Alteration of phenolic profiles and antioxidant capacities of common buckwheat and tartary buckwheat produced in China upon thermal processing

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Abstract

BACKGROUND: Buckwheat products are receiving increasing attention for their high nutritive values and significant health-promoting properties. In this study, a total of 15 buckwheat products grown in different parts of China were investigated. Representative common or tartary buckwheat samples were further subjected to soaking, roasting, microwave cooking, boiling, and steaming treatments. Colorimetric analyses and HPLC analyses were performed to determine the phenolic profiles and antioxidant capacities of the raw and thermally processed buckwheat samples, respectively. RESULTS: Tartary buckwheat exhibited remarkably higher total phenolic content (TPC), total flavonoids content (TFC), DPPH free radical scavenging activity (DPPH), and ferric reducing antioxidant power (FRAP) than that of common buckwheat, but there were no significant differences between their ABTS free radical scavenging capacity. All thermal treatments, particularly microwave cooking contributed to the uppermost losses of phenolics and antioxidant capacities in the common buckwheat sample, while boiling and steaming usually resulted in the lowest losses. For the tartary buckwheat sample, all thermal treatments (except roasting), especially boiling and

steaming led to significant increases in TPC, TFC, DPPH, FRAP, and ABTS. However, HPLC analyses indicated that all thermal treatments, especially microwave cooking gave rise to the uppermost losses of the sum content of 14 phenolic acids and 3 flavonoids, while boiling led to the minimum losses.

CONCLUSION: Both steaming and boiling treatments are recommended when preparing common or tartary buckwheat food products, since they can minimize thermal degradation or promote their phenolic compounds and antioxidant capacities at the most extent.

Keywords: Thermal processing; HPLC; phenolic compounds; antioxidant capacities; common buckwheat; tartary buckwheat.

Abbreviations

ABTS: 2, 2′-azino-di-(3-ethylbenzthiazoline sulfonic acid); BHT: butylated hydroxytoluene; DPPH: 2-diphenyl-1-picryhydrazyl; FRAP: ferric reducing antioxidant capacity; GA: gallic acid; HPLC: high performance liquid chromatography; TFC: total flavonoid content; TPC: ferric reducing antioxidant capacity; TPTZ: 2,4,6-tri (2-pyridyl)-s-triazine; Trolox: hydroxy-2,5,7,8-tetramethlchroman-2-carboxylic acid.

INTRODUCTION

Buckwheat is a gluten-free crop that belongs to the Polygonaceae family, which is traditionally cultivated in Asia, Central and Eastern Europe.[1] According to statistical data from United Nations Food and Agriculture Organization, [2] Russia (1,186,333 tons), China (404,259 tons), and Ukraine (176,430 tons) are the major buckwheat producers in 2016. In China, the common buckwheat *Fagopyrum esculentum* Moench with the light green kernel is widely distributed in the northern part, while the tartary buckwheat *F. tataricum* (L.) Gaertn with the brown kernel is majorly cultivated in the southwestern mountainous regions.[3] Comparing with common buckwheat, tartary

buckwheat has much bitterer taste, mainly due to their higher phenolic and flavonoid compounds, such as rutin, quercetin and quercitrin.[4]

Buckwheat groats are commonly consumed as porridge in Western Asia and Eastern Europe. Buckwheat noodles are also the traditional staple food for people living in Qinghai-Tibetan Plateau for centuries. Nowadays, buckwheat becomes increasingly popular in Korea, Italy, China, United States, and Canada. Novel buckwheat products such as buckwheat tea, bread, cake, cookies, and gluten-free buckwheat beer have been developed due to their high nutritive values and significant health-promoting properties .[5, 6]

As grain crop, both common and tartary buckwheat are remarkable for their rich amino acids, vitamins, minerals, and unsaturated fatty acids contents.[7-10] It is more noteworthy that buckwheat grains also contain certain prophylactic components, such as functional proteins, resistant starch and dietary fiber, which may help to reduce plasma cholesterol level, [11] lose weight, [12, 13] as well as decrease the risk of getting diabetes, [14] stroke, [15] hypertension, [16] and coronary heart disease. [17] Not only that, buckwheat is also rich in various phytochemicals, such as flavonoids, phenolic acids, condensed tannins, and phytosterols, which contribute to its noticeable antioxidative, antihemorrhagic, and blood vessel protecting properties.[5, 7, 18]

To enhance palatability and nutrient availability, buckwheat seeds need to be cooked before consumption. Appropriate cooking methods, such as roasting, microwave cooking, boiling and steaming have been proven to be effective in inactivating the trypsin inhibitors and improving the vitro protein digestibility in buckwheat.[19] Recent years, many studies have been performed to investigate the phenolic profiles and antioxidant capacities of buckwheat.[20-22] However, almost all the previous studies focus on the buckwheat flour, rather than intact buckwheat seeds. Not only that, studies exploring the effect of boiling treatment on the phenolic profiles and antioxidant capacities were scarcely reported, although buckwheat was mainly consumed in the form of intact seeds when preparing buckwheat porridge (kasha).

Therefore, the objective of this study is to investigate the effects of roasting, microwaving, boiling, and steaming on phenolic profiles (total phenolic content and the total flavonoids content) and antioxidant capacities (DPPH, FRAP, and ABTS) in both common and tartary buckwheat seeds by colorimetric analyses. Corresponding high performance liquid chromatography (HPLC) analyses were also performed to explore the fate of phenolic acids and flavonoids before and after thermal processing. The results of this study may help to optimize the processing temperature and time, so

as to maximize the health benefits of various buckwheat-based products during home cooking or industrial manufacturing.

MATERIALS AND METHODS

Chemicals and reagents

In this study, absolute ethanol, acetone, hydrochloric acid, glacial acetic acid, ferric chloride hexahydrate, ferrous sulfate heptahydrate, potassium persulfate ($K_2S_2O_8$), and sodium acetate were delivered by Tianjin Damao Chemical Reagent Co., Ltd (Tianjin, China). 2,2′-Azino-di-(3-ethylbenzthiazoline sulfonic acid) (ABTS), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), 2-diphenyl-1-picryhydrazyl (DPPH), butylated hydroxytoluene (BHT), Folin–Ciocalteu reagent, quercetin, quercitrin, rutin, and vanillin were supplied by Shanghai Yuanye Biological Technology Co., Ltd (Shanghai, China). Aluminum chloride hexahydrate was dispatched from Aladdin Industrial Corporation (Shanghai, China). (+)-Catechin and trifluoroacetic acid were obtained from Sigma-Aldrich Co., Ltd (Shanghai, China). Gallic acid (GA) was got from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 6-Hydroxy-2,5,7,8-tetramethlchroman-2-carboxylic acid (Trolox) was acquired from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). HPLC-grade methanol and acetonitrile were delivered by Merck

KGaA Co., Ltd (Darmstadt, Germany). All chemicals were of analytic grade unless specially mentioned.

Buckwheat samples

A total of 15 kinds of dry buckwheat products were collected from different provinces in China. Among all buckwheat samples, ten kinds of products belonged to common buckwheat species *F. esculentum* Moench (samples 1-10), the rest samples came from tartary buckwheat species *F. tataricum* (L.) Gaertn (samples 11-15). All buckwheat products were harvested in 2016 crop year. The commercial product name, species, as well as their producing province were listed in **Table 1**.

