Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 11 (6): 285-293, 2012
ISSN 1682-3974 / DOI: 10.3923/ajps.2012.285.293

Phytochemical Content and Antioxidant Activity of Colored and Non-colored Thai Rice Cultivars

[1]K. Chakuton, [1,2]D. Puangpronpitag and [1]M. Nakornriab
[1]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham, 44150, Thailand
[2]Faculty of Medicine, Mahasarakham University, Mahasarakham, 44000, Thailand

Abstract: Rice is one of the important foods of the world. Particularly, countries in Asia are popular eating rice daily food rather than in order regions of the world. Rice is sources of many bioactive non-nutrient compounds, known as phytochemicals. This study aimed to determine phytochemicals value such as, total phenolic compound, total anthocyanin, phytic acid, gamma (γ)-oryzanol composition and antioxidant activity of rice seed extracts of twelve colored and non-colored native Thai rice cultivars in Surin province, Thailand. The results showed that methanolic extracts of those rice seeds produced the highest extraction yield (5.45%). The colored cultivar 53 showed the highest Total Phenolic Content (TPC) and anthocyanins content by Folin-Ciocalteu method and pH-differential, respectively (7.40 mg Gallic Acid Equivalent (GAE)/100 g and 1045.12 mg malvidin/100 g, respectively). Moreover, there is a similarity in the γ-oryzanol content which detected by HPLC, colored rice seed extracts was statistically significant higher ($p<0.05$) than those non-colored. There were 4 major components of oryzanol (cycloartenol ferulate, 24-methylene cycloartenol ferulate, campesteryl ferulate and β-sitosteryl ferulate) which were successfully identified. Antioxidant activity of rice crude extracts was examined by DPPH scavenging method. Colored rice seed cultivar number 98 showed the highest activity (IC_{50} = 0.545 mg mL^{-1}). While, phytic acid content in non-colored-rice seed cultivar number 236 showed the highest content (9.94 mg/100 g). From these results suggested to the potential of Thai rice seeds which may be further developed in food industry, cosmetic and health product.

Key words: Anthocyanin, gamma-oryzanol, Thai rice cultivars, phenolic compound, phytic acid

INTRODUCTION

Rice (*Oryza sativa* L.) is the principle cereal consumed in Asia and the primary staple food being consumed by nearly half of the world's population (Zhai *et al*., 2001). It is also the main export product of Thailand. There are different varieties of rice that contain color pigments. The cultivars of pigmented rice have long history for people consumption, especially in Southeastern Asia (Hu *et al*., 2003). In addition, pigmented rice composed of high content of phenolic compounds and it contains anthocyanin pigments with notable antioxidant (Hu *et al*., 2003; Oki *et al*., 2002).

Rice contains a high level of several phytochemicals, e.g., gamma (γ)-oryzanol (Xu and Godber, 1999), tocopherols, tocotrienols (Tai-Sun and Godber, 1994) and phenolic compounds (Butsat and Siriamornpun, 2010). In addition, it has reported that phenolic compounds, from methanolic extracts of rice husk, exhibit high antioxidant activity against scavengers of singlet oxygen and inhibit high hydrogen peroxide-induced damage to cellular deoxyribonucleic acid (DNA) in human lymphocytes (Jeon *et al*., 2006). There is evidence that phenolic substances act as antioxidants by preventing the oxidation of LDL-lipoprotein, platelet aggregation and damage of red blood cells (Cheynier, 2005). Additionally, phenolics act as: (i) metal chelators, (ii) antimutagens and anticarcinogens, (iii) antimicrobial agents and (iv) clarifying agents (Anli and Vural, 2009; Proestos *et al*., 2005). These compounds are a part of the everyday diet and also used as medicines or supplements.

Early studies showed that polyphenols such as plant anthocyanins are beneficial to cardiovascular health (Stoclet *et al*., 2004; Curin and Andriantsitohaina, 2005). *Oryza sativa*, one of the anthocyanin pigmented rice varieties, is well known for its taste and health-improving properties. Cyanidin-3-O-β-D-glucopyranoside is the most abundant pigment in purple rice (Ryu *et al*., 1998). It also has been known to have diverse physiological effects, protection against cytotoxicity (Chen *et al*., 2005),

Corresponding Author: Darunee Puangpronpitag, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science and Faculty of Medicine, Mahasarakham University Mahasarakham, 44150, Thailand

