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Short communication

Partial decomposition of Tartary buckwheat and grape seed flavonoids during the preparation of pasta

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Abstract

Background and purpose: *Grain of Tartary buckwheat (*Fagopyrum tataricum (L.) Gaertn.*) is rich in phenolic substances, especially flavonoid rutin. The grains are used in Asia and Europe mainly to make pasta products. Grape (*Vitis vinifera L.*) processing, mainly for wine, results in by-products, including seeds, rich in secondary plant metabolites. Main grape seed flavonoids are catechin and epicatechin. There is a need for applying grape seed flavonoids in human nutrition. As is known, flavonoids could be subjected to metabolic changes during dough processing.*

Materials and methods: *Combined Tartary buckwheat/vinegrape pasta was produced in pasta machine. Starting seed flours and pasta were analyzed by LC-MS performed on UltiMate 3000 UHPLC system, coupled with a triple quadrupole/linear ion trap mass spectrometer. Methanol (Chromasolv LC-MS grade, Fluka, Switzerland) and purified water were used for the preparation of mobile phases, and formic acid (Fluka) was used as modifier. An analytical HPLC column Hypersil GOLD Aq, Thermo 150x2.1, 3 µm, was used with the flow rate of 0.3 mL min^{-1}. A mobile phase consisting of methanol and water, both modified with 0.1% formic acid was used throughout the work.*

Results: *In the present research, it was established that in the dough from the mixture of Tartary buckwheat and grape seed flours it is decomposed a considerable part of Tartary buckwheat flavonoid rutin, and grape seed flavonoids catechin and epicatechin.*

Conclusion: *Procedures for minimizing the loss of flavonoids during the manipulation of combined Tartary buckwheat and grape seed flour dough and preparing pasta are needed.*

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INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is a plant known as a rich source of nutritionally important flavonoids, especially rutin *(1)*. Flavonoids are in all parts of the Tartary buckwheat plants. Tartary buckwheat originates in Himalayan areas *(2)*. Due to the high altitude, a wealth of polyphenol content has developed in Tartary buckwheat. Phenolic substances protect plants from strong UV-B radiation and other environmental impacts *(3)*. By human migration, Tartary buckwheat was spread from Himalayas to Chinese regions of Sichuan and Yunnan, and further to Inner Mongolia and Europe *(4)*. Among the phenolic compounds in plants, quercetin and its derivatives, including rutin, are very important *(5)*. Phenolic substances, like quercetin, have been isolated from stool samples after the ingestion of plant food, rich in phenolic substances. These phenols may exert beneficial

functions in target tissues *(6)*. Quercetin can cross the blood-brain barrier and has important protective functions even in the brain *(6)*. The beneficial effects of Tartary buckwheat extract, mainly composed of polyphenols, include the exertion of the lipid-lowering effects of dishes *(7)*. Potential drawbacks of buckwheat phenolic substances are not reported, except for phototoxic fagopyrin, which is mainly in buckwheat flowers and green parts and not in grain *(5)*.

Buckwheat is used in different flour mixtures for food products *(8)*. Buckwheat flour is the basis for important functional food products with significant effects on health *(9)*. Buckwheat flavonoids are allocated in diverse milling fractions *(10)*.

Tartary buckwheat flavonoids rutin and quercetin have effects on the reduction in serum levels of myeloperoxidase and cholesterol *(11, 12)*. They have as well important antigenotoxic effects *(13)* and an antiproliferative impact on human breast cancer cells *(14)*. Buckwheat does not contain gluten proteins so that it could be a source of gluten-free dishes suitable for people who have celiac disease *(15)*.

During the preparation of dough, rutin could be transformed to quercetin and other phenolic molecules *(16)*. The transformation is mediated by the rutin degrading enzymes *(17)*. They could be deactivated by the hydrothermal treatment of buckwheat grain or flour products *(18)*. Tartary buckwheat gene FtRDEs promotes the concentration of quercetin by converting rutin to quercetin through strong activity in rutin hydrolysis. Besides the hydrolysis of rutin, this gene promotes the early phases of synthesis of flavonoids as well *(19)*. However, quercetin has important effects in inhibiting alpha-glucosidase activity and thus protecting diabetic patients *(20)*.

