Fagopyrum 37(2):37-40 (2020)

Short communication

Extraction of rutin and quercetin from Tartary buckwheat grains, hydrothermally treated at different temperatures

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DOI https://doi.org/10.3986/fag0017

Received: July 26, 2019; accepted September 25, 2019.
Keywords: Tartary buckwheat, rutin, quercetin

ABSTRACT

Tartary buckwheat grains were hydrothermally treated to establish the conditions under which rutin remains in the grain. Tartary buckwheat grains were soaked in water at the temperatures 51 °C, 61 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C, and a control group at 21 °C. During 20 minutes soaking at 51 °C or 61 °C the concentration of rutin decreased. This effect was mostly pronounced by soaking at 70 °C and 75 °C, where instead of missing amount of rutin, some quercetin appeared. After soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C concentration of rutin was not significantly different in comparison to the concentration of rutin after soaking 20 minutes at 21°C. It is suggested that exposure to water at 21°C is similar to natural conditions, where rutin degrading enzymes remain mainly inactive and in grain separated from its potential substrate. Further is suggested that at the soaking temperatures 51 °C, 61 °C, 70 °C and 75 °C, grain structures are partly degraded and rutin degrading enzymes got contact to the substrate. By soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C, rutin degrading enzymes lose their activity. Thus wet treatment of Tartary buckwheat grains for 20 minutes at temperature at 80 °C or above, this threshold is enough to preserve the content of rutin in the samples. This is of importance for nutritional quality of Tartary buckwheat food products.

INTRODUCTION

Flavonoid rutin and rutin degrading enzymes are in different structures of buckwheat grain. Rutin is in embryo, in the middle of the grain, while rutin degrading enzymes are located in the peripheral part of the grain. After crushing and milling of the grain, in the wet environment, rutin degrading enzymes are in the direct contact with their substrat, and the concentration of rutin may begin to decrease (MetaCyc, 2011). Harvested buckwheat grain contain among flavonoids mainly rutin, and just a low concentration of quercetin. During dough and bread making, due to transforming of rutin to quercetin, the concentration of rutin decreases and quercetin concentration increases (MetaCyc, 2011; Lukšič et al., 2016).

Quercetin is a flavonoid, frequently present in foods and drinks, with bitter taste (Anand et al., 2016).

Tartary buckwheat contains in its leaves and grain higher concentrations of rutin in comparison to common buckwheat. Bitter taste of Tartary buckwheat is due to the high concentration of rutin and quercetin (Bonafaccia et al., 2003). Buckwheat cultivation decreased for years, but recently it is coming back because of knowledge of health promoting properties (Li et al., 2001).

The aim of this investigation was to establish the temperatures, at which the activity of rutin degrading enzymes are hindered.

MATERIAL AND METHODS

Preparation of samples

Tartary buckwheat (*Fagopyrum tataricum*, cv. Zlata) grains were obtained from Mill Rangus, Šentjernej, Slovenia. Grains were soaked in water for 20 minutes at temperatures 21 °C, 51 °C, 61 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C. After hydrothermal treatment samples were dried for 24 hours at 40 °C. Dry samples were milled in a coffee grinder (Gorenje, SMK 202, Velenje). Samples (1 g per 25 mL) were extracted for 20 min in a 80 % (v/v) methanol in horizontal shaker (Phoenix Instrument, RS-OS 5, Garbsen, Germany). After the extraction solutions were filtered (Agilent Econofilters, PTFE 25 mm 0.2 µm, Santa Clara, USA). Three independent samples were analyzed for each hydrothermal treatment.

Determination of rutin and quercetin by HPLC

Standard chemicals (rutin and quercetin), methanol (Chromasolv for HPLC), acetonitrile (LC-MS Chromasolv) and phosphoric acid (ACS grade) were purchased by Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steiheim, Germany). Deionized water (dH_2O) was treated in a deionization system DI 425 TK-0.10425 (Thermo Scientific, Waltham, USA).

Preparation of calibration solutions and samples solutions were described by Lukšič et al. (2016).

