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Effect of slightly acidic electrolyzed water on amino acid and phenolic profiling of germinated brown rice sprouts and their antioxidant potential

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ABSTRACT

This study investigated the effectiveness of slightly acidic electrolyzed water (SAEW ACC-10 ppm) on the accumulation of total flavonoids content, total phenolic content, antioxidants, and bioactive compounds over the tap and distilled water during germination. Germination was carried out with moisture content (20–30%) at 35 ± 1 °C in Co_2 incubator using tap water, distilled water, and SAEW pH-5.5, ACC-10 ppm. SAEW was used to decontaminate natural microbiota from the rice outer layer and to check its effect on rice bioactive components. Nevertheless, after 48 h, SAEW germination showed detrimental effects on germination potential and germination rate in brown rice. The germination enhanced GABA content from 1.8 mg/L to 7.35 mg/L showing an increase of about three times. HPLC-FLD-MS/MS and UHPLCQ-TOF- MS/MS approaches were applied to analyze amino acids & phenolics in brown rice samples. In our study, germination using SAEW affected GABA content and enhanced other amino acids, antioxidants, and phenolic compounds such as ferulic acid, p-coumaric acid, quercetin, and ascorbic acid as compared with the raw, tap, and distilled water germination. Results indicated that SAEW in germination might enhance the bioactive compounds of grains so it can be utilized for germination and functional food production safely in the food sector.

1. Introduction

Rice is a widely consumed staple meal and the major source of carbohydrates in the diet. Nowadays, consumers have been lured to the healthy and natural components in meals, which can help maintain their immune system and prevent cardiovascular diseases (Meydani, 2000). Brown rice (BR) consists of several nutrients, including fiber, minerals, vitamins, and a variety of phytochemicals like phenolic acids, gamma-aminobutyric acid (GABA), flavonoids, gamma-oryzanol, phytic acids, and tocopherols that can promote health (Tyagi, Yeon, et al., 2021). GABA is an amino acid, non-protein present in every living being, from bacteria to plants and mammals, functioning as a major inhibitory neurotransmitter in the central nervous system. Because of its strong and unique effects have been widely researched (Diana, Quílez, & Rafecas, 2014). It has been claimed that GABA improves memory functions by assisting blood flow in the brain, alleviates depressive illnesses, and reduces blood pressure (Kalueff & Nutt, 1996; Vaiva et al., 2004).

GABA in natural foods research has focused chiefly on germinated brown rice. Because activated hydrolytic enzymes during germination break down high molecular weight polymers to create bio-functional compounds. Therefore, brown rice germinated and immersed in water to begin blossoming is more nutritious and extensively consumed (Lin, Pao, Wu, & Chang, 2015). The stored carbohydrates, lipids, and proteins are destroyed and utilized as energy sources and developmental materials during the germination process. Raw BR has a modest amount of GABA in its original form; however, germination boosts its GABA concentration by endogenous synthesis. Furthermore, germination physical stress like simple temperature control and oxygen shortage has been found to increase GABA and other bioactive compounds production in BR.

Generally, GABA production in plants requires a combination of polyamine degradation and GABA shunt. Biologically, it is produced by glutamate decarboxylation catalyzed by glutamate decarboxylase enzymes (GAD) (Bouché, Lacombe, & Fromm, 2003). The production of GABA in plant tissues requires GAD since it transforms excess glutamic acid into GABA. GAD is activated in rice bran and germs during brown rice sprouting, especially under moderate, acidic, and warm circumstances (35–40 °C and pH 5.5) (Ohtsubo, AsANO, Sato, & Matsumoto, 2000; Zhang, Yao, Chen, & Wang, 2007). The synthesis of GABA in plant tissue may also be enhanced by environmental stresses such as aerobic

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storage, water addition, temperature fluctuations, and a lack of light (Poojary et al., 2017). Stressful conditions, in general, might lead to a drop in Ca^{2+} cytosolic, H^{+} or calmodulin levels and a rise in GAD activity (Komatsuzaki et al., 2007).

Since 2002 electrolyzed oxidizing (EOW) water has been an approved food additive in Japan as it reduces microbiological pollutants, particularly in vegetables (Suzuki, Nakamura, Kokubo, & Tomita, 2005). Diverse sectors have been described for electrolyzed oxidizing (EO) water, acidic electrolyzed water (AEW) treatment and were used to eliminate or disable foodborne microorganisms (Jadeja & Hung, 2014; Kim, Hung, & Brackett, 2000). It is a novel disinfectant because EO water generates an environmentally favorable and broad spectre of antibacterial effects by electrolysis of the dilute sodium chloride solution. A hydrochloride acid electrolysis or anode-cathode combination generates slightly acidic electrolyzed water (SAEW). Due to its near-neutral pH (5.0–6.5), slightly acidic electrolyzed water (SAEW) is one of the most extensively used types, as it is less harmful to humans and equipment while also being environmentally benign (Hao, Li, & Zhao, 2021). SAEW has shown promise in the fruits and vegetable business, with excellent disinfection efficacy and minimal damage to the produce's physiochemical qualities and reducing enzymatic browning (Ding et al., 2015). Hypochloric acid is the primary free chlorine form at near-neutral pH, leading to increased microbial inactivation.

Most research on GABA synthesis in rice has used fermentation or germination. The need for an extended length of time and creating an unpleasant odor from the metabolic process or microbial contamination are significant disadvantages of those methods. Fewer studies have been reported in boosting GABA synthesis in brown rice utilizing stress treatments like SAEW, reducing processing time, and reducing the development of unpleasant odors by inhibiting microbial contamination.

Apart from GABA, most amino acids play a role in various biological processes, including cell communication, cellular metabolism, protein synthesis, immunological response, and certain antioxidant qualities (Wu, 2009). Moreover, phenolic compounds are extensively dispersed in plants, and their antioxidant activity and free radical scavenging capacity have recently attracted a lot of attention due to their potential health benefits. Amino acids are identified mainly by reversed-phase high-pressure liquid chromatography (RP-HPLC) using diode array detector (DAD), ultra-violet (UV), and fluorescence (FLD), which is performed mainly after pre-column derivatization (Wang et al., 2010). Additionally, Ultra-high-performance liquid tandem chromatography quadrupole flight mass spectrometry (UHPLCQ-TOF- MS/MS), a novel technique for evaluating and quantifying phenolic metabolites with a greater sensitivity on a similar LC-MS approach, was also becoming popular (Yang et al., 2018).