anti-neuro-degenerative activity (Kim *et al*., 2005), glycogen phosphorylase inhibition (Jakobs *et al*., 2006), antioxidative activity (Kano *et al*., 2005; Nam *et al*., 2006). Furthermore, phytic acid (inositol hexaphosphoric acid, IP6), one of phytochemicals is associated with rice brans; some brans can contain over 5% phytic acid. IP6 has a strong ability to chelate multivalent metal ions, specially zinc, calcium and iron. The binding can result in very insoluble salts with poor bioavailability of minerals (Zhou and Erdman, 1995). Thus, IP6 may be considered a natural antinutrient substance (Ko and Godin, 1990; Patearroyo *et al*., 1995). However, negative properties of IP6 which complexes with iron may be bring about a favorable reduction in the formation of hydroxyl radicals in the colon (Graf and Eaton, 1993). But, true mechanisms of the interactions between phytic acid and minerals are not yet understood. In addition, IP6 has also been reported as an anti-cancer properties in soft tissue, colon, prostate, metastatic and mammary cancers (Dost and Tokul, 2006). γ-oryzanol is another one of the phytochemicals that found at high concentration in rice bran, including sterols and ferulic acid, has been reported to exhibit more antioxidant activity than vitamin E as six fold and other health beneficial properties (Xu *et al*., 2001). Their important bioactivities include anti-inflammatory activity (Saenjum *et al*., 2012), enhancement of the immune system (Choi *et al*., 2007), heart disease, cardiovascular disease, glycemic control, diabetes and inhibit tumor promotion (Liu, 2007; Schatzkin *et al*., 2007; Shirakawa *et al*., 2006; Nam *et al*., 2005; Anderson, 2004; Montonen *et al*., 2003).

However, there is no study so far about these phytochemicals value such as polyphenols, anthocyanin, phytic acid, γ-oryzanol, including the antioxidant activity in the colored and non-colored native Thai rice cultivars. Therefore, this study was interest to further investigate this point. The results would be preliminary information that worth to further studied for uses native Thai rice cultivars in food industry, health product, pharmaceutical and medicinal applications.

MATERIALS AND METHODS

Plant material: Twelve cultivar numbers of colored (53, 97, 98, 202, 203, 206, 208 and 209) and non-colored (210, 233, 235 and 236) native Thai rice seeds (Table 1) were collected from Surin Rice Research Center, Northeastern Thailand, in May 2010. Rice seeds were cracked to remove husk and ground into powder.

Solvent extraction: Seven gram of rice seed powder (12 cultivars) was extracted with 21 mL of various organic solvents (methanol, distilled water, hexane and ethyl acetate) in electrical shaker (Platform Shaker Universal PSU-20, Biosan), speed 170 rpm at room temperature for 1 h and then filtered through Whatman No.1 filter paper. The solvent with the highest efficiency of extraction was used in subsequent experiments. The residues were re-extracted under the same conditions and supernatants from both extractions were combined and removed solvent with rotary evaporator *in vacuo* (Buchi Rotavapor R-200, USA) at 50 °C under vacuum. The evaporative dried extracts were weighed for calculating % yield (dry matter basis, db) and then stored at -20 °C until using.

Table 1: Twelve cultivars of Thai rice from Surin province

Cultivar No.	Gene specific No.	Cultivars name
Colored rice cultivar		
53	621	Neawdam
97	13213	Pa-mia
98	13214	Wongwan
202	NRM 09001	Neawdam 1
203	NRM 09002	Neawdam 2
206	NRM 09005	Malii-dang 2
208	NRM 09006	Jaowdam
209	NRM 09008	Jaowdam
Non-colored rice cultivars		
210	3896	Na-rok
233	3379	E-ton
235	3384	Pla-kaeng
236	3386	Panlak

Determination of the yield of rice seed extracts: The evaporative dried rice seed extracts were weighed for calculating the yield by the following equation:

$$\text{Yield (\% dry basis; \%db)} = \frac{W_1}{W_2} \times 100$$

Where:
W_1 = Weight of rice seed extract after evaporation
W_2 = Weight of rice seed powder before extract

Determination of total phenolic content: The total phenolic content of the rice seed extracts was determined using the Folin-Ciocalteu method as described by Bonoli *et al*. (2004) and Puangpronpitag *et al*. (2008) with some modifications, using gallic acid as a standard. A 0.1 mL of the extract solution (5 mg mL^{-1}) was mixed with 1.5 mL of 10% sodium carbonate solution and then 3 mL of 10% Folin-Ciocalteu's reagent were added, stand for 30 min in the dark. Absorbance at 760 nm was measured in a Lamda 25 UV-Vis spectrophotometer (Thermo Spectronic, GENESYS™, USA). All measurements were determined triplicate and the data were expressed as mg Gallic Acid Equivalent (GAE) per 100 g of dried extract, based on the calibration curve of gallic acid (0.1-3 mg mL^{-1}).

DPPH radical-scavenging assay: Antioxidant activity of the rice extracts was measured using DPPH radical-scavenging assay by modified version of Liyana-Pathirana and Shahidi (2007). Butylated Hydroxyanisole (BHA) was used as the standard (2 mg mL^{-1}). All measurements were done in triplicate. The scavenging activity was derived following:

$$\text{DPPH scavenging activity (\%)} = 1 - \frac{A_{sample}}{A_{control}} \times 100$$

Where:

A_{sample} = Absorbance of sample (Rice extract or standard BHA+DPPH)

$A_{control}$ = Absorbance of control (Methanol+DPPH)

The scavenging activity of rice bran extract was expressed as 50% inhibition concentration, IC_{50} (mg mL^{-1}) and was obtained by interpolation from linear regression analysis. BHA was used for comparison.

