

Cold plasma decontamination of brown rice grains: Impact on biochemical and sensory qualities of their corresponding seedlings and aqueous tea infusions

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ARTICLE INFO

Keywords:

Corona discharge plasma
brown rice
Decontamination
Germination
Sensory quality

ABSTRACT

Germinated brown rice is nutritionally superior to its ungerminated counterpart. However, germination may allow multiplication of grain-associated contaminants. Therefore, in the present study, the suitability of corona discharge plasma jet (CDPJ) as a disinfectant prior to germination was tested. CDPJ was generated under atmospheric pressure conditions using a 20 kV DC power source and at 1.5 A current. CDPJ treatment of brown rice was found effective in the decontamination of grain contaminants, namely aerobic bacteria as well as yeasts and molds. Moreover, the treatment unaffected germination rate and positively affected the weight and length of their (treated grains) corresponding seedlings. DPPH and ABTS radical scavenging activities as well as total phenolic content of the seedlings were positively influenced by CDPJ treatment. Also, the plasma treatment did not alter the sensory qualities of corresponding aqueous tea infusions. In conclusion, CDPJ could potentially be used for the improvement of microbial quality and safety of brown rice grains, with positive effects on biochemical qualities and no negative effects on sensory characteristics of their corresponding seedlings and aqueous tea infusions.

1. Introduction

Diets rich in whole grains have received greater attention owing to their potential to lower the risk of chronic diseases (Shi et al., 2018). Brown rice as a whole grain is a rich source of bioactive compounds that are readily water-soluble such as polyphenols, γ -aminobutyric acid (GABA), dietary fiber, vitamins, and trace minerals (Cho & Lim, 2016). However, poor taste as well as hard texture of brown rice hardly meet the requirements of consumers when treat as a staple food (Shi et al., 2018). Germination or sprouting is a popular approach to enhance the nutritional value and texture of brown rice. During germination of cereal grains including rice, hydrolytic enzymes (e.g. α -amylase) are activated and they degrade starch, protein, and non-starch polysaccharides, which leads to the increase of oligosaccharides and amino acids (Manna, Naing, & Pe, 1995; Ohtsubo, Suzuki, Yasui, & Kasumi, 2005). Compared with regular brown rice, germinated brown rice (GBR) contains relatively high levels of certain nutrients, such as phenolic compounds, γ -oryzanol, and GABA (Caceres, Martinez-Villaluenga, Amigo, & Frias, 2014). In addition, the concentrations of dietary fiber, lysine, ferulic acid, inositols, phytic acid, minerals (magnesium, zinc and potassium), vitamins (including vitamin B1, B6,

niacin and vitamin E), and prolyl endopeptidase inhibitor have been shown to improve significantly during the brown rice germination (Kayahara, 2001, pp. 4–6; Kayahara & Tsukahara, 2000). Phenolic compounds have free radical scavenging potentialities and antioxidant properties, and are known to exert different physiological effects in humans, such as decreasing the risk of coronary heart disease and cancer, preventing oxidative damage of lipid and low-density lipoproteins, and inhibiting platelet aggregation (Tian, Nakamura, & Kayahara, 2004). To estimate antioxidant activity, 2,2'-diphenylpicrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays are the popular ones because of their speed, ease, sensitivity, and the usage of stable radicals (Olszowy & Dawidowicz, 2018).

On the other hand, the multiplication of rice grain-associated microorganisms has been shown in germination process due to the occurrence of warm and humid environment (Lu et al., 2010). The occurrence of both beneficial and harmful microorganisms in brown rice has been shown (Panneerselvam, Binodh, Kumar, Sugitha, & Anandan, 2017, pp. 159–181). It has also been shown that the microbial load of brown rice was higher than that of white rice (Martinez et al., 2013). Brown rice was shown to be contaminated with total mesophilic

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bacteria, total coliforms, and *Staphylococcus aureus* in the levels of 3.7, 1.3, and 2.5 log CFU/g, respectively (Chang, Han, Song, Chung, & Shin, 2004). In another study, the counts of total mesophilic bacteria, total coliforms, thermotolerant coliforms, enterococci, and yeast and mold in brown rice seeds were reported to be 7.45, 3.08, 2.28, 1.30, and 4.41 log CFU/g, respectively (Piernas & Guiraud, 1997).

Different strategies to control microbial growth have been suggested. Periodical washing, soaking, and atmospheric germination have been recommended to suppress off-flavor formation and microbial growth (Panneerselvam et al., 2017, pp. 159–181). Rinsing in electrolyzed water or chitosan solution was suggested in order to control microbial growth (Lu et al., 2010; Oh & Choi, 2000). On the other hand, extended period of germination is undesirable since it may lead to excessive growth of root and sprout as well as microbial growth. The application of controlled germination (which refers to the controlled application of novel physical energy forms [e.g. acoustic energy, electric energy, hydrostatic pressure, light, magnetic fields, and nonthermal plasma] to stimulate germinating seeds, for the purpose of enhancing the production and accumulation of health-promoting compounds) is of emerging interest (Ding & Feng, 2019). In the present study, the applicability of corona discharge plasma treatment was tested to reduce the microbial load of brown rice grains; and the effects of the treatment on germination rate, seedling growth, biochemical characteristics, and sensory qualities of the corresponding sprouts and teas were evaluated.

