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RESEARCH ARTICLE

The effect of cold plasma pretreatment on GABA, γ-oryzanol, phytic acid, phenolics, and antioxidant capacity in brown rice during germination

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Abstract

Background and Objectives: Germination pretreatment is an effective way to improve the nutritional quality and sensory quality of germinated brown rice (GBR). Cold plasma pretreatment (CPP) has been demonstrated to improve the physicochemical properties of GBR. The effects of CPP on reducing phytic acid and improving nutrient composition in GBR have not been evaluated, and there are few studies on the changes of phytic acid and phytase, especially the changes of the forms and compositions of γ-oryzanol, phenolics, and flavonoids in GBR with CPP. Therefore, this study evaluated the changes of phytase, phytic acid, γ-aminobutyric acid (GABA), γ-oryzanol, flavonoids, phenolics, and antioxidant activity in GBR with or without CPP.

Findings: The phytic acid content in CPP-treated GBR for germination of 72 h was lower (7.60 mg/g, dry basis weight [DW]) than that in untreated GBR (9.01 mg/g DW). At the same germination time, the phytase activity and GABA, total γ-oryzanol contents in CPP-treated GBR were higher than those in untreated GBR. However, total flavonoids and phenolics levels, flavonoid compositions and phenolic acids contents, and T-AOC and DPPH antioxidant capacity in CPP-treated GBR were lower than those of untreated GBR.

Conclusions: These results indicated that CPP for brown rice was an effective method for decreasing the phytic acid and enhancing GABA and γ-oryzanol in GBR compared with non-CPP of brown rice for germination.

Significance and novelty: CPP is beneficial to reduce phytic acid and improve the GABA and γ-oryzanol contents of GBR, which provides a theoretical basis for producing functional and nutritious GBR foods.

KEYWORDS

γ-oryzanol, cold plasma pretreatment, GABA, germinated brown rice, phenolics and antioxidant activity, phytic acid

1 | INTRODUCTION

As a nutritious whole grain, brown rice may be beneficial to prevent varieties of diseases, including hyperlipidemia, diabetes, and hypertension (Wu et al., 2022). However, it is hard to cook and unpalatable to eat brown rice because of its germ and bran, thus the utilization of brown rice is limited in the food industry. Germination can enhance edible quality and improve a lot of nutrients of brown rice. Therefore, germinated brown rice (GBR) has become a new type of germinated grain foods due to its greatly improved nutrition and texture (Nelson et al., 2013).

Some large molecules in brown rice are hydrolyzed during germination, such as proteins and carbohydrates, which leads to the synthesis of biochemical and nutritional substances (R. Li et al., 2022). Several previous reports have demonstrated that the regular intake of GBR may prevent chronic diseases owing to the enhancement of γ-oryzanol, vitamins, phenolic acids, γ-aminobutyric acid (GABA), and other active substances during the germination process (Cho & Lim, 2015; Nelson et al., 2013). Therefore, the application of controlled grain germination processes has gained great attention. Traditional pretreatment methods were used to promote the synthesis of nutrients in GBR. For example, soaking pretreatment was mainly used to control soaking time and temperature. Additionally, some modern pretreatment methods have been demonstrated to promote nutritional levels of GBR, such as electric, magnetic fields, microwaves, and ultrasonic pretreatments (Goussous et al., 2010). However, these methods may cause the degradation of some heat-sensitive nutrients during the treatment process. Therefore, in recent years, cold plasma technology has been used in whole grain processing due to its characteristics of low temperature and short processing time.

Plasma is made up of negative and positive ions, free radicals, electrons, and various active groups, which is regarded as a neutral ionized gas (Pankaj & Keener, 2017). In the last decade, cold plasma pretreatment (CPP) has been shown to improve seed viability and growth in plants such as brown rice, soybean, and wheat (Meng et al., 2017). Zargarchi and Saremnezhad (2019) found that CPP could improve the germination rate of paddy rice. The levels of total phenolic, vitamin E, total γ-oryzanol in brown rice were enhanced by applying cold plasma (100–200 W and 25–300 s) for pretreatment before germination (Yodpitak et al., 2019). Additionally, Chen et al. (2016) found that CPP (1–3 kV for 10 min) could increase GABA content and α-amylase activities in GBR. However, there are few studies on the changes of phytic acid and phytase, especially the changes of the forms and compositions of flavonoids, phenolics, and γ-oryzanol, in GBR with CPP.

Consequently, the purpose of this study was to evaluate the impact of CPP of brown rice on GABA, phytase, phytic acid, and γ-oryzanol compositions, the forms and compositions of phenolics and flavonoids, and antioxidation in GBR. This study may contribute to enhance the accumulation of nutrients and reduce antinutritional factors in GBR with CPP.

2 | MATERIALS AND METHODS

2.1 | Materials

The Brown rice (Tongnuo 2) used in the experiment was obtained from Beijing Siensaier Biotechnology Co., LTD. The HD-300 type cold plasma equipment was purchased from Changzhou Zhongke Changtai Plasma Technology Co., LTD. All phenolic acid and flavonoid standards were provided by Shanghai Yuanye Biotechnology Co., Ltd. Total antioxidant capacity (T-AOC) of the samples was measured using a method of test kit obtained from the Nanjing Institute of Bioengineering. The phytase activity in the sample was determined using a method of ELISA kit purchased from Jiangsu Meimian Industrial Co., Ltd. Other chemicals used in the work were analytically pure.

2.2 | CPP of brown rice

Brown rice was put into a 100 cm acrylic reactor. The acrylic reactor was placed in a vacuum reactor and the pressure was adjusted to 40–50 Pa. The reactor was filled with helium when the vacuum system reached the appropriate pressure. The equipment was operated at 400 W for 5 min after reaching 130 Pa in the reactor. In our previous tests, CPP powers of 100, 200, 300, 400, and 500 W, treatment time of 1, 2, 3, 4, and 5 min were selected as treatment conditions, and untreated brown rice was used as control. It was observed that phytic acid in GBR was the lowest for the CPP at 400 W and 5 min, compared with the control sample. Accordingly, the CCP condition was selected at 400 W for 5 min in this study.

2.3 | Germination procedure

CPP-treated brown rice and untreated brown rice were soaked in deionized water (30°C, 24 h). After soaking, the samples were drained and distributed in a disposable petri dish. The germination conditions of the samples were for 18, 36, 54, and 72 h at 30°C in the dark. The

sample was smashed, passed a 60-mesh sieve, and stored at −18°C.

2.4 | Measurement of phytase and phytic acid

The contents of phytic acid in samples were measured using a previous method by Haug and Lantzsch (1983). HCl solution (0.2 mol/L, 20 ml) and sample (0.5 g) were mixed into centrifuge tube. The sample was extracted by vibration for 3 h at 25°C. Extracted solution (1 ml) and ammonium ferric sulfate solution (0.2 g/L, 2 ml) were mixed in the plastic tube after centrifugation (15 min, 3500 r/min). The resulting solution was incubated in a boiling water bath (30 min) and an ice water bath (15 min). The supernatant (1 ml) and the dipyridine solution (1 g/100 ml, 1.5 ml) were mixed in the plastic tube after centrifugation (10 min, 3500 r/min), which was vibrated and mixed. The distilled water was as blank, and the absorbance at 519 nm was measured.

