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*Full Length Research Paper*

# Study on phenolic compounds and their antioxidant activities in Iranian rice varieties

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**The analytical method for the determination of phenolic compounds in Iranian rice varieties was developed. The method consisted of extraction of phenolic compounds from rice before analysis by high performance liquid chromatography (HPLC). Reversed phase HPLC equipped with photodiode array detection was used and the separation condition was optimized. Under the optimum condition, twelve phenolic compounds were separated within 24 min. Pressurized liquid extraction (PLE) was used to extract free phenolic compounds from rice with the optimum extraction condition of 70% methanol and extraction time of 15 min. While bound phenolic compounds were extracted using alkaline hydrolysis for 15 min, six varieties of Iranian rice including pigment and non-pigment rice in their brown and polished forms were investigated. All of the 12 phenolic compounds were detected as free phenolic compounds in all samples. Ferulic acid was the most abundant free phenolic compounds in all samples, while ferulic acid and p-coumaric acid were found as bound phenolic compounds in some samples. The content of phenolic compounds, total flavonoid and antioxidant activity detected in pigment rice and brown form were higher than non-pigment rice and polished form.**

**Key words:** Phenolic compounds, antioxidant activities, rice.

## INTRODUCTION

Rice (*Oryza sativa L*) is the most important cereal crop in the world, either directly as human foods or indirectly as animal feeds. It is the staple food of over half the world's population (Zhai et al., 2001). Similar to cereal grains, rice is rich in many nutrient components including carbohydrates, proteins, certain fatty acids and micronutrients (vitamins and trace minerals). They are also sources of many bioactive non-nutrient compounds, known as antioxidant, including phenolic compounds (Frei et al., 2004). Phenolic compounds are the secondary metabolites with a large range of structures and functions, but generally possess an aromatic ring bearing one or more hydroxyl substituents (Liu, 2007). They are widely distributed in the medicinal plants, spices, vegetables, fruits, grains, pulses and other seeds (Stratil et al., 2007).

Phenolic compounds exhibit a wide range of physiological properties such as anti-allergenic, anti-artherogenic, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effect (Pupponen-Pimiä et al., 2001). It has been recognized that health benefits can be achieved from consuming high levels of fruits and vegetables. The beneficial effects derived from phenolic compounds could be a major determinant of antioxidant potentials of foods (Heim et al., 2002) and could therefore be a natural source of antioxidants. Phenolic compounds in plants exist either in the free form or the bound form. Generally, the free phenolic compounds are proanthocyanidins or flavonoids, while the bound

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phenolic compounds are ester-linked to cell-wall polymers (Bonoli et al., 2004). Phenolic compounds commonly present in whole grains are phenolic acids and flavonoids (Al-Farsi and Lee, 2008). The common phenolic acids found in whole grains are ferulic acid, vanillic acid, caffeic acid, syringic acid and p-coumaric acid (Sosulski et al., 1982), while flavonoids are flavonols, flavan-3-ols, flavones and flavanones (Lin and Tang, 2007). In addition, cereal grains have been reported to contain special phenolic compounds, such as ferulic acid and diferulates, which are not present in significant quantities in fruits and vegetables (Tian et al., 2005). Ferulic acid has been known as an antioxidant which is effective toward anti-inflammation and inhibition of tumor initiation and as a preservative (Adom et al., 2003). Therefore, consumption of large amounts of rice is considered to result in both nutritional (calories) and health benefits. There are a number of literature reports on phenolic compounds and their antioxidant activities in cereal, fruit and vegetables. However, there are few literature reports on rice (Tian et al., 2005; Zhou et al., 2004).

The analysis and determination of phenolic compounds in plants is usually performed in three steps. Firstly, extraction of phenolic compounds from samples, either in free or bound form. Next, clean up of the extracts to eliminate interferences or, in some cases, to pre concentrate the phenolic compounds. Finally, analysis of the phenolic compounds in the extracts is performed. All steps are important to provide accurate and precise results.

Isolation of phenolic compounds from sample matrices is generally based on extraction. A wide variety of extraction techniques have been used to extract free phenolic compounds from plant materials, such as ultrasonic assisted extraction (Hung and Morita, 2008), the shake-flask technique (Madhava et al., 2008), supercritical fluid extraction (Martins et al., 2006), soxhlet extraction (Schantz, 2006), microwave assisted extraction (Palma and Taylor, 1999) and pressurized liquid extraction (Bonoli et al., 2004). An automated system of pressurized liquid extraction (PLE) becomes an interesting alternative to conventional time-consuming solideliquid extraction because it utilizes organic solvent at high temperature and pressure to extract analytes (Govindarajan et al., 2007). PLE is done in an inert atmosphere and is protected from light. This is very convenient for the purposes of automation, shorter extraction time, lower solvent consumption and on-line coupling of the extraction and separation techniques (Chirinos et al., 2008). On the other hand, bound phenolic compounds can be extracted from plant materials using alkaline hydrolysis and acid hydrolysis.

