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Rutin, quercetin, and free amino acid analysis in buckwheat (*Fagopyrum*) seeds from different locations

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ABSTRACT. In this study, five common buckwheats and nine tartary buckwheats grown at different locations were analyzed for the contents of rutin, quercetin, and amino acids by high-performance liquid chromatography and spectrophotometry. The rutin content was higher than quercetin in buckwheat seeds. Rutin content was in the range from 0.05 (0.05 g per 100 g dry seeds) to 1.35% of buckwheat seeds. Quercetin content varied from 0.01 to 0.17% and in some common buckwheats it was even difficult to detect. Comparatively, tartary buckwheat seeds contained more rutin and quercetin than common buckwheat seeds. Meanwhile, the bran has higher rutin content than the farina in tartary buckwheat seeds, with a respective content of 0.45 to 1.19% and 0.14 to 0.67%. It was found that amino acid contents were around 1.79 to 12.65% (farina) and 5.74 to 7.89% (bran) in common buckwheats, and 1.73 to 5.63% (farina) and 2.64 to 16.78% (bran) in tartary buckwheat seeds. The highest total rutin content was found to be 1.35% in tartary buckwheat seeds from Sichuan, China. The highest total amounts of amino acid were detected to be 20.13% in tartary buckwheat seeds from Changzhi, Shanxi Province (China). Our

results suggested that food products made of whole-buckwheat flour are healthier than those made of fine white flour.

Key words: Buckwheat; Farina; Bran; Rutin; Quercetin; Amino acid

INTRODUCTION

Buckwheat, which belongs to the family Polygonaceae, genus *Fagopyrum*, has been a popular health food in Asian and European countries for a long time. The most widely grown buckwheat species include common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) (Zhang et al., 2012). Buckwheat is a unique dual-use cereal crop and has attracted more and more attention due to both its nutritional and medicinal values in recent years (Guo et al., 2011). Buckwheat contains many important bioactive compounds such as protein rich in essential amino acids, oil rich in essential fatty acids (Horbowicz and Obendorf, 1992; Steadman et al., 2001a), starch with a low glycemic index, polyphenol compounds (including rutin, quercetin, orientin, vitexin, isovitexin and isoorientin) (Danihelová and Šturdík, 2012; Sharma et al., 2012), and many essential minerals (Steadman et al., 2001b). Studies have revealed that buckwheat can cure chronic human diseases, decrease blood cholesterin, inhibit mammary cancer, prevent gallstones and many others (Tomotake et al., 2000). As a result, buckwheat has been listed as health protection food in many countries, and many nutritional meals related to buckwheat have been developed, such as buckwheat bread, noodles, soft drinks, beverages, tea, and buckwheat sprouts (Xiao, 2003).

Although a natural vasorelaxant compound was identified from rutin-free tartary buckwheat extracted by Matsui et al. (2010), it is well known that the many unique physiological functions of buckwheat are mainly attributed to its high content of flavonoids, especially rutin (Jiang et al., 2007). Quercetin is an important factor for plant secondary metabolism; it has a wide range of biological activity and is also a promising candidate for prevention and treatment of various cancers (Joanna et al., 2011; Wang et al., 2012). The quercetin glycosides and free quercetin in buckwheat flowers, leaves, stems, and achenes were discovered by Dadakova in 2010. Plant polyphenol content is influenced by many factors such as variety, location, harvest month and others (Zou et al., 2012; Gunaratne et al., 2013; Zhang et al., 2013). Meanwhile, the phenolic contents may vary in different tissues (Pugliese et al., 2013). Amino acid content is an important factor to evaluate nutritional value. Many plants have been investigated for their content or biodiversity of free amino acids (Pratta et al., 2011; Elfalleh et al., 2012). However, little is known about the comprehensive assessment of buckwheat seeds based on the contents of rutin, quercetin, and free amino acids. In order to determine these beneficial components in buckwheat seeds, fourteen samples collected from different locations were studied by means of spectrophotometry and HPLC in the present study. The results may guide people to choose appropriate material (buckwheat fine flour or whole flour) and food processes.

MATERIAL AND METHODS

Apparatus and materials

High-performance liquid chromatography (HPLC; HITACHI, Japan), Labsystems Multiskam MK3 ELISA (Thermo, USA), Vacuum Filter (Waters, USA).

All reagents used for HPLC and LC-MS were of HPLC purity (Tianjin Secondary Factory for Chemical Agent, China). The other chemicals were of analytical grade and were filtered with a vacuum through a 0.45-μm membrane (Millipore, USA). Standards of rutin (95%) and quercetin were purchased from Sigma (USA). Amino Acid Quantitation Kit was purchased from Nanjing Jiancheng Biological Ltd. (China).

