Rutin Content in Buckwheat Enriched Bread and Influence of its Consumption on Plasma Total Antioxidant Status

T. BOJŇANSKÁ[1], H. FRANČÁKOVÁ[1], P. CHLEBO[3] and A. VOLLMANNOVÁ[2]*

[1]Department of Storing and Processing Plant Products; [2]Department of Chemistry, Faculty of Biotechnology and Food Sciences; [3]Department of Human Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovak Republic

**E-mail: tatiana.bojnanska@uniag.sk*

Abstract: The goal of this study is the evaluation of enriched bread prepared with an addition of buckwheat as a source of biologically active components in nutrition. Experimental pastry was baked using buckwheat wholegrain flour blended with wheat flour in different portions (10%, 20%, 30%, 40% and 50%) in order to observe the influence of buckwheat addition on dough processing and the quality of the final baked products. Taking into account the overall acceptability rating, it was concluded that bread with an addition of up to 30% of buckwheat could be baked with satisfactory evaluation results. The content of rutin in loaves in a consumable form prepared with a buckwheat addition varied between 7.76 mg/kg and 26.90 mg/kg. The clinical study which followed the baking experiment was based on the consumption of enriched bread (30% buckwheat, between 34.7 mg/kg and 38.2 mg/kg rutin content in dry matter) by a group of volunteers during a period of four weeks. The results approved the increase of the total antioxidant status thanks to the buckwheat enriched bread consumption from the value of 1.135 ± 0.066 mmol/dm^3 at the beginning of the study to 1.46 ± 0.083 mmol/dm^3 at the end of the study, i.e. significant differences.

Keywords: total antioxidant status; buckwheat enriched bread; rutin

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench.) is rich in vitamins, especially those of the B group (Fabjan *et al.* 2003), and is an important source of microelements (Zn, Cu, Mn, Se) as well as macroelements (K, Na, Ca, Mg) (Bonafacia & Fabjan 2003; Stibilj *et al.* 2004). It also contains high quality proteins (Kreft *et al.* 1996; Watanabe 1998; Guo & Yao 2006; Christa & Soral-Smietana 2008), but of a relatively low true digestibility (Skrabanja *et al.* 2000). Buckwheat products may have an important content of retrograded starch (Skrabanja & Kreft 1998) as well as a resistant starch content (Christa & Soral-Smietana 2008). Buckwheat grains and hulls contain some components with healing properties and biological activity, such as flavonoids and flavon, phenolic acid, condensed tannins, phytosterols and fagopyrins. Flavonoids are phytonutrients acting as antioxidants and with chelating properties. Their cardioprotective effects stem from the ability to inhibit lipid peroxidation, chelate redox-active metals, and attenuate other processes involving reactive oxygen species (Heim *et al.* 2002). The propensity of a flavonoid to inhibit free-radical mediated events is governed by its chemical structure. Buckwheat contains many flavonoid compounds, known for their effectiveness in reducing blood cholesterol, keeping capillaries and arteries strong and flexible, and helping the prevention of a high blood pressure (Santos *et al.* 1999). Rutin is a flavonol glycoside composed of flavonol quercetin and disaccharide rutinose. The antioxidant power of rutin was corroborated by several studies. Some studies however report its pro-oxidant activity and the ability to generate reactive oxygen species is ascribed to aglycon

quercetin (Watanabe *et al.* 1997; Watanabe 1998; Quettier-Deleu *et al.* 2000; Cotelle 2001; Rice-Evans 2001). Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as antimutagenity, anticarcinogenity and antiaging, among others, originate from this property (Cook & Samman 1996). The biological activities of flavonoids are related to their antioxidative effects. The determination of total antioxidant status (TAS) is a valuable and reproducible method to detect the actual antioxidant status (Lantos *et al.* 1997). If antioxidant activities are important, the elucidation of how such properties *in vitro* relate to the potential for conjugates and metabolites *in vivo* to act as antioxidant is required (Rice-Evans 2001).

The content of vitamins, protein, minerals, fibre, starch with reduced speed of digestion, rutin and other flavonoids made buckwheat products favorable for healthy nutrition. Buckwheat is used for pasta products, for blended bread (in combination with wheat, corn and other cereals) and for different types of other flour foods. In this study we investigated the responses in serum total antioxidant capacity following the consumption of bread with an addition of buckwheat.

