

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 (C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>) 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<br>Dark flour                      | 19 mg/kg DM<br>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<br>(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).

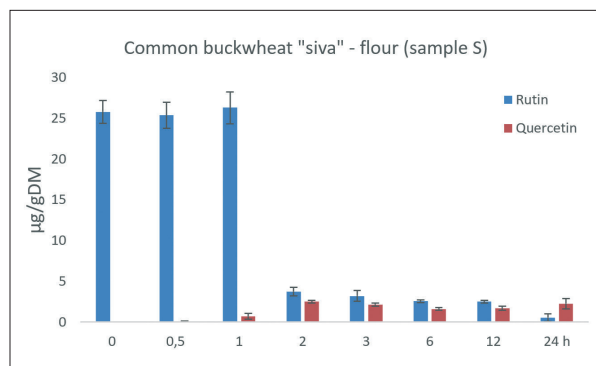
- b) Tartary buckwheat flour and dough were analysed at the Department of Chemistry, Slovak Agricultural University in Nitra, Slovakia, as described by Vomberg et al. (2010).

## RESULTS

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

Common buckwheat flour contained  $25.8 \pm 1.4$  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  $11.67 \pm 0.09$  mg rutin/g DM, in the flour it was as well  $0.63 \pm 0.03$  mg quercetin/g DM.

Concentration of rutin dropped down already after 5 min of the contact with water (to  $0.79 \pm 0.01$  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  $5.65 \pm 0.01$  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<br>(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 |

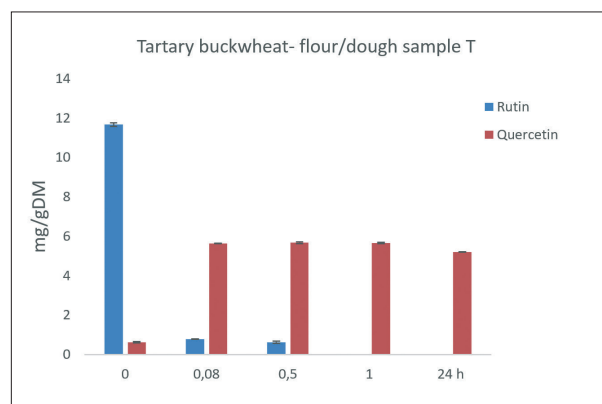
$n = 3$

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.