



INFLUENCE OF PU-ERH TEA EXTRACT ON PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF GERMINATED BROWN RICE

Jeong-Ho Kim¹, Yong-Han Yoon¹, Il-Doo Kim², Sanjeev Kumar Dhungana³, Dong-Hyun Shin^{4✉}

¹ Department of Green Technology Convergence, Konkuk University, Chungju 27478, Korea

² International Institute of Research & Development, Kyungpook National University, Daegu 41566, Korea

³ Department of Southern Area Crop Science, National Institute of Crop Science, Rural Development Administration, Miryang 50424, Korea

⁴ School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea
✉ dhshin@knu.ac.kr

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ABSTRACT

Brown rice is prepared by removing the hull of the rice kernel. Despite the high nutritional value of normal brown rice, it is not widely accepted as it cannot be cooked easily in a conventional rice cooker. However, germinated brown rice (GBR) is easy to cook and the texture is also softer than that of the normal brown rice. The objective of this study was to investigate the physicochemical and antioxidant potential of GBR produced by soaking in different concentrations (0.5–3%, w/v) of Pu-erh tea extracts. The lightness value of the extract-treated GBR was reduced, however, the redness and yellowness values were significantly increased with the concentration of the tea extracts. The GBR samples grown with the extract treatment were enriched with some amino acids such as γ -aminobutyric acid although the amount of total free amino acid was reduced in the treated samples. Similarly, the amount of total minerals and DPPH free radical scavenging potential of the extract-treated samples were higher than that of the untreated one. The total polyphenol and/or flavonoid levels of some of the tea-treated GBR samples were also improved. The results indicated that the nutritional and functional properties of brown rice could be enhanced by Pu-erh tea extract treatments.

1. Introduction

Rice supplies daily calories for about half of the world's population. Rice literally means white rice, also known as polished rice. Brown rice is prepared by removing only the outermost layer, the hull, of the rice kernel, and is the least damaging to its nutritional value. However, white rice is produced by further milling and removing the bran and most of the germ layer from brown rice. Brown rice is healthier than polished rice (Dinesh Babu *et al.*, 2009). Brown rice has high dietary fiber, rich in vitamin B complex and minerals, and high in fat. Also, it has been reported that brown rice contains high phytic acid, antioxidant, and anti-cancer; it

decreases serum cholesterol; and it is considered a low glycemic index food.

Although normal brown rice has high nutritional value, its popularity is low because it cannot be cooked in a conventional rice cooker, however, germinated brown rice (GBR) is easily cooked and the texture is softer than that of brown rice (Komatsuzaki *et al.*, 2007; Patil and Khan, 2011). Therefore, GBR could become a popular healthy food. Several studies on GBR indicate that during the process of germination, nutrients in the brown rice change drastically. Not only the contents of existing nutrients are modified but new components are also released due to germination (Spanier *et al.*, 2001). GBR

is more beneficial than normal brown rice and white rice, particularly in the prevention of some diet-related diseases, including obesity, type 2 diabetes, and colorectal cancers (Imam *et al.*, 2014). An intake of GBR instead of white rice is good for the control of postprandial blood glucose concentration without increasing insulin secretion in subjects with hyperglycemia (Ito *et al.*, 2005). Intake of GBR is suggested to protect cell proliferation and apoptosis as well as to prevent heart failure owing to myocardial ischemia (Petchdee *et al.*, 2020).

The nutrients like γ -aminobutyric acid (GABA), lysine, vitamin E, dietary fiber, niacin, magnesium, vitamin B1, and vitamin B6 are significantly increased in GBR (Spanier *et al.*, 2001). They found that regular intake of GBR is beneficial for preventing headaches, relieving constipation, preventing colon cancer, regulating blood sugar level and preventing heart disease. Intake of GABA suppresses blood pressure and improves sleeplessness and autonomic disorder observed during the menopausal or presenile period (Okada *et al.*, 2000).