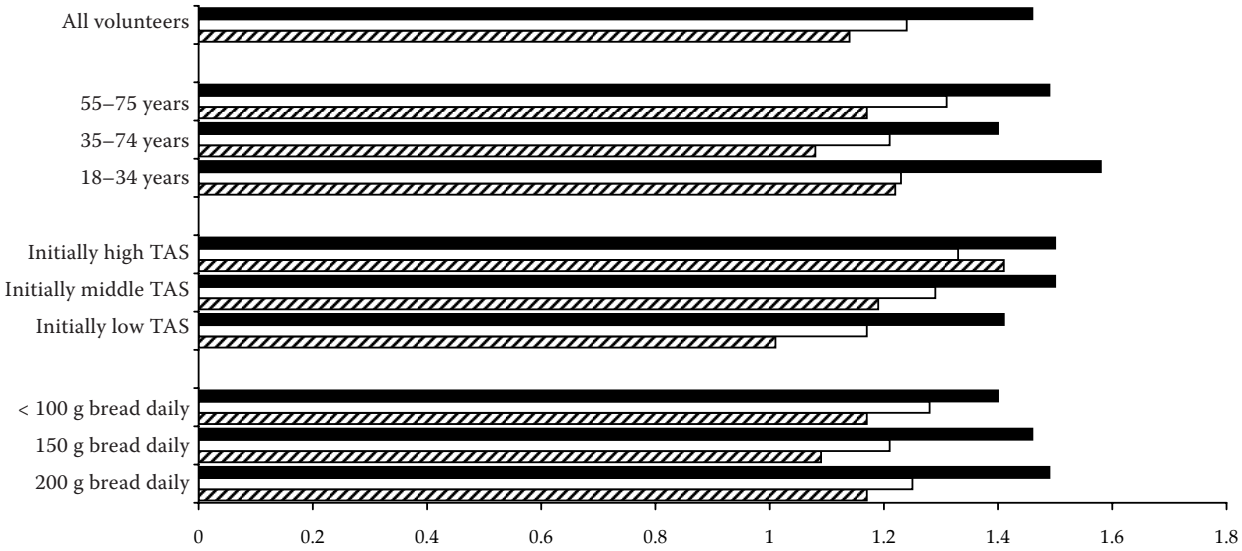
MATERIAL AND METHODS

The buckwheat wholegrain flour (from winnowed seeds) was mixed with wheat flour in different portions (10%, 20%, 30%, 40% and 50%) for producting buckwheat bread. The selected parameters (loaf volume (cm^3), specific loaf volume (cm^3/100 g loaf), volume efficiency (cm^3/100 g flour), crude protein content (N × 5.7, %) by Kjeldahl, ash content (%) and rutin content (mg/kg) by a chromatograph have been evaluated during the experimental baking test. The analysis of the rheological property changes with different portions of buckwheat has been provided by means of Farinograph-E and Extenzograph-E (Brabender OhG, Duisburg). The sensory parameters of the baked loaves have been evaluated by an intensive and hedonic scale. The plasma TAS was measured on a LISA 200 (Biocode-Hycel) analyser with a Randox reagent set. A clinical study based on a daily consumption of 200 g of enriched bread (30% buckwheat) was conducted on the group of volunteers 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.