Most researchers determined the bound phenolic compounds in cereal flours by alkaline hydrolysis (Bonoli et al., 2004; Tian et al., 2004; Zhou et al., 2004). The reactions were left to proceed at room temperature for 15 min up to overnight. Some investigations have reported that the reactions are carried out in the dark, as well as under an inert atmosphere, that is, argon or nitrogen gas. In only a few reports, the bound phenolic compounds of cereals have been determined by using acid hydrolysis (Bonoli et al., 2004; Kosar et al., 2003). Reversed phase-high performance liquid chromatography (RP-HPLC) has been accepted as the most useful tool for the qualitative and quantitative analyses of phenolic compounds (Bonoli et al., 2004; Zhou et al., 2004). Various detectors are equipped with RP-HPLC including Ultraviolet-visible spectroscopy (UV-Vis or UV/Vis) (Zhou et al., 2004), photodiode array (PDA) (Bonoli et al., 2004), fluorescence (Aoun et al., 2005) and mass spectrometer (MS) (Tian et al., 2005). Since phenolic compounds possess antioxidant activities, it is worthwhile to investigate their antioxidant activities along with their quantities. DPPH assay is the commonly used method for the evaluation of free radical scavenging activity (Chew et al., 2008; Herrera and Luque de Castro, 2005). The method is simple, polarity-independent, very rapid and reproducible (Tian et al., 2004). In addition, no expensive reagents or sophisticated instruments are required (Nara et al., 2006).

Therefore, the aim of this study is to develop an analytical method for the determination of phenolic compounds and their antioxidant activities in rice. The method comprises extraction of phenolic compounds using pressurized liquid extraction for free phenolic compounds and alkaline hydrolysis for bound phenolic compounds, and analysis of phenolic compounds by RP-HPLC.

## MATERIALS AND METHODS

### Chemicals and reagents

Phenolic compound standards of highest purity were used. Gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid and caffeic acid were obtained from Acros (USA), whereas syringic acid, 3-hydroxybenzaldehyde, p-coumaric acid, guaiacol, p-cresol, o-cresol and 3,5-xylenol were purchased from Fluka (Switzerland). Ferulic acid was obtained from Sigma (Germany). Deionized water obtained from RiOs type I Simplicity 185 (MilliporeWaters, USA) with the resistivity of 18.2 MU cm was used throughout the experiments. Methanol and acetonitrile of HPLC grade were obtained from Lab-Scan Asia (Thailand). Acetic acid of analytical reagent (AR) grade was obtained from Carlo Erba (Italy). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was from Sigma (USA). The mobile phase was filtered through a 0.45 mm membrane filter (Millipore) and degassed prior to use.

### Instrumentation

The HPLC system comprised a Waters 600E multisolvent

**Table 1.** The varieties and abbreviations of rice samples studied.

| Variety | Abbreviation | Description |
|---|---|---|
| Hashemi rice | PH | Polished Hashemi rice |
| | BH | Brown Hashemi rice |
| Sadri rice | PS | Polished Sadri rice |
| | BS | Brown Sadri rice |
| Neda rice | PN | Polished Neda rice |
| | BN | Brown Neda rice |
| Red tarom rice | BRT | Brown Red Tarom rice |
| Glutinous rice (Ali kazemi) | BA | Brown Glutinous rice |
| Glutinous rice (Tarom) | BGT | Brown Glutinous rice |

delivery system, a Waters in-line degasser AF, a Rheodyne injector with a sample loop of 20 mL, a Waters 2996 photodiode array detector and a Waters temperature control system. The Empower software was used for data acquisition. Separations were performed on a Symmetry column (3.9 mm i.d.150 mm, 5 mm particle diameter) coupled to a guard column. A Pressurized Liquid Extractor ASE-200 (Dionex, Sunnyvale, CA, USA) was used for the extraction. The extraction cell volume was 11 mL, the collection vial was 30 mL and cellulose filters were used.

**Rice samples**

Six varieties of Iranian rice which were harvested in 2013 were obtained from the market of Gilan Province. Sadri, Neda and Ali kazemi varieties are non-pigment (white) rice, while Tarom variety is red rice and Tarom variety is brown rice. These grains were milled using Satake THU35A (Japan) to separate husk from brown rice. The brown rice was then polished to obtain polished rice. The samples were stored at 4°C in vacuum package. Table 1 summarizes the varieties and abbreviations used in this study.

**Sample preparation**

The rice grain was ground into a fine flour using Moulinex Optiblend 2000 blender prior to analysis.

**Extraction of free phenolic compounds**

Two Whatman No. 42 filter papers were placed on a cellulose filter (P/N 049458) at the outlet end of the extraction cell. An accurately weighed rice flour (2.5 g) and silica gel (6 g ) were loaded into the cell and then a filter paper No. 42 was placed on the top of the sample. The PLE condition was 70% (v/v) methanol, extraction time of 15 min at room temperature under pressure of 1500 psi. The PLE extract was evaporated and the residue was adjusted to pH 2 using 6 mol/L HCl. The solution was made to a final volume of 10.0 mL and then passed through a solid phase cartridge.