To investigate the effects of thermal processing on phenolic profiles and antioxidant capacities of both common and tartary buckwheat samples, one representative common buckwheat product from Inner Mongolia (Lehuoshi), and one representative tartary buckwheat product from Sichuan Province (Huantai) were further soaked, thermally processed and then determined by colorimetric methods and HPLC as described below.

Soaking and determination of hydration rate

Determination of the hydration rate of raw buckwheat seeds was performed according to the method described by Tang *et al*.[23] Briefly, 50 g of the buckwheat seeds were rinsed and then soaked in 350 mL of tap water for 24 hours at room temperature. Within the first 3 hours, the weight of the soaked buckwheat samples was measured every half hour. Between the 3rd to the 6th hour, the weights of samples were determined for each hour. The final measurement was performed at the 24th hour. At each time of measurement, the soaked buckwheat seeds were filtered and gently wiped by tissue paper to remove excess water. The net hydration rate was obtained as the ratio of net weight gain and the original weight of dry buckwheat. A desired soaking time or hydration rate was then selected and applied in the buckwheat samples ready for thermal treatments.

Roasting and determination of roasting time

Roasting of buckwheat seeds was conducted by a domestic roasting oven (Galanz, KWS1528LQ-F2 (SS), 1500 W, 38 L, Zhongshan, China). Before roasting, the oven was preheated for 10 min to reach 120°C inside. Afterward, pre-soaked buckwheat (100 g in dry weight) was evenly roasted at 120°C for 20 min for common buckwheat (Lehuoshi) and 30 min for tartary buckwheat (Huantai), respectively. The roasting time was designated according to the preliminary experiments, when over 90% of the

buckwheat seeds were found to be crispy and edible. Finally, the roasted buckwheat was ground into powder and ready for extraction by 70% acidic acetone solutions (acetone/water/acetic acid, 70:29.5:0.5, v/v/v).

Microwaving and determination of processing time

Microwaving was conducted using a domestic microwave oven. Pre-soaked buckwheat (100 g in dry weight) was evenly radiated under 100 W for 7 min 56 s for common buckwheat and 12 min 56 s for tartary buckwheat, respectively. The microwaving time was designated according to the preliminary experiments, when over 90% of the buckwheat seeds were found to be crispy and edible. Finally, the microwave radiated buckwheat was ground into powder, and ready for extraction by 70% acidic acetone solutions.

Boiling and determination of boiling time

Boiling of buckwheat seeds was carried out in a domestic cooker. Pre-soaked buckwheat (100 g in dry weight) was immersed into 400 mL of boiling water for 10 min. The boiling time and the volume of boiling water were designated according to the preliminary experiments, when over 90% of the buckwheat seeds were found to be

edible and no water was left after cooling. Finally, the boiled buckwheat was freeze-dried, ground into powder, and ready for extraction by 70% acidic acetone solutions.

Steaming and determination of steaming time

Steaming of buckwheat seeds was performed by a domestic steamer. Within the steamer, the pre-soaked buckwheat (100 g in dry weight) was steamed over 400 mL of boiling water for 10 min. After steaming, the buckwheat was cooled to room temperature for 1 h. The steaming time was designated according to the preliminary experiments, when over 90% of the buckwheat seeds were found to be edible. Finally, the steamed buckwheat was freeze-dried, ground into powder, and ready for extraction by 70% acidic acetone solutions.

Colorimetric analyses of phenolic profiles and antioxidant capacities

Extraction of phenolics from buckwheat samples

According to Tahidul *et al*.,[24] samples ready for colorimetric determination were prepared as below. Precisely, 0.5 gram of dry ground buckwheat sample powders was accurately weighed in a set of centrifuge tubes. The centrifuge tubes were then added with 5 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v) extraction solvent, and shaken on an orbital shaker (250 rpm) for 3 h. Later, the mixtures were extracted for

another 12 h by standing in the dark environment. After extraction, the mixtures were centrifuged (high-speed refrigerated centrifuge, H1850R, Changsha, China) at 955 *g* for 10 min, the supernates were then collected and transferred into new centrifuge tubes. The residues in the centrifuge tubes were then extracted again by adding another 5 mL extraction solvent. Finally, the two supernates were combined and stored at 4 °C in the dark ready for analyses.

Determination of total phenolic content (TPC)

TPC was determined using a Folin-Ciocalteu assay according to the method described by Tang *et al*.[25] Absorbance was measured at 765 nm using a visible spectrophotometer (722s, Shanghai Jingmi Kexue Co., Ltd., China). In accordance with the calibration curve of gallic acid, whose linearity range varied between 50-750 μg mL^{-1} (R^2 = 0.9992), the TPC of each buckwheat sample (expressed as mg of gallic acid equivalent (GAE)/g sample) was recorded.

Determination of total flavonoid content (TFC)

According to Xu *et al*., [26] TFC was measured by using a colorimetric method. The absorbance at 510 nm was determined using the visible spectrophotometer. According to the calibration curve of (+)-Catechin, whose linearity range varied between 25-300 μg

mL^{-1} (R^2 = 0.9997), the total flavonoid content of each buckwheat sample (expressed as mg of catechin equivalent (CAE)/g sample) was worked out.

Determination of DPPH free radical scavenging capacity (DPPH)

In accordance with the method described by Luo *et al*., [27] the DPPH free radical scavenging capacity of buckwheat extract was determined using a colorimetric method. The DPPH discoloration rate was calculated as %Discoloration = ($A_{Control}$-A_{Sample})/$A_{Control}$*100, where A refers to absorbance. Based on the calibration curve of Trolox, whose linearity range varied between 20-500 μmol L^{-1} (R^2 = 0.999), the DPPH free radical scavenging capacity of each buckwheat sample (expressed as μmol of Trolox equivalent (TE)/g sample) was calculated.

Determination of ferric reducing antioxidant capacity (FRAP)

The FRAP assay was conducted based on the description of Tang *et al*.[23] The absorbance of the sample or blank was measured using the visible spectrophotometer at 593 nm. In accordance with the calibration curve of Fe^{2+}, whose linearity range varied between 200-1000 μmol L^{-1} (R^2 = 0.9995), the FRAP of each buckwheat sample (expressed as mmol of Fe^{2+} equivalent (Fe^{2+} E)/100g sample) was calculated.

Determination of ABTS radical scavenging activity (ABTS)

The ABTS assay was carried out according to Tahidul *et al*.[24] The absorbance was measured using the visible spectrophotometer at 734 nm. According to the calibration curve of Trolox, whose linearity range varied between 200-800 μmol L^{-1} (R^2 = 0.9995), the ABTS free radical scavenging capacity of each buckwheat sample (expressed as μmol of Trolox equivalent/g sample) was calculated.

HPLC analysis of phenolic acids and flavonoids in buckwheat samples

Extraction of phenolic acids in buckwheat samples

The extraction of phenolic acids was performed according to Xu and Chang,[28] 0.5 gram of ground buckwheat samples were extracted by 5 mL of extraction solvent (methanol/water/acidic acid/BHT=85:15:0.5:0.2, v/v/v/w) for twice on an orbital shaker at 250 rpm for 3 h. After centrifuged at 3821 *g* for 20 min, the supernatant was filtered and evaporated to dryness on a porcelain evaporating dish in a vacuum oven at 35°C overnight. The residues in the evaporating dishes were then dissolved in 2.5 mL of 25% methanol. An aliquot of the sample solution was filtered through a 0.2 μm PTFE syringe filter prior to HPLC assay.

