PHILIPP AGRIC SCIENTIST
Vol. 100 No. 2, 190–199
June 2017

ISSN 0031-7454

Comparative Evaluation of 2,2-Diphenyl-1-Picryl-Hydrazylhydrate (DPPH) Free Radical- and Oxygen Radical Absorbance Capacity (ORAC) Assays in Measuring the Antioxidant Capacities of Pigmented Rice Varieties

Xiaoqiong Chen[1,2,*], Kohei Irifune[2], Dingqian Yang[1], Norio Nagao[2], Yuuki Chikawa[2], Xianjun Wu[1] and Tomio Itani[2,*]

[1]Rice Research Institute, Sichuan Agricultural University, 211 Huimin Road, 611130 Wenjiang, China
[2]Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, 562 Nanatsuka, Shobara 727-0023, Japan
[*]Authors for correspondence; e-mail: xiaochenq777@126.com; itani@agr.ryukoku.ac.jp

This study aimed to compare different experimental approaches for measuring antioxidant capacities of pigmented rice varieties. Samples of red, black, and white rice varieties were analyzed. The anti-oxidative activities of the rice samples were assessed by the 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) free radical and oxygen radical absorbance capacity (ORAC) assays, respectively. The total phenolic contents and the extraction efficiencies of the methanol and ethanol solvents were compared. Although the DPPH free radical and ORAC assays yielded different results, the same trends were observed with regard to their antioxidant capacities, with ranges of 1492.7–2065.8 (highest value), 713.7–1587.4, and 23.9–92.5 µmol Trolox 100 mg^{-1} corresponding to red, black, and white varieties, respectively. The most efficient extraction solvent was 1% HCl in methanol, which yielded extracts with the highest antioxidant capacity and total phenolic content. Extraction with 1% HCl in methanol was found to be suitable for analyzing antioxidant compounds and total phenolic contents.

Key Words: antioxidant capacity, DPPH, phenolic content, pigmented rice, solvent extraction

Abbreviations: AOA – antioxidant activity, CUPRC – copper reduction, DPPH – 2,2-diphenyl-1-picryl-hydrazylhydrate, FRAP – ferric reducing ability of plasma, MES – 2-morpholino ethane sulfonic acid, ORAC – oxygen radical absorbance capacity, TEAC – Trolox equivalent antioxidant capacity, TPC – total phenolic content, TRAP – oxygen radical absorbing, TSPC – total soluble phenolic compounds

INTRODUCTION

Rice (*Oryza sativa* L.) is a fundamental food for a great proportion of the population worldwide. In addition to the predominant white rice, several varieties with colors are well known, including black, red, and green cultivars. The black and white rice varieties have been intensively studied for their beneficial properties, such as their antioxidant capacity, contribution to a decreased cardiovascular risk, aside from their protein, vitamin and mineral content (Itani et al. 2002; Suzuki et al. 2004; Lee et al. 2008; Chen et al. 2012; Kazemzadeh et al. 2014). Extracts from pigmented rice bran have also been shown to inhibit allergic reactions *in vitro* (Choi et al. 2007). Antioxidants from plant materials can be obtained by different techniques and solvents, depending on the natural diversity of these compounds and their unique distribution in plants (Antolovich et al. 2000; Sultana et al. 2009). Extraction by using solvents, such as acetone, methanol, ethanol, and aqueous mixtures, is the most frequently used technique for analyzing antioxidants in fruits, vegetables, grains, medicinal plants and

agricultural waste products (Bonoli et al. 2004; Shahid et al. 2006; NIIR Board of Consultants and Engineers 2006; Sultana et al. 2009).

Many studies have assessed the antioxidant capacity of pigmented rice varieties by using 1% HCl in methanol as an extraction solvent (Hu et al. 2003; Hiemori et al. 2009). Recently, analysis of antioxidant activities of pigmented rice varieties by mass spectrometry-based metabolite profiling revealed that the black and red rice seeds have higher activity than the white rice seeds (Kim et al. 2014). However, although several studies have compared the antioxidant capacities of rice varieties by using different extraction solvents and antioxidant-activity evaluation methods, the overall number of these reports is considered minimal (Anwar and Przybylski 2012; Farahmandfar et al. 2015; Ghasemzadeh et al. 2015). Moko et al. (2014) demonstrated that colored rice varieties possess greater antioxidant properties compared with their non-colored counterparts by using phenolic, flavonoid, alkaloid, steroid, triterpenoid and saponin tests. Interestingly, the use of antioxidants has demonstrated health benefits and the importance of extraction methods that maximize their yield. In this connection, comparing and assessing the antioxidant capacities of the pigmented rice varieties would require selection of suitable extraction solvents and measurement methods.

Antioxidant capacity is routinely determined by two methods: electron transfer reaction and hydrogen atom transfer reaction assays. The electron transfer reaction assays include the Trolox equivalent antioxidant capacity (TEAC), the ferric reducing ability of plasma (FRAP), the 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the copper reduction (CUPRC) assays. The oxygen radical absorbing (TRAP) and the oxygen radical absorbing capacity (ORAC) assays are hydrogen atom transfer reaction methods. The assessment of the antioxidant capacities of phenolic compounds in beverages such as green tea, orange juice, vegetable juice and apple juice has been demonstrated by previous studies that utilized the DPPH, ORAC and TEAC methods (Natella et al. 2002).

