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Comparison of phenolic compositions between common and tartary buckwheat (*Fagopyrum*) sprouts

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Abstract

The phenolic compositions of non-germinated/germinated seeds and seed sprouts (at 6–10 day-old) of common (*Fagopyrum esculentum* Möench) and tartary (*Fagopyrum tataricum* Gaertn.) buckwheats were investigated. Phenolic compounds, including chlorogenic acid, four *C*-glycosylflavones (orientin, isoorientin vitexin, isovitexin), rutin and quercetin, were determined in the seed sprouts by high-performance liquid chromatography (HPLC). In the edible parts of common buckwheat sprouts, individual phenolics significantly increased during sprout growth from 6 to 10 days after sowing (DAS), whereas in tartary buckwheat sprouts they did not. While the sum contents of phenolic compounds in the edible part (mean 24.4 mg/g DW at 6–10 DAS) of tartary buckwheat sprouts were similar to those of common buckwheat sprouts, rutin contents in the non-germinated/germinated seeds (mean 14.7 mg/g DW) and edible parts (mean 21.8 mg/g DW) of tartary buckwheat were 49- and 5-fold, respectively, higher than those of common buckwheat. Extracts of the edible parts of both species showed very similar free radical-scavenging activities (mean 1.7 μmol trolox eq/g DW), suggesting that the overall antioxidative activity might be affected by the combination of identified phenolics and unidentified (minor) components. Therefore, buckwheat seed sprouts are recommended for their high antioxidative activity, as well as being an excellent dietary source of phenolic compounds, particularly tartary buckwheat sprouts, being rich in rutin.

Keywords: Buckwheat sprouts; DPPH assay; Functional food; Phenolic compounds and flavonoids

1. Introduction

Several recent studies in Korea and Japan have focussed on the development of buckwheat (*Fagopyrum* spp.) as a potential "functional food" material, particularly with respect to its seed sprouts (Kim et al., 2006, 2001, Kim, Kim, & Park, 2004; Watanabe & Ito, 2003). Both seeds and seed sprouts of common buckwheat (*Fagopyrum esculentum* Möench) are rich in nutrients and phenolic compounds, and show a good balance of amino acids and minerals (Kim et al., 2004; Pomeranz & Robbins, 1972; Steadman, Burgoon, Lewis, & Edwardson, 2001).

The two main species of buckwheat, namely common and tartary buckwheats (*Fagopyrum tataricum* Gaertn.), are consumed all around the world. Common buckwheat seeds contain between 12.6 and 35.9 mg rutin/100 g DW, thus representing a major dietary source of rutin (Kitabayashi, Ujihara, Hirose, & Minami, 1995), a flavonoid which can protect buckwheat herbs from the induction of ultraviolet radiation damage (Kreft, Štrukelj, Gaberščik, & Kreft, 2002). By contrast, Fabjan et al. (2003) measured rutin content of tartary buckwheat seed at between 800 and 1700 mg rutin/100 g DW, together with only traceable amounts of quercetin, the aglycone of rutin. Moreover, Kim et al. (2006) determined rutin content of green seed sprouts of tartary buckwheat to range between 5000 and

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6000 mg rutin/100 g DW, some 2.2-fold greater than that in similarly produced common buckwheat sprouts.

Flavonoids are the most common and widely distributed group of plant phenolic compounds, but cannot be synthesized by humans or animals (Cook & Samman, 1996). They have received considerable attention because of their beneficial health (antioxidant, antitumor, anti-inflammatory) and pharmacological effects (antituberculosis, antimalarial, antimicrobial, antiviral). The primary activity of plant flavonoids is believed to reside in their free radical-scavenging capacity and their antioxidant activity generally increases with an increase in the number of hydroxyl groups that they bear and a decrease in their glycosylation (Rice-Evans, Miller, & Paganga, 1996). The flavonoids most commonly found in common buckwheat are anthocyanins and four *C*-glycosylflavones (orientin, isoorientin vitexin and isovitexin), along with rutin and quercetin (Margna & Margna, 1978; Watanabe & Ito, 2002). Rutin is a UV–B absorbing secondary plant metabolite, synthesized in higher plants in order to afford them protection from the harmful effects of such radiation. At 28 days after sowing (DAS), rutin concentration and rutin glucosidase (rutin-degrading enzyme) activity in the leaves of tartary buckwheat were 122% and 363% greater, respectively, under UV–B radiation than under ambient illumination (Suzuki, Honda, & Mukasa, 2005).

