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Front page photo: Marbled bread. See paper of Vombergar et al., this issue, pages 35-42.

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Research paper

The disappearance of vitexin from Tartary buckwheat flour-water mixtures after the hydrothermal treatment

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ABSTRACT

Vitexin is an apigenin flavone glucoside with known biological functions. This research reported the effects of initial temperature treatments of Tartary buckwheat flour mixture with water and time of methanol extraction from the cooked doughs on the amount of extractable vitexin. The mixtures of flour and water were initially hydrothermally treated at temperatures from 25 °C to 95 °C. Afterward the mixtures were cooked at 95 °C for 20 min, and vitexin extracted at room temperature with 80% aqueous methanol for 20 min, 2 h and 8 h. The extractable vitexin was extracted during the same extraction times for the control in the nonhydrothermally treated Tartary buckwheat flour-water mixture samples. For the cooked dough samples, the hydrothermal treatments were important in terms of the extractability of vitexin. The extractable vitexin persisted in the control samples during the extraction time up to 8 hours, while in the hydrothermally treated and cooked dough samples, there remained none detectable vitexin. The high-temperature initial treatments during dough preparation appeared not to prevent the degradation of vitexin in Tartary buckwheat flour.

INTRODUCTION

Vitexin is a vital nutraceutical known for suppressing renal cell carcinoma and wound healing effects (Kim et al., 2005; He et al., 2016; Li et al., 2020; Bektas et al., 2020; Peng et al., 2021). Abbasi et al. (2021) reported the neuroprotective effects of vitexin, the experiments with rats have shown that vitexin has anticonvulsant effects in the brain.

Ganesan and Xu (2017) reviewed comprehensive information on various cancers and therapeutic possibilities of vitexin and isovitexin. According to Ganesan and Xu (2017), both, in vitro and in vivo studies are suggesting that vitexin and its isomere isovitexin are compounds with chemopreventive activity against various cancers, as they are included in proapoptotic events and/or autophagy. Li et al. (2020) reported the suppression of renal cell carcinoma by vitexin. Peng et al. (2020) reviewed recent advances in studies of absorption, metabolism, and bioactivity of vitexin. Bektas et al. (2020) recently evaluated the wound healing effect of chitosan-based gel formulation containing vitexin.

This activity is possibly through interaction at the benzodiazepine site of the gamma-aminobutyric acid type A receptor complex (Abbasi et al., 2012). Vitexin has shown free radical scavenging activity in ultraviolet B-irradiated cultured human dermal fibroblasts (Dong et al., 2011).

Due to important effects on human health, Tartary buckwheat has become more widely used for preparing

gluten-free foods (Kreft 2016, Costantini *et al.* 2014, Capraro *et al.* 2018). Tartary buckwheat contains phenolic compounds that are reported to have antioxidant properties (Holasoava *et al.* 2002;).

Hydrothermal treatments comprised heating with steam or hot water and followed by slow cooling and drying. The present study aimed to define the effects of temperature and moisture in Tartary buckwheat dough preparation on the concentration of extractable vitexin.

MATERIAL AND METHODS

The Tartary buckwheat flour (cv. 'Zlata') was obtained from Rangus Mill (Šentjernej, Slovenia). Flour (100 g) containing 8% moisture was mixed for 1 min with 200 g water at 25 °C, 45 °C, 60 °C, 80 °C, or 95 °C. The resulting doughs were kept in a chamber for 20 min at their respective temperatures. After this treatment, each dough sample was heated to 95 °C for a further 20 min. After cooling these cooked dough samples to room temperature, they were frozen (-18 °C) until they were lyophilized. The complete procedures were performed independently as three repetitions. The freeze-dried samples were milled and used in the methanol extractions for HPLC analyses. Extraction and HPLC analyses were performed according to the methods described by Germ et al. (2019).

The normal distribution of the data was tested using Shapiro-Wilk tests. Differences between treatments were tested using one-way analysis of variance followed by Duncan multiple range test. The level of significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

The extractability of vitexin using 80% aqueous methanol from the control Tartary buckwheat flour samples and following the cooking (95 °C, 20 min) of the hydrothermally treated (25, 45, 60, 80, 95 °C) Tartary buckwheat dough samples are illustrated in Table 1. In the controls, all of the extractable vitexin was extracted within the first 20 min, which remained similar after the extraction times of 2 h and 8 h. Thus, vitexin was easily extractable from the untreated Tartary buckwheat flour using 80% aqueous methanol.