As regards the assessment of the phenolic compounds in rice, the data reported in the literature indicate that notably DPPH and ORAC are the methods selected for the evaluation of the total antioxidant capacity (Qiu et al. 2009; Min et al. 2011). In the present study, DPPH and ORAC assays were chosen for the comparative analysis of the antioxidant capacities in various rice varieties. This choice was derived from earlier studies indicating that the different varieties of pigmented rice contain distinct antioxidant capacities, possibly due to the contribution of several environmental factors, such as sunshine and temperature (Jun et al. 2012). Interestingly, black rice has a higher phenolic content, compared with red rice (Chen et al. 2012). Thus, the present study aimed (1) to compare the extraction efficiencies of methanol and ethanol solvents in terms of the antioxidant capacities and the total phenolic content of pigmented rice varieties, and (2) to assess the contributions of light and culture environment as factors in the synthesis of total phenolic content in the rice varieties.

MATERIALS AND METHODS

Materials

The red rice varieties used in this study were Tsukushiakamochi (glutinous) and Beniroman (non-glutinous), and the black rice varieties were Asamurasaki (glutinous) and Okunomurasaki (non-glutinous). The white rice variety was solely the Koshihikari (non-glutinous) variety. The rice plants were cultivated in the experimental fields of the Faculty of Life and Environmental Sciences, at the Prefectural University of Hiroshima during the period 2005–2011. Planting and harvesting were done in the same calendar year. Seeds were stored at 5 °C and dehulled before use.

AAPH (2,2-azobis2-amidino-propanedi-hydrochloride), Folin-Ciocalteu's reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carbonsaure), and DPPH (1,1-diphenyl-2-picrylhydrazyl) were obtained from Sigma Chemicals (St. Louis, MO, USA). MES [2-(N-morpholino) ethane-sulfonic acid] was purchased from Nacalai Tesque Inc. (Nacalai Tesque, Kyoto, Japan). Gallic acid was purchased from Biomedicals (Illkirch, France). The other chemicals used in this study were of the highest grade available and were purchased from Wako Pure Chemical Industries Ltd. (Hiroshima, Japan).

Sample Preparation

The hulled grains (caryopsis) were randomly selected in each variety and were milled with an ultracentrifuge l mill (Iwatani, Japan). The milled rice

powder (0.2 g) was soaked in 50 mL of the following solvents: 1% HCl in methanol, 80% ethanol and 100% methanol (Wako Pure Chemical Industries Ltd., Hiroshima, Japan). The powder was sequentially extracted for 24 h at room temperature with continuous shaking. The total volume of the extraction was adjusted to 10 mL with additions of the extraction solvent (1% HCl in methanol, 80% ethanol and 100% methanol). The resulting extracts were used for the measurement of the phenolic content and the DPPH assay. The extracts that were obtained with 1% HCl in methanol were also used for the oxygen radical absorbing capacity (ORAC) assay. All extracts were stored at −20 °C until further use.

Climate Indexes

The climate indexes were obtained from the Hiroshima Prefecture Bureau of Meteorology, Syobara City, Japan in December 2012 (Table 1). The cumulative actual sunshine hours and average temperatures were recorded between the heading of each variety and 30 d later.

Color Measurements

The color of the hulled rice was determined by the Hunters reflected color method (McGuire 1992). The hulled rice grains were placed in plastic bags (0.04 mm thick), and the Hunter L*, a*, and b* reflected color values were measured, with 10 repetitions, using a colorimeter (CR-221, Minolta Co. Ltd., Osaka, Japan).

DPPH Assay

DPPH scavenging activity was evaluated as described previously (Oki et al. 2001). Briefly, 150 μL of the aforementioned sample (see 'Sample preparation') were mixed with 50 μL of 100% ethanol, 50 μL of 2-morpholinoethanesulfonic acid (MES) buffer (pH 6.0, 0.2 M), and 20% of ethanol in a 96-well microplate. The reaction was initiated by the addition of 50 μL of 800 μM mL^{-1} DPPH in ethanol. The reaction mixture was vortexed and incubated at room temperature for 20 min in the dark. Finally, the absorbance was measured by spectrophotometry at 510 nm, using a dual-wavelength flying spot scanning densitometer (CS9300PC, Shimadzu Co., Ltd., Kyoto, Japan). The DPPH radical-scavenging activity was estimated by the decrease in absorbance at 510 nm, and is expressed in Trolox equivalent per 100 mg of sample, using a Trolox standard curve.

ORAC Assay

Assessment of the ORAC assay was carried out using a Lab systems Fluoroskan Ascent FL plate reader (Sigma Chemical Co.), according to a procedure described by Wu et al. (2004). The compound 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH) was used as the peroxyl radical generator, the compound Trolox as the standard, and the compound fluorescein as the fluorescent probe. An excitation wavelength of 485 nm was selected using appropriate filters, and the fluorescence emission was measured at 520 nm every 2 min over a 90-min period. The measurements were carried out in triplicate.

The final ORAC results were standardized compared with the Trolox. The fluorescence analysis system automatically generated a Trolox standard curve using a net fluorescence (background-subtracted values) value. The relative Trolox equivalent ORAC value was calculated as follows:

Relative ORAC value = (Area under the curve (AUC) sample - AUC blank) micromoles of Trolox equivalent per gram of dry weight/(AUC Trolox - AUC blank) micromoles of Trolox equivalent per gram of dry weight.

Table 1. Climate index between heading and following 30 d.

