Evaluation of antioxidant properties of bran and grain in 10 genotypes of rice (*Oryza Sativa* L)

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Abstract

This study aimed to appraise the chemical features such as total phenol (TP), total flavonoid (TF), and total antioxidant capacity based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays of bran and grain in 10 genotypes of rice (*Oryza sativa* L.) grown in Iran. Based on two procedures of DPPH and FRAP, the results demonstrated that in terms of the amount of antioxidants in the bran of genotypes of the tested rice, genotype G4 achieved the highest and genotype G6 achieved the lowest mean. Also, the examination of the amount of the antioxidant in genotypes grain of the tested rice showed that genotype G4 had the highest and genotype G6 had the lowest mean in the antioxidant of the grain. Besides, the examination of phenol and flavonoid levels of grain and the bran of the tested genotypes indicated that genotype G3 had the highest TP level and the genotype G4 had the highest flavonoid level in this experiment and they were placed in group A in the mean comparison table. The correlation result between phenolic compounds and antioxidant procedures confirmed that there are significant correlations among traits in this study.

Keywords: Antioxidant capacity; flavonoids; phenol; phytochemicals; rice

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Introduction

Rice is one of the basic cereals of ordinary diet in people's life which is known as vastly cultivated food crops in the world. It is the primary staple food for the population of at least half of the world, for the most part in developing countries (Iqbal and et al., 2005; Monks and et al., 2013). As Deng et al. (2013) argued, rice turns out to be increasingly significant because of the exclusive compounds such as phenolics that have been proved to have benefits for human health. This has caused a multitude of research studies for comprehensive analysis of these compounds in the grain (Walter et al., 2013; Abdelnaser et al., 2017).

In cereal grains, phenolic compounds are placed among the phytochemicals that are typically taken into account as natural antioxidants (Tian and et al., 2004). The most recognized phenolics founded in rice consist of the phenolic acids which contain mostly ferulic acid

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(FA) and diferulates, which exist in kernels of NPR (Tian et al., 2004) while ferulic and p-coumaric acids are the basic elements found in the rice bran (Wanyo and et al., 2014).

Rice bran is considered as an abundant source of natural antioxidants that can be utilized as the free radical scavengers. It is broadly perceived that large numbers of today's diseases are attributable to the oxidative stress that can be explained as a result of an inconsistency between the formation and neutralization of free radicals. The rice bran oil antioxidants have large effects in decreasing low-density lipoprotein and total serum cholesterol (Moldenhauer et al., 2003; Butsat et al., 2009; Jung, 2010; Raghavendra, 2010).

Increasing interest in utilization of natural antioxidants and the high efficiency of plant compounds in the creation of antioxidant properties due to the presence of phenolic compounds especially phenolic acids and flavonoids (Chao et al., 2018) have led us to investigate further the antioxidant properties of rice cultivars in the present study.

Materials and Methods

Extraction

To start, the bran and grain from 10 genotypes of rice (*Oryza sativa* L.) grown in Iran (Table 1) were weighed separately and then powdered. Then, 10 grams of powdered sample was mixed with 200 extraction solvent containing methanol 80%. After centrifugation for 15 min at 1000 rpm, the specimens were preserved at room temperature for 12 hours. Subsequently, the supernatant of the samples was gently removed and stashed away at −20 °C, until it was required for analysis (Jun and et al., 2012).

DPPH free radical scavenging activity

In order to calculate and determine the activity of DPPH free radical scavenging, first, the prepared extract (50 µl) was blended with methanolic DPPH solution (1000 µl of 6×10^{-5} mol L^{-1}) which was then left in a dark environment at room temperature for 30 minutes. Finally, the absorbance was calculated by means of a spectrophotometer (515 nm). Based on the following equation, the decrease in the percentage and DPPH reduction was estimated (Nakajima and et al., 2004):

$$\%\ Inhibition\ of\ DPPH = \frac{Abs\ control - Abs\ sample}{\text{Abs control}} * 100$$

where Abs control is the absorbance of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution without the extract.