2.5 | Measurement of GABA

The GABA levels in samples were performed following a modified method (Ding et al., 2018). Ethanol solution (10 ml, 80%) and sample (1.0 g) were mixed into a test tube and extracted by ultrasonic treatment for 0.5 h. The sample was then oscillated in a vortex mixer for 2 min and stood for 5 min. Centrifugation was performed for 10 min at 5000 r/min. 4-dimethylamine-azobenzene 4-sulfonyl chloride (2 g/L, 400 μl), sodium bicarbonate solution (0.04 g/ml, 0.2 ml), and supernatant (1 ml) were mixed into a plastic tube and derived in a water bath under for 20 min at 70°C. Filtration for the sample was carried out with 0.22 μm organic filter membrane (0.22 μm) before determination. The high-performance liquid chromatography (HPLC) (Thermo Scientific U3000) conditions were set as follows. The UV-visible detector was at 254 nm, the column was Agilent plus-C18 (100 mm × 4.6 mm, 3.5 μm). The elution solvent was sodium acetate trihydrate (6.8 g/L) and acetonitrile (65:35, v/v). Elution was carried out at 1.0 ml/min for 10 min. The GABA standard was regarded as a control to draw the standard curve and calculate GABA content in the samples.

2.6 | Measurement of γ-oryzanol

γ-oryzanol levels in samples were examined by a previously described method (D. Li et al., 2020). Methanol (10 ml) and sample (3 g) were mixed in a plastic tube, and ultrasound treatment at 25°C for 1 h. The extract was transferred into a rotary evaporation flask and steamed (50°C, 50 r/min) to dry after centrifugation at 3500 r/min for 15 min, methanol was accurately added to constant volume (6 ml), then was mixed by vortex shaker for 5 min. The resulting solution in the rotary evaporation bottle was passed through 0.45 μm organic microporous membrane. HPLC (Thermo Scientific U3000) system conditions were operated at UV-visible detector of 325 nm, injection volume of 10 μl, and C18 column (Agilent, 5 μm, 150 mm × 4.6 mm). Elution time was 35 min with a flow rate of 1.4 ml/min. Elution solvents were methanol, acetic acid, acetonitrile (54:3:44 v/v/v). γ-oryzanol standard was regarded as a control to draw the standard curve and calculate
γ-oryzanol content in all samples.

2.7 | Extraction of phenolics

The extraction of free and bound phenolics for samples was performed using the method of Wu et al. (2018). Briefly, samples were mixed with chilled acidified methanol and then homogenized in an ice bath using a homogenizer for 5 min and centrifuged for 10 min. Free phenolic was obtained from the supernatant by rotary evaporation at 45°C. The residue was digested with NaOH for 1 h under dark conditions, neutralized with concentrated hydrochloric acid, and then extracted five times with ethyl acetate. Bound phenolic was obtained from the supernatant by rotary evaporation at 45°C.

2.8 | Measurement of total flavonoids and phenolic

The measurement of total flavonoids and phenolic contents in the sample was performed following the method of Wu et al. (2018). Briefly, 100 μl of the extraction were mixed with 100 μl of methanol and 250 μl of Folin-Ciocalteu for 6 min, and 2.5 ml of 7 g/100 ml Na_2CO_3 and 2 ml of distilled water were added. The reaction time was 90 min at room 25°C, and the absorbance of the mixture was measured at 765 nm. The total phenolic content of the samples was expressed as gallic acid equivalents per 100 g dry weight of the sample (mg GAE/100 g DW). The 0.1 ml extract was mixed with 0.2 ml of 5 g/100 ml $NaNO_2$ solution for 6 min, 0.25 ml of 10 g/100 ml $Al(NO_3)_3$ solution for 6 min, and 2 ml of 4 g/100 ml NaOH was added. The reaction time was 15 min at 25°C, and the absorbance of the

solution was measured at 510 nm. The total flavonoids content of the sample extract was expressed as rutin equivalent per 100 g dry weight of the sample (mg RE/100 g DW).

2.9 | Flavonoid and phenolic compositions determination

Phenolic acids in all samples were measured following the method of Ti et al. (2014). Briefly, HPLC (Thermo Scientific U3000) system conditions were operated at UV-visible detector of 280 nm, injection volume of 20 μl, and C18 column (Agilent, 5 μm, 250 mm × 4.6 mm). Elution time was 55 min with a flow rate of 1.0 ml/min. Elution solutions A and B were 0.4% aqueous and acetonitrile. The elution condition: 0 min, 95% A; 0–40 min, 95%–75% A; 40–45 min, 75%–60% A; 45–50 min, 60%–50% A; 50–55 min, 95% A.

Flavonoids compositions in the sample were measured following the method of Pradeep and Sreerama (2015). HPLC (Thermo Scientific U3000) system conditions were operated at UV-visible detector of 342 nm, injection volume of 20 μl, and C18 column (Agilent, 5 μm, 250 mm × 4.6 mm). Elution time was 55 min with a flow rate of 1.0 ml/min. Elution solutions A and B were formic acid (pH 2.8) and acetonitrile, respectively. The elution condition: 0–5 min, 90% A; 5–31 min, 90%–77% A; 31–43 min, 77%–65% A; 43–55 min, 65%–0% A.

2.10 | Antioxidant capacity determination

The 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) assay in samples was measured using the previously described method by Xi et al. (2011). Briefly, the sample dilution was mixed with 3 ml, 0.1 mm/L DPPH methanol solution, and the absorbance was measured at 517 nm after reaction for 20 min. Water soluble vitamin E (Trolox) was used as the standard sample to prepare the standard curve of methanol solution. The results were expressed as the number of micromoles containing Trolox equivalent in 100 g dry weight (μmol Trolox/100 g).

2.11 | Statistical analysis

Experiments were replicated for at least three times. Data were reported by mean ± SD ($n = 3$ or 4). Different samples were analyzed by Duncan's test and one-way analysis of variance ($p < .05$) with SPSS 25.0 (SPSS Inc.) Software.

3 | RESULTS AND DISCUSSION

3.1 | Reduction of phytic acid and improvement of phytase in GBR with and without CPP

Changes of phytase and phytic acid in GBR with and without CPP for different germination hours are shown in Figure 1a,b. With the increase of germination time (18–72 h), a decreased trend was observed for the content of phytic acid in untreated and CPP-treated GBR. The phytic acid level in GBR was decreased by the CPP of brown rice before germination. During whole germination, the phytic acid contents in untreated GBR and CPP-treated GBR were 9.01–12.61 mg/g DW and 7.60–11.73 mg/g DW, respectively. At the same germination time, phytic acid levels in untreated GBR were higher than those in CPP-treated GBR. Additionally, at the same germination time except for 18 h germination (Figure 1b), phytase activity in CPP-treated GBR was higher than that in untreated GBR.