Variety	Mean Daily Temperature (°C)			Total Sunshine/h		
	2005	2007	2011	2005	2007	2011
White rice						
Koshihikari	22.22 ± 0.5	23.82 ± 0.7	24.52 ± 0.9	111.9 ± 2.2	159.0 ± 5.8	127.8 ± 4.5
Red rice						
Tsukushiakamochi	23.08 ± 0.4	22.60 ± 1.0	21.97 ± 0.8	120.1 ± 1.6	155.2 ± 2.3	157.6 ± 4.8
Beniroman	22.07 ± 0.7	21.26 ± 0.5	21.77 ± 0.7	127.9 ± 2.4	147.7 ± 3.2	160.6 ± 2.1
Black rice						
Asamurasaki	24.5 ± 0.6	23.82 ± 0.8	24.84 ± 0.5	108.7 ± 2.3	153.6 ± 2.3	137.8 ± 1.9
Okunomurasaki	24.5 ± 0.6	23.82 ± 0.8	24.84 ± 0.5	108.7 ± 2.3	153.6 ± 2.3	137.8 ± 1.9

Measurement of Total Phenolic Content (TPC)

TPC was determined by the Folin-Ciocalteu method as described previously (Zhang et al. 2010). A standard curve was constructed using a stock concentration of 1 mg/mL of gallic acid dissolved in 80% methanol. The aliquots were prepared at a volume range of 0.025, 0.05, 0.10, 0.20, 0.4 and 0.6 mL of the stock solution and were mixed with 1.25 mL of Folin-Ciocalteu's reagent and 3.75 mL of 20% Na_2CO_3 solution. The resulting solution was transferred to cuvettes and was incubated at 30 °C for 2 h in the dark.

The absorbance of each sample was measured at 760 nm (JascoUbest-30 spectrophotometer, Tokyo, Japan) for the determination of the total phenolic content, while a solution without gallic acid monohydrate was used as negative control. The Folin-Ciocalteu assay relies on the electron transfer in alkaline medium from phenol compounds to phosphotungstic acid complexes that are determined spectrophotometrically at 760 nm (Ainsworth and Gillespie 2007).

Statistical Analysis

Descriptive statistics was used for the analysis. The differences between the three groups of rice varieties were examined using one-way analysis of variance (one-way ANOVA) with regard to the determination of antioxidant capacity and climate index. The data are presented as the mean ± SD and the statistical analysis was carried out using SPSS statistics software 22.0 (SPSS Inc. Chicago, IL). A *P*-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Physical Properties of Hulled Rice Grains in Different Varieties

The hulled rice grains were examined for their colors using a colorimeter (Table 2). The white rice had the highest L* value, followed by the red and black varieties. The red rice grains showed the highest a* value, and the white rice the lowest. The b* value was greater for the red rice varieties, with the exception of the Beniroman variety. The white rice variety exhibited a greater b* value compared with the Beniroman variety and the black rice variety. The red rice Tsukushiakamochi had the greatest c* value, as evaluated by Metricchroma, followed by the white and black varieties.

The analysis by the Metric hue angle yielded the greater h* value for the white rice, followed by the red and black rice varieties. Interestingly, the value of each a*, b*, c*, and L* parameter, respectively, was significantly different between the red and black rice varieties; the white varieties exhibited greater L* color value, followed by the red and the black varieties. With regard to the a* color value, the black and white varieties showed comparable efficacies, whereas the red variety indicated the greatest a* value.

The hue of the pigmented rice was not correlated with the sunshine and the temperature (as indicated by the climatic index shown in Table 1). For example, there was no significant difference in Asamurasaki's color properties between 2005 (sunshine 108.7 h, L* 17.2) and 2007 (sunshine 153.6 h, L* 17.9). Thus, it is

Table 2. Hunters-reflected color values of hulled grains; L* = lightness, a* = bluish- green/red – purple hue component, b* = yellow/blue hue component; metric chroma: $c^* = \sqrt{[(a^*)^2 + (b^*)^2]}$; metric hue angle: h* = arc tan (b*/a*).

Variety		Hunters-Reflected Color (Mean ± Standard Deviation)				
		L*	a*	b*	c*	h*
White rice						
Koshihikari	2005	62.67 ± 2.74	4.39 ± 0.59	24.75 ± 1.01	25.1	251.1
	2007	63.64 ± 2.10	4.41 ± 0.71	25.13 ± 1.58	25.5	251.5
	2011	62.62 ± 2.30	4.46 ± 0.54	24.55 ± 0.76	25.0	250.4
Red rice						
Tsukushiakamochi	2005	41.97 ± 2.43	19.07 ± 0.72	29.03 ± 0.69	34.7	178.1
	2007	41.19 ± 3.12	17.19 ± 0.92	27.70 ± 1.39	32.6	182.8
	2011	44.54 ± 2.96	14.39 ± 1.45	27.39 ± 1.26	30.9	195.7
Beniroman	2005	34.68 ± 1.50	13.50 ± 1.37	19.75 ± 1.68	23.9	174.8
	2007	34.62 ± 1.80	15.53 ± 0.81	20.93 ± 0.90	26.1	167.8
	2011	38.12 ± 2.78	13.24 ± 0.66	21.74 ± 1.12	25.5	184.3
Black rice						
Asamurasaki	2005	17.17 ± 2.36	4.06 ± 1.56	1.09 ± 0.65	4.2	47.2
	2007	17.88 ± 2.51	4.13 ± 1.55	1.28 ± 0.51	4.3	54.1
	2011	18.34 ± 2.14	4.10 ± 1.67	1.95 ± 2.48	4.5	79.9
Okunomurasaki	2005	18.16 ± 2.11	5.30 ± 1.37	2.75 ± 1.59	6.0	86.2
	2007	17.01 ± 2.07	3.23 ± 0.57	0.97 ± 0.54	3.4	52.5
	2011	18.37 ± 2.31	4.77 ± 1.07	2.42 ± 1.32	5.3	84.5

reasonable to assume that the rice color is determined mainly by the genetic background, rather than by the environment. However, the general appearance of the rice may be a cumulative effect of the hue and the various defects that may contribute to the color.

