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EFFECT OF THERMAL TREATMENT ON RUTIN CONTENT IN SELECTED BUCKWHEAT PRODUCTS USING CALCIUM AS AN INTERNAL TRACER

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ABSTRACT

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used for rutin (quercetin-3-rutinoside) determination in selected buckwheat products (whole meal flour, broken seeds, seed hulls, herbs and baked cereal breads). The effect of various thermal procedures on content of rutin was evaluated using calcium as an internal tracker to correct changes in mass and composition of the buckwheat products. These factors are very seldom taken into account. The results show non-significant changes in rutin levels obtained in whole meal flour and broken seed samples after thermal treatment up to 150°C. Higher temperature already caused sudden fall in the observed rutin concentrations. The evaporation of some volatile compounds and degradation products can decrease the mass of the samples and formally increase the content of rutin (35.5 ±4.7 mg per 100 g for whole meal flour and 10.2 ±0.4 mg per100 g for broken seeds at 150°C). Serious decrease of rutin contents at elevated temperatures (>150°C) can be explained by its degradation (by breaking off the C-C bond in quercetin-3-rutinoside moiety) and/or evaporation (24.3 ±1.4 mg per 100 g for whole meal flour and 3.06 ±0.3 mg per100 g for broken seeds at 180°C). In case of baked cereal breads the level of rutin changed in dependence on the ratio between buckwheat and corn flour. Longer time leaching and higher temperature implicate higher rutin content in infusions prepared from buckwheat seed hulls and herbs.

Keywords: rutin; buckwheat; *Fagopyrum esculentum* Moench; effect of thermal treatment; calcium; internal tracer

INTRODUCTION

Basic chemical composition of nutritionally important plants differs with respect to its origin, vegetative cycle or growing conditions. As expected, the level of biologically active compounds is directly influenced by many factors such as weather, maturing, stress or fertilization. However, indispensable influence is also caused by technological processes for food production and/or culinary practices.

Various treatment processes have significant effects on chemical composition of food matrix. Degradation of thermo labile compounds and evaporation of volatile compounds (organic compounds, alkylated metals etc.) can cause losses of mass of the matrices. On the other hand, absorption of water, frying oil, oxidation processes and other reactions among constituents can increase the mass of matrix. Technological and/or culinary procedures also influence accuracy (overestimate or underestimate) of the final results. Thus, the presence of internal/external tracers in a sample matrix could be applied to correct the changes in mass (volume) and/or composition of the matrix.

Rutin, a representative of flavonoids, is synthesized in higher plants as a response on stress invoked by diseases, predators or ultraviolet radiation. It was recognized that rutin (quercetin-3-rutinoside) has significant anti-carcinogenic and anti-inflammatory properties **(Sharma et al., 2013)**, acts as α-glucoside inhibitor **(Li et al., 2009)** and shows lipid peroxidation and antioxidant activity. More, rutin antagonizes the increase of capillary fragility associated with hemorrhagic disease or hypertension in humans **(Shanno, 1946)**. It also decreases the permeability of the blood vessels and has an anti-oedema effect, reduces the risk of arteriosclerosis. Nowadays, rutin has been reveled as a very interest constituent of functional foods. Common buckwheat (*Fagopyrum esculentum Moench*) and tartary buckwheat (*Fagopyrum tataricum*) are well-known as nutritious gluten-free ingredient and very valuable pseudocereal sources of rutin **(Zhang et al., 2012; Ahmed et al., 2014)**.

Generally, buckwheat is a rich source of phenolic acids and polyphenolic compounds **(Guo et al., 2011; Inglett et al., 2011; Guo et al., 2012)**. Several papers are focused on evaluation of nutrient quality, antioxidant properties and rutin content in various foodstuff enriched with buckwheat flour such as instant noodles **(Choy et al., 2013)** or cereal

bread **(Lin et al., 2009)**. The effect of pseudocereal flour addition to antioxidant properties and sensory value of breads was also studied in **Chlopicka et al. (2012)**. Content of rutin in buckwheat raw material and some food product was deeply investigated by **Kreft et al. (2006)**. **Fessas et al. (2008)** evaluated nutritional properties of wheat and buckwheat flour (namely polysaccharides and proteins composition) by thermogravimetry and differential scanning calorimetry. They showed how de-hulling step and addition of buckwheat fraction affect crumb structure and bread properties.

