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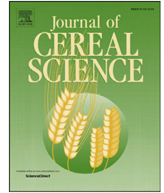

Hydrothermal treatment of Tartary buckwheat grain hinders the transformation of rutin to quercetin

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ABSTRACT

The impact of hydrothermal treatment on extractability of flavonoids from starchy matrix was investigated. Tartary buckwheat grain was hydrothermally treated (HT) and milled to yield HT flour. In not hydrothermally treated (NHT) Tartary buckwheat flour, most of extractable rutin (8 mg of rutin per g DM (dry matter)) was extracted during the first 20 min of extraction. In contrast, in HT Tartary buckwheat flour only 4 mg of rutin per g DM was extracted in 20 min, and 6 mg and 7 mg of rutin per g DM within 2 and 8 h, respectively. This indicates that, during the hydrothermal treatment, rutin becomes embedded in the flour matrix, becoming less readily extractable. Slowly extracted rutin was protected from transformation to quercetin during dough and bread making. Thus, from an initial 7 mg of extractable rutin per g DM in HT buckwheat flour, the resulting Tartary buckwheat bread contained 2 mg of rutin per g DM, and 6 mg of quercetin per g DM appeared. No other Tartary bread making technology which would be able to conserve such an amount of rutin from flour through the process to the final bread product is known to our knowledge.

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1. Introduction

Buckwheat is a popular food source because of the balanced amino-acid composition of its proteins and the content of fiber, retrograded starch, trace elements, vitamins and antioxidants, including flavonoids (Holasova et al., 2002; Bonafaccia et al., 2003; Fabjan et al., 2003; Pongrac et al., 2016). Another reason for its popularity is its relative resistance to pest and diseases, ability to repress weeds depending on agro-environmental conditions and on the weather (Juszczak et al., 2009), suitability for biological cultivation, and ability to grow at high altitudes, exposed to strong UV-B radiation. Buckwheat could enhance food diversity and it resembles the tradition, and in some way the heritage, of »the good old days«. Buckwheat does not contain gluten proteins, so it is safe for people suffering from gluten in their diet. However, because of absence of gluten, it is difficult to make bread solely from buckwheat flour (Costantini et al., 2014).

Tartary buckwheat flour is more and more used in preparing dishes, due to its much higher content of rutin (up to about 14.7 mg per g DM) than in common buckwheat (up to about 0.1 mg per g DM) (Fabjan et al., 2003; Lukšič et al., 2016; Kreft, 2016). Otherwise, the composition of common and Tartary buckwheat is quite similar in terms of proteins, their amino acid composition, and lipids (Bonafaccia et al., 2003).

Hydrothermal treatment (a process that involves heating with hot water or steam, followed by slow cooling and drying) is used in buckwheat to produce buckwheat groats (husked buckwheat or kasha) by the traditional technology known and still applied in Slovenia, Croatia, Poland, Ukraine and Russia (Kreft, 2003). The appearance of retrograded starch in hydrothermally treated (HT) common buckwheat has been studied (Skrabanja et al., 1998, 2000). HT groats can be further milled to obtain instantized pregelatinized buckwheat flour.

Traditional bread making procedure, usually using about 70% wheat flour and 30% buckwheat flour, involves scalding the buckwheat part of the flour before mixing and kneading the dough. Recently it was established that Tartary buckwheat bread making is

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feasible without any addition of wheat or gluten, by using the sour bread starter procedure (Costantini et al., 2014; Lukšič et al., 2016).

The aim of the present study was to assess the extractability of rutin and quercetin from Tartary buckwheat materials as affected by hydrothermal treatment of Tartary buckwheat grain, and by the sour bread making procedure. To study the transformation of rutin in HT buckwheat flour during bread making and baking, Tartary buckwheat flour was used because of its high concentration of rutin.

2. Material and methods

2.1. Materials and sample preparation

Non-hydrothermally treated (NHT) and HT Tartary buckwheat flours were prepared in a Rangus mill (Šentjernej, Slovenia) using Tartary buckwheat grain from a domestic strain that originated in Luxemburg (Bonafaccia et al., 2003). Grains were processed hydrothermally by soaking in water at 95 °C for 20 min, followed by drying by warm air (40 °C) ventilation to 20% moisture in a grain dryer (A. Rangus, Šentjernej, Slovenia).

Hydrothermal and drying procedures facilitate removal of firm buckwheat husks from grains. Dry husked Tartary buckwheat grain was milled in a stone mill (A. Rangus, Šentjernej, Slovenia) to give HT Tartary buckwheat flour. NHT Tartary buckwheat flour was obtained by milling untreated Tartary buckwheat grain in the same type of mill. The first pass was with a gap of 3 mm between both milling stones, after the husk was removed by sifting off with a 3 mm round hold sieve, the second pass was the 2 mm gap between the stones.