Smut was shown to impart a gray hue to rice grains, whereas heat damage may have caused a color change. Furthermore, the color may indicate other features such as rice quality. It has been shown that brown rice and white rice have different amounts of protein content that in turn affects their hepatic lipid content and weight gain (Zhang et al. 2011). In addition, the composition of rice in terms of protein, carbohydrate and nitrate levels is affected by the environment and growth conditions such as nutrient solution, CO_2 level and photosynthetic photon flux (McKeehen et al. 1996).

Comparison of the Antioxidant Capacities of the Different Rice Varieties using the DPPH Method

The DPPH scavenging effects of the different rice varieties were assessed using various extraction solvents (Table 3). The antioxidant capacities of the rice varieties that were examined in the present study were significantly different. The greatest values were obtained for the red varieties (1492.7–2065.8 μmol Trolox 100 mg^{-1}), followed by the black (713.7–1587.4 μmol Trolox 100 mg^{-1}) and the white (23.9–92.5 μmol Trolox 100 mg^{-1}) varieties. Yao et al. (2010) compared the red, black, and purple rice varieties and reported a more pronounced radical scavenging activity in the black rice, compared with the red rice by the DPPH method. Similar results were reported by Laokuldilok et al. (2011) in terms of antioxidant activity. These findings corroborate the data published by Oki et al. (2002) and Chen et al. (2012) which indicated that the DPPH radical scavenging activity and the oxygen radical absorbing capacity (ORAC) of the red rice is greater than that of either the black or the white rice varieties.

The antioxidant capacities of the extracts from all five rice varieties were significantly lower with the 100% methanol-extraction method, compared with the 80% ethanol- or the 1% HCl/methanol-extraction method. The extracts obtained using the 1% HCl/ methanol extraction method exhibited the greatest DPPH scavenging activity, compared with the 80% ethanol- and the 100% methanol-extraction solvent method. The DPPH scavenging activity that was obtained by using 100% methanol and 80% ethanol as extraction solvents did not reveal a statistically significant difference. An antioxidant capacity of 2065.8 μmol Trolox 100 mg^{-1} was obtained for the Tsukushiakamochi (red rice) variety when the 1% HCl/methanol extraction method was used, whereas 1562.3 and 1505.8 μmol Trolox 100 mg^{-1} were obtained for the 80% ethanol- and the 100% methanol-extraction method, respectively. Similarly, the DPPH values for the Asamurasaki (black rice) variety indicated significant variations depending on the extraction solvent that was used.

The use of 1% HCl in methanol yielded greater antioxidant capacity (1383.8 μmol Trolox 100 mg^{-1}) compared with the use of 80% ethanol (955.9 μmol Trolox 100 mg^{-1}) and 100% methanol (748.3 μmol Trolox 100 mg^{-1}) (Table 3). These findings corroborate previous data reported by the study of Anwar and Przybylski (2012) in flaxseeds. Their study used four different solvents (80% ethanol, 100% methanol, 80% methanol and 100% ethanol solvents) and the greatest antioxidant capacity was noted for the 80% ethanol solvent, followed by the 100% methanol solvent, as demonstrated by the DPPH method (Anwar and Przybylski 2012). Furthermore, Zhao et al. (2006) reported that the extraction solvent

Table 3. Antioxidant capacity in five rice varieties planted in 2007, as determined by the 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) assay following extraction with different solvents.

Variety	μmol Trolox 100 mg^{-1}		
	80% Ethanol	100% Methanol	1% HCl in Methanol
White rice			
Koshihikari	76.8 ± 1.1^{aC}	23.9 ± 1.2^{bC}	92.5 ± 21.1^{aC}
Red rice			
Tsukushiakamochi	1562.3 ± 285.5^{bA}	1505.8 ± 26.7^{bA}	2065.8 ± 240.3^{aA}
Beniroman	1492.7 ± 75.4^{aA}	1633.5 ± 239.4^{aA}	1740.3 ± 195.3^{aAB}
Black rice			
Asamurasaki	955.9 ± 93.9^{bB}	748.3 ± 57.2^{bB}	1383.8 ± 239.8^{aB}
Okunomurasaki	1023.6 ± 272.8^{bB}	713.7 ± 95.2^{cB}	1587.4 ± 417.1^{aB}

Values are indicative of the mean ± standard deviation of three measurements. Identical superscript capital letters denote no significant difference of the variety using the same extraction solvent. Identical superscript small letters denote no significant difference of the extraction solvent in the same variety.

significantly affects the DPPH scavenging activity, based on their assessment of barley extraction with acetone, 80% ethanol and 80% methanol. The study by Zhao et al. (2006) reported that 80% acetone extract contained highest levels of (-)-catechin, and ferulic, caffeic, vanillic, and coumaric acids, while 80% methanol extract had the highest levels of (-)-epicatechin and syringic acid. The water extracts exhibited the greatest levels of protocatechuic and gallic acids (Zhao et al. 2006). The use of acetone as an extraction solvent was found to selectively enhance the yield of catechin and proanthocyanidin (Bonoli et al. 2004; Bonoli et al. 2004).