Extraction of flavonoids in buckwheat samples

The extraction of flavonoids was manipulated according to Xu and Chang.[28] Specifically, 0.5 g sample was extracted with 10 mL of extraction solvent (70% acetone/

29.5% water/ 0.5% acetic acid, v/v/v) on an orbital shaker at 250 rpm for 3 h. Later, the slurry was centrifuged at 3210 *g* for 20 min. A total of 5 mL of the supernatant was filtered and evaporated to dryness on a porcelain evaporating dish in a vacuum oven at 35 °C overnight. After that, the residues in the evaporating dishes were dissolved in 2 mL of 80% methanol, the sample solution was then filtered through a 0.2 μm PTFE syringe filter before HPLC assay.

HPLC analysis of phenolic acids and flavonoids in buckwheat samples

The concentrations of 14 phenolic acids and 3 flavonoids were quantified by HPLC in according with the settings listed in **Supplemental Tables 1** and **2**. The maximum detection wavelengths, regressive equations and correlation coefficients for phenolic acids and flavonoids standards were listed in **Supplemental Table 3**.

Statistical analysis

All samples and standard solutions were prepared in triplicates. The results were reported as mean ± standard deviation. One-way ANOVA (In the case of homogeneity of variances) and 2 Independent Sample Nonparametric Test (In the case of heterogeneity of variances) was implemented by SPSS 17.0 to determine significant differences between each treatment. Correlation analyses among phenolic compounds and

antioxidant capacities were done via Pearson Correlation Tests. Statistical significance is defined as $p < 0.05$.

RESULTS

Soaking and hydration rate

As it was presented in **Fig. 1**, the water absorption rate of the common buckwheat sample increased rapidly at the first half hour, and then increased moderately to the saturation point at the 2nd hour. As far as the tartary buckwheat sample was concerned, it exhibited a rapid water absorption rate at the first hour, and finally entered the equilibrium phase at the 5th hour. According to one-way ANOVA, there were no significant differences in their net water absorption rates at the first half hour ($p = 0.352$). But at the saturation point, the net water absorption rate of the tartary buckwheat sample (approximately 125%) was significantly ($p < 0.0001$) higher than that of the common buckwheat sample (around 60%). Such remarkable differences may be explained by their distinct osmolarities that generated by their diverse macro- (proteins, starches, and fibers) and micro- (vitamins and minerals) nutrient contents.[29-30]

Apart from that, it was noticeable that the color of the soaking water for the common buckwheat sample was pink, but for the tartary buckwheat sample, it was yellow-green. This indicated that some water-soluble components including phenolic

compounds and flavonoids had dissolved into the soaking water.[31] In order to decrease or minimize the potential losses of aforementioned antioxidant components during soaking, and meanwhile, to save time and energy for cooking, common buckwheat sample and tartary buckwheat sample was designated to be soaked for one hour and two hours before cooking, respectively.

Phenolic profiles and antioxidant capacities in buckwheat samples

Phenolic profiles of 15 raw buckwheat samples

The phenolic compounds of 15 raw buckwheat samples, including the total phenolic content (TPC, expressed in mg gallic acid equivalent (GAE)/g), as well as the total flavonoid content (TFC, expressed in mg catechin equivalent (CAE)/g) were exhibited in **Table 2**.

Briefly, there were no intraspecific differences among the TPC or TFC in both common or tartary buckwheat samples. However, the average value of TPC and TFC of the common buckwheat samples (TPC: 5.32±0.37 mg GAE/g; TFC: 1.22±0.23 mg CAE/g) were significantly ($p < 0.0001$) lower than the tartary buckwheat samples (TPC: 6.67±0.57 mg GAE/g; TFC: 3.71±0.39 mg CAE/g), even if these buckwheat samples were collected from different producing provinces with variable growth environments.

Antioxidant capacities of 15 raw buckwheat samples

The antioxidant capacities of 15 raw buckwheat samples including DPPH free radical scavenging activity (expressed in μmol Trolox equivalent (TE)/g), FRAP (expressed in mmol Ferrous equivalent (Fe^{2+}E)/100g) and ABTS radical scavenging activity (expressed in μmol TE/g) were summarized in **Table 2**.

To be specific, the common buckwheat samples showed remarkably lower average DPPH (common buckwheat: 12.59±1.18 μmol TE/g; tartary buckwheat: 16.92±0.30 μmol TE/g) and FRAP (common buckwheat: 5.51±0.54 mmol Fe^{2+}E/100g; tartary buckwheat: 6.40±0.71 mmol Fe^{2+}E/100g) value than the tartary buckwheat samples ($p < 0.0001$). However, as far as ABTS was concerned, in accordance with 2 Independent Sample Nonparametric Test, there were no significant differences between the 10 common (42.78±4.39 μmol TE/g) and 5 tartary buckwheat samples (39.48±6.50 μmol TE/g) ($p = 0.067$).

Effect of thermal processing on phenolics and antioxidant capacities of the common buckwheat sample

As displayed in **Fig. 2** and **Fig. 3**, all the thermal treatments, especially microwaving contributed to the uppermost losses of TPC (-50.00%), TFC (-44.06%), DPPH (-37.1%), FRAP (-26.4%), and ABTS values (-47.0%) than that of raw common buck-

wheat samples ($p < 0.05$), followed by roasting, boiling. While steaming treatments usually gave rise to the minimal losses of TPC (-30%), TFC (-23.8%), DPPH (-12.1%), FRAP (-5.3%), and ABTS (-15.2%) values. As a result, thermal degradation of phenolic compounds and antioxidant capacities in common buckwheat samples was ranked as the following descending order: Raw > Steaming ≥ Boiling ≥ Roasting > Microwave radiation. For those people who take common buckwheat as the staple food, steaming or boiling treatment is therefore recommended.

Effect of thermal processing on phenolics and antioxidant capacities of the tartary buckwheat sample

As displayed in **Fig. 2** and **Fig. 3**, roasting treatment resulted in a dramatic reduction of TPC (12.1%), TFC (15.5%), DPPH (1.78%), FRAP (9.54%), and ABTS values (23.6%) than that of raw tartary buckwheat samples. However, all other thermal treatments, especially boiling treatment gave rise to a significant ($p < 0.05$) increase of TPC (55.1%), TFC (54.6%), DPPH (4.28%), FRAP (78.2%), and ABTS (20.5%), as compared to the raw tartary buckwheat sample. Overall, thermal promotion of phenolic compounds and antioxidant capacities in tartary buckwheat samples was ranked as the following ascending order: Roasting ≤ Raw < Microwave radiation ≤ Steaming

≤ Boiling. As a result, steaming or boiling treatment was recommended when preparing tartary buckwheat food products.

Phenolic acids and flavonoids contents in buckwheat quantified by HPLC

Phenolic acids and flavonoids contents in raw buckwheat samples

Tables 3 and **4** summarized the concentrations of 14 phenolic acids and 3 flavonoids in both the raw common (Lehuoshi) and tartary (Huantai) buckwheat samples, respectively. The HPLC chromatogram of 14 phenolic acids is displayed in **supplemental Fig. 1**.