Determination of total anthocyanin content: Total anthocyanin content was determined in anthocyanin-pigmented rice seed extract of cultivar numbers 53, 97, 98, 202, 203, 206, 208 and 209 by the pH differential method with some modifications (Hosseinian *et al*., 2008). The methanolic rice extract was diluted in 0.025 M potassium chloride buffer, pH 1.0 (0.4: 3.6, v/v) and stand in dark room temperature 30 min. Absorbance was measured at 520 and 700 nm, respectively. The extract was repeat measured by mixed with 0.4 M sodium acetate buffer, pH 4.5 and measured absorbance at 520 and 700 nm, respectively. The difference in absorbance between pH values and wavelengths was calculated as following below:

$$A = (A_{520nm}-A_{700nm})_{pH\ 1.0}-(A_{520nm}-A_{700nm})_{pH\ 4.5}$$

The concentration of Total Anthocyanin (TA) content was calculated in terms of malvidin-3-O-glucoside following equation:

$$\text{TA (mg malvidin/100 g rice powder)} = \frac{A \times MW \times DF \times 1000}{MA \times L}$$

Where:

A = Absorbance of sample
MW = molecular weight (malvidin-3-O-glu = 493.5)
MA = molar absorptivity (28,000)
DF = dilution factor for sample (0.4 mL)

Determination of phytic acid: Phytic acid was determined by following the method of Febles *et al.* (2002) with some modifications. The rice ground samples (3 g) were extracted by shaking with 20 mL of 0.1 M HCl at 200 rpm for 2 h at room temperature and centrifugation at 3000 rpm for 20 min, then supernatant was used for analysis. In this procedure, the decrease in iron (determined calorimetrically with 2,2'-bipyridine) in the supernatant was measured as the content of phytic acid. Five hundred microliter of supernatant was precipitated with 1 mL of ferric solution. The mixture was boiled at 100°C for 30 min in a water bath. After cooling, samples was transferred into Eppendorf tubes and centrifuged at 5000 rpm for 10 min. One microliter of supernatant was mixture with 2 mL of 2,2'-bipyridine solution. After incubation for 5 min at room temperature, the absorbance was measured at 519 nm. The method was calibrated with standard phytic acid solutions for each set of analysis and the results were expressed as mg phytic acid equivalents per 100 g of sample.

Determination of γ-oryzanol content: Rice seed power was stabilized according to the method of Ramezanzadeh *et al*. (2000). Then, the sample was mixed homogeneously and microwaves again for another 1 min. The sample was allowed to cool at room temperature before it was put in dark plastic bags and stored at 20°C till use. Then, content of γ-oryzanol in stabilized rice sample was detected on a Reverse-phase High Performance Liquid Chromatography (RP-HPLC) model Hitachi L-6200 (Column C18, 150×2.1 mm, 5 μm). The mobile phase was the mixture of methanol, acetonitrile, dichloromethane and acetic acid (50:44:3:3 by volume) and eluted at a flow rate of 1.4 mL min^{-1} at ambient temperature. The detector wavelength was set at 330 nm. The injection volume for the sample was 50 μL. The data were produced in three replicates and in reference to the peak area-concentration calibration curve of γ-oryzanol standards (Wako Pure Chemical Industries Ltd., Osaka, Japan).

Statistical analysis: All experiments were conducted in triplicate and the results are expressed as Mean±SD. Analysis of the variance was used to determine any difference in antioxidant activities resulting from the methods. Significant differences ($p \leq 0.05$) among treatments were detected using Duncan' multiple range test.

RESULTS

Extraction yields: Twelve cultivar numbers of colored (53, 97, 98, 202, 203, 206, 208 and 209) and non-colored (210, 233, 235 and 236) native Thai rice seeds from Surin Rice Research Center, in northeastern Thailand were extracted using methanol, distilled water, hexane and ethyl acetate.

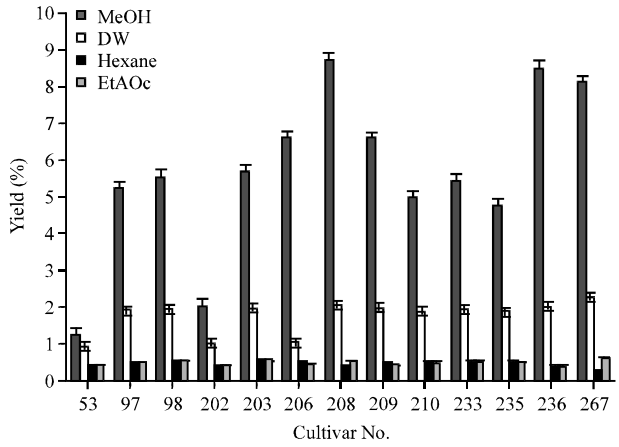

Fig. 1: The extraction yield of rice seeds with various organic solvents (n = 3), MeOH: Methanol, DW: Distilled water, EtAOc: Ethyl acetate

