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A Process for Preventing Enzymatic Degradation of Rutin in Tartary Buckwheat (*Fagopyrum tataricum* Gaertn) Flour

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Abstract The use of tartary buckwheat flour as a source of dietary rutin has been limited because of the enzymatic degradation of rutin during the dough-making process, which results in a bitter taste. A variety of pretreatment regimes, including heating, steaming, boiling, and extruding, were evaluated in relation to the inactivation of the rutin-degrading enzyme responsible for rutin loss and color change during dough-making. Steaming (120 sec), boiling (90 sec) buckwheat grains, or extruding (180 rpm/min at 140°C) the flour resulted in the retention of >85% of the original rutin and eliminated the bitter taste in the hydrated flours. In contrast, dry heating at 140°C for 9 min or microwaving at 2,450 MHz for 3 min did not reduce the rutin loss, and the bitter taste remained. Unlike in the flour, the rutin degradation in water-soaked grains was insignificant at room temperature. Moreover, the samples treated by steaming, boiling, or extrusion were darker and more reddish in color.

Keywords: bitter taste, rutin, rutin-degrading enzyme, tartary buckwheat

Introduction

Agriculturally, buckwheat is classified as either common buckwheat (*Fagopyrum esculentum* Moench) or tartary buckwheat [*Fagopyrum tataricum* (Linn) Gaertn] (1). Common buckwheat is grown worldwide, whereas tartary buckwheat is only found in western China, northern India, Nepal, Bhutan, and a small part of northwestern Europe (2,3). Tartary buckwheat originated in China, in the south of Sichuan Province and the north of Yunnan Province.

Buckwheat is known to relieve diabetes, obesity, hypertension, hypercholesterolemia, and constipation (4). Tartary buckwheat seeds contain 100-times more rutin (quercetin 3-*O*-rutinoside) than common buckwheat seeds (5), and have been identified as a potential anticancer agent (6,7) in humans. Specially, buckwheat contains rutin that has been prescribed to treat capillary fragility and also shown to improve collagen synthesis (8), protecting against collagen breakdown (9). As a phenolic antioxidant, the rutin has been demonstrated to scavenge superoxide radicals (10) by attaching to the iron ion, Fe^{2+}, preventing it from binding to hydrogen peroxide, which leads to highly reactive free radicals that may damage cells (11,12). Therefore, rutin may play an important role in inhibiting some cancers (13,14). Rutin also improves glucose homeostasis (15) and retinal function recovery (16), and may consequently reduce the symptoms of diabetes (17,18). In addition, rutin ameliorates lipid metabolism (19,20) and maintains cardiac function (21,22), thereby helping to lower the risk of hyperlipidemia and heart disease.

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Although tartary buckwheat, with its high-rutin content, is promoted as a healthy food, its consumption is not widespread. One of the major reasons is the strong bitter taste, which may be derived from quercetin (23,24). Free quercetin is only present in 0.01-0.05% of tartary buckwheat seeds, but glycosylated quercetins, such as rutin, are more common in seeds. Furthermore, da Silva *et al.* (25) demonstrated that, in addition to its bitter taste, quercetin is more genotoxic than rutin *in vivo*.

Two types of enzymes related to rutin catabolism have been isolated from tartary buckwheat. Yasuda and Nakagawa (26) obtained 2 rutin-degrading enzymes (RDEs I and II) from the seeds; both enzymes had the same optimal pH of 5, the same Km value for rutin, and were completely inactivated by incubation at 80°C for 30 min in buffer solution. Yasuda and Shinoyama (27,28) also found that the RDEs convert rutin into bitter quercetin in tartary buckwheat flour during the dough-forming process. Suzuki *et al.* (29) found that flavonol 3-glucosidase (F3G) consists of 2 isozymes in the tartary buckwheat testa that degrade rutin into quercetin. However, they are shown to have different molecular weight and kinetic constants. Tartary buckwheat flour showed only a trace of the bitter taste after heating at 85°C for 24 hr to inactivate most of the F3G, but the strong bitter taste was still found in heated tartary buckwheat flour after the addition of purified F3G (30).

The bitter components of tartary buckwheat seeds can also be eliminated by isoelectric precipitation, which improves the taste of the tartary buckwheat protein (31). In addition, we previously found that tartary buckwheat flour tastes less bitter after the flavonoids have been removed using ethanol or acetone (32). Here, we investigated several physical pretreatment (e.g., heating, steaming) procedures to produce tartary buckwheat products with a less bitter taste while retaining a high-rutin content.