Phytic acid is considered to be an antitrophic factor that negatively affects mineral absorption in the body. Therefore, reducing phytic acid content in whole grains is beneficial to promote mineral absorption in the human body. The phytic acid level in GBR (72 h) was decreased by 54.40% compared with ungerminated brown rice (Liang et al., 2008). The decrease of phytic acid content in GBR could be explained by the enhancement of phytase activity during the germination processes (Towo et al., 2006). Therefore, it was demonstrated that phytic acid content was reduced by CPP with stimulating phytase activity in GBR.

3.2 | Increase of GABA level in GBR with and without CPP

Increase in GABA level in GBR with and without CPP for different germination hours is presented in Figure 1c. With the increase of germination time (0–72 h), GABA level in GBR was boosted, and GABA level in GBR was increased by CPP. During whole germination, GABA contents in untreated GBR and CPP-treated GBR were 13.89–27.75 mg/100 g DW and 17.21–42.42 mg/100 g DW, respectively. At the same germination time, GABA levels in untreated GBR were lower than those in CPP-treated GBR.

The influence of CPP on GABA level in GBR was investigated by Chen et al. (2016), who showed that GABA level in GBR was increased by about 1.5 times compared with that in untreated samples after treatment at 3 kV for 10 min. Additionally, it was reported that CPP increased GABA level in germinated paddy (Zargarchi &

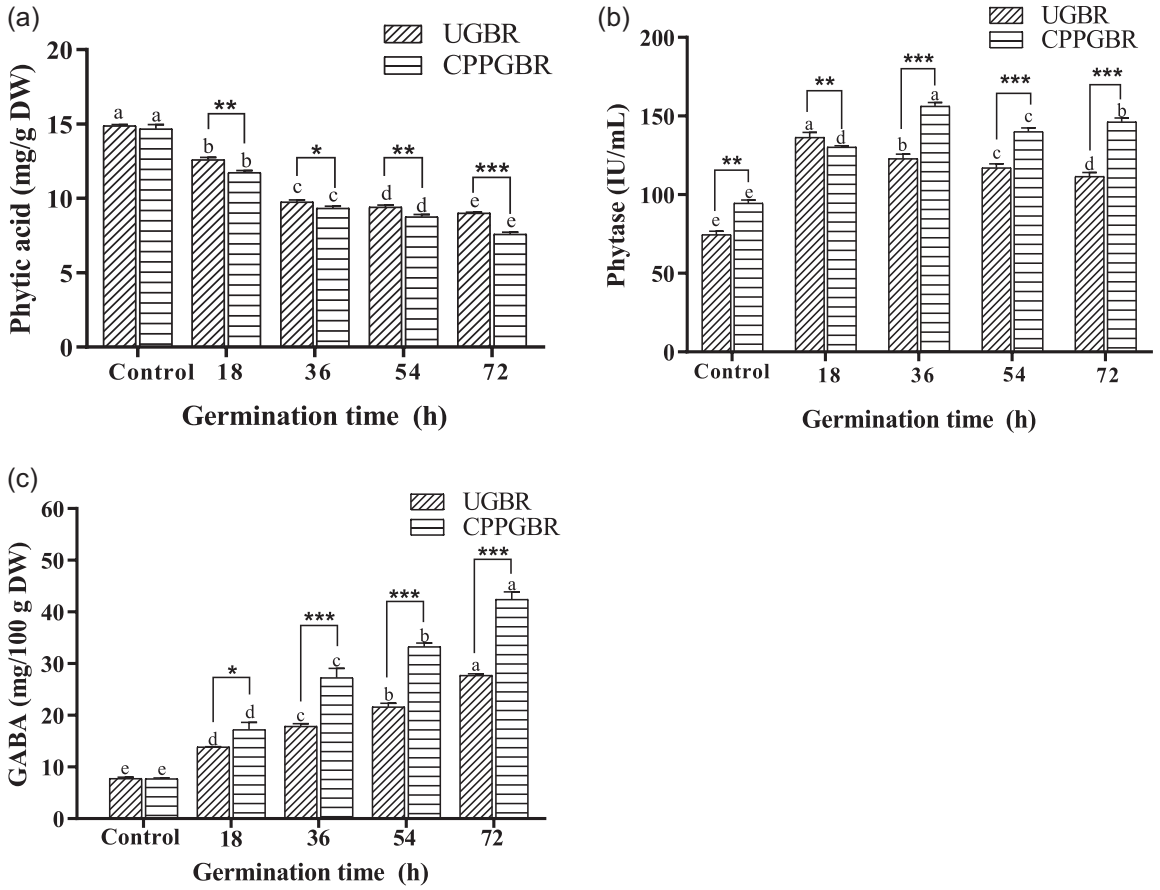

FIGURE 1 The contents of phytic acid, GABA, and phytase activity in germinated brown rice with and without cold plasma pretreatment for different germination hours. (a) Phytic acid, (b) phytase, (c) GABA. *$p < .05$, **$p < .01$, and ***$p < .001$ were considered statistically significant, highly significant, and extremely significant between UGBR and CPPGBR at the same germination time, respectively. Different letters at the top of the columns indicate significant differences ($p < .05$, $n = 3$). CPPGBR, germinated brown rice with cold plasma pretreatment; DW, dry basis weight; UGBR, germinated brown rice without cold plasma pretreatment.

Saremnezhad, 2019). The synthesis of GABA in plants is mainly due to the decarboxylation of L-glutamic acid, and the activity of glutamate decarboxylase (GAD) is positively correlated with GABA level (Ando & Nakamura, 2016). Therefore, the enhancement of GABA level in GBR might be related to the activation of GAD after CPP.

3.3 | Increase of γ-oryzanol level in GBR with and without CPP

Changes in the level of γ-oryzanol and steryl ferulates in GBR with and without CPP are shown in Table 1. The contents of 24-methylenecycloartanyl ferulate were the highest in GBR, followed by level of cycloartenyl ferulate, sitosteryl ferulate, and campesteryl ferulate. During whole germination processes, steryl ferulates and total γ-oryzanol levels in GBR increased first, then decreased, reaching the highest contents at 36 h. Total γ-oryzanol contents in CPP-treated GBR and untreated GBR were 95.68–152.66 μg/g DW and 73.77–137.99 μg/g DW, respectively, in the whole germination period. At the same germination time, total γ-oryzanol levels in untreated GBR were lower than those in CPP-treated GBR.

The influence of CPP on γ-oryzanol level in GBR was evaluated by Yodpitak et al. (2019), who found that γ-oryzanol level was first increased during germination, then gradually decreased, and γ-oryzanol levels in GBR were increased by CPP. The reduction of total γ-oryzanol level after reaching a maximum value at germination for 36 h might be owing to the factors, mainly including CPP time, CPP temperature, and CPP energy.

