Effect of Ultrasonic Extraction Regimes on Phenolics and Antioxidant Attributes of Rice (*oryza sativa* L.) Cultivars

Zubair, Muhammad*[+•]

Department of Chemistry, University of Gujrat, PAKISTAN

Anwar, Farooq[••]

Department of Chemistry, University of Sargodha, Sargodha, PAKISTAN

Nadeem Zafar, Muhammad; Faizan Nazar, Muhammad

Department of Chemistry, University of Gujrat, PAKISTAN

Ijaz Hussain, Abdullah

Department of Chemistry, Govt. College University Faisalabad, PAKISTAN

Mughal, Ehsan Ullah

Department of Chemistry, University of Gujrat, PAKISTAN

ABSTRACT: *The ultrasound-assisted magnetic stirrer was used as an effective extraction technique for the evaluation of total phenolic contents (TPC) and antioxidant potential of ten Pakistani brown rice cultivars. For this purpose, ultrasonic (240W frequency 50/60 Hertz) assisted magnetic stirrer coupled with three solvents methanol, ethanol, and isopropanol in pure and aqueous fractions (80:20) were used for the extraction of brown rice material. The extract yields of brown rice were obtained from 1.62 g/100g to 3.67 g/100g for all the varieties. Aqueous isopropanol preferably and methanol (80:20) showed best extraction yields on the dry mass basis of brown rice. The contents of total phenolics were determined as highest (496.9 mg GAE/kg) in Basmati Pak while lowest (137.7 mg GAE /kg) in non-basmati Irri-6 variety. For antioxidant activity, brown rice extracts of Basmati Pak showed the best potential at* IC_{50} *2.19 mg/mL against DPPH radical scavenging. Reducing the power of Basmati Pak was found highest at 0.85. Brown rice extract of Basmati 515 showed best ferrous ion metal chelation at 5.78 Eq. EDTA mg/100g.*

KEYWORDS: *Basmati; Extraction; Antioxidant; Phenolics.*

***** *To whom correspondence should be addressed.*

+ E-mail: muhammad.zubair@uog.edu.pk

● Other Address: Department of Chemistry, University of Agriculture, Faisalabad, PAKISTAN

●● Other Address: Department of Pharmaceutical Chemistry, Prince Sattam bin Abdulaziz University,Al-Kharj, Saudi Arabia

1021-9986/2018/1/109-119 11/$/6.01

DOI:

INTRODUCTION

Recovery and purification of active ingredients from plant materials require appropriate extraction process [1]. Usually, the traditional techniques require longer time and produce low extraction yields. Moreover, many bioactive compounds are thermally decomposed and may degrade while thermal extraction. Nowadays, use of extraction processes, such as maceration, solvent extraction, steam or hydro-distillation, cold pressing, squeezing etc in nutraceutical, pharmaceutical, cosmetic industries does not produce the desired results. Moreover, energy crises and increase in greenhouse emissions of food processing and chemical industries are current issues of developing countries to find new technologies in order to reduce energy consumption, controlled emissions and cost reduction with quality as well as functionality [2]. Selection of correct and appropriate solvent and the use of agitation to increase extraction yield is an important step to finding better results. The green process of extraction using solvent has applicability in many areas of research as a selective process of separation for food and nutrition, environmental, health and some other fields of importance and interest.

Recent research has revealed the application of power ultrasound in the extraction of numerous bioactive, such as phenolics, flavonoids, vitamins, peptides and essential oils, from different parts of plant and plant seeds [3, 4]. Cereals, being a rich source of phenolics and other antioxidant components, not only provide valuable nutrients for human diet but also play a vital role in protecting against oxidative stress-related diseases [5]. Phenolics and other minor bioactives are mainly distributed in the seed pericarp tissues of cereals and thus contribute to the physiochemical parameters (appearance, taste, odor and oxidative stability) of the end-user products [6]. The amount of such components is also affected due to post-harvest factors (storage conditions and processing) as well as due to changes in sample preparation and analysis regimes [7-9].

Antioxidants from natural sources such as cereals, vegetables, and fruits have become an alternative potential to avoid oxidative stress. Rice provides about 22% of the global food supply. According to estimation 90% rice is produced and consumed in the Asian region. The degrees of milling differentiate brown rice from white rice. Brown rice is mechanically converted into white rice removing bran from the surface. During the process of milling brown rice lost chemical compositions of many beneficial components of rice bran including sterols, oryzanols, tocopherols, tocotrienols, and phenolic compounds.

Brown rice has also exhibited health beneficial antioxidative and antimutagenic activities, which play an important role in maintaining health [10, 11]. Brown rice is a good source of natural antioxidants including phenolic compounds. Both, phenolic and flavonoids compounds have potential as antioxidants to act as free radical scavengers, reducing agents, and/or metal ion chelators, thus providing various human health benefits. An enhanced formation of free radicals than scavenging capacities leads to oxidative stress for the extensive deterioration of all cell components including lipids, proteins, and DNA, resulting into chronic effects such as carcinogenic, cardiovascular, atherosclerosis, aging and inflammation [8]. Although some synthetic antioxidants have been developed, the toxic effects of the antioxidants have been observed in animal [12].

The analysis of the antioxidant activities of rice bran using various solvents systems and traditional extraction has been made earlier [13-16] followed by the qualitative and quantitative measurement of phytoxidants. However, there are few studies, which clearly report green process for the extraction of antioxidant compositions of cereals and grains with various solvent systems. The present research on green extraction process has got importance world over because of more sustainable results [2, 4, 17].

Pakistan is rich in rice production with an estimate of 6.5 million tons/year. Therefore, it is also used as second cereal grain all over Pakistan. Pakistani basmati varieties have unique aroma for which they are highly demanded by the world community to fulfill their nutritional requirements. Therefore, the objective of this study was to use an ultrasonic assisted extraction process for the evaluation of phenolic contents and antioxidant properties of the brown rice using different solvents.

EXPERIMENTAL SECTION

Collection of Rice (Oryza sativa L.) Samples

Ten approved varieties of Pakistan Agriculture Research Board (PARB) namely; Basmati Super, Basmati 515, Basmati 198, Basmati 385, Basmati 2000, Basmati 370, Basmati Pak, KSK-133, KS 282 and IRRI-6 were tested. Samples of paddy rice were collected,

from Rice Research Centre, Kala Shah Kako, Lahore, Pakistan. Sample amount of 5.0 kg/variety was obtained for research analysis.