In the present study, extraction with 1% HCl in methanol yielded the highest antioxidant capacity, followed by extraction with 80% ethanol. Methanol (100%) was a less favorable extraction solvent, since it yielded the extract with the lowest antioxidant capacity. The differences in the extraction efficiencies of various solvents can be attributed to the differences in their properties. Therefore, it is speculated that the use of a strong polar solvent may promote the extraction of antioxidant compounds. Consequently, the present study confirms the use of selected solvents in order to obtain a greater yield of antioxidant compounds.

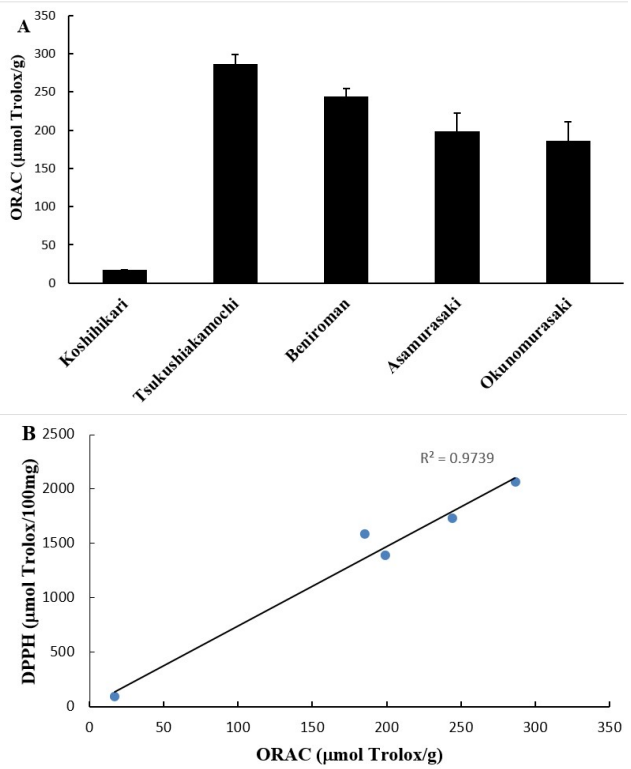

Fig. 1. A: Evaluation of the antioxidant capacities by the ORAC assay in five rice varieties planted in 2007. The letters a, b, c and d indicate statistical significance ($P< 0.05$). **B**: The correlation between DPPH and ORAC in the five rice varieties planted in 2007. Identical superscript letters denote no significant difference at $P< 0.01$. (DPPH – 2,2-diphenyl-1-picryl-hydrazylhydrate, ORAC – oxygen radical absorbance capacity).

Antioxidant Capacities of Different Rice Varieties using the ORAC Assay

Plants cultivated in 2007 were selected for assessing the antioxidant capacities of the various rice varieties using the ORAC assay (Fig. 1A). The year 2007 was selected as the mid time-point of the experimental setting, which is likely to yield representative data. The samples were extracted with 1% HCl in methanol, and the antioxidant capacity was evaluated by using the DPPH assay. Although the same trends were obtained for the antioxidant capacities between the DPPH and the ORAC assays, the values were significantly lower in the ORAC assay compared with the DPPH method. The Tsukushiakamochi variety showed an antioxidant capacity of 286.1 µmol Trolox g m^{-1} in the ORAC assay, which was 70-fold lower than the value obtained in the DPPH assay (20658 µmol Trolox g m^{-1}). Similar results were obtained for the other varieties tested. However, a significant positive pair-wise correlation was noted between the data derived from the DPPH and the ORAC assays R^2= 0.0974 (Fig. 1B). Natella et al. (2002) reported that the crocin test that utilizes the same peroxy radical generator (AAPH) and the same antioxidant standard (Trolox) as the ORAC assay was more efficient in the determination of the antioxidant activities of tea and other beverages compared with the TRAP test (Natella et al. 2002).

In the present study, the difference observed in the correlation coefficient between the ORAC and DPPH assays might have been caused by the sample composition differences, including the phenolic compound types and ratios. In addition, it is plausible that the differences observed between the DPPH and ORAC values resulted from the distinct reaction conditions. For example, DPPH is an electron reaction, whereas the ORAC is based on hydrogen atom transfer. Thus, the various analysis methods should be examined to determine the rice varieties with the strongest antioxidant capacity.

Effect of Planting Year on Antioxidant Capacities of Different Rice Varieties

The DPPH assay was used to evaluate the antioxidant capacities of different rice varieties harvested in 2005, 2007, and 2011 (Table 4). No statistically significant difference was obtained among the values for the planting years examined. However, the Beniroman variety was an exception to this trend, showing a significant difference between the years 2005 and 2007 (Table 4). The mean value of the antioxidant capacity of the Okunomurasaki variety was greater for the year 2007, compared with the year 2005, although the results were not statistically significant (Table 4). Taken together, these results indicate that antioxidant capacities differ among rice varieties.

