Relationships between antioxidant compounds and antioxidant activities of tartary buckwheat during germination

Xiaoli Zhou · Tingfeng Hao · Yiming Zhou · Wen Tang ·
Ying Xiao · Xiaoxiao Meng · Xiang Fang

Revised: 12 December 2013 / Accepted: 10 February 2014 / Published online: 26 February 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract Relationships of changes between major non-enzymatic antioxidant compounds and antioxidant capacities of tartary buckwheat during germination were evaluated by means of correlation analysis and principal component analysis in this paper. The changes of antioxidant compounds, including vitamin C, vitamin E, flavonoids, carotenoids, and chlorophyll, and antioxidant activities were detected. A good accumulation in the content of vitamin C (0.71 mg/g), total flavonoids (19.53 mg rutin/g), and rutin (11.34 mg/g) was found after 7-day germination, but germination decreased the vitamin E activity. Germination improved the activities of buckwheat extracts to scavenge DPPH, ABTS, and superoxide free radicals by 107, 144, and 88 %, respectively. Furthermore, the correlation and principal component analysis showed that the vitamin C, total flavonoids, and rutin contents were closely related positively with free radicals scavenging properties, indicating that the compounds which play a key role in the elevated antioxidant activities during germination consisted of vitamin C, total flavonoids, and rutin, but not vitamin E and quercetin.

Keywords Tartary buckwheat · Germination · Antioxidant activity · Principal component analysis

Introduction

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn) is one of the traditional crops cultivated in many countries. It has a strong adaptability to adverse environments with a very short

growing span. A large variety of buckwheat foods have been produced. Buckwheat now has been used and will be better used as an important raw material for functional food production (Fabjan et al. 2003). A lot of studies have been conducted in the functionalities and properties of buckwheat proteins, vitamins, flavonoids, phytosterols, thiamin-binding proteins, and other rare compounds in buckwheat seed. Of these nutrient compounds, vitamin C, vitamin E, rutin, quercetin, other flavonoids, and chlorophyll in buckwheat seeds have antioxidative activity (Fabjan et al. 2003; Kreft et al. 2006). Free radicals play harmful roles in pathological changes. Therefore, intake of food rich in antioxidant compounds, which act as radical scavengers, has been expected to be effective in attenuating or preventing many diseases (Al-Gubory et al. 2010).

However, the content of available nutrients and the digestibility of some large molecular nutrients in buckwheat are low. Germination as seedling treatment is one of useful methods for nutrients improvement. Some previous reports demonstrated that if the buckwheat seeds were germinated, their biological utilization rates could be greatly improved (Kim et al. 2004; Lin et al. 2008). Several studies found that germination increased strongly rutin and quercetin contents of tartary buckwheat (Kim et al. 2007b; Suzuki et al. 2007). Lin et al. (2008) optimized the bioactive compounds in buckwheat sprouts and examined the effect on blood cholesterol in hamsters. Furthermore, novel biological activities of buckwheat sprouts were emerged after germination. Some studies found that flavonoids from buckwheat sprouts have anti-stress effects and anti-inflammatory in mice and in vitro (Ishii et al. 2008; Watanabe and Ayugase 2008). Additionally, Kim et al. (2007a) identified some anthocyanins in the sprouts of common and tartary buckwheat sprouts. Watanabe (2007) evaluated the contribution of anthocyanin compounds of commercial buckwheat sprouts to antioxidant capacity. However, the relationships between antioxidant compounds and antioxidant

X. Zhou · T. Hao · Y. Zhou · W. Tang · Y. Xiao (✉) · X. Meng · X. Fang

School of Perfume and Aroma Technology, Shanghai Institute of Technology, 100 Haiquan Road, Shanghai 2001418, People's Republic of China
e-mail: xiaoying@sit.edu.cn

activities of tartary buckwheat during germination have rarely been reported in the previous studies.

