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Rutin and quercetin transformation during preparation of buckwheat sourdough bread

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ABSTRACT

Sourdough bread was prepared from flour of the common buckwheat (*Fagopyrum esculentum*) and of Tartary buckwheat (*Fagopyrum tataricum*) to follow the transformation of rutin and quercetin in these sourdoughs. During Tartary buckwheat sourdough fermentation, there was conversion of rutin to quercetin. The Tartary buckwheat flour contained 14.6 mg/g rutin and 1.9 mg/g quercetin as dry matter. The sourdough starter contained 1.5 mg/g rutin and an unexpectedly high 12.5 mg/g quercetin. The sourdough contained 3.2 mg/g rutin and 8.1 mg/g quercetin. In the Tartary buckwheat sourdough bread there was no rutin, whereas there was 5.0 mg/g quercetin. Thus, during the sourdough fermentation, the rutin was completely degraded. However, despite the long fermentation time (sourdough, 10 h; bread dough, 5 h), most of the quercetin remained in the dough and appeared in the baked bread. In contrast to Tartary buckwheat bread, neither rutin nor quercetin was present in common buckwheat bread. Information on the persistence of quercetin in sourdough bread is important for designing breads with high concentrations of flavonoids and good functional value.

1. Introduction

Common buckwheat (CB) (*Fagopyrum esculentum* Moench) and Tartary buckwheat (TB) (*Fagopyrum tataricum* (L.) Gaertn.) are used in different parts of the world to make various food products. The grain of both of these cultivated buckwheat species contains up to 27% fibre (Bonafaccia et al., 2003). Buckwheat groats are a prebiotic food because they can, for example, increase the lactic acid bacteria in the intestine because of their content of resistant starch (Skrabanja et al., 1998, 2001). Buckwheat is also an important source of anti-oxidant activity in functional foods (Holasova et al., 2002). This arises from the presence of the flavonoids rutin and quercetin in the buckwheat grain and products, because of their anti-oxidant and anti-inflammatory effects. It has been reported that TB contains about 100-fold more rutin than does CB (Fabjan et al., 2003). Rutin and quercetin are also present in baked biscuits made from CB and TB (Wieslander et al., 2011). Buckwheat products decrease cholesterol levels and improve lung capacity in humans (Wieslander et al., 2011; Sikder et al., 2014; Yang et al., 2014). Extracts from CB and TB can also protect DNA from damage caused by hydroxyl radicals (Cao et al., 2008; Vogrinčič et al., 2010).

One of the ways to prepare bread involves sourdough fermentation, which can be accompanied by the formation of flavour compounds such as lactic acid and acetic acid that have an impact on dough processing and the preparation of sourdough bread (Michalska et al., 2008). However, the combined effects of sourdough fermentation and the baking process on the flavonoid concentrations and anti-oxidant properties of CB and TB sourdough starter, bread dough and sourdough bread have not yet been

Abbreviations: a*, range of colour between red and green; ANOVA, analysis of variance; b*, range of colour between yellow and blue; CB, common buckwheat; DM, dry mass; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HPLC, high performance liquid chromatography; L*, brightness of colour; ND, not detected; $ORAC_{FL}$, oxygen radical absorbing capacity; PCL, photochemiluminescence; PCR, polymerase chain reaction; TB, Tartary buckwheat.

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studied. Therefore, the objective of the present study was to investigate the effects of sourdough fermentation and the baking process on rutin and quercetin content and on the anti-oxidant activities of CB and TB. More precisely, the aim was to determine whether sourdough fermentation and thermal processing affect the conversion of rutin to quercetin. We were also interested in determining how much rutin and quercetin remain in CB and TB products after sourdough fermentation and the baking process. These findings will contribute to a better understanding of the value of buckwheat products for human consumption.

2. Materials and methods

2.1. Materials and sample preparation

The CB and TB flours were prepared in a Rangus mill (Vrhpolje near Šentjernej, Slovenia) using the CB grain cv. 'Pyra' and TB grain from a domestic strain that originated in Luxembourg (Bonafaccia et al., 2003). The CB and TB were harvested in 2012. The initial sourdough starter was obtained from Sweden (Bageriet Andreas Östlund, Uppsala, Sweden), and was stored in a glass jar in a refrigerator (5 °C). Before use in this study, this sourdough starter was refreshed twice weekly for 6 months by addition of an equal volume of TB flour suspension (1:1; v/v; TB flour: preboiled and cooled tap-water). This was done to adapt the starter to the flavonoid-rich material. The sourdough starter was kept in a refrigerator (5–8 °C).