Pretreatment of Samples

Samples of paddy rice were air dried at the ambient condition to avoid any loss of ingredients. The dried rice samples were processed for husk removal using a Satake Rice Huller (Model THO35A), yielding brown rice. The brown rice samples (with bran layer) were ground to fine powder and packed tightly into polyethylene bags and stored at 4°C in a freezer for later extraction and analysis

Ultrasonic Assisted Extraction

For the extraction of powder rice materials, the ultrasonic system (Utech Cleaner Model 04677867, NY, USA) equipped with P-240W frequency range 50/60 Hertz was employed for 60min minutes assisted with a magnetic stirrer with the medium of six different solvents systems (Methanol, Ethanol, Isopropanol) in pure and in 80:20 ratio [16]. Briefly, 20.0 g powdered brown rice materials, was extracted separately with 100 mL of each solvent for 1.0 hrs at 37°C. The process of extraction was carried out thrice to obtain maximum yield. All the three extractions were pooled to make a uniform system. To concentrate the extracts, an excess of the solvent was evaporated using a rotary vacuum evaporator (N–N Series, Eyela Tokyo, Japan). Finally obtained crude extracts were dried with pure nitrogen gas flush, weighed and stored at - 4°C in a refrigerator until used for further analysis.

Total Phenolic Contents

Total phenolic contents of rice extracts were evaluated according to the method described in [18] where they used Folin-Ciocalteu reagent with a slight modification of temperature are 37^0C.

Antioxidant Activity

DPPH Radical Scavenging Assay

The rice extracts were tested for DPPH radical scavenging activity by the method of [14, 18]. The complete protocol was carried out at 37^0C.

Reducing Power

The reducing power of the brown rice extracts was examined following the method of [18, 19] with modification of temperature 37^0C .

Metal Chelating Activity

Metal chelating (Ferrous ion)-chelating activity of different brown rice, extracts were determined according to the modified method of [18, 20, 21].

Statistical Analysis

Three different samples of brown rice for each variety were prepared and analyzed for various assays and data is reported as mean (n = 3 x 3) ± SD (n = 3 x 3). Analysis of variance (ANOVA) in two ways for solvent and varieties was used to determine significant differences considering a level of significance at less than 5% ($P< 0.05$) by using the statistical software.

RESULTS AND DISCUSSION

Extraction order revealed solvents used as Isopropanol > MeOH > EtOH for brown rice materials of different varieties (Table 1). Extraction yield of six solvents used with sonication assisted magnetic stirrer technique varied over a wide range of 1.62 to 3.67 g/100g (Table 1). Overall, employing ultrasonically assisted protocol of extraction isopropanol 80% was established to be the best solvent for the extraction of brown rice. In comparison to conventional extraction technique yields obtained were about 10% higher in ultrasonically assisted protocol [18]. Literature revealed that brown rice material of basmati varieties not preferably used for extraction to explore vital compounds whereas wild rice has been extracted with methanol, ethanol, and ethyl acetate with extraction yields 1.0–3.9 % which shows similar extraction yield [22]. As for as solvents are concerned Rohrer and Siebenmorgen used hexane for the extraction of rice [23] also revealed a similar yield but was lower than that (< 20 %) of isopropanol extracts at a temperature (40 to 60°C) [24]. In another study, Devi and Arumughan reported extract yields of rice ranged from 1.0% to 4.9% [25]. Extraction of plant materials supports mostly aqueous methanol as one of the suitable solvents whereas aqueous isopropanol also has significant results. The present research also signifies the extraction yield in aqueous isopropanol and methanol with the ultrasonic application. All the results were analyzed in two ways among solvents and varieties using ANOVA at <5% significant level. It was revealed that there is a significant difference among varieties and solvents (Table 4). There is no level of interaction found in the extraction process of solvents and varieties.

Table 1: Extract yield (g/100g) of antioxidant components from different varieties of rice (Oryza sativa L.) using sonication–assisted magnetic stirrer

Varieties	Methanol		Ethanol		Isopropanol	
	100%	80%	100%	80 %	100%	80%
Basmati Super	$2.52 \pm 0.1_{ab}^{b}$	$2.65 \pm 0.1_{a}^{ef}$	$1.88 \pm 0.1_{c}^{ab}$	$1.92 \pm 0.1_{c}^{de}$	$2.73 \pm 0.1_{a}^{e}$	$2.76 \pm 0.1_{a}^{a}$
Basmati 515	$2.17 \pm 0.1_{c}^{de}$	$2.80 \pm 0.1_{ab}^{cde}$	$1.62 \pm 0.1_{d}^{c}$	$1.88 \pm 0.1_{c}^{bcde}$	$3.45 \pm 0.2_{a}^{b}$	$3.48 \pm 0.2_{a}^{de}$
Basmati 198	$1.92 \pm 0.1_{c}^{f}$	$2.55 \pm 0.1_{ab}^{f}$	$1.79 \pm 0.1_{d}^{abc}$	$2.04 \pm 0.1_{bc}^{abc}$	$2.92 \pm 0.2_{a}^{d}$	$2.95 \pm 0.1_{a}^{e}$
Basmati 385	$2.22 \pm 0.1_{bc}^{cd}$	$2.91 \pm 0.1_{b}^{bcd}$	$1.93 \pm 0.1_{c}^{a}$	$1.98 \pm 0.1b_{c}^{cde}$	$3.12 \pm 0.2_{a}^{c}$	$3.15 \pm 0.2_{a}^{bc}$
Basmati 2000	$2.76 \pm 0.1_{ab}^{a}$	$3.19 \pm 0.1_{a}^{a}$	$1.71 \pm 0.1_{d}^{bc}$	$2.05 \pm 0.1_{c}^{ab}$	$2.59 \pm 0.1_{ab}^{e}$	$2.62 \pm 0.1_{ab}^{de}$
Irri 6	$2.39 \pm 0.1_{bc}^{bc}$	$2.97 \pm 0.1_{ab}^{bc}$	$1.78 \pm 0.1_{d}^{abc}$	$1.99 \pm 0.1_{c}^{abcd}$	$3.27 \pm 0.2_{a}^{c}$	$3.30 \pm 0.2_{a}^{f}$
KSK 133	$2.46 \pm 0.1_{bc}^{b}$	$2.93 \pm 0.2_{ab}^{bcd}$	$1.86 \pm 0.1_{d}^{ab}$	$1.81 \pm 0.1_{d}^{e}$	$3.26 \pm 0.2_{a}^{c}$	$3.23 \pm 0.2_{a}^{bc}$
KS 282	$2.28 \pm 0.1_{ab}^{cd}$	$2.90 \pm 0.1_{ab}^{bcd}$	$1.73 \pm 0.1_{c}^{bc}$	$1.87 \pm 0.1_{c}^{cde}$	$2.76 \pm 0.1d_{a}^{e}$	$2.79 \pm 0.2_{a}^{b}$
Basmati 370	$2.28 \pm 0.1_{bc}^{cd}$	$3.01 \pm 0.2_{ab}^{b}$	$1.84 \pm 0.1_{c}^{ab}$	$2.16 \pm 0.1_{bc}^{a}$	$3.63 \pm 0.2_{a}^{a}$	$3.67 \pm 0.2_{a}^{cd}$
Basmati Pak	$2.04 \pm 0.1_{bc}^{ef}$	$2.76 \pm 0.2_{ab}^{de}$	$1.79 \pm 0.1_{d}^{abc}$	$2.05 \pm 0.1_{bc}^{ab}$	$3.49 \pm 0.2_{a}^{ab}$	$3.52 \pm 0.2_{a}^{a}$