The aim of the present paper was to evaluate the relations of changes between major non-enzymatic antioxidant compounds in tartary buckwheat, including vitamin C, vitamin E, flavonoids, carotenes, and chlorophyll, and antioxidant capacities during germination by means of multiple correlation analysis and principal component analysis. This study helped to understand further role of the germinated tartary buckwheat in antioxidant activity and to improve fully its biological utilization rate and activity.

Materials and methods

Chemicals

3-Ethylbenzthiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), nicotinamide adenine dinucleotide (NADH), rutin, quercetin, vitamin C, tocopherols, and other chemicals were purchased from Shanghai Aladdin regents Co. (China). All reagents were analytical or HPLC grade.

Materials and germination treatment

The tartary buckwheat seeds (Shanxi heifeng 1[#]) were obtained from Shanxi Long Qiao Co. Ltd (China). The buckwheat seeds were sterilized by soaking in 0.1 % aqueous sodium hypochlorite for 25 min, and then rinsed with distilled water until the pH was neutral. Next, the seeds were soaked in distilled water for 6 h at 30 °C and then were transferred to a flat. The flats were covered with a wet laboratory paper and introduced into the germination machine (BTI 3000, Shanghai Boxun Industry & Commerce CO. LTD, China). The condition of germination was controlled temperature (30±2 °C) and humidity (85±10 %) with a 12-h light/12-h dark cycle. The seeds were germinated for 1, 2, 3, 4, 5, 6, and 7 days, respectively. After germination, the seeds and the sprouts were freeze-dried, milled, and passed through a sieve of 0.5 mm. The flour obtained was stored in plastic bags and stored at −80 °C until used for assay.

Chlorophyll and carotenoids contents determination

Chlorophyll concentrations were measured following the procedure by Mirecki and Teramura (1984). 0.2 g of sample was employed, which was left overnight at 4 °C in a 10 ml acidified ethanol [80/20 v/v, (CH₃OH/H₂O)] to extract chlorophyll. The extracts were diluted by six folds of the acidified methanol and analyzed by spectrometric method at wavelength of 300 nm.

Carotenoids extracted from the sprouts or seeds were determined according to the procedure of Lim et al. (2012). The 0.2 g dried sample was mixed with 6 ml of ethanol containing 0.1 % BHT, and the sealed mixture solution was pre-incubated at 85 °C for 5 min. After additional of 120 µl of 80 % KOH, the reaction solution was incubated at 85 °C for 10 min. The reaction solution was then immediately placed on ice, and 3 ml of distilled water and 3 ml of hexane were added. After centrifugation, the absorbance of the hexane layer was measured at 450 nm. A standard curve was prepared by using β-carotene, and the absorbance was converted to carotenoids content in terms of mg of β-carotene equivalent (CE) per g of dry weight (DW).

Vitamin C and vitamin E content analysis

The vitamin C quantification in raw buckwheat seeds or sprouts was carried out by HPLC (Agilent Hewlett-Packard series 1100, CA, USA) according to the procedure described by Ali et al. (2011). HPLC was equipped with Hibar-LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany). The tocopherol isomers (α-tocopherol, γ-tocopherol, and δ-tocopherol) determination was carried out by HPLC according to the method of Frias et al. (2005). The content of tocopherol isomers were expressed in µg/g DW. Vitamin E activity was calculated as a-TE µg/g DW: (mg α-tocopherol×1.0) + (mg γ-tocopherol×0.1) + (mg δ-tocopherol×0.03).

Total flavonoids, rutin, and quercetin content analysis

Finely ground buckwheat seed samples (0.02–0.2 g) were transferred into a 25 ml glass bottle, 8 ml of 70 % methanol was added, and the samples were mixed for 24 h and centrifuged. The supernatants were combined and made up to a total volume of 10 ml with 70 % methanol for a quantitative analysis of the total flavonoids, quercetin, and rutin.