The assay of Fferric-reducing antioxidant power (FRAP)

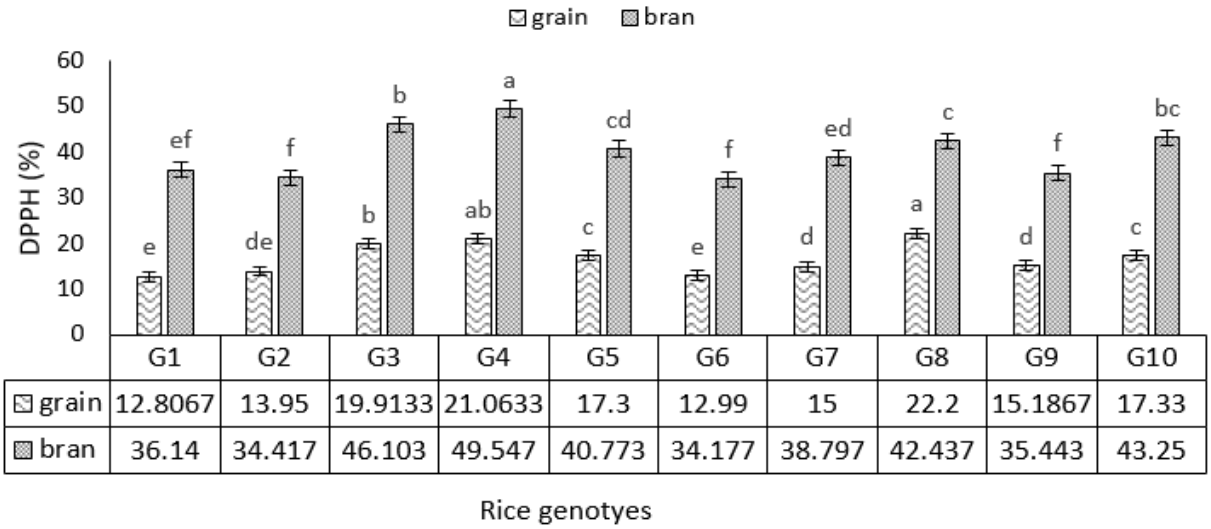
Before measurements, the FRAP reagent was quickly prepared and formulated by mixing 100 mM acetate buffer (pH 3.6), 10 mM 4,6-tripryridyls-triazine (TPTZ) in 40 mM HCl, and 20 mM ferric chloride in a ratio 10:1:1 (by volume). One hundred microliters of extracts were mixed with 4.9 ml of FRAP reagent and incubated at 37 °C. After 10 minutes, the absorbance of samples was scrutinized at 593 nm and the outcomes were displayed as mg Fe^{2+}/100 g (Benzie and et al, 1996).

Total phenolic (TP) determination

Folin–Ciocalteu colorimetric method was selected to assay the TP content in extracts according to Slinkard and Ingleton (1977). The resultants were calculated and represented as mg of GAE/100g sample.

