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## EFFECT OF RUTIN IN CONSUMED BREAD ON BLOOD PARAMETERS OF VOLUNTEERS VPLYV KONZUMÁCIE CHLEBA S RUTÍNOM NA VYBRANÉ PARAMETRE KRVÍ PROBANTOV

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The study aims at the evaluation of enriched bread prepared with the addition of buckwheat (*Fagopyrum aesculentum* Moench.) as a source of biologically active components in nutrition. The effect of consumption of bread enriched by 30 % of buckwheat on healthy state of consumers has been observed. The parameters from blood samples of clinical study of participants have been analysed. The study was based on a daily consumption of 200 g enriched bread. The addition of buckwheat provide for daily intake of 4.2 mg of rutin during four weeks. Among the evaluated elements it was found out that calcium and magnesium content in blood of volunteers has been decreased as follows: calcium in average from 2.81 mmol l<sup>-1</sup> to 2.65 mmol l<sup>-1</sup>, and magnesium from 0.91 mmol l<sup>-1</sup> to 0.85 mmol l<sup>-1</sup>, which represent statistically significant differences. The positive finding was the increase of iron content in blood which was statistically significant (in average from 30.04 µmol l<sup>-1</sup> to 48.41 µmol l<sup>-1</sup>). Regarding other evaluated elements decrease of their values was expected due to buckwheat consumption. Decrease of triglycerides in blood from 2.15 mmol l<sup>-1</sup> to 1.78 mmol l<sup>-1</sup> as well as decrease of creatinine from 71.94 µmol l<sup>-1</sup> to 61.21 µmol l<sup>-1</sup> is considered as highly positive. Based on blood analyses after a four-week period of consumption of bread enriched with buckwheat it is not feasible to conclude that total cholesterol amount in blood of volunteers showed values significantly lower then before consumption. The small decrease occurred but this was not of significant values (from 5.96 mmol l<sup>-1</sup> to 5.78 mmol l<sup>-1</sup>).

**Key words:** rutin, buckwheat, enriched bread, clinical study

Polyphenols are the most abundant antioxidants in the human diet. They show a considerable structural diversity, which largely influences their bioavailability. All polyphenols are reducing agents. As such, they may scavenge free radicals, participate in the regeneration of the other antioxidants such as vitamin E and protect cell constituents against oxidative damage (Scalbert et al., 2002).

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described, and they are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids (Guardia et al., 2000). Flavonoids interact with cellular signal pathways controlling the cell cycle, differentiation and apoptosis. Their potentially antineoplastic effects include antioxidant activity, induction of Phase II enzyme activity, inhibition of protein kinases and interactions with Type II estrogen binding sites. Naturally occurring polyphenolic compounds may play a role in the protective effects of fruits and vegetables against cancers in general, and they appear to have considerable potential for pharmaceutical uses as chemopreventive agents against neoplastic changes in the alimentary tract (Gee and Johnson, 2001).

Rutin is a flavonoid with a wide range of biological activities and is used in many countries as vasoprotectans and is an ingredient of numerous multivitamin preparations and herbal remedies (Erlund et al., 2000). Clinical studies that support the use of rutin in the treatment of the above-mentioned conditions are few, but at least two studies indicate that buckwheat, which contains high amounts of rutin, is useful in the treatment of chronic venous insufficiency (Ihme et al. 1996; Koscielny et al., 1996). More comprehensive data on the vasoprotectant effects of flavonoids are available for the hydroxyethyl rutosides, which are synthetic derivatives of rutin (Shami et al., 1997).

Buckwheat is introduced into the diet as an alternative crop of renewed interest due to its nutritive and health-promoting

value. Content of vitamins, protein, minerals, fibre, and starch with reduced speed of digestion, rutin and other flavonoids make buckwheat products favourable for healthy nutrition. Buckwheat has been utilized in food products as groats or flour for pasta products, for blended bread (in combination with wheat, corn and other cereals) and for different types of other flour foods. Flour contains mainly central endosperm, while the bran milling fraction has seed coat and some embryo tissues. Rutin is concentrated in the hull of common buckwheat and its concentration is low in groats but higher in bran containing hull fragments (Steadman et al., 2001).