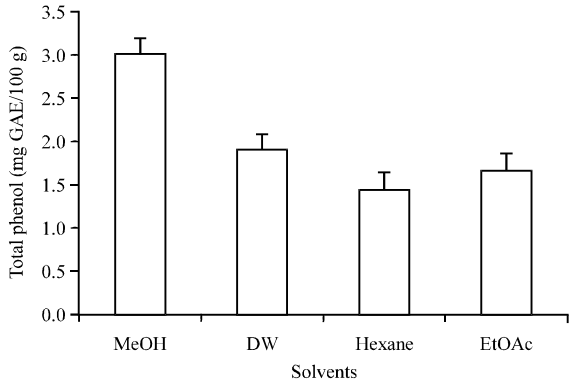

Fig. 2: Total phenolic content of the rice seeds with various organic solvents, MeOH: Methanol, DW: Distilled water, EtAOc: Ethyl acetate

The results (Fig. 1) showed that methanolic extracts of those 12 rice seeds produced the highest percent yield in all rice seeds (5.45%) and cultivar number 208 showed the highest extraction yield (8.729%). The yield of extracted rice seed by methanol was much significantly higher ($p<0.01$) than that of distilled water, ethyl acetate and hexane, respectively.

Total phenolic content: The Total Phenolic Content (TPC) of the rice seeds with various organic solvents were evaluated to again verify the first-rate solvent extraction and expressed as Gallic Acid Equivalent (GAE) in milligrams per 100 gram dry material by calibrating with standard curve of gallic acid. Similar results to extraction yields, it showed that TPC of all cultivars with methanolic extract had the highest value (2.02-4.77 mg GAE/100 g) when compared with distill water, hexane and ethyl acetate (Fig. 2). Thus, methanol was used for all subsequent extraction of those rice seeds.

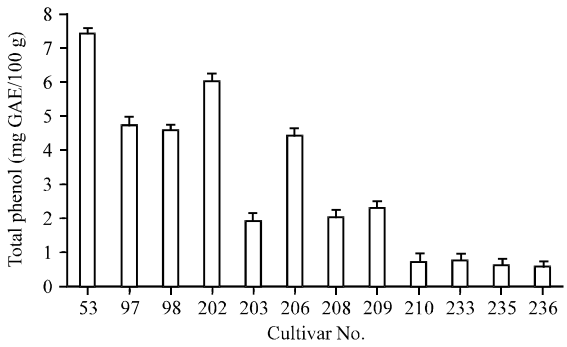

Fig. 3: Total phenolic content of methanolic extract in seed of 12 Thai rice cultivars

Among 12 cultivars which extracted with methanol, cultivar 53 showed the highest TPC (7.40 mg GAE/100 g dried extract), then cultivar 202, 97, 98 (6.03, 4.77, 4.57 mg GAE/100 g dried extract) and lowest TPC in cultivar 236 (0.53 mg GAE/100 g dried extract) (Fig. 3).

DPPH scavenging activity: Similar in the TPC, DPPH scavenging activity of colored-rice seed extracts was higher significantly different ($p<0.05$) than non-colored extracts. However, free radical scavenging activity of all rice seed extracts was significantly lower ($p<0.01$) than the standard antioxidant, BHA. The IC_{50} of rice seed extracts in 12 cultivar numbers; colored (53, 97, 98, 202, 203, 206, 208 and 209) and non-colored (210, 233, 235 and 236) was 0.54-49.75 mg mL^{-1}, compared with IC_{50}= 0.079 mg mL^{-1} for BHA (Table 2).

Total anthocyanin content: Total anthocyanin content of colored-rice seed extracts was 2.10-1045.12 mg malvidin/100 g rice powder). These rice seeds have a

Table 2: Scavenging activity of rice seed extracts in methanol

Cultivars No.	IC_{50} (mg mL^{-1})
53	0.913±0.002
97	1.034±0.001
98	0.535±0.008
202	2.691±0.016
203	7.039±0.002
206	49.746±0.001
208	16.567±0.002
209	24.731±0.001
210	48.644±0.006
233	24.331±0.004
235	24.783±0.003
236	24.710±0.003
BHA	0.079±0.003

Table 3: Total anthocyanin content from colored rice seed extracts

Cultivar No.	Total anthocyanin (mg malvidin/100 g)
53	1045.12±4.421
97	2.10±2.023
98	9.02±0.962
202	9.23±2.383
203	9.02±3.846
206	nd
208	54.34±4.096
209	42.38±17.288

nd: Not detect

characteristic dark purple color. The anthocyanin of cultivar number 53 had the highest value (1045.12 mg malvidin/100 g rice powder), then cultivar numbers 208, 209, 202 (54.34, 42.38, 9.23 mg malvidin/100 g rice powder) (Table 3).

Phytic acid content: Phytic acid content of 12 cultivars of rice seed extracts were evaluated by calibrating with standard curve of phytic acid. A linear standard curve was obtained by plotting the decrease in absorbance at 519 nm against phytate concentration. The range for analysis was from 25 to 250 μg mL^{-1} phytate phosphorus. The value of the correlation coefficient (r = -0.9967) of the calibration curve represented a strong negative relationship between absorbance and concentration of phytate phosphorus. As the value of the absorbance increased, the amount of phytate phosphorus decreased. The total content of phytic acid in rice bran was determined using the linear equation, y = -0.0034x+1.0786. Rice seed extracts in 12 cultivar numbers showed phytic acid content was 2.70±0.0436 to 9.94±0.0055 mg/100 g; cultivar number 236 showed the highest phytic acid content (9.94 mg/100 g), then cultivar numbers 233, 203, 97 (9.61, 9.57, 9.55 mg/100 g) and lowest phytic acid content in cultivar number 202 (2.70 mg/100 g) (Fig. 4).