For the hydrothermal treatments of the doughs at 25, 40, and 60 °C, the vitexin extracted from the cooked doughs using 20% aqueous methanol was lower than detection limits. Control samples contained about 5.4 to

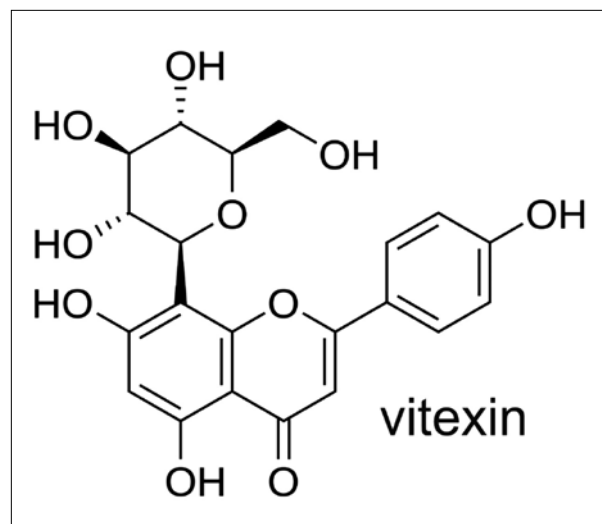


Figure 1. Vitexin molecule. The upper part of the vitexin molecule is glucose, the lower part of the molecule is apigenin.

Table 1. Time-courses of vitexin extraction (mg vitexin·kg⁻¹ dry matter) with 20% (v/v) aqueous methanol for Tartary buckwheat flour (C; control) and following cooking (95 °C, 20 min) of the hydrothermally treated (25, 45, 60, 80, 95 °C) Tartary buckwheat dough samples. Data are means ± standard deviation (n = 3), except for treatments where vitexin concentration was under the limit of detection. <LOD means under the limit of detection

Extraction time	Control	25°C	40°C	60°C	80°C	95°C
20 min	5.4 + 1.2 ^a	<LOD	<LOD	<LOD	<LOD	<LOD
2 hours	6.3 + 0.4 ^a	<LOD	<LOD	<LOD	<LOD	<LOD
8 hours	6.4 + 0.4 ^a	<LOD	<LOD	<LOD	<LOD	<LOD

Means followed by the same superscript letters are not significantly different at $p < 0.05$ (n = 3). Legend: LOD – under detection limit

6.4 mg vitexin·kg⁻¹ dry matter (Table 1). Also, after all of the dough hydrothermal treatments (i.e., 25, 40, 60, 80, 95 °C), there were no more detectable concentrations of vitexin extracted from the cooked doughs in any of the times of extraction. Thus, in one scenario vitexin might be degraded by the conditions of hydrothermal treatment of the Tartary buckwheat doughs. Alternatively, vitexin might form insoluble complexes.

In nonhydrothermally treated Tartary buckwheat flour samples, extraction of vitexin with 80% aqueous methanol was complete within 20 min of extraction.

The sugar part of vitexin is glucose (Figure 1). The aglicone part is apigenin.

For the difference to rutin, which is O glucoside (sugar part and the flavonoid part of the molecule are connected by oxygen), is vitexin C glicoside (sugar and flavonoid part are connected by carbon-carbon bond). C glycosides are more stable than O glycosides, and they are less likely to be split apart. However, it is possible.

One possibility for disappearance of vitexin after hydrothermal treatment is its oxidation, and by additional –OH group there could appeared orientin. But no orientin was detected in the samples.

According to our knowledge, this is the first report that Tartary buckwheat contains in grain and flour a flavonoid vitexin, which is stable during the prolong extraction by methanolic extraction medium, but not stable when the mixture of flour with water is exposed to hydrothermal condition. Regarding importance of vitexin in health-preserving nutrition it is a challenge to study further the appearance and disappearance of vitexin and related metabolites in Tartary buckwheat.

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IZVLEČEK

Vpliv hidrotérmičnega tretiranja na vsebnost viteksina v testu iz tatarske ajdove moke

Viteksin je flavonski glukozid z apigeninom. Viteksinu pripisujejo pomembne biološke učinke. V članku avtorji poročajo o vplivu predhodnega tretiranja mešanice moke tatarske ajde in vode pri različnih temperaturah na ekstrakcijo viteksina iz tako pripravljenega testa. Mešanica tatarske ajdove moke in vode je bila 20 minut tretirana pri temperaturah od 25 °C do 95 °C in nato še 20 minut pri 95 °C. Rkstrakcija viteksina je potekala pri sobni temperaturi (80% vodnim metanolom) 20 minut, 2 uri oziroma 8 ur. Na enak način je bil termično netretiran viteksin ekstrahiran tudi iz kontrolne mešanice tatarske ajdove moke. Na kuhane vzorce testa (mešanice moke in vode) je imelo hidrotérmično tretiranje pomemben vpliv. Medtem ko je bila pri kontrolnih vzorcih količina viteksina nespremenjena še po 8 urah ekstrakcije, je bil pri hidrotérmično tretiranih vzorcih viteksina pod mejo detekcije. Hidrotérmično tretiranje testa tatarske ajde torej ni preprečilo degradacije viteksina v tatarski ajdovi moki.