2.2. Determination of sourdough starter microorganisms

To identify the bacteria present, diluted sourdough starter was plated onto deMan, Rogosa and Sharpe (MRS) agar medium (Sigma–Aldrich, St. Louis, Missouri, US) and incubated for 3 days at 30 °C under anaerobic conditions. Each type of morphologically different colony was further purified to isolate the pure strains. The isolates were grown in liquid MRS medium, and the biomass was harvested and used for DNA isolation using NucleoSpin Tissue kits (Macherey–Nagel, Düren, Germany). The 16S rRNA bacterial genes were specifically amplified by polymerase chain reaction (PCR) using the primers 16S_27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16S_rH1542 (5′-AAGGAGGTGATCCAGCCGCA-3′). The PCR products were cleaned up using NucleoSpin gel and PCR Clean-up kits (Macherey–Nagel, Düren, Germany) and their partial sequences were determined by Eurofins Genomics. The sequences were analysed using the Blastn programme available from the National Center for Biotechnology Information (NCBI) database. The isolates were assigned to the species to which the 16S rRNA gene sequences showed the highest identity. In this way, two morphologically different species were isolated and identified as *Lactobacillus heilongjiangensis* and *Pediococcus parvulus*. The sequences were deposited in the EMBL/GenBank/DDBJ database under accession numbers LN650641 (*L. heilongjiangensis*) and LN650642 (*P. parvulus*). Fresh baker's yeast was purchased from Bonopan (Logatec, Slovenia).

2.3. Sourdough bread production

The sourdough bread was prepared in two steps. On the first day, a mixture of 50 g sourdough starter, 90 g flour and 150 g tap-water at room temperature (25 °C) was prepared. The mixture was stored in a refrigerator (5 °C) and allowed to ferment for 10 h. On the second day, 25 g tap-water, 8 g sucrose, 5 g salt, 4 g fresh baker's yeast and 150 g flour were added to the mixture. The dough was placed in baking forms on baking paper and left to rise for 5 h. The bread was then baked in an oven at 200 °C for 1 h. Four bread loaves of each type of bread (CB or TB) were included in the study, made independently from the beginning (including making the sourdough out of four different starter samples). All four were used for the measurement of anti-oxidant activity, and three randomly selected loaves were used for other measurements.

2.4. Measurement of loaf volumes

For the determination of loaf volumes, the rapeseed displacement method was used according to AACC method number 10-05.01 (AACC International, 2008). Specific loaf volumes were obtained by dividing the volume by the loaf weight (expressed as g/cm^3).

2.5. Colour measurements

Colour measurements were performed on the bread crumb and crust. The lightness (L*) and red (+a*) and yellow (+b*) colorimetric indices were established according to the CIELAB colour system (CIE, 1986) using a Chroma Meter CR 300 Minolta (Konica Minolta, Inc., Tokyo, Japan) equipped with a pulsed xenon lamp and illuminant D65. A white plate (X = 91.98, Y = 93.97, Z = 110.41) was used to standardize the instrument. Three independently prepared loaves of each type of bread were measured.

2.6. Preparation of methanol extracts

The concentrations of rutin and quercetin and the anti-oxidant activity were determined for the CB flour, TB flour, sourdough starter, 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 the two different types of sourdough bread (i.e., CB, TB). Bread samples were prepared at room temperature (25 °C). After preparing the dough and baking the bread, the loaves (four each of the two types of bread) were cooled and cut into pieces, which were stored for analysis in a freezer at −20 °C. All of the samples prepared were freeze-dried and milled for analysis. To prepare the methanol extracts, 50 mL 80% aqueous methanol (HPLC grade, Sigma–Aldrich Corporation, St. Louis, MO, USA) was added to 1 g of each milled sample. The mixtures were shaken at room temperature for 8 h (250 rpm). The samples were then filtered through filter paper (130 g/m^2; Filtrak, Thermalbad Wiesenbad, Germany) and kept at 5 °C for further analysis. The methanol extracts of each type of bread were made using three independently prepared loaves.

2.7. Anti-oxidant activity assays

The anti-oxidant activities were determined on the basis of photochemiluminescence (PCL) and fluorescence ($ORAC_{FL}$) assays. The PCL assay was used to measure the anti-oxidant activity of extracts against superoxide anion radicals generated from luminol (a photosensitizer) when exposed to UV light, assessed with a Photochem instrument (Analytik Jena, USA Inc., Delaware, OH, USA). The anti-oxidant activities of the sample extracts were measured using integral anti-oxidant capacity of water-soluble substances kits from Analytik Jena (USA Inc., Delaware, OH, USA). Chemiluminescence evolution was monitored using the PCL soft control and analysis software. The lag time (expressed in seconds) was used as a measure of radical-scavenging activity. The anti-oxidant capacities were estimated by comparison with a Trolox standard (0.5–3 nmol of Trolox) and are expressed as μg/mg Trolox equivalents. The anti-oxidant index was obtained by dividing the anti-oxidant capacity by the lag time and multiplying by 1000 (anti-oxidant activity/lag time × 1000).