Reports show that different pretreatments and/or cultivation techniques have been employed to enhance the quality of GBR. Apoptotic pathway is found to be activated in the *Lactobacillus acidophilus*-fermented GBR that may prevent preneoplastic lesions of the colon (Li *et al.*, 2019). Gamma oryzanols contents are increased in the cold plasma-treated GBR compared to the untreated GBR (Yodpitak *et al.*, 2019). Gamma oryzanols is reported to increase the muscle strength (Eslami *et al.*, 2014). The GABA content is substantially increased following the cellulase solution treatment to GBR (Zhang *et al.*, 2019). Germination of brown rice in red onion solution increases the antioxidant capacity and GABA content as well as makes the rice slightly softer and stickier than that germinated in water (Nakamura *et al.*, 2020).

Pu-erh tea is receiving increased attention due to its health benefits for a variety of hypolipidemic, antiobesity, antimutagenic, antioxidative, antitumor, free radical

scavenging, and toxicity suppressing activities (Lee and Foo, 2013). Extracts obtained from plant sources have been found to increase the quality of soybean sprouts (Chaikina *et al.*, 2009; Kim *et al.*, 2017). So far, no reports on the effect of phytochemical-rich Pu-erh tea on GBR have been published. Considering the health benefits of Pu-erh tea and GBR, this study was conducted to investigate the effect of Pu-erh tea on the nutritional and functional values of GBR. This study will provide an insight into the effects of functional food material, such as Pu-erh tea on the quality of GBR.

2. Materials and methods

2.1. Chemicals and materials

Folin-Ciocalteu phenol reagent, quercetin, gallic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Sigma-Aldrich Corp, St. Louis, MO, USA) and amino acid standards were obtained from Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). All the chemicals used in this study were of analytical grade.

Brown rice (*Oryza sativa* L.) obtained from a Korean rice cultivar Ilpum Byeo was used in this study. A typical wild Pu-erh tea, produced in the Yunnan province of China, was considered to prepare tea extracts.

2.2. Production of germinated brown rice and sample powder

One kilogram of brown rice was washed with tap water and soaked in tap water alone or three different concentrations (0.5, 1.5, and 3%; w/v) of Pu-erh tea extracts prepared in tap water for 1 h. The sample soaked in tap water alone, 0.5, 1.5, and 3% Pu-erh tea extracts was named GBR-0, GBR-0.5, GBR-1.5, and GBR-3, respectively. After soaking for 1 h, the brown rice samples were put into an incubator (35°C) for 36 h to allow germination. The rice samples were kept in net bags and moistened every 1 h by brief dipping in the respective solutions used for the pre-soaking.

The freshly harvested GBR samples were kept at -70 °C for 24 h before freeze-drying. The freeze-dried samples were ground into powder

using a commercial grinder (HIL-G-501, Hanil Co., Seoul, Korea) and filtered through a 100-mesh sieve.

2.3. Color measurement

The color values of powdered samples were determined following the Hunter's color measurement system as described earlier (Kim *et al.*, 2014). The 'L' (lightness), 'a' (redness), and 'b' (yellowness) values were measured using a Chroma Meter (CR-300, Minolta Corp, Tokyo, Japan). A calibration plate (Minolta Corp.; YCIE = 94.5, XCIE = 0.3160, YCIE = 0.330) and a standard plate (Hunter Associates Laboratory Inc., Reston, VA, USA; 'L'= 97.51, 'a'= -0.18, 'b'= 1.67) were considered for standardizing the instrument with D65 illuminant.

2.4. Determination of free amino acid

The free amino acids were analyzed following the method described by Je *et al.* (2005). The powdered sample (1.5 g) was homogenized (12000 rpm, 2 min) with 10 mL of ice-cold 6% (v/v) perchloric acid in an ice bath using an ACE homogenizer (Nissei AM-7, Nihonseikei Kaisha Ltd, Tokyo, Japan) and then kept in ice for 30 min before centrifugation (5000 rpm, 15 min). The supernatant was filtered using a filter paper (Whatman No. 41). The pH of the filtrate was adjusted to 7 using a KOH solution (33%, w/v), and centrifuged (5000 rpm, 10 min). After centrifugation, the precipitate of potassium perchlorate was removed and the pH of the mixture was adjusted to 2.2 using 10 M HCl and then the final volume of the mixture was made 50 mL with distilled water. Two milliliters of the mixture were mixed with 1 mL lithium citrate buffer (pH 2.2) and the free amino acid profile was analyzed using an automatic amino acid analyzer (Biochrom-20, Pharmacia Biotech Co., Uppsala, Sweden).