The solid phase cartridge (Oasis HLB) was first conditioned with 5 mL of methanol, followed by 5 mL of water and then the extract solution was loaded. The cartridge was washed first with 0.1 mol/L HCl (5 mL) and the phenolic compounds were then eluted with 2.0 mL of aqueous methanol (1:9 v/v). The solution was evaporated to dryness. The dry residue was dissolved in 2 mL methanol. The obtained solution was divided for further analysis.

For HPLC analysis, internal standard (IS) of 150 mL was added and the volume was made up to 2 mL with methanol and was then analyzed by HPLC after filtering through a 0.2 mm nylon filter. For the determination of total phenolic compounds, total flavonoid and antioxidant activity, the solution was diluted with methanol to the final volume of 10 mL before analysis. All analyses were performed in triplicate.

**Extraction of bound phenolic compounds**

Bound phenolic compounds were extracted using the method of Nara et al. (2006) with some modification. Accurate weight of rice flour (10 g) was shaken with 70% (v/v) methanol for 24 h at ambient temperature. The supernatant was filtered through a Whatman No. 42 filter paper. The rice residue was washed with acetone and then dried, resulting into the alcohol-insoluble solid (AIS). Weight of AIS (2.5 g) was shaken with 2 mol/L NaOH (30 mL) at room temperature under nitrogen gas for 15 min. The mixture was acidified to pH 2 with 6 mol/L HCl and then extracted three times with 30 mL of hexane to remove lipid. The solution was finally extracted three times with 30 mL ethyl acetate. The organic fractions were pooled and evaporated to dryness. The dry residue was analyzed in the same manner as described in the

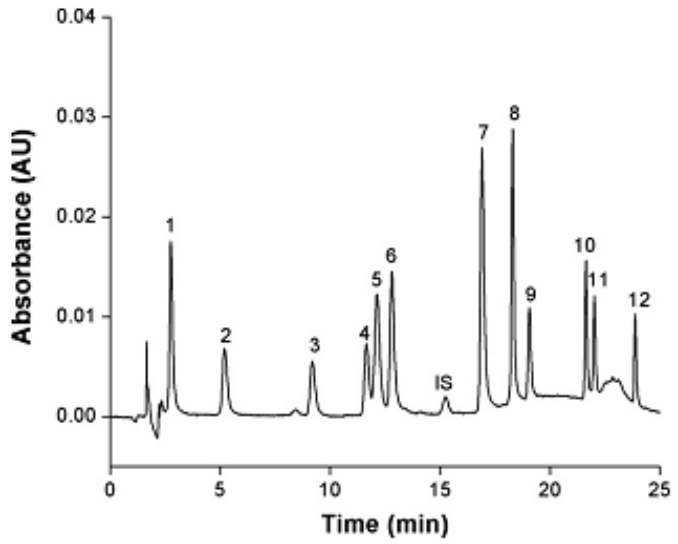


**Figure 1.** Typical chromatogram of standard phenolic compounds (3.0 mg/L each).
Conditions: symmetry C18 column (3.9 mm_150 mm), gradient elution of ACN and 1.0% (v/v) acetic acid (see in text) with a flow rate of 0.8 mL/min, injection volume of 20 mL, separation temperature of 30°C and detection at 280 nm.
Peak assignments: 1, gallic acid; 2, protocatechuic acid; 3, p-hydroxybenzoic acid; 4, vanillic acid; 5, caffeic acid; 6, syringic acid; IS (internal standard), 3-hydroxybenzaldehyde; 7, p-coumaric acid; 8, ferulic acid; 9, guaiacol; 10, p-cresol; 11, o-cresol; and 12, 3,5-xylenol.

foregoing.

### Determination of total phenolic content (TPC)

TPC was determined by Folin-Ciocalteu method (Bonoli et al., 2004). The extracts (free and bound phenolic compounds) of 0.10 mL were introduced into test tubes and then 0.50 mL of Folin–Ciocalteu reagent (FCR) and 6 mL of deionized water were added. After incubation for 2 min, 2 mL of 15% sodium carbonate was added, after which it was left for 0.5 min and its volume was made to 10 mL with water. The absorbance was measured at 755 nm after incubation for 2 h. Gallic acid was used as chemical standard of calibration. The TPC was expressed as mg gallic acid equivalent (GAE) per 100 g of rice (mg GAE/100 g).

### Determination of total flavonoid content (TFC)

Total flavonoid content of samples was determined by using a colorimetric method (Sakanaka et al., 2005). Aliquot (1 mL) of the extract (free and bound phenolic compounds) was placed in a 10 mL volumetric flask containing 5 ml of deionized water. Then 0.3 mL of 5% sodium nitrite was added, after 5 min, 0.3 mL of 10% aluminum chloride was added. After 6 min, 2 mL of 1 mol/L sodium hydroxide was added and diluted to volume with deionized water. The absorbance was measured immediately at 510 nm. The content of total flavonoids was calculated from the calibration curve of catechin standard. Measurements were calibrated to a standard curve of prepared catechin solution, and were expressed as mg catechin equivalent (CE) per 100 g rice (mg CE/100 g).

