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Determination of Important Phenolic Compounds in Pakistani Brown Rice Varieties in Controlled, Germinated and Fermented Conditions by High Performance Liquid Chromatography

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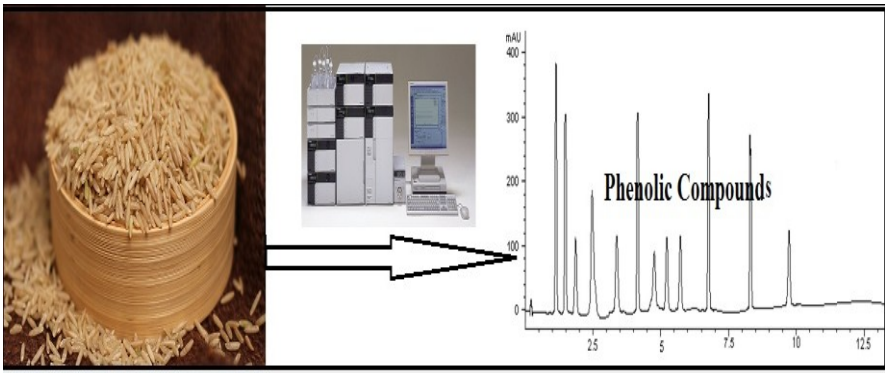
GRAPHICAL ABSTRACT



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ABSTRACT

A simple and novel high performance liquid chromatography (HPLC) method has been developed for the simultaneous determination and quantification of phenolic compounds in brown whole grain rice varieties of Pakistani origin. The accumulation of these compounds was studied during germination stages and fermentation process, thereby, providing a reliable and rapid method for their quantification in food samples. Calibration curves for the standard phenolic compounds showed good linear regression values (r^2 =0.996-0.998) within the test ranges. The limit of detection and the limit of quantification were found in the range of 0.04-0.06 µg/mL and 0.166-0.205µg/mL, respectively. Precision (%RSD) of the method was found in the range of 0.05-5.25 and 0.05-0.58 for inter-day (n=3) and intra-day (n=5), respectively. The robustness (%RSD) was found in the range of 1.05-2.65. Excellent recoveries were attained within the range of 93%-106%.The average amount of phenolic compounds was found to be 0.185 g/100g in controlled condition whereas, further accumulation of these compounds was noticed during germination and fermentation phases. The maximum average amount of phenolic compounds after germination period of 120 hours and fermentation process was found to be 0.284g/100gand 0.565 g/100g, respectively.

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1. Introduction

Rice grains are among the major components of the daily human diet. The production and consumption of cereal grains including rice is very high worldwide, especially in Asia. Brown rice is a functional food obtained by removing husk from the paddy. Brown rice contains more nutritional components then the milled or polished rice because many bio-functional compounds, such as, phenolic compounds are present mainly in the germ and bran layer. The whole grains also contain many other beneficial components like dietary fiber, vitamin, minerals and other secondary metabolites[1, 2]. Food processing such as milling and pearling processes affect the distribution of phenolic compounds and thus their properties vary among the milling fractions[3].Phenolic acid content also varies significantly among the varieties due to several factors, such as environmental conditions and agronomic practices [4, 5].

Phenolic compounds have great importance for human consumption and also play an important role in plants. Brown rice contains a wealthy amount of phenolic compounds, especially, in outer shell which is considered highly beneficial for human's consumption. Phenolic compounds have also been reported in food items such as cereals, tea, juices, coffee, and wines [6, 7]. The appearance, taste, odor and oxidative stability of the food, and thus the commercial value, are thought to be affected by their presence [8].

Phenolic compounds are derived in two from with respect to their core structures one is hydroxy cinnamic acid form and other is hydroxy benzoic acid form. The derivatives of hydroxy cinnamic acid include ferulic, caffeic, p-coumaric and sinapic acids, while the derivatives of hydroxy benzoic acid constitute gallic, vanillic, syringic and protocatechuic acids [9, 10]. Phenolic compounds also show inhibition of peroxidation and chelation of transition metals [11]. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-diabetic, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects [12-14]. The health benefits and diverse commercial applications of phenolic compounds have prompted the interest of the scientists to increase their concentrations in plant by using different techniques such as genetic engineering [15].