Values are mean ± SD for three samples of each variety, analyzed individually in triplicate (n = 3x3)
Means with different superscript letters within the same column indicate significant differences ($p < 0.05$) among varieties tested
Means with different subscript letters within the same row indicate significant differences ($p < 0.05$) among solvents used

To find the effect of solvent, Tukey test was employed which shows the clear difference of the effect of the solvents on yield (Table-5).

Extracts (mg/mL) of various brown rice materials of basmati varieties were evaluated for Total Phenolic Content (TPC). Extracts of brown rice of basmati varieties using ultrasonic energy depict TPC values minimum as 137.7 mg/kg in pure ethanol and 496.9 mg/kg in 80% methanol solvent. Previously given values revealed that non-Basmati Irri-6 has lowest TP content whereas Basmati Pak has highest TPC respectively (Table 2).

A significant difference was found among varieties and the highly significant difference was found among solvents (Table 5). Similarly, aqueous methanol and isopropanol showed leading results of TPCs Fig-2 clearly depicting the trend of solvents and varieties with mean values. In the similar research work, total phenol content of black rice was determined as 83.01±0.51µg GAE/mg extract using Folin-Ciocalteu reagent [26]. Results reported are quite similar showing the potential of rice varieties for phenolic as well for antioxidants. In another study, the total phenolic concentration was 18.2±0.59 and 13.3±0.31 mg GAE/g was determined for whole grains from two black rice species [27]. Different results may be due to extraction conditions, kind of solvent, properties of soil, climate conditions and where grain was grown in areas [27]. Hodzic *et al.* conducted a research work and revealed results of total phenolic compounds ranged from 295–2035 mg GAE on 20ºC and 429–3065 mg GAE on 40ºC in different extracts; rye, oats, barley, corn, wheat, and rice, respectively [28]. After an extensive research work using three different varieties, the values of total phenolic compared and it was concluded that the content of total phenols in brown rice is higher than in white rice [28].

The scavenging assay of 2,2-diphenyl-1-picrylhydrazyl(DPPH·) radical is commonly employed for the assessment of antioxidant activity of plant materials. DPPH radical scavenging activity (IC_{50} value) of extracts obtained from different rice varieties by using six different solvent systems, ranged from weakest 6.26 mg/mL (Basmati 515) to strongest 2.19 mg/mL (Basmati 370). Among the different extraction solvents used, 80% methanol and 80% isopropanol offered the best results of DPPH radical scavenging activity (IC_{50} value 2.19 mg/mL) Table 3. As for as effectiveness of ultrasonic extraction technique is concerned about the considerable DPPH radical scavenging activity (IC_{50} value) was observed for the extracts which showed promising values over the conventional orbital shaker [18]. The superior DPPH radical scavenging activity of 80% isopropanol and 80% methanol extracts may be linked to the polar nature of these solvents as well as their efficacy for polar

Table 2: Total phenolic contents (GAE mg/kg) of different varieties of rice (Oryza sativa L.) extracted using sonication–assisted Magnetic stirrer.

