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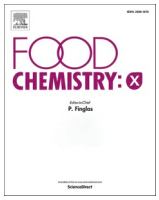

Changes of polyphenols and their antioxidant activities in non-pigmented, red and black rice during *in vitro* digestion

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ABSTRACT

Polyphenols, a major bioactive constituent in rice grain, require processing and digestion before being absorbed by human body. Free and bound phenolics, flavonoids and their antioxidant activities in non-pigmented, red and black rice after cooking and INFOGEST digestions of oral, gastric and intestinal phases were investigated. It showed that cooking caused great losses of polyphenols and antioxidant activity. Free ferulic, isoferulic and *p*-coumaric acid in most rice were highest at intestinal phase ($p < 0.05$). Bound ferulic acid in three colored rice, bound *p*-coumaric acid in black rice and catechin in red rice were higher at oral and/or gastric phase. After cooking, total flavonoids of non-pigmented and pigmented rice were highest at intestinal and gastric phase, respectively. Cyanidin-*3-O*-glucoside, peonidin-*3-O*-glucoside and quercetin peaked at intestinal phase in black rice. It suggested that black rice has a greater potential to be used in meal balance and functional product development.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important crops that feeds approximately half of the world's population. Most rice available in the market is milled or polished or white rice, which is obtained by removing the embryo and bran layers of brown rice. Compared to brown rice, polished rice exhibits superior sensory properties and higher storage stability. However, the eating of polished rice might result in a lack of essential vitamins, polyphenols, fibers, minerals, γ-oryzanols and other phytochemical compounds (Bagchi et al., 2021). It is reported that the consumption of whole rice grain is associated with the reduction in risks of developing non-communicable chronic disease such as obesity, cardiovascular diseases, type II diabetes and certain cancers (Khan et al., 2022). Therefore, the whole brown rice is a food with high nutritional value, which is gradually being accepted in developed and developing countries (Goufo & Trindade, 2014).

Polyphenols, a group of important bioactive phytochemicals in rice grains, contains multiple phenolic hydroxyl groups in their molecular structure including phenolic acids, flavonoids, anthocyanins and tannins (Lang et al., 2024; Wojtunik-Kulesza et al., 2020). They are present in plants mainly in glycosylated, polymerized, and esterified forms. Phenolic acids in rice mainly exist in free and bound forms, and the content of bound phenolic acids is significantly higher than that of free ones (Khan et al., 2022; Shao et al., 2018). Ferulic and *p*-coumaric acid are the main bound phenolic acids in whole rice grains, and high amounts of *2,5*-dihydroxybenzoic, protocatechuic, and vanillic acid are also detected in colored rice (Shao et al., 2018). Additionally, pigmented rice is rich in flavonoids. Cyanidin-*3-O*-glucoside and peonidin-*3-O*-glucoside are primarily found in black rice, whereas the building blocks of catechin and epicatechin of proanthocyanidins exist in red rice (Chen et al., 2022). It is reported that the polyphenol extract from *Salvia haenkei* rich in luteolin can down-regulate gene expressions and reverse gene expression patterns associated with aging by interfering with p16-CDK6 interactions in mice (Sara et al., 2024). The addition of tea

Abbreviations: TPC, total phenolic content; TFC, total flavonoids content; BR, brown raw rice; CBR, cooked brown rice; OBR, oral-digested cooked brown rice; GBR, gastric-digested cooked brown rice; IBR, intestinal-digested cooked brown rice; FA, ferulic acid; IFA, isoferulic acid; VA, vanillic acid; *p*-CA, *p*-coumaric acid; *p*-HA, *p*-hydroxybenzoic acid; SIA, sinapic acid; GA, gallic acid; PA, protocatechuic acid; SYA, syringic acid; CHA, chlorogenic acid; *trans*-CA, *trans*-cinnamic acid; C3G, cyanidin-*3-O*-glucoside; P3G, peonidin-*3-O*-glucoside; CAT, catechin; QUE, quercetin; LEUC, leucocyanidin; ERI, eriodictyol; NAR, naringenin; KAE, kaempferol..

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polyphenols can increase the abundance of beneficial bacteria in the intestines of rats and Ningxiang pigs, which create a more favorable environment for lipid metabolism and excretion, and have the potential to improve lipid deposition and reduce the risks of metabolic diseases (Wang et al., 2024). Cyanidin-3-*O*-*β*-glucoside purified from black rice can effectively protect mice from carbon tetrachloride induced liver fibrosis by inhibiting the activation of hepatic stellate cells and exerting its antioxidant and anti-inflammatory effects (Jiang et al., 2015). Overall, these polyphenol compounds may affect biological functions individually or synergistically.

Free phenolic acids are more easily digested. Bound phenolic acids are released from the cell wall in the large intestine in the form of glycosidic ligand by the activity of bacteria or enzymes, and then reformed into glucoside, which is used by the human body *via* the glucose transporter in the cell (Khan et al., 2022). Flavonoids exist mainly in soluble (free) form in rice, and their glycosides and glucosylated forms can be absorbed by passive diffusion or active transport in the small intestine (Wojtunik-Kulesza et al., 2020). Rice, as a staple food, needs to be cooked before it can be digested, absorbed and utilized by the human body. It is indicated that cooking causes significant losses of polyphenols ($p < 0.05$) in brown rice, and *in vitro* digestion can improve the antioxidant capacity (Ti et al., 2015). However, another study shows that *in vitro* digestion causes great losses of individual phenolic compounds (Nignpense et al., 2022). Therefore, the effect of *in vitro* digestion on polyphenols in rice grains are still controversial. On the other hand, some studies investigating the effect of *in vitro* digestion on phenolic compounds rarely consider the actual situation of rice consumption and ignore the effect of cooking before digestion (Fu et al., 2024; Rocchetti et al., 2022). In addition, the study on the differences in digestion of different colored rice grains during oral, gastric, and intestinal phases is still largely unknown.Therefore, it is necessary to systematically study the change patterns of polyphenols and their potential antioxidant capacity in different color rice during the digestion of oral, gastric, and intestinal phases after cooking.

In this study, we aimed to investigate the variations in free and bound total phenolic, flavonoids, and their antioxidant activities, as well as the phenolic acids and flavonoid compositions, in 2 non-pigmented, 2 red, and 2 black rice grains after cooking and during *in vitro* digestions of oral, gastric, and intestinal phases. It would contribute to a deeper understanding of the health mechanisms of polyphenols in different colored rice grains, and provide practical implications for optimizing healthy dietary patterns based on the whole rice grain and promoting the development of functional rice products.

2. Materials and methods

2.1. Rice samples and in vitro digestion

Six rice genotypes (*Oryza sativa* L.) were selected in this study: Nongken2021 (NK2021), Zhongzao35 (ZZ35), Caofeihong (CFH), Yanzhidao (YZD), Binhei (BH), and Heixiangdao (HXD). Among these varieties, NK2021 and ZZ35 were non-pigmented rice grains, CFH and YZD were red rice grains, and BH and HXD were black rice grains. NK2021 was planted at a farm located in Jiamusi city, Heilongjiang province, China. ZZ35 was grown at a farm belonging to the China National Rice Research Institute in Hangzhou, China. All the other genotypes were grown at a farm belonging to Institute of Coastal Agriculture, Hebei Academy of Agriculture and Forestry Sciences in Tangshan, China. All the samples were planted in 2022. After maturing, the grains were harvested, sun-dried to a moisture content of approximately 12 %, stored in airtight plastic bags at room temperature for three months, and then stored in the dark at 4 °C before analysis. The brown rice grain was obtained by de-husking of rough rice grain using a Satake Rice Machine (Satake, Tokyo, Japan). The brown rice grain was soaked at room temperature for two hours, and then boiled using an electric pressure rice cooker (CUCKOO, Korea) with a rice-to-water ratio of 1:1.8 (*w/v*). *In vitro* digestion was performed following the procedure described by Brodkorb et al. (2019). The simulated digestive reserve fluids of oral (1.25 × SSF), gastric (1.25 × SGF) and intestinal phases (1.25 × SIF) were composed of KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2$, $(NH_4)_2CO_3$, $CaCl_2$, HCl, and ddH_2O. The simulated salivary fluid (SSF) contained 1.25 × SSF, α-amylase, and ddH_2O. The simulated gastric fluid (SGF) was comprise of 1.25 × SGF, pepsin, HCl, and ddH_2O. The simulated intestinal fluid (SIF) consisted of 1.25 × SIF, pancreatin, bile, NaOH and ddH_2O. Prior to *in vitro* digestion, the cooked rice was crushed using a food blender (Vitamix, USA) according to Fernandes et al. (2020) with slight modifications.

For the oral digestion, about 5.0 g cooked rice was added into 5.0 mL SSF at a ratio of 1:1 (*w*/w) and incubated at pH 7.0 and 200 r/min for 2 min in an incubator. After oral digestion, about 10 mL SGF (oral bolus: SGF = 1:1, *v*/v) was added, and then incubated at pH 3.0 and 200 r/min for 2 h. Following this, about 20 mL SIF (gastric chime: SIF = 1:1, v/v) was added, and then incubated at pH 7.0 and 200 r/min for an additional 2 h. The entire digestion process was conducted under nitrogen atmosphere at 37 °C. A vortex (M3 Basic, IKA, German) was used for a short time to mix the rice with the digestive liquid in every stages. Digestive tubes containing the whole samples from the end points of the oral, gastric, and intestinal phases were inactivated using an ice bath (−20 °C) and subsequently vacuum freeze-dried. The dried samples were milled to pass through a 100-mesh sieve using a grinder (Spex SamplePrep, USA) and stored at −20 °C until analysis. Brown raw rice, cooked brown rice, and the samples digested in the oral, gastric, and intestinal phases were labeled as BR, CBR, OBR, GBR, and IBR, respectively. Each sample was digested in triplicate.

2.2. Chemicals

Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis-(3-ethylbenzonthiazoline-6-sulfonic acid) diammonium salt), Trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylicacid), vanillin, α-amylase, porcine pepsin, and porcine pancreatin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hydrochloric acid, sodium chloride, potassium chloride, potassium dihydrogen phosphate, sodium bicarbonate, magnesium chloride, calcium chloride, ammonium carbonate, sodium hydroxide, sodium nitrite, sodium carbonate, potassium persulfate and Tris (Tris (hydroxymethyl) aminomethane) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The HPLC grade methanol and ethyl acetate were purchased from Merck (Darmstadt, Germany) and Tedia (Fairfield, OH, USA), respectively. The standards of GA (gallic acid), PA (protocatechuic acid), CHA (chlorogenic acid), *p*-HA (*p*-hydroxybenzoic acid), VA (vanillic acid), SIA (sinapic acid), SYA (syringic acid), *p*-CA (*p*-coumaric acid), FA (ferulic acid), IFA (isoferulicacid), *trans*-CA (*trans*-cinnamic acid), C3G (cyanidin-*3*-*O*-glucoside), P3G (peonidin-*3*-*O*-glucoside), CAT (catechin), LEU (leucocyanidin), ERI (eriodictyol), NAR (naringenin), QUE (quercetin), KAE (kaempferol) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC grade acetic and formic acid was purchased from Macklin (Shanghai, China). The certified Maltose standards were obtained from the National Institute Center of Standards (Beijing, China).

2.3. Extraction of free and bound phenolics

Free and bound phenolics were extracted separately according to the methods described by Shao et al. (2018). Briefly, about 0.5 g of rice flour was extracted with 10 mL of 80 % methanol on a shaker. After 20 min, the mixture was centrifuged (Thermo Scientific, Sorvall ST 8R, Germany) at 9400 ×*g* for 10 min at 4 °C. After twice extraction, the supernatants were collected together, and then concentrated using a nitrogen evaporator (Organomation, 24 Position N-EVAP, U.S.A.) at 37 °C. The concentrated extracts were hydrolyzed with 4 M NaOH for 2 h, followed by adjusting the pH to 1.5–2.0. After extracting with ethyl

acetate and drying using a nitrogen evaporator, the extracts were dissolved in 5 mL of 50 % methanol and labeled as free phenolics extracts.

