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Dual effects of slightly acidic electrolyzed water (SAEW) treatment on the accumulation of γ-aminobutyric acid (GABA) and rutin in germinated buckwheat

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ABSTRACT

In the present study, the dual effects of slightly acidic electrolyzed water (SAEW) treatment on γ-aminobutyric acid (GABA) and rutin accumulation of germinated buckwheat were evaluated during germination. The results showed that SAEW treatment (pH 5.83, ACC of 20.3 mg/L) could promote the accumulation of GABA and rutin in germinated buckwheat. The GABA and rutin contents of SAEW-germinated buckwheat reached 143.20 and 739.9 mg/100 g respectively, which is significantly higher than those of control ($P < 0.05$). Moreover, SAEW treatment could increase the activity of glutamic acid decarboxylase (GAD) and phenylalanine ammonialyase (PAL) and thus result in the GABA and rutin accumulation of germinated buckwheat. The results suggested that SAEW treatment could promote the rutin accumulation of germinated buckwheat by influencing phenylpropanoid secondary metabolic pathway instead of the inhibition of rutin degrading enzyme (RDE) activity. In addition, SAEW treatment had no adverse impact on the sprouts growth and could reduce the microbial populations of germinated buckwheat during germination.

1. Introduction

Being the only cereal contains rutin in seeds, buckwheat (*Fagopyrum spp.*), which has two main species including common buckwheat (*Fagopyrum esculentum Moench*) and Tartary buckwheat (*Fagopyrum tataricum Gaertn.*), is concerned increasingly due to its medicinal and edible values (Bonafaccia, Marocchini, & Kreft, 2003; Suzuki, Honda, Funatsuki, & Nakatsuka, 2002). The flavonoids (mainly rutin) of buckwheat have positive effects on reducing capillary vessel brittleness, improving microcirculation, and strengthening body immunity (Liu & Zhu, 2007). Moreover, buckwheat grains and its products had been demonstrated to possess high antioxidant activity due to their high contents of rutin (Guo et al., 2011; Zhang, Chen, Li, Pei, & Liang, 2010). Therefore, the accumulation or maintenance of rutin in buckwheat attract many researcher's interests during the development of buckwheat food.

In food processing, buckwheat has been used to make various healthful foods such as noodles, herb tea and crackers in Asian countries (Ren, Wu, Ren, & Zhang, 2013). During germination, many significant changes were observed in the biochemical and physical aspects that resulted in the reduction of undesirable substances and the improvement of nutritional values. Because buckwheat germination can enhance the digestibility of buckwheat protein as well as improve the quality of protein and the nutritional value of fatty acid composition, the buckwheat sprout has been considered as a new vegetable (Ghimeray et al., 2014; Ren et al., 2013). Moreover, the buckwheat sprouts are rich in flavonoid compounds (mainly rutin and quercetin), phenolic acids, amino acids, minerals, vitamins and crude fibers (Kim et al., 2008; Liu, Chen, Yang, & Chiang, 2008). The γ-aminobutyric acid (GABA), considered to have positive effects on humans and animals including lowering of blood pressure and inhibiting the proliferation of cancer cell (Xie, Xia, & Le, 2014), could accumulate as one typical functional substance during germination of some plant grain such as brown rice, beans, oats, millet, barely and so on (Bai et al., 2009; Chung, Jang, Cho, & Lim, 2009; Liao, Wang, Shyu, Yu, & Ho, 2013; Tanida, 1996; Zhang et al., 2014). However, little information on

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GABA accumulation of germinated buckwheat was reported in the previous studies.

The buckwheat sprout is easy to produce, but it is important to avoid microorganism contamination during germination, which is a common problem when producing sprouts (Liu et al., 2013). Hot water treatment at 90 °C for 90 s was effective in eliminating pathogens in artificially inoculated seeds, but the germination yield decreased significantly (Bari, Nei, Enomoto, Todoriki, & Kawamoto, 2009). Pre-soaking the seeds followed by high hydrostatic pressure treatment enhanced the inactivation of *Salmonella* on artificially contaminated alfalfa seeds but at the expense of seed viability (Neetoo & Chen, 2010). Dry heat followed by a 1.0-kGy dose of irradiation completely eliminated *Escherichia coli* O157:H7 from mung bean seeds, but decreased the length of the mung bean sprouts (Bari et al., 2009). Treatment with $Ca(ClO)_2$ was also effective for reducing the populations of *Salmonella* and non-*Salmonella* micro flora on alfalfa seeds, but the use of high concentrations of chlorine generates concerns for worker safety (Kim, Hung, Brackett, & Lin, 2003). Generally, more researches need to be conducted on methods of reducing food-borne pathogens without affecting seed viability.