RESULTS AND DISCUSSION

Buckwheat is not commonly used as a bakery raw material because there is no glutenin and gliadin fraction for gluten formation. The influence of the addition of buckwheat on dough processing and on the quality of the baked goods was observed. The rheological properties of dough changed when the amount of buckwheat in the blend was increased. The farinograph curve confirmed the prolongation of the dough development time and the raise of the energy input demands for kneading dough with an optimal consistency after increasing the addition of buckwheat. The extensograph indicated the decrease of the dough resistance and its stability during mixing. The baking test confirmed this, too. The loaves prepared with an addition of buckwheat were evaluated to be of a lesser quality from the technological viewpoint when compared with pure wheat loaves. The additions negatively influenced the loaf volume, specific volume, and bulk productivity of the bakery product. From the nutritive point of view the addition of buckwheat has increased the content of protein, minerals, fibre as well as the important flavonoid rutin. The major protein fractions of the grains are water-soluble and salt-soluble albumins and globulins and the amino acid composition of buckwheat proteins is well balanced and is rich in arginine and lysine, the primary aminoacids limiting the content of proteins in cereals (Watanabe 1998; Guo & Yao 2006; Christa & Soral-Smietana 2008). The protein content in buckwheat bread was higher (13.63%) in comparison to the control bread (11.36%) and the protein was of a higher nutritive quality. The organoleptic evaluation of the bread revealed that as the portion of buckwheat flour was increased, the bread crust colour changed from light brown to chestnut brown. The crust texture, flavour and taste also changed. Taking into account the overall acceptability rating, it was concluded that bread with an addition of up to 30% of buckwheat could be baked with satisfactory results. Such enriched bread is considered to have a high nutritive value and to be acceptable from

a sensory point of view. The baking experiment was followed by a clinical study during which the bread enriched by 30% of buckwheat was prepared and consumed on a daily basis. The content of rutin in buckwheat crush added to bread dough amounted to 79.9 mg/kg in dry matter (DM), what represents a significant nutritive content although according to Holasova *et al*. (2002), the content of rutin in winnowed buckwheat seeds might reach 184 mg/kg DM. The content of rutin in the prepared bread increased accordingly with the addition of buckwheat from 14.1 mg/kg DM in bread with an addition of buckwheat of 10% to 48.9 mg/kg DM in bread with an addition of buckwheat of 50%. We should bear in mind though that the bread was not consumed in a dry state and that the amounts of rutin in fresh bread are lower. The loaves with an addition of buckwheat of 30% contained 3.8 g rutin in 200 g of bread in a consumable form; this equals the daily dosage of rutin in the clinical study provided that the experimental subjects consume the complete dosage. The total antioxidant status (TAS) when taking the first blood sample varied before any consumption of enriched bread from 0.92 to 1.48 mmol/dm^3 and only four experimental subjects showed a value above the minimal reference level (1.3–1.77 mmol/dm^3). The average value of 1.14 mmol/dm^3 is considered to be a low TAS level. After four weeks of consuming enriched bread the average level of TAS reached 1.24 mmol/dm^3. Although this is still below the minimal reference value of 1.3 mmol/dm^3, already 10 out of 33 experimental persons reached that value. The marked increase of TAS was found only during the following weeks. When taking the last blood sample the average TAS reached 1.46 mmol/dm^3, this is within the scale of reference values and 29 experimental persons reached the optimal TAS (values varied between 1.04 and 1.93 mmol/dm^3). The values obtained during the different measurements by means of the T test showed significant differences. According to Holasová *et al*. (2002) the relation between antioxidant activities and total phenolics, rutin and tocol content indicate, that statistically significant relationships were observed between total phenolics and antioxidant activity and between rutin content and antioxidant activity. In contrary to this Oomah and Mazza (1996) found in buckwheat only a nonsignificant correlation between the rutin and tocol content and the antioxidant activity. The



	Bread daily			Initially TAS			Years			All voluntees
	200 g	150 g	< 100 g	low	middle	high	18–34	35–54	55–75	
■ 3th measuremen	1.49	1.46	1.40	1.41	1.50	1.50	1.58	1.40	1.49	1.46
□ 2nd measuremen	1.25	1.21	1.28	1.17	1.29	1.34	1.23	1.21	1.31	1.24
▨ 1st measuremen	1.17	1.21	1.17	1.01	1.19	1.41	1.22	1.08	1.17	1.14

Figure 1. Total oxidant status all volunteers and groups of volunteers by age, initial TAS and bread daily dosage

most remarkable increase of the TAS values was found with experimental persons, who consumed the complete daily dosage. The ones who did not consume the complete daily dosage showed lower final TAS values. 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. Based on the Kruskal-Wallis test of the final measurement of the level of significance alfa = 0.1 it can be said that the age of the experimental persons was a factor influencing the final values of TAS. Our data suggested that buckwheat was a significant antioxidant as TAS in human plasma and that increased TAS by doses of buckwheat bread could be useful as a free radical scavenger. It appeared that TAS of the plasma of volunteers who consumed daily buckwheat enriched bread during the period of four weeks was significantly higher than before its consumption.

