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Distribution of Vitamin E, Squalene, Epicatechin, and Rutin in Common Buckwheat Plants (*Fagopyrum esculentum* Moench)

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Buckwheat leaves and young parts of the plant are consumed in some countries as a vegetable. Green flour, obtained by milling of the dried plants, is used as a natural food colorant. The distribution of vitamin E, squalene, epicatechin, and rutin (as the most important antioxidants) within buckwheat plants, as well as changes of their content within leaves during the growing season, were determined by GC-MS and HPLC analyses. α-Tocopherol was found as the main component of vitamin E in all parts of the plant; epicatechin and squalene were also detected. For the use of buckwheat as an antioxidant source in the human diet, the most suitable part of the plants seems to be the leaves and the flowers at the stage of full flowering due to the considerable amounts of rutin and epicatechin. α-Tocopherol content correlates positively with temperature, drought, and duration of solar radiation. Certain differences appear among varieties of buckwheat, especially in their squalene and rutin contents.

KEYWORDS: α-Tocopherol; developmental changes; epicatechin; *Fagopyrum esculentum*; plant parts; rutin; squalene; varieties

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) is an alternative pseudocereal belonging to the Polygonaceae family. Buckwheat hulled achenes have received renewed interest due to their high nutritive value, flavonoid content, and suitability for a gluten-free diet. The use of the herb as a medicinal plant is less well-known. An infusion made of *Fagopyri herba* has been administered against high blood pressure. Herb extracts have been efficiently used against leg edema, and they can protect against diabetic retinopathy, as well (*1*). The leaves and delicate buckwheat shoots are consumed as a salad vegetable or as a heat-processed food, prepared similarly to spinach (*2*). In China, India, and Nepal, the leaves are also used as a dried or pickled vegetable. In Japan, buckwheat inflorescences are utilized as a functional food, due to their high rutin content. A green flour obtained by milling the dried flowering buckwheat plants is added as a natural food colorant to pasta, ice cream, and other products in Japan and South Korea. Recently, a new vegetable—buckwheat sprouts—was introduced (*3*).

It is known that the achenes of buckwheat can be stored for a long time without any symptoms of chemical change. This is due to the content of several natural antioxidants stabilizing the grain during storage (*4*, *5*). The following hierarchy of antioxidant activity was provided for 80% methanol extracts originating from whole grain cereals: buckwheat > barley > oat > wheat = rye (*5*). The antioxidant activity of buckwheat parts decreased in the following order: buckwheat leaves > buckwheat hulled seeds > buckwheat seeds > buckwheat hulls > buckwheat straws (*6*). Antioxidants, including flavonoids, tocopherols, and phenolic acids, play an important role in preventing undesirable changes in the nutritional quality of foods, and they have an important role in the prevention of human diseases, as well.

Total tocopherol concentrations in buckwheat grains ranged from 14.3 to 21.7 mg/kg (*7*). High levels of vitamin E intake have been associated with a reduction in cardiovascular disease (*8*), lowering the risk of Alzheimer's disease and prostate cancer, improving the immune system, and delaying both age-related cataracts and age-related macular degeneration (*9*). Buckwheat achenes have been also recognized as an important resource of vimamins B_1 (thiamin, 3.3 mg/kg), B_2 (riboflavin, 10.6 mg/kg), B_3 (niacin, 18.0 mg/kg), B_5 (pantothenic acid, 11.0 mg/kg), and B_6 (pyridoxine, 1.5 mg/kg) (*1*). In buckwheat sprouts vitamins B_1 and B_6 and vitamin C were described (*7*).

Squalene is an isoprenoid compound having six isoprene units that possesses antioxidant activities, and it is widely produced in plants. Squalene protects cells against radicals, strengthens the immune system, and decreases the risk of various cancers (*10*).

Four catechins [(−)-epicatechin, (+)-catechin-7-*O*-β-D-glucopyranoside, (−)-epicatechin-3-*O*-*p*-hydroxybenzoate, and (−)-epicatechin-3-*O*-(3,4-di-*O*-methyl)gallate] were isolated from the

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Table 1. Basic Meteorological Data during the Period 2004–2005

	May	June	July	Aug	Sept	year
mean air temperature (°C), 2004	12.5	16.3	18.3	19.2	13.7	8.9
mean air temperature (°C), 2005	14.4	17.7	19.0	16.8	14.8	8.8
total precipitation (mm), 2004	65.7	101.4	52.3	47.5	48.9	655.5
total precipitation (mm), 2005	64.7	68.3	162.3	157.3	98.3	798.3
sunshine duration (h), 2004	188.6	195.1	206.4	246.1	246.1	1726.8
sunshine duration (h), 2005	248.5	240.7	206.1	178.4	171.3	1778.1