### Determination of antioxidant activity by DPPH method

In this study, DPPH method (Brand-Williams et al., 1995; Chew et al., 2008) was used to determine free radical scavenging property of phenolic compounds. For each solution, different concentrations were tested using ascorbic acid as a standard for calibration and also calculated as mg ascorbic acid equivalent per 100 g rice (mg AA/100 g). Aliquot of 3 mL of $5 \times 10^{-5}$ mol/L DPPH solution was added into the extract of 1 mL. Subsequently, the mixture was kept for 30 min in the dark, and the absorbance was measured at 515 nm. Antioxidant activity of the sample was expressed as $EC_{50}$ and ascorbic acid equivalent. $EC_{50}$ is defined as the amount of antioxidant required to reduce the initial DPPH concentration by 50%.

### Statistical analysis

All experiments were conducted in triplicates and the results were expressed as mean±SE. Significance of mean differences between chemical compositions and bioactive compounds within different rice varieties were statistically compared using an analysis of variance (ANOVA) at the 5% probability level ($p \leq 0.05$).

## RESULTS AND DISCUSSION

### Chromatographic separation of phenolic compounds

The chromatographic separation was carried out using gradient elution of acetonitrile (ACN) and 1% (v/v) acetic acid with a flow rate of 0.8 mL/min. The gradient condition was performed as follow: 7% ACN (initial step), ramped to 15% ACN (0 - 10 min), ramped to 35% ACN (10 - 15 min), and then ramped to 55% CAN (15 - 20 min). After that, ramped to 100% ACN (20 - 25 min) and was held for 15 min for washing the column. The chromatograms were recorded at 280 nm. Using this condition, twelve studied phenolic compounds and 3-hydroxybenzaldehyde (IS) were successfully separated within 25 min (Figure 1) with the order of elution: gallic acid (GAL), protocatechuic acid (PRO), p-hydroxybenzoic acid (p-HYD), vanillic acid (VAL), caffeic acid (CAF), syringic acid (SYR), 3-hydroxybenzaldehyde (IS), p-coumaric acid (p-COU), ferulic acid (FER), guaiacol (GUA), p-cresol (p-CRE), o-cresol (o-CRE), and 3,5-xylenol (XYL), respectively. The other works used more complicate mobile phases than this work such as Zhou et al. (2004) who used ternary mixture mobile phase with 55 min-gradient cycle to separate 6 phenolic compounds.

**Table 2.** Analytical characteristics for determination of phenolic compounds.

| Peak | Phenolic compound | Linear range (mg/L) | Linear equation | $R^2$ | LOD (mg/L) | Intra-day, RSD (%), n = 5 | | Inter-day, RSD (%), n = 3×5 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $t_R$ | Peak area | $t_R$ | Peak area |
| 1 | Gallic acid (GAL) | 0.050-6.0 | Y =6.26 _ 104× _6.74 _ $10^3$ | 0.997 | 0.050 | 0.37 | 0.30 | 0.91 | 9.4 |
| 2 | Protocatechuic acid (PRO) | 0.080-9.0 | Y =3.06 _ 104× _3.48 _ $10^3$ | 0.994 | 0.080 | 0.32 | 0.35 | 0.75 | 5.47 |
| 3 | p-Hydroxybenzoic acid (p-HYD) | 0.15-9.0 | Y =3.17 _ 104× 2.57 _ $10^3$ | 0.997 | 0.080 | 0.15 | 0.26 | 0.79 | 4.64 |
| 4 | Vanillic acid (VAN) | 0.080-6.0 | Y =3.13 _ 104× 2.57 _ $10^3$ | 0.999 | 0.040 | 0.09 | 0.40 | 0.29 | 6.77 |
| 5 | Caffeic acid (CAF) | 0.040-6.0 | Y =4.61 _ 104× _6.30 _ $10^2$ | 0.999 | 0.09 | 0.09 | 0.31 | 0.34 | 2.48 |
| 6 | Syringic acid (SYR) | 0.15-6.0 | Y=5.88 _ 104× _5.98 _ $10^3$ | 0.998 | 0.15 | 0.09 | 0.22 | 0.22 | 2.36 |
| IS | 3-Hydroxybenzaldehyde[a] | -- | | | | 0.03 | 0.51 | 0.21 | 7.19 |
| 7 | p-Coumaric acid (p-COU) | 0.10-6.0 | Y =1.29 _ 105× _5.76 _ $10^3$ | 0.998 | 0.10 | 0.04 | 0.61 | 0.17 | 2.88 |
| 8 | Ferulic acid (FER) | 0.10-6.0 | Y =6.87 _ 104× 2.75 _ $10^3$ | 0.999 | 0.10 | 0.05 | 0.80 | 0.13 | 6.25 |
| 9 | Guaiacol (GUA) | 0.010-6.0 | Y =2.92 _ 104× 1.53 _ $10^3$ | 0.998 | 0.010 | 0.03 | 0.58 | 0.12 | 8.44 |
| 10 | p-Cresol (p-CRE) | 0.070-9.0 | Y =2.42 _ 104× 5.92 _ $10^2$ | 0.999 | 0.070 | 0.01 | 0.26 | 0.14 | 2.47 |
| 11 | o-Cresol (o-CRE) | 0.070-9.0 | Y =1.81 _ 104× 1.20 _ $10^3$ | 0.999 | 0.070 | 0.01 | 0.26 | 0.14 | 2.56 |
| 12 | 3,5-Xylenol (XYL) | 0.070-9.0 | Y =1.50 _ 104× 1.90 _ $10^2$ | 0.999 | 0.070 | 0.01 | 0.26 | 0.13 | 6.52 |