In this study we investigated possibilities of buckwheat use at bread production and influence of such bread consumption on protective effect on blood components such as chosen microelements content, such as cholesterol, triglycerides and other important components.

### Material and methods

Rutin was determined by a chromatograph, column Lichrospher 100RP-18, 250-4, 5 µm Clinical study based on daily consumption of 200 g of enriched bread with an addition of buckwheat (wholegrain flour from winnowed seeds) of 30 % during the period of four weeks, after which the selected parameters in blood were evaluated. Three intravenous blood samples were taken: before the clinical study, immediately after it (after four weeks of consuming enriched bread) and after another four-week period. The blood parameters (Ca, Mg, Fe, creatinine, urea, chloride, cholesterol, and triglyceride level) were measured on analyser LISA 200 (Biocode-Hycl). Statistical analysis of data was performed by the means of Student's t-test. Differences were considered statistically significant in case of P < 0.01.

The study population consisted of 33 healthy volunteers. Subject characteristics were as follows: age 46.03 years (average), range 27 – 61 years and body mass index 25.34 kg m<sup>-3</sup> (average), range 18.8 – 32.9 kg m<sup>-3</sup>. All subjects gave written informed consent before participating in the study. Volunteers consumed daily bread enriched with buckwheat; otherwise their diet had not been changed.

## Results and discussion

Common buckwheat is a typical low input crop rich in minerals and with high nutritional quality of proteins. Content of fibre, starch with reduced speed of digestion and rutin made buckwheat products favourable for healthy nutrition, for patients with diabetes and celiac disease (Guo and Yao, 2006; Kreft et al., 1997). Buckwheat contains many flavonoid compounds, known for their effectiveness in reducing the blood cholesterol, keeping capillaries and arteries strong and flexible, and assisting in prevention of high blood pressure (Santos et al., 1999), they inactivate free radicals, inhibit peroxidation of membrane phospholipids, lower the portion of oxidised LDL cholesterol, decrease fragility and permeability of blood capillaries, support the stability of vein walls, they have antibacterial, antiviral, antiparasitic as well as hepatoprotective effects (Mikulajová et al. 2007). They are also responsible for high antioxidant activity of buckwheat (Watanabe 1998; Quettier-Deleu et al., 2000; Holasova et al., 2002).

Bojňanská and Fikselová (2010) and Bojňanská and Chlebo (2010) published the results on the influence of the buckwheat enriched bread consumption on total antioxidant status. The results confirmed the increase of the total antioxidant status thanks to the buckwheat enriched bread consumption from the value of  $1.135 \pm 0,066 \text{ mmol dm}^{-3}$  at the beginning of the study to  $1.46 \pm 0,083 \text{ mmol dm}^{-3}$  at the end of the study, i.e. significant differences. The most significant increase in comparison to the initial state was found with experimental persons with an initially low TAS, the increase reached nearly 40 %. The highest TAS level (initial as well as final) was found with younger experimental persons between 18 and 34 years old, the lowest with people between 35 and 54 years old (Bojňanská et al., 2009).

According to Holasova et al. (2002) the relations between antioxidant activity and the content of total polyfenols, rutin and

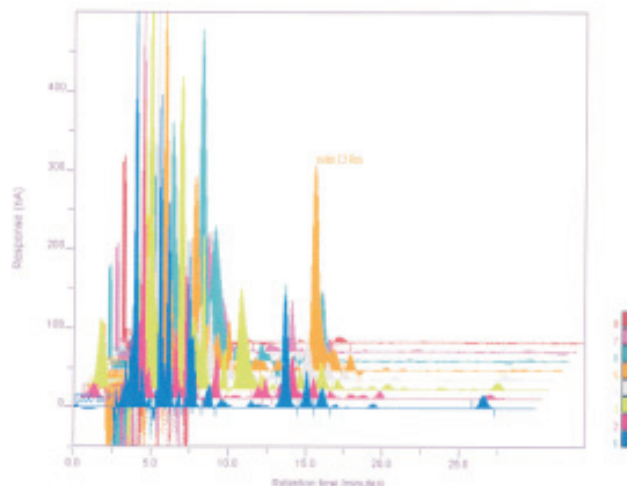


Figure 2 Rutin content in buckwheat wholegrain flour

tocopherols in buckwheat are statistically significant. However, the other authors (Oomah and Mazza, 1996) found out only minor relation between the content of flavonoids and antioxidant activity in buckwheat.