Among these phenolic acids, 12 kinds of phenolic acids including gallic acid, protocatechuic acid, 2,3,4-trihydroxybenzoic acid, protocatechualdehyde, *p*-hydroxybenzoic acid, gentisic acid, chlorogenic acid, vanillic aicd+caffeic acid, syringic acid, *p*-coumaric acid, and syringaldehyde were found in both the common and tartary buckwheat samples. It was worth mentioning that ferulic acid and sinapic acid were only detected in the common buckwheat samples, while salicylic acid was merely discovered in the tartary buckwheat samples. In the case of the total concentration of 14 phenolic acids, the tartary buckwheat samples (615.33±24.65 mg kg^{-1}) exhibited 5.5 times higher than that of the common buckwheat samples (111.98±3.49 mg kg^{-1}) ($p < 0.05$). Protocatechualdehyde (25.44±0.40 mg kg^{-1}, 22.7%) and vanillic aicd+caffeic acid (31.44±0.72 mg kg^{-1}, 28.1%) in the common buckwheat sample, and

salicylic acid (554.57±19.57 mg kg^{-1}, 90.1%) in the tartary buckwheat sample were the predominant phenolic acid, respectively.

As far as flavonoids were concerned, the tartary buckwheat samples presented 122.58, 2.81 and 4.77 times higher of rutin, quercitrin and quercetin concentrations than that of the common buckwheat samples, respectively. Among three flavonoids, rutin is the predominant flavonoid compound in both the common and the tartary samples, which account for 63.75% (62.19±1.33 mg kg^{-1}) and 98.0% (7,623.26±1,110.19 mg kg^{-1}) of the sum three flavonoid concentrations, respectively.

Effect of thermal processing on 14 phenolic acids in buckwheat samples

As shown in **Table 3**, all thermal treatments, especially microwaving (-51.9%) led to the uppermost losses of the total phenolic acid concentration in the common buckwheat sample, followed by roasting (-33.3%), steaming (-23.5%), and boiling (-10.5%). However, some noticeable increases in certain phenolic acids were observed after thermal processing. To be precise, all thermal treatments, particularly roasting resulted in 3.56 times higher concentration of syringic acid than the raw common buckwheat sample. Boiling also gave rise to 0.21 and 0.88 times higher content of *p*-hydroxybenzoic acid and gentisic acid, respectively. Apart from that, steaming also contributed to 0.56 and 0.44 times higher content of vanillic aicd+caffeic acid and *p*-coumaric acid+syringaldehyde, respectively.

While in the tartary buckwheat sample, microwaving (-58.0%) also gave rise to the biggest losses of the total phenolic acid content, followed by roasting (-36.2%), steaming (-32.6%), and boiling (-14.5%). After thermal treatments, the concentrations of gallic acid, syringic acid, and salicylic acid were remarkably deducted. However, the contents of protocatechuic acid (+35.1%) and vanillic acid+caffeic acid (+70.9%) were significantly increased after boiling treatment. Noticeable increases in 2,3,4-trihydroxybenzoic acid, protocatechualdehyde, *p*-hydroxybenzoic acid, gentisic acid, chlorogenic acid, and *p*-coumaric acid+syringaldehyde were also noticed after both boiling and steaming treatment.

Effect of thermal processing on three flavonoids in buckwheat samples

As shown in **Table 4**, in the common buckwheat samples, microwaving contributed to the uppermost losses of rutin (-32.5%) and quercitrin (-85.7%) contents, while boiling gave rise to the biggest losses of quercetin content (-15.5%). Interestingly, microwaving and steaming treatment increased the quercetin contents at the level of 11.4% and 5.45%, respectively. However, overall, all thermal treatments resulted in significant reductions in the sum flavonoid contents (rutin, quercitrin, and quercetin), the reductions were ranked as the following descending order: Raw < Steaming (-14.30%) < boiling (-17.7%) < Microwave radiation (-24.9%) < Roasting (-27.9%).

While in the tartary buckwheat sample, only microwaving resulted in a dramatic increase in the quercitrin content (+415.53%). In total, all thermal treatments resulted in remarkable decreases in the sum flavonoid contents, the losses were ranked as the following descending order: Raw < Boiling (-8.56%) < Steaming (-19.5%) < Roasting (-31.1%) < Microwave radiation (-42.4%).

DISCUSSION

Phenolic profiles and antioxidant capacities of 15 raw buckwheat samples

In accordance with the colorimetric analyses, tartary buckwheat samples exhibited remarkably higher TPC and TFC than that of common buckwheat samples; these results were further confirmed by HPLC analyses, in which the total concentration of 14 phenolic acids and 3 flavonoids were significantly higher than that of common buckwheat. Such results were also in good consistency with previous studies.[32-33]

In the case of antioxidant capacities, the reason why tartary buckwheat exhibited remarkably stronger DPPH and FRAP values could be explained by their rich rutin concentration (122.6 times higher than common buckwheat), since there was a significant correlation between the rutin content and antioxidant capacities identified in tartary buckwheat.[32] The research performed by Morishita *et al*. and Zielinski *et al*. also pointed that tartary buckwheat grains displayed over 100-fold of rutin content, as well

as 3-4 times higher antioxidant capacities than that of common buckwheat. [4, 34] These data suggested the differences in antioxidants capacities between tartary and common buckwheat might be contributed by their different rutin contents. Not only that, common buckwheat groats also contain some other phytochemicals such as vitexin, isovitexin, catechin, and epicatechin gallate,[35] which exert higher antioxidant capacity than rutin under the same concentration.[36] The existence of such specific phytochemicals may help to explain why the average ABTS radical scavenging activity of common buckwheat was comparable with tartary buckwheat. [28]

Effect of thermal processing on phenolic profiles and antioxidant capacities in the common buckwheat sample

In terms of the thermal effects on phenolic profiles and antioxidant capacities in the common buckwheat sample, some noticeable increases in certain phenolic acids were observed in this study. For instance, the content of gallic acid was incredibly increased (+239.1%) after the roasting treatment, which was in good consistency with Beitāne *et al*., who reported that roasting treatment gave rise to a 36% higher gallic acid content than the raw buckwheat flour.[37] Apart from that, all four thermal treatments, particularly the roasting treatment led to 3.56 times higher syringic acid content in the common buckwheat seeds. These data were in good agreement with Hen-

ryk *et al*, who reported that extrusion at 120, 160 and 200°C could give rise to as much as 5 times higher syringic acid contents in common buckwheat groats.[38]

However, according to HPLC analyses, the sum concentrations of 14 phenolic acids (particularly protocatechuic acid, 2,3,4-trihydroxybenzoic acid, protocatechualdehyde, chlorogenic acid, ferulic acid, and sinapic acid), and the sum concentrations of three flavonoids (especially rutin and quercitrin) were all significantly ($p < 0.05$) reduced after any of the thermal treatment. Declines of these phytochemicals thereby resulted in significantly ($p < 0.05$) lower phenolic profiles (TPC and TFC) and radical scavenging capacities (DPPH, FRAP or ABTS) in the common buckwheat samples. These data were in good consistency with previous studies, in which thermal treatments, like roasting, [4, 39, 40] microwaving,[19] boiling,[19, 41] and steaming [4, 40] contributed to significant decomposition of TPC, TFC, DPPH, FRAP, and ABTS in the common buckwheat groats.