Tian et al. (2005) used binary mixture of acetonitrile and 0.25% tri fluoroacetic acid with 38 min gradient cycle for the separation of eleven phenolic compounds at 38°C. For the validation of HPLC method, linearity, precision and accuracy were investigated. The calibration graphs were constructed by plotting concentration of the phenolic compounds (mg/L) against the ratio of peak area of each phenolic compound and internal standard. The analytical data obtained from the calibration graphs of 12 standard phenolic compounds are listed in Table 2. The limit of detection (LOD) of individual compound was evaluated by the concentration giving the signal-to-noise ratio of 3 (S/N = 3). The precision of the method was demonstrated by replicating analyses (n = 5) and calculating the relative standard deviation (RSD) of peak area and retention time. The results showed the LOD ranging from 0.01 to 0.15 mg/L and good precision (RSD less than 1.0%) for both peak area and retention time.

**Free phenolic compounds in rice samples**

Free phenolic compounds were extracted using PLE. The condition for PLE was optimized using central composite design (CCD) (data not shown). The accuracy of the PLE method was investigated by adding phenolic standards (2 mg/L) to 2.5 g of rice flour samples. The average recoveries of triplicate for 12 phenolic compounds ranged from 84 to 99%. The phenolic compounds in free form detected in rice samples and their contents are summarized in Table 3. All the studied phenolic compounds (12 compounds) were detected in all samples, except BS, PH and PS in which only 9 and 6 compounds were found (Table 3). A typical chromatogram obtained from rice sample is shown in Figure 2. The results revealed that the pigment rice samples (BGT and BRT) had higher content of phenolic compounds than non-pigment samples (BA, PH, BH, PS, BS, PN and BN). In addition, the phenolic compounds were rich in brown rice than the polished rice which showed that the results are in good agreement with those of the other works (Tian et al., 2004; Wojdyło and Oszmiański, 2007). Ferulic acid and p-coumaric acid were detected in all the samples with higher amounts compared to the other compounds. The phenolic compounds found in this study were comparable to those reported by Tian et al. (2005) (Table 4).