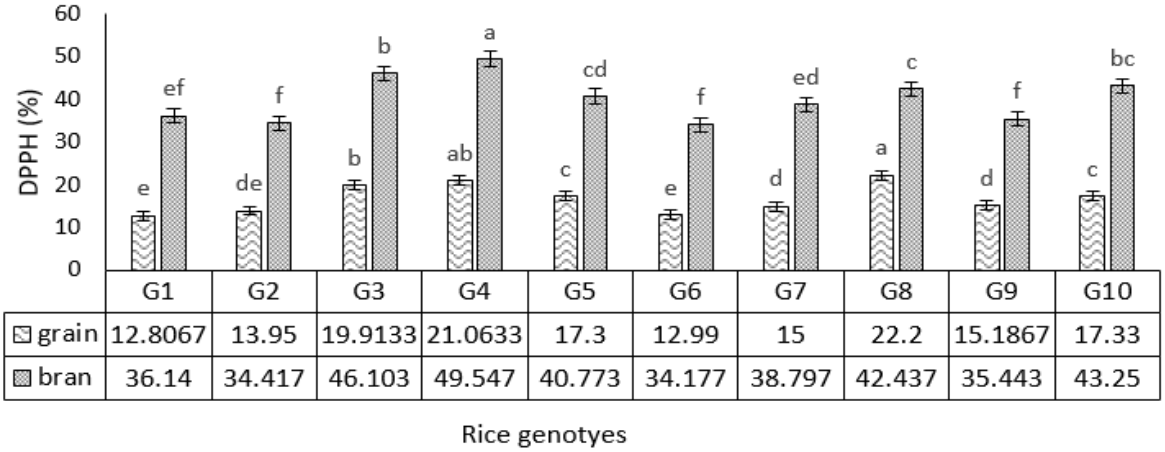
Table 1
Name of genotypes

Genotypes name	number of Genotypes
Tarom Mahali	G1
Domsiyahe Mashhad	G2
Hashemi	G3
Anbarboo Ilam	G4
Salari	G5
Sangju	G6
Shahpasand	G7
Gharib	G8
Zire Bandepey	G9
Ghashanghe	G10



	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
grain	12.8067	13.95	19.9133	21.0633	17.3	12.99	15	22.2	15.1867	17.33
bran	36.14	34.417	46.103	49.547	40.773	34.177	38.797	42.437	35.443	43.25

Fig. I. DPPH assays of free radical scavenging activity in rice bran and grain.; values are significantly different at p<0.01.



	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
grain	12.8067	13.95	19.9133	21.0633	17.3	12.99	15	22.2	15.1867	17.33
bran	36.14	34.417	46.103	49.547	40.773	34.177	38.797	42.437	35.443	43.25

Fig. II. Values for FRAP assays of rice grain and bran free radical scavenging activity are significantly different at p<0.01.

Total flavonoid (TF) determination

Bound and free extracts (0.2 mL) were blended with both sodium nitrite (0.15 mL 5% w/v) and aluminium chloride hexahydrate (0.15 mL 10% w/v). The blend was kept in a place for 5 minutes, and then NaOH (1 mol/L) was added (Bao, and et al., 2005). The absorbance was recorded at 510 nm after 30 minutes. Mg of catechin equivalents (mg CATE) per 100 g sample were selected to express the results.

Statistical Analysis

A completely randomized design was selected to conduct this study. Analysis of variance was performed and means were calculated for all data and separated by Duncan's multiple range test ($p<0.01$) using SAS (Version 9.2). Inferential analysis including factor analysis and correlation were done using SPSS (Version 22).

Results

DPPH radical scavenging activity

Currently, by expanding the study on the antioxidant activity in the field of nutrition and food science, the evaluation of antioxidant capacity has necessarily increased based on different assay systems. The evaluation of the capacity of antioxidant in rice grain and bran according to FRAP and DPPH assays showed that there are differences between two methods in the evaluation of antioxidant content of the studied rice cultivars (Figs. I and II).

Table 1
Total phenol (TP) and total flavonoid (TF) contents of bran and grain of rice (*Oryza sativa* L.)

genotypes	TP(grain)	TP(bran)	TF (grain)	TF (bran)
G1	96.71^{f}	177.66^{ef}	41.73^{d}	65.50^{g}
G2	98.86^{def}	188.77^{d}	44.69^{cd}	71.90^{f}
G3	123.43^{a}	306.66^{a}	56.08^{a}	81.03^{b}
G4	114.85^{b}	295.26^{b}	57.35^{a}	88.46^{a}
G5	105.86^{c}	189.33^{d}	42.067^{d}	75.93^{de}
G6	98.37^{ef}	173.47^{f}	51.36^{b}	76.77^{cd}
G7	88.33^{g}	182.84^{de}	47.66^{c}	73.06^{ef}
G8	112.84^{b}	185.58^{de}	55.58^{a}	79.12^{bcd}
G9	102.93^{cde}	173.83^{f}	51.91^{b}	70.62^{f}
G10	103.62^{cd}	271.17^{c}	43.56^{d}	79.99^{bc}

Values in the same column with different lowercase letters are significantly different at $p<0.01$.