TPC Values Obtained with Various Extraction Methods in Different Rice Varieties

Several solvents were used to extract the total phenolic compounds in the different rice varieties. There were significant differences in the TPC values depending on the extraction solvent used. The greatest TPC was obtained with 1% HCl/methanol, followed by the 100% methanol and the 80% ethanol solvent extraction methods (Table 5). This trend was the same in all the varieties tested. Zhao et al. (2006) reported the following decreasing ranking order for the TPC values in the solvent extraction of the same barley variety: 80% acetone, 80% ethanol, 80% methanol, and water. In another study, the TPC value in flax seed was the greatest with the 80% ethanol-extraction method (3260 mg 100 g^{-1}), followed by the 100% methanol-extraction (2700 mg 100 g^{-1}) and the 80% methanol-extraction method (2020 mg 100 g^{-1}) (Anwar and Przybylski 2012). Similarly, Sultana et al. (2007) reported that the 80% aqueous ethanol extraction method was the most effective solvent for the total phenolic components from the bark of some plants. A study by Walter et al. (2013) showed that the total soluble phenolic compounds (TSPC) and the antioxidant activities (AOA) of the rice grains with the brown, the red, and the black pericarps were significantly different, and the greatest values were obtained in the red and black rice varieties (Walter et al. 2013). Furthermore, a positive significant correlation between TSPC concentrations and AOA was demonstrated, although the different extraction methods were not taken into account (Walter et al. 2013).

In the present study, it was shown that the TPC values vary according to the extraction solvents. The 1% HCl/methanol solvent extraction method yielded the greatest values followed by the 100% methanol and the 80% ethanol solvent extraction methods. The TPC values in the white rice were 43.2, 12.7, and 11.3 mg/100 g^{-1}, respectively, when 1% HCl/methanol, 100% methanol and 80% ethanol were used. These values were significantly lower than the corresponding levels noted for the black and the red

Table 4. Antioxidant capacity and total phenolic content in five rice varieties planted in different years, as determined by the 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) method and the extraction method of 1% HCl/ methanol, respectively.

Test Analysis	Variety	µmol Trolox 100 mg^{-1}		
		2005	2007	2011
Antioxidant capacity	White rice			
	Koshihikari	85.8 ± 21.8^{aC}	92.5 ± 21.1^{aC}	95.67 ± 12.2^{aC}
	Red rice			
	Tsukushiakamochi	1806.2 ± 340.6^{aA}	2065.8 ± 240.3^{aA}	2145.1 ± 186.3^{aA}
	Beniroman	1442.1 ± 117.4^{bB}	1740.3 ± 195.3^{aAB}	1722.6 ± 52.7^{aA}
	Black rice			
	Asamurasaki	1411.3 ± 10.4^{aB}	1383.8 ± 239.8^{aB}	1703.4 ± 118.4^{aA}
	Okunomurasaki	1324.9 ± 86.1^{aB}	1587.4 ± 417.1^{aB}	1384.8 ± 65.1^{aB}
Total phenolic content				
	White rice			
	Koshihikari	36.7 ± 1.2^{bD}	43.3 ± 0.6^{bC}	48.9 ± 0.7^{aD}
	Red rice			
	Tsukushiakamochi	240.7 ± 14^{cC}	416.5 ± 3.5^{bB}	500.6 ± 22^{aA}
	Beniroman			
	Black rice	337.2 ± 0.6^{bB}	404.2 ± 11.2^{aB}	389.8 ± 4.0^{aB}
	Asamurasaki			
	Okunomurasaki	509.1 ± 5.5^{aA}	477.5 ± 11.2^{bA}	534.6 ± 12.1^{aA}
		305.4 ± 11.7^{bB}	403.2 ± 4.1^{aB}	295.7 ± 1.7^{bC}

Values are mean ± standard deviation of three independent measurements. Identical superscript capital letters denote no significant difference with the variety using the same extraction solvent. Identical superscript small letters denote no significant difference with the extraction solvent in the same variety.

Table 5. Total phenolic content, as measured by UV spectrophotometry following extraction with different solvents in five rice varieties planted in 2007.

Variety	mg/100 g		
	80% Ethanol	100% Methanol	1% HCl in Methanol
White rice			
Koshihikari	11.3 ± 1.2^{bD}	12.7 ± 0.8^{bC}	43.2 ± 0.6^{aC}
Red rice			
Tsukushiakamochi	186.4 ± 13.1^{cB}	251.2 ± 4.1^{bA}	416.5 ± 3.6^{aB}
Beniroman	231.7 ± 5.2^{cA}	295.4 ± 11.3^{bA}	404.2 ± 1.0^{aB}
Black rice			
Asamurasaki	130.5 ± 3.4^{bC}	127.2 ± 17.9^{bB}	477.5 ± 0.8^{aA}
Okunomurasaki	101.3 ± 12.1^{bC}	128.9 ± 7.5^{bB}	403.2 ± 4.1^{aB}

Values are indicative of the mean ± standard deviation of three measurements. Identical superscript capital letters denote no significant difference among the different varieties using the same extraction solvent. Identical superscript small letters denote no significant difference within the same variety when different extraction solvents were used.

rice varieties. However, no significant difference in the TPC contents was noted between the black and the red rice varieties using 1% HCl/methanol as the extraction solvent. The results of the present study corroborated the findings of Sompong et al. (2011) and Chen et al. (2012) that demonstrated greater TPC contents in the black and the red rice varieties compared with the white rice. Thus, the use of a strong polar solvent for extraction results in a greater TPC value, due to the high solubility of the phenolic compounds.