2.5. Determination of mineral content

The amount of mineral elements was measured using an inductively coupled plasma atomic emission spectrometer (ICP AES: Varian Vista, Victoria, Australia) as described earlier (Skujins, 1998). Five hundred milligrams of

powdered sample was digested in a mixture of 65% HNO₃ (15.0 mL) and 35% H₂O₂ (2 mL). The mixture was diluted with an equal volume of distilled water. The amount of different mineral elements was measured after calibrating the ICP AES with known standards.

2.6. Preparation of sample extracts for antioxidant assays

One gram of the powdered sample was extracted in 10 mL of absolute methanol using a shaking incubator (250 rpm, 25°C) for 2 h and the mixture was centrifuged (1660 × g, 15 min). The supernatant was filtered through a syringe filter (0.2 μm) and the filtrate extract was used for further analyses.

2.7. Determination of DPPH radical scavenging activity

The DPPH free radical scavenging potential of GBR samples was measured following the methods described earlier (Blois, 1958; Dhungana *et al.*, 2015). One hundred microliters of sample extract and freshly prepared 0.05% (w/v) methanolic solution of DPPH were mixed in wells of the 96-well microplate and incubated at room temperature for 30 min under dark condition. After 30 min of incubation, the absorbance values of the reaction mixtures were measured at 517 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific Oy, Vantaa, Finland). The free radical scavenging potential was calculated as follows.

$$\text{Scavenging potential (\%)} = [1 - (S - S_0)/(C - C_0)] \times 100$$

where S, S₀, C, and C₀ are the absorbance values of the sample and DPPH, sample and methanol, methanol and DPPH, and methanol, respectively.

2.8. Determination of total polyphenol content

The total polyphenol content (TPC) of GBR samples was determined according to the Folin-Ciocalteu method (Singleton *et al.*, 1999) as described by Dhungana *et al.* (2016). Fifty microliters of the sample extracts and 1 mL of 2% (w/v) aqueous Na₂CO₃ were mixed in

microtubes and allowed to react at room temperature for 3 min. After 3 min, fifty microliters of 1 N Folin-Ciocalteu reagent was mixed into the mixture and incubated at room temperature for 30 min under dark condition. The absorbance value of the reaction mixtures was measured at 750 nm using a microplate spectrophotometer (Multiskan GO; Thermo Fisher Scientific). The total polyphenol content of the samples was calculated using the calibration curve drawn using gallic acid (GA) as standard.

2.9. Flavonoid content analysis

The flavonoid content of GBR samples was measured following the procedure described earlier (Zhishen *et al.*, 1999; Dhungana *et al.*, 2016). One hundred microliters of the sample extracts, 500 μ L absolute methanol, 50 μ L 10% $AlCl_3$, 50 μ L 1 M HCl, and 300 μ L distilled water were mixed in microtubes and incubated as in the TPC determination method. After the 30 min incubation, absorbance values of the reaction mixtures were measured at 510 nm using a microplate spectrophotometer (Multiskan GO; Thermo Fischer Scientific). The flavonoid content of the samples was determined using the calibration curve plotted using quercetin as a standard.

2.10. Statistical analysis

Statistical analysis was performed through the analysis of variance using SAS 9.4 (SAS Institute, Cary, NC, USA). The significant differences between samples were determined using the Tukey test ($p < 0.05$). The average values of three replicates are reported unless otherwise mentioned.

3. Results and discussions

3.1. Color value of germinated brown rice

The Hunter's color values of GBR were significantly affected by Pu-erh tea extract treatment (Table 1). The lightness value of GBR was the highest in GBR-0 (82.7) and was significantly reduced with the concentration of tea extracts (73.96–78.88). On the contrary, the redness and yellowness values were the highest for GBR-0 (0.82 and 9.14) and significantly

increased in the extract-treated samples (1.84–2.77 and 9.47–9.77) with the concentration of the tea extracts, respectively.