Table 2. Comparison of α-Tocopherol and Squalene Contents in Leaves of Three Buckwheat Varieties at the Stage of Branching and before Harvest of Achenes (Milligrams per Kilogram)

	α-tocopherol				squalene			
	2004		2005		2004		2005	
variety	DM[a]	FW[b]	DM	FW	DM	FW	DM	FW
at stage of branching								
Emka	572.15	109.56	257.96	49.68	nf[c]	nf	16.38	3.15
Pyra	958.29	211.49	436.59	80.98	nf	nf	16.94	3.14
Krupinka	440.51	96.88	308.62	64.48	nf	nf	54.20	11.32
before harvest								
Emka	4205.05	1185.19	1860.80	454.62	nf	nf	84.50	20.64
Pyra	3145.14	1084.11	298.66	149.18	nf	nf	91.53	15.58
Krupinka	2515.32	903.93	1090.14	414.73	nf	nf	98.57	37.50

[a] Dry matter. [b] Fresh weight. [c] Not found.

ethanol extracts of buckwheat groats (*11*); (−)-epicatechin-3-*O*-gallate, procyanidin B2, and procyanidin B2-3′-*O*-gallate were found in buckwheat callus and hairy root cultures (*12*). The antioxidant activity of these catechins was higher than that of rutin (*11*). Both (+)-catechin and (−)-epicatechin may act as inhibitors of low-density lipoprotein oxidation, and they have antiangiogenic activity as well as the potential to inhibit cell proliferation and to modulate carcinogen metabolism (*13*).

There is insufficient information about the composition of the green parts of the buckwheat plant. Buckwheat leaves and flowers are the plant parts richest in rutin (*2*) and, therefore, a potential source for the industrial extraction of this compound. Rutin has cardioprotective, anti-inflammatory, and anticarcinogenic activities and also has effects on the relaxation of smooth muscles (*14*). Compared to 18 other antioxidant compounds (including quercetin, gallic acid, and α-tocopherol), rutin has the highest activity (*15*). Vitamin E, squalene, and catechins were not quantified and had not, to date, been described in the vegetative parts of buckwheat.

The aim of the present study was to determine the distribution of vitamin E, epicatechin, and rutin, as the most important antioxidants, in buckwheat plants and to determine the changes in their contents within buckwheat leaves at the beginning, in full flower, and at the end of the growing season.

MATERIALS AND METHODS

Materials. The seeds of three genotypes—Pyra (Czech Republic), Emka (Poland), and Krupinka (Russia)—of the common buckwheat (*F. esculentum* Moench) species, examined in this study, were obtained from the Plant Germplasm Collection held at the Research Institute of Plant Production, Prague, Czech Republic. They were grown in four repeated cycles on plots at the experimental farm of the University of South Bohemia in Ceske Budejovice (380 masl, sand-loam soil, pH 6.4, mean light intensity = 3750 MJ/m^2) from the second week of May until the first week of September in 2004 and 2005. Basic meteorological data are given in **Table 1**. Seeds were sown in lines 12.5 cm wide, with the growth density being 200 plants/m^2. During the vegetation period there were no mechanical or chemical treatments of the growths.

The materials for analysis (the whole plants of buckwheat) were collected at the beginning of branching (June 25, 2004, and June 23, 2005), at the stage of full flowering (July 20, 2004, and July 13, 2005), and at harvest time (Sept 3, 2004, and Sept 19, 2005) and then were manually separated into parts for analysis (flowers, stems, leaves). These materials were immediately frozen and freeze-dried (24 h, −46 °C, 0.25 mbar). The dried materials were ground into a powder in a laboratory mill (Retsch MM200, Germany) and stored in closed containers in a freezer (−18 °C).

Chemicals. Standards of α-tocopherol, γ-tocopherol, squalene, epicatechin, trifluoroacetic acid, and ethyl acetate were purchased from Sigma-Aldrich; the acetonitrile and methanol standards were purchased from Merck (Czech Republic).

Sample Preparation. Lyophilized plant materials (stems, leaves, roots) (0.25 g) were extracted with 3 mL of 90% methanol for 30 min at laboratory temperature. After centrifugation, the sediment was washed twice with 1 mL of 90% methanol. Supernatants were collected, stored in a freezer, and finally analyzed by HPLC. The samples of the leaves, stems, and roots of the buckwheat plants were also extracted in ethyl acetate using the same procedure as described above for methanol extraction; ethyl acetate extracts were dried over anhydrous sodium sulfate, and the final extracts were analyzed by GC-MS.

