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ALTERATION OF BIOCHEMICAL PARAMETERS AND MICROSTRUCTURE OF FAGOPYRUM ESCULENTUM MOENCH GRAIN IN PROCESS OF GERMINATION

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ABSTRACT

Biochemical parameters alteration of *Fagopyrum esculentum Moench* grain in process of germination was studied. It was found that during germination of *Fagopyrum esculentum Moench* grain within 24 hours the content of ascorbic acid, thiamine, nicotinic acids, pantothenic acids and routine was increased. The peptide composition of *Fagopyrum esculentum Moench* grain was studied by gel electrophoresis. The most significant alteration of reserve globulins structure are observed during germination phase from 20 till 24 hours. New low-molecular polypeptides appear during above mentioned period, that indicates embryonic awakening and synthesis of new protein compounds, mainly hydrolases. The process of proteolysis during germination of *Fagopyrum esculentum Moench* grain promotes a content increase of soluble fractions and sum of albumins and globulins. There is a significant decrease of insoluble protein residue during germination phase change. Chromatographic method was used to determine the change of carbohydrate composition of *Fagopyrum esculentum Moench* grains during germination. It was established that the content of total carbohydrates amount in grain extracts increases. Electron scanning microscopy revealed that after 12 hours germination of *Fagopyrum esculentum Moench* grain, swelling of starch grains and minor damage of their packaging in endosperm are observed. After 24 hours, endosperm of germinated grain significantly changed microstructure: starch grains and components of protein matrix had a vague outline, grain disintegration was observed. Evaluation of antioxidant activity of alcohol extract from *Fagopyrum esculentum Moench* grain germinated during 24 hours showed that percentage of DPPG free radicals inhibition increases with process prolongation. Thus, *Fagopyrum esculentum Moench* grain germinated within 24 hours is characterized by a high content of biologically active substances and can be used in food technologies for functional products development.

Keywords: *Fagopyrum esculentum Moench* grain; germination; vitamins; peptide composition; carbohydrate composition; microstructure; antioxidant activity

INTRODUCTION

The nutritional value of *Fagopyrum esculentum Moench* in terms of protein, vitamins, minerals, organic acids, amino acid and dietary fibers content similar to wheat and triticale, so *Fagopyrum esculentum Moench* is used for wheat substitution in bakery, pasta production and confectionery **(Wronkowska et al., 2010; Marijana et al., 2015)**. Whole-grain buckwheat flour has a positive effect on the physical, chemical and sensory properties of bread and enhances its functional and antioxidant properties **(Li and Zhang, 2001; Lin et al., 2009; Yıldız and Bilgiçli, 2012)**. The antioxidant activity of *Fagopyrum esculentum Moench* grain primarily associated with the content of polyphenols and tocopherols. Polyphenols in buckwheat flour exist in free and bound forms, concentration of free polyphenols can be from 48% up to 64%. Buckwheat flavonoids have positive effects in hypotension treatment, have an inflamotor and anti-allergic effect **(He et al., 1995; Kim et al., 2003; Kim et al., 2004; Wloch et al., 2016)**. Buckwheat proteins are characterized by a unique amino acid composition that helps lower blood cholesterol levels and improve patient's conditions with constipation and obesity **(Huff and Carroll, 1980; Ahmed et al., 2014; Sikder et al., 2014; Sakač et al., 2015)**. Proteins of *Fagopyrum esculentum Moench* grain are characterized by an overestimated amino acid content in terms of tryptophan **(Chao et al., 2002)**. Buckwheat is the best source of magnesium, potassium, phosphorus, zinc, manganese and cuprum than other cereals. Among the vitamins, pyridoxine is the most prevalent in buckwheat, and presented phytosterols are useful to lower cholesterol level in the blood. Whole grains contain 7% of fiber. Outer layers of buckwheat grain contain main part of effective prophylactic compounds **(Danihelová and Šturdík 2012)**.

Buckwheat is a potentially safe source of gluten-free products for patients with celiac disease and chronic enteropathy **(Sedej et al., 2011; Katar et al., 2016)**. *Fragopyrum esculentum Moench* grain extracts have antibacterial and immunostimulating effects **(Čabarkapa et al., 2008; Świątecka et al., 2013)**. Germination of *Fagopyrum esculentum Moench* grain is considered as an effective method to improve nutritional properties. This is a complex process with significant changes in biochemical and sensory characteristics due to the activation of enzymes. Germinated grain or sprouts have bigger nutritional value than their original grain in terms of protein content and starch digestibility **(Nonogaki et al., 2010)**. A significant increase in total amount of phenolic compounds and a higher antioxidant activity are observed after 64 hours germination, while heating leads to decrease in total amount of phenolic compounds and antioxidant activity. Germinated buckwheat has a better nutritional value and antioxidant activity and it is an excellent natural source of flavonoids and phenolic compounds, especially rutin and C-glycosylflavones. Therefore, germinated *Fagopyrum esculentum Moench* grain can be used as a promising functional nutrition for health promotion **(Koyama et al., 2013; Zhang et al., 2015; Terpinc et al., 2016)**.

