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Evolution of nutrient ingredients in tartary buckwheat seeds during germination

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ABSTRACT

Evolution of nutrient components and the antioxidative activity of seed sprouts of tartary buckwheat (*Fagopyrum tataricum L. Gaertn*) were investigated in the course of germination. Results showed that the contents of total flavonoids increased with germination time and leveled off after the third germination day with the changing trend of rutin and quercetin opposite to each other. The decrease of total protein and total sugar contents in the germinated seeds was accompanied respectively by an increase of amino acid and reducing sugar contents. The contents of vitamin C (V_c) and B_1(V_{B1}) exhibited a minimum with no appreciable changes found for vitamin B_2 (V_{B2}) and B_6 (V_{B6}). The contents of total chlorophyll, chlorophyll A and B all exhibited a maximum on the fifth germination day. The contents of fatty acids had no regular changing trend with germination time. The free radical-scavenging activities of the seeds increased with germination time and were caused by an increase in their antioxidative activity.

1. Introduction

Tartary buckwheat is one of the traditional crops cultivated in central and east Europe and Asia. A large variety of buckwheat foods have been produced for centuries. Now it becomes a raw material for healthy food, owing to its anti-oxidative, anti-inflammatory and anti-hypertensive effects.

The buckwheat seeds are rich in nutrient compounds, such as proteins, dietary fibers, resistant starch, flavonoids, polyunsaturated fatty acids, vitamin B_1, C and B_2 (Bonafaccia, Marocchini, & Kreft, 2003; Javornik, Eggum, & Kreft, 1981; Kitabayashi, Ujihara, Hirose, & Minami, 1995).

Of these nutrient compounds, the rutin, qucertin and other flavonoids in buckwheat seed (Kreft, Fabjan, & Yasumoto, 2006) cannot be synthesized by humans and have antioxidative activity. No rutin was found in cereals and pseudocereals except buckwheat (Oomah & Mazza, 1996). And it was found that flavonoids in buckwheat seeds could be increased by germination (Kim, Kim, & Park, 2004). The proteins in buckwheat seeds have a high and balanced essential amino acid content, which are nutritionally superior to that of cereal grains (Pomeranz & Robbins, 1972). But the digestibility of buckwheat seed proteins is relatively low in human body, owing possibly to the existence of tannins, phytic acid and protease inhibitors. Starch and edible fibers account for 60–70 wt.% in buckwheat seeds. The chemical compositions of buckwheat starch are similar to those in corn. It was reported that buckwheat starch contained 21–26 wt.% amylase. It has a high biological value, but its digestibility is also relatively low, which is ascribed to its structure and constituent characteristics (Skrabanja, Laerke, & Kreft, 1998).

In summary, the digestibility of some large molecular nutrients in buckwheat, such as proteins and starch, is low. But if they were fragmented by enzymes in germination, their biological utilization rate should be improved greatly. Until now, there is no clear information for these aspects as far as authors know. In this paper, the dynamic changes of nutrient components and antioxidant activity in buckwheat seeds during germination were investigated to make full use of buckwheat and to improve its biological utilization rate and activity.

2. Materials and methods

2.1. Materials and germination treatment

The tartary buckwheat seeds (shanxi heifeng 1[#]) were obtained from Shanxi Long Qiao Co. Ltd. The seeds were soaked, washed with distilled water and put in flats lined with moist paper towels. The flats were covered with aluminum foil and the seeds were germinated in the dark at 37 °C for 1, 2, 3, 4, 5, 6 and 7 days. The germinated sprouts were analyzed each day according to the following methods.

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2.2. Protein

The protein of the sprouts was analyzed, following the method given by Bradford (Bradford, 1976).

2.3. Amino acids

The contents of amino acid in the sprouts were determined by an amino acid analyzer (L-8500, Hitachi, Tokyo, Japan) after the samples were hydrolyzed in the presence of 6 N HCL at 110 °C for 24 h. Methionine and cystine were oxidized by perchloric acid before hydrolysis.