Review paper

New trends in preparing buckwheat dishes in Slovenia

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ABSTRACT

Slovenian cuisine is traditionally related to the use of buckwheat, especially common buckwheat. At the Education Centre Piramida Maribor there are developing possibilities of using common and Tartary buckwheat in modern gastronomy. This paper presents buckwheat dishes, which were developed and upgraded according to the trends in modern nutrition, along with market responses. Present varieties of buckwheat bread made with different leavening agents (e.g. yeast, sourdough, baking powder, cream of tartar, mineral water and without rising agents), confectionery (e.g. cakes, pies, cream-pies, strudels, biscuits) from Tartary buckwheat, common buckwheat and other flours and also Tartary buckwheat pasta are presented. Buckwheat (either as gruel or as flour) can also be used as an ingredient in filling the chocolate pralines or in ice cream. In addition various buckwheat dishes with a touch of contemporary trends and culinary art are introduced. Furthermore, the possibilities of using common and Tartary buckwheat flour for gluten-free confectionery and for a variety of sweet and savory gluten-free dishes in the culinary will be presented. The latest speciality will be the presentation of buckwheat bread, confectionery and pasta combined with edible flowers.



Fig. 1. Marbled bread. Left: Tartary buckwheat part (the dark greenish one, 30% Tartary buckwheat flour, with wheat flour), the grey part is 30% common buckwheat flour, with wheat flour, and the white part is 100% wheat flour. Right: the grey part is 30% common buckwheat flour, with wheat flour, the white part is 100% wheat flour, and yellow part is made from corn flour (30% corn flour, with wheat flour).

INTRODUCTION

Slovenian cuisine is traditionally related to the use of buckwheat. We know several Slovenian national dishes, such as buckwheat groats, buckwheat 'žganci', buckwheat

'krapec', buckwheat black sausage, buckwheat 'bizejski kolač', 'ajdnik', etc.. According to demands and responses of the consumers authors from the Education Centre Piramida Maribor developed and upgraded bread, confectionery, pasta, ice cream and some other buckwheat dishes.



Fig. 2. Tartary buckwheat bread (30% to 50% Tartary buckwheat flour with wheat flour) with added walnuts.



Fig. 3. Bread with 30% of Tartary buckwheat flour, and wheat flour, with the addition of dry common buckwheat flowers.

TECHNIQUES, TECHNOLOGIES AND PRODUCTS

Various techniques and technologies for the manufacture of our products were used, and their sensory quality or acceptance were monitored. Mixtures of buckwheat flour or Tartary buckwheat flour with wheat flour were used in the production (Kreft et al., 2012; Vombergar et al., 2012; 2013; 2015; 2016; 2018; 2020). However, use of Tartary buckwheat in Slovenian gastronomy is less known. At EC Piramida over the past years, more than 100 different products and dishes from Tartary



Fig. 4. Tartary buckwheat milk bread (50% Tartary buckwheat flour, and 50% of wheat flour), prepared with milk, eggs, butter and sugar, with the addition of elder (*Sambucus nigra L.*) flowers.



Fig. 5. Salted Tartary buckwheat pretzels (mixture of Tartary buckwheat flour (30%) and wheat flour)

buckwheat were developed, and using in gastronomy (Figs. 1 – 18). A positive market response to innovative products was seen. The properties of various sorts of buckwheat dough or mixtures of flours, which were added to the Tartary buckwheat flour, were examined and several technological procedures for the production of buckwheat bread, confectionery and pasta were developed. In the pilot production of **yeasted dough for bread** mixtures of buckwheat flour with wheat flour were produced in different proportions (30-50% buckwheat flour, and the remainder wheat flour). Various leavening agents were used (for example, yeast, yeast starter, sourdough, baking powder, cream of tartar and mineral water). **In confectionery** a mixture of buckwheat and wheat flour in a ratio of 1: 1 was used for the puffy pastry dough (strudels, cream-pies, etc.), puffy pastry yeast dough and a leavened sweet milky dough. The same blend of flour can be used for all kinds of confectioneries, cakes, pies, sponge cakes and biscuits. The preparation of **buckwheat pasta** is simple by using a mixture



Fig. 6. Braided bread with 30% to 50% Tartary buckwheat flour, with wheat flour. Sprinkled with sesame or poppy seeds.



Fig. 7. Tartary buckwheat braided bread with cheese and wasabi cheese filling, made by Marko Kržan, student of EC Piramida Maribor, Slovenia.



Fig. 8. „Potica“ baked cake with walnuts cream filling (to the right), and with poppy seeds filling, in this case made from the mixture of Tartary buckwheat (50%) and wheat flour.



Fig. 9. „Piramida“ Tartary apple cake. Gluten-free, in the dough 100% Tartary buckwheat flour, without any addition of wheat flour.

of buckwheat and wheat flour in a ratio of 1:1 (Fig. 12). Rolled and extruded pasta (wide and thin noodles, etc.) were produced. Up to 75% of Tartary buckwheat flour can be used in the production of extruded pasta. Pasta is greenish brown, slightly brighter after drying, while after boiling it becomes dark brown.

The demand for **gluten-free products** in Slovenia and Europe is increasing. Good sensory properties have for example buckwheat sponge cake and buckwheat shortbread biscuits and it is possible to use buckwheat flour or Tartary buckwheat flour as the only flour without the addition of any wheat flour. Buckwheat gingerbread



Fig. 10. Buckwheat „brizganci“. Yellow part is made from mixture of Tartary buckwheat flour (if desired with the addition of some wheat flour), the gray parts are made from common buckwheat flour (if desired with the addition of some wheat flour).