The study of buckwheat flour blending with wheat flour in different ratios for cookies production showed a significant change in the physico-chemical and functional properties of blended flour. The overall acceptability of cookies according to sensory analysis was at the highest level at 40% mixing. This study showed that buckwheat addition to wheat flour can not only improve the physicochemical and functional properties of mixed flour, but also increase the nutraceutical potential of product made of it **(Jan et al., 2015)**.

The target of the study was to study alteration of biochemical parameters and microstructure of *Fagopyrum esculentum Moench* grain during germination.

Scientific hypothesis

Germination process has a significant effect on biochemical parameters change of *Fagopyrum esculentum Moench* grain.

MATERIAL AND METHODOLOGY

The parameters of grain's technological qualities for 2 local varieties and 26 breeding varieties of different morphotype representing the main stages of selection of *Fagopyrum esculentum Moench* were analyzed. As a result of analysis *Fagopyrum esculentum Moench* variety Dikul (laboratory of cereal crops selection of the Institute of Leguminous and Cereal Cultures, Russian Federation) was selected for further research. *Fagopyrum esculentum Moench* grain was separated from various impurities and washed with a large amount of cold water. Then it was poured with water at a ratio of grain:water = 1 : 1, soaked for 12 hours at 25 °C, distributed in equal layer and germinated in incubation chamber at an air humidity of 60 – 70%, temperature 18 – 25 °C for 12 – 24 hours.

Vitamin content determination was carried out by HPLC on a Milichrom-5 device (NJSC Nauchpribor, Russia). An aqueous buckwheat grane extract (pH 3) was used, eluent of the composition was acetonitrile: an aqueous solution of sodium heptanesulfonate and potassium phosphate monosubstituted (pH 3.0, 20 : 80 ratio); mobile phase flow rate was 1 $cm^3.min^{-1}$; elution mode was isocratic, detection was carried out in wavelength range 200 – 400 nm, analysis time 12 – 25 min, sample volume 2 – 6 μL.

The polypeptide composition of the total buckwheat grain protein was determined by one-dimensional DDS-Na electrophoresis on gel plates with an acrylamide concentration gradient of 10 – 20% in resolving gel (pH 8.8) and 6% acrylamide in concentrated gel (pH 6.8). A Helicon camera was used for vertical electrophoresis with Elf-4 power supply unit (OOO Helikon Company, Russia).

The concentration of low-molecular carbohydrates in grain samples was determined by chromatographic method using electrochemical detection on an Agilent 1100 liquid chromatograph with an ESA Coulochem III electrochemical detector (Agilent Technologies, USA). Separation of the sugar mixture was carried out on an anion exchange column with a grafted aminophase followed by electrochemical detection.

Microstructural studies were carried out using an electronic scanning microscope ZEISS EVO LS (Carl Zeiss Industrielle Messtechnik GmbH, Germany) with an accelerated voltage of 15 kV.

The complex of phenolic compounds was determined by HPLC on a Milichrom-5 device (NJSC Nauchpribor, Russia). An alcohol extract of grain was used, eluent of the composition was acetonitrile: an aqueous solution of trifluoroacetic acid (pH 2.5, 15 : 85 ratio); elution mode was isocratic, analysis time is 12 – 25 minutes, sample volume was 2 – 6 μL.

Antioxidant activity was determined by spectrophotometric method in an alcohol extract described by **Silva et al. (2005)** based on percentage of inhibition of DPPH radical (2,2-diphenyl-1-picrylhydrazyl). We determined the optical density of solutions in the interaction DFPG with extractive substances of plants by spectrophotometer "Specord M40" (Carl Zeiss Industrielle Messtechnik GmbH, Германия) at a wavelength of 515 nm.

Statisic analysis

T-statistics (a two-sample t-test for independent samples) was used to assess reliability of test differences, Analysis were conducted to a significance level p <0.05 using Statistica 7.0 software (StatSoft Inc., USA).

RESULTS AND DISCUSSION

The study of water-soluble vitamins accumulation process by *Fagopyrum esculentum Moench* grain was carried out during period of swelling and germination, which is characterized by especially intensive metabolism. During above mentioned period, the reserve substances are transformed into vital compounds used by sprout to form new tissues. The content of water-soluble vitamins in *Fagopyrum esculentum Moench* grain was determined by

HPLC before germination and after 36 hours of incubation. Chromatograms are shown on Figures 1 and 2.

