

Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable

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Received 6 October 2003; accepted 11 December 2003

Abstract

By using the mass production system, we could harvest the buckwheat sprouts that had their pericarps completely removed. As seeding days progressed, monosaccharides in buckwheat sprouts were rapidly increased, while di-, tri-, and tetrasaccharides were gradually decreased. Linoleic acid (C18:2) was found to be the major fatty acid of buckwheat sprouts and increased up to 52.1% at 7 days after seeding (DAS), and total unsaturated fatty acid composition was greater than 83%. Free amino acid contents in buckwheat sprouts were almost four-times higher than those of buckwheat seeds. Based on these results, it was concluded that the abundance of lysine, γ -amino-*n*-butyric acid (GABA) and sulfur containing amino acids in buckwheat sprouts provides a high nutritional value as a new vegetable. Rutin (quercetin-3-*O*-rutinoside), quercitrin (quercetin-3-*O*-rhamnoside), chlorogenic acid, and two unknown compounds were presented in both buckwheat seeds and sprouts. As seeding days progressed, rutin, quercitrin, and unknown compound B (431 *m/z*) were notably increased, while chlorogenic acid and unknown compound A (451 *m/z*) were moderately increased. Among the water-soluble vitamins, vitamin B₁ + B₆, vitamin C, and two kinds of unidentified compounds were presented in the buckwheat sprouts. Vitamin C contents of buckwheat sprouts were increased and its maximum content (171.5 mg/100 g) was observed at 7 DAS. On the other hand, vitamin B₁ + B₆ contents were moderately increased. Buckwheat sprouts not only have the soft and slightly crispy texture, and attractive fragrance, but also have abundant nutrients.

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Keywords: Buckwheat sprouts; Free sugars; Fatty acids; Free amino acids; Rutin; Quercitrin; Chlorogenic acid; Water-soluble vitamins

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is not a cereal but is usually grouped with cereals due to its ways of cultivation and utilization. Buckwheat has been used both as a food and a traditional medicine (Marshall & Pomeranz, 1982; Xiaoling, Xie, Na, & Jinliang, 1992). Buckwheat grown in Korea is milled into flour and is used largely for noodles and curds, in addition its pericarp is used for pillow stuffing materials (Choi, Kim, & Kim, 1996). Buckwheat is abundant in nutrients, such as protein, amino acids, and minerals (Ikeda, Yamashita, & Murakami, 1995; Pomeranz, Marshall, Robbins, &

Gilbertson, 1975). Buckwheat is cited as an origin plant of rutin (Couch, Naghski, & Krewson, 1946), which is a kind of flavonol glycoside compounds used in preventing edema, haemorrhagic diseases, and stabilizing high blood pressure due to its effectiveness in controlling blood vessel (Havsteen, 1983; Xiaoling et al., 1992).

In our previous report, we have introduced the buckwheat sprouts as a new vegetable for the first time (Kim et al., 2001). Buckwheat sprouts have a soft and slightly crispy texture and an attractive fragrance, but do not have beany flavor as soybean sprouts do. Buckwheat sprouts are abundant in amino acids, minerals, and crude fiber. We noted that lysine contents of buckwheat sprouts were notably higher than those of buckwheat seeds and other cereals. Moreover the rutin contents of buckwheat sprouts were higher than those of buckwheat seeds. Based on these properties, we have recommended the buckwheat sprouts as a new source of

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vegetable. Recently, we have successfully developed the new cultural method and the mass production system for the cultivation of buckwheat sprouts. In the present study, we discuss about the changes in free sugars, fatty acids, free amino acids, water-soluble vitamins, and flavonoids during cultivation to evaluate the nutritional properties of buckwheat sprouts.

2. Materials and methods

2.1. Plant materials

‘Dasanmaemil’, a new buckwheat variety developed for the usage of buckwheat sprouts (Fig. 2(a)) at the National Institute of Crop Science in Korea, was tested in this study. As briefly shown in Fig. 1, buckwheat sprouts were cultured by using a mass production system which was constructed to control the temperature and water-spray at a regular interval (Fig. 2(b)), and the culture vessels were specially designed to remove the pericarps from buckwheat sprouts (Fig. 2(c)). General characteristics of buckwheat sprouts were measured according to days after seeding (DAS). Randomly collected buckwheat sprouts were immediately frozen in -70°C refrigerator. After freeze-dried, the buckwheat sprouts they were finely grounded to be used for preparing the samples of chemical analysis.

2.2. Analysis of free sugars

Free sugars were analyzed using YMC-Pack Polyamine II column $5\text{ }\mu\text{m}$ ($250 \times 4.6\text{ mm}$) from YMC Co., Ltd. (Kyotyo, Japan). Twenty milliliters of 20% ethanol solution was added to 1.0 g of freeze-dried

sprouts and shaken for 60 min at 35°C water-bath. It was then centrifuged at $5000g$ for 5 min. The collected supernatant was filtered with Sep-Pak NH_2 solid-phase extraction cartridge (Waters, USA), then 1.0 ml of filtrate was evaporated to dryness at 50°C dry-bath by blowing with N_2 gas. The residue was dissolved in 0.2 ml of water. Then $20\text{ }\mu\text{l}$ was injected into HPLC equipped with Waters 510 Pump, Waters 410 R.I. Detector, and Waters 746 Integrator. The operating conditions were as follows: column temperature 35°C ; detector temperature 39°C ; mobile phase acetonitrile:water (65:35, v/v); flow rate 0.7 ml min^{-1} . The simple sugars and oligosaccharides were obtained from Sigma (St. Louis, USA).