Therefore, the present study intended to evaluate GABA with other amino acids, antioxidant activities, and phenolic or flavonoid compounds of germinated brown rice to explore tap water, distilled water, and SAEW (10 ppm or 10 mg/L) ability to enhance bioactive compounds during germination as compared with raw BR. According to our understanding, this is the first time germination approaches using BR are compared between low ACC (10 ppm) slightly acidic electrolyzed water (SAEW), tap water, and distilled water.

2. Materials and methods

2.1. Rice samples

BR samples were procured from a local grain market in Chuncheon-City, South Korea. After bioconversion technology (germination), BR is crushed into powder and filtered through mesh 40 using an electric mill. Samples were stored at −20 °C for further analysis.

2.2. Chemicals and cultures

Analytical grade chemical reagents were used in all experiments. Daejung chemicals and metals Co., Ltd. supplied ethanol, methanol, and other organic solvents. TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), gallic acid, ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferulic, quercetin, catechin and p-Coumaric acids, Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) were purchased from Sigma, South Korea.

2.3. Sample preparation

2.3.1. Preparation of slightly acidic electrolyzed water (SAEW)

In the electrolyzed oxidizing water (EOW) generator, SAEW electrolyzes a 0.03 g/100 mL of NaCl solution was prepared. Before the experiment, the airtight container was used to collect EOW, and the ACC, ORP, and pH were established. A Thermo scientific pH meter (ORION STAR, A211, Thermo Fisher Scientific Korea Co., Ltd.Seoul) with a pH electrode and an ORP electrode was used to measure the pH and ORP. SAEW has a pH of 5.5–6.0 and an ORP of 940–968 mV. Low ACC content (10 ppm or 10 mg/L) was used for this study as we were working on food materials.

2.3.2. Brown rice germination

The germination of BR was carried out by the method reported previously (Zhang, Xia, Li, & Hung, 2018). Briefly, BR seeds (30–40 g) were washed 3–4 times with distilled water, tap water, and SAEW separately. Later, seeds were soaked separately in the respective treatment water for 12 h at 30 ± 1 °C, 70% relative humidity (RH). Every 6 h, the soaking solution was replaced. Germination was carried out in the dark for 48 h (85% RH) at 35 ± 1 °C (DAIHAN LABTECH. Co., Ltd., Incubator, South Korea). To maintain humidity during germination, each treatment solution was sprayed separately over BR (Fig. S1). Afterward, the germinated BR were dried for 6 h at 55 °C in a blast drying oven (OF-22GW, JEIO Tech Instrument Co., Ltd., South Korea). After drying, the different geminated samples were crushed into a fine powder and sorted through mesh 40 using an electric mill, and stored at −20° till further use.

2.3.3. Preparation of ethanolic extracts

Extraction was done using our previous protocol (Tyagi, Shabbir, et al., 2021). BR powder (1 g) was shaken for 4 h in an electric shaker at 50 °C with 20 mL of ethanol (10 mL ethanol in 10 mL distilled water) (1:20 w/v). Then the extracts were centrifuged and the supernatant collected for about 10 min at 4000 *g* before the residue was extracted again. The same procedure was repeated three times. The supernatants were filtered, evaporated (ethanol evaporation) at 50–55 °C, and freeze-dried. Later the freeze-dried samples were kept at −20 °C until further use. A stock solution of the sample is made at a concentration of 1 mg/mL. This is the stock that will be utilized throughout the analysis.

2.4. Detection of total phenolic content (TPC)

TPC was carried out using the previously mentioned protocol (Chang et al., 2018) with slight changes. Briefly, for a short duration of 6 min, 100 μL of sample extract or standard solution of gallic acid was treated with 200 μL of Folin-Ciocalteu reagent. The mixture was then alkalized with 1 mL of 700 mM Na_2CO_3. The absorbance was measured using the SpectraMax i3 plate reader at 760 nm after 90 min (Molecular Devices Korea, LLC). TPCs were calculated using the standard gallic acid curves as mg gallic acid equivalent per 100 g (mg GAE/100 g) of the sample.

2.5. Detection of total flavonoid content (TFC)

TFC of ethanol extracts was calculated using the microplate (24-well) method as defined previously (Apea-Bah, Minnaar, Bester, & Duodu,

Table 1
Total antioxidants DPPH, ABTS, FRAP, TPC and TFC of raw and different brown rice germinated samples.

S·NO	Sample	DPPH (mg Trolox equivalent 100 g, DW)	ABTS(mg Trolox equivalent 100 g, DW)	FRAP(mg Trolox equivalent 100 g, DW)	TPC(mg Gallic acid equivalent 100 g, DW)	TFC(mg catechin equivalent 100 g, DW)
1	Raw BR	15.53 ± 0.45^{d}	17.06 ± 0.33^{d}	16.11 ± 1.98^{d}	13.75 ± 0.25^{d}	14.72 ± 0.90^{d}
2	Tap water germinated BR	104.40 ± 0.56^{b}	110.12 ± 0.67^{b}	122.16 ± 1.61^{b}	99.17 ± 0.50^{b}	77.62 ± 1.13^{b}
3	Distilled water germinated BR	98.02 ± 1.4^{c}	103.37 ± 1.58^{c}	118.24 ± 1.34^{c}	85.70 ± 0.82^{c}	74.88 ± 2.32^{c}
4	SAEW (10 ppm) germinated BR	145.99 ± 0.29^{a}	152.21 ± 0.12^{a}	166.61 ± 2.10^{a}	115.94 ± 0.25^{a}	99.85 ± 0.84^{a}

BR-Brown rice, SAEW-slightly acidic electrolyzed water, ppm-parts per million (10PPM- 10 mg/L).
The findings are shown as the average SD of triplicates. Statistically, various alphabet letters in each column indicate significant variances (Tukey and Duncan test $p \leq 0.05$) DW, sample for dry weight.

2016) with slight modifications. Briefly, 200 μL of our sample extracts were added to 1 mL of distilled water and 75 μL of $NaNO_2$ (50 g L^{-1}). The reaction mixture was administered after 5 min of incubation with 75 μL $AlCl_3$ (100 g L^{-1}). 600 μL of distilled water and 500 μL of 1 M NaOH were added later after 7 min. At 510 nm, the SpectraMax i3 reader was used to read the absorbance (Molecular Devices Korea, LLC). The results were shown as the equivalent of milligram catechin for 100 g of samples (mg CE/100 g). Catechin was utilized as a baseline.

