Short Communication

Fagopyrin and flavonoid contents in common, Tartary, and cymosum buckwheat

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A B S T R A C T

Fagopyrin is a phototoxic substance found in buckwheat products in varying amounts. The aim of this study was to determine the fagopyrin, rutin, and quercetin contents of plant samples taken from 3 buckwheat species, namely common (Fagopyrum esculentum), Tartary (F. tataricum), and cymosum buckwheat (F. cymosum). HPLC was optimized for fagopyrin analysis using a fluorescence detector, and for other components a UV/VIS detector, while hypericin, rutin, and quercetin were used as standards. Common buckwheat leaves contained high levels of fagopyrin (322–2300 µg g⁻¹) while even higher levels were found in other samples such as the flowers (≤4830 µg g⁻¹). The highest fagopyrin content (20,700 µg g⁻¹) was found in the flowers of F. cymosum. Two cymosum samples had a high proportion of fagopyrin relative to rutin (∼200 µg fagopyrin mg⁻¹, rutin), whereas this proportion was lower (15–90 µg fagopyrin mg⁻¹ rutin) in other samples.

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1. Introduction

Buckwheat contains numerous beneficial nutrients and phytochemicals (Bonafaccia et al., 2003; Brindzova et al., 2009; Kalinova and Vrchotova, 2011; Zhang et al., 2012) and it is used both as a food and as a medicinal plant (Wieslander and Norbäck, 2001; Guo et al., 2012; Gulpinar et al., 2012). Buckwheat herb tea or tablets contain rutin, and are used to treat vein diseases (Ihme et al., 1996) and retinopathy (Archimowicz-Cyrylowska et al., 1996). The use of fresh buckwheat is not common in Europe, whereas in Asia the young herb is extensively used as a vegetable, and green leaf flour is used as an additive or a natural food coloring (Kreft et al., 2006). Three main species of buckwheat are utilized for food, i.e. common buckwheat (Fagopyrum esculentum), Tartary buckwheat (F. tataricum), and cymosum (F. cymosum). Cymosum buckwheat is listed as a medicinal plant in Nepal, India, Thailand and China. The young wild plants are used as cattle feed in Nepal and Bhutan (Ohnishi, 2003) and as a vegetable for human consumption in India (Tahir and Farooq, 1989).

The rutin and quercetin levels in buckwheat vary depending on the species and cultivation conditions (Kalinova and Vrchotova, 2011). The rutin content is highest in the flowering stage of buckwheat (Metzger et al., 2010). Compared with common buckwheat seeds, Tartary buckwheat seeds contain more rutin and they are an important health-promoting food. Trace amounts of quercetin are present in Tartary buckwheat seeds, but not in common buckwheat (Fabjan et al., 2003). The presence of quercetin in Tartary buckwheat samples is probably caused by the degradation of rutin (Vogrinčič et al., 2010; Yoo et al., 2012).

In addition to its beneficial compounds, buckwheat contains fagopyrin, which is a naftodianthrone related to hypericin (Brockmann et al., 1950, 1952). This substance provokes phototoxic effects known as fagopyrism (Wender et al., 1943). In China, there are records dating from the 7th century A.D. reporting that eating uncooked buckwheat leaves can cause skin irritation, while one from the 13th century reports that the consumption of too many buckwheat leaves in the spring may cause breathlessness, faintness, loss of hair, dermatitis, and other adverse symptoms (Zhang et al., 2003).

More recently, problems associated with consuming the green parts of buckwheat plants have been reported in Western literature. Fagopyrin is reported to be less phototoxic than hypericin (Theurer et al., 1997). In the past, fagopyrism was found in cattle and other farm animals, but it also occurs in humans. The most common symptoms are redness, swelling, and
inflammation of hairless skin areas after exposure to sunlight (Chick and Ellinger, 1941). Fagopyrin is produced from proto-
flower and similar substances after exposure to daylight (Habermann, 2000), which is similar to hypericin formation from protho-
potentand that (Alali et al., 2004). Data on the fagopyrin content of buckwheat is scarce and there is no information on the
phototoxic dose in humans.

