Journal of Medicinal Plants Research Vol. 5(4), pp. 572-578, 18 February, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Antioxidative properties of hydrated ethanol extracts from tartary buckwheat grains as affected by the changes of rutin and quercetin during preparations

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Accepted 26 November, 2010

Rutin and its hydrolyzing enzymes naturally occur in tartary buckwheat grains. However, it is not known whether these enzymes affect the preparations of tartary buckwheat flavonoids. In this study, water followed by ethanol (3:7), or premixed 70% ethanol, was used to extract flavonoids from flours or hulls of tartary buckwheat grains. The flavonoid compositions of hydrated ethanol extracts were analyzed by HPLC, and their antioxidative capacities were evaluated by free radical scavenging test and reducing power assay. The premixed 70% ethanol inhibited the activity of rutin hydrolyzing enzymes to produce the extract of high rutin content, while the addition of water followed by ethanol led to the degradation of rutin to produce the extract of high quercetin content. The antioxidative activities of high quercetin extracts were more than those of high rutin extracts.

Key words: Tartary buckwheat, rutin, quercetin, antioxidative activity.

INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum*), a highly nutritious crop, is mainly grown in the southwest of China, and used in many traditional foods by Yi nationality. Compared with its congener, common buckwheat (*Fagopyrum esculentum*), tartary buckwheat has a lower planted acreage and more bitter taste. Nevertheless, tartary buckwheat, until recently a largely forgotten crop, is attracting increasing interest from food technologists and consumers for its significant health benefits in the relieves of diabetes, obesity, and constipation (Li et al., 2009).

Tartary buckwheat is rich in flavonoids, including the predominant flavonol rutin and minor flavonols quercetin 3-O-rutinoside-3′-O-β-glucopyranoside, kaempferol 3-O-rutinoside and quercetin (Li et al., 2010). Among these flavonoids, the rutin has been prescribed to strengthen the capillaries and reduce the symptoms of haemophilia. Dietary intake of rutin is associated to make the blood thinner and improve circulation. Quercetin is the aglycone and bioactive part of rutin.

Flavonoid extracts from tartary buckwheat have also been shown to possess special medicinal properties of antihyperglycemia (Qi et al., 2003), antihypercholesterolemia (Qi, 2003) and anticancer (Ren et al., 2003, 2001). Recently, tartary buckwheat flavonoids were also found to have neuroprotection functions (Huang et al., 2006). As a result of these beneficial effects on human health, dietary supplement based on tartary buckwheat flavonoids is available in some countries.

Processing methods have been found to affect the polyphenol concentration of tartary buckwheat foods (Vogrincic et al., 2010). From the recent studies, some

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thermal treatments were shown to reduce flavonoids content and antioxidative activities of tartary buckwheat flour (Zhang et al., 2010). However, data on the composition and antioxidative properties of flavonoid extracts from tartary buckwheat as affected by their preparation procedures are limited.

The preparation of tartary buckwheat flavonoids often includes two steps involving hydrated ethanol extraction and resin purification (Liu et al., 2007). However, the rutin-degrading enzyme (RDE) (Yasuda et al., 1992) and flavonol 3-glucosidase (F3G) (Suzuki et al., 2002), also naturally occur in tartary buckwheat. The purified RDE and F3G were found to be thermostable, and able to hydrolyze rutin into quercetin even in the presence of some organic solvents. In order to explore the effect of these enzymes on flavonoids during preparations, tartary buckwheat grains were extracted with water followed by ethanol, or premixed 70% ethanol in this study. The flavonoid composition and antioxidative activity of the hydrated ethanol extracts were determined.

MATERIALS AND METHODS

Chemicals

Rutin and quercetin were purchased from Zelang Medical Technology (Nanjing, Jiangsu, China). DPPH (2, 2-diphenyl-1-picrylhydrazyl) was obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol was obtained from Fisher Scientific (Pittsburgh, PA, USA). Water was prepared using a double distilled water system (BSZ-2, Botonyc, Shanghai, China). All other chemicals used were of reagent grade and were purchased from Sinopharm Chemical Reagent (Shanghai, China).

