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Free Amino Acids, Fatty Acids, and Phenolic Compounds in Tartary Buckwheat of Different Hull Colour

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Abstract

Peng L.-X., Zou L., Tan M.-L., Deng Y.-Y., Yan J., Yan Z.-Y., Zhao G. (2017): Free amino acids, fatty acids, and phenolic compounds in Tartary buckwheat of different hull colour. Czech J. Food Sci., 35: 214–222.

In this paper, free amino acids, fatty acids, and phenolic compounds in buckwheat of different hull colour were quantified by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), gas chromatography-mass spectrometry (GC-MS), and high performance liquid chromatography-ultraviolet detector (HPLC-UV), respectively. A total of 20 free amino acids, 8 fatty acids, and 6 phenolic compounds were detected in Tartary buckwheat flour and bran. The data on concentrations were subjected to common chemometric analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), to gain better understanding of the differences between the tested samples. Results indicated that most of the free amino acids, fatty acids, and phenolic compounds were higher in bran than in flour, and there is no significant difference in respect to the hull colour. Our results may be helpful for quality control in Tartary buckwheat and its products in the future.

Keywords: *Fagopyrum tataricum* L.; activity compounds; seed colour; different part

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn) is an important crop widely planted in many countries such as China, northern India, Bhutan, and Nepal (Zhao & Zou 2012). Buckwheat is mainly cultivated in the cold highland area, and contains many beneficial components such as phenolic compounds, amino acids, fatty acids, as well as vitamins and minerals (Zhao & Shan 2008). It was reported that amino acids in buckwheat are more balanced than in many other crops (Pomeranz & Robbinas 1972). As amino acids are the main participant in the Maillard reaction, they are important for the quality of Tartary buckwheat and its products. Fatty acids are present in the oil of buckwheat seed, among them, oleic acid and linoleic acid possess the highest concentration (Gulpinar *et al*. 2012). These unsaturated fatty acids have been proved to be important for the health (Wang *et al*. 2008). Phenolic compounds, very important in functional biochemistry of buckwheat, are also attracting more and more researchers (Zhao & Zou 2012).

The hull colour of Tartary buckwheat mainly includes black and brown, therefore 'black Tartary buckwheat' and 'yellow (brown) Tartary buckwheat'

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are distinguished in China. 'Black Tartary buckwheat' is traditionally deemed to have higher quality than 'brown Tartary buckwheat'. There is a report that analysed the relationship between flour colour, hull colour, and the antioxidant activity (Fujita *et al*. 2014). However, the comprehensive analysis of the functional biochemistry such as amino acids, fatty acids, and phenolic compounds between different types of hull colour has not been reported. Concentrations of amino acids in buckwheat were reported by some studies (Zhang *et al*. 2005; Wang 1995). Amino acid analyser, capillary electrophoresis, gas chromatography, liquid chromatography are widely used in amino acid quantification (Jiang *et al*. 2013), and recently, UPLC-MS/MS has been certified as a method of sufficient sensitivity, selectivity, and robustness for amino acid analysis (Kivrak *et al*. 2014). As to our best knowledge, there is no report at present that detects amino acids in Tartary buckwheat using UPLC-MS/MS. For the fatty acids, Gulpinar *et al*. (2012) found that buckwheat (*Fagopyrum esculentum* Moench) oil was dominated by oleic, linoleic, and palmitic acids, this result also agrees with report of Mazza (1988). Dorrell (1971) identified eighteen fatty acids in buckwheat, among them, palmitic, stearic, oleic, linoleic, linolenic, arachidic, behenic, and lignoceric acids accounted for over 93%. Many other reports (Taira *et al*. 1986; Tsuzuki *et al*. 1987) published similar results. GC-MS technology is widely used for the fatty acid analysis in food science literature. The preparation of samples for GC usually involves two separate procedures: extraction and methylation. This procedure is time and material consuming. In this paper, we aim to identify the differences in functional chemistry including free amino acids, fatty acids, and phenolic compounds between flour and bran, and also to clarify whether or not these compounds correspond to the hull colour. In order to quantify free amino acids, fatty acids, and phenolic compounds accurately, UPLC-MS/MS, GC-MS, and HPLC-UV were used, respectively. Then, for distinguishing the difference in different types of hull colour of Tartary buckwheat, the data on concentrations were subjected to common chemometric analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), to gain better understanding of the differences between the tested samples.