2.3. Analysis of fatty acids

The fatty acid was analyzed as described by Rafael and Mancha (1993). The procedure were as follows: 0.5 g of freeze-dried buckwheat sprouts was heated with a reagent containing methanol:heptane:benzene:2,2-dimethoxypropane: H_2SO_4 (37:36:20:5:2, v/v). The simultaneous digestion and lipid transmethylation took place in a single phase at 80°C . After they were cooled at the room temperature, the upper phases containing the fatty acid methyl esters (FAMES) were prepared 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 (cross-linked polyethylene glycol) column ($0.25\text{ }\mu\text{m} \times 30\text{ m}$). The initial temperature of 150°C was raised to the final temperature of 280°C at a rate of $4^{\circ}\text{C min}^{-1}$. Carrier gas was nitrogen at a flow rate of 10 ml min^{-1} . During the analysis, the temperatures of inlet and detector were maintained at 250 and 300°C , respectively. The stan-

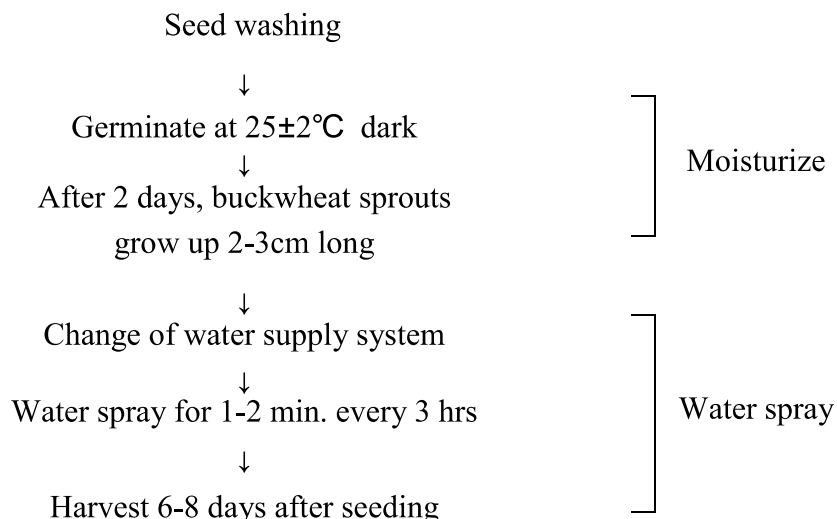


Fig. 1. Procedure for the mass production of buckwheat sprouts by automatic culture system.

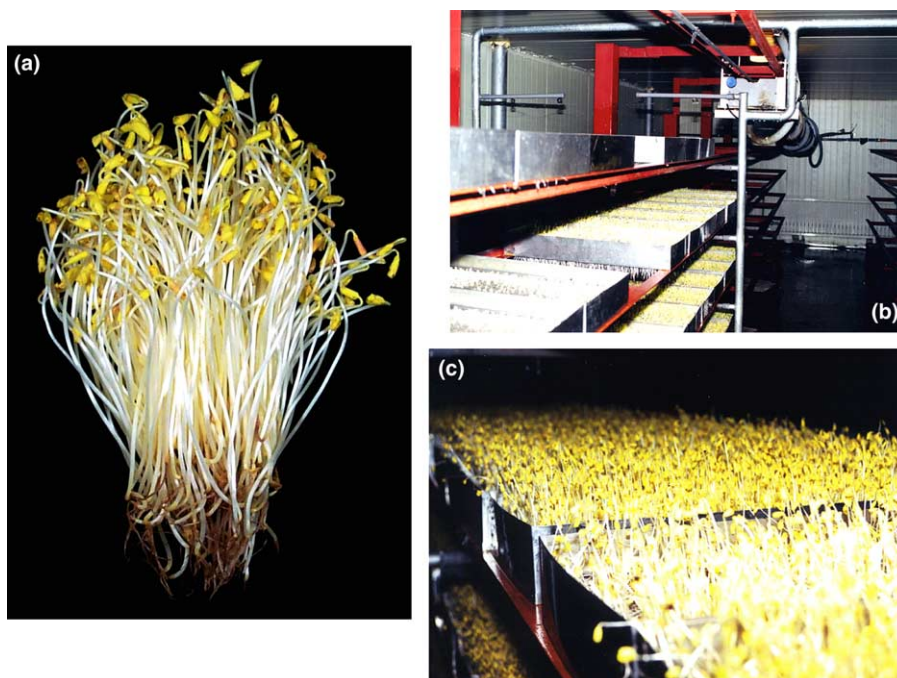


Fig. 2. Buckwheat sprouts (a) and mass production system (b,c).

dard FAME mix (C14–C22) was obtained from Supelco (Bellefonte, USA).

2.4. Analysis of free amino acids

Free amino acid (FAA) contents were determined by L-8800 high-speed amino acid analyzer (Hitachi, Japan). The 1.0 g of freeze-dried buckwheat sprouts was diluted with 10 μ l of 3% trichloroacetic acid solution. The sample was left at the room temperature for 1 h, centrifuged at 10,000g for 15 min. The collected supernatant was filtered with Millipore 0.45 μ m syringe filters (Milford, USA). The filtrate was loaded on amino acid analyzer. The standard amino acid solutions, type AN-II and type B, were obtained from Wako (Wako-shi, Japan).

