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Comparison of chemical and functional components of different *indica* brown and germinated rice

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Abstract

Nowadays brown rice (BR) and germinated brown rice (GBR) have aroused great interest of consumers, due to the higher nutrition compared with polished rice. In this study, the nutritional and bioactive compositions of BR and GBR samples prepared by 6 early *indica* rice cultivars and 12 middle *indica* rice cultivars from three producing areas were investigated. The free amino acid (215.75 mg/100 g), γ-aminobutyric acid (18.04 mg/100 g) and ferulic acid (195.9 μg/ g) content in early *indica* rice cultivars after germinated were significantly higher ($P < 0.05$) than those of other cultivars. In addition, principal component analysis and orthogonal partial least squares discriminant analysis models were able to identify BR and GBR, quercetin and *p*-coumaric acid were the most differential metabolites of all tested rice samples after germinated. The results provided the potential of early *indica* rice on functional food processing.

Keywords: *indica* brown rice; germinated brown rice; phenolics; multivariate statistics analysis.

Practical Application: Functional compositions comparation of different rice cultivars is need to help improve rice commodity value. In this study, we have investigated the chemical compositions and polyphenolic components of eighteen *indica* brown rice and germinated rice. Multivariate statistical analysis suggests that rice samples after germinated are easier to discriminate. This study provides a data basis for high added-value utilization of *indica* rice.

1 Introduction

As the most important staple food for over half of the world's population, rice (*Oryza sativa* L.) has been cultivated for nearly 6, 000 years (Bao, 2012; Maleki et al., 2020). Nowadays, growing health awareness prompt people to choose brown rice (BR) instead of polished rice (Lee et al., 2019; Mir et al., 2020), since BR is whole-grain rice which retains the embryo and bran layers, and contains more bioactive components, such as starch, protein, γ- aminobutyric acid (GABA), phenolics and so on (Saleh et al., 2019; Waewkum & Singthong, 2021). Moreover, germination is an effective method to enhance the nutritive value of BR (Sibian et al., 2017; Liu et al., 2018; Luo et al., 2022). Numerous studies illustrate that the BR and germinated brown rice (GBR) have potential health benefits including antidiabetic, antioxidant and cholesterol-lowering (Goufo & Trindade, 2014; Owolabi et al., 2019; Tyagi et al., 2022; Wang et al., 2022b).

Meanwhile, elicitation strategy aimed on improve the nutritional functions of BR and GBR is also a hot topic (Choe et al., 2021; Seong & Kim, 2021). For example, high hydrostatic pressure treatment significantly increases the total phenolic acid content nearly two times (Kim et al., 2017). Choe et al. (2021) indicated that $CaCl_2$ treatment during germination greatly increase the bioactive compounds accumulation by approximately 130%. Moreover, the yield of bioactive compounds is strongly influenced by rice variety and geographical origin (Chinma et al., 2015; Chao et al., 2021). Currently, China rice yield especially *indica* rice ranks in the top of worldwide (Kwon et al., 2018), for better utilize the abundant rice cultivars resource, comparation on the functional compositions in different rice cultivars is need to help improve rice commodity value (Choi & Lee, 2021; Jia et al., 2022).

In this study, six early *indica* rice cultivars and twelve middle *indica* rice cultivars harvested from three producing areas of China were collected to prepare BR and GBR, their functional components including protein, amino acids and phenolics diversity were studied. Accordingly, principle component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) models were built to further evaluate their overall quality attributes. In this manner, we hope to provide a data basis for high added-value utilization of *indica* rice.

2 Material and methods

2.1 Rice materials and pre-treatment

Eighteen *indica* rice cultivars (Table 1) were harvested at maturity in 2019 from three areas of China. Raw rice grains were sun-dried until the moisture content reached nearly 12%. Then the paddy grains were dehusked to obtained brown rice samples (BRSs). For germinated rice samples (GRSs), 10 g BR grains were surface sterilized with 0.1% sodium hypochlorite solution for 10 min, steeped in 60 mL deionized water at 25 °C for 16 h after washing three times. Then the grains were drained

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Table 1. Information of tested rice cultivars.

No.	Varieties	Sown/Harvest	Groups
NN_1	hua5950	Apr/mid-July	Nanning, early *indica* rice
NN_2	xiaoliangyou15		
NN_3	jingxiangyou7675		
NN_4	longfengyou1549		
NN_5	liangliangyou534		
NN_6	hongliangyou128		
HZ_1	liangliangyou70122	Apr/early-Sep	Hanzhong, middle *indica* rice
HZ_2	hongliangyousizhan		
HZ_3	1002liangyou883		
HZ_4	yueliangyou8612		
HZ_5	jingliangyou8612		
HZ_6	zhenliangyoutaisi		
CS_1	long8you5298	May/late-Aug	Changsha, middle *indica* rice
CS_2	Hliangyou570		
CS_3	jingliangyoudizhan		
CS_4	yueliangyou8549		
CS_5	341liangyou1597		
CS_6	jingliangyouyuzhan		

and germinated at 30 °C for 30 h. Finally, the BRSs and GRSs were immediately freeze-dried (Gold-Sim, China) and stored at -20 °C for subsequent analysis.