Previous studies have shown that the flowers of common buckwheat contained 0.64 mg g⁻¹ fagopyrin, leaves contained 0.4–
0.6 mg g⁻¹, and the stems contained 0.04–0.12 mg g⁻¹. The hulls contained 0.02 mg g⁻¹ but no fagopyrin was found in buckwheat
groats (Ozbolt et al., 2008; Eguchi et al., 2009). Chick and Ellinger (1941) fed rats with different parts of buckwheat and found that
flowers induced the most severe photosensitivity. Swelling occurred in rats at doses of 2.5–3.0 g of flowers kg⁻¹ body weight (equivalent
to approximately 2.5–3.0 mg kg⁻¹ fagopyrin) (Chick and Ellinger, 1941).

Analyses of fagopyrin content have been reported in only a few
recent papers (Ozbolt et al., 2008; Eguchi et al., 2009; Hinneburg et al., 2009). Flavonoids can protect from naftodanthrone
phototoxicity (Wilhelm et al., 2001), so it is important to know the
fagopyrin content and its level relative to flavonoids.

The aim of this investigation was to determine the fagopyrin,
rutin, and quercetin content in the plant parts of three buckwheat
species, which are used in traditional food products, or novel ones
that are available on the market.

2. Materials and methods

2.1. Plant material

The following samples were collected from three buckwheat
species:

A. Fagopyrum cymosum leaves, collected in the vicinity of Lugu
Lake, Junnan, China, 2700 m altitude.
B. F. cymosum stems, collected in the vicinity of Lugu Lake, Junnan,
China, 2700 m altitude.
C. F. cymosum herb collected by Prof. C. H. Park in Chunghung area,
South Korea.
D. F. cymosum flowers, collected in Slovenia (Selo, Žirovnica),
seeds obtained from Shillong, India.
E. F. cymosum leaves, collected in Slovenia (Selo, Žirovnica), seeds
obtained from Shillong, India.
F. Buckwheat tea (herb) commercial sample from Bosnia and
Herzegovina.
G. Buckwheat tea (herb) from Biodynamic farm A. Č., (Slovenia).
H. Buckwheat tea (herb) from the traditional herbalist T. N.
(Ljubljana, Slovenia).
I. Buckwheat tea, (herb) producer I. (Czech Republic).
J. Product J; (herb) tea bags.
K. Product K; tea bags.
L. Product L; tea bags.
M. Buckwheat tea (herb), producer M. (Austria).
N. Leaves of buckwheat, cv. Siva, cultivated on field 1 in Šentjernej
(Slovenia).
O. Leaves of buckwheat, cv. Siva, cultivated on field 2 in Šentjernej
(Slovenia).
P. F. tataricum leaves, cultivated in Sichuan, Xichan region, China,
at 2700 m altitude.
Q. F. tataricum seeds (with hulls), cultivated in Sichuan, Xichan
region, China, at 2700 m altitude.

Samples A–E and N–R were collected in the fields following
local custom. Samples F–H were purchased in the local markets,
and samples J–M in food shops. Some samples are similar (sample
K and L and samples N and O) and could be considered as two lots
of one sample. The weight of samples varied from 30–100 g.

2.2. Extraction

Sample material was homogenized using a mill (Blender
8010EB, model HGBTW, Waring Commercial, Stamford, Connecti-
icut, USA) or a pestle and mortar. Fagopyrin, rutin, and quercetin
were extracted with methanol (HPLC grade, Sigma–Aldrich, St.
Louis, Missouri, USA). Dry samples (200 mg) were suspended in
methanol (10 mL) and thoroughly mixed for 1 min. The suspensions
were then incubated for 4 h at 65 °C. Samples were mixed for 1
min and centrifuged at 1000 rpm for 3 min at 25 °C. Next, 900 μL
aliquots of the clear supernatants were dispensed into 2 mL plastic
test tubes (TPP, Trasdingen, Switzerland), diluted with 900 μL of
methanol, and filtered through membrane filters (Milllex-GN
filters; pore size = 0.2 μm, Millipore Corporation, Billerica, Mas-
sachusetts, USA).