2. Materials and methods

2.1. Brown rice

Packaged brown rice was purchased from a local grocery store (emart) in Seongnam, Korea. The rice was stored at room temperature (25 °C) for 1 week before use in experiments.

2.2. Detection of microbial contaminants

Into a stomacher bag (3M Microbiology, St. Paul, MN, USA) containing 3 g of brown rice grains, 27 mL of sterile saline (0.85% w/v NaCl) solution was added. Then, using a paddle blender (IUL Instruments, Barcelona, Spain), the mixture was homogenized for 3 min. Under aseptic conditions, 1.0 mL aliquots from the stomacher bag filtrate were serially diluted (seven 10-fold serial dilutions) using sterile saline solution, followed by plating in Petri dishes (pour plate method) containing melted agar culture media (general-purpose media or selective media) (~15 mL each) and finally incubated at 37 °C for 24–48 h. The same procedure was used to estimate the level of microbial contaminants during seedling stage.

Two types of microbial growth media, namely general and selective, were used to detect contaminants. Viable microbial counts were enumerated by the standard plate count method (KFDA, 2011). General-purpose media, namely plate count agar (PCA) and potato dextrose agar (PDA), were used for the detection of mesophilic aerobic bacteria and molds and yeast, respectively. Selective enrichment media (Difco, Becton Dickinson and Co., Sparks, USA) used for contaminant detection include: mannitol-egg yolk-polymyxin agar, eosin-methylene blue agar, Baird-Parker agar, and xylose-lysine-deoxycholate agar for *B. cereus*, *E. coli*, *S. aureus*, and *Salmonella* spp., respectively.

2.3. Plasma treatment

CDPJ was generated using a high voltage power source (20 kV DC voltage, 58 kHz frequency, and 1.5 A current) under atmospheric pressure conditions, as described in earlier studies (Kim, Puligundla, & Mok, 2015; Puligundla, Kim, & Mok, 2017a). The plasma treatment was conducted as follows: brown rice grains taken in Petri plates (1 g each) were treated using the plasma for predetermined durations (0–10 min). A gap of 2.5 cm was maintained between the sample plate and the

plasma electrode tip during the treatment. For the enumeration of residual microbes, plasma-treated grains (1 g for each treatment condition) were placed into individual sterile stomacher bags, and was followed by the addition of 0.85% sterile saline solution (9 mL to each bag). Later, the samples were homogenized using a paddle mixer for 3 min, and viable microorganisms were detected and enumerated according to the aforementioned procedures.

2.4. Germination of brown rice

The grains of brown rice (both control and CDPJ-treated) were allowed to imbibe water by immersion in distilled water (grain-to-water ratio of 1:10) at room temperature for 24 h. Then, the untreated and treated grains were placed separately in Petri dishes and covered with two layers of filter paper (Whatman No. 2), and allowed to germinate in a plant growth chamber (Hannaek Science, Bucheon, Korea) for 4 days. Temperature and humidity during the germination were maintained at 25 °C and 95%, respectively. Samples were collected at 24-h intervals.

2.5. Sprout growth measurement

The length and weight of brown rice seedlings were randomly measured. The length of the head and stem were measured together and the weight was measured by removing excess water with tissue paper (Yuhan Kimberly, Seoul, Korea). The germination percentage (%) of the seeds was determined by the ratio of the number of germinated seeds to the number of incubated seeds after 4 days of incubation.

2.6. Preparation of aqueous infusion (tea) of brown rice

Sprouted grains were dried at 55 °C for 24 h and cooled down to room temperature. Then, samples (untreated and CDPJ-treated brown rice grains as well as un-germinated and germinated subsequently) were roasted individually by gradual increase in temperature [from room temperature (25 °C) to 245 °C] over 30 min to obtain roast aroma and pulverized for 3 s using a blender (HR 2860, Koninklijke Philips NV, Nevada, USA). For each tea sample preparation, about 5 g of ground sample was put in a bag (T & CE, Gunpo, Korea) and allowed to leach in 50 mL of boiling water for 5 min.

2.7. Modeling of inactivation

Modeling of inactivation pattern provides information needed for quantitative microbial risk assessment. It also provides tools to compare the impact of different process technologies on the reduction of microbial populations (FDA, 2000). The inactivation patterns of contaminants following CDPJ treatment were modeled using the Singh and Heldman model, a pseudo-first-order kinetics, as recommended in an earlier report (Kim et al., 2015).