Fig. 11. Common buckwheat „krapec“ with edible daisy (*Bellis perennis L.*) flowers, produced from common buckwheat flour, and some cottage cheese and sour cream.

is also an excellent gluten-free product. Tartary sponge cake (for cakes) and pie dough are also interesting for introducing gluten-free dishes into the gastronomy. The dough is darker, and after baking it becomes dark brown with a greenish-yellowish shade. However it is much more difficult to use buckwheat flour as the only raw material in breadmaking, for bakeries and 'potica', Slovenian traditional product. Technologies are explored in this direction and we are persistently looking for suitable solutions (Figs. 9, 13, 14).



Fig. 12. Tartary buckwheat pasta, in the dough are incorporated dandelion (*Taraxacum officinale* F.H. Wigg) florets.

Some other cereals (e.g., barley, millet, oats, rice) were also tested and we already have some results. All combinations depend also on the quantity and quality of proteins and starch in different flours.

There are many ideas for an interesting offer of gluten-free products and foods. Tartary or common buckwheat groats can be cooked and served with vegetables or mushrooms as side dishes. Buckwheat groats can be also served for dessert, for example with yogurt and fruit. For flavoring you can also add it to ice creams, puddings and yoghurts. Groats can also be baked in an oven after cooking, with the addition of cottage cheese, sour cream, eggs, sugar and butter, or maybe fruits, nuts, etc., but it can also be combined with millet porridge and oat flakes.

In similar way chocolate pralines with Tartary buckwheat and roasted buckwheat flour, with some wine additives for the excellent taste were developed. Tartary buck-



Fig. 13. Gluten-free common buckwheat sponge cake with poppy seeds.



Fig. 14. Tartary buckwheat honey hearts made of Tartary buckwheat flour (alternatively some replacement with wheat flour) among other ingredients are butter, sugar, buckwheat honey or forest honey (Vombergar et al., 2018).

wheat tea can be sweetened with honey; the best choice would certainly be buckwheat honey. Buckwheat 'žganci' can be prepared from common or Tartary buckwheat flour, and polenta by using maize and Tartary buckwheat flours. The characteristic of Tartary buckwheat products is their bitterness. That can be somehow annoying for an unwitting consumer. Nevertheless, bitterness can be partly softened in the preparation process by adding spices, herbs, chocolate, cocoa, or other ingredients.



Fig. 15. Special cooked buckwheat rolls („štruklji“), greenish-yellow layer with Tartary buckwheat flour (and 50% wheat flour), grey layer with common buckwheat flour (and 50% wheat flour) and poppy seeds filling.



Fig. 16. Cooked Tartary buckwheat rolls („štruklji“), with poppy seed filling.

Recently the upgrading the offer of buckwheat products by using or adding **edible flowers** to the products is performed (Figs. 3, 4, 11, 12). This trend is becoming more and more popular, and it presents also an excellent decoration. Edible flowers can also affect human health in different ways. Buckwheat bread, confectionery and pasta with the addition of edible flowers, e.g. buckwheat, chamomile, and apricot blossoms, primroses, daisies, violets, etc. could be presented. Learning about and following the trends in nutrition and also market requirements are important catalysts for the offer of novel buckwheat products.



Fig. 17. Cooked Tartary buckwheat rolls ("štruklji"), with walnuts filling.

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Fig. 18. Tartary buckwheat snack sticks (50% Tartary buckwheat flour, and wheat flour).

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IZVLEČEK

Novi trendi pripravljanja ajdovih jedi v Sloveniji

Za slovensko tradicionalno kulinariko so značilne nekatere ajdove jedi in izdelki. Predvsem je znana uporaba navadne ajde, ne pa tudi tatarske ajde. Na Izobraževalnem centru Piramida Maribor so razvili več vrst pekovskih in slašičarskih izdelkov iz navadne in tudi tatarske ajde. Pri tem so sledili trendom in smernicam sodobne gastronomije in povpraševanjem potrošnikov po zdravih in zanimivih jedeh. Uporabljene so bile različne tehnologije izdelave (različni deleži navadne in tatarske ajdove moke v testu, različna vzhajalna in rahljajalna sredstva - kvas, kislo testo, pecilni prašek, vinski kamen, mineralna voda, brez kvasa). Predstavljeno je več vrst kruhov (tatarski ajdovi kruhi, pisani kruhi, zmesni kruhi, pletenice iz tatarske ajde), slaščic (torte, pite, kremne rezine, zavitki, biskviti) in testenin iz navadne in tatarske ajde ter tudi drugih mok. Praženo ajdovo moko so uporabili tudi za polnilo za čokoladne pralineje, kuhano ajdovo kašo pa za sestavino v sladoledu. Predstavljena je tudi možnost uporabe navadne in tatarske ajde za izdelavo kruhov in sladic brez glutena, saj se v sodobnih gastronomskih trendih zaznava povpraševanje po izdelkih brez glutena. Pripravljenih je več ajdovih izdelkov (kruhov, slaščic in testenin) z užitnimi cvetovi.