We have independently developed a mass production system, with an automatic water-supply, for green (non-etiolated) seed sprouts of buckwheat species, particularly those of tartary buckwheat. In the present study, hull-free seed sprouts of common buckwheat were also investigated because the seed sprouts with removed pericarp (seed coat) from the cotyledons are preferred when presented as fresh vegetables in commercial markets (Kim et al., 2004). However, little is known about phenolic levels and their antioxidative activities on the growth of buckwheat seed sprouts. Therefore, the aim of this study was to evaluate the influence of buckwheat sprout growth on phenolic components and to assess free radical-scavenging abilities by monitoring their abilities to quench 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2. Materials and methods

2.1. Plant materials and growing conditions

Seed sprouts of the common buckwheat cultivar, Kitawase, with or without pericarp (namely "hull-on or -free Kitawase" obtained by removing the hull using an impact dehuller) and tartary buckwheat breeding line, Hokkai T 9, were cultivated by a mass production system at the National Agricultural Research Center for the Hokkaido region (Memuro, Hokkaido; longitude, 143°03′E; latitude, 42°55′N).

On September 2, 2005, buckwheat seeds (8 g, 264, 352 and 400 grains for hull-on Kitawase, hull-free Kitawase and Hokkai T 9, respectively), surface-sterilized by soaking for 3 h in aq. 10% (v/v) sodium hypochlorite (NaClO), were sown in perforated plastic Kaiware-daikon (Japanese radish sprout) pots (65 × 65 × 150 mm), packed with polyurethane (Araikasei, Toyohashi, Japan). Twenty-eight pots were prepared per type/species, representing seven treatments with four replicates each. The seeds were germinated in a growth chamber for two days in the dark at 25 °C and approximately 60% relative humidity. Germinated whole seeds with pericarps were harvested 1 and 2 DAS (germinated seeds), and the remaining seeds/pots were transferred to a glass-house at the Memuro Research Station, National Agricultural Research Center for the Hokkaido region. The growth benches (2 × 1 m) were shaded with a plastic netting, which allowed roughly 5% of natural light to reach the developing seedlings (mean light intensity: under the netting, 16 μmol/s/m; above the netting, 504 μmol/s/m). Sprouting seeds were maintained at a mean temperature and humidity of 27 °C and 87%, respectively, and automatically sprayed with de-ionized water (mean pH 6.0) for 5 min (at 2 l/min from a total of 4 nozzles) every hour. Every morning, from 6 to 10 DAS, the sprout portions (shoot and cotyledons) of seed sprouts were harvested by cutting them at the upper surface of the growing medium and removing the pericarp, where appropriate. Plant materials, non-germinated seeds, 1 and 2 DAS germinated seeds, and 6–10 DAS seedlings, cutting off root parts, were immediately lyophilized, ground in an IFM-180G Miller (Iwatani International Co., Tokyo, Japan) and individually stored in a closed plastic bottle in a desicator prior to analysis.

2.2. Solvents and reagents

HPLC-grade acetonitrile (CH_3CN), methanol (MeOH) and ethanol (EtOH) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Chlorogenic acid and flavonoids, including *C*-glycosylflavones (orientin, isoorientin vitexin, isovitexin), rutin and quercetin, which served as external standards, were obtained from Extrasynthèse (Genay, France). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was supplied by Nacalai Tesque (Kyoto, Japan), and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.3. Measurement of sprout growth

Buckwheat sprout growth (fresh and dry weights, FW and DW, and water content) of the edible parts was monitored from 6 to 10 DAS. Sprouts harvested by cutting off the roots with scissors, were then thoroughly washed with flowing Milli-Q water, and their fresh weight per pot recorded. After freeze-drying, their dry weight was recorded, and their former water contents calculated.

