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Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products

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Abstract

The rutin content of buckwheat products was compared to the rutin content in their raw materials, in order to evaluate their value for producing functional foods. There is much less rutin in noodles (78 mg/kg, d.w.b. – dry weight basis), than in the dark buckwheat flour (218 mg/kg, d.w.b.) from which they are produced. One of the possible explanations is the presence of the rutin degrading enzyme. In raw (uncooked) groats there is 230 mg/kg (d.w.b.) of rutin and in precooked groats, 88 mg/kg (d.w.b.). In buckwheat beer and vinegar there is a negligible content of rutin. Buckwheat leaf flour contains about 2700 mg/kg (d.w.b.) rutin, and is thus a suitable material for enriching functional foods, giving it the potential for preventive nutrition.

Keywords: Buckwheat; Rutin; Flavonoids; Nutraceutics

1. Introduction

Rutin (quercetin-3-rutinosid) is a flavonol glycoside synthesized in higher plants as a protectant against ultraviolet radiation and disease (Gaberščik, Vončina, Trošt, Germ, & Björn, 2002; Rozema et al., 2002). The rutin present in our food and drinks has many interesting effects. The phenolic part of the molecule is linked with sugar – a hydrophilic part of the molecule. This reduces the biological effect a little but makes the molecule more soluble. Rutin is a secondary plant metabolite that antagonizes the increase of capillary fragility associated with haemorrhagic disease, reduces high blood pressure (Abeywardena & Head, 2001; Schilcher, Patz, & Schimmel, 1990), decreases the permeability of the blood vessels and has an anti-oedema effect, reduces the risk of arteriosclerosis (Wojcicki, Barcew-Wiszniewska, Samochowiec, & Rozewicka, 1995) and has antioxidant activity (Holasova et al., 2001; Krkošková & Mrázová, 2005; Park et al., 2000; Watanabe, 1998).

Rutin is widely present in plants but is relatively rare in their edible parts. It was first detected in *Ruta graveolens* which gave the common name to this pharmaceutically important substance (Chen, Huang, Huang, Wang, & Ou, 2001). Among fruits, vegetables and grain crops, grapes and buckwheat are the most important rutin containing foods. No rutin was found in cereals and pseudocereals except buckwheat, which can be used as a good source of dietary rutin (Kreft, Knapp, & Kreft, 1999; Ohsawa & Tsutsumi, 1995; Park et al., 2000; Watanabe, 1998). Different cultivars of buckwheat may have different contents of rutin (Ohsawa & Tsutsumi, 1995). Different parts of plants contain different concentrations of rutin. Most rutin is accumulated in the inflorescence (up to 12%, d.w.b. – dry weight basis), in stalks (0.4–1.0%, d.w.b.) and in upper leaves (8–10%, d.w.b.) (Hagels, 1999). Ecological factors, such as UV irradiation

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may also have a great influence on rutin content (Kreft, Strukelj, Gaberscik, & Kreft, 2002).

Finally, buckwheat is an important functional food. Besides various polyphenols (Luthar, 1992), it contains proteins with high biological value and balanced amino acid composition (Eggum, 1980; Eggum, Kreft, & Javornik, 1980; Ikeda, Kusano, & Yasumoto, 1986; Kayashita, Shimaoka, Nakajoh, Kishida, & Kato, 1999; Liu et al., 2001; Tomotake et al., 2000), relatively high fibre content (Bonafaccia, Marocchini, & Kreft, 2003; Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001), retrograded starch in groat products (Kreft & Skrabanja, 2002; Skrabanja, Laerke, & Kreft, 1998; Skrabanja & Kreft, 1998; Skrabanja, Liljeberg, Kreft, & Björck, 2001), high contents of available zinc, copper and manganese (Ikeda, Edotani, & Naito, 1990; Ikeda & Yamaguchi, 1993; Ikeda & Yamashita, 1994) and dietary selenium (Stibilj, Kreft, Smrkolj, & Osvald, 2004).