2.2 Nutrient composition analyses

The protein content (N × 5.95) was measured using a Kjeltec8400 auto-analyzer (Foss, Sweden). Free amino acid (FAA) was analyzed by L8900 amino acid analyzer (Hitachi, Japan) (Wang et al., 2022a). Briefly, 2.0 g rice powder was mixed thoroughly with 50 mL 0.02 mol/L HCl and was oscillated for 30 min. The supernatant fluid was transferred and diluted with 0.02 mol/L HCl to 100 mL for FAA analysis.

2.3 Free and bound phenolic compounds extraction

The extraction method was following the reported method (Shen et al., 2015) with some modifications. Initially, 1.0 g rice powder was blend with 5 mL n-hexane and ultrasonic treatment for 30 min to remove ester. Centrifugation to remove n-hexane, the remaining part was ultrasonic extracted twice with 5.0 mL methanol. The two methanol supernatants were mixed and dried under nitrogen at 40 °C, then dissolved with 1.0 mL methanol. Adjusted pH to 2.0 with HCl, the solution was extracted twice with 2.0 mL ethyl acetate. Pooled the ethyl acetate layer containing free phenolic extracts and dried under nitrogen, then dissolved with 2 mL methanol and stored at −20 °C. Total 10 mL 4 mol/L NaOH was added in the residues and shaken for 4 h in the dark. The supernatants were adjusted pH to 2.0 with HCl, and extracted twice with 5 mL ethyl acetate. Pooled the ethyl acetate layer containing bound phenolic extracts and dried under nitrogen, then dissolved with 2 mL methanol and stored at −20 °C.

2.4 Total phenolic content analysis

Total phenolic content (TPC) was determined based on the Folin-Ciocalteu colorimetric method (Chen et al., 2015). The reaction mixtures containing 0.3 mL phenolic extraction, 5.7 mL deionized water, 0.5 mL Folin-Ciocalteu reagent and 1.5 mL of 20% Na_2CO_3 were incubated at 30 °C for 1 h in the dark. The absorbance was measured at 760 nm by using a UV2000 spectrophotometer (Hitachi, Japan). Ferulic acid was used as standard (0-200 mg/L, $R^2 = 0.993$), TPC was expressed as milligram ferulic acid equivalents (mg FAE/g).

2.5 Phenolic components analyses

The phenolic components were analyzed by Waters ACQUITY UPLC I-class system (Waters, Milford, USA) equipped with BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) and PDA eλ detector (Bagchi et al., 2021). A gradient elution (Table S1) was performed with the solvent A (1.0% formic acid in water) and solvent B (methanol) with a flow rate of 0.25 mL/min and an injection volume of 5 μL. The chromatographic column and samples temperature were maintained at 40 °C and 16 °C respectively, the phenolics were monitored at 260 nm, 320 nm and 370 nm. Individual phenolics in rice samples was identified by the retention time and UV spectra of standard compounds (0.5-40 μg/mL, Solarbio, China), the quantitative value was calculated from their linear calibration curves (Table S2).

2.6 Statistical analysis

Each sample was replicated three experimental times, data were expressed as mean ± standard deviation (SD). Significant differences were determined by Duncan's test ($P < 0.05$) using SPSS 16.0. Heatmap was drawn with Z-score standardized data by R software. The PCA and OPLS-DA were established by SIMCA 13.0.

3 Results and discussion

3.1 Protein and free amino acid

Protein and FAA comparison results were presented in Table 2. The protein content in all tested samples differed largely, while there was no clear upward or downward trend after germinated. Previous study illustrated that the most protein were storage in the endosperm (Kim et al., 2012). The shorter germination time may not enough to highlight the difference.

The FAA content of different BRSs ranged from 37.98 mg/100 g to 76.97 mg/100 g, while in 18 GRSs, it increased significantly from 114.54 mg/100 g to 270.13 mg/100 g. Shen et al. (2015) reported that FAA content after germinated was 1.5 times higher, in this study, the FAA content after germinated in Nanning group was 4.1 times higher in average. The GRS of Jingxiangyou7675 (NN_3) had the highest FAA content (270.13 mg/100 g) among the tested rice samples.