HPLC Analysis. Samples were analyzed by HPLC HP 1050 (Hewlett-Packard); DAD detector (HP 1040, Hewlett-Packard); Phenomenex Luna C18(2), 3 μm, 2 × 150 mm column. The mobile phase A was 5% acetonitrile plus 0.15% trifluoroacetic acid; mobile phase B was 80% acetonitrile plus 0.15% trifluoroacetic acid. The gradient was from 5% B to 35% B in 55 min and from 35% B to 60% B for 5 min; flow was 0.25 mL/min. Rutin and epicatechin were detected at 220 nm.

GC-MS Analysis. Gas chromatography−mass spectrometry analyses were performed on a Finnigan GCQ instrument, using a Zebron ZB-5 column (30 m, i.d. = 0.25 mm, stationary phase thickness = 0.25 μm), with the following temperature program: initial temperature, 60 °C for 1 min; then gradient of 20 °C/min to 180 °C; followed by a gradient of 1.5 °C/min to 275 °C. The linear inlet helium velocity was set to 40 cm/s.

Each peak in the chromatogram was evaluated using Xcalibur mass spectrometry software, and the mass spectra obtained were compared with the spectra from the NIST library. Only those measured spectra having the highest probability of a match with the library spectra were

Table 3. Comparison of Epicatechin and Rutin Contents in Leaves of Three Buckwheat Varieties at the Stage of Branching and before Harvest of Achenes (Milligrams per Kilogram)

	epicatechin				rutin			
	2004		2005		2004		2005	
variety	DM[a]	FW[b]	DM	FW	DM	FW	DM	FW
at stage of branching								
Emka	1115.12	234.96	600.01	115.54	57493.47	12114.51	54801.41	10552.99
Pyra	457.04	96.30	625.97	116.11	67468.69	14216.39	66783.00	12387.26
Krupinka	1503.54	316.80	642.29	134.19	66715.67	14056.99	57908.59	12098.31
before harvest								
Emka	600.19	197.46	1049.26	256.35	37311.58	12262.03	88541.88	21631.91
Pyra	675.81	222.34	816.67	407.92	35840.44	11778.55	98194.03	49047.17
Krupinka	729.90	240.14	1233.41	469.23	45242.24	14868.35	87615.84	33332.11

[a] Dry matter. [b] Fresh weight.

Table 4. Distribution of α-Tocopherol and Squalene in the Buckwheat Plant at the Stage of Full Flowering, Emka Variety (Milligrams per Kilogram)

	α-tocopherol				squalene			
	2004		2005		2004		2005	
plant part	DM[a]	FW[b]	DM	FW	DM	FW	DM	FW
leaves	2982.24	850.40	652.55	118.17	123.74	35.29	120.96	21.91
stems	102.32	24.78	56.87	6.11	302.71	73.31	56.20	6.04
flowers	173.37	60.06	62.13	11.57	121.60	42.13	60.20	11.22
roots	46.89	17.02	nf[c]	nf	55.10	20.00	37.83	9.56

[a] Dry matter. [b] Fresh weight. [c] Not found.

recorded. On the basis of the described procedures we have found some phenolic compounds and vitamin E. Standards of α-tocopherol and γ-tocopherol were used for the determination of vitamin E components. For the quantitative analysis of α-tocopherol, the external calibration curve was used.

Statistical Analysis. Statistical analyses were performed using Statistica 6.0 (StatSoft) software. The LSD test was used to determine significant differences between the contents of the selected compound at each of the different buckwheat growth stages. The method of cluster analysis (Euclidean distances) was used for the evaluation of the varietal differences. This tree clustering method uses distances between objects when forming the clusters. Similar objects are connected in the cluster. The statistical significance of the simple linear correlation was determined between compounds and weather parameters.

RESULTS AND DISCUSSION

α-Tocopherol. The vitamin E family includes four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ), which differ in their methyl substitutions and saturation. According to Kim et al. (*7*), tocopherol concentrations of buckwheat grains decreased in the order γ > α > δ, and γ-tocopherol was found to be the major tocopherol. We found that the major tocopherol of leaves, roots, and flowers is α-tocopherol. The highest α-tocopherol content was determined in the leaves (**Table 4**). This is probably because α-tocopherol is the major vitamin E compound found in leaf chloroplasts (*16*). Many studies have found that α-tocopherol has the highest biological activity among the tocopherols. If the activity of α-tocopherol is designated 100, the relative activities for the other nutritionally important compounds are 25−50 for β-tocopherol, 10−35 for γ-tocopherol, and 30 for β-tocotrienol (the ranges are due to the different types of assays) (*17*).