Study results shows that after 24 hours of incubation concentration of ascorbic acid in seeds increased by 821%, thiamine by 664%, nicotinic acid by 571%, pantothenic acid by 574%, routine by 706%. According to published data, during *Fagopyrum esculentum Moench* grain germination, a gradual increase of vitamin B1 content is observed, which corelates with our data. The amount of vitamins B2 and B6 does not change while content of vitamin C decreases in the early stages of germination, and then on the third day of buckwheat grains germination increases dramatically **(Yiming et al., 2015)**. According to other researchers **(Kim et al., 2004)**, the content of vitamin C in buckwheat grains increases rapidly, as well as in our studies, and for B1 and B6, a relatively moderate increase is noticed.

During study of biochemical processes in germinating seeds used for food purposes, one of the tasks was to determine the technological characteristics of reserve proteins. During germination some proteins are synthesized using a series of biochemical reactions. Meanwhile, other proteins can be hydrolyzed by an activated protease. The protein content in the grain during germination is determined by both the effect of proteolysis and the rate of protein molecules synthesis. Therefore, a change of protein content during germination of grain is a dynamic process. In previous studies about effect of germination on proteins in cereals and legumes, conflicting data were obtained. **(Uppal and Bains, 2012; Zhang et al., 2015)** indicate an increase of protein content during grain germination. At the same time **(Yiming et al., 2015)** found that the protein content decreases during germination of buckwheat grain. In our case, a decrease of protein nitrogen content in *Fagopyrum esculentum Moench* grain during germination is also observed (Table 1). The contradictory nature of the data may be related with specific features of plants and germination conditions. Structural changes in the protein complex at germination stages were examined by gel electrophoresis of peptide composition of *Fagopyrum esculentum Moench* grain. The electrophoregrams are shown on Figure 3.

Three main groups of peptides with molecular weights of 20 – 25, 40 – 45 and 65 kDa have been allocated. Computer processing by program "Biotest-D" of obtained electrophoregrams by luminosity of peptides was carried out, which confirmed the changes in their quantitative content. The most significant changes in structure of reserve globulins were observed during the germination phase after 20 – 24 hours.

New low-molecular polypeptides appear in this period, which indicates the embryonic awakening of the embryo and the synthesis of new protein compounds. This group of protein includes mainly hydrolases (lipases, proteases, peptidases, amylases, phytases) aimed at hydrolysis of all reserve nutrients. The electrophoregram of *Fagopyrum esculentum Moench* grain proteins after 36 hours of germination indicates unrestricted soaking and dissolution of peptides. This is probably due to the deep hydrolysis and decomposition of reserve proteins.

Obtained electrophoregrams of buckwheat grain protein correspond to the published data. Proteins with molecular weights of 15 and 22 kDa were identified as the main allergic proteins **(Morita et al., 2006)**. According to other data, buckwheat grain with a molecular weight of 24, 19, 16 and 9 kDa are the main allergens **(Park et. Al., 2000)**. The results of our research show that low-molecular proteins of this fraction were destroyed during germination process and *Fagopyrum esculentum Moench* germinated grain can be used for the production of food with a low content of allergens.

For food technology, usage of sprouted buckwheat grain is most acceptable, due to peptides biological activity. Therefore, further studies of biochemical parameters were carried out for a germinated within 24 hours grain.

Table 1 shows the change in the composition of *Fagopyrum esculentum Moench* grain protein fractions during germination.

Proteolysis process during *Fagopyrum esculentum Moench* grain germination promotes an increase of soluble fractions content, sum of albumins and globulins: in soaked grain by 12.8% (t =4.37, p =0.02), in sprouted grain – by 27.2% (t =8.18, p =0.001). There is a significant reduction of insoluble protein residue during germination phases change from 45.5 (t =11.82, p =0.001) up to 56.0% (t =15.35, p =0.001).

Determination of carbohydrate composition change in *Fagopyrum esculentum Moench* grain during germination was made by chromatographic method. The results are shown in Table 2.

It was found that hydrolysis of glycosidic bonds in polysaccharide molecules occurred under the influence of own carbohydrases, and substances with low molecular weight and high solubility were formed. The content of carbohydrates in grain extracts increases in the process of germination. This indicates the biochemical processes occurring in the molecules of starch, due to activation of enzymes.

The content of carbohydrates in grain extracts increases during germination by 27.79% in soaked grains and by 38.97% in sprouted grain. Differences are significant and statistically significant t =3.92, p =0.02 for soaked grain and t =10.18, p =0.001 for sprouted.

Reducing of carbohydrates content in buckwheat grain during germination was observed **(Colmenares De Ruiz and Bressani, 1990)**. Germination of legumes increas digestibility of carbohydrates, this is associated with the degradation of starch into smaller fragments and the formation of reducing sugars **(Kelkar et al., 1996)**.