2.5. Analysis of phenolic compounds

The 1.0-g of freeze-dried buckwheat sprouts was extracted with 30 ml of methanol for 1 h at 80 °C. After filtration with Sep-Pak C₁₈ solid-phase extraction cartridge, the filtrate was used for HPLC.

Analysis of rutin and phenolic compounds in buckwheat sprouts was performed on a Waters 2690 separation module (Waters Milford, USA) equipped with a photodiode array detection system Waters 996. The column used was an XTerra MS C₁₈, 5 μ m (150 $\times$ 2.1 mm) from Waters. The column was operated at the temperature of 25 °C. The mobile phase consisted of 2% acetic acid in water (eluent A) and of 0.5% acetic acid in

water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 10% B to 55% B (50 min), 55% B to 100% B (10 min), 100% B to 10% B (5 min). The injection volume for all samples was 2 μ l. Spectra were recorded from 200 to 450 nm at a flow-rate of 0.2 ml min⁻¹. HPLC mass spectrometry was carried out with a micromass electrospray interface ZMD 4000 (Micromass, Manchester, UK). Nitrogen was used as nebulizing gas. Source block and desolvation temperature were 150 and 250 °C, respectively. Mass condition: electrospray ionization (negative mode), cone voltage ramp 10–50 V; acceleration lens potential 0.5 kV; scan rate 0.4 scans s⁻¹, multiplier voltage 650 V. Chlorogenic acid, quercitrin, and rutin were obtained from Sigma (St. Louis, USA).

2.6. Analysis of water-soluble vitamins

The 1.0-g of freeze-dried buckwheat sprouts was diluted with 20 ml of Milli-Q water (Millipore, USA), and then sonicated for 10 min. The resulting mixture was then centrifuged at 5000g for 15 min. The supernatant was filtered through a 0.4 μ m membrane filter. Twenty microliters of the filtrate was injected into the HPLC equipped with dual wavelength detector (Waters 2487) (Milford, USA) and detection was monitored at 254 nm. A reversed-phase Nova-Pack C₁₈ (150 $\times$ 3.9 mm) column was used for the analysis of water-soluble vitamins at room temperature. The ion-pair mobile phase of the HPLC system consisted of 5 mM tetrabutylammonium phosphate:acetonitrile (75:25, v/v) was used at a flow

rate of 0.6 ml min⁻¹. Acquisition of chromatographic data was made with an Autochro-WIN data module (Younglin instrument, Korea). Vitamin B₁ (thiamin), B₆ (pyridoxine), and vitamin C (ascorbic acid) were obtained from Sigma (St. Louis, USA).

2.7. Statistical analysis

There were three replicates for all measurements. The data obtained from the analysis were statistically analyzed using SAS release ver. 6.12 for Windows (Statistical Analysis Systems Institute Inc., Raleigh, NC, USA).

3. Results and discussion

3.1. Buckwheat sprouts

Buckwheat sprouts (Fig. 2(a)) have a light-yellow colored cotyledon and a bright-white colored hypocotyls, making their appearance similar to soybean sprouts.

By using the mass production system (Fig. 2(b) and (c)), approximately 8.9 kg of buckwheat sprouts were harvested from the 1.0 kg of buckwheat seeds. Furthermore, we observed a high germination ratio (85% <, data not shown) and obtained the buckwheat sprouts that had their pericarps completely removed (Fig. 2(a)).

Pericarp shedding rate is one of the important quality related characteristics of buckwheat sprouts because the pericarp-remained sprouts are not only difficult to be removed, but also to become an obstacle in using them as fresh vegetables.

In general, the pericarp shedding of buckwheat sprouts begin from at 5 DAS and their shedding rate reaches approximately 87% at 8 DAS. In the previous report, we suggested that water-spray culture is more efficient than other methods for accelerating the pericarp shedding of buckwheat sprouts. However, it was also suggested that the more effective one should be developed to lessen the defect. Therefore, the new culture method and system were developed to remove the pericarps from buckwheat sprouts (Figs. 1 and 2), and by using the new culture system, we could harvest the buckwheat sprouts that have their pericarps were almost completely removed.

In Korea, buckwheat sprouts are approximately two times more expensive than soybean sprouts and other types of sprouts, due to the higher price of buckwheat seeds and technical difficulties in buckwheat sprouts production. We hope that the new cultivation method would be reduced the amount of the cost in buckwheat sprouts production and consumer's price.

3.2. Free sugar contents

Eight kinds of free sugars, such as rhamnose, fructose, glucose, sucrose, maltos, raffinose, DP3 (degree of polymerization), and DP4 were observed in buckwheat seeds (Fig. 3). Sucrose and raffinose were presented as the major free sugars in buckwheat seeds, while small quantities of rhamnose, fructose, glucose, maltose, DP3, and DP4 were detected by the HPLC system. As seeding days progressed, the contents of monosaccharide (fructose and glucose) in buckwheat sprouts were markedly increased (Fig. 3(a)), while disaccharides (sucrose and maltose), trisaccharides (raffinose and DP3), and tetrasaccharide (DP4) were rapidly decreased (Fig. 3(b)). Maltose, DP3, and DP4 were observed only until 2 DAS of buckwheat sprouts, while sucrose was continuously decreased until 6 DAS. However, rhamnose was presented until 8 DAS and its changing pattern was relatively moderated compared to other free sugars.