The rice flour residue (after the extraction of 80 % methanol) was used to extract bound phenolics. The protocols for bound phenolics extracts were similar to those for free phenolics, involving hydrolysis by 4 M NaOH, pH adjustment, extraction by ethyl acetate, drying and dissolution. All extractions were performed in triplicate and stored at −20 °C in dark before analysis.

2.4. Determination of total phenolic content (TPC)

Free and bound TPC were measured by the Folin-Ciocalteu assay as reported by Shao et al. (2018). Briefly, 0.2 mL of the free or bound phenolics extract was mixed with 1.5 mL of 0.1 N Folin-Ciocalteu reagent, and then neutralized with 1.5 mL of saturated sodium carbonate. After incubation for 90 min in the dark, the absorbance was recorded at 725 nm using a spectrophotometer (Shimadzu, UV- 2600, Japan). A calibration curve was prepared using gallic acid solutions as standards. The results were expressed as milligrams of gallic acid equivalent per 100 g of rice flour on a dry weight basis (mg GAE/100 g). Each extract was measured in duplicate. The TPC of free and bound phenolics extract was recorded as free TPC and bound TPC, respectively. Total TPC was calculated by adding the free TPC and the bound TPC.

2.5. Determination of total flavonoids content (TFC)

Flavonoids (soluble flavonoids) were extracted and measured according to the methods reported by Shao et al. (2018) with minor modifications. Briefly, flavonoids from rice flour were extracted by formic acid methanol solution (methanol: 0.5 % formic acid = 8: 2, *v*/v) using ultrasonography (Kunshan Ultrasonic Instruments Co., LTD, KQ-300TDE, China) for 30 min at room temperature. The mixture was centrifuged at 9400 ×*g* for 10 min at 4 °C. After twice extraction, the supernatants were pooled together. All extractions were performed in triplicate and stored at −20 °C in the dark before analysis.

The flavonoids extract (0.5 mL) was mixed with 0.15 mL of 5 % $NaNO_2$ for 5 min. After adding 0.15 mL of 10 % $AlCl_3 \bullet 6H_2O$ for another 5 min, 1 mL of 1 M NaOH and 3 mL of ddH_2O were added. The mixture was incubated for 10 min and measured at a wavelength of 510 nm. A calibration curve was prepared using catechin solution as standards. TFC was expressed as milligrams of catechin equivalent per 100 g of rice flour on a dry weight basis (mg CE/100 g). Each extract was measured in duplicate.

2.6. Determination of antioxidant activity

The antioxidant activity was measured using the ABTS and DPPH radical scavenging assays as reported by Pang et al. (2018). The free ABTS and DPPH (free ABTS and DPPH radical scavenging capacity, respectively) were tested by using the free phenolics extracts, the bound ABTS and DPPH (bound ABTS and DPPH radical scavenging capacity, respectively) were measured by using the bound phenolics extracts, and the flavonoids DPPH and ABTS (flavonoids ABTS and DPPH radical scavenging capacity, respectively) were determined by using the flavonoids extracts. The results were expressed as micromoles of Trolox equivalent antioxidant activity per gram of rice flour on a dry weight basis (μM TE/g). Each extract was measured in duplicate.

2.7. HPLC analysis of phenolic acids

The phenolic acids were analyzed by an HPLC system (Agilent Technologies 1260 Infinity, Calif., U.S.A.). A C18 column of 250 × 4.6 mm with 5 μm particles (Agilent Eclipse Plus), Calif., U.S.A.) was used for separation. The mobile phase consisted of A (0.1 % acetic acid in water) and B (0.1 % acetic acid in methanol). The flow rate was set at 0.5 mL/min. A 35 min linear gradient was applied as follows: 0–1 min, 9–25 % B; 1–4 min, 25–35 % B; 4–8 min, 35–45 % B; 8–10 min, 45–46 % B; 10–12 min, 46–47 % B; 12–14 min, 47–48 % B; 14–16 min, 48–48.5 % B; 16–16.5 min, 48.5–49 % B; 16.5–17.5 min, 49–49.1 % B; 17.5–18 min, 49.1–49.5 % B; 18–20 min, 49.5–50 % B; 20–25 min, 50–60 % B; 25–30 min, 60–9 % B; 30–35 min, 9 % B. The injection volume was 3 μL. The column temperature was maintained at 35 °C. Extracts were filtered through 0.45 μm membrane filters before analysis.

GA, PA, *p*-HA, VA, SYA, and *trans*-CA were detected at a wavelength of 280 nm, while CHA, *p*-CA, FA, SIA, and IFA were measured at 320 nm. All the phenolic acids were quantified using external calibration curves based on the retention time of phenolic acid standards (Supplementary Fig. S1). The results were expressed as micrograms per gram of rice flour on a dry weight basis (μg/g). All extracts were analyzed in duplicate.

2.8. HPLC analysis of flavonoids compositions

The HPLC system and the column described above were also employed for the analysis of flavonoids compositions. The mobile phase consisted of A (0.1 % acetic acid in water) and B (Acetonitrile), and the flow rate was set at 0.8 mL/min. The injection volume was 10 μL. A 18 -min linear gradient was set as follows: 0–6 min, 10–35 % B; 6–10 min, 35–40 % B; 10–12 min, 40–45 % B; 12–14 min, 45–50 % B; 14–18 min, 50–10 % B; 18–25 min, 10 % B. C3G, P3G, CAT, LEU, ERI, and NAR were detected at a wavelength of 280 nm, while QUE and KAE were measured at 360 nm. External calibration curves were used to quantify the contents of flavonoids compositions (Supplementary Fig. S2). The results were expressed as micrograms per gram of rice flour on a dry weight basis (μg/g). All the extracts were analyzed in duplicate.

2.9. Statistical analysis

All parameters were measured in triplicate, and the results were reported as means ± standard deviation (SD). Differences among different rice genotypes and digestion phases were analyzed using ANOVA, followed by Tukey multiple comparison tests. Pearson's correlation test was performed for the correlation analysis. Statistical significance was set at a level of $p < 0.05$. Principal component analysis (PCA) was used to study the different digestion characteristics of polyphenols and antioxidant activities among non-pigmented, red, and black rice grains. The graphs were generated using Origin software (OriginPro 2021, Northampton, USA).

3. Results

3.1. Total phenolic, flavonoids content and their antioxidant activity

The effects of cooking and *in vitro* digestion on TPC and TFC of non-pigmented, red, and black rice are shown in Table 1. Free, bound, total TPC, and TFC in BR ranged from 16.99 to 68.06 mg GAE/100 g, 42.10–113.32 mg GAE/100 g, 59.09–249.40 mg GAE/100 g, and 29.20–249.40 mg CE/100 g, respectively. Red rice had higher free TPC (except for CFH) and TFC than black rice, and non-pigmented rice had the lowest ($p < 0.05$). Bound and total TPC of black rice were significantly higher than those of red and non-pigmented rice, with red rice exhibiting higher contents than non-pigmented rice. After cooking, free TPC, bound TPC, total TPC and TFC decreased by 54.7–84.3 %, 8.0–60.0 %, 23.6–70.7 % and 13.9–76.1 %, respectively. Great losses of TPC and TFC were observed especially in red rice. Comparing with CBR, the *in vitro* oral digestion caused a significant increase of free TPC in non-pigmented rice by 31.9–36.9 %. Free, bound and total TPC in CFH (red rice) increased significantly after oral digestion, but their contents in YZD decreased significantly ($p < 0.05$). After gastric digestion phase, free TPC of non-pigmented rice remained at similar levels compared to OBR (except for NK2021, $p < 0.05$) (Table 1). Free TPC of red and black rice increased or stayed at similar levels, while bound and total TPC decreased or stayed at similar levels (except for YZD). Interestingly, a

Table 1
Free, bound and total TPC, and TFC in BR, CBR, OBR, GBR and IBR of six rice grains [a].

	Samples	BR	CBR	OBR	GBR	IBR
Free TPC (mg GAE/ 100 g)	NK2021	23.43 ± 0.56c(b)	10.25 ± 0.40d (d)	14.03 ± 0.86c(c)	9.98 ± 0.17e(d)	26.86 ± 1.17c(a)
	ZZ35	16.99 ± 0.30d(b)	6.39 ± 0.10e(d)	8.43 ± 0.30e(c)	7.49 ± 0.10f (cd)	21.85 ± 0.98d(a)
	CFH	61.01 ± 0.56b(a)	9.57 ± 0.13d (d)	11.35 ± 0.41d(c)	12.37 ± 0.86d(c)	24.20 ± 0.78d (b)
	YZD	68.06 ± 3.16a(a)	15.51 ± 0.45c(c)	9.37 ± 0.59e(d)	14.06 ± 0.49c(c)	32.70 ± 0.31b (b)
	BH	59.15 ± 0.67b(a)	26.81 ± 1.02a(d)	25.36 ± 0.51a(d)	32.69 ± 0.69a(c)	44.98 ± 1.29a(b)
	HXD	58.96 ± 3.32b(a)	23.39 ± 0.30b(c)	22.99 ± 0.70b(c)	26.22 ± 0.72b(c)	43.76 ± 1.41a(b)
Bound TPC (mg GAE/ 100 g)	NK2021	56.96 ± 1.23d(a)	49.66 ± 1.45d (b)	50.70 ± 1.63d (b)	47.55 ± 0.29c(b)	42.68 ± 1.84c(c)
	ZZ35	42.10 ± 1.69e(a)	38.74 ± 0.84e(a)	42.10 ± 1.90e(a)	42.43 ± 1.12c(a)	32.40 ± 0.39d (b)
	CFH	77.53 ± 2.45c(a)	31.02 ± 0.74f(d)	39.38 ± 0.93e(c)	32.18 ± 1.10d (d)	53.92 ± 0.21b (b)
	YZD	87.63 ± 1.95b(a)	63.99 ± 0.36c(c)	56.36 ± 0.55c(d)	70.80 ± 0.48b (b)	54.74 ± 1.5b(d)
	BH	112.24 ± 3.56a (a)	98.14 ± 2.26a(b)	97.82 ± 3.53a(b)	90.92 ± 4.21a(c)	65.28 ± 0.11a(d)
	HXD	113.32 ± 4.00a (a)	72.36 ± 0.10b (b)	75.14 ± 3.55b (b)	70.37 ± 1.89b (b)	62.49 ± 0.66a(c)
Total TPC (mg GAE/ 100 g)	NK2021	80.39 ± 0.67d(a)	59.91 ± 1.05d (d)	64.73 ± 2.49c(c)	57.54 ± 0.12d (d)	69.54 ± 0.67e(b)
	ZZ35	59.09 ± 1.38e(a)	45.12 ± 0.74e(d)	50.52 ± 1.60d(c)	49.92 ± 1.02e(c)	54.26 ± 0.59f(b)
	CFH	138.54 ± 3.01c (a)	40.59 ± 0.61f(e)	50.73 ± 1.34d(c)	44.54 ± 1.96f(d)	78.12 ± 0.99d (b)
	YZD	155.70 ± 1.21b (a)	79.50 ± 0.81c(d)	65.73 ± 0.04c(e)	84.87 ± 0.01c(c)	87.44 ± 1.19c(b)
	BH	171.39 ± 4.23a (a)	124.95 ± 3.27a (b)	123.18 ± 4.05a (b)	123.61 ± 3.52a (b)	110.26 ± 1.18a (c)
	HXD	172.28 ± 0.68a (a)	95.75 ± 0.40b(c)	98.13 ± 4.25b(c)	96.59 ± 1.17b(c)	106.25 ± 2.08b (b)
TFC (mg CE/ 100 g)	NK2021	40.73 ± 0.68e(b)	23.72 ± 1.64d(e)	26.45 ± 0.81e(d)	31.02 ± 1.22e(c)	52.97 ± 1.58e(a)
	ZZ35	29.20 ± 1.98e(b)	17.29 ± 1.49e(c)	17.95 ± 1.20f(c)	28.13 ± 0.38e(b)	48.91 ± 0.88e(a)
	CFH	205.46 ± 4.51b (a)	63.51 ± 2.77c(d)	58.50 ± 1.81d (d)	97.50 ± 1.78d (b)	87.15 ± 2.88d(c)
	YZD	249.40 ± 17.29a (a)	59.71 ± 1.20c(c)	74.96 ± 1.90c(c)	112.71 ± 1.43c (b)	96.55 ± 4.58c(b)
	BH	155.45 ± 11.74c (b)	133.84 ± 2.95a (c)	126.22 ± 0.79a (c)	180.88 ± 8.83a (a)	140.03 ± 3.83a (c)
	HXD	125.60 ± 3.40d (b)	97.73 ± 1.96b(c)	97.46 ± 1.05b(c)	143.92 ± 3.40b (a)	121.65 ± 5.98b (b)