2.3. HPLC analyses of fagopyrin, rutin, and quercetin

The extracts were analyzed by HPLC, according to the method of
Eguchi et al. (2009) with some modifications. In our laboratory, we
used the following HPLC system and settings: UFCL XR Shimadzu
O2AD XR Kyoto, Japan; injection volume: 20 μL; column: Ascentis
Express C18 (2.7 μm, 10 cm × 4.6 mm; Supelco, Sigma–Aldrich
group, St. Louis, Missouri, USA) at 40 °C; flow rate: 2 mL min⁻¹;
mobility phase: A (distilled water with 0.1% trifluoroacetic acid
(HPLC grade, Carlo Erba, Rodano, Italy) and B (acetonitrile
(HPLC grade, J. T. Baker, Deventer, Netherlands) with 0.1% trifluoroacetic
acid); elution gradient: 0–1 min at 0% B, 1–2 min at 0–60% B, 2–
8 min at 60–100% B, 8–15 min at 100% B, and 15.01–18 min at 0% B.
Fagopyrin was detected using a fluorescence detector (Shimadzu
RF–10A XL, Kyoto, Japan;) at an excitation wavelength of 330 nm
and an emission wavelength of 590 nm. The fagopyrin content was
calculated using hypericin standards because a fagopyrin standard
was not available. Rutin (p.a., Carl Roth, Karlsruhe, Germany),
quercetin (p.a., Sigma–Aldrich, St. Louis, Missouri, USA), and other
compounds were determined using a UV/VIS detector (Shimadzu
SPD–M20A, Kyoto, Japan;) at 353 nm, and their content was
calculated using rutin and quercetin standards. The detectors were
connected in series, UV–vis first and fluorescence second. The
volume of the tubes between the detectors (80 μL) resulted in
slight (2.5 s) shift of the retention times.

The identification of peaks was based on their fluorescence
spectra and by comparing the retention time with the measure-
ments of Eguchi et al. (2009). Similar to Eguchi et al. (2009),
several peaks in the fagopyrin region of the chromatogram (Fig. 1) were
observed. We considered that all of these peaks represented
fagopyrin derivatives, and all of the peaks were integrated
together. No differences were observed in the ratios of the
fagopyrin derivatives in different samples. The total content of
fagopyrin was determined relative to the hypericin content,
because its spectral characteristics and structure are similar to
fagopyrin (Ozbolt et al., 2008; Hinneburg et al., 2005).

Several unidentified peaks (with flavonoid-like absorbance
spectra) were observed in some samples. Two independent
extractions and analyses were performed for each sample. The
averaged results are presented in Table 1.

3. Results and discussion

A selective, reproducible and sensitive method for simulta-
neous determination of fagopyrin, rutin and quercetin was
optimized. Fagopyrin, rutin and quercetin UV absorptions do not
interfere since the retention times are sufficiently different. Retention time of fagopyrin was 6–7 min. Retention time of rutin was 3.85 min, and of quercetin, 4.15 min. Limit of quantification (LOQ) for the HPLC method determined as concentration with signal-to-noise ratio 10:1 was 0.5 μg mL$^{-1}$ for hypericin, 0.0002 μg mL$^{-1}$ for rutin and 0.001 μg mL$^{-1}$ for quercetin. In combination with the applied sample preparation method, this corresponds to the LOQ in buckwheat samples of 50 μg g$^{-1}$ for hypericin, 0.02 μg g$^{-1}$ for rutin and 0.1 μg g$^{-1}$ for quercetin. Linearity of calibration curve was adequate ($R > 0.999$ for hypericin, rutin and quercetin).