The total flavonoids content was measured using a modified colorimetric method (Pilerood and Prakash 2011). The absorption was measured at 415 nm against the same mixture and was compared to that of a standard rutin. The quercetin and rutin content was assayed by HPLC. The solvents for HPLC were acetonitrile and methanol mixture (1:2) named as A, and 0.75 % aqueous H₃PO₄ named as B. The initial solvent was 100 % B, which was changed linearly to a mixture of 60 % A and 40 % B in 20 min, then to 100 % A within another 20 min, and finally to 100 % B for 10 min equilibration. The effluent compounds were detected at 380 nm and identified by a comparison of each compound with the retention time of the relevant standard solution (Fabjan et al. 2003).

Antioxidant activity assays

Antioxidant activity of the 60 % aqueous ethanolic extracts from the buckwheat sprouts or seeds was evaluated by scavenging effects of ABTS, DPPH, and superoxide radical according to the method of Mantena (Mantena et al. 2003) with a slight modification. The 60 % aqueous ethanol was selected for extraction, because the proportion of the antioxidant compounds in the extracts was similar to those in seeds or sprouts.

The ABTS radical was produced by the oxidation of 7 mM ABTS with 2.45 mM potassium persulfate solution. The mixture was allowed to stand in the dark at room temperature for 24 h before use, and then the ABTS solution was diluted with phosphate buffered saline (PBS) at pH 7.4 and equilibrated at 25 °C to give an absorbance of 0.70 ± 0.02 at 734 nm. After 30 μ L of sample solutions were mixed with 1.5 mL of the ABTS preparation at 25 °C for 5 min, the absorbance values were measured at 734 nm. Trolox was used to obtain the standard curve. The results were expressed as micromole trolox equivalent per gram of DW (μ mol TE/g DW).

A 0.5 ml sample was added to react with 1.5 ml DPPH solution (0.5 mM in absolute ethanol) in the dark, and absorbance value was measured at 510 nm after 20 min of reaction at 25 °C. DPPH radical scavenging capacity was expressed as μ mol of TE per g of DW.

One milliliter of nitroblue tetrazolium solution (150 μ M in 100 mM PBS, pH 7.4), 1 ml NADH solution (468 μ M in 100 mM PBS, pH 7.4) and 0.1 ml extracts were mixed. The reaction was initiated by adding 100 μ L of phenazine methosulphate solution (60 μ M in 100 mM PBS, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 50 min, and the absorbance at 560 nm was measured against blank samples. A decreased absorbance of the reaction mixture was indicative of an increased superoxide anion scavenging activity. Results were expressed as μ mol of TE per g of DW.

Statistical analysis

All above analysis was repeated independently for three times, by which averaged data were obtained as summarized below. Data were expressed as means $\pm$ standard deviation (SD). Comparisons across groups were performed by one-way analysis of variance with the Duncan multiple range test for significance at $P < 0.05$. ANOVA analysis, multiple correlations, and principal component analysis was done with SPSS 15 (SPSS, Inc., Chicago, IL, USA).

Results and discussion

Vitamins C and vitamin E

The effect of the germination time on the vitamin C and tocopherols (vitamin E) content is shown in Table 1. It was found that the vitamin C content was increased sharply during the first 5 days of germination, which is similar to some findings reported by Kim et al. (2004). The vitamin C content was about 14 times as large as that of control when germinated for 7 days.

However, it was observed that germination caused a significant ($P < 0.05$) decrease in the γ -, δ -tocopherol content and in vitamin E activity but a significant increase in α -tocopherol content after 3 days. α -Tocopherol is traditionally recognized as the most active form of vitamin E in humans, and the highest value of α -tocopherol content (7.2 μ g/g DW) was obtained after 7 days of germination. Our findings on the changes in tocopherol isomers and vitamin E activity after germination are similar to some previous findings (Fernandez-Orozco et al. 2006, 2008). However, the mechanism for the decreased vitamin E activity in germinated seed has not been clear. Grain seeds are rich in vitamin E which is a major biological antioxidant and quenches free radicals. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination (Sattler et al. 2004). Generally, the fat content was decreased in sprout after germination (Hahm et al. 2009). Therefore, the reduced vitamin E activity may still have an enough preventive effect against lipid peroxidation, accompanied with the decreased fat content. Additionally, the change in vitamin E activity may be related with different cultivars. The further investigation is needed to validate the above-mentioned possible reason.