2.6. Antioxidant assays

2.6.1. DPPH radical scavenging activity

DPPH assay was determined as described earlier (Chen, Yu, Wang, Gu, & Beta, 2016) with some modification. Briefly, a 24-well microplate was used with 200 μL sample extracts were mixed with 1 mL of freshly formulated 100 μM DPPH radical solution (100 mL 95 percent v/v methanol used to dissolve 4 mg DPPH). The absorbance at 515 nm was determined using the spectrophotometer (SpectraMax i3) after 60 min of incubation in the dark at room temperature. Standard curves were utilized for the Trolox concentrations plot with radical DPPH activity. DPPH was reported as the equivalent mg Trolox (TE) for 100 g sample DW.

2.6.2. ABTS radical scavenging activity

ABTS activity was measured as described earlier (Świeca, 2016) with some modifications. The ABTS radical cation reagent was produced using a 7.2 mmol/L ABTS stock with a stock solution of 2.5 mmol/L of potassium persulphate (1:1, v/v). The solution was stored in the dark at room temperature for 12–16 h before prior use. The ABTS•+ was then mitigated with methanol at 734 nm to get absorbance around 0.700 ± 0.02. Later 100 μL of diluted crude extracts or standards were merged into 1 mL of ABTS radical solution. The spectrophotometer (SpectraMax i3 plate reader Molecular Devices Korea, LLC) was used to monitor absorption at 734 nm after 40 min of incubation at room temperature in dark conditions. A reference curve was utilized for Trolox concentrations with ABTS radical scavenging rate. Results were reported as mg TE/100 g, DW.

2.6.3. Ferric reducing antioxidant power (FRAP)

FRAP was analyzed using the previously documented method (Zeng et al., 2019) with some improvements. Briefly, 0.1 mL extracts were combined with a FRAP reagent of 3.9 mL using a buffer of 50 mL (0.3 M, pH 3.6), 5 mL of tripyridyl triazine (TPTZ) solution (10 mmol/L TPTZ in 40 mmol/L HCl), and 5 mL of $Fecl_3 \cdot 6H_2O$ (20 mmol/L) the FRAP reagent was developed and set at 37 °C for a total of 10 min. The results were shown as mg TE/100 g, DW.

2.7. Identification of amino acid and phenolic compounds

2.7.1. HPLC-FLD-MS/MS identification of amino acid

An HPLC system with a PerkinElmer Series 200 binary pump and autosampler separated amino acids (Shelton, CT, USA). An Eppendorf TC-50 controller with a CH-30 column heater and a Gastorr TG-14 inline degasser (GenTech Scientific Inc., USA) (Westbury, NY, USA). A 1260 Infinity fluorescence detector was used for detection, controlled by an Instant Pilot odel G4208A. (Agilent Technologies, Santa Clara, CA, USA). A PE Nelson 900 interface and a PE Nelson 600 Link box were used to aligning the system. The excitation and emission wavelengths for fluorescence were tuned to 262 nm and 425 nm, respectively. 5 mL acetonitrile with 95 mL water and HPLC grade pure acetonitrile were used in the A and B mobile phases. The following was the gradient condition: 0 min = 0% B, 1 min = 15% B, 4 min = 24% B, 5 min = 29% B, 7 min = 33% B, 9 min = 38% B, 10 min = 47% B, 12 min = 51% B, 13 min = 54% B, 15 min = 80% B, 18 min = 100% B, and 20 min = 100% B. The injection volume was 10 μL, and the flow rate was 1.0 mL/min. The temperature of the column was fixed at 35 °C.

The derivatization technique was automated; prior to sample injection, the autosampler collected aliquots of 100 μL of OPA and added them to HPLC vials holding rice samples or amino acid standards solution. At 25 ± 2 °C, the OPA-sample combination was incubated for 2 min. HPLC-FLD was used to examine the reaction mixture right away. The retention time (RT) was used to define chromatographic peaks, then recognized using online MS. Ionized electrospray ionization source in positive ion mode, spray pressure 60 psi, dry gas flow rate 9 L/min, dry gas temperature 350 °C, Vap temperature 450 °C, and capillary voltage 3500 V were the MS conditions.

2.7.2. UHPLC Q-TOF-MS/MS identification of phenolic compounds

UHPLCQ-TOF-MS/MS was used to identify phenolic phytochemicals in BR samples. Like all the above experimental analyses, stock samples (1 mg/mL) were used for phenolic detection. Millex syringe filters with 0.25-μm pore size (Merck KGaA Darmstadt, Germany) were used to filter the samples, and samples (1 mL) were separated and transferred to LC-MS vials for further analysis. For UHPLC and mass spectrometric analyses (LC-MS/MS), a SCIEX ExionLC AD machine (MA, USA) equipped with a controller, AD pump, degasser, AD autosampler, AD column oven, and photodiode array (PDA) detector (ExionLC) was used, along with a quadrupole time-of-flight mass spectrometer (X500R Q-TOF). The protocol from our previous research was used in the detection methodology (Tyagi, Yeon, et al., 2021), both positive (ESI+) and negative (ESI) modes of mass spectrometric analysis were utilized.

2.8. Statistical analysis

All of the collected data was analyzed with Graphpad Prism 8.0. SPSS program was used to compute the differences in mean values between raw and germinated BR samples (raw BR, tap-water, distilled water, and SAEW germinated BR) using one-way variance analysis (ANOVA) followed by a significant $p < 0.05$ Tukey's test. The findings were presented with an average ± standard deviation (SD). All the experiments were conducted in triplicates from each group (Raw BR, tap water germinated BR, distilled water germinated BR, and SAEW germinated