2.4. Fatty acids

The fatty acids in the sprouts were analyzed as described by Rafael and Mancha (Garcés & Mancha, 1993). 0.5 g of freeze-dried buckwheat sprouts was heated with a reagent mixture containing methanol: heptane: benzene: 2,2-dimethoxypropane: H_2SO_4 (37:36:20:5:2, v/v). A simultaneous digestion and lipid transmethylation took place during the heating. After the samples were cooled at the room temperature, the upper layers containing fatty acid methyl esters (FAMEs) were ready for the capillary GC analysis. The GC analysis was performed on a HP 6890 system (HP Co., USA) equipped with a FID by using a HP-Innowax capillary column (0.25 mm × 30 m). The temperature of column was raised from 150 to 280 °C at a rate of 4 °C min^{-1}. The flow rate of carrier gas (nitrogen) was 10 mL min^{-1}. During the analysis, the temperatures of inlet and detector were maintained at 250 and 300 °C, respectively. The standard FAME mixture (C_{14}–C_{22}) was obtained from Supelco (Bellefonte, USA).

2.5. Chlorophyll

Chlorophyll concentrations were measured following the procedure by Mirecki and Teramura (Mirecki & Teramura, 1984). 0.2 g of fresh samples was employed, which was left overnight at 4 °C in a 10 mL acidified methanol [79/20/1 v/v/v, (CH_3OH/H_2O/HCL)] to extract chlorophyll. The extracts were diluted by sixfolds of the acidified methanol and analyzed by spectrometric method at wavelength of 300 nm.

2.6. Vc, V_{B1}, V_{B2}, V_{B6}, total sugar and reducing sugar

The analysis of Vc, V_{Bs}, total sugar, reducing sugar in buckwheat sprouts was performed according to the standard AOAC methods (AOAC, 1997).

2.7. Total flavonoids, rutin and qucertin

Finely ground dehulled buckwheat seed samples (0.02–0.2 g) were transferred into a 25 mL glass bottle and 8 mL of 80% methanol was added, which were mixed for 24 h and centrifuged. The supernatants were combined and made up to a total volume of 10 mL with 80% methanol for a quantitative analysis of the total flavonoids, quercetin and rutin.

HPLC was performed using a Spectra-Physics (Hewlett-Packard series 1100, Agilent Technologies, Inc., Santa Clara, CA, USA) instrument Spectra System P4000, equipped with Hibar – LiChrospher 100, RP-18 (5 μm) column (E. Merck, Darmstadt, Germany, 250 mm × 4.6 mm). The solvents for HPLC were acetonitrile and methanol mixture (1:2) named as A, and 0.75% aq. H_3PO_4 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 340 nm (rutin), 370 nm (qucertin) and identified by a comparison of each compound with the retention time of the relevant standard solution.

All above analysis was repeated independently for three times, by which averaged data were obtained as summarized below.

2.8. Determination of superoxide anion scavenging activity

Superoxide anion scavenging activity of the ethanolic extract from the buckwheat sprout was based on the method described by Robak and Gryglewski (Robak & Gryglewski, 1988) with a slight modification. The ethanol solutions of the buckwheat sprout extracts (0-7d) and ascorbic acid were prepared. One milliliter of NBT solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4), and 0.1 mL of the ethanolic extract from buckwheat sprout were mixed. The reaction was initiated by adding 100 μL of phenazine methosulphate (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture, the reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against blank samples. A decreased absorbance of the reaction mixture is indicative of an increased superoxide anion scavenging activity. The inhibition degree to the generation of superoxide anion was calculated using the following formula:

$$\% \text{ scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the buckwheat sprout extract.