[a] The results are present as means ± SD ($n = 3$), and different lowercase letters inside and outside brackets indicate differences among different digestion phases and varieties, respectively ($p < 0.05$). TPC, total phenolic content; TFC, total flavonoids content; BR, brown raw rice; CBR, cooked brown rice; OBR, oral-digested cooked brown rice; GBR, gastric-digested cooked brown rice; IBR, intestinal-digested cooked brown rice.

large amount of TFC was released, which increased by 17.3–56.7 % and 43.3–66.7 % in non-pigmented and pigmented rice, respectively ($p < 0.05$). Free TPC of non-pigmented, red, and black rice after intestinal digestion was 91.7–169.1 %, 95.6–132.6 %, and 37.6–66.9 % higher than that after gastric digestion, respectively. On the contrary, the bound TPC decreased by 10.2–28.2 % (except for CFH). The TFC of non-pigmented rice after intestinal digestion increased by 70.8–73.9 % as comparing with that after gastric digestion, but a decrease of 10.6–22.6 % was observed in pigmented rice.

The DPPH and ABTS antioxidant activities of free and bound phenolics, and flavonoids extracts in BR, CBR, OBR, GBR and IBR of the six rice grains are shown in Table 2. Consistent with the variation trends observed in TPC and TFC in three colored rice grains, the DPPH and ABTS antioxidant activities exhibited higher levels in red and black rice, whereas they were lower in non-pigmented rice ($p < 0.05$). The ABTS and DPPH of flavonoids extracts in red rice were higher than those in black rice. Although DPPH and ABTS significantly decreased (especially for red rice) after cooking, they rebounded during gastric and/or intestinal phases. Interestingly, TFC in pigmented rice decreased significantly after intestinal digestion, but their DPPH and ABTS increased. High correlations between free TPC, bound TPC, total TPC, TFC, and their DPPH or ABTS antioxidant activity were observed in six rice grains during cooking and *in vitro* digestions (Fig. 1). The value of correlation coefficient between free ABTS and free TPC ($r = 0.9448$) was higher than that between free DPPH and free TPC ($r = 0.8102$) (Fig. 1 A). The correlation coefficient between bound DPPH and bound TPC was similar as that between bound ABTS and bound TPC ($r = 0.9803$, Fig. 1 B), which was also observed between total DPPH (or ABTS) and total TPC, and between flavonoids DPPH (or ABTS) and TFC (Fig. 1 C and D).

3.2. Phenolic acids

A total of 8 free phenolic acids in raw, cooked, and three digestion phases of the six rice grains are presented in Table 3. For BR, the total phenolic acids in non-pigmented, red, and black rice ranged from 36.63 to 79.28 μg/g, from 129.24 to 187.32 μg/g, and from 220.95 to 655.19 μg/g, respectively. PA (81.33–117.03 μg/g) and VA (31.57–50.43 μg/g) were highest in black rice, and *p*-HA (19.43–20.16 μg/g) was abundant in red rice. Cooking caused significant decreases of total phenolic acids by 40–79 %. Among them, PA, *p*-HA, SYA, *p*-CA and FA decreased significantly after cooking in all rice samples. However, VA and SIA (in NK2021, ZZ35 and HXD) increased or stayed at similar levels as comparing with raw rice samples. Total phenolic acids remained unchanged after oral digestion, except for YZD which decreased from 39.74 (CBR) to 13.79 μg/g (OBR). *p*-HA, *p*-CA, SIA and IFA in OBR stayed the same levels as those in CBR in all rice samples, except for NK2021. Interestingly, VA decreased after oral digestion in rice samples except for HXD. The total phenolic acids in GBR of the six rice genotypes ranged from 20.64 to 767.12 μg/g, with similar levels in non-pigmented and red rice and the higher levels in black rice ($p < 0.05$). Total phenolic acid contents in NK2021 decreased after gastric digestion, but that in BH increased. Compared with OBR, the GBR of NK2021 had lower contents of *p*-HA, SYA and SIA, and the GBR of BH had higher *p*-HA, VA, SYA and IFA. It was worth noting that *p*-HA, VA, SYA, *p*-CA and FA in YZD increased after gastric digestion. The total free phenolic acid contents of non-pigmented and pigmented rice grains in IBR were 71.6–113.8 % and 189.9–368.1 % higher than those in GBR, respectively (except for HXD). This is primarily attributed to the increased content of free *p*-CA, FA, and IFA in all three rice varieties, along with elevated levels of PA and *p*-HA

Table 2
Antioxidant activity of free and bound phenolic, and flavonoids extracts in BR, CBR, OBR, GBR and IBR of six rice grains (μM TE/g) [a].

Antioxidant activity	Samples	BR	CBR	OBR	GBR	IBR
Free DPPH	NK2021	0.74 ± 0.03c (b)	0.63 ± 0.01c (bc)	0.61 ± 0.05b (c)	0.52 ± 0.02 cd(c)	2.45 ± 0.05a (a)
	ZZ35	0.66 ± 0.05c (b)	0.35 ± 0.04d (c)	0.42 ± 0.07c (c)	0.35 ± 0.03e (c)	0.80 ± 0.02d (a)
	CFH	0.79 ± 0c(b)	0.41 ± 0.01d (c)	0.54 ± 0.06bc (c)	0.45 ± 0.02d (c)	1.02 ± 0.08c (a)
	YZD	2.61 ± 0.04b (a)	0.68 ± 0.07c (c)	0.40 ± 0.03c (d)	0.58 ± 0.06c (c)	1.08 ± 0.04c (b)
	BH	2.88 ± 0.08a (a)	1.18 ± 0.05a (c)	1.15 ± 0.07a (c)	1.43 ± 0.04a (b)	1.44 ± 0.07b (b)
	HXD	2.56 ± 0.07b (a)	0.99 ± 0.06b (d)	1.10 ± 0.06a (cd)	1.22 ± 0.06b (bc)	1.36 ± 0.05b (b)
Bound DPPH	NK2021	2.11 ± 0.04e (a)	1.84 ± 0.04d (b)	1.89 ± 0.01d (b)	1.84 ± 0.04d (b)	1.73 ± 0.03d (c)
	ZZ35	1.29 ± 0.06f (b)	1.37 ± 0.05f (b)	1.52 ± 0.05e (a)	1.54 ± 0.05e (a)	1.11 ± 0.05e (c)
	CFH	3.49 ± 0.05d (a)	1.49 ± 0.06e (d)	1.89 ± 0.05d (c)	1.44 ± 0.04f (e)	2.37 ± 0.05b (b)
	YZD	3.66 ± 0.03c (a)	2.66 ± 0.02c (b)	2.32 ± 0.04c (d)	2.55 ± 0.03c (c)	2.11 ± 0.05c (e)
	BH	4.25 ± 0.05b (a)	3.83 ± 0a(c)	3.91 ± 0.06a (b)	3.93 ± 0.03a (b)	2.48 ± 0.06ab (d)
	HXD	4.65 ± 0.03a (a)	3.13 ± 0.02b (b)	3.13 ± 0.04b (b)	2.93 ± 0b (c)	2.51 ± 0.05a (d)
Total DPPH	NK2021	2.85 ± 0.08d (b)	2.47 ± 0.03d (cd)	2.50 ± 0.04d (c)	2.36 ± 0.02d (d)	4.18 ± 0.02a (a)
	ZZ35	1.95 ± 0.11e (a)	1.72 ± 0.01f (b)	1.94 ± 0.02e (a)	1.89 ± 0.08e (ab)	1.91 ± 0.03e (ab)
	CFH	4.28 ± 0.05c (a)	1.90 ± 0.07e (d)	2.43 ± 0.11d (c)	1.89 ± 0.03d (d)	3.39 ± 0.02c (b)
	YZD	6.26 ± 0.01b (a)	3.34 ± 0.09c (b)	2.72 ± 0.01c (d)	3.13 ± 0.04c (c)	3.19 ± 0.09d (c)
	BH	7.12 ± 0.03a (a)	5.01 ± 0.04a (c)	5.05 ± 0.13a (c)	5.36 ± 0.01a (b)	3.92 ± 0.01b (d)
	HXD	7.21 ± 0.04a (a)	4.12 ± 0.05b (b)	4.24 ± 0.1b(b)	4.16 ± 0.06b (b)	3.87 ± 0b(c)
Flavonoids DPPH	NK2021	3.13 ± 0.02e (b)	1.78 ± 0.12e (d)	2.41 ± 0.11d (c)	2.21 ± 0.11d (c)	4.89 ± 0.07d (a)
	ZZ35	2.24 ± 0.03f (b)	1.21 ± 0.01f (d)	1.59 ± 0.1e(c)	1.73 ± 0.01e (c)	4.13 ± 0.06e (a)
	CFH	10.79 ± 0.05a (a)	4.32 ± 0.04c (e)	5.01 ± 0.05c (d)	5.14 ± 0c (c)	7.73 ± 0.01c (b)
	YZD	10.58 ± 0.04b (a)	3.42 ± 0.05d (d)	5.07 ± 0.01c (c)	5.17 ± 0.08c (c)	8.33 ± 0.15b (b)
	BH	9.68 ± 0.03c (a)	7.33 ± 0.09a (c)	7.74 ± 0.06a (b)	7.28 ± 0.03a (c)	9.77 ± 0.1a(a)
	HXD	9.36 ± 0.1d(b)	6.44 ± 0.1b(e)	7.14 ± 0.08b (c)	6.93 ± 0.09b (d)	9.56 ± 0.12a (a)
Free ABTS	NK2021	0.77 ± 0.07d (a)	0.34 ± 0.01bc (bc)	0.32 ± 0.06bc (c)	0.33 ± 0.02d (bc)	0.44 ± 0.01d (b)
	ZZ35	0.60 ± 0.04d (a)	0.21 ± 0.01c (c)	0.24 ± 0.03c (c)	0.22 ± 0e (c)	0.36 ± 0.02e (b)
	CFH	2.10 ± 0.04ab (a)	0.36 ± 0.04bc (b)	0.43 ± 0.04b (b)	0.47 ± 0.09c (b)	0.51 ± 0.01c (b)
	YZD	2.47 ± 0.30a (a)	0.67 ± 0.1a(b)	0.38 ± 0.04b (b)	0.55 ± 0c (b)	0.80 ± 0.01b (b)
	BH	1.65 ± 0.09c (a)	0.89 ± 0.05a (bc)	0.87 ± 0.04a (c)	1.06 ± 0.02a (b)	1.00 ± 0.06a (bc)
	HXD	1.72 ± 0.11bc (a)	0.63 ± 0.26ab (b)	0.76 ± 0.11a (b)	0.92 ± 0.02b (b)	0.99 ± 0.02a (b)
Bound ABTS	NK2021	2.10 ± 0.06c (a)	1.83 ± 0.06c (b)	1.87 ± 0 cd(b)	1.84 ± 0.04c (b)	1.77 ± 0.08c (b)
	ZZ35	1.94 ± 0.01c (a)	1.56 ± 0.12c (bc)	1.66 ± 0.08de (b)	1.69 ± 0.06d (b)	1.40 ± 0.06d (c)
	CFH	2.60 ± 0.22b (a)	1.21 ± 0.17d (d)	1.61 ± 0.01e (c)	1.42 ± 0e (cd)	1.95 ± 0.07b (b)
	YZD	2.79 ± 0.19ab (a)	2.26 ± 0.02b (bc)	2.07 ± 0.13c (c)	2.46 ± 0.03b (b)	1.99 ± 0.02b (c)
	BH	3.07 ± 0.22a (a)	2.81 ± 0.06a (b)	2.79 ± 0.2a(b)	2.73 ± 0.07a (b)	2.15 ± 0.03a (c)
	HXD	4.16 ± 0.06b (b)	4.24 ± 0.1b(b)	5.14 ± 0c (c)	7.28 ± 0.03a (c)	6.93 ± 0.09b (d)

