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Short Communication

New red rice transgressive variants with high antioxidant capacity

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Abstract

Red coloured rice, has been shown to contain high levels of bioactive properties. The aim of this study was to quantify the major antioxidant compounds in the whole grain of two new red rice transgressive variants together with their parents which was determined by the oxygen radical absorbance capacity (ORAC) method, measured in methanol extract. A Thailand commercial red rice was used as a control. Although, the ORAC values for some red rice samples were similar, they were higher than light brown rice control, MR219. The antioxidant capacity was also evaluated by ferric-reducing antioxidant power assay. FRAP result was well correlated with ORAC (r = 0.94).

Introduction

Recently, a great attention has been paid to plants and foods rich in natural antioxidants, which have been associated with reduced risks of cardiovascular diseases in human. Phytochemicals are non-nutrient, bioactive, and naturally occurring plant compounds found in vegetables, fruits, and whole grains (Liu, 2004). Phytochemicals which are found in whole grains showed antioxidant activity, which results to the whole grain consumption health benefits (Liu, 2007). Whole-grain cereals cover a much higher level of compounds with remarkable antioxidant effect than do refined cereals (Fardet *et al*., 2008).

Rice is the most studied cereal in animal models, both its outer layer fractions (which are mainly rich in anthocyanin), or rice bran oil which has a high tocotrienol content (Fardet *et al.*, 2008). Rices with a red bran layer are termed red rice. In spite of the problem related with red rice as a weed, the pigment is of interest for nutritional value. It acts as powerful antioxidant that showed to decrease atherosclerotic plaque formation, a risk factor linked to cardiovascular disease (Ling *et al*., 2001). In Malaysia, few researches are being carried out to develop rice cultivar with red pericarp grain. Several varieties of rice, including coloured varieties, are expected to have a greater antioxidant capacity than white rice. Procyanidins (anthocyanin pigments) are the main compounds engaged in the antioxidant activity of red rice (Oki *et al*., 2002).

The antioxidant capacity of whole grains has been determined using many different antioxidant capacity assays. The oxygen radical absorbance capacity (ORAC) assay measures a sample's ability to prevent the oxidation of fluoroscein by a peroxyl radical compared to the ability of various concentrations of Trolox (Ou *et al*., 2002) and has been applied as a standard antioxidant capacity assay to measure the antioxidant activity of foods (Prior *et al*., 2005). Another method called FRAP assay was originally developed by Benzie and Strain (1996) which measures reduction of ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to a coloured product.

In our previous paper (Fasahat *et al*., 2012), we observed that red rice samples showed higher antioxidant activity based on ferric-reducing antioxidant power (FRAP) assay. We also found out that total phenolic content was higher in red rice samples but for vitamin E content genotype G37 was statistically at par with light brown rice control MR219. The present study was aimed at quantifying antioxidant compounds and investigation on relationship of two different antioxidant capacity measurement methods. Based on previous study (Lai *et al*., 2009), methanol extraction produced significantly higher yield and total content in phenolic compounds and tocols. Thus, it was used

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for antioxidant extraction.

Materials and Methods

Materials

Two red rice transgressive variants together with their parents (a commercial light brown rice named MR219 and a wild rice, *Oryza rufipogun*) and a commercial Thailand red rice were selected for this study (Fasahat *et al*., 2012). Chemicals used for FRAP analysis including 2,4,6,-Tris (1-pyridyl)-5-triazine (TPTZ) was purchased from Sigma (Steinheim, Germany), and ferrous sulphate was obtained from R&M Chemicals (Essex, UK). Chemicals used for ORAC including 2,2-Azobis (2-methyl propionamidine dihydrochloride) (AAPH), fluorescein sodium salt and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Co. (Malaysia).

Total antioxidant capacity assays

The antioxidant capacity of the extracts was assessed using the ORAC and FRAP assays. The ORAC assay was conducted according to Huang *et al.* (2002). The ORAC assay employs the area under the curve of the magnitude and time to the oxidation of fluorescein owing to peroxyl radicals produced by the addition of AAPH. The assay was carried out on a $FLUO_{star}$ OMEGA (BMG LABTECH, Germany) microplate reader utilising fluorescence filters with 485 nm excitation and 520 nm emission wavelength. All data were expressed as µmol of TE/kg.

The FRAP assay determines the capacity of antioxidants as reductants in a redox-linked colorimetric reaction of the reduction of Fe^{3+}-2,4,6-tri-pyridyl-S-triazine to a blue-coloured Fe2+ complex at low pH that is measured spectrophotometrically at 593 nm (Benzie and Strain, 1996). The extracts were incubated at room temperature with the FRAP reagent and the absorbance recorded after 1 h (Fasahat *et al*., 2012). The reducing power is expressed as µmol$FeSO_4$/g.