Effect of thermal processing on phenolic profiles and antioxidant capacities in the tartary buckwheat sample

While in the tartary buckwheat samples, the colorimetric analyses indicated that the roasting treatment (120°C for 30 min) led to significant reductions in TPC, TFC, DPPH, FRAP, and ABTS free radical scavenging activities, and these findings were further confirmed by the HPLC analyses. Similar research performed by Zhang *et al*.

also reported that roasting at 80°C for 20 or 40 min contributed to significant decreases of TPC and TFC in tartary buckwheat flour, respectively.[20]

In respect to other thermal treatments, the colorimetric analyses indicated that microwaving (100 W for 16 min 55 s), boiling (10 min), and steaming (normal pressure, 10 min) gave rise to significantly ($p < 0.05$) higher TPC, TFC, DPPH (except steaming treatment, $p = 0.207$), FRAP, and ABTS free radical scavenging activities. However, these data were not supported by the corresponding HPLC analyses, in which microwaving contributed to the greatest reductions in the sum concentrations of 14 phenolic acids and 3 flavonoids, followed by boiling and steaming treatments ($p < 0.05$). Although microwaving led to a dramatic increase (+415.5%) in the quercitrin concentration, while steaming contributed to some noticeable increases in 2,3,4-trihydroxybenzoic acid, protocatechualdehyde, *p*-hydroxybenzoic acid, gentisic acid, chlorogenic acid, and *p*-coumaric acid+syringaldehyde content. Except that, the obtained results by the colorimetric analyses were either not consistent with previous research. In an earlier study, Zhang *et al*. reported that both microwaving (700 W for 10 min) and pressure steaming (0.1 MPa and 0.2 MPa for 20 min and 40 min) resulted in significantly ($p < 0.05$) lower TPC, TFC, DPPH, FRAP, and ABTS values in tartary buckwheat flour.[20] Xu and Chang also noticed that regular boiling (90, 120 and 150

min) led to remarkable reductions in TPC and DPPH values in green pea, yellow pea, and chickpea seeds ($p < 0.05$).[42]

Comparison of the colorimetric analyses, HPLC analyses and previous studies on tartary buckwheat

The reason why the colorimetric analyses were contradicted with HPLC analyses and previous studies is discussed below. The first and foremost, HPLC analyses only determined 14 kinds of phenolic acids and three kinds of flavonoids in this study, rather than total phenolic content (TPC) and total flavonoid content (TFC) by colorimetric analyses. As a result, content fluctuations among some undetermined phytochemicals, for instance, flavan-3-ols [e.g., (+)-catechin, (+)-epicatechin, epicatechin-gallate], flavonols or flavones (e.g., myricetin, luteolin, apigenin, kaempferol, kaempferol-3-glucoside), condensed tannin content (CTC), and monomeric anthocyanin content (MAC, such as 3-O-β-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin) may gave rise to significantly different results between the colorimetric and HPLC analyses.[28]

In the present research, the raw tartary buckwheat whole grain samples were soaked for 2 h prior to any thermal treatments. While in the previous studies, the phenolic profiles and antioxidant capacities were determined in the buckwheat flour without soaking treatment. According to Xu and Chang, [42] TPC, DPPH, oxygen radi-

cal absorbing capacity (ORAC) in the soaked green pea, yellow pea, and chick pea were all significantly increased as the hydration rate increased from 70% to 85%. This phenomenon may be attributed by the enhanced phenylalanine ammonialyase (PAL) activities in the buckwheat seeds, which can promote the catabolism of *L*-phenylalanine into phenolic acids (e.g., cinnamic acid, *p*-coumaric, ferulic, and caffeic acid), flavonoids and tannins, thereby resulted in significant improvements of antioxidant capacities.[43]

Apart from that, the intact structure of buckwheat seeds and the water absorbed from soaking treatment can protect the phenolic profiles from thermal degradation through increased penetration time and water evaporation. But these features cannot prevent buckwheat seeds from thermal degradation during microwave radiation, since microwaves can penetrate the seeds, excite polar molecules (e.g., water and fat) to rotate, thereby generate evenly thermal energy throughout the buckwheat seeds, rather than heat the buckwheat seeds through thermal conduction. [19] That is the reason why microwave radiation contributed to the uppermost losses of the sum concentration of 14 phenolic acids and 3 flavonoids in the tartary buckwheat sample.

Not only that, the low-power/temperature/pressure-short-time thermal treatments in this study also played a predominant role in protecting the potent antioxidants from heat decomposition. Since similar research [42, 44] on the green pea, yellow pea, chick-

pea, and black bean seeds reported that pressurized steaming treatment (5 psi, 70 min) attributed to significantly low TPC and DPPH than that of regular steaming treatment (70 min). Both pressurized boiling (5 psi for 30 min, or 15 psi for 15 min) and pressurized steaming (5 psi or 15 psi for 15 min) also gave rise to significant reduction in TPC and DPPH than that of regular boiling (30, 45 or 60 min) or steaming (15 min) treatments in lentil seeds, respectively.[42] While in our study, all the thermal treatments, especially boiling and steaming were performed under normal pressure condition. As a result, the thermal decomposition of phenolic profiles and antioxidant capacities in the current study may not be as high as previous studies.

It is well known that different extraction methods could give rise to significantly different concentrations of phenolic compounds and antioxidant activities. In an earlier study, [45] Xu and Chang reported that acidic 70% acetone solution gave rise to significantly higher TPC, TFC, CTC in lentil, black soybean, red kidney bean, and black beans, as compared to 70% methanol solution. While in the current research, samples for the colorimetric and HPLC analyses were extracted by acidic 70% acetone solution (acetone/water/acetic acid, 70:29.5:0.5, v/v/v) and acidic 85% methanol solution (methanol/water/acidic acid/BHT=85:15:0.5:0.2, v/v/v/w), respectively. The differences of the extraction solvents may help to explain the contrasting results between the colorimetric and HPLC analyses. One the key reasons why we used different ex-

traction methods (e.g., extraction solvents, centrifuge settings, and re-dissolving solvents) was for easier comparison with previous studies.[23, 24, 28, 31, 42, 44, 45] Since each of these extraction methods was used as the standard method for the colorimetric analyses (TPC, TFC, DPPH, FRAP, and ABTS) or the HPLC analyses (phenolic acids, and flavonoids) in food crops. Using the same extraction methods may underestimate or even fail to measure the actual levels of interested indexes. For instance, it may not be practical to apply the extraction method of the HPLC analyses on the colorimetric analyses, since its final solution volume (2.5 mL for phenolic acids analyses, and 2.0 mL for flavonoids analyses) will not be enough for the analyses of TPC, TFC, DPPH, FRAP, and ABTS (each assay require at least 1 mL sample solution).

The results obtained in this study also showed that heating treatments could promote the contents of some phenolic acids, which were speculated due to the transition of some phytochemicals into other phenolic acids or flavonoid compounds.[28, 31] In yellow beans, regular boiling (120 min) and steaming (100 min) gave rise to significant lower TPC, but remarkably higher TFC, condensed tannin content (CTC). Not only that, pressurized steaming (15 psi, 60 min) also attributed to significantly higher TPC (including free benzoic acids like gallic acid, protocatechuic acid, 2,3,4-trihydroxybenzoic acid, protocatechualdehyde, and vanillic acid), TFC (including total isoflavone content), CTC, DPPH, FRAP, and ORAC in yellow soybeans.[31] These

data suggested that thermal treatments may break down the conjugated phenolic acids into free phenolic acids, thus increased the contents of some of the phenolic acids like gentisic acid, which can be further transformed into flavonoid compounds such as quercitrin and quercetin.[28]

Last but most important, it is well known that food processing such as cooking can give rise to significant losses in many antioxidants. However, the overall antioxidant properties of food can be maintained and even enhanced by other chemical changes.[46] For instance, Millard reaction, which occurs when sugar condenses with free amino acids, peptides or proteins, and then resulted in the formation of a wide variety of antioxidant compounds (e.g., melanoidin), which cannot be detected during TPC (Folin-ciocalteu method) or TFC determination. That's one of the key reasons why HPLC analyses noticed remarkable reductions in the sum contents of 3 flavonoids and 14 phenolic profiles, but the antioxidant capacities (DPPH, FRAP, ABTS) of the tartary buckwheat sample were significantly promoted after thermal treatments (except roasting). As a result, differences in the buckwheat cultivar, growth environment, composition and concentration of the amino acids and reducing sugar might contribute to totally different antioxidant capacities after thermal treatments.[46]

Considering the fluctuated content of some undetermined phytochemicals, the promoting effect of soaking treatment, the protection effect of absorbed water and the

intact structure of buckwheat seeds (as compared to buckwheat flour in previous studies), the mild (low working power/temperature/pressure-short time) thermal treatments applied in this study, the different extraction solvent applied in the colorimetric analyses or HPLC analyses, the enhanced effect of thermal treatments on phenolic profiles, as well as the antioxidant capacities induced by Millard reaction products, the thermal degradation of phenolic profiles and antioxidant capacities might be counteracted and even overwhelmed by the newly generated antioxidants (including phenolic profiles), that's why the obtained results were contradicted with HPLC analyses and previous studies.

