Note

Antioxidative Activities in Rutin Rich Noodles and Cookies Made with a Trace Rutinosidase Variety of Tartary Buckwheat (*Fagopyrum tataricum* Gaertn.), ‘Manten-Kirari’

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A Tartary buckwheat variety, ‘Manten-Kirari’, which is rich in rutin but lacks bitterness because of trace rutinosidase activity, was recently developed. Rutin content was 1,269 and 1,421 mg/100 g DW in dried noodles and cookies made with 50% ‘Manten-Kirari’ flour, respectively. The residual ratio of rutin during preparation of dried noodles and cookies was 99.3% and 80.6%, respectively. Hydrophilic antioxidant capacity (H-ORAC) was 149.8 μmol TE/g DW and 209.2 μmol TE/g DW in dried noodles and cookies, respectively. The contribution ratio of rutin to H-ORAC was 96.2% and 69.8% in dried noodles and cookies, respectively. These results indicated that the antioxidative activities of dried noodles and cookies made with ‘Manten-Kirari’ flour were mainly due to the hydrophilic antioxidant rutin. Tartary buckwheat ‘Manten-Kirari’ is a potentially excellent source of antioxidative food, and is characterized by a high rutin content and minimal bitterness.

Keywords: Tartary buckwheat, rutin, rutinosidase, quercetin, ORAC

Introduction

Buckwheat is cultivated in many countries, including China, Nepal, Russia, Europe and Japan. Buckwheat is a rutin-rich plant and is the only known cereal to contain rutin in the seeds. Rutin is a flavonoid and has many biological functions, such as antioxidative (Jiang et al., 2007; Awatsuhara et al., 2010), strengthening of blood capillaries (Griffith et al., 1944; Shanno, 1946), antihypertensive (Matsubara et al., 1985), α-glucosidase inhibitory (Li et al., 2009) and anti-metabolic syndrome (Choi et al., 2006; Hsu et al., 2009; Panchal et al., 2011) activities. Among buckwheat species, Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) contains an extremely high rutin content in its seeds, 0.5–2% w/w, which is approximately 100-fold higher than that in common buckwheat (*Fagopyrum esculentum* Moench) (Jiang et al., 2007; Yasuda et al., 1992; Suzuki et al., 2002; Guo et al., 2011). Tartary buckwheat seeds have been identified as a potential rutin-rich material for food products (Yasuda et al., 1992; Suzuki et al., 2002; Guo et al., 2011); however, with its extremely high rutinosidase activity, rutin-containing buckwheat flour can be hydrolyzed within minutes after the addition of water (Yasuda et al., 1992; Suzuki et al., 2002; Guo et al., 2011; Yasuda et al., 1994). Tartary buckwheat is also known as ‘bitter buckwheat’; it has been reported to contain at least three bitter compounds, and the bitterness is the major reason for the limited use of Tartary buckwheat. Rutin hydrolysis is the major contributing factor to bitterness, with quercetin, a rutin hydrolysate, as one of the bitter compounds (Suzuki et al., 2014a; Kawakami et al., 1995). Recently, we developed a novel Tartary buckwheat variety, ‘Manten-Kirari’, with trace-rutinosidase activity (Suzuki et al., 2014b); the rutinosidase activity in the flour is about two or three
orders of magnitude less than in other Tartary buckwheat varieties. Additionally, the flour of this variety lacks bitterness. Therefore, we propose that rutin-rich and non-bitter foods can be produced using ‘Manten-Kirari’ buckwheat flour.