Buckwheat also contains some antinutritional factors. Allergenic reactions may be caused by ingesting buckwheat and its products or by exposure to buckwheat dust. The hypersensitive symptoms involve asthma and asthmatic attacks, urticaria and gastrointestinal disorders (Li & Zhang, 2001; Wieslander & Norbäck, 2001). As buckwheat does not contain gluten, it is a common supplement for patients with coeliac disease. Buckwheat intolerance is rare among patients with gluten intolerance alone, but more common in those with coeliac disease combined with other food allergies. Allergic reactions are caused by ingestion of allergenic buckwheat proteins (Wieslander & Norbäck, 2001). Ingestion of the entire plant can cause serious photosensitization. Phototoxity of fagopyrin is connected with sensitivity to ultraviolet rays (Li & Zhang, 2001). Polyphenols in buckwheat may also inhibit the action of certain enzymes (Eggum et al., 1980).

Buckwheat is grown in many countries in Asia, Europe and South Africa, in Canada, USA, Brazil and in certain other places around the world. Consequently, a large variety of buckwheat foods have been produced traditionally for centuries. Dishes made from buckwheat seed are generally classified into two groups, flour dishes and groats dishes (Ikeda, 1997; Ikeda, 2002; Kreft, 1995). The most popular food is buckwheat noodles, very popular in Japan, China and Italy, made from buckwheat flour–water dough. Increasing awareness and care for health has led to the production of high quality food products. The presence of rutin in buckwheat plants and foods is one of the main reasons for the production of different kinds of buckwheat foods. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) contains more rutin than common buckwheat (Suzuki, Yutaka, Funatsuki, & Nakatsuka, 2002). Other products made from buckwheat are buckwheat floral honey (Nagai, Sakai, Inoue, Inoue, & Suzuki, 2001; Paradkar & Irudayaraj, 2002), green buckwheat tea, buckwheat sprouts, buckwheat vinegar, buckwheat beer, buckwheat spirit and fresh green plant parts used as a vegetable (Kreft, 1994; Kreft, 1995).

The aim of this study was to compare the rutin contents of buckwheat materials and of their respective products, and to evaluate the value of the latter as possible functional foods.

2. Material and methods

2.1. Buckwheat samples

Common buckwheat (*Fagopyrum esculentum* Moench) grain cv. Siva II, grown in Dolenjska, Slovenia, was used to obtain dark buckwheat flour (59.6% extraction rate), and bran. Air dried dark buckwheat flour contained 11.2% w/w of moisture. Dough was made by the addition of the same amount of distilled water to the buckwheat flour in a bowl. Fresh buckwheat noodles were made according to the method described by Udesky (1995), using 70% dark buckwheat flour and 30% wheat flour. Dry buckwheat noodles were obtained by drying fresh buckwheat noodles for 24 h.

Pre-cooked buckwheat groats were obtained using the method described by Skrabanja et al. (1998); to mimic the traditional hydrothermal treatment, the groats were cooked at 100 °C for 60 min. Uncooked (raw) buckwheat groats were produced by dehusking buckwheat grain by hand.

Groats-tea is a commercial instant-tea product in Japan, produced from dehusked buckwheat grain, and declared as a rutin rich product. Leaf flour is a commercial raw material, produced by a local producer in Toyama-ken, Japan by milling dried buckwheat plants, harvested at flowering time. Buckwheat beer (Harpe noir, St-Servant, Rennes, France) is a French product, the raw material containing about 10% crushed buckwheat grain. Buckwheat vinegar is a Chinese commercial product (Shanxi Vinegar Factory, Taiyuan, China), made from tartary buckwheat grain. Prior to extraction, the liquid of buckwheat beer and vinegar was evaporated under the reduced pressure. Dry matter was determined in samples by drying at 105 °C until constant weight.

2.2. Extraction

Samples (250 mg), (solid samples milled by Udy-Tecator mill, Landskrona, Sweden), were extracted with 5 ml methanol/water (67:33) at room temperature, by shaking for 40 min, unless stated differently in the results and discussion section. Prior to extraction, the liquid of buckwheat beer and vinegar was evaporated under the reduced pressure, and the solid part of samples extracted with 5 ml methanol/water (67:33) at room temperature, by shaking for 40 min.

