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An improved process for high nutrition of germinated brown rice production: Low-pressure plasma

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ABSTRACT

Brown rice was exposed to low-pressure plasma ranging from 1 to 3 kV for 10 min. Treatment of brown rice in low-pressure plasma increases the germination percentage, seedling length, and water uptake in laboratory germination tests. Of the various treatments, 3-kV plasma exposure for 10 min yielded the best results. In germinating brown rice, α-amylase activity was significantly higher in treated groups than in controls. The higher enzyme activity in plasma-treated brown rice likely triggers the rapid germination and earlier vigor of the seedlings. Low-pressure plasma also increased gamma-aminobutyric acid (GABA) levels from ~19 to ~28 mg/100 g. In addition, a marked increase in the antioxidant activity of brown rice was observed with plasma treatments compared to controls. The main finding of this study indicates that low-pressure plasma is effective at enhancing the growth and GABA accumulation of germinated brown rice, which can supply high nutrition to consumer.

1. Introduction

Germination is an effective and common process used to improve the nutritional quality of cereals consumed around the world (Lee et al., 2007). During germination, some seed reserves are degraded and used for the respiration and synthesis of new cell constituents for the developing embryo, thereby causing significant changes in the biochemical, nutritional, and sensory characteristics of the cereal seed (López-Amorós, Hernandez, & Estrella, 2006). After soaking brown rice in water for several hours it is incubated under certain temperature and humidity conditions until the germinated brown rice is harvested. Germinated brown rice is popular because it contains considerably more gamma-aminobutyric acid (GABA; a non-protein amino acid which has a high biological activity) than brown rice (Liu et al., 2013).

GABA acts as an inhibitory neurotransmitter in the brain and spinal cord of mammals (Manyam, Katz, Hare, Kanifefski, & Tremblay, 1981) and shows a series of functions, such as regulation of blood pressure and heart rate, and alleviation of pain and anxiety; it is also a strong secretagogue of insulin from the pancreas (Adeghate & Ponery, 2002; Mody, De Koninck, Otis, & Soltesz, 1994). The consumption of GABA-enriched foods can also inhibit cancer cell proliferation (Park & Oh, 2007) and improve memory and learning abilities in rats (Miura et al., 2006).

Modern agricultural efforts are now in search of an efficient ecofriendly production technology based on physical treatment of seeds to increase the seedling vigor and crop establishment (Vashisth & Nagarajan, 2010). The germination, growth, yield, and quality of crops are determined by the properties of the seed material, which can be improved by a pre-sowing treatment, such as electric fields, magnetic fields, lasers, and microwaves (Aladjadjiyan, 2007). Rice seeds exposed to a weak electromagnetic field for 12 h showed significantly increased germination as well as shoot and root length of seedlings (Alexander & Doijode, 1995).

Recently, plasma-processing procedures result in an etching of brown rice surface, which allows water to be easily absorbed by the rice kernel during soaking. After plasma treatment, the cooking time of brown rice is reduced, and the cooked brown rice has a soft texture and is easier to chew (Chen, Chen, & Chang, 2012). Such methods include the use of glow discharge for sterilization, deposition and etching of thin films, and increasing the surface energy of materials. Glow discharge plasma can produce high energy electrons and other highly active species at room temperature (Zou, Liu, & Eliasson, 2004). Other applications have been conducted at pressures below 1333 Pa, where stable glow discharge plasmas were easily generated by the application of a DC electrical field to the gap between two metal electrodes (Gadri et al., 2000).

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It was previously reported that the germination time of mung beans was shortened using a thermal atmospheric plasma field (Lein, Wang, Yu, & Hurng, 2008), but no other studies of plasma have been applied to enhancing the germination of crops. Therefore, the objective of this study was to evaluate the effectiveness of low-pressure plasma on the germination and the GABA content of germinated brown rice. We look forward to utilizing this novel processing method, low-pressure plasma technology, for enhancing the germination and GABA accumulation of germinated brown rice.

2. Materials and methods

2.1. Rice sample

Taikeng 9 (TK9) brown rice, a japonica cultivar hybrid derived from the Japanese Koshihikari cultivar, was taken from the first crop of 2011 and obtained from Union Rice Co. Ltd. (New Taipei City, Taiwan). Immediately upon receipt, the brown rice was vacuum packed and stored at 4 °C.

2.2. Exposure of brown rice to plasma and rice storage

The design of low-pressure plasma is based on a previous method (Chen et al., 2012). Samples of brown rice (20 g) were placed in a 15-cm acrylic reactor. Rice samples were fairly evenly distributed in the reactor, and a vacuum system provided a pressure of 800 Pa. After reaching a suitable vacuum in the reactor, a DC high voltage supply was operated at 1–3 kV with a constant current of 1.2 mA for 10 min. Air was used as the discharge gas. In our pre-tests, a treatment time of 10 min improved the germination of the rice. Therefore, the process time was fixed at 10 min, and the influence of different voltages on the germination of brown rice was explored.