Materials and Methods

Chemicals Rutin and quercetin standards were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). HPLC grade methanol was obtained from Fisher Scientific Co. (Pittsburgh, PA, USA). Water was prepared using the Milli-Q plus Ultra-Pure Water System (Millipore, Billerica, MA, USA). All other chemicals used were of reagent grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Sample preparation Whole grains of tartary buckwheat from China were ground using a mill (model 880200; Brabender GmbH & Co., Duisburg, Germany). The same mill was used to grind tartary buckwheat grains that had been soaked in water at 25°C for 8 hr and then aerated at 25°C overnight. Endosperm flour (protein 8.78%, starch 79.31%, lipids 1.20%, and rutin 0.12%) and embryo flour (protein 24.02%, starch 52.77%, lipids 6.08%, and rutin 2.44%) were obtained by sieving through 120 and 80 mesh, respectively. Whole flour (protein 11.70%, starch 73.60%, lipids 2.60%, and rutin 1.11%) is a mixture of endosperm flour and embryo flour.

Steaming Whole grains or embryo flour were spread 0.5 cm thick on a 60-mesh sifter of the same diameter as a kitchen boiler, and placed over the boiler in 10 cm from the surface of boiling water, and covered for 0-5 min. The steamed grains or flour were aerated at 25°C overnight to remove some of the water. Whole flour from the treated grains was obtained using an 80 mesh and the Brabender mill.

Heating Embryo flour (moisture 11.03%) was spread 0.5 cm thick on a glass plate and heated in an oven to 100°C for 1 or 3 hr, or at 120 or 140°C for 9 min.

Microwaving Embryo flour (moisture 11.03%) was spread 0.5 cm thick on a glass plate and microwaved (Galanz WD-750BS microwave oven; 220 V, 750 W, Galanz Group Co., Ltd., Guangdong, China) at 2,450 MHz for either 2 or 3 min.

Boiling Whole grains of tartary buckwheat were packed in gauze and placed in boiling water for 0-5 min. The boiled grains were aerated at 25°C overnight to remove water, and then ground to 80 mesh using a Brabender mill.

Extrusion Whole flour of tartary buckwheat was extruded using a twin-screw extruder (Creusot, Loire, France) with a 4 mm outlet diameter at 120°C at 150 rpm/min, 140°C at 180 rpm/min, 140°C at 120 rpm/min, or 160°C at 150 rpm/min. The extruded products were crushed to 80 mesh using an FSF-type pulverizer (Jiading Cereal & Oil Instrument Manufacture Co., Ltd., Shanghai, China).

Color analysis A colorimeter (TG-PII; Beijing Optical Instrument Plant, Bejing, China), calibrated using standard black and white boards, was used to determine the color of all the samples, according to the CIE L*a*b*-system. The L*, a*, and b* values were recorded and the differences between pretreated tartary buckwheat and natural tartary buckwheat were calculated as ΔL^*, Δa^*, and Δb^* (33).

Sensory evaluation Samples were coded and arranged randomly. A panel of 5 volunteers from Jiangnan University evaluated the taste of the coded samples and scored them as either bitter (+) or not bitter (−).

Flavonoid extraction The pretreated buckwheat flours were hydrated by adding 0.2 g of the sample to 1.5 mL of water in a 25-mL flask and shaken for 10 min at 25°C to start the rutin degrading reaction, followed by the addition of 23.5 mL methanol for extraction. The control samples were treated directly with only 25 mL of 94% methanol at room temperature. The flask was placed in water in an ultrasonic cleaner (USC202; Bolong Electronic Equipment Co., Ltd., Shanghai, China) and sonicated at 56 KHz for 1 hr at 25°C (34). The sample was then filtered through a 0.45 μm PVDF membrane (Millipore) prior to flavonoid analysis.

HPLC determination of flavanoids A Varian 5000 HPLC system (Palo Alto, CA, USA) with a Supelcosil™ LC-18-DB column (25 cm×4.6 mm, 5 μm; Supelco, Bellefonte, PA, USA) was used to determine flavonoid concentrations by detection at 350 nm (35). The samples were chromatographed using an isocratic solvent system consisting of 55 % methanol and 45% acetic acid (1%, v/v) at a flow rate of 1.0 mL/min. The concentrations of flavonoids in the samples were calculated from rutin and quercetin standard curves. Rutin content in the treated samples after water addition divided by that in the control samples gave the retention rate of rutin.

Statistical analysis All data were the mean values of 3 parallel measurements. Rutin content of hydrated extrusion flours was further analyzed by Student's *t*-test. The tests were considered statistically significant at $p<0.01$.