The separation and quantification of phenolic compound has been widely reported previously by several research groups using analytical techniques such as U/HPLC, GC, TLC and CE [16-18]. Pakistan is a major producer and exporter of many high value rice varieties. These varieties are considered among world best in terms of their taste and aroma. However, previous studies lack the comprehensive research focusing the analysis of phenolic compounds in rice varieties of Pakistani origin [19]. We suggest that the method developed during this study can be used for investigation and separation of important phenolic compounds in prominent brown rice varieties. This study could also be helpful for monitoring the accumulation of phenolic compounds during different germination intervals, after fermentation process and in soaked form of brown rice to check and control the quality of food products based upon them.

2. Experimental

2.1. Chemicals and Reagents

Gallic acid, caffeic acid, 4-hydroxybenzoic acid, syringic acid, ferulic acid, salicylic acid, phloroglucinol, cinnamic acid, catechin, quercetin, vanillin, phloridzin, and phloretin were purchased from Sigma-Aldrich (USA) and Merck (Darmstadt, Germany). All other chemicals and solvents used in this study were supplied by Sigma-Aldrich (USA) and were of analytical grads. De-ionized water was used from Milli-Q water purification system (Millipore MA, USA) during the study.

2.2. Standard Solutions

A stock solution of the mixture of standard compounds was prepared by dissolving accurately weighed portions of the standards in methanol, transferring the solution to a 5 mL volumetric flask, and then adding methanol to make up the volume. The concentration of each compound in stock solution was 0.57 (gallic acid), 0.52 (caffeic acid), 0.53 (4-hydroxybenzoic acid), 0.42 (syringic acid), 0.32 (ferulic acid), 0.45 (vannillic acid), 0.42 (p-cumeric), 0.35 (cinnamic acid), 0.39 (catechin), 0.36 (quercetin), 0.40 (protocatechuic acid), 0.36 (Phloridzin) mg/mL. The stock solutions were diluted to provide different concentration ranges. The calibration curves for each compound were plotted with at least five appropriate concentrations of each standard in triplicate. The solutions were brought to room temperature and filtered through a 0.45 µm membrane filter and an aliquot of 2.5 µL was injected into HPLC for analysis. The detection of phenolic compounds was recorded by UV detection at 280 nm, the regression equations were calculated in the form of $Y = A \times X + B$ where Y and X were peak area and amount of compound injected respectively whereas A is slope of the equation. The calibration curves were obtained by Microsoft Excel 2010.

2.3. Samples Preparation

Three brown rice varieties including long grain basmati, long grainirri-6 and long grain irri-9 were collected from National Agricultural Research Centre, Islamabad, Pakistan, and Rice Research Institute, Dokri, Sindh, Pakistan. Freshly collected samples were divided into three parts and stored in sealed clear polyethylene plastic bags at -40°C until they were used. The first part of collected samples was used as controlled whereas the remaining two parts of the stored samples were used for germination and fermentation studies. All the three parts were freeze-dried and stored at -40°C for at least 60 hours. Samples were milled to fine powder using a grinder and passed through a fine sieve (45 Mesh) to achieve uniform particle size rice flour.

2.3.1 Germination of Rice Samples

Germination of rice samples was performed using the previously reported method by Hayat et al. with slight modifications. [20, 21] Briefly, 150 grams of all brown rice varieties were individually soaked in1000 mL Erlenmeyer flasks containing 500 mL distilled water provided with aerobic conditions at room temperature. Water in the flasks was changed after every 24 hours. 20 grams of each sample was drawn after 24, 48, 72, 96 and 120 hours for incubation at 50°Cto achieve approximately 10% of moisture content. The dry germinated samples were grounded finely (45 Mesh) using a laboratory grinder before extraction. All samples were analyzed in triplicates after extraction followed by HPLC separation and quantification.