Sample preparation

Buckwheat grains harvested in 2009 were provided by the 11th International Symposium of Buckwheat. The plant materials were dried and stored under refrigerated conditions. Common buckwheat from five locations and tartary buckwheat from nine locations were used (Table 1). Hulled buckwheat seeds were ground and then sieved with a number 40 mesh screen in order to obtain farina and bran samples. Accurately weighed farina and bran samples were defatted in Soxhlet extraction with diethyl ether as the extraction agent for 40 min. Prepared samples were then extracted with 40 mL 95% ethanol at 80°C for 4 h. After concentration, the extraction was transferred into a 10 mL measuring flask and filtered through a cellulose acetate filter (0.45 μm; Millipore, USA) for quantification.

Table 1. Common buckwheat and tartary buckwheat obtained from different locations.

Sample	1	2	3	4	5	6	7	8
Cultivar	CB	CB	CB	CB	CB	TB	TB	TB
Location	Hebei, China	Yulin, Shanxi, China	Taiyuan, Shanxi, China	Czech	German	Bhutan1	Mexico	German
Sample	9	10	11	12	13	14		
Cultivar	TB	TB	TB	TB	TB	TB		
Location	US	Jiangxi, China	Changzhi, Shanxi, China	Guizhou, China	Sichuan, China	Yunnan, China		

[a]Common buckwheat (CB), tartary buckwheat (TB).

Determination of rutin and quercetin

HPLC analysis was carried out to determine the content of rutin and quercetin using an HPLC system (HITACHI, Japan) equipped with an L-2130 Pump fitted with an L-2200 Automatic Sampling machine. The analysis was performed on a C18 column (4.6 mm x 200 mm, 5 μm particle size) (Dalian Elite YPERSIL, China). The mobile phase, a mixture of buffer (0.4% phosphoric acid), and methanol (50:50 v/v) was filtered through a 0.45 μm membrane filter and degassed by sonication. HPLC analysis was performed at 30°C with a flow rate of 1.0 mL/min. The column effluent was monitored at 360 nm. Quantification was performed by comparing the peak areas obtained from the samples with those of standards. Serial volumes (1, 2.5, 5, 10, 15, 20 μL) of 0.12 mg/mL standard rutin solution and serial volumes (3, 5, 10, 15, 20, 25, 30, 35, 40 μL) of 4 x 10^{-3} mg/mL standard quercetin solution were injected, respectively, for an HPLC analysis. The standard curve was developed with peak area as y and sample volume as x.

Validation

The method was validated for precision, repeatability, stability, and accuracy. Instrument precision was checked by injecting 10 μL farina sample #1 solution three times and measuring the peak areas of rutin and quercetin, then the RSD (%) was calculated. Repeatability was tested by preparing three samples farina extract #6 (1.5 g per sample); the measurement according to the

chromatography condition is mentioned above. Stability was studied by analyzing the contents of rutin and quercetin of sample #8 farina extract on five different days, and calculating the RSD (%) of daily averages. Accuracy of the method was studied using the method of standard addition. Standard rutin and quercetin solutions were added to the extract and the percent recovery was determined. The amounts of rutin and quercetin were determined and the percent recovery was calculated.

Determination of amino acid

Amino acid was determined according to the Amino Acid Quantitation Kit instructions. Briefly, the reaction system contained 0.5 mL sample, 1.0 mL reaction buffer, and 0.5 mL developer solution. The blank control and standard group were 0.5 mL distilled water and 0.5 mL standard amino acid solution, respectively, instead of 0.5 mL sample. The reaction system was mixed and centrifuged for 10 min (3500 rpm^{-1}), and then the supernatant was measured for the absorption value at 630 nm. The total amino acid content can be calculated by the equation from standard amino acid.

Statistical analysis

Data are reported as means ± standard deviation (SD) for at least three replicates for each sample. Analysis of variance and least significant difference tests were conducted to identify differences among means using the SPSS 18.0 software. Correlation analyses were performed using a Pearson correlation test. Statistical significance was considered at $P < 0.05$.

RESULTS

Determination of rutin and quercetin in buckwheat farina and bran by HPLC

Chromatography conditions

HPLC analysis was performed according to the following conditions: Dalian Elite YPERSIL 5 µm 4.6 x 200 mm C18 column (China), methonal-0.4 phosphoric acid water solution (50:50) as the mobile phase, with a detection wavelength of 360 nm. Flow rate is 1.00 mL/min and column temperature is 30°C. The chromatography graphs of rutin and quercetin are shown in Figure 1 (A: standard, B: sample). Each peak in the sample was identified by comparing its retention time with that of the standard, which was carried out under the same chromatography conditions. The retention times of rutin and quercetin standards were 3.32 and 6.82 min respectively, while rutin and quercetin in samples were determined with retention times of 3.36 and 6.82 min, respectively.