Rutin and quercetin were determined using an Agilent 1100 Series high performance liquid chromatograph (Agilent Technologies, Santa Clara, USA) with quaternary solvent pump (G1311A) coupled with degasser (G1379A), sample manager (G1329A), column manager (G1316A), autosampler (G1329A) and DAD detector (G1315B). All HPLC analyses were performed on a Zorbax Eclipse XDB-C18 column (4.6 mm x 250 mm x 5 µm) (Agilent Technologies, Santa Clara, USA).

The mobile phase consisted of acetonitrile (gradient) (A) and 0.1% phosphoric acid in dH_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 25 °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 proceed using Agilent Chemstation 9.01 software.

Statistical evaluation

The data are expressed as means, and standard deviation, from three independently prepared samples. ANOVA was performed, the data were considered to be significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Results are presented in Fig. 1.

During 20 minutes soaking at 51 °C or 61 °C the concentration of rutin decreased. This effect was most pronounced by soaking at 70 °C and 75 °C, where instead of missing amount of rutin, some quercetin appeared. After soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C concentration of rutin was not significantly different in comparison to the concentration of rutin after soaking 20 minutes at 21°C. It is suggested that exposure to water at 21°C is similar to natural conditions, where rutin degrading enzymes remain mainly inactive and in

grains separated from its potential substrate. Further is suggested that at the soaking temperatures 51 °C, 61 °C, 70 °C and 75 °C, grain structures are partly degraded and rutin degrading enzymes got contact to the substrate. By soaking at 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C, rutin degrading enzymes lose their activity. Thus wet treatment of Tartary buckwheat grains for 20 minutes at temperature at 80 °C or above this threshold is enough to preserve the content of rutin in the samples. This is of importance for nutritional quality of Tartary buckwheat food products.

CONCLUSION

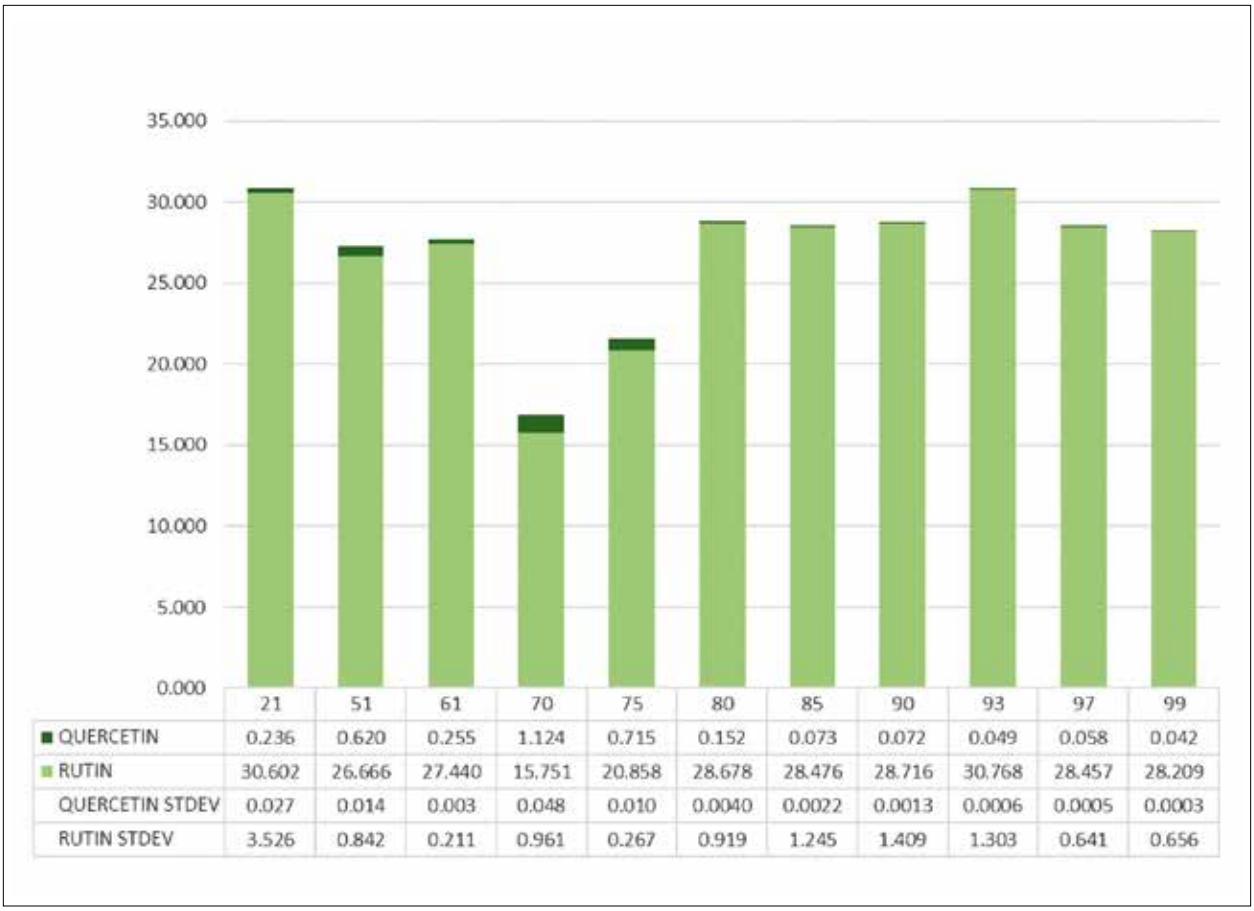
Rutin degrading enzymes were most active during soaking Tartary buckwheat grain at the temperatures 70 °C and 75. At temperatures 80 °C, 85 °C, 90 °C, 93 °C, 97 °C and 99 °C concentration of rutin was not significantly decreased, and no quercetin appeared, meaning that the rutin degrading enzymes were under this condition inactivated.