CONCLUSIONS

Based on the study it can be concluded that the consumption of bread enriched by an addition of buckwheat of 30% (an amount acceptable from the technological point of view based on rheological tests and a baking experiment) as well as from the sensory point of view containing antioxidant flavonoid compounds (mainly rutin), can have a positive increase on the serum antioxidant capacity in humans. We can recommend such bread for daily consumption having a positive effect on health.

Acknowledgements: The study was carried out with the financial support of the Scientific Grant Agency (VEGA) of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (Grant No. 1/4436/07).

References

Bonafacia G., Fabjan N. (2003): Nutritional comparison of tartary buckwheat with common buckwheat and minor cereals. Research Reports Biotechnological Faculty of the University of Ljubljana, **81**: 349–355.

Christa K., Soral-Smietana M. (2008): Buckwheat grains and products – nutritional and prophylactic value of their components – a review. Czech Journal of Food Sciences, **26**: 153–162.

Cook N.C., Samman S. (1996): Flavonoids – chemistry, metabolism, cardioprotective effects, and dietary sources. Journal of Nutritional Biochemistry, **7**: 66–76.

Cotelle N. (2001): Role of flavonoids in oxidative stress. Current Topics in Medicinal Chemistry, **1**: 569–590.

Fabjan N., Rode J., Kosir I.J., Zhang Z., Kreft I. (2003): Tartary buckwheat (*Fagopyrum tartarikum* Gaertn.) as a source of dietary rutin and quercetin. Journal of Agricultural and Food Chemistry, **51**: 6452–6455.

Guo X., Yao H. (2006): Fractionation and characterization of tartary buckwheat flour proteins. Food Chemistry, **98**: 90–94.

Heim K.E., Tagliaferro A.R., Bobilya D.J. (2002): Flavonoid antioxidant: chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry, **13**: 572–584.

Holasova M., Fiedlerova V., Smrcinova H., Orsak M., Lachman J., Vavreinova S. (2002): Buckwheat – the source of antioxidant activity in functional foods. Food Research International, **35**: 207–211.

Kreft I., Skrabanja V., Ikeda S., Bonafaccia G. (1996): Dietary value of buckwheat. Research Reports Biotechnological Faculty of the University of Ljubljana, **67**: 73–78.

Lantos J., Röth E., Czopf L., Nemes J., Gál I. (1997): Monitoring of plasma total antioxidant status in different diseases. Acta Chirurgica Hungarica, **36**: 188–189.

Oomah B.D., Mazza G. (1996): Flavonoids and antioxidative activities in buckwheat. Journal of Agricultural and Food Chemistry, **44**: 1746–1750.

Rice-Evans C. (2001): Flavonoid antioxidants. Current Medicinal Chemistry, **8**: 797–807.

Quettier-Deleu C., Gressier B., Vasseur J., Nemes J., Gál I. (2000): Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum aesculentum* Moench.) hulls and flour. Journal of Ethnopharmacology, **72**: 35–42.

Santos K.F.R., Oliveira T.T., Nagem T.J., Pinto A.S., Oliveira M.G.A. (1999): Hypolipidaemic effects of narigenin, rutin, nicotinic acid and their associations. Pharmaceutical Research, **40**: 493–496.

Skrabanja V., Kreft I. (1998): Resistant starch formation following autoclaving of buckwheat (*Fagopyrum aesculentum* Moench.) groats. An *in vitro* study. Journal of Agricultural and Food Chemistry, **46**: 2020–2023.

Skrabanja V., Nygaard L.H., Kreft I. (2000): Protein-polyphenol interactions *in vivo* digestibility of buckwheat groat proteins. Pflügers Archiv European Journal of Physiology, **440**: R129–R131.

Stibilj V., Kreft I., Smrkolj P., Osvald J. (2004): Enhanced selenium content in buckwheat (*Fagopyrum aesculentum* Moench.) and pumpkin (*Cucurbita pepo* L.) seeds by foliar fertilization. European Food Research Technologies, **219**: 142–144.