MATERIAL AND METHODS

Tartary buckwheat seeds were obtained from the Coarse Cereal Processing Center (CCPC) of Chengdu University (China) (Table 1). The seeds were harvested in Sichuan (China) during July 2014. Buckwheat seeds were ground in a blender, and separated into the bran and the flour on different sieves, the flour under the 65-mesh sieves and the bran between 24–65 mesh sieves. The samples were then freeze-dried and kept in a deep freezer (below –70°C) before analysis. 12 isotope-labelled amino acids including $^{2}H_{4}$, 5-^{13}C-arginine, $^{2}H_{3}$-leucine, $^{2}H_{3}$-methionine, $^{13}C_{6}$-phenylalanine, $^{2}H_{3}$-aspartic acid, $^{2}H_{2}$-citrulline, $^{2}H_{3}$-glutamic acid, $^{2}H_{8}$-valine, $^{13}C_{6}$-tyrosine, $^{2}H_{4}$-alanine, $^{2}H_{3}$-ornithine, ^{15}N,2-,^{13}C-glycine were purchased from Cambridge Isotope Laboratories, Inc. Amino acids [Glycine (Gly), Alanine (Ala), Beta-Alanine (Beta-Ala), GABA, Serine

Table 1. Sources of the buckwheat samples

No.	Cultivar	Abbreviation		Locality	Hull colour
		flour	bran		
K1	Chuanqiao No.1	cq1-f	cq1-b	Chengdu University	black
K2	Chuanqiao No.2	cq2-f	cq2-b		black
K3	Guyuan No.1	gy1-f	gy1-b		black
K4	Xiqiao No.1	xq1-f	xq1-b		black
K5	Yunqiao No.1	yq1-f	yq1-b		black
K6	Xiqiao No.3	xq3-f	xq3-b		brown
K7	MiqiaoNo.1	mq1-f	mq1-b	Yanyuan, Sichuan	brown
K8	Jinqiao No.4	jq4-f	jq4-b	Chengdu University	brown
K9	Jinqiao No.2	jq2-f	jq2-b		brown
K10	Fenghuang	fh-f	fh-b		brown

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(Ser), Proline (Pro), Valine (Val), Threonine (Thr), Leucine (Leu), Isoleucine (Ile), Asparagine (Asn), Aspartic acid (Asp), Lysine (Lys), Glutamic acid (Glu), Methionine (Met), Histidine (His), Phenylalanine (Phe), Arginine (Arg), Tyrosine (Tyr)), Tryptophan (Trp)] and fatty acids were purchased from Sigma-Aldrich. The external standards caffeic acid, vitexin, rutin, quercitrin, quercetin, and kaempferol were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China). Acetonitrile (HPLC-grade) was purchased from Fisher Scientific Co (USA). Other chemicals and solvents used in the study were of analytical grade.

Analysis of amino acids. Amino acids were extracted and quantified according to Li *et al*. (2013) with some modifications. Briefly, buckwheat samples (100 mg) were transferred into a 1.5 ml centrifuge tube, 2000 µl of 50% pre-cooled ethanol was then added to the tube and vortexed for 30 seconds. The tube was placed into an ultrasound machine for 60 min at 40°C, and then centrifuged for 15 min at 8000 rpm. 800 µl supernatant was transferred into a new Eppendorf tube. After blow drying with a moderate nitrogen gas stream, 150 µl of 50% ethanol solution (4°C) was then added to the sample tube, centrifuged at 13000 rpm for 15 minutes. The supernatant was collected. 20 µl of amino acid standards, 80 µl of the supernatant were transferred into a 1.5 ml centrifuge tube, 150 µl of 12 isotope-labelled amino acids (dissolved using methanol) internal standard was then added to the tube and blended, and drying with a moderate nitrogen gas stream. For derivatisation, 150 µl of derivatisation reagent [hydrochloric acid/n-butyl alcohol (1 : 3, v/v)] was added into the mixture and reacted for 30 minutes. Then, the sample was separated and analysed by UPLC-MS/MS.

The UPLC-MS/MS instrument consisted of a Waters (USA) Acquity Ultra Performance LC with a Waters binary system manager coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer equipped with an electro spray ionisation (ESI) probe. Samples were separated by Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 mm particle size). The mobile phase consisted of a mixture of (A) water containing 0.1% formic acid (v/v) and (B) acetonitrile containing 0.1% formic acid (v/v) and 0.01% heptafluorobutyric acid (v/v). The gradient program was set as: 0–1.5 min, 95% solvent A; 1.5–2 min, 95–80% solvent A; 2–7 min, 80–70% solvent A; 7–8.5 min, 70–2% solvent A; 8.5–10.5 min, 2% solvent A; 10.5–11 min, 2–95% solvent A, 11–12.5 min, 95% solvent A (total 12.5 min). The column oven temperature was 40°C. The flow rate was set at 0.4 ml/min, and injection volume was 5 µl.

The ESI source was used in positive mode by multiple reaction monitoring (MRM) mode with the following conditions: the ion source capillary voltage 3.0 kV, cone voltage 20 V, and desolvation temperature 380°C, desolvation flow rate of nitrogen was 600 l/hour. Data analysis and quantitation were executed using the Waters MassLynx and TargetLynx software.