Varieties	Methanol		Ethanol		Isopropanol	
	100%	80%	100%	80 %	100%	80%
Basmati Super	$216.9 \pm 7.9_{bc}^{b}$	$220.7 \pm 10.8_{bc}^{de}$	$191.2 \pm 9.9_{c}^{a}$	$196.2 \pm 8.6_{c}^{b}$	$325.6 \pm 12.4_{ab}^{d}$	$382.9 \pm 10.1_{a}^{de}$
Basmati 515	$141.2 \pm 5.2_{d}^{f}$	$213.9 \pm 9.1_{bc}^{ef}$	$170.1 \pm 8.4_{c}^{c}$	$177.5 \pm 4.1_{c}^{cd}$	$288.7 \pm 9.7_{b}^{e}$	$414.2 \pm 11.6_{a}^{c}$
Basmati 198	$203.3_{b} \pm 10.1^{c}$	$180.5 \pm 7.1c^{h}$	$181.3 \pm 6.7_{c}^{abc}$	$184.3 \pm 6.6_{c}^{c}$	$232.1 \pm 10.2_{bc}^{f}$	$307.2 \pm 9.3_{a}^{g}$
Basmati 385	$198.7 \pm 8.6_{bc}^{c}$	$195.1 \pm 8.1_{bc}^{gh}$	$170.3 \pm 8.1_{c}^{c}$	$179.6 \pm 7.5_{c}^{bc}$	$278.4 \pm {}_{b}9.8^{e}$	$371.1 \pm 11.7_{a}^{e}$
Basmati 2000	$149.7 \pm 7.5_{d}^{ef}$	$229.8 \pm 8.8_{bc}^{cd}$	$173.3 \pm 7.9_{c}^{bc}$	$185.2 \pm 4.4_{c}^{bc}$	375.4 ± 10.3^{c}	$402.2 \pm 14.4_{a}^{cd}$
Irri 6	$137.7 \pm 5.2_{d}^{f}$	$205.3 \pm 9.1_{bc}^{fg}$	$174.2 \pm 6.3_{c}^{bc}$	$178.2 \pm 4.1_{c}^{cd}$	$321.4 \pm 8.7_{a}^{d}$	$311.4 \pm 13.1_{a}^{g}$
KSK 133	$160.8 \pm 5.6_{d}^{e}$	$200.9 \pm 8.4_{bc}^{fg}$	$174.9 \pm 7.2_{c}^{bc}$	$175.4 \pm 6.1_{c}^{cd}$	$446.84 \pm 11.2_{a}^{b}$	$350.6 \pm 12.0_{ab}^{f}$
KS 282	$185.5 \pm 6.8_{c}^{d}$	$279.1 \pm 9.2_{b}^{b}$	$188.2 \pm 6.5_{c}^{a}$	$167.5 \pm 7.3_{d}^{d}$	$475.9 \pm 9.1_{a}^{a}$	$469.5 \pm 13.9_{a}^{b}$
Basmati 370	$197.8 \pm 7.8_{c}^{cd}$	$236.4 \pm 8.5_{b}^{c}$	$187.6 \pm 8.7_{c}^{ab}$	$178.9 \pm 7.6_{c}^{c}$	$391.8 \pm 13.4_{ab}^{c}$	$417.2 \pm 10.3_{a}^{c}$
Basmati Pak	$242.6 \pm 8.9_{bc}^{a}$	$296.8 \pm 9.3_{b}^{a}$	$197.4 \pm 9.2_{c}^{a}$	$219.2 \pm 7.8_{bc}^{a}$	$476.9 \pm 12.3_{a}^{a}$	$496.9 \pm 11.9_{a}^{a}$

Values are mean ± SD for three samples of each variety, analyzed individually in triplicate (n = 3x3).
Means with different superscript letters within the same column indicate significant differences ($p < 0.05$) among varieties tested.
Means with different subscript letters within the same row indicate significant differences ($p < 0.05$) among solvents used.

Table 3: DPPH radical scavenging activity (IC50 mg/mL) of extracts of different varieties of rice (Oryza sativa L.) produced by sonication–assisted Magnetic Stirrer.

Varieties	Methanol		Ethanol		Isopropanol	
	100%	80%	100%	80 %	100%	80%
Basmati Super	$4.36 \pm 0.21_{ab}^{bc}$	$3.28 \pm 0.16_{c}^{c}$	$4.77 \pm 0.28_{a}^{de}$	$4.37 \pm 0.25_{ab}^{ab}$	$4.63 \pm 0.11_{a}^{bc}$	$3.07 \pm 0.13_{c}^{f}$
Basmati 515	$5.35 \pm 0.27_{ab}^{a}$	$4.46 \pm 0.14_{b}^{a}$	$6.12 \pm 0.26_{a}^{a}$	$4.08 \pm 0.20_{b}^{bc}$	$4.89 \pm 0.21_{b}^{ab}$	$3.23 \pm 0.11_{c}^{ef}$
Basmati 198	$3.70 \pm 0.18_{b}^{d}$	$2.58 \pm 0.15_{d}^{fg}$	$4.85 \pm 0.25_{a}^{de}$	$4.23 \pm 0.18_{b}^{b}$	$4.95 \pm 0.14_{a}^{a}$	$4.49 \pm 0.13_{b}^{a}$
Basmati 385	$4.19 \pm 0.25_{b}^{c}$	$2.79 \pm 0.13_{d}^{ef}$	$4.66 \pm 0.22_{a}^{e}$	$4.22 \pm 0.24_{b}^{b}$	$3.29 \pm 0.16_{b}^{d}$	$3.40 \pm 0.10_{b}^{de}$
Basmati 2000	$5.56 \pm 0.32_{a}^{a}$	$4.45 \pm 0.12_{b}^{a}$	$5.65 \pm 0.32_{a}^{b}$	$4.18 \pm 0.22_{b}^{b}$	$4.39 \pm 0.17_{b}^{c}$	$3.51 \pm 0.14_{c}^{cd}$
Irri 6	$4.15 \pm 0.21_{b}^{c}$	$3.10 \pm 0.17_{c}^{cd}$	$5.65 \pm 0.27_{a}^{b}$	$4.08 \pm 0.19_{b}^{bc}$	$4.39 \pm 0.14_{b}^{c}$	$3.98 \pm 0.14_{c}^{b}$
KSK 133	$4.74 \pm 0.22_{a}^{b}$	$3.69 \pm 0.15_{c}^{b}$	$4.91 \pm 0.24_{b}^{de}$	$4.15 \pm 0.18_{b}^{b}$	$4.63 \pm 0.14_{a}^{bc}$	$3.52 \pm 0.16_{c}^{cd}$
KS 282	$4.17 \pm 0.18_{b}^{c}$	$3.03 \pm 0.16_{c}^{de}$	$5.47 \pm 0.24_{a}^{bc}$	$4.10 \pm 0.20_{b}^{b}$	$4.68 \pm 0.17_{b}^{bc}$	$3.63 \pm 0.11_{c}^{c}$
Basmati 370	$3.13 \pm 0.25_{c}^{e}$	$2.19 \pm 0.11_{d}^{h}$	$5.54 \pm 0.26_{a}^{bc}$	$4.72 \pm 0.23_{b}^{a}$	$3.31 \pm 0.11_{c}^{d}$	$2.79 \pm 0.11_{d}^{g}$
Basmati Pak	$3.71 \pm 0.20_{c}^{d}$	$2.53 \pm 0.14_{d}^{g}$	$5.18 \pm 0.22_{a}^{cd}$	$3.73 \pm 0.18_{c}^{c}$	$4.88 \pm 0.19_{b}^{ab}$	$3.50 \pm 0.12_{c}^{cd}$

Values are mean ± SD for three samples of each variety, analyzed individually in triplicate (n = 3x3).
Means with different superscript letters within the same column indicate significant differences ($p < 0.05$) among varieties tested.
Means with different subscript letters with in the same row indicate significant differences ($p < 0.05$) among solvents used IC_{50}
Value of BHT = 0.03 mg/mL.