The content of α-tocopherol in leaves, flowers, and stems was higher than the content of total tocopherols in achenes. According to Keli et al. (*18*), the average content of total tocopherols in achenes was 14.2 mg/kg. Most of the crop seeds

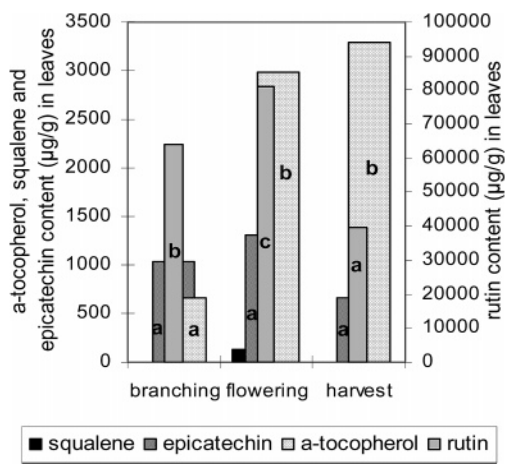

Figure 1. Changes of α-tocopherol, squalene, rutin, and epicatechin contents in buckwheat leaves during development in 2004. a–c: differences after LSD test.

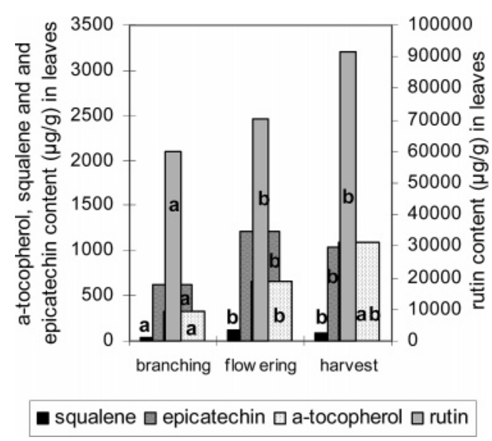

Figure 2. Changes of α-tocopherol, squalene, rutin, and epicatechin contents in buckwheat leaves during development in 2005. a–c: differences after LSD test.

do not contain any major amounts of α-tocopherol; for example, wheat seeds have only 20% of total tocopherol as α-tocopherol (*19*). We can assume the same in buckwheat. Tocopherols other than α commonly are not detected in measurable amounts in most green plant parts (*19*).

α-Tocopherol content in the leaves increases during vegetation (**Figures 1** and **2**). The content of α-tocopherol was on average 4 times higher in dry matter and 6 times higher in fresh weight in the 16th week compared to the 5th week. The same tendency was described by Hollander-Czytko et al. (*20*) in leaves of *Arabidopsis thaliana*. They found an increase of α-tocopherol

Table 5. Distribution of Epicatechin and Rutin in the Buckwheat Plant at the Stage of Full Flowering, Emka Variety (Milligrams per Kilogram)

	epicatechin				rutin			
	2004		2005		2004		2005	
plant part	DM[a]	FW[b]	DM[a]	FW[b]	DM[a]	FW[b]	DM[a]	FW[b]
leaves	936.71	281.86	990.79	179.43	67468.69	20301.50	78301.24	14179.97
stems	222.89	54.63	186.69	20.06	10323.83	2530.60	8788.94	944.45
flowers	2050.16	735.19	1755.26	326.99	41694.29	14950.57	52185.56	9721.76
roots	386.91	137.82	197.39	49.90	1279.18	455.71	532.56	134.63

[a] Dry matter. [b] Fresh weight.

Table 6. Correlations between α-Tocopherol, Rutin, and Epicatechin Contents in Buckwheat Leaves and the Daily Average Temperature, Sum of Precipitation, and Hours of Sunshine for the Given Developmental Stage (Correlation Coefficients)

	average temperature		precipitation		hours of sunshine	
	2004	2005	2004	2005	2004	2005
α-tocopherol	0.924 ***	0.817 *	–0.939 ***	–0.886 **	0.754 *	0.878 **
squalene		0.812 *		0.343 ns		0.671 ns
epicatechin	0.474 ns[a]	0.702 ns	–0.563 ns	0.385 ns	0.183 ns	0.694 ns
rutin	–0.366 ns	0.774 *	0.164 ns	0.433 ns	–0.694 ns	0.697 ns

[a] Nonsignificant; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

content from 15 to 20 mg/kg of fresh weight in the 5th week, as compared to 60 mg/kg in the 13th week.