Buckwheat is often cultivated in the same areas, suitable for growing vine grape (*Vitis vinifera* L.) and producing wine. This is the case as well in Slovenia. Grape seeds are waste from wine production, they are rich in polyphenolic secondary metabolites *(21)*. Niculescu et al. *(22)* suggested the implementation of the sustainable circular economy, with the use of side products of the wine industry, including the use of grape seeds. Andabaka et al. *(23)* established that among the most abundant secondary metabolites in grape seeds are catechin and epicatechin, with some differences among grape varieties. Grape seeds contain substances with antimicrobial activity, which could be applied in food production, without chemical preservatives *(24)*. Grape seeds are important sources of catechin and epicatechin. These substances are able to enter the blood circulation after the meals with grape seed extracts *(25)*. This investigation aims to establish if and how much of valuable flavonoids from Tartary buckwheat flour and grape seed flour persist through the Tartary buckwheat noodle-making procedure.

MATERIAL AND METHODS

Preparing grape seed flour

Preparation of grape seed flour: After pressing juice from grapes (*Vitis vinifera* L., cv. Rumeni muškat, location Spodnji Duplek, Slovenia) at a temperature of around 12.5 °C, the seeds were isolated from the skin in a rotating metal cylinder by mixing with a hatch rotating in the opposite direction to the cylinder. Seeds were removed from the cylinder through the holes in the cylinder. Grape seeds were dried at 45 °C on sieves for 24 hours. Dried seeds were cleaned by the air flow to remove the rest of the grape skins. Until the milling, the grape seeds were stored in paper bags in a dark dry place at 20 °C. Undamaged grape seeds were cold pressed to remove the oil, by processing on a screw press, which crushes the kernels, with initial temperature 22 °C, which at the end of the process due to the pressure raised to 35–45 °C, and immediately cooled down to 20 °C (Press Prototip, Kokol, Spodnji Duplek, Slovenia). The seeds were, after pressing, milled on a stone mill A 700 MSM Naxos (Naxos, Greece), at 230 turns/min, to obtain the flour.

Tartary buckwheat samples

Tartary buckwheat grain and Tartary buckwheat whole grain flour (*Fagopyrum tataricum* (L.) Gaertn., cv. Zlata) were obtained from Rangus Mill (Rangus, Dolenje Vrhpolje, Šentjernej, Slovenia). Used wheat flour was Tip 400 ostra (Žito, Ljubljana, Slovenia).

Making pasta

Tartary buckwheat/vinegrape pasta was produced in four repetitions in pasta dough machine Dolly (Italy Food equipment Imperia Monferina, Termoli, Italy), by mixing 150 grams of Tartary buckwheat flour, 50 g of vinegrape seeds flour, 350 grams of wheat flour, 168 g of eggs (containing 42 g dry matter, 126 g water), and 50 g water. Pasta was produced by pasta Machine Dolly – (Italy Food equipment, Termola, Italy) with bronze dies, kneading capacity 2.5 kg, kneading time 30 min, no resting time . Extruded fresh pasta production (4.6 Kg/h, power 0.75 kW) resulted in flat noodles, dimensions 100 mm x 10 mm x 3 mm. Pasta was air dried at room temperature (22 °C) on specially made sieves measuring 800 x 570 mm and with hole sizes of 1.5 x 1.5 mm (EC Piramida Maribor, prototype) for 48 hours to reach a moisture content of 13.5%.

Extraction of phenolic compounds

For extraction 80 mg of each lyophilized and ground sample was weighed and mixed with 8 mL of 80% methanol (HPLC purity). The mixture was incubated for 10 minutes in an ultrasonic bath (ASonic, China). After incubation, the samples were centrifuged at room temperature for 6 min at 1,360 g. The supernatant was filtered

through a cellulose acetate filter with 0.2 µm pores. Extracts were stored in vials and kept in the refrigerator until measurements.