2.3. HPLC analyses

HPLC was performed using a Spectra-Physics (Mountain View, California, USA) instrument Spectra System P4000, Hibar – LiChrospher 100, RP-18 (5 μm) column (E. Merck, Darmstadt, Germany, 250 mm × 4 mm).

The solvents for HPLC were A acetonitrile and methanol (1:2), and B 0.75% aq. H_3PO_4. Initial condition was 100% B. The samples were run on a linear gradient to 60% A and 40% B in 20 min; and then a linear gradient to 100% A and 0% B for a further 20 min, and finally 10 min equilibration (100% B). The compounds were detected at 380 nm and identified by comparison of the retention time with the retention time of the standard solutions.

All analyses were performed in triplicate in three independent samples.

2.4. Statistical analysis

The data were analyzed statistically using STATG (Statgraphics 5.0, Statistical Graphics Corporation, USA).

3. Results and discussion

3.1. Rutin extraction efficiency

In Fig. 1, is shown the result of rutin extracted from buckwheat flour, using different solvents. Methanol/water (67:33) is a better extraction medium for buckwheat rutin than 100% methanol. This is probably due to the presence of a more hydrophilic sugar moiety attached to the quercetin part of molecule. In buckwheat raw material, rutin is non-covalently associated with other molecules, and included in the buckwheat plant's cellular structures. The solvent has to enter the structures by imbibition, in order to reach the rutin molecules and break the non-covalent bonds between rutin and other cellular substances. Having solubilized the rutin, the solvent is separated from the mainly insoluble cell material by centrifugation, leaving the rutin in the supernatant. Other molecules may also be extracted, which can counteract the solubility or persistence of rutin molecules. In the case of an aqueous solvent, rutin degrading enzymes may be extracted, which would lower the yield of extracted rutin.

The relation of the amount of rutin extracted to the extraction time is shown in Fig. 2. Thirty minutes is enough to extract rutin from dry noodles, but not from the light flour, and especially not from the dark flour. The experiment was repeated with the same ratio of solvent to rutin in all three kind of samples. However, the quantity of rutin extracted was not significantly different from that obtained in the experiment performed with the same amount of solvent for each sample. Thus, saturation of solvent with rutin was excluded as a possible reason for the longer optimal rutin extraction time required for dark flour than for noodles produced from this dark flour. A possible explanation is that, during the dough making, some rutin is removed from its original location in the flour particles and dissolves in the water that is added to flour for dough making. When the noodles are dried, rutin is probably deposited on the surface of particles, possibly less strongly bound than on the original site in cellular structures. However, there is much less rutin in noodles than in the corresponding amount of dark flour from which they were produced.

3.2. Rutin content of buckwheat materials and products

The rutin content of three buckwheat grain samples is given in Table 1. The findings of Ohsawa and Tsutsumi (1995), that there are inter-varietal variations of rutin content in common buckwheat, were confirmed by our results.

The rutin content in noodles is compared with the content in dark flour (Table 2). As noodles contain only 70% of buckwheat flour, the expected amount of rutin in

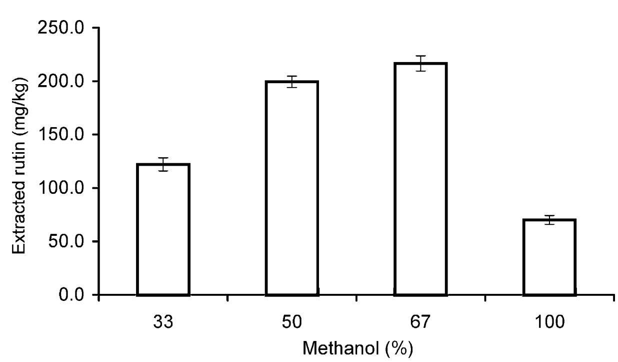

Fig. 1. Amount of rutin extracted from buckwheat flour, with different proportions of methanol in the methanol/water solvents.