Table 1. Hunter's color values of germinated brown rice (GBR) produced after soaking in different concentration of Pu-erh tea extracts

| Sample ¹⁾ | Color value ²⁾ | | |
|----------------------|--------------------------------|------------------|------------------|
| | L (lightness) | a (redness) | b (yellowness) |
| GBR-0 | 82.7 \pm 1.67a ³⁾ | 0.82 \pm 0.16d | 9.14 \pm 0.54d |
| GBR-0.5 | 78.88 \pm 0.39b | 1.84 \pm 0.09c | 9.47 \pm 0.10c |
| GBR-1.5 | 75.06 \pm 0.43c | 2.50 \pm 0.03b | 9.51 \pm 0.07b |
| GBR-3 | 73.96 \pm 0.28d | 2.77 \pm 0.03a | 9.77 \pm 0.07a |

¹⁾ GBR-0: GBR produced after soaking the rice in tap water for 1 h; GBR-0.5: GBR produced after soaking the rice in 0.5% (w/v) Pu-erh tea extract for 1 h; GBR-1.5: GBR produced after soaking the rice in 1.5% (w/v) Pu-erh tea extract for 1 h; and GBR-3: GBR produced after soaking the rice in 3% (w/v) Pu-erh tea extract for 1 h. ²⁾ L: lightness (100, white; 0, black); a: redness (–, green; +, red); b: yellowness (–, blue; +, yellow). ³⁾ Values are presented as mean \pm standard deviation of three replicates. Values followed by different letters (a, b, c, and d) in the same column indicate significant difference ($p < 0.05$, ANOVA, Tukey test).

The effects of Pu-erh tea extracts on the physicochemical characteristics and antioxidant potentials of GBR were investigated in the present study. Visible traits such as the color of a food product are determining factors that affect the willingness of consumers to buy the product (Udomkun *et al.*, 2018). The reasons for the variation in the color value of the GBR due to the tea extract treatments were not well understood in the present study.

3.2. Free amino acid content

The effect of tea extract treatments on GBR was less significant across samples when considered the free amino acid content (Table 2). A total of 25 (8 essential, 8 non-essential, and 9 other) amino acids were detected, whereas the amounts of 11 amino acids were non-detectable. The amounts of the essential, non-essential, and total amino acids were higher in the tea extract-untreated GBR than in the treated samples. However, the amount of some amino acids such as GABA was significantly higher in GBR-0.5 (0.59 mg/g) and GBR-1.5 (0.59 mg/g) than in GBR-0 (0.52 mg/g) and GBR-3 (0.51 mg/g).

Calcium plays a role in the activation of diamine oxidase activity that subsequently influences GABA synthesis (Wang *et al.*, 2016). High calcium content in Pu-erh tea might have increased the GABA content in two of the tea extract-treated GBR samples. However, the reason for reduced GABA content in GBR-3 could not be justified. Amino acids such as GABA and glycine are associated with learning and memory enhancement; stroke and neurodegenerative disease control; anxiety,

sedation, and anticonvulsant relief; and muscle relaxation functions (Mody *et al.*, 1994; Oh and Oh, 2004). The GABA-rich foods are beneficial for regulating blood cholesterol and pressure, decreasing insomnia and depression, and relieving pain (Dhakal *et al.*, 2012) inhibiting sleeplessness and autonomic disorder observed during the menopausal or presenile period (Okada *et al.*, 2000). GABA is also found as advantageous to control diabetes (Reeds, 2000).

Table 2. Free amino acid composition (mg/g of dry weight) of germinated brown rice (GBR) produced after soaking in different concentration of Pu-erh tea extracts