**Determination of antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) in rice samples**

In this study, the antioxidant activity is expressed in terms of $EC_{50}$

**Table 3.** Free phenolic compounds in rice samples.

| Phenolic cpd. | Amount of phenolic compounds in rice samples (mg/100 g dry weight); n=3 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Polished rice | | | Brown rice | | | | | | Tian et al. (2004a) |
| | PH | PS | PN | BH | BS | BN | BA | BRT | BGT | -- |
| GAL | 0.19 ±0.02 | ND | 0.19 ±0.08 | 0.21 ±0.01 | 0.22 ±0.11 | 0.20 ±0.04 | ND | 0.50 ±0.13 | 2.03 ±0.49 | -- |
| PRO | ND | ND | ND | ND | 0.05 ±0.01 | ND | 0.75±0.21 | 0.68 ±0.19 | 2.50 ±0.22 | 0.01 _0.05 |
| p-HYD | ND | ND | ND | 0.36 ±0.04 | ND | 0.14 ±0.02 | 0.52 ±0.18 | 0.88 ±0.08 | ND | 0.01 _0.04 |
| VAN | ND | ND | 0.04±0.01 | 0.16 ±0.02 | ND | ND | 0.77 ±0.31 | 0.95 ±0.21 | 1.37 ±0.10 | 0.03 _0.07 |
| CAF | ND | ND | 0.15 ±0.02 | 0.39 ±0.16 | ND | 0.22 ±0.03 | 0.97 ±0.23 | 1.06±0.13 | 1.84±0.25 | 0.02 _0.05 |
| SYR | 0.19 ±0.02 | ND | 0.12 ±0.02 | 0.23 ±0.06 | 0.56 ±0.03 | 0.49 ±0.03 | 1.85 ±0.12 | 0.66±0.37 | 3.12 ±0.13 | 0.01 _0.03 |
| p-COU | 0.09±0.01 | 0.03 ±0.01 | 0.07±0.02 | 0.35 ±0.11 | 0.16 ±0.05 | 0.28 ±0.02 | 7.69 ±0.23 | 1.02 ±0.13 | 1.06 ±0.06 | 0.02 _0.12 |
| FER | 0.80 ±0.02 | 1.15±0.17 | 0. 50 ±0.01 | 1.18 ±0.14 | 0.71 ±0.08 | 0.89 ±0.02 | 2.20 ±0.24 | 3.01 ±0.16 | 5.27 ±0.28 | 0.07 _0.48 |
| GUA | 0.09 ±0.03 | 0.09 ±0.03 | ND | 0.10 ±0.02 | 0.10±0.04 | 0.14 ±0.03 | 0.47 ±0.12 | ND | 0.53 ±0.13 | -- |
| p-CRE | 0.13 ±0.01 | 0.11 ±0.08 | 0.49 ±0.02 | 0.19 ±0.02 | 0.16±0.06 | 0.80 ±0.13 | ND | 0.83 ±0.24 | 1.11 ±0.25 | -- |
| o-CRE | 0.16 ±0.02 | 0.06 ±0.01 | 0.11 ±0.02 | 0.25 ±0.04 | 0.15 ±0.04 | 0.19±0.05 | 1.03 ±0.27 | 1.52 ±0.14 | 1.56±0.14 | -- |
| XYL | ND | 0.26 ± 0.15 | 0.15± 0.03 | 0.11 ± 0.03 | 0.15 ± 0.03 | 0.18 ± 0.05 | 0.38 ± 0.10 | 1.12 ± 0.20 | 1.80 ±0.20 | -- |

ND: not detected; a: Ethanolic extracted by magnetic stirring; e: not reported.

and ascorbic acid equivalent. High value of ascorbic acid equivalent or low value of $EC_{50}$ means high antioxidant activity. It is clearly seen that the methanolic extracts (free form of phenolic compounds) of all rice samples had higher antioxidant activity than those of alkaline extracts (bound form of phenolic compounds). In conclusion, the rice samples contained high TPC and TFC, and it showed high antioxidant activity. Similar to the study of Oki et al. (2002), pigment rice had higher antioxidant activity than non-pigment rice. In addition, the brown rice samples contained higher TPC, TFC and antioxidant activity than their polished form. Table 5 summarizes TPC, TFC and antioxidant activity of rice samples studied in this work.

## Conclusion

This study demonstrates a reliable method to extract and analyze phenolic compounds in rice. The PLE used short extraction time (only 15 min) and the chromatographic condition which was mild used binary mixture mobile phase in which 12 phenolic compounds were separated within 25 min. From this study, database for the presence of phenolic compounds and their amounts as well as antioxidant activity in six varieties of Iran rice were reported. For methanolic extracts (free phenolic compounds) of all the studied samples, twelve phenolic compounds were detected. Ferulic acid, p-coumaric acid and syringic acid were detected in large amount as compared to the other phenolic acid. Only ferulic acid and p-coumaric acid were found as bound form in alkaline hydrolyses.

TPC and TFC for both free and bound forms were also determined using spectrophotometry. TPC found in rice samples were in good agreement with the results obtained from HPLC, that is, pigment and brown rice samples had higher content than non-pigment and polished rice samples.

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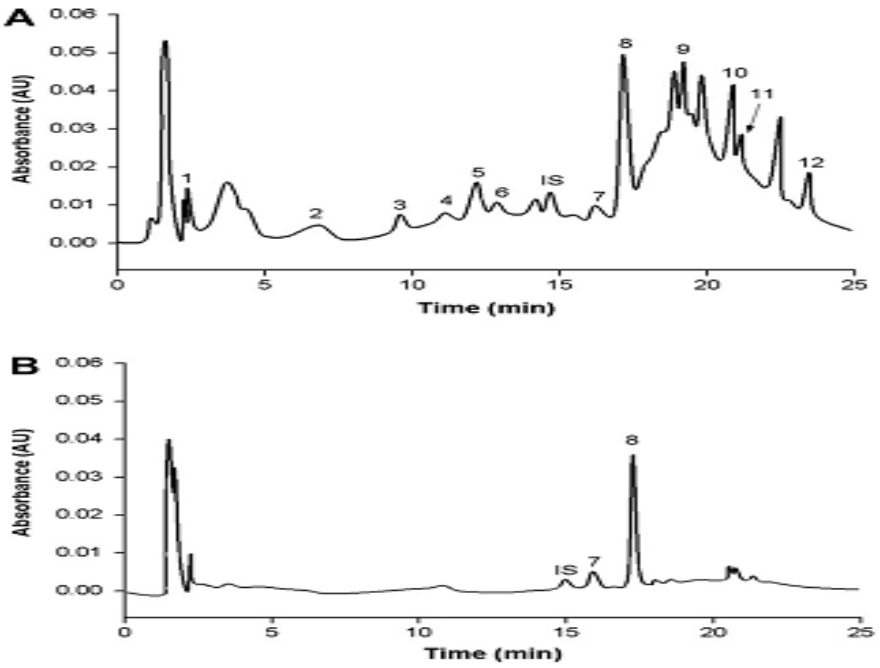


**Figure 2.** (A) Chromatogram of free phenolic compounds from brown black glutinous rice (BBG), and (B) chromatogram of bound phenolic compounds from brown black glutinous rice sample (BBG).
Chromatographic condition and peak assignment are described in Figure 1.