In the frame of presented clinical study the content of rutin in the bread consumed by volunteers was determined on a level of  $39.9 \pm 0.6 \text{ mg kg}^{-1}$  dry mass (DM). (Figure 1) what represents after changing into a consumable form the value of  $21.01 \text{ mg kg}^{-1}$ . Taking into account that the daily portion was 200 g of bread, the volunteers consumed in buckwheat enriched bread 4.2 mg of rutin per day. The content of rutin in buckwheat wholegrain flour was  $79.9 \text{ mg kg}^{-1}$  DM (Figure 2).

Prior to consumption the blood sample was taken and later on compared with blood samples taken after four and eight weeks to see changes in evaluated elements influenced by the buckwheat enriched bread consumption.

Regarding evaluated elements, we found out the decrease of calcium and magnesium content in blood of volunteers: calcium in average from  $2.81 \pm 0.16 \text{ mmol.l}^{-1}$  to  $2.65 \pm 0.13 \text{ mmol l}^{-1}$ , and magnesium from  $0.91 \pm 0.06 \text{ mmol l}^{-1}$  to  $0.85 \pm 0.07 \text{ mmol l}^{-1}$ , which represent statistically significant differences (at significance level  $\alpha = 0.01$ ). Calcium has an important influence on permeability of cell membrane and activation of enzymes (Keller et al. 1993) and its amount in blood should be between  $2.25 - 2.75 \text{ mmol l}^{-1}$ , thus the decrease showed in our study was not from the health point of view important or risky. The situation was similar with magnesium where average values were in the limits of recommended dosages ( $0.66 - 1.03 \text{ mmol l}^{-1}$ ).

The positive influence of buckwheat bread consumption can be seen in increase of iron content in blood which was statistically significant after four weeks of consumption and continued to increase until the end of clinical study. Shortage of iron represents the most common deficiency disease affecting wide population which can be evolved into iron deficiency anaemia. Iron amount in organism depends on its intake by food and its biological disposability influenced by its chemical form, existence of other biologically active elements in food as well as the way of meals preparation (Žourek et al., 2007). Based on findings, buckwheat consumed in a form of enriched bread might be considered as a suitable source of acceptable iron.

Regarding other evaluated elements decrease of their values was expected due to buckwheat consumption. Statistically significant decrease of creatinine content was confirmed with decrease of values from  $71.94 \pm 13.05 \text{ } \mu\text{mol l}^{-1}$

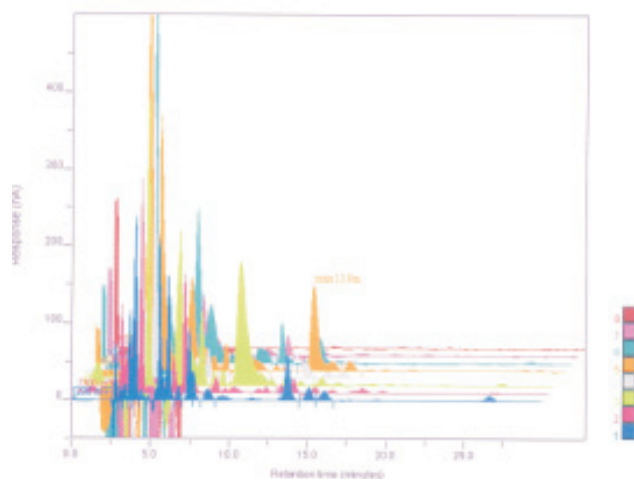


Figure 1 Rutin content in consumed bread (30 % buckwheat)

to  $64.56 \pm 12.78 \mu\text{mol l}^{-1}$  (second sample taking) to  $61.21 \pm 10.36 \mu\text{mol l}^{-1}$  (third sample taking) which is thanks to the positive influence of buckwheat consumption. Creatinine indicates worsened function of kidneys, renal, most often caused by metabolic diseases (diabetes) and high blood pressure (Dzúrik and Spustová, 1999).