Table 4: Analysis of Variance for Extraction Yields.

Source	DF	SS	MS	F	P
Varieties	9	0.6480	0.0720	3.61	0.002
Solvent	5	15.2664	3.0533	153.10	0.000
Error	45	0.8974	0.0199		
Total	59	16.8118			

Table 5: Tukey Simultaneous Tests for Differences of Means of Extraction Yields.

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	P-Value
100% Iso - 100% E	1.1967	0.0757	(0.9730, 1.4203)	15.82	0.000
100% M - 100% E	0.6606	0.0757	(0.4370, 0.8842)	8.73	0.000
80 % E - 100% E	0.4362	0.0757	(0.2126, 0.6598)	5.77	0.000
80% Iso - 100% E	1.2699	0.0757	(1.0463, 1.4935)	16.79	0.000
80% M - 100% E	1.4010	0.0757	(1.1774, 1.6246)	**18.52**	0.000
100% M - 100% Iso	-0.5361	0.0757	(-0.7597, -0.3124)	-7.09	0.000
80 % E - 100% Iso	-0.7605	0.0757	(-0.9841, -0.5368)	-10.05	0.000
80% Iso - 100% Iso	0.0733	0.0757	(-0.1504, 0.2969)	0.97	0.926

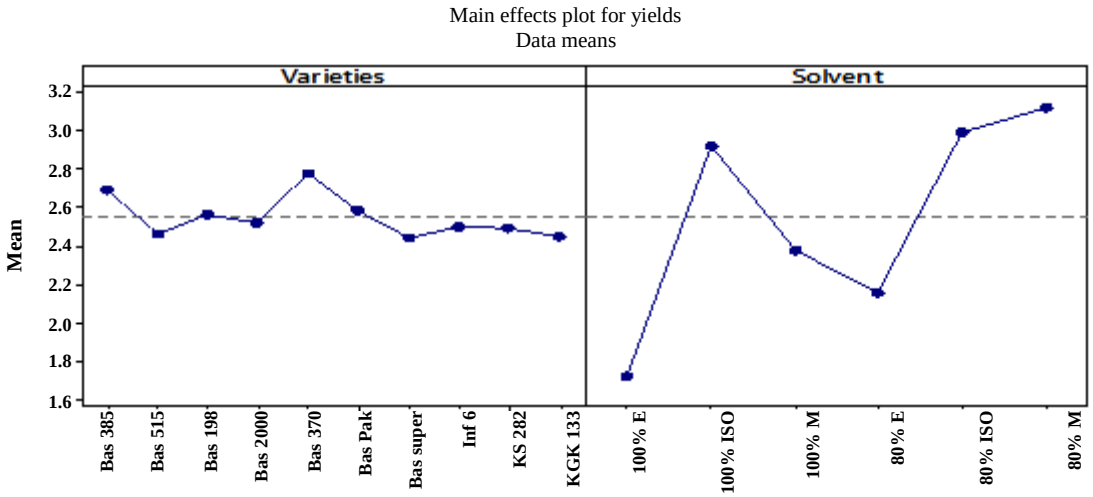

Fig. 1: Comparison of varieties and solvents with their effect on yield.

extractable antioxidant components [29]. To ensure the efficacy of results two ways ANOVA was applied viz varieties and solvents for IC_{50} value of DPPH radical. Results revealed a very significant difference of values at <5% significant level (Table 7). Similarly, comparison of varieties and solvents as highlighted in Fig-3 showing the promising difference in solvents effect rather than varieties change. Moreover, it was revealed that there are interaction factors among varieties and solvents. Overall, the present results indicated that aqueous methanol and aqueous isopropanol extracts exhibited better free radical scavenging activity (IC_{50} value) in sonication-assisted magnetic stirrer technique. With increasing the concentration of phenolic compounds or the degree of hydroxylation of phenolic compounds, DPPH radical scavenging activity also increases, thus correlating

Table 6: Analysis of Variance for Total Phenolics.

Source	DF	SS	MS	F	P
Varieties	9	72528	8059	4.75	0.000
Solvent	5	543051	108610	64.01	0.000
Error	45	76356	1697		
Total	59	691935			

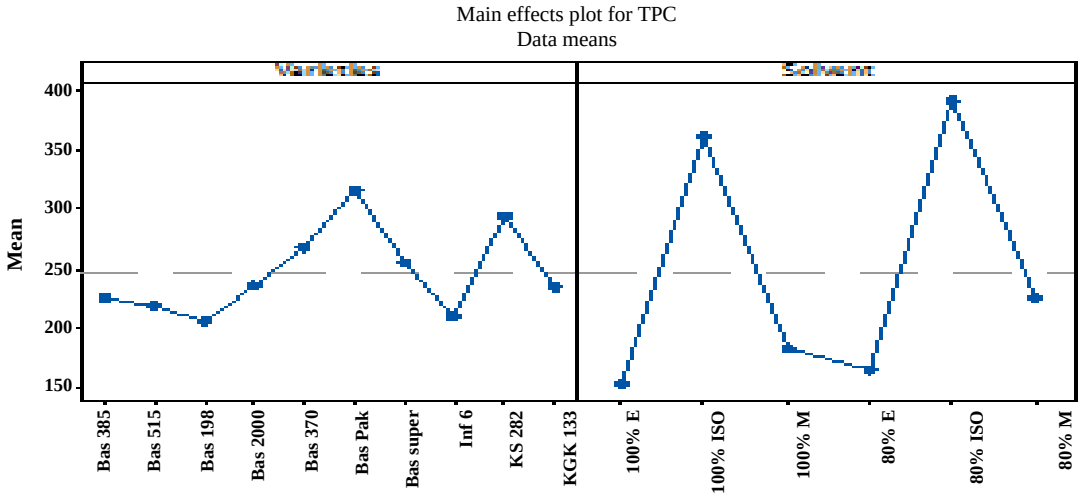

Fig. 2: Comparison of varieties of and solvents for total phenolic (TPC).