Chlorophyll and carotenoids

Dietary chlorophyll and carotenoids as pigment have antioxidant and antimutagenic activities (Ferruzzi et al. 2006). Table 1 shows the chlorophyll and carotenoids contents in tartary buckwheat during the germination. It was clearly observed that the contents of total chlorophyll and carotenes exhibited a maximum on the 5th day of germination. However, the chlorophyll concentration started to decrease after 5 days of germination, but Suzuki et al. (2007) reported that the chlorophyll concentration reached the highest on the 8th day, which does not fully coincide with the present findings. In addition, the dynamic changes were similar pattern between carotenes and chlorophyll contents during germination.

Table 1 Changes in contents of antioxidant compounds in tartary buckwheat during germination

Germination days	Vitamin C (mg/g)	α -tocopherol (μ g/g)	γ -tocopherol (μ g/g)	δ -tocopherol (μ g/g)	Vitamin E activity (a-TE μ g/g)	Chlorophyll (mg/g)	Carotenes (mg CE/g)	Total flavonoids (mg rutin/g)	Rutin (mg/g)	Quercetin (mg/g)
0	0.05 $\pm$ 0.03 ^a	2.1 $\pm$ 0.3 ^a	117.8 $\pm$ 8.2 ^f	7.3 $\pm$ 0.7 ^c	14.1 $\pm$ 1.2 ^c	0.05 $\pm$ 0.01 ^a	3.6 $\pm$ 0.5 ^a	3.72 $\pm$ 0.51 ^a	2.78 $\pm$ 0.31 ^b	0.49 $\pm$ 0.13 ^a
1	0.15 $\pm$ 0.03 ^b	3.2 $\pm$ 0.4 ^b	103.4 $\pm$ 6.7 ^e	5.4 $\pm$ 0.5 ^b	13.7 $\pm$ 0.8 ^c	0.07 $\pm$ 0.01 ^a	6.3 $\pm$ 0.6 ^b	7.98 $\pm$ 0.62 ^b	1.66 $\pm$ 0.21 ^a	6.04 $\pm$ 0.71 ^{cd}
2	0.18 $\pm$ 0.03 ^b	5.8 $\pm$ 0.3 ^c	65.5 $\pm$ 5.4 ^d	5.2 $\pm$ 0.6 ^b	12.5 $\pm$ 0.8 ^c	0.14 $\pm$ 0.02 ^b	7.9 $\pm$ 0.7 ^b	8.97 $\pm$ 0.59 ^b	1.47 $\pm$ 0.28 ^a	7.19 $\pm$ 0.53 ^d
3	0.34 $\pm$ 0.05 ^c	6.2 $\pm$ 0.7 ^c	32.8 $\pm$ 2.1 ^c	4.1 $\pm$ 0.6 ^a	9.6 $\pm$ 0.5 ^b	0.15 $\pm$ 0.02 ^b	11.3 $\pm$ 0.6 ^{cd}	14.85 $\pm$ 0.87 ^c	6.41 $\pm$ 0.42 ^c	6.84 $\pm$ 0.80 ^{cd}
4	0.56 $\pm$ 0.04 ^d	6.4 $\pm$ 0.5 ^c	23.7 $\pm$ 1.8 ^b	4.4 $\pm$ 0.4 ^{ab}	8.9 $\pm$ 0.5 ^{ab}	0.19 $\pm$ 0.02 ^{bc}	14.2 $\pm$ 0.5 ^e	16.43 $\pm$ 1.25 ^c	8.93 $\pm$ 0.39 ^d	5.07 $\pm$ 0.67 ^c
5	0.62 $\pm$ 0.07 ^e	6.4 $\pm$ 0.4 ^c	16.8 $\pm$ 0.5 ^a	4.0 $\pm$ 0.8 ^a	8.2 $\pm$ 0.6 ^a	0.22 $\pm$ 0.03 ^c	14.6 $\pm$ 0.8 ^e	16.79 $\pm$ 1.14 ^c	8.69 $\pm$ 0.53 ^d	5.53 $\pm$ 0.54 ^c
6	0.60 $\pm$ 0.05 ^e	6.7 $\pm$ 0.4 ^{cd}	16.9 $\pm$ 0.5 ^a	3.7 $\pm$ 0.5 ^a	8.5 $\pm$ 0.4 ^a	0.19 $\pm$ 0.02 ^{bc}	12.7 $\pm$ 0.5 ^d	19.45 $\pm$ 1.02 ^{cd}	9.55 $\pm$ 0.60 ^e	6.39 $\pm$ 0.58 ^{cd}
7	0.71 $\pm$ 0.12 ^e	7.2 $\pm$ 0.5 ^d	16.8 $\pm$ 0.8 ^a	3.9 $\pm$ 0.7 ^a	9.0 $\pm$ 0.4 ^{ab}	0.14 $\pm$ 0.01 ^b	10.7 $\pm$ 0.6 ^c	19.53 $\pm$ 0.96 ^d	11.34 $\pm$ 0.57 ^f	3.98 $\pm$ 0.42 ^b