(continued on next page)

Table 2 (*continued*)

Antioxidant activity	Samples	BR	CBR	OBR	GBR	IBR
Total ABTS	HXD	3.11 ± 0.17a (a)	2.29 ± 0.13b (b)	2.41 ± 0.01b (b)	2.41 ± 0.04b (b)	2.20 ± 0.01a (b)
	NK2021	2.87 ± 0.13c (a)	2.17 ± 0.04c (b)	2.18 ± 0.06d (b)	2.17 ± 0.05d (b)	2.21 ± 0.07d (b)
	ZZ35	2.53 ± 0.05d (a)	1.77 ± 0.13d (b)	1.90 ± 0.11e (b)	1.91 ± 0.06e (b)	1.76 ± 0.04e (b)
	CFH	4.70 ± 0.26b (a)	1.57 ± 0.13d (d)	2.03 ± 0.03de (c)	1.88 ± 0.09e (c)	2.46 ± 0.06c (b)
	YZD	5.27 ± 0.11a (a)	2.92 ± 0.08b (bc)	2.45 ± 0.09c (c)	3.01 ± 0.04c (b)	2.79 ± 0.03b (b)
	BH	4.72 ± 0.13b (a)	3.69 ± 0.11a (b)	3.66 ± 0.24a (b)	3.78 ± 0.06a (b)	3.15 ± 0.03a (c)
	HXD	4.83 ± 0.07b (a)	2.92 ± 0.13b (c)	3.17 ± 0.12b (bc)	3.33 ± 0.02b (b)	3.19 ± 0.03a (bc)
Flavonoids ABTS	NK2021	1.68 ± 0.07c (b)	1.16 ± 0.05d (d)	1.50 ± 0.04d (bc)	1.28 ± 0.04c (cd)	4.19 ± 0.13d (a)
	ZZ35	1.15 ± 0.02c (b)	0.67 ± 0.03e (d)	0.96 ± 0.05e (c)	0.95 ± 0.01c (c)	3.88 ± 0.07d (a)
	CFH	7.90 ± 0.16a (a)	2.69 ± 0.06c (e)	3.11 ± 0.03c (d)	3.57 ± 0.18b (c)	5.27 ± 0c(b)
	YZD	7.97 ± 0.09a (a)	2.50 ± 0c(e)	3.33 ± 0.17c (d)	4.04 ± 0.04b (c)	5.24 ± 0.37c (b)
	BH	7.42 ± 0.12ab (a)	5.32 ± 0.05a (b)	5.57 ± 0.06a (b)	5.76 ± 0.71a (b)	7.21 ± 0.05a (a)
	HXD	6.96 ± 0.63b (a)	4.45 ± 0.38b (c)	4.74 ± 0.11b (bc)	5.43 ± 0.2a (b)	6.52 ± 0.06b (a)

[a] The results are present as means ± SD (n = 3), and values with different letters are significantly different ($p < 0.05$); lowercase letters inside and outside brackets indicate differences among different phases of digestion and different varieties, respectively. Free DPPH and bound DPPH: DPPH radical scavenging activity of free and bound phenolics extracts, respectively; Free ABTS and bound ABTS: ABTS radical scavenging activity of free and bound phenolics extracts, respectively; total DPPH: the plus of free and bound DPPH; total ABTS: the plus of free and bound ABTS; Flavonoids DPPH: DPPH radical scavenging activity of flavonoids extracts; Flavonoids ABTS: ABTS radical scavenging activity of flavonoids extracts; other abbreviations are shown in Table 1.

in non-pigmented and red rice, and an increase in SIA content specifically in red and black rice ($p < 0.05$). Interestingly, the releases of PA (8.81–9.60 μg/g) and IFA (3.57–6.91 μg/g) in non-pigmented during intestinal digestion was higher than those lost during cooking (4.19–4.43 and 1.64–5.11 μg/g, respectively).

A total of 11 bound phenolic acids were quantified, and their contents in BR, CBR, OBR, GBR, and IBR of the six rice grains are shown in Table 4. The total bound phenolic acids in BR ranged from 361.30 to 797.35 μg/g, with the highest content found in the two black rice and the lowest in ZZ35. Among all the bound phenolic acids, FA was the most abundant (191.75–469.25 μg/g), which accounted for 51.4–58.9 % of total phenolic acids, and followed by IFA (65.75–227.24 μg/g) and *p*-CA (46.48–68.43 μg/g). Moreover, black rice had higher contents of PA (120.91–144.78 μg/g) and VA (46.56–57.54 μg/g), and red rice had higher contents of *p*-HA (17.49–25.28 μg/g) and PA (13.11–21.96 μg/g). Cooking significantly reduced the contents of total bound phenolic acids in NK2021 and CFH, which primarily due to the decrease of the contents of FA and *p*-CA in NK2021and FA, *p*-CA, IFA, *p*-HA, PA, and CHA in CFH. The contents of total bound phenolic acids in OBR remained at similar levels to those in CBR in rice samples except for YZD. In YZD, the contents of FA, IFA and PA in OBR were 22.8 %, 25.1 % and 41.0 % lower than those in CBR, respectively. In non-pigmented and black rice, the content of total phenolic acids in GBR remained at a similar level to that in OBR. In red rice, the total phenolic acid content of GBR in CFH was 65.35 μg/g lower than that of OBR, while the total phenolic acid content of GBR in YZD was 140.16 μg/g higher than that of OBR. This was mainly attributed to the decrease of FA, IFA and *p*-CA in CFH, and their increase in YZD. The total bound phenolic acids in IBR decreased by 16.1 % to 30.5 % compared to GBR except for CFH, and PA, FA and *p*-HA decreased by 20.2–100 % in most rice samples. The increase of total phenolic acids of IBR in CFH mainly due to the increase of FA, *p*-CA, IFA, SIA and CHA. For black rice, the contents of SIA and IFA in IBR of HXD were lower than those in GBR, and the contents of SIA and IFA in IBR of BH was higher than those in GBR ($p < 0.05$).However, the content of *p*-CA was higher in IBR of HXD and lower in IBR of BH. It was notable that the contents of VA and SYA almost remained at similar levels during cooking and *in vitro* digestion.

3.3. Flavonoids compositions

The contents of flavonoids compositions in BR, CBR, OBR, GBR and IBR of six rice grains are shown in Table 5. A total of 8 flavonoid compositions were detected, including C3G, P3G, CAT, LEUC, ERI, QUE, NAR, and KAE. The total contents of flavonoids compositions was highest in black rice (679.28–1023.46 μg/g), followed by red rice (34.56–42.72 μg/g), and non-pigmented rice had the lowest content (2.47–3.13 μg/g). In non-pigmented rice, only CAT was detectable. In red rice, CAT and LEUC could be detected, with the former ranging from 17.24 to 25.59 μg/g and the latter ranging from 17.13 to 17.32 μg/g. In black rice, all eight flavonoid compositions were detected, among which C3G (546.02–877.32 μg/g) and P3G (63.98–95.25 μg/g) were the highest, and ERI (2.32–3.01 μg/g), NAR (Nd - 1.85 μg/g), and KAE (1.96–4.02 μg/g) were the lowest. Cooking caused significant losses of flavonoids in black rice (about 80 % lower than those of BR), which was mainly due to the decrease of C3G, P3G and CAT. In red rice, the total flavonoids content increased by 18.8 % to 26.0 % after cooking, which was attributed to the increase of CAT (from 17.24 to 25.59 to 41.07–53.80 μg/g). For non-pigmented and red rice, only CAT was found at oral and gastric phases, and its content peaked at gastric digestion phase. For black rice, C3G was the main flavonoids at oral and gastric digestion phases (102.15–129.90 μg/g), followed by LEUC (17.89–20.47 μg/g) and QUE (12.87–15.27 μg/g), and P3G was not detectable (except for GBR of HXD). From gastric to intestinal digestion, the contents of C3G, P3G, and NAR in black rice increased by 342.7–412.9 %, 177.7 %, and 44.5 %, respectively. Small amounts of ERI (1.97–4.64 μg/g) and KAE (2.55–3.03 μg/g) were detected in non-pigmented and red rice after intestinal digestion.

3.4. Principal component analysis

The principal component analysis of free, bound and total TPC, TFC, and their antioxidant activity, phenolic acids, and flavonoids compositions in cooking and *in vitro* digestion of six rice grains is shown in Fig. 2.

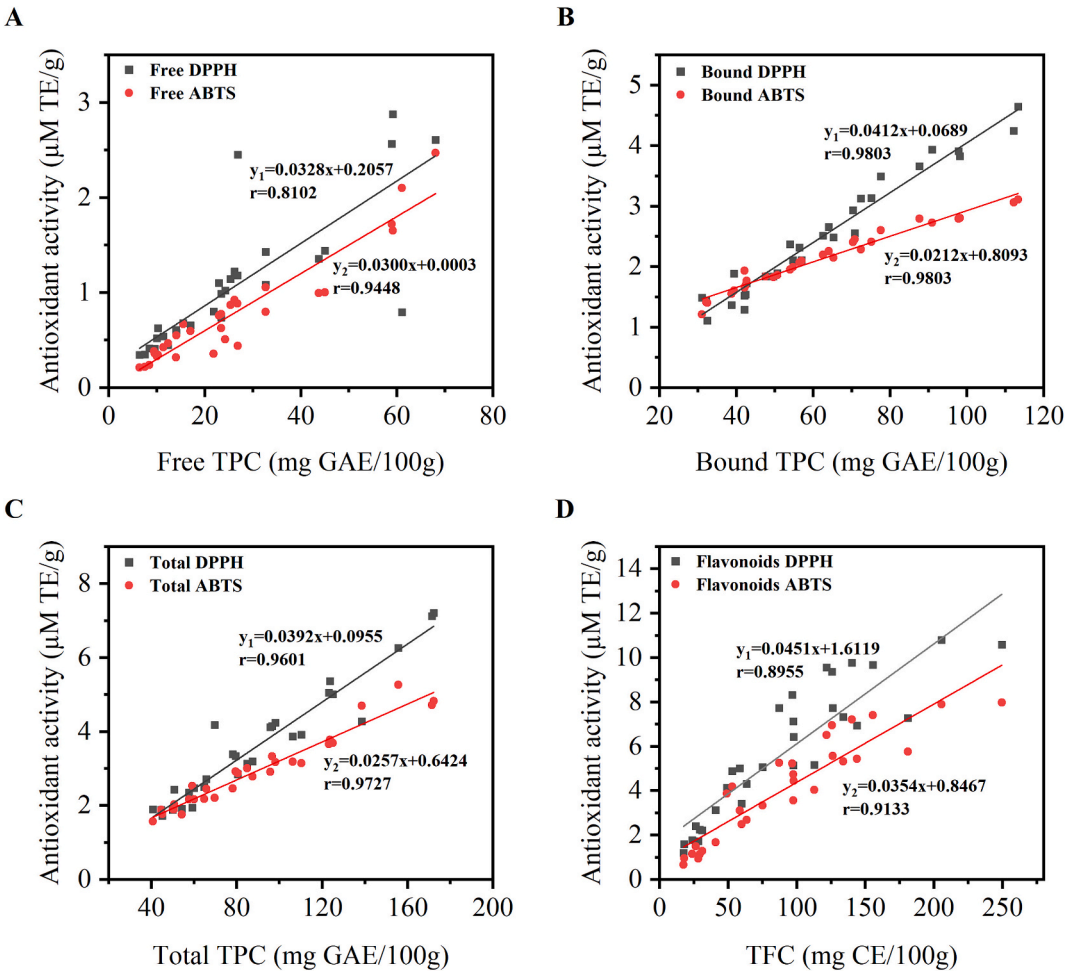

Fig. 1. Correlation analysis of polyphenols and antioxidant activity in BR, CBR, OBR, GBR and IBR of six rice grains.