Correlation between phenolic compounds and antioxidant capacities

In the case of the colorimetric analyses, correlations analyses between phenolic compounds and antioxidant capacities were conducted. In both common and tartary buckwheat samples, significant ($p < 0.001$) correlations were found among phenolic compounds and antioxidant capacities. The results proved that all antioxidant assay methods were well-correlated. It was also worth mentioning that the correlation coefficients (r) between TPC, TFC, DPPH, and FRAP were all higher than 0.80, which provided a strong demonstration that TPC and TFC are the major contributors to the DPPH and FRAP in both common and tartary buckwheat species. As for the relatively lower correlation coefficient observed between TPC and ABTS ($r = 0.66$, $p < 0.0001$),

or TFC and ABTS ($r = 0.44$, $p < 0.0001$), it could be explained by some undetermined phytochemicals, for example, condensed tannins content (CTC) and monomeric anthocyanin content (MAC), which might give rise to predominant ABTS scavenging capacity, [28] thereby resulted in a relatively lower correlation coefficient between TPC or TFC with ABTS.

CONCLUSIONS

In summary, tartary buckwheat exhibited remarkably higher TPC, TFC, DPPH, and FRAP than that of common buckwheat. The colorimetric analyses and HPLC analyses indicated that all thermal treatments especially microwaving contributed to the uppermost losses of phenolic profiles and antioxidant capacities in the common buckwheat sample, boiling and steaming usually resulted in the lowest losses. While in the tartary buckwheat sample, the colorimetric analyses noticed that all thermal treatments (except roasting), especially boiling and steaming led to significant increases in TPC, TFC, DPPH, FRAP, and ABTS. But HPLC analyses indicated that all thermal treatments, especially microwaving gave rise to the uppermost losses of the sum concentration of 14 phenolic acids and 3 flavonoids, while boiling led to the minimum losses. Such contradicts might be explained by some undetermined phytochemicals, the difference between extraction solvents, the transition of some phytochemicals into

other phenolic acids or flavonoid compounds, as well as the antioxidants induced by Millard reaction products. To conclude, both steaming and boiling treatments are recommended when preparing common or tartary buckwheat food products, since they can minimize thermal degradation or promote their phenolic compounds and antioxidant capacities at the most extent.

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Conflict of Interest

There is no conflict of interest in this article.

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Figure legends

Figure 1. The net water absorption curves of dry common (Lehuoshi) and tartary (Huantai) buckwheat samples. The net hydration rate of dry beans was considered as 0. Desired hydration ratio of buckwheat samples was calculated by calibration through a quadratic fit equation of net hydration curve.

Figure 2. Effect of thermal processing on total phenolics (**A**, Total phenolic content (TPC, mg gallic acid equivalent/g); and **B**, Total flavonoid content (TFC, mg Catechin Acid Equivalent/g) of common (Lehuoshi) and tartary (Huantai) buckwheat sample. Bar data are expressed as mean ± standard deviation (n = 3) on a dry weight basis. Values marked above the same color bar with the same letter are not significantly different ($p < 0.05$). R, roasting at 120°C for 20 min on common buckwheat and for 35 min on tartary buckwheat; M, microwaving at 100 W for 9 min 40 s on common buckwheat and for 16 min 55 s on tartary buckwheat; B, boiling at 100°C for 10 min on both common and tartary buckwheat; S, steaming at 100°C for 10 min on both common and tartary buckwheat.

Figure 3. Effect of thermal processing on antioxidant capacities (**A**, DPPH free radical scavenging activity (DPPH, μmol Trolox equivalent/g); **B**, ferric reducing antioxidant power (FRAP, mmol of Ferrous equivalent/100g); and **C**, ABTS free radical scavenging activity (ABTS, μmol Trolox equivalent/g)) of common (Lehuoshi) and tartary (Huantai) buckwheat sample. Data are expressed as mean ± standard deviation (n = 3) on a dry weight basis. Values marked above the same color bar with the same letter are not significantly different ($p < 0.05$). R, roasting at 120°C for 20 min on common buckwheat and for 35 min on tartary buckwheat; M, microwaving at 100 W

for 9 min 40 s on common buckwheat and for 16 min 55 s on tartary buckwheat; B, boiling at 100°C for 10 min on both common and tartary buckwheat; S, steaming at 100°C for 10 min on both common and tartary buckwheat.

Table 1. Buckwheat samples collected in China

Sample number	Sample name	Species	Origin
1	Jiulihu	*F. esculentum* Moench	Shanxi
2	Lehuoshi	*F. esculentum* Moench	Inner Mongolia
3	Dingbian	*F. esculentum* Moench	Shannxi
4	Yangguang	*F. esculentum* Moench	Heilongjiang
5	Guxing	*F. esculentum* Moench	Inner Mongolia
6	Sanfulai	*F. esculentum* Moench	Ningxia
7	Siping	*F. esculentum* Moench	Jilin
8	Qiqihaer	*F. esculentum* Moench	Heilongjiang
9	Tiandi	*F. esculentum* Moench	Liaoning
10	Xianxiang	*F. esculentum* Moench	Beijing
11	Jialv	*F. tataricum* (L.) Gaertn	Shanxi
12	Huantai	*F. tataricum* (L.) Gaertn	Sichuan
13	Guguan	*F. tataricum* (L.) Gaertn	Yunnan
14	Qinggao	*F. tataricum* (L.) Gaertn	Shanxi

15	Qining	*F. tataricum* (L.) Gaertn	Guizhou

Table 2. Phenolic profiles and antioxidant capacities of raw buckwheat samples

Sample Number	Samples Name	Phenolic compounds		Antioxidant capacities		
		TPC	TFC	DPPH	FRAP	ABTS
1	Jiulihu	5.52±0.45 ab	1.39±0.11 ab	13.08±0.20 c	5.80±0.44 cde	45.90±0.58 fg
2	Lehuoshi	5.90±0.36 bc	1.43±0.10 ab	14.28±0.70 d	6.06±0.27 de	43.71±1.10 ef
3	Dingbian	5.06±0.43 a	1.17±0.04 a	11.35±0.30 ab	4.94±0.09 a	40.90±0.66 de
4	Yangguang	5.25±0.52 ab	1.65±0.13 bc	12.00±0.45 b	5.11±0.14 ab	40.97±1.00 de
5	Guxing	5.26±0.32 ab	1.39±0.07 ab	11.63±0.10 ab	5.13±0.28 ab	37.66±2.30 bc
6	Sanfulai	5.11±0.16 a	1.26±0.10 a	11.19±0.10 a	5.39±0.16 abc	37.10±2.66 bc