Sample preparation

Twenty gram of tartary buckwheat grains from Bijie of Guizhou Province was powdered using a pulverizer, and then sieved into 4.33 g of hull and 15.09 g of flour through 80 mesh.

Next, water and ethanol were added into hull or flour for extraction by two different procedures.

Procedure 1: Extraction with water followed by ethanol (3:7). The hull or flour was hydrated by adding 0.2 g of the sample to 3 ml of water in a 10-ml flask and shaken for 5 min at room temperature, followed by the addition of 7 ml ethanol.

Procedure 2: Extraction with premixed 70% ethanol. The hull or flour was mixed directly with 10 ml of 70% ethanol in a 10-ml flask at room temperature.

Then, the flasks were placed in water in an ultrasonic cleaner (KQ300VDE; Ultrasonic Instrument Co., Ltd., Kunshan, China) and sonicated at 56 KHz for 30 min at room temperature (Li et al., 2008). The sample was then filtered through a 0.45-µm PVDF membrane (Millipore, Billerica, MA, USA) for HPLC analysis and antioxidative test. All samples were stored at 4°C until use.

HPLC analysis

Flavonoids in the hydrated ethanol extracts were analyzed by a Dionex Summit HPLC (Sunnyvale, CA, USA) using a Waters Nova-Pak C18 column (150 × 3.9 mm i.d., 4 µm particle size, Milford, MA, USA) and recorded at 350 nm with a UV detector. A gradient solvent system consisting of solvents A (methanol/water/formic acid, 20:80:0.1, v/v/v) and B (methanol/water/formic acid, 80:20:0.1, v/v/v) was used. In 0 to 30 min, solvent A was decreased gradually from 100 to 0% and solvent B was increased gradually from 0 to 100% at a flow rate of 0.8 ml/min. The concentrations of rutin and quercetin were calculated from their standard curves.

Evaluation of the free radical scavenging capacity

The capacity to scavenge the ‘‘stable’’ free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of Choi et al. (2003) with minor modifications.

Four hundred and sixty microlitre of the diluted extraction solution and 540 µl of DPPH (0.2 mM) in ethanol were mixed vigorously and then kept in a dark place. After 20 min, the absorbance at 517 nm was recorded using a spectrophotometer. The free radical scavenging rate (SR) of the reaction solution was calculated as a percentage of DPPH discolouration using the equation:

$\%SR = 100 \times (1-A_c/A_D)$, where A_c is the absorbance of the solution when the sample has been added at a particular level, and A_D is the absorbance of the DPPH solution.

Evaluation of the reducing power

The reducing power was determined according to the method of Oyaizu (1986). Two hundred microlitre of the diluted extraction solution was mixed with 200 µl of 0.2 M sodium phosphate buffer (pH 6.6) and 200 µl of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 200 µl of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 5000 rpm for 10 min. Five hundred microlitre of the supernatant was mixed with 500 µl deionised water and 100 µl of 0.1% (w/v) of ferric chloride, and the absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power.

RESULTS

Flavonoid composition of hydrated ethanol extracts from tartary buckwheat grains

Low molecular weight alcohols, ethanol and methanol, are often used as the extraction solvents of flavonoid compounds.

In this study, 70% ethanol was selected in consideration of its low toxicity and suitable polar, and added into the powdered hulls or flours with two different procedures: water followed by ethanol (3:7), or premixed 70% ethanol, respectively. The ultrasonic extractions were performed at room temperature.

The flavonoid compounds in the hydrated ethanol extracts were analyzed with HPLC (Figure 1), and their contents were calculated (Table 1). The results showed that quercetin is the predominant flavonoid and rutin is the minor flavonoid in the hydrated ethanol extracts of hull or flour with the procedure of water followed by ethanol (3:7). On the contrary, rutin is the predominant flavonoid and quercetin is the minor flavonoid in the