Results demonstrated that the maximum capacities of bran and grain antioxidant were observed in genotype G4 (Anbarboo Ilam) (22.2%) and (49.547%) based on the DPPH assay, respectively. Also, the lowest grain and bran antioxidant capacities were observed in G6 (Sangju) genotype (12.323%) and (34. 177%) based on the DPPH assay, respectively.

Ferric-reducing antioxidant power (FRAP) assay

As the findings indicated in the Fig. II, according to the FRAP assay, the maximum capacities of antioxidant in rice bran and grain were observed in G4 (Anbarboo Ilam) genotype (95.51%) and (44.87%), respectively. In addition, the lowest capacities of antioxidant in bran and grain were observed in G6 (Sangju) genotype (58.71, 33.25%, respectively based on the FRAP assay.

Total Phenol (TP)

As it is demonstrated in Table 2, the TF and TP contents variations of the genotypes under study were statistically different ($p<0.01$). Findings indicated that the maximum TP contents in rice bran and grain were 306.667 and 123.433 mg GAE/100 g, respectively observed in G3 (Hashemi) genotype. Also, the minimum TP contents in the rice bran and grain were 173.473 and 88.333 mg GAE/100 g respectively, which were detected in G6 (Sangju) and G7 (Shahpasand) genotypes.

Total Flavonoid (TF)

Based on the results (Table 2) the maximum TF content of grain was 57.537 mg CAT/100 g, recorded in G4 (Anbarboo Ilam) genotype and the minimum grain TF content was 41.700 mg CAT/100 g observed in G1 (Tarom Mmahali) genotype. Besides, the results also showed that the maximum bran TF content was obtained in G4 (Anbarboo Ilam) genotype (88.467 mg CAT/100 g) and the minimum TF content of the bran was recorded in G1 (Tarom Mahali) genotype (65.503 mg CAT/100 g). The mean value of TF contents of bran and grain in the study were 76.242 and 49.198 mg CAT/100 g, respectively.

The correlation

As Forde et al., (1975) argued, the correlation among traits can be utilized in breeding programs. Table 3 demonstrates a significant correlation between antioxidant methods and phenolic compounds. There were positive significant correlations between TP (grain), and TP (bran) (0.713*), DPPH (grain) (0.812**), DPPH (bran) (0.742*), FRAP (grain) (0.837**), TF (grain) (0.640**), and TF (bran) (0.684**). In addition, DPPH (grain) had a positive significant correlation with FRAP (grain) (0.881**), FRAP (bran) (0.792*), DPPH (bran) (0.869**), TF (grain) (0.640*), and TF (bran) (0.783**). Albeit, DPPH (grain), FRAP (grain), FRAP (bran), and TF (grain) did not correlate with TP (bran). Formerly, a positive correlation was established between

Table 2
Pearson's correlation coefficients of antioxidant capacity (DPPH and FRAP assays), total phenols (TP), and total flavonoids (TF) in bran and grain of rice (*Oryza sativa* L.)

	TP (grain)	TP (bran)	DPPH (grain)	DPPH (bran)	FRAP (grain)	FRAP (bran)	TF (grain)	TF (bran)
TP (grain)	1	0.71*	0.81**	0.74*	0.83**	0.47 ns	0.64*	0.68*
TP (bran)		1	0.60 ns	0.85**	0.57 ns	0.52 ns	0.41 ns	0.76**
DPPH (grain)			1	0.86**	0.88**	0.79**	0.64*	0.78**
DPPH (bran)				1	0.79**	0.80**	0.50 ns	0.84**
FRAP (grain)					1	0.66*	0.62 ns	0.55 ns
FRAP (bran)						1	0.26ns	0.58 ns
TF (grain)							1	0.63*
TF (bran)								1

ns, no significant; *, significant at p≤0.05; **, significant at p≤0.001

phenolic content of rice straw extract and DPPH radical scavenging activity.