3. Results and discussion

3.1. Sugar content

Fig. 1 shows the changing trend of total sugar content and reducing sugar content with time respectively during the germination of tartary buckwheat. It was found that the total sugar content decreased with germination time in general while the reducing sugar content increased remarkably with germination time. The sugar consumption was increased in germination and the reducing sugar was accumulated in the seeds for germination as a result of a degradation of total sugar. It can be inferred that the large molecular of sugar be hydrolyzed to small molecular reducing sugar during germination to provide sprouting energy and other requirements for buckwheat. Therefore, monosaccharides were accumulated increasingly in buckwheat sprouts during germination, making the buckwheat sprouts good source of foodstuff in food industry.

3.2. Protein and amino acids

Fig. 1 shows the evolution of the protein content with time during the germination of tartary buckwheats. It was found that the protein content declined with germination time.

The effect of the germination time on the amino acid contents is tabulated in Table 1. It was found that the amino acid content decreased first and then slowly increased with the germination time as compared with the control. But the arginine content was lower than that of control within germination days investigated.

The data showed that an increase of the amino acid accumulation was accompanied by a decrease of protein contents. This indicated that the amino acids were accumulated as a result of a degradation of protein, which increased the digestibility of buckwheat protein.

Buckwheat protein has hypocholesterolemic, anti-constipation and antiobesity activities and chemopreventive activity against mammary tumorigenesis (Kayashita, Shimaoka, Nakajoh, Kishida, & Kato, 1999). These functions appear to be caused by its lower

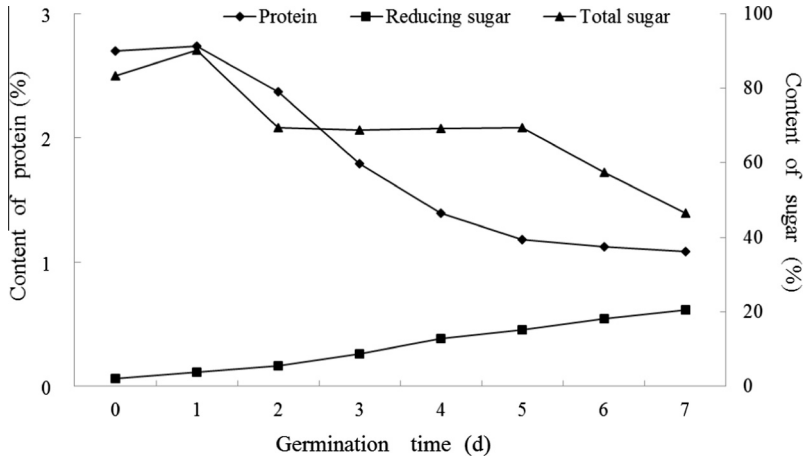

Fig. 1. The changing trend of the total-sugar, reducing-sugar and protein content with time during the germination of tartary buckwheat.

Table 1
The contents of amino acids during the germination of tartary buckwheat (%, single AA/total AA).

Time (d)	1	2	3	4	5	6	7
Asp	1.09	1.03	1.10	1.28	1.24	1.27	1.21
Thr	0.45	0.43	0.48	0.53	0.58	0.57	0.59
Ser	0.61	0.58	0.62	0.66	0.68	0.67	0.68
Glu	2.01	1.87	2.08	2.29	2.08	2.23	2.12
Pro	0.40	0.39	0.36	0.31	0.54	0.34	0.52
Gly	0.73	0.67	0.64	0.72	0.73	0.73	0.72
Ala	0.57	0.56	0.63	0.75	0.78	0.76	0.78
Cys	0.11	0.11	0.08	0.06	0.07	0.04	0.07
Val	0.54	0.52	0.66	0.78	0.72	0.80	0.71
Met	0.19	0.18	0.19	0.14	0.09	0.12	0.10
Ile	0.47	0.45	0.51	0.60	0.60	0.62	0.59
Leu	0.83	0.80	0.92	1.06	1.11	1.11	1.09
Tyr	0.33	0.31	0.29	0.32	0.34	0.32	0.39
Phe	0.56	0.53	0.55	0.62	0.64	0.65	0.63
Lys	0.75	0.71	0.83	0.99	1.02	1.02	0.99
His	0.33	0.30	0.34	0.40	0.42	0.42	0.42
Arg	1.15	1.04	0.97	0.99	0.94	0.90	0.90

digestibility. The physiologic functions of dietary buckwheat protein appear to be similar to those of some dietary fibers. So the functions will be decreased after germination but the nutritional value will be increased. Forevermore, it has been considered that a higher arginine/lysine ratio may lead to a lower cholesterol. But this function of buckwheat protein would also be declined for the decrease of arginine content after germination.