Research paper

Rutin and quercetin in common and Tartary buckwheat flour and dough

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ABSTRACT

The concentration of flavonoids rutin and quercetin in flours of common and Tartary buckwheat was investigated. In Tartary buckwheat, concentration of rutin is much higher compared to common buckwheat. In Tartary buckwheat it was measured 1.17 to 1.75% rutin in dry matter, while in common buckwheat it was only 0.003%. After direct contact of buckwheat flour with water, different biochemical activities in Tartary buckwheat developed with rutin. After the time (5 minutes or two hours), the concentration of rutin is in the flour-water mixtures much lowered, and quercetin appeared. However, after quick initial changes, some rutin remained in flour-water mixtures even after 24 hours. In any way, after 24 hours of direct contact of flour particles with water, the concentration of quercetin is higher than that of rutin. It is established that the concentration of rutin in flour-water mixtures is the result of two processes. One is the release of rutin from grain structures and its dissolving in water, and the second is the release of rutin degrading enzymes from grain structures and their activity in solution.

INTRODUCTION

Knowledge about the concentration of rutin and other phenolic substances in buckwheat is important for the use of buckwheat in healthy food products. About the rutin content in buckwheat are many publications, some of them are dating from the middle of the previous century (Couch et al., 1946), and some are dated from more than 25 years ago. Since 1990, about considerable amounts of rutin and other polyphenolic substances reported some Slovenian authors (Luthar, 1992a; Kreft and Luthar, 1993; Kreft et al., 1994), there are as well many other reports, especially since the year 2000 (Kim et al., 2001; Kreft et al., 2006; Kim et al., 2008; Bonafacia et al., 2009; Stojilkovski et al., 2013).

Phenolic substances are important for plants, as well as in human nutrition. They protect against viruses, bacteria, and against herbivores; they are protecting plants from UV radiation. In plants, the concentration of phenolic substances depends on species, cultivar, growing conditions, climate (temperature, light, precipitation) and agrotechnical factors (Häkkinen et al., 1999).

Flavonoids (for example, rutin, kaempferol and quercetin) are secondary metabolites of plants that deserve considerable attention because of their potential antioxidative, antiviral, antiallergic and other health-protecting effects (Griffith et al., 1944; Arima et al., 2002; Kawa et al., 2003; Russo et al., 2004; Anthoni et al., 2008). They are used in medicine, pharmacy, cosmetics, and nutrition (Bian et al., 2004; Lee et al., 2005; Anthoni et al., 2008; Vogrinčič et al., 2010; Costantini et al., 2014; Merendino et al., 2014; Lukšič et al., 2016a,b).

Rutin (C₂₇H₃₀O₁₆) is a quercetin-3-rutinoside. It is a product, produced by higher plants, protecting them against UV radiation (Gaberšček et al., 2002; Rozema et al., 2002). It exists in many plants, but only a few of them are of importance in human nutrition. Environmental factors, like UV radiation, are important in triggering its synthesis in plants (Kreft et al., 2002; Regvar et al., 2012). Rutin and its aglycone quercetin have an antioxidative impact *in vitro* and *in vivo*. Rutin degrading enzymes degrade rutin to quercetin and rutinose.

RUTIN IN BUCKWHEAT

In buckwheat rutin is the main flavonoid. It is located in different plant parts. There are different concentrations of rutin among buckwheat species and cultivars (Table 1). Ohsawa and Tsutsumi (1995), Kitabayashi et

al. (1995) and Ghimery et al. (2009) discussed these differences. The concentrations depend on buckwheat plant genotype, development phases, growing conditions, weather, and differences among the years of cultivation.

Rutin is located in different parts of the plants, including the grain. In dark buckwheat flours, there is more rutin than in the light ones. Experiments revealed that Tartary buckwheat has more rutin in all parts of the plants than in common buckwheat (Briggs et al., 2004). About the differences in buckwheat, content reported several authors (Fabjan et al., 2003; Park et al., 2004a,b; Suzuki et al., 2004; Asami et al., 2007; Fabjan, 2007; Brunori and Végvári, 2007; Ghimeray et al., 2009). Even in groats and flour of Tartary buckwheat, there is more rutin in Tartary buckwheat in comparison to common buckwheat (Steadman et al., 2001). The concentration of rutin in buckwheat milling fractions was studied by Kreft et al. (1999), Hung and Morita (2008).

Literature reports that Tartary buckwheat contains from 30 to 150-times higher concentration of rutin than common buckwheat (Yasuda et al., 1994; Suzuki et al., 2002; Vombergar, 2010 and 2020), and that it has strong glucosidase activity because of the rutin degrading enzyme (Suzuki et al., 2002). It is supposed that rutin and the enzyme protect buckwheat grain from UV radiation during the maturation and some other activities (Suzuki et al., 2005).

Kreft et al. (2006) reported on rutin content in the grain of common buckwheat 'darina', 'darja' and 'siva II'. They find that rutin content in hydrothermally treated buckwheat (porridge) is significantly lower than in raw buckwheat. In addition, light buckwheat flour from the inside of the grains (endosperm) has a lower rutin content than darker flour from embryo and the outer layers of the grains.

