (BioMate5, Thermo Spectronic) against blanks. DPPH free radical-scavenging ability was calculated using the following formula:

> Scavenging ability (%) = [Absorbance at 517 nm of the control – Absorbance at 517 nm of the sample]/Absorbance at 517 nm of the control x 100.

The scavenging activity of rice fraction extracts was expressed as the 50% effective concentration, EC_{50} (µg/mL), which was obtained by interpolation from linear regression analysis. A lower EC_{50} value indicates higher antiradical activity (Brand-Williams et al., 1995). BHT (99.0% purity, Rankem, India), a synthetic antioxidant was used as reference.

2.5 Statistical analysis

The analysis of variance was performed using Statistical Package for the Social Sciences (SPSS, version 17.0, 2008). Duncan's multiple range test ($p<0.05$) was used to detect differences among treatment means. Moreover, Pearson's correlation coefficient value was determined (significance level $p<0.05$).

3. Results and Discussion

3.1 Nutritional properties and antioxidants content of five dehulled Thai rice varieties

Five popular Thai rice varieties, including KDML105, PT1, SPT, RD31 and DSK, were dehulled and assessed for their nutrient contents. Table 2 shows the nutritional composition of the dehulled rice samples. Protein influences the nutritional value of rice. All rice samples contained the same level of protein ($p\geq0.05$), in the range of 5.52-6.63% dry matter (DM). The protein contents of rice in our study were relatively low (< 7%). Lipid, crude fiber, ash and carbohydrate contents were significantly different amongst the varieties. The PT1 and DSK varieties contained the highest lipid content (1.77 and 1.83% DM), while the lowest was found in KDML105 (1.43%

DM). The crude fiber content in PT1 was significantly lower than in the other strains ($p<0.05$). The ash content of the rice samples was in the range of 1.05-1.75% DM, which is similar to ash content in rice reported by Juliano (1985). DSK showed the highest ash content while the lowest was found in the SPT variety. The carbohydrate content in the rice samples was relatively high. In this study, rice samples contained 84.84-86.65% DM, which was higher than the level shown in a study by Sompong et al. (2011), who reported the carbohydrate content of rice as 71.99-79.27% DM. The nutritional composition of rice is influenced by variety, the nature of the soil, environmental conditions and fertilizer use (Juliano et al., 1964).

Table 2.Nutritional composition of dehulled rice.

Composition (%DM)	Variety				
	KDML105	PT1	SPT	RD31	DSK
Protein^{ns}	6.63±0.12	5.52± 0.11	6.27 ±0.01	6.31 ±0.23	6.15 ±0.21
Lipid*	1.43^{c} ±0.02	1.77^{a} ±0.05	1.64^{b} ±0.04	1.67^{b} ±0.03	1.83^{a} ±0.02
Crude fiber*	2.98^{ab} ±0.14	2.56^{b} ±0.13	3.35^{a} ±0.16	3.11^{ab} ±0.16	2.82^{ab} ±0.14
Ash*	1.61^{b} ±0.05	1.41^{c} ±0.04	1.05^{d} ±0.10	1.47^{c} ±0.03	1.75^{a} ±0.04
Carbohydrate*	84.91^{b} ±0.14	86.65^{a} ±0.13	84.99^{b} ±0.16	84.84^{b} ±0.16	85.27^{b} ±0.14

* Mean values within a row superscripted by the same letter are not significantly different at $p<0.05$, ns = not significant.

Rice is well-known to be a source of many antioxidants. The total phenolic, γ-oryzanol, α-tocopherol and anthocyanin contents of the dehulled rice samples are shown in Table 3. The results show that γ-oryzanol and phenolic compounds were the major antioxidants found in all rice samples, whereas α-tocopherol was found in a small amount. No anthocyanin was found in non-pigmented rice samples, while DSK showed a high level of anthocyanin. DSK (a glutinous black rice variety) contained 1.13 mg/g of anthocyanins, whichis much higher than the levels found in colored corn and wheat, i.e. 0.05-0.61 mg/g total anthocyanins,but lower than the previous reported in black rice, i.e. 3.27 mg/g (Abdel-Aal et al., 2006). According to a study by Kallithraka et al. (2005), DSK contains a similar anthocyanin content as grape (0.73 mg/g fresh berry weight). DSK contained the highest level of total phenolics ($p<0.05$), which was up to five-fold higher than non-pigmented rice samples. The phenolic acid composition of pigmented rice was reported by Laokuldilok et al. (2011), who found that black rice contained a higher level of total and soluble phenolic acids, which may be involved in the biosynthesis process of anthocyanin in rice grains.