Determination of flavonoids by LC-MS

Experimental LC-MS part: LC-MS analysis was performed on UltiMate 3000 UHPLC system (Thermo Scientific, U.S.A.) coupled with a triple quadrupole/linear ion trap mass spectrometer (4000 QTRAP LC-MS/MS System; Applied Biosystems/MDS Sciex, Ontario, Canada). Methanol (Chromasolv LC-MS grade, Fluka, Switzerland) and water purified on a Milli-Q system from Millipore (Bedford, MA, USA) were used for the preparation of mobile phases, and formic acid from Fluka was used as modifier. An analytical HPLC column Hypersil GOLD Aq, Thermo 150x2.1, 3 µm, was used with the flow rate of 0.3 mL min^{-1}. A mobile phase consisting of methanol and water, both modified with 0.1% formic acid was used throughout the work. Injection volume and column temperature were 10 µL and 30 °C, respectively.

Quantification and calibration procedure

Quantification of analytes was performed using external calibration curves prepared from standard solutions in the concentration range of 0.01 µg/mL to 50 µg/mL. Calibration standards were obtained from Fluka, Sigma Aldrich, and ABCR. Each analyte's concentration in the samples was determined based on the corresponding calibration curve. Due to the dilution of the samples prior to the analysis, matrix effects were not expected and, therefore, matrix-matched calibration was not applied. The assumption of negligible matrix effects was confirmed by the linearity and consistency of the calibration responses. Limits of detection (LOD) and limits of quantification (LOQ) were not directly determined in this study; however, any analyte concentrations reported as below LOD correspond to the values far below the lowest point of the calibration curve, and no measurable signal was observed.

Statistical analysis

Data were represented as mean value and standard deviation of four independent repetitions for each sample. Statistical analysis was made using the statistical software XL Stat for Excel (Version 2023.3.0, Addinsoft). The normal distribution of data was checked with Shapiro–Wilk test. Homogeneity of variance from the means was assessed using Levene's tests. Differences between treatments were tested using one-way analysis of variance followed by Duncan testing. Data for gallic acid were analyzed with a t-test to distinguish between the content in Tartary buckwheat and grape seed flour pasta and content in grape seed flour. The level of significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

In the analyzed pasta, made from the combination of Tartary buckwheat flour and grape seed flour (with the addition of wheat flour, eggs, and water), there were 0.97 mg/g of rutin originating mainly from Tartary buckwheat flour (Table 1). From the content of rutin in the ingredients it could be expected in pasta about 4.04 mg/g rutin in dry matter.

Different letters in columns indicate significant differences between the different samples ($p < 0.05$). Mean±SD. Concentrations of gallic acid in TB grain and TB flour were under the detection limit.

The result obtained for pasta is much below the expected value. It is known that in buckwheat dough, rutin could be degraded *(16, 18)*. The content of quercetin in pasta would be expected at the level about 0.1 mg/g in dry matter, but the obtained value is higher, 0.73 mg/g (Table 1). If all the missing amount of rutin (around 3 mg/g) is transformed into quercetin, it would be about 1.5 mg/g quercetin in pasta. Namely, quercetin molecules are after the separation of the sugar part from rutin smaller in comparison to the rutin molecules. Rutin molecules are degraded partly to quercetin, and a smaller portion of the quercetin is degraded further to other phenolic molecules.

Likewise to rutin, originating from Tartary buckwheat, most catechin and epicatechin, originating from grape seed flour, are degraded during the preparation of pasta. Instead of expected 1.06 mg/g catechin in dry matter, it is only 0.62 mg/g in pasta. Instead of the expected 0.67 mg/g epicatechin in dry matter, it is only 0.47 mg/g epicatechin in dry matter in pasta (Table 1). Thus, the amount 0.20 mg/g in pasta dry matter is missing. During the dough processing, catechin and epicatechin are partly destroyed by some degradation process. So, not only a part of Tartary buckwheat flavonoid metabolites but also

Table 1. *Flavonoid metabolites in Tartary buckwheat (TB) and grape seed samples and pasta (mg/g in dry matter).*