2.3.2 Fermentation of Rice Samples

The fermentation of rice seeds was carried out using method previously reported by Hayat et al. and Inagaki et al., with slight modifications.[20, 22] 50 grams of brown whole grain rice seed from each variety was taken into 500 mL Erlenmeyer flasks individually and added with 200 mL of distilled water. Flasks were autoclaved at 121 °C for 20 min for steam-cooking and then cooled to room temperature. A 2.5 mL of pure culture suspension (106 Spores/mL) of *Rhizopusoryzaespores* (ATCC No. 11145) obtained from the Department of Biotechnology, University of Karachi were then evenly inoculated to each flask and placed in a fermentation chamber at 35 °C for 72 hours with controlled humidity and aerobic conditions. The fermented mass was then autoclaved (121 °C, 10 min) and dried in an oven at 50 °C for 12 hours to obtain approximately 10% moisture. The dried fermented substrates were grinded in a laboratory grinder and extracted prior to HPLC analysis.

2.4 Extraction of Phenolic Compounds

After homogenization, milling, grinding and sieving, 2 g of each rice flour sample was added with the mixture of ethanol and water (70:30) and sonicated for 30 min at room temperature followed by centrifugation at 4000 rpm for 10 min. The supernatant was filtered through whatman filter paper No. 42 (Sigma Aldrich, USA). The samples were extracted thrice and evaporated using Rota-evaporator. The dried extracts were re-dissolved in 1 mL methanol, filtered through 0.45 µm filter and stored until analysis.

2.5 HPLC Analysis

The HPLC analysis was performed at 35°Cusing a C_{18} column UHC-250(2×50 mm id, MAC-MOD Analytical, Inc., USA particle size 5µm)at 280 nm on Agilent 1200 RRLC system (Agilent Technology Inc., Wilmington, DE) equipped with an auto sampler, degasser, binary pump and diode array detection (DAD) system. The Chemstation software was used for the data acquisition.The mobile phase consisted of 0.25% trifluoroacetic acid in Mili Q water (A) and methanol (B) at a flow rate of 0.6mL/min. Gradient elution was performed as follows: 8% solvent B from 0 to 4 min, 10% solvent B from 4 to 6 min, 12% solvent B from 6 to 8 min, 15% solvent B from 8 to 10min, 10% solvent B from 10 to 12 min and 8% solvent B from 12 to 13 min. All samples were analyzed in triplicate and phenolic compounds in the samples were identified by comparing their relative retention time and UV spectra with authentic compounds and were detected using an external standard or spike method and the results were calculated using a standard curve.

3 Results

It is well known that the elution order of phenolic compounds in RP-HPLC is closely related to their polarity, with the most polar ones eluting first, followed by the less polar ones. Once the analytes were identified, the parameters affecting HPLC retention performance such as sample solvents, mobile phase composition, column temperature, and flow rate were optimized for the detection of phenolic compound using this method. Several gradient systems were tried to attain the maximum separation among the analytes. The chromatogram showed the phenolic compound eluting at the retention times of 1.03, 1.42,1.75, 2.47, 3.45, 4.32, 4.74, 5.19, 5.56, 6.05, 8.81, and 9.85 min for gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, vinallic acid, phloridzin, p-cumaric acid, syringic acid, catechin, caffic

acid, cinnamic acid, quercetin, ferulic acid , respectively, as shown in Figure 1.

