



# Low concentration of sodium bicarbonate improves the bioactive compound levels and antioxidant and $\alpha$ -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 ( $\text{NaHCO}_3$ ) on the accumulation of flavonoids, total phenolics and D-chiro-inositol (DCI), as well as the antioxidant and  $\alpha$ -glucosidase inhibitory activities, in tartary buckwheat sprouts. Treatment with low concentrations of  $\text{NaHCO}_3$  (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  $\alpha$ -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  $\alpha$ -glucosidase inhibition activities, were observed in tartary buckwheat sprouts treated with 0.05%  $\text{NaHCO}_3$  for 96 h. These results indicated that appropriate treatment with  $\text{NaHCO}_3$  could improve the healthy benefits of tartary buckwheat sprouts.

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## 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, celeria 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, quercitrin 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).  $\alpha$ -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  $\alpha$ -glucosidase inhibitors that suppress carbohydrate digestion. Given the gastrointestinal side effects of  $\alpha$ -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 tartary buckwheat; phlorotannins and fatty acids in edible seaweed; and functional components in sweet potato leaf) as  $\alpha$ -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<sub>3</sub> (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  $\alpha$ -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<sub>3</sub>, 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<sub>3</sub> 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<sub>3</sub> (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  $\mu$ m 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  $\mu$ L. 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 Labortechnik 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  $\mu$ m PTFE syringe filter (Membrana, Germany) for immediate HPLC-ELSD (evaporative light scattering detector) analysis. An alltech prevail carbohydrates

ES 5  $\mu\text{m}$  column (4.6 mm  $\times$  250 mm, Alltech, Deerfield, IL) was used. The injection volume was 10  $\mu\text{L}$ . The mobile phase was 80% acetonitrile, the flow rate was set at 1 mL/min, and eluant was sent to an ELSD (Alltech, USA). The temperature of the drift tube was set at 95  $^{\circ}\text{C}$ , the nebulizing gas flow rate was 2.2 L/min, and the gain was 1.

## 2.6. DPPH radical-scavenging activity

The antioxidant activity of the total phenolic compounds extract was evaluated using a DPPH radical scavenging assay (Hirose, Fujita, Ishii, & Ueno, 2010). The phenolic extracts solution was diluted 67.5 times (2 mL) and mixed with 2 mL of methanol solution containing DPPH radicals (0.2 mM). The reaction mixtures were vortexed and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a methanol blank. The scavenging activity was calculated using the following equation:

$$\text{DPPH radical-scavenging capacity (\%)} = [(A - B)/A] \times 100. \quad (1)$$

where A = absorbance of DPPH radical + methanol and B = absorbance of DPPH radical + total phenolic compound extract.

## 2.7. $\alpha$ -Glucosidase inhibitory activity

The  $\alpha$ -glucosidase inhibition activity in DCI extracts from tartary buckwheat sprouts was determined according Nishioka, Wabata, and Aoyama (1998) with slight modifications. The  $\alpha$ -glucosidase solution was prepared as follows: rat intestinal acetone powder (500 mg) was mixed with 15 mL of 0.1 M phosphate buffer (pH 7.0) and extracted 12 times with ultrasound at 4  $^{\circ}\text{C}$  for 30 s. The extracts were combined and centrifuged at 3500g for 5 min, and the supernatant collected. This DCI-extract (50  $\mu\text{L}$ ) was diluted 125-fold and mixed with 100  $\mu\text{L}$  of  $\alpha$ -glucosidase solution before incubation in 96-well plates at 37  $^{\circ}\text{C}$  for 10 min. After pre-incubation, 50  $\mu\text{L}$  of 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 7.0) was added and the mixture incubated at 37  $^{\circ}\text{C}$  for 5 min. The absorbance was measured at 490 nm using a Smart SpecTM Plus Spectrophotometer. Buffer solution (50  $\mu\text{L}$ ) was used as a blank control. The  $\alpha$ -glucosidase inhibitory activity was expressed as percentage (%) inhibition and calculated using the following equation:

$$\alpha\text{-Glucosidase inhibitory activity (\%)} = [(A - B)/A] \times 100. \quad (2)$$

where A = absorbance of 0.1 M phosphate buffer +  $\alpha$ -glucosidase solution + 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside solution and B = absorbance of DCI extracts +  $\alpha$ -glucosidase solution + 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside solution.