The first three principal components explained 74.3 % of total variance, with the first (PC1), second (PC2), and third principal component (PC3) explaining 46.5 %, 18.0 %, and 9.8 % of total variance, respectively (Supplementary Table S1). PC1 represented total FA, total TPC, total DPPH antioxidant activity, and bound VA; PC2 was mainly attributed to bound *p*-HA, total *p*-HA, and bound CHA; PC3 corresponded to total SIA, free SIA, bound IFA, and total IFA (Supplementary Table S2). The PCA results revealed distinct differences in polyphenol content and antioxidant activity among non-pigmented, red and black rice during cooking and *in vitro* digestion. Although the polyphenols and antioxidant activity in red and non-pigmented rice were completely different, their change patterns during *in vitro* digestion (OBR, GBR, and IBR) were relatively similar (Fig. 2). The *in vitro* digestion products of black rice were positioned in the positive part of PC1, while those of red and non-pigmented rice were located in the negative part of PC1. The majority of *in vitro* digestion products from the two black rice varieties were situated in the positive and negative parts of PC2, respectively, exhibiting significant genotypic differences. However, little differences were observed between non-pigmented and red rice varieties.

4. Discussion

TPC, TFC and their antioxidant activities, phenolic acids and flavonoids compositions were higher in pigmented rice than those in non-pigmented rice (Table 1–5), which was similar to previous studies (Fracassetti et al., 2020; Shao et al., 2018). For pigmented rice, red rice (YZD) had higher free TPC than black rice, but relatively lower total free phenolic acids. It was largely due to the differences among rice genotypes. On the other hand, TPC was measured by the Folin-Ciocalteu assay which could be interfered by other substances. It was well-known that proanthocyanidins and anthocyanins was specially found in most red and black rice, respectively (Shao & Bao, 2015). Proanthocyanidins in red rice could be extracted by methanol and ethyl acetate, but anthocyanins was water soluble and could not be extracted by ethyl acetate. Therefore, the free TPC in red rice was higher, and its phenolic acids might be not.

The effect of hydrothermal treatment on free and bound TPC, and TFC was consistent with previous reports (Bhawamai et al., 2016; Fracassetti et al., 2020; Pinto et al., 2024). In hydrothermal treatment, the crystalline structure of starch granules was destroyed. As the temperature of the cooked rice dropped gradually, the starch chains transformed from a disordered and unfolded state to a new ordered crystalline state through hydrogen bonds and hydrogen-bond interactions among proteins, amylose, amylopectin, polyphenols, and other substances (Lu et al., 2023). Red rice was rich in proanthocyanidins (or high-density tannins) which could interact with the helical structures during starch retrogradation after heat-thermal treatment (Lu et al., 2023). The formation of insoluble complexes made the extraction of phenolics and flavonoids difficult, which might be a factor in the decline of polyphenols after cooking. Simultaneously, the absorption of water and heat processing could break rice bran and the ester bonds between polyphenols and cell walls, expose some polyphenols to the air, and accelerate their oxidative degradation (Bagchi et al., 2021; Oghenerukevwe et al., 2023). For free phenolic acids, FA, PA, *p*-HA, *p*-CA, and SYA decreased in most samples after cooking, but SIA in non-pigmented rice, and IFA in black rice increased (Table 3). This trend was similar to

Table 3
Free phenolic acids in BR, CBR, OBR, GBR and IBR of six rice grains (μg/g) [a].

	Samples	BR	CBR	OBR	GBR	IBR
PA	NK2021	4.19 ± 0.09e(b)	Nd	Nd	Nd	13.79 ± 0.81b(a)
	ZZ35	4.43 ± 0.48e(b)	Nd	Nd	Nd	13.24 ± 0.09b(a)
	CFH	10.78 ± 0.18d(b)	3.88 ± 0.15b(c)	Nd	Nd	13.58 ± 0.55b(a)
	YZD	17.44 ± 0.84c(a)	Nd	Nd	Nd	17.57 ± 2.51a(a)
	BH	81.33 ± 2.75b(a)	13.66 ± 9.48ab (bc)	18.16 ± 4.93a (bc)	24.87 ± 5.37a(b)	7.33 ± 0.27c(c)
	HXD	117.03 ± 3.43a (a)	26.55 ± 5.02a(b)	18.11 ± 0.56a(c)	17.71 ± 0.35a(c)	7.77 ± 1.17c(d)
p-HA	NK2021	8.10 ± 0.16b(a)	5.01 ± 0.28a(d)	6.08 ± 0.26a(c)	4.93 ± 0.04a(d)	7.13 ± 0.11b(b)
	ZZ35	2.37 ± 0.37d(a)	Nd	Nd	Nd	3.12 ± 0.24d(a)
	CFH	19.43 ± 2.58a(a)	2.63 ± 0.15c(b)	2.27 ± 0.22d(b)	2.38 ± 0d (b)	4.84 ± 0.08c(b)
	YZD	20.16 ± 0.56a(a)	4.37 ± 0.05b(c)	2.63 ± 0.14c(d)	4.59 ± 0.03ab(c)	9.94 ± 0.13a(b)
	BH	6.85 ± 0.16bc (a)	3.05 ± 0.04c(c)	3.11 ± 0.07c(c)	4.21 ± 0.21b(b)	3.21 ± 0.21d(c)
	HXD	4.45 ± 1.11 cd (a)	4.49 ± 0.17b(a)	3.78 ± 0.11b (ab)	3.34 ± 0.48c(ab)	2.54 ± 0.27e(b)
VA	NK2021	2.03 ± 0.08d(b)	2.56 ± 0.13 cd (a)	1.44 ± 0.06c(c)	1.44 ± 0.08c(c)	1.24 ± 0.01d(c)
	ZZ35	3.09 ± 0.05d(a)	2.68 ± 0 cd(ab)	1.92 ± 0.07c(d)	2.13 ± 0.07c(cd)	2.52 ± 0.30d (bc)
	CFH	5.54 ± 0.23c(a)	3.8 ± 0.77c(b)	2.73 ± 0.07c(c)	2.35 ± 0.15c(c)	4.95 ± 0.42c(a)
	YZD	2.18 ± 0.51d(a)	1.12 ± 0d(b)	Nd	1.17 ± 0.06c(b)	2.70 ± 0.02d(a)
	BH	31.57 ± 0.68b(b)	28.49 ± 0.75b(c)	25.6 ± 0.28b(d)	30.5 ± 1.49b(bc)	37.75 ± 0.78b(a)
	HXD	50.43 ± 0.67a(a)	47.86 ± 1.42a(a)	52.36 ± 2.34a(a)	50.70 ± 5.45a(a)	48.11 ± 1.26a(a)
SYA	NK2021	1.20 ± 0.09c(a)	1.03 ± 0b(b)	1.19 ± 0.01c(a)	1.02 ± 0.07b(b)	Tr
	ZZ35	2.07 ± 0.43b(a)	Tr	1.11 ± 0.12c(a)	Tr	1.05 ± 0.09e(a)
	CFH	4.14 ± 0.18a(a)	1.15 ± 0.41b(b)	1.18 ± 0.20c(b)	1.15 ± 0.04b(b)	1.36 ± 0.12d(b)
	YZD	2.11 ± 0.38b(b)	1.00 ± 0.20b(c)	Tr	1.09 ± 0.01b(c)	8.73 ± 0.03a(a)
	BH	3.39 ± 0.09a(a)	1.93 ± 0.22a(c)	2.13 ± 0a(c)	2.13 ± 0a (c)	3.13 ± 0.01b(b)
	HXD	1.93 ± 0.22bc (a)	1.71 ± 0.26ab (a)	1.87 ± 0.10b(a)	1.92 ± 0.15a(a)	1.99 ± 0.06c(a)
p-CA	NK2021	2.95 ± 0.52d(d)	3.73 ± 0.89b (cd)	5.51 ± 0.16b (ab)	4.55 ± 0.12bc (bc)	6.06 ± 1.10d(a)
	ZZ35	Tr	Nd	Tr	Nd	2.55 ± 0.27e
	CFH	4.94 ± 0.63c(a)	1.09 ± 0.27c(b)	1.06 ± 0.31c(b)	Nd	1.56 ± 0.09e(b)
	YZD	12.46 ± 0.59b(a)	3.46 ± 0.08b(c)	Tr	3.49 ± 0.73c(c)	10.00 ± 1.00c(b)
	BH	2.98 ± 0.21d(c)	4.42 ± 0.33b (bc)	5.02 ± 0.07b (bc)	6.11 ± 0.39b(b)	35.98 ± 1.90a(a)
SIA	HXD	37.23 ± 0.56a(a)	16.83 ± 0.16a(c)	15.75 ± 2.57a(c)	17.10 ± 0.74a(c)	21.62 ± 1.60b(b)
	NK2021	3.06 ± 0.15c(c)	7.29 ± 0.93b(b)	9.13 ± 1.15b(a)	6.59 ± 0.49b(b)	3.79 ± 1.21c(c)
	ZZ35	Nd	5.73 ± 2.15b(a)	7.72 ± 0.53b(a)	6.46 ± 0b (a)	Tr
	CFH	19.62 ± 0.88b(a)	1.07 ± 0.41c(b)	1.26 ± 0.40c(b)	Nd	2.33 ± 0.81c(b)
	YZD	43.75 ± 6.24a(a)	5.04 ± 0.65b(b)	2.32 ± 0.55c(b)	5.54 ± 0.49b(b)	38.05 ± 5.13a(a)
	BH	51.87 ± 1.47a(a)	1.53 ± 0.02c(d)	1.42 ± 0.39c(d)	4.96 ± 0.59b(c)	16.73 ± 0.87b(b)
	HXD	Nd	33.50 ± 1.56a(a)	40.40 ± 3.92a(a)	46.30 ± 5.55a(a)	Nd
FA	NK2021	35.91 ± 0.4c(a)	11.49 ± 1.16b(c)	11.66 ± 0.82b(c)	11.83 ± 1.16b(c)	19.15 ± 0.44c(b)
	ZZ35	12.63 ± 0.65d(a)	3.69 ± 0.61b(c)	4.60 ± 0.52b(c)	3.95 ± 0.28b(c)	8.42 ± 1.09c(b)
	CFH	46.31 ± 0.85bc (a)	7.42 ± 1.57b (bc)	7.29 ± 1.23b (bc)	5.82 ± 0.18b(c)	10.99 ± 1.50c(b)
	YZD	59.23 ± 2.31b(a)	11.13 ± 1.54b(c)	2.19 ± 0.13b(d)	7.48 ± 0.47b(c)	23.81 ± 0.60c(b)
	BH	42.96 ± 0.36bc (b)	17.22 ± 1.86b(c)	16.49 ± 0.01b(c)	20.59 ± 1.54b(c)	266.62 ± 11.41b (a)
	HXD	389.33 ± 18.1a (a)	281.43 ± 27.18a (b)	296.19 ± 27.77a (b)	277.71 ± 40.03a(b)	303.46 ± 11.58a (b)
IFA	NK2021	21.84 ± 1.41b(b)	16.73 ± 0.83b(c)	16.20 ± 0.56b(c)	14.26 ± 0.33b(c)	25.41 ± 1.87c(a)
	ZZ35	12.03 ± 1.54b(b)	10.39 ± 0.21b(b)	11.43 ± 1.05b(b)	10.77 ± 1.27b(b)	18.94 ± 1.00c(a)
	CFH	18.48 ± 7.01b (ab)	10.86 ± 2.16b (bc)	11.59 ± 0.64b (abc)	8.94 ± 0.45b(c)	20.21 ± 0.18c(a)
	YZD	29.99 ± 1.55b(a)	13.62 ± 6.95b(b)	6.64 ± 0.44b(b)	12.33 ± 0.10b(b)	25.71 ± 2.53c(a)
	BH	Nd	12.36 ± 0.49b (bc)	10.15 ± 1.04b(c)	16.12 ± 1.84b(b)	141.74 ± 1.10b (a)
	HXD	54.79 ± 14.16a (c)	293.93 ± 27.6a (ab)	332.05 ± 49.69a (ab)	352.33 ± 71.53a(a)	201.79 ± 8.16a (b)
Total	NK2021	79.28 ± 2.08d(a)	47.83 ± 4.24b (bc)	51.21 ± 1.38b(b)	44.62 ± 0.44b(c)	76.58 ± 0.04d(a)
	ZZ35	36.63 ± 2.56e(b)	22.49 ± 2.97b(c)	26.79 ± 1.92b(c)	23.31 ± 0.92b(c)	49.85 ± 2.54d(a)
	CFH	129.24 ± 12.53c (a)	31.92 ± 5.58b(c)	27.38 ± 0.99b(c)	20.64 ± 0.83b(c)	59.82 ± 2.5d(b)
	YZD	187.32 ± 11.88b (a)	39.74 ± 9.31b(c)	13.79 ± 0.98b(d)	35.70 ± 1.67b(cd)	136.51 ± 6.88c (b)
	BH	220.95 ± 2.59b (b)	82.66 ± 5.82b(d)	82.08 ± 3.66b(d)	109.49 ± 7.72b(c)	512.49 ± 12.04b (a)
	HXD	655.19 ± 29.84a (a)	706.30 ± 63.38a (a)	760.50 ± 86.84a (a)	767.12 ± 124.27a (a)	587.28 ± 21.20a (a)