3.4 | Changes of free, bound, and total phenolic contents in GBR with and without CPP

Table 2 shows the changes of bound, free, and total phenolic levels in GBR with and without CPP. In the whole

TABLE 1 Changes in the total γ-oryzanol contents and compositions of steryl ferulates of untreated and cold plasma-pretreated germinated brown rice during 72 h germination (μg/g DW)

Sample	Time (h)	Cycloartenyl ferulate	24-Methylenecycloartanyl ferulate	Campesteryl ferulate	Sitosteryl ferulate	Total γ-oryzanol
UGBR	Control	33.17 ± 1.56^{cE}	47.83 ± 2.77^{bD}	14.74 ± 0.30^{bcD}	15.49 ± 0.30^{bC}	111.22 ± 4.57^{cE}
	18	37.54 ± 0.70^{bD}	53.76 ± 4.91^{abCD}	16.33 ± 1.28^{abCD}	16.61 ± 0.21^{bBC}	124.23 ± 6.72^{bcD}
	36	42.50 ± 1.94^{aC}	59.47 ± 4.57^{aC}	17.62 ± 1.13^{aC}	18.41 ± 1.09^{aAB}	137.99 ± 5.81^{aC}
	54	38.43 ± 2.54^{bD}	58.56 ± 3.79^{aC}	15.88 ± 1.77^{abCD}	15.56 ± 0.45^{bC}	128.43 ± 8.04^{abCD}
	72	21.53 ± 1.63^{dG}	28.31 ± 0.98^{cF}	12.27 ± 0.35^{cE}	11.66 ± 0.42^{cD}	73.77 ± 2.70^{dG}
CPPGBR	Control	32.93 ± 0.96^{dE}	48.64 ± 1.52^{dD}	15.06 ± 0.39^{cD}	15.35 ± 0.23^{bcC}	111.98 ± 2.30^{cE}
	18	44.56 ± 1.38^{cC}	69.10 ± 1.37^{cB}	20.59 ± 0.32^{bB}	18.41 ± 1.82^{abAB}	152.66 ± 4.86^{bB}
	36	53.47 ± 1.67^{aA}	82.13 ± 1.86^{aA}	23.05 ± 0.74^{aA}	20.97 ± 1.99^{aA}	179.62 ± 6.22^{aA}
	54	50.03 ± 1.91^{bB}	78.16 ± 1.51^{bA}	22.34 ± 0.50^{aAB}	20.63 ± 1.90^{aA}	171.16 ± 5.74^{aA}
	72	27.80 ± 1.16^{eF}	40.95 ± 0.94^{eE}	12.77 ± 0.08^{dE}	14.16 ± 0.65^{cC}	95.68 ± 1.85^{dF}

Note: Values are presented as mean ± standard deviation ($n = 3$). Different lowercase letters indicate significant differences of the data among the different germination hours in the same group (UGBR group or CPPGBR group) ($p < .05$), and different capital letters indicate significant differences in the same column ($p < .05$).

Abbreviations: CPPGBR, germinated brown rice with cold plasma pretreatment; DW, dry basis weight; UGBR, germinated brown rice without cold plasma pretreatment.

TABLE 2 Changes in the phenolic contents of untreated and cold plasma-pretreated germinated brown rice during 72 h germination

		Phenolics (mg GAE/100 g DW)			Flavonoids (mg RE/100 g DW)		
Sample	Time (h)	Free	Bound	Total	Free	Bound	Total
UGBR	Control	86.17 ± 0.90^{bB}	20.03 ± 0.96^{dH}	106.20 ± 1.54^{eE}	23.37 ± 0.76^{bB}	20.46 ± 1.18^{cD}	43.83 ± 0.86^{cD}
	18	85.62 ± 2.67^{bB}	29.19 ± 1.41^{cG}	116.43 ± 3.43^{dD}	23.82 ± 0.36^{bB}	29.63 ± 1.06^{bB}	53.46 ± 1.34^{bB}
	36	101.98 ± 1.05^{aA}	41.38 ± 1.37^{bD}	143.36 ± 1.85^{aA}	22.25 ± 0.36^{bB}	35.43 ± 0.13^{aA}	57.68 ± 0.41^{aA}
	54	84.11 ± 1.72^{bB}	43.09 ± 1.10^{bC}	127.19 ± 0.83^{bB}	28.39 ± 0.99^{aA}	29.38 ± 2.17^{bB}	57.77 ± 3.09^{aA}
	72	71.43 ± 1.25^{cD}	48.76 ± 0.61^{aA}	120.19 ± 1.45^{cC}	24.54 ± 3.83^{bB}	30.89 ± 1.72^{bB}	55.43 ± 3.70^{abAB}
CPPGBR	Control	85.95 ± 1.88^{aB}	20.26 ± 0.34^{eH}	106.21 ± 1.88^{dE}	22.64 ± 1.08^{bB}	20.89 ± 0.30^{cD}	43.53 ± 1.28^{cD}
	18	86.97 ± 1.93^{aB}	35.34 ± 0.37^{cF}	122.32 ± 2.13^{aC}	22.55 ± 0.75^{bB}	27.23 ± 0.31^{bC}	49.78 ± 0.96^{bC}
	36	79.13 ± 1.84^{bC}	34.52 ± 0.25^{dF}	113.64 ± 1.97^{cD}	27.68 ± 0.19^{aA}	26.48 ± 0.20^{bC}	54.16 ± 0.19^{aB}
	54	77.33 ± 1.05^{bC}	39.55 ± 0.67^{bE}	116.88 ± 1.57^{bD}	23.16 ± 0.23^{bB}	27.10 ± 0.70^{bC}	50.26 ± 0.60^{bC}
	72	62.73 ± 0.47^{cE}	45.02 ± 0.22^{aB}	107.75 ± 0.34^{dE}	14.45 ± 1.02^{cC}	30.35 ± 0.55^{aB}	44.79 ± 0.51^{cD}

Note: Values are presented as mean ± standard deviation ($n = 4$). Different lowercase letters indicate significant differences of the data among the different germination hours in the same group (UGBR group or CPPGBR group) ($p < .05$), and different capital letters indicate significant differences in the same column ($p < .05$).

Abbreviations: CPPGBR, germinated brown rice with cold plasma pretreatment; DW, dry basis weight; GAE, gallic acid equivalent; RE, rutin equivalent; UGBR, germinated brown rice without cold plasma pretreatment.

germination period, total and free phenolic contents in GBR were increased first, then decreased, while the contents of bound phenolic were increased. Free phenolic levels in untreated GBR and CPP-treated GBR were 71.43–101.98 mg GAE/100 g DW and 62.73–86.97 mg GAE/100 g DW, respectively, after 18–72 h germination. Bound phenolic contents in CPP-treated GBR and untreated GBR were 34.52–45.02 mg GAE/100 g DW and 29.19–48.76 mg GAE/100 g DW, respectively, in the whole germination period. During the whole germination, the contents of total phenolic in untreated GBR and CPP-treated GBR were 116.43–143.36 mg GAE/100 g DW and 107.75–122.32 mg GAE/100 g DW, respectively. At the same germination time (36–72 h), total, free, and bound phenolic contents in untreated GBR were higher than those in CPP-treated GBR.

These findings were similar to the results of Ti et al. (2014), who demonstrated an increased trend in the content of phenolics in GBR, and total, bound and free phenolic levels in GBR were improved by 63.16%, 36.50%, and 76.67%, respectively, for germination processes (0–48 h), respectively. The biosynthesis of phenolic compounds and release of aglycones from glucosides during germination might have promoted to the enhancement of phenolic level in germinated grains (Pradeep & Sreerama, 2015). Zargarchi and Saremnezhad (2019) found a lower total phenolic content in cold plasma-treated germination paddy rice. However, the higher phenolic level in CPP-treated GBR was found by Yodpitak et al. (2019), who considered that cold plasma treatment might provide a stress stimulation that could boost the accumulation of phenolics in GBR. It was attributed to many factors, including the decomposition of phenolic and inactivation of phenylalanine ammonia-lyase (PAL) in GBR owing to application of CPP (Gómez-Favela et al., 2017).