Results and Discussion

Rutin loss during dough-making Rutin content in embryo flour was determined by HPLC to be 2.44% (Fig. 1a). The rutin of the dough dramatically decreased when the embryo flour was mixed with 50% water for 1 min at room temperature (Fig. 1b), and 96.46% of the rutin in the flour was degraded into quercetin. These results are in accordance with those reported by Kawakami *et al.* (31).

However, after whole tartary buckwheat grains were soaked in water at room temperature for 8 hr, the embryo flour contained less than 0.2% quercetin (Fig. 1c), suggesting that rutin and the rutin-degrading enzyme are present in different regions of tartary buckwheat seeds.

Suzuki *et al.* (29) also showed that the major part of the rutin was found in the embryo of tartary buckwheat seeds, and almost all the f3g activity was detected in its testa.

The above mentioned experiment further proved that rutin in the embryo of whole seeds is not affected by the rutin-degrading enzyme in the testa, and is only available to the enzyme when seeds are turned into flour. The results imply rutin-degrading enzyme in tartary buckwheat grains is involved in the degradation of rutin to quercetin.

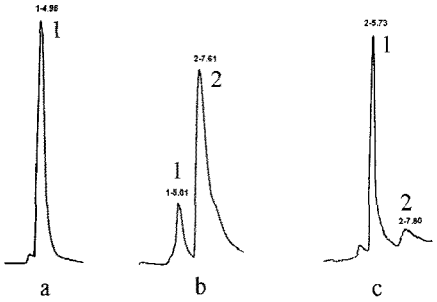

Fig. 1. HPLC chromatograms of rutin (peak 1) and quercetin (peak 2) in tartary buckwheat flour with detection at 350 nm. a) CK, b) 1 min after water (2×) addition, c) 8 hr after grains were soaked in water. The numbers indicate the retention times in min.

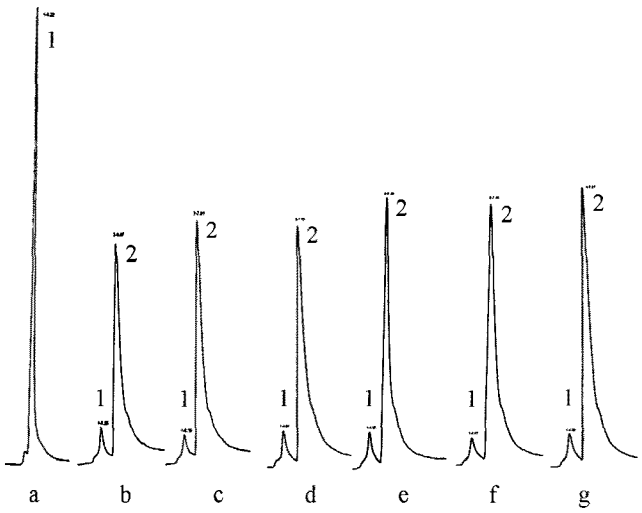

Fig. 2. HPLC chromatograms of rutin (peak 1) and quercetin (peak 2) in tartary buckwheat flour at 350 nm without (a) or with (b-g) the addition of water. a, 100°C for 3 hr; b, 100°C for 1 hr; c, 100°C for 3 hr; d, 120°C for 9 min; e, 140°C for 9 min; f, 2,450 MHz for 2 min; g, 2,450 MHz for 3 min.

Heating and microwaving pretreatments The rutin-degrading enzyme in tartary buckwheat flour was not deactivated by heat treatment at 100-140°C for 9 min to 3 hr or by microwaving at 2,450 MHz for 2-3 min. More than 95% of rutin was degraded into quercetin after the addition of water (Fig. 2b-g). The results imply that the rutin-degrading enzyme is highly stable in xerothermic conditions.

Steaming and boiling pretreatments The retention of rutin in steamed tartary buckwheat after the addition of water increased with pretreatment time (Fig. 3-5). Steam-treating the flour for 10-50 sec and the grains for 1-5 min reduced the activity of the rutin-degrading enzyme.

Steam-treating tartary buckwheat flour for 60 sec resulted in the retention of 100% of the rutin in the presence of water, although water content increased more than 2%. However, over steaming caused the starch in the flour to gelatinize and form agglomerations of various sizes, which is undesirable for storage and transport of the flour. Therefore, pre-treating grains by steaming for 2-5 min was found to be the most suitable treatment for manufacturing a tartary buckwheat flour that maintains a high rutin content during dough-making.