Decrease of triglycerides between first sample taking and last one after eight weeks (from  $2.15 \pm 0.91 \text{ mmol l}^{-1}$  to  $1.78 \pm 0.71 \text{ mmol l}^{-1}$ ) is similarly to creatinine content a very positive decrease mainly taking into account the influence of high level of triglycerides on development of coronary heart disease (Patrick, 2007). Considering health point of view, the level of triglycerides should not reach over  $2.2 \text{ mmol l}^{-1}$ . The values of this parameter varied with individual volunteers in the scale between  $1.21 \text{ mmol l}^{-1}$  to  $6.13 \text{ mmol l}^{-1}$ .

High level of blood cholesterol is one of the most important risk factor related with coronary heart disease and atherosclerosis, too. Total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels in blood were determined. Average decrease of total cholesterol content in volunteers' blood was insignificant; however, the decrease of HDL level was significant what is considered as non desirable. HDL values should varied above  $1.6 \text{ mmol l}^{-1}$ , what decreases the risk of coronary heart disease and atherosclerosis. This finding was rather surprising as the experiments with hamsters (Lin et al., 2008) confirmed that the level of serum low-density lipoprotein cholesterol was significantly suppressed by all buckwheat meals and serum high-density lipoprotein cholesterol levels were increased, however, insignificantly. The total cholesterol, triglyceride and LDL cholesterol levels were significantly decreased in rats administrated with rutin compared to control group (Lim et al., 2006; Ziaee et al., 2009), too. López-Revuelta et al. (2006) analysed the influence of rutin and quercetin on cholesterol content in rabbits with positive results. Despite insignificant decrease of cholesterol the results showed we can recommend the buckwheat meal consumption mainly because of its high antioxidant effect.

Based on blood analyses after a four-week period of consumption of buckwheat enriched bread it is not feasible to conclude that total cholesterol amount in blood of volunteers showed values significantly lower then before consumption. Although the small decrease occurred it was not of significant values. Out of 33 volunteers, 24 (nearly 73 %), had in the beginning of the clinical study the level of total cholesterol higher than  $5.2 \text{ mmol l}^{-1}$ , which is quite alarming result. After

four weeks of consuming buckwheat enriched bread 21 volunteers had still the level of total cholesterol higher than  $5.2 \text{ mmol l}^{-1}$  and after four following weeks even 22 volunteers. Despite the information appearing often in literature on positive effect of buckwheat consumption on lowering the level of cholesterol in blood, we have to disagree with this statement as we did not observe such effect in our volunteers. The same results, i.e. no significant changes were observed with values of LDL and HDL (Figure 3).

The highest decrease of total cholesterol was observed in the group of the youngest volunteers (18 – 34 years old), the values decreased in average from  $5.46 \text{ mmol l}^{-1}$  to  $5.20 \text{ mmol l}^{-1}$ , however after further four weeks the level increased to  $5.37 \text{ mmol l}^{-1}$ . With volunteers 35 to 54 years old we observed after four weeks a slight increase of total cholesterol but after additional four weeks the values went back to the original ones. With the volunteers 55 – 75 years old the amount of total cholesterol decreased with each sample taking ( $6.9 \text{ mmol l}^{-1}$  –  $6.87 \text{ mmol l}^{-1}$  –  $6.64 \text{ mmol l}^{-1}$ ), but the values were still high above the limit.

With volunteers who consumed daily required 200 g of bread we observed more significant decrease of total cholesterol than with volunteers who did not consume the exact quantity, but nevertheless the results were not satisfactory in either group.

