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Full Length Research Paper

Phenolic content and antioxidant activities in red unpolished Thai rice prevents oxidative stress in rats

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Radicals cause cellular damage and eventually progress to chronic diseases. Phenolic compounds play a crucial role in radicals scavenging. In this study, we investigated total anti-oxidant activities, total phenolic content and profiles in color strains of unpolished Thai rice. The level of malondialdehyde was also assayed in rats that consumed unpolished Thai rice. Red color strain had the highest antioxidant activities in all tests. It was also showed the highest phenolic content. Interestingly, total phenolic content was strongly correlated with all anti-oxidant in the methods used: 1,1-diphenyl-2-picrylhydrazyl ($r = 0.958$, $p < 0.01$), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($r = 0.966$, $p < 0.01$) and ferric reducing antioxidant power ($r = 0.992$, $p < 0.01$). Malondialdehyde level in high and low dose treated groups were significantly lower than that in the control group of rats that consumed unpolished Thai rice. Red color of unpolished Thai rice, source of phenolic compounds, may play a crucial role in oxidative stress prevention.

Key words: Unpolished Thai rice, phenolic compounds, antioxidant, oxidative stress.

INTRODUCTION

Pro-oxidants and anti-oxidants maintain a ratio of biomolecular function (Valko et al., 2006). The shift in this ratio towards pro-oxidants gives rise to oxidative stress (Halliwell and Whiteman, 2004). An imbalance between reactive oxygen species and biological anti-oxidant plays a role in oxidative stress (Sies, 1997). Excess of radicals was implicated on lipid, protein, and DNA damage (De and Van, 2004; Finkel and Holbrook, 2000). Oxidative stress eventually caused cellular damage (Halliwell, 1987) and developed chronic diseases (Valko et al., 2006). In previous studies reported that the phytochemical nutrition including phenolic compounds, carotenoids, ascorbic acid, *α*-tocopherol play a crucial role in the radical scavenging activities (Azzi and Stocker, 2000; Kim et al., 2002; Polyakov et al., 2001; Suwannalert et al., 2007; Velioglu et al., 1998; Xu et al., 2001).

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Unpolished rice is the whole grain of rice that the germ and outer layers enclosing the bran have not been removed (Smith and Dilday, 2003). It did not only contain carbohydrate and energy source but also source for phytochemicals that affect the radical scavenging activities (Adom and Liu, 2002; Lloyd et al., 2000). Unpolished rice also contained ferulic, *p*-coumaric, hydroxycinnamic, hydroxybenzoic, protocatechuic, vanillic, syringic, chlorogenic, caffeic, and sinapinic acids (Adom and Liu, 2002; Tian et al., 2004; Zhou et al., 2004). Phenolic compounds were reported to exert an anti-oxidative stress effect (Lima et al., 2006; Montilla et al., 2006).

Up to now, the relationship between color strains of unpolished Thai rice and radical scavenging activity has not been reported. In this study, 9 strains of unpolished Thai rice were used to investigate the antioxidant activities by standard methods: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP). The phenolic profiles and phenolic contents of 9 samples were assayed. In

addition, the level of serum oxidative stress marker, malondialdehyde (MDA) was investigated in rats consuming the unpolished Thai rice.

MATERIALS AND METHODS

Unpolished Thai rice

Nine color strains of unpolished Thai rice were obtained from the "Organic Project Sukhothai Airport, Sukhothai, Thailand". Unpolished rice samples could be defined into three groups: 1). Red color strains: found in Hawm Deang Sukhothai 1 and Red Rose. 2). Black color stains: found in Luem- Phua, Klam, Hawm Nil, Hawm Dum Sukhothai 2, and Black Rose 3). White color strain: found in RD 2 and Khao Dawk Mali 105. All samples were grounded by an electric blender, and extracted with 95% ethanol, 1:4 (w/v) and shaked at 150 rpm, 24 h. The mixed extract was centrifuged at 3000 rpm for 15 min and filtered by polytetrafluoroethylene (PTFE) filter nylon 0.45 µm before use.