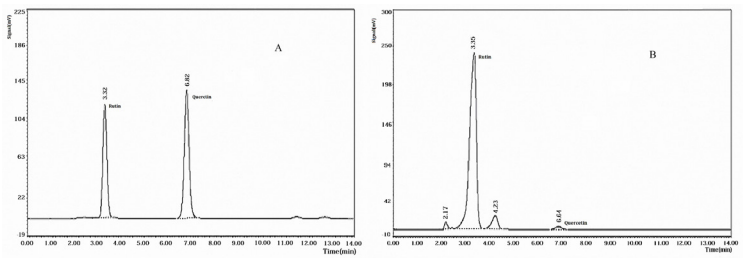

Figure.1 HPLC chromatograms of rutin and quercetin. **A.** Standard. **B.** Sample.

Method validation

A linear relationship between the peak area and the amount of standard rutin was obtained for rutin in the range of 0.120-2.400 µg. The equation of linear regression curve obtained was y = 1432.3x - 112.57, where y = (peak area), x = (volume of rutin), with a correlation coefficient (r^2) of 0.9994. The linearity range of standard quercetin was 0.012-0.160 µg and the equation of linear regression was y = 1.9404x + 1.8326, where y = (peak area), x = (volume of quercetin), with a correlation coefficient (r^2) = 0.9999. The precision (RSD) was found to be 3.14% and the repeatability (RSD) of the method was 1.50%. Values of inter-day precision (RSD) was found to be 3.28%. The accuracy of the method was evaluated by recovery. The results of recovery analysis are shown in Table 2. The recovery of rutin and quercetin was 100.89 and 94.91%, respectively, and the corresponding RSD was 1.97 and 0.26%, respectively.

Table 2. Results of recovery analysis of rutin and quercetin by HPLC.

	Preanalyzed sample (mg)	Amount of standard to preanalyzed sample (mg)	Total amount found	Recovery (%)	Mean recovery (%)	RSD (%)
Rutin	0.1095	0.2286	0.3256	94.53	94.91	1.97
	0.1095	0.2286	0.3265	94.93		
	0.1095	0.2286	0.3273	95.28		
Quercetin	5.3378	7.5000	12.9240	101.15	100.89	0.26
	5.3378	7.5000	12.7471	98.79		
	5.3378	7.5000	13.0433	102.74		

Amounts of rutin and quercetin in buckwheat from different locations

Samples from different locations are shown in Table 1. The rutin and quercetin contents of buckwheat seeds are shown in Figures 2 and 3. The rutin content ranged from 0.05% (0.05 g per 100 g dry seeds) to 1.35%. Obviously, tartary buckwheat seeds had higher rutin content than common buckwheat seeds (Figure 2). Similar difference in the flavonoid content was listed in the work of Pankaja et al. (2012). In their work, the sprouts, microgreens, and leafy greens of common and tartary buckwheat were compared for the phenolic contents. It was found that tartary buckwheat samples expressed higher total phenolic and flavonoid contents compared to the common buckwheat. Meanwhile, the bran had higher rutin content than the farina in tartary buckwheat seeds, with a respective content of 0.45 - 1.19% and 0.14 - 0.67%. These results are consistent with the results of Cho et al. (2014). It was also found that buckwheat bran contained a distinctly higher content of rutin, compared to hull and flour. Tartary buckwheat from Sichuan (China) had the highest rutin content (1.35%), followed by tartary buckwheat from Guizhou (China) out of the fourteen samples tested.

As Figure 3 shows, quercetin content was extremely low. No quercetin was detected in common buckwheat bran and farina. Quercetin was only found in some samples of tartary buckwheat and varied from 0.01 to 0.17%. Similar results were recorded in the work of Ren and Sun (2014). In their work, no quercetin was detected in common buckwheat flour, bran, or sprouts from 1 to 9 days and quercetin was only found in tartary buckwheat sprouts during the early stages of germination. However, in the study of Kim et al. (2008), a trace of quercetin was found in non-germinated seeds while quercetin was not detected in the seed sprouts. It is important to emphasize that quantities of quercetin in buckwheat seeds were relatively low and close to the detection limit of HPLC, leading to the obscure observed results (Kim et al., 2004). Similar to rutin, it was found

that bran contained higher amounts of quercetin than farina in tartary buckwheat. Concerning quercetin, the highest value (0.17%) was determined in tartary buckwheat from Changzhi (Shanxi province, China) and varied from 0.01 to 0.17%.

