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Degradation of Rutin and Polyphenols during the Preparation of Tartary Buckwheat Bread

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The impact of bread making and baking procedure on rutin, quercetin and polyphenol concentration and antioxidant activity of tartary buckwheat (Fagopyrum tataricum) bread and breads made of mixtures of tartary buckwheat and wheat flour was studied. A decrease in polyphenol concentration through baking was observed in all samples. The high DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity in mixed breads (32−56%) and in tartary buckwheat bread (85−90%) decreased slightly through the bread making process, while an increase of antioxidant activity in bread made of 100% wheat flour during bread making was observed. With the addition of water to mixtures containing tartary buckwheat during the preparation of the dough, rutin concentration decreased, while quercetin concentration increased. The rutin concentration continued to decrease during the bread baking process, while the concentration of quercetin remained stable. After baking, rutin (0.47 mg/g) was present only in bread made of 100% tartary buckwheat flour along with quercetin (4.83 mg/g).

KEYWORDS: Tartary buckwheat (*Fagopyrum tataricum*); wheat (*Triticum aestivum*); bread; baking; rutin; quercetin; antioxidant activity; total polyphenols; DPPH; Folin−Ciocalteau assay; HPLC

INTRODUCTION

Pseudocereals have received increased interest in recent years due to the growing awareness of the need for healthy diets. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is a pseudocereal rich in dietary beneficial components. It is predominantly cultivated in southwest China, northern India, Bhutan, and Nepal (*1*). Tartary buckwheat contains relatively high amounts of fibers, vitamins B1, B2, and B6, and proteins with a balanced amino acid composition and high biological value (*2*). In addition, it has significant amounts of other bioactive components such as phytosterols, squalene, fagopyritols, and polyphenols (*3*, *4*). Tartary buckwheat can be recommended for patients with celiac disease since it does not contain gluten. Tartary buckwheat was widely grown in the territory of Slovenia since the beginning of 19th century. During the 20th century, the cultivation of tartary buckwheat gradually decreased, and nowadays, only common buckwheat is grown in a relatively small growing area. Hard-boiled tartary buckwheat mush was one of the staple foods made traditionally, but there is no information about tartary buckwheat bread use. However, common buckwheat bread was very popular in the past, and its use is reviving in present times because of the growing awareness of healthy diets and functional foods.

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Polyphenols have antioxidant properties that may inhibit lipid peroxidation (*5*), decrease capillary fragility associated with hemorrhagic changes (*6*), and reduce high blood pressure and the risk for arteriosclerosis (*7*). They are also secondary plant metabolites that play a role in the protection of plants against ultraviolet radiation, pathogens, and herbivores (*8*). Flavonol glycosides, including rutin, quercetin, and kaemferol-3-rutinoside, as well as trace amounts of flavanol triglycoside have been identified in methanol extracts of buckwheat (*9*). Tartary buckwheat contains higher concentrations of rutin compared to that in common buckwheat, other grain crops, and most fruits and vegetables (*5*, *10*−*12*). Rutin (quercetin-3-rutinosid) is a flavonol glycoside that is synthesized in higher plants and used as a mechanism for protection against ultraviolet radiation and diseases (*13*, *14*). Although polyphenols are known antioxidants, some data suggest that flavonoid compounds can also behave as prooxidants, depending on the concentration and free radical source (*15*).

Processing methods can modify the polyphenol content of food in several ways (*16*). Thermal processing of common buckwheat was shown to have a detrimental effect on flavonoid content (*17*). In addition, Sensoy et al. (*18*) reported that extrusion had no effect on the antioxidant activity of buckwheat, in contrast to roasting, which caused a slight decrease of antioxidant activity. An analysis of roasting, pressure steam-heating, and microwave heating methods showed a decrease in phenolic content and antioxidant activity of tartary buckwheat whole-meal flour (*19*). In contrast, other data have shown that cooking, steaming, and

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Table 1. Ingredients of Dough and Bread Types[a]

		ingredients (g)				
bread types	Tf/Wf ratio (%)	Tf	Wf	water	yeast	salt
T_0	0:100		300	200	4	5
T_30	30:70	90	210	240	4	5
T_50	50:50	150	150	250	4	5
T_100	100:0	300		300	4	5

[a] Tf: tartary buckwheat flour. Wf: wheat flour.

microwaving have no deleterious effects on the total polyphenolic content and antioxidant activity of some vegetables (*20*). Moreover, moderate heat treatment may increase the phenolic content and antioxidant activity (*20*) due to the occurrence of Maillard reactions, which can lead to the synthesis of substances with antioxidant properties (*21*).