Technological and/or culinary treatments influence food product properties such as hardness, integrity, color and/or representation of analytes. As showed in **Blanszczak et al. (2013)**, high-pressure treatment of buckwheat groats resulted in decreasing nearly 20% of antioxidant activity and rutin looses almost 50% in comparison with raw material. Pressured-steam heating, roasting and microwave irradiation heating were applied to whole meal flour processing and the effect on polyphenolic content was followed in **Zhang et al. (2010)**. Similarly, **Zielinski et al. (2009**) evaluated the effect of roasting on antioxidants of buckwheat seeds and **Vogrincic et al. (2010)** studied looses of rutin and other polyphenolic compounds caused by bread baking procedure. They also followed changes in antioxidant activity of bread samples prepared by mixing of buckwheat and wheat flour. Other studies, reported by **Biney a Beta (2014) and Verardo et al. (2011)** illustrated effect of cooking of buckwheat pasta to antioxidant properties and amount of phenolic compounds. In all mentioned publications related with thermal treatment authors observed significant decrease of antioxidant characteristics and looses of phenolic compounds content.

Basic chemical composition of products studied in this work has been previously described in **Vojtíšková et al. (2014)**. The aim of this study was to evaluate effect of various thermal treatment procedures on rutin content in selected products (cereal breads, whole meal flour, broken seeds, seed hulls and herbs) made from common buckwheat (*Fagopyrum esculentum Moench*) cultivated in the Czech Republic. Applications of calcium as an internal tracer to compensate the changes in mass (volume) and/or composition of the matrix were also tested.

Statistical hypothesis

Thermal procedures has an significant effect on rutin content in selected products.

MATERIAL AND METHODOLOGY

2.1. Sample preparation

Selected buckwheat products were made from common buckwheat (*Fagopyrum esculentum* Moench) cultivated in the region of Slezské Rudoltice, Czech Republic. Baking of breads was realized on 300 g flour samples (mixtures of corn and buckwheat flours from 0% up to 100% with steps of 10%) applying high speed dough mixing and a short fermentation time. Dough was prepared from flour (up to 100%), 1.5% dry yeast, 1.5% salt, 1.9% sugar and 0.005% ascorbic acid with addition of water to optimum consistency. Baking of breads was realized in accordance with ICC Standard No. 131 **(ICC, 1980)**. The final breads were dried, ground to a fine powder and sieved through 1 mm mesh. Buckwheat whole meal flour, broken seeds, seed hulls and buckwheat herbs were obtained from Pohankový mlýn Šmajstrla (Frenštát p. R., Czech Republic).

2.2 Chemicals

Acetonitrile (ACN), methanol (both Chromasolv, gradient grade) and Folin-Ciocalteau reagent were purchased from Sigma-Aldrich (Steinheim, Germany). Acetic acid was obtained from Penta (Chrudim, Czech Republic). Sodium carbonate, potassium persulphate and gallic acid from Lachema (Brno, Czech Republic) and 2,2´-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS) from Fluka (Steinheim, Germany) were used. Rutin hydrate was purchased from Dr. Ehrenstorfer GmbH (Ausburg, Germany). All used reagents were of the analytical grade and all solutions were prepared in double distilled water (Heraeus Quarzschmelze, Hanau, Germany).