2.4. Determination of phenolic contents

Dried plant powder (10 mg) was placed in a 2 ml microcentrifuge tube and extracted with 1 ml of MeOH containing 10% phosphoric acid [0.1% (v/v)]. The mixture was

vigorously vortexed for 5 min at ambient temperature and stored for 3 h at 37 °C in an incubator with 5 min of vortexing after each hour. After centrifugation at 1000 g for 5 min, the supernatant was filtered through a disposable syringe filter (PTFE, 0.5 μm, hydrophobic; Advantec, Tokyo, Japan) (Suzuki, Honda, Funatsuki, & Nakatsuka, 2002). The filtrate was then analyzed by an HPLC (class-VP chromatography data system; Shimadzu, Kyoto, Japan) equipped with a Capcell PAK ODS column (250 × 4.6 mm i.d., particle size 5 μm; Shiseido, Tokyo, Japan). Absorbance was monitored at 350 nm, and the column oven temperature was set at 40 °C. The injection sling was 20 μl. The solvent system was delivered at a rate of 1.0 ml/min and consisted of a mixture of (A) MeOH: water: acetic acid (5: 92.5: 2.5 v/v/v) and (B) MeOH: water: acetic acid (95: 2.5: 2.5 v/v/v). The initial mobile phase composition was 0% solvent B, followed by a linear gradient from 0% to 80% of solvent B in 48 min, then holding at 0% solvent B for an additional 10 min (Watanabe & Ito, 2002). Quantification of the different phenolic compounds were based on peak areas and calculated as equivalents of seven representative standard compounds. All contents were expressed as milligrammes per g dry weight.

2.5. Free radical-scavenging activity by DPPH assay

The DPPH superoxide scavenging activity of 6–10 DAS seedlings was evaluated according to the method of Kim, Lee, Lee, and Lee (2002), with minor modifications. A dried sample was extracted in 80% EtOH so as to result in a final concentration of 10 mg DW/ml. To a 50 μl-sample solution, 50 μl of 20% EtOH, 50 μl of MES–NaOH buffer (pH 5.5, 0.2 M), and 50 μl of 400 μM DPPH–EtOH solution were added. After shaking the mixture vigorously for 20 s, the absorbance was monitored at 492 nm, using a UV-2100PC UV–vis spectrophotometer (Shimadzu, Kyoto, Japan). The solution was then allowed to stand for 30 min at room temperature in the dark, and the absorbance was again measured. The free radical-scavenging activity by DPPH assay was evaluated as the difference in absorbance between the initial and final measurements, and expressed as micromoles of trolox equivalents per g dry weight of sample.

2.6. Statistical analysis

Data were analyzed by application of the Tukey's multiple range test at $P \leqslant 0.05$, using Esumi Statistical Software (version 5.0, 2003; Esumi Inc., Tokyo, Japan). The data shown in all the Tables are the means of four replicates. Correlations of DPPH × rutin and × all phenolic amounts determined in this study were also determined.

3. Results and discussion

3.1. Sprout growth

Sprout growth (fresh and dry weights and water content) was measured from 6 to 10 DAS (Table 1). For Kitawase, the lowest fresh and dry weights were found at 6 DAS and at 10 DAS, respectively, whereas the fresh weight of hull-free Kitawase was not significantly affected by sprout age, though its dry weight decreased significantly. In contrast, the fresh and dry weights of Hokkai T 9 tended to follow patterns similar to those of hull-on Kitawase, with water content increasing significantly with sprout age. Hull-free Kitawase sprouts are thus more desirable than hull-on Kitawase or tartary sprouts in terms of producing a greater quantity of fresh vegetable mass. As a result, we recommend that buckwheat sprouts of both species be harvested at 7 or 8 DAS for the species studied here. While, for common buckwheat, the fresh weight basis yield of 8-DAS etiolated sprouts is roughly 10-fold that of the original seeds (Kim et al., 2001), for light-grown (green) sprouts the disparity in sprout-seed weight was only 2.7-, 4.3- and 2.3-fold in the case of hull-on Kitawase, hull-free Kitawase and Hokkai T 9, respectively. It is our experience that the yield of soil-grown green sprouts of common buckwheat is roughly 5-fold that of the seeds. This suggests that, in the current study, the germination rate was reduced by the high density/quality of seeds, by the growth conditions employed (sun or artificial light, temperature, humidity)

Table 1
Plant growth (g per radish sprout pot)[A,B] of 6–10 DAS seedlings in the edible parts of common and tartary buckwheat sprouts

