The effect of extraction conditions on antioxidant activities and total phenolic contents of different processed Thai Jasmine rice

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Abstract

Germination and parboiling process are techniques for enhancing nutrients and phytochemicals in rice. The objective of this research was to optimize extraction conditions (ethanol concentrations, extraction temperatures and extraction times) in relatedness to antioxidant activities and total phenolic contents (TPCs) using rice samples that passed through different processes. According to the results, rice extracted with 40% (v/v) aqueous ethanol under 50°C of extraction temperature exhibited the highest antioxidant activities and TPCs. However, varied extraction times (1–16 hours) could not differentiate antioxidant activities and TPCs. Under the optimized extraction conditions, parboiled germinated brown rice significantly exhibited the highest TPC (72±2 mg GAE/100 g dry weight) and antioxidant activities measured by ORAC (3343±137 µmole TE/100 g dry weight), DPPH radical inhibition (71% inhibition) and FRAP (420±41 µmole TE/100 g dry weight) assays, followed by those of germinated brown rice, brown rice and white rice, respectively. These results suggested that bioactive compounds and phenolics could be lost due to rice polishing process, while significant amount of nutrients were promoted during parboiling process.

Introduction

Rice (*Oryza sativa*) is a plant developed as food that has become staple crop for supply over half of the world's population, especially in Asia. Almost all population consumes rice in a form of milled rice or white rice. However, bran and germ layer are significant sources of bioactive compounds such as lipids, proteins, minerals, vitamins, phytin and lectin (Orthoefer, 2005), which are normally removed during milling process. Thus, many techniques are developed to produce value–added rice such as partial milling, parboiling and germination. Unmilling or partial milling process is able to maintain bran and germ layer, thus more nutrients are remained in brown rice than in milling white rice. Likewise, germination produces chemicals and physicals modification with enzyme being activated to create hydrolyzed bioactive compounds that may possess bio–functional activity (Osman, 2007). This process induces an increase in γ–aminobutyric acid (GABA), γ–oryzanol, dietary fibre, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium and zinc (Xu *et al*., 2001). Moreover, clinical study had confirmed that consumption of brown or pregerminated brown rice could decrease fasting blood glucose, total cholesterol and triglyceride level (Hsu *et al*., 2008; Ho *et al*., 2012). Parboiling process is hydrothermal reaction that requires steaming and drying paddy rice before dehulling. This process causes starch gelatinization, resulting in prevention of broken head of paddy rice. Thus, parboiling process is capable of maintaining nutrients in bran layer (mainly soluble vitamin and minerals), because such nutrients can transfer into rice endosperm (Luh, 1991). Even though many researches have been reported antioxidant capacities and TPCs of white rice, brown rice, germinated brown rice and parboiled brown rice, information regarding antioxidant activities and TPCs of parboiled germinated brown rice is currently unavailable. Thus, the objective of this study was to optimize extraction conditions in relatedness to antioxidant activities and TPCs of parboiled germinated brown rice in comparison to its counterpart white, brown and germinated brown rice.

Materials and Methods

Rice samples

Raw Thai Jasmine parboiled germinated brown rice (PGBR) and its counterpart germinated brown rice (GBR), brown rice (BR) and white rice (WR) were obtained from RCK Agri Marketing Co., Ltd. (Thailand). Parboiling process of germinated brown

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rice consists of two steps; germination process of BR with husk and then dehusking, which was prepared as follows. Rice paddy (80 kg) was soaked in water twice its weight for 18 hours. Water was then changed every 4 hours in order to prevent the increase in temperature and also to prevent the foul smell that may occur during the process of germination. The soaking process was continued until the moisture content of paddy increased by 30%, followed by separation of paddy from water. This steeped paddy was germinated for 42-48 hours, followed by steaming for 60 minutes in vacuum environment. Parboiled rice was then dried at 70°C–75°C for 2 hours in vacuum environment. In order to reduce moisture content, PGBR was left to dry in a hot air oven at 40°C until the moisture content was reduced to 13%. Finally, rice sample was left for 7 days in order to distribute the moisture evenly throughout the rice grain prior to dehusking. The samples were freeze dried before being grounded into fine powder by a cyclotex sample mill (series 1903 with 200–240V and 50/60 Hz from FOSS, Höganäs, Sweden). The moisture content after freeze dried was determined using Association of Official Analytical Chemists (AOAC) method (2000), which was found in range of 4%–6%. All samples were kept in vacuum bag and stored at –20°C.