2.3. Germination tests

To determine the germination percentage, 100 kernels of whole brown rice were placed on each Petri dish and covered with wet filter paper. The Petri dishes were placed in an incubator set at 25 °C and the ratio of germinated grains was counted after 12, 18, and 24 h. After germination, samples were lyophilized, kept at 4 °C, and protected from light prior to further analysis (Chen & Sung, 2001).

The seedling length of the brown rice was measured using a Vemier caliper, and 25 samples from each treatment were measured. All measurements were conducted in triplicate.

The lyophilized grains were pulverized into rice flour in a mill. The rice flour was used for further analysis.

2.4. Seed water uptake

Seed water uptake was determined by the modified method (Chen et al., 2012). For each treatment, seed moisture at 12, 18, and 24 h of imbibition was measured. For this purpose, three replicates of 100 kernels/replicate were placed in Petri dishes, as described for the germination experiments, removed at 12, 18, and 24 h after initiation of imbibition, drained, blotted with absorbent paper, and weighed. Seed moisture was determined as [(total weight − initial weight)/initial weight] × 100. All measurements were conducted in triplicate.

2.5. Assay of α-amylase

Activity of α-amylase was estimated following the method described by Bernfeld (1955). One gram of germinating seed was ground in 10 mL of 0.1 M acetate buffer, pH 4.75, homogenized for 30 min, and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was used for the enzyme assay. Enzyme extract (0.25 mL) was taken and diluted to 1 mL. One milliliter of a 0.01% (w/v) starch solution was added and incubated for 60 min at 37 °C. Two milliliters of DNS reagent (1% dinitrosalicylic acid dissolved in 0.2 M NaOH and 30% sodium potassium tartrate) were added and kept in a boiling water bath for 5 min. After cooling in water, absorbance was measured at 540 nm. For the controls, 1 mL double-distilled water was used in place of the enzyme extract. A standard graph was prepared using glucose, and the amount of amylase present in the samples was calculated from the standard curve and expressed as mg glucose/g fresh weight. All measurements were conducted in triplicate.

2.6. GABA content measurement

One gram of rice flour was placed in a screw-top tube containing 5 mL distilled water. The mixture was vigorously mixed at 125 rpm for 90 min at 70 °C and centrifuged at 8000*g* at 4 °C for 5 min. The supernatant (200 μL) was freeze-dried, and the residue was dissolved in 40 μL of an ethanol–water–triethylamine (2:2:1) solution, and 60 μL of an ethanol–water–triethylamine–phenyl isothiocyanate (PITC) solution (7:1:1:1) was added. This mixture remained at room temperature for 20 min to allow the formation of PITC-GABA and was then filtered through a 0.45-μm filter and analyzed by HPLC according to the method described by Cho, Chang, and Chang (2007) with slight modifications as follows: A Shimadzu HPLC instrument (Shimadzu Co., Kyoto, Japan) was equipped with a Mightysil RP-18 column (GP250-4.6, 5 μm, Kanro Chemical, Tokyo, Japan), LC-10AD pumps, and an SPD-10AV UV–visible detector at 254 nm. The elution solvent system was (A) 1.4 mM sodium acetate, 0.1% triethylamine, and 6% acetonitrile (pH 6.1), and (B) 60% acetonitrile. The column was eluted with a linear gradient of 0–100% at a flow rate of 1.0 mL/min with (B) for 50 min. Authentic GABA was used as a control and to make a standard curve for calculating the amount of GABA in the samples. All measurements were conducted in triplicate.

2.7. Measurement of total phenolic compounds (TPCs)

The rice flour (approximately 2 g dry matter) was extracted with 20 mL of 80% ethanol for 30 min at room temperature and then centrifuged at 6000 rpm and 4 °C for 30 min. After centrifugation, the supernatant (0.1 mL) was mixed with 2 mL of 2% Na_2CO_3 for 3 min. After adding 0.1 mL of 50% Folin–Ciocalteau reagent, the final mixture was left for 30 min before reading the absorbance at 750 nm with a UV–visible spectrophotometer (CT-2200, ChromTech, Singapore). All measurements were conducted in triplicate, and the data were expressed as mg gallic acid equivalents (GAE)/gram dry matter, based on the calibration curve of gallic acid (Taga, Miller, & Pratt, 1984).

2.8. Antioxidant capacity

The antioxidant capacity was evaluated by ABTS and DPPH radical scavenging activity. The ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature prior to use. The $ABTS^{+\cdot}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (CT-2200 spectrophotometer, ChromTech, Singapore). For the

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