Days after sowing	Fresh weight (g/pot)			Dry weight (g/pot)			Water content (%)		
	Common		Tartary	Common		Tartary	Common		Tartary
	Hull-on Kitawase	Hull-free Kitawase[C]	Hokkai T 9	Hull-on Kitawase	Hull-free Kitawase	Hokkai T 9	Hull-on Kitawase	Hull-free Kitawase	Hokkai T 9
6 d	19 ± 1.0	33 ± 6.0	14 ± 0.6	2.8 ± 0.2	4.0 ± 0.2	2.5 ± 0.2	85 ± 0.7	88 ± 1.6	82 ± 1.3
7 d	22 ± 0.7	33 ± 4.6	17 ± 0.7	3.0 ± 0.2	3.3 ± 0.3	2.4 ± 0.2	87 ± 0.7	90 ± 0.3	86 ± 0.7
8 d	23 ± 4.8	36 ± 3.1	20 ± 2.2	2.7 ± 0.6	3.2 ± 0.2	2.5 ± 0.3	88 ± 0.3	91 ± 0.5	88 ± 0.2
9 d	24 ± 3.9	33 ± 4.6	21 ± 3.3	2.8 ± 0.6	2.9 ± 0.6	2.1 ± 0.4	88 ± 0.6	91 ± 0.9	90 ± 0.7
10 d	21 ± 3.7	37 ± 5.2	21 ± 5.8	2.1 ± 0.5	3.0 ± 0.5	2.0 ± 0.5	90 ± 1.2	92 ± 0.6	91 ± 0.3
Mean ($n = 20$)	22 ± 3.4	34 ± 4.6	19 ± 3.8	2.7 ± 0.5	3.3 ± 0.5	2.3 ± 0.4	88 ± 1.8	90 ± 1.6	87 ± 3.1

[A] Within each column, values followed by the same letters are not significantly different at $P \leqslant 0.05$, using Tukey's multiple range test.
[B] Mean values ± SD ($n = 4$).
[C] Hull-free Kitawase: pericarp removed before sowing.

and by the mass production system's water supply, all of which should be improved in order to produce a greater yield.

3.2. Phenolic content in seed sprout

The quantity of flavonoids, including four *C*-glycosylflavones (orientin, isoorientin, vitexin and isovitexin), rutin and quercetin, along with chlorogenic acid in non-germinated/germinated seeds and edible portions of common and tartary buckwheats is shown in Table 2. In an experiment conducted by Watanabe and Ito (2002), the four *C*-glycosylflavones were the most common flavonoids present in common buckwheat seedlings up to 18 DAS. Rutin is a flavonoid commonly present in buckwheat organs, including leaves, stems, flowers (Kreft, Knapp, & Kreft, 1999) and seedling cotyledons (Margna, Margna, & Paluteder, 1990) and has been detected at low concentrations in groats (hull-free mature achenes of common buckwheat; Steadman et al., 2001). A comparison of HPLC chromatograms show a distinct difference in elution profiles [retention times (min): chlorogenic acid, 15.8; orientin, 23.9; isoorientin, 24.6; vitexin, 25.9; isovitexin, 27.7; rutin, 28.5; quercetin, 35.9 min] between non-germinated seeds and edible parts and between common and tartary buckwheat (data not shown). In both species and types of buckwheat, only

Table 2
Chlorogenic acid and *C*-glycosylflavone content (mg/g DW)[A,B] of ungerminated seeds, 1 or 2 days after sowing (DAS) of germinated seeds and 6–10 DAS seedlings of hull-on and hull-free Kitawase and Hokkai T 9 sprouts