	Rutin	Quercetin	Catechin	Epicatechin	Gallic acid
TB grain	15.3 ± 0.8 ^{b}	0.29 ± 0.08 ^{b}	0.012 ± 0.001 ^{c}	0.03 ± 0.003 ^{c}	
TB flour	18.2 ± 1.8 ^{a}	0.45 ± 0.35 ^{ab}	0.013 ± 0.001 ^{c}	0.02 ± 0.002 ^{d}	
Grape seed flour	0.01 ± 0.003 ^{d}	0.004 ± 0.003 ^{c}	14.33 ± 0.263 ^{a}	9.06 ± 0.30 ^{a}	0.29 ± 0.02 ^{b}
Pasta	0.97 ± 0.03 ^{c}	0.73 ± 0.08 ^{a}	0.62 ± 0.010 ^{b}	0.47 ± 0.01 ^{b}	0.042 ± 0.003 ^{a}

grape seed secondary metabolites could be partly destroyed during the noodle making process.

During the milling and production of Tartary buckwheat flour, the concentration of rutin is decreased *(10)*. It is further decreased as a result of the degradation of rutin to quercetin *(16)*. So, in the experimental pasta there is more quercetin in comparison to either Tartary buckwheat flour or grape seed flour. Instead of about 0.3 mg/g it is 0.7 mg/g rutin in dry matter in combined pasta (Table 1).

In Tartary buckwheat flour, there are only traces (0.013 mg/g in dry matter) of catechin, while grape seed flour contains 14.3 mg/g of catechin (Table 1). Instead of expected about 1.4 mg/g of catechin in pasta, we established only 0.6 mg/g. This points to catechin degradation during dough and pasta making and drying.

Epicatechin is in Tartary buckwheat flour in an amount of about 0.02 mg/g and in grape seed flour 9.02 mg/g (Table 1). Instead of expected about 0.9 mg/g, there is only 0.6 mg/g of epicatechin in pasta, which shows the possibility of some epicatechin degradation during pasta preparation. So, epicatechin is probably subjected to similar degradation process as catechin during pasta preparation.

In contrast to other studied metabolites, gallic acid is present only in grape seed flour and in this case no detectable amounts are in Tartary buckwheat flour. The concentration of gallic acid in grape seed flour is at the level of 0.3 mg/g (Table 1). The amount 0.021 mg/g in pasta dry matter should be expected from grape seed flour. However, pasta contained 0.042 mg/g dry matter of gallic acid (Table 1). The content of gallic acid in pasta could appear as one of the intermediate steps of the degradation of other phenolic substances during pasta dough manipulation or from wheat flour *(26–28)*. Gallic acid, which is not contained in buckwheat samples, may, together with grape seed, improve *in vitro* starch digestibility, antioxidant and eating quality in buckwheat dish *(29)*.

The possible content of chlorogenic acid, neochlorogenic acid, caffeic acid, vitexin, isovitexin, emodin and orientin was investigated in studied samples of Tartary buckwheat, grape flour, and pasta, but none of them was confirmed in studied samples. Traces of resveratrol (about 0.0045 mg/g) and piceid (about 0.013 mg/g) were found in grape seed samples, but none were found in detectable amounts in Tartary buckwheat grain, flour, or pasta samples.

The effects of combined pasta could be mainly attributed to the interactions among phenolic substances, resistant starch and proteins in the flour *(5)*. Polyphenols have an impact on protein digestibility after hydrothermal treatment. Their interaction reduces the digestion of protein through the small and large intestines. Microbial processes in the colon enhance the digestibility of the grain protein and starch, which are otherwise blocked by polyphenols in processed buckwheat food products.

Tartary buckwheat pasta has been rising in popularity, especially among consumers conscious in food quality. This provides the possibility to develop new, more "modern", food products based on old culinary traditions, with re-evaluation through contemporary scientific knowledge of the quality and potential of Tartary buckwheat and grape seeds *(5)*.

CONCLUSIONS

As the result of this investigation, it appears that in using the mixture of Tartary buckwheat flour and grape seed flour for pasta, the decomposition of nutritionally valuable flavonoids must be prevented. This could be done either by hydrothermal treatment of Tartary buckwheat flour and grape seed flour or by the use Tartary buckwheat flour with the genetically reduced amount of flavonoid degrading enzymes.

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