The degradation of anthocyanins may require a higher of phenolic content in rice. At a pH value higher than 7, anthocyanins are unstable and are degraded to aldehydes and phenolic acids (Castaneda-Ovando et al., 2009). Many studies have shown significant levels of phenolics in pigmented rice compared to non-pigmented rice. The highest γ-oryzanol content was found in the KDML105 variety (0.65 mg/g), while the lowest was found in SPT (0.38 mg/g). Rice samples showed α-tocopherol contents of 0.18-0.22 mg/100 g; however, PT1 contained a very small amount of α-tocopherol, which was below the detection limit of our instrument.

Table 3. Antioxidants content of dehulled rice.

Active compound	Variety				
	KDML105	**PT1**	**SPT**	**RD31**	**DSK**
Total phenolics* (mg GAE/g)	0.23^{b} ±0.02	0.20^{bc} ±0.09	0.23^{b} ±0.03	0.24^{b} ±0.09	1.04^{a} ±0.12
γ-Oryzanol* (mg/g)	0.65^{a} ±0.04	0.60^{ab} ±0.03	0.38^{c} ±0.03	0.54^{b} ±0.05	0.57^{b} ±0.04
α-Tocopherol^{ns} (mg/100g)	0.22±0.07	N.D.	0.21±0.05	0.18±0.08	0.20±0.04
Anthocyanins (mg/g)	N.D.	N.D.	N.D.	N.D.	1.13±0.06

* Mean values within a row superscripted by the same letter are not significantly different at $p<0.05$, N.D.=not detectable

3.2 Relationship between milling time and DOM

Dehulled rice samples were successively milled for 0, 10, 20, 30, 40, 50 and 60 sec to obtain white rice with various DOMs. The relationship between milling time and the DOM of five rice varieties is presented in Fig. 1. After 60 sec of milling, KDML105 showed the highest DOM (19.9%), while the lowest was found for DSK (18.2%). Increasing the milling time increased the DOM in all rice samples. However, the relationship between milling time and DOM was not linear (Fig. 1 and 2). In the first 30 sec of milling, the DOM increased sharply, but after this point, the increase in DOM was slower. The similar relationship between DOM and milling time of rice was reported by Lui et al. (2009) and Liang et al. (2008). The differences in the DOM of the rice samples resulted from the differences in shape, length, width, porosity and hardness of each rice variety (Singh et al., 2000 and Lui et al., 2008). The curves indicated that hardness was not

homogenously distributed in rice grains. The outer layer of rice grains was softer than the inner endosperm. Regression analysis was performed using these data, and the obtained equation was $y=9.156E-5x^3-0.015x^2+0.895x-0.104$ ($R^2=0.971$), where y is DOM (%) and x is time (sec). The results show that almost the entire bran layer of rice was removed within the first 30 sec of milling. Further milling may have resulted in overmilling and a reduction in white rice yield.