γ-oryzanol content: The typical HPLC chromatograms of standard γ-oryzanol was shown in Fig. 5 with

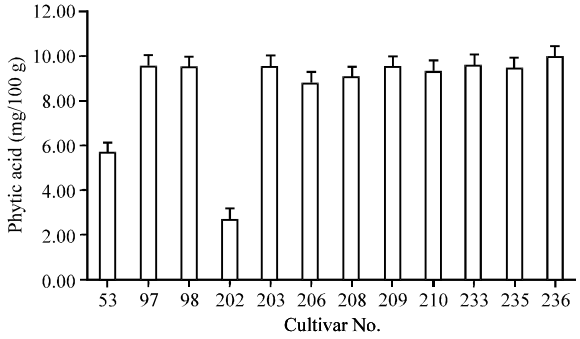

Fig. 4: Phytic acid content of 12 cultivar of Thai rice

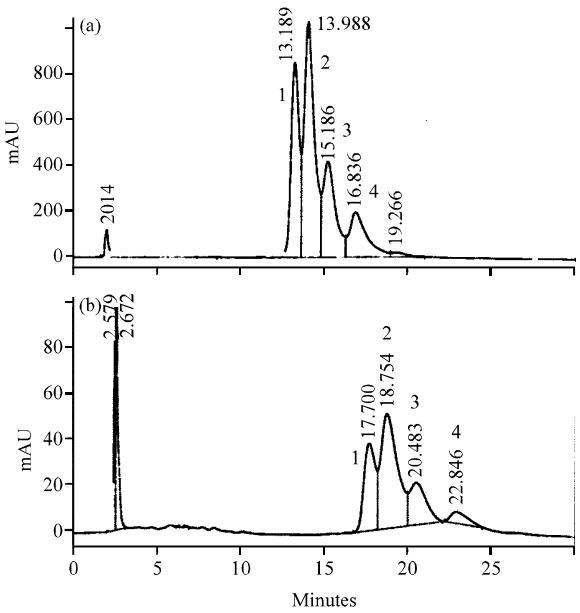

Fig. 5(a-b): High-performance liquid chromatography pattern of oryzanol components (a): Pure oryzanol and (b): Crude rice seed (cultivar number 97) extract in methanol. Peak identities: 1 = cycloartenol ferulate; 2 = 24-methylene cycloartenol ferulate; 3 = campesteryl ferulate; 4 = β-sitosteryl ferulate. Numbers on the peaks represent elution times, in minutes

comparing to previous study (Azrina *et al*., 2008; Krishna *et al*., 2001), there were 4 major isomers detected namely; cycloartenol ferulate, 24-methylene cycloartenol ferulate, campesteryl ferulate and β-sitosteryl ferulate.

γ-oryzanol content of rice bran in 12 cultivar numbers was found in the value of 0 to 44.73 mg mL^{-1} and rice bran cultivar number 97 showed the highest γ-oryzanol content (44.73 mg mL^{-1}), then cultivar numbers 203, 98 (17.10 and 4.31 mg mL^{-1}, respectively) (Fig. 6).

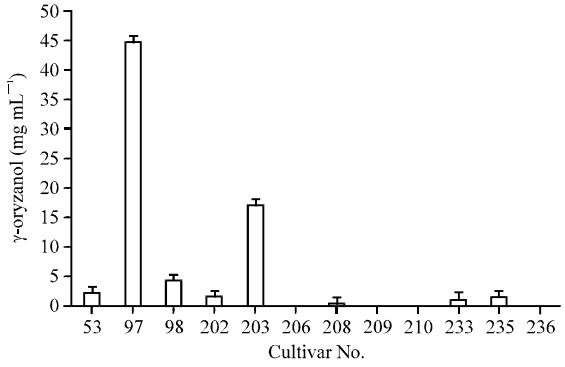

Fig. 6: γ-Oryzanol content of 12 Thai rice cultivars

DISCUSSION

Rice have concentrate of bioactive compounds such as, phenolic compounds, anthocyanin, phytic acid, γ-oryzanol and others bioactive compounds which were not determined in this study, were found in greater amounts in germ, bran in outer layers, but that were milled to white rice that have little bioactive compounds. Colored rice cultivars have demonstrated a higher antioxidant activity than non-colored cultivar due to have a high phenolic compound. Rice used in this study is native cultivars from Surin province which antioxidant activity may also depending on season harvest, topography and environment in around. Solvent extraction of colored and non-colored Thai rice cultivars with various organic solvents; methanolic extracts of those rice seeds produced the highest percent yield in all rice seeds. This is consistent with a previous report (Renuka Devi and Arumughan, 2007) showed that methanol was the most effective extractant. According to Chen and Bergman (2005), the better extractability of phytochemicals such as oryzanol and tocols with methanol compared to hexane could be attributed to the hydroxyl group of the chroman rings of vitamin E homologs and of the benzene ring of ferulate part of oryzanol that might make these compounds more extractable with polar solvents. Besides, relatively higher polarity further facilitates ferulic acid to remain in the defatted rice bran while the hexane extracted rice bran oil does not contain ferulic acid, adds further credence to this assumption.