Types/species	Edible portion	Days after sowing	Chlorogenic acids	Orientin	Isoorientin	Vitexin	Isovitexin	Rutin	Quercetin	Sum
Hull-on Kitawase	Seed	0d	N.D.[C]	N.D.	N.D.	0.0 ± 0.0b[E]	0.0 ± 0.0b[E]	0.2 ± 0.0b	N.D.	*n.d.*
	Germinated seed	1d	N.D.	0.0 ± 0.0b[E]	N.D.	0.0 ± 0.0b[E]	0.0 ± 0.0b[E]	0.2 ± 0.0b	N.D.	*n.d.*
		2d	N.D.	0.2 ± 0.0a	0.1 ± 0.0	0.3 ± 0.0a	0.2 ± 0.0a	0.5 ± 0.0a	N.D.	*n.d.*
		Mean ($n = 12$)	*n.d.*[D]	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	*n.d.*	*n.d.*
	Edible part	6d	0.8 ± 0.2b	4.3 ± 1.0b	5.3 ± 1.3b	2.7 ± 0.7b	3.7 ± 0.9b	4.1 ± 1.0b	N.D.	20.8 ± 5.8b
		7d	0.8 ± 0.1b	4.4 ± 0.4b	5.4 ± 0.5b	2.8 ± 0.2b	3.9 ± 0.3b	4.0 ± 0.3b	N.D.	21.3 ± 1.7b
		8d	0.9 ± 0.1b	4.9 ± 0.4b	6.0 ± 0.5b	3.0 ± 0.2b	4.2 ± 0.3b	4.4 ± 0.3b	N.D.	23.5 ± 1.8b
		9d	1.0 ± 0.1a	6.8 ± 1.2a	8.4 ± 1.5a	4.5 ± 0.2a	6.2 ± 1.1a	5.9 ± 0.4a	N.D.	32.7 ± 5.5a
		10d	1.0 ± 0.4a	6.5 ± 2.5a	7.9 ± 3.0a	4.2 ± 1.4a	5.8 ± 1.8a	6.0 ± 1. 1a	N.D.	31.3 ± 11.3ab
		Mean ($n = 20$)	0.9 ± 0.2	5.4 ± 1.6	6.6 ± 2.0	3.5 ± 1.0	4.7 ± 1.4	4.9 ± 1.4	*n.d.*	25.9 ± 5.1
Hull-free Kitawase	Seed	0d	N.D.	N.D.	N.D.	N.D.	N.D.	0.2 ± 0.0b	N.D.	*n.d.*
	Germinated seed	1d	N.D.	0.0 ± 0.0b[E]	N.D.	0.0 ± 0.0b[E]	0.0 ± 0.0b[E]	0.2 ± 0.0b	N.D.	*n.d.*
		2d	N.D.	0.5 ± 0.0a	0.3 ± 0.0	0.7 ± 0.1a	0.7 ± 0.1a	0.6 ± 0.0a	N.D.	*n.d.*
		Mean ($n = 12$)	*n.d*	0.3 ± 0.3	0.3 ± 0.0	0.4 ± 0.4	0.4 ± 0.4	0.3 ± 0.2	*n.d.*	*n.d.*
	Edible part	6d	1.1 ± 0.9a	5.8 ± 0.1b	5.3 ± 0.1b	3.4 ± 0.1b	3.6 ± 0.1b	2.9 ± 0.1c	N.D.	22.1 ± 0.2b
		7d	1.4 ± 0.1a	6.6 ± 0.5b	6.1 ± 0.5b	3.9 ± 0.3b	4.1 ± 0.3b	3.4 ± 0.2bc	N.D.	25.4 ± 1.9ab
		8d	1.5 ± 0.1a	7.5 ± 0.5ab	6.9 ± 0.4ab	4.4 ± 0.2ab	4.7 ± 0.2ab	3.9 ± 0.3ab	N.D.	28.8 ± 1.5ab
		9d	1.7 ± 0.9a	10.8 ± 3.8a	10.0 ± 3.6a	6.3 ± 2.2a	6.6 ± 2.4a	5.4 ± 1.2a	N.D.	40.9 ± 13.9a
		10d	1.1 ± 0.2a	7.0 ± 0.5ab	6.5 ± 0.5ab	4.1 ± 0.4ab	4.3 ± 0.4ab	3.5 ± 0.3b	N.D.	26.5 ± 2.2ab
		Mean ($n = 20$)	1.4 ± 0.4	7.5 ± 2.4	6.9 ± 2.2	4.4 ± 1.4	4.7 ± 1.5	3.8 ± 1.3	*n.d.*	28.7 ± 6.4
Hokkai T 9	Seed	0d	N.D.	N.D.	N.D.	0.1 ± 0.0b	N.D.	14.1 ± 0.8a	N.D.	*n.d.*
	Germinated seed	1d	N.D.	N.D.	N.D.	0.1 ± 0.0b	N.D.	14.6 ± 1.3a	0.1 ± 0.0a	*n.d.*
		2d	N.D.	N.D.	N.D.	0.1 ± 0.0a	N.D.	15.6 ± 1.7a	0.1 ± 0.0a	*n.d.*
		Mean ($n = 12$)	*n.d*	*n.d.*	*n.d.*	0.1 ± 0.0	*n.d.*	14.7 ± 1.4	0.1 ± 0.0	*n.d.*
	Edible part	6d	1.1 ± 0.1a	0.2 ± 0.0a	0.1 ± 0.0a	0.6 ± 0.0a	0.5 ± 0.0a	23.8 ± 1.1a	0.1 ± 0.0a	26.3 ± 1.2a
		7d	1.1 ± 0.2a	0.2 ± 0.0a	0.1 ± 0.0a	0.5 ± 0.1a	0.5 ± 0.1a	21.9 ± 2.1a	0.1 ± 0.0a	24.4 ± 4.6ab
		8d	1.2 ± 0.1a	0.2 ± 0.0a	0.1 ± 0.0a	0.5 ± 0.1a	0.5 ± 0.1a	22.1 ± 2.3a	0.1 ± 0.0a	24.6 ± 2.5ab
		9d	1.2 ± 0.1a	0.2 ± 0.0a	0.1 ± 0.0a	0.5 ± 0.1a	0.5 ± 0.1a	21.4 ± 2.2a	0.1 ± 0.0a	24.0 ± 2.5ab
		10d	1.2 ± 0.1a	0.1 ± 0.0a	0.1 ± 0.0a	0.5 ± 0.1a	0.5 ± 0.1a	20.1 ± 2.4a	0.1 ± 0.0a	22.6 ± 2.6b
		Mean ($n = 20$)	1.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	21.8 ± 2.6	0.1 ± 0.0	24.4 ± 1.2