Yasuda (2001) reported that buckwheat grain contains a large amount of rutin and enzymes that break down rutin. He noted that rutin is rapidly broken down into quercetin when water is added to flour. Many buckwheat dishes are prepared by mixing buckwheat flour and water. The dough, which is usually used for bread, pastries, or pasta, can rest for a specific time to develop the appropriate texture and technological properties. Several authors have reported the content of rutin in bread and pasta (Vogrinčič et al., 2010 and 2013; Costantini et al., 2014; Merendino et al., 2014). The use of Tartary buckwheat flour as a source of rutin is limited due to the enzymatic degradation of rutin in the dough preparation pro-

Table 1: Rutin and quercetin content in common buckwheat flour and Tartary buckwheat flour

Source	Sample	Rutin content	Quercetin content	References
Common buckwheat	Fine flour	0.155 g/kg DM	0.002 g/kg SS	Steadman et al. (2001)
Common buckwheat 'siva'	Light flour Dark flour	19 mg/kg DM 168 mg/kg DM		Kreft et al. (1999)
Common buckwheat	Flour	380–1010 mg/kg DM		Qian et al. (1999)
Common buckwheat	Flour	98 mg/kg		Quettier-Deleu et al. (2000)
Common buckwheat 'siva', 'darja'	Flour	305–322 mg/kg DM	0	Fabjan (2007)
Common buckwheat	Flour	10–20 mg/100 g	cca. 1 mg/100 g	Asami et al. 2007
Common buckwheat	Light flour	19–168 mg/kg DM		Škrabanja et al. (2004)
Common buckwheat	Light flour	112.8 mg/kg DM		Kreft et al. (2006)
Common buckwheat	Coarse dark flour	57–77 mg/kg DM		Škrabanja et al. (2004)
Common buckwheat	Dark flour	218 mg/kg		Kreft et al. (2006)
Tartary buckwheat Lux01	Flour	6315 mg/kg DM	0	Fabjan (2007)
Tartary buckwheat Lux05	Flour	5049 mg/kg DM	0	Fabjan (2007)
Tartary buckwheat	Flour	30000 mg/kg		Mukasa et al. (2009)
Tartary buckwheat	Flour	1200 mg/100 g	cca. 1 mg/100 g	Asami et al. (2007)
Tartary buckwheat	Flour	20421 mg/kg DM		Soon-Mi et al. (2006)
Tartary buckwheat	Flour and water mixture (less than 100 min)	5000 mg/kg		Mukasa et al. (2009)
Tartary buckwheat (Luxemburg)	Bread	traces	4.99 mg/g	Germ et al. (2009)

cess, which also results in a bitter taste (Li et al., 2008). To determine the potential inactivation of enzymes that degrade rutin and cause a decrease in rutin content in products and discoloration during dough preparation, various authors studied different pre-treatment procedures of buckwheat (heating, steaming, cooking, extrusion). When boiling, cooking and extruding, most of the rutin can be retained, and the bitter taste does not appear. The effect of fermentation on rutin and other polyphenols, enzyme activity, linkages to pH, and other factors was studied by Han et al. (2002) and Krahl et al. (2008). Research is also on the interactions between proteins and flavonoids (Arts et al., 2002).

MATERIAL AND METHODS

Material

Two buckwheat samples were investigated, common buckwheat (*Fagopyrum esculentum* Moench) – cv. 'siva' from Slovenia (sample S), and a sample of Tartary buck-

wheat (*F. tataricum* Gaertn.) from Germany, originated from Luxemburg (sample T). Common buckwheat was obtained as grain, and Tartary buckwheat as a flour, after milling in Slovenia, 42% of flour gain from grain (sample T).

For the determination of rutin and quercetin, common husked buckwheat grain were milled on a Udy-Tecator Mill (Landskrona, Sweden) with a sieve with openings 0.7 mm, to obtain whole grain flour. For the determination of rutin and quercetin in Tartary buckwheat flour it was used the flour obtained.

Dough (flour-water mixture) was made by mixing 5 g of flour with 8 mL of distilled water, at the temperature 20°C. Duration of mixing was 20 seconds. Before the analyses dough was waiting covered at 20 ± 1°C for given time. After the given time, samples were frozen and freeze-dried. All analyses were performed in three independent samples.

HPLC analyses

a) Common buckwheat flour and dough were analysed at Biotechnical Faculty, University of Lju-

bljana, according the method described by Kreft, Fabjan in Yasumoto (2006).

- b) Tartary buckwheat flour and dough were analysed at the Department of Chemistry, Slovak Agricultural University in Nitra, Slovakia, as described by Vomberg et al. (2010).

RESULTS

a) Rutin and quercetin in common buckwheat flour and dough (sample S)

Common buckwheat flour contained 25.8 ± 1.4 µg rutin/g DM (dry matter) of sample; there were detected no traces of quercetin.

The highest concentration of rutin was in the dough 0.5 h respectively one hour after the beginning of contact of flour with water. After two hours of dough resting, it contained only about 3.8 µg/g rutin (in DM). Before the mixing of flour with water, it was no quercetin established in common buckwheat flour (Table 2 and Fig. 1). Quercetin appeared in the flour-water mixture after one hour, and the maximal value about 2.5 µg quercetin/g DM was reached after two hours from the beginning of flour-water contact. It is supposed that quercetin is the result of the decomposition of rutin. After 24 hours of dough resting, there were only traces of rutin (0.54 µg rutin/g DM), but appeared 2.3 µg/g DM quercetin (Table 2, Fig. 1).