All values are on dry weight basis

Data are mean values $\pm$ SD ($n=3$)

Means with different superscript letters, within a column, are significantly different at $P<0.05$

Flavonoids

Dietary flavonoids are important in an optimal human diet, which give their free radicals scavenging activities by virtue of the hydrogen donating capacity of their phenolic groups (Harborne and Williams 2000). Tartary buckwheat has been known as a source of dietary flavonoids (Fabjan et al. 2003). As shown in Table 1, the total flavonoids content of tartary buckwheat was accumulated by gradual increase during the first 6 days. The content of total flavonoids was significantly increased by around 4.2 folds after 7 days of germination. Several similar results have shown that germination improved strongly flavonoids contents (Lim et al. 2012; Suzuki et al. 2007; Watanabe 2007).

Flavonoids in tartary buckwheat include rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin. Rutin and quercetin are major flavonoids compounds in tartary buckwheat. No rutin is present in cereals and pseudocereals except buckwheat (Liu et al. 2008). The presence of rutin in buckwheat plants is one of the main reasons for the production of different kinds of buckwheat foods. Therefore, the changes in contents of rutin and quercetin were assayed, and other flavonoids compounds were not addressed in this study due to their relative lower contents.

The rutin and quercetin contents of buckwheat during germination are given in Table 1. The rutin content was decreased when germinated for the first 2 days of germination, accompanied with the markedly increased quercetin content. We presumed that this observation might be attributed to the increased activity of rutin-degrading enzyme which catalyzes hydrolysis of rutin to quercetin (Kreft et al. 2006). However, a significant increase in rutin content was found from the third day to the 7th day, and rutin content rose by about 300 % (11.34 mg/g DW) after 7 days of germination. Quercetin content had no regular changing trend during germination

process. Quercetin content reached to a maximum (7.19 mg/g DW) on the second day of germination, but its content was significantly decreased on the 7th day of germination, suggesting that germination had not good effect of quercetin accumulation.

The present findings did not fully agree with several previous findings that germination is a good way for accumulation of bioactive compounds, such as vitamins and quercetin (Kim et al. 2007b; Kreft et al. 2006; Suzuki et al. 2007). Additionally, their changing trends during germination were differently exhibited. We presumed that the different cultivars, parts of buckwheat, and growing conditions could result in exhibiting different changing trends of antioxidant compounds contents. More explanations for this speculation through other direct further evidences will be necessary in future studies.