[a] The results are shown as means ± SD (n = 3), and different lowercase letters inside and outside brackets indicate differences among different digestion phases and varieties, respectively ($p < 0.05$). PA, protocatechuic acid; *p*-HA, *p*-hydroxybenzoic acid; VA, vanillic acid; SYA, syringic acid; *p*-CA, *p*-coumaric acid; SIA, sinapic acid; FA, ferulic acid; IFA, isoferulic acid; Tr, detected in trace amounts (< 1 μg/g); Nd, not detectable; other abbreviations are shown in Table 1.

Table 4
Bound phenolic acids in BR, CBR, OBR, GBR and IBR of six rice grains (μg/g) [a].

	Samples	BR	CBR	OBR	GBR	IBR
GA	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Tr	Nd	Nd
	CFH	Nd	Nd	Nd	Nd	Nd
	YZD	Tr	Nd	Nd	Tr	Nd
	BH	4.85 ± 0.23a(a)	3.61 ± 0.69a (bc)	2.82 ± 0.08b(c)	4.37 ± 0.59a (ab)	4.80 ± 0.18a(a)
	HXD	4.62 ± 0.14a (ab)	3.79 ± 0.21a (bc)	3.90 ± 0.17a (bc)	3.58 ± 0.44a(c)	5.40 ± 0.50a(a)
PA	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Nd	Nd	Nd
	CFH	13.11 ± 0.27c(a)	4.28 ± 0.72c(c)	8.47 ± 0.17c(b)	5.21 ± 0.41c(c)	5.18 ± 0.32b(c)
	YZD	21.96 ± 1.41c(a)	11.28 ± 0.20c(b)	6.66 ± 0.83c (cd)	9.15 ± 1.06bc (bc)	4.26 ± 0.52b(d)
	BH	120.91 ± 1.87b (a)	110.69 ± 6.25a (ab)	93.60 ± 1.96a(c)	107.74 ± 6.18a (b)	7.68 ± 0.11a(d)
	HXD	144.78 ± 9.38a (a)	27.29 ± 2.55b(b)	18.90 ± 0.56b(b)	18.28 ± 0.94b (bc)	7.60 ± 0.43a(c)
CHA	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Nd	Tr	Nd
	CFH	23.62 ± 0.10a(a)	7.41 ± 0.26a(d)	10.13 ± 1.75a(c)	6.12 ± 1.61b(d)	13.56 ± 1.64a(b)
	YZD	25.92 ± 3.88a(a)	16.93 ± 1.49a(b)	18.01 ± 0.51a(b)	20.83 ± 2.58a (ab)	5.89 ± 0.43a(c)
	BH	Nd	Nd	Nd	Nd	Nd
	HXD	Nd	Nd	Nd	Nd	Nd
p-HA	NK2021	2.66 ± 0.16d(c)	3.55 ± 0.22c(a)	3.44 ± 0.09b (ab)	2.89 ± 0.02c (bc)	1.59 ± 0.35c(d)
	ZZ35	4.04 ± 0.11 cd (a)	2.47 ± 0.36d(b)	1.94 ± 0.07c(c)	1.40 ± 0d(d)	Nd
	CFH	17.49 ± 0.78b(a)	2.78 ± 0.64 cd (c)	3.94 ± 0.86b(b)	2.85 ± 0.66c(c)	3.82 ± 0.08a (bc)
	YZD	25.28 ± 2.01a(a)	7.44 ± 0.11a (bc)	6.7 ± 0.45a (cd)	9.44 ± 0.58a(b)	4.38 ± 0.03a(d)
	BH	5.47 ± 0.55c(b)	6.70 ± 0.36a(a)	6.27 ± 0.35a(a)	5.56 ± 0.4b(b)	2.49 ± 0.06b(c)
	HXD	5.89 ± 0.16c(a)	4.69 ± 0.31b(b)	4.08 ± 0.46b(b)	3.69 ± 0.41c(b)	2.54 ± 0.27b(c)
VA	NK2021	2.62 ± 0.45c(a)	2.27 ± 0d(a)	2.31 ± 0.06c(a)	2.07 ± 0.01c(a)	2.18 ± 0.06 cd (a)
	ZZ35	1.66 ± 0.16c(a)	1.42 ± 0.14e(a)	1.59 ± 0.27c(a)	1.41 ± 0.19c(a)	1.34 ± 0.29d(a)
	CFH	2.83 ± 0.75c(a)	1.37 ± 0.34e(a)	1.95 ± 0.07c(a)	1.50 ± 0.50c(a)	2.65 ± 0.90 cd (a)
	YZD	2.23 ± 0c(c)	2.91 ± 0.08c (abc)	2.59 ± 0.70c (bc)	3.74 ± 0.03c(a)	3.39 ± 0.02c (ab)
	BH	46.56 ± 0.68b(a)	46.28 ± 0.27b(a)	43.64 ± 1.37b (ab)	40.93 ± 3.76b(b)	40.10 ± 0.63b(b)
	HXD	57.54 ± 0.15a(a)	49.57 ± 0.46a (bc)	53.68 ± 0.75a (ab)	52.33 ± 3.14a (bc)	48.45 ± 0.36a(c)

Table 4 (*continued*)

	Samples	BR	CBR	OBR	GBR	IBR
SYA	NK2021	1.48 ± 0.04ab (a)	1.08 ± 0.08c(b)	1.11 ± 0.04b(b)	Tr	Tr
	ZZ35	1.32 ± 0.1ab(a)	1.12 ± 0.04c(a)	Tr	Tr	Tr
	CFH	1.51 ± 0.18ab (a)	Tr	Tr	Tr	1.10 ± 0.24c(a)
	YZD	1.07 ± 0.04b (ab)	1.26 ± 0.09c (ab)	1.04 ± 0.41b(b)	1.61 ± 0.09a(a)	1.22 ± 0.09c (ab)
	BH	2.01 ± 0.62a(a)	2.92 ± 0.09a(a)	2.58 ± 0.56a(a)	2.34 ± 0.46a(a)	3.30 ± 0.01a(a)
	HXD	Tr	1.82 ± 0.05b(a)	1.98 ± 0.06ab (a)	1.95 ± 0.11a(a)	2.05 ± 0.06b(a)
p-CA	NK2021	63.22 ± 5.04a(a)	46.29 ± 3.05c(b)	44.63 ± 2.24b(b)	41.83 ± 3.02b(b)	39.96 ± 1.44c(b)
	ZZ35	58.58 ± 3.59ab (a)	45.75 ± 2.72c(b)	48.56 ± 1.30b(b)	50.35 ± 0.74b(b)	30.02 ± 1.96d(c)
	CFH	64.67 ± 1.51a(a)	25.33 ± 0.70d(c)	30.41 ± 2.01c(c)	24.76 ± 1.73c(c)	41.67 ± 7.92bc (b)
	YZD	66.79 ± 6.75a(a)	58.46 ± 1.55b(a-c)	47.64 ± 3.65b(c)	61.06 ± 6.24a (ab)	50.76 ± 0.71a (bc)
	BH	68.43 ± 6.33a(a)	67.96 ± 3.43a(a)	65.79 ± 4.41a(a)	64.51 ± 5.12a(a)	49.49 ± 0.92ab (b)
	HXD	46.48 ± 3.47b(a)	19.51 ± 0.63d(c)	20.93 ± 2.27d(c)	20.21 ± 0.33c(c)	27.80 ± 0.2d(b)
SIA	NK2021	24.47 ± 0.89a(c)	38.22 ± 3.36a (ab)	42.34 ± 7.91a (ab)	49.37 ± 1.27a(a)	30.93 ± 3.39a (bc)
	ZZ35	15.97 ± 0.87b(b)	26.77 ± 2.30b(a)	20.48 ± 3.98bc (ab)	30.10 ± 1.61b(a)	21.99 ± 5.3b(ab)
	CFH	8.78 ± 0.59c(b)	14.22 ± 2.80c(b)	13.06 ± 1.22c(b)	10.07 ± 1.81c(b)	19.98 ± 4.58b(a)
	YZD	Nd	15.83 ± 0.19c(c)	12.26 ± 2.73c(c)	32.26 ± 2.55b(a)	23.54 ± 1.34b(b)
	BH	Nd	Nd	Nd	Nd	19.62 ± 2.24b
	HXD	Nd	40.49 ± 2.57a(a)	31.62 ± 9.14ab (a)	49.51 ± 2.64a(a)	Nd
FA	NK2021	340.48 ± 6.36b (a)	257.40 ± 15.31c (b)	260.11 ± 2.95c (b)	241.76 ± 14.71c (b)	148.89 ± 11.24c (c)
	ZZ35	191.75 ± 31.62c (a)	179.16 ± 9.94d (a)	185.71 ± 1.62d (a)	188.43 ± 3.47d (a)	124.00 ± 6.14d (b)
	CFH	302.76 ± 7.02b (a)	128.04 ± 17.48e (c)	136.52 ± 10.67e (c)	102.83 ± 17.34e (c)	178.20 ± 11.94b (b)
	YZD	281.24 ± 30.52b (a)	232.63 ± 0.97c (bc)	179.70 ± 11.87d (d)	250.11 ± 18.91c (ab)	199.55 ± 5.7b (cd)
	BH	469.25 ± 22.90a (ab)	489.18 ± 7.14a (a)	474.10 ± 22.13a (ab)	447.37 ± 17.21a (b)	305.79 ± 8.03a (c)
	HXD	443.57 ± 3.08a (a)	314.32 ± 11.2b (bc)	324.61 ± 0.22b (b)	304.86 ± 6.33b (c)	318.49 ± 1.08a (bc)
IFA	NK2021	227.24 ± 4.69a (b)	220.75 ± 8.62b (b)	239.14 ± 15.59b (ab)	269.82 ± 17.16b (a)	213.99 ± 7.77a (b)

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Table 4 (*continued*)