3.5 | Changes of free bound and total flavonoid levels in GBR with and without CPP

Table 2 shows the changes of total, bound, and free flavonoid contents in GBR with and without CPP. In the whole germination period, total, bound, and free flavonoid levels in GBR were first enhanced, then decreased. During whole germination, free flavonoid contents in CPP-treated GBR and untreated GBR were 14.45–27.68 mg GAE/100 g DW and 22.25–28.39 mg RE/100 g DW, respectively. During the whole germination, bound flavonoid contents in CPP-treated GBR and untreated GBR were 26.48–30.35 mg RE/100 g DW and 29.38–35.43 mg RE/100 g DW, respectively. During the whole germination, total flavonoid levels in CPP-treated GBR and untreated GBR were 44.79–54.16 mg RE/100 g DW and 53.46–57.77 mg RE/100 g DW, respectively. At the same germination time, total, bound, and free flavonoid contents in untreated GBR were higher than those in CPP-treated GBR.

Ti et al. (2014) obtained that total, bound, and free flavonoid levels in GBR were increased by 23.62%, 23.63%, and 23.40%, respectively, with the increase of germination time (0–48 h). The phenylpropyl metabolic pathway might activate the synthesis flavonoid pathway during seed germination, thus promoting the further formation of acetyl coenzyme A esters (CoA) into flavonoid (J. Wang et al., 2020). This could explain the increase of flavonoids in brown rice during germination. During germination processes, the synthesis of flavonoids in grain seeds was mainly regulated by PAL and chalcone isomerase (CHI), and other key enzymes, and a specific flavonoid would be synthesized in seeds under the action of certain enzymes (Kashmir et al., 2009). L. Wang et al. (2013) demonstrated that the activities of CHI and PAL in germination grain seeds were positively associated with the content of total flavonoids. Thus, a negative influence of CPP on flavonoids might be owing to the factors, including the decomposition of flavonoids in GBR and the inactivation of CHI and PAL as a key enzyme in flavonoids biosynthesis.

3.6 | Changes of phenolic acids compositions in GBR with and without CPP

Eleven phenolic compositions were detected in bound and free phenolics in GBR with and without CPP (Table 3). CPP changed the level of free phenolic acid in GBR. During whole germination, the free protocatechuic acid contents in CPP-treated GBR and untreated GBR were 10.58–17.26 μg/g DW and 15.28–18.83 μg/g DW, respectively. The free ferulic acid contents in untreated GBR and CPP-treated GBR were 10.31–11.46 μg/g DW and 11.49–12.43 μg/g DW, respectively, during the whole germination. At the same germination time, free ferulic acid and protocatechuic acid contents in untreated GBR were higher than that in CPP-treated GBR. Additionally, free *o*-coumaric acid was not found in either the CPP-treated or untreated groups. For bound phenolic acids, bound *p*-coumaric acid contents in CPP-treated GBR and untreated GBR were 9.95–19.33 μg/g DW and 36.41–61.57 μg/g DW, respectively, in the whole germination period. During whole germination, bound ferulic acid contents in untreated GBR and CPP-treated GBR were 65.95–104.58 μg/g DW and 48.55–72.86 μg/g DW, respectively. At the same germination time, bound ferulic acid and *p*-coumaric acid levels in untreated GBR were higher than that in CPP-treated GBR. Additionally, bound chlorogenic acid was not detected in either the CPP-treated or untreated groups.

In this study, most of the coumaric acids and ferulic in GBR were present in bound forms, which was similar to the findings shown by Zhou et al. (2004). Most free phenolic acids content in brown rice increased sharply after germination, which might be due to the activation of endogenous esterase, thus promoting the production of free phenolic acids (Tian et al., 2004). Interestingly, after germination, bound *p*-coumaric acid and ferulic acid were significantly boosted. Ti et al. (2014) suggested

TABLE 3 Changes in the phenolic compositions contents of untreated and cold plasma-pretreated germinated brown rice during 72 h germination