Obtained results illustrated that before the hydrolysis of starch is fully activated in buckwheat seeds, di-, tri- and tetrasaccharide were promptly degraded to simple carbohydrates or monosaccharides such as fructose and glucose to provide the energy and synthetic requirements of buckwheat sprouts. Therefore, buckwheat sprouts increasingly accumulated a monosaccharide during germination and these properties of buckwheat sprouts will enable us to use buckwheat sprouts as a good source of vegetable.

3.3. Fatty acid composition

The fatty acid composition of buckwheat seeds indicates that buckwheat seeds are abundant in unsaturated fatty acids, with only lower amounts present in saturated fatty acids (Table 1). The composition rates of unsaturated fatty acids, such as oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) are 36.8%, 38.1%, and 2.7%, respectively. Meanwhile, major saturated fatty acid of buckwheat seeds is palmitic acid (16:0) which comprised approximately 17.7% of total fatty acids. Accordingly, only small quantities of myristic acid (14:0), stearic acid (18:0), arachidic acid (20:0), and behenic acid (22:0) were observed. As seeding days progressed, however, all the saturated fatty acids were rapidly decreased and myristic and stearic acid were not present in 1 DAS of buckwheat sprouts, while arachidic and behenic acid were not detected in 3 DAS of buckwheat sprouts. Among the unsaturated fatty acids, oleic acid was gradually decreased, while linoleic and linolenic acid were increased as germination days progressed. Total unsaturated fatty acid (oleic + linoleic + linolenic acid) in 6–8 DAS of buckwheat sprouts was greater than 83%, and the composition rate of linoleic acid was comprised approximately 50–52%.

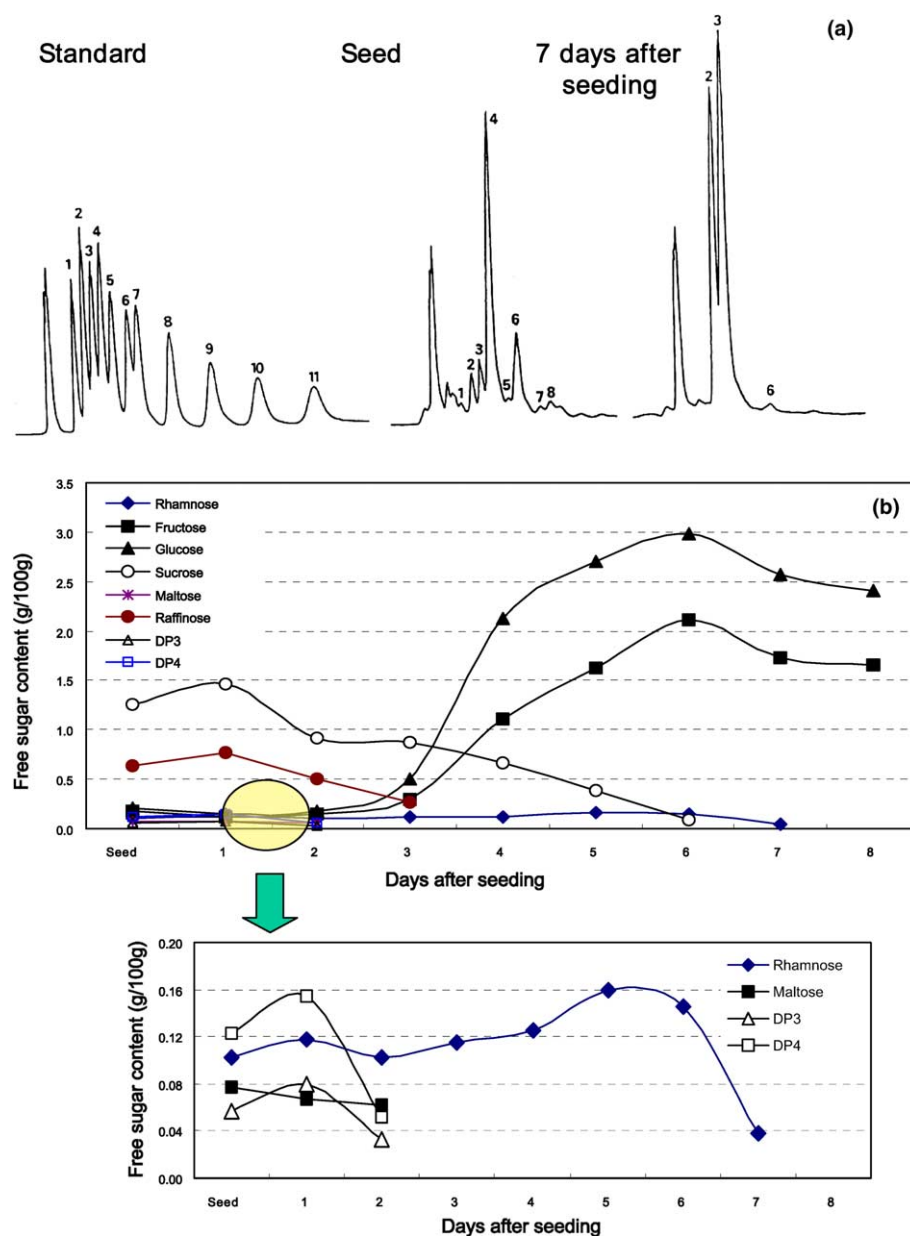


Fig. 3. Comparison of HPLC chromatogram of free sugars between buckwheat seeds and sprouts (a), and the changes of free sugar contents in buckwheat sprouts according to days after seeding (b). (1) rhamnose; (2) fructose; (3) glucose; (4) sucrose; (5) maltose; (6) raffinose; (7) DP3; (8) DP4; (9) DP5; (10) DP6; (11) DP7. Values are the means of three samples.