Germination is effective to improve the GABA quality. Notably, the average GABA content of early *indica* GRSs was 15.1 times higher than it in BRSs, but it was 12.2 times and 2.5 times higher in Hanzhong and Changsha groups respectively, which were all belong to middle *indica* rice. Furthermore, longfengyou1549 (NN_4) and liangliangyou534 (NN_5) could be considered as GABA-rich foods.

3.2 Total phenolic content

Phenolics are known to be abundant in cereals and exhibited distinctive benefits (Bagchi et al., 2021). The free phenolic content (FPC), bound phenolic content (BPC) and total phenolic content (TPC) were displayed in Table 3. Generally, the contents of phenolics after germinated were significantly increased ($P < 0.01$) than it in BRSs, which agreed with previous studies in the comparison of brown and germinated rice (Kim et al., 2021). Study illustrated that phenolics exist in free and bound forms in BR (Wu et al., 2018). In this study, the BPC before and after germination were similar, but FPC significantly increased ($P < 0.05$) about 1.1 to 2.2 times. Moreover, the FPC were lower than BPC in all test rice cultivars, which was consistent with

Table 2. Protein and amino acid contents of tested rice samples.

Cultivars	Protein (g/100 g)		GABA (mg/100 g)		FAA (mg/100 g)	
	BRS	GRS	BRS	GRS	BRS	GRS
NN_1	6.69 ± 0.10^{efg}	6.72 ± 0.04^{def}	0.87 ± 0.08^{gh}	16.16 ± 1.42^{de}	49.27 ± 1.47^{fg}	196.48 ± 7.60^{cd}
NN_2	5.87 ± 0.07^{i}	5.74 ± 0.13^{h}	0.98 ± 0.14^{fgh}	14.99 ± 0.74^{e}	45.93 ± 3.63^{g}	221.52 ± 11.66^{b}
NN_3	6.23 ± 0.33^{hi}	6.26 ± 0.04^{g}	1.47 ± 0.17^{cd}	15.17 ± 1.28^{e}	52.15 ± 1.67^{ef}	270.13 ± 11.21^{a}
NN_4	6.65 ± 0.03^{fgh}	7.02 ± 0.37^{de}	1.43 ± 0.22^{ef}	25.02 ± 1.57^{a}	67.42 ± 1.53^{b}	268.09 ± 14.98^{a}
NN_5	6.23 ± 0.12^{hi}	6.38 ± 0.15^{fg}	1.03 ± 0.08^{fgh}	20.21 ± 1.30^{b}	49.64 ± 2.14^{fg}	177.53 ± 7.95^{ef}
NN_6	6.23 ± 0.14^{hi}	6.43 ± 0.20^{fg}	1.56 ± 0.16^{e}	16.67 ± 0.96^{cde}	57.84 ± 2.52^{cde}	160.78 ± 6.04^{fg}
HZ_1	6.87 ± 0.16^{cdefg}	7.00 ± 0.14^{de}	0.76 ± 0.07^{h}	12.50 ± 0.85^{f}	37.98 ± 2.54^{h}	114.54 ± 8.92^{j}
HZ_2	7.43 ± 0.08^{ab}	7.61 ± 0.19^{bc}	6.83 ± 0.54^{c}	18.28 ± 0.83^{c}	76.97 ± 3.21^{a}	182.04 ± 11.46^{de}
HZ_3	6.50 ± 0.07^{gh}	6.67 ± 0.16^{efg}	1.52 ± 0.16^{e}	16.71 ± 1.16^{cde}	48.04 ± 5.54^{fg}	165.93 ± 6.92^{ef}
HZ_4	7.85 ± 0.13^{a}	8.18 ± 0.33^{a}	1.35 ± 0.11^{efg}	17.65 ± 1.02^{cd}	58.16 ± 3.86^{cd}	205.23 ± 10.10^{bc}
HZ_5	7.18 ± 0.13^{bcd}	7.23 ± 0.05^{cd}	1.47 ± 0.07^{ef}	20.46 ± 1.16^{b}	48.56 ± 2.55^{fg}	136.56 ± 12.14^{hi}
HZ_6	7.17 ± 0.05^{bcde}	7.52 ± 0.36^{bc}	1.41 ± 0.09^{ef}	21.76 ± 0.61^{b}	53.49 ± 2.97^{def}	163.53 ± 6.92^{efg}
CS_1	6.85 ± 0.30^{defg}	6.81 ± 0.04^{def}	5.17 ± 0.17^{a}	11.02 ± 0.97^{fg}	57.97 ± 2.69^{cd}	130.70 ± 8.53^{hij}
CS_2	7.83 ± 0.13^{a}	7.76 ± 0.04^{ab}	3.61 ± 0.31^{c}	9.22 ± 0.66^{gh}	53.65 ± 4.01^{def}	140.46 ± 11.81^{hi}
CS_3	7.19 ± 0.27^{bcd}	7.02 ± 0.17^{de}	2.73 ± 0.33^{d}	9.51 ± 0.55^{gh}	50.37 ± 1.31^{fg}	120.75 ± 11.10^{ij}
CS_4	6.75 ± 0.41^{defg}	6.71 ± 0.04^{efg}	4.29 ± 0.29^{b}	6.13 ± 0.42^{i}	53.28 ± 4.71^{def}	127.90 ± 15.57^{hij}
CS_5	7.03 ± 0.50^{bcdef}	6.93 ± 0.36^{de}	3.59 ± 0.55^{c}	9.10 ± 0.67^{h}	59.99 ± 2.18^{c}	146.20 ± 11.04^{gh}
CS_6	7.31 ± 0.36^{bc}	7.21 ± 0.10^{cd}	3.17 ± 0.38^{c}	9.58 ± 0.75^{gh}	52.09 ± 3.01^{ef}	145.47 ± 13.71^{gh}
Mean ± SD	6.88 ± 0.53	6.96 ± 0.57	2.40 ± 1.71	15.01 ± 5.18	54.04 ± 8.54	170.77 ± 46.37