**Table 4.** Amount of bound phenolic compounds in rice samples extracted by alkaline hydrolysis.

| Ref. | Rice sample | Amount of phenolic compounds in rice samples (mg/100 g dry weight); n=3 | |
|---|---|---|---|
| | | p-Coumaric acid | Ferulic acid |
| This work[a] | **Polished** | | |
| | PH | 0.31 ± 0.31 | 2.41 ± 0.28 |
| | PS | 0.19 ± 0.08 | 0.95 ± 0.89 |
| | PN | 0.15 ± 0.15 | 0.18 ± 0.05 |
| | **Brown** | | |
| | BH | 0.82 ± 0.15 | 3.31 ± 0.61 |
| | BS | 0.64 ± 0.46 | 3.58 ± 0.96 |
| | BN | 1.21 ± 0.42 | 3.58 ± 0.59 |
| | BA | 0.64 ± 0.28 | 1.13 ± 0.36 |
| | BRT | 1.25 ± 0.11 | 4.03 ± 0.98 |
| | BGT | 0.62 ± 0.44 | 5.54 ± 0.59 |
| Zhou et al. (2004)[b] | Milled rice | 0.66 | 5.20 |
| | Brown rice | 7.10 | 25.90 |
| Tian et al. (2004)[c] | White rice | 0.34 ± 0.01 | 5.26 ± 0.14 |
| | Brown rice | 2.10 ± 0.08 | 15.19 ± 0.52 |
| | Germinated brown rice | 3.05 ± 0.02 | 20.04 ± 0.77 |

a: methanolic extracted and washed with acetone before being subject to alkaline hydrolysis; b: direct alkaline extracted; c: ethanolic extracted and alkaline hydrolysis.

**Table 5.** Total phenolic content, total flavonoid content, and antioxidant activity in the studied rice samples (n=3).

| Rice | Total phenolic content | | Total flavonoid content | | Total antioxidant activities $EC_{50}$ (g/mL) ± S.D. | | Ascorbic acid equivalent (mg AA/100 g) ± S.D. | |
|---|---|---|---|---|---|---|---|---|
| | Free form (mg GAE/100 g) ±S.D. | Bound form (mg GAE/100 g) ±S.D. | Free form (mg CE/100 g) ±S.D. | Bound form (mg CE/100 g) ±S.D. | Free form | Bound form | Free form | Bound form |
| **Polished** | | | | | | | | |
| PH | 26.38±0.55 | 0.84±0.33 | 7.74±0.23 | 0.35±0.18 | 27.00±7.87 | 47.63±8.89 | 6.24±0.09 | 1.67±0.05 |
| PS | 6.80±0.35 | 0.96±0.15 | 0.69±0.12 | 0.23±0.17 | 61.25±7.22 | 142.91±6.67 | 5.97±0.04 | 1.38±0.04 |
| PN | 4.60±0.79 | 0.96±0.15 | 0.86±0.15 | 0.13±0.10 | 63.51±8.52 | 79.76 ±7.44 | 5.36±0.19 | 1.14±0.04 |
| **Brown** | | | | | | | | |
| BH | 36.87±0.71 | 1.64±0.16 | 11.79±0.14 | 0.61±0.15 | 13.77±4.51 | 40.83±3.33 | 7.99±0.51 | 2.24±0.07 |
| BS | 12.39±0.35 | 1.83±0.37 | 1.03±0.22 | 0.21±0.12 | 40.83±3.33 | 110.64±5.52 | 6.76±0.14 | 1.72±0.06 |
| BN | 12.32±0.41 | 1.48±0.32 | 1.15±0.24 | 0.20±0.11 | 39.42±5.29 | 72.97±6.72 | 6.67±0.17 | 1.70±0.06 |
| BA | 34.14±0.56 | 0.98±0.07 | 10.15±0.63 | 0.37±0.12 | 14.41±4.76 | 40.35±2.94 | 7.06±0.48 | 1.78±0.05 |
| BRT | 53.79±0.63 | 2.53±0.25 | 38.08±0.43 | 1.66±0.15 | 13.14±3.67 | 39.42±5.29 | 8.25±0.55 | 3.02±0.08 |
| BGT | 60.60±0.85 | 3.16±0.656 | 42.96±0.38 | 1.73±0.56 | 11.39±5.35 | 35.36±7.82 | 9.95±0.70 | 3.85±0.08 |

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## REFERENCES


Adom KK, Sorrells ME, Liu RH (2003). Phytochemical profiles and antioxidant activity of wheat varieties. J. Agric. Food Chem., *51*(26):7825-34

Al-Farsi MA, Lee CY (2008). Optimization of phenolic and dietary fiber extraction from date seeds. Food Chem., 108: 977-985.