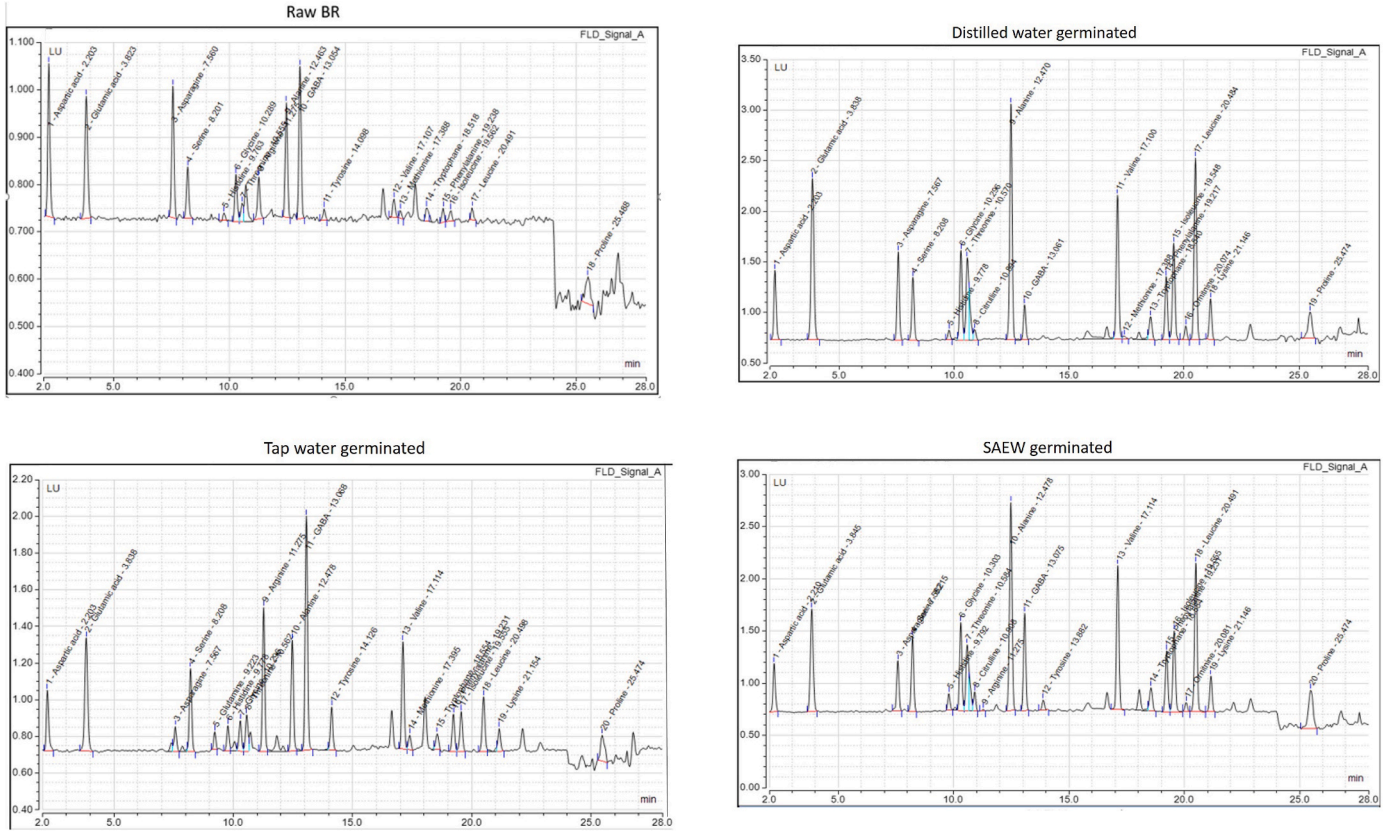

Fig. 1. Raw BR, Tap water germinated, distilled water germinated and SAEW germinated BR samples amino acid chromatographs.

BR). The findings were represented as the average SD of triplicates.

ClustVis (http://biit.cs.ut.ee/clustvis/) has been utilized for multivariate statistics and to draw heat maps (Metsalu & Vilo, 2015). Principal component analysis (PCA) was used to compare the changes among germinated BR samples. Origin software 2021 was used to analyze PCA. Heat maps and PCA were drawn using concentrations of samples by clustVis (Pinkard et al., 2020) and Origin software 2021.

3. Results and discussion

3.1. TPC and TFC of germinated samples

Phenolics are phytochemicals containing one or more hydroxyl groups from aromatic rings and exhibit antioxidant properties (Van Hung, 2016). Table 1 shows the TPC and TFC of all four samples (raw BR, tap water, distilled water, and SAEW germinated BR) ($p \leq 0.05$). TPC content varied from 13.75 ± 0.25 to 115.94 ± 0.25 mg GAE/100 g, DW. TPC levels were found lowest in raw BR samples 13.75 ± 0.25 and highest in SAEW germinated BR samples 115.94 ± 0.25 mg GAE/100 g. This research shows that enzyme hydrolysis typically increases total phenolic content during germination. TPC lifts highest with SAEW germination (115.94 ± 0.25 mg GAE/100 g, DW) compared with other germinated samples using tap and distilled water. This study found that BR had a greater TPC concentration after germination than previously reported (Gong et al., 2020; Ilowefah, Bakar, Ghazali, & Muhammad, 2017).

Flavonoids are phenolic compounds consisting of two aromatic rings (A and B) connected in an oxygenated heterocyclic ring by a three-carbon structure (C ring). They have high antioxidant activity and are linked to a lower risk of chronic illnesses. In our study, TFC was also higher in SAEW germinated BR 99.85 ± 0.84 mg catechin equivalent 100 g, DW, followed by tap and distilled water germinated BR (Table 1). Whereas we found higher TPC concentration in BR samples than TFC, the overall TFC in this research was higher than previously reported (Huang & Ng, 2012).

After germination, the phenolic content of brown rice was significantly increased ($p < 0.05$) in our study. Other cereals, such as wheat, rice, barley, oats, and rye, have also been reported to have comparable characteristics (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012) after germination. Different researchers' BR values may differ owing to genotype, cultivation landscape, and climatic circumstances. Furthermore, various extraction solvents and methods might substantially impact phenolic concentration. Additionally, increased TPC and TFC content in SAEW germinated BR samples might result from external stress, in which case cellular metabolism could be altered during germination, enabling changes in the chemical composition of sprouts while responding to stressful conditions (Aruna & Baskaran, 2010).

3.2. Antioxidant assay (DPPH, ABTS, FRAP)

Antioxidants are exogenous or endogenous substances that decrease or diminish any kind of oxidative damage. Various methods have been explored, including lipid peroxidation inhibition, chelation of metal ions, and free radical scavenging, to explain how rice extracts can be utilized as an efficient antioxidant (Ghasemzadeh, Jaafar, Juraimi, & Tayebi-Meigooni, 2015). This work assessed the antioxidant activity of different germinated BR samples using DPPH, ABTS, and FRAP tests. Antioxidant values for FRAP, ABTS, and DPPH are represented in Table 1.

DPPH radical by spectrophotometry is among the most widely used antioxidant activity tests on natural materials. The highest DPPH scavenging activity in the SAEW germinating sample has been detected (145.99 ± 0.29 mg Trolox equivalent 100 g, DW), following tap water and distilled water germinated (104.40 ± 0.56, 98.02 ± 1.4 mg Trolox equivalent 100 g, DW). Raw BR (15.53 ± 0.45 mg Trolox equivalent 100 g, DW) showed the lowest results.