3.3 Effect of milling time on nutritional and antioxidant composition of rice bran

Rice bran obtained from milling at 10, 20 and 30 sec was assessed for its nutritional and antioxidant composition. The DOMs of five rice samples after 10, 20 and 30 sec of milling are shown in Table 4. The protein, fat, fiber, ash and carbohydrate contents of rice bran samples are shown in Table 5. The protein, crude fiber, ash and carbohydrate contents of rice bran were significantly influenced by milling time ($p<0.05$), whereas the fat content was not influenced by milling time ($p\geq0.05$). Longer milling time decreased the contents of protein, fiber and ash, while the carbohydrate content was increased. Rice bran samples contained 14.49-22.61% DM of fat, which contains considerable amounts of essential fatty acids (Wang, 1986). The relatively high fat content of the bran samples compared to that of rice is due to the fact that, during milling, most of the fat is removed as it is contained in the bran (Grist, 1975). The rice bran samples contained 10.60-12.30% DM of protein. Crude protein levels of 10.6–13.4% DM (ICAR, 1964) and 12–17% DM (Bhattacharya, 1988) have been reported in other studies. Rice bran obtained after 10 and 20 sec of milling contained higher contents of protein and crude fiber than rice bran obtained after 30 sec of milling ($p<0.05$). The ash content of bran is an important component in determining the quality of milled rice. The DOM of rice grain can be determined based on the content of ash and crude fat, the degree of whiteness and the yield of polished rice (Hogman and Deobald, 1961). The ash content in rice bran obtained after 10 sec of milling was significantly higher than 20 and 30 sec milled rice bran ($p<0.05$). In this study, the ash content in rice bran was 8.08-9.74% DM, which similar to levels reported in a study by Juliano and Bechtel (1985), who reported the ash content of rice bran in the range of 6.6-9.9% DM. A longer milling time increased the carbohydrate content of rice bran, resulting in the dilution of other nutrients. The highest content was found in DSK rice bran after 30 sec of milling. These results indicate that rice bran obtained after 10 sec of milling had the greatest nutritional value and contained the highest contents of protein, crude fiber and ash, whereas the carbohydrate content was lowest.

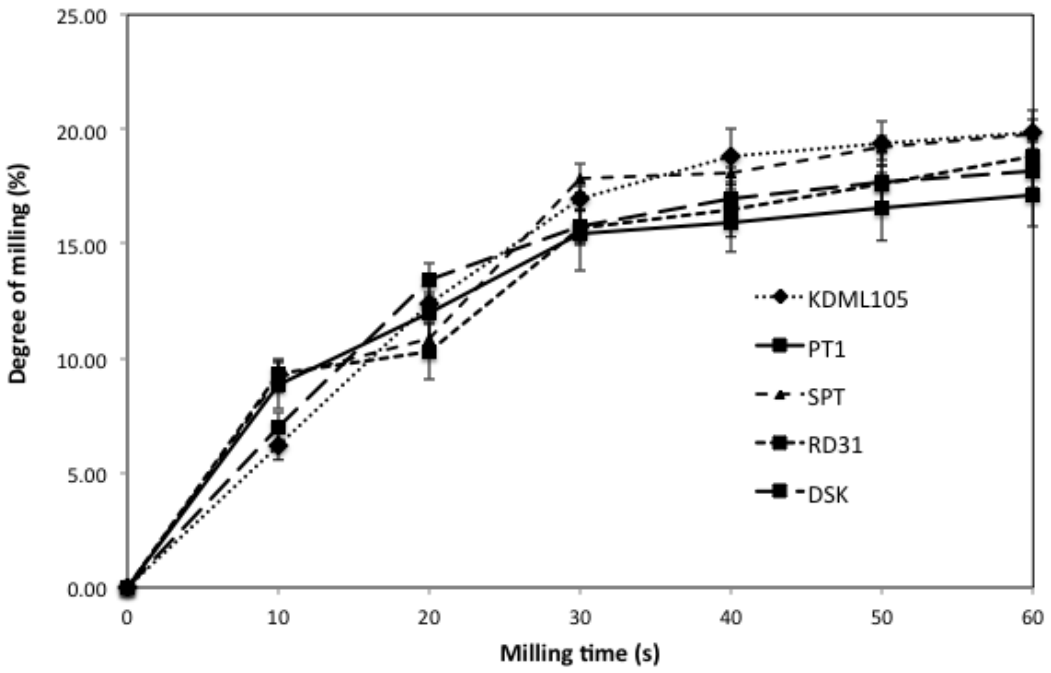

Figure 1. Relationship between milling time and degree of milling of 5 Thai rice varieties.