[A] Within each column, values followed by the same letters are not significantly different at $P \leqslant 0.05$, using Tukey's multiple range test.
[B] Mean values ± SD ($n = 4$).
[C] N.D., Not detected.
[D] *n.d.*, Not determined.
[E] Trace amounts, less than 0.05 mg/g DW.

vitexin and/or isovitexin, rutin, and a trace of quercetin were found in non-germinated seeds whereas, by contrast, chlorogenic acid, the four *C*-glycosylflavones and rutin were present in the seed sprouts, while quercetin, the aglycone of rutin, was not detected. However, it needs to be emphasized that the quantities of quercetin detected in seeds were close to the limit of detection and separability of the HPLC conditions (Kim et al., 2004; Watanabe & Ito, 2003).

In hull-on Kitawase seeds, vitexin, isovitexin and rutin were present at very low levels whereas, in the germinated seeds, orientin and isoorientin appeared, together with vitexin, isovitexin, and rutin. Even at 2 DAS, levels of the four *C*-glycosylflavones and rutin in the germinated seeds, though very low (0.1–0.3 mg/g DW and 0.5 mg/g DW, respectively), were significantly greater than those of seeds. Their levels in the edible part increased with sprout age such that, at their peak at 9 and 10 DAS, contents of 1.0, 4.2–8.4, and 5.9–6 mg/g DW were recorded for chlorogenic acid, the four *C*-glycosylflavones and rutin, respectively. The high level of such compounds in green sprouts likely serves as a protection system against light damage though, up to 5 DAS, *C*-glycosylflavones are higher in etiolated sprouts than in light-grown ones (Watanabe & Ito, 2003).

For the hull-free Kitawase sprouts, changes in phenolic compounds with sprout age followed a pattern similar to that of hull-on Kitawase sprouts (Table 2). In the edible parts of hull-free sprouts, rutin content (mean 3.8 mg/g DW) was 27% lower; chlorogenic acid, orientin, and vitexin contents were higher and isoorientin and isovitexin contents similar to than in hull-on Kitawase at 6–10 DAS. The rutin contents of edible parts, in both hull-on and hull-free Kitawase at 6–10 DAS, increased substantially (mean 13- and 15-fold, respectively) compared to those of seeds or germinated seeds. For common buckwheat, chlorogenic acid content of green sprouts (approximately 0.75 mg/g DW at 8 DAS) was twice that of etiolated seed sprouts (Kim et al., 2004), indicating that the formation of more highly oxidized flavonoids is accelerated by natural light (Yao et al., 2004).

On the other hand, in non-germinated/germinated seeds of Hokkai T 9, only vitexin, rutin and quercetin (0.1, 15.6, and 0.1 mg/g DW, respectively, at 2 DAS) were found (Table 2). In the edible parts at 6–10 DAS, chlorogenic acid content (mean 1.2 mg/g DW) was much higher than that of any other flavonoid, except rutin. While the four *C*-glycosylflavones present in Hokkai T 9 occurred at very low levels compared to those of hull-on and hull-free Kitawase, rutin levels (21.8 mg/g DW) in Hokkai T 9 were 25- and 11-fold higher than those in their respective Kitawase counterparts. Individual phenolic components were not significantly affected by sprout age.