2.3. Chromatographic method for rutin determination

HPLC method for rutin determination was based on **Deineka *et al*. (2004).** All experiments were carried out on Shimadzu 10AVP chromatographic system (Shimadzu, Tokyo, Japan) comprising a SCL-10AVP system controller, two LC-10AVP pumps, CTO-10ASVP column owen, Rheodyne 7120 injection valve (20 μL sample loop) and SPD-M10AVP photodiode-array detector and degasser GT-154. Analyses were provided using HPLC column Waters C18 (Waters Corp., Milford, MA, USA, 75 x 4.6 mm, 5 μm pore size). The temperature of column was set to 25°C. Mobile phase composed of 2% acetic acid, acetonitrile and methanol (75/15/10, v/v/v) was used under isocratic elution at the flow rate of 1 mL min^{-1}. Chromatograms were collected at 355 nm and evaluated using the VP-Class analytical software (version 6.13 SP2). Limit of detection for rutin was 0.06 μg mL^{-1}, limit of quantification was 0.2 μg mL^{-1}. Retention time of rutin was 3 min and all analyses were realized in triplicate.

2.4. Analysis of whole meal breads

Breads consisted of various buckwheat and corn flour ratios (100/0, 90/10, 80/20, 70/30. 60/40, 50/50, 40/60. 30/70, 20/80, and 10/90) were baked in accordance with ICC Standard No. 131 **(ICC, 1980).** The dehydrated breads were crushed, ground to a fine powder and sieved through 1 mm mesh. Weighed ground samples (2 g ±0.001 g) were extracted with acetic acid/methanol/water mixture (1/50/50, v/v/v). After sonication and shaking, test-tubes were centrifuged at 4000 rpm for 5 minutes and supernatant was filtrated through 0.45 μm filter (Millipore, Bedford, MA, USA) and analyzed for rutin content. The extracts were also used for spectrophotometric determination of total polyphenolic content and antioxidant activity. All spectrophotometric measurements were carried out on spectrophotometer UNICAM 5625 (Spectronic Unicam Cambridge, UK) in a quartz cuvette, 10 mm optical path.

2.4.1. Determination of total polyphenolic content

Total phenolic content (TPC) was measured according to Folin-Ciocalteau method (**Singleton a Rossi, 1965**). The procedure was as follows: 1.58 mL of distilled water was mixed in the test tubes with 0.1 mL of Folin-Ciocalteau

reagent and 0.02 mL of calibration standards (0, 50, 100, 250 and 500 mg L^{-1} of gallic acid) or properly diluted extracts. After 5 minutes, 0.3 mL of sodium carbonate solution (200 g L^{-1}) was added and the mixtures were kept 2 hours in the dark at 20°C. The absorbance was measured three times against blank at 765 nm. The results were expressed as gallic acid equivalent (GAE) per 1 g of dry matter (DM).

2.4.2. Determination of total antioxidant activity

Antioxidant activity (TAA) measurements were carried out using ABTS reagent (**Re et al., 1999**). Standard stock solution of cation radical $ABTS^{+}$ consisted of 7 mM ABTS and 2.4 mM potassium persulphate was mixed and 2 hours incubated at 50°C. The solution was 50-times diluted and used as working ABTS solution. Then, 1.99 mL of ABTS working solution was mixed in the test tubes with 0.01 mL of calibration standards of gallic acid (0, 25, 50, 100 and 250 mg L^{-1}) or suitably diluted samples. After 10 minutes, the absorbance was measured in triplicate at 734 nm against blank and the results were expressed as gallic acid equivalents (GAE) per 1 g of dry matter (DM).

2.5. Calcium as an internal tracer

Calcium as a non-volatile and most abundant element **(Vojtíšková et al., 2014)** was selected as an internal standard to correlate any changes in composition and in weight of the samples during the thermal treatment. Precisely weighed ground samples of wholemeal flour and broken seeds (0.3 to 0.5 g ±0.001 g) were decomposed in a microwave owen Ethos SEL (Milestrone, Sorisole, Italy) using concentrated HNO_3 (5 mL conc. HNO_3 + 5 mL of deionized H_2O) at a temperature of 210°C for 30 min. The final solution was transferred into 25 mL volumetric flasks after cooling to a room temperature. The flasks were filled to the mark. Calcium contents as an internal standard were determined by flame AAS (acetylene-air) on an atomic absorption spectrometer AA 30 (Varian A.G., Australia) at 422.7 nm. Strontium nitrate at a concentration of 1 g L^{-1} was used as a spectral buffer to suppress the flame emission. Concentrations were determined by the calibration curve method and the integration of peak area.