When we compare the α-tocopherol content of buckwheat with other plants (*19*, *21*), buckwheat leaves had an α-tocopherol content similar to that of nettle, for which values varied from 240 mg/kg of dry matter (50 mg/kg of fresh weight) in March through 1070 mg/kg (215 mg/kg of fresh weight) in June−July to 550 mg/kg (105 mg/kg of fresh weight) in November. The recommended daily intake of tocopherols is 10 mg (*17*). This means that 10 g of buckwheat leaves can supply from $^1/_{10}$ of the recommended daily intake to the whole intake. However, large losses of tocopherols arise during processing; for example, the drum-drying of steamed rolled oats resulted in an almost complete loss of both tocopherols and tocotrienols (*22*), but buckwheat leaves as a vegetable can be a good source of natural vitamin E for human nutrition.

According to Munné-Bosch (*16*), α-tocopherol is a part of the compensatory mechanism, which helps plants to withstand environmental stress. A plethora of plant reactions exist to circumvent the potentially harmful effects caused by light, drought, extreme temperatures, and other stresses. This antioxidant deactivates photosynthesis-derived reactive oxygen species and prevents the propagation of lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes. Our established correlations indicate that the amount of α-tocopherol in buckwheat leaves increases with temperature and amount of sunshine and decreases with the amount of precipitation (**Table 6**). The effect of temperature on the tocopherol content of vegetables has been described in several works; for example, during the seed maturation of soybeans, elevated temperatures and drought led to large (2−3-fold) increases in α-tocopherol (*23*). The results suggest that weather or climate can significantly affect not only the α-tocopherol in the seeds but also the α-tocopherol in the plants. This information can be useful in the growing of crops and in their breeding. The α-tocopherol content in 2004 was 2 times higher than the content in 2005. Weather in the second half of the growing season of 2004 was considerably hot and dry (**Table 1**).

Keli et al. (*18*) reported that the tocopherol concentration of buckwheat grain in Chinese germplasm varied from 0.009 to 0.815 mg/kg (Tibet location). On the basis of cluster analysis (**Figure 3**), the Emka genotype was separated from the group with a significantly lower content of α-tocopherol, which contains the Krupinka and Pyra varieties. However, for further differentiation in the variants, another test is necessary. Specific varieties and environment interactions are also sources of variations.

Squalene. The content of squalene in buckwheat plants is high at the stage of full flowering (**Table 4**). The richest resources for squalene from the vegetative plant parts are usually the leaves. In those of *Amaranthus* sp., for example, on average, the content is 41 mg/kg of the dry weight and *A. albus* had the highest squalene yield of 143 mg/kg of dry leaf (*24*). In the case of buckwheat, the stems were the richest part in 2004 and the leaves in 2005. This difference could have been caused by the rapid growth of plants in 2005, which was rich in precipitation. The plants were ≈300 mm higher than in 2004, and they had very well-developed root systems. In 2005 the squalene content was lower in all plant parts except leaves. A positive correlation was found between the amount of squalene in buckwheat leaves and temperature.

The content of squalene changed during the buckwheat growing season. The highest amount of squalene was established at the stage of full flowering. On the contrary, He and Corke (*24*) did not observe any apparent overall variation trend in the squalene concentrations at the different growth stages of *Amaranthus*.

In 2004 a comparison of buckwheat varieties, however, cannot be done because the values were under the limit of detection (**Table 2**). In 2005 Krupinka was different from the others (**Figure 3**), as it had the highest squalene content.

Epicatechin. Catechins are found particularly in fruits, but dark chocolate contains the highest levels of catechins. Vegetables and legumes are poor dietary sources of catechins; only rhubarb, broad beans, and marrowfat peas contained catechins (*25*). Rhubarb belongs to the Polygonaceae family, as does buckwheat. It is possible that a certain content level of catechin is characteristic and typical for the whole Polygonaceae family. Rhubarb contained 5.1 ± 3.30 mg/kg of (−)-epicatechin (*25*).

Buckwheat flowers had a higher content of epicatechin (**Table 5**) than did dark chocolate (*25*) (327−502 mg/kg in dark chocolate). The epicatechin content in buckwheat leaves is