2.8. Biochemical analyses

2.8.1. Alpha-amylase activity

Dried (55 °C for 24 h) test samples were pulverized using a blender. Alpha-amylase activity was measured as reported previously (Kim & Koh, 2004). Briefly, 20 mL of 0.5% saline solution were added to 1 g of pulverized sample and the mixture was subjected to extractive incubation on a shaker (MX-RL-Pro, Proneer, Gunpo, Korea) for 3 h, followed by centrifugation at 3200 rpm for 30 min. The resulting supernatant was used as enzyme solution.

To prepare starch substrate for α -amylase, 2 g of soluble starch was dissolved in 100 mL of boiling water to prepare a 2% soluble starch solution, and then mixed with 0.1 N acetic acid buffer solution (pH 6.0) in a 1:1 ratio to prepare a 1% starch solution having a pH of 6.0.

Later, 0.1 mL of the 1% starch solution and 0.1 mL of 40 mM phosphate buffer (pH 6.0) were added to a test tube and preheated in a

constant temperature (37 °C) water bath (Ultra, SejongPlus, Ilsan, Korea). Thereafter, 50 µL of the enzyme solution was added to the test tube and incubated at 37 °C for 5 min, and then 1 mL of 0.1 N HCl was added to stop the reaction. Finally, glucose content of the reaction solution was determined by dinitrosalicylic acid (DNS) method (Miller, 1959). The activity of α-amylase was expressed as units per gram of dry weight (dw) of sample.

2.8.2. Beta-amylase activity

Beta-amylase activity was measured as reported previously (Kim, 1985). Briefly, 20 mL of 0.5% saline solution was added to 1 g of the ground sample and the resultant mixture was subjected to extractive incubation on a shaker (MX-RL-Pro, Proneer, Gunpo, Korea) for 3 h, followed by centrifugation at 3200 rpm for 30 min. The resulting supernatant was used as enzyme solution. To prepare starch substrate for β-amylase, 6 g of soluble starch was dissolved in 100 mL of boiling water to prepare a 6% soluble starch solution. Then, 7.5 mL of 0.1 M acetate buffer solution (pH 5.0) and 0.5 mL of the enzyme solution were added to a test tube and incubated at 50 °C in a water bath for 30 min, followed by the addition of 0.1 N HCl 1 mL to stop the reaction. Finally, 0.5 mL of this mixture was taken to quantify formed reducing sugars by the Nelson and Somogyi method (Marais, de Wit, & Quicke, 1996). The activity of β-amylase was expressed as units per gram of dry weight of sample.

2.8.3. Extract preparation

Extracts were prepared as described previously (Park, Kang, Kang, & Shin, 2018). Briefly, fresh samples (10 g) were milled for 3 min using a blender (HR2860, Philips, Korea), followed by freeze-drying for 24 h using a lyophilizer (Ilshin Lab Corporation, Yangju, Korea). Each freeze-dried sample was pulverized in a grinder and was added to 10 times its weight of distilled water, and then extracted for 3 h in a constant temperature bath at 70 °C. The solution was filtered through filter paper (Whatman No. 2) to use the filtrate as a sample.

2.8.4. DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of samples was measured according to the method of Blois (1958). Briefly, sample (extract or tea infusion) and DPPH solution (0.2 mM in ethanol) were mixed in 1:1 ratio and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm using methanol as blank. The percentage DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(A_{517\text{blank}} - A_{517\text{sample}})}{A_{517\text{blank}}} \times 100$$

2.8.5. ABTS radical scavenging activity

Antioxidant activity was determined by 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, as reported previously (Re et al., 1999). Briefly, 7.4 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed in 1:1 ratio and incubated in the dark for 24 h to generate ABTS radicals from the ABTS salt. Then the solution was diluted to an absorbance of 0.700 ± 0.02 using phosphate-buffered saline (pH 7.4). Thereafter, for each measurement, 1.9 mL of diluted ABTS radical solution was mixed with in 50 µL of sample (extract or tea infusion), and incubated in the dark for 10 min. Finally, the absorbance of each incubated sample was measured at 734 nm.

2.8.6. Total phenolic content

Total phenolic content was determined according to the previous method (Jalili, Heydari, Sadeghzade, & Alipour, 2012). Briefly, 0.5 mL of sample (extract or tea infusion) and 2.5 mL of 10% Folin solution prepared using Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis,

MO, USA) were mixed in a test tube. To this solution, 2 mL of 7.5% Na₂CO₃ was added and incubated at 45 °C for 15 min. The absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated using a gallic acid (G7384, Sigma Chemical Co., St. Louis, MO, USA) standard curve and expressed in terms of mg gallic acid equivalent (GAE) per 100 mL.

2.9. Sensory evaluation

Sensory evaluation was conducted as reported previously (Yeh et al., 1998). Briefly, 12 students who majored in food engineering were involved in the sensory evaluation of teas made from grains treated with CDPJ. The temperature of the beverages was maintained at 25 °C, and turbidity, color, flavor, and overall acceptance characteristics were rated using the 9-point hedonic scale. (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

2.10. Statistical analyses

Results were presented as mean ± standard deviation (SD) of three replicates. Statistical analyses were conducted with the SAS software package (version 9.2; SAS Institute Inc., Cary, NC). The statistical significance ($p < 0.05$) of biochemical and sensory evaluation data was analyzed by a one-way analysis of variance (ANOVA) test (Duncan's multiple range test).