Although some research has been conducted on common buckwheat, data on the antioxidant properties and rutin content of tartary buckwheat flour products are limited. Kreft et al. (*22*) recently reported on the degradation of rutin in common buckwheat products. Noodles made of dark common buckwheat flour (flour extraction rate 60%) contained approximately one-third the concentration of rutin found in flour (0.08 mg of rutin/g vs 0.218 mg of rutin/g, respectively). However, proper assessment of the antioxidant potential of tartary buckwheat is dependent on understanding how processing impacts these compounds, and to date, these data have been scarce. In the present study we examined the nutritional quality, i.e., the rutin, quercetin, and total polyphenol content as well as the antioxidant activity of tartary buckwheat flour and bread that contained different ratios of tartary buckwheat and wheat flour. Specifically, we wanted to assess how these factors are affected before and after the bread making and baking processes.

MATERIALS AND METHODS

Materials. Tartary buckwheat seeds (local domestic variety from Luxemburg, known as Wëllkar) were purchased from Lothar Kails (Lützkampen, Germany, near the border to Luxemburg) and milled by Katić (Leskovec pri Krškem, Slovenia) to obtain tartary buckwheat flour (particle size ≤236 μm; 42% yield, this relatively low flour yield is normal in tartary buckwheat since after milling, over 50% are husks and bran). Tartary buckwheat was harvested in the year 2008. Wheat flour (type 850) and dry yeast purchased from Mlinotest (Ajdovščina, Slovenia) and Podravka (Koprivnica, Croatia), respectively, were also used in the process.

Total polyphenol, rutin, and quercetin concentrations and antioxidant activity were analyzed in buckwheat flour (Tf), wheat flour (Wf), dry yeast, dough, and bread made from tartary buckwheat and wheat flour in the following ratios: 100:0 (T_0), 70:30 (T_30), 50:50 (T_50), and 0:100 (T_100). The bread making procedure was as follows: 5 min of kneading, 30 min of rising, an additional 1 min of kneading, and 29 min of a second rising. Dough sampling was carried out at 35 and 60 min after the commencement of the first kneading. Breads were baked in triplicate, and volumes were measured. Baking was performed in an oven with hot air circulation. The temperature was initially 200 °C for 10 min and then subsequently lowered to 180 °C for an additional 30 min. Samples of crust (0 to 10 mm under the bread surface) and bread inside were taken from the loaves of baked bread. After 5 h of cooling at room temperature, the samples were frozen at −20 °C, freeze-dried, and milled for future analyses. The ingredients of bread types are presented in **Table 1**.

Preparation of Methanol Extracts. To prepare methanol extracts, 25 mL of 80% methanol (HPLC grade; Sigma-Aldrich Corporation, St. Louis, USA) was added to 1 g of each of the milled sample. The mixture was shaken at room temperature for 8 h at 250 rpm. Samples were then filtered through filter paper (130 g/m^2, Filtrak, Thermalbad Wiesenbad, Germany) and kept at 8 °C for further analysis. Methanol extracts of all samples were prepared in duplicate.

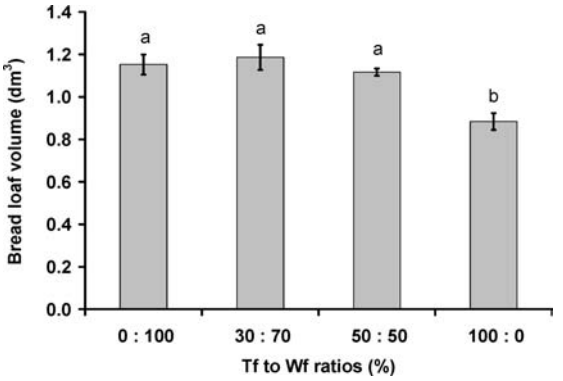

Figure 1. Volumes of bread loaves baked with different tartary buckwheat (Tf) to wheat (Wf) flour ratios (values marked with the same letter are not significantly different at $P < 0.05$).