Interestingly, quercitrin (the monosaccharide rhamnose bound to a quercetin aglycone) was not detected in the sprouts of either species, with or without hulls, as had been previously reported (Fabjan et al., 2003). In sprouts, it was found to gradually increase from 1 to 8 DAS, reaching a peak value of 23 mg/g DW at 8 DAS (Kim et al., 2004). Besides the fact that its content is influenced by genetic factors, such as species, and environmental conditions, such as light intensity (Fabjan et al., 2003; Kim et al., 2004; Yao et al., 2004), it is considered that quercitrin could not detected by the HPLC analysis applied here and this result is in good agreement with a previous report (Watanabe & Ito, 2003). Furthermore, Suzuki et al. (2002) developed an analytical method for the measurement of rutin and isoquercitrin in fresh leaves or cotyledons of buckwheat which is both rapid (11 min/sample) and does not require complex pre-treatments. However, besides involving the somewhat cumbersome process of using liquid nitrogen and a hand homogenizer to process samples, it tends to overestimate some flavonoids, particularly rutin (approximately 54 mg/g DW at 3 DAS in dark), in seed sprouts of tartary buckwheat (Kim et al., 2006). This occurs because the short separation time is likely insufficient to separate and distinguish isovitexin and rutin in the presence of many types of phenolic substances (e.g., *C*-glycosylflavones), present in the seed sprouts (Watanabe & Ito, 2002). Therefore, in the present study we have used a combination of the analytical methods of Watanabe and Ito (2002) and Suzuki et al. (2002) to quantity those compounds in seed sprouts, though it is much more time-consuming (58 min/sample) for multiple samples.

The rutin content in the edible portions of green Hokkai T 9 sprouts (mean 21.8 mg/g DW at 6–10 DAS) was 50% greater than that in non-germinated/germinated seeds, but less than that in 6–8 DAS etiolated seed sprouts (27–29 mg/g DW) (Kim et al., 2002). Interestingly, rutin content (mean 14.7 mg/g DW) in the non-germinated/germinated seeds of Hokkai T 9 was 48- and 51-fold greater than in hull-on and hull-free Kitawase, respectively; by contrast, in the case of the edible parts at 6–10 DAS it was only 4.5- and 5.7-fold greater, respectively. This suggests that, at seed germination, the enzymic glycosylation of quercetin to rutin (quercetin 3-rutinoside) in tartary buckwheat sprouts is less active than in common buckwheat sprouts (Barber, 1962; Yasuda, Masaki, & Kashiwagi, 1992). These results concur with those of Fabjan et al. (2003), who showed rutin content in tartary buckwheat seeds (0.8–1.7% DW) to be greater than that in common buckwheat seeds (0.01% DW). Moreover, in tartary buckwheat sprouts cultured under natural light, rutin was present at up to 5.0% DW whereas, in common buckwheat sprouts, it was half that (2.6% DW) at 6 DAS (Kim et al., 2006). Similarly, rutin content in the roots of Hokkai T 9 (0.3% DW) was much greater than that in hull-on or hull-free Kitawase roots, accounting for 0.01 and 0.03% DW, respectively (data not shown).

The sum contents of phenolic compounds in the different buckwheat sprouts produced at 6–10 DAS in this study are presented in Table 2. In the seed sprouts of hull-on and hull-free Kitawase, mean ($n = 20$) sums of phenolic contents ranged from 20.8 to 32.7 and 22.1 to 40.9 mg/g

DW, respectively, in the growth period, with the greatest levels being attained at 9 DAS. At 6–10 DAS, the total phenolic content of hull-free Kitawase sprouts (mean 28.7 mg/g DW) was 15% greater than that of Hokkai T 9 sprouts (24.4 mg/g DW), in which it was similar to that of Hull-on Kitawase. However, the total phenolic content of hull-free Kitawase sprouts at 6–10 DAS was markedly lower than that of younger (1–5 DAS) common buckwheat sprouts, which ranged from 26 to 55 mg/g DW (Watanabe & Ito, 2003). In both hull-on and hull-free Kitawase sprouts, the percent of total phenolic contents not attributable to rutin exceeded 80% whereas, for Hokkaido T 9, it was only about 10% (Fig. 1). This suggests that, even though the functionality of individual *C*-glycosylflavones has not yet been investigated in detail, the nutritional (functional) value of buckwheat seed sprouts, particularly in the case of common buckwheat, should be assessed on the basis of total phenolic content, when research nutritionists recommend them as a source of dietary rutin (Fabjan et al., 2003; Kim et al., 2004).