Table 2
Amino acids discovered in the free fractions of Raw BR, Tap water, distilled water and SAEW germinated BR samples by HPLC-FLD-MS/MS.

S. No	Sample Name	Retention Time (min)	Area LU* min	Concentration (mg/L)	Relative Area %	Relative Height %	Amino Acid
1	Raw BR	2.203	0.037	2.83	14.42	15.98	Aspartic acid
	Tap water germinated BR	2.210	0.055	4.20	3.25	3.65	
	Distilled water germinated BR	2.203	0.040	3.08	4.70	4.88	
	SAEW germinated BR	2.203	0.080	6.14	4.38	4.83	
2	Raw BR	3.823	0.037	3.32	14.56	12.74	Glutamic acid
	Tap water germinated BR	3.838	0.141	12.64	8.40	7.75	
	Distilled water germinated BR	3.838	0.091	8.15	10.70	9.10	
	SAEW germinated BR	3.845	0.229	20.55	12.58	11.19	
3	Raw BR	7.560	0.032	2.50	12.76	13.73	Asparagine
	Tap water germinated BR	7.567	0.058	4.47	3.46	3.78	
	Distilled water germinated BR	7.567	0.017	1.27	1.95	2.01	
	SAEW germinated BR	7.582	0.103	7.89	5.64	6.13	
4	Raw BR	8.201	0.013	0.69	5.13	5.39	Serine
	Tap water germinated BR	8.208	0.088	4.06	5.26	5.65	
	Distilled water germinated BR	8.208	0.055	2.87	6.43	6.68	
	SAEW germinated BR	8.215	0.077	4.64	4.24	4.39	
5	Raw BR	9.763	0.001	0.24	0.57	0.81	Histidine
	Tap water germinated BR	9.778	0.019	3.05	1.11	1.27	
	Distilled water germinated BR	9.778	0.016	2.63	1.88	2.05	
	SAEW germinated BR	9.792	0.012	1.89	0.63	0.70	
6	Raw BR	10.289	0.013	0.69	5.13	5.39	Glycine
	Tap water germinated BR	10.296	0.102	3.55	6.09	6.64	
	Distilled water germinated BR	10.296	0.021	0.72	2.44	2.46	
	SAEW germinated BR	10.303	0.110	3.82	6.02	6.24	
7	Raw BR	10.555	0.005	0.31	2.03	1.99	Threonine
	Tap water germinated BR	10.562	0.086	5.19	5.11	4.97	
	Distilled water germinated BR	10.570	0.025	1.53	2.97	2.93	
	SAEW germinated BR	10.584	0.099	5.98	5.42	5.73	
8	Raw BR	ND	ND	ND	ND	ND	Citrulline
	Tap water germinated BR	ND	ND	ND	ND	ND	
	Distilled water germinated BR	10.894	0.020	1.73	1.22	1.44	
	SAEW germinated BR	10.908	0.013	1.11	0.72	0.77	
9	Raw BR	11.275	0.011	0.84	4.39	4.41	Arginine
	Tap water germinated BR	11.275	0.001	0.11	0.09	0.13	
	Distilled water germinated BR	ND	ND	ND	ND	ND	
	SAEW germinated BR	11.275	0.090	6.78	10.59	11.58	
10	Raw BR	12.463	0.029	1.22	11.30	11.94	Alanine
	Tap water germinated BR	12.478	0.267	11.35	15.91	15.67	
	Distilled water germinated BR	12.470	0.072	3.06	8.47	8.91	
	SAEW germinated BR	12.478	0.289	12.29	15.86	16.40	
11	Raw BR	13.054	0.038	1.84	15.00	15.88	GABA
	Tap water germinated BR	13.068	0.153	5.39	2.24	2.43	
	Distilled water germinated BR	13.061	0.041	4.36	17.96	18.81	
	SAEW germinated BR	13.075	0.041	7.96	6.66	7.34	
12	Raw BR	14.098	0.003	0.25	1.16	1.23	Tyrosine
	Tap water germinated BR	14.126	0.028	1.00	0.69	0.78	
	Distilled water germinated BR	ND	ND	ND	ND	ND	
	SAEW germinated BR	13.882	0.012	2.44	3.34	3.48	
13	Raw BR	17.107	0.005	0.24	1.91	1.94	Valine
	Tap water germinated BR	17.114	0.071	8.58	10.24	10.87	
	Distilled water germinated BR	17.100	0.178	3.54	8.34	8.63	
	SAEW germinated BR	17.114	0.172	8.90	9.78	9.99	
14	Raw BR	17.388	0.002	0.12	0.73	0.85	Methionine
	Tap water germinated BR	17.395	0.010	0.63	1.19	1.19	
	Distilled water germinated BR	17.388	0.004	0.23	0.21	0.22	
	SAEW germinated BR	ND	ND	ND	ND	ND	
15	Raw BR	18.518	0.004	0.53	1.67	1.41	Tryptophan
	Tap water germinated BR	18.554	0.012	4.04	1.79	1.61	
	Distilled water germinated BR	18.540	0.033	1.53	1.46	1.29	
	SAEW germinated BR	18.554	0.033	4.08	1.97	1.77	
16	Raw BR	19.238	0.004	0.26	1.38	1.47	Phenylalanine
	Tap water germinated BR	19.231	0.076	5.55	4.53	4.63	
	Distilled water germinated BR	19.217	0.025	1.85	2.99	3.01	
	SAEW germinated BR	19.231	0.080	5.82	4.38	4.40	
17	Raw BR	19.562	0.003	0.18	1.28	1.13	Isoleucine
	Tap water germinated BR	19.555	0.029	5.60	6.13	6.11	
	Distilled water germinated BR	19.548	0.103	1.50	3.40	3.22	
	SAEW germinated BR	19.555	0.1232	6.72	6.76	6.71	
18	Raw BR	20.491	0.003	0.16	1.18	1.28	Leucine
	Tap water germinated BR	20.498	0.040	2.22	4.75	4.42	
	Distilled water germinated BR	20.498	0.189	10.39	11.27	11.22	
	SAEW germinated BR	20.491	0.233	12.79	12.78	12.63	
19	Raw BR	25.488	0.014	1.68	5.51	2.84	Proline
	Tap water germinated BR	25.474	0.089	10.70	5.33	2.92	

(*continued on next page*)

Table 2 (*continued*)