Antioxidant Activity. For the analysis of free radical scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used according to the protocol in Brand-Williams et al. (*23*). To obtain a stock solution, 0.025 g of DPPH (Sigma-Aldrich Corporation, St. Louis, USA) was diluted to 100 mL with methanol (Spectranal, Ridel de Haën, Hanover, Germany) and kept in a cool and dark place. Immediately before the analysis, a 1:10 dilution of the stock was made with methanol. For the analysis, 3.9 mL of the DPPH working solution was added to a cuvette and the absorbance at 515 nm was measured (A_0) with a Shimadzu spectrophotometer (710, Shimadzu, Kyoto, Japan). Subsequently, 0.1 mL of the extract was added to the cuvette with DPPH, and the absorbance was measured after 5 min (A_5) and 10 min (A_{10}). An increasing amount of antioxidants present in the methanol extract of the sample reduced DPPH and faded the color of the solution in a correlation proportional to the antioxidant concentration. The percentage of DPPH inhibition was measured according to the following equation:

$$\text{inhibition}(\%) = [(A_0 - A_{t(t=5,10)})/A_0] \times 100$$

Total Polyphenol Content Estimation. The amount of total phenolics was determined using Folin−Ciocalteau reagent (FCR) (Merck, Darmstadt, Germany) according to Lachman et al. (*24*). Sample extract (0.05 to 1 mL according to the expected polyphenol content), 2.5 mL of FCR, and 3−5 mL of H_2O were added to a 50 mL flask. After 3 min, 7.5 mL of Na_2CO_3 (20%) were also added to the flask and diluted to 50 mL with H_2O. The mixture was then incubated for 2 h and the absorbance was measured at 765 nm on a Shimadzu spectrophotometer (710, Shimadzu, Kyoto, Japan) against a blank (sample extract replaced with 80% methanol). The amount of total phenolics was calculated as gallic acid equivalents (GAE) in milligrams per gram of dry sample.

Determination of Rutin and Quercetin Concentrations. Sample extracts filtered through filter paper were additionally filtered through a syringe filter unit (0.22 μm, Millipore, Billerica, USA). The filtrate was injected into a high performance liquid chromatography (HPLC) system that consisted of an HPLC chromatograph (Alliance 2695, Waters USA), a LiChroCART Purospher RP C18 column (5 μm, 250 × 4.6 mm; Merck, Darmstadt, Germany), and a DAD 2996 UV detector (Waters, Milford, USA). The column temperature was 30 °C. A gradient elution of the mobile phase was used with a flow rate of 1 mL/min. Solvent A was acetonitril, and solvent B was 0.1% phosphoric acid. The solvent gradient was as follows: the concentration of solvent A was 40% for the first 3 min, 5% for the next 5 min, and 5% for an additional 2 min. The concentration of solvent B was 60% for the first 3 min, 95% for the next 5 min, and 95% for an additional 2 min. The presence of rutin and quercetin was detected at 365 nm, and the content was calculated on the basis of the calibration curve of rutin and quercetin standards (Acros Organics, Waltham MA 02454, USA) prepared in methanol (gradient elution grade; Sigma-Aldrich Corporation, St. Louis, USA). Results were expressed as mg/g of dry weight.

Statistical Analysis. All of the data were expressed as the mean ± standard deviation of two replications and four measurements, except for the volume estimations of breads, which were determined once for each

Table 2. Antioxidant Activity and Total Polyphenol, Rutin, and Quercetin Concentrations of Yeast, Tartary Buckwheat (Tf) and Wheat (Wf) Flour[a]

	DPPH scavenging capacity (%)				
	after 5 min	after 10 min	total polyphenols (mg GAE/g)	rutin (mg/g)	quercetin (mg/g)
Wf	−0.29 ± 0.39 a	−1.01 ± 0.47 a	0.94 ± 0.12 a	ND a	ND a
Tf	86.91 ± 0.53 b	89.03 ± 0.44 b	13.08 ± 0.47 b	11.67 ± 0.09 b	0.63 ± 0.03 b
yeast	8.61 ± 0.49 c	11.38 ± 0.52 c	1.10 ± 0.05 a	ND a	ND a

[a] Results are given as an average of 4 measurements ± standard deviation. Extracts of all samples were prepared in duplicate. Mean values marked with the same letter in the same column are not significantly different at $P < 0.05$. ND: not detected.