| Amino acid | Sample ¹⁾ | | | |
|-----------------------------------|--------------------------|-------------|-------------|-------------|
| | GBR-0 | GBR-0.5 | GBR-1.5 | GBR-3 |
| Essential amino acid | | | | |
| L-Threonine | 0.04±0.01a ²⁾ | 0.04±0.01a | 0.04±0.01a | 0.03±0.01a |
| L-Valine | 0.11±0.01a | 0.09±0.02ab | 0.09±0.01ab | 0.07±0.01b |
| L-Methionine | 0.03±0.01a | 0.01±0.01a | 0.02±0.01a | 0.02±0.01a |
| L-Isoleucine | 0.06±0.01a | 0.05±0.01a | 0.05±0.01a | 0.04±0.01a |
| L-Leucine | 0.08±0.01a | 0.07±0.01ab | 0.06±0.02ab | 0.05±0.01b |
| L-Phenylalanine | 0.08±0.01a | 0.07±0.02ab | 0.06±0.01ab | 0.05±0.01b |
| L-Lysine | 0.07±0.01a | 0.06±0.02a | 0.06±0.01a | 0.05±0.01a |
| L-Histidine | 0.09±0.02a | 0.07±0.01a | 0.06±0.01a | 0.06±0.01a |
| Sub-total | 0.56 | 0.46 | 0.44 | 0.37 |
| Non-essential amino acid | | | | |
| L-Aspartic acid | 0.04±0.01a | 0.03±0.01a | 0.04±0.01a | 0.04±0.02a |
| L-Serine | 0.09±0.01a | 0.08±0.02a | 0.10±0.01a | 0.09±0.01a |
| L-Glutamic acid | 0.31±0.03ab | 0.29±0.02b | 0.35±0.01a | 0.32±0.02ab |
| Glycine | 0.02±0.01a | 0.02±0.01a | 0.02±0.01a | 0.02±0.01a |
| L-Alanine | 0.15±0.02a | 0.14±0.01a | 0.15±0.01a | 0.14±0.01a |
| L-Tyrosine | 0.10±0.01a | 0.07±0.02b | 0.06±0.01b | 0.05±0.01b |
| L-Arginine | 0.17±0.01a | 0.12±0.01b | 0.11±0.02bc | 0.09±0.01c |
| Proline | 0.05±0.01a | 0.05±0.01a | 0.04±0.01a | 0.04±0.01a |
| Sub-total | 0.93 | 0.80 | 0.87 | 0.79 |
| Other amino acid | | | | |
| O-Phospho-L-serine | ND ³⁾ | ND | ND | ND |
| Taurine | ND | ND | ND | ND |
| O-Phospho ethanol amine | 0.02±0.01a | 0.02±0.01a | 0.02±0.01a | 0.02±0.01a |
| L-Sarcosine | ND | ND | ND | ND |
| L- α -Amino asipic acid | 0.01±0.01a | 0.01±0.01a | 0.01±0.01a | 0.01±0.01a |
| L-Citrulline | ND | ND | ND | ND |
| L- α -Amino-n-butyric acid | 0.01±0.01a | 0.01±0.01a | 0.01±0.01a | 0.01±0.01a |
| L-Cystine | ND | ND | ND | ND |

| | | | | |
|-------------------------------------|------------------|------------------|------------------|------------------|
| Cystathionine | ND | ND | ND | ND |
| β -Alanine | 0.03 \pm 0.01a | 0.03 \pm 0.01a | 0.03 \pm 0.01a | 0.03 \pm 0.01a |
| D,L- β -Amino isobutyric acid | 0.02 \pm 0.01a | 0.02 \pm 0.01a | 0.04 \pm 0.01a | 0.02 \pm 0.01a |
| γ -Amino-n-butyric acid | 0.52 \pm 0.01b | 0.59 \pm 0.02a | 0.59 \pm 0.01a | 0.51 \pm 0.02b |
| Ethanolamin | 0.01 \pm 0.01a | 0.02 \pm 0.01a | 0.03 \pm 0.01a | 0.01 \pm 0.01a |
| Hydroxylysine | ND | ND | ND | ND |
| L-Ornithine | 0.01 \pm 0.01a | 0.01 \pm 0.01a | 0.01 \pm 0.01a | 0.01 \pm 0.01a |
| 1-Methyl-L-histidine | 0.03 \pm 0.01a | 0.02 \pm 0.01a | 0.02 \pm 0.01a | 0.02 \pm 0.01a |
| 3-Methyl-L-histidine | ND | ND | ND | ND |
| L-Anserine | ND | ND | ND | ND |
| L-Carnosine | ND | ND | ND | ND |
| Hydroxy proline | ND | ND | ND | ND |
| Sub-total | 0.66 | 0.73 | 0.76 | 0.64 |
| Total free amino acid | 2.15 | 1.99 | 2.07 | 1.80 |

¹⁾ Samples are defined in Table 1. ²⁾ Values are expressed as mean \pm standard deviation of two replicates. Values followed by different letters (a, b, c, and d) in the same row are significantly different ($p < 0.05$, ANOVA, Tukey test). ³⁾ Non-detected.