## 2.8. Statistical analysis

Results are expressed as means  $\pm$  standard deviations of triplicate determinations. Multiple comparisons of means were performed using the LSD (least significance difference) test. Probability values of  $<0.05$  were considered statistically significant. All computations were performed with statistical software (SAS, version 9.1.3).

**Table 1**  
The total flavonoid content of tartary buckwheat sprouts treated with different concentrations of  $\text{NaHCO}_3$  (mg/g DW).

Treatment	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h
Water	13.11 $\pm$ 0.21a	14.96 $\pm$ 0.07a	14.61 $\pm$ 0.31c	17.04 $\pm$ 0.19b	17.37 $\pm$ 0.12d	18.06 $\pm$ 0.19b	18.5 $\pm$ 0.13c	19.52 $\pm$ 0.32c	25.20 $\pm$ 0.39b	21.44 $\pm$ 0.45c	20.76 $\pm$ 0.31c
0.05% $\text{NaHCO}_3$	13.11 $\pm$ 0.21a	15.27 $\pm$ 0.12a	18.18 $\pm$ 0.12a	18.25 $\pm$ 0.58a	19.07 $\pm$ 0.06a	19.99 $\pm$ 0.30a	21.39 $\pm$ 0.31a	22.24 $\pm$ 0.07a	26.69 $\pm$ 0.40a	25.14 $\pm$ 0.60a	23.76 $\pm$ 0.06a
0.1% $\text{NaHCO}_3$	13.11 $\pm$ 0.21a	15.13 $\pm$ 0.20a	16.80 $\pm$ 0.13b	17.15 $\pm$ 0.07b	18.71 $\pm$ 0.27b	19.27 $\pm$ 0.79ab	20.24 $\pm$ 0.19b	20.59 $\pm$ 0.77b	25.87 $\pm$ 0.14ab	24.25 $\pm$ 0.13b	21.22 $\pm$ 0.06b
0.2% $\text{NaHCO}_3$	13.11 $\pm$ 0.21a	15.10 $\pm$ 0.54a	15.08 $\pm$ 0.06c	17.09 $\pm$ 0.12b	17.75 $\pm$ 0.07c	19.16 $\pm$ 0.32ab	19.53 $\pm$ 0.26b	20.37 $\pm$ 0.42bc	25.81 $\pm$ 0.19ab	21.80 $\pm$ 0.21c	20.80 $\pm$ 0.20c

Data are expressed as mean  $\pm$  standard deviation (n = 3). Means in each column followed by different letters are significantly different ( $p < 0.05$ ).