directly to the antioxidant efficacy of a typical plant material [30]. Because these radicals are very sensitive to the presence of hydrogen donors, the whole system operates at a very low concentration; hence, a large number of samples can be tested in a short time [31]. *Sompong et al.*, have reported similar results on DPPH• scavenging activity of different black rice varieties showing a higher level of activity against white rice analyzed in the present research. The EC_{50} values of the tested extracts of black rice and BHT were 0.29 mg/mL and 0.11 mg/mL respectively. The lower EC_{50} value indicates higher antioxidant activity [32]. Results of IC50 values of DPPH radical were found highly significant in solvents where is less significant in varieties (Table 7). Moreover no significant interactions were found among the values of radical scavenging (Fig-3)

For another assay, the antioxidant potential of the selected varieties of rice was evaluated using reducing power assay [33]. Reducing the power of the extracts is relative value against BHT standard used for color formation. Results of reducing the power of different brown rice varieties are showing the effects of six solvents with ultrasonic-assisted agitation Fig-4. The reducing powers of the rice extracts (1.0 mg/mL) tested ranged from 0.22 to 0.95 for Basmati-370 and KSK-133. The results revealed that isopropanol extracts offered the highest values of reducing powers (0.95) while ethanol extracts the lowest (0.22). Of the extraction techniques employed, sonication-assisted magnetic stirrer showed better efficacy towards recovering antioxidant components of potent reducing nature as compared with an orbital shaker. *Nam et al.* investigated rice cultivars for reducing powers and reported that pigmented or brown rice has greater potential than that of white rice [34]. It has been examined that reducing the power of rice extracts increased in a concentration-dependent manner [34, 35]. *Iqbal et al.* measured the reducing potential of methanol extracts of different rice bran and established an increasing trend for reducing powers with increasing the concentration [15].

Metal (Iron) chelating activity has a reasonable correlation with antioxidant properties as iron complexion could provide defense against oxidative harm [36].

Table 7: Analysis of Variance for IC50 of DPPH.

Source	DF	SS	MS	F	P
Varieties	9	4.5180	0.5020	1.81	0.093
Solvent	5	33.7188	6.7438	24.29	0.000
Error	45	12.4949	0.2777		
Total	59	50.7317			

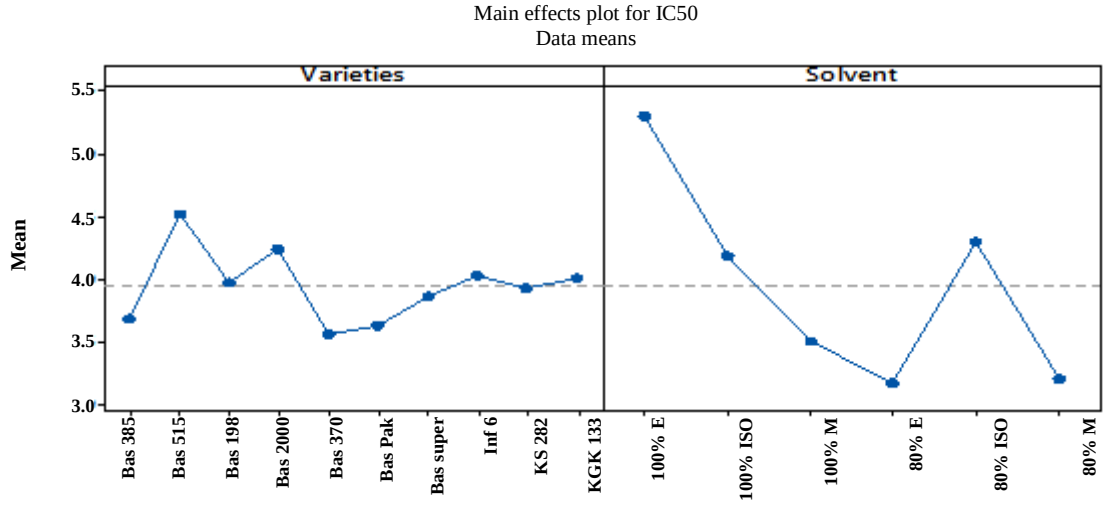

Fig. 3: Comparison of varieties and solvents for IC_{50} of DPPH.

In the present research work, the metal chelating activity of selected varieties of brown rice was determined against Fe^{2+} and reported as EDTA equivalents. Mostly, a considerable ($p<0.05$) variation was observed in metal chelating activity among the tested varieties. Metal chelating activities (EDTA Eq.) of rice extracts (1.0 mg/mL), obtained using sonication-assisted protocol is shown in Fig. 2. As expected, the highest results of metal chelating activity were observed for isopropanol extracts and the lowest for ethanol using. Ferrous ions have a major application in the food system is the most effective pro-oxidants so high metal chelating powers of methanol extracts of rice are beneficial [37]. In support of our present analysis, several other studies also indicate that rice extracts have promising metal chelating activity [38, 39].

The metal chelating activity attributed in another research revealed to the presence of phytic acid in colored rice [27]. The similar research studies have also reported that rice extracts have excellent antioxidant activity [32, 40].

These results could help rice producers to promote brown rice. It can be concluded on the basis of results revealed of selected varieties of Pakistani brown rice that Pakistani rice varieties are not only famous for their unique aroma but also have good antioxidant activity. These results may establish opportunities for rice producers and eventually commercial stakeholders, to nourish the production of brown rice enriched with enhanced levels of the natural antioxidants and nutraceuticals. Brown rice rich in some potent phytoxidants may be used into value-added functional foods.

CONCLUSIONS

The use of ultrasonic assisted extraction in aqueous methanol and isopropanol for brown rice of Pakistan showed promising antioxidant activity over the conventional process, particularly Isopropanol. Basmati varieties were superior in terms of antioxidant attributes compared to non-Basmati varieties. For growers and consumers, Basmati 370 and basmati Pak are preferable according to their potential of antioxidants activity. These varieties could better play their role for health care cereals.