S. No	Sample Name	Retention Time (min)	Area LU* min	Concentration (mg/L)	Relative Area %	Relative Height %	Amino Acid
	Distilled water germinated BR	25.474	0.029	3.42	3.36	2.09	
	SAEW germinated BR	25.474	0.053	6.36	2.92	1.83	
20	Raw BR	ND	ND	ND	ND	ND	Lysine
	Tap water germinated BR	21.154	0.043	7.16	2.57	2.70	
	Distilled water germinated BR	21.146	0.016	2.65	1.88	1.84	
	SAEW germinated BR	21.146	0.050	8.25	2.73	2.83	
21	Raw BR	ND	ND	ND	ND	ND	Ornithine
	Tap water germinated BR	ND	ND	ND	ND	ND	
	Distilled water germinated BR	20.074	0.012	3.06	0.71	0.73	
	SAEW germinated BR	20.081	0.017	4.38	0.93	0.98	
22	Raw BR	ND	ND	ND	ND	ND	Glutamine
	Tap water germinated BR	9.223	0.010	0.85	1.19	1.43	
	Distilled water germinated BR	ND	ND	ND	ND	ND	
	SAEW germinated BR	ND	ND	ND	ND	ND	

ND-NOT DETERMINED.

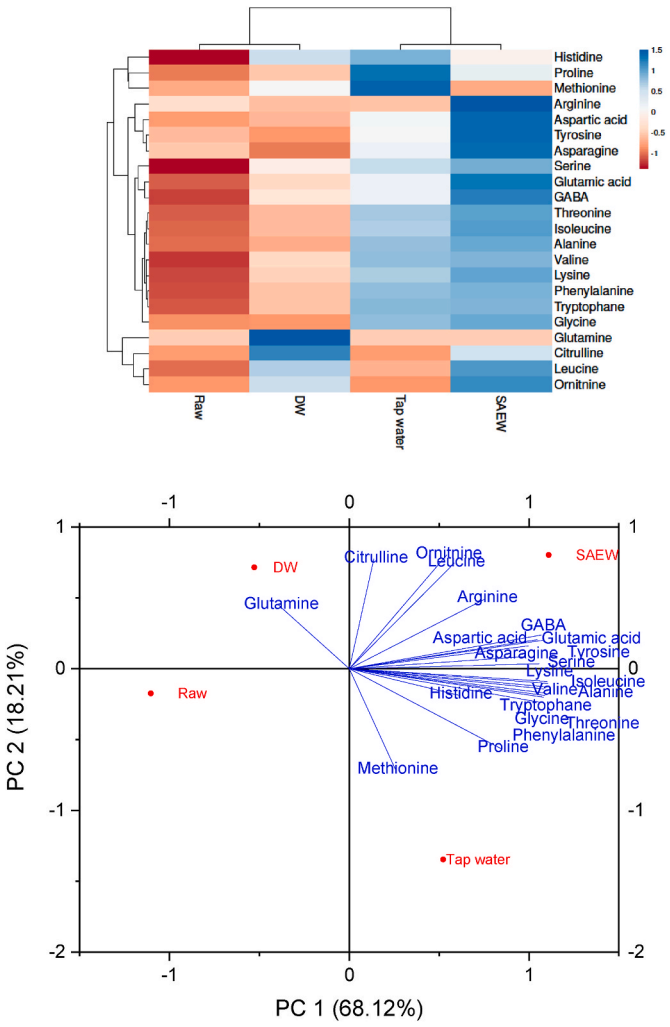

Fig. 2. Amino acid levels in Raw BR, distilled water (DW), tap water, and SAEW germinated samples of BR. (A) The heat map displays varying amounts of amino acid, from blue to red in samples reflects a decreasing quantity of amino acids (B) Principal component analysis (PCA) of Raw BR, distilled water (DW), tap water and SAEW germinated BR samples was shown by comparing PC 1 with PC2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

A similar tendency with DPPH has been seen in our ABTS and FRAP assessments study. ABTS scavenging activity was also highest in SAEW germinated sample (130.52 ± 0.97 mg Trolox equivalent 100 g, DW) followed by tap and distilled water germinated BR (Table 1). In ABTS, also lowest activity was observed by raw BR. In a similar way, FRAP was highest in the SAEW sample (111.16 ± 1.83 mg Trolox equivalent 100 g, DW).

In germinated BR samples, there were favorable associations between antioxidant ability and TPC and TFC. It was concluded that SAEW treatment increased the antioxidant activity of brown rice sprouts primarily by encouraging the formation of phenolic components (including flavonoids and phenolic acids). High amounts of phenolics and antioxidants can accumulate in plant tissues under various adverse situations, such as water stress and salt stress. Regarding germination, SAEW could be regarded as a form of stress, explaining why antioxidants and phenolics accumulate in SAEW-germinated brown rice. Similarly, when SAEW was employed, Hao and colleagues (Hao et al., 2021) discovered substantial positive correlations between buckwheat sprouts' DPPH radical scavenging ability and the TPC concentration. We observed higher findings than earlier reports (Cho & Lim, 2018) for germinated BR.

3.3. *Identification of amino acid and phenolic compounds*

HPLC-FLD-MS/MS was employed to detect amino acid content in raw and different germinated BR samples.

3.3.1. *Amino acid level in brown rice*

Amino acids have a vital function in the growth and development of organisms and can help improve the taste of food. In BR, a total of 22 amino acids were identified in this investigation (Fig. 1, Table 2). Raw BR has the lowest amino acid content, while SAEW and tap water germination improve amino acid content. During germination, certain molecules get destroyed, allowing for respiration and the synthesis of new cell components, resulting in substantial modification of grains' sensorial, biochemical, and nutritional properties. A cluster of amino acids depending on their concentrations (raw BR, tap water, distilled water, and SAEW germinated BR) were analyzed by the heat-map (Fig. 2A) with the color scheme spanning from blue to red, indicating a decreasing concentration. The highest levels of amino acids were observed in the SAEW germinated sample, which might be due to the SAEW external stress leading to the activation of numerous latent enzymes during the germination process that enhances free amino acids in BR. Stress also initiates a signal transduction cascade in plants, where glutamate decarboxylase, γ-aminobutyric acid transaminase, succinic semialdehyde dehydrogenase, and intracellular and intercellular transportation all play a role in the accumulation of gamma-aminobutyric acid. It suggests that the physical treatment might have activated GAD and other enzymes involved in amino acid modifications after SAEW treatment. A low level in the raw sample, on the other hand, could be due to more bound amino acids with the parent molecules. Except for histidine and methionine, the concentrations of certain essential amino

Table 3
Phenolic compounds identified in raw BR, tap water germinated BR, distilled water germinated BR and SAEW germinated BR by UHPLC-Q-TOF-MS/MS.

