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 $\mu\text{mol TE/g DW}$ and 209.2 $\mu\text{mol TE/g DW}$ 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

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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 (Bojnanska *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 (Ghimera *et al.*, 2014; Xu *et al.*, 2014; Cho *et al.*, 2014; Zhou *et al.*, 2015). However, there are very few reports on the antioxidant 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 rutinoidase 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 rutinoidase 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-amidinopropane) 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-rutinoidase 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 μ m 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 μ m 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 μ m 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 x 2 mm i.d., 3 μ m, 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 μ L. 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

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

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

Data are presented as mean ± SE (n=8). n.d., not detected.

weight (DW). Residual rutin rates after preparation of dried noodles and cookies were calculated as the molar ratio of rutin to the sum of rutin and quercetin.

DPPH radical scavenging activity DPPH radical scavenging activity was assayed as previously described (Ishiguro *et al.*, 2007) with the following modifications: samples (100 mg) were vigorously mixed in 10 mL of 80% ethanol and heated in a boiling water bath for 5 min. The extract was diluted with 80% ethanol to various concentrations. Diluted samples (50 µL) were transferred to a 96-well microplate, and 50 µL of 2-morpholinoethanesulfonic acid (MES) buffer (200 mM MES, pH 6.0), 50 µL of 20% ethanol and 50 µL of DPPH solution (800 µM in ethanol) were added. After allowing the reaction to proceed for 20 min, absorbance at 520 nm was measured using a microplate reader (Infinite M200 PRO; TECAN Japan Co., Ltd., Kanagawa, Japan). Reaction solutions without DPPH were used as color controls, and Trolox was used as a standard. A_{520} values were plotted against the diluted sample, and a linear decrease in A_{520} of the DPPH radical was recorded. DPPH radical scavenging activity was expressed as µmol Trolox equivalents (TE) per gram of DW.

Hydrophilic and lipophilic oxygen radical absorbance capacities (H-ORAC and L-ORAC) Lipophilic and hydrophilic components in noodles and cookies were extracted according to the method of Wu *et al.* (2004) with slight modification. Five grams of sea sand were added to 1 g of either noodle or cookie sample, and initial extraction was performed with 10 mL of hexane/dichloromethane to extract the lipophilic components. Supernatants were collected after centrifugation at 3,000 g for 10 min. This step was repeated again before the supernatants were dried by rotary evaporator and redissolved in 10 mL of acetone. The extracts were then diluted 10- and 20-times by 7% MCD, and used in the L-ORAC assays. The precipitates, after hexane/dichloromethane extraction, were dried under nitrogen flow before the addition of 10 mL of acetone/water/acetic acid (70:29.5:0.5, AWA), and vortexing for 30 s, followed by sonication at 37°C for 5 min. After cooling at room temperature for 10 min, samples were centrifuged at 3,000 g for 10 min. This extraction step was repeated before supernatants were collected and made up to a final volume of 25 mL. The AWA extracts were diluted 100-, 200-, 400- and 800-times using assay buffer (75 mM potassium phosphate buffer (pH 7.4)) for use in the H-ORAC assays. H-ORAC and L-ORAC

assays were measured according to the method of 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:

$$\text{Contribution (\%)} = (\text{H-ORAC value of rutin or quercetin} \times \text{rutin or quercetin content in sample}) / (\text{H-ORAC value of sample}) \times 100$$

.....Eq. 1

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 rutinoidase 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 rutinoidase 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 rutinoidase 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 rutinoidase 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 2. DPPH radical scavenging activities, H-ORAC and L-ORAC values in dried noodles and cookies containing 50% Tartary buckwheat ‘Manten-Kirari’

Foods	DPPH radical scavenging ($\mu\text{mol TE/g DW}$)	H-ORAC ($\mu\text{mol TE/g DW}$)	L-ORAC ($\mu\text{mol TE/g DW}$)
Dried noodle	38.3 ± 0.5	149.8 ± 2.9	–
Cookie	50.7 ± 0.8	209.2 ± 10.5	6.8 ± 0.5

Data are presented as mean $\pm$ SE (n=8). –, below limit of detection.

Fig. 1. Residual ratio of rutin, quercetin and H-ORAC value of cooked noodles after boiling for 6 min. The boxes represent the relative contents of rutin and quercetin, or relative H-ORAC value. Vertical bars represent SE (n=5). ** Indicates statistical differences between raw and cooked noodles at a 1% level, according to the Student's *t*-test; n.s., not significant.