Table 3. Antioxidant Activity of Dough and Bread Made Using Different Tartary Buckwheat (Tf) and Wheat (Wf) Flour Ratios Measured after 5 and 10 min[a]

		DPPH scavenging capacity (%)			
		dough		bread loaf	
Tf/Wf ratio (%)	time point	35 min	60 min	inside	crust
0:100 (T_0)	5 min	0.19 ± 0.15 a	0.62 ± 0.22 b	2.88 ± 0.85 c	4.67 ± 0.75 d
	10 min	−0.19 ± 0.19 a	0.54 ± 0.32 b	2.76 ± 0.62 c	4.16 ± 0.79 d
30:70 (T_30)	5 min	32.30 ± 0.53 a	32.55 ± 1.42 a	33.98 ± 0.77 a	33.99 ± 5.51 a
	10 min	37.65 ± 1.17 a	37.45 ± 1.15 a	39.47 ± 0.89 a	36.99 ± 3.10 a
50:50 (T_50)	5 min	52.63 ± 1.60 a	54.16 ± 4.82 a	51.02 ± 1.45 a	52.81 ± 7.68 a
	10 min	59.58 ± 1.84 a	59.67 ± 1.36 a	55.88 ± 1.27 b	56.20 ± 7.18 b
100:0 (T_100)	5 min	85.84 ± 2.55 a	81.67 ± 2.10 b	81.32 ± 2.15 b	79.64 ± 1.70 b
	10 min	90.07 ± 2.17 a	87.44 ± 1.62 b	85.24 ± 1.71 b	84.25 ± 1.48 c

[a] Results are given as an average of 4 measurements ± standard deviation. Extracts of samples were prepared in duplicate. Mean values marked with the same letter in the same row are not significantly different at $P < 0.05$.

Table 4. Total Polyphenol (mg GAE/g), Rutin (mg/g), and Quercetin (mg/g) in Dough and Bread Loaves Made Using Different Tartary Buckwheat (Tf) and Wheat (Wf) Flour Ratios[a]

		dough		bread loaf	
Tf/Wf ratio (%)		35 min	60 min	inside	crust
0:100 (T_0)	polyphenols	0.70 ± 0.03 a	0.82 ± 0.01 a	0.64 ± 0.02 a	0.61 ± 0.06 a
	rutin	ND a	ND a	ND a	ND a
	quercetin	ND a	ND a	ND a	ND a
30:70 (T_30)	polyphenols	4.04 ± 0.03 a	3.48 ± 0.03 b	3.40 ± 0.11 b	3.50 ± 0.11 b
	rutin	0.32 ± 0.01 a	ND b	ND b	ND b
	quercetin	1.26 ± 0.15 a	1.50 ± 0.03 b	1.53 ± 0.01 b	1.52 ± 0.01 b
50:50 (T_50)	polyphenols	8.59 ± 0.27 a	7.11 ± 0.33 b	5.11 ± 0.34 c	4.72 ± 0.24 d
	rutin	0.54 ± 0.02 a	0.31 ± 0.02 b	ND c	ND c
	quercetin	2.47 ± 0.05 a	2.65 ± 0.04 a	2.50 ± 0.05 a	2.54 ± 0.05 a
100:0 (T_100)	polyphenols	10.99 ± 0.44 a	12.66 ± 0.06 b	7.84 ± 0.37 c	7.63 ± 0.39 c
	rutin	1.01 ± 0.02 a	0.64 ± 0.01 b	0.44 ± 0.01 c	0.47 ± 0.02 c
	quercetin	5.13 ± 0.03 a	5.12 ± 0.07 a	5.00 ± 0.09 b	4.83 ± 0.06 c

[a] Results are given as an average of 4 measurements ± standard deviation. Extracts of samples were prepared in duplicate. Mean values marked with the same letter within the same row are not significantly different at $P < 0.05$. ND: not detected.

bread (baked in triplicate). One factor ANOVA was used for the statistical analysis, and the values were considered to be significantly different when $P < 0.05$.