Alteration results of buckwheat grain carbohydrate composition in germination process are coordinated with the data of electron scanning microscopy. Figure 4 shows microphotographs of cut surface structure of dry buckwheat grain and sprouted for 12 and 24 hours.

In the process of *Fagopyrum esculentum Moench* grain germination, there is a soaking of grain starch and a slight damage to their packaging in endosperm after 12 hours.

In the process of *Fagopyrum esculentum Moench* grain germination, there is a soaking of grain starch and a slight damage to their packaging in endosperm after 12 hours. After 24 hours, endosperm of germinated grain underwent significant changes in microstructure: grain starch and components of protein matrix had a vague outline, grain disintegration was observed.

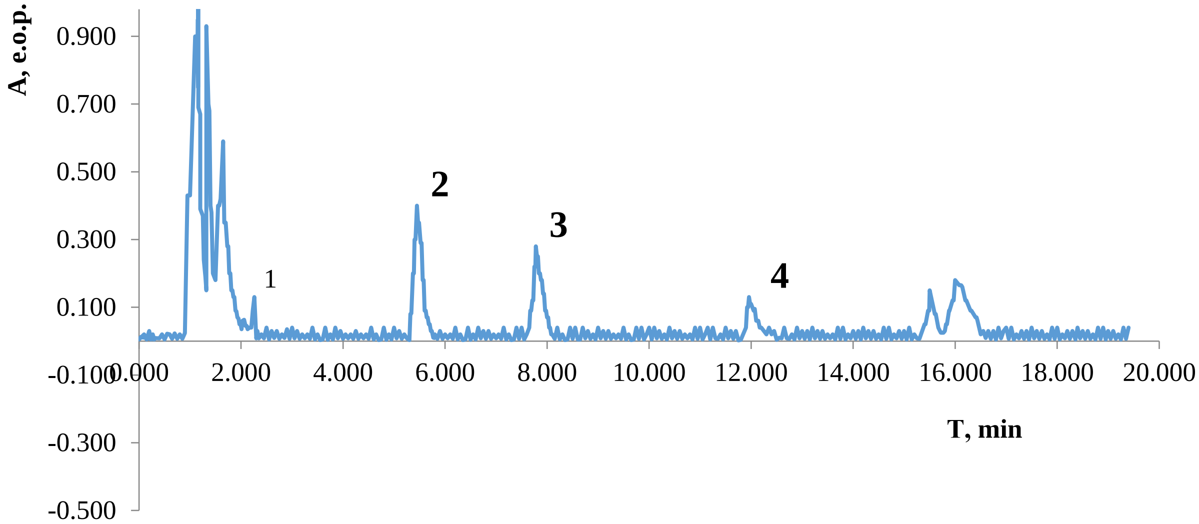


Figure 1 Chromatogram of extract from native buckwheat : 1 – ascorbic acid (C), 2 – thiamine (B_1), 3 – nicotinamide (PP), 4 – pantothenic acid (B_3).

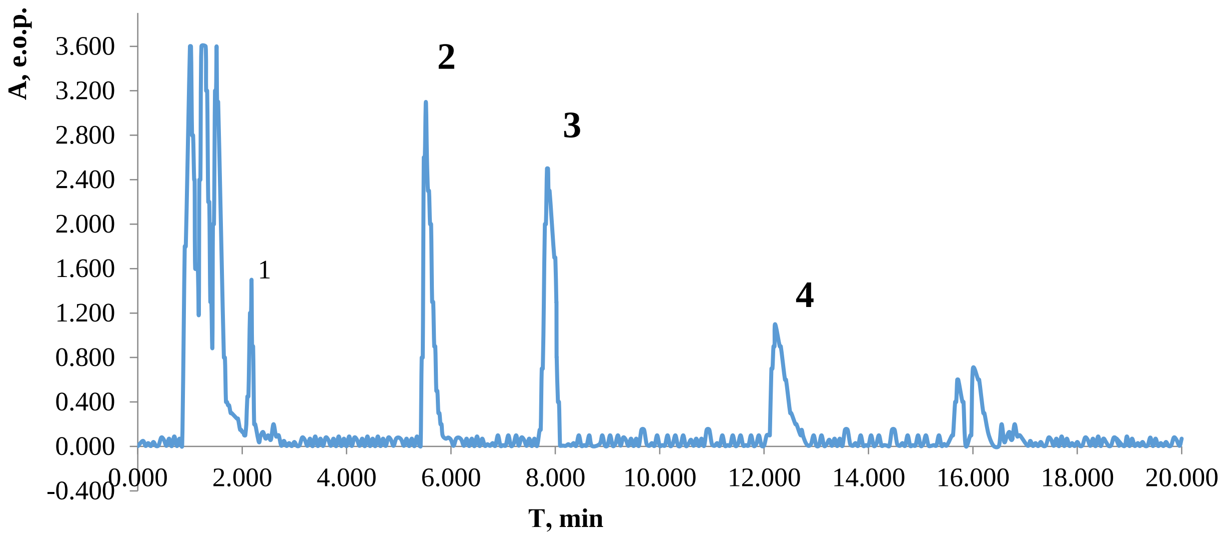


Figure 2 Chromatogram of extract from buckwheat after 24 hours of germination: 1 – ascorbic acid (C), 2 – thiamine (B_1), 3 – nicotinamide (PP), 4 – pantothenic acid (B_3).