1,1-Diphenyl-2-picrylhydrazyl (DPPH)

The DPPH radical scavenging activity was determined by modified method of Gamez et al., 1998. The extract samples 20 µl were mixed with 167 µM of DPPH in 180 µl ethanol. The mixture was incubated at 37°C for 30 min. Absorbance of the mixed reaction was measured by using a Multimode Detector (Beckman, DTX 880, Australia) at 540 nm. DPPH radical scavenging activity of sample was expressed as L-ascorbic acid (Vitamin C) equivalent antioxidant capacity (VEAC).

2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

The working ABTS solution was freshly prepared by mixing 7 mM ABTS and 2.4 mM potassium persulfate in equal quantities and allowed to react for 12 h at room temperature in the dark condition. The mixed solution was diluted with ethanol to obtain an absorbance of 0.95 ± 0.01 units at 734 nm. The extracted sample 70 µl were reacted with 630 µl of the mixed solution. After 30 min, the absorbance was measured at 734 nm spectrophotometrically (Shimadzu, UV-Vis 2450, Japan). Trolox was used as reference.

Ferric reducing antioxidant power (FRAP)

FRAP reagent was prepared by mixing solution of 40 mM 2,4,6-tripyridyl-s-triazine (TPTZ solution), 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$), and 25 ml acetate buffer (pH 3.6). Briefly, 20 µl of sample or blank were added to 180 µl freshly prepared FRAP reagent and incubated for 10 min in the dark. The absorbance of ferrous complex, the end product of reaction was determined at 595 nm by using a Multimode Detector (Beckman, DTX 880, Australia). Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was used as a reference.

Phenolic profiles and total phenolic content

Phenolic profiles of 9 unpolished Thai rice were determined by the spectophotometric scanning analysis (Shimadzu, UV-Vis 2450, Japan). Total phenolic content was assayed by standard method that modified from Ferreira et al., 2004 (Ferreira et al., 2004). The Folin-Denis reagent was prepared by mixing 50 g of sodium tungstate dihydrate, 10 g of sodium molibdate, 25 ml of phosphoric acid and 400 ml of water. Then, the reaction was refluxed for 3 h and made up to 500 ml with water and kept in the cool temperature for stock solution. Sample 10 µl was added to 790 µl distilled water and then mixed thoroughly with 50 µl of Folin-Denis reagent. Afterthat, 150 µl of 7.5% (w/v) sodium carbonate was added. The mixed reaction was incubated for 30 min and measured at 725 nm by a spectrophotometrically (Shimadzu, UV-Vis 2450, Japan). The concentration of total phenolic contents was expressed as milligrams of gallic acid equivalents (GAE) per gram of unpolished rice sample.

Animal experiment

Sprague–Dawley rats from National Laboratory Animal Centre, Mahidol University, Nakhon Pathom, Thailand, weighing 200 - 300 g at the beginning of the experiment were used. The experimental procedures were approved by the Animal Ethics Committee of Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand in protocol number 31/2551.

The rats consuming the mixture of unpolished Thai rice with commercial standard of animal feeding in 0, 10 and 70% for control, low dose and high dose groups. After two months, the blood samples were kept at -80°C until use.

Serum malondialdehyde

Malonaldehyde bis-dimethyl acetal (1,1,3,3-tetramethoxy propane: TMP) was used as a reference standard. One hundred microliters of serum or standard solution were added to 50 µl of 7.2% butyrated hydroxytoluene (BHT). Next, 750 µl of 0.44 M phosphoric acid, 250 µl thiobarbituric acid (TBA) and 200 µl 8.1% SDS were added. The mixture was incubated at 90°C for 30 min and then cooled down at 4°C for 10 min. Afterthat, 3 ml of n-butanol was added and centrifuged at 3,000 rpm for 15 min. The fluorescence intensity of supernatant was measured by a spectrofluorometer (Jasco, FP-777, Japan) at 532 nm excitation and 552 nm emission.

Statistical analysis

All data were presented as means ± SD. One-way analysis of variance (ANOVA), LSD tests was carried out to test any significant differences between treatments. Pearson's correlation coefficient (r) was also applied to establish specific correlations. A $p < 0.05$ was considered as statistically significant.