Watanabe M. (1998): Catechins as antioxidants from buckwheat (*Fagopyrum aesculentum* Moench.) groats. Journal of Agricultural and Food Chemistry, **46**: 839–845. Watanabe M., Ohshita Y., Tsushida T. (1997): Antioxidant compounds from buckwheat (*Fagopyrum aesculentum* Moench.) hulls. Journal of Agricultural and Food Chemistry, **45**: 1039–1044.

Effects of Infrared Radiation on Protein Solubility and Antioxidants Content in Maize Flour

S. ZILIC, V. HADZITASKOVIS-SUKALOVIC, M. MILASINOVIC, D. TERZIC and M. MAKSIMOVIC

[1]Maize Research Institute &quot, Zemun Polje-quot, Belgrade-Zemun, Serbia; [2]Military Medical Academy, Institute of Hygiene, Belgrade, Serbia, E-mail: szilic@mrizp.co.yu

Abstract: The objective of these studies was to observe the effects of the process of micronisation on the protein solubility, as well as, on contents of antioxidants and vitamins in whole maize flour. Maize hybrids differing in the kernel colour developed at the Maize Research Institute, Zemun Polje, Belgrade, Serbia, were selected for these studies. The semi-flint hybrid ZP 633 has pronounced yellow kernels; the pericarp of ZP Rumenka is dark red, while the hybrid ZP 551b is characterised by a white colour of kernels. Grain of selected genotypes was used in the production of flour. Whole flours were produced by milling of intact maize fresh grain in a stone grinder. In order to determine the effect of the process of micronisation on nutritive properties of maize flour, intact kernels of selected ZP hybrids were subjected to the process of micronisation at the temperature of 145oC. Infrared rays are used to cook maize kernels, which were then flaked under the pressure of rolls. Maize flakes were also ground in the stone grinder and produced flour was used for analyses. Differences in nutritive values of produced flours were established by the analysis of standard chemical composition (ash, total proteins, oil, cellulose, starch), soluble proteins (albumin, globulin, zein, glutelin), ?- and ?+?-tocopherol, total antioxidants, total phenols and ?-carotene. According to our results, white maize flour does not contain ?-carotene. Raw whole flour of yellow maize, produced by milling of grain of the hybrid ZP 633, had a high content of ?-carotene (5.43 ?g/g), while this content was somewhat lower in raw whole flour produced from red maize (2.56 ?g/g). A micronisation heat treatment caused a significant reduction in the ?-carotene content in yellow micronised maize flour (23%). In the present study, the highest ?-tocopherol content was estimated in raw whole flour made from yellow maize (20.94?g/g). A high temperature of infrared rays caused the reduction of the ?-tocopherol content, hence it amounted to 16.94?g/100g in flour made by milling of micronised yellow maize. This reduction was somewhat lower in flour made by milling of micronised red-seeded maize and it amounted to 35%. Flour produced from raw and micronized red maize grain had the highest antioxidant activity. According to our results 3.27mg of whole flour made from raw! red maize grain inhibits 50% activities of DPPH radicals. Some of Maillard reaction products which are formed during process of micronisation have an antioxidant activity, which could be a reason for the increased antioxidant activity in flour produced by milling of micronised white and red maize grain. 2.34mg of red micronized flour inhibits 50% activities of DPPH radicals. The antioxidant activity in white micronised flour was higher by 40% than of flour produced from raw white kernels. The highest content of total phenols was determined in flour produced by milling of raw and micronised grain of red maize (2.80 mg catechin/g on the average). Heating of plant protein sources leads to decreasing of the protein solubility as well as content of tryptophan.

Keywords:

References

Singleton, V.L., Rossi J.A. (1965): Colorimetry a total phenolics with phosphomolibdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, **16**: 144–158.

Abe N., Murata T., Hirota A. (1998): Novel DPPH radical scavengers, bisorbicillinol and demethyltrichodimerol, from a fungus. Bioscience, Biotechnology, and Biochemistry. **62**: 661–666.

Oufnac D.S., Xu Z., Sun T., Sabliov C., Prinyawiwatkul W., Godber J.S. (2007): Extraction of antioxidants from wheat bran using conventional solvent and microwave-assisted methods. Cereal Chemistry, **84**: 125–129.