	Samples	BR	CBR	OBR	GBR	IBR
	ZZ35	85.6 ± 11.46c (b)	120.11 ± 12.87c (ab)	130.48 ± 17.87c (a)	137.96 ± 0.02c (a)	107.89 ± 16.10d (ab)
	CFH	122.08 ± 14.72b (a)	72.36 ± 16.71d (b)	87.95 ± 5.30d(b)	73.75 ± 15.67d (b)	143.29 ± 12.65bc (a)
	YZD	84.24 ± 3.3c(b)	124.26 ± 1.75c (a)	93.01 ± 6.66d(b)	120.46 ± 11.87c (a)	132.69 ± 5.19c (a)
	BH	77.89 ± 16.34c (b)	90.16 ± 7.39d(b)	80.80 ± 2.55d(b)	114.29 ± 20.76 cd(b)	163.82 ± 3.98b (a)
	HXD	65.75 ± 0.55c(d)	342.13 ± 0.09a (b)	380.13 ± 7.47a (a)	401.38 ± 15.95a (a)	212.08 ± 2.93a (c)
trans -CA	NK2021	Nd	2.35 ± 0.58b(a)	2.59 ± 0.57a(a)	2.98 ± 0.16a(a)	1.94 ± 0.32a(a)
	ZZ35	2.38 ± 0.36a(b)	3.37 ± 0.15a(a)	3.25 ± 0.08a(a)	3.88 ± 0.54a(a)	2.12 ± 0a(b)
	CFH	1.52 ± 0.14b(a)	Nd	Tr	Tr	1.13 ± 0.03b(a)
	YZD	2.15 ± 0.10ab (a)	1.97 ± 0.04bc (ab)	1.95 ± 0.02a (ab)	1.06 ± 0.07b(c)	1.74 ± 0.21a(b)
	BH	1.98 ± 0.12ab (a)	1.35 ± 0.08c(b)	Tr	Tr	1.12 ± 0.08b(b)
	HXD	Tr	Tr	Tr	1.03 ± 0.09b(b)	2.04 ± 0.03a(a)
Total	NK2021	662.18 ± 14.95b (a)	571.91 ± 30.06b (b)	595.66 ± 18.80c (b)	610.71 ± 0.82b (ab)	439.47 ± 23.93b (c)
	ZZ35	361.30 ± 48.27d (ab)	380.17 ± 27.50d (a)	392.02 ± 14.48d (a)	413.53 ± 5.44d (a)	287.37 ± 29.21c (b)
	CFH	558.37 ± 6.04c (a)	255.79 ± 4.69e (cd)	292.43 ± 11.45e (c)	227.08 ± 38.91e (d)	410.57 ± 40.07b (b)
	YZD	510.88 ± 33.66c (a)	472.96 ± 1.85c (ab)	369.55 ± 27.82d (c)	509.71 ± 36.69c (a)	427.42 ± 12.5b (bc)
	BH	797.35 ± 9.93a (a)	818.85 ± 12.87a (a)	769.61 ± 33.24b (a)	787.11 ± 42.12a (a)	598.21 ± 3.06a (b)
	HXD	768.63 ± 2.13a (c)	803.62 ± 11.57a (bc)	839.83 ± 18.70a (ab)	856.83 ± 26.97a (a)	626.43 ± 4.04a (d)

[a] The results are present as means ± SD (n = 3), and different lowercase letters inside and outside brackets indicate differences between different digestion phases and varieties, respectively ($p < 0.05$). GA, gallic acid; CHA, chlorogenic acid; *trans*-CA, *trans*-cinnamic acid; other abbreviations are shown in Table 1 and Table 3.

previous studies (Oghenerukevwe et al., 2023; Yu et al., 2020), and partially consistent with other studies (Ramos et al., 2022; Sęczyk et al., 2020), as both genotype and cooking method were major influencing factors. Furthermore, the loss of anthocyanins could produce bioactive phenolic metabolites such as *p*-CA, VA, and FA (Nignpense et al., 2022). For bound phenolic acids, FA, *p*-CA, PA, and CHA were susceptible to degradation during thermal processing, and VA and *trans*-CA exhibited resistance to rapid thermal degradation (Table 4), which were similar to other study (Bagchi et al., 2021) and inconsistent with another study (Ti et al., 2015). These differences might be caused by the varieties and cooking techniques. The anthocyanins in black rice decreased greatly after cooking (Table 5). It might be due to the thermal sensitivity of anthocyanins, and their interactions with starch granules which made them difficult to be extracted. CAT in red rice increased significantly after cooking (Table 5), which might be due to the release of CAT from

Table 5
The flavonoids compositions in BR, CBR, OBR, GBR and IBR of six rice grains (μg/g) [a].

	Samples	BR	CBR	OBR	GBR	IBR
C3G	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Nd	Nd	Nd
	CFH	Nd	Nd	Nd	Nd	Nd
	YZD	Nd	Nd	Nd	Nd	Nd
	BH	546.02 ± 9.60b(a)	102.15 ± 0.05b (c)	103.80 ± 0.09b (c)	113.56 ± 26.74a (c)	502.66 ± 9.89a (b)
	HXD	877.32 ± 23.42a(a)	118.96 ± 0.82a (c)	129.90 ± 0.82a (c)	106.29 ± 4.59a (c)	545.17 ± 3.76a (b)
P3G	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Nd	Nd	Nd
	CFH	Nd	Nd	Nd	Nd	Nd
	YZD	Nd	Nd	Nd	Nd	Nd
	BH	63.98 ± 1.60b(a)	Nd	Nd	Nd	19.86 ± 1.46a(b)
	HXD	95.25 ± 1.00a(a)	Nd	Nd	7.94 ± 0.19(c)	22.04 ± 0.17a(b)
CAT	NK2021	3.13 ± 0.19d(bc)	2.75 ± 0c(cd)	3.49 ± 0.15e(b)	8.79 ± 0.16e(a)	2.58 ± 0.01d(d)
	ZZ35	2.47 ± 0.02d(c)	2.10 ± 0.02c(d)	2.49 ± 0.10e (bc)	4.95 ± 0.01e(a)	2.70 ± 0.11d(d)
	CFH	17.24 ± 0.85c(d)	41.07 ± 0.13b(c)	42.96 ± 0.57b (bc)	51.86 ± 0.19b(a)	44.79 ± 2.34b(b)
	YZD	25.59 ± 1.20b(d)	53.8 ± 1.39a(c)	66.07 ± 0.34a(a)	66.10 ± 3.10a(a)	60.59 ± 1.19a(b)
	BH	35.65 ± 0.49a(a)	3.88 ± 0.57c(d)	4.69 ± 0.76d(d)	14.41 ± 0.32d(b)	6.50 ± 0.24c(c)
	HXD	23.99 ± 1.41b(a)	3.94 ± 0.83c(d)	5.91 ± 0.59c (cd)	19.73 ± 1.46c(b)	7.88 ± 0.64c(c)
LEUC	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Nd	Nd	Nd
	CFH	17.32 ± 0.35a	Nd	Nd	Nd	Nd
	YZD	17.13 ± 0.68a	Nd	Nd	Nd	Nd
	BH	13.92 ± 0.23b(c)	17.89 ± 0.86a(b)	18.14 ± 0.23b(b)	46.71 ± 1.86a(a)	16.19 ± 0.22a(b)
	HXD	13.30 ± 0.03b(d)	18.82 ± 0.46a(b)	20.47 ± 0.03a(b)	44.24 ± 1.54a(a)	16.26 ± 0.08a(c)
ERI	NK2021	Nd	Nd	Nd	Nd	4.64 ± 0.21b
	ZZ35	Nd	Nd	Nd	Nd	1.97 ± 0.08d
	CFH	Nd	Nd	Nd	Nd	2.18 ± 0.15d
	YZD	Nd	Nd	Nd	Nd	2.73 ± 0c
	BH	3.01 ± 0.16a(d)	5.22 ± 0.37a(c)	5.28 ± 0.31a(c)	6.95 ± 0.16a(b)	9.67 ± 0.36a(a)
	HXD	2.32 ± 0.10b(c)	5.10 ± 0.12a(b)	5.07 ± 0.31b(b)	5.41 ± 0.18a(b)	9.15 ± 0.15a(a)
QUE	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Nd	Nd	Nd
	CFH	Nd	Nd	Nd	Nd	Nd
	YZD	Nd	Nd	Nd	Nd	Nd
	BH	10.83 ± 0.13a(c)	14.96 ± 0.12a(a)	15.27 ± 0.11a(a)	15.20 ± 0.35a(a)	12.03 ± 0.59a(b)
	HXD	9.32 ± 0.22a(c)	12.87 ± 0.29b (ab)	13.23 ± 0.09b(a)	13.37 ± 0.29a(a)	12.08 ± 0.70a(b)

(*continued on next page*)

Table 5 (*continued*)

	Samples	BR	CBR	OBR	GBR	IBR
NAR	NK2021	Nd	Nd	Nd	Nd	Nd
	ZZ35	Nd	Nd	Nd	Nd	Nd
	CFH	Nd	Nd	Nd	Nd	Nd
	YZD	Nd	Nd	Nd	Nd	Nd
	BH	1.85 ± 0.01(c)	2.71 ± 0.08(b)	2.66 ± 0.02(b)	2.71 ± 0.18(b)	3.92 ± 0.33a(a)
	HXD	Nd	Nd	Nd	Nd	6.86 ± 0.23a
KAE	NK2021	Nd	Nd	Nd	Nd	2.74 ± 0.08d
	ZZ35	Nd	Nd	Nd	Nd	2.55 ± 0.14d
	CFH	Nd	Nd	Nd	Nd	2.61 ± 0.28d
	YZD	Nd	Nd	Nd	2.12 ± 0.01c(b)	3.03 ± 0.09c(a)
	BH	4.02 ± 0.10a(c)	4.33 ± 0.02a(b)	4.29 ± 0.03a(b)	4.18 ± 0a(bc)	4.50 ± 0.03a(a)
	HXD	1.96 ± 0.01b(d)	2.37 ± 0.13b(c)	2.66 ± 0.02b(c)	3.14 ± 0.01b(b)	3.57 ± 0.28b(a)
Total	NK2021	3.13 ± 0.19d(cd)	2.75 ± 0e(d)	3.49 ± 0.15e(c)	8.79 ± 0.16c(b)	9.96 ± 0.13e(a)
	ZZ35	2.47 ± 0.02d(c)	2.1 ± 0.02e(d)	2.49 ± 0.10e(c)	4.95 ± 0.01c(b)	7.22 ± 0.18e(a)
	CFH	34.56 ± 0.50c(c)	41.07 ± 0.13d(b)	42.96 ± 0.57d(b)	51.86 ± 0.19b(a)	49.58 ± 2.76d(a)
	YZD	42.72 ± 1.89c(c)	53.8 ± 1.39c(b)	66.07 ± 0.34c(a)	68.22 ± 3.10b(a)	66.36 ± 1.27c(a)
	BH	679.28 ± 11.34b(a)	151.13 ± 0.82b (d)	154.13 ± 0.81b (d)	203.71 ± 28.96a (c)	575.33 ± 11.95b (b)
	HXD	1023.46 ± 20.93a (a)	162.06 ± 2.38a (d)	177.25 ± 1.59a (cd)	200.12 ± 5.33a (c)	623.01 ± 2.54a (b)

[a] The results are present as means ± SD (n = 3), and different lowercase letters inside and outside brackets indicate differences between different digestion phases and varieties, respectively ($p < 0.05$). C3G, cyanidin-*3*-*O*-glucoside; P3G, peonidin-*3*-*O*-glucoside; CAT, catechin; LEUC, leucocyanidin; ERI, eriodictyol; QUE, quercetin; NAR, naringenin; KAE, kaempferol; Nd, not detectable; other abbreviations are shown in Table 1.