A number of health benefits of buckwheat foods have been reported. The consumption of buckwheat-enriched bread increased the serum antioxidant capacity in humans (Bojanska et al., 2009), while rutin-enhanced buckwheat noodles reduced the levels of serum total cholesterol and free fatty acids (Qu et al., 2013). In addition, eating buckwheat cookies made with common buckwheat and Tartary buckwheat was associated with a reduction in cholesterol levels, nasal irritation and headache (Wieslander et al., 2011; Wieslander et al., 2012), and intake of Tartary buckwheat cookies with high levels of rutin reduced the serum levels of myeloperoxidase and fatigue symptoms (Wieslander et al., 2011; Wieslander et al., 2012). The effects of buckwheat foods could be largely due to rutin as an antioxidant, since reactive oxygen species (ROS) are implicated in some human diseases, including cancer, atherosclerotic heart disease and Alzheimer’s disease. (Ames, 1995; Diaz et al., 1997; Engelhart et al., 2008). Oxygen radical absorbance capacity (ORAC) is a frequently used method in the evaluation of antioxidation (Wu et al., 2004; Watanabe et al., 2012). Previous studies have reported on the antioxidant capacity of Tartary buckwheat and its food products (Ghimeray et al., 2014; Xu et al., 2014; Cho et al., 2014; Zhou et al., 2015). However, there are very few reports on the antioxidation evaluation of buckwheat foods by ORAC (Zielinski et al., 2009), and the antioxidant capacity of buckwheat foods made from Tartary buckwheat ‘Manten-Kirari’ with trace rutinosidase activity has not been investigated.

In this study, we prepared two kinds of foods, noodles and cookies, using the Tartary buckwheat ‘Manten-Kirari’ with trace rutinosidase activity. The rutin and quercetin contents in these foods were assessed. Antioxidative activities using the ORAC method as well as DPPH radical scavenging activity were assayed. Contribution ratios of rutin to H-ORAC were also evaluated in dried noodle and cookies.

Materials and Methods
Reagents and chemicals Fluorescein (sodium salt) (FL) and 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) were from Sigma-Aldrich (St. Louis, MO, USA). 1,1-Diphenyl-2-picrylhydrazyl Free Radical (DPPH) was from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Methyl-β-cyclodextrin (MCD) was obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). Rutin, quercetin, 2,2’-azobis (2-aminodipropane) dihydro-chloride (AAPH) and all other reagents were purchased from Wako Pure Chemical Ind. (Osaka, Japan).

Flour preparation The Tartary buckwheat variety ‘Manten-Kirari’ (trace-rutinosidase variety) was grown in Oumu, Hokkaido, Japan (longitude, 142°58E; latitude, 44°35N) in 2013. Seeds were sown in June and harvested in late August. Harvested seeds were dried at 40°C for one week and then threshed and stored at 4°C until used for milling. Tartary buckwheat seeds were milled by the Minami milling company (Sapporo, Japan) using a roll mill. Tartary buckwheat flour was stored at −20°C until used for experiments.

Preparation of noodles and cookies Dried noodles containing 50% Tartary buckwheat flour to total fresh weight were prepared by Kobayashi Shokuhin Co., Ltd., as follows: 25.0 kg of Tartary buckwheat ‘Manten-Kirari’ flour, 23.5 kg of hard wheat flour and 1.5 kg of wheat protein (Japan Colloid, Co., Ltd., Tokyo, Japan) were combined. Salt (0.5 g) and water (15 – 16 g) were added using a mixer (Tokyo Menki Co., Ltd., Saitama, Japan) for 10 min. After mixing, the crumbly dough mixture was transferred to a noodle-sheeting machine (Tokyo Menki Co., Ltd.). The sheet was cut into noodles, and the cut noodles were air dried until a water content of 15.0% was reached. They were milled and passed through a 250 μm sieve before storage at −30°C until analysis.

Dried noodles were boiled in 4 L of water for 6 min and then rinsed with cold water. Boiled noodles were freeze-dried before storage at −30°C until their use in the rutin and quercetin content and ORAC assays.

