Research Note

Effects of Millimeter Wave Treatment on the Germination Rate and Antioxidant Potentials and Gamma-aminobutyric Acid of the Germinated Brown Rice

Dong-Ho Seo, Mi-Seon Kim, Hyun-Wook Choi, Jung-Min Sung, Jong-Dae Park, and Jun-Seok Kum*

Korea Food Research Institute, Seongnam, Gyeonggi 13539, Korea

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*Corresponding Author
Tel: +82-31-780-9385
Fax: +82-31-780-9036
E-mail: jskum@kfri.re.kr

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Abstract Many studies have been conducted to promote germination in brown rice in order to improve its nutritional value in terms of enzymes, protein quality, and micronutrient content. The purpose of this study was to investigate the effect of millimeter wave on germination. Millimeter wave is a form of electromagnetic radiation with frequency between 30 and 300 GHz, and is also called ‘biomicrowave’. Millimeter wave significantly stimulated germination. Total polyphenol content and DPPH radical scavenging activity also increased, especially at high frequency and prolonged dose. However, γ -aminobutyric acid content was diminished.

Keywords: millimeter wave, brown rice, germination, antioxidant, γ -aminobutyric acid

Introduction

Upon germination, the nutritional value of brown rice improves in terms of enzymes, protein quality, and micronutrient content (1). Furthermore, germination significantly stimulates production of γ -aminobutyric acid, a compound well known to prevent hypertension and obesity, and to facilitate neurotransmission and sedation (2). Thus, germinated brown rice offers enhanced healthful benefits. Generally, brown rice is germinated by soaking in warm water (30–35°C) for 1 to 2 days, with germination rates increasing with soak time. However, fermentation occurs when seeds are soaked for over 36 h, with consequent production of undesirable odors and reduction in nutritional quality. Therefore, several studies have been conducted to promote germination. For instance, Liu *et al.* (3) demonstrated that acidic electrolyzed water promoted growth better than tap water. Treatments with ultrasonic and electrical stimuli, as well as high-voltage electric fields, also accelerated germination (4,5).

In particular, electromagnetic radiation has been demonstrated to be strongly associated with plant growth. Indeed, irradiation with low-power microwave, which does not generate heat, stimulated in a dose-dependent manner the germination of wheat (*Triticum aestivum*), bengal gram (*Cicer arietinum*), green gram (*Vigna radiate*), and moth bean (*Vigna aconitifolia*) (6). On the other hand, exposure to an extremely low-frequency electromagnetic field (50 Hz, 15 μ T) for 13 weeks significantly enhanced the growth of cork oak (*Quercus*

suber), while treatment of duckweed (*Lemna minor* L.) for 2 h with an electromagnetic field (23 V/m electric field of 900 MHz) strongly inhibited growth (7,8).

Millimeter wave is a form of electromagnetic radiation with frequency between 30 and 300 GHz and is also called ‘biomicrowave’ (9,10). Investigators have examined the application of millimeter wave in biology and medicine, and its effect on growth has been debated for over 30 years (10–12). The purpose of this study was to investigate the effect of millimeter wave on the germination of brown rice.

Materials and Methods

Millimeter wave treatments Brown rice samples were exposed to single-frequency millimeter wave in an instrument (Nizhniy Novgorod, Russia) consisting of a control system, oscillator, and transit generator (Fig. 1). Brown rice samples were germinated and irradiated with 60 and 70 GHz millimeter waves simultaneously. Non-irradiated samples were used as control.

Preparation of germinated brown rice Fresh brown rice was purchased from Agricultural and Marine Products Wholesale Market (Seongnam, Korea), sterilized in 0.01% sodium hypochlorite for 30 min, and then extensively washed in distilled water. To germinate, 300 g rice was placed in a stainless wicker tray, covered with wet

Fig. 1. Block diagram of millimeter wave system.

gauze, and incubated at 30°C for 24 h with millimeter wave irradiation. Thereafter, seeds were soaked in distilled water every 4 h. Germinated brown rice was ground to powder and stored at −20°C in airtight containers until analysis.

Germination rate One hundred seeds were carefully selected and placed on two layers of moistened filter paper disks in 10 cm Petri dishes, to which 20 mL distilled water was subsequently added. Samples were germinated at 30°C in the dark for 24 h. Germination rate was calculated as percent of seeds that have germinated, a seed being considered germinated when it has grown to >2 mm in length.