S. No	Sample Name	Retention Time (min)	Adduct/Charge	Concentration (μg/g)	Formula	Name
1	Raw BR	N/A	N/A	N/A	$C_6H_8O_6$	Ascorbic acid
	Tap water germinated BR	1.03	[M + H]+	103		
	Distilled water germinated BR	N/A	N/A	N/A		
	SAEW germinated BR	1.01	[M + H]+	224.4		
2	Raw BR	16.08	[M − H]−	3.74	$C_9H_8O_3$	p-Coumaric acid
	Tap water germinated BR	16.09	[M − H]−	19.6		
	Distilled water germinated BR	N/A	N/A	N/A		
	SAEW germinated BR	16.07	[M − H]−	53.8		
3	Raw BR	N/A	N/A	N/A	$C_{15}H_{14}O_6$	Catechin
	Tap water germinated BR	N/A	N/A	N/A		
	Distilled water germinated BR	N/A	N/A	N/A		
	SAEW germinated BR	N/A	N/A	N/A		
4	Raw BR	16.72	[M − H]−	4.46	$C_{10}H_{10}O_4$	Ferulic acid
	Tap water germinated BR	16.73	[M − H]−	40.5		
	Distilled water germinated BR	16.7	[M − H]−	4.07		
	SAEW germinated BR	16.71	[M − H]−	127.2		
5	Raw BR	19.35	[M + H]−	2.82	$C_{15}H_{10}O_7$	Quercetin
	Tap water germinated BR	19.38	[M − H]−	0.80		
	Distilled water germinated BR	19.33	[M − H]−	7.17		
	SAEW germinated BR	19.38	[M − H]−	0.86		

N/A-not applicable.

acids such as valine, phenylalanine, tryptophan, threonine, leucine, and lysine rose dramatically in SAEW germinated sample. Certain conditionally essential amino acids (serine, ornithine, tyrosine, and arginine) were also higher in the SAEW germinated BR sample. Besides GABA, changes in the composition of other amino acids might be significant to determine brown rice health and nutritional role.

Like plant and animal cells, germinating seeds go through the same cellular respiration processes. Glycolysis, Krebs cycle, and the Electron Transport Chain are the three steps of cellular respiration. Cellular respiration produces ATP molecules, which give seed germination energy and fuel the cell-building activities that eventually create the plant body. The effect of exogenous ATP on H_2O_2 levels was demonstrated in a study done by Chen and colleagues, leading to increased antioxidant enzyme activity. The mechanism could be linked to ATP signaling, which in stressed plants can activate the Ca^{2+}, NO, and ROS signal pathways, and could also be a possible reason for the enhancement of antioxidants, amino acids, and other bioactive compounds during SAEW germination (Chen et al., 2018).

Moreover, compared to the distilled water approach, the tap water germinated strategy also improves the amino acid level. Another investigation using brown rice revealed a similar increase in total amino acid content during their research (Oh, Kim, Lim, & Reddy, 2019). PCA is a valuable approach for identifying the primary metabolites in high-throughput profiles. As a result, PCA analysis was used to screen the major metabolites and identify metabolic differences between raw and germinated samples (raw BR, tap water, distilled water, and SAEW germinated BR). The separation of variables was investigated, and PCA was used to emphasize the discriminative metabolites. The first two principal components (PCs) explained 86.33 percent of the total data (PC1: 68.12 percent; PC2: 18.21 percent), indicating that the model predicted the data correctly (Fig. 2B). The plot in Fig. 2B exhibited the well separation of all four samples and metabolites. The amino acid profile of the raw BR sample was found divergent from all other samples, showing more diversity of amino acids towards SAEW germinated sample followed by tap water germination. Distilled water germinated samples can be seen rich only in a few amino acids. In contrast, tap and SAEW germinated samples were found more comparable. As presented by heatmap, the PCA graph represents a similar abundance of metabolites in the germinated samples.

3.3.2. *Phenolic compounds in Brown rice samples*

The phenolic phytochemicals have been identified and characterized utilizing the UHPLC-Q-TOF-MS/MS to compare quickly with the authentic standards (Table 3 and Fig. S2). Nowadays, UHPLC-Q-TOF-MS/MS approach is becoming a popular and reliable approach by various researchers in different fields for metabolites detection.

It has been proposed that phenolic compounds are responsible for the health advantages of rice consumption in the prevention of chronic illnesses such as stress-related disorders, obesity, cardiovascular disease, type II diabetes, oxidative stress, and cancer (Okarter & Liu, 2010). Substantial variations were found after comparing each sample's levels of phenolic compounds. Ascorbic acid (224.4 μg/g), p-Coumaric acid (53.8 μg/ml), and ferulic acid (127.2 μg/g) were found most abundant in SAEW (10 ppm) germinated BR. Catechin was not detected in any of the germinated samples. At the same time, distilled water germinated BR was found abundant in quercetin (7.17 μg/g) among all germinated samples. Heat map analysis was performed (raw BR, tap water, distilled water, and SAEW germinated BR) to cluster phenolic chemicals based on their concentrations (Fig. 3A). The color scheme ranged from grey to red, indicating concentration in decreasing order. The maximum phenolics were found in a low ACC 10 ppm SAEW germinated BR sample, followed by a tap water germinated sample. Phenolic compounds are not easily accessible as they often exist in an esterified state connected to the grain wall matrix of cereals (Dai et al., 2019). This could be the reason for the least phenolic detection in the raw BR sample. Germination is thought to be a viable approach for increasing amino acids, releasing insoluble bound phenolic compounds, and improving the low bioavailability of grain phenolics. In this study, phenolic compounds like ascorbic acid (Berretta et al., 2020), p-coumaric acid (Daroi, Dhage, & Juvekar, 2021), and ferulic acid were identified as higher in SAEW germinated BR. All of these compounds have already been reported in the literature as potent antioxidants and aid in preventing oxidative stress-related illnesses.

We checked the early metabolic pathways for phenolic compounds identified in our investigation to identify the most relevant ways associated with the germination procedure using the KEGG databases (https://www.genome.jp/kegg/pathway.html). The few pathways that govern the production of these phenolic compounds are the vitamin digestion and absorption pathway, the phenylpropanoid biosynthesis pathway, and the flavones and flavonols biosynthetic pathway. Changes in germination samples and levels of phenolic compound in different germinated samples may be linked to variations in the transition of specific genes to critical sites in the metabolic pathway. In addition, metabolic pathways may be employed to confirm the transformation genes of specific phytochemicals triggering genes and provide suggestions for the future selection and acquisition of exceptional variants.