3.3. Mineral content

Although the amount of total free amino acids was lower in the Pu-erh tea extract-treated GBR samples, the treatment significantly increased many mineral elements along with the total mineral content (Table 3). Out of the eight minerals measured, five were higher in one of the tea extract-treated GBR than in the untreated sample. The amounts of two minerals Ca and Cu were significantly higher in the tea extract-untreated GBR (35.35 and 31.57 mg/kg) than in the extract-treated samples (21.75–26.31 and 12.54–30.39 mg/kg), respectively. K (1417.57–1759.23 mg/kg) was the most abundant mineral in the GBR samples. The amount of total mineral content in the tea extract-treated GBR was substantially higher than that in the untreated sample.

Table 3. Mineral contents (mg/kg of dry weight) of germinated brown rice (GBR) produced after soaking in different concentration of Pu-erh tea extracts

| Element | Sample ¹⁾ | | | |
|---------|---------------------------------|----------------------|---------------------|----------------------|
| | GBR-0 | GBR-0.5 | GBR-1.5 | GBR-3 |
| Ca | 35.64 \pm 3.87a ²⁾ | 26.31 \pm 2.00b | 25.69 \pm 0.86b | 21.75 \pm 1.70c |
| Cu | 31.57 \pm 0.26a | 21.49 \pm 0.39c | 30.39 \pm 0.26b | 12.54 \pm 0.09d |
| Fe | 35.54 \pm 0.16b | 34.97 \pm 0.52b | 43.99 \pm 1.48a | 22.62 \pm 0.16c |
| K | 1417.57 \pm 21.13d | 1644.99 \pm 10.29c | 1674.45 \pm 4.56b | 1759.23 \pm 10.21a |
| Mg | 1025.00 \pm 11.78a | 1043.35 \pm 8.95a | 967.16 \pm 5.01b | 984.63 \pm 1.76b |
| Mn | 19.45 \pm 0.32c | 22.05 \pm 0.31b | 22.16 \pm 0.01b | 23.89 \pm 0.04a |
| Na | 181.23 \pm 0.23b | 179.91 \pm 1.51b | 186.86 \pm 0.82a | 167.19 \pm 2.63c |
| Zn | 35.12 \pm 0.23b | 22.88 \pm 0.11c | 41.34 \pm 0.31a | 20.35 \pm 0.08d |
| Total | 2781.12 | 2995.95 | 2992.04 | 3012.23 |

¹⁾ Samples are defined in Table 1. ²⁾ Values are expressed as mean \pm standard deviation of two replicates. Values followed by different letters (a, b, c, and d) in the same row are significantly different ($p < 0.05$, ANOVA, Tukey test).

Like the GABA content, the mineral content of the GBR samples was increased by

soaking brown rice in the mineral-rich Pu-erh tea (McKenzie *et al.*, 2010). Similar results of higher zinc content were found in the zinc sulfate-applied soybean sprouts (Xu *et al.*, 2012; Zou *et al.*, 2014). Minerals, including Fe and Zn, which were increased in some of the Pu-erh tea extract-treated GBR samples, are some of the most commonly lacking elements in human diets (White and Broadley, 2009). Minerals Mg and K are beneficial against hypertension (Houston and Harper, 2008); Fe plays roles in oxygen transport, energy metabolism, mitochondrial respiration, DNA synthesis, and cellular growth and differentiation (Ganz, 2013); Zn contributes to the growth, development, differentiation, DNA synthesis, RNA transcription, and cellular apoptosis (MacDiarmid, 2000).