Table 2 Free radicals scavenging activities of extracts from tartary buckwheat

Germination days	DPPH (μ mol TE/g)	ABTS	O ₂ ^{•-}
0	2.6 $\pm$ 0.4 ^a	0.9 $\pm$ 0.05 ^a	5.8 $\pm$ 0.6 ^a
1	3.5 $\pm$ 0.3 ^b	0.8 $\pm$ 0.07 ^a	4.7 $\pm$ 0.8 ^a
2	3.3 $\pm$ 0.5 ^b	0.8 $\pm$ 0.06 ^a	6.6 $\pm$ 0.8 ^{bc}
3	3.9 $\pm$ 0.4 ^{bc}	0.9 $\pm$ 0.09 ^a	6.1 $\pm$ 1.0 ^b
4	4.4 $\pm$ 0.5 ^c	1.2 $\pm$ 0.07 ^b	8.4 $\pm$ 0.9 ^c
5	5.2 $\pm$ 0.7 ^{cd}	1.5 $\pm$ 0.10 ^b	8.2 $\pm$ 1.1 ^{bc}
6	4.9 $\pm$ 0.4 ^{cd}	1.9 $\pm$ 0.15 ^{bc}	10.5 $\pm$ 0.9 ^d
7	5.4 $\pm$ 0.4 ^d	2.2 $\pm$ 0.12 ^c	10.9 $\pm$ 0.8 ^d

All values are on dry weight basis

Data are mean values $\pm$ SD ($n=3$)

Means with different superscript letters, within a column, are significantly different at $P<0.05$

Table 3 Pearson correlation between antioxidant composition and free radical scavenging activity

	VC	VE	Chlorophyll	Carotenes	Total flavonoids	Rutin	Quercetin
DPPH	0.974 ^b	−0.897 ^a	0.775 ^a	0.833 ^a	0.954 ^b	0.901 ^b	0.290
ABTS	0.878 ^b	−0.717	0.511	0.580	0.831 ^a	0.880 ^b	−0.049
O ₂ ^{•−}	0.889 ^b	−0.765 ^a	0.623	0.662	0.854 ^b	0.883 ^b	0.014

^a Correlation is significant at the 0.05 level^b Correlation is significant at the 0.01 level

Antioxidative activity

The scavenging effects of extracts from tartary buckwheat sprouts or seeds to free radicals including DPPH, ABTS, and superoxide radicals (O₂^{•−}) are shown in Table 2. The results showed that an increase in scavenging abilities of buckwheat extracts to O₂^{•−}, DPPH, and ABTS was observed during germination, but O₂^{•−} and ABTS scavenging abilities were not improved significantly during the first 2 days of germination. The 7-day germinated buckwheat sprouts extracts possessed powerful scavenging effects to free radicals, presumably resulting from the accumulation of antioxidative compounds, such as flavonoids and vitamins. The antioxidative compositions in buckwheat included antioxidative substances and antioxidative enzymes. Except for the antioxidative substances, the enzymes could also contribute to the scavenging effects to free radicals.

Correlation and principal components analysis

The Pearson correlation analysis and principal components analysis was firstly used to evaluate the relations of changed antioxidant compositions with free radicals scavenging properties. The Pearson correlation coefficients (*r*) between the non-enzymatic antioxidant compositions contents (vitamin C, vitamin E activity, chlorophyll, carotenoids, total flavonoids, rutin, and quercetin) and the free radicals scavenging properties (DPPH, ABTS, and O₂^{•−}) of the extracts from tartary buckwheat sprouts are presented in the Table 3. The vitamin C and rutin contents were significantly (*p*<0.01) positively correlated with the free radicals scavenging activities. A strong positive correlation of the total flavonoids content was also observed to the free radicals scavenging effects to DPPH (*p*<0.01), ABTS (*p*<0.05), and O₂^{•−} (*p*<0.01). Additionally, the chlorophyll and carotenes contents were only related with DPPH radical scavenging effect (*p*<0.05). In contrast, a negative correlation of the vitamin E activity was found to the DPPH and ABTS radicals scavenging activities. However, this finding did not mean that vitamin E had a reverse contribution to antioxidant activities of buckwheat during germination. This negative correlation might be due to the reduced vitamin E activity after germination in spite of the increased α -tocopherol content. Additionally, the

quercetin content was not significantly correlated with the free radicals scavenging effects.