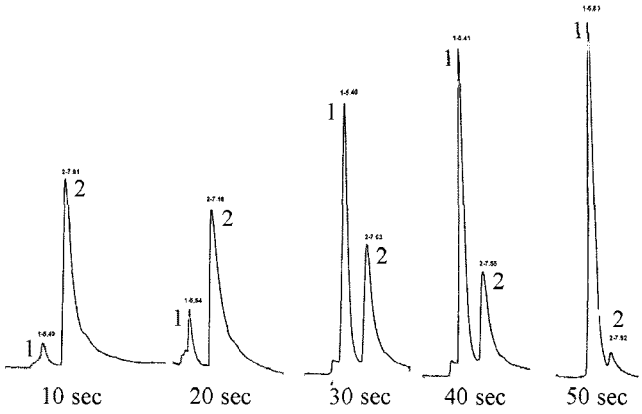

Fig. 3. HPLC chromatograms of rutin (peak 1) and quercetin (peak 2) in tartary buckwheat flour after steam treatment for 10, 20, 30, 40, or 50 sec. The rutin and quercetin contents were determined following the addition of water.

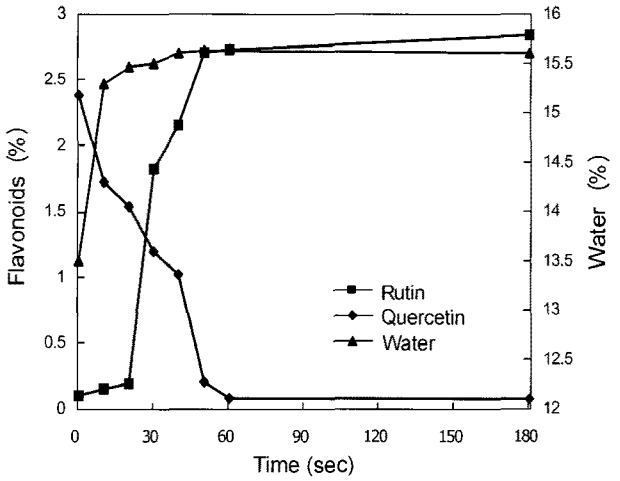

Fig. 4. Flavonoid contents of steamed tartary buckwheat flour after the addition of water.

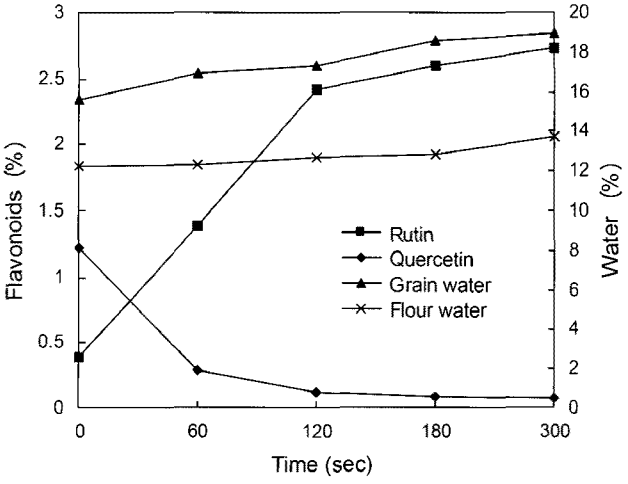

Fig. 5. Flavonoid contents of tartary buckwheat flour made from steamed grains after the addition of water.

Whole grains of tartary buckwheat were boiled for 0-120 sec. The rutin content in the flours made from the

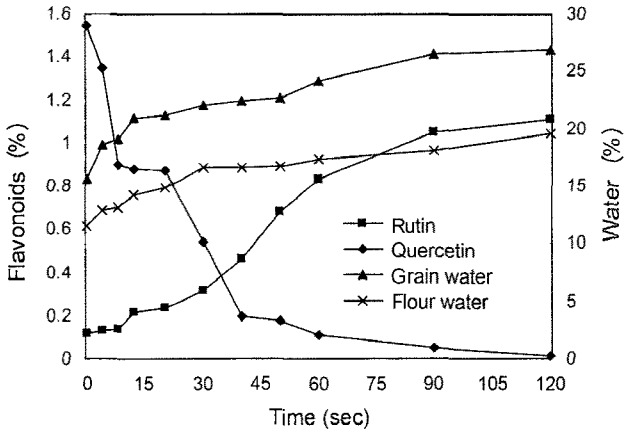

Fig. 6. Flavonoid contents of tartary buckwheat flour made from boiled grains after the addition of water.