3.3. Fatty acid composition

Table 2 shows the change of fatty acid contents in buckwheat seeds with the germination days. The data showed that the contents of palmitic acid, stearic acid, eicosenoic and arachidic acid increased, but that of linoleic acid decreased. Polyunsaturated fatty acids have been shown to lower the risk of heart attacks (Dorrell, 1971). Therefore, fatty acid change during germination was also two-sided in terms of their functions.

3.4. Vitamins

The effect of the germination time on the Vc content was determined and the data are shown in Fig. 2. It was found that the Vc content decreased firstly and then increased sharply in the germination period compared with the control. The Vc content was 3.5 times as large as that of control at the 7th day.

Table 2
The contents of fatty acids during the germination of tartary buckwheat (relative content, %).

Time (d)	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	Eicosenoic	Arachidic acid
0	14.6	27.9	53.8	2.6	0.8	0.4
1	16.3	21.5	54.3	3.0	2.2	1.1
2	16.7	20.0	59.1	2.0	1.4	0.7
3	15.1	35.3	36.6	4.3	3.5	1.9
4	11.5	10.6	60.7	13.4	1.7	1.4
5	15.3	26.0	51.8	2.0	2.5	0.9
6	13.8	23.5	50.3	6.9	1.9	1.0
7	15.8	14.9	61.4	6.7	0.6	0.4

Fig. 2 shows the changing trend in the contents of V_{B1}, V_{B2}, V_{B6} during the germination of tartary buckwheats. It was found that the V_{B1} contents increased with germination time from 1 to 7 days. But the contents of V_{B2}, V_{B6} had no apparent change.

3.5. Flavonoids

The rutin content of non-germinated/germinated buckwheat seeds is given in Fig. 3. The rutin and quercetin content had no regular changing trend with germination and the changing pattern of the former was rightly opposite to that of the latter. The contents of total flavonoids increased with germination time and leveled off after the 4th day. The content of total flavonoids was higher than the sum of rutin and quercetin content, indicating that there were other flavonoids present in the sprouts, which might be synthesized or transformed from other compounds during germination.

Rutin is widely present in plants but is relatively rare in their edible parts. No rutin is present in cereals and pseudocereals except buckwheat. The presence of rutin in buckwheat plants and foods is one of the main reasons for the production of different kinds of buckwheat foods. Different cultivars of buckwheat may have different rutin contents. Different parts of plants contain different concentrations of rutin. The Fig. 3 indicated that germination is a good way for accumulation of bioactive flavonoids, such as rutin.

3.6. Chlorophyll

Fig. 3 shows the changing trend of the chlorophyll contents during the germination of tartary buckwheat. It was found that the contents of total chlorophyll, chlorophyll B and chlorophyll A all