ACKNOWLEDGEMENT

Authors are thankful to prof. Ivan Kreft (ARRS project L4-9305, co-financed by Ministry of Agriculture, Forestry and Food), to Mr. Anton Rangus (Mill Rangus, Šentjertnej, Slovenia) and to Gimnazija Novo mesto. This research was performed in the frame of activities for talented students of Gimnazija Novo mesto, Novo mesto »Razmišljam, iščem, (se) razvijam«, supported by EU and MIZŠ, in the educational project »RaST – Razvojno središče talentov«, coordinated by II. gimnazija Maribor (number of project 20.02054).

Fig. 1

Concentration of rutin and quercetin in Tartary buckwheat samples (in mg/g dry mass), treated at given temperatures



	21	51	61	70	75	80	85	90	93	97	99
QUERCETIN	0.236	0.620	0.255	1.124	0.715	0.152	0.073	0.072	0.049	0.058	0.042
RUTIN	30.602	26.666	27.440	15.751	20.858	28.678	28.476	28.716	30.768	28.457	28.209
QUERCETIN STDEV	0.027	0.014	0.003	0.048	0.010	0.0040	0.0022	0.0013	0.0006	0.0005	0.0003
RUTIN STDEV	3.526	0.842	0.211	0.961	0.267	0.919	1.245	1.409	1.303	0.641	0.656

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IZVLEČEK

Ekstrakcija rutina in kvercetina iz hidrotermično obdelanih zrn tatarske ajde pri različnih temperaturah

Namen raziskave je bil določiti pogoje, pod katerimi se rutin ohrani v ajdi pri hidrotermični obdelavi zrn tatarske ajde. Zrna tatarske ajde so bila hidrotermično obdelana v vodi pri temperaturah 51 °C, 61 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 93 °C, 97 °C in 99 °C s kontrolno skupino pri 21 °C. Pri 51 °C in 61 °C se je koncentracija rutina znižala. Pri 70 °C in 75 °C se je znaten del rutina pretvoril v kvercetin. Pri 80 °C, 85 °C in 90 °C se je pretvorba rutina v kvercetin zmanjšala predvidoma zaradi denaturacije encimov, ki razgrajujejo rutin. Pri višjih temperaturah 93 °C, 95 °C in 99 °C je bila koncentracija kvercetina izrazito manjša, ohranil pa se je velik delež rutina. Glede na ugotovljeno imajo hidrotermično obdelana zrna tatarske ajde višjo koncentracijo rutina, kar je pomembno s stališča prehrane.