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Enhancement of gama-aminobutyric acid (GABA) and other health-related metabolites in germinated red rice (*Oryza sativa* L.) by ultrasonication

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ABSTRACT

Red rice (*Oryza sativa* L.) that has a red (reddish brown) bran layer in de-hulled rice is known to contain rich biofunctional components. Germination is an effective technique to improve the nutritional quality, digestibility, and flavor of de-hulled rice. Ultrasonication, a form of physical stimulation, has been documented as a novel approach to improve the nutritional quality of plant-based food. This study was undertaken to test the use of ultrasound to enhance the nutritional value of red rice. Ultrasonication (5 min, 16 W/L) was applied to rice during soaking or after 66 h germination. Changes of metabolites (amino acids, sugars, and organic acids) in red rice treated by ultrasonication were determined using a GC/MS plant primary metabolomics analysis platform. Differential expressed metabolites were identified through multivariate statistical analysis. Results showed that γ-aminobutyric acid (GABA) and riboflavin (vitamin B_2) in red rice significantly increased after germination for 72 h, and then experienced a further increase after treatment by ultrasound at different stages during germination. The metabolomics analysis showed that some plant metabolites, i.e. GABA, O-phosphoethanolamine, and glucose-6-phosphate were significantly increased after the ultrasonic treatment (VIP $>$ 1.5) in comparison with the untreated germinated rice. The findings of this study showed that controlled germination with ultrasonic stress is an effective method to enhance GABA and other health-promoted components in de-hulled rice.

1. Introduction

Rice (*Oryza sativa* L.), as a staple food crop that has nourished human civilization for thousands of years, is widely grown in more than 100 countries, especially in Asia, Latin America, Caribbean, and Africa [1]. De-hulled rice has a variety of natural colors such as brown, red (reddish brown), and black (purplish black), depending on the anthocyanin content in the episperm [2]. Red rice is reported to be rich in nutritional and biofunctional components such as proanthocyanidins, γ-aminobutyric acid (GABA), γ-oryzanol, dietary fibers, vitamins, and minerals compared with common brown rice [2,3]. Higher contents of flavonoids and polyphenols are often linked with higher antioxidant activities [4]. Therefore, whole-grain red rice is regarded as a good source of nutritional grain-based foods.

Germination is a biochemical process that can effectively improve the nutrients in whole grains [5–7]. Germinated grain products have been used as new ingredients in the food industry for enhancing nutritional value, mineral absorption, taste and flavor [6]. Among the health-promoting micronutrients that are documented in the germinated de-hulled rice, GABA has drawn more interests because of its special bioactivity in reducing blood pressure [8], inducing relaxation and enhancing immunity [9], improving brain function, and postponing intelligence degradation [10]. In plants, GABA is primarily metabolized via a short pathway named GABA-shunt, where GABA is derived from L-glutamate catalyzed by glutamate decarboxylase (GAD); however, it can also be converted reversibly to succinic semi aldehyde (SSA) by GABA transaminase (GABA-T) [11,12].

Most vitamins are synthesized from amino acids as precursors in plants, thus are linked to plant nitrogen metabolism. Riboflavin (i.e. vitamin B_2) derived from guanosine-5′-triphosphate (GTP) is reported

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to promote increase of coenzyme pyridoxal phosphate (PLP) and vitamin B_6 [13]. In plants, PLP-dependent enzymes play key roles in many pathways including GABA shunt, and abiotic stresses result in significant increases in PLP levels [13]. In human, PLP is beneficial to reducing blood sugar and lipids, whereas vitamin B_6 has the potential to reduce cardiovascular risks and is involved in the synthesis of a number of neurotransmitters including GABA [13,14]. Riboflavin plays an important role in human vision, and its deficiency leads to night blindness and circadian rhythms disorders [15]. Riboflavin has been recorded to increase by 135.5% in wheat grain after sprouting at 28 °C for 96 h [16].

Efforts have been made in recent years to enhance GABA and other nutrients in germinating de-hulled rice. Ng et al. reported an increase in GABA, total tocopherols, total tocotrienols and γ-oryzanol, when de-hulled rice was pre-soaked at 30 °C and 25 °C [17]. Since GABA functions as a signal to induce higher resistance of plants to abiotic stresses, elicitation has been used to enhance GABA, and phenolics and antioxidants as well in seeds during the germination process. In a recent study, Ding et al. found that GABA content was significantly increased in dehulled rice after controlled germination, especially under hypoxia (CO_2) for 6 h [18]. Other health-beneficial and/or flavor-related compounds such as lysine and D-mannose were also increased after the hypoxic treatment.

Ultrasonic waves ranging in frequency from 20 to 100 kHz have been long used as an industrial surface-cleaning tool, with other potential applications in the food industry reported in recent years [19–21]. Ultrasound, as an emerging non-thermal processing technology, could be used to enhance production of bioactive compounds including primary and secondary metabolites in plant-based foods [22,23]. Yang et al. showed that GABA content was increased by 43.4% in soybean sprouts when ultrasonic treatment (40 kHz, 300 W) was applied during the soaking process for 30 min [24]. Rodriguez et al. reported that ultrasonic treatment (25 kHz, 5.1 W/L) for 10–30 min enhanced vitamins B_1, B_2, B_3 in pineapple [25]. In our previous study, ultrasound (25 kHz, 26 W/L) improved the antioxidant capacity and overall quality of Romaine lettuce [26]. However, little is known about the effect of power ultrasound on the accumulation of GABA, vitamins, and other health-related metabolites in germinated de-hulled rice. In this study, we reported a new strategy to enhance GABA and riboflavin in red rice during germination by ultrasonic stimulation. The metabolic responses of the germinating red rice to ultrasound was also explored.