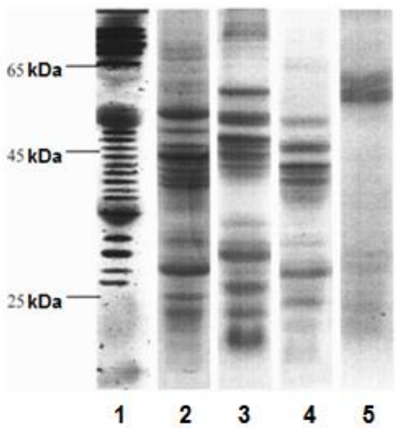


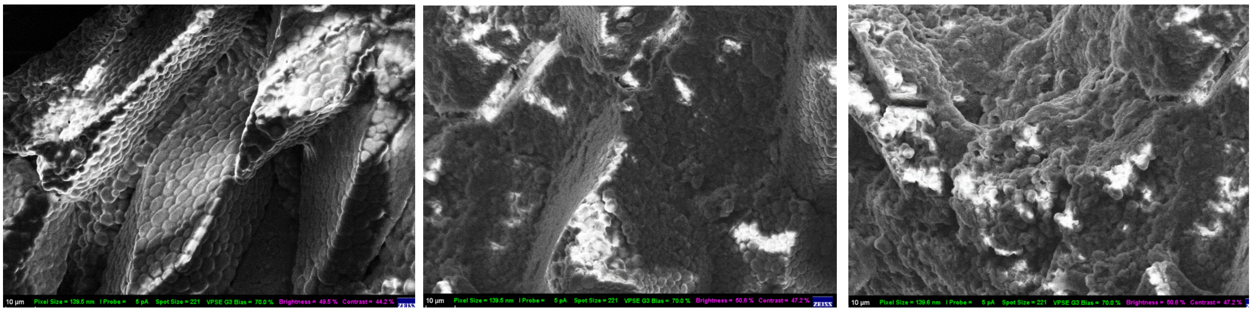
Figure 3 The electrophoregrams of buckwheat seeds protein (1 – standart, 2 – native grain; 3 – germinated (12 h); 4 – sprouting (24 h), 5 - sprouted (36 h).

Table 1 Fractional composition of *Fagopyrum esculentum Moench* grain protein during germination.

Indicators	**Physiological state of grain**		
	Resting	**Swollen after 12h of germination**	**Sprouted after 24h of germination**
Total nitrogen, %	2.42 ±0.42	2.12 ±0.18	2.15 ±0.20
Protein nitrogen, %	2.26 ±0.21	1.90 ±0.15	1.76 ±0.19
Fractions composition (compared to total extracted nitrogen in %):			
Albumins	22.12 ±0.80	24.50 ±0.98	27.83 ±1.10
Globulins	42.34 ±1.95	48.24 ±1.63	54.16 ±2.22
Glutelins	11.25 ±0.41	12.92 ±0.32	10.72 ±0.33
Insoluble residue	23.15 ±0.79	12.73 ±0.39	10.18 ±0.30

Table 2 Alteration of carbohydrate composition of *Fagopyrum esculentum Moench* grain during germination, g.L^{-1}.

Type of carbohydrate s	**Sprouting time**		
	0 h	**12 h**	**24 h**
Dextrins of the 2nd order	28.85 ±2.24	39.60 ±2.18	39.71 ±2.09
Dextrins of the 1nd order	5.23 ±0.42	7.62 ±0.51	13.26 ±0.81
Rafinose	2.17 ±0.30	2.98 ±0.38	2.09 ±0.25
Maltose	9.28 ±0.68	18.55 ±0.72	18.45 ±0.69
Glucose	17.85 ±0.72	23.29 ±0.98	27.50 ±0.90
Fructose	13.96 ±0.84	6.79 ±0.65	6.47 ±0.52
Total carbohydrates	77.34 ±2.88	98.83 ±4.66	107.48 ±0.69



1 2 3

Figure 4 Microstructure of cut surface of *Fagopyrum esculentum moench* grain (1 – dry grain, 2 – grain sprouted in 12 hours, 3 – grain, sprouted within 24 hours), x 2000 magnification. Photo: S. Motyleva, 2017.