RESULTS AND DISCUSSION

Bread Loaf Volumes. No significant difference in volumes among T_0, T_30, and T_50 was observed, but the T_100 bread, made of 100% tartary buckwheat flour, was darker in color and had a significantly smaller volume compared to that of other breads ($P < 0.05$) (**Figure 1**). It is known that loaf volume of common buckwheat/wheat mixed bread decreases in correlation with the higher percentage of common buckwheat flour in the flour mixture (*25*). However, loaf volumes of tartary buckwheat bread are clearly higher than those of common buckwheat, and the loaf volume decrease with a higher percentage of tartary buckwheat flour in the flour mixture is not as obvious as with common buckwheat. But the reason for this has not yet been studied in detail.

Effect of the Bread Making Process on Antioxidant Activity. Our results clearly show the higher antioxidant activity of tartary buckwheat flour compared to that of wheat flour (approximately 89% and −1%, respectively) (**Table 2**). However, bread made of 100% wheat flour had a significantly higher antioxidant activity (inside and crust, 2.76% and 4.16%, respectively) in comparison to that of wheat flour (−1.01%) and the dough itself (0.54%) ($P < 0.05$) (**Table 3**). These results could be due to Maillard reactions that occur during bread baking and can result in the synthesis of substances with antioxidant properties (*21*). Turkmen et al. (*20*) reported an increase of antioxidant activity in vegetables as a result of moderate heat treatment.

In buckwheat dough and breads, the overall antioxidant activity increased with a growing percentage of tartary buckwheat flour used (**Table 3**), i.e., the highest antioxidant activity was observed in bread made of 100% tartary buckwheat flour. The antioxidant activities were approximately 35%, 55%, and 85% in dough and breads containing 30%, 50%, and 100% of tartary buckwheat flour, respectively. In contrast to increasing antioxidant activity in 100% wheat flour bread, a decrease of antioxidant activity was observed in bread made of 100% tartary buckwheat flour through the bread making process (**Table 3**).

In contrast, the DPPH scavenging capacity of T_30 mixed bread (with 30% tartary buckwheat flour) remained stable throughout the bread making process. This could be due to the high percentage of wheat flour in the flour mixture (70%) since it is seen that the antioxidant activity of 100% wheat bread increases during the bread making process (**Table 3**). In the T_50 mixed bread (with 50% tartary buckwheat flour), only a slight decrease of DPPH scavenging capacity was noticed (**Table 3**).

The study by Zhang et al. (*19*) showed a significant decrease of antioxidant activity in raw tartary buckwheat flour due to various thermal treatments (roasting, pressure steam-heating, and microwaving); in contrast, Sensoy et al. (*18*) reported only a slight decrease of antioxidant activity in common buckwheat flour that was roasted for 10 min at 200 °C. In addition, no change in antioxidant activity was observed after extrusion (170 °C).

Effect of Bread Making on Total Polyphenol Concentrations. The highest concentration of polyphenols was noticed in tartary buckwheat flour (13.08 ± 0.47 mg GAE/g) (**Table 2**). In dough and breads, the total polyphenol content increased with the growing percentage of tartary buckwheat flour used (**Table 4**). Results presented in **Table 4** show a decrease in total polyphenol content as a result of heat treatment during the baking process. During the kneading and rising of bread, we observed a slight increase of total polyphenol concentration in wheat (100%) and buckwheat (100%) bread, and a slight decrease in mixed breads (**Table 4**).

Results presented in our study are in agreement with the results of Alvarez-Jubete et al. (*26*) that showed a significant decrease in total polyphenol concentration in bread made of common buckwheat (0.65 mg GAE/g) compared to that in buckwheat seeds (3.23 mg GAE/g). The results of Zhang et al. (*19*) also showed a degradation of polyphenols due to the thermal treatment of tartary buckwheat flour.