Total phenolic content of colored-rice seed extracts were significantly different from non-colored extracts ($p<0.05$). This is consistency to Oki *et al*. (2002) and Clifford (2002) that reported that pigmented rice composed of high content of phenolic compounds. This is in agreement with Vichapong *et al*. (2010) that showed the content of phenolic compounds; total flavonoids and antioxidant activity detected in pigment rice and brown from were higher than non-pigment rice and polished from as well. Another research discovered that rice bran contains a phenolic content (2.51-3.59 mg g^{-1}) higher than wheat bran (Iqbal *et al*., 2005).

Phenolic compounds were antioxidants that have potential to reduce risk of several diseases such as, inhibiting platelet aggregation (Daniel *et al*., 1999), reducing the risk of coronary heart disease and cancer (Martinez-Valverde *et al*., 2000). In addition, it has also reported that phenolic compounds, from methanolic extracts of rice husk, exhibit high antioxidant activity (Jeon *et al*., 2006). All rice seed extracts showed significant antioxidant activity in a concentration-dependent manner through the scavenging of DPPH radicals. DPPH assay is the commonly used method for the evaluation of free radical scavenging activity (Chew *et al*., 2008). The method is simple, polarity-independent, very rapid and reproducible (Tian *et al*., 2004). In addition, no expensive reagents or sophisticated instruments are required. This experiment, scavenging activity of color and non-color rice crude extracts showed consistency with the study of Hu *et al*. (2003) which showed a significant positive correlation between in the black rice extract and their antioxidant activity. In this analysis, the possible mechanisms suggest that the radical-scavenging effects of rice seed extracts might be due to the hydroxyl groups in the antioxidants of the extracts. For the colored extracts showed a higher scavenging activity because colored extracts had the higher level of anthocyanins which a potent reducing agent and possesses strong radical scavenging activity (Nam *et al*., 2006; Ryu *et al*., 1998). Because pigmented rice shows anticipated the greater functional dietary potential than that of the white rice (Nam *et al*., 2006). Total anthocyanin content was verified in colored- rice seed extract of cultivar numbers 53, 97, 98, 202, 203, 206, 208 and 209 which are expressed in terms of malvidin-3-O-glu (mg malvidin/100 g rice powder). The results showed that total anthocyanin in the extracts were 2.10-1045.12 mg malvidin/100 g rice powder). Additionally, many studies have been reported that black rice contains rich of anthocyanin and other polyphenolic compound much more abundantly than white rice (Zhang *et al*., 2006; Ryu *et al*., 1998).

The present study show that there was no statistically significantly difference ($p>0.05$) between colored and non-colored of rice seed extracts in phytic acid content. Although, non-colored-rice seed cultivar

number 236 showed the highest content (9.94 mg/100 g). However, highest content of phytic acid in this study exhibited lower than the other studied such as the studied conducted by Liu (2007) reported that phytic acid content in japonica rice cultivars ranged from 0.685 to 1.03% which was collected from different areas of China.

The determination of γ-oryzanol content by HPLC, rice bran cultivar number 97 showed the highest content (44.73 mg mL^{-1}). γ-oryzanol content in colored of rice seed extracts was statistically significant higher ($p<0.05$) than those non-colored. Through the HPLC methods, it has been clearly established that γ-oryzanol is a mixture of several components (Diack and Saska, 1994; Norton, 1995). However, depending on the chromatographic approach, different numbers of individual components have been identified. The present study is successfully identified only 4 major components of oryzanol (cycloartenol ferulate, 24-methylene cycloartenol ferulate, campesteryl ferulate and β-sitosteryl ferulate) in colored and non-colored rice seed extracts. This finding is important as γ-oryzanol is a potent and high value antioxidant compound and locally produced bran could be the source of this compound.

These results suggested that the methanolic rice seed extracts, especially colored cultivars contained high phenolic and anthocyanins, resulting in potent scavenging activity which may be used as preliminary information for further investigation. Furthermore, the methanolic rice seed extracts represented possible source of γ-oryzanol which is a component of plants cell walls, acts as an efficient natural antioxidant in the oil. Therefore, these native Thai rice seed extracts may have health benefit for consumption and should be developed further to be used in food industry, cosmetic, health products, pharmaceutical and medicinal applications. Future work may be isolation and structural characterization of the bioactive compounds and biological (*in vivo*) studies to investigate mechanism of these extracts.

ACKNOWLEDGMENTS

Financial support from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education is gratefully acknowledged. The authors wish to thanks Division of Research Facilitation and Dissemination, Mahasarakham University for financial support and the Surin Rice Research Center, Surin province, Thailand for providing the rice seed cultivars.

REFERENCES

Anderson, J.W., 2004. Whole grains and coronary heart disease: The whole kernel of truth. Am. J. Clin. Nutr., 80: 1459-1460.