Discussion

Form total phenols' perspective, the difference among rice cultivars is due to discordance in their cultivars and genotypes. Previous studies have also suggested the effects of climate change on the amount of total phenols (Miller and Engel, 2006; Kadir et al, 2009; Laokuldilok et al, 2011). As Dykes and Rooney (2007) put, the genotypic variety of some phytochemicals in rice bran layers has been broadly determined. For example, Bergman and Xu (2003) stated the effects of genotype and environment on rice tocopherol, tocotrienol, and g-oryzanol contents. Another report by Miller and Engel (2006) showed the g-oryzanol contents in brown rice (Miller and Engel, 2006).

As Ahmad et al., (2015) stated, plants due to oxidative conditions in their environment have different types of antioxidants with a verity of structures and functions, therefore, it is not possible to utilize a single prediction method in the parameters involved in oxidative capacity. Consequently, both DPPH and FRAP methods were practiced in the study and the findings showed a difference between the two methods in the rice antioxidant contents, which is similar to studies reported on other plants (Chao Ding and et al., 2018).

The results of the present study demonstrated that there is statistically a significant difference between the studied genotypes in total phenol, flavonoid, and antioxidant contents due to the differences in genotypes and their environmental conditions. Moreover, these results can be utilized for applying in the breeding programs and presenting superior genotypes (Chao Ding and et al., 2018).

Among the rice genotypes studied, G3 (Hashemi) and G4 (Anbarboo Ilam) were identified as superior genotypes due to their high antioxidant capacity and phenolic and flavonoid compounds. The differences between genotypes for phenol and flavonoid could be due to genetic differences between genotypes and differences in their origin and environmental conditions. Previous studies have confirmed the role of habitat as an effective factor in the accumulation of secondary metabolites in some plants. In view of the correlation between phenol and antioxidant levels, a significant difference was observed in the antioxidant content of rice genotypes (Hemmati et al., 2006; Gohari et al., 2011; Saboura et al., 2014).

In this study, it was observed that the antioxidant capacity and phenolic and flavonoid compounds in rice bran were higher than those in rice grain, which is similar to the results of the study carried out by Chao et al. (2018) and the finding could be attributed to genetics. The diversity of the antioxidant activity of different organs of a plant has also been observed in previous studies and has been attributed to the presence of natural antioxidants such as polyphenols that vary depending on the action of the organs (Matkowski et al., 2008; Megdiche et al., 2009). It should be noted that the existence of this difference may be due to genetic diversities among different rice varieties or the

environmental conditions in which the cultivar is present. Shun et al. (2005) in his study reported that differences are to be effective between sampling time and the plant tissue analyzed. Since rice is a staple food for more than half of the world's population, specifically Asia, it is recommended to use mature rice for optimum use of this crop with higher antioxidant capacity and more phytochemicals compounds.

Conclusion

DPPH and FRAP methods were different in the bran and grain rice antioxidant contents. Also, a significant interaction effect of phenol and flavonoid antioxidant was found in rice. G3 (Hashemi) and G4 (Anbarboo Ilam) genotypes were recognized as superior genotypes owing to their high antioxidant capacity and phenolic and flavonoid compounds. Also, the results of this study suggest the use of two varieties of Anbarboo Ilam and Hashemi for further studies in improving nutrition and providing nutrients needed for human body. It is recommended to develop stepped-up programs by agricultural and governmental organizations as well as the collaboration of nutritionists to efficient consumption of whole rice in the human diet. Implementation of such a strategy will help to protect consumers' health and reduce many of the problems associated with nutrition.

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