The cookies containing 50% Tartary buckwheat flour to total fresh weight were prepared as follows: 38 g of Tartary buckwheat ‘Manten-Kirari’ flour, 13 g of sugar, 19.5 g of raw egg, 4.65 g of fermented butter without salt and 0.25 g of salt were mixed. The dough was stored at 4°C for 1 hour before being cut into cookies weighing 15 g each, which were then baked at 160°C for 15 min. Baked cookies were milled and passed through a 250 μm sieve and stored at −30°C until analysis.

Rutin and quercetin contents Rutin and quercetin were extracted from 1.0 g of samples with 50 mL of 80% ethanol at 80°C for two hours with intermittent mixing. After centrifugation at 3,000 g for 10 min, the supernatant was diluted 100 times with 80% ethanol and filtered through a 0.45 μm membrane; the rutin and quercetin contents were determined by HPLC (Suzuki et al., 2002). The HPLC system consisted of two pumps (model LC-20AD; Shimadzu, Kyoto, Japan), an autoinjector (model SIL-20AC; Shimadzu), a column oven (model CTO-20AC; Shimadzu), and a photodiode array detector (model SPD-M20A; Shimadzu). A reversed phase column was used (150 × 2 mm i.d., 3 μm, Cadenza CD-C18; Imtakt, Co., Ltd., Kyoto, Japan). The mobile phase consisted of 7.5% (v/v) acetonitrile containing 0.1% (v/v) TFA (A) and 50% (v/v) acetonitrile containing 0.1% (v/v) TFA (B). Elution was performed using a 35% solution of B, at a flow rate of 0.3 mL/min for 18 min. The column oven was set at 40°C and the injection volume was 2 μL. Rutin and quercetin were identified by the retention time and the UV-vis spectra of standards. Quantification of rutin and quercetin was carried out according to an external standard method using calibration curves based on detection at 360 nm. Rutin and quercetin were quantified as mg per 100 g of dry flour.
Dried noodle and cookie preparations, respectively (Table 1). Only small amounts of quercetin were produced by hydrolyzation of rutin during noodle and cookie preparation because of the trace amount of rutinosidase in the ‘Manten-Kirari’ flour; quercetin was not originally present in flour. A statistical comparison of rutin and quercetin contents of noodles and cookies containing 50% Tartary buckwheat ‘Manten-Kirari’ flour retained 99.3% and 80.6% of rutin during preparation, respectively (Table 1). Only small amounts of quercetin were produced by hydrolyzation of rutin during noodle and cookie preparation because of the trace amount of rutinosidase in the ‘Manten-Kirari’ flour; quercetin was not originally present in flour. Mikami-Konishide et al. (2013) Thirty-five microliters of diluted extracts were transferred, in duplicate, to 96-well microplates, followed by the addition of 115 μL of 110.7 nM FL solution in assay buffer. After a pre-incubation of 15 min at 37°C, fluorescence intensity (Ex: 485 nm, Em: 530 nm) was measured using a microplate reader (Infinite M200 PRO; TECAN Japan Co., Ltd.). For H-ORAC, 50 μL of 31.7 mM AAPH assay buffer and for L-ORAC, 50 μL of 63.4 mM AAPH was added to each sample before measurement of fluorescence intensity for 90 min for H-ORAC and 120 min for L-ORAC at 2 min intervals. Data are expressed as μmol TE per gram. The contribution ratio of rutin or quercetin to the H-ORAC value was calculated as follows:

\[
\text{Contribution} \% = \left( \frac{\text{H-ORAC value of rutin or quercetin x rutin or quercetin content in sample}}{\text{H-ORAC value of sample}} \right) \times 100
\]

Statistical analysis Eight replications were performed for all analyses and data are shown as average ± standard error. Student’s t-test was used to show the differences between the rutin, quercetin and H-ORAC values before and after cooking the dried noodles.