Anli, R.E. and N. Vural, 2009. Antioxidant phenolic substances of Turkish red wines from different wine regions. Molecules, 14: 289-297.

Azrina, A., I. Maznah and A.H. Azizah, 2008. Extraction and determination of oryzanol in rice bran of mixed herbarium UKMB; AZ 6807: MR 185, AZ 6808: MR 211, AZ6809: MR 29. ASEAN Food J., 15: 89-96.

Bonoli, M., E. Marconi and M.F. Caboni, 2004. Free and bound phenolic compounds in barley (*Hordeum vulgare* L.) flours: Evaluation of the extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry. J. Chromatogr. A, 1057: 1-12.

Butsat, S. and S. Siriamornpun, 2010. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. Food Chem., 119: 606-613.

Chen, M.H. and C.J. Bergman, 2005. A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. J. Food Comp. Anal., 18: 319-331.

Chen, P.N., S.C. Chu, H.L. Chiou, C.L. Chiang, S.F. Yang and Y.S. Hsieh, 2005. Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis *in vitro* and suppress tumor growth *in vivo*. Nutr. Cancer, 53: 232-243.

Chew, Y.L., Y.Y. Lim, M. Omar and K.S. Khoo, 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci. Technol., 41: 1067-1072.

Cheynier, V., 2005. Polyphenols in foods are more complex than often thought. Am. J. Clin. Nutr., 81: 223S-229S.

Choi, Y., H.S. Jeong and J. Lee, 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem., 103: 130-138.

Clifford, M.N., 2002. Anthocyanins nature, occurrence and dietary burden. J. Food Sci. Agric., 80: 1063-1072.

Curin, Y. and R. Andriantsitohaina, 2005. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacol. Rep., 57: 97-107.

Daniel, O., M.S. Meier, J. Schlatter and P. Frischknecht, 1999. Selected phenolic compounds in cultivated plants: Ecologic functions, health implications and modulation by pesticides. Environ. Health Perspect., 107: 109-114.

Diack, M. and M. Saska, 1994. Separation of vitamin E and γ-oryzanols from rice bran by normal-phase chromatography. J. Am. Oil Chem. Soc., 71: 1211-1217.

Dost, K. and O. Tokul, 2006. Determination of phytic acid in wheat and wheat products by reverse phase high performance liquid chromatography. Anal. Chim. Acta, 558: 22-27.

Febles, C.I., A. Arias, A. Hardisson, C. Rodriquez-Alvarez and A. Sierra, 2002. Phytic acid level in wheat flours. J. Cereal Sci., 36: 19-23.

Graf, E. and J.W. Eaton, 1993. Suppression of colonic cancer by dietary phytic acid. Nutr. Cancer, 19: 11-19.

Hosseinian, F.S., W. Li and T. Beta, 2008. Measurement of anthocyanins and other phytochemicals in purple wheat. Food Chem., 109: 916-924.

Hu, C., J. Zawistowski, W. Ling and D.D. Kitts, 2003. Black rice (*Oryza sativa* L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J. Agric. Food Chem, 51: 5271-5277.

Iqbal, S., M.I. Bhanger and F. Anwar, 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem., 93: 265-272.

Jakobs, S., D. Fridrich, S. Hofem, G. Pahlke and G. Eisenbrand, 2006. Natural flavonoids are potent inhibitors of glycogen phosphorylase. Mol. Nutr. Food Res., 50: 52-57.

Jeon, K.I., E.J. Park, H.R. Park, Y.J. Jeon, S.H. Cha and S.C. Lee, 2006. Antioxidant activity of far-infrared radiated rice hull extracts on reactive oxygen species scavenging and oxidative DNA damage in human lymphocytes. J. Med. Food, 9: 42-48.

Kano, M., T. Takayanagi, K. Harada, K. Makino and F. Ishikawa, 2005. Antioxidant activity of anthocyanins from purple sweet potato, *Ipomoera batatas* cultivar Ayamurasaki. Biosci. Biotechnol. Biochem., 69: 979-988.

Kim, D.O., H.J. Heo, Y.J. Kim, H.S. Yang and C.Y. Lee, 2005. Sweet and sour cherry phenolics and their protective effects on neuronal cells. J. Agric. Food Chem., 53: 9921-9927.

Ko, K.M. and D.V. Godin, 1990. Ferric ion-induced lipid peroxidation in erythrocyte membranes: Effects of phytic acid and butylated hydroxytoluene. Mol. Cell. Biochem., 95: 125-131.

Krishna, A.G.G., S. Khatoon, P.M. Shiela, C.V. Sarmandal, T.N. Indira and A. Mishra, 2001. Effect of refining of crude rice bran oil on the retention of oryzanol in the refined oil. J. Am. Oil Chem. Soc., 78: 127-131.

Liu, R.H., 2007. Whole grain phytochemical and health. J. Cereal Sci., 46: 207-219.

Liyana-Pathirana, C.M. and F. Shahidi, 2007. Antioxidant and free radical scavenging activities of whole wheat and milling fractions. Food Chem., 101: 1151-1157.