GABA = γ aminobutyric acid; FAA = free amino acid; BRS = brown rice sample; GRS = germinated rice sample. Mean value with a different lowercase in the same column was significantly different ($P < 0.05$).

Table 3. Phenolic content of tested rice samples.

Cultivars	FPC (mg FAE/g)		BPC (mg FAE/g)		TPC (mg FAE/g)	
	BRS	GRS	BRS	GRS	BRS	GRS
NN_1	0.07 ± 0.01^{gh}	0.11 ± 0.01^{ef}	0.60 ± 0.01^{cd}	0.67 ± 0.00^{ef}	0.68 ± 0.02^{de}	0.78 ± 0.01^{g}
NN_2	0.11 ± 0.01^{ef}	0.12 ± 0.01^{e}	0.64 ± 0.02^{a}	0.72 ± 0.01^{bc}	0.75 ± 0.02^{ab}	0.85 ± 0.01^{cde}
NN_3	0.09 ± 0.01^{g}	0.17 ± 0.01^{cd}	0.64 ± 0.01^{a}	0.67 ± 0.01^{def}	0.73 ± 0.01^{bc}	0.84 ± 0.01^{cde}
NN_4	0.08 ± 0.01^{gh}	0.10 ± 0.01^{fg}	0.65 ± 0.03^{a}	0.78 ± 0.03^{a}	0.77 ± 0.03^{a}	0.88 ± 0.03^{bc}
NN_5	0.10 ± 0.00^{f}	0.16 ± 0.01^{d}	0.64 ± 0.01^{ab}	0.70 ± 0.01^{cd}	0.74 ± 0.02^{ab}	0.86 ± 0.02^{cd}
NN_6	0.11 ± 0.01^{ef}	0.16 ± 0.01^{d}	0.53 ± 0.01^{e}	0.62 ± 0.01^{ghi}	0.63 ± 0.02^{fg}	0.78 ± 0.01^{g}
HZ_1	0.05 ± 0.01^{j}	0.10 ± 0.00^{fg}	0.61 ± 0.01^{bcd}	0.68 ± 0.04^{de}	0.65 ± 0.04^{ef}	0.77 ± 0.04^{g}
HZ_2	0.06 ± 0.00^{ij}	0.12 ± 0.01^{ef}	0.55 ± 0.01^{e}	0.60 ± 0.01^{hij}	0.63 ± 0.03^{fgh}	0.72 ± 0.01^{h}
HZ_3	0.06 ± 0.00^{ij}	0.13 ± 0.01^{e}	0.53 ± 0.01^{e}	0.57 ± 0.02^{k}	0.60 ± 0.02^{h}	0.70 ± 0.03^{hi}
HZ_4	0.07 ± 0.01^{hi}	0.16 ± 0.01^{d}	0.60 ± 0.02^{d}	0.62 ± 0.02^{gh}	0.66 ± 0.02^{def}	0.79 ± 0.03^{fg}
HZ_5	0.06 ± 0.01^{ij}	0.09 ± 0.01^{g}	0.55 ± 0.01^{e}	0.58 ± 0.02^{jk}	0.61 ± 0.01^{gh}	0.68 ± 0.02^{i}
HZ_6	0.07 ± 0.01^{hi}	0.11 ± 0.01^{fg}	0.59 ± 0.02^{d}	0.61 ± 0.01^{ghij}	0.67 ± 0.03^{def}	0.72 ± 0.03^{h}
CS_1	0.18 ± 0.01^{a}	0.21 ± 0.02^{ab}	0.49 ± 0.01^{f}	0.59 ± 0.02^{ijk}	0.68 ± 0.01^{de}	0.80 ± 0.02^{fg}
CS_2	0.12 ± 0.01^{de}	0.15 ± 0.01^{d}	0.54 ± 0.01^{e}	0.66 ± 0.03^{ef}	0.66 ± 0.01^{def}	0.81 ± 0.03^{efg}
CS_3	0.13 ± 0.01^{cd}	0.18 ± 0.01^{c}	0.53 ± 0.01^{e}	0.64 ± 0.02^{fg}	0.65 ± 0.01^{ef}	0.83 ± 0.02^{def}
CS_4	0.11 ± 0.01^{ef}	0.16 ± 0.01^{d}	0.63 ± 0.01^{abc}	0.75 ± 0.02^{ab}	0.73 ± 0.02^{bc}	0.91 ± 0.02^{b}
CS_5	0.13 ± 0.01^{c}	0.20 ± 0.02^{b}	0.60 ± 0.02^{cd}	0.75 ± 0.02^{ab}	0.74 ± 0.02^{ab}	0.96 ± 0.02^{a}
CS_6	0.16 ± 0.00^{b}	0.23 ± 0.01^{a}	0.55 ± 0.01^{e}	0.64 ± 0.01^{fg}	0.70 ± 0.02^{cd}	0.87 ± 0.03^{c}
Mean ± SD	0.10 ± 0.03	0.15 ± 0.04	0.58 ± 0.05	0.66 ± 0.06	0.68 ± 0.05	0.81 ± 0.08