Sample	Time (h)	Phenolic compositions (μg/g DW)										
		Gallic acid	Protocatechuic acid	P-hydroxybenzonic acid	Chlorogenic acid	Vanillic acid	Caffeic acid	Syringic acid	*p*-coumaric acid	Ferulic acid	Singicylic acid	*o*-coumaric acid
Free												
UGBR	Control	9.39 ± 0.06^{bBCD}	9.53 ± 0.32^{dE}	1.60 ± 0.01^{dE}	0.00 ± 0.00^{cF}	1.81 ± 0.01^{bB}	8.76 ± 0.01^{aB}	16.41 ± 0.03^{aA}	1.87 ± 0.04^{aA}	11.37 ± 0.23^{cC}	0.00 ± 0.00^{eF}	ND
	18	9.23 ± 0.04^{dDEF}	15.39 ± 0.14^{cC}	1.64 ± 0.01^{cD}	0.00 ± 0.00^{cF}	1.73 ± 0.00^{dB}	8.53 ± 0.01^{bC}	0.00 ± 0.00^{dD}	1.54 ± 0.03^{bD}	11.55 ± 0.03^{cC}	8.10 ± 0.01^{dD}	ND
	36	9.30 ± 0.04^{cCDE}	18.83 ± 0.76^{aA}	1.69 ± 0.02^{bC}	0.00 ± 0.00^{cF}	1.79 ± 0.00^{cB}	0.00 ± 0.00^{cD}	0.00 ± 0.00^{dD}	1.77 ± 0.05^{aB}	12.08 ± 0.09^{bB}	8.49 ± 0.05^{aA}	ND
	54	9.48 ± 0.03^{aABC}	17.63 ± 0.34^{bB}	1.73 ± 0.02^{aB}	25.50 ± 0.03^{aC}	1.77 ± 0.01^{cB}	0.00 ± 0.00^{cD}	15.94 ± 0.02^{bB}	1.54 ± 0.01^{bD}	11.49 ± 0.20^{cC}	8.25 ± 0.01^{cC}	ND
	72	9.11 ± 0.04^{eF}	15.28 ± 1.15^{cC}	1.58 ± 0.01^{dEF}	25.18 ± 0.01^{bE}	1.83 ± 0.02^{aAB}	0.00 ± 0.00^{cD}	15.74 ± 0.02^{cC}	1.58 ± 0.02^{bD}	12.43 ± 0.17^{aA}	8.38 ± 0.09^{bB}	ND
CPPGBR	Control	9.09 ± 0.13^{dF}	9.27 ± 0.27^{dE}	1.56 ± 0.02^{cFG}	0.00 ± 0.00^{dF}	1.76 ± 0.03^{cB}	8.56 ± 0.12^{bC}	16.01 ± 0.23^{aB}	1.76 ± 0.07^{aB}	10.05 ± 0.15^{dF}	0.00 ± 0.00^{cF}	ND
	18	9.13 ± 0.11^{cdF}	10.58 ± 0.07^{cD}	1.55 ± 0.01^{cFG}	25.63 ± 0.01^{bB}	0.00 ± 0.00^{dC}	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bD}	1.74 ± 0.02^{aBC}	10.57 ± 0.04^{bD}	0.00 ± 0.00^{cF}	ND
	36	9.65 ± 0.15^{aA}	17.26 ± 0.39^{aB}	1.68 ± 0.02^{bCD}	26.40 ± 0.03^{aA}	1.93 ± 0.17^{aA}	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bD}	1.71 ± 0.04^{aBC}	11.46 ± 0.14^{aC}	0.00 ± 0.00^{cF}	ND
	54	9.37 ± 0.21^{bcBCD}	14.90 ± 0.34^{bC}	2.17 ± 0.03^{aA}	25.62 ± 0.01^{bB}	1.77 ± 0.01^{bcB}	8.64 ± 0.02^{bBC}	0.00 ± 0.00^{bD}	1.67 ± 0.06^{aC}	10.31 ± 0.02^{cE}	8.43 ± 0.02^{aB}	ND
	72	9.56 ± 0.06^{abAB}	14.58 ± 0.70^{bC}	1.53 ± 0.02^{cG}	25.33 ± 0.09^{cD}	1.91 ± 0.01^{abA}	9.01 ± 0.19^{aA}	15.89 ± 0.08^{aB}	1.41 ± 0.02^{bE}	11.35 ± 0.05^{aC}	8.02 ± 0.01^{bE}	ND
Bound												
UGBR	Control	9.30 ± 0.05^{bBCD}	7.23 ± 0.04^{aA}	2.02 ± 0.06^{aA}	ND	1.87 ± 0.03^{aA}	9.00 ± 0.03^{aA}	17.91 ± 0.26^{bC}	42.94 ± 0.15^{cB}	79.99 ± 5.72^{bBC}	17.49 ± 0.22^{aA}	3.01 ± 0.01^{aA}
	18	9.24 ± 0.04^{bcBCDE}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{cD}	ND	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bC}	17.58 ± 0.06^{cD}	57.26 ± 5.04^{bA}	104.58 ± 5.55^{aA}	8.54 ± 0.12^{bC}	0.00 ± 0.00^{bD}
	36	9.44 ± 0.01^{aABC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{cD}	ND	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bC}	19.16 ± 0.08^{aB}	61.57 ± 1.63^{aA}	100.27 ± 2.36^{aB}	0.00 ± 0.00^{cE}	0.00 ± 0.00^{bD}
	54	9.21 ± 0.06^{cCDE}	0.00 ± 0.00^{bC}	1.55 ± 0.03^{bB}	ND	1.75 ± 0.00^{bC}	0.00 ± 0.00^{bC}	16.97 ± 0.07^{dE}	43.36 ± 5.32^{cB}	87.28 ± 2.15^{bC}	0.00 ± 0.00^{cE}	0.00 ± 0.00^{bD}
	72	9.10 ± 0.02^{dDE}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{cD}	ND	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bC}	16.92 ± 0.03^{dE}	36.41 ± 5.05^{cC}	65.95 ± 4.91^{cDE}	0.00 ± 0.00^{cE}	0.00 ± 0.00^{bD}
CPPGBR	Control	9.13 ± 0.18^{bcDE}	7.10 ± 0.07^{aB}	1.50 ± 0.02^{cC}	ND	1.82 ± 0.02^{aB}	8.72 ± 0.14^{aB}	17.72 ± 0.25^{cCD}	40.57 ± 1.20^{aBC}	79.52 ± 2.04^{aC}	17.01 ± 0.14^{aB}	3.03 ± 0.05^{aA}
	18	9.00 ± 0.04^{cE}	0.00 ± 0.00^{bC}	1.51 ± 0.01^{bcBC}	ND	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bC}	18.97 ± 0.09^{bB}	16.56 ± 0.97^{cD}	62.70 ± 2.92^{cE}	0.00 ± 0.00^{cE}	0.00 ± 0.00^{bD}
	36	9.63 ± 0.06^{aA}	0.00 ± 0.00^{bC}	1.54 ± 0.01^{abBC}	ND	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bC}	24.22 ± 0.07^{aA}	19.33 ± 2.68^{bD}	72.86 ± 5.51^{abCD}	0.00 ± 0.00^{cE}	0.00 ± 0.00^{bD}
	54	9.12 ± 0.03^{bcDE}	0.00 ± 0.00^{bC}	1.55 ± 0.02^{aB}	ND	1.75 ± 0.01^{bC}	0.00 ± 0.00^{bC}	17.53 ± 0.04^{cD}	14.27 ± 0.04^{cDE}	66.83 ± 7.26^{bcDE}	0.00 ± 0.00^{cE}	0.00 ± 0.00^{bD}
	72	9.49 ± 0.40^{abAB}	7.06 ± 0.06^{aB}	1.55 ± 0.03^{aB}	ND	0.00 ± 0.00^{cD}	0.00 ± 0.00^{bC}	17.14 ± 0.02^{dE}	9.95 ± 0.55^{dE}	48.55 ± 1.13^{dF}	8.07 ± 0.00^{bD}	0.00 ± 0.00^{bD}

Note: Values are presented as mean ± standard deviation ($n = 4$). Different lowercase letters indicate significant differences of the data among the different germination hours in the same group (UGBR group or CPPGBR group) ($p < .05$), and different capital letters indicate significant differences in the same column ($p < .05$).

Abbreviations: CPPGBR, germinated brown rice with cold plasma pretreatment; DW, dry basis weight; ND, not detectable; UGBR, germinated brown rice without cold plasma pretreatment.

that the increase of bound coumaric acids and ferulic acids in GBR might be due to polymerization from free phenolic. The increase of phenylaldehydes ammino-lyase activity during germination was the main reason to boost the accumulation of phenolic compounds (Dixon & Paiva, 1995). Therefore, CPP might inhibit the accumulation of phenolic compounds by passivating key enzymes activity such as phenylaldehylyase.

3.7 | Changes of flavonoid compositions in GBR with and without CPP

Eight flavonoid compositions were measured in bound and free fractions of GBR with and without CPP (Table 4). Free kaempferol contents in CPP-treated GBR and untreated GBR were 3.81–4.28 μg/g DW and 10.25–11.44 μg/g DW, respectively, for 18–72 h germination. Free naringenin contents in CPP-treated GBR and untreated GBR were 18.10–20.44 μg/g DW, and 24.94–34.74 μg/g DW, respectively, after 18–72 h germination. Free apigenin contents in CPP-treated GBR and untreated GBR were 2.13–4.01 μg/g DW and 8.37–14.78 μg/g DW, respectively, after 18–72 h germination. At the same germination time, free kaempferol, apigenin, and naringenin contents in untreated GBR were higher than that in CPP-treated GBR. For bound flavonoid, bound naringenin contents in CPP-treated GBR and untreated GBR were 17.89–18.73 μg/g DW and 17.91–28.32 μg/g DW, respectively, after 18–72 h germination. Bound rutin contents in CPP-treated GBR and untreated GBR were 5.29–8.81 μg/g DW and 6.25–44.64 μg/g DW, respectively, for 18–72 h germination. At the same germination time, bound rutin and naringenin in untreated GBR were higher than that in CPP-treated GBR.