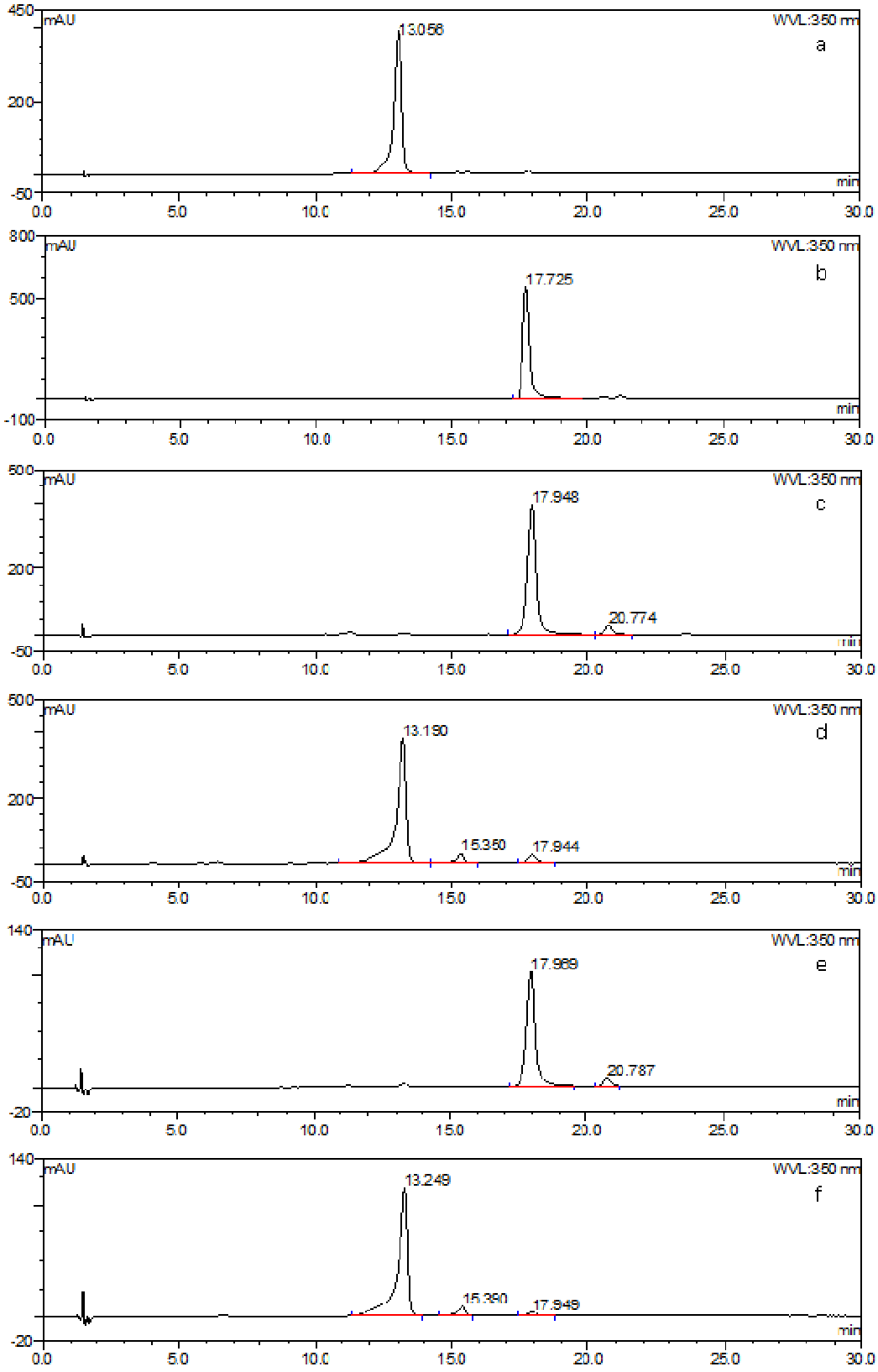

Figure 1. HPLC chromatograms of hydrated ethanol extracts from tartary buckwheat grains. (a). Rutin standard, (b). quercetin standard, (c). flour extract with water followed by ethanol, (d). flour extract with premixed 70% ethanol, (e). hull extract with water followed by ethanol and (f). hull extract with premixed 70% ethanol.

Table 1. Flavonoid composition of hydrated ethanol extracts from tartary buckwheat grains (mM). (Average ± standard deviation, number of repeats = 3.).