3. Results and discussion

3.1. Microbial contaminants

Aerobic bacteria as well as yeasts and molds were found to be predominant contaminants in brown rice grains; their levels were 6.24 ± 0.02 and 6.16 ± 0.04 log CFU/g, respectively. Common foodborne pathogenic bacteria, including *Bacillus cereus*, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, were not detected in the grains.

As aforementioned, the levels of aerobic bacteria and yeasts and molds in brown rice grains vary widely. The levels of contamination by these contaminants were found to be moderately high in the present study. During germination, these counts may further increase due to favorable growth conditions. In a previous study, aerobic bacterial population was shown to increase during germination; from initial 3.91 to 6.52 log CFU/g after 36 h (Lu et al., 2010). In another study, microbial population after germination for 48 h has been shown to be increased; final counts of mesophilic aerobic bacteria, total coliforms, thermotolerant coliforms, enterococci, and yeast and molds were 8.92, 7.32, 7.00, 6.51, and 5.77 log CFU/g, respectively (Piernas & Guiraud, 1997). Such a high multiplication rate of microbes could render GBR unsuitable to use as a ready-to-eat food (e.g. tea infusion). According to the New Zealand Ministry of Health, the acceptable limit of mesophilic aerobic microflora in ready-to-eat foods is below 10^6 CFU/g (MoH, 1995). Therefore, lower levels of contaminants in seeds prior to their germination would be beneficial.

3.2. Inactivation of contaminants

Upon CDPJ exposure, the contaminants of the grains were inactivated in a time-dependent manner. Nearly 1.0 log reduction of both aerobic bacteria and yeasts and molds was noted within 5 min of the treatment. In 0–10 min treatment, the viable counts of aerobic bacteria and yeasts and molds were decreased by 1.74 and 1.53 log CFU/g, respectively, compared with untreated controls. The microbial susceptibility to the plasma seems to vary widely, depending primarily on the structure and chemical composition of microbes. In an earlier study, bacteria have been shown to be more susceptible to low temperature plasma compared with fungi (Soušková et al., 2011). Similar results

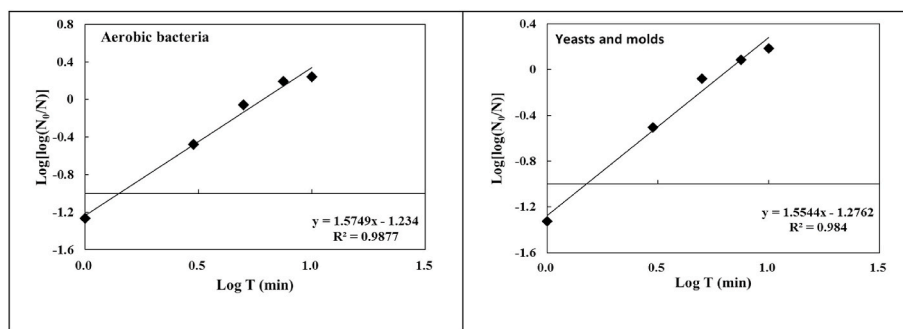


Fig. 1. Inactivation kinetics of brown rice contaminants upon treatment with the CDPJ (based on Singh-Heldman model).

have been reported in a previous study using CDPJ (Puligundla, Kim, & Mok, 2018).

Corona discharges generated in air under atmospheric pressure exhibit a strong antibacterial effect. These discharges are known to produce chemically active species such as reactive oxygen species and other charged species having strong oxidation potential (Deng et al., 2007). To control microorganisms during processing of GBR, novel disinfectants, namely ultraviolet irradiation, high-pressure treatment of rice and disinfections by electrochemical treatment, have been used (Komatsuzaki et al., 2007).

3.2.1. Modeling of inactivation

For kinetic modeling of the inactivation patterns of the contaminants, a pseudo-first-order kinetic model, namely Singh-Heldman model, was successfully applied. The assigned model exhibited a good fit to rate of inactivation, as given in Fig. 1. The coefficients of determination (R^2) closer to 1.0 indicate that the inactivation patterns could be better explained using the pseudo-first-order kinetics.

3.2.2. D' -values

D' -value is similar to, if $n = 1$, decimal reduction time (D -value) in case of heat sterilization, which is the time required at a certain temperature to kill 90% of the organisms being studied. The aerobic bacterial contaminants seem relatively more sensitive to CDPJ as they were inactivated relatively quickly compared with yeasts and molds. D' -values of the detected aerobic bacteria and yeasts and molds following CDPJ treatment were calculated to be 6.07 and 6.70 min, respectively.