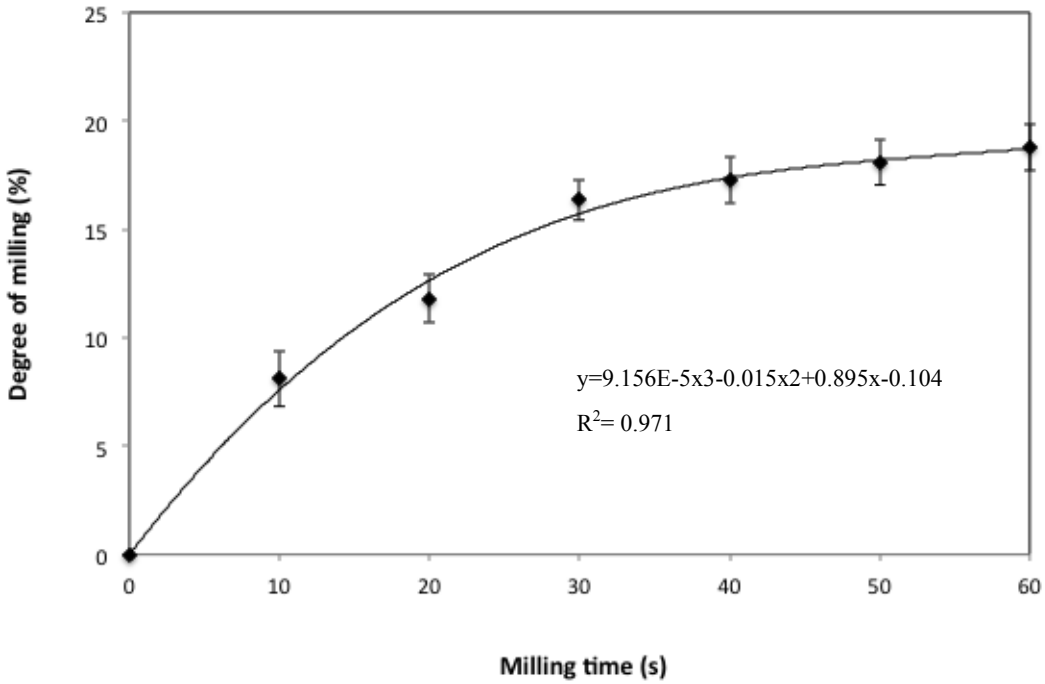

Figure 2. The milling curve and regression equation (averaged from 5 Thai rice varieties).

Table 4. Effect of milling time on degree of milling of 5 Thai rice varieties.

Milling time	Varieties				
(sec)	KDML105	PT1	SPT	RD31	DSK
10	6.20 ±0.63	8.83 ±1.03	9.17 ±0.64	9.33 ±0.61	6.97 ±0.65
20	12.40 ±0.49	12.01 ±1.42	10.86 ±0.75	10.29 ±0.21	13.43 ±0.73
30	17.00 ±0.57	15.43 ±1.60	17.86 ±0.63	15.71 ±0.77	15.76 ±0.71

Rice bran consists of many antioxidant compounds. Total phenolics, γ-oryzanol and α-tocopherol are known to be common rice antioxidants. Fig. 3 shows the influence of milling time on the total phenolic content in rice bran. In this study, a decrease in the total phenolic content was observed in the rice bran of all rice varieties as a function of milling time. Bran samples showed a high total phenolic content of 1.85-3.56 mg GAE/g, which is approximately 6.4-fold higher than the level in brown rice. The highest total phenolic content was found in DSK bran after 10 sec of milling. This result indicates that black rice bran is a good source of phenolic compounds. Milling duration influenced the total phenolic content in bran. On average, bran after 10 sec of milling contained 8.9% and 21.5% more total phenolics than rice bran after 20 and 30 sec of milling, respectively. The γ-oryzanol content of rice bran samples is shown in Fig. 4. HPLC analysis showed that the brans contained 1.40-2.60 mg/g of γ-oryzanol. A decrease in the γ-oryzanol level in bran from KDML105, PT1, RD31 and DSK was observed with an increase in milling duration, while the γ-oryzanol content of the SPT sample was not decreased. These results suggested that γ-oryzanol was concentrated in the outer layer of the grain in KDML105, PT1, RD31 and DSK, whereas it would homogenously distribute in the SPTgrain. The ferulate esters that comprise oryzanol are located primarily in the outer pericarp, seed coat and nucellus layer (Bechtel and Pomeranz, 1997), which are milled in the first 10 sec. This result is similar to the results of a study by Rohrer and Siebenmorgen (2004), who reported that bran collected after the first 10 sec of milling contained a greater γ-oryzanol content than after 20 and 30 sec of milling.