2.6. Thermal treatment of whole meal flour and broken seed

The influence of thermal treatment on rutin content was further studied with buckwheat flour and broken seeds. Precisely weighed samples of selected buckwheat products (0.5 ±0.001 g) were heated in a laboratory oven with temperature control on glass dishes. Small portions of samples (approximately 10 %) were taken at specific temperatures 25°C, 60°C, 90°C, 120°C, 150°C, 180°C and 210 °C in the time intervals of 20 min, extracted with 10 ml of mixture consisted of acetic acid:methanol:water (1:50:50, v/v/v). After cooling and shaking, the extracts were centrifuged and filtrated through 0.45 μm filter. The content of rutin was measured using HPLC method.

2.7. Thermal treatment of buckwheat seed hulls and herbs

Buckwheat seed hulls and herbs were analyzed as a material for tea preparation and the effect of water temperature and leaching time were evaluated. Precisely weighed samples (1 g ±0.001 g) of hulls and herbs were mixed with 100 ml distilled water of 80°C, 90°C or 100°C. Water macerates were sampled in intervals 5, 10, 15 and 20 min, filtrated through 0.45 μm filter and analyzed by HPLC method.

2.8. Statistical evaluation

All results were statistically evaluated using the variation statistics (ANOVA, StatSoft, Prague, Czech Republic). Correlation matrices and regression functions were calculated using the statistical package Unistat, v. 5.5 (Unistat Ltd., England).

RESULTS AND DISCUSSION

3.1. Whole meal breads analysis

Kreft *et al.* (2006) in their study presented the value of rutin concentration in various buckwheat material and products such as dark flour (218.5 mg kg^{-1}) and light flour (112.8 mg kg^{-1}). We studied cereal breads consisted of various buckwheat and corn flour ratios. Depending on rising portion of buckwheat flour, increasing trend in rutin content was found **(Figure 1)**. While in 10% buckwheat flour bread the concentration 2.7 ±0.2 mg per 100 g was observed, in 100% whole meal buckwheat flour bread the rutin concentration was 10-times higher (28.2 ±2.9 mg per100 g). Convex form of the relationship between rutin concentration and content of buckwheat flour is evident over the 70/30 ratios.

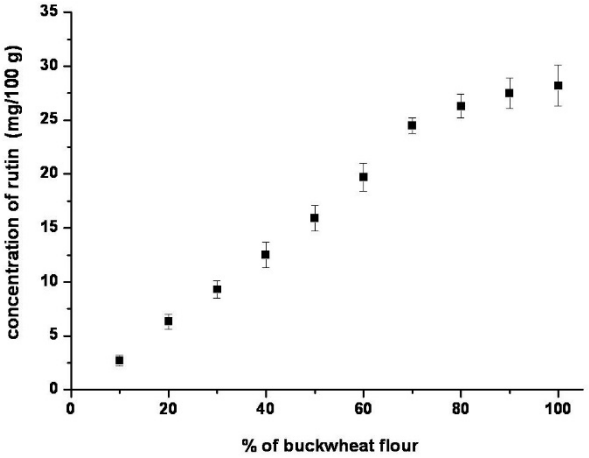

Figure 1 Content of rutin (mg/100g) in breads baked of various buckwheat and corn flour ratios

Total polyphenolic content (TPC) and total antioxidant activity (TAA) give information about expected antioxidant properties. As observed previously, addition of buckwheat flour to semolina flour significantly (p <0.001) increase antioxidant properties and polyphenolic content **(Biney et al., 2014)**. Similar trend p <0.001 was noticed in our measurements. TAA was calculated as total activity of hydrophilic and lipophilic antioxidants using $ABTS^{\cdot+}$ cation radical and the higher values of TPC and TAA were observed in samples with higher portion of buckwheat flour. However, observed trend did not correlate linearly with buckwheat flour content **(Table 1)** due to the more remarkable destruction of rutin in samples with higher contents of buckwheat flour.