3.3. Free radical-scavenging activity by DPPH assay

The edible parts of sprouts were analyzed for their free radical-scavenging activity by DPPH assay. The radical-scavenging activities on the edible parts of green seed sprouts showed very similar values, mean 1.7 μmol trolox eq/g DW at 6–10 DAS among different buckwheat types/species (Table 3). The lowest levels were found at 6 DAS in all buckwheat types/species while the highest in hull-free Kitawase was found at 8 DAS, but there were no significant differences at 7–10 DAS either in hull-on Kitawase or Hokkai T 9. Given their differences in phenolic levels, this suggests that overall antioxidative activity might be affected by the combination of both minor compounds and identified compounds (Hinneburg & Neubert, 2005). In general, tissues with high flavonoid contents have high antioxidant activities (Wang & Zheng, 2001). Moreover, a relationship between total phenolics and antioxidant activity is positive and highly significant in plant materials (Velioglu, Mazza, Gao, & Oomah, 1998) and rutin alone can generally serve as a very good estimator of antioxidant effects (Pulido, Bravo, & Saura-Calixto, 2000; Watanabe & Ito, 2002). However, no correlation was found between the total phenolics, or rutin, and antioxidative activity in the present study. Therefore, assessments of antioxidative activities of common buckwheat sprouts must involve the role of their total phenolic substances in order to avoid an underestimation of free radical-scavenging activity.

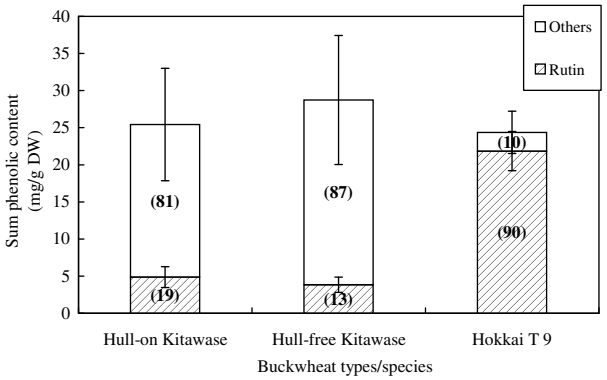

Fig. 1. Percent (on average from 6 to 10 days after sowing) of total phenolic content attributable to rutin in edible parts of buckwheat sprouts of different types and species. Numbers given in parentheses on bars are values of the percent to total phenolic content.

Table 3
Radical-scavenging activity (μmol trolox eq./g DW)[A,B] on the edible part of common and tartary buckwheat sprouts by DPPH assay

Days after sowing	Common		Tartary
	Hull-on Kitawase	Hull-free Kitawase	Hokkai T 9
6d	1.5 ± 0.2b	1.5 ± 0.2c	1.5 ± 0.2b
7d	1.7 ± 0.1ab	1.7 ± 0.1ab	1.7 ± 0.1a
8d	1.8 ± 0.1a	1.9 ± 0.1a	1.7 ± 0.1a
9d	1.7 ± 0.2 ab	1.6 ± 0.3bc	1.7 ± 0.1a
10d	1.8 ± 0.1a	1.7 ± 0.1b	1.7 ± 0.2a
Mean ($n = 20$)	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
Correlation (*t*-test)			
DPPH × rutin	NS	NS	NS
DPPH × sum[C]	NS[D]	NS	NS

[A] Within each column, values followed by the same letters are not significantly different at 5% level, using Tukey's multiple range test.
[B] Mean values ± SD ($n = 4$).
[C] Sum: all phenolic amounts identified from the seed sprouts.
[D] NS: non-significant.

4. Conclusions

In conclusion, the total quantities of phenolic compounds in green seed sprouts of common and tartary buckwheats were similar. However, rutin represented about 90% of the total content of phenolics in tartary sprouts, but only 20% of that in common sprouts. Even though rutin content is regarded as an indicator of the nutritional quality of buckwheat seeds, total phenolics should also be evaluated in seed sprouts of different buckwheat species. Significant differences were not observed between common and tartary buckwheat sprouts. This suggests that, in estimating antioxidative activity of whole seed sprouts of different buckwheat species, one should be concerned, not only with such major phenolic compounds as rutin and *C*-glycosylflavones, but also other minor unidentified phenolic constituents or non-phenolic metabolites such as ascorbic acid. Seed sprouts of both common and tartary buckwheats represent excellent nutritional sources of these compounds and show potential antioxidative activity.

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