Table 5. Effect of milling on nutritional composition of rice bran.

Composition (%DM)	Milling time (sec)	Variety					Mean
		KDML105	PT1	SPT	RD31	DSK	
Protein*	10	10.57 ± 0.34	10.89 ± 0.18	11.37 ± 1.03	11.92 ± 0.21	12.30 ± 0.20	**11.49**^{a}
	20	10.97 ± 0.25	10.76 ± 0.32	11.40 ± 0.32	11.70 ± 0.18	11.87 ± 0.35	**11.34**^{ab}
	30	11.22 ± 0.23	10.60 ± 0.03	1.22 ± 0.25	11.41 ± 0.25	11.04 ± 0.20	**11.10**^{b}
Fat^{ns}	10	16.30 ± 0.01	19.28 ± 0.36	19.79 ± 0.14	22.61 ± 3.04	17.92 ± 0.49	**19.16**
	20	16.82 ± 1.02	20.29 ± 0.11	20.08 ± 0.02	21.61 ± 2.31	15.89 ± 1.45	**18.94**
	30	15.47 ±0.02	19.72 ± 0.17	20.55 ± 0.64	22.15 ± 4.53	14.49 ± 0.34	**18.48**
Crude fiber*	10	16.51 ± 3.64	16.35 ± 0.23	19.07 ± 0.06	18.64 ± 0.36	16.68 ± 0.06	**17.45**^{a}
	20	18.05 ± 4.18	13.43 ± 1.05	18.05 ± 0.08	16.95 ± 0.07	15.78 ± 0.16	**16.45**^{a}
	30	15.08 ± 1.31	11.12 ± 0.97	15.82 ± 0.24	14.18 ± 0.18	13.48 ± 0.12	**13.94**^{c}
Ash*	10	9.78 ± 0.28	9.74 ± 0.06	9.48 ± 0.58	9.56 ± 0.44	8.33 ± 0.08	**9.38**^{a}
	20	9.48 ± 0.23	9.14 ± 0.14	9.17 ± 0.09	9.35 ± 0.17	8.14 ± 0.13	**9.06**^{bc}
	30	9.76 ± 0.04	9.05 ± 0.04	9.54 ± 0.19	9.70 ± 0.15	8.08 ± 0.03	**9.22**^{bc}
Carbohydrate*	10	46.74 ± 3.05	43.73 ± 0.85	40.29 ± 1.41	37.38 ± 2.92	44.77 ± 0.71	**42.52**^{b}
	20	44.68 ± 5.68	46.38 ± 0.47	41.29 ± 0.13	40.38 ± 2.23	48.32 ± 1.38	**44.21**^{b}
	30	48.46 ± 1.60	49.50 ± 0.73	42.86 ± 0.01	42.56 ± 4.45	52.90 ± 0.68	**47.26**^{a}

* Mean values within a column superscripted by the same letter are not significantly different at $p<0.05$, ns=not significant.