Preparation of rice extract

Rice powder (4 g) was extracted with solvent (20 mL) in a water bath sonicator a water bath sonicator (model B1510, 40 KHz; Bransonic Bransonic® Ultrasonic, Danbury, CT) for 10 minutes before being shaken in a shaker (Memmert GmbH, Wisconsin, USA) at 100 rpm. The mixture was centrifuged (model Z 400K; HERMLE Labortechnik GmbH, Wehingen, Germany) at 1190xg for 5 minutes. The supernatant was collected and filtered through Whatman No. 1 filter paper. The filtrate was then kept at 4°C for analysis. In order to optimize bioactive compounds extracted from rice samples, various concentrations of solvent (0%, 20%, 40%, 60%, 80% and 100% (v/v) ethanol in deionized water), extraction temperatures (30°C, 50°C and 70°C) and shaking times (0.5, 1, 2, 4, 8 and 16 hours) were investigated.

Determination of oxygen radical scavenging activity

Oxygen radical scavenging activity in rice extracts was analyzed using oxygen radical antioxidant capacity (ORAC) method (Ou *et al.*, 2001). The assay is based on the reactions between peroxyl radical (ROO•) generated from thermal decomposition of 2, 2–azobis (2–amidinopropane) dihydrochloride (AAPH) and sodium fluorescein

probe to produce non–fluorescent compound. The antioxidant capacity is measured by the decrease of non–fluorescein production as a result of antioxidant that captures AAPH to protect the fluorescein probe from oxidative damage. The reaction mixture consisted of rice extract (0.2 g/mL), fluorescein solution (30 mM) and AAPH solution (19.1 mM) in 75 mM potassium phosphate buffer (KPB) (pH 7.4). Rice extract and fluorescein solution were incubated at 37°C for 15 minutes prior to addition of AAPH regent. The fluorescence intensity was monitored for 90 min using a microplate reader (Synergy HT multi–detection microplate reader, Bio–Tek Instruments, Inc., Winooski, VT) with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Trolox, a water–soluble analogue of vitamin E (0–100 μM), was used as a standard control. The results were expressed as μmol trolox equivalence (TE) per 100 grams dry weight sample.

Determination of chelating ability on ferrous ion

The chelating ability on ferrous ion in rice extract was determined by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996) with some modifications. This assay was measuring ability of antioxidant that reduces ferric tripyridyltriazine (FeIII–TPTZ) into ferrous tripyridyltriazine (FeII–TPTZ) in FRAP reagent. Rice sample (20 μL) was mixed with FRAP reagent (150 μL) that was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ (2, 4, 6–tripyridyl–s–triazine, 10 mM in 40 mM HCl) and $FeCl_3 \cdot 6H_2O$ solution (20 mM) in a ratio of 10:1:1. The mixture was incubated for 8 minutes at 37°C before measuring absorbance at 600 nm. The FRAP value was determined using trolox (0–100 μM) standard curve and reported as TE per 100 grams dry weight sample.

Determination of 2,2–diphenyl–1–picrylhydrazyl radical scavenging activity

The assay was performed using a colorimetric assay (Fukumoto and Mazza, 2000) with some modifications. Free radical scavenging capacity was detected by measuring the loss of absorbance at 520 nm as a result of reduced 2, 2–diphenyl–1–picrylhydrazyl (DPPH) radical by antioxidant. The assay mixture that consisted of rice extracts and DPPH (152 μM in 95% (v/v) aqueous ethanol) was incubated in dark at room temperature for 30 minute before being measured absorbance at a wavelength of 520 nm. DPPH radical scavenging activity was presented as a percent calculated by following equation;

$$\% \text{ radical scavenging activity} = 100 \times \{1 - [\text{Abs}_{sample}/\text{Abs}_{reagent\ blank}]\},$$