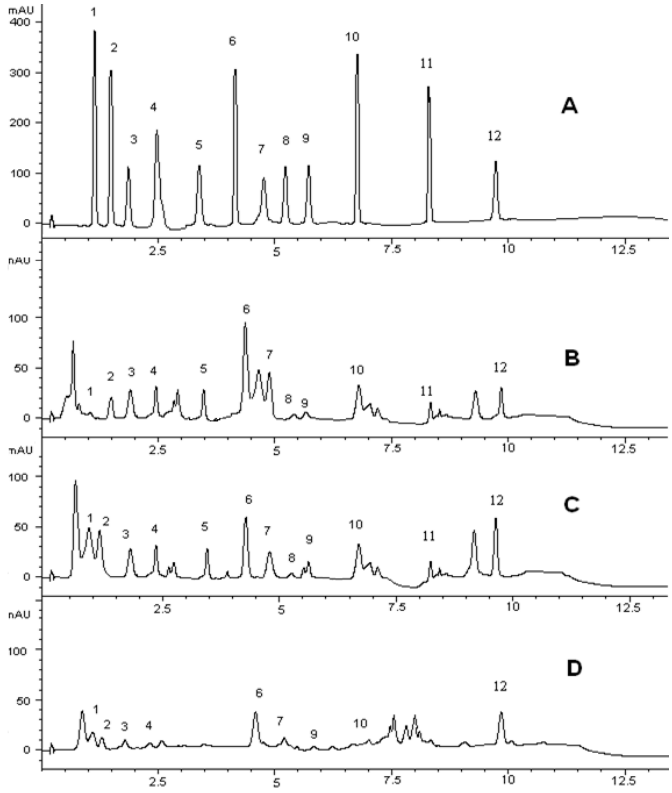

Fig. 1. HPLC chromatogram of phenolic compounds for (A) Standard analytes (B) Fermented Brown Rice Extract (C) Brown Rice Extract after 120 hours Germination (D)Brown Rice Extract in controlled conditions. Peaks Identification: (**1**) Gallic acid (**2**) Protocatechuic acid (**3**) 4-Hydroxybenzoic acid (**4**) Vinallic acid(**5**) Phloridzin (**6**) P-coumaric acid (**7**) Syringic acid (**8**) Catechin (**9**) Caffic acid (**10**)Cinnamic acid (**11**) Quercetin, and(**12**) Ferulic acid.

3.1 Method Performance

Baseline separation was achieved within 10 min time by using the optimized gradient elution system. The dilute stock solution of the standard phenolic compounds was further diluted with methanol to give a series of concentrations for determining the limits of detection (LOD) and quantification (LOQ).The LODs and LOQs were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively. All calibration curves showed good linear regression values (r^2 =0.996-0.998) within the calibration ranges. The limit of detection (S/N = 3) and the limit of quantification (S/N = 10) were found in the ranges of 0.04-0.06 µg/mL and 0.166-0.205µg/mL, respectively.

3.2 Method Validation

The assessment of repeatability and reproducibility of the method was performed by measurement of intra-day and inter-day variability of the results. The intra-day variability was performed under optimal conditions by monitoring five replicate determinations of a mixed standard solution of all phenolic compounds. The relative standard deviation (RSD, %) values for retention time and peak area of each compound were calculated. The inter-day variability was examined over three days by monitoring five replicate determinations in each day. Robustness of method was observed with respect to flow rate, column temperature and concentration of TFA in mobile phase.

As demonstrated in **Tables 1, 2 and 3** that this HPLC-DAD method is precise, accurate, and sensitive enough for the simultaneous separation, quantitative and evaluation of the phenolic compounds in Pakistani brown rice varieties. Validation studies of this method indicates that this method has good reproducibility as shown in **Table 2**, the overall precision as RSD (%) inter-day (n=3) intra-day (n=5)with variations of the peak area and retention time is less than 5% for all phenolic compounds. The robustness of the method with respect to flow rate, Concentration of TFA as mobile phase and column temperature also has RSD (%) value less than 5%. The average percentage recovery of phenolic compounds in brown rice varieties were found within the range 93%-106% C.V. 3.25% (n=3).The standards of phenolic compounds were also examined for stability in every week for two month time period and found to consistent in their retention time and peak area.

4 Discussion

The results demonstrated that the content of phenolic compounds was found significantly higher in germinated and fermented brown rice than in controlled conditions. Although phenolic compounds may also bind to carbohydrates and proteins as described by Pinelo et al., during soaking binding becomes weaker thus accumulation may occur during the germination process of the rice seeds. [23] The amount of phenolic compounds after fermentation was also found to be increased,however, the increase was not as higher as reported in rice bran which ispreviously described by Shao et al., Whereas, they have also described that the increasing order of total content of phenolic compounds in whole grain is endosperm, embryo, and bran, each accounting for 1-23%, 3-23% and 60-93%,respectively[24].