Effect of the Cultivation Year on TPC Values in Different Rice Varieties

The TPC of the different rice varieties was estimated in the samples from the corresponding cultivation years following the extraction with 1% HCl/ methanol. The red rice cultivar Tsukushiakamochi planted in 2005, 2007, and 2011 showed TPC values of 240.7, 416.5 and 500.6 mg 100 g^{-1}, respectively (Table 4), indicating that the TPC values in 2011 were 2-fold greater than those obtained in 2005. The black rice cultivar Okunomurasaki exhibited TPC of 305.4, 403.2, and 295.7 mg 100 g^{-1}, respectively, in 2005, 2007, and 2011. These data indicate that the TPC is further affected by meteorological and cultivation conditions.

Meteorological Conditions Affect TPC

Sunshine hours had a positive effect on the TPC in the black and the red rice. For example, in the Tsukushiakamochi variety, the TPC was 240.7, 416.5, and 500.6 mg 100 g^{-1}, respectively, during 120.1, 155.2, and 157.6 h of exposure to sunshine (Tables 1 and 4). Similarly, Okunomurasaki showed a TPC value of 403.2 mg 100 g^{-1} following 153.6 h of exposure to sunshine, which was 97 mg 100 g^{-1} greater than that obtained by 108.7 h of exposure to sunshine (305.4 mg 100 g^{-1}). In contrast to these observations, the daily temperature increase negatively affected the TPC. For example, the TPC value obtained in the Beniroman variety was 337.2, 404.2, and 389.8 mg 100 g^{-1}, respectively, at a temperature of 22.07, 21.26 and 21.77 °C (Tables 1 and 5). Thus, exposure to sunshine apparently promotes the synthesis of phenolics, while their accumulation is hampered by higher temperatures. These findings further demonstrated that the cultivation environment of rice plants is important in determining their antioxidant capacity.

TPC Correlates with Antioxidant Capacity

A high correlation was previously reported between the content of total phenolic compounds and the antioxidant capacity in bayberry (Zhou et al. 2009) and rice (Walter et al. 2013). Despite the evidence presented in the latter reports, a study by Chen et al. (2012) demonstrated that there is little correlation between the TPC and the antioxidant capacity in the black and the red rice varieties, which is in agreement with our results (Chen et al. 2012). For example, the TPC value in the Tsukushiakamochi variety (416.5 mg 100 g^{-1}) was 61 mg 100 g^{-1} lower than that noted in the Asamurasaki variety (477.5 mg 100 g^{-1}), although the corresponding DPPH value was 682.0 μmol Trolox 100 mg^{-1} greater in the same extract (Table 4). In contrast to these observations, a significant positive correlation in the same variety between the TPC and the antioxidant capacity was observed. For example, the Tsukushiakamochi variety planted in 2011 showed a higher TPC value (500.6 mg 100 g^{-1}) and a more pronounced antioxidant capacity (2145.1 μmol Trolox 100 mg^{-1}) compared with the rice planted in 2005. The antioxidant capacity of the latter was 240.7 mg 100 g^{-1} and 1806.2 μmol Trolox 100 mg^{-1}, respectively (Table 4).

SUMMARY AND CONCLUSION

The present study revealed significant differences in the TPC and the antioxidant capacity of rice cultivars depending on the extraction solvent that was used in the method. Using polar extraction solvents was found to be most suitable for analyzing the antioxidant compounds and the TPC. Furthermore, there was a significant positive correlation between the ORAC and DPPH methods regarding measurement of their antioxidant capacity, thus indicating that both assays are equally suitable for determining the antioxidant capacity.

REFERENCES CITED

AINSWORTH EA, GILLESPIE KM. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Prot 2: 875–877.

ANTOLOVICH M, PRENZLER P, ROBARDS K, RYAN D. 2000. Sample preparation in the determination of phenolic compounds in fruits. Analyst 125: 989–1009.

ANWAR F, PRZYBYLSKI R. 2012. Effect of solvent extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). Acta Sci Pol Technol Aliment 11: 293–301.

BONOLI M, MARCONI E, CABONI MF. 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.

BONOLI M, VERARDO V, MARCONI E, CABONI MF. 2004. Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. J Agric Food Chem 52: 5195–5200.

CHEN XQ, NAGAO N, ITANI T, IRIFUNE K. 2012. Anti-oxidative analysis, and identification and quantification of anthocyanin pigments in different coloured rice. Food Chem 135: 2783–2788.

CHOI SP, KANG MY, KOH HJ, NAM SH, FRIEDMAN M. 2007. Antiallergic activities of pigmented rice bran extracts in cell assays. J Food Sci 72: S719–726.

FARAHMANDFAR R, ASNAASHARI M, SAYYAD R. 2015. Comparison of antioxidant activity of Tarom Mahali, rice bran extracted from different extraction methods and its effect on canola oil stabilization. Food Sci Technol 52(10): 6385–6394.

GHASEMZADEH A, JAAFAR HZE, JURAIMI AS, TAYEBI-MEIGOONI A. 2015. Comparative evaluation of different extraction techniques and solvents for the assay of phytochemicals and antioxidant activity of Hashemi rice bran. Molecules 20(6): 10822–10838.

HIEMORI M, KOH E, MITCHELL AE. 2009. Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. japonica var. SBR). J Agric Food Chem 57: 1908–1914.

HU C, ZAWISTOWSKI J, LING W, KITTS DD. 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.

ITANI T, TAMAKI M, ARAI E, HORINO T. 2002. Distribution of amylose, nitrogen, and minerals in rice kernels with various characters. J Agric Food Chem 50: 5326–5332.