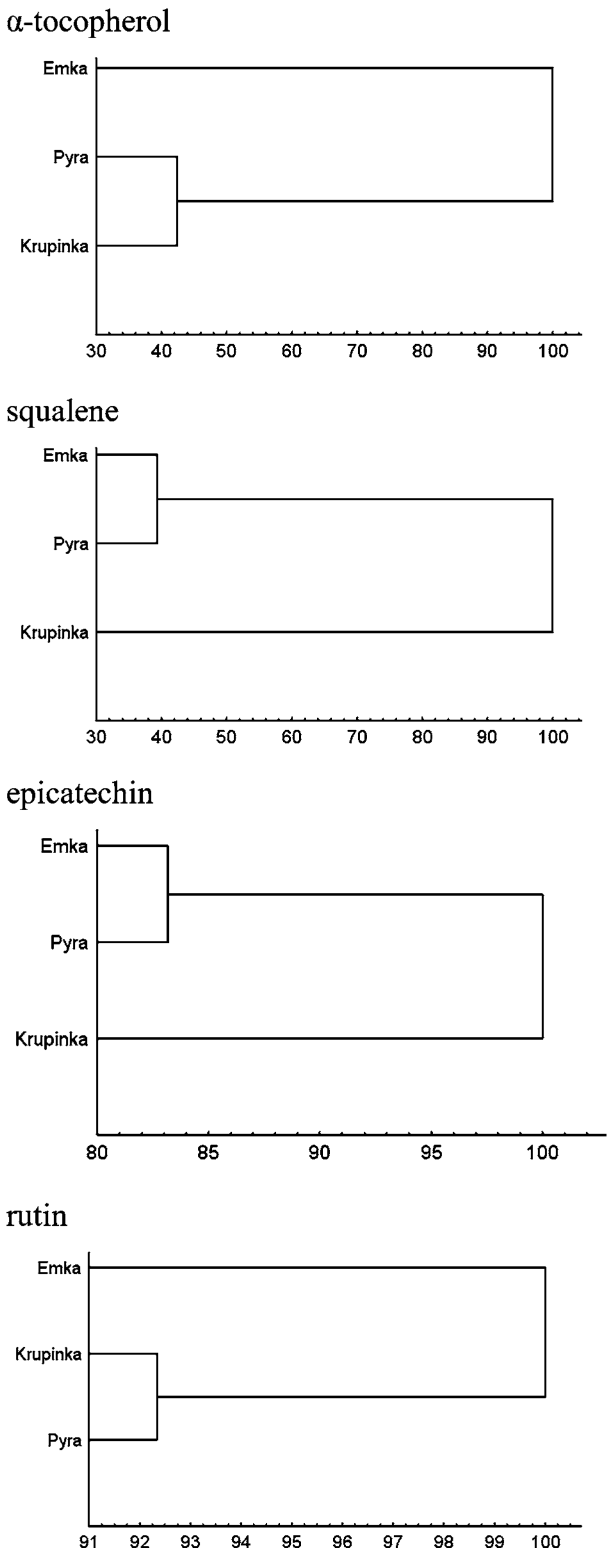

Figure 3. Tree diagrams for comparison of buckwheat varieties (D_{link}/D_{max} × 100).

similar to the content determined by Ilja et al. (*25*) in the broad bean *Vicia faba* L. (225.1 ± 184.78 mg/kg). Quettier-Deleu (*26*) found only 3.395 mg/kg of epicatechin in buckwheat flour.

The epicatechin concentrations in the buckwheat stalks were lower than in the leaves of buckwheat, similar to those determined and described by Hanefeld and Herrmann (*27*) in the leaves and stems of rhubarb. Mayer et al. (*28*) described first an increase and then a decrease of epicatechin in apples during their maturation. The results of this study indicate an increase to the full flowering stage and then a decrease; however, the differences were significant for a considerable variability of the values only in year 2005 (**Figures 1** and **2**). Hanefeld and Herrmann (*27*) described an decrease of epicatechin content in rhubarb stalks and leaves during plant growth.

The epicatechin occurs in plants except the monomer structure like a polymer-condensed tannin. The main concerns about tannins are their astringency, reducing food palatability, and their antinutritional ability. However, tannins can protect proteins against degradation by rumen microbes (*1*). The distribution of epicatechin in the buckwheat plant parts was similar to the distribution of tannins. According to Kreft et al. (*29*) the highest tannin content was found in flowers (5.8% of dry weight), followed by leaves (1.1% of dry weight) and stems (0.1% of dry weight).

According to Kirokosyan et al. (*30*), drought and cold stress treatments caused increases in the levels of (−)-epicatechin in *Crataegus laevigata* and *Crataegus monogyna*. However, we did not find any correlation between the epicatechin content and temperature, precipitation, or duration of sunshine (**Table 6**). The values of epicatechin content in both years were similar.

By comparison of the differences among the varieties (**Figure 3**), cluster analysis separated the genotype Krupinka as having higher values of epicatechin than the other varieties.

Rutin. It is evident from the literature, for example, Dietrych-Szostak and Oleszek (*4*), that the greatest amount of rutin is contained in buckwheat flowers at the stage of full flowering. On the contrary, we have found that the leaves were the richest plant parts (**Table 3**). The difference can be caused by the influence of variety, year, or place. In 2005 the content of rutin was highest at the end of the growing season. This untypical response can be due to the very low number of leaves per plant in this year, which could cause an accumulation of rutin in the remaining leaves. According to Munné-Bosch (*16*) flavonoids help plants to withstand environmental stress, and they are a part of a compensatory mechanism to protect the photosynthetic apparatus. The plants were sampled about 14 days later due to heavy rains during the last month of the growing season. The rains supported defoliation. The tetraploid variety (Emka) was different from the others (**Figure 3**), as it had a lower rutin content (**Table 3**).