Table 1. Antioxidant capacities and TPCs in rice samples extracted with various ethanol concentrations

| *Rice samples | Ethanol concentration (% v/v) | Antioxidant Capacities | | | TPCs (mg GAE/100g) |
		ORAC (μmole TE/100 g)	% DPPH radicals inhibition	FRAP (μmole TE/100g)	
WR	0	247 ± 18[c]	11 ± 1[b]	45 ± 1[b]	14 ± 1[a]
	20	271 ± 9[c]	8 ± 1[c]	49 ± 1[ab]	10 ± 1[b]
	40	346 ± 3[b]	10 ± 1[bc]	57 ± 3[a]	11 ± 0[b]
	60	434 ± 5[a]	16 ± 0[a]	50 ± 2[ab]	10 ± 1[b]
	80	327 ± 15[b]	11 ± 1[b]	35 ± 1[c]	14 ± 1[a]
	100	147 ± 12[d]	3 ± 0[d]	16 ± 0[d]	4 ± 0[c]
BR	0	776 ± 31[d]	39 ± 2[cd]	142 ± 7[a]	54 ± 1[a]
	20	1320 ± 96[ab]	41 ± 2[bc]	141 ± 9[a]	51 ± 2[a]
	40	1314 ± 90[b]	45 ± 1[ab]	158 ± 6[a]	52 ± 2[a]
	60	1501 ± 55[a]	46 ± 1[a]	162 ± 16[a]	55 ± 2[a]
	80	1010 ± 57[c]	36 ± 1[d]	113 ± 11[b]	51 ± 2[a]
	100	557 ± 55[e]	15 ± 0[e]	106 ± 7[b]	45 ± 2[b]
GBR	0	1217 ± 86[c]	49 ± 3[b]	166 ± 4[cd]	46 ± 2[ab]
	20	1256 ± 86[bc]	57 ± 1[a]	186 ± 17[bc]	43 ± 3[b]
	40	1435 ± 59[b]	60 ± 1[a]	245 ± 11[a]	53 ± 5[a]
	60	1724 ± 88[a]	54 ± 4[ab]	197 ± 6[b]	50 ± 5[ab]
	80	1051 ± 96[c]	49 ± 0[b]	156 ± 7[d]	43 ± 1[b]
	100	429 ± 14[a]	12 ± 0[c]	174 ± 11[bcd]	45 ± 2[ab]
PGBR	0	1357 ± 134[b]	46 ± 1[b]	181 ± 8[c]	48 ± 2[c]
	20	1673 ± 71[a]	49 ± 2[b]	229 ± 14[b]	59 ± 2[b]
	40	1742 ± 62[a]	57 ± 3[a]	312 ± 6[a]	71 ± 4[a]
	60	1769 ± 93[a]	62 ± 2[a]	281 ± 20[a]	72 ± 2[a]
	80	1103 ± 108[c]	36 ± 1[c]	153 ± 3[c]	42 ± 2[c]
	100	397 ± 38[d]	16 ± 0[d]	114 ± 7[d]	34 ± 2[d]

*Rice samples (4 g) were extracted under various extraction solvent systems (20 mL) but fixed extraction temperature of 50°C and extraction time of 2 hours.
Each value was represent as mean ± SD (n = 3). Mean within a column in each tested condition was shown with difference superscript letters, which were significantly different (P < 0.05).

where Abs_{sample} is the detention of assay with sample, and $Abs_{reagent}$ blank is the detection of assay without sample.

Determination of total phenolic content

Total phenolic content was analyzed using Folin–Ciocalteu reagent (Ainsworth and Gillespie, 2007). The samples were mixed with 10% (v/v) Folin–Ciocalteu reagent in the 96–well flat–bottom microplate. After 5 minutes incubation, saturated sodium bicarbonate (7.5% (w/v), 200 μL) was added, and the reaction was mixed well. The mixture solution was then incubated in dark at room temperature for 2 hours. The absorbance at 765 nm was measured using the microplate reader. Gallic acid (0–200 μg/mL) was used as a standard control. The total phenolics content in the samples was expressed in gallic acid equivalents per 100 grams sample (GAE mg/100 g sample).