FPC = free phenolic content; BPC = bound phenolic content; TPC = total phenolic content; FAE = ferulic acid equivalents; BRS = brown rice sample; GRS = germinated rice sample. Mean value with a different lowercase in the same column was significantly different ($P < 0.05$).

some researches (Gao et al., 2018). While there were some reports indicated that the phenolics existed in of black and *indica* rice bran were mainly free form (Wu et al., 2018), the difference may be caused by extraction method and variety.

It had been proved that germination could increase rice nutritional components (Sibian et al., 2017). In this study, after germinated, the highest FPC and TPC were observed in Changsha group, which was come from the lower Yangtze River. The variety yueliangyou8549 (CS_4) showed the highest TPC (0.96 mg FAE/g) after germinated. Hence geographical conditions could consider as an important condition in phenolic production optimization.

3.3 Phenolic composition

The individual phenolics in free and bound phenolic extracts of tested BRSs and GRSs were detected by UPLC. The UPLC chromatograms of phenolics standard mixture and one rice sample were showed in Figure S1. Generally, six phenolic compositions including *p*-hydroxybenzonic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid and quercetin were detected and presented in Figure 1.

Ferulic acid (Figure 1A) and *p*-coumaric acid (Figure 1B) were the most abundant phenolic acids in all the tested samples, which was in accord with previous studies (Sumczynski et al., 2017).