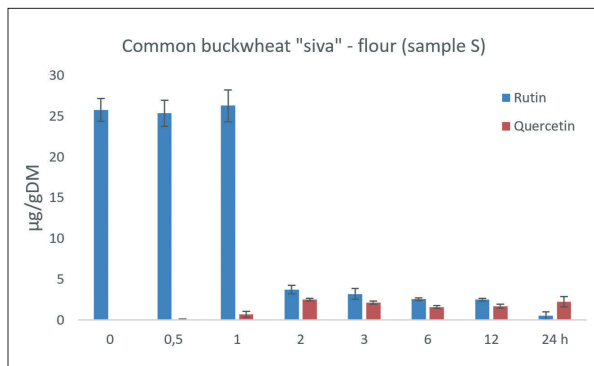


Figure 1: Rutin and quercetin extracted from common buckwheat flour and from flour dough in the 24- hour period of dough resting (sample S)

b) Rutin and quercetin in Tartary buckwheat flour and dough (sample T)

Tartary buckwheat flour (sample T) contained 11.67 ± 0.09 mg rutin/g DM, in the flour it was as well 0.63 ± 0.03 mg quercetin/g DM.

Concentration of rutin dropped down already after 5 min of the contact with water (to 0.79 ± 0.01 mg/g DM), after 24 hours no rutin was detected in the dough of Tartary buckwheat flour. In the dough of Tartary buckwheat flour it appeared after 5 min of flour-water contact 5.65 ± 0.01 mg quercetin/g DM. Quercetin is supposed to be

Table 2: Rutin and quercetin concentrations in common buckwheat flour and in buckwheat dough (sample S) during the dough resting time

Sample S (common buckwheat 'siva')	Dough				
	Time of contact of flour and water until measurement (h)	Rutin (µg/g DM)	SD	Quercetin (µg/g DM)	SD
	0	25.8	1.40	UDL	-
	0.5	25.39	1.61	0.10	0.01
	1	26.32	1.96	0.71	0.36
	2	3.75	0.53	2.50	0.18
	3	3.21	0.68	2.14	0.17
	6	2.56	0.18	1.61	0.18
	12	2.50	0.19	1.71	0.25
	24	0.54	0.46	2.25	0.61

n = 3

S - common buckwheat; flour, dough

UDL - under detection limit

SD - standard deviation

DM - dry matter

Table 3: Rutin and quercetin concentrations in Tartary buckwheat flour (sample T) during the 24- hour period of dough resting

Tartary buckwheat (sample T)	Dough			
	Rutin (mg/g DM)	SD	Quercetin (mg/g DM)	SD
Time of contact of flour and water until measurement (h)				
0	11.67	0.09	0.63	0.03
0.08	0.79	0.01	5.65	0.01
0.5	0.63	0.05	5.68	0.04
1	UDL	-	5.66	0.03
24	UDL	-	5.21	0.01

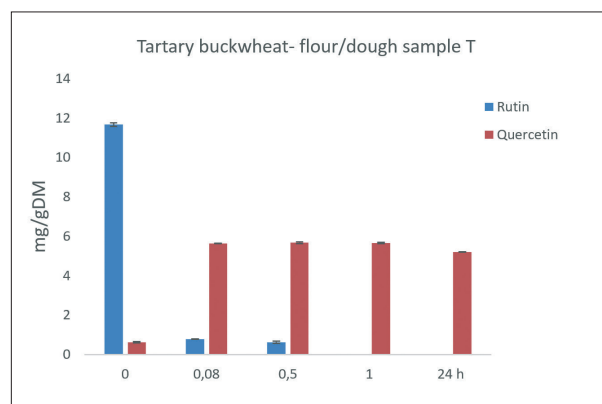
$n = 3$

T - Tartary buckwheat from Luxembourg (flour, dough)

UDL - under detection limit

SD - standard deviation

DM - dry mass

**Figure 2:** Rutin and quercetin extracted from Tartary buckwheat flour and buckwheat flour dough in the 24-hour period of dough resting (sample T)

the result of decomposition of rutin in the dough. After 24 hours of dough resting, the concentration of rutin is under the limit of detection, but there appeared about 5 mg quercetin/g DM (Table 3, Fig. 2).

DISCUSSION

It is known that beside the main flavonoid rutin, in Tartary buckwheat grain is as well some quercetin and quercitrin, rutin is responsible for about 85–90 % of antioxidative activity (Morishita et al., 2007; Liu and Zhu, 2007). Recent results show that in investigated sample of Tartary buckwheat rutin is important part of flavonoid content (T: flour 1.17 % rutin/DM). Previous investigations showed results in the similar range 0.24–4.47 % of flavonoids (Vombergar, 2010; Vombergar and Luthar, 2018; Vombergar et al., 2018; Vombergar, 2020). Similar results about higher content of flavonoids (mainly rutin) in Tartary buckwheat were reported by Fabjan et al. (2003), Briggs et al. (2004), Asami et al. (2007), Fabjan (2007), Jiang et al. (2007), Yu and Li (2008) and other. In the present investigation, it was established that our sample of Tartary buckwheat flour contained 400 times higher concentration of rutin than common buckwheat flour sample (Table 4).