3.3. Effect of CDPJ grain treatment on their germination, seedlings growth

The treatment of brown rice grains using the CDPJ for 1–10 min had no significant effect on their germination rate, as shown in Supplementary Fig. 1. The germination rates (%) of brown rice grains treated by the CDPJ for 2.5, 5.0, and 10.0 min were 97.40 ± 1.02 , 97.60 ± 0.49 , and 97.20 ± 1.17 , respectively. In the case of untreated grains, the germination rate was $98.00 \pm 0.65\%$. However, in our previous studies, it has been shown that CDPJ treatment of radish and pak choi seeds exerted a positive effect initially (up to 2-min plasma treatment) on their germination rate, but the rate was negatively impacted on further prolongation of treatment time (Puligundla et al., 2018; Puligundla, Kim, & Mok, 2017b).

CDPJ treatment of brown rice grains (up to 10 min) positively influenced the weight and length of their corresponding seedlings. Grains treated for 5 min exhibited relatively high weight and length of their corresponding seedlings on the 1st day of germination. However, on the 4th day of germination, seedlings from grains treated by CDPJ for 10 min exhibited relatively better weight and length compared with others, as shown in Fig. 2. The average weight and length of seedlings grown from the CDPJ-treated (10 min) grains were 0.050 ± 0.006 g/seedling and 1.30 ± 0.25 cm/seedling, respectively; whereas the weight and length of seedlings grown from untreated grains were

0.046 ± 0.006 g/seedling and 0.96 ± 0.11 cm/seedling, respectively.

3.4. Effect of CDPJ grain treatment on their amylase activities

During germination up to 3 days, CDPJ-treated (2.5, 5.0, and 10.0 min) grains exhibited relatively higher levels of α - and β -amylase activities compared with untreated controls, as shown in Fig. 3. CDPJ treatment time positively correlated with both α - and β -amylase levels. However, during the 4th day of germination, abrupt reductions in both α - and β -amylase activities were noted in seedlings grown from CDPJ-treated (2.5, 5.0, and 10.0 min) grains compared with seedlings grown from untreated grains. Several factors such as steeping time, steep moisture level, amylose and amylopectin composition, and germination temperature are known to influence amylase activities during cereal germination (Guzmán-Ortiz et al., 2019).

3.5. Effect of CDPJ treatment on biochemical properties

3.5.1. DPPH radical scavenging activity

CDPJ treatment positively correlated with DPPH radical scavenging activity both in ungerminated and germinated brown rice grains. Compared with controls, a significant ($p < 0.05$) improvement in the activity was noted in ungerminated grains upon CDPJ treatment for 5.0 or 10 min (Table 1). During germination (from 1st to 4th day), DPPH radical scavenging activities of corresponding seedlings of CDPJ-treated grains (at all tested treatment times) as well as untreated grains were increased significantly ($p < 0.05$) compared with grains (both untreated and CDPJ-treated) at the beginning of germination (ungerminated). The maximum DPPH radical scavenging activity was noted on the 3rd day of germination in CDPJ-treated grains and decreased thereafter. These findings indicated promising antioxidant activity of extracts of germinated brown rice grains, especially CDPJ-treated grains, compared with untreated and ungerminated grains (controls). The improved DPPH radical scavenging activity could be due to the softening of seed coat by CDPJ-derived oxidants. Seed coat is known to act as barrier to germination by preventing water absorption and conferring mechanical resistance to the protrusion of embryo. Etching effect on the seed coat due to cold plasma-induced reactions could be possible, and then some active species would penetrate into the seeds, which might benefit physiological reactions (Li et al., 2017).

3.5.2. ABTS radical scavenging activity

Similar to DPPH radical scavenging activity, CDPJ treatment positively correlated with ABTS radical scavenging activity both in ungerminated and germinated brown rice grains. Compared with untreated controls, a significant ($p < 0.05$) improvement in ABTS radical scavenging activity was noted in ungerminated grains upon CDPJ treatment for 5.0 or 10 min (Table 1). During germination (from 1st to 4th day), ABTS radical scavenging activities of corresponding seedlings of CDPJ-treated grains (at all tested treatment times) as well as untreated grains were increased significantly ($p < 0.05$) compared with

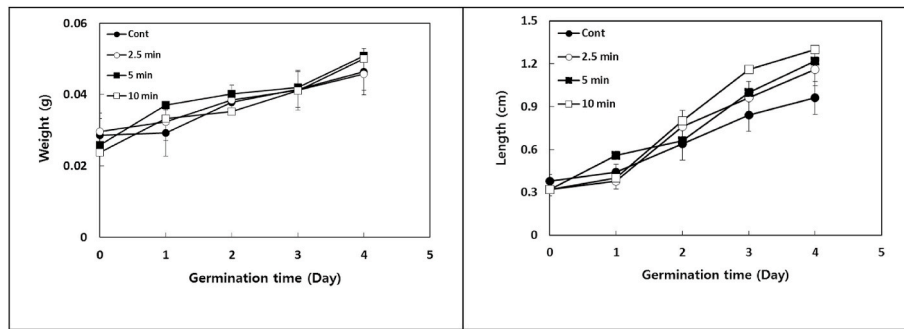


Fig. 2. Effect of CDPJ treatment of brown rice grains on the weight and length of their corresponding seedlings.