DPPH radical scavenging activity, total phenolic and flavonoid contents Germinated brown rices were ground using a JL-1000 grinder (Hibell Inc., Hwaseong, Korea), and sifted through a 100-mesh sieve. Powdered sample (10 g) was extracted for 24 h at 45°C with shaking (BS-21; JEIO TECH Co., Ltd., Daejeon, Korea) in 50 mL 70% ethanol. Extracts were centrifuged at 3,000×g for 30 min, and supernatants were stored at −20°C until analysis.

Total phenolic content was measured by the Folin Ciocalteu colorimetric method (13). An aliquot of 0.125 mL extract was mixed with 0.5 mL distilled water and then with 0.125 mL Folin Ciocalteu reagent. After 6 min, 1.25 mL of 7% Na₂CO₃ in water was added, and water was used to bring the total volume up to 3 mL. Mixtures were incubated for 120 min at room temperature, and absorbance at 760 nm was measured using a V-650 UV-Vis spectrophotometer (JASCO Corporation, Tokyo, Japan). Gallic acid was used as standard, and total phenolic content is reported as mg gallic acid equivalents per 100 g dry sample.

DPPH radical scavenging activity was determined using the

method described by Blois (14). An aliquot of 0.2 mL extract was mixed with 0.8 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH). The suspension was incubated in the dark at room temperature for 30 min and absorbance was measured at 517 nm. Scavenging activity was calculated according to the following formula: DPPH radical scavenging activity (%) = 1 − (absorbance of sample/absorbance of control) × 100%. Results are reported as IC₅₀ values, the concentration required to achieve 50% scavenging activity.

Total flavonoid content was determined using a published colorimetric method (13), with modifications. An aliquot of 0.3 mL extract was diluted in 1.5 mL of distilled water, and mixed with 0.09 mL of 5% NaNO₂. After 5 min, 0.18 mL of 10% AlCl₃·6H₂O was added, and 0.6 mL of 1 M NaOH was added at 11 min. Distilled water was used to bring up the total volume to 3 mL, and absorbance at 510 nm was measured immediately using a UV-Vis spectrophotometer. Total flavonoid content is expressed as mg (+)-catechin equivalents per 100 g dry sample.

γ-Aminobutyric acid content Powdered sample (10 g) was extracted for 24 h at 4°C in 1 mL of distilled water. Extracts were centrifuged at 3,000×g for 30 min and supernatants were stored at −20°C until reacted with *O*-phthalaldehyde (15). γ-Aminobutyric acid was measured using a Waters e2695 HPLC system (Waters Co., Milford, MA, USA) equipped with an autosampler, a Zorbax Eclipse-AAA column (4.6×150 mm, Agilent Technology, Santa Clara, CA, USA), a Zorbax Eclipse-AAA guard column (4.6×12.5 mm), and a photodiode array detector set at 338 nm. The mobile phase contained 40 mM Na₂HPO₄ pH 7.8 or 45% acetonitrile and 45% methanol in deionized water. Samples were separated at 40°C and 1.5 mL/min.

Table 1. Germination rates, total polyphenols, total flavonoid and DPPH radical scavenging activity of brown rice irradiated with millimeter waves at varying frequency and dose

Sample	Germination rate (%) ¹⁾	Total polyphenols (mg/100 g dry sample) ²⁾	Total flavonoids (mg/100 g dry sample) ²⁾	DPPH radical scavenging activity (IC ₅₀ , mg/mL) ²⁾
Untreated	80.00±4.08 ^b	45.17±1.53 ^{bc}	11.45±3.20 ^a	156.15±1.16 ^a
60 GHz, 6 h	91.50±4.04 ^a	43.68±2.99 ^c	12.04±1.95 ^a	155.26±3.35 ^{ab}
60 GHz, 12 h	93.75±2.50 ^a	46.16±1.64 ^{abc}	11.06±1.41 ^a	149.93±3.79 ^{bc}
70 GHz, 6 h	93.75±2.06 ^a	49.39±0.53 ^a	10.60±1.85 ^a	146.21±2.80 ^c
70 GHz, 12 h	94.75±3.59 ^a	47.98±0.70 ^{ab}	13.35±3.75 ^a	149.99±3.63 ^{bc}

¹⁾Data are mean±standard error of the mean (n=100).

²⁾Data are mean±standard error of the mean (n=18). Values with different letters within a column differ significantly, *p*<0.05.

Statistical analysis Data are reported as mean±standard deviation of triplicate experiments, and were analyzed using SPSS 12.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA). Pearson correlation test was used to analyze correlation between variables. Significance levels were defined at *p*<0.05.