Statistical analysis

All experiments were expressed as mean of triplicate assays ± standard deviation (SD). One way analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed to determine the significant differences between values. Significance of difference was defined at p<0.05. All statistical analysis was carried out using SPSS statistics version 17 for Windows (SPSS Inc., Chicago, USA).

Results and Discussion

Different types of processed rice including WR, BR, GBR and PGBR were investigated in term of optimized extraction conditions for antioxidant activities and TPCs. The concentrations of extraction solvent, extraction temperatures and extraction times were examined as external factors that might affect the quantity of bioactive compounds with biological properties from rice.

Effect of different ethanol concentrations

Antioxidant capacities and TPCs were varied according to rice extracted with difference ethanol concentrations (0%, 20%, 40%, 60%, 80% and 100% (v/v) aqueous ethanol) (Table 1). Under fixed extracted temperature (30°C) and time (2 hours), the extracted rice samples with 40%–60% (v/v) aqueous ethanol exhibited the highest antioxidant activities as being detected by ORAC, FRAP and DPPH radical

Table 2. Antioxidant capacities and TPCs in rice samples extracted with various temperatures

*Rice samples	Temperature (°C)	Antioxidant Capacities			TPCs (mg GAE/100g)
		ORAC (µmole TE/100 g)	% DPPH radicals inhibition	FRAP (µmoleTE /100g)	
WR	30	168 ± 16[b]	12 ± 0[c]	48 ± 2[c]	10 ± 1[b]
	50	239 ± 7[a]	18 ± 1[a]	57 ± 1[a]	18 ± 0[a]
	70	189 ± 10[b]	16 ± 0[b]	52 ± 2[b]	17 ± 1[a]
BR	30	1281 ± 28[b]	52 ± 3[b]	244 ± 12[b]	41 ± 2[a]
	50	1719 ± 85[a]	60 ± 1[a]	310 ± 16[a]	52 ± 0[a]
	70	1587 ± 60[a]	57 ± 1[ab]	281 ± 6[ab]	52 ± 1[a]
GBR	30	1311 ± 85[b]	52 ± 3[b]	214 ± 14[b]	41 ± 2[b]
	50	1803 ± 104[a]	53 ± 1[b]	229 ± 15[ab]	53 ± 1[a]
	70	1466 ± 38[b]	59 ± 1[a]	245 ± 2[a]	52 ± 1[a]
PGBR	30	1925 ± 5[b]	65 ± 2[c]	417 ± 26[b]	64 ± 3[b]
	50	2337 ± 58[a]	79 ± 1[b]	553 ± 8[a]	84 ± 4[a]
	70	2259 ± 42[a]	84 ± 3[a]	548 ± 7[a]	88 ± 1[a]

*Rice samples (4 g) were extracted under various extraction temperatures but fixed extraction solvent of 40% (v/v) aqueous ethanol (20 mL) and extraction time of 2 hours.
Each value was represent as mean ± SD (n = 3). Mean within a column in each tested condition was shown with difference superscript letters, which were significantly different (P < 0.05).

scavenging assays. Pure deionized water (0% (v/v) aqueous ethanol) and high ethanol concentrations (80%–100% (v/v) aqueous ethanol) decreased antioxidant activities. From the nature of solid–liquid extraction, solvent likely diffuses into solid material and solubilizes compounds with similar charge (polarity). Both water and ethanol are polar solvents, which present polarity index values of 9.0 and 5.2, respectively. Thus, addition of ethanol into water may cause a decrease in polarity. It was previously found that the polarity index values of 25%, 50% and 75% (v/v) aqueous ethanol are 8.1, 7.1 and 6.2, respectively (Cahyo et al., 2009). Thus, the extracts with different ethanol concentrations likely contain bioactive compounds with different charges. For example, solvents with higher polarity can extract a significantly higher quantity of total isoflavones (Lin and Giusti, 2005). As a result, the mixture of aqueous ethanol is a better solvent than absolute ethanol or pure water for extracting both antioxidants and phenolics from rice. This information is corresponded to the previous research (Mijanur et al., 2013), in which the maximum polyphenol and flavonoid contents in Centella asiatica were extracted with 50% (v/v) aqueous ethanol (polarity index of 7.1). Moreover, ethanol can inactivate some enzymes such as polyphenol oxidase (polyphenol degraded enzyme) that is released into solvent when cell wall is disrupted during extraction processes. Thus, phenolics possess a better chance to maintain in ethanol solution rather than in pure water (Tiwari et al., 2011).