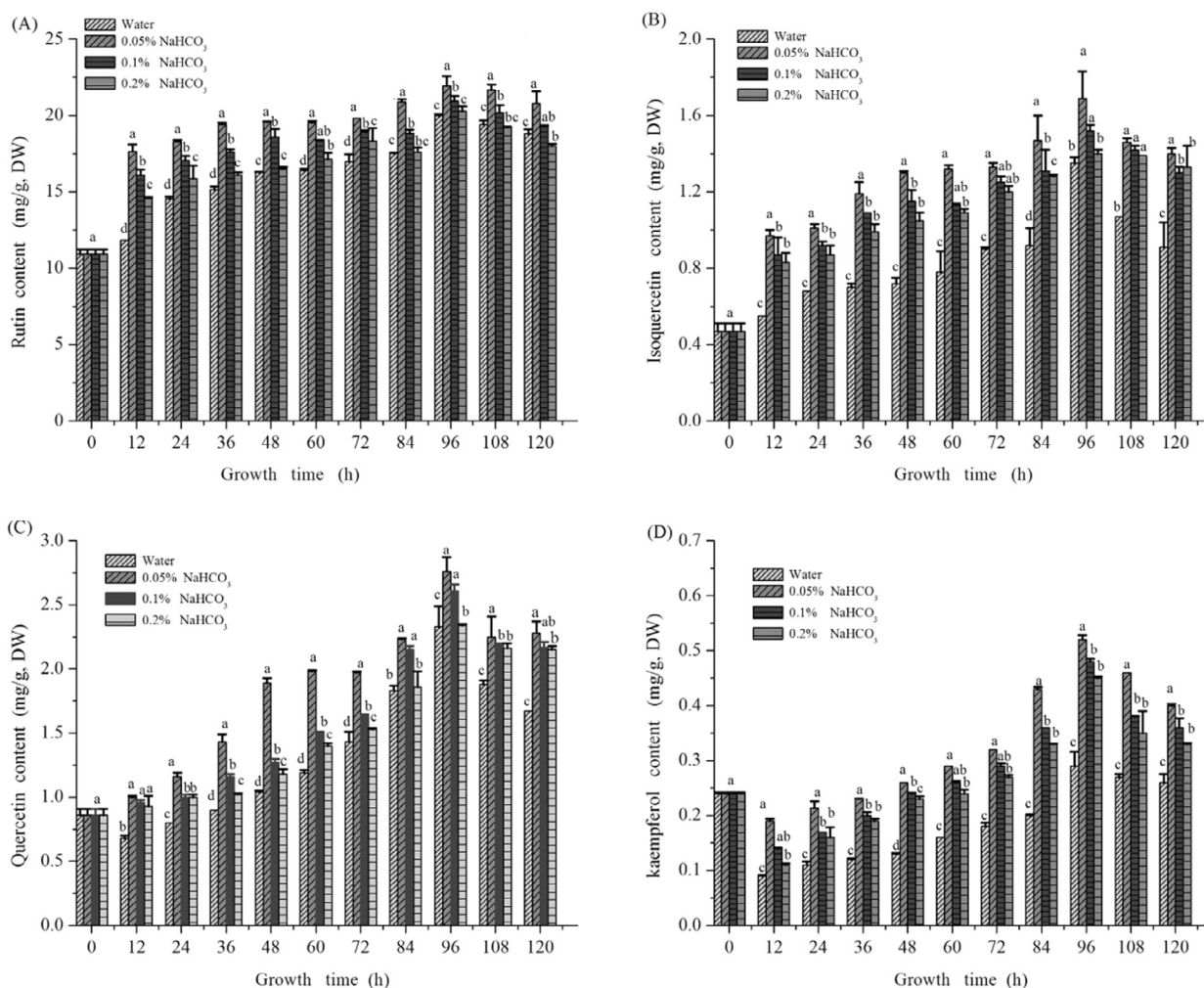
### 3. Results and discussion

#### 3.1. Effect of sodium bicarbonate on flavonoids and total phenolic compound accumulation

Changes in total flavonoid content during germination of tartary buckwheat treated with different concentrations of  $\text{NaHCO}_3$  were determined using the aluminium chloride colorimetric method (Table 1). Total flavonoid content increased with time and reached a maximum at 96 h under all treatments (0, 0.05, 0.1, and 0.2%  $\text{NaHCO}_3$ ). The increase was greatest in tartary buckwheat sprouts treated with low concentrations of  $\text{NaHCO}_3$  (0.05, 0.1, and 0.2%) compared with tartary buckwheat sprouts grown in water (control). Compared to control and 0.2%  $\text{NaHCO}_3$ , 0.05 and 0.1%  $\text{NaHCO}_3$  enhanced total flavonoid significantly ( $P < 0.05$ ). In addition, the content of all four flavonoids investigated increased with time, but decreased after 96 h under all treatments (Fig. 1). The rutin and isoquercitrin concentrations in tartary buckwheat sprouts treated with 0.05 and 0.1%  $\text{NaHCO}_3$  were significantly ( $P < 0.05$ ) higher than those found in water- and 0.2%  $\text{NaHCO}_3$ -treated tartary buckwheat sprouts after 96 h. The quercetin and kaempferol concentrations in tartary buckwheat sprouts treated with 0.05, 0.1, and 0.2%  $\text{NaHCO}_3$  were significantly ( $P < 0.05$ ) higher than the control after 96 h.