In 2005 a positive correlation between the rutin content and temperature was established. No other correlation, regarding weather characteristics and rutin content, was observed in either year (**Table 6**), whereas the results obtained by Orsak et al. (*31*) in model experiments showed that UV irradiation enhances rutin content in buckwheat.

Therapeutic doses of rutin, derived from buckwheat, were reported in amounts between 180 and 350 mg (*4*). Consumption of 10 g of fresh leaves would cover almost such a dose. A recommended daily intake of rutin has not yet been established. The common intake of flavonoids is from 2.6 to 13 mg/day (*1*).

Buckwheat also contains fagopyrin, a phototoxic derivative of hypericin, which belongs to the group of photodynamic substances. Its concentration in the fresh leaves and flowers is 0.02−0.08% w/w, whereas dried parts contain only traces (*32*). According to Muhler and Schiebel-Scholsser (*32*), the limit dose is 1 mg of fagopyrin/kg of body weight. If we assume 30% as the content of dry mass in the leaves, the limit for the consumption of buckwheat leaves could be ≈235 g.

Hence, buckwheat can be an important source of antioxidants, especially of epicatechin and rutin, and it has a very good potential to be a material for functional food development, such as foods fortified with buckwheat flour from the leaves or other parts.

There are certain differences in the contents of α-tocopherol, epicatechin, and rutin among varieties. Among the evaluated varieties, the genotype Krupinka was the best from the point of view of antioxidant content.