Therefore, different types of bioactive compounds could be maximally extracted under different solvent systems, depending on polarity and availability to maintain in a particular environment. As a result, the mixture of aqueous ethanol could optimize extracted antioxidants and phenolics from all rice samples with a higher quantity than those under pure water and high ethanol concentration. Since the rice samples extracted with 40% (v/v) aqueous ethanol exhibited higher overall antioxidant activities and TPCs than those of the samples extracted with other solvents, this extraction solvent was chosen for further investigation on the effects of extraction temperatures and times.

Effect of different extraction temperatures

The quantity of TPCs and antioxidant activities in all rice samples (extracted under fixed extraction solvent of 40% (v/v) aqueous ethanol and extraction time of 2 hours) were greater under high extraction temperature (50°C –70°C) than those of the samples extracted under low temperature (30°C) (Table 2). An increase in extracted bioactive compounds may occur through greater solubility and mass transfer rate (diffusion coefficient) (Spigno and de Faveri, 2011). It was previously suggested that high temperature could accelerate extraction rate (especially initial extraction rate) (Bucić–Kojić et al., 2007; Linares et al., 2010; Qu et al., 2010), then the rate would be slowing down and eventually reaching equilibrium (Qu et al., 2010). Not only that high temperature could accelerate rate of reaction, it also induces compound degradation process (Kahyo et al., 2009). It was previously reported that an increase in temperature up to 74°C could increase quantity of extracted phenolics; however, it also decreased the quantity of extracted anthocyanins (Cacace and Mazza, 2003). Thus, the

Table 3. Antioxidant capacities and TPCs in rice samples extracted with various times

| *Rice samples | Time (hrs) | Antioxidant Capacities | | | TPCs (mg GAE/100g) |
		ORAC (µmole TE/100 g)	% DPPH radicals inhibition	FRAP (µmole TE/100g)	
WR	0.5	414 ± 36[a]	7 ± 1[b]	35 ± 1[c]	13 ± 1[b]
	1	465 ± 29[a]	8 ± 1[ab]	36 ± 1[c]	14 ± 1[ab]
	2	418 ± 35[a]	9 ± 0[ab]	36 ± 1[bc]	15 ± 0[abc]
	4	477 ± 21[a]	14 ± 1[ab]	41 ± 1[a]	15 ± 0[abc]
	8	385 ± 23[a]	15 ± 2[a]	37 ± 1[bc]	16 ± 1[ab]
	16	414 ± 33[a]	15 ± 1[a]	40 ± 2[ab]	16 ± 1[a]
BR	0.5	1600 ± 130[ab]	41 ± 0[a]	226 ± 1[a]	39 ± 1[b]
	1	1389 ± 10[bc]	42 ± 4[a]	234 ± 10[a]	46 ± 1[a]
	2	1689 ± 5[a]	44 ± 1[a]	229 ± 10[a]	44 ± 1[a]
	4	1738 ± 101[a]	43 ± 1[a]	234 ± 9[a]	43 ± 1[a]
	8	1540 ± 18[ab]	46 ± 2[a]	216 ± 6[a]	46 ± 1[a]
	16	1277 ± 68[c]	41 ± 1[a]	222 ± 2[a]	46 ± 1[a]
GBR	0.5	1492 ± 45[ab]	44 ± 1[ab]	201 ± 9[a]	39 ± 1[b]
	1	1387 ± 73[bc]	46 ± 1[ab]	224 ± 4[a]	44 ± 2[a]
	2	1614 ± 121[a]	48 ± 1[a]	217 ± 7[a]	44 ± 3[a]
	4	1613 ± 121[a]	46 ± 2[ab]	209 ± 7[a]	44 ± 2[a]
	8	1221 ± 58[c]	44 ± 2[ab]	211 ± 4[a]	45 ± 1[a]
	16	1315 ± 75[bc]	43 ± 1[c]	214 ± 3[a]	48 ± 1[a]
PGBR	0.5	2122 ± 133[a]	79 ± 0[a]	363 ± 5[ab]	80 ± 6[a]
	1	2099 ± 104[a]	77 ± 0[ab]	323 ± 20[b]	73 ± 0[a]
	2	2306 ± 21[a]	76 ± 1[b]	328 ± 28[b]	76 ± 1[a]
	4	2088 ± 87[a]	77 ± 2[ab]	344 ± 20[ab]	77 ± 3[a]
	8	1950 ± 174[a]	77 ± 0[ab]	347 ± 9[ab]	76 ± 5[a]
	16	1930 ± 182[a]	79 ± 1[ab]	378 ± 13[a]	73 ± 0[a]