The trend of total phenolic compound accumulation in tartary buckwheat sprouts was the same as that for flavonoids (Table 2). The total phenolic compound content increased with time up to 96 h; after that time, the total phenolic compound content in tartary buckwheat sprouts treated with 0.05, 0.1, and 0.2%  $\text{NaHCO}_3$  was significantly ( $P < 0.05$ ) higher than that of the control. Thus, the salinity stress described here improved accumulation of phenolic compounds during germination, as observed in a similar study of common buckwheat (Lim et al., 2012).

Flavonoids are the most common and widely distributed group of plant phenolic compounds. These secondary metabolites are derived from the phenylpropanoid pathway, and perform a variety of essential functions in higher plants. Previous studies found that biotic and abiotic stress could affect the generation of phenolic compounds (Giorgi, Mingozi, Madeo, Speranza, & Cocucci, 2009; Wang et al., 2013). The pathway starts with the conversion of phenylalanine to cinnamic acid, which is catalysed by phenylalanine ammonia-lyase (PAL). Enzymes such as chalcone-synthase (CHS), chalcone isomerase (CHI), dihydroflavonol-reductase (DFR) and glycosyltransferases (GT) are involved in this pathway (Peng et al., 2008; Treutter, 2001). Ren and Sun (2014) reported a positive linear relationship between PAL activity and flavonoid accumulation in common buckwheat and tartary buckwheat sprouts, suggesting that changes in PAL activity were probably involved in



**Fig. 1.** Changes in the contents of individual flavonoids of tartary buckwheat sprouts treated with different concentrations of  $\text{NaHCO}_3$ : (A) rutin; (B) isoquercitrin; (C) quercetin; (D) kaempferol. Different letters denote significant differences ( $p < 0.05$ ).



**Table 2**  
The total phenolic compounds content of tartary buckwheat sprouts treated with different concentrations of NaHCO<sub>3</sub> (mg/g DW).

Treatment	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h
Water	13.66 ± 0.38a	13.68 ± 0.15c	14.43 ± 0.22b	15.42 ± 0.03c	16.05 ± 0.10c	17.76 ± 0.03b	18.76 ± 0.97c	21.63 ± 0.16c	24.36 ± 0.14d	24.14 ± 1.36b	22.16 ± 0.49c
0.05%NaHCO <sub>3</sub>	13.66 ± 0.38a	16.17 ± 0.21a	16.34 ± 0.06a	17.16 ± 0.18a	18.08 ± 0.13a	18.97 ± 0.21a	20.99 ± 0.20a	23.17 ± 0.34a	29.31 ± 0.45a	28.65 ± 0.03a	26.59 ± 0.06a
0.1%NaHCO <sub>3</sub>	13.66 ± 0.38a	14.81 ± 0.21b	14.59 ± 0.08b	15.66 ± 0.42b	16.18 ± 0.12b	18.65 ± 0.20a	20.09 ± 0.03ab	22.11 ± 0.24b	27.50 ± 0.29b	24.07 ± 0.03b	22.82 ± 0.14b
0.2%NaHCO <sub>3</sub>	13.66 ± 0.38a	13.70 ± 0.26c	14.51 ± 0.08b	15.56 ± 0.64b	16.30 ± 0.05b	18.07 ± 0.50ab	19.55 ± 0.03bc	21.84 ± 0.06bc	24.95 ± 0.37c	23.68 ± 0.29b	22.21 ± 0.06c

Data are expressed as mean ± standard deviation (n = 3). Means in each column followed by different letters are significantly different (p < 0.05).

phenolic (or flavonoid) accumulation. Therefore, we hypothesize that salinity stress (NaHCO<sub>3</sub>) might also enhance PAL activity during tartary buckwheat germination. Further studies are needed to test this hypothesis.