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 rutinoidase 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 $\mu\text{mol TE/g DW}$) and quercetin (27,045 $\mu\text{mol TE/g DW}$). 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 $\mu\text{mol TE/g}$ 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 $\mu\text{mol TE/g FW}$ and from 1.58 to 33.47 $\mu\text{mol TE/g FW}$, respectively. The average H-ORAC values of the 23 vegetables and 13 fruits was 6.95 $\mu\text{mol TE/g FW}$ and 12.23 $\mu\text{mol TE/g FW}$, respectively. The H-ORAC values of raw whole seeds and groats of common buckwheat were 124.55 $\mu\text{mol TE/g DW}$ and 95.01 $\mu\text{mol TE/g DW}$, respectively (Zielinski *et al.*, 2009). In comparison, the H-ORAC values of noodles (136.3 $\mu\text{mol TE/g FW}$) and cookies (189.0 $\mu\text{mol TE/g FW}$) 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 $\mu\text{mol TE}$ per day, which is about 10% lower than the Japanese government recommendations of 4,879 $\mu\text{mol TE/g FW}$. Our results show that

boiled noodles containing 50% 'Manten-Kirari' flour provides 65.5 $\mu\text{mol TE/g FW}$. 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 $\mu\text{mol TE}$ of hydrophilic antioxidants. We also estimate that 50 g (4 pieces) of cookies containing 50% 'Manten-Kirari' flour could provide 7,365 $\mu\text{mol TE}$ 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 rutinoidase 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

- Ames, B.N., Gold, L.S., and Willet, W.C. (1995). The causes and prevention of cancer. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 5258-5265.
- Awatsuhara, R., Harada, K., and Maeda, T. (2010). Antioxidative activity of the buckwheat polyphenol rutin in combination with ovalbumin. *Molecular Med. Rep.*, **3**, 121-125.
- Bojnanska, T., Francakova, H., Chlebo, P., and Vollmannova, A. (2009). Rutin content in buckwheat enriched bread and influence of its consumption on plasma total antioxidant status. *Czech J. Food Sci.*, **27**, s236-s240.
- Cho, Y.J., Bae, I.Y., Inglett, G.E., and Lee, S. (2014). Utilization of tartary buckwheat bran as a source of rutin and its effect on the rheological and antioxidant properties of wheat-based products. *Ind. Crop Prod.*, **61**, 211-216.
- Choi, I., Park, Y., Choi, H., and Lee, E.H. (2006). Anti-adipogenic activity of rutin in 3T3-L1 cells and mice fed with high-fat diet. *BioFactors*, **26**, 273-281.
- Diaz, M.N., Frei, B., and Keaney, J.F., Jr. (1997). Antioxidants and atherosclerotic heart disease. *New Engl. J. Med.*, **337**, 408-416.
- Engelhart, M.J., Geerlings, M.I., Ruitenbergh, A., van Swieten, J.C., Hofman, A., Witteman, J.C.M., and Breteler, M.M.B. (2008). Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA*, **287**, 3223-3229.
- Ghimeray, A.K., Sharma, P., Phoutaxay, P., Salitxay, T., Woo, S.H., Park, S.U., and Park, C.H. (2014). Far infrared irradiation alters total polyphenol, total flavonoid, antioxidant property and quercetin production in tartary buckwheat. *J. Cereal Sci.*, **59**, 167-172.
- Griffith, J.Q., Couch, J.F., and Lindauer, A. (1944). Effect of rutin on increased capillary fragility in man. *Proceedings of the Society for Exp. Biol. Med.*, **55**, 228-229.
- Guo, X.D., Ma, Y.J., Parry, J., Gao, J.M., Yu, L.L., and Wang, M. (2011). Phenolics content and antioxidant activity of tartary buckwheat from different locations. *Molecules*, **16**, 9850-9867.
- Hsu, C.L., Wu, C.H., Huang, S.L., and Yen, G.C. (2009). Phenolic compounds rutin and 0-coumaric acid ameliorate obesity induced by high-fat diet in rats. *J. Agric. Food Chem.*, **57**, 425-481.
- Ishiguro, K., Yahara, S., and Yoshimoto, M. (2007). Changes in polyphenolic content and radical-scavenging activity of sweetpotato (*Ipomoea batatas* L.) during storage at optimal and low temperatures. *J. Agric. Food Chem.*, **55**, 10773-10778.
- Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J.A., and Briggs, C.J. (2007). Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Res. Int.*, **40**, 356-364.
- Kawakami, A., Kayahara, H., and Ujihara, A. (1995). Properties and elimination of bitter components derived from Tartary buckwheat (*Fagopyrum tataricum*) flour. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **42**, 892-898 (in Japanese).
- Kreft, I., Fabjan, N., and Yasumoto, K. (2006). Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. *Food Chem.*, **98**, 508-512.
- Li, Y.Q., Zhou, F.C., and Gao, F. (2009). Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. *J. Agric. Food Chem.*, **57**, 11463-11468.
- Matsubara, Y., Kumamoto, H., Iizuka, Y., Murakami, T., Okamoto, K., Miyake, H., and Yokoi, K. (1985). Structure and hypotensive effect of flavonoid glycosides in *Citrus unshiu* peelings. *Agric. Biol. Chem.*, **49**, 909-914.
- Mikami-Konishida, I., Murakami, S., Nakanishi, K., Takahashi, Y., Yamaguchi, M., Shioya, T., Watanabe, J., and Hino, A. (2013). Antioxidant capacity and polyphenol content of extracts from crops cultivated in Japan, and the effect of cultivation environment. *Food Sci. Technol. Res.*, **19**, 69-79.
- Ohara, T., Ohinata, H., Muramatsu, N., and Matsushashi, T. (1989). Determination of rutin in buckwheat foods by high performance liquid chromatography. *Nippon Shokuhin Kogyo Gakkaishi*, **36**, 114-120 (in Japanese).
- Panchal, S.K., Poudyal, H., Arumugam, T.V., and Brown, L. (2011). Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. *J. Nutr.*, **141**, 1062-1069.
- Qu, Y., Yasuda, T., Nakajima, K., Hiwatashi, A., Moroi, C., Sanada, H., and Egashira, Y. (2013). Effect of rutin in buckwheat noodle on lipid