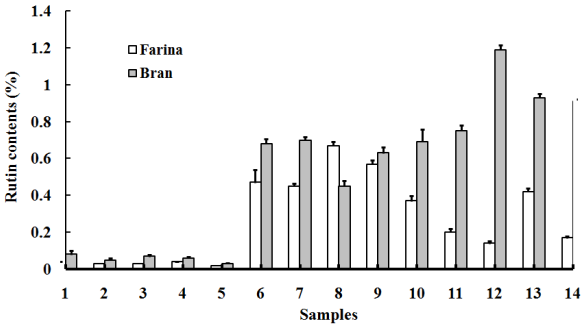

Figure 2. Rutin content of buckwheat collected from 14 locations determined in bran and farina.

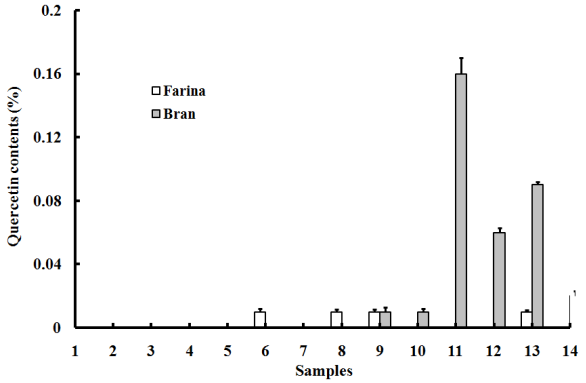

Figure 3. Quercetin content of buckwheat collected from 14 locations determined in bran and farina.

Content of amino acids in buckwheat from different locations

The content of amino acids is shown in Figure 4. As the results show, amino acid contents were around 0.18-1.27% in common buckwheat and 0.17-1.68% in tartary buckwheat seeds. Tartary buckwheat from Changzhi (Shanxi Province, China) had the highest amino acid content, followed by common buckwheat from Hebei (China).

Reported contents of amino acids varied greatly depending on the material sources. Additionally, different extraction and dilutions may have a significant impact on the relative quantities of amino acid (Mehdizadeh et al., 2015). In this study, the obtained results are in accordance with those of literature. According to Kivrak et al. (2014), the total free amino acids were 0.20% in the giant puffball mushroom (*Calvatia gigantea*) using UPLC-MS/MS. Content of free amino acids in hawthorns at different maturation stages was 0.32 to 0.35% (Li et al., 2015). In the seeds of vegetable soybeans the amount of the free amino acids ranged from 0.46 to 1.02% (Song et al., 2013) and the contents of cocoa samples varied between 0.52 and 1.81% (Marseglia et al., 2014).

Higher levels of free amino acid was detected in rambutan seed (9.73%) and blueberry (18.7%) (Zhang et al., 2014; Mehdizadeh et al., 2015).

More free amino acids were detected in bran than in farina in both common buckwheat and tartary buckwheat seeds in this study. These results are in accordance with literature. Different fractions and products of wheat, rye, oats, and barley were analyzed by Mustafa et al. (2007), and it was found that bran contained more free amino acids than did the other analyzed fractions of cereals.

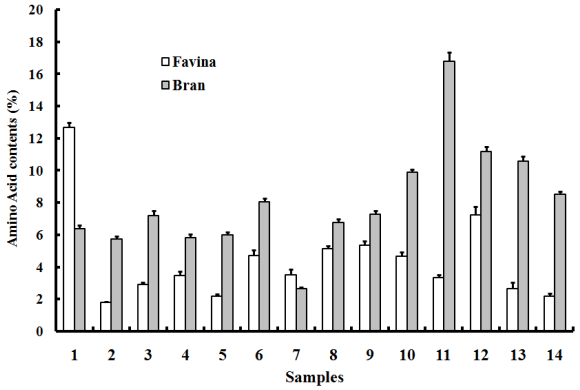

Figure 4. Amino acid content of buckwheat collected from 14 locations determined in bran and farina.

DISCUSSION

A precise and accurate HPLC method was established for determination of rutin and quercetin in buckwheat seeds. The method is fast, relatively economical, and can be applied to other grains. The results show significant variations in buckwheat seeds. The contents of rutin, quercetin, and amino acids in common buckwheat seeds were lower than those in tartary buckwheat seeds. Quercetin could not be detected in some samples of common buckwheat in this study. The relatively high rutin content in both common and tartary buckwheat implies that buckwheat may serve as an excellent dietary source of rutin, which is known to have beneficial health effects such as reduced blood pressure, lowered blood sugar concentration, and increased antioxidant activity (Hernández-Herrero and Frutos, 2015).

The bran has higher rutin, quercetin, and amino acids than the farina in common and tartary buckwheat, suggesting that food products made of whole-buckwheat flour is more healthful than that of fine white flour. However, the texture of whole-wheat flour food is usually not as desirable. Therefore, it is necessary to improve the processing methods of buckwheat foods to meet the demand for both texture and nutritional value. Furthermore, our results highlight the possibility of using buckwheat bran, a by-product of buckwheat flour production, as a source of rutin for both dietetic and industrial application.

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