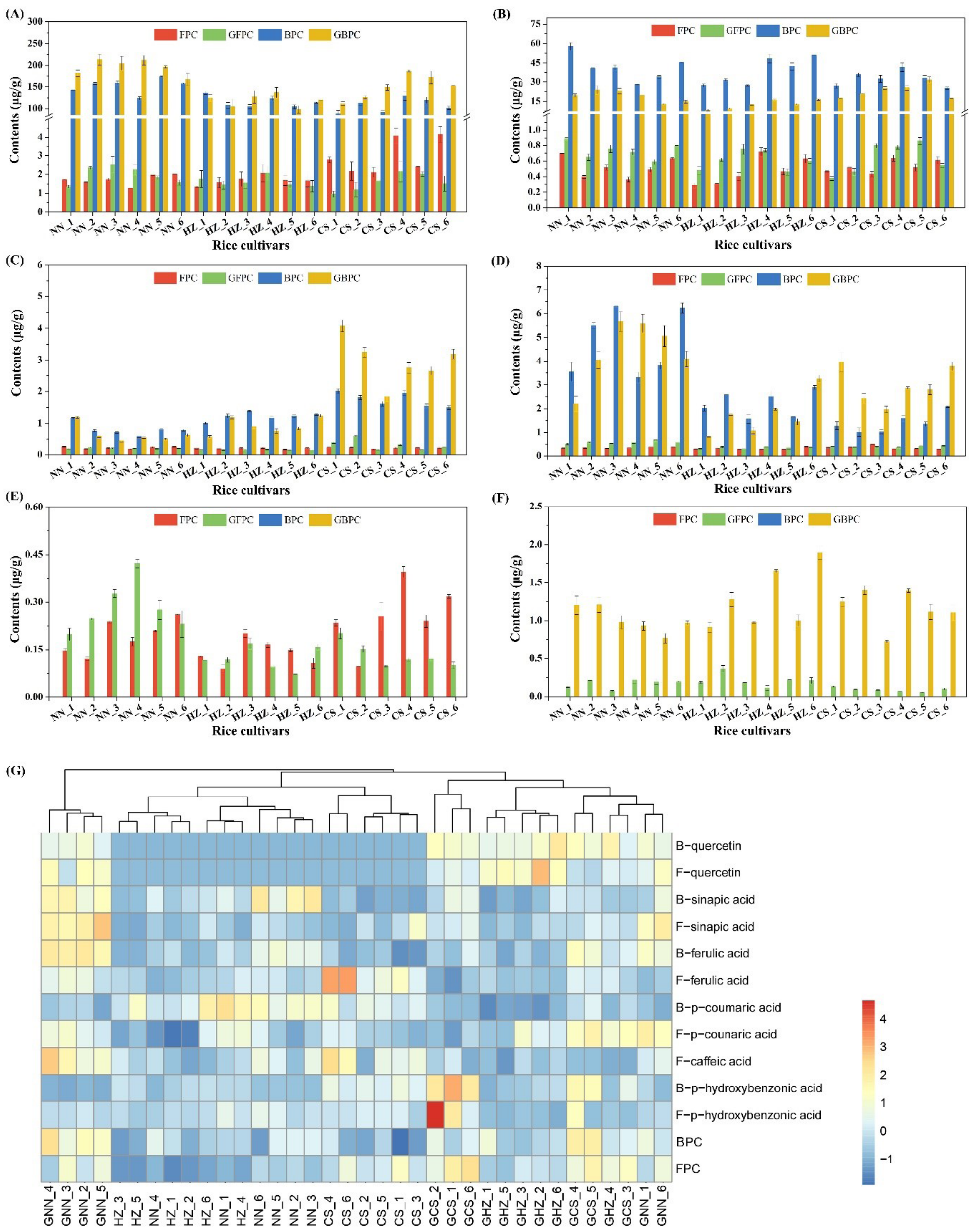

Figure 1. Comparison of the phenolic components in the tested BRSs and GBRs. (A) Ferulic acid. (B) *p*-coumaric acid. (C) *p*-hydroxybenzonic acid. (D) Sinapic acid. (E) Caffeic acid. (F) Quercetin. Bars represent means ± SD, n = 3. FPC and GFPC indicated the FPC of BRS and GRS respectively; BPC and GBPC indicated the BPC of BRS and GRS respectively. (G) Heatmap according to the phenolics. GNN, GHZ and GCS indicated the corresponding GRS. The phenolic name added a prefix F or B indicated the free or bound phenolic content respectively.

The bound ferulic acid increased significantly in most GRSs ($P < 0.05$), on the contrary, the bound *p*-coumaric acid decreased significantly. The highest bound ferulic acid content, found in Nanning group, was 195.9 μg/g in average after germination, which was significantly higher than Hanzhong (118.7 μg/g) and Changsha groups (149.5 μg/g).

In addition, the yield of *p*-hydroxybenzonic acid and sinapic acid after germinated exhibited significant increase in the rice cultivars from Changsha group (Figure 1C). The highest content of sinapic acid (Figure 1D) was the BRSs from Nanning group (4.78 μg/g, in average), but most of them were decreased after germinated except longfengyou1549 (NN_4) and liangliangyou534 (NN_5).

Only free caffeic acid (Figure 1E) with poor content was detected in all tested samples, but it showed an obvious increase from 0.19 to 0.28 μg/g in the GRSs from Nanning group. Interestingly, quercetin (Figure 1F) was only detected in the extracts of GRSs, and the bound quercetin content was 10 times higher than the free form.

The heatmap regarding the phenolics was shown in Figure 1G. It was evident that the 18 BRSs possessed similar phenolic contents, and were classified into an individual cluster. After germinated, four GRSs from Nanning group possessed the highest amounts and gathered, while GNN_1 and GNN_6 were present in similar contents with other GRSs from Hanzhong and Changsha groups. Notably, GCS_2 exhibited the relative highest content of *p*-hydroxybenzonic acid, but the actual content was lower than 1 μg/g.