Table 1. Validation results Precision (Inter-day n=3 and intra-day n=5) and Robustness (n=3)

Analyte	**Precision RSD (%)**				**Robustness RSD (%)**					
	Inter day (n=3)		Intra day (n=5)		Flow Rate mL/min		Column Temp. (C)		TFC Conc. (% *v/v*)	
	Retention Time	Peak Area	Retention Time	Peak Area	**5.0**	**6.0**	**30**	**35**	**0.20**	**0.25**
Gallic acid	0.35	2.35	0.15	0.35	1.05	0.95	2.15	1.84	1.25	1.12
Caffeic acid	0.25	1.42	0.05	0.42	1.25	1.05	2.45	1.98	2.45	2.15
4-hydroxybenzoic acid	0.45	3.45	0.05	0.55	1.14	1.05	1.95	1.45	1.85	1.45
Syringic acid	0.55	4.52	0.15	0.48	1.58	1.45	1.84	1.46	1.47	1.25
Ferulic acid	0.35	4.25	0.05	0.38	1.23	1.15	1.65	1.33	2.15	2.08
P-coumaric acid	0.25	5.25	0.15	0.54	1.32	1.20	2.25	1.84	2.14	1.95
Protocatechuic acid	0.28	3.85	0.20	0.35	1.22	1.11	2.15	1.85	1.92	1.55
Cinnamic acid	0.55	4.25	0.25	0.25	1.25	1.15	2.45	2.15	1.85	1.75
Catechin	0.50	5.10	0.20	0.35	1.65	1.25	2.58	2.22	1.74	1.65
Quercetin	0.44	5.24	0.24	0.45	2.45	1.95	2.47	2.45	1.55	1.45
Vanillic acid	0.68	4.58	0.08	0.52	1.45	1.25	2.65	2.24	2.15	1.05
Phloridzin	0.65	4.25	0.05	0.58	2.21	1.85	2.14	1.85	1.56	1.35

Table 2. Regression data, LODs and LOQs for the standard phenolic compounds

Analyte	**Leaner Range (µg/mL)**	**r^2**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**RSD (%)**
Gallic acid	1.4-18.5	0.998	0.041	0.137	1.95
Caffeic acid	0.5-15.58	0.997	0.045	0.151	1.98
4-hydroxybenzoic acid	0.5-12.5	0.996	0.034	0.113	1.89
Syringic acid	0.75-18.5	0.998	0.057	0.139	1.97
Ferulic acid	0.5-10.5	0.998	0.033	0.110	1.22
P-coumaric acid	1.25-10	0.998	0.038	0.127	2.31
Protocatechuic acid	0.5-12.5	0.997	0.044	0.145	1.95
Cinnamic acid	0.5-15	0.997	0.068	0.226	1.56
Catechin	1.25-15.5	0.996	0.055	0.183	4.56
Quercetin	1.5-20	0.996	0.025	0.085	4.26
Vanillic acid	1.0-15	0.997	0.038	0.126	1.85
Phloridzin	1.25-18.5	0.998	0.062	0.206	2.85

Table 3. Phenolic compounds Content of Brown Rice, Germinated Brown Rice and Fermented Brown Rice (mg/100g of Rice Flour)