7	Siping	5.06±0.25 a	1.13±0.04 a	11.83±0.10 ab	5.57±0.26 bcd	41.55±3.80 de
8	Qiqihaer	5.28±0.21 ab	1.34±0.03 a	12.80±0.71 c	5.78±0.45 cde	42.07±0.70 e
9	Tiandi	5.34±0.06 ab	1.29±0.06 a	14.25±0.30 d	4.90±0.28 a	50.97±1.03 i
10	Xianxiang	5.38±0.32 ab	1.77±0.12 c	13.52±0.70 c	6.37±0.27 ef	47.01±1.68gh
11	Jialv	6.19±0.32 cd	3.18±0.26 d	16.84±0.20 e	5.86±0.30 cde	34.53±1.80 ab
12	Huantai	6.72±0.34 de	3.81±0.16 fg	16.84±0.20 e	5.87±0.34 cde	43.25±2.31 ef
13	Guguan	6.96±0.36 e	3.64±0.21 ef	17.08±0.20 e	6.73±0.30 f	38.40±0.69 cd
14	Qinggao	7.32±0.57 e	3.98±0.36 g	17.16±0.30 e	7.43±0.60 g	49.12±2.21hi
15	Qining	6.14±0.23 cd	3.43±0.26 de	16.68±0.30 e	6.13±0.40 de	32.10±1.03 a

Data were expressed as mean ± standard deviation (n = 3). The data in the same column marked with different small case letters are significantly ($p < 0.05$) different. Total phenolic content (TPC, mg gallic acid equivalent/g); total flavonoid content (TFC, mg catechin equivalent/g); DPPH free radical scavenging activity (DPPH, μmol Trolox equivalent/g); ferric reducing antioxidant power (FRAP, mmol of Fe^{2+} equivalent/100g); ABTS free radical scavenging activity (ABTS, μmol Trolox equivalent/g).

Table 3. The Phenolic acids content of raw and thermal processed common and tartary buckwheat samples

	Common buckwheat					Tartary buckwheat				
Phenolic acids	Raw common (mg kg^{-1})	Roasted (mg kg^{-1})	Microwaved (mg kg^{-1})	Boiled (mg kg^{-1})	Steamed (mg kg^{-1})	Raw tartary (mg kg^{-1})	Roasted (mg kg^{-1})	Microwaved (mg kg^{-1})	Boiled (mg kg^{-1})	Steamed (mg kg^{-1})
Gallic acid	2.84±0.20 c	9.63±1.08 a	1.93±0.01 c	2.71±0.56 c	4.93±0.50 b	6.52±0.59 a	0.81±0.01 c	0.48±0.07 c	0.66±0.00 c	4.45±0.17 b

Protocatechuic acid	4.04±0.37 a	0.42±0.07 c	ND	1.62±0.06 b	1.32±0.12 b	10.04±1.06 b	8.62±0.57 b	4.65±0.49 c	13.56±0.47 a	9.68±0.84 b
2,3,4- trihydroxybenzoic acid	5.36±0.21 a	ND	ND	ND	ND	6.85±0.05 a	4.60±0.01 b	5.32±0.00 b	7.94±0.02 a	7.79±1.23 a
Protocatechual-dehyde	25.44±0.40 a	4.84±0.39 b	3.28±0.41 c	5.23±0.62 b	5.82±0.56 b	1.96±0.16 a	2.02±0.20 a	1.09±0.02 b	2.65±0.17 a	2.38±0.41 a
P-hydroxybenzoic acid	1.88±0.22 b	0.75±0.02 d	2.01±0.09 b	2.27±0.07 a	1.52±0.02 c	5.69±0.33 b	3.60±0.23 c	2.16±0.19 c	6.10±0.12 a	6.86±1.54 a
Gentisic acid	11.51±0.35 b	ND	2.87±0.32 c	21.64±6.00 a	2.05±0.25 c	1.95±0.12 b	ND	1.97±0.33 b	3.34±0.18 a	2.82±0.79 ab

Chlorogenic acid	11.51±0.33 a	3.22±0.16 e	2.36±0.32 d	3.90±0.04d c	4.76±0.35 b	5.69±0.94 ab	4.39±0.26 bc	3.08±0.01 c	7.16±0.33 a	7.60±1.27 a
Vanillic acid + caffeic acid	31.44±0.72 c	29.87±1.43 c	23.79±1.80 d	43.34±0.27 b	48.90±2.13 a	18.68±1.38 b	19.61±1.68 b	9.87±0.59 c	31.92±0.70 a	16.12±3.04 b
Syringic acid	3.65±0.46 d	16.65±1.97 a	10.26±0.97 c	13.13±0.25 b	8.71±1.12 c	1.90±0.33 a	0.41±0.04 b	0.69±0.10 b	1.39±0.17 a	1.59±0.30 a
P-coumaric acid + Syringaldehyde	1.83±0.17 a	1.78±0.15 a	1.50±0.04 a	2.12±0.01 a	2.64±0.10 a	1.48±0.12 bc	1.76±0.22 bc	1.27±0.02 c	1.87±0.01 b	2.40±0.34 a
Ferulic acid	8.94±0.12 a	5.78±0.50 b	4.46±0.34 c	3.07±0.20 d	3.45±0.45 d	ND	ND	ND	ND	ND
Sinapic acid	3.54±0.29 a	1.77±0.10 b	1.41±0.02 b	1.24±0.05 b	1.52±0.09 b	ND	ND	ND	ND	ND
Salicylic acid	ND	ND	ND	ND	ND	554.57±19.57 a	346.99±35.23 c	227.75±17.61 d	449.61±45.79 b	353.16±29.85 c

Total	111.98±3.49	74.71±5.8	53.87±4.32	100.27±8.13	85.62±5.69	615.33±24.65	392.81±38.45	258.33±19.43	526.20±47.96	414.85±39.78

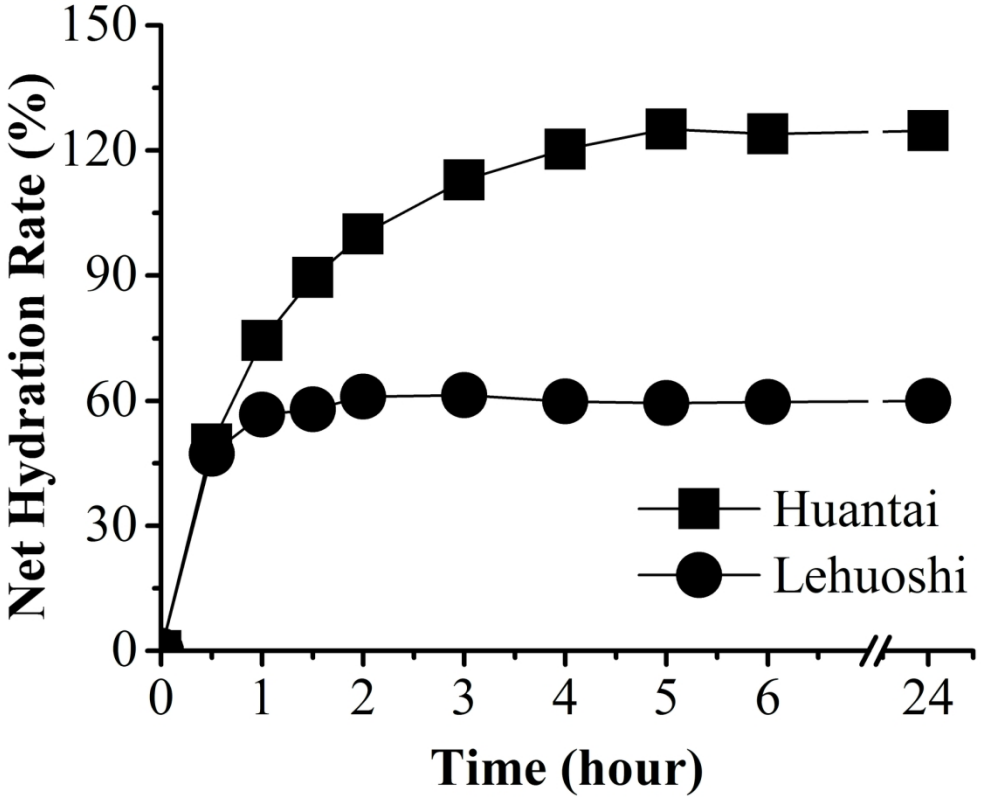
Data were expressed as mean ± standard deviation (n = 3). The data in the same column marked with different small case letters are significantly ($p < 0.05$) different.