A previous study showed that naringin and kaempferol levels in millet after germination were significantly decreased by 63.26 and 36.66 μg/g, respectively, while apigenin levels were enhanced by 69.28 μg/g (Pradeep & Sreerama, 2015). This was similar to the results of this study. It was found that naringin was an important precursor of flavonoid biosynthesis catalyzed by many enzymes (Winkel-Shirley, 2001). Therefore, the decrease of naringin content and the increase of apigenin content might be caused by the conversion of naringin to apigenin under the action of flavone synthase enzyme during brown rice germination. Furthermore, the study indicated that the content of flavonoids in GBR decreased under CPP. Suzuki et al. (2002) found that the change of flavonoid content during seed germination might be related to the release of bound flavonoid or the transformation and biosynthesis of flavonoid during seed germination. Therefore, the activities of key enzymes such as glucosidase were inhibited and flavonoids were decomposed under CPP, thus reducing the content of flavonoids in GBR.

3.8 | Changes of antioxidant activities in GBR with and without CPP

The results showed that antioxidant capacity was increased continuously and then decreased with the increase of germination time (Table 5). During whole germination, DPPH antioxidant activities of free phenolics in untreated GBR and CPP-treated GBR were 69.33–80.79 μmol Trolox/100 g DW and 50.42–73.94 μmol Trolox/100 g DW, respectively. DPPH antioxidant capacities of bound phenolics in untreated GBR and CPP-treated GBR were 20.76–32.45 μmol Trolox/100 g DW and 21.19–27.61 μmolTrolox/100 g DW, respectively, during the whole germination. DPPH antioxidant activities of total phenolics in untreated GBR and CPP-treated GBR were 90.10–113.24 μmol Trolox/100 g DW and 72.91–97.77 μmol Trolox/100 g DW, respectively, in the whole germination period. At the same germination time (18–54 h), DPPH antioxidant activities of total, bound, and free phenolics in untreated GBR were higher than those in CPP-treated GBR. T-AOC of free phenolics in CPP-treated GBR and untreated GBR were 27.19–37.79 U/100 mg DW and 34.99–41.95 U/100 mg DW, respectively, in the whole germination period. During whole germination, the T-AOC of bound phenolics in CPP-treated GBR and untreated GBR were 6.87–11.06 U/100 mg DW and 4.65–11.68 U/100 mg DW, respectively. The T-AOC of total phenolics in CPP-treated GBR and untreated GBR were 35.75–47.00 U/100 mg DW and 39.65–51.05 U/100 mg DW, respectively, in the whole germination period. At the same germination time (36–72 h), T-AOC of total and free phenolics in untreated GBR were higher than those in CPP-treated GBR.

Cevallos-Casals and Cisneros-Zevallos (2010) demonstrated that germinated grains were an excellent source of phenolic antioxidants. Yodpitak et al. (2019) observed that the antioxidant activity of phenolics in six kinds of GBR indicated a trend of first enhancing, then gradually decreasing during germination. Additionally, Chen et al. (2016) reported an enhancement in DPPH antioxidant activity of phenolic in GBR during germination (0–24 h). CPP reduced the antioxidant capacity of phenolic in GBR, which coincided with the findings of Zargarchi and Saremnezhad (2019), who reported that the DPPH clearance rate of phenolic in germinated paddy was decreased by 10% after CPP. In this study, the changes of DPPH free radical scavenging and T-AOC of GBR were consistent with the changes of phenolic content. Therefore, the activities of PAL and other key enzymes

TABLE 4 Changes in the flavonoid compositions contents of untreated and cold plasma-pretreated germinated brown rice during 72 h germination

Sample	Time (h)	Flavonoid compositions (μg/g DW)							
		Kaempferol	Naringenin	Myricetin	Quercetin	Daidzin	Luteolin	Rutin	Apigenin
Free									
UGBR	Control	14.34 ± 0.71^{aA}	33.01 ± 1.45^{aB}	2.68 ± 0.04^{aA}	19.49 ± 0.04^{bB}	ND	0.50 ± 0.03^{bC}	9.12 ± 0.51^{cdEF}	13.97 ± 0.29^{bB}
	18	10.71 ± 0.42^{bcCD}	24.94 ± 1.02^{cE}	2.45 ± 0.03^{bB}	18.77 ± 0.06^{cD}	ND	0.45 ± 0.01^{cD}	11.68 ± 0.10^{bB}	10.94 ± 0.18^{cD}
	36	10.66 ± 0.40^{bcCD}	34.47 ± 0.68^{aA}	0.00 ± 0.00^{cC}	19.85 ± 0.18^{aA}	ND	0.62 ± 0.02^{aA}	9.63 ± 0.26^{cDE}	10.52 ± 0.75^{cD}
	54	10.25 ± 0.82^{cD}	25.47 ± 1.28^{cE}	0.00 ± 0.00^{cC}	18.58 ± 0.00^{dE}	ND	0.00 ± 0.00^{dF}	15.77 ± 0.25^{aA}	14.78 ± 0.33^{aA}
	72	11.44 ± 0.31^{bC}	29.16 ± 0.45^{bD}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{eF}	ND	0.00 ± 0.00^{dF}	8.69 ± 0.48^{dF}	8.37 ± 0.23^{dE}
CPPGBR	Control	12.95 ± 0.92^{aB}	31.08 ± 0.90^{aC}	2.49 ± 0.03^{aB}	19.02 ± 0.24^{aC}	ND	0.53 ± 0.04^{aB}	8.72 ± 0.47^{cF}	11.94 ± 0.52^{aC}
	18	3.98 ± 0.15^{bE}	18.10 ± 0.12^{dG}	2.45 ± 0.03^{aB}	0.00 ± 0.00^{cF}	ND	0.00 ± 0.00^{cF}	10.25 ± 0.41^{bC}	3.31 ± 0.09^{cG}
	36	4.28 ± 0.08^{bE}	18.95 ± 0.05^{cdG}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{cF}	ND	0.00 ± 0.00^{cF}	9.88 ± 0.06^{bCD}	2.13 ± 0.08^{dH}
	54	4.23 ± 0.07^{bE}	20.44 ± 0.19^{bF}	2.44 ± 0.05^{aB}	18.73 ± 0.02^{bDE}	ND	0.40 ± 0.01^{bE}	6.87 ± 0.20^{dG}	4.01 ± 0.30^{bF}
	72	3.81 ± 0.08^{bE}	18.66 ± 0.12^{cdG}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{cF}	ND	0.00 ± 0.00^{cF}	11.49 ± 0.42^{aB}	2.52 ± 0.30^{dH}
Bound									
UGBR	Control	6.93 ± 0.43^{bB}	22.64 ± 0.52^{bB}	12.45 ± 0.89^{aA}	24.31 ± 0.69^{aB}	17.11 ± 1.29^{aA}	0.73 ± 0.14^{aCD}	27.18 ± 1.00^{cC}	6.22 ± 0.57^{bB}
	18	3.69 ± 0.05^{cD}	18.12 ± 0.04^{cDE}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{dF}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{bF}	44.64 ± 3.51^{aA}	0.00 ± 0.00^{cD}
	36	3.75 ± 0.13^{cD}	18.60 ± 0.01^{cCD}	0.00 ± 0.00^{cC}	19.33 ± 0.03^{cD}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{bF}	38.68 ± 0.75^{bB}	0.00 ± 0.00^{cD}
	54	11.07 ± 0.63^{aA}	28.32 ± 0.69^{aA}	2.72 ± 0.06^{bB}	23.07 ± 0.20^{bC}	3.76 ± 0.09^{bB}	0.00 ± 0.00^{bF}	40.44 ± 1.85^{bB}	9.28 ± 0.75^{aA}
	72	3.60 ± 0.04^{cD}	17.91 ± 0.01^{cE}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{dF}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{bF}	6.25 ± 0.12^{dD}	0.00 ± 0.00^{cD}
CPPGBR	Control	6.41 ± 0.26^{aC}	23.00 ± 0.28^{aB}	13.04 ± 0.69^{aA}	23.77 ± 0.38^{bB}	17.11 ± 1.29^{aA}	0.83 ± 0.02^{bcBC}	27.51 ± 0.37^{aC}	6.28 ± 0.51^{aB}
	18	3.59 ± 0.05^{cD}	17.89 ± 0.02^{dE}	0.00 ± 0.00^{cC}	18.63 ± 0.05^{cE}	0.00 ± 0.00^{bC}	0.42 ± 0.04^{dE}	7.77 ± 0.21^{bD}	1.84 ± 0.07^{bC}
	36	3.85 ± 0.06^{bD}	18.73 ± 0.05^{bC}	0.00 ± 0.00^{cC}	19.19 ± 0.01^{cDE}	0.00 ± 0.00^{bC}	0.94 ± 0.30^{bB}	6.93 ± 0.48^{bD}	1.99 ± 0.06^{bC}
	54	4.06 ± 0.14^{bD}	18.48 ± 0.11^{cCD}	2.72 ± 0.06^{bB}	18.75 ± 0.02^{cDE}	0.00 ± 0.00^{bC}	0.57 ± 0.14^{cdDE}	8.81 ± 4.59^{bD}	2.19 ± 0.11^{bC}
	72	4.07 ± 0.08^{bD}	18.30 ± 0.06^{cCDE}	0.00 ± 0.00^{cC}	27.76 ± 0.72^{aA}	0.00 ± 0.00^{bC}	4.09 ± 0.11^{aA}	5.29 ± 0.14^{bD}	2.22 ± 0.08^{bC}