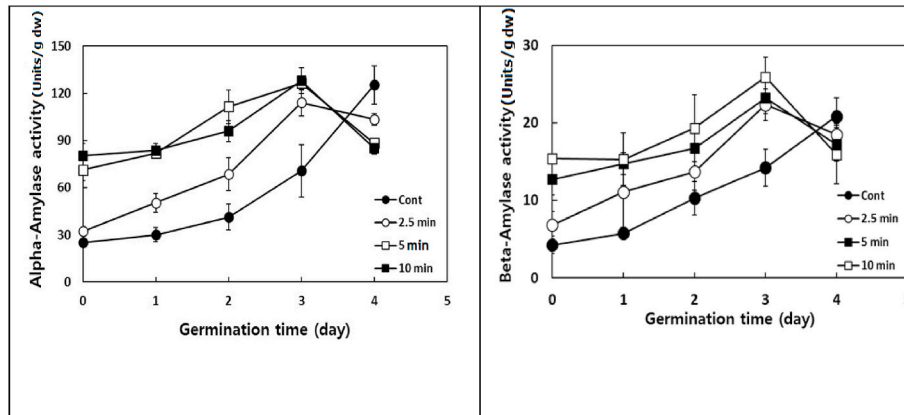


Fig. 3. Effect of CDPJ treatment of brown rice grains on α - and β -amylase activities of their corresponding seedlings.

grains (both untreated and CDPJ-treated) at the beginning of germination (ungerminated). The maximum ABTS radical scavenging activity was noted on the 3rd day of germination in CDPJ-treated grains and decreased thereafter. Mechanism similar to the aforementioned could be responsible for relatively higher ABTS radical scavenging activity in CDPJ-treated germinated grains.

3.5.3. Total phenolic content

CDPJ treatment positively correlated with total phenolic content both in ungerminated and germinated brown rice grains. Compared with untreated controls (5.18 ± 0.39 mg GAE/100 g), a significant ($p < 0.05$) improvement in phenolic content was noted in ungerminated grains upon CDPJ treatment for 5.0 min (5.93 ± 0.51 mg GAE/100 g) or 10 min (6.23 ± 0.63 mg GAE/100 g) (Table 1). During germination (from 1st to 4th day), total phenolic contents of

Table 1

Effect of CDPJ treatment of brown rice grains on DPPH radical scavenging activity (%), ABTS radical scavenging activity (%), and total phenolic contents (mg GAE/100 g) of their corresponding seedlings.

Germination time (day)	Treatment time (min)			
	Control	2.5	5	10
DPPH radical scavenging activity (%)				
0	16.16 ± 2.60^{Bc}	16.28 ± 1.16^{Cc}	19.72 ± 0.35^{Db}	22.22 ± 0.52^{Da}
1	20.95 ± 2.43^{Ab}	23.08 ± 1.50^{Bab}	23.20 ± 1.79^{Cab}	25.12 ± 0.93^{Ca}
2	23.00 ± 4.63^{Ab}	23.24 ± 0.58^{Bb}	27.50 ± 1.50^{Ba}	29.05 ± 1.16^{Ba}
3	22.34 ± 0.23^{Ac}	25.00 ± 0.75^{Ab}	31.67 ± 3.36^{Aa}	30.73 ± 1.22^{Aa}
4	22.22 ± 1.56^{Ac}	24.75 ± 0.75^{Ab}	28.72 ± 1.97^{Ba}	29.05 ± 0.35^{Ba}
ABTS radical scavenging activity (%)				
0	19.11 ± 2.31^{Db}	20.81 ± 3.52^{Cb}	21.80 ± 0.70^{Cab}	24.72 ± 1.00^{Ba}
1	22.23 ± 1.31^{Cb}	22.66 ± 0.90^{CB}	22.37 ± 3.92^{Cb}	25.92 ± 1.51^{ABa}
2	23.65 ± 0.50^{Cb}	23.30 ± 1.00^{BCb}	24.22 ± 1.10^{BCb}	27.13 ± 2.61^{Aa}
3	26.21 ± 1.71^{Ba}	27.06 ± 2.51^{Aa}	28.27 ± 2.01^{Aa}	27.77 ± 0.30^{Aa}
4	28.27 ± 0.20^{Aa}	24.15 ± 2.21^{Bb}	26.70 ± 2.41^{ABa}	25.92 ± 1.10^{ABab}
Total phenolic content (mg GAE/100 g)				
0	5.18 ± 0.39^{Cc}	5.48 ± 0.04^{Cbc}	5.93 ± 0.51^{Dab}	6.23 ± 0.63^{Da}
1	5.98 ± 0.35^{Cb}	5.98 ± 0.12^{Cb}	7.37 ± 0.75^{Ca}	7.76 ± 0.51^{Ca}
2	8.43 ± 0.82^{Bc}	8.90 ± 0.55^{Bbc}	10.12 ± 1.34^{Bab}	10.37 ± 0.90^{Ba}
3	10.46 ± 0.94^{Ab}	11.21 ± 0.67^{ABab}	11.54 ± 0.43^{Aa}	11.68 ± 0.86^{Aa}
4	10.96 ± 0.71^{Aa}	11.34 ± 0.16^{Aa}	9.46 ± 0.47^{Bb}	9.62 ± 0.79^{Bb}