RESULTS

Hawm Deang Sukhothai 1 strain showed the highest antioxidant activities in DPPH, ABTS, and FRAP. Strains of Hawm Deang Sukhothai 1, Red Rose and Luem Phua exhibited high activity in DPPH test: $3.51 \pm 5.88(10^{-2})$, $1.75 \pm 7.18(10^{-3})$ and $1.41 \pm 1.76(10^{-2})$ VEAC, respectively (Table 1). Similar pattern of total anti-oxidant activity was presented in ABTS test: Hawm Deang Sukhothai 1 [$5.10 \pm 3.62(10^{-2})$ TEAC], Red Rose [$3.18 \pm 1.81(10^{-2})$ TEAC] and Luem Phua [$2.78 \pm 7.89(10^{-2})$ TEAC] (Table 1). In addition, strain of Hawm Deang Sukhothai 1 was also shown highest antioxidant activity in FRAP method, $23.13 \pm 4.21(10^{-1})$ µmol (Table 1).

High level of phenolic content in Hawm Deang Sukhothai 1, Luem Phua and Red Rose strains were

Table 1. Total phenolic content and antioxidant activities in color strains of unpolished Thai rice.

Rice	Color coated seed	Total phenolic (GAE) (mg gallic acid /g sample)	Antioxidant capacity		
			DPPH (VEAC) (mg ascorbic acid /g sample)	ABTS (TEAC) (mg trolox /g sample)	FRAP (µmol Fe^{2+} /g sample)
Hawm Deang Sukhothai 1	Red	$2.01 \pm 2.57(10^{-2})$	$3.51 \pm 5.88(10^{-2})$	$5.10 \pm 3.62(10^{-2})$	$23.13 \pm 4.21(10^{-1})$
Red Rose	Red	$1.37 \pm 3.64(10^{-2})$	$1.75 \pm 7.18(10^{-3})$	$3.18 \pm 1.81(10^{-2})$	$15.01 \pm 1.43(10^{-1})$
Luem Phua	Black	$1.54 \pm 2.43(10^{-2})$	$1.41 \pm 1.76(10^{-2})$	$2.78 \pm 7.89(10^{-2})$	$17.77 \pm 2.27(10^{-1})$
Klam	Black	$0.94 \pm 1.38(10^{-2})$	$0.54 \pm 6.00(10^{-2})$	$1.85 \pm 1.05(10^{-2})$	$11.53 \pm 2.00(10^{-1})$
Hawm Dum Sukhothai 2	Black	$0.85 \pm 2.58(10^{-2})$	$0.38 \pm 5.18(10^{-3})$	$0.45 \pm 1.05(10^{-2})$	$10.18 \pm 1.68(10^{-1})$
Hawm Nin	Black	$0.82 \pm 1.63(10^{-2})$	$0.28 \pm 1.14(10^{-2})$	$0.42 \pm 1.46(10^{-3})$	$8.37 \pm 3.26(10^{-1})$
Black Rose	Black	$0.80 \pm 2.32(10^{-2})$	$0.25 \pm 1.57(10^{-2})$	$0.33 \pm 1.09(10^{-3})$	$7.69 \pm 3.38(10^{-1})$
Khao Dawk Mali 105	White	$0.58 \pm 2.49(10^{-2})$	$0.13 \pm 3.10(10^{-3})$	$0.17 \pm 2.35(10^{-3})$	$6.58 \pm 2.29(10^{-1})$
RD 2	White	$0.52 \pm 5.65(10^{-3})$	$0.13 \pm 4.64(10^{-3})$	$0.18 \pm 5.43(10^{-4})$	$6.24 \pm 4.50(10^{-1})$

