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Low concentration of sodium bicarbonate improves the bioactive compound levels and antioxidant and α-glucosidase inhibitory activities of tartary buckwheat sprouts

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ABSTRACT

This study aimed to investigate the effects of different concentrations of sodium bicarbonate ($NaHCO_3$) on the accumulation of flavonoids, total phenolics and D-*chiro*-inositol (DCI), as well as the antioxidant and α-glucosidase inhibitory activities, in tartary buckwheat sprouts. Treatment with low concentrations of $NaHCO_3$ (0.05, 0.1, and 0.2%) resulted in an increase in flavonoids, total phenolic compounds and DCI concentrations, and improved DPPH radical-scavenging and α-glucosidase inhibition activities compared with the control (0%). The highest levels of total flavonoids (26.69 mg/g DW), individual flavonoids (rutin, isoquercitrin, quercetin, and kaempferol), total phenolic compounds (29.31 mg/g DW), DCI (12.56 mg/g DW), as well as antioxidant and α-glucosidase inhibition activities, were observed in tartary buckwheat sprouts treated with 0.05% $NaHCO_3$ for 96 h. These results indicated that appropriate treatment with $NaHCO_3$ could improve the healthy benefits of tartary buckwheat sprouts.

1. Introduction

Originating in East Asian countries, seed sprouts, which are considered functional vegetables with high nutritional and putative healthy benefits have spread to other parts of the world, such as Europe, Australia and the United States (Kim et al., 2007; Sharma, Demirci, Beuchat, & Fett, 2002; Weiss & Hammes, 2003). Many edible sprouts can be found in China, including soybean, mung bean, pea, peanut, buckwheat, garlic, radish, endive, cedrela sinensis, Chinese prickly ash, mustard, and alfalfa. In recent years, Buckwheat and, in particular, buckwheat sprouts have received attention as functional vegetables because of their beneficial nutritional contents, including high unsaturated fatty acids, amino acids, peptides, flavonoids and other phenolic compounds, anthocyanin, 2″-hydroxynicotianamine etc. (Kim, Kim, & Park, 2004; Kim et al., 2007, 2008; Koyama, Nakamura, & Nakamura, 2013; Suzuki et al., 2009).

There are two main species of cultivated buckwheat, common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn]. Buckwheat contains high concentrations of potentially beneficial components, such as monosaccharides, unsaturated fatty acid, free amino acid, vitamins and polyphenols including rutin, quericitrin and chlorogenic acid, which increase after 6–8 days of germination (Kim et al., 2004). The rutin content in the edible portion of tartary buckwheat sprouts is 3- to 31-fold greater than that in the roots or pericarp, and the free radical scavenging activity in the edible portions significantly increased during germination (Kim, Zaidul, et al., 2007). Day 8 buckwheat sprouts have the most nutrients and bioactivities, and exhibit potent hypocholesterolemic and hypotriglyceridemic activities as well as antioxidant capacity (Lin, Peng, Yang, & Peng, 2008).

As the biosynthesis of many secondary metabolites in plants is a defence response, their accumulation can be stimulated by a variety of abiotic stresses, including light, temperature, water, salt and hypoxia as well as internal factors. Such stressful abiotic conditions are considered one of the most effective strategies for improving functional metabolite production in plant tissue culture (Smetanska, 2008; Bai, Yang, Zhang, & Gu, 2013; Goyal, Siddiqui, Upadhyay, & Soni, 2014). Recently, the effects of abiotic stress, including those caused by trace elements, exogenous inducers (e.g., methyl jasmonate and salicylic acid), UV-B radiation, light conditions, salinity (NaCl), L-phenylalanine, nutrient fertilization and phytohormones, on the accumulation of phytonutrients in buckwheat and its seed sprouts have been studied more

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extensively (Hsu, Chiang, Chen, Yang, & Liu, 2008; Kim, Park, & Lim, 2011; Lim, Park, Kim, Jeong, & Kim, 2012; Park et al., 2013; Tsurunaga et al., 2013; Lee et al., 2014; Seo, Arasu, Kim, Park, & Kim, 2015). Lim et al. (2012) evaluated the effect of different concentrations of NaCl on the phenolic compounds, carotenoids and antioxidant activity in common buckwheat sprout.

Compared to the sprouts of common buckwheat, those of tartary buckwheat have received greater attention as a potential functional food because of their higher rutin content (Mukoda, Sun, & Ishiguro, 2001). However, to the best of our knowledge, there are no published reports on the effects of salinity stress on the bioactive compound accumulation and the health benefits of tartary buckwheat during germination; a deeper understanding of these topics might lead to strategies further improving the bioactive compound content and functional activities of tartary buckwheat.

Type 2 diabetes mellitus, a metabolic disorder characterized by high blood glucose levels, which accounts for approximately 90% of cases, is a major cause of ill health worldwide (Zhang et al., 2016). α-Glucosidase is one of the most important enzymes in carbohydrate digestion. A promising approach for preventing and managing of type 2 diabetes is to control postprandial blood glucose using α-glucosidase inhibitors that suppress carbohydrate digestion. Given the gastrointestinal side effects of α-glucosidase inhibition drugs, such as acarbose, miglitol and voglibose (Joshi et al., 2015), the use of potential functional food components (such as D-*chiro*-inositol [DCI], vitexin and isovitexin in mung bean sprout; flavonoids and DCI in tartarty buckwheat; phlorotannins and fatty acids in edible seaweed; and functional components in sweet potato leaf) as α-glucosidase inhibitors has attracted increasing attention (Li, Zhou, Gao, Bian, & Shan, 2009; Liu, Kongstad, Wiese, Jager, & Staerk, 2016; Wang, Li, Niu, Wang, & Chen, 2013; Yao, Chen, Wang, Wang, & Ren, 2008; Zhang et al., 2016).