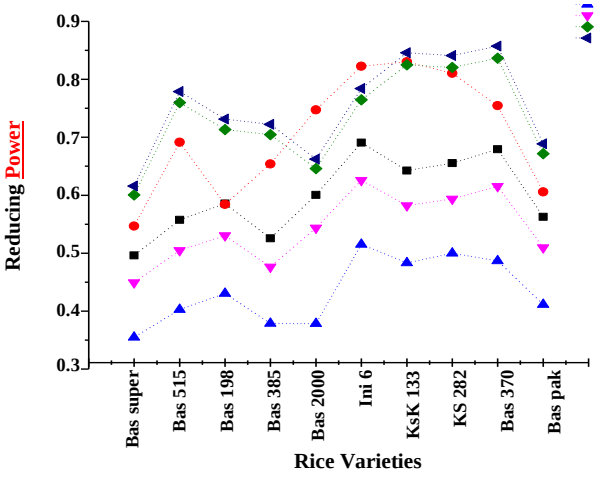

Fig. 4: Reducing power of extracts of different varieties of rice (Oryza sativa L.) produced by sonication–assisted magnetic stirrer. M = Methanol, E = Ethanol, Iso = Isopropanol.

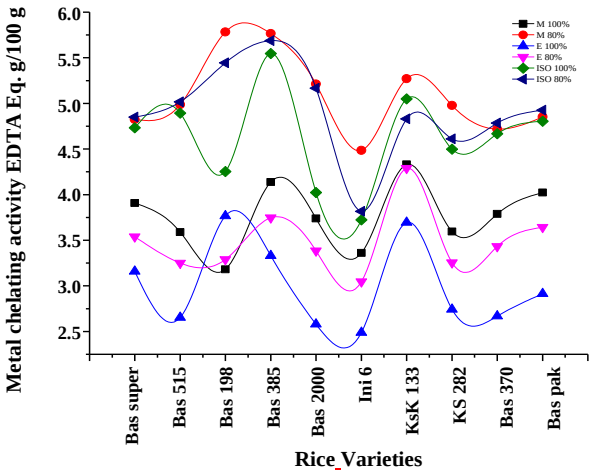

Fig. 5: Metal chelating activity (EDTA Eq. g/100g) of extracts from different varieties of rice (Oryza sativa L.) produced by different solvents using sonication–assisted magnetic stirrer. M = Methanol, E = Ethanol, Iso = Isopropanol.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

The corresponding author is thankful to the Higher Education Commission (HEC) of Pakistan for providing financial support to carry out this research work.

Received: Nov. 5, 2016 ; Accepted: Jul. 3, 2017

REFERENCES

[1] Do Q.D., Anqkawijaya A.E., Tran-Nguyen P.L., Huynh L.H., Soetaredjo F.E., Ismadji S., Ju Y.H., Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of Limnophila Aromatica, *Journal of Food and Drug Analysis*, **22**(3): 296-302 (2014).

[2] Chemat F., Rombaut N., Sicaire A.G., Meullemiestre A., Fabiano-Tixier A.S., Abert-Vian M., Ultrasound Assisted Extraction of Food and Natural Products. Mechanisms, Techniques, Combinations, Protocols and Applications, *A review. Ultrasonics Sonochemistry*, **34**: 540-560 (2017).

[3] Li Y., Fabiano-Tixier A.S., Tomao V., Cravotto G., Chemat F., Green Ultrasound-Assisted Extraction of Carotenoids Based on the Bio-Refinery Concept Using Sunflower Oil as an Alternative Solvent, *Ultrasonics Sonochemistry*, **20**(1): 12-18 (2013).

[4] Jacotet-Navarro M., Rombaut N., Deslis S., Fabiano-Tixier A.S., Pierre F.-X., Bily A., Chemat F., Towards a "dry" Bio-Refinery without Solvents or Added Water Using Microwaves and Ultrasound for Total Valorization of Fruit and Vegetable by-Products, *Green Chemistry*, **18**(10): 3106-3115 (2016).

[5] Slavin J.L., Whole Grains and Health: Separating the Wheat from the Chaff, *Nutrition Today*, **29**(4): 6-10 (1994).

[6] Han X., Shen T., Lou H., Dietary Polyphenols and Their Biological Significance, *International Journal of Molecular Sciences*, **8**(9): 950-988 (2007).

[7] Hamauzu Y., Chachin K., Effect of High Temperature on the Postharvest Biosynthesis of Carotenes and α-Tocopherol in Tomato Fruit, *Journal of the Japanese Society for Horticultural Science*, **63**(4): 879-886 (1995).

[8] Ruperez F.J., Martin D., Herrera E., Barbas-Journal C., Chromatographic Analysis of α-Tocopherol and Related Compounds in Various Matrices, *Journal of Chromatography A*, **935**(1): 45-69 (2001).

[9] Ronald R., Eitenmiller J.L., Vitamin E: "Food Chemistry, Composition, and Analysis", CRC Press. 540 Florida, USA (2004).

[10] Lin Y.T., Pao C.C., Wu S.T., Chang C.Y., Effect of Different Germination Conditions on Antioxidative Properties and Bioactive Compounds of Germinated Brown Rice. *BioMed Research International*, **2015**: 10 (2015).

[11] Imam M.U., Azmi N.H., Bhanger M.I., Ismail N., Ismail M.,, Antidiabetic Properties of Germinated Brown Rice: A Systematic Review, *Evidence-Based Complementary and Alternative Medicine*,**2012**: 12-(2012).

[12] El-Gharably A.M.A., Ashoush I.S., Utilization Impact of Adding Pomegranate Rind Powder and Red Beet Powder as Natural Antioxidant on Quality Characteristics of Beef Sausage, *World Journal of Dairy & Food Sciences*, **6**(1):. 86-97 (2011).

[13] Chen M.-H., Bergman C., A Rapid Procedure for Analysing Rice Bran Tocopherol, Tocotrienol and γ-oryzanol Contents, *Journal of Food Composition and Analysis*, **18**(4): 319-331 (2005).

[14] Chotimarkorn C., Benjakul S., Silalai N., Antioxidant Components and Properties of Five Long-Grained Rice Bran Extracts from Commercial Available Cultivars in Thailand, *Food Chemistry*, **111**: 636-641 (2008).

[15] Iqbal S., Bhanger M.I., Anwar F., Antioxidant Properties and Components of Some Commercially Available Varieties of Rice Bran in Pakistan, *Food Chemistry*, **93**: 265-272 (2005).

[16] Hu W., Wells J.H., Shin T., Godber J.S., Comparison of Isopropanol and Hexane for Extraction of Vitamin E and Oryzanols from Stabilized Rice Bran, *Journal of the American Oil Chemists' Society*, **73**(12): 1653-1656 (1996).