Statistical analysis

The data obtained were means ± S.D. of three determinations, and followed by the Student's t-test. Differences were considered to be statistically significant if $p < 0.05$. Pearson's correlation tests were performed to reveal possible associations between the antioxidant capacity assays, using statistical software package (SAS version 9.1.3, SAS Institute Inc. 2003).

Results

In this study, all the rice sample extracts could be analyzed using the ORAC assay. The antioxidant capacity of the rice sample extracts based on hydrogen atom transfer reaction was assayed using 2,20-azo-bis (2-amidinopropane) dihydrochloride (AAPH) as peroxyl radicals source and fluorescein sodium salt as molecular probes. The data presented in Figure 1 demonstrate the ability of the $FLUO_{star}$ OMEGA reader to perform the ORAC assay and measure antioxidant activity of samples. The dynamic curves of several different concentrations of Trolox standard displays different amounts of protection of fluorescein against oxidation that results in the loss of fluorescence. The highest concentration tested (50 µM) provided effectively full protection for approximately 240 minutes, before fluorescence intensity began to reduce, however the lowest concentration tested (3.125 µM) provided only slight protection above the buffer only control.

When net AUC are calculated from these dynamic curves (five standard concentration of Trolox) and plotted against Trolox concentration, a linear relationship was observed. The equation of the calibration curve was y = 234880.9x + 941019.8. By means of a least means squared linear regression analysis a coefficient of determination (r^2) of 0.99 was obtained. As a consequence, the determination Trolox equivalents of rice samples can be made with confidence.

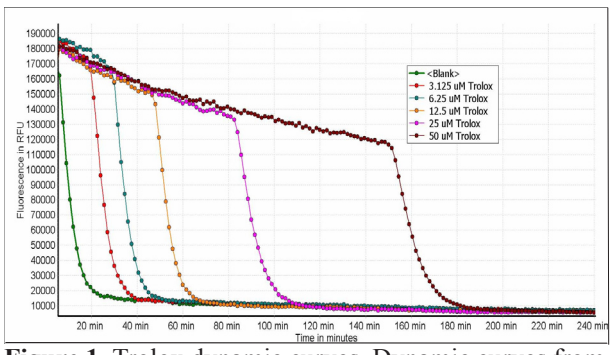

Figure 1. Trolox dynamic curves. Dynamic curves from an ORAC assay of Trolox antioxidant standards ranging from 0 to 50 µM were plotted using MARS Data analysis software (v. 2.10 R3). The reactions were initiated by the addition of 25 µl of AAPH (153 mM) solution.

The standard curve then inserted to quantitate rice samples. The results of determinations were expressed as Trolox equivalents per kg. The ORAC assay, as explained before, estimates the potential antioxidant of the sample under study from the area under the plotted curve of fluorescence versus time. The ORAC curves in Figure 2, present a clear lag

phase during which essentially no consumption of the fluorescein indicator dye occurs. This continues until the Trolox is consumed and the fluorescein is then also quickly oxidized. Antioxidants present in red rice samples completely neutralize the peroxyl radicals produced in the system, which slows down the decay of the fluorescence curve until a certain time. The length of the lag phase and the total area under the curve is proportional to the concentration of Trolox. The results obtained appear in Table 1.

Table 1. Contents of ORAC and FRAP in five rice genotypes (mean ± SD, n=3)

Sample	ORAC (µmol of TE/kg)	FRAP (µmol$FeSO_4$/g)
G33*(R14-3-66-4-B-B)†	4668.0 ± 1.97^c	107.3 ± 0.86^c
G37 (R19-2-93-3-B-B)	5650.6 ± 1.91^b	90.9 ± 0.99^d
MR219	3062.0 ± 0.71^d	69.7 ± 0.68^e
Thailand rice	5516.0 ± 1.00^b	111.5 ± 1.18^b
Oryza rufiogon	9550.0 ± 1.19^a	254.3 ± 1.89^a

*Code used in this study
†Mardi rice Gene Bank designation
Mean values within a column superscripted by the same letter are not significantly different at $p < 0.05$

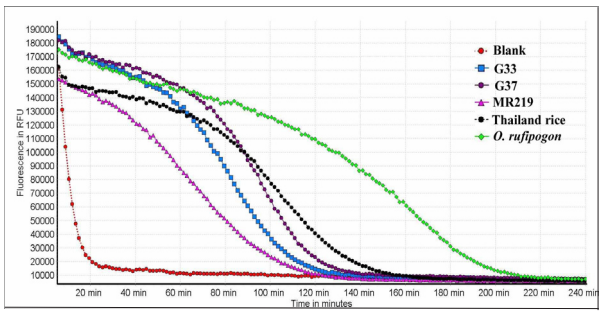

Figure 2. Fluorescence decay curve during the ORAC assay in the presence of various rice samples. The greater the extent of florescent decay, the smaller the expected area under curve value would be.