Samples of common buckwheat leaves contained a high level of fagopyrin (322–2300 μg g$^{-1}$) (Table 1). A very high content was found in common buckwheat samples, especially the flowers (≤4830 μg g$^{-1}$ fagopyrin). The highest fagopyrin (20,700 μg g$^{-1}$) content was found in flower samples from F. cymosum. Two cymosum plant samples had a relatively high proportion of fagopyrin relative to rutin (∼200 μg fagopyrin mg$^{-1}$ rutin), whereas this proportion was much lower in the other buckwheat herb samples we investigated (15–90 μg fagopyrin mg$^{-1}$ rutin). Tartary buckwheat seed contained more rutin (12.9 mg g$^{-1}$) than leaf samples from the same plants (9.9 mg g$^{-1}$). There was a much lower fagopyrin content (68 μg g$^{-1}$) in the seed than the leaves (512 μg g$^{-1}$) and the seed contained a low proportion of fagopyrin relative to rutin (∼5.3 μg fagopyrin mg$^{-1}$ rutin). Thus, Tartary buckwheat seed is suitable for making products with a high rutin content and a relatively low fagopyrin content.

Samples of inflorescences (flowers) generally had a high rutin content and a higher level of fagopyrin relative to rutin compared with leaf samples (Table 1). Thus, flowers contained a higher concentration of rutin, which is desirable, but also a high level of fagopyrin, which is not. The highest rutin level was found in a cymosum buckwheat flower sample, which also had the highest concentration of fagopyrin. It is not known whether fagopyrin might have therapeutic or other desirable effects, similar to products containing hypericin.

**Fig. 1.** Chromatograms obtained at HPLC analysis of buckwheat flowers using fluorescence (top) or absorbance (bottom) detector.
Table 1
Content of fagopyrin (μg·g⁻¹ of product dry matter), rutin (mg/g of product dry matter), fagopyrin/rutin ratio (μg·mg⁻¹), and quercetin (mg·g⁻¹ of product dry matter) in buckwheat samples.

