LWT - Food Science and Technology 79 (2017) 423–427

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Determination of fagopyrins, rutin, and quercetin in Tartary buckwheat products

Nina Kočevar Glavač [a, *], Katja Stojilkovski [a], Samo Kreft [a], Cheol Ho Park [b], Ivan Kreft [c]

[a] Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia
[b] Department of Bio-Health Technology, College of Biomedical Science, Kangwon National University, 200-701 Chunchon, South Korea
[c] Institute of Nutrition, Tržaška 40, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form
19 January 2017
Accepted 24 January 2017
Available online 25 January 2017

Keywords:
Tartary buckwheat
Fagopyrum tataricum
Fagopyrin
Rutin
Quercetin

Chemical compounds studied in this article:
Fagopyrin (PubChem CID:5488537)
Rutin (PubChem CID:5280805)
Quercetin (PubChem CID:5280343)

ABSTRACT

Buckwheat grain is a valuable source of various phytochemicals: health promoting flavonoids (rutin), as well as substances such as phototoxic fagopyrin. In this study, total fagopyrins, rutin, and quercetin were determined in Tartary buckwheat grains, milled fractions, and food products. HPLC was used for analyses; fagopyrins were detected by fluorescence, flavonoids by UV/VIS. After steaming, the fagopyrin content in the grain decreased. The remaining fagopyrins were unequally distributed; minor amounts were found in groats and higher concentration in hulls. Similarly, the concentration of rutin decreased, although to a lesser extent. Unlike fagopyrins, rutin was concentrated in groats. In the bread making procedure, a minor decrease of fagopyrins was seen comparing its concentration in grains, dough, and bread, while large amounts of rutin were degraded into quercetin during baking. Our results may stimulate further research on edible parts and products of buckwheat, particularly, the quantitative evaluation of total fagopyrin content in relation to flavonoids, providing information about the treatment process needed to prepare buckwheat products with the highest utilization value.

1. Introduction

Buckwheat grain is rich in valuable nutrients and phytochemicals (Bonafaccia, Marocchini, & Kreft, 2003; Choi, Morrison, Hughes, Marriott, & Small, 2013; Holasová et al., 2002; Sedej et al., 2011). It has been recognized as an important part of a healthy diet, mainly because of its high polyphenol content, especially rutin. Rutin is used to treat vein diseases (Ihme et al., 1996), retinopathy (Archimowicz-Cyryłowska, Adamek, Droździk, Samochowiec, & Wójcicki, 1996), serum holesterol (Wieslander et al., 2011), and fatigue symptoms (Wieslander et al., 2012). Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) contains more rutin in comparison to common buckwheat (*Fagopyrum esculentum* Moench) (Kim et al., 2008; Zielińska, Turemko, Kwiatkowski, & Zieliński, 2012). Minor amounts of quercetin found in Tartary buckwheat seeds (Fabjan et al., 2003) are the result of rutin degradation (Vogrinčič, Timoracka, Melichacova, Vollmannová, & Kreft, 2010).

In addition to valuable polyphenols, buckwheat contains fagopyrins, phototoxic naftodianthrones similar to hypericin (Brockmann, Weber, & Pampus, 1952). Phototoxic effects caused by fagopyrins following exposure to sunshine are known as fagopyrism (Wender, Gortner, & Inman, 1943). Fagopyrism was first documented in China in the 7th century when it was noted that eating uncooked buckwheat leaves could cause skin itching; and in the 13th century, it was reported that consuming large amounts of buckwheat leaves caused dermatitis, breathlessness, fainting, and hair loss (Zhang, Wang, & Zhao, 2003). In 1941, phototoxicity was confirmed *in vivo* in unpigmented rats and mice, the reported symptoms included inflammation of skin (ears, nose, paws, tail) and mucous membranes (conjunctivitis, diarrhoea), and disorders of the central nervous system (convulsions) (Chick & Ellinger, 1941). However, fagopyrin toxicity is considered less severe than hypericin toxicity (Theurer, Gruetzner, Freeman, & Koetter, 1997). The phototoxic dose of fagopyrins for humans is still unknown. In rats, fagopyrin doses in the range of 2.5–3 mg/kg body weight caused health problems (Theurer et al., 1997). It has been shown that

* Corresponding author.
E-mail address: nina.kocevar.glavac@ffa.uni-lj.si (N. Kočevar Glavač).

http://dx.doi.org/10.1016/j.lwt.2017.01.068
0023-6438/

flavonoids have the ability to protect from naftodianthrone phototoxicity (Wilhelm, Biel, & Siegers, 2001). Other effects of fagopyrins remain mostly unknown. However, its antifungal activity has been reported only recently (Sytar et al., 2016).

Fagopyrins are formed from protofagopyrin and other related substances following exposure to sunlight (Habermann, 2000); similarly, hypericin is a metabolite produced from protohypericin (Alali, Tawaha, & Al-Eleimat, 2004). HPLC analyses of Tartary buckwheat revealed that fagopyrins are composed of at least 8 similar substances that were characterized by UV–Vis absorption, NMR spectroscopy, and mass spectrometry (Eguchi, Anase, & Osuga, 2009; Tavčar Benković, Žigon, Friedrich, Plavec, & Kreft, 2014). Most recently, Tavčar Benković, & Kreft, 2015 elucidated structures of two new fagopyrins (A and E). Despite numerous research attempts, an efficient preparative method for isolation of single fagopyrins to obtain reference standards has not yet been reported (Tavčar Benković, & Kreft, 2015). Consequently, the phototoxic potential of a single fagopyrin compound remains unknown. Therefore, we considered it reasonable to analyse fagopyrins together as a group, i.e. as total fagopyrins.