Table 3 Flavonol content and antioxidant activity of *Fagopyrum esculentum Moench* seeds after germination.

Type of raw material	**Total content of flavonols on basis of rutin, %**	**Antioxidant activity, inhibition % of DPPG radical**
Dry buckwheat grains	0.26 ±0.02	29.0 ±0.90
Buckwheat grains after 12 hours of germination	0.55 ±0.04	47.0 ±0.72
Buckwheat grains after 24 hours of germination	0.80 ±0.04	63.0 ±0.94

A lot of works have been published based on definition of antioxidant activity of germinating grain of *Fagopyrum esculentum Moench*, and grain of other plants, **(Lan-Sook Lee et al., 2016; Bolnvar, Cevallos-Casals and Cisneros-Zevallos, 2010)**. These studies demonstrate increase of polyphenols content and antioxidant activity during grain germination. According to **(Jiang et al., 2007)**, the antioxidant activity of *Fagopyrum esculentum Moench* grain correlates with the total content of flavonoids and routine. Other authors did not observe significant correlations between flavonoids and the measured antioxidant effect **(Danihelová and Šturdík, 2013)**.

Determination of antioxidant activity (Table 3) of alcohol extract from buckwheat grain germinated for 24 hours showed that inhibition percentage of DPPG free radicals increases with duration of the process. The total content of flavonols in recalculation on routine increased by 2.11 (t =6.48, p =0.01) times in 12 hours of germination and in 3.07 (t =12.07, p =0.001) times in 24 hours. The obtained values of the total content of flavonols and antioxidant activity of grain *Fagopyrum esculentum Moench* correspond to those established by other authors **(Morishita et al., 2007)**.

Experimental data show that antioxidant activity of buckwheat grain increases with growth of total flavonols content during germination.

CONCLUSION

It is experimentally established that in the early stages of germination process of *Fagopyrum esculentum Moench* grain, its biochemical parameters change. The process of proteolysis during *Fagopyrum esculentum Moench* grain germination promotes an increase of soluble fractions content, the amount of albumins and globulins: in the soaked grain by 12.8%, in the germinated grain by 27.2%, the amount of insoluble protein decrease from 45.5 to 56.0%. During germination, the amount of low-molecular carbohydrates in grain extracts increases by 27.79% in soaked grain and by 38.97% in sprouted grain, and the total content of flavonols in terms of routine amounts increases in 2.11 and 3.07 times, respectively. Differences between measurements of the chemical composition in the germination process are statistically significant. It is shown that in the process of *Fagopyrum esculentum Moench* grain germination content of water-soluble vitamins C, B1, PP, B3 increases, the microstructure of the endosperm surface changes - the elements of packing of starch grains disintegrate and they lose clear outlines. During the germination of *Fagopyrum esculentum Moench* grain, antioxidant activity increases after 24 hours.

Thus, sprouted within 24 hours grain of *Fagopyrum esculentum Moench* is characterized by a high content of biologically active substances and has indicators of high nutritional value. In the sprouted grains of buckwheat, there are no major protein allergens. Such a grain can be used in food technology to create products with a functional purpose with a low content of allergens. Based on the sprouted grain of *Fagopyrum esculentum Moench*, we created a complex food supplement, which additionally includes the green mass of macrophyte *Lemna minor*, succinic acid and yeast *Saccharomyces boulardi*. This additive can be used in the production of bakery and confectionery products, as well as dairy products.

REFERENCES

Ahmed, A., Khalid, N., Ahmed, A., Abbasi, N. A., Latif, M. S. Z., Randhawa, M. A. 2014. Phytochemicals and biofunctional properties of buckwheat: a review. *Journal of Agricultural Science*, vol. 152, no. 3, p. 349-369. https://doi.org/10.1017/S0021859613000166

Butenko, L. I., Ligay, L. V. 2013. Study of the chemical composition of germinated seeds of buckwheat, oats, barley and wheat. *Fundamental research*, no. 4-5, p. 1128-1133.

Čabarkapa, I. S., Sedej, I. J., Sakać, M. B., Šarić, L. C., Plavšić, D. V. 2008. Antimicrobial activity of buckwheat (*Fagopyrum esculentum Moench*) hulls extract. *Food Processing Quality Safety*, vol. 35, no.4, p. 159-163.

Cevallos-Casals, B. A, Cisneros-Zevallos, L. 2010. Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. *Food Chemistry*, vol. 119, no. 4, p. 1485-1490. https://doi.org/10.1016/j.foodchem.2009.09.030

Chao, P. L., Hsiu, S., Hou, Y. 2002. Flavonoids in herbs: biological fates and potential interactions with xenobiotics. *Journal of Food and Drug Analysis*, vol. 10. p. 219-228.

Colmenares de Ruiz, A. S., Bressani, R. 1990. Effect of germination on the chemical composition and nutritive value of amaranth grain. Cereal Chem, vol. 67, no. 6, p. 519-522.