<table>
<thead>
<tr>
<th>Sample mark</th>
<th>Species</th>
<th>Country</th>
<th>Part of the plant</th>
<th>Fagopyrin (μg·g⁻¹ of product)</th>
<th>Rutin (mg·g⁻¹ of product)</th>
<th>Fagopyrin rutin ratio (μg·mg⁻¹)</th>
<th>Quercetin (mg·g⁻¹ of product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F. cymosum</td>
<td>China</td>
<td>Leaves</td>
<td>936</td>
<td>28.8</td>
<td>32.5</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>F. cymosum</td>
<td>China</td>
<td>Stems</td>
<td>282</td>
<td>17.6</td>
<td>16.0</td>
<td>0.25</td>
</tr>
<tr>
<td>C</td>
<td>F. cymosum</td>
<td>Korea</td>
<td>Leaves and thin stems</td>
<td>1930</td>
<td>9.6</td>
<td>201</td>
<td>0.16</td>
</tr>
<tr>
<td>D</td>
<td>F. cymosum</td>
<td>Slovenia</td>
<td>Flowers</td>
<td>20,800</td>
<td>113</td>
<td>183</td>
<td>0.39</td>
</tr>
<tr>
<td>E</td>
<td>F. cymosum</td>
<td>Slovenia</td>
<td>Leaves</td>
<td>947</td>
<td>41.6</td>
<td>22.8</td>
<td>0.30</td>
</tr>
<tr>
<td>F</td>
<td>F. esculentum</td>
<td>Bosnia-Herzegovina</td>
<td>Flowers, leaves, stems</td>
<td>4840</td>
<td>53.5</td>
<td>90.4</td>
<td>0.20</td>
</tr>
<tr>
<td>G</td>
<td>F. esculentum</td>
<td>Slovenia</td>
<td>Flowers, leaves, stems</td>
<td>3750</td>
<td>54.3</td>
<td>69.2</td>
<td>0.10</td>
</tr>
<tr>
<td>H</td>
<td>F. esculentum</td>
<td>Slovenia</td>
<td>Leaves and flowers</td>
<td>1600</td>
<td>26.6</td>
<td>60.1</td>
<td>0.37</td>
</tr>
<tr>
<td>I</td>
<td>F. esculentum</td>
<td>Czech Republic</td>
<td>Leaves</td>
<td>432</td>
<td>14.4</td>
<td>30.1</td>
<td>0.43</td>
</tr>
<tr>
<td>J</td>
<td>F. esculentum</td>
<td>Germany</td>
<td>Leaves</td>
<td>1580</td>
<td>27.2</td>
<td>58.1</td>
<td>1.49</td>
</tr>
<tr>
<td>K</td>
<td>F. esculentum</td>
<td>Germany</td>
<td>Leaves</td>
<td>1410</td>
<td>33.4</td>
<td>42.3</td>
<td>0.95</td>
</tr>
<tr>
<td>L</td>
<td>F. esculentum</td>
<td>Germany</td>
<td>Leaves</td>
<td>1850</td>
<td>43.3</td>
<td>42.8</td>
<td>0.99</td>
</tr>
<tr>
<td>M</td>
<td>F. esculentum</td>
<td>Austria</td>
<td>Leaves</td>
<td>2310</td>
<td>31.2</td>
<td>73.9</td>
<td>0.53</td>
</tr>
<tr>
<td>N</td>
<td>F. esculentum</td>
<td>Slovenia</td>
<td>Leaves</td>
<td>533</td>
<td>34.1</td>
<td>15.7</td>
<td>1.19</td>
</tr>
<tr>
<td>O</td>
<td>F. esculentum</td>
<td>Slovenia</td>
<td>Leaves</td>
<td>322</td>
<td>15.3</td>
<td>21.0</td>
<td>0.68</td>
</tr>
<tr>
<td>P</td>
<td>F. tataricum</td>
<td>China</td>
<td>Leaves</td>
<td>512</td>
<td>9.9</td>
<td>51.7</td>
<td>0.74</td>
</tr>
<tr>
<td>R</td>
<td>F. tataricum</td>
<td>China</td>
<td>Seed</td>
<td>68</td>
<td>13.0</td>
<td>5.3</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The highest fagopyrin content was in cymosum buckwheat samples, which also had a very high concentration of rutin, i.e. the flowers sample contained 11.2% rutin in the dry matter (Table 1 and Fig. 2). Hinneburg et al. (2005) suggested that an extract with a high phenolic content and a low content of the phototoxic fagopyrin can be obtained by adapting the extraction procedure (Wilhelm et al., 2001; Hinneburg et al., 2005). Differences in the rutin content have also been reported when using common buckwheat herbs with different origins (Hinneburg and Neubert, 2005).

We found a negligible amount of quercetin in the buckwheat samples analyzed in this study, where the dry matter content was mainly <1 mg·g⁻¹, and only one sample exceeded this concentration, i.e. ~1.5 mg·g⁻¹ in a sample from Germany, while ~1 mg·g⁻¹ was detected in two other samples from the same country and in a sample from Slovenia. Additional unidentified substances with typical flavonoid UV spectra were found in some flower samples (<12 mg·g⁻¹ expressed as rutin).

The results on variability of fagopyrin content in samples, obtained on the market, between buckwheat species and within the species, are interesting as an orientation for further and more detailed analyses of the samples, which are expected to show either high or low content of fagopyrin, respectively, according to specific use of products. A further point of interest is the question of how much rutin content is expected to be lost when samples with as low a content of fagopyrin as possible are chosen.

4. Conclusions

We found variable amounts of fagopyrin and rutin in plant part samples taken from common, Tartary, and cymosum buckwheat,
and the levels were very high in flower samples, especially cymosum buckwheat flowers. The herb samples taken from common, Tartary, and cymosum buckwheat had a less favorable proportion of fagopyrin relative to rutin compared with Tartary buckwheat seed. The results of this study will help in preparing a healthful diet with acceptable fagopyrin and rutin content. However, further development of analytical methods for fagopyrin analyses are needed.

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