For each characteristic, values in the same column followed by the same uppercase letters and in the same row followed by same lowercase letters are not significantly different ($p > 0.05$).

corresponding seedlings of CDPJ-treated grains (at all tested treatment times) as well as untreated grains were increased significantly ($p < 0.05$) compared with grains (both untreated and CDPJ-treated) at the beginning of germination (ungerminated).

In a previous study, the reactions of plasma active species (penetration of active species through the porous seed coat inside the carpopses where they react with plant cells) have been shown to be responsible for changes in phenolic compounds contents in wheat and oat seeds following a discharge plasma treatment (Sera, Spatenka, Sery, Vrchotova, & Hruskova, 2010). These authors hypothesize possible changes in metabolism processes of growing plants after plasma treatment.

Relatively high concentrations of phenolic compounds in GBR could be due to the release of cell wall-bound phenolic compounds—since the breakdown of the cell wall occurs during germination (Gujral, Sharma, Kumar, & Singh, 2012).

3.6. Antioxidant activities (DPPH and ABTS radical scavenging activities) and phenolic content of aqueous tea infusions prepared from brown rice grains

DPPH and ABTS radical scavenging activities as well as total phenolic content of aqueous tea infusions prepared from brown rice grains exhibited trends similar to the extract of brown rice. The DPPH radical scavenging activities of tea infusions prepared from ungerminated brown rice were in the range of 25.13 ± 1.88 to $36.69 \pm 2.08\%$ (Table 2). The maximum DPPH radical scavenging activity observed in teas from untreated grains was $57.21 \pm 1.46\%$ on the 4th day of germination, which was significantly ($p < 0.05$) higher than control. The maximum DPPH radical scavenging activities of tea infusions prepared from CDPJ-treated grains (2.5, 5.0, and 10.0 min) were $59.81 \pm 2.91\%$, $60.96 \pm 1.46\%$, and $61.38 \pm 6.46\%$, respectively. These maximum values were observed on the 3rd day of germination.

The ABTS radical scavenging activities of tea infusions prepared from ungerminated brown rice were in the range of 37.36 ± 0.40 to $40.77 \pm 1.21\%$. The maximum ABTS radical scavenging activity observed in teas from untreated grains was $45.17 \pm 0.20\%$ on the 4th day of germination, which was significantly ($p < 0.05$) higher than control. The maximum ABTS radical scavenging activities of tea infusions prepared from CDPJ-treated grains (2.5, 5.0, and 10.0 min) were $46.52 \pm 0.30\%$, $50.14 \pm 0.80\%$, and $51.70 \pm 0.60\%$, respectively.

Table 2

Effect of CDPJ treatment of brown rice grains on DPPH radical scavenging activity (%), ABTS radical scavenging activity (%), and total phenolic contents (mg GAE/100 mL) of their corresponding aqueous tea infusions (which were prepared following germination and roasting).

Germination time (day)	Treatment time (min)			
	Control	2.5	5	10
DPPH radical scavenging activity (%)				
0	25.13 ± 1.88^{Ec}	30.13 ± 3.54^{Cb}	32.42 ± 2.29^{Eb}	36.69 ± 2.08^{Ba}
1	29.50 ± 2.29^{Dc}	30.75 ± 4.37^{Cc}	35.23 ± 2.08^{Db}	41.17 ± 3.54^{Ba}
2	35.65 ± 2.91^{Cc}	45.23 ± 5.01^{Bb}	52.42 ± 3.13^{Ca}	55.96 ± 4.37^{Aa}
3	49.19 ± 2.50^{Bb}	59.81 ± 2.91^{Aa}	60.96 ± 1.46^{Aa}	61.38 ± 6.46^{Aa}
4	57.21 ± 1.46^{Aa}	58.67 ± 1.05^{Aa}	56.48 ± 1.24^{Ba}	57.73 ± 3.75^{Aa}
ABTS radical scavenging activity (%)				
0	38.58 ± 1.10^{Dd}	37.36 ± 0.40^{Dc}	39.28 ± 0.70^{Db}	40.77 ± 1.21^{Ea}
1	38.78 ± 0.80^{Cc}	40.13 ± 0.10^{Cc}	41.76 ± 1.81^{Ca}	43.04 ± 0.40^{Da}
2	40.77 ± 1.21^{Bd}	44.25 ± 0.90^{Bc}	47.23 ± 1.10^{Bb}	49.50 ± 2.31^{Ba}
3	43.96 ± 1.31^{Ad}	46.52 ± 0.30^{Ac}	50.14 ± 0.80^{Ab}	51.70 ± 0.60^{Aa}
4	45.17 ± 0.20^{Aa}	43.68 ± 1.10^{Bb}	46.24 ± 0.50^{Ba}	45.74 ± 1.00^{Ca}
Total phenolic content (mg GAE/100 mL)				
0	16.25 ± 0.57^{Db}	16.27 ± 0.51^{Eab}	16.81 ± 0.44^{Da}	16.69 ± 0.41^{Dab}
1	17.35 ± 0.30^{Cb}	18.16 ± 0.78^{Dab}	18.45 ± 1.08^{Ca}	18.51 ± 1.08^{Ca}
2	18.48 ± 0.74^{Bc}	19.65 ± 0.37^{Cb}	20.54 ± 0.41^{Ba}	20.73 ± 0.24^{ABa}
3	20.34 ± 0.81^{Ab}	21.50 ± 0.17^{Aa}	21.64 ± 0.84^{Aa}	21.35 ± 0.14^{Aa}
4	20.57 ± 0.68^{Aab}	20.79 ± 0.44^{Ba}	20.00 ± 0.61^{Bb}	20.49 ± 0.44^{Bab}