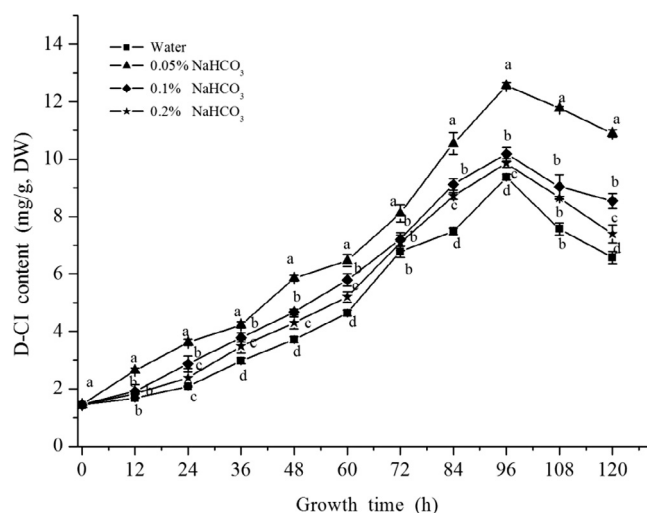
### 3.2. Effect of sodium bicarbonate on DCI accumulation

The DCI content in tartary buckwheat sprouts was determined using a simple, rapid method based on HPLC linked to an evaporative light-scattering detector (HPLC-ELSD) at different germination times (Fig. 2). As in previous studies, germination was found to increase DCI in tartary buckwheat sprouts (Jia et al., 2015; Wang et al., 2013). The DCI content increased dramatically with increasing germination time up to 96 h; afterwards, the DCI concentrations in tartary buckwheat sprouts treated with 0, 0.05, 0.1, and 0.2% NaHCO<sub>3</sub> were 546.21, 766.21, 655.86, and 602.07% higher than in tartary buckwheat seeds. The DCI content in tartary buckwheat seeds was 1.45 mg/g, which is similar to our previous study (Yang & Ren, 2008). Compared to the control (distilled water), treatment with low concentrations of NaHCO<sub>3</sub> during germination could significantly (P < 0.05) improve DCI concentrations in tartary buckwheat sprouts, as with flavonoids and total phenolic compounds.

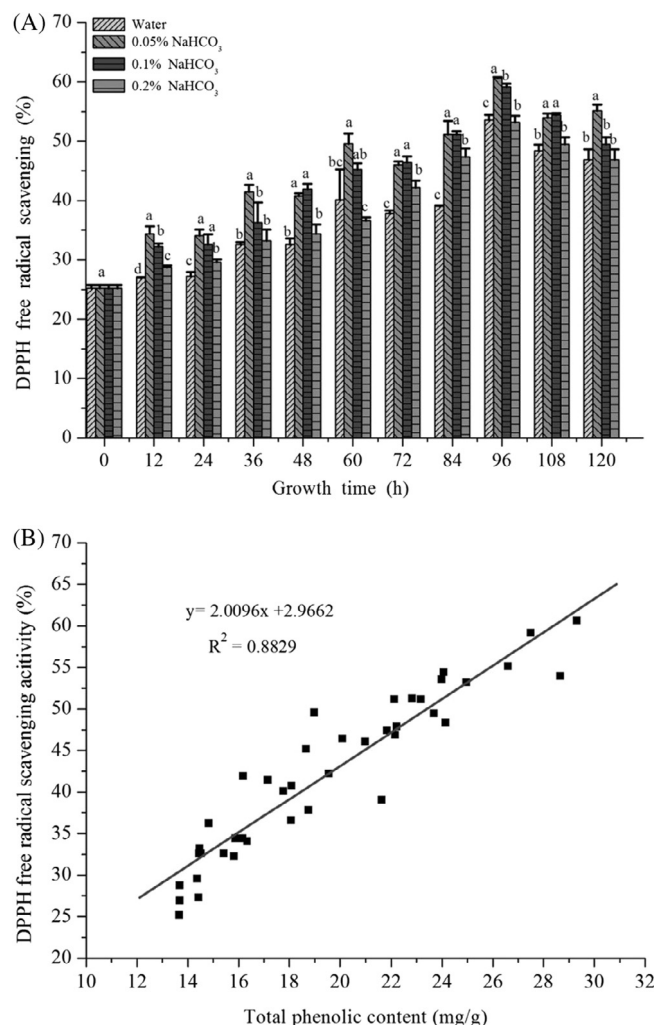
Wang et al. (2013) found that the germination temperature, which plays an essential role in tartary buckwheat germination, could significantly influence DCI levels. Also, metallic additives significantly improved DCI accumulation in tartary buckwheat sprouts (Wang et al., 2013). In this study, treatment with salinity stress during germination enhanced the content of the free form of DCI in tartary buckwheat.