3.4 Classification by PCA and OPLS-DA

PCA according to phenolics was performed to classify the tested cultivars from different groups. Figure 2 showed that the first two principal components explain 51.4% of the data variation. The score plot (Figure 2A) indicated that BRS and GRS were separated into three groups clearly by PC1. The first group was composed of 18 BRSs, the loading plot (Figure 2B) indicated their higher level of bound *p*-coumaric acid and free ferulic acid, but lower level of BPC, bound ferulic acid and free sinapic acid. Moreover, the GRSs from early *indica* rice and

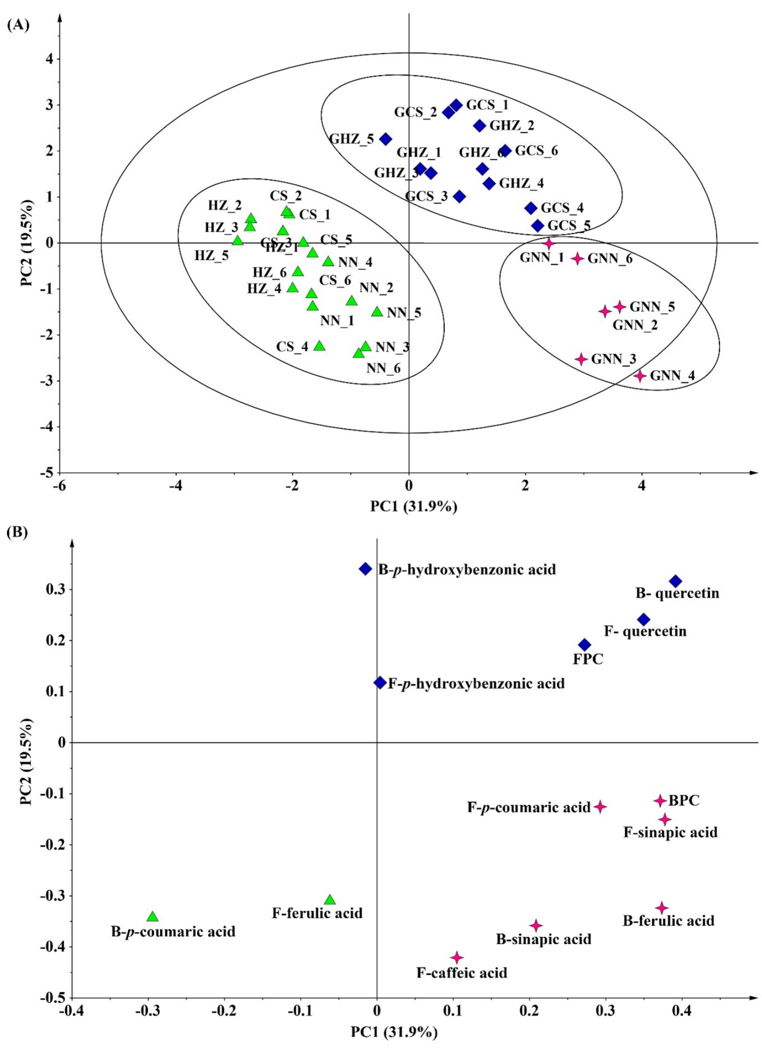

Figure 2. PCA score plot (A) and loading plot (B) according the phenolics. Green triangle: BRSs; blue diamond: GRSs of middle *indica* rice; red star: GRSs of early *indica* rice. Phenolics with a prefix F or B indicated the free or bound phenolic content respectively.