Table 1 Total polyphenolic content (TPC) and total antioxidant activity (TAA) of buckwheat (F) and corn (S) flour mixture (F:S) expressed as gallic acid equivalent per 1 gram of dry matter (DM).

	TPC	TAA
F:S	mg GAE g^{-1} of DM	mg GAE g^{-1} of DM
100:0	3.95 ±0,02	4.16 ±0.04
90:10	3.81 ±0.02	4.09 ±0.02
80:20	3.63 ±0.02	3.38 ±0.04
70:30	3.03 ±0.02	2.42 ±0.05
60:40	2.88 ±0.05	2.36 ±0.02
50:50	2.53 ±0.05	2.05 ±0.05
40:60	2.26 ±0.02	1.57 ±0.02
30:70	1.67 ±0.02	1.18 ±0.04
20:80	1.38 ±0.07	0.91 ±0.02
10:90	1.23 ±0.05	0.97 ±0.05

3.2. Evaluation of thermal treatment on rutin content in whole meal flour and broken seed

The changes in rutin concentration after heating of whole meal flour and broken seeds are depicted in **Figure 2**.

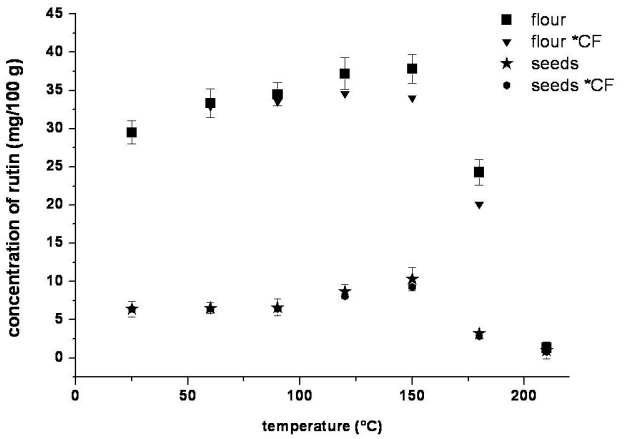

Figure 2 Effect of thermal treatment on rutin content in the whole meal flour and broken seeds. Corrected values correspond to the multiplying with corrected factor CF (25°C CF=1.00, 60°C CF=0.99, 90°C CF=0.97, 120°C CF=0.93, 150°C CF=0.90, 180°C CF=0.86 and 210°C CF=0.81)

Units or tens of mg of rutin per 100 g of buckwheat product samples were determined and approximately 5-times lower levels of rutin were found in broken seeds compare to whole meal flour ($p < 0.001$). Very steady increase of rutin content can be attributed to the destruction of the sample particles and easier extraction of rutin from their structure (the destruction is more evident especially in the case of broken seeds). Another reason can be explained by partial destruction of samples by overheating and degradation of some compounds of flour or seeds. The evaporation of some volatile compounds and degradation products can decrease the mass of the samples and formally increase the content of rutin (35.5 ±4.7 mg per 100 g for whole meal flour and 10.2 ±0.4 mg per100 g for broken seeds at 150°C). Serious decrease of rutin contents at elevated temperatures (>150°C) can be explained by its degradation and/or evaporation (24.3 ±1.4 mg per 100 g for whole meal flour and 3.06 ±0.3 mg per100 g for broken seeds at 180°C). Chromatograms of whole meal flour and broken seed extracts with peak of rutin are shown in **Figure 3**.