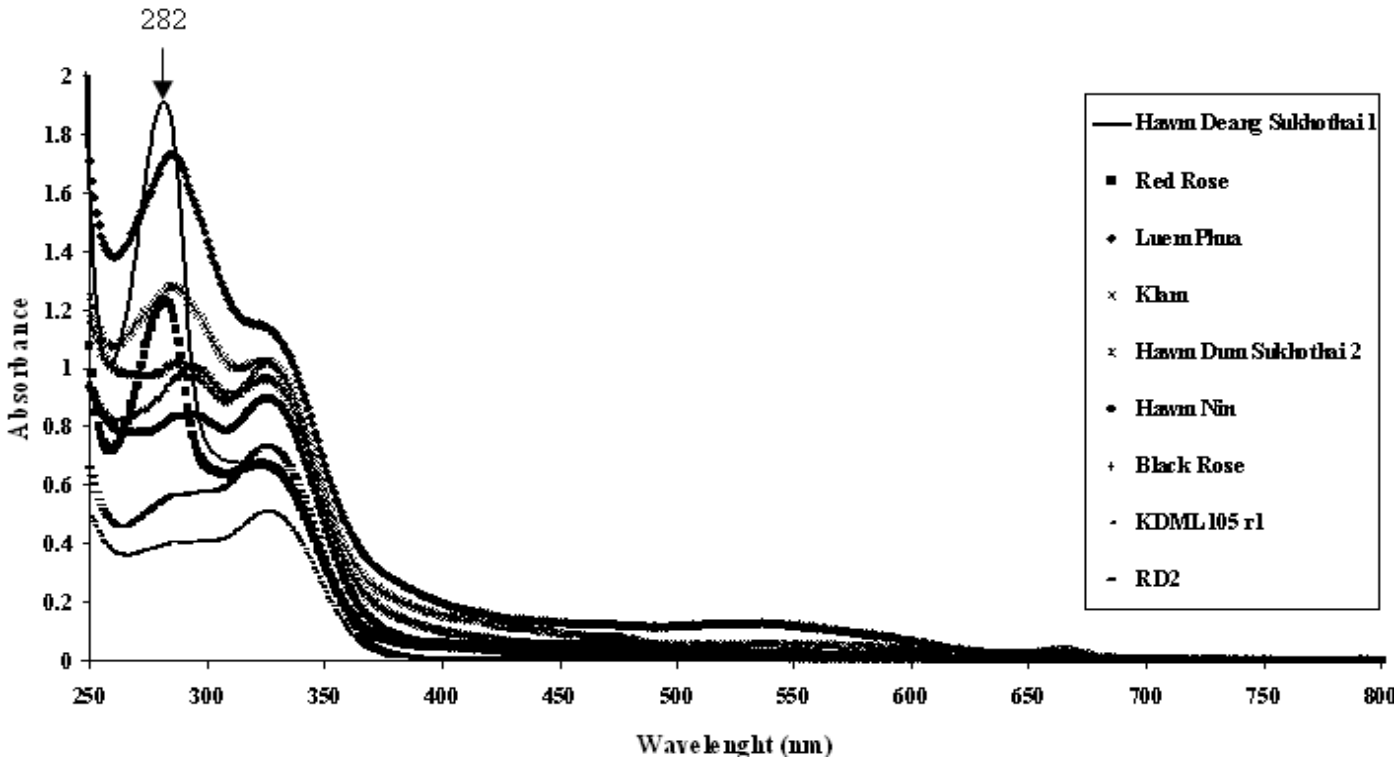
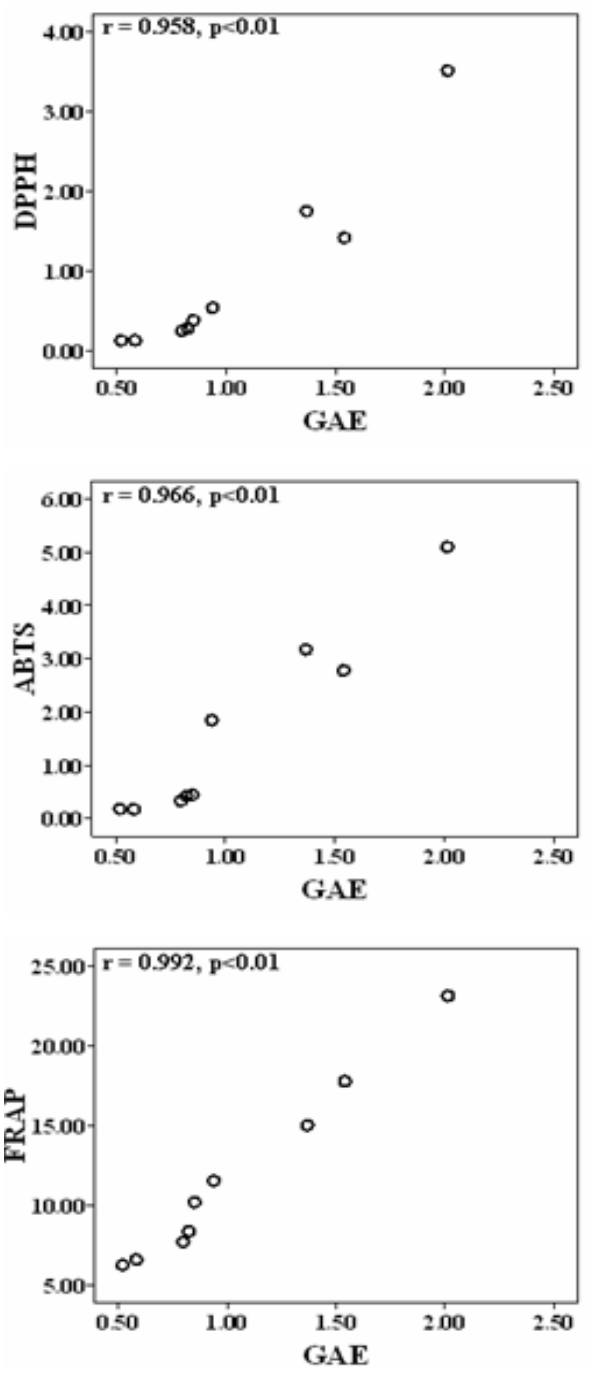