*Rice samples (4 g) were extracted under various extraction times but fixed extraction solvent of 40% (v/v) aqueous ethanol (20 mL) and extraction temperature of 50°C.
Each value was represent as mean ± SD (n = 3). Mean within a column in each tested condition was shown with difference superscript letters, which were significantly different ($P < 0.05$).

suitable extraction temperature is a significant factor to determine the quantity and quality of bioactive compounds (i.e., antioxidants and phenolics) from each sample. In this case, the extraction temperature of 50°C that could optimize antioxidant activities and TPCs as being detected in most cases was chosen for further investigation of extraction time.

Effect of different extraction times

It was found that extraction times exhibited trivial effect on all antioxidant capacities and TPCs (Table 3), suggesting that bioactive compounds in all rice samples were not significantly degraded in 40% (v/v) aqueous ethanol under 50°C extraction temperature. These results were corresponded to the previous research (Spigno and de Faveri, 2007), which indicated that the extraction time shared trivial influence on antioxidants extracted from grape stalks and marc model. Thus, the extraction time of 2 hours was employed for further investigation on the comparison of antioxidant activities and TPC on rice regarding its polished, germinated and parboiled processes.

Comparison of antioxidant activities and TPCs in rice extracts

Under the optimized extraction conditions, all rice samples including WR, BR, GBR and PGBR were extracted with 40% (v/v) aqueous ethanol at extraction temperature of 50°C and extraction time of 2 hours. It was shown that PGBR exhibited the highest quantity of TPC (72±2 mg GAE/100 g dry weight) and antioxidant activities detected by ORAC (3343±137 µmole TE/100 g dry weight), FRAP (420±41 µmole TE/100 g dry weight) and DPPH radical scavenging (71% inhibition) assays, followed by those of GBR ~ BR and WR, respectively (Figure 1). Since phenolics are well known for their antioxidant activities due to hydroxyl moieties, they can act as reducing agent, hydrogen donors, singlet oxygen quenchers to scavenge free radicals and also metal chelators (Potterat, 1997). Therefore, it was highly possible that the major compounds that acted as antioxidants in all rice extracts were phenolics.

As results, rice with retaining bran and germ layer significantly affects antioxidant activities as well as TPCs. Milling process that is a character of WR causes the most loss of those bioactive compounds, which are contributed to decrease in antioxidant activities and TPCs. Thus, PGBR, GBR and BR with retaining bran and germ layer exhibited higher antioxidant activities and TPCs than those of WR.