JUN HI, SONG GS, YANG EI, YOUN Y, KIM YS. 2012. Antioxidant activities and phenolic compounds of pigmented rice bran extracts. J Food Sci 77: C759–C764.

KAZEMZADEH M, SAFAVI SM, NEMATOLLAHI S, NOURIEH Z. 2014. Effect of brown rice consumption on inflammatory marker and cardiovascular risk factors among overweight and obese non-menopausal female adults. Int J Prev Med 5: 478–488.

KIM GR, JUNG ES, LEE S, LIM SH, HA SH, LEE CH. 2014. Combined mass spectrometry-based metabolite profiling of different pigmented rice (*Oryza sativa* L.) seeds and correlation with antioxidant activities. Molecules 19: 15673–15686.

LAOKULDILOK T, SHOEMAKER CF, JONGKAEWWATTANA S, TULYATHAN V. 2011. Antioxidants and antioxidant activity of several pigmented rice brans. J Agric Food Chem 59: 193–199.

LEE JC, KIM JD, HSIEH FH, EUN JB. 2008. Production of black rice cake using ground black rice and medium-grain brown rice. Int J Food Sci Tech 43: 1078–1082.

LING WH, CHENG QX, MA J, WANG T. 2001. Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. J Nutr 131: 1421–1426.

McGUIRE RG. 1992. Reporting of objective color measurements. HortScience 27: 1254–1255.

McKEEHEN JD, MITCHELL CA, WHEELER RM, BUGBEE B, NIELSEN SS. 1996. Excess nutrients in hydroponic solutions alter nutrient content of rice, wheat and potato. Adv Space Res 18: 73–83.

MIN B, McCLUNG AM, CHEN MH. 2011. Phytochemicals and antioxidant capacities in rice brans of different color. J Food Sci 76(1): 117–126.

MOKO E, PURNOMO H, KUSNADI J, IJONG F. 2014. Phytochemical content and antioxidant properties of colored and non-colored varieties of rice bran from Minahasa, North Sulawesi, Indonesia. Int Food Res J 21: 1017–1023.

NATELLA F, NARDINI M, GIANNETTI I, DATTILO C, SCACCINI C. 2002. Coffee drinking influences plasma antioxidant capacity in humans. J Agric Food Chem 50: 6211–6216.

NIIR Board of Consultants & Engineers. 2006. Wheat, Rice, Corn, Oat, Barley, Maize and Sorghum Processing Handbook (Cereal Food Technology). New Dehli, India: Asia Pacific Business Press, Inc. 464 p.

OKI T, MASUDA M, FURUTA S, NISHIBA Y, SUDA I. 2001. Radical scavenging activity of fried chips made from purple-fleshed sweet potato. J Jpn Soc Food Sci Tech 48: 926–932.

OKI T, MASUDA M, KOBAYASHI M, NISHIBA Y, FURUTA S, SUDA I, SATO T. 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. J Agric Food Chem 50: 7524–7529.

QIU Y, LIU Q, BETA T. 2009. Antioxidant activity of commercial wild rice and identification of flavonoid. J Agric Food Chem 57(16): 7543–7551.

SHAHID C, SHAHZAD A, ANWAR F, MANZOOR M, BAJWA R JAWAD-UR. 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. Grasas y Aceites 57: 328–335.

SOMPONG R, SIEBENHANDL-EHN S, LINSBERGER-MARTIN G, BERGHOFER E. 2011. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. Food Chem 124: 132–140.

SULTANA B, ANWAR F, ASHRAF M. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 14: 2167–2180.

SULTANA B, ANWAR F, PRZYBYLSKI R. 2007. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chem 104: 1106–1114.

SUZUKI M, KIMURA T, YAMAGISHI K, SHINMOTO H, YAMAKI K. 2004. Comparison of mineral contents in 8 cultivars of pigmented brown rice. J Jpn Soc Food Sci Tech 51(8): 424–427.

WALTER M, MARCHESAN E, MASSONI PFS, DA SILVA LP, SARTORI GMS, FERREIRA RB. 2013. Antioxidant properties of rice grains with light brown, red and black pericarp colors and the effect of processing. Food Res Int 50: 698–703.

WU X, BEECHER GR, HOLDEN JM, HAYTOWITZ DB, GEBHARDT SE, PRIOR RL. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J Agric Food Chem 52: 4026–4037.

YAO Y, SANG W, ZHOU M, REN G. 2010. Antioxidant and alpha-glucosidase inhibitory activity of colored grains in China. J Agric Food Chem 58: 770–774.

ZHANG H, BARTLEY GE, MITCHELL CR, ZHANG H, YOKOYAMA W. 2011. Lower weight gain and hepatic lipid content in hamsters fed high fat diets supplemented with white rice protein, brown rice protein, soy protein, and their hydrolases. J Agric Food Chem 59: 10927–10933.

ZHANG MW, ZHANG RF, ZHANG FX, LIU RH. 2010. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. J Agric Food Chem 58: 7580–7587.

ZHAO H, DONG J, LU J, CHEN J, LI Y, SHAN L, LIN Y, FAN W, GU G. 2006. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). J Agric Food Chem 54: 7277–7286.

ZHOU S, FANG Z, LÜ Y, CHEN J, LIU D, YE X. 2009. Phenolics and antioxidant properties of bayberry (*Myrica rubra* Sieb. et Zucc.) pomace. Food Chem 112: 394–399.