Impact of Bread Making on Rutin and Quercetin Content. Tartary buckwheat flour was the only ingredient containing rutin and quercetin (11.67 and 0.63 mg/g, respectively) (**Table 2**). Rutin and quercetin concentrations increased with the growing percentage of tartary buckwheat flour used. Dough and bread made without tartary buckwheat did not contain any of these two compounds (**Table 4**). Tartary buckwheat dough made of 100% tartary buckwheat flour had a lower concentration of rutin and a higher concentration of quercetin compared to those in the respective flour alone (**Tables 2** and **4**); 0.0175 mmol of rutin was degraded with the addition of water and yeast to tartary buckwheat flour, and 0.0149 mmol of quercetin was gained at the same time. This indicates that 85% of rutin was transformed to quercetin with the addition of water and yeast to the flour. Degradation could be caused by the rutin degrading enzymes found in buckwheat (*27*−*30*). Rutin degrading enzymes are stable and active at pH 5 to 7 and below 40 °C (*31*). We have measured the pH of the dough, and it was between 5.5 and 6.1. The concentration of rutin continued to decrease during the bread rising process. In addition, the rutin concentration in dough after 60 min of rising was lower as it was after 35 min of rising (**Table 4**). After the baking process, some rutin remained present only in bread made of 100% tartary buckwheat flour, while it was undetectable in all other samples. On the basis of a comparison of concentration levels (**Table 4**), quercetin seemed to be more stable than rutin during the bread rising and baking processes. There were no significant differences in rutin and quercetin concentrations between the inside of the bread and the crust.

ABBREVIATIONS USED

DM, dry matter; Tf, tartary buckwheat flour; Wf, wheat flour; T_0, tartary buckwheat to wheat flour ratio 0:100; T_30, tartary buckwheat to wheat flour ratio 30:70; T_50, tartary buckwheat to wheat flour ratio 50:50; T_100, tartary buckwheat to wheat flour ratio 100:0; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FCR, Folin−Ciocalteau reagent; GAE, gallic acid equivalents; HPLC, high performance liquid chromatography; UV, ultraviolet.

LITERATURE CITED

(1) Fabjan, N.; Rode, J.; Kosir, I. J.; Wang, Z. H.; Zhang, Z.; Kreft, I. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. *J. Agric. Food Chem.* **2003**, *51*, 6452–6455.

(2) Bonafaccia, G.; Marocchini, M.; Kreft, I. Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chem.* **2003**, *80*, 9–15.

(3) Steadman, K. J.; Burgoon, M. S.; Lewis, B. A.; Edwardson, S. E.; Obendorf, R. L. Buckwheat seed milling fractions: description, macronutrient composition and dietary fibre. *J. Cereal Sci.* **2001**, *33*, 271–278.

(4) Wijngaard, H. H.; Arendt, E. K. Buckwheat. *Cereal Chem.* **2006**, *83*, 391–401.

(5) Jiang, P.; Burczyński, F.; Campbell, C.; Pierce, G.; Austria, J. A.; Briggs, C. J. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum* and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Res. Int.* **2007**, *40*, 356–364.

(6) Yildzogle-Ari, N.; Altan, V.; Altinkurt, O.; Ozturk, Y. Pharmacological effects of rutin. *Phytother. Res.* **1991**, *5*, 19–23.

(7) Hellerstein, H. K.; Orbison, J. L.; Rodbard, S.; Wilburne, M.; Katz, L. N. The effect of rutin in experimental malignant hypertension. *Am. Heart J.* **1951**, *42*, 271–283.

(8) Harborne, J. B.; Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481–504.

(9) Tian, Q.; Li, D.; Patil, B. S. Identification and determination of flavonoids in buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) by high performance liquid chromatograpy with electrospray ionization mass spectrometry and photodiode array ultraviolet detection. *Phytochem. Anal.* **2002**, *13*, 251–256.

(10) Kreft, S.; Knapp, M.; Kreft, I. Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. *J. Agric. Food Chem.* **1999**, *46*, 47–50.

(11) Ohsawa, R.; Tsutsumi, T. Inter-varietal variation of rutin content in common buckwheat flour (*Fagopyrum esculentum* Moench). *Euphytica* **1995**, *86*, 183–189.

(12) Park, C. H.; Kim, Y. B.; Choi, Y. S.; Heo, K.; Kim, S. L.; Lee, K. C.; Chang, K. J.; Lee, H. B. Rutin content in food products processed from groats, leaves and flowers of buckwheat. *Fagopyrum* **2000**, *17*, 63–66.

(13) Gaberščik, A.; Vončina, M.; Trošt, T.; Germ, M.; Björn, L. O. Growth and production of buckwheat (*Fagopyrum esculentum*) treated with reduced, ambient and enhanced UV-B radiation. *J. Photochem. Photobiol., B* **2002**, *66*, 30–36.