Note: Values are presented as mean ± standard deviation ($n = 4$). Different lowercase letters indicate significant differences of the data among the different germination hours in the same group (UGBR group or CPPGBR group) ($p < .05$), and different capital letters indicate significant differences in the same column ($p < .05$).

Abbreviations: CPPGBR, germinated brown rice with cold plasma pretreatment; DW, dry basis weight; ND, not detectable; UGBR, germinated brown rice without cold plasma pretreatment.

TABLE 5 Changes in the DPPH and T-AOC antioxidant activities of free, bound, and total phenolic extracts from untreated and cold plasma-pretreated germinated brown rice during 72 h germination

		DPPH (μmolTrolox/100 g DW)			T-AOC (U/100 mg DW)		
Sample	**Time (h)**	**Free**	**Bound**	**Total**	**Free**	**Bound**	**Total**
UGBR	Control	60.45 ± 1.35^{dF}	13.01 ± 1.50^{eE}	73.46 ± 2.77^{dFG}	27.27 ± 0.99^{eG}	5.01 ± 0.27^{dF}	32.28 ± 1.04^{eH}
	18	80.79 ± 1.86^{aA}	32.45 ± 1.45^{aA}	113.24 ± 3.30^{aA}	34.99 ± 0.61^{dD}	4.65 ± 0.15^{eG}	39.65 ± 0.73^{dEF}
	36	75.18 ± 2.56^{bB}	29.44 ± 1.98^{bB}	104.62 ± 2.74^{bB}	41.95 ± 0.47^{aA}	9.10 ± 0.14^{bC}	51.05 ± 0.35^{aA}
	54	71.47 ± 1.46^{cCD}	23.46 ± 0.45^{cCD}	94.93 ± 1.07^{cCD}	38.24 ± 0.26^{bB}	11.68 ± 0.17^{aA}	49.91 ± 0.13^{bB}
	72	69.33 ± 1.93^{cD}	20.76 ± 1.57^{dD}	90.10 ± 3.50^{cE}	36.56 ± 0.21^{cC}	7.02 ± 0.06^{cE}	43.58 ± 0.18^{cD}
CPPGBR	Control	59.09 ± 1.36^{cF}	12.94 ± 1.44^{cE}	72.03 ± 1.14^{dG}	27.67 ± 0.80^{dG}	4.42 ± 0.19^{eG}	32.09 ± 0.89^{eH}
	18	63.57 ± 1.68^{bE}	27.61 ± 1.54^{aB}	91.18 ± 3.23^{bDE}	29.13 ± 0.21^{cF}	11.06 ± 0.10^{aB}	40.19 ± 0.28^{bE}
	36	73.94 ± 1.53^{aBC}	23.83 ± 1.84^{bC}	97.77 ± 3.19^{aC}	31.78 ± 0.32^{bE}	6.87 ± 0.19^{dE}	38.65 ± 0.49^{cF}
	54	55.91 ± 1.75^{dG}	21.19 ± 1.24^{bCD}	77.10 ± 2.68^{cF}	37.79 ± 0.29^{aB}	9.21 ± 0.12^{bC}	47.00 ± 0.40^{aC}
	72	50.42 ± 1.53^{eH}	22.49 ± 1.88^{bCD}	72.91 ± 1.83^{cdFG}	27.19 ± 0.57^{dG}	8.55 ± 0.27^{cD}	35.73 ± 0.83^{dG}

Note: Values are presented as mean ± standard deviation ($n = 4$). Different lowercase letters indicate significant differences of the data among the different germination hours in the same group (UGBR group or CPPGBR group) ($p < .05$), and different capital letters indicate significant differences in the same column ($p < .05$).

Abbreviations: CPPGBR, germinated brown rice with cold plasma pretreatment; DW, dry basis weight; T-AOC, total antioxidant capacity; UGBR, germinated brown rice without cold plasma pretreatment.

decreased under CPP, thus reducing the content of phenolic in GBR.

4 | CONCLUSIONS

The study evaluated the impact of CPP of brown rice on GABA, phytase, phytic acid, flavonoids, phenolics, γ-oryzanol, and antioxidant capacity in GBR. These results indicated that the levels of γ-oryzanol and GABA in CPP (400 W, 5 min) treated GBR were the highest at germination time 36 and 72h , respectively, which were higher than those in untreated GBR. However, under the same conditions of cold plasma treatment, the levels of phytic acid, flavonoids, phenolics, and antioxidant capacity in CPP (400 W, 5 min) treated GBR were the lowest at germination time was 72 h, which were lower than those of untreated GBR. Accordingly, the application of CPP for the germination of brown rice may be a novel approach to degrade phytic acid and improve GABA and γ-oryzanol in brown rice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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