3.4. DPPH inhibition activities and total polyphenol and flavonoid contents

The antioxidant potentials of the GBR samples were determined through DPPH inhibition activities as well as total polyphenol and flavonoid contents (Table 4). The DPPH free radical scavenging potentials of GBR-1.5 (72.49%) and GBR-3 (76.18%) were significantly higher than that of GBR-0 (62.56%) and GBR-0.5 (62.58%). The total polyphenol content was highest in GBR-0.5 (164.19 $\mu\text{g GAE/g}$) followed by GBR-0 (152.58 $\mu\text{g GAE/g}$), GBR-1.5 (143.38 $\mu\text{g GAE/g}$), and GBR-3 (130.53 $\mu\text{g GAE/g}$). The flavonoid contents of GBR-1.5 (36.17 $\mu\text{g QE/g}$) was the significantly lowest among the samples (39.69–41.48 $\mu\text{g QE/g}$).

Several reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radical, and singlet oxygen cause oxidative damage in lipids, proteins, and DNA (Santos *et al.*, 2003). Production of excessively high levels of the ROS may harm the cells by lipids peroxidation, proteins oxidation, nucleic acids destruction, enzyme inhibition, programmed cell death activation pathway, and eventually cells death (Mishra *et al.*,

2011; Srivastava and Dubey, 2011). The higher levels of biosynthesis of antioxidants in the tea extract-treated samples might be resulted from the high contents of elements like calcium (McKenzie *et al.*, 2010) and/or phenolic compounds (Zhang *et al.*, 2012; Chen *et al.*, 2017) in Pu-erh tea.

Table 4. DPPH inhibition activities and total polyphenol and flavonoid contents of germinated brown rice (GBR) produced after soaking in different concentration of Pu-erh tea extracts

| Sample ¹⁾ | DPPH (% inhibition) | Total polyphenol ($\mu\text{g GAE}^{2)/\text{g}$) | Flavonoid ($\mu\text{g QE}^{3)/\text{g}$) |
|----------------------|---------------------------------|---|---|
| GBR-0 | 62.56 \pm 1.22c ⁴⁾ | 152.58 \pm 3.26b ⁴⁾ | 39.69 \pm 1.88a |
| GBR-0.5 | 62.58 \pm 2.05c | 164.19 \pm 0.72a | 41.35 \pm 0.68a |
| GBR-1.5 | 72.49 \pm 1.64b | 143.38 \pm 2.68c | 36.17 \pm 1.59b |
| GBR-3 | 76.18 \pm 2.12a | 130.53 \pm 0.77d | 41.48 \pm 2.60a |

¹⁾ Samples are defined in Table 1. ²⁾ GAE: gallic acid equivalents. ³⁾ QE: quercetin equivalents. ⁴⁾ Values are expressed as mean \pm standard deviation of three replicates. Values followed by different letters (a, b, c, and d) in the same column are significantly different ($p < 0.05$, ANOVA, Tukey test).

The results of this study are in agreement with that of previous reports of high phenolic contents in the germinated brown rice when the rice was treated with high phenolic-containing onions (Gennaro *et al.*, 2002; Griffiths *et al.*, 2002; Nakamura *et al.*, 2020). Phenolic compounds have antioxidant potentials (Rice-evans *et al.*, 1995; Yang *et al.*, 2015) that can scavenge harmful free radicals. In addition to phenolic compounds, various enzymatic and non-enzymatic antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, vitamin C, vitamin E, polyphenols, and carotenoids possess the free radical scavenging potentials. The antioxidant potential of foods is a multifaceted outcome of several factors, such as the partitioning properties of specific

antioxidants, oxidation conditions, and the physical state of the oxidizable substrate (Frankel and Meyer, 2000). Thus, a visible difference in the level of an individual antioxidant such as total polyphenol and/or flavonoid might not always account for higher antioxidant activity, as in the GBR samples with higher DPPH radical scavenging potential but lower total polyphenol and/or flavonoid.

4. Conclusions

The influence of Pu-erh tea extracts on GBR was investigated considering the physicochemical characteristics and antioxidant potentials. The color values of GBR were significantly modified with the tea extract treatments. Although the total free amino acids of the extract-treated GBR samples were lower than the untreated one, the amount of some amino acids like GABA were increased. In addition, the amount of total minerals and antioxidant potentials of the extract-treated GBR samples were higher than that of the untreated one. Overall, the nutritional and functional properties of brown rice could be enhanced by soaking it in Pu-erh tea extracts (0.5–3%, w/v).

5. References

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