The results indicated that the antioxidant capacity varied significantly among the samples. Red rice samples were likely to be associated with high total antioxidant capacity. ORAC results showed similar trends to FRAP values for the different rice samples studied (Table 1). The ORAC values of rice samples ranged from 3062 to 9550 µmol of TE/kg for MR219 and *O. rufiogon* cultivars, respectively (Table 1). The ORAC was in the order of *O. rufiogon* > G37 > Thailand > G33 > MR219.

Data of Table 1 show that the ferric-reducing antioxidant power and ORAC value showed a similar pattern of change. Our results showed a correlation coefficient value of 0.94 between both methods for samples extract studied.

Discussion

The determination of antioxidant activity is generally, based on two main reactions, single electron transfer (SET), and hydrogen atom transfer (HAT) (Ou *et al.*, 2002; Huang *et al.*, 2005; Prior *et al.*, 2005). According to recommendations for antioxidant capacity methods standardization, it is obvious that only one antioxidant capacity assay will not really display the "total antioxidant capacity". The total antioxidant capacity demands at least two methods for determination of antioxidant capacity owing to reflect and differentiate both single electron transfer (radical reducing) and hydrogen atom transfer (radical quenching) (Prior *et al.*, 2005). As a result, the present study used two methods (FRAP and ORAC assay) which are used in most of rice studies (Butsat and Siriamornpun, 2010; Min *et al.*, 2011) with different procedure to determine the antioxidant capacity. FRAP assay was based on single electron transfer whereas ORAC assay was based on hydrogen atom transfer (Prior *et al.*, 2005).

ORAC values obtained in this study corresponded well with those found by others but were somewhat lower. In a study by Chanphrom (2007), wide range of variations for antioxidant capacity in purple rice varieties were observed which ranged from 37.91 to 239.29 µmole TE/g in ORAC assay; While, antioxidant capacity of glutinous pigmented rice ranged from 46.93 to 79.21 µmole TE/g in FRAP assay and 127.91 to 207.23 µmole TE/g in ORAC assay. The ORAC activity in the present study was higher than those reported by Aguilar-Garcia *et al.* (2007) in which three varieties of brown rice was about 14-18 µmole TE/g dry basis. The higher values in this study might be due to the colour of the samples (Chanphrom, 2007). According to Liyana-Pathirana and Shahidi (2007), the ORAC value of two wheat cultivar whole grain varied from 95 ± 5 to 100 ± 1 µmol TE/g (defatted sample).

In this study red rice had higher antioxidant capacity than light brown rice control. Previous studies showed that coloured varieties of cereals, such as rice, have more antioxidant capacity than non-coloured varieties (Hu *et al.*, 2003). In a study by Qiu *et al.* (2010), the white rice control had the lowest ORAC value (2534 µmol TE/100 g) and for wild rice samples, the ORAC values varied from 8346 to 11903 µmol TE/100 g. The ORAC-index has previously shown to depend on the target molecule used (López-Alarcón and Lissi, 2006). Methanolic extracts of grains consumed in Korea (red sorghum and black rice) were also shown to have higher antioxidant activities than white rice, brown rice,

foxtail millet, proso millet and barley (Fardet *et al*., 2008).

Correlation between FRAP and ORAC

It has been reported before that antioxidant capacity determined by in vitro assays differs (Ou *et al*., 2002; Wu *et al*., 2004; Wootton-Bearda *et al*., 2010). As such, it is not uncommon that the FRAP and ORAC assays may or may not correlate depending on the food system being tested (Aguilar-Garsia *et al*., 2007). For example, Ou *et al*. (2002) found no correlation of antioxidant activity between the FRAP and ORAC assays among most of the 927 freeze-dried vegetable samples, while these methods revealed high correlation in blueberry fruit (Connor *et al*., 2002) and in berries (Moyer *et al*., 2002). The results of this study showed highly significant correlation between ORAC assay and FRAP assay with $r = 0.94$ at $p \leq 0.01$ similar to Chanphrom (2007) ($r = 0.87$ at $p \leq 0.01$). Thaipong *et al*. (2006) also found high correlation coefficient among ORAC and FRAP assays in guava fruit extracts ($r = 0.74$, $P < 0.05$).

Conclusion

The methanol extracts of red rice displayed up to 2 times higher antioxidant capacity than light brown rice control by using FRAP and ORAC methods, which signifies that more health benefits may be achieved by consuming whole grain red rice than white rice.

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