Analysis of fatty acids. Fatty acids were extracted and quantified according to Araujo's and Garcés's methods (Garcés & Mancha 1993; Araujo *et al*. 2008) with some modifications. Buckwheat samples (10 mg of bran, 20 mg of flour) were transferred into a centrifuge tube, 2 ml methanol containing 1% H_2SO_4 was added for methyl-esterification reaction for 30 min at 80°C, then 2 ml *n*-hexane was added for extraction, and 5 ml deionised water was used for washing. 500 µl methyl salicylate (50 ppm) as internal standard was added into 1 ml *n*-hexane extract liquor and then shaken up. After the above reactions, samples were separated by an Agilent DB-WAX capillary column (30 m × 0.25 mm i. d.; Agilent J&W Scientific, USA) and determined for contents of fatty acids using the 7890A GC/5975C MS system (Agilent, USA). The split ratio was set at 10 : 1. The injection volume was 1 µl, the injector temperature was 280°C, ion source temperature 230°C, transmission line temperature was 250°C. The program of temperature rise was followed by initial temperature of 50°C for 3 min, 10°C/min rate up to 220°C, and staying at 220°C for 20 minutes. The flow rate of nitrogen was 1.0 ml/minute. Mass spectrometry was determined by the full-scan method with a range from 35 to 780 (*m/z*).

Quantification of phenolic compounds. Phenolic compounds were extracted and quantified according to Li *et al*. (2012) with slight modifications. Briefly, 10 mg samples were extracted with 1 ml of MeOH containing 10% phosphoric acid [0.1% (v/v)], vortexed at room temperature for 5 min, and stored at 37°C for 3 h 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 0.45 µm PTFE syringe filter (Tianjin Jinteng Experiment Equipment Co., Ltd, China) for HPLC analysis. A Kromasil 100-5 C18 (250 mm × 4.6 mm × 5 µm) column was operated at 40°C. The mobile phase 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 gradient program was set as: 0–55 min, 0–80% B; 55–65 min, 0–0% B. The quantification wavelength

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was set at 350 nm. The flow rate was 1.0 ml/min and injection volume was 20 µl. The phenolic content was calculated by comparing the HPLC peak area with authenticated standards.

Statistical analysis. All treatments were performed in triplicate, and the results were represented by their mean values and standard deviations (SD). The contents of amino acids, fatty acids, and phenolic compounds were imported into the Simca-P software (version 11.5; http://www.umetrics.com/simca) for principal component analysis (PCA) to clarify differences between 'black Tartary buckwheat' and 'brown Tartary buckwheat', bran and flour. Significant differences between buckwheats of different hull colour were tested using the *t*-test. Hierarchical cluster analysis (HCA) and *t*-test were performed by the SPSS 19.0 software (IBM Corp., USA).

RESULTS AND DISCUSSION

Amino acids in buckwheat. Some studies have focused on the free amino acid composition of buckwheat. Woo *et al*. (2013) reported the distribution of free amino acids in Tartary buckwheat and common buckwheat in different parts; results indicated that Val is the most abundant amino acid in the leaf of Tartary and common buckwheat sprouts, however, is very low in stem and root. Our research showed different distribution of free amino acids in bran and flour. The amino acids in buckwheat were detected by an amino acid analyser or pre-column derivatisation high-pressure liquid chromatography in most of the present studies (Woo *et al*. 2013; Xia *et al*. 2015), the quantitative accuracy needs further improvements. In our study, a total of 20 free amino acids were examined in Tartary buckwheat bran and flour using UPLC–MS/MS. The chromatographic and MRM method parameters for free amino acids are listed in Table S1 (in electronic supplementary material – EMS). MRM chromatograms of some free amino acids are shown in Figure 1. All the essential amino acids, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine, phenylalanine, and all the non-essential amino acids were detected and quantified. The results are summarised in Table 2, and Tables S3 and S4 (in ESM). The concentration of Glu was highest (173.5 and 589.4 mg/100 g in flour and bran, respectively) and the Ile content was lowest (0.01 and 0.09 mg/100g in flour and bran) in Tartary buckwheat. Almost all the free amino acids have similar concentrations in two different colour types of buckwheat (Table 2). GABA, an inhibitory neurotransmitter found in the nervous systems of widely divergent species, is an important active compound in buckwheat. GABA could