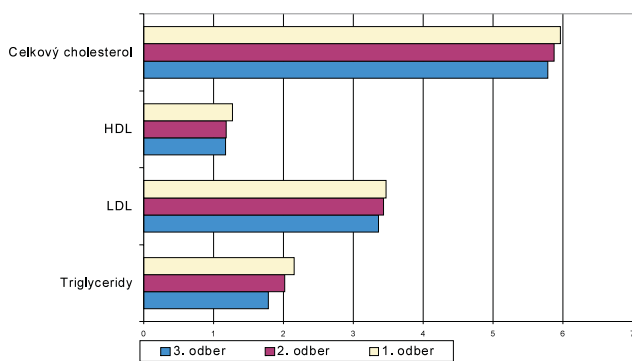
## Conclusions

From the nutritive point of view the addition of buckwheat has increased the content of the important flavonoid rutin. The content of rutin in the prepared bread was determined on  $39.9 \pm 0.6 \text{ mg kg}^{-1}$  dry mass (DM), what represents after changing into a consumable form the value of  $21.01 \text{ mg kg}^{-1}$ . Taking into account that the daily portion was 200 g of bread, the volunteers consumed in buckwheat enriched bread 4.2 mg of rutin per day. Regular consuming of bread with this rutin content influenced following blood parameters: Ca, Mg, Fe, creatinine, cholesterol, and triglyceride level.

Daily consumption of buckwheat enriched bread during the clinical study by volunteers significantly increased the iron level in blood and significantly decreased calcium and magnesium. Significant decrease of HDL cholesterol level was surprising as well as non desirable. On the other hand expected and welcome decrease of total cholesterol was statistically insignificant. Among the positive changes there was a significant decrease of triglyceride and creatinine.

## Súhrn

Pohánka (*Fagopyrum esculentum* Moench.) je významným zdrojom flavonoidov a jej konzumácia má pozitívny vplyv na zdravotný stav konzumentov. Bola sledovaná účinnosť konzumácie chleba obohateného o pohánku (30 %) na parametre analyzované z krvných vzoriek účastníkov klinickej štúdie založenej na pravidelnej konzumácii 200 g pohánkou obohateného chleba denne. Prídavok pohánky do chleba zabezpečil denný príjem rutínu v množstve 4,2 mg v priebehu štyroch týždňov, kedy prebiehala klinická štúdia. Z hodnotených prvkov bolo zistené zníženie obsahu vápnika a horčíka v krvi probandov: pri vápniku z priemerne  $2,81 \text{ mmol.l}^{-1}$  na  $2,65 \text{ mmol.l}^{-1}$  a pri horčíku z  $0,91 \text{ mmol.l}^{-1}$  na  $0,85 \text{ mmol.l}^{-1}$ , čo boli štatisticky preukazné rozdiely. Pozitívnym bolo zvýšenie obsahu železa (priemerne z  $30,04 \mu\text{mol.l}^{-1}$  na  $48,41 \mu\text{mol.l}^{-1}$ ), ktoré bolo štatisticky preukazné. Pri ostatných



**Figure 3** Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol and triglycerides levels in blood volunteers

hodnotených ukazovateľoch bolo očakávané, v dôsledku pravidelnej konzumácie pohánkového chleba, zníženie ich hodnôt. Za veľmi pozitívne je možné považovať zníženie množstva triglyceridov v krvi z 2,15 mmol.l<sup>-1</sup> na 1,78 mmol.l<sup>-1</sup> a zníženie kreatinínu z 71,94 μmol.l<sup>-1</sup> na 61,21 μmol.l<sup>-1</sup>. Na základe výsledkov krvných analýz po štvortýždňovej konzumácii chleba s pohánkou nie je možné urobiť záver, že množstvo celkového cholesterolu pri probandoch bolo preukazne nižšie ako pred konzumáciou. K určitému zníženiu síce prišlo, ale bolo nevýznamné (z 5,96 mmol.l<sup>-1</sup> na 5,78 mmol.l<sup>-1</sup>).

**Kľúčové slová:** rutin, pohánka, obohatený chlieb, klinická štúdia

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