Extracts	Rutin	Quercetin	Total flavonoids
Flour extract with water followed by ethanol	0.5 ± 0.1	30.2 ± 0.5	30.7 ± 0.5
Flour extract with 70% ethanol	27.3 ± 0.4	2.5 ± 0.2	29.8 ± 0.5
Hull extract with water followed by ethanol	0.2 ± 0.0	8.2 ± 0.3	8.4 ± 0.3
Hull extract with 70% ethanol	8.6 ± 0.3	0.9 ± 0.2	9.5 ± 0.4

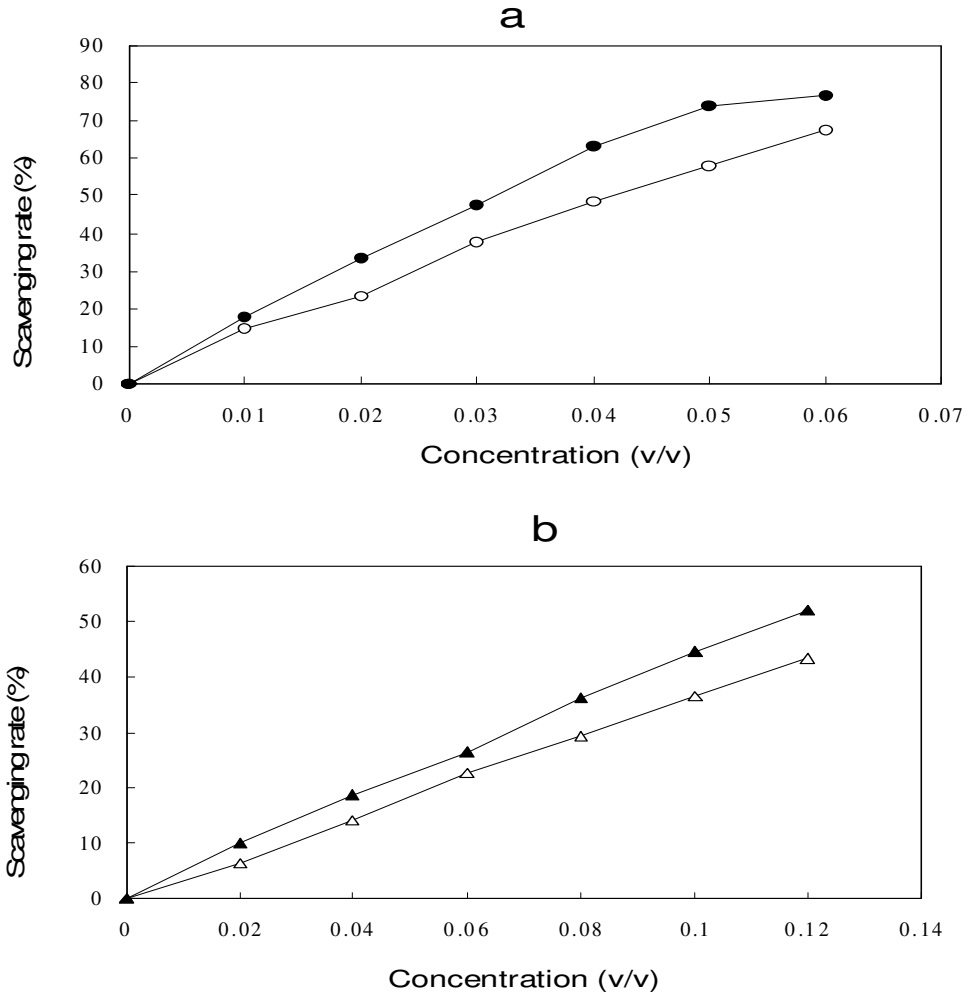

Figure 2. DPPH free radical scavenging rate of hydrated ethanol extracts from tartary buckwheat grains. (a). Flour extracts with water followed by ethanol (●), and premixed 70% ethanol (○). (b). Hull extracts with water followed by ethanol (▲), and premixed 70% ethanol (△).

hydrated ethanol extracts of hull or flour with the procedure of premixed 70% ethanol. The total flavonoids content of flour extracts was three times more than that of hull extracts.

These results revealed that RDE or F3G could hydrolyze rutin into quercetin in water, but not in the premixed 70% ethanol during the preparation of tartary buckwheat flavonoids.