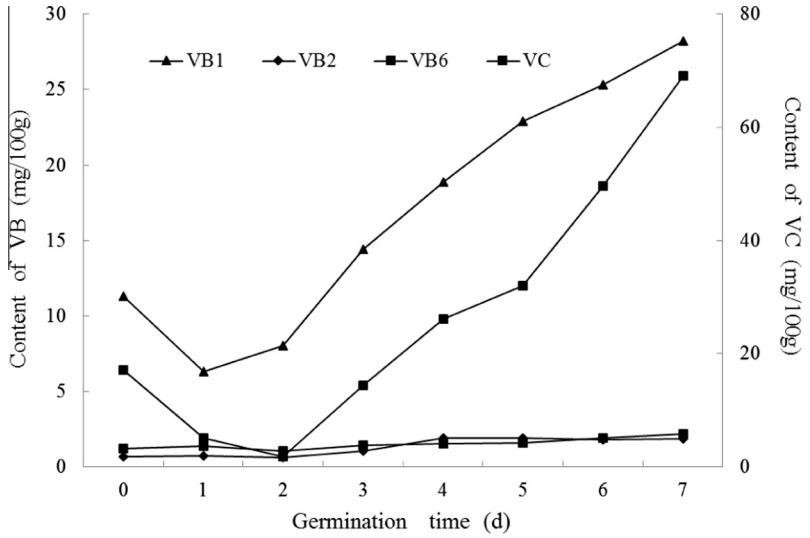

Fig. 2. The evolution of the Vc and B group vitamins contents during the germination of tartary buckwheat.

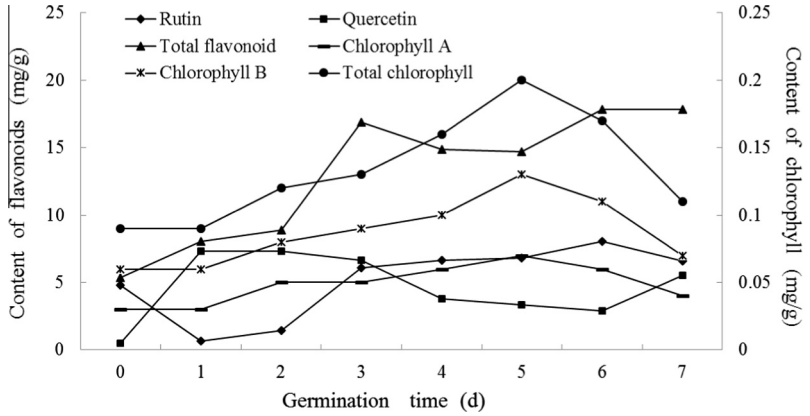

Fig. 3. The changing trend of the flavonoid and chlorophyll contents during the germination of tartary buckwheat.

exhibited a maximum on the 5th day. Moreover, the accumulation of chlorophyll A was always less than that of the chlorophyll B during the germination period. On the other hand, the content of total chlorophyll was higher than the sum content of chlorophyll A and B, indicating that other pigments maybe present in the germination.

3.7. Antioxidative activity

The scavenging effects of buckwheat sprouts extracts to superoxidant radicals was investigated by colorimetry method as shown in Fig. 4. The result showed that the buckwheat sprouts extracts possessed powerful scavenging effects to superoxidant radicals, which were all better than ascorbic acid. This might be caused by the accumulation of antioxidative compounds after germination, such as rutin, fatty acids, vitamins that have scavenging effects. The antioxidative compositions in buckwheat seeds included antioxidative substances and antioxidative enzymes. Except the antioxidative substances analyzed in this paper, the enzymes could have contributions to the scavenging effects for free radicals. The germination of buckwheat seeds could improve their antioxidative activity.

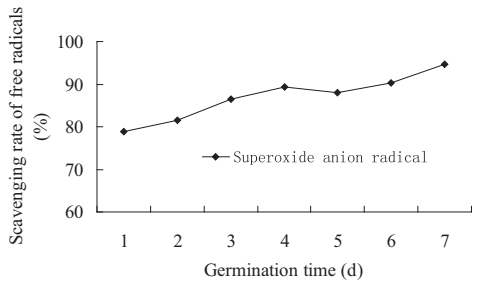

Fig. 4. The changing trend of the antioxidative activity during the germination of tartary buckwheat.

4. Conclusions

Buckwheat has high nutritional and pharmaceutical value. Buckwheat sprouts have a soft and slightly crispy texture and are attractive fragrance. Furthermore, the nutritional values of fatty acids, amino acids, reducing sugar and flavonoids, vitamins in buckwheat seeds were improved by germination. The antioxidative activity also increased with germination time from 1 to 7 days. All of these provided helpful information for a better use of buckwheat in food industry.

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