Further, in order to get a better insight in the relationships between the antioxidant compositions and antioxidant activities of the germinated buckwheat, the principal component analysis (PCA) calculation was on the correlation matrix between the values of the characteristics. PCA was applied to detect the relative importance of individual antioxidant-related variables for determining the data structure, which meant that the contribution of each variable was independent of the range of its value (Komes et al. 2011). PCA showed that the first two principal components accounted for 93.23 % of the total variability, of which 75.57 % was along the first principal component (PC1), and 14.51 % was along PC2.

Figure 1 represents contribution of the antioxidant-related variables of germinated tartary buckwheat on the bidimensional space formed by rotated PC 1 and 2. Most of the variables were positively correlated with the PC 1 (vitamin C=0.991, total flavonoids=0.985, DPPH=0.968, rutin=0.951, O₂^{•−}=0.901, and ABTS=0.879) and negatively only with vitamin E (−0.954). It was presumed that PC 1 should reflect free radical radicals scavenging properties. PC 2 was positively correlated with quercetin (0.930). Samples with similar values for the variables explained by the PC appeared close together. Thereby, it was clear that vitamin C, total flavonoids, rutin, DPPH, O₂^{•−}, and ABTS were categorized

Fig. 1 Plot of contribution of antioxidant-related variables of germinated tartary buckwheat on the bidimensional space formed by rotated principal component 1 and 2 (VC vitamin C, VE vitamin E activity)

as one class (Fig. 1). Vitamin C has been recognized as a very effective antioxidant in biological systems, protecting other substances from oxidative injury and regenerating small molecule antioxidants, such as tocopherol and carotenes (Kumar et al. 2010). Rutin and other flavonoids have powerful antioxidant capacities against various free radicals systems in vitro (Harborne and Williams 2000; Yang et al. 2008). Therefore, we presumed that the increased contents of primary compounds, including vitamin C, total flavonoids, and rutin, in tartary buckwheat during germination could be important to act as free radicals scavenging properties, base on the PCA analysis.

In conclusion, our results showed that germination improved significantly the antioxidative activities of tartary buckwheat. Further, the correlation and principal component analysis demonstrated that the accumulated contents of vitamin C and total flavonoids, especially rutin but not quercetin, could play a key role in the elevated activities of free radicals scavenging in tartary buckwheat during germination.

Acknowledgments The authors are gratefully for the financial support from the National Natural Science Foundation of China (31071527 and 31371761).