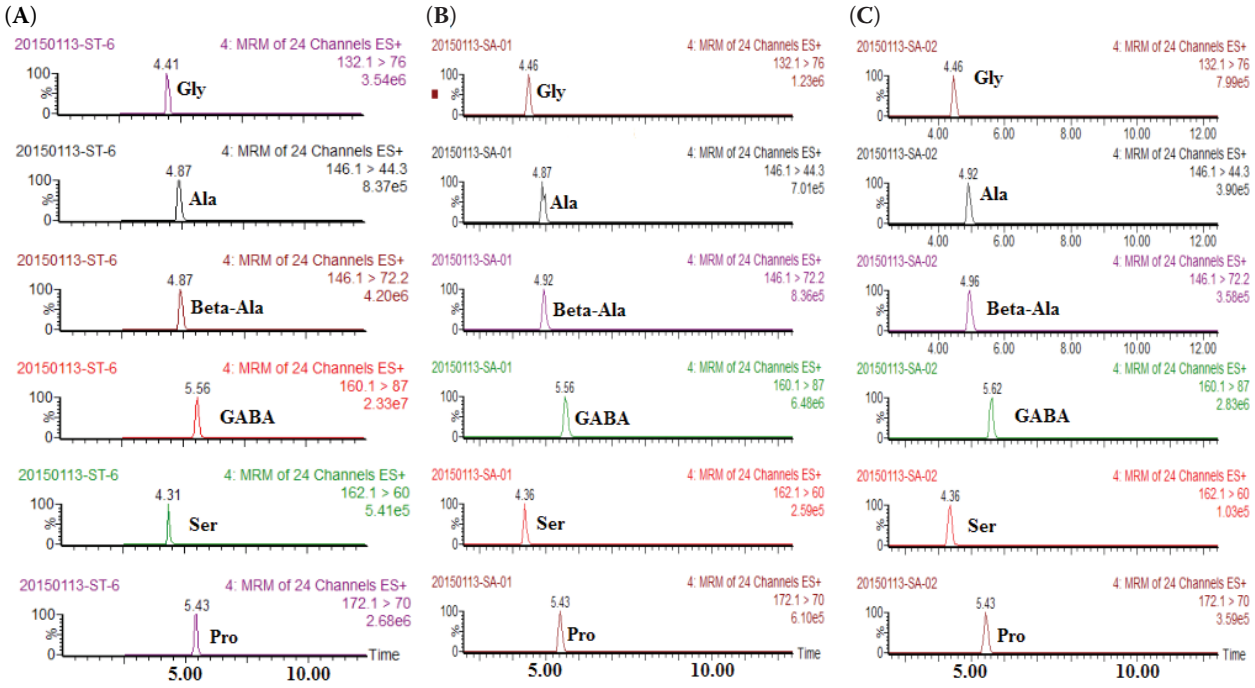

Figure 1. MRM chromatograms of some free amino acids in Tartary buckwheat: (**A**) amino acid standards, (**B**) amino acids in bran, and (**C**) amino acids in flour

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reduce appetite and reduce blood sugar in diabetics (http://www.hmdb.ca/metabolites/HMDB00112). GABA in bran relatively increased about 2.80 fold, suggesting that brans possess a higher nutritional value. The detected amino acids in bran possessed 2.2–22.5 times higher content than that in flour, which indicated that the bran should be a good foodstuff for the development of functional food.

Fatty acids in buckwheat. A total of 8 fatty acids including myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2n-6c), linolenic acid (C18:3n-3), arachidic acid (C20:0), and cis-11-eicosenoic acid (C20:1) were examined and quantified in Tartary buckwheat bran and flour using GC-MS (Table 2 and Tables S3 and S4 in ESM). GC-MS total ion chromatograms for fatty acids identified in Tartary buckwheat are shown in Figure 2. Calibration linearity (R2), limit of detection (LOD), and precision for fatty acids are listed in Table S2 (in ESM). Correlation coefficients (R2) of linear regression analysis from calibration curves were > 0.99, between 0.995 and 0.999. The limit of detection for sensitivity was evaluated in the range of 0.013–0.870 mg/l. RSD values corresponding to