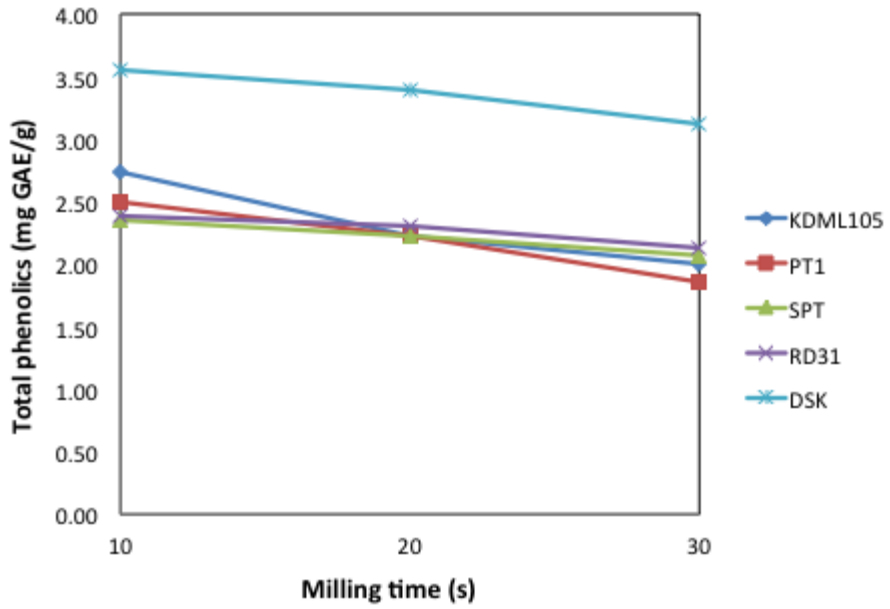

Figure 3. Total phenolics content (mg GAE/g) in rice bran with different milling time.

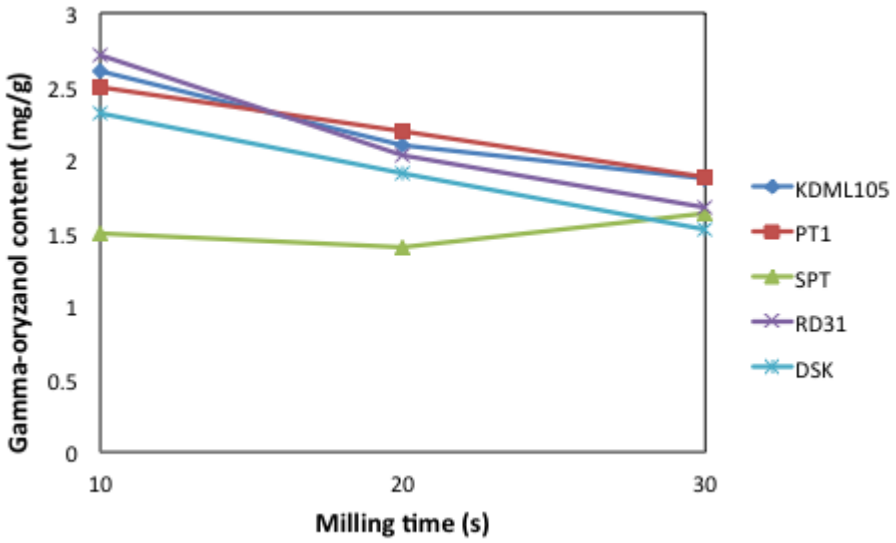

Figure 4. Gamma-oryzanol content (mg/g) in rice bran with different milling time.

The content of α-tocopherol in rice bran is shown in Fig. 5. The α-tocopherol content in rice bran from KDML105, SPT and DSK decreased with longer milling, in contrast, but an increase in the α-tocopherol content was found in PT1 and RD31 samples. The results can be explained by the non-homogenous distribution of α-tocopherol in rice grain. Tocopherols are concentrated at

the mid-thickness of the rice milling fraction (Rohrer and Siebenmorgen, 2004), which resulted in a variation in the α-tocopherol content in our samples. In this study, rice bran contained α-tocopherol in the range of 1.24-25.13 µg/g, which is relatively low compared with other antioxidants.