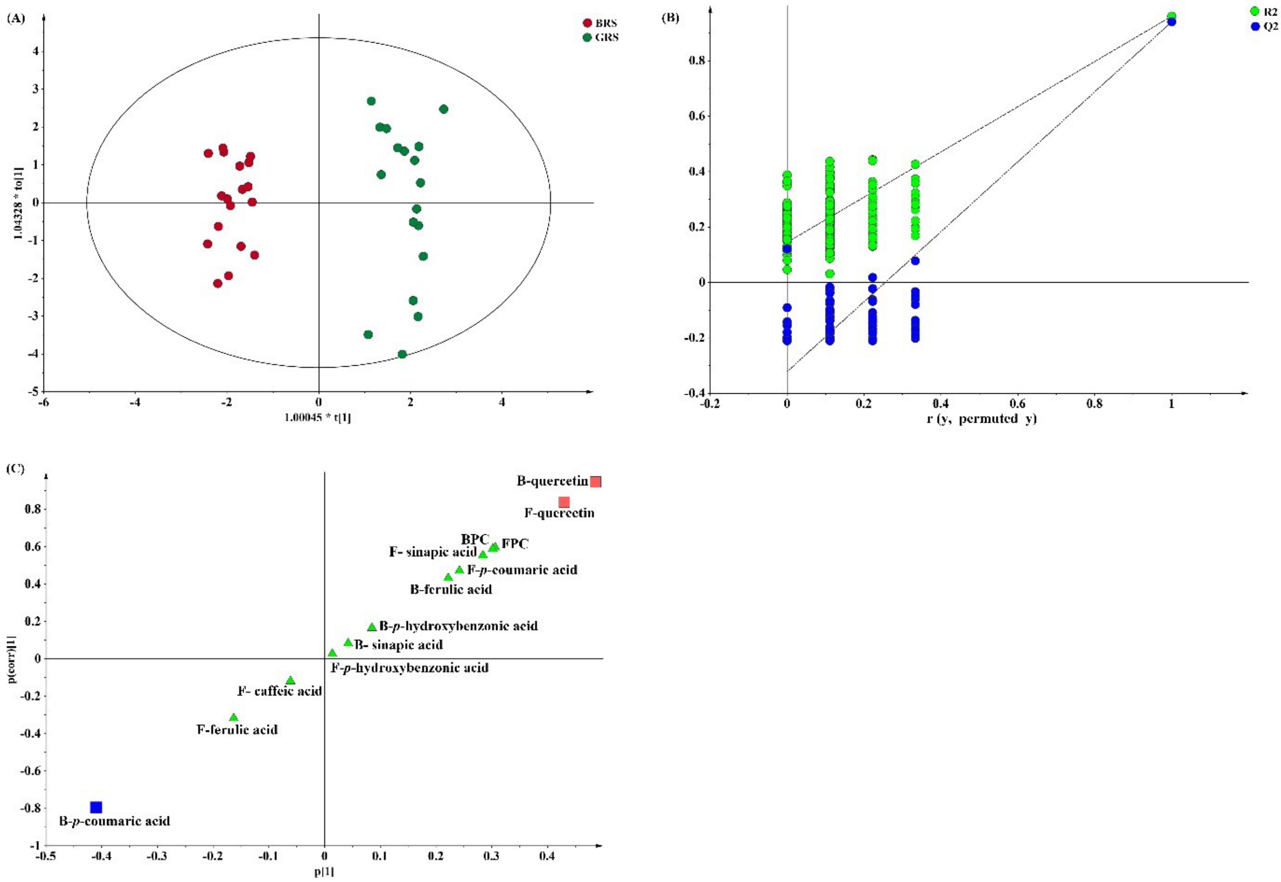

Figure 3. OPLS-DA analysis with tested rice samples. (A) Score plot. Red dots: BRSs; green dots: GRSs. (B) 200 permutation tests. R^2: fit ability. Q^2: predictive ability. (C) S-plot. Variables with $|p| \geq 0.05$ and $|p(corr)| \geq 0.5$ are considered statistically significant. Phenolics with a prefix F or B indicated the free or bound phenolic content respectively.

middle *indica* rice were reasonably separated by PC2. The second group consisted of 12 GRSs from Hanzhong and Changsha, which were more concentrated in bound *p*-hydroxybenzonic acid and quercetin. Six GRSs from Nanning were gathered as the third group, which were all belong to the early *indica* rice cultivars. Although GNN_4 keeping a distance from each other, the difference may cause by the obviously higher free caffeic acid.

Furthermore, the supervised discriminant analysis was performed by OPLS-DA. BRSs and GRSs obviously clustered on the left and right side of score plots (Figure 3A). The fit ability ($R^2 = 0.509$) and predictive ability ($Q^2 = 0.944$) were measured and the results showed good predictability. A 200-time permutation test (Figure 3B) was conducted and proved predictive OPLS-DA model did not overfit, since all blue Q^2-values to the left were lower than the original points to the right. Finally, s-plot (Figure 3C) demonstrated that quercetin and *p*-coumaric acid were the greatest difference compounds between BRSs and GRSs ($|p(corr)| \geq 0.8$), which further illustrated that quercetin increased significantly, while bound *p*-coumaric acid decreased significantly after germinated.

4 Conclusion

In this study, the nutrients and phenolics in BRSs and GRSs of 18 early and middle *indica* rice cultivars obtained from three areas were analyzed. The results indicated significant differences among the rice cultivars before and after germination. It was seen that the GRSs of early *indica* rice were rich in FAA and most phenolics. Besides, multivariate statistical analysis suggested that rice samples after germinated were easier to discriminate, the biggest compound differences were quercetin and *p*-coumaric acid between BRSs and GRSs. The results reveal the potential of early *indica* rice on functional food processing, and provide help for the evaluation of the overall quality of Chinese rice resources.

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Supplementary material

Supplementary material accompanies this paper.

Table S1. The phenolics elution with a gradient system.

Table S2. Linear calibration curves of phenolic standard compounds.

Figure S1. The UPLC chromatograms of phenolic standards and the rice sample.

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