DCI is a compound with an insulin-like bioactivity that in its free form has been shown to decrease plasma glucose in obese rhesus monkeys with spontaneous insulin resistance, and tartary buckwheat is an important natural source of DCI (Horbowicz, Brenac, & Obendorf, 1998; Ortmeyer, Bodkin, Lilley, Larner, & Hansen, 1993). Previous studies reported that most DCI in buckwheat exists as fagopyritols, and these can be converted to free DCI during germination (Jia, Hu, Chang, & Gao, 2015; Wang et al., 2013; Yang & Ren, 2008).

The aim of this study was to evaluate the effects of $NaHCO_3$ (baking soda), which is widely used as a leavening agent during processing of cake, pastries, and baked products, on the accumulation of flavonoids and other phenolics as well as the antioxidant activity of tartary buckwheat sprouts. Moreover, changes in the DCI content and α-glucosidase inhibition activity, during germination, were investigated.

2. Materials and methods

2.1. Chemicals and reagents

Rutin, kaempferol, quercetin, isoquercitrin, gallic acid, DCI, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and rat intestinal acetone powder were purchased from Sigma-Aldrich (Shanghai, China). HPLC grade acetonitrile and trifluoroacetic acid (TFA, 99%) were purchased from the Fisher Institute of Agricultural Products Processing, Chemicals (Shanghai, China). $NaHCO_3$, methanol and other analytical reagents were purchased from Beijing Chemical Works (Beijing, China).

2.2. Plant materials and growth conditions

Tartary buckwheat seeds were immersed in 40 °C water for 15 min and then soaked in 0 (distilled water, control), 0.05, 0.1, or 0.2% $NaHCO_3$ at room temperature for 5 h. Afterwards, the seeds were sown in plastic boxes (170 × 110 × 80 mm) with aluminium foil on top (to exclude light) and gauze at the bottom (for moisture) in a controlled environment for 120 h at 25 °C. The sprouts were sprayed with different concentrations of $NaHCO_3$ (0, 0.05, 0.1, and 0.2%) and harvested every 12 h. The harvested sprouts were dried using an electrothermal drying box at 50 °C, and then dehulled and ground in a Cyclotec™ 1093 Sample Mill (FOSS Tecator, Hoganas, Sweden) before they were stored at 4 °C until further use.

2.3. Analysis of flavonoid compounds

The extraction of flavonoid compounds was performed according to the NY/T 1295–2007 method (NY/T, 2007). The dried tartary buckwheat sprouts powders (500 mg) were mixed with 25 mL of 70% methanol at 65 °C in a conical flask for 2 h with continuous shaking; then, the mixtures were passed through Whatman #4 filter paper. The residue was re-extracted and re-filtered as above. The supernatants from these extract steps were combined and the volume adjusted to 50 mL with 70% methanol prior to analysis.

The total flavonoid content was determined using an aluminium chloride colorimetric assay (Chang, Yang, Wen, & Chern, 2002) and expressed as milligrams rutin equivalents per gram dry weight. After the samples were passed through a 0.45 μm PTFE syringe filter (Membrana, Germany), the individual flavonoid contents, including rutin, isoquercitrin, quercetin and kaempferol, were analysed by HPLC (Shimadzu LC-20A series HPLC, Tokyo, Japan). The system was equipped with a YMC ODS-A column (4.6 × 250 mm, YMC Co., Ltd., Kyoto, Japan) and the UV-detector set at 375 nm. The injection volume was 20 μL. The mobile phase was delivered at a rate of 0.8 mL/min and consisted of a mixture of 0.05% TFA (solvent A) and acetonitrile (solvent B). The gradient program was as follows: 0–8 min, 25% solvent B; 8–18 min, 25–50% solvent B; 18–30 min, 50–100% solvent B; 30–35 min, 100% solvent B; 35–45 min, 100–25% solvent B; and finally 25% solvent B for 5 min (total 50 min), which was modified from our previous study (Qin, Wu, Yao, & Ren, 2013).

2.4. Analysis of total phenolic compounds

Total phenolic compounds were extracted using the methods of Hung and Morita (2008), with a slight modification. The sample (0.5 g) was extracted twice with 15 mL of 80% ethanol at 37 °C for 30 min in a water shaker and passed through Whatman #4 filter paper. The supernatants were combined and dried using a rotary evaporator (Rotavapor R-210, Buchi Labrortechnik AG, Flawil, Switzerland) at a maximum of 60 °C. The samples were dissolved in 25 ml of methanol and stored at 4 °C until use.

The total polyphenol content was determined using the Folin-Ciocalteus method (Emmons & Peterson, 1999) and the results expressed as milligrams of gallic acid equivalents per gram dry weight of the sample.

2.5. Analysis of D-chiro-inositol

DCI in tartary buckwheat sprouts was determined according to a method established by our laboratory (Yang & Ren, 2008). In brief, 1 g of sample was mixed with 20 mL of 50% ethanol and incubated at room temperature for 30 min in a water bath with continuous shaking. The extract was passed through Whatman #4 filter paper, and 1 mL of supernatant was transferred into a vial and dried at 50 °C in an oven. The dried extract was re-dissolved in 1 mL of methanol and passed through a 0.45 μm PTFE syringe filter (Membrana, Germany) for immediate HPLC-ELSD (evaporative light scattering detector) analysis. An alltech prevail carbohydrates