Results and Discussion

Effect of millimeter wave on germination of brown rice Table 1 lists the germination rate of brown rice irradiated with different doses of millimeter waves of varying frequency. Irradiation increased the germination rate from 80.0 to 91.5–94.8%, although frequency and dose did not affect the germination rate. Many studies have been conducted to promote plant germination by external stimuli (4–6, 16, 17). Lee *et al.* (4) demonstrated that ultrasonic treatments at 28, 40, and 60 kHz stimulated germination in brown rice. In particular, they found that the germination rate increased with dose at low frequency, but not at high frequency (4). On the other hand, tomato seeds exposed to an AC electric or magnetic field and cultured for 7 days germinated at an accelerated rate, although there was no correlation between germination rate and dose or field intensity (16). Additionally, when barley seeds exposed to 61.5 GHz millimeter wave, cell division increased by 36 and 50% at 28 and 8°C, respectively (17). It is believed that growth stimulation by electromagnetic stimuli is due to activation of enzymes, ions, and other polar substances (5, 6, 16).

Total polyphenols, flavonoids, and DPPH radical scavenging activity

Total polyphenols, flavonoids, and DPPH radical scavenging activity of brown rice treated with millimeter wave are summarized in Table 1. Total polyphenols and DPPH radical scavenging activity increased with increasing frequency and dose. In particular, rice irradiated at 70 GHz contained 48.0–49.5 mg total polyphenols per 100 g dry sample, which is significantly higher than the total polyphenols in untreated brown rice (45.2 mg/100 g dry sample). On the other hand, 70 GHz radiation significantly decreased the IC₅₀ of DPPH radical scavenging activity to 146.2–150.0 mg/mL from 156.2 mg/mL in untreated brown rice. However, total flavonoids were comparable

Table 2. γ -Aminobutyric acid (GABA) content in rice exposed to different doses of millimeter waves of varying frequency

Sample	GABA contents ¹⁾ (mg/100g)
Untreated	2.80±0.08 ^{ab}
60 GHz, 6 h	2.67±0.06 ^b
60 GHz, 12 h	2.86±0.02 ^a
70 GHz, 6 h	1.75±0.05 ^d
70 GHz, 12 h	2.09±0.11 ^c

¹⁾Data are mean±standard error of the mean (n=18). Values with different letters within a column differ significantly, *p*<0.05.

between irradiated (10.6–13.4 mg/100 g dry sample) and untreated brown rice (11.5 mg/100 g dry sample). Production of biological components including antioxidants in plants was strongly related to light sources such as UV, light-emitting diodes, and electron beams (18–20). However, stimulation of antioxidant activity by electromagnetic waves has been rarely reported. Total phenols and antioxidant activity of tomato plants treated with 290–400 nm UV was increased (18, 21), as well as of baby leaf lettuce exposed to light-emitting diodes (19). It is believed that plants synthesize phenolic compounds as a response to reactive oxygen species generated during treatment (18, 21, 22). We also believe that millimeter waves induce oxidative stress, generate reactive oxygen species, and elicit similar antioxidant responses.

γ -Aminobutyric acid contents Millimeter wave significantly reduced γ -aminobutyric acid, the major active component in germinated brown rice (Table 2). Untreated brown rice accumulated γ -aminobutyric acid to 27.96 mg/100 g dry sample. However, in brown rice irradiated at 70 GHz for 6 h, the level significantly decreased to 17.46 mg/100 g dry sample. Notably, brown rice irradiated at the same frequency for 12 h contained a higher amount of γ -aminobutyric acid at 20.96 mg/100 g dry sample. On the other hand, levels were 26.69 and 28.55 mg/100 g dry sample, respectively, in seeds exposed at 60 GHz for 6 and 12 h, confirming that 12 h irradiation reverses some of the loss in γ -aminobutyric acid, as observed at 70 GHz. Depending on species and conditions at germination, brown rice typically contains 2–10 mg γ -aminobutyric acid/100 g dry sample (23, 24). Levels we observed in our experiments are within this range. γ -Aminobutyric

acid is generated by glutamate decarboxylase, which is sensitive to environmental factors such as mechanical stimuli and oxygen deficiency (23,25). Because millimeter waves appear to generate reactive oxygen species, as indicated by increased total phenols, we believe that the oxidative stress also inhibits glutamate decarboxylase, resulting in reduced γ -aminobutyric acid content. Indeed, oxygen radicals have been known to significantly suppress enzyme activity (26-28).

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