Table 1. Rutin content of extruded tartary buckwheat flours after the addition of water

Extrusion temp. (°C) /screw speed (rpm)	Rutin[2] (mg/g)	Retention rate (%)
CK[1]	1.20±0.0721 D	10.81
120/150	9.98±0.0436 B	87.42
140/180	10.32±0.0529 A	90.20
140/120	7.89±0.0361 C	69.40
160/150	10.09±0.0200 B	88.30

[1]Untreated flour.
[2]Values are means±SD (n=3), Student's *t*-test at $p<0.01$.

boiled grains after the addition of water increased with pretreatment time, and 94.6% of the rutin was retained with a boiling pretreatment of 90 sec (Fig. 6). The results imply that the rutin-degrading enzyme is highly unstable in hot moist conditions.

Extrusion pretreatment Extrusion-cooking continues to be used by the food and feed industry (36). Twin-screw extruders can be used as effective continuous reactor (37). A high screw speed was used to investigate the effect of extrusion treatment on the retention of rutin. At an extrusion temperature of 140°C, the higher screw speed of 180 rpm was more advantageous than the lower speed of 120 rpm for the manufacture of tartary buckwheat flour with high rutin content after the addition of water. At a screw speed of 150 rpm, the effect of extrusion temperatures of 120 and 160°C on the retention of rutin did not differ greatly. The hydrated product obtained using a screw speed of 180 rpm at 140°C contained 90.20% rutin. It is possible

Table 2. Rutin loss and bitterness in tartary buckwheat flours with different pretreatment regimes

Treatment[1]	Rutin retention rate	Bitter taste[2]
CK, H140, M2450, S20, B8	3.54-13.85	+++++
B12, B30	19.82-28.83	++++
S'60, B40	41.44-49.06	+++
S30, B50, E120	61.26-69.40	++
S40, B60	74.77-75.87	+
S50, S'120, B90, E180, E150	85.22-96.31	-

[1]CK, Untreated flour; S20-50, flour steamed for 20-50 sec; S' 60-120, grains steamed for 60-120 sec; B8-90, boiling for 8-90 sec; E120-180, extrusion at 120-180 rpm/min; H140, heating at 140°C for 9 min; M2450, microwaving at 2,450 MHz for 3 min.
[2]5 volunteers; -, non-bitter; + bitter.

that increased rutin retention is achieved by extrusion treatment at high-screw speeds that may deactivate the rutin-degrading enzyme.

Color and taste The taste of the tartary buckwheat products was evaluated after the various pretreatment regimes. A reduction in rutin loss, i.e., high rutin retention and low quercetin formation (Fig. 1-6), resulted in a less bitter taste (Table 2). The results suggest that no significant bitter taste was produced by steam treatment of flour or grain for 50 or 120 sec, boiling for 90 sec, or extrusion at 150 or 180 rpm/min, all of which resulted in a rutin retention of 85-96%. However, heat treatment at 140°C for 9 min or microwaving at 2,450 MHz for 3 min resulted in significant rutin loss and did not reduce the bitter taste of tartary buckwheat. Additional research is needed to confirm the potential effects of rutin retention on the elimination of bitter taste.

The pretreated tartary buckwheat flour was darker than the untreated flour (Table 3). Steaming and boiling treatments resulted in high red and blue values. In contrast, heating or microwaving changed the color of the products in the opposite direction. Extrusion increased the red and yellow values. This may be explained by non-enzymatic browning reactions at low moisture and high temperature, as suggested by Parker *et al.* (38).

The present results indicate that pretreatment procedures such as steaming, boiling, and extruding of tartary buckwheat grain and flour significantly contributed to the reduction of rutin loss caused by rutin-degrading enzymes. High-rutin retention and low-quercetin formation in the treated samples appeared to result in a less bitter taste. Thus, the methods mentioned in this study may be used to help produce and market tartary buckwheat as a functional

Table 3. Color of selected tartary buckwheat flours with different treatments[1,2]

Color scale	CK	S50	S'120	B90	E180	H140	M2450
L*(L*)	54.29(0)	44.45(-9.84)	44.36(-9.93)	43.31(-10.98)	45.26(-9.03)	50.43(-3.86)	50.4(-3.89)
a*(A*)	-1.70(0)	-0.57(1.13)	-0.72(0.98)	-0.60(1.10)	1.85(3.55)	-3.43(-1.73)	-3.33(-1.63)
b*(B*)	13.56(0)	12.96(-0.60)	12.78(-0.78)	11.25(-2.31)	19.90(6.34)	15.25(1.69)	14.17(0.61)

[1]CK, Untreated flour; S50, steaming flour for 50 sec; S'120, steaming grains for 120 sec; B90, boiling for 90 sec; E180, extrusion at 140°C and 180 rpm/min; H140, heating at 140°C for 9 min; M2450, microwaving at 2,450 MHz for 3 min.
[2]Values in parenthesis were the color difference between treated samples and control sample.

food material that has a high-rutin content and reduced bitter taste.

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