Rice Variety	Germination Time (hrs)	Phenolic Compounds											
		4-HA	CA	CTH	FA	GA	p-CA	QUT	PA	VA	SA	CNA	PHZ
Basmati Super	Controlled	0.24±0.03	0.26±0.02	nd*	0.25±0.03	0.25±0.02	0.21±0.02	nd	0.16±0.02	0.19±0.02	0.24±0.03	0.18±0.02	nd
	24	0.25±0.04	0.27±0.03	nd	0.28±0.04	0.24±0.02	0.20±0.03	nd	0.18±0.03	0.21±0.03	0.22±0.04	0.21±0.03	tc
	48	0.26±0.03	0.31±0.04	tc**	0.31±0.05	0.27±0.03	0.21±0.02	tc	0.22±0.02	0.23±0.02	0.24±0.03	0.15±0.02	0.09±0.01
	72	0.28±0.06	0.32±0.05	0.13±0.01	0.35±0.03	0.28±0.04	0.23±0.03	0.12±0.01	0.24±0.03	0.26±0.04	0.26±0.05	0.24±0.03	0.12±0.02
	96	0.29±0.05	0.34±0.04	0.21±0.02	0.38±0.03	0.32±0.05	0.20±0.02	0.14±0.02	0.23±0.03	0.29±0.05	0.28±0.04	0.22±0.03	0.15±0.02
	120	0.29±0.05	0.36±0.04	0.21±0.02	0.45±0.06	0.33±0.05	0.24±0.02	0.21±0.02	0.26±0.04	0.31±0.05	0.32±0.05	0.25±0.03	0.18±0.02
	Fermented	0.98±0.09	0.74±0.08	0.44±0.05	0.98±0.08	0.84±0.07	0.34±0.03	0.24±0.02	0.38±0.04	0.75±0.06	0.54±0.06	0.32±0.04	0.24±0.02
Irri-6	Controlled	0.25±0.03	0.27±0.02	nd*	0.26±0.03	0.24±0.02	0.22±0.02	nd	0.17±0.02	0.21±0.02	0.22±0.03	0.15±0.02	nd
	24	0.26±0.04	0.28±0.03	nd	0.29±0.04	0.23±0.02	0.22±0.03	nd	0.19±0.03	0.22±0.03	0.23±0.02	0.19±0.03	tc
	48	0.27±0.03	0.33±0.04	tc**	0.33±0.05	0.26±0.03	0.23±0.02	tc	0.24±0.02	0.26±0.02	0.26±0.03	0.18±0.02	0.06±0.01
	72	0.28±0.06	0.35±0.05	0.12±0.01	0.38±0.03	0.26±0.04	0.25±0.03	0.14±0.01	0.26±0.03	0.27±0.04	0.27±0.03	0.20±0.03	0.14±0.02
	96	0.31±0.05	0.36±0.04	0.19±0.02	0.39±0.03	0.31±0.05	0.24±0.02	0.16±0.02	0.27±0.03	0.32±0.05	0.29±0.03	0.21±0.03	0.16±0.02
	120	0.32±0.05	0.38±0.04	0.20±0.02	0.46±0.06	0.32±0.05	0.26±0.02	0.23±0.02	0.26±0.04	0.34±0.05	0.36±0.04	0.22±0.03	0.21±0.02
	Fermented	0.94±0.08	0.76±0.08	0.42±0.05	1.09±0.08	0.83±0.07	0.36±0.03	0.28±0.03	0.42±0.04	0.85±0.06	0.58±0.05	0.31±0.04	0.25±0.03
Irri-9	Controlled	0.22±0.03	0.22±0.02	nd*	0.22±0.03	0.23±0.02	0.18±0.02	nd	0.15±0.02	0.16±0.02	0.26±0.03	0.11±0.02	nd
	24	0.23±0.04	0.25±0.03	nd	0.25±0.04	0.23±0.02	0.20±0.03	nd	0.16±0.03	0.18±0.03	0.22±0.04	0.12±0.03	Tc
	48	0.25±0.03	0.29±0.04	tc**	0.30±0.05	0.25±0.03	0.21±0.02	tc	0.20±0.02	0.21±0.02	0.28±0.03	0.12±0.02	0.10±0.01
	72	0.26±0.06	0.30±0.05	0.11±0.01	0.32±0.03	0.26±0.04	0.22±0.03	0.12±0.01	0.22±0.03	0.25±0.04	0.32±0.03	0.16±0.03	0.12±0.02
	96	0.27±0.05	0.31±0.04	0.15±0.02	0.33±0.03	0.32±0.05	0.23±0.02	0.13±0.02	0.24±0.03	0.27±0.05	0.33±0.04	0.18±0.03	0.16±0.02
	120	0.27±0.06	0.33±0.04	0.18±0.02	0.41±0.06	0.34±0.05	0.24±0.02	0.19±0.02	0.25±0.04	0.32±0.05	0.35±0.04	0.20±0.03	0.18±0.02
	Fermented	0.92±0.08	0.72±0.07	0.35±0.05	0.95±0.08	0.84±0.07	0.32±0.03	0.22±0.03	0.35±0.05	0.70±0.06	0.55±0.05	0.35±0.04	0.23±0.03

nd*: Not detected; tc**: trace

Results mentioned here are the mean±standard deviations of all five replicates.