Free radical scavenging capacity of hydrated ethanol extracts from tartary buckwheat grains

The hydrated ethanol extracts were mixed with DPPH and their free radical scavenging capacities were evaluated by the bleaching reaction. Figure 2 showed that all of the samples were able to scavenge DPPH free radicals, and their scavenging capacities were positively

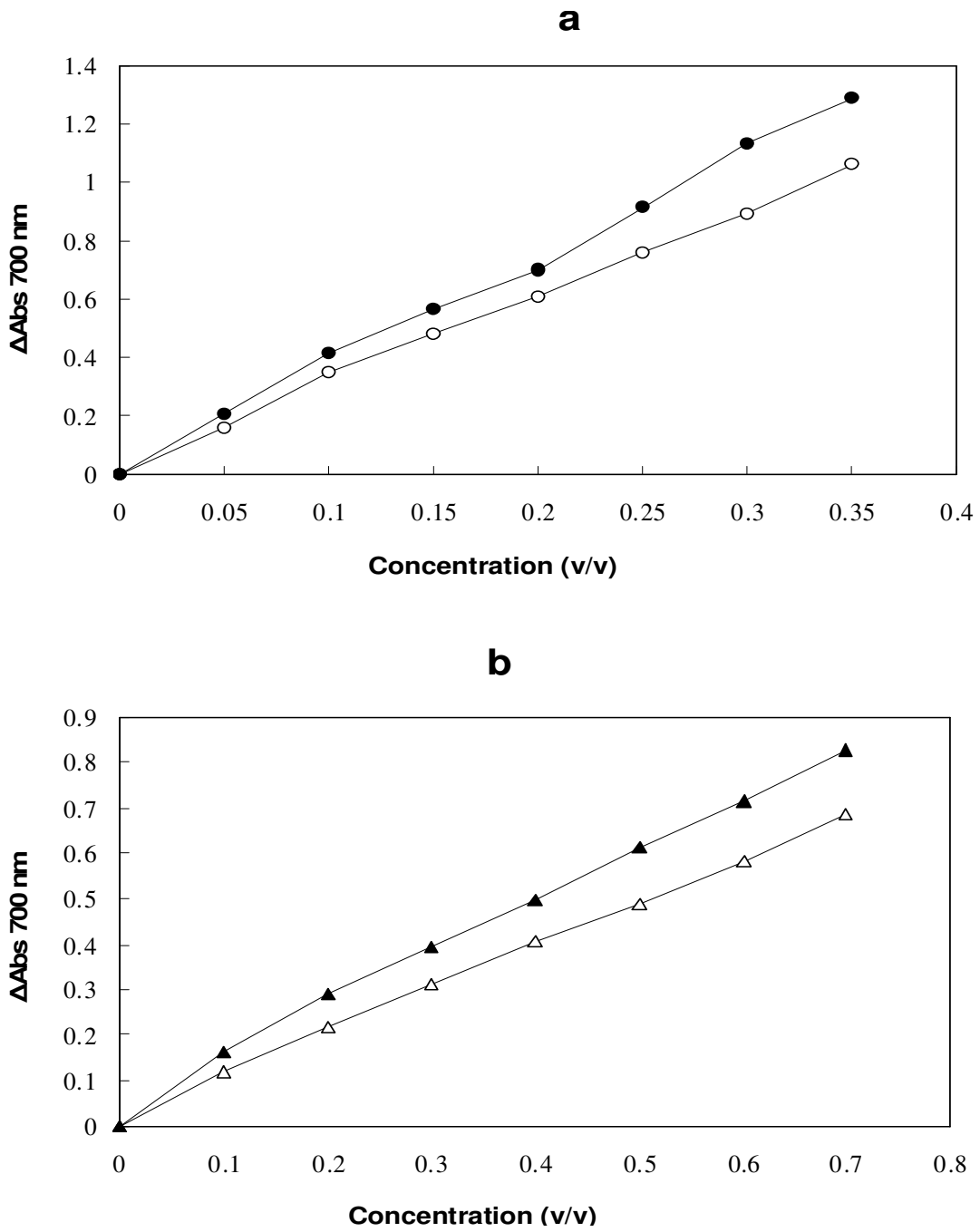

Figure 3. Reducing power of hydrated ethanol extracts from tartary buckwheat grains. (a). Flour extracts with water followed by ethanol (●), and premixed 70% ethanol (○). (b). Hull extracts with water followed by ethanol (▲), and premixed 70% ethanol (△).

correlated with their concentration in the system. At the same concentration, the free radical scavenging capacity of extract with water followed by ethanol (3:7) were more than those of extract with premixed 70% ethanol. With the increase of test concentration, the difference of free radical scavenging rate of two extracts increased gradually.