### 3.3. Effect of sodium bicarbonate on the antioxidant activity

The effect of salinity stress (NaHCO<sub>3</sub>) on antioxidant activity of tartary buckwheat sprouts was determined using the DPPH radical scavenging activity. As with the change in phenolic compound and DCI contents, the radical scavenging activity of the sprouts increased in response to growing time up to 96 h (Fig. 3A). Treatment with low concentrations of NaHCO<sub>3</sub> significantly (P < 0.05) increased the radical scavenging activity of tartary buckwheat sprout extracts. The DPPH radical scavenging activity in extracts from tartary buckwheat sprout treated with 0.05% NaHCO<sub>3</sub> at 96 h was highest, followed by 0.1 and 0.2% NaHCO<sub>3</sub>. The lowest



**Fig. 2.** Changes in DCI content of tartary buckwheat sprouts treated with different concentrations of NaHCO<sub>3</sub>. Different letters denote significant differences (p < 0.05).

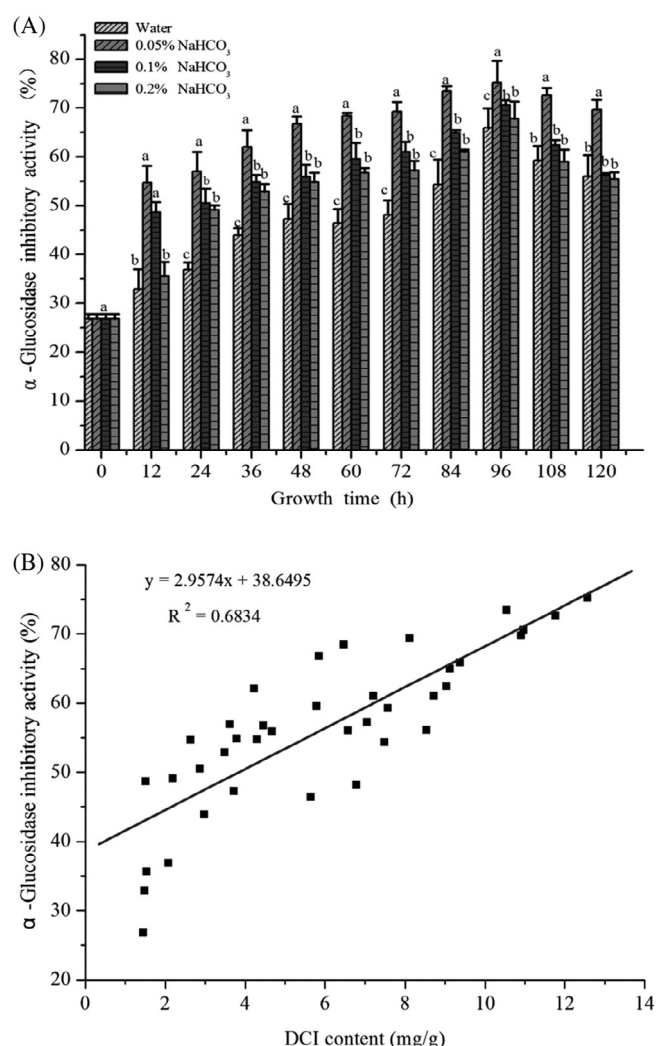


**Fig. 3.** DPPH free radical scavenging activity of total phenolic compounds extracts of tartary buckwheat sprouts treated with different concentrations of NaHCO<sub>3</sub> (A), and the correlation between scavenging activity and total phenolic compounds content (B). Different letters denote significant differences ( $p < 0.05$ ).