(14) Rosema, J.; Björn, L. O.; Bornman, J. F.; Gaberščik, A.; Häder, D. P.; Trošt, T.; Germ, M.; Klisch, M.; Gröniger, A.; Sinha, R. P.; Lebert, M.; He, Y. Y.; Buffoni-Hall, R.; de Bakker, N. V. J.; van de Staaij, J.; Meijkamp, B. B. The role of UV-B radiation in aquatic and terrestrial ecosystems: An experimental and functional analysis of the evolution of UV-absorbing compounds. *J. Photochem. Photobiol., B* **2002**, *66*, 2–12.

(15) Methodiewa, D.; Jaiswal, A. K.; Cenas, N.; Dickancaite, E.; Aguilar, J. S. Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinoidal product. *Free Radical Biol. Med.* **1999**, *26*, 107–116.

(16) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.

(17) 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.

(18) Sensoy, I.; Rosen, R. T.; Ho, C.; Karwe, M. V. Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chem.* **2006**, *99*, 388–393.

(19) Zhang, M.; Chen, H.; Li, J.; Pei, Y.; Liang, Y. Antioxidant properties of tartary buckwheat extracts as affected by different thermal processing methods. *LWT Food Sci. Technol*. **2010**, *43*, 181–185.
(20) Turkmen, N.; Sari, F.; Velioglu, Y. S. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chem*. **2005**, *93*, 713–718.
(21) Lindenmeier, M.; Hofmann, T. Influence of baking conditions and precursors supplementation on the amounts of the antioxidant pronyl-L-lysine in bakery products. *J. Agric. Food Chem*. **2004**, *52*, 350–354.
(22) Kreft, I.; Fabjan, N.; Yasumoto, K. Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. *Food Chem*. **2006**, *98*, 508–512.
(23) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol*. **1995**, *28*, 25–30.
(24) Lachman, J.; Hejtmánková, A.; Dudjak, E.; Fernández, E.; Pivec, V. Content of Polyphenolic Antioxidants and Phenolcarboxylic Acids in Selected Parts of Yacon [*Smallanthus sonchifolia* (Poepp. et Endl.) H. Robinson]. In *Vitamins 2003*; Sbornik conference; Univerzita Pardubice: Pardubice, 2003; pp 89−97.
(25) Kreft, I. Nährwert des Buchweizens: Grütze - Mehl - Brot - Gemüse - Tee - Essig - Honig. In *Das Buchweizen Buch: mit Rezepten aus aller Welt. 2. überarbeitete und erweiterte Aufl*; Kreft, I., Ries, C., Zewen, C., Eds.;Islek ohne Grenzen EWIV: Arzfeld, Germany, 2007; pp 92−104.
(26) Alvareze-Jubete, L.; Wijngaard, H.; Arendt, E. K.; Gallagher, E. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa, buckwheat and wheat as affected by sprouting and baking. *Food Chem*. **2010**, *119*, 770–778.
(27) Yasuda, T.; Nakagawa, H. Purification and characterization of rutin-degrading enzymes in tartary buckwheat seeds. *Phytochemistry* **1994**, *37*, 133–136.
(28) Suzuki, T.; Honda, Y.; Funatsuki, W.; Nakatsuka, K. Purification and characterization of flavonol 3-glucosidase, and its activity during ripening in tartary buckwheat seeds. *Plant Sci*. **2002**, *163*, 417–423.
(29) Suzuki, T.; Honda, Y.; Mukasa, Y. Effects of UV-B radiation, cold and desiccation stress on rutin concentration and rutin glucosidase activity in tartary buckwheat (*Fagopyrum tataricum*) leaves. *Plant Sci*. **2005**, *168*, 1303–1307.
(30) Suzuki, T.; Kim, S. J.; Takigawa, S.; Mukasa, Y.; Hashimoto, N.; Saito, K.; Noda, T.; Matsuura-Endo, C.; Zaidul, I. S. M.; Yamauchi, H. Changes in rutin concentration and flavonol-3-glucosidase activity during seedling growth in tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *Can. J. Plant Sci*. **2007**, *87*, 83–87.
(31) Yasuda, T.; Shinoyama H.; Nakagawa H. Purification and Characterization of the Rutin-Degrading Enzymes in Tartary Buckwheat Seeds and the Activity of the Enzymes in the Presence of Organic Solvents. *Fagopyrum, Proceedings of the VII International Symposium on Buckwheat, Advances in Buckwheat Research*, Winnipeg, Manitoba, Canada, **1998**; pp 30−39.

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