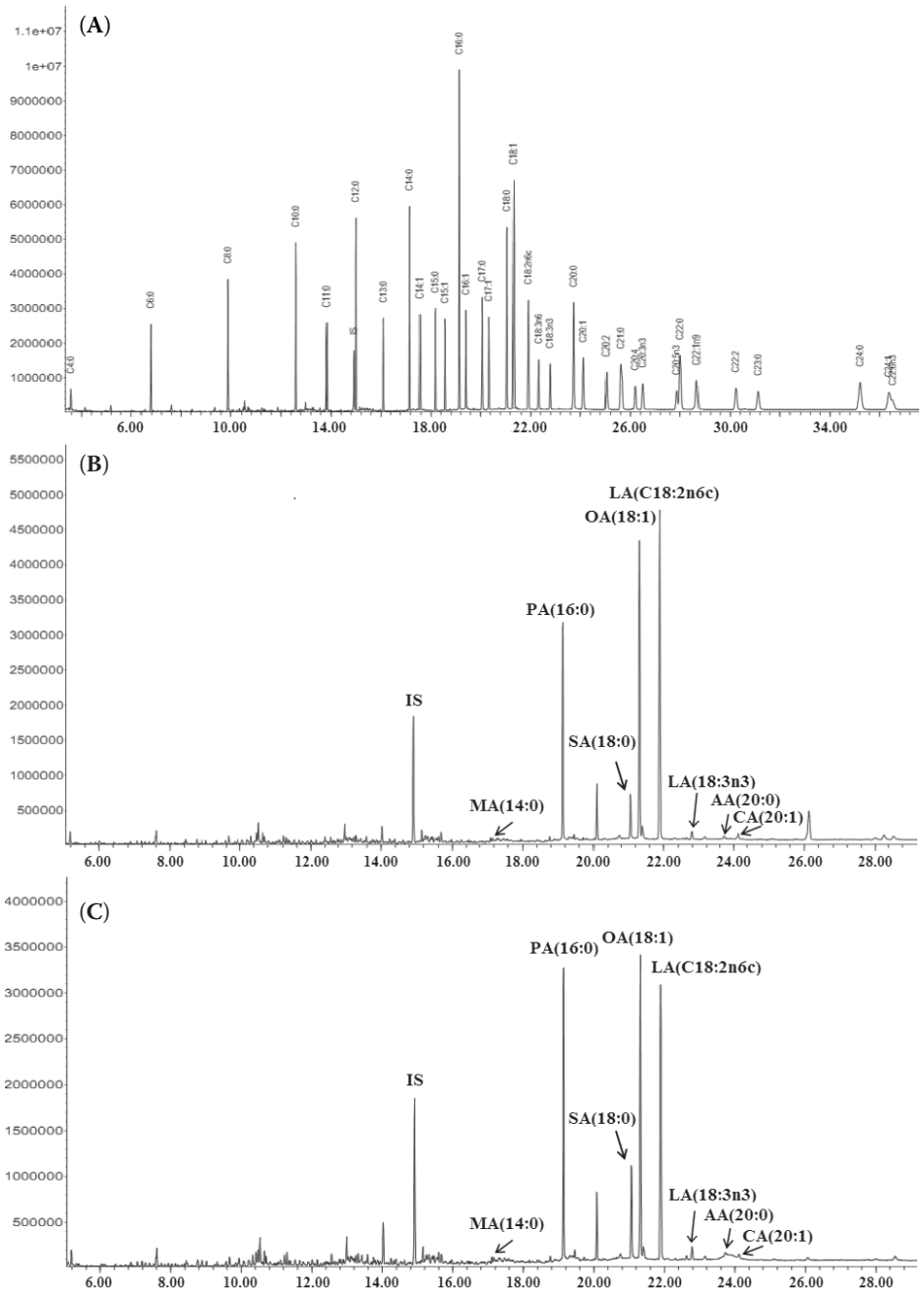

Figure 2. GC-MS total ion chromatograms for fatty acids identified in Tartary buckwheat: (**A**) fatty acid standards, (**B**) fatty acids in bran, and (**C**) fatty acids in flour

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Table 2. Concentration of amino acid, fatty acid, and phenolic compounds in buckwheat (in mg/100 g)