Quantitative data on fagopyrins in buckwheat is limited and mainly refers to its concentration in the green parts of the plant (Eguchi et al., 2009; Hinneburg & Neubert, 2005; Ožbolt, Kreft, Kreft, Germ, & Stibilj, 2008; Stojilkovski, Kočevar Glavač, Kreft, & Kreft, 2013). The total fagopyrin content of the common buckwheat herb is 3.7–4.8 mg/g; the content of the common buckwheat and Tartary leaf is 0.3–2.3 mg/g and 0.5 mg/g, respectively; and the total fagopyrin content of the Tartary buckwheat grain is 0.07 mg/g (Stojilkovski et al, 2013). It has been shown that compositions of different grain milled fractions differ considerably as the milled fractions reflect the composition of corresponding seed tissues (Park et al., 2000; Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001a). Data are available on nutrients in different processed fractions, such as carbohydrates, proteins, lipids (Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001b), and rutin (Park et al., 2000). However, the total fagopyrin content has never been studied in detail.

The aim of this study was to determine the content of total fagopyrins and two flavonoids, rutin and quercetin, in various traditional Tartary buckwheat grain products, as well as in novel products available in Korean and Slovenian markets. This information is important and necessary for designing food products and adapting new technology for grain or food processing.

2. Materials and methods

2.1. Production of Tartary buckwheat grain tea and respective side products

The Tartary buckwheat grain was dehulled in a small private company (Chunchon, Kangwon Region, South Korea) that regularly produces and sells an Asian buckwheat grain tea that is rich in rutin (Table 1, sample A). The authors (C. H. P. and I. K.) followed technological procedure and took samples for analyses at different steps of the procedure (samples A-I). The procedure includes mechanical cleaning of the grains, steaming for 30 min, drying, dehulling, sieving and consequent sorting by air flow to separate hulls and other particles, drying, storage, and packing.

2.2. Products made of Tartary buckwheat sprouts and herb

Two additional samples (samples J and K) were produced and collected in the above mentioned South Korean company. Tartary buckwheat grains were grown for 10 days in a dark place at room temperature, the sprouts were then harvested, dried, and pressed into tablets (sample J). Sample K, experimental expanded briquettes, was obtained by harvesting the flowering Tartary buckwheat plants and expanding the material under pressure using a single screw press to obtain a dry product used for food supplementation.

2.3. Preparation of flour, dough, and bread from Tartary buckwheat

Tartary buckwheat grains (sample L) were milled into semolina (sample M), flour (sample N), and four hull fractions (samples O, P, and R). Yeasted bread dough (sample S) and bread (sample T) were prepared using the fine flour fraction according to the traditional procedure for yeasted bread but without the addition of wheat flour, as described in details by Vogrinčič et al. (Vogrinčič et al., 2010).

2.4. Extraction

Homogenized samples (400 mg) were extracted with 5 mL of methanol for 4 h at 65 °C, according to the method previously optimized and validated by Stojilkovski et al. (Stojilkovski et al, 2013). The extraction method was optimized for extraction of fagopyrins (temperature and time of extraction, successive extraction steps, and ultrasonic extraction were studied), but not for rutin. The starch bound fraction of rutin might not be extracted using methanol (Kreft, Knapp, & Kreft, 1999), therefore, all results are expressed as the extractable fraction of rutin. Experimental data showed that rutin is stable during the extraction process (data not shown).

2.5. HPLC

The HPLC method was based on the work of Eguchi et al (Eguchi et al., 2009). and optimized in our laboratory as described by Stojilkovski et al. (Stojilkovski et al, 2013). In brief, sample extracts (20 μL) were injected into an HPLC system (UFCL XR Shimadzu 02AD XR, Japan) using Ascentis Express C18 column (2.7 μm, 10 cm × 4.6 mm, Supelco, Pennsylvania), at 60 °C, and a flow rate of 2 mL/min. Fagopyrins were detected using a fluorescence detector (Shimadzu RF-10A XL), at an excitation wavelength of 330 nm, an mission wavelength of 590 nm, and the content was calculated using the hypericin standard because the fagopyrin standard is not available. Fagopyrins' peaks were identified by their fluorescence spectra and by comparing their retention time with measurements reported by Eguchi et al. (Eguchi et al., 2009). Rutin and quercetin were detected by an UV/Vis detector (Shimadzu SPD-M20A) at 353 nm, and their content was calculated using rutin (Carl Roth, Germany) and quercetin (Sigma-Aldrich, Germany) standards. Retention time of hypericin was 10.15 min, retention time of fagopyrins was 6–7 min, of rutin 3.85 min, and of quercetin 4.15 min. Limit of quantification (LOQ), determined as concentration with signal-to-noise ratio 10:1, was 0.5 mg/mL for hypericin, 0.0002 mg/mL for rutin, and 0.001 mg/mL for quercetin. Linearity of calibration curve was adequate ($R > 0.999$ for hypericin, rutin, and quercetin).

3. Results and discussion

3.1. Steaming and roasting

The total fagopyrin content of the Tartary buckwheat grain (Table 2, sample A) decreased by 3-fold after steaming (sample B), from an initial concentration 53.70 μg/g to 17.11 μg/g. The remaining fagopyrins were unequally distributed in the grain. In groats (steamed grains without hulls), its concentration was only 3.35 μg/g (sample C). Thus, most of the fagopyrins were located in