2. Materials and methods

2.1. Preparation of germinated red rice (GRR) samples

Red rice cv. Gangteyou 37 grown and harvested in the experimental farm of the Huanggang Academy of Agricultural Sciences located at Yingcheng City (113°57′ E, 30°93′ N), Hubei Province, China was used in this study. The de-hulled red rice was shown in Fig. S1. Following the method of Ding et al. [18], the de-hulled red rice was soaked in 0.1% NaOCl solution for 10 min for surface sterilization and then rinsed with sterile water. After soaking in water at 26 ± 2 °C for 12 h, the rice sample was placed in germination trays in a growth chamber at 28 ± 1 °C with moisture supplied by an ultrasonic humidifier (> 95%). Ultrasound was applied to the red rice soaked for 12 h or the GRR (germinated red rice) at 66 h of germination for 5 min, respectively. The ultrasonic treatment protocol and set up are shown in Section 2.2. Following the treatments, the samples were placed back in the same growth chamber for germination up to 72 h. The GRR sample without ultrasonic treatment was used as the control. After treatments, all the samples were frozen in liquid nitrogen immediately, freeze-dried for 3 days, ground to powder, then stored at −80 °C for future analysis.

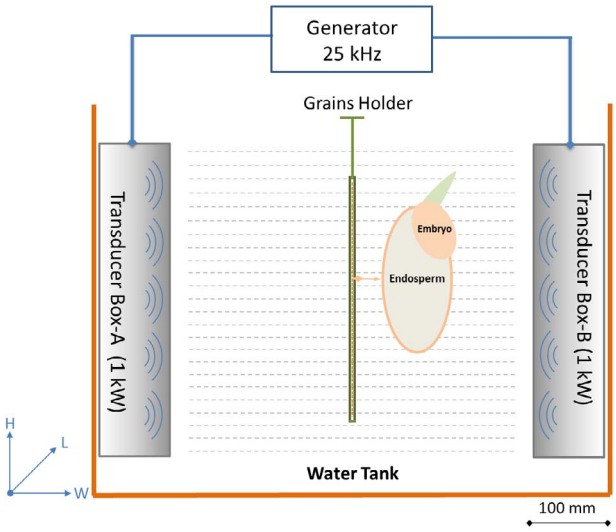

Fig. 1. Schematic diagram of the lab-scale ultrasonic tank system.

2.2. Ultrasonic treatment

The method described by Yu et al. was used with slight modifications to treat red rice samples [26]. As shown in Fig. 1, the system consisted of an ultrasound generator (25 kHz) and a stainless-steel water tank (height 500 mm × length 660 mm × width 460 mm). Two transducer boxes (1 kW each, height 400 mm × width 400 mm × thickness 90 mm) were installed face to face to the inner walls of the tank in an upright position, with a spacing of 480 mm between them. A specially designed grain sample holder (height 250 mm × width 400 mm × thickness 8 mm) was used to hold the grain samples, which was parallel with the transducer boxes. This setup allowed the samples to be exposed to a relative uniform ultrasound field in the room temperature water. Germinated red rice samples were continuously treated for 5 min (acoustic power density is 16 W/L by volume and 1.25 W/cm^2 by area, amplitude 100%, temperature 23–24 °C).

2.3. Quantitative analysis of riboflavin and GABA

Riboflavin content was determined according to the protocol of Kim et al. [27]. GABA content was analyzed following the method of Yang et al. [28] using a Waters e2695 high performance liquid chromatography (HPLC) with a ZORBAX Eclipse AAA reversed-phase column (3.5 μm), 4.6 mm × 150 mm. The HPLC run time for the separation of GABA from other compounds was less than 30 min with a column temperature of 35 °C. To extract GABA, 1 mL of sample was mixed with 1 mL of $NaHCO_3$ (1 M, pH 9) and 1 mL dansyl chloride (1 mg/mL, in acetone) in the umber tube, and reacted at 69 °C for 10 min. The reaction was stopped by putting the tubes into an ice bath. Two mobile phases were used: 0.045 mol/L CH_3COONa (pH 4) and acetonitrile at a flow rate of 0.5 mL/min. The GABA peak and peak area were detected and quantified at 425 nm using a Waters 2489 UV/Visible detector.

2.4. GC/MS analysis of metabolomics

Metabolomics analysis was performed following the methods of Roessner et al. [29] and Ding et al. [18] with minor modifications. Metabolites in the samples were extracted by ultrasound (30 s pulse ON followed by 45 s pulse OFF) for 5 min using a QSonica Q700 ultrasonic homogenizer (QSonica LLC, CT, USA) with 1 mL of methanol: chloroform (1:2) in a tube. After centrifugation (15,000g for 5 min), the supernatant was dried under vacuum and then extracted by ultrasound for the second time with 1 mL of 70% methanol following by centrifugation