Martinez-Valverde, I., M.J. Periago and G. Ros, 2000. Nutritional importance of phenolic compounds in the diet. Arch. Latinoam. Nutr., 50: 5-18.

Montonen, J., P. Knekt, R. Jarvinen, A. Aromaa and A. Reunanen, 2003. Whole-grain and fiber intake and the incidence of type 2 diabetes. Am. J. Clin. Nutr., 77: 622-629.

Nam, S.H., S.P. Choi, M.Y. Kang, H.J. Koh, N. Kozukue and M. Friedman, 2005. Bran extracts from pigmented rice seeds inhibit tumor promotion in lymphoblastoid B cells by phorbol ester. Food Chem. Toxicol., 43: 741-745.

Nam, S.H., S.P. Choi, M.Y. Kang, H.J. Koh, N. Kozukue and M. Friedman, 2006. Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chem., 4: 613-620.

Norton, R.A., 1995. Quantitation of steryl ferulate and p-coumarate esters from corn and rice. Lipids, 30: 269-274.

Oki, T., M. Masuda, M. Kobayashi, Y. Nishiba, S. Furuta, I. Suda and T. Sato, 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. J. Agric. Food Chem., 50: 7524-7529.

Patearroyo, M.A., A. Fernandez-Quintela and C. Cid, 1995. Antinutritive substances in plant-derived foods: Its meaning in human nutrition. Alimentaria, 6: 115-120.

Proestos, C., A. Bakogianis, C. Psarianos, A.A. Koutinas, M. Kanellaki and M. Komaitis, 2005. High performance liquid chromatography analysis of phenolic substances in Greek wines. Food Control, 16: 319-323.

Puangpronpitag, D., P. Areejitranusorn, P. Boonsiri, M. Suttajit and P. Yongvanit, 2008. Antioxidant activities of polyphenolic compounds isolated from *Antidesma thwaitesianum* Mull. Arg. seeds and marcs. J. Food Sci., 73: C648-C653.

Ramezanzadeh, F.M., R.M. Rao, P. Witoon, W.E. Marshall and M. Windhauser, 2000. Effects of microwave heat, packaging and storage temperature on fatty acid and proximate compositions in rice bran. J. Agric. Food Chem., 48: 464-467.

Renuka Devi, R. and C. Arumughan, 2007. Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. Bioresour. Technol., 98: 3037-3043.

Ryu, S.N., S.Z. Park and C.T. Ho, 1998. High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. J. Food Drug Anal., 6: 729-736.

Saenjum, C., C. Chaiyasut, S. Chansakaow, M. Suttajit and B. Sirithunyalug, 2012. Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. J. Med. Plants Res., 6: 1070-1077.

Schatzkin, A., T. Mouw, Y. Park, A.F. Subar and V. Kipnis *et al*., 2007. Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and health study. Am. J. Clin. Nutr., 85: 1353-1360.

Shirakawa, H., T. Koseki, K. Ohinata, K. Hashizume and M. Komai, 2006. Rice bran fractions improve blood pressure, lipid profile and glucose metabolism in stroke-prone spontaneously hypertensive rats. J. Agric. Food Chem., 54: 1914-1920.

Stoclet, J.C., T. Chataigneau, M. Ndiaye, M.H. Oak, J. El Bedoui, M. Chataigneau and V.B. Schini-Kerth, 2004. Vascular protection by dietary polyphenols. Eur. J. Pharmacol., 500: 299-313.

Tai-Sun, S. and J.S. Godber, 1994. Isolation of four tocopherols and four tocotrienols from a variety of natural sources by semi-preparative high-performance liquid chromatography. J. Chromatogr. A, 678: 49-58.

Tian, S., K. Nakamura and H. Kayahara, 2004. Analysis of phenolic compounds in white rice brown rice and germinated brown rice. J. Agric. Food Chem., 52: 4808-4813.

Vichapong, J., M. Sookserm, V. Srijesdaruk, P. Swatsitang and S. Srijaranai, 2010. High performance liquid chromatographic analysis of phenolic compounds and their antioxidant activities in rice varieties. LWT-Food Sci. Technol., 43: 1325-1330.

Xu, Z. and J.S. Godber, 1999. Purification and identification of components of γ-oryzanol in rice bran oil. J. Agric. Food Chem., 47: 2724-2728.

Xu, Z., N. Hua and J.S. Godber, 2001. Antioxidant activity of tocopherols, tocotrienols and gamma-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis(2-methylpropionamidine) dihydrochloride. J. Agric. Food Chem., 49: 2077-2081.

Zhai, C.K., C.M. Lu, X.Q. Zhang, G.J. Sun and K.J. Lorenz, 2001. Comparative study on nutritional value of Chinese and North American wild rice. J. Food Compos. Anal., 14: 371-382.

Zhang, M.W., B.J. Guo, R.F. Zhang, J.W. Chi and Z.C. Wei *et al*., 2006. Separation, purification and identification of antioxidant compositions in black rice. Agric. Sci. China, 5: 431-440.

Zhou, R.J. and J.W. Jr. Erdman 1995. Phytic acid in health and disease. Crit. Rev. Food Sci. Nutr., 35: 495-508.