In the past, the strong disinfection efficacy of electrolyzed oxidizing water (EOW) and its application in food industry was widely reported and reviewed (Hricova, Stephan, & Zweifel, 2008; Huang, Hung, Hsu, Huang, & Hwang, 2008). EOW has two main types including acidic electrolyzed water (AEW, 2.3 < pH < 2.8, available chlorine concentration (ACC) of 60–100 mg/L) and slightly acidic electrolyzed water (SAEW, 5.5 < pH < 6.5, ACC of 5–30 mg/L). Due to its neutral pH and lower ACC (5–30 mg/L), SAEW showed a promising prospect in the agricultural and food industry (Cao, Zhu, Shi, Wang, & Li, 2009). It is found that SAEW has an equivalent or high disinfectant efficacy on fresh cut cabbage and spinach compared to NaClO solution (Koide, Takeda, Shi, Shono, & Atungulu, 2009; Rahman, Ding, & Oh, 2010b). Our previous results also demonstrated SAEW had strong disinfection ability to reduce the microbial population of fresh-cut cilantro and could be an alternative of AEW and NaClO solutions (Hao et al., 2011). Moreover, our previous studies found that SAEW was effective on reducing the microbial load on brown rice and could enhance the GABA accumulation of germinated brown rice (Liu et al., 2013). In addition, electrolyzed water was demonstrated not only to reduce the quantity of microorganism on the surface of mung bean sprouts, but also to promote the growth of sprouts in our previous study (Rui, Jianxiong, Haijie, & Lite, 2011).

As endogenous phytochemicals, rutin and GABA could be found in many plants, but their contents are always very low. In the present study, the dual effects of SAEW treatment on rutin and GABA accumulation of germinated buckwheat were investigated during germination. Considering that the sprouts growth could influence on the rutin and GABA contents, the impact of SAEW on the growth of germinated buckwheat was also investigated. Moreover, the effectiveness of SAEW on reducing total bacterial counts during germination was evaluated.

2. Materials and methods

2.1. Materials

The cultivar "Heiqiao 1" of tartary buckwheat (*F. tataricum Gaertn.*) was used in this study. The buckwheat was harvested at Duolun county, Neimenggu province of China on September 10th, 2013. The dried buckwheat grains were dehusked in an experimental rubber roll sheller (THU class 35A, Satake rice machine, Tokyo, Japan). The buckwheat was sealed in plastic bags and stored at 4 °C until use.

Standard GABA and rutin were purchased from Sigma (St. Louis, MO). Analytical grade chemicals and distilled water were used in this study.

2.2. Preparation of SAEW

SAEW was prepared by using a flow type electrolysis apparatus (non-membrane electrolytic cell, model AQUACIDO NDX-250KMS, OSG Company Ltd., Japan). After preparation, SAEW was stored in polypropylene containers, and immediately used for the measurement. The pH value of SAEW was measured by a pH meter (Model 86802, Orion Inc. America) and the available chlorine concentration (ACC) was measured by the iodometric method. The pH value and ACC of SAEW used in the present study are 5.95 ± 0.1 and 20.25 ± 0.45 mg/L respectively.

2.3. Production of germinated buckwheat

300 g of dehusked buckwheat grains were washed for 2 h with 3000 mL treatment solutions (SAEW and tap water as control). After washing, the buckwheat was soaked in the treatment solution (1:3, m/v) for 12 h. The soaked dehusked buckwheat was then drained and spread on sterile cheesecloth in a plastic box with holes in the bottom in a constant temperature humidity chamber (model HWS, Ningbo Jiangnan Instrument Factory, China) at 20 ± 1 °C and relative humidity 85–90%. The same treatment solutions (1000 mL) were used to water the treated germinated buckwheat once every day for 8 days.

The GABA contents, rutin content and phenylalanine ammonialyase (PAL) activity were evaluated on 0, 1, 2, 4, 6 and 8 days respectively. The glutamic acid decarboxylase (GAD) activity and rutin degrading enzyme (RDE) activity were evaluated on 1, 2, 4, 6 and 8 days respectively. The total bacterial counts were evaluated on 0, 3, 5 and 8 days. The morphological measurements were carried out every day.

2.4. Morphological measurements of the germinated buckwheat

The sprouts length, hypocotyl length and hypocotyl diameter of germinated buckwheat were measured by a vernier caliper, and 30 sprouts from each treatment were measured during germination. The hundred-grain weight was weighted by an electronic balance (0.0001 g, model Sartorius BSA224S, Sartorius Ltd. Germany) and 100 grains from each treatment were measured. The germination rate was counted per 100 grains from each treatment. Each measurement was conducted three times.

2.5. Microbiological analysis

To enumerate the total bacterial counts, 10 g of germinated buckwheat sample was homogenized for 3 min by a homogenizer (Model DY89-1, Ningbo Xinzhi Company, China). Following homogenizing, the homogenate was combined with 90 ml of sterile 0.85% sodium chloride solution and agitated for 2 min at low speed. The aliquot was used for various serial dilutions. The total bacteria counts were determined by spreading 0.1 ml of diluted sample onto Plate Count Agar (Aoboxing Bioscience Inc, Beijing, China). The plates were incubated at 35 °C for 48 h and the colonies were counted. In this study, the total bacterial counts on germinated buckwheat samples were expressed in $\log_{10}$CFU g^{-1}.

2.6. Evaluation of GABA content of germinated buckwheat during germination

The samples were freeze-dried and ground to powder prior to analysis for the GABA content. The freeze-dried powder sample