Danihelová, M., Šturdík, E. 2012. Nutritional and health benefits of buckwheat. *Potravinarstvo*, vol. 6, no. 3, p. 1-9. https://doi.org/10.5219/206

Danihelová, M., Šturdík, E. 2013. Antioxidant and antiproteinase effects of buckwheat hull extracts. *Potravinarstvo*, vol. 7, no. 1, p. 89-94. https://doi.org/10.5219/272

Fesenko, A., Kuznetsova, E., Polehina, N., Shipulin,O., Fesenko, N., Selifonova, N. 2015. Comparative analysis of grain quality of buckwheat varieties with different breeding history. *Technology and the study of merchandise of innovative foodstuffs*, vol. 4, no. 33, p. 76-86.

He, J., Klag, M. J., Whelton. P. K., Mo, J. P., Chen, J. Y., Qian, M. C., Mo, P. S., He, G. Q. 1995. Oats and buckwheat intakes and cardiovascular disease risk factors in an ethnic minority of China. *The American Journal of Clinical Nutrition*, vol. 61, no. 2, p. 366-372. https://doi.org/10.1093/ajcn/61.2.366
PMid:7840076

Huff, M. W., Carroll, K. K. 1980. Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits. *Journal of Lipid Research*, vol. 21, no. 5, p. 546-558. PMid:7400686

Jan, U., Gni, A., Ahmad, M., Shah, U., Baba, W. N., Masoodi, F. A., Magsood, S., Gani, A., Wani, I. A., Wani, S. M. 2015. Characterization of cookies made from wheat flour blended with buckwheat flour and effect on antioxidant properties. *Journal of Food Science and Technology-Mysore*, vol. 52, no. 10, p. 6334-6344. https://doi.org/10.1007/s13197-015-1773-8
PMid:26396378

Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J. A., Briggs, C. J. 2007. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Research International*, vol. 40, no. 3, p. 356-364. https://doi.org/10.1016/j.foodres.2006.10.009

Katar, D., Olgun, M., Turan, M. 2016. Analysis of morphological and biochemical characteristics of buckwheat (*Fagopyrum esculentum Moench*) in comparison with cereals. *Cyta-journal of food*, vol. 14, no. 2, p. 176-185. https://doi.org/10.1080/19476337.2015.1076522

Kelkar, K, Shastri, P, Rao, B. Y. 1996. Effect of processing on *in vitro* carbohydrate digestibility of cereals and legumes. *Journal of Food Science and Technology – Mysore*, vol. 33, no. 6, p. 493-497.

Kim, C. D., Lee, W. K., No, K. O., Park, S. K., Lee, M. H., Lim, S. R., Roh, S. S. 2003. Anti-allergic action of buckwheat (*Fagopyrum esculentum Moench*) grain extract. *International Immunopharmacology*, vol. 3, no. 1, p. 129-136. https://doi.org/10.1016/S1567-5769(02)00261-8

Kim, S. L., Kim, S. K., Park, C. H. 2004. Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International*, vol. 37, no. 4, p. 319-327. https://doi.org/10.1016/j.foodres.2003.12.008

Koyama, M., Nakamura, C., Nakamura, K. 2013. Changes in phenols contents from buckwheat sprouts during growth stage. *Journal of Food Science Technology*, vol. 50, no. 1, p. 86-93. https://doi.org/10.1007/s13197-011-0316-1

Lee, L. S., Choi, E. J., Kim, C. H., Sung, J. M., Kim, Y. B., Seo, D. H., Choi, H. W., Choi, Y. S., Kum, J. S., Park, J. D. 2016. Contribution of flavonoids to the antioxidant properties of common and tartary buckwheat. *Journal of Cereal Science*, vol. 68, p. 181-186. https://doi.org/10.1016/j.jcs.2015.07.005

Li, S. Q., Zhang, Q. H. 2001. Advances in the development of functional foods from buckwheat. *Critical Reviews in Food Science and Nutrition*, vol. 41, no. 6, p. 451-464. https://doi.org/10.1080/20014091091887
PMid:11592684

Lin, L., Liu, H., Yu, Y., Lin, S., Mau, J. 2009. Quality and antioxidant property of buckwheat enhanced wheat bread. Food Chemistry, vol. 112, no. 4, p. 987-991. https://doi.org/10.1016/j.foodchem.2008.07.022

Morishita, T., Yamaguchi, H., Degi, K., 2007. The contribution of polyphenols to antioxidative activity in common buckwheat and tartary buckwheat grain. *Plant Production Science*, vol. 10, no. 1, p. 99-104. https://doi.org/10.1626/pps.10.99