Table 1

Fatty acid composition of buckwheat sprouts according to days after seeding

Days after seeding	Composition of fatty acids (%)							
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
Seeds	0.33 ± 0.0*	17.7 ± 1.9	1.8 ± 0.1	36.8 ± 2.1	38.1 ± 2.5	2.7 ± 0.1	1.1 ± 0.3	1.6 ± 0.2
1	—	18.7 ± 1.8	1.7 ± 0.1	35.4 ± 1.9	39.9 ± 2.5	2.9 ± 0.2	—	1.5 ± 0.0
2	—	18.6 ± 2.0	1.5 ± 0.2	33.6 ± 2.0	41.6 ± 2.9	3.4 ± 0.1	—	1.3 ± 0.1
3	—	19.1 ± 1.9	—	31.3 ± 2.2	45.6 ± 3.1	4.0 ± 0.2	—	—
4	—	18.0 ± 1.5	—	28.7 ± 1.7	45.1 ± 3.0	8.2 ± 0.3	—	—
5	—	18.4 ± 0.9	—	25.9 ± 2.1	46.7 ± 3.2	9.1 ± 0.3	—	—
6	—	17.5 ± 0.8	—	19.5 ± 1.6	50.7 ± 3.6	12.3 ± 0.6	—	—
7	—	16.9 ± 1.0	—	16.9 ± 1.9	52.1 ± 2.7	14.1 ± 0.9	—	—
8	—	14.6 ± 0.8	—	15.4 ± 1.4	51.1 ± 3.0	18.9 ± 0.8	—	—

* Means ± SE, $n = 3$.

Polyunsaturated fatty acids such as linoleic acid (18:2), linolenic acid (18:3), and arachidonic acid (20:4) are called essential fatty acids (EFA) because of their necessity in the human body (Wassef, 1985). Linolenic acid is a known enhancer for transporting bioactive compounds into the skin, and it is converted to arachidonic acid which serves as a precursor for powerful hormone-like compounds (Wassef, 1985). Most parts of the fatty acids in buckwheat sprouts are unsaturated, with linoleic acid being the major fatty acid in buckwheat sprouts.

3.4. Free amino acids

Table 2 shows the FAA contents in buckwheat seeds and in 3, 5, and 7 DAS of buckwheat sprouts. The 32

kinds of FAA including NH_3 were found in buckwheat seeds, while the 34 kinds of FAA were found in 7 DAS of buckwheat sprouts. Most of FAA contents in buckwheat sprouts were increased as seeding days progressed and total FAA contents in buckwheat sprouts were almost four-times higher than those of buckwheat seeds. Cystine, β -amino isobutyric acid, 1-methylhistidine, and 3-methylhistidine were not presented in buckwheat seed, while sarcosine and carnosine were not present in 7 DAS. However, anserin was present only in 5 DAS of buckwheat sprouts.

We have discussed in the previous report that total amino acid (TAA) contents of buckwheat sprouts (acid hydrolysis with 6 N HCl) were approximately 28–38% higher than those of buckwheat seeds, and that 14 kinds of amino acids were notably increased (Kim et al.,

Table 2
Free amino acid contents of buckwheat sprouts according to days after seeding