The changes in chemical composition and mass of heated samples can be compensated using correction factor (CF) that is equal to the ratio of calcium concentration at the start of heating ($c_{Ca\text{-}0}$) and calcium concentration at each heating temperature t ($c_{Ca\text{-}t}$), i.e. CF = $c_{Ca\text{-}0}/c_{Ca\text{-}t}$. The CF is equal to 1 at the lowest temperatures and it rises values <1 at the elevated temperatures for matrices with losses of mass or values >1 for matrices with increased mass. Multiplying the determined concentrations of rutin by CF we can compensate some changes in mass and chemical composition of matrices expecting thermal stability of calcium ions (CF equal to 1.00, 0.99, 0.97, 0.93, 0.90, 0.86 and 0.81 for 25°C, 60°C, 90°C, 120°C, 150°C, 180°C and 210°C, respectively).

3.3. Evaluation of thermal treatment of buckwheat seed hulls and herbs

More dissimilar results were obtained after boiling of seed hulls and herbs for preparation of buckwheat infusions. In comparison, rutin content detected in hulls was 100-times lower than in herbs ($p < 0.001$). It is in agreement with the findings of **Kreft et al. (2006)** and **Vojtíšková et al. (2014)** that the leaves and flowers of

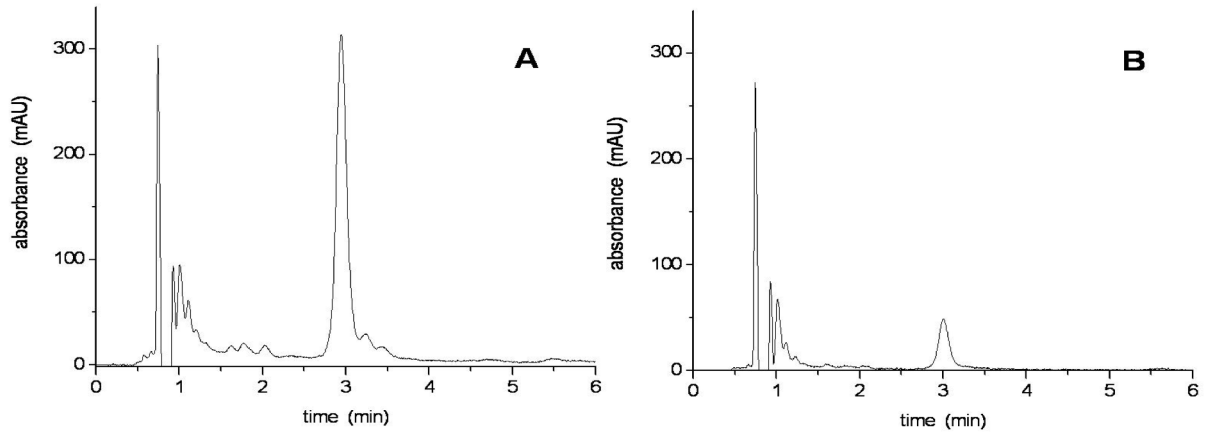

Figure 3 Chromatogram of the whole meal flour extract (A) and the broken seeds extracts (B) at 355 nm.

buckwheat plant are the richest sources of rutin. Observed amounts of rutin in seed hulls ranged from 7.2 mg per 100 g to 14.9 mg per100 g while concentrations in buckwheat herbs infusions varied from 716 mg per 100 g to 1302 mg per100 g. In both cases, increasing tendency related with warmer water and longer time of maceration **(Figure 4)** due to the higher efficacy of the maceration process.

CONCLUSION

Buckwheat for human nutrition is used in several forms. People use mainly seeds for making a gluten-free meal. Nowadays, a broad range of buckwheat products is available on a market, such as pasta, crunchy products, flour, tea (mixture of peels and milled aerial part of plants) *etc.* In this work, the highest content of rutin was obtained in tea prepared from buckwheat herbs while the lowest amount of rutin was determined in buckwheat broken seeds. Depending on increasing portion of buckwheat flour in cereal breads, nonlinear rising tendency in rutin content and antioxidant characteristics due to the more evident degradation of rutin was also observed. Serious decrease of rutin contents at elevated temperatures (>150°C) can be explained by its degradation and/or evaporation. Thus, classical mechanical milling must be preferred against thermal treatment and all cooking practices can avoid high temperature (t >100°C) treatment.

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