Interestingly, GBR and BR exhibited similar antioxidant activities and TPCs, suggesting that germination might not affect the extraction of these bioactive compounds. The germination is often employed for increasing quantity and quality of nutrients and bioactive compounds. This process also improves antioxidant activity, not only for rice, but also for other grains. It was previously found
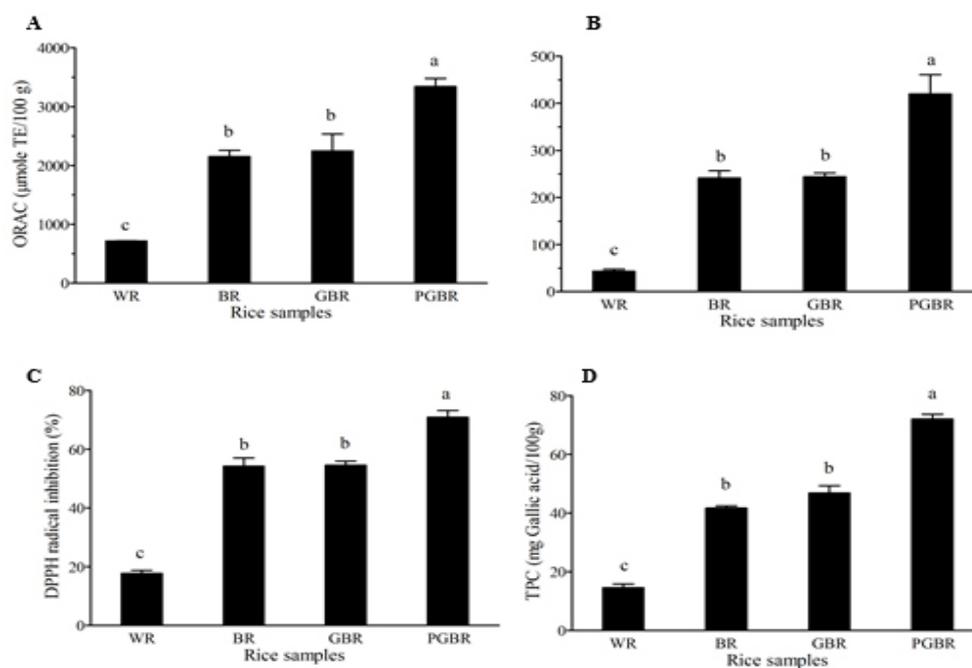
Figure 1. The antioxidant activities of WR, BR, GBR and PGBR were detected by (A) ORAC, (B) FRAP and (C) DPPH radical scavenging assays. Their TPC values were also detected using Folin–Ciocalteu procedure (D)

that the germination of sesame (*Sesamum indicum L.*) for 4 days could increase various minerals and vitamins (Hahm *et al.*, 2009). Sweet lupin seeds (*Lupinus angustifolius L.*) after germination for 9 days exhibited double antioxidant activity than ungerminated seed and also provided higher TPCs and vitamin C and E contents (Fernandez–Orozco *et al.*, 2006). However, the germination that was unlikely useful for improving antioxidants and TPCs in rice were observed in our study. It was previously found that the quantity of γ–oryzanol, α–tocopherol and TPCs in germinated brown rice are greatly impacted by germination times (Moongngarm and Saetung, 2010). Germination duration for 2–4 days exhibited significant nutrients such as γ–tocopherol, α–tocopherol and α–tocotrienol, γ–oryzanol and also TPCs (Fernandez–Orozco *et al.*, 2006). The highest DPPH radicals scavenging activity was also observed after germination for 1–2 days; however, the activity began to decline continuingly afterward. Thus, similar antioxidant activities and TPCs of GBR and BR observed in our experiments might be affected by the germination time (2 days).

Parboiling process is a considerable factor that contributed antioxidants retaining in rice. In our experiments, it was found that parboiling process could significantly increase antioxidant activities and TPCs. These results suggested the possible transferring of nutrients and bioactive compounds from bran layer into gelatinized starch; therefore, this process prevents the loss of nutrients during milling

(Luh, 1991), resulting in higher antioxidant activities and phenolics.

Conclusion

Ethanol concentration and extraction temperature provided the great impacts on antioxidant activities and TPCs in rice. On the other hand, extraction time only provided trivial effect on these factors. The optimized extraction conditions for rice were 40%–60% (v/v) aqueous ethanol and 50°C–70°C extraction temperature. Under these optimized extraction conditions, PGBR exhibited the highest antioxidant activities and TPCs, followed by those of GBR~BR and WR, respectively. Milling process was considered an important factor that causes a loss of bioactive compounds. On the other hand, parboiling process can significantly increase antioxidant activities and phenolics, while the germination only provided trivial effect on those contents.

Acknowledgements

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