Different methods of milling, use of different mills, obtaining of fraction with different granulation may have

Table 4: Rutin and quercetin content in common buckwheat (sample S) and Tartary buckwheat (sample T3)

Sample of flour	Rutin %/DM	Quercetin %/DM
Common buckwheat (S)	0.0026	UDL
Tartary buckwheat (T)	1.1670	0.063

UDL – under detection limit

impact on amount and speed of extraction of phenolic substances from milling fractions. Size of flour particles is very important for flour properties. Smaller particles have bigger contact surface with water, so the action of enzymes may be different. Impact of enzymes on small flour particles can be bigger. Phenolic substances are part of several grain and cell components. Their extraction could be thus different.

In previous research, investigated the common and Tartary buckwheat samples on the content of total flavonoids, we established similar trends after the contact of flour particles with water (Vombergar, 2010; Vombergar et al., 2018). Flavonoids concentration raised in milling fractions after 5 min of contact with water (in comparison to concentration in flour), up to 2 times or more, but later the concentration of total flavonoids was lower, especially after 24 hours of the flour-water contact it was lower.

Investigation established that the concentration of rutin in flour-water mixtures the result of two processes. One is extraction of rutin from grain structures and its dissolving in water, and the second is the relaxation of rutin degrading enzymes from grain structures and their activity in solution. However, the details of these processes are not yet known, it is a possibility of further investigation.

Very interesting is, that some rutin remains in the dough even after 24 hours of flour-water contact. We do not know, if this is due to the deactivation or decomposition of enzymes, or the remaining rutin is available for the extraction, but not available for the transformation by enzymes.

CONCLUSION

In Tartary buckwheat, we determined a much higher concentration of rutin in comparison to common buckwheat. In Tartary buckwheat, we determined from

1.17 to 1.75% rutin in dry matter, while it was in common buckwheat only 0.003%. After direct contact of buckwheat flour with water, different biochemical activities in Tartary buckwheat occur. After the time (5 minutes or two hours), the concentration of rutin is in the flour-water mixtures much lowered, and quercetin appeared. However, after quick initial changes, some rutin remained in flour-water mixtures even after 24 hours. In any way, after 24 hours of direct contact of flour particles with water, the concentration of quercetin is higher than that of rutin.

Investigation established that the concentration of rutin in flour-water mixtures is the result of two processes. One is extraction of rutin from grain structures and its dissolving in water, and the second is the relaxation of rutin degrading enzymes from grain structures and their activity in solution.

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IZVLEČEK

Vsebnost rutina in kvercetina v moki in testu navadne in tatarske ajde

Izvedena je bila primerjava vsebnosti rutina in kvercetina v mokah navadne in tatarske ajde. Tatarska ajda ima bistveno višjo vsebnost rutina kot navadna ajda. Vsebnost rutina v tatarski ajdi je 1,17–1,75 % v suhi snovi, v navadni ajdi 'siva' pa le 0,003 %. V tatarski ajdovi moki ugotavljamo torej okoli 400x več rutina kot v navadni ajdovi moki. Pri neposrednem stiku ajdove moke z vodo težko najdemo vzporednice med tatarsko in navadno ajdo ter dogajanja v povezavi z rutinom v testu. Koncentracija rutina v testu se po določenem času (različen čas pri navadni in tatarski ajdi – 5 minut do 2 uri) močno zniža, pojavi se kvercetin. Ugotavljamo, da kljub burni začetni reakciji razgradnje rutina v testih, rutin ne razpade popolnoma, ampak se ga minimalna količina ohrani v testu tudi po 24 urah. Vsebnost kvercetina v testu je po 24 urah višja kot vsebnost rutina. Dosedanje raziskovanje je pokazalo, da je vsebnost rutina v zmesi mlevske frakcije zrn ajde in vode rezultanta dveh procesov. Na eni strani je to izločanje rutina iz struktur zrna in njegovo raztapljanje v tekočini. Drugi proces je sproščanje encimov, ki razgrajujejo rutin.

The first announcement on the 3rd European Buckwheat Symposium, September 14-15, 2021, online

Crop Research Institute (CRI, Prague, Czech Republic) is pleased to announce the **3rd European Buckwheat Symposium** to be held on **14-15 September 2021 online**.

Registration will open on **1 July 2021**.

More information about the schedule and virtual symposium will be available in early July on the website: www.euroibra2021.eu.

To whom the symposium is aimed to?

All scientists, growers, processors and students in buckwheat research, production and processing from Europe are welcome. Buckwheat colleagues from outside Europe are also welcome to attend if the time zone allows being present online.

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This symposium is supported by the ECOBREED project, European Union's Horizon 2020 research and innovation programme, grant agreement No 771367.

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