For each characteristic, values in the same column followed by the same uppercase letters and in the same row followed by same lowercase letters are not significantly different ($p > 0.05$).

(Table 2). These maximum values were observed on the 3rd day of germination. In a previous study, an increased ABTS radical scavenging activity was observed in germinated rough rice tea with increasing roasting temperature, roasting time, and leaching times (Lee et al., 2009).

The total phenolic contents of tea infusions prepared from ungerminated brown rice were in the range of 16.25 ± 0.57 to 16.69 ± 0.41 mg GAE/100 mL. The maximum phenolic concentration observed in teas from untreated grains was 20.57 ± 0.68 mg GAE/100 mL on the 4th day of germination, which was significantly ($p < 0.05$) higher than control. The maximum phenolic contents of tea infusions prepared from CDPJ-treated grains (2.5, 5.0, and 10.0 min) were 21.50 ± 0.17 , 21.64 ± 0.84 , and 21.35 ± 0.14 mg GAE/100 mL, respectively (Table 2). These maximum levels were observed on the 3rd day of germination.

In a previous study, 6'-O-(E)-feruloylsucrose and 6'-O-(E)-sinapoylsucrose have been shown to be the major soluble phenolic compounds in brown rice (Tian et al., 2004). During germination, a significant increase in free phenolic acid content and ferulic acid content of brown rice has been observed. The increase in free phenolic compound content in GBR suggests that it has a higher potential bioavailability and thus a higher antioxidant potential (Tian et al., 2004).

3.7. Sensory evaluation

CDPJ treatment of brown rice grains did not negatively affect the turbidities of their corresponding tea infusions (prepared from both ungerminated and germinated grains). Similar trends were observed for both color and flavor characteristics, with minor exceptions (Supplementary Table 1). Therefore, overall acceptance scores of tea infusions prepared from CDPJ-treated grains (ungerminated or germinated) did not significantly differ from untreated controls.

Brown rice, under roasting, could form a unique flavor and increase the favorite degree of consumers. It has been shown that the development of characteristic flavor in roasted brown rice is due to reactions between amino compounds and carbonyl compounds under high temperature conditions (Shi et al., 2018). Furans and pyrazines have been shown to be the predominant contributors to the aroma of roasted brown rice (Shi et al., 2018).

4. Conclusions

Aerobic bacteria and yeasts and molds were observed as contaminants in brown rice grains, and the grains are free from pathogenic bacterial contaminants. On application of CDPJ for 10 min, >1.5 log CFU/g reductions were noted for both the contaminants. CDPJ treatment of the grains did not negatively affect germination rate and positively affected the weight and length of their corresponding seedlings. Treatment using the CDPJ positively affected both α - and β -amylase levels in the grains up to 3 days of germination. The plasma treatment enhanced DPPH and ABTS radical scavenging activities as well as total phenolic content in ungerminated and germinated brown rice grains. Similar trends were observed for these biochemical parameters in aqueous tea infusions prepared from CDPJ-treated brown rice grains. CDPJ treatment of brown rice grains did not negatively affect the turbidities of their corresponding tea infusions (prepared from both ungerminated and germinated grains).

CRedit authorship contribution statement

Hyesung Park: Methodology, Investigation. **Pradeep Puligundla:** Writing - original draft, Writing - review & editing. **Chulkyoon Mok:** Conceptualization, Supervision.

Declaration of competing interest

We declare that we have no conflict of interest.

Acknowledgements

This research was supported by a Grant (18163MFD025-1) from the Ministry of Food and Drug Safety, Korea, in 2019.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2020.109508>.

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