Reducing power of hydrated ethanol extracts from tartary buckwheat grains

The antioxidative activity of hydrated ethanol extract was also measured by their ability to reduce the [Fe.sup.3+]/ ferricyanide complex by forming ferrous products. Figure 3 showed that all of the samples were able to reduce

ferric ion to ferrous ion, and their reducing power was positively correlated with their content in the system. At the same test concentration, the reducing power of extract with water followed by ethanol (3:7) were more than those of extract with premixed 70% ethanol. With the increase of test concentration, the difference of reducing power of two extracts increased gradually.

DISCUSSION

Two types of rutin hydrolyzing enzymes, RDE (Yasuda et al., 1992) and F3G (Suzuki et al., 2002), related to rutin catabolism into quercetin have been isolated from tartary buckwheat grain respectively. And these enzymes and flavonoids have been proved to be located in the different regions of tartary buckwheat grain since the major part of the rutin was found in the embryo and almost all rutin hydrolyzing activity was detected in its testa. The degradation of rutin in water-soaked grains was insignificant at room temperature (Li et al., 2008).

In this study, grains were ground into powder. This resulted in a mix of rutin and its hydrolyzing enzymes. Although rutin hydrolyzing enzymes are thermostable and RDE is also active in the low concentration of ethanol, rutin degradation during the preparation of tartary buckwheat flavonoids from the powdered hulls or flour did not take place if the premixed 70% ethanol was used as the extraction solvent. This indicated that the high concentration of ethanol completely inhibited the activity of rutin hydrolyzing enzymes.

However, almost all rutin was degraded into quercetin if water followed by ethanol (3:7) was successively added into the powdered hulls or flour of tartary buckwheat grains. These results indicated that rutin hydrolyzing enzymes had high activity in the powdered hull and flour in the presence of water. The similar changes of rutin and quercetin were also observed during the preparation of tartary buckwheat dough (Vogrincic et al., 2010; Li et al., 2008).

Tartary buckwheat has been considered as the dietary source of rutin and quercetin (Fabjan et al., 2003). However, quercetin is more bitter (Li et al., 2008) and genotoxic than rutin *in vivo* (da Silva et al., 2002). From this study, rutin-rich fraction could be extracted using premixed 70% ethanol. Quercetin-rich fraction could be obtained using water followed by ethanol (3:7).

Although, flavonoids only account for a part of hydrated ethanol extracts of tartary buckwheat grain, the changes of their composition in this study led to different antioxidative activities.

Free radical scavenging capacity and reducing power of hydrated ethanol extracts were positive correlated with the quercetin content. Those might result from the extra OH group located in the C ring of quercetin. It could directly contribute to the reactivity of quercetin so that quercetin is more reactive and has more reactive centers than rutin (Aliaga et al., 2004).

Conclusions

The mature grains of tartary buckwheat contain much rutin. At the same time, the activity of rutin hydrolyzing enzymes could be detected in either its flour or its powdered hulls. During the preparation of tartary buckwheat flavonoids, high concentration of ethanol (for instance, the premixed 70% ethanol) could completely inhibit the activity of rutin hydrolyzing enzymes and produce high rutin extract. However, the degradation of rutin took place and high quercetin extracts were produced if water followed by ethanol were successively mixed with the above-mentioned materials. The antioxidative activities of high quercetin extract were more than those of high rutin extract.

ACKNOWLEDGMENTS

This work was supported in part by "the eleventh five" research projects of science and technology (2008192), education department of Jilin province government. We thank Cui Fan, Chenshuai Fu and Sicheng Dai, the undergraduate students from the department of food science and engineering at Changchun University, for assisting us with the samples preparation and antioxidative analysis.

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