ND, not detectable.

Table 4. The flavonoids content of raw and thermal processed common and tartary buckwheat samples

Flavonoids	Common buckwheat					Tartary buckwheat				
	Raw common (mg kg^{-1})	Roasted (mg kg^{-1})	Microwaved (mg kg^{-1})	Boiled (mg kg^{-1})	Steamed (mg kg^{-1})	Raw tartary (mg kg^{-1})	Roasted (mg kg^{-1})	Microwaved (mg kg^{-1})	Boiled (mg kg^{-1})	Steamed (mg kg^{-1})
Rutin	62.19±1.33 a	43.21±4.02 d	41.98±0.74 d	55.79±1.83 b	52.61±1.14 c	7,623.26±1, 110.19 a	5,286.94±27 2.1 b	4,271.86±62 3.5 b	6,975.48±57 0.05 a	6,191.02±57 6.97 a
Quercitrin	8.41±0.29 [a]	2.04±0.05 c	1.20±0.04 d	1.74±0.15 c	2.57±0.17 b	23.63±1.87 b	21.55±2.86 b	121.82±16.6 1 a	18.67±2.60 b	11.73±0.20 b
Quercetin	26.95±1.14 b	25.02±0.97 c	30.01±0.28 a	22.78±0.20 d	28.42±0.99 ab	128.56±23.2 3 a	49.86±8.24 b	83.22±10.90 b	115.50±0.65 a	53.92±5.93 b

Data were expressed as mean ± standard deviation (n = 3). The data in the same column marked with different small case letters are significantly ($p < 0.05$) different.



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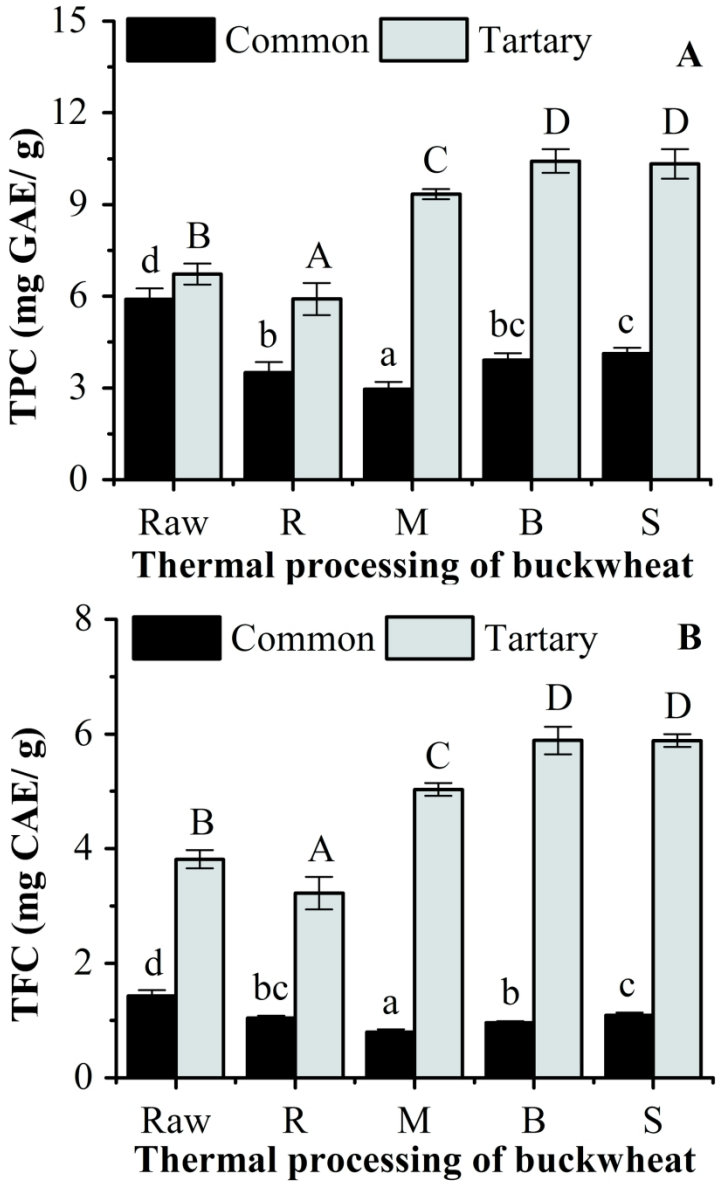

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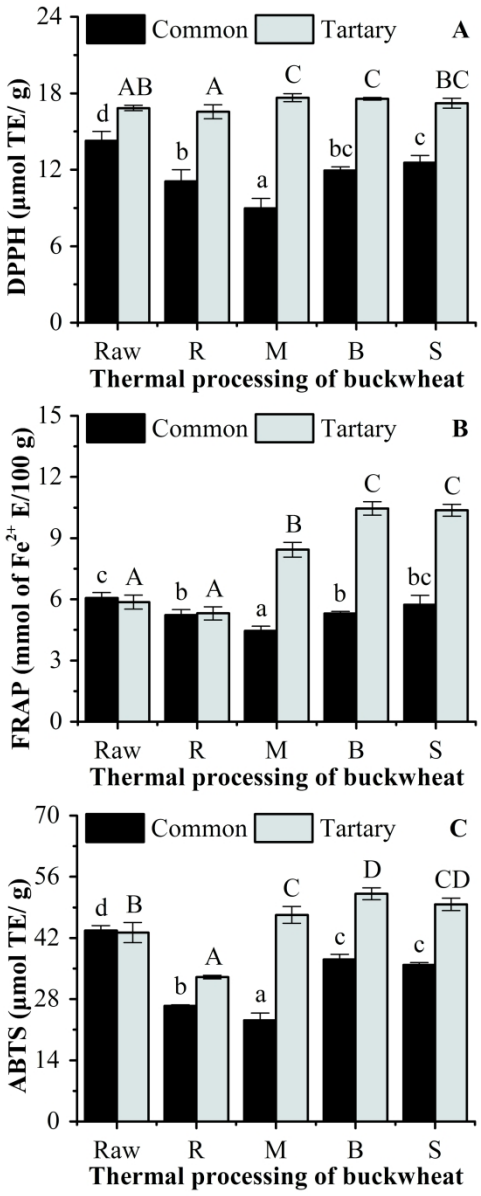

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