Free amino acids	Seed	Day after seeding		
		3	5 (mg/100 g, DW)	7
Phosphoserine	4.3 $\pm$ 0.2*	6.1 $\pm$ 0.2	10.3 $\pm$ 0.2	36.2 $\pm$ 2.3
Taurine	2.1 $\pm$ 0.1	2.3 $\pm$ 0.0	4.3 $\pm$ 0.8	1.6 $\pm$ 0.0
Phospho ethanol amine	1.5 $\pm$ 0.0	2.4 $\pm$ 0.3	5.2 $\pm$ 1.0	11.3 $\pm$ 2.4
Urea	37.8 $\pm$ 3.3	27.3 $\pm$ 2.5	65.9 $\pm$ 5.2	10.4 $\pm$ 2.1
Aspartic acid	24.7 $\pm$ 2.2	26.5 $\pm$ 2.4	50.7 $\pm$ 2.3	38.0 $\pm$ 3.2
Threonine	12.4 $\pm$ 1.7	26.7 $\pm$ 1.9	46.7 $\pm$ 3.6	94.9 $\pm$ 7.6
Serine	10.3 $\pm$ 0.9	30.7 $\pm$ 2.7	51.5 $\pm$ 3.1	114.6 $\pm$ 10.3
Glutamic acid	58.8 $\pm$ 5.2	99.3 $\pm$ 5.2	158.3 $\pm$ 8.8	72.8 $\pm$ 5.2
Sarcosine	2.7 $\pm$ 0.7	3.1 $\pm$ 0.2	4.1 $\pm$ 0.7	–
α -Amino adipic acid	9.1 $\pm$ 1.1	11.6 $\pm$ 1.1	19.0 $\pm$ 2.1	21.0 $\pm$ 1.3
Glycine	8.6 $\pm$ 1.0	18.7 $\pm$ 2.0	30.1 $\pm$ 3.2	47.5 $\pm$ 4.5
Alanine	8.1 $\pm$ 1.2	47.0 $\pm$ 3.2	64.6 $\pm$ 6.0	55.6 $\pm$ 6.1
Citrulline	0.6 $\pm$ 0.0	3.3 $\pm$ 0.2	9.4 $\pm$ 2.5	27.0 $\pm$ 2.1
α -Amino- <i>n</i> -butyric acid	0.5 $\pm$ 0.0	1.2 $\pm$ 0.1	1.6 $\pm$ 0.1	2.5 $\pm$ 0.6
Valine	26.3 $\pm$ 2.3	40.6 $\pm$ 3.5	64.0 $\pm$ 4.5	119.7 $\pm$ 7.9
Cystine	–	1.1 $\pm$ 0.0	8.2 $\pm$ 1.6	31.9 $\pm$ 2.1
Methionine	3.7 $\pm$ 0.1	7.5 $\pm$ 1.0	8.8 $\pm$ 2.2	14.7 $\pm$ 3.0
Cystathionine	47.5 $\pm$ 3.2	56.1 $\pm$ 3.3	63.5 $\pm$ 2.6	46.9 $\pm$ 1.8
Isoleucine	9.6 $\pm$ 1.5	14.6 $\pm$ 2.1	31.1 $\pm$ 2.0	68.3 $\pm$ 4.1
Leucine	6.8 $\pm$ 1.4	22.7 $\pm$ 1.9	38.4 $\pm$ 3.0	74.9 $\pm$ 3.9
Tyrosine	4.9 $\pm$ 0.8	18.4 $\pm$ 2.0	31.3 $\pm$ 2.5	37.7 $\pm$ 2.3
Phenylalanine	3.7 $\pm$ 0.3	17.6 $\pm$ 2.1	26.6 $\pm$ 3.3	68.6 $\pm$ 3.2
β -Alanine	1.1 $\pm$ 0.1	2.3 $\pm$ 0.1	6.5 $\pm$ 1.0	13.3 $\pm$ 0.8
β -Amino isobutyric acid	–	–	0.6 $\pm$ 0.0	0.6 $\pm$ 0.0
γ -Amino- <i>n</i> -butyric acid	2.5 $\pm$ 0.1	16.5 $\pm$ 2.4	81.9 $\pm$ 5.5	78.8 $\pm$ 6.2
Ethanol amine	0.9 $\pm$ 0.0	4.6 $\pm$ 0.3	19.6 $\pm$ 1.5	55.1 $\pm$ 4.5
NH_3	3.6 $\pm$ 0.3	7.6 $\pm$ 1.0	55.8 $\pm$ 3.4	80.7 $\pm$ 6.4
Hydroxylysine	3.9 $\pm$ 0.6	4.4 $\pm$ 0.2	3.7 $\pm$ 0.8	0.9 $\pm$ 0.0
Ornithine	3.1 $\pm$ 0.2	3.6 $\pm$ 0.0	5.4 $\pm$ 1.1	4.9 $\pm$ 0.7
Lysine	7.0 $\pm$ 1.1	22.0 $\pm$ 0.5	41.4 $\pm$ 3.2	56.2 $\pm$ 2.6
1-Methylhistidine	–	–	2.2 $\pm$ 0.1	2.3 $\pm$ 0.2
Histidine	3.7 $\pm$ 0.3	19.1 $\pm$ 1.1	56.2 $\pm$ 3.6	144.3 $\pm$ 10.2
3-Methylhistidine	–	–	0.6 $\pm$ 0.0	0.9 $\pm$ 0.0
Anserine	–	–	2.1 $\pm$ 0.0	–
Carnosine	4.6 $\pm$ 0.6	2.2 $\pm$ 0.3	1.0 $\pm$ 0.0	–
Arginine	73.0 $\pm$ 5.2	115.6 $\pm$ 11.0	224.5 $\pm$ 12.1	146.0 $\pm$ 7.8
Proline	2.9 $\pm$ 0.4	11.3 $\pm$ 1.3	22.8 $\pm$ 2.4	16.2 $\pm$ 1.6
Total	389.9 $\pm$ 35.2 (100)	694.1 $\pm$ 56.7 (178.0)	1317.9 $\pm$ 91.0 (338.0)	1596.3 $\pm$ 115.5 (409.4)

* Means $\pm$ SE, $n = 3$.

2001). In this study, FAA contents in buckwheat sprouts were markedly increased and almost four-times higher than seeds. It was considered that abundance of lysine, γ -amino-*n*-butyric acid (GABA), and sulfur containing amino acids such as cystine, methionine, and cystathionine in buckwheat sprouts will provide a high nutritional value because lysine is the first limiting amino acid in the various protein sources, and GABA and sulfur containing amino acids are evaluated as the functional amino acids. Furthermore, the increment in FAA content is favorable as the protein quality of vegetable depends not only on its amino acids but also on the availability of these amino acids (Hamad & Field, 1979).