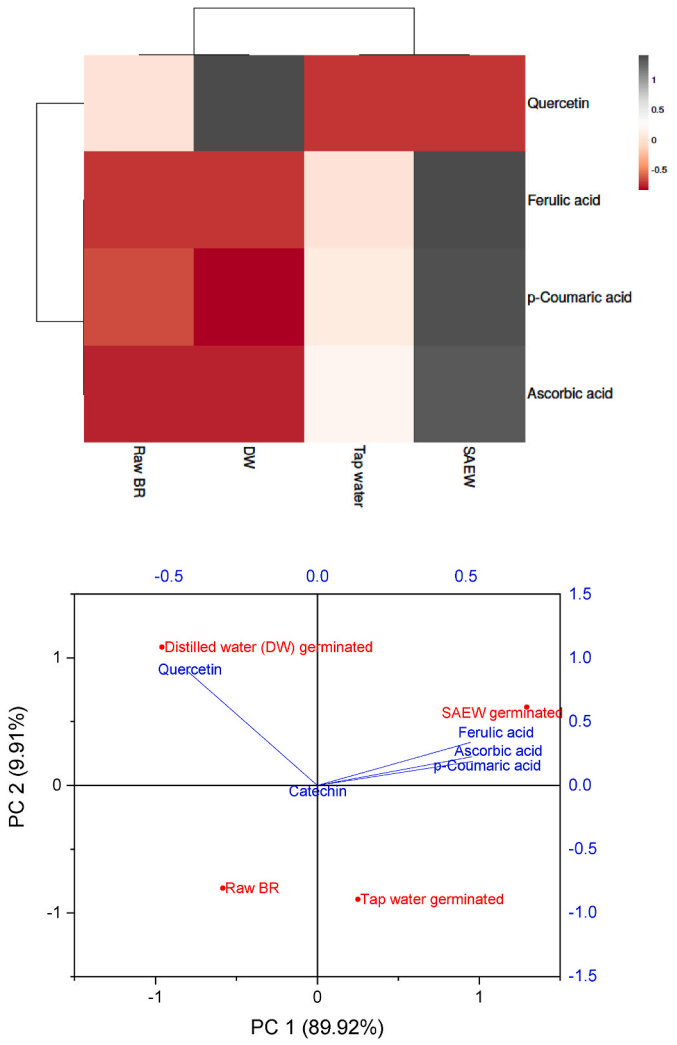

Fig. 3. Levels of phenolic Compounds in Raw, distilled water (DW), tap water, and SAEW germinated BR samples. (A) The heat map displays varying amounts of phenolic compounds, from grey to red in samples reflects a decreasing quantity of phenolic (B). Principal component analysis (PCA) of Raw BR, distilled water (DW), tap water, and SAEW germinated BR samples was shown by comparing PC 1 with PC2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PCA was applied for better interpretations and to avoid multi-collinearity. Moreover, as per the heat map interpretations, a similar trend was observed in the PCA analysis of all four samples (raw BR, tap water, distilled water, and SAEW germinated BR). The graph demonstrated that the data from four groups were well split into distinct clusters. The first two principal components (PCs) explained 99.83% of the total data (PC1: 89.92 percent; PC2: 9.91 percent), indicating that the model predicted the data correctly (Fig. 3B). Distilled water germinated samples can be seen rich in quercetin. In contrast, most identified phenolic compounds were higher in SAEW germinated samples except catechin, which was not detected in any of the samples (Fig. 3B). Additionally, all the groups were divergent due to the concentrations differences in the phenolic compounds. After comparing different approaches, we examined that germination can enhance bioactive compounds as well as bioavailability and bio-accessibility of various grains like brown rice, mung beans, etc. (Tang, Dong, Guo, Li, & Ren, 2014; Ti et al., 2014).

4. Conclusion

In this research, SAEW's impact on antioxidants, amino acids, and phenolic compounds during BR germination was examined over tap and distilled water. According to our knowledge, this is the first time when the comparison between low ACC (10 ppm) slightly acidic electrolyzed water (SAEW), tap, and distilling water germination efficiency is conducted utilizing BR. SAEW germinated BR with lower ACC levels (10 ppm) exhibited the highest antioxidant activity, total phenolic content, and total flavonoid levels in germination BR germs among all treatments.SAEW germinated BR group was also found rich in amino acids and phenolic compounds. Enzyme production and kernel alteration occur during the germination phase, which might increase intrinsic phenolic compounds and antioxidant activity (Kaukovirta-Norja, Wilhelmson, & Poutanen, 2004). Germinated BR has recently become one of the most popular germinated cereal products, owing to its enhanced texture and nutritional characteristics compared to BR. We provide results demonstrating that SAEW germinated BR contains a high concentration of phenolics and flavonoids and has significant antioxidant properties.

The introduction of SAEW in germination might be able to minimize microbial contamination, and mild stress of moisture content, pH, and ACC content may activate different latent enzymes. These enzymes could further enhance the production of amino acids, antioxidants, and other phenolic or bioactive compounds (Aruna & Baskaran, 2010; Chen et al., 2018; Zhang et al., 2018). This data indicates that consuming SAEW germinated brown rice high in antioxidant phytonutrients might give good health advantages. Furthermore, SAEW germinated BR can be used as a suitable source for the production of functional foods with high antioxidant and oxidative stress-reducing properties. However, *in-vivo* investigations on the capacity of SAEW germinated BR to reduce oxidative stress-related diseases are needed further to validate their health-promoting benefits and functional food development.

CRediT authorship contribution statement

Akanksha Tyagi: Conceptualization, Formal analysis, Methodology, Validation, Visualization, Writing – original draft. **Xiuqin Chen:** Methodology, Writing – review & editing. **Umair Shabbir:** Software, Formal analysis, Writing – review & editing. **Ramachandra Chelliah:** Writing – review & editing. **Deog Hwan Oh:** Supervision, Data curation, Funding acquisition, Resources, Investigation.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in study design, data collection, and analysis, decision to publish, or manuscript preparation.

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Abbreviations

BR	Brown rice
Tap	Tap water
DW	Dry weight
SAEW (10 ppm)	Slightly acidic (ACC 10 PPM) electrolyzed water
EOW	Electrolysed oxidizing water
TPC	Total phenolic content
TFC	Total flavanoid content
PCA	Principal component analysis
ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid

DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
GABA	Gamma aminobutyric acid
GAD	glutamate decarboxylase enzymes

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113119.

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