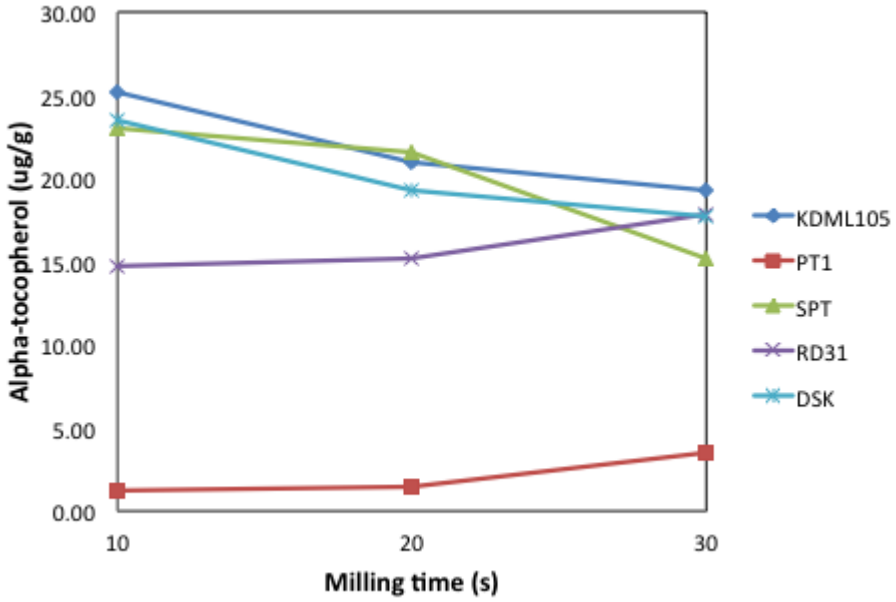

Figure 5. Alpha-tocopherol content (µg/g) in rice bran with different milling time.

DSK bran samples were assessed for their anthocyanin content; the results are shown in Fig. 6. Bran from DSK (a glutinous black rice variety) was a valuable source of anthocyanin, the contents of which were 3.09-3.82 mg/g. DSK rice bran anthocyanin levels were much higher than those reported in purple wheat bran (1.155 mg/g) (Li et al., 2007). The anthocyanin content of the bran was influenced by milling time; a longer milling time reduced the concentration of anthocyanin in rice bran. Bran after 10 sec of milling contained 19.0% and 23.6% more anthocyanins than bran after 20 and 30 sec of milling, respectively.

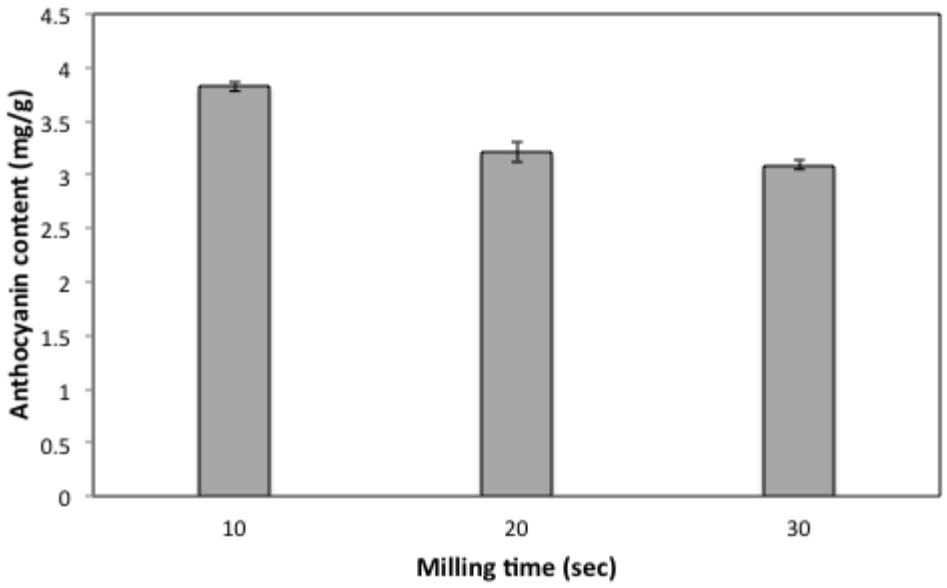

Figure 6. Anthocyanin content (mg/g) in DSK rice bran with different milling time.

3.4 DPPH radical scavenging activity of rice bran extract

Rice bran samples were defatted and then extracted twice by methanol. After evaporation, these extracts were diluted in methanol and assessed for their scavenging activity. The results, expressed as the EC_{50} value (μg/mL), are shown in Fig. 7. The results show that all rice bran samples contained the primary antioxidants that donate hydrogen atoms to free radicals to the terminate free radical chain reaction. Many antioxidants in rice are extracted by methanol, such as tocopherols, tocotrienols, γ-oryzanol, free phenolic compounds and anthocyanins (Chotimarkorn et al., 2008). Regarding the EC_{50} value, the DPPH radical scavenging activity of the DSK extract was higher than the other bran samples. The DPPH radical activity of the extract from DSK bran was approximately three-fold higher than the activity of the KDML105, SPT, PT1 and RD31 extracts. This may be due to the high content of phenolics and anthocyanins in pigmented rice bran. A longer milling duration reduced the antiradical activity of rice bran extracts due to the dilution of the antioxidant content. The highest DPPH radical scavenging activity was found in DSK bran milled for 10 sec. However, the DPPH radical scavenging activity of all extract was less than of BHT, a synthetic antioxidant.