Morita, N., Maeda, T., Sai, R., Miyake, K., Yoshioka, H., Urisu, A., Adachi, T. 2006. Studies on distribution of protein and allergen in graded flours prepared from whole buckwheat grains. *Food Research International*, vol. 39, no. 7, p. 782-790. https://doi.org/10.1016/j.foodres.2006.02.005

Nonogaki, H., Bassel, G. W., Bewley, J. D. 2010. Germination – still a mystery. *Plant Science*, vol. 179, no. 6, p. 574-581. https://doi.org/10.1016/j.plantsci.2010.02.010

Oomah, B. D., Mazza, G. 1996. Flavonoids and antioxidative activities in buckwheat. *Journal of Agricultural and Food Chemistry*, vol. 44, no. 7, p. 1746-1750. https://doi.org/10.1021/jf9508357

Park, J. W., Kang D. B., Ким, C. W., Ko, S. H., Yum, H. Y., Kim, K. E., Hong, C. C., Lee, K. Y. 2000. Identification and characterization of the major allergens of buckwheat. Allergy. *European Journal of Allergy and Clinical Immunology*, vol. 55, no. 11, p. 1035-1041.

Sakač, M. B., Sedej, I. J., Mandić, A. I., Mišan, A. Č. 2015. Antioksidativna svojstva brašna od heljde – Doprinos funkcionalnosti pekarskih, testeničarskih i brašneno-konditorskih proizvoda. *Hemijska Industrija*, vol. 69, no. 5, p.469-483.

Sedej, I., Sekač, M., Mandić, A., Mišan, A., Pestorić, M., Šimurina, O., Čanadanović-Brunet, J. 2011. Quality assessment of gluten-free crackers based on buckwheat flour. *LWT – Food Science and Technology*, vol. 44, no. 3, p. 694-699. https://doi.org/10.1016/j.lwt.2010.11.010

Sikder, K., Kesh, S. B., Das, N., Manna, K., Dey, S. 2014. The high antioxidative power of quercetin (aglycone flavonoid) and its glycone (rutin) avert high cholesterol diet induced hepatotoxicity and inflammation in Swiss albino mice. *Food and Function*, vol. 5, no. 6, p. 1294-1303. https://doi.org/10.1039/c3fo60526d

Świątecka, D., Markiewicz, L. H., Wróblewska, B. 2013. *In vitro* evaluation of the effect of the buckwheat protein hydrolysate on bacterial adhesion, physiology and cytokine secretion of Caco-2 cells. *Central European Journal of Immunology*, vol. 38, no. 3, p. 317-327. https://doi.org/10.5114/ceji.2013.37753

Terpinc, P., Cigić, B., Polak, T., Hribar, J., Požrl, T. 2016. LC-MS analysis of phenolic compounds and antioxidant activity of buckwheat at different stages of malting. *Food Chemistry*, vol. 210, p. 9-17. https://doi.org/10.1016/j.foodchem.2016.04.030 PMid:27211614

Uppal, V., Bains, K. 2012. Effect of germination periods and hydrothermal treatments on *in vitro* protein and starch digestibility of germinated legumes. *Journal of Food Science Technology*, vol. 49, no. 2, p. 184-191. https://doi.org/10.1007/s13197-011-0273-8 PMid:23572840

Wloch, A., Strugala, P., Pruchnik, H., Żyłka, R., oszmiański, J., Kleszczyńska, H. 2016. Physical effects of buckwheat extract on biological membrane *in vitro* and its protective properties. *Journal of Membrane Biology*, vol. 249, no. 1-2, p. 155-170. https://doi.org/10.1007/s00232-015-9857-y

Wronkowska, M., Zielińska, D., Szawara-Nowak, D., Troszyńska, A., Soral-Śmietana, M. 2010. Antioxidative and reducing capacity, macroelements content and sensorial properties of buckwheat-enhanced gluten-free bread. *International Journal of Food Science and Technology*, vol. 45, p. 1993-2000. https://doi.org/10.1111/j.1365-2621.2010.02375.x

Yıldız, G., Bilgiçli, N. 2012. Effects of whole buckwheat flour on physical, chemical, and sensory properties of flat bread, lavaş. *Czech Journal of Food Science*, vol. 30, no. 6, p. 534-540. https://doi.org/10.17221/10/2012-CJFS

Yiming, Z., Hong, W., Linlin, C., Wen, T., Xinli, S. 2015. Evolution of nutrient ingredients in tartary buckwheat seeds during germination. Food Chemistry, vol. 186, p. 244-248. https://doi.org/10.1016/j.foodchem.2015.03.115 PMid:25976817

Zhang, G., Xu, Z., Gao, Y., Huang, X., Zou, Y., Yang, T. 2015. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. *Journal of Food Science*, vol. 80, no. 5. p. 1111-1119. https://doi.org/10.1111/1750-3841.12830 PMid:25858540

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