Figure 1. Phenolic profiles in color strains of unpolished Thai rice.

found to be $2.01 \pm 2.57(10^{-2})$, $1.54 \pm 2.43(10^{-2})$ and $1.37 \pm 3.64(10^{-2})$ GAEs/g of sample, respectively (Table 1). We found that red and black colors of unpolished Thai rice contained high level of phenolic profiles especially, in Hawm Deang Sukhothai 1 that showed the highest level of phenolic absorption peak at 282 nm (Figure 1).

Interestingly, the level of total phenolic content in unpolished Thai rice was strongly correlated with all anti-oxidant found in the standard methods: DPPH ($r = 0.958$, $p < 0.01$) (Figure 2a), ABTS ($r = 0.966$, $p < 0.01$) (Figure 2b) and FRAP methods ($r = 0.992$, $p < 0.01$) (Figure 2c).

The rats consumed unpolished Thai rice, serum MDA level in the control, low dose and high dose groups were 137.56 ± 14.80, 114.07 ± 14.02 and 71.23 ± 18.36 nM, respectively. The level in the high dose group was lower than those in the low dose and the control groups (Figure 3). The mean values of MDA in high dose and low dose groups were significantly lower than that in the control at $p < 0.01$ and $p < 0.01$, respectively. Moreover, MDA level in the high dose group was also significant lower than



2a. Correlation between total phenolic content and DPPH

2b. Correlation between total phenolic content and ABTS

2c. Correlation between total phenolic content and FRAP

Figure 2. Correlation between total phenolic content and antioxidant activities in unpolished Thai rice.

that in the low dose group ($p < 0.01$) (Figure 3).

DISCUSSION

Free radicals can cause cellular damage and eventually lead to chronic diseases (Halliwell, 1987; Sies, 1997). Many studies found that phenolics compounds play a crucial role in oxidative scavenging (Mateos et al., 2005; Olas et al., 2003). Unpolished rice is the main sources of phenolic compounds (Tian et al., 2004). In the present study, we found that the red color strain of Hawm Deang Sukhothai 1 had the highest antioxidant activities in all standard method tested: DPPH, ABTS, and FRAP (Table 1).