Buckwheat allergies are not common, however, it is considered to be a very potent allergen. Ingestion of small amounts has been found to produce anaphylactic reactions, particularly in children and susceptible hu-

mans (Davidson, Passero, & Settupane, 1992; Horesh, 1972). Identification and characterization of the major allergen(s) in buckwheat are currently underway, and proteins with molecular masses around 14 and 18 kDa were identified as the major allergenic proteins in the buckwheat-allergic patients' sera (Yoshimasu, Zhang, Hayakawa, & Mine, 2000). Therefore, special attention is need for testing the potential allergins in buckwheat sprouts.

3.5. Phenolic compounds

Buckwheat seeds contain the small quantities of rutin, quercitrin, and chlorogenic acid (Fig. 4(a) and (b)). By using ESI mass, we could observe two unknown compounds (A and B) and successfully identify their molecular weights; 451 *m/z* and 431 *m/z*, respectively. ESI

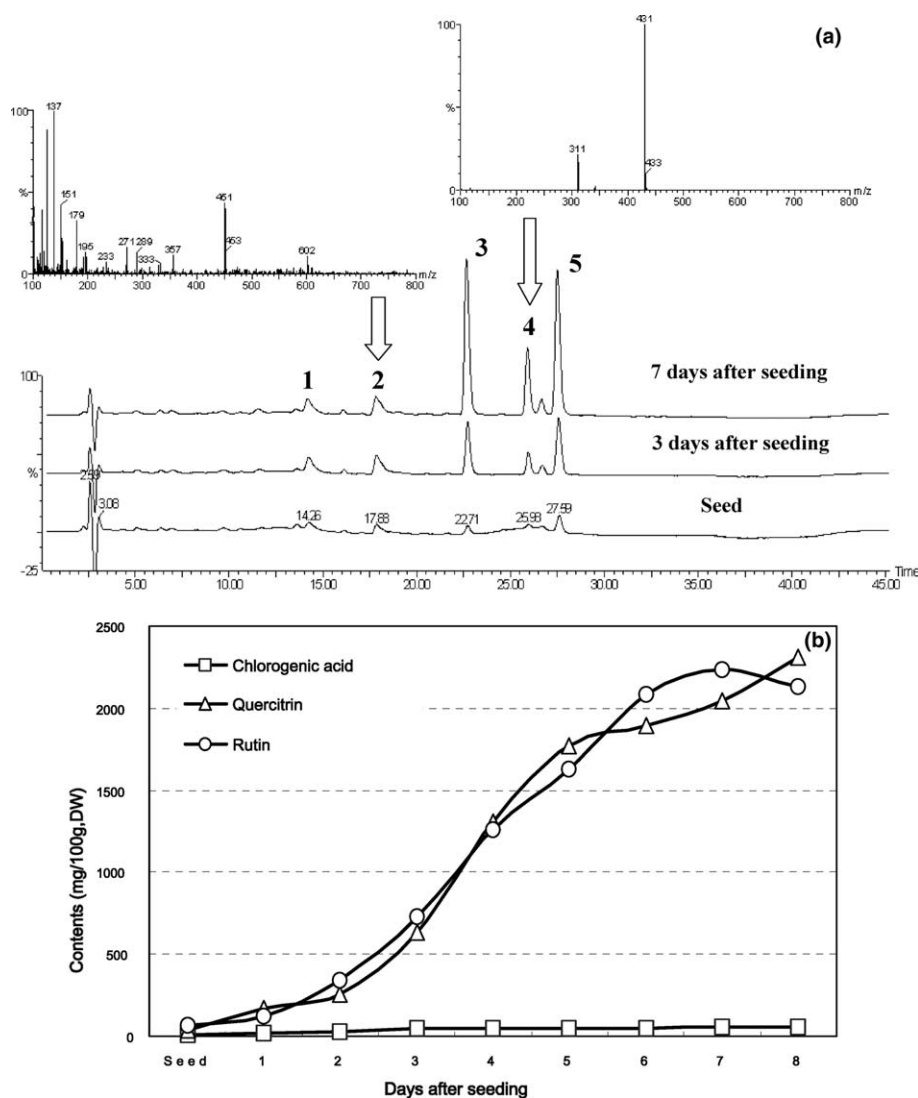


Fig. 4. Comparison of HPLC chromatogram of phenolic compounds between buckwheat seeds and sprouts (a), and the changes of chlorogenic acid, quercitrin, and rutin contents in buckwheat sprouts according to days after seeding (b). (1) chlorogenic acid; (2) 451 *m/z*; (3) quercitrin; (4) 431 *m/z*; (5) rutin. Values are the means of three samples.

mass spectra were recorded in the negative ion mode. Due to the ionization conditions applied, $[M - H]^-$ ions and minimum fragmentations were obtainable (Fig. 4(a)).

As seeding days progressed, the contents of rutin (quercetin-3-*O*-rutinoside) and quercitrin (quercetin-3-*O*-rhamnoside) were gradually increased. The maximum contents of rutin and quercitrin were observed in 7 and 8 DAS of buckwheat sprouts, and their levels were 2236.7 and 2312 mg/100 g, respectively. Rutin (at 7 DAS) and quercitrin (at 8 DAS) contents were approximately 35 and 65 times higher than those of buckwheat seeds (rutin 63.3, quercitrin 35.4 mg/100 g). The result come out interesting that among two unknown compounds, the content of unknown compound B (431 *m/z*) showed notable increase as germination days progressed, while unknown compound A (451 *m/z*) and chlorogenic acid showed slight increment.