	Flour			Bran			FC_1	FC_2
	cq1-f[§]	xq3-f	cq2-f	cq1-b	xq3-b	cq2-b		
C14:0	11.7 ± 0.5	12.4 ± 0.6	11.0 ± 0.4	23.4 ± 0.8	23.0 ± 0.9	23.0 ± 0.8	1.0	1.0
C16:0	206.1 ± 10.3	238.9 ± 11.2	184.2 ± 8.9	423.8 ± 17.5	412.6 ± 18.3	380.6 ± 15.4	1.1	0.9
C18:0	82.6 ± 3.8	100.4 ± 5.8	67.4 ± 3.4	105.4 ± 5.1	125.4 ± 5.8	124.8 ± 7.2	1.1	0.9
C18:1	283.9 ± 16.3	264.7 ± 11.2	272.6 ± 12.3	722.2 ± 20.1	615.6 ± 19.6	547.2 ± 17.8	0.9	1.0
C18:2n-6c	328.8 ± 15.3	258.3 ± 11.3	329.9 ± 12.3	1032.4 ± 27.6	809.8 ± 23.6	793.0 ± 22.7	0.9	0.9
C18:3n-3	26.1 ± 1.2	24.9 ± 1.8	21.9 ± 1.9	44.0 ± 2.1	49.4 ± 1.9	38.4 ± 2.4	1.1	1.0
C20:0	16.1 ± 0.9	15.2 ± 0.8	1 6.5 ± 0.9	34.4 ± 1.4	35.6 ± 1.1	35.0 ± 1.3	1.0	1.0
C20:1	8.8 ± 0.3	12.1 ± 0.4	8.9 ± 0.4	31.8 ± 0.9	29.2 ± 1.1	26.0 ± 0.7	1.1	0.9
Ala	6.2 ± 0.2	16.9 ± 0.6	9.7 ± 0.5	23.1 ± 1.3	32.9 ± 2.1	28.2 ± 1.7	1.2	0.9
Arg	82.9 ± 4.8	85.9 ± 4.3	116.8 ± 7.2	284.3 ± 14.3	397.9 ± 17.8	356.9 ± 16.3	1.6	1.0
Asn	5.9 ± 0.2	5.8 ± 0.3	8.8 ± 0.7	25.5 ± 1.6	19.5 ± 1.3	29.2 ± 1.9	0.9	0.7
Asp	2 5.2 ± 1.8	28.7 ± 1.9	44.0 ± 2.1	124.6 ± 7.4	88.5 ± 4.1	156.5 ± 8.1	1.0	0.8
B-Ala	5.7 ± 0.7	9.3 ± 0.7	10.0 ± 0.8	25.7 ± 1.6	20.5 ± 1.2	28.4 ± 1.9	1.0	0.8
GABA	42.6 ± 3.1	112.5 ± 8.9	79.3 ± 5.6	114.5 ± 6.3	195.5 ± 11.8	324.1 ± 18.9	1.1	0.9
Glu	137.6 ± 8.4	158.6 ± 7.8	374.0 ± 19.2	528.3 ± 29.5	462.3 ± 21.3	1195.9 ± 54.1	0.9	0.8
Gly	4.3 ± 0.8	11.2 ± 1.1	5.1 ± 0.6	10.5 ± 0.8	18.9 ± 0.9	12.4 ± 0.7	1.1	0.8
His	1.8 ± 0.1	2.1 ± 0.2	4.4 ± 0.5	7.2 ± 0.6	6.8 ± 0.7	13.5 ± 0.9	1.1	0.9
Ile	nd	nd	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.03	0.0	0.5**
Leu	3.5 ± 0.2	5.0 ± 0.4	10.4 ± 1.1	14.3 ± 0.9	14.8 ± 1.2	24.7 ± 1.5	0.8	0.7
Met	0.3 ± 0.04	0.4 ± 0.03	1.7 ± 0.1	1.0 ± 0.1	1.5 ± 0.2	4.6 ± 0.3	0.7	0.7
Phe	4.4 ± 0.3	5.8 ± 0.4	7.5 ± 0.6	16.7 ± 1.1	21.5 ± 1.8	24.2 ± 1.6	0.9	0.8
Pro	5.9 ± 0.4	8.3 ± 0.5	16.4 ± 1.3	13.6 ± 1.2	11.7 ± 1.0	45.9 ± 2.8	0.7	0.6
Ser	1.4 ± 0.1	2.9 ± 0.2	5.7 ± 0.4	3.9 ± 0.2	4.5 ± 0.3	11.8 ± 0.8	0.8	0.7
Thr	0.7 ± 0.08	0.9 ± 0.07	1.1 ± 0.1	1.7 ± 0.2	1.9 ± 0.2	3.2 ± 0.3	0.9	0.8
Trp	3.5 ± 0.4	3.2 ± 0.3	4.7 ± 0.3	12.5 ± 1.1	10.6 ± 0.8	15.2 ± 1.1	1.0	0.9
Tyr	2.5 ± 0.2	1.9 ± 0.3	3.8 ± 0.3	70.0 ± 5.1	79.3 ± 4.1	76.1 ± 5.6	0.6*	0.6
Val	2.2 ± 0.2	4.4 ± 0.3	5.4 ± 0.3	7.6 ± 0.6	7.7 ± 0.5	11.8 ± 0.7	0.9	0.7
Lys	2.7 ± 0.2	3.2 ± 0.3	9.4 ± 0.6	7.1 ± 0.4	8.0 ± 0.6	183 ± 0.9	0.5	0.6
Caffeic acid	22 ± 1	43 ± 0	47 ± 4	65 ± 3	103 ± 9	119 ± 4	0.8	0.8
Vitexin	1 ± 0	3 ± 0	3 ± 0	4 ± 0	9 ± 3	8 ± 1	0.8	1.0
Rutin	849 ± 21	1486 ± 40	1177 ± 20	4079 ± 44	5186 ± 79	4682 ± 206	1.1	0.9
Quercitrin	43 ± 1	59 ± 1	68 ± 1	146 ± 12	263 ± 62	291 ± 18	1.1	1.0
Quercetin	26 ± 5	24 ± 1	72 ± 7	62 ± 5	80 ± 11	111 ± 5	0.7	0.8
Kaempferol	1 ± 0	1 ± 0	3 ± 0	2 ± 1	4 ± 2	3 ± 0	0.7	1.1