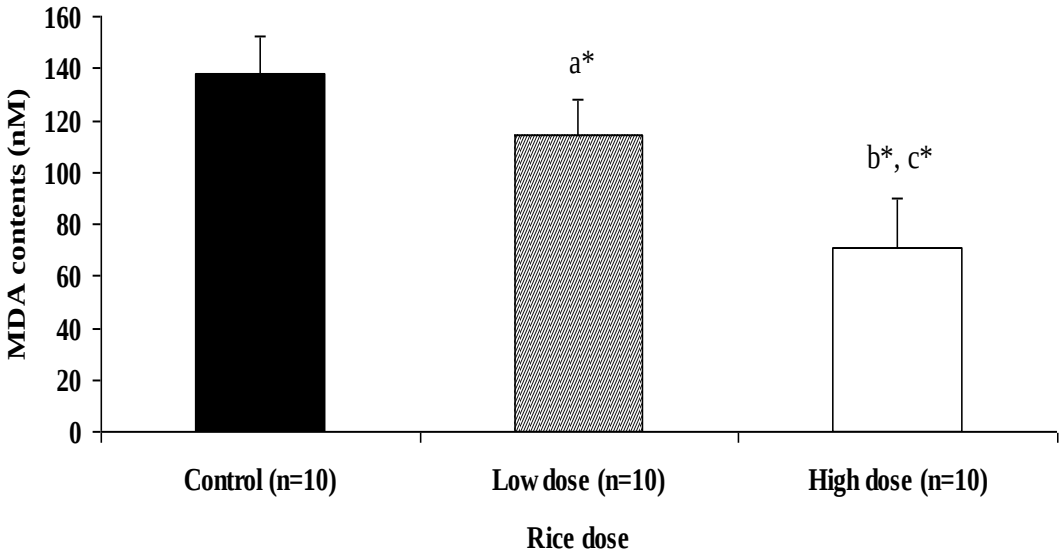


Figure 3. MDA level in rats consume unpolished Thai rice, hawm deang Sukhothai 1.
a = p-value of low dose and control groups, b=*p*-value of high dose and control groups, c = p-value of high dose and low dose groups, * = statistically significant at $p < 0.01$

Additionally, the red color strain of Hawm Deang Sukhothai 1 also showed the highest level of phenolic content (Table 1). Red color in plants is the high source of phenolic compounds (Gould et al., 2009; Yawadio and Morita, 2007) and these compounds has a crucial role in anti-oxidant activities (Kong and Lee, 2010). Moreover, phenolic compounds have been reported for the prevention of aging, cardiovascular disease, diabetes, and cancer (Kris-Etherton et al., 2002; Kwon et al., 2008; Mellen et al., 2008).

The red strain of Hawm Deang Sukhothai 1 showed the highest level of phenolic absorption peak at 282 nm (Figure 1). This area mainly indicated the Phenolics (Gould et al., 2009). The high level of phenolic compound was also presented in the strains of Red Rose and Luem Phua which coded with the red and black colors (Figure 1). Red and black colors of the unpolished rice were represented high phenolic sources (Kong and Lee, 2010). Interestingly, total phenolic content in unpolished Thai rice was strongly correlated with all anti-oxidant results in the standard methods used in this study (Figure 2a-c).

MDA is a marker of oxidative stress (Mateos et al., 2005). Previous study reported that anti-oxidative stress was inversely related with MDA level (Ahmad et al., 2008; Suwannalert et al., 2007). MDA level in the high dose group was lower than those of both low dose and control groups in rats consuming red color, Hawm Deang Sukhothai 1 (Figure 3). In addition, mean value of MDA was decreased in a dose dependent manner with high percentage of red color unpolished rice. These results may indicate the red color strain of unpolished Thai rice, a high source of phenolic compounds, plays a crucial role in the oxidative stress prevention.

Conclusion

The red color of unpolished Thai rice was the high source of phenolic compounds and potent in antioxidant activities. It plays a beneficial role in oxidative stress prevention.

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