The initial sourdough starter was obtained from Bageriet Andreas Östlund (Uppsala, Sweden), and was stored in refrigerator (5 °C). Before use in this study, it was refreshed each 3–4 days for 6 months by addition of an equal volume of Tartary buckwheat flour suspension (TB flour: boiled and cooled tap water; 1:1; v/v). The sourdough starter contained *Lactobacillus heilongjiangensis* and *Pediococcus parvulus*, details described by Lukšič et al. (2016). Fresh baker's yeast was purchased from Bonopan (Logatec, Slovenia).

2.2. Sourdough bread production

Sourdough bread was prepared with HT Tartary buckwheat flour in two steps. A mixture of 50 g sourdough starter, 90 g flour, and 150 g tap water at room temperature (25 °C) was prepared on the first day. The mixture was fermented in a refrigerator (5 °C) for 10 h. On the following day, 150 g of HT Tartary buckwheat flour, 25 g tap water, 8 g sugar, 5 g salt, 4 g fresh baker's yeast were added to the dough. It was placed in baking models on baking paper and left to rise for 5 h. Loaves were baked for 1 h in an oven at 200 °C.

2.3. Preparation of methanol extracts

Antioxidant activities were determined for the NHT Tartary buckwheat flour, HT Tartary buckwheat flour, sourdough starter, and intermediate products of further bread making steps using HT Tartary buckwheat flour: sourdough before and after the 10-h incubation in a refrigerator (5 °C), bread dough before and after the 5-h rising at room temperature, and in sourdough bread. After preparing the dough and baking the bread, the loaves were cooled and cut into pieces. Samples were freeze dried and milled for analysis. Methanol extracts were produced, by adding 25 mL 80% aqueous methanol (HPLC grade, Sigma-Aldrich, Germany) to 1 g of each sample. The mixtures were shaken on a horizontal shaker (250 rpm) at room temperature for 20 min, 2 h or 8 h. The samples were then filtered through filter paper (84 g m^{-2}; Munktell, Germany) and kept at 5 °C for further analysis. Methanolic extracts of all of the samples were prepared from three independently prepared bread samples.

2.4. Assay of antioxidant activity

The antioxidant activity of each extract was determined by photochemiluminescence (PCL) and fluorescence ($ORAC_{FL}$) assays. In the PCL assay antioxidant activity of extracts was measured, using a Photochem instrument (Analytik Jena, USA Inc., Delaware, OH, USA), against superoxide anion radicals generated from luminol photosensitizer under the UV light as described by Lukšič et al. (2016).

2.5. Determination of rutin and quercetin by HPLC-DAD

2.5.1. Chemicals

Standard chemicals (rutin and quercetin), methanol (HPLC grade), acetonitrile (gradient HPLC grade) and phosphoric acid (ACS grade) were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steiheim, Germany). Double deionized water (ddH_2O) was treated (0.054 μS cm^{-1}) in a Simplicity 185 purification system (Millipore SAS, Molsheim, France).

2.5.2. Preparation of calibration solutions and samples

Standard solutions (rutin, quercetin) were prepared by dissolving 0.5 mg each of them with methanol in 10 mL. The lyophilized samples (2 g) were after homogenization in a mortar extracted with 20 mL of 80% methanol at laboratory temperature for 20 min, 2 h and 8 h by horizontal shaker (Unimax 2010; Heidolph Instrument GmbH, Germany). Extract was filtered through Munktell No 390 paper (Munktell & Filtrac, Germany) and stored in closed 20 mL vial tubes. Prior to injection the standard solutions and extracts were filtered through syringe filter Q-Max (0.22 μm, 25 mm; Frisenette ApS, Knebel, Denmark).

2.5.3. HPLC analyses

Rutin and quercetin were determined using an Agilent 1260 Infinity high performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) with quaternary solvent manager coupled with degasser (G1311B), sample manager (G1329B), column manager (G1316A) and DAD detector (G1315C). All HPLC analyses were performed on a Purosphere reverse phase C18 column (4 mm × 250 mm × 5 μm) (Merck, KGaA, Darmstadt, Germany).

The mobile phase consisted of acetonitrile (gradient) (A) and 0.1% phosphoric acid in ddH_2O (B). The gradient elution was as follows: 0–1 min isocratic elution (20% A and 80% B), 1–5 min linear gradient elution (25% A and 75% B), 5–15 min (30% A and 70% B) and 20–25 min (40% A and 60% B). The initial flow rate was 1 mL min^{-1} and the injection volume was 10 μL. Column oven temperature was set up to 30 °C and the samples were kept at 4 °C in the sample manager. The detection wavelengths were conducted at 265 nm (rutin) and 372 nm (quercetin). The data were collected and processed using Agilent OpenLab ChemStation software for LC 3D Systems. Limit of detection for rutin and quercetin were 1.09 and 0.99 μg mL^{-1}, respectively. Limit of quantification for rutin and quercetin were 3.59 and 3.27 μg mL^{-1}, respectively.

2.6. Statistical analysis

Data are expressed as means ± standard deviation of the results on three independently prepared bread loaves. ANOVA was performed, and the data were considered to be significantly different when $P < 0.05$.

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