[17] Boukroufa M., Boutekedjiret C., Petigny L., Rakotomanomana N., Chemat F., Bio-Refinery of Orange peels Waste: A New Concept Based on Integrated Green and solvent free extraction processes using Ultrasound and Microwave Techniques to Obtain Essential Oil, Polyphenols and Pectin, *Ultrasonics Sonochemistry*, **24**: 72-79 (2015).

[18] Zubair M., Anwar F., Shahid S.A., Effect of Extraction Solvents on Phenolics and Antioxidant Activity of Selected Varieties of Pakistani Rice (*Oryza sativa*), *International Journal of Agriculture & Biology*, **14**(6): 935−940 (2012).

[19] Yen G.C., Duh P.D., Tsai C.L., Relationship between Antioxidant Activity and Maturity of Peanut Hulls, *Journal of Agricultural and Food Chemistry*, **41**: 67-70 (1993).

[20] Decker F.A., Welch B., Role of Ferritin as a Lipid Oxidation Catalyst in Muscle Food, *Journal of Agricultural and Food Chemistry*, **38**: 674-677 (1990).

[21] Shimada K., Fujikawa K., Yahara K., Nakamura T., Antioxidative Properties of Xanthan on the Autoxidation of Soybeen Oil in Cyclodextrin Emulsion, *Journal of Agriculture and Food Chemistry*, **40**: 945-948 (1992).

[22] Wu X., Beecher G.R., Hoden J.M., Haytowiz D.B., Gebhardt S.E., Prior R.L., Lipophilic and Hydrophilic Anti-Oxidant Capacities of Common Foods in the United States, *Journal of Agriculture and Food Chemistry*, **52**: 4026-4037 (2004).

[23] Rohrer C.A., Siebenmorgen T.J., Nutraceutical Concentrations within the Bran of Various Rice Kernel Thickness Fractions, *Biosystems Engineering*, **88**: 453-460 (2004).

[24] Saenjum C., Chaiyasut C., Chansakaow S., Suttajit M., Sirithunyalug B., Antioxidant and Anti-Inflammatory Activities of Gamma-Oryzanol Rich Extracts from Thai Purple Rice Bran, *Journal of Medicinal Plants Research*, **6**(6): 1070-1077 (2012).

[25] Devi R.R., Arumughan C., Phytochemical Characterization of Defatted Rice Bran and Optimization of a Process for Their Extraction and Enrichment, *Bioresource Technology*, **98**(16): 3037-3043 (2007).

[26] Isbilir S.S., Tuncay D., A Study on Bioactive Content, Antioxidant Activity, and α-Amylase Inhibition of Black Rice Grown in Turkey, *European International Journal of Science and Technology*, **3**(7): 51-60 (2014).

[27] Kong S., Lee J., Antioxidants in Milling Fractions of Black Rice Cultivars, *Food Chemistry*, **120**(1): 278-281 (2010).

[28] Hodzic Z., Pasalic H., Memisevic A., Srabovic M., Saletovic M., Poljakovic M., The Influence of Total Phenols Content on Antioxidant Capacity in the Whole Grain Extracts, *European Journal of Scientific Research*, **28**(3): 471-477 (2009).

[29] Shona M.Y., Choi, Sang D.O., Kahng G.G., Nam S.H., Sung N.J., Antimutagenic, Antioxidant and Free Radical Scavenging Activity of Ethylacetate Extracts from White, Yellow and Red Onion, *Food and Chemical Toxicology*, **42**: 659-666 (2004).

[30] Roginsky Vi., Lissi E.A., Review of Methods to Determine Chain Breaking Antioxidant Activity in Food, *Food Chemistry*, **92**: 235-254 (2005).

[31] Cheng Z., Su L., Moore J., Zhou K., Luther M., Yin J.J., Yu L.L., Effect of Postharvest Treatment and Heat Stress on Availability of Wheat Antioxidants, *Journal of Agriculture and Food Chemistry*, **54**: 5623-5629 (2006).

[32] Sompong R., Siebenhandl-Ehn S., Linsberger-Martin G., Berghofer E., Physicochemical and Antioxidative Properties of Red and Black Rice Varieties from Thailand, *China and Sri Lanka*. *Food Chemistry*, **124**: 132-140 (2011).

[33]Yen G.-C., Duh P.-D., Chuang D. Y., Antioxidant Activity of Anthraquinones and Anthrone, *Food Chemistry*, **70**(4): 437-441 (2000).

[34] Nam S.H., Choi Su., Kang M., Koh H.J., Kozukue N., Friedman M., Antioxidative Activities of Bran Extracts from Twenty one Pigmented Rice Cultivars, *Food Chemistry*, **94**(4): 613-620 (2006).

[35] Rao A.S., Reddy S.G., Babu P.P., Reddy A.R., The Antioxidant and Antiproliferative Activities of Methanolic Extracts from Njavara Rice Bran, *BMC Complementary and Alternative Medicine*, **10** (4): 2-9 (2010).

[36] Yoshimura O., Tsutomu I., Takaho W., Hiroyuki N., Antioxidative Effect of Maillard Reaction Products Using Glucose-Glycine Model System, *Journal of Agricultutre and Food Chemistry*, **45**: 4106-4109 (1997).

[37] Yamauchi R., Tatsumi Y., Asano M., Kato K., Ueno Y., Effect of Metal Salts and Fructose on the Autoxidation of Methyl Linoleate in Emulsions, *Agricultutral Biology and Chemistry*, **52**: 849-850 (1988).

[38] Chang S.T., Wu J.H., Wang S.Y., Kang P.L., Yang N.S., Shyur L.F., Atioxidant activity of Extracts from Acacia Confusa Bark and Heartwood, *Journal of Agriculture and Food Chemistry*, **49**: 3420-3424 (2001).

[39] Yang D.S., Lee K., Jeong O., Kim K., Stanley J.K., Characterization of Volatile Aroma Compounds in Cooked Black Rice, *Journal of Agriculture Food Chemistry*, **56**: 35-240 (2008).

[40] Sam, Y., Kim, S., Isolation of Anthocyanin from Black Rice (Heugjinjubyeo) and Screening of Its Antioxidant Activities, *Korean Journal of Microbiology and Biotechnology*, **36**: 55-60 (2008).