References

- Al-Gubory KH, Fowler PA, Garrel C (2010) The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol* 42:1634–1650
- Ali L, Alsanius BW, Rosberg AK, Svensson B, Nielsen T, Olsson ME (2011) Effects of nutrition strategy on the levels of nutrients and bioactive compounds in blackberries. *Eur Food Res Technol* 234:33–44
- Fabjan N, Rode J, Kosir IJ, Wang Z, Zhang Z, Kreft I (2003) Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. *J Agric Food Chem* 51:6452–6455
- Fernandez-Orozco R, Piskula M, Zielinski H, Kozłowska H, Frias J, Vidal-Valverde C (2006) Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. zapaton. *Eur Food Res Technol* 223:495–502
- Fernandez-Orozco R, Frias J, Zielinski H, Piskula MK, Kozłowska H, Vidal-Valverde C (2008) Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emerald, *Glycine max* cv. jutro and *Glycine max* cv. merit. *Food Chem* 111:622–630
- Ferruzzi MG, Böhm V, Courtney PD, Schwartz SJ (2006) Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assays. *J Food Sci* 67:2589–2595
- Frias J, Miranda LM, Doblado R, Vidal-Valverde C (2005) Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. multolupa. *Food Chem* 92:211–220
- Hahm TS, Park SJ, Martin Lo Y (2009) Effects of germination on chemical composition and functional properties of sesame (*Sesamum indicum* L.) seeds. *Bioresour Technol* 100:1643–1647
- Harborne JB, Williams CA (2000) Review: advances in flavonoid research since 1992. *Phytochemistry* 55:481–504
- Ishii S, Katsumura T, Shiozuka C, Ooyauchi K, Kawasaki K, Takigawa S, Fukushima T, Tokuji Y, Kinoshita M, Ohnishi M, Kawahara M, Ohba K (2008) Anti-inflammatory effect of buckwheat sprouts in lipopolysaccharide-activated human colon cancer cells and mice. *Biosci Biotechnol Biochem* 72:3148–3157
- Kim SL, Kim SK, Park CH (2004) Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Res Int* 37:319–327
- Kim SJ, Maeda T, Sarker MZ, Takigawa S, Matsuura-Endo C, Yamauchi H, Mukasa Y, Saito K, Hashimoto N, Noda T, Saito T, Suzuki T (2007a) Identification of anthocyanins in the sprouts of buckwheat. *J Agric Food Chem* 55:6314–6318
- Kim SJ, Zaidul ISM, Maeda T, Suzuki T, Hashimoto N, Takigawa S, Noda T, Matsuura-Endo C, Yamauchi H (2007b) A time-course study of flavonoids in the sprouts of tartary (*Fagopyrum tataricum* Gaertn.) buckwheats. *Sci Hortic* 155:13–18
- Komes D, Belščak-Cvitanović A, Horžić D, Rusak G, Likić S, Berendika M (2011) Phenolic composition and antioxidant properties of some traditionally used medicinal plants affected by the extraction time and hydrolysis. *Phytochem Anal* 22:172–180
- Kreft I, Fabjan N, Yasumoto K (2006) Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. *Food Chem* 98:508–512
- Kumar V, Rani A, Dixit AK, Pratap D, Bhatnagar D (2010) A comparative assessment of total phenolic content, ferric reducing-antioxidative power, freeradical-scavenging activity, vitamin C and isoflavones content in soybean with varying seed coat colour. *Food Res Int* 43:323–328
- Lim JH, Park KJ, Kim BK, Jeong JW, Kim HJ (2012) Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. *Food Chem* 135:1065–1070
- Lin LY, Peng CC, Yang YL, Peng RY (2008) Optimization of bioactive compounds in buckwheat sprouts and their effect on blood cholesterol in hamsters. *J Agric Food Chem* 56:1216–1223
- Liu CL, Chen YS, Yang JH, Chiang BH (2008) Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. *J Agric Food Chem* 56:173–178
- Mantena SK, Jagadish PC, Badduri SR, Siripurapu KB, Unnikrishnan MK (2003) In vitro evaluation of antioxidant properties of *Cocoa nucifera* Linn. water. *Nahrung/Food* 47:126–131
- Mirecki R, Teramura AH (1984) Effects of ultraviolet-B irradiance on soybean V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. *Plant Physiol* 74:475–480
- Pilerood SA, Prakash J (2011) Evaluation of nutritional composition and antioxidant activity of Borage (*Echium amoenum*) and Valerian (*Valerian officinalis*). *J Food Sci Technol*. doi:10.1007/s13197-011-0573-z
- Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M, DellaPenna D (2004) Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *Plant Cell* 16:1419–1432
- Suzuki T, Kim SJ, Takigawa S, Mukasa Y, Hashimoto N, Saito K, Noda T, Matsuura-Endo C, Zaidul ISM, Yamauchi H (2007) Changes in rutin concentration and flavonol-3-glucosidase activity during seedling growth in tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *Can J Plant Sci* 87:83–87
- Watanabe M (2007) An anthocyanin compound in buckwheat sprouts and its contribution to antioxidant capacity. *Biosci Biotechnol Biochem* 71:579–582
- Watanabe M, Ayugase J (2008) Anti-stress effects of flavonoids from buckwheat sprouts in mice subjected to restraint stress. *Food Sci Technol Res* 14:253–260
- Yang J, Guo J, Yuan J (2008) In vitro antioxidant properties of rutin. *LWT Food Sci Technol* 41:1060–1066