- metabolism in rats. *Food Sci. Technol. Res.*, **19**, 1011-1018.
- Shanno, R. (1946). Rutin: A new drug for the treatment of increased capillary fragility. *Am. J. Med. Sci.*, **211**, 539-543.
- Suzuki, T., Honda, Y., Funatsuki, W., and Nakatsuka, K. (2002). Purification and characterization of flavonol 3-glucosidase, and its activity during ripening in tartary buckwheat seeds. *Plant Sci.*, **163**, 417-423.
- Suzuki, T., Morishita, T., Mukasa, Y., Takigawa, S., Yokota, S., Ishiguro, K., and Noda, T. (2014a). Discovery and genetic analysis of non-bitter Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) with trace-rutinosidase activity. *Breeding Sci.*, **64**, 339-343.
- Suzuki, T., Morishita, T., Mukasa, Y., Takigawa, S., Yokota, S., Ishiguro, K., and Noda, T. (2014b). Breeding of 'Manten-Kirari', a non-bitter and trace-rutinosidase variety of Tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *Breeding Sci.*, **64**, 344-350.
- Suzuki, T., Morishita, T., Takigawa, S., Noda, T., and Ishiguro, K. (2015). Characterization of rutin-rich bread made with 'Manten-Kirari', a trace-rutinosidase variety of Tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *Food Sci Technol.*, **21**, 733-738.
- Takebayashi, J., Oki, T., Watanabe, J., Yamasaki K., Chen J., Sato-Furukawa, M., Tsubota-Utsugi, M., Taku, K., Goto, K., Matsumoto, T., and Ishimi, Y. (2013). Hydrophilic antioxidant capacities of vegetables and fruits commonly consumed in Japan and estimated average daily intake of hydrophilic antioxidants from these foods. *J. Food Compos. Anal.*, **29**, 25-31.
- Watanabe, J., Oki, T., Takebayashi, J., Yamasaki, K., and Tsushida, T. (2009). Approaches to establish the standardized methods for the determination of antioxidant capacities of foodstuffs. *Kagaku to Seibutsu*, **47**, 237-243 (in Japanese).
- Watanabe, J., Oki, T., Takebayashi, J., Yamasaki, K., Takano-Ishikawa, Y., Hino, A., and Yasui, A. (2012). Method validation by interlaboratory studies of improved hydrophilic oxygen radical absorbance capacity methods for the determination of antioxidant capacities of antioxidant solutions and food extracts. *Anal. Sci.*, **28**, 159-165.
- Wieslander, G., Fabjan, N., Vogrincic, M., Kreft, I., Janson, C., Spetz-Nyström, U., Vombergar, B., Tagesson, C., Leanderson, P., and Norbäck D. (2011). Eating buckwheat cookies is associated with the reduction in serum levels of myeloperoxidase and cholesterol: A double blind crossover study in day-care center staffs. *Tohoku J. Exp. Med.*, **225**, 123-130.
- Wieslander, G., Fabjan, N., Vogrincic, M., Kreft, I., Vombergar B., and Norbäck, D. (2012). Effect of common and tartary buckwheat consumption on mucosal symptoms, headache and tiredness: A double-blind crossover intervention study. *J. Food Agric. Environ.*, **10**, 107-110.
- Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., and Prior, R.L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.*, **52**, 4026-4037.
- Xu, F.Y., Gao, Q.H., Ma, Y.J., Guo, X.D., and Wang, M. (2014). Comparison of tartary buckwheat flour and sprouts steamed bread in quality and antioxidant property. *J. Food Quality*, **37**, 318-328.
- Yasuda, T., Masaki, K., and Kashiwagi, T. (1992). An enzyme degrading rutin in Tartary buckwheat seeds. *Nippon Shokuhin Kogyo Gakkaishi*, **39**, 994-1000 (in Japanese).
- Yasuda, T. and Nakagawa, H. (1994). Purification and characterization of rutin-degrading enzymes in Tartary buckwheat seeds. *Phytochem.*, **37**, 133-136.
- Zhou, X.L., Hao, T.F., Zhou, Y.M., Tang, W., Xiao, Y., Meng, X.X., and Fang, X. (2015). Relationships between antioxidant compounds and antioxidant activities of tartary buckwheat during germination. *J. Food Sci. Tech. Mys.*, **52**, 2458-2463.
- Zielinski, H., Michalska, A., Amigo-Benavent, M., Del Castillo, M.D., and Piskula, M.K. (2009). Changes in protein quality and antioxidant properties of buckwheat seeds and groats induced by roasting. *J. Agric. Food Chem.*, **57**, 4771-4776.