Aoun E, Rima J, Chidiac G, Hanna K (2005). High-performance liquid chromatographic and spectrofluorometric determination of a-tocopherol in a natural plant: Ferula hermonis (Zalooh root). J. Food Comp. Analy., 18: 607-615.

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. Chrom., 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.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technologie, 28: 25-30.

Chew YL, Lim YY, Omar M, Khoo KS (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. Food Scie. Tech., 41: 1067-1072.

Chirinos R, Campos D, Costa N, Arbizu C, Pedreschi R, Larondelle Y (2008). Phenolic profiles of Andean mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers: identification by HPLC-DAD and evaluation of their antioxidant activity. Food Chemi., 106: 1285-1298.

Frei M, Becker K (2004). Agro-biodiversity in subsistence-oriented farming systems in a Philippine upland region: nutrition considerations. Biodiver. and Conser., 13:1591-1610.

Govindarajan R, Singh DP, Rawat AKS (2007). High-performance liquid chromatographic method for the quantification of phenolics in 'Chyavanprash' a potent Ayurvedic drug. J. Pharm. Biom. Anal., 43: 527-532.

Heim KE, Tagliaferro AR, Bobilya DJ (2002). Flavonoid antioxidants: chemistry, metabolism and structure activity relationships. J. Nutri. Bioch., 13:572-584.

Herrera MC, Luque de Castro MD (2005). Ultrasound-assisted extraction of phenolic compounds from strawberries prior liquid chromatographic separation and photodiode array ultraviolet detection. J. Chroma. A., 1100: 1-7.

Hung PV, Morita N (2008). Distribution of phenolic compounds in the graded flour milled from whole buckwheat grains and their antioxidant capacities. Food Chem., 109: 325-331.

Kosar M, Dorman HJD, Bachmayer O, Baser KHC, Hiltunen R (2003). An improved on-line HPLC-DPPH_ method for the screening of free radical scavenging compounds in water extracts of Lamiaceae plants. Chem. Natura. Compo., 39: 161-166.

Lin J Y, Tang CY (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulator effects on mouse splenocyte proliferation. Food Chem., 101: 140-147.

Liu RH (2007). Whole grain phytochemicals and health. J.Cereal Scie., 46: 207-219.

Madhava NM, Sulochanamma G, Sampathu S R, Srinivas V (2008). Studies on extraction and antioxidant potential of green coffee. Food Chem., 107: 337-384.

Martins TD, Ferreira Patão R, Varela Coalho A, Teixeira da Costa C (2006). Comparison between sample disruption methods and solid liquid extract (SLE) to extract phenolic compounds from Ficus carica leaves. J. Chrom. A, 1103: 22-28.

Nara K, Miyoshi T, Honma T, Koga H (2006). Antioxidant activity of bound from phenolics in potato peel. Biosci. Biotech. Biochem., 70: 1489-1491.

Oki T, Masuda M, Kobayashi M, Nishiba Y, Furuta S, Suda I(2002). Polymeric procyanidins as radical-scavenging components in red-hulled rice. J. Agric. Food Chem., 50: 7524-7529.

Palma M, Taylor LT (1999). Extraction of poly phenolic compounds from grape seed with near critical carbon dioxide. J. Chrom. A, 849: 117-124.

Pupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia A (2001). Antimicrobial properties of phenolic compounds from berries. J. Appl. Micro., 90; 494-507.

Sakanaka S, Tachibana Y, Okada Y (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem., 89: 569-575.

Schantz MM (2006). Pressurized liquid extraction in environmental analysis. Analyt. Biol. analyt. Chem., 386: 1043-1047.

Sosulski F, Krygier K, Hogge L (1982). Free, esterified, and insoluble-bound phenolic acids 3 composition of phenolic acids in cereal and potato flours. J. Agric. Food Chem., 30: 337-340.

Stratil P, Klejdus B, Kubán V (2007). Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Talanta, 71: 1741-1751.

Tian S, Nakamura K, Cui T, Kayahara H (2005). High performance liquid chromatographic determination of phenolic compounds in rice. J. Chrom. A, 1063: 121-128.

Tian S, Nakamura K, Kayahara H (2004). Analysis of phenolic compounds in white rice, brown rice and germinated brown rice. J. Agric. Food Chem., 52: 4808-4813.

Zhai C K, Lu C M, Zang Q, Sun G J, Lorenz K J (2001). Comparative study on nutritional value of Chinese and North American wild rice. J. Food Compo. Analy., 14: 371-382.

Zhou Z, Robards K, Helliwell S, Blanchard C (2004). The distribution of phenolic acids in rice. Food Chem., 87: 401-406.