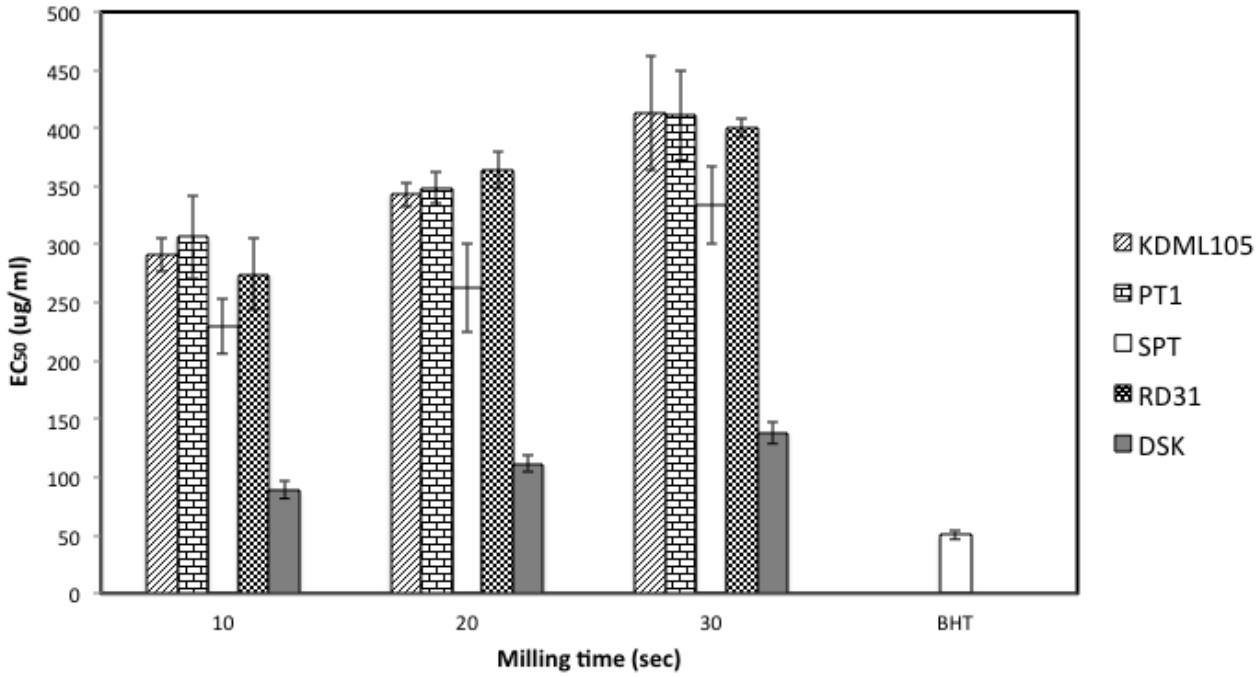

Figure 7. DPPH radical activity expressed as EC_{50} value (μg/mL) of rice bran extracts with different milling time compared with BHT.

4. Conclusion

Milling for 30 sec was sufficient to remove almost all of bran fraction from rice grain. The further milling resulted in the dilution of nutrients and antioxidant compounds in rice bran and may also reduce the white rice yield by overmilling. Pigmented rice bran (DSK) showed the highest phenolic and anthocyanin contents, and this extract also possessed the highest DPPH radical scavenging activity. This study provides evidence that clearly demonstrates that a longer milling duration decreases both the nutritional value and antioxidant content in rice bran. Moreover, pigmented rice bran, after 10 sec of milling, is a rich source of nutrients and antioxidant compounds, suggesting that it may have the potential for utilization as a novel product that is rich in natural antioxidants.

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