### Results and Discussion

**Rutin and quercetin contents of noodles and cookies containing Tartary buckwheat flour with trace rutinosidase activity** Dried noodles and cookies containing 50% Tartary buckwheat ‘Manten-Kirari’ flour retained 99.3% and 80.6% of rutin during preparations, respectively (Table 1). Only small amounts of quercetin were produced by hydrolyzation of rutin during noodle and cookie preparation because of the trace amount of rutinosidase in the ‘Manten-Kirari’ flour; quercetin was not originally present in the flour. Suzuki et al. (2015) reported that the residual rutin ratio in dough prepared from ‘Manten-Kirari’ after the addition of water slightly and slowly decreased with increasing time and water to flour ratio, while in another Tartary buckwheat flour with normal rutinosidase activity, most of the rutin rapidly decomposed to quercetin on addition of water. It is suggested that rutin degradation in the dough is minimized by the trace rutinosidase activity of ‘Manten-Kirari’ during dried noodle and cookie preparation. The higher water content and increased time of dough preparation could have resulted in the increased degradation of rutin in cookies compared with noodles.

### Table 1. Rutin and quercetin contents in flour, dried noodles and cookies containing 50% Tartary buckwheat ‘Manten-Kirari’

<table>
<thead>
<tr>
<th>Foods</th>
<th>Rutin content (mg/100 g DW)</th>
<th>Quercetin content (mg/100 g DW)</th>
<th>Residual rutin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>2,712 ± 51.7</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>Dried noodle</td>
<td>1,269 ± 8.6</td>
<td>4.4 ± 2.9</td>
<td>99.3 ± 0.4</td>
</tr>
<tr>
<td>Cookie</td>
<td>1,421 ± 39.8</td>
<td>169 ± 15.1</td>
<td>80.6 ± 1.8</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE (n=8). n.d., not detected.
A substantial amount of rutin present in dried noodles made using common buckwheat was reduced by boiling (Ohara et al., 1989). Therefore, the ratios of residual rutin and quercetin were evaluated after cooking dried noodles made using Tartary buckwheat ‘Manten-Kirari’ in boiling water. The rutin content significantly reduced to 51.3% and quercetin content reduced to 61.3% (Fig. 1). The reductions were considered to be due to losses to the boiling water during cooking.

Previous reports measured the rutin content of noodles made with 70% common buckwheat flour as 7.84 mg/100 g DW and precooked groats as 8.79 mg/100 g DW (Kreft et al., 2006); bread containing 30% common buckwheat had between 3.47 mg/100 g DW and 3.82 mg/100 g DW rutin (Bojnanska et al., 2009), while the concentrations of rutin and quercetin were 253 mg/100 g DW and 163 mg/100 g DW, respectively, in cookies made with 50% other Tartary buckwheat flour (Wieslander et al., 2011). The foods made with ‘Manten-Kirari’ flour in this study had much higher rutin contents compared with foods made with common and other Tartary buckwheats.

Antioxidative activities of noodles and cookies containing Tartary buckwheat flour with trace rutinosidase activity In order to evaluate the antioxidative activities of the noodles and cookies made with Tartary buckwheat ‘Manten-Kirari’, DPPH radical scavenging activity and ORAC assays were performed. The H-ORAC value was about 4 times higher than the DPPH radical scavenging activity (Table 2). The L-ORAC values in dried noodles were below the limit of detection, and those in cookies were considerably low compared with H-ORAC (Table 2). Most of the antioxidants showed higher antioxidative activity in the H-ORAC assay compared to the DPPH assay (Watanabe et al., 2009), and the same trend in the difference between DPPH and H-ORAC assays was observed in other foodstuffs (Takebayashi et al., 2013). This trend is probably due to the difference in the antioxidative activity of the antioxidants (rutin and quercetin) in dried-noodles and cookies containing ‘Manten-Kirari’ flour between H-ORAC and DPPH assays. The contribution ratio of rutin to H-ORAC was 96.2% and that of quercetin was 0.8% in dried noodles, derived from the ORAC values of rutin (10,240 μmol TE/g DW) and quercetin (27,045 μmol TE/g DW). In cookies, the contribution of rutin was 69.8% and that of quercetin was 22.6%. These results confirmed that the antioxidative activities in dried noodles and cookies made with Tartary buckwheat ‘Manten-Kirari’ flour were mainly due to the hydrophilic antioxidant rutin.