FC1 – fold changes of biochemistry in flour (calculated using the formula $\log_2^{(black/brown)}$); FC2 – fold changes of biochemistry in bran (calculated using the formula $\log_2^{(black/brown)}$); [§]for abbreviations see Table 1; nd – not detected; *$P < 0.05$, **$P < 0.01$ – evaluated by Student's *t*-test

precision were between 0.99 and 7.89%. The major fatty acids of Tartary buckwheat were C18:2n-6c, C18:1, and C16:0. Several studies were performed on the fatty acid composition of buckwheat. In Dorrell's report (1971), palmitic, stearic, oleic, linoleic, linolenic, arachidic, behenic, and lignoceric acids were found to be the fatty acids in buckwheat seeds. Tsuzuki *et al.* (1987) reported that palmitic, stearic, arachidic, and behenic acids are present in buckwheat seedlings, Taira *et al.* (1986) also found that oleic and linoleic acids are the main fatty acids in buckwheat. These studies reported the relative contents

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of individual fatty acids by their percentage of the peak area. In our study, we calculated the absolute amount of fatty acid using the standard curve. Our research also indicated that oleic and linoleic acids are the dominant fatty acids in buckwheat, the concentration of which is 257.2, 281.6 mg/100 g in flour and 620.6, 818.7 mg/100g in bran, respectively. Palmitic acid was found to be the main saturated fatty acid. Similar to free amino acids, almost all the fatty acids are not significantly different between 'black' and 'brown' Tartary buckwheat. On the other hand, the detected fatty acids in bran were 1.4–3.1 times higher than in flour. Linoleic acid and linolenic acid are important compounds for human health, their concentration in bran was 2.9 and 1.7 times higher than in flour, respectively, and it also reflects the higher nutrition in Tartary buckwheat bran.

Phenolic compounds in buckwheat. In this study, six phenolic compounds including caffeic acid, vitexin, rutin, quercitrin, quercetin, and kaempferol were quantified in Tartary buckwheat bran and flour. The concentration of rutin was highest among all the samples compared with other phenolic compounds (> 85% of the detected phenolic compounds). On the other hand, the concentration of total phenolic compounds in bran was significantly higher than that in flour (3.39–5.54 times among different samples), which mean that bran may possess higher antioxidant activity and other functions than flour. Phenolic compounds in the flour of black Tartary buckwheat are 1134 ± 285 mg/100 g, while in brown Tartary buckwheat they are 1260 ± 285 mg/100 g, which is not a significant difference ($P > 0.05$). Similar results were obtained for phenolic compounds in the bran of black Tartary buckwheat and brown Tartary buckwheat, also without significant difference (5057 ± 506 mg/100 g in black Tartary buckwheat, 4602 ± 700 mg/100 g in black brown buckwheat, $P > 0.05$). Results indicated that the concentration of functional chemistry in buckwheat is not in respect to the hull colour.