value was under control conditions. These results are in accordance with a previous study that showed salinity stress improved the antioxidant activity of common buckwheat sprouts (Lim et al., 2012). There was also a positive correlation between the DPPH radical scavenging activity and total phenolic compound content, with an  $r$ -squared value of 0.8829 (Fig. 3B). Similarly, a correlation between total phenolic compound content and antioxidant activity was reported previously for tartary buckwheat treated with thermal processing (Qin et al., 2013) and common buckwheat sprouts treated with NaCl (Lim et al., 2012).

### 3.4. Effect of sodium bicarbonate on the $\alpha$ -glucosidase inhibitory activity

DCI extracts from tartary buckwheat sprouts were assessed for  $\alpha$ -glucosidase inhibitory activity and the results are shown in Fig. 4A. As with the change in DCI content,  $\alpha$ -glucosidase inhibitory activity in DCI extracts increased dramatically with increased germination time up to 96 h. The levels of  $\alpha$ -glucosidase inhibitory activity in extracts from tartary buckwheat sprouts treated with 0, 0.05, 0.1, and 0.2% NaHCO<sub>3</sub> were 145.35, 180.09, 162.92, and 152.49% higher than that from tartary buckwheat seeds. The tartary buckwheat sprouts treated with 0.05% NaHCO<sub>3</sub> showed the



**Fig. 4.**  $\alpha$ -Glucosidase inhibitory activity of DCI extracts of tartary buckwheat sprouts treated with different concentrations of NaHCO<sub>3</sub> (A), and the correlation between  $\alpha$ -glucosidase inhibitory activity and DCI content (B). Different letters denote significant differences ( $p < 0.05$ ).

greatest  $\alpha$ -glucosidase inhibitory activity, followed by 0.1 and 0.2% NaHCO<sub>3</sub>. The lowest value was sprouts under control conditions. The  $\alpha$ -glucosidase inhibitory activity was positively correlated with DCI content, and the  $r$ -squared value was 0.6834 (Fig. 4B). Wang et al. (2013) also found that addition of Cu<sup>2+</sup> and Zn<sup>2+</sup> during the germination of tartary buckwheat increased  $\alpha$ -glucosidase inhibitory activity, and analysis of  $\alpha$ -glucosidase inhibitory activity and DCI content also demonstrated a significant correlation. Our previous study suggested that mung bean sprout extracts containing DCI, vitexin and isovitexin exerted an anti-diabetic effect in type 2 diabetic mice (Yao et al., 2008), which suggests that the DCI from tartary buckwheat sprouts might have potential as a natural  $\alpha$ -glucosidase inhibitor for diabetic patients.

## 4. Conclusions

In summary, the levels of flavonoids, total phenolic compounds, DCI accumulation, and DPPH radical-scavenging and  $\alpha$ -glucosidase inhibitory activities were investigated in tartary buckwheat sprouts treated with different concentrations of NaHCO<sub>3</sub> (0, 0.05, 0.1, and 0.2%). Treatment with low NaHCO<sub>3</sub> concentrations increased accumulation of flavonoids, total phenolic compounds

and DCI, and enhanced the antioxidant and  $\alpha$ -glucosidase inhibitory activities of extracts from tartary buckwheat sprouts. Tartary buckwheat seeds treated with 0.05% of  $\text{NaHCO}_3$  during germination contained the highest levels of flavonoids, total phenolic compounds and DCI, and they had the greatest antioxidant and  $\alpha$ -glucosidase inhibitory activities at 96 h. Tartary buckwheat sprouts treated with an appropriate concentration of sodium bicarbonate could be a functional food for preventing and managing of diseases related to oxidation stress and hyperglycaemia. Further studies are needed to evaluate the hypoglycemic activity of tartary buckwheat sprouts in a diabetic model, such as mice.

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