Supporting Information Available: Chromatogram of ethyl acetate extract of the buckwheat leaves, α-tocopherol standard, and comparison of the mass spectrum of α-tocopherol in the extract and α-tocopherol standard (D). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Belton, P. S.; Taylor, R. N. *Pseudocereals and Less Common Cereals. Grain Properties and Utilization Potential*; Springer-Verlag: Berlin, Germany, 2002; 270 pp.
(2) Kreft, I.; Chang, J.; Choi, Y. S.; Park, C. H. *Ethnobotany of Buckwheat*; Jinsol Publishing: Seoul, Republic of Korea, 2003; 150 pp.
(3) Kim, S. L.; Soon, Y. K.; Hwang, J. J.; Kim, S. K.; Hur, H. S.; Park, C. H. Development and utilization of buckwheat sprouts as functional vegtables. *Fagopyrum* **2001**, *18*, 49−54.
(4) Dietrych-Szostak, D.; Oleszek, W. Effect of processing on the flavonoid content in buckwheat (*Fagopyrum esculentum* Moench) grain. *J. Agric. Food Chem.* **1999**, *47*, 4384−4387.
(5) Zielinski, H.; Kozowska, H. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *J. Agric. Food Chem.* **2000**, *48*, 2008−2016.
(6) Holasova, M.; Fiedlerova, V.; Smrcinova, H.; Orsak, M.; Lachman, J.; Vavreinova S. Buckwheat—the source of antioxidant activity in functional foods. *Food Res. Int.* **2002**, *35*, 207−211.
(7) Kim, S. L.; Kim, S. K.; Park, C. H. Comparisons of lipid, fatty acids and tocopherols of different buckwheat species. *Food Sci. Biotechnol.* **2002**, *11*, 332−336.
(8) Jha, P.; Flather, M.; Lonn, E.; Farkouh, M.; Yusuf, S. The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. *Ann. Intern. Med.* **1995**, *123*, 860−872.
(9) Vinson, J. A.; Al Kharrat, H.; Andreoli, L. Effect of *Aloe vera* preparations on the human bioavailability of vitamins C and E. *Phytomedicine* **2005**, *12*, 760−765.
(10) Das, B.; Yeger, H.; Baruchel, H.; Freedman, M. H.; Koren, G.; Baruchel S. In vitro cytoprotective activity of squalene on a bone marrow versus neuroblastoma model of cisplatin-induced toxicity: implications in cancer chemotherapy. *Eur. J. Cancer* **2003**, *39*, 2556−2565.
(11) Watanabe, M. Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. *J. Agric. Food Chem.* **1998**, *46*, 839−845.
(12) Trotin, F.; Moumou, Y.; Vasseur, J. Flavanol production by *Fagopyrum esculentum* hairy and normal root cultures. *Phytochemistry* **1993**, *32*, 929−931.
(13) Demeule, M.; Michaud-Levesque, J.; Annabi, B.; Gingras, D.; Boivin, D.; Jodoin, J. Green tea catechins as novel antitumor and antiangiogenic compounds. *Curr. Med. Chem. Anti-Cancer Agents* **2002**, *2*, 441−463.
(14) Krishna, K. M.; Annapurna, A.; Gopal, G. S. Partial reversal by rutin and quercetin of impaired cardiac function in streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol.* **2005**, *83*, 343−355.
(15) Sawa, T.; Nakao, M.; Akaike, T.; Ono, K.; Maeda, H. Alkylperoxyl radical scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor promoter effect of vegetables. *J. Agric. Food Chem.* **1999**, *47*, 397−402.
(16) Munné-Bosch, S. The role of α-tocopherol in plant stress tolerance. *J. Plant Physiol.* **2005**, *162*, 743−748.
(17) National Research Council. Food and Nutrition Board. *Recommended Dietary Allowances*, 10th ed.; National Academy Press: Washington, DC, 1989.
(18) Keli, Y.; Dabiao, L.; Genjiu, L. The quality appraisal of buckwheat germplasm resources in China. *Proceedings of the 5th International Symposium on Buckwheat*; Agricultural Publishing House: Taiyuan, China, 1992; pp 90−97.
(19) DellaPenna, D. A decade of progress in understanding of vitamin E synthesis in plants. *J. Plant Physiol*. **2005**, *182*, 729−737.
(20) Hollander-Czytko, H.; Grabowski, J.; Sandorf, I.; Weckermann, K.; Weiler, E. W. Tocopherol content and activities of tyrosine aminotransferase and cystine lyase in *Arabidopsis* under stress conditions. *J. Plant Physiol*. **2005**, *162*, 767−770.
(21) Booth, V. H.; Bradford, M. P. Tocopherol contents of vegetables and fruits. *Br. J. Nutr.* **1963**, *17*, 575−580.
(22) Bryngelsson, S.; Dimberg, L. H.; Kamal-Eldin, A. Effects of commercial processing on levels of antioxidants in oats (*Avena sativa* L.). *J. Agric. Food Chem.* **2002**, *50*, 1890−1896.
(23) Britz, S. J.; Kremer, D. F. Warm temperatures or drought during seed maturation increase free r-tocopherol in seeds of soybean (*Glycine max* [L.] Merr.). *J. Agric. Food Chem.* **2002**, *50*, 6058−6063.
(24) He, H. P.; Corke, H. Oil and qualene in *Amaranthus* grain and leaf. *J. Agric. Food Chem*. **2003**, *51*, 7913−7920.
(25) Ilja, C.; Arts, W.; van de Putte, B.; Hollman, P. C. H. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods and processed foods. *J. Agric. Food Chem.* **2000**, *48*, 1746−1751.
(26) Quettier-Deleu, Ch.; Gressier, B.; Vasseur, J.; Dine, T.; Brunet, C.; Luyck, M.; Cazin, M.; Cazin, J. C.; Bailleul, F.; Trotin, F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J. Ethnopharmacol.* **2000**, *72*, 35−42.
(27) Hanefeld, M.; Herrmann, K. On the occurrence of proanthocyanidins, leucoanthocyanidins and catechins in vegetables. *Z. Lebensm. Unters. Forsch.* **1976**, *161*, 243−248.
(28) Myar, U.; Treutter, D.; Santos-Buelga, C.; Bauer, H.; Feucht, W. Developmental changes in the phenol concentrations of 'Golden Delicious' apple fruits and leaves. *Phytochemistry* **1995**, *38*, 1151−1155.
(29) Kreft, S.; Strukelj, B.; Gaberscik, A.; Kreft, I. Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method. *J. Exp. Bot.* **2002**, *53*, 1801−1804.
(30) Kirakosyan, A.; Seymour, E.; Kaufman, P. B.; Warber, S.; Bolling, S.; Chang, S. C. Antioxidant capacity of polyphenolic extracts from leaves of *Crataegus laevigata* and *Crataegus monogyna* (hawthorn) subjected to drought and cold stress. *J. Agric. Food Chem.* **2003**, *51*, 3973−3976.
(31) Orsak, M.; Lachman, J.; Vejdova, M.; Pivec, V.; Hamouz, K. Changes of selected secondary metabolites in potatoes and buckwheat caused by UV, gamma- and microwave irradiation. *Rostl. Vyroba* **2001**, *47*, 493−500.
(32) Muhler, A.; Schiebel-Schlosser, G. *Buchweizen: Botanik, Inhaltsstofe, Analytik, Pharmakologie, Toxikologie, Klinik*; Wiss. Verl.-Ges.: Stuttgart, Germany, 1998; 111 pp.

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