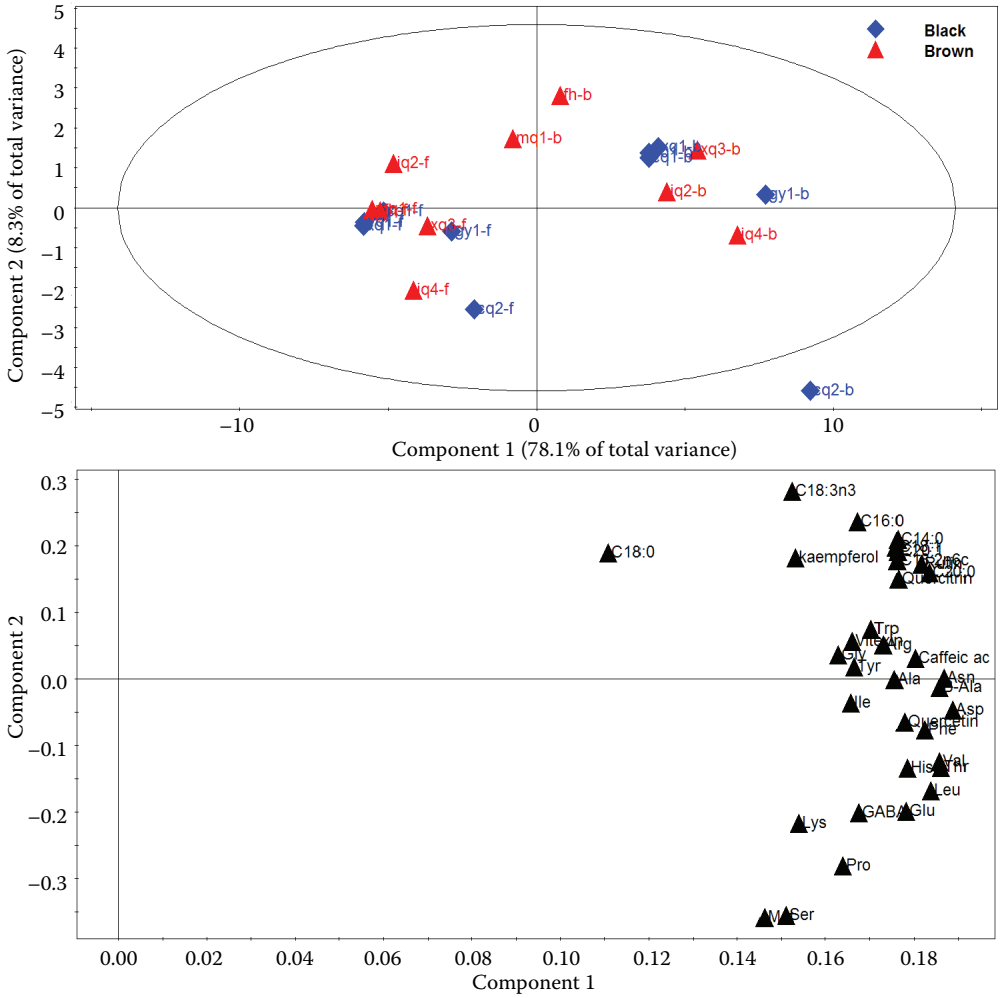

Figure 3. Principal component analysis (PCA) of amino acids, fatty acids, and phenolic compounds in Tartary buckwheat

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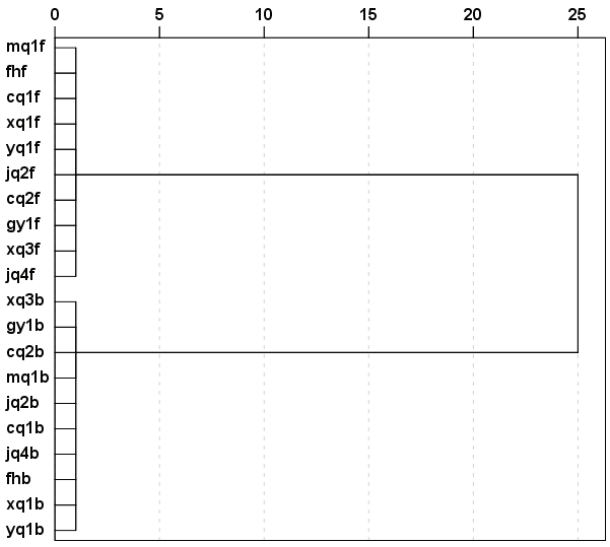

Figure 4. Dendrogram of HCA for buckwheat

Principal component analysis. To highlight the relationships between 20 amino acids, 8 fatty acids, and 6 phenolic compounds, the data was subjected to PCA. From Figure 3, a marked separation between flour and bran was achieved, however, not between 'black Tartary buckwheat' and 'brown Tartary buckwheat'. The first two principal components explained 86.4% of the total variance in the data. The loadings expressed how well the principal components correlated with the old variables. The first principal component explained 78.1% of variance. The second principal component explained 8.3% of variance. The similarities and correlations could be defined between two compounds by their loading values. The compounds with high loadings have a greater influence on the data structure. Buckwheat flour and bran were well separated on PC1, and the loading plot of PC1 indicated that bran had higher amounts of amino acids, fatty acids, and phenolic compounds. However, 'black Tartary buckwheat' cannot be separated from 'brown Tartary buckwheat' on PC1 and PC2, meaning that the functional chemistry does not significantly correspond to the hull colour.

Hierarchical cluster analysis. The amino acid, fatty acid, and phenolic compound concentrations were further analysed by HCA, which is the most widely used unsupervised pattern recognition technique in chemometrics. The Ward's method of clustering was applied with the Squared Euclidean distance in our calculation. As shown in Figure 4, the flour and bran were separated into two clusters very well. Results of HCA indicated that amino acids, fatty acids, and phenolic compounds of buckwheat were mainly regulated by the buckwheat part, not the hull colour of buckwheat. In addition, the HCA results showed very good agreement with the PCA results.

CONCLUSIONS

In this study, we accurately quantified 20 free amino acids, 8 fatty acids, and 6 phenolic compounds in buckwheat flour and bran using UPLC-MS/MS and GC-MS. The data on concentrations were subjected to common chemometric analyses to gain better understanding of the differences between the tested samples. Results indicated that free amino acids, fatty acids, and phenolic compounds were not significantly different between buckwheat samples of the black and brown hull colour; the difference in biochemistry and function between different cultivars of Tartary buckwheat with different hull colour needs further study. Bran possesses higher free amino acids, fatty acids, and phenolic compounds than flour, and may be a healthy foodstuff for development of Tartary buckwheat products. Our results are also helpful for quality control in Tartary buckwheat and its products in the future.

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