Mikami-Konishida et al. (2013) reported that H-ORAC values ranged from 1.94 to 298.3 μmol TE/g fresh weight (FW) in 71 crops cultivated in Japan. The higher ORAC values were mainly measured in leaf and stem vegetables, such as mulukhiya (Corchorus olitorius) and perilla (Perilla frutescens). Takebayashi et al. (2013) reported that the H-ORAC values of 23 vegetables and 13 fruits commonly consumed in Japan ranged from 1.63 to 66.07 μmol TE/g FW and from 1.58 to 33.47 μmol TE/g FW, respectively. The average H-ORAC values of the 23 vegetables and 13 fruits was 6.95 μmol TE/g FW and 12.23 μmol TE/g FW, respectively. The H-ORAC values of raw whole seeds and groats of common buckwheat were 124.55 μmol TE/g DW and 95.01 μmol TE/g DW, respectively (Zielinski et al., 2009). In comparison, the H-ORAC values of noodles (136.3 μmol TE/g FW) and cookies (189.0 μmol TE/g FW) containing 50% Tartary buckwheat ‘Manten-Kirari’ flour were extremely high, even though in boiled noodles the value significantly decreased to 60.5% of that in dried noodles, with a corresponding decrease in rutin and quercetin (Fig. 1).

Takebayashi et al. (2013) reported that the estimated daily intake of hydrophilic antioxidants was 4,423 μmol TE per day, which is about 10% lower than the Japanese government recommendations of 4,879 μmol TE/g FW. Our results show that

<table>
<thead>
<tr>
<th>Foods</th>
<th>DPPH radical scavenging (μmol TE/g DW)</th>
<th>H-ORAC (μmol TE/g DW)</th>
<th>L-ORAC (μmol TE/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried noodle</td>
<td>38.3±0.5</td>
<td>149.8±2.9</td>
<td>–</td>
</tr>
<tr>
<td>Cookie</td>
<td>50.7±0.8</td>
<td>209.2±10.5</td>
<td>6.8±0.5</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE (n=8). -, below limit of detection.
boiled noodles containing 50% ‘Manten-Kirari’ flour provides 65.5 μmol TE/g FW. Hence, the intake of 100 g of dried noodles made with 50% ‘Manten-Kirari’ flour after boiling would result in the consumption of 6,550 μmol TE of hydrophilic antioxidants. We also estimate that 50 g (4 pieces) of cookies containing 50% ‘Manten-Kirari’ flour could provide 7,365 μmol TE of hydrophilic antioxidants. These results indicate that noodles and cookies made from the novel Tartary buckwheat variety ‘Manten-Kirari’ could contribute to an increase in antioxidant intake.

Rutin is the main hydrophilic antioxidant in noodles and cookies made using the Tartary buckwheat ‘Manten-Kirari’. Trace rutinosidase activity in ‘Manten-Kirari’ results in a higher rutin residual ratio and non-bitterness in the foods. Intake of abundant hydrophilic antioxidants (mainly rutin) from Tartary buckwheat ‘Manten-Kirari’ would be relatively easily accomplished, since soba-noodle is a popular menu choice in Japan. In addition, the non-bitter taste of this noodle would spur consumption. Dried-noodles made with ‘Manten-Kirari’ are now available to purchase, and human intervention studies of these foods have just started. Beneficial health effects of foods containing the Tartary buckwheat ‘Manten-Kirari’ are expected.

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References

Qu, Y., Yasuda, T., Nakajima, K., Hiwatashi, A., Moroi, C, Sanada, H., and Egashira, Y. (2013). Effect of rutin in buckwheat noodle on lipid...