Buckwheat is known as an origin plant of rutin known for its pharmacological effects (Havsteen, 1983). Rutin (quercetin-3-*O*-rutinoside), quercitrin (quercetin-3-*O*-rhamnoside), and unknown compound B (431 *m/z*) contents in buckwheat sprouts were rapidly increased as seeding days progressed. It was reported that chlorogenic acid has a same biosynthesis pathway as quercetin-3-glycoside and cyanidin-3-galactoside (Lancaster, 1992). Mohamed, Patricia, and Anthon (2001) suggested that accumulation of chlorogenic acid is apparently not affected by the synthesis of quercetin-3-glycoside and cyanidin-3-galactoside. However, the exact reason responsible for the observed results regarding contents of the flavonoids and phenolic compounds in buckwheat sprouts remains obscure, so it was considered that special attention should be paid to study it further. The dietary and health value of rutin in buckwheat seeds has received an increased attention in recent years. Chlorogenic acid and quercitrin are lesser known than rutin, but since they are present in the buckwheat seeds and sprouts, it could also be interesting as seen in this study results. Rutin and quercitrin have the same aglycone, quercetin. Quercetin is a flavonoid molecule ubiquitous in nature. A number of its actions make it a potential anti-cancer agent, including cell cycle regulation, interaction with type II estrogen binding sites, and tyrosine kinase inhibition. In addition, it appears to be associated with little toxicity (Davis & Matthew, 2000).

3.6. Water-soluble vitamins

Fig. 5 shows the water-soluble vitamin contents in the buckwheat sprouts of 3, 5, and 7 DAS. Among the water-soluble vitamins, vitamin B₁ + B₆, vitamin C, and two kinds of unidentified compounds were observed in buckwheat sprouts, while they were not present in

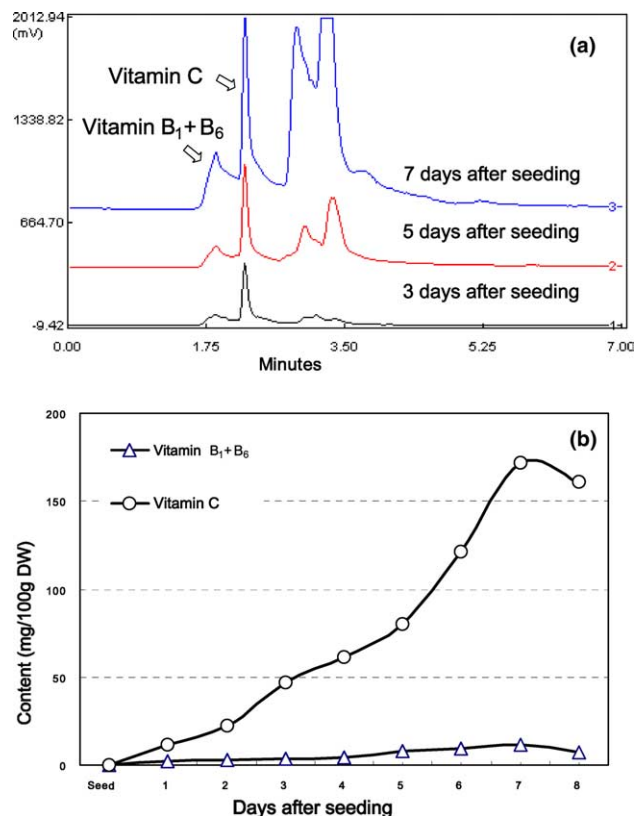


Fig. 5. Comparison of HPLC chromatogram of water-soluble vitamins between buckwheat seeds and sprouts (a), and the changes of vitamin B₁ + B₆ and vitamin C contents in buckwheat sprouts according to days after seeding (b). Values are the means of three samples.

buckwheat seeds. Although vitamins B₁ and B₆ were not separated from each other, and they were overlaid at one peak under this study HPLC conditions (Fig. 5(a)). As seeding days progressed, vitamin B₁ + B₆ contents showed relatively moderate increment reaching their maximum (11.8 mg/100 g) at 7 DAS. However, vitamin C contents increased more rapidly as seeding days progressed and reaching their maximum (171.5 mg/100 g) at 7 DAS. Vitamin B₁ (thiamin) is important for energy metabolism and in initiation of nerve impulses. Vitamin B₆ (pyridoxine) is necessary for proper function of enzymes and for carbohydrate and fat metabolism (Zanzen, Botzer, Zanger, & Shainberg, 1998). Vitamin C (ascorbic acid) is well known as a powerful antioxidant, protecting against oxidative damage to DNA, membrane lipids and proteins. It is also involved in the synthesis of numerous substances (Dekkers, van Doornen, & Kemper, 1996; Fraga, Motchnik, & Shigenaga, 1991; Frei, 1991). Obtained results showed that buckwheat sprouts are abundance in vitamin B₁ + B₆ and vitamin C. Furthermore, the seeding days progressed the contents of two unidentified compounds were markedly increased. Therefore, it was suggested that special attention be paid to determine the characteristics of the compounds.

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