4-HA (4-hydroxybenzoic acid), CA (Caffic acid), CTH (Catechin), FA (Ferullic acid), GA (Gallic acid), p-CA (p-Cumaric acid), QUT (Quercetin), PA (Protocatechuic acid), VA (Vinallic acid), SA (Syrrangic acid) CNA (Cinnamic acid) and PHZ (Phloridzin)

rice representative amount of phenolic compounds was found, thus, its accumulation is also higher accordingly. So we can conclude from here that the phenolic content must higher in purely separated bran and lesser in purely separated endosperm of the grain, in comparison to brown rice.

All the phenolic compounds were detected and quantified in controlled, germinated and fermented brown rice samples except catechin, quercetin and phloridzine, which were found to be below detection limit in samples of controlled conditions. The average amount of phenolic compounds was found to be 0.185 g/100g in controlled condition. In the germination samples, the highest accumulation occurred during germination period of 120 hours resulted in the average content of 0.284 g/100g. While in the fermentation samples the average highest phenolic content detected was 0.565 g/100g. Evident from the results, during germination and fermentation the increase in the amount of phenolic content was observed. The most abundant compound was found to be ferulic acid. The highest amount of ferulic acid content of brown rice was found to be 0.45 mg/100 g of flour after 120 hours germination period and 0.98 mg/100g in fermentation samples, in comparison to 0.25 mg/100 g of flour observed in samples at controlled condition. The detailed composition of all phenolic compounds in controlled, germinated and fermented conditions was demonstrated in **Table 3**.

Recovery studies were also conducted to evaluate the extraction efficiency of the developed method in real samples. The recoveries of all compounds were determined by adding accurately known amounts of the standards to the sample extract before performing analysis. The analysis was performed in 5 replicates with three levels of added concentrations. The recoveries were found to be within the range of 93%-106%. The average recoveries and relative standard deviations (RSD %) were determined and are presented in **Table 4**.

Table 4. Recoveries of the Phenolic compounds in Pakistani brown rice (n=5)

Analyte	Contained (mg)	Added (mg)	Found ± SD (mg)	Recovery (%)
Gallic acid	1.57	5	6.51±0.45	99.16
		10	11.31±0.77	97.82
Caffeic acid	2.45	5	7.36±0.58	98.84
		10	12.40±0.43	99.65
4-hydroxybenzoic acid	1.54	5	6.51±0.27	99.576
		10	11.47±0.84	99.47
Syringic acid	1.45	5	6.21±0.25	96.31
		10	11.50±0.54	100.52
Ferulic acid	1.48	5	6.31±0.64	97.51
		10	11.21±0.37	97.66
P-coumaric acid	2.58	5	7.73±0.32	102.07
		10	13.04±0.24	103.69
Protocatechuic acid	3.25	5	7.71±0.15	93.48
		10	12.22±0.48	92.23
Cinnamic acid	1.25	5	6.20±0.25	99.22
		10	11.11±0.13	98.81
Catechin	1.26	5	5.95±0.75	95.06
		10	10.89±0.68	96.80
Quercetin	1.35	5	6.47±0.19	101.94
		10	11.64±0.51	102.63
Vanillic acid	2.25	5	7.35±0.95	101.42
		10	12.53±0.75	102.30
Phloridzin	2.45	5	7.41±0.54	99.49
		10	12.41±0.63	99.74

Results are mean of five replicates of each brown rice variety n=5(120 h germination time) used in this study

4 Conclusions

We present here a reliable, selective and validated method for the analysis of important phenolic compounds in brown rice varieties. The developed analytical method was validated in terms of linearity (r2 =0.996-0.998 10–350 mg/L), precision (both intra-day and inter-day RSD ≤ 5.24%), robustness RSD <5%, accuracy (92.23%–103.69%), specificity, limit of detection and quantification. The selectivity, sensitivity and reproducibility of the method were found to be suitable for the determination and separation of selected phenolic compounds from brown rice seeds in controlled, germinated and fermented conditions in less than 10 minutes. Pakistani brown rice varieties were found to possess significant amounts of phenolic compounds. The analysis suggested the accumulation of phenolic compounds may occur during germination and fermentation of brown rice. This method would be useful for the rapid quantification of phenolic compounds in rice samples as well as its by-product like husk, bran and in the rice based commercial products formed by soaking or producing drinks after fermentation.

Conflict of Interest

The authors declare that they have no conflict of interest.

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