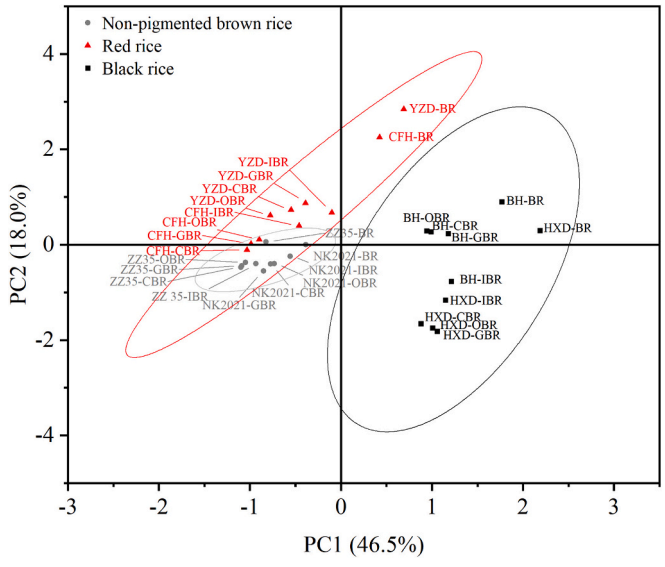

Fig. 2. Biplot of principal component analysis of polyphenols and their antioxidant activity in BR, CBR, OBR, GBR and IBR of six rice grains.

the building blocks of proanthocyanidins (Shao & Bao, 2015).

In oral digestion phase, saliva which was rich in amylase played a crucial role in enhancing the solubility of polyphenols (Wojtunik-Kulesza et al., 2020). Since the digestion time of the oral phase was relatively short, the interaction between polyphenols (especially high-density tannins) and macromolecules (starch, protein, etc) was of particular importance, which was very necessary to be studied in further. During gastric digestion phase, the lack of starch-digesting enzymes, coupled with the oxidative degradation, resulted in a decreased or maintenance of phenolic levels in most rice samples, as compared to OBR (Tables 1, 3, 4). In addition, the enzyme and pH conditions might promote the destruction of high molecular weight phenols bound to proteins or fiber (Lucas-Gonzalez et al., 2016). The rising trend of TFC in all samples (Table 1) was consistent with a previous study using non-pigmented *japonica* brown rice as materials, which showed a 27.5 % increase in TFC after gastric digestion (Liu et al., 2021). In this study, we found that TFC increased by 17.3 % and 56.7 %, respectively, in non-pigmented *japonica* (NK2021) and *indica* rice (ZZ35) after gastric digestion, and the pigmented rice showed an increase ranging from 43.3 % to 66.7 %. In stomach, pepsin could facilitate the release of flavonoids from matrix (Fan et al., 2024), and the acidic environment provides a relatively stable environment for flavonoids (Ramos et al., 2022). Therefore, the contents of CAT and LEUC significantly increased after gastric digestion (Table 5). It was also reported that the strongly interactions between high-molecular-weight tannins and proteins could be destroyed by pepsin (Wojtunik-Kulesza et al., 2020).

During intestinal digestion, free TPC, DPPH, ABTS, and some phenolic acids (FA, IFA, *p*-CA) in most rice samples increased, and those in bound form decreased (Table 1–4). This might be due to the digestion of most starches and proteins by pancreatin, which resulted in the liberation of certain bound phenols into free forms. It suggested that the changes in the existence forms of phenolics depended primarily on the degree of digestion of the rice matrix (Fu et al., 2024). Moreover, the release of free phenolics after intestinal digestion might originate from the degradation of other polyphenols, for example, glycosides could be hydrolyzed by bacteria to aglycones, which were then transformed into various acids through the action of *β*-glucosidase, *β*-rhamnosidase, and esterases during intestinal digestion (Wojtunik-Kulesza et al., 2020). Interestingly, intestinal digestion could release more flavonoid compounds, such as C3G, P3G, KAE, ERI, and NAR, in black rice (Table 5). It might be due to several reasons: (1) the breakdown of residual matrix (starch, protein, and fat) by intestinal enzymes could increase the content of some flavonoids; (2) these flavonoid compounds were more stable during pH transition from acidic to neutral environments, while CAT, LEUC, and QUE were chemically reactive in neutral conditions and might be degraded or isomerized under certain conditions (Wojtunik-Kulesza et al., 2020); (3) proanthocyanidins were hydrolyzed to monomeric units (CAT) during the gastric digestion phase under strong acidic conditions, and catechin units and flavanols could strongly bind to fiber or other components *via* covalent bonds in the intestine (Lingua et al., 2018).

The free phenolic acids would be absorbed in the stomach and/or small intestine and distributed throughout to the whole body for their health benefits (Shao et al., 2018), while the bound phenolics were partially released under the action of digestive enzymes and the gastrointestinal environment, and the remaining phenolics were transferred into the colon to be utilized by microorganisms (Hu et al., 2024). Some bound polyphenols might be degraded by gut bacterial communities to produce substances such as hydroxy phenylpropionic acid, hydroxy phenylacetic acid derivatives, hydroxybenzaldehyde, and acetaldehyde (Possemiers et al., 2011; Wojtunik-Kulesza et al., 2020). One of the most important factors in determining bioavailability was the release and dissolution from the food matrix during digestion, which was a prerequisite for intestinal absorption. It was estimated that 48 % of total phenolics were digested in the small intestine in non-pigmented rice (Wojtunik-Kulesza et al., 2020). In this study, the potential

bioavailability of phenolics in non-pigmented, red and black rice grain was 38.6–40.3 %, 31.0–37.4 %, and 40.8–41.2 % after small intestinal digestion, respectively. They were slightly lower than the above study (48 %) (Wojtunik-Kulesza et al., 2020), which might be attributed to the high-pressure cooking process in this study, whereas no cooking was performed in the previous study. Non-pigmented rice released more polyphenols through the degradation of starches and proteins, due to its high degree of starch digestion, which occurred without the influences of proanthocyanidins and anthocyanins (Pinto et al., 2024; Rocchetti et al., 2022). It was suggested that the losses of anthocyanins and proanthocyanins might be attributed to nonenzymatic degradation in the intestine, among which the degradation of proanthocyanins was more (Lucas-Gonzalez et al., 2016; Nignpense et al., 2022). The major flavonoids in black rice were water-soluble anthocyanins including C3G and P3G, which were demonstrated to be potent inhibitors of amylases and glucosidases *in vitro* (Ou et al., 2023). Polyphenols released during digestion could perform their specific biological functions, and the effect of the total assembly of polyphenols on the health effects could be higher than that of a single component. It was reported that the released *p*-CA have the ability to decrease the resistance of low-density lipoprotein to cholesterol oxidation, and exerted its potential protective function against cardiovascular diseases (Khan et al., 2022). The released FA was found to be associated with the inhibition of cell growth by modulating cell cycle phases in colonic cancer cells (Khan et al., 2022). For red rice, in contrast to catechin, the polymerization degree of proanthocyanidin significantly influenced their fate in the body, which was affected by poor absorption through the gut barrier and limited metabolism by the intestinal microflora (Gonthier et al., 2003).

The results of antioxidant activity of polyphenols show that the values of DPPH generally higher than those of ABTS, especially for flavonoids extracts (Table 2). It might be because that DPPH• + was more easily affected by soluble substances such as proteins, carbohydrates, and amino acids (Wojtunik-Kulesza et al., 2020), coupled with the presence of 3-OH or 5-OH groups in flavonoids that could accelerate the reaction with electron-deficient radicals (Li et al., 2023). In addition, flavonoids might be oxidized to more active forms for scavenging free radicals and chelating ionic iron in gastrointestinal conditions (Pinacho et al., 2015; Wojtunik-Kulesza et al., 2020). Therefore, TFC decreased but the antioxidant activity increased in intestinal digestive products compared with gastric digestive products (Table 1). It was also reported that pH values of 3.5 and 7.4 could enhance the antioxidant activities of cinnamic acids (caffeic, cinereic, and ferulic acid) more than those of benzoic acids (gallic, syringic, and vanillic acid). Therefore, the multiple assays were needed to comprehensively evaluate total antioxidant capacity of polyphenols during *in vitro* digestions. The high correlations between antioxidant activity (ABTS and DPPH) and phenolic content (free, bound and total TPC, and TFC) was similar to those reported in raw rice grains (Pinto et al., 2024; Shao & Bao, 2015). In black rice grain, the antioxidant activity of flavonoids was mainly attributed to the content of anthocyanins during *in vitro* digestion (Lucas-Gonzalez et al., 2016). It was demonstrated that acute intake of black rice grains with TPC and TFC higher than 149 mg GE/100 g and 240 mg CE/100 g, respectively, could significantly improve the plasma antiradical capacity of healthy volunteers (Vitalini et al., 2020). In mice, daily intake of C3G could prevent liver fibrosis progression induced by CCl_4 through inhibiting the activation of hepatic stellate cells (Jiang et al., 2015).

Although cooking caused significant losses of free TPC and free phenolic acids (FA, PA, *p*-HA, *p*-CA, and SYA), intestinal digestion could release some free phenolic acids (FA, IFA, *p*-CA) and exert their potential biological effects. In non-pigmented rice, free TPC and TFC in IBR accounted for 114.6–128.6 % and 130.1–167.5 % of the initial content in BR, respectively. In black rice, the percentages were 76.0–74.2 % for free TPC and 90.1–96.9 % for TFC. While, they were lowest in red rice, which accounted for 39.7–48.0 % and 42.4–38.7 %, respectively. It suggested that the polyphenols in non-pigmented and black rice had higher bioavailability during high-pressure cooking and *in vitro* digestion, even though the non-pigmented contained lower levels of polyphenols. After small intestinal digestion, food would enter into large intestine for further digestion, where polyphenols esterified to fiber could be fermented, decomposed, and utilized by intestinal microorganisms (Liang et al., 2024; Rechner et al., 2001; Wojtunik-Kulesza et al., 2020). It was reported that free phenolic acids might be generated by the release of 4-OH benzoic acid from fiber, or by biotransformation of other phenolic compounds in grains due to the action of gut microbiota (Oghenerukevwe et al., 2023). Therefore, the effects of intestinal microorganisms on phenolic compositions in whole rice during *in vitro* digestion should be further clarified.

5. Conclusions

Free and bound TPC, TFC and their antioxidant activities and compositions in non-pigmented and pigmented rice during cooking and *in vitro* digestion were investigated. Cooking significantly reduced polyphenols and antioxidant activities, while *in vitro* digestion enhanced their releases at different stages, with the gastric environment releasing TFC and the intestinal environment releasing TPC. Strong correlations were observed between TPC, TFC, and DPPH and ABTS antioxidant activities ($r = 0.7052–0.9860$). Bound TPC was converted to free TPC during digestion, especially during intestinal digestion phase. Intestinal digestion released substantial amounts of free isoferulic and ferulic acid, while vanillic and *p*-coumaric acid remained relatively constant throughout the digestion process. Bound protocatechuic and chlorogenic acid were abundant in pigmented rice and decreased significantly after intestinal digestion ($p < 0.05$). C3G, P3G and naringenin were only found in black rice and peaked at the intestinal phase after cooking. Red rice exhibited a higher content of catechin with a notably increased concentration observed after gastric digestion. Non-pigmented and red rice had similar digestion characteristics of polyphenols, and black rice had higher polyphenols and antioxidant activities. The results suggested that people could get more bioactive compounds by consuming the cooked black rice, and the food enterprises could use black rice grain as raw material to produce more functional foods. The influence of gut microbiota on polyphenols after intestinal digestion remains an understudied area of this study. Therefore, the complex mechanism of short-term oral digestion and gut flora affecting polyphenols and their antioxidant activity would be an interesting direction of future study.

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CRediT authorship contribution statement

Jing Yu: Writing – original draft, Investigation, Data curation, Conceptualization. **Xin Zheng:** Validation, Resources, Methodology. **Dawei Zhu:** Validation, Resources, Conceptualization. **Qingyu Xu:** Methodology, Investigation. **Feifei Xu:** Software, Formal analysis. **Mingxue Chen:** Funding acquisition. **Lingqi Meng:** Resources. **Yafang Shao:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101821.

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