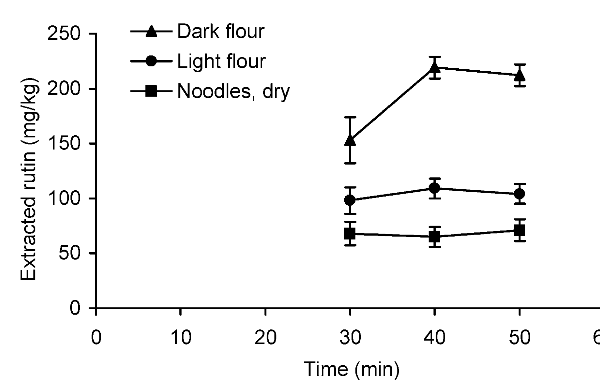

Fig. 2. Rutin extracted from buckwheat samples as a function of the extraction time (mg/kg, sample dry-weight basis ±SD, $n = 3$).

Table 1
Rutin content in buckwheat grain of three different cultivars (mg/kg, dry-weight basis ± SD[a])

Cultivar	Rutin
Darina	181.3 (±5.7)[a]
Darja	115.9 (±6.1)
Siva II	181.9 (±7.0)

[a] Standard deviation for three independent determinations.

Table 2
Rutin content in buckwheat materials and products (mg/kg, dry-weight basis ± SD[a])

Sample	Rutin
Dark flour	218.5 (±7.1)[a]
Noodles, expected	153.0
Noodles, fresh	78.4 (±11.5)
Noodles, dry	67.6 (±8.9)

[a] Standard deviation for three independent determinations.

Table 3
Rutin content in buckwheat materials and products (mg/kg, dry-weight basis ± SD[a])

Sample	Rutin
Groats (raw)	230.1 (±11.4)
Groats (precooked)	87.9 (±10.7)

[a] Standard deviation for three independent determinations.

Table 4
Rutin content in buckwheat materials and products (mg/kg, dry-weight basis ± SD[a])

Sample	Rutin
Light flour	112.8 (±7.7)
Groats tea	204.3 (±18.4)
Leaf flour	2692.0 (±37.5)
Buckwheat beer	<1
Buckwheat vinegar	<1

[a] Standard deviation for three independent determinations.

noodles would be about 153 mg/kg (d.w.b.). However, the actual quantity extracted from the noodles is much less. A possible explanation is the presence and activity of the rutin degrading enzyme flavonol 3-glucosidase. The enzyme was originally isolated from tartary buckwheat (Suzuki et al., 2002; Yasuda & Nakagawa, 1994), but was recently found as well in common buckwheat grain, mainly in the testa (Suzuki et al., 2002).

The impact on the rutin content of the hydrothermal treatment of buckwheat grain during the production of groats, is obvious from the results presented in Table 3. It is possible that rutin was degraded or combined with some other molecules, in such a way that it became insoluble in the solvent. The influence of the hydrothermal procedure, similar to that, traditional in central and eastern European countries, for producing precooked groats, has thus an important effect on the content of the extractable rutin (Table 3). This would not be expected, but Dietrych-Szostak and Oleszek (1999) reported a drastic effect of temperature on the flavonoid content in de-hulled buckwheat groats. Dimberg, Molteberg, Solheim, and Frölich (1996) also reported a decrease of content of phenolic substances in heat processed oats.

The rutin content in some other buckwheat materials and products is presented in Table 4. Light flour and groats tea has the expected content of rutin, not very different from that in dark buckwheat flour. The content of rutin in leaf flour is much higher. This flour is used in Japan as an additive to some food products, for example ice cream, for the attractive green colour of the products, and to declare the functionality of the product, dependent on the rutin content. In buckwheat beer and vinegar, there is only a small content of rutin, if any, possibly due to the long production procedure, which is obviously not suitable for maintaining the original content of rutin in the products.

Buckwheat materials have potential, at least in regard to the rutin content, as a functional food. However, attention should be paid, during processing, to the factors which may lower the rutin content.

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