UDC: 633.12:664.788.3

BIOACTIVATED BUCKWHEAT IN TERMS OF ITS NUTRITIONAL VALUE

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DOI: https://doi.org/10.15673/fst.v15i2.2030

Article history

Received 11.02.2021
Reviewed 22.03.2021
Revised 17.05.2021
Approved 08.06.2021

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Cite as Vancouver style citation

Zenkova M. Bioactivated buckwheat in terms of its nutritional value. Food science and technology. 2021;15(2):4-10. DOI: https://doi.org/10.15673/fst.v15i2.2030

Цитування згідно ДСТУ 8302:2015

Zenkova M. Bioactivated buckwheat in terms of its nutritional value // Food science and technology. 2021. Vol. 15, Issue 2. P. 4-10 DOI: https://doi.org/10.15673/fst.v15i2.2030

Abstract. Buckwheat is a gluten-free pseudocereal crop consumed as functional food in some regions. Traditionally, buckwheat grain is used to make buckwheat groats and flour. Bioactivation improves the nutritional value of buckwheat grain by activating hydrolytic enzymes that make nutrients available for the plant's growth and for the human body. The article contains research on the content of nutrients in bioactivated buckwheat grain of the Kupava variety grown in Belarus. The study examined hulled buckwheat kernels (not steamed) of the botanical variety Kupava, cream-coloured with a greenish hue, with the humidity 12.0%, and with the proportion of sound kernels 99.4%. At the first stage of germination, bioactivated buckwheat grain was obtained in 48 hours. It had the following characteristics: humidity (39.2±1.0)%, sprout size (2.0±0.5)mm. According to the organoleptic parameters, the grain had a pleasant sweetish taste and a subtle nutty aftertaste. The components of bioactivated buckwheat grain that provide a person with the largest amounts of macronutrients are starch (31.84±0.6)%, sugars (5.1±0.3)%, and protein (5.67±0.02)%. Bioactivated buckwheat grain contains all essential amino acids (30.5% of the total quantity of amino acids). The limiting amino acid is threonine (amino acid score 31.7%). Of nonessential amino acids, there is a high content of glutamic acid (1.0972g per 100g). The difference ratio of the amino acid score is 54.75%, the potential biological value of the protein is 45.25%. 100g of sprouted buckwheat grain contains 48.5% of the daily requirement of manganese, 24.0% of copper, 18.9% and 34.0% of iron (for women and for men respectively), about 23.0% of the daily requirement of vitamin B1, and 12.7% of the daily requirement of vitamin E. Besides, bioactivated buckwheat grain contains fibre (6.5% of the daily requirement on average). Bioactivated buckwheat grain can be consumed as a meal in its own right, as a side dish, as an additive to salads and fermented dairy products, or as a dessert when mixed with fruit purée, honey, or dried fruit.

Keywords: buckwheat, bioactivated grain, sprouted grain, macronutrients, micronutrients.

Introduction. Formulation of the problem

The growing popularity of sprouted grain products around the world is attributed to the need to prevent diabetes, cardiovascular, gastrointestinal, and other diseases. This is caused by the fact that people have been using plants for a long time not only as a source of food, but also as a remedy for many diseases. According to the optimal nutrition principles, a human diet should include a wide range of food products. They should contain various combinations of nutrients necessary to restore cells and tissues, provide energy, and regulate numerous metabolic processes [1]. The usefulness of natural plant raw materials is associated with a well-balanced combination of all its components and their synergistic effect on the body. However, people consume food not only in its natural form, but in processed form, too. When raw materials are processed into food, they change physically and chemically. These changes are accompanied by loss of nutrients. A widespread approach to make up for the loss is enriching food products with micronutrients they lack or using bioactive supplements [2]. According to some scientists, the use of sprouted (bioactivated) grains effectively improves the nutrient composition of a diet. The important role of sprouted grains consists in the fact that even after heat treatment, their nutrient composition is more useful than that of unsprouted grains [3-5]. Sprouted (bioactivated) grains can be used as a separate dish for breakfast or as a side dish for lunch. They can be introduced as a dietary supplement into various consumer products to give them new sensory qualities and useful properties [6].

Analysis of recent research and publications

Food-enriching sprouted grain is widely used internationally [7-10]. For this purpose, there is much research on the processes occurring in grain during germination and on the changes in the chemical composition of grain. New methods are being developed that allow preparing germinated grain and introducing it into food products with minimal changes to the recipe or technology [6,11-12]. Buckwheat grain is of interest for its content of bioactive compounds useful for human health [13,14]. Compared with other grain crops such as wheat, rice, or maize, buckwheat grain has higher nutritional value [15,16]. Germination of buckwheat grains causes significant changes in their protein complex, makes sugars and free amino acids (including lysine) more available, results in accumulation of γ-aminobutyric acid and phenolic compounds, and increases the antioxidant activity [17-19]. The nutritional value of grain increases with controlled sprouting. Thus, under the action of the enzyme phytase, minerals become available for intestinal absorption and vitamins are synthesised and accumulated [18,20]. However, so far, there is no generally accepted definition and description of the characteristics of sprouted and bioactivated grains. In scientific articles, as described in [18,19], a whole grain is considered to be sprouted if it has a sprout not exceeding the length of the grain, and such grain is intended to be eaten whole. However, consumers traditionally believe that sprouted grains include germinated cereal grains prior to the appearance of green shoots and also microgreens. Obviously, the nutritional value of grains will differ depending on the degree of sprouting. Bioactivated grains are obtained from a short controlled sprouting process, which takes place in the presence of water, heat, and air, and is, in fact, the beginning of grain sprouting [21]. With this process, a seedling only starts forming and does not exceed 2.5mm. The process of bioactivation can be called the first stage of grain sprouting. It is assumed that bioactivated grain differs from sprouted grain by the low activity of amylolytic and proteolytic enzymes. However, bioactivated grain, like sprouted grain, can have a positive effect on human cholesterol and glucose levels, blood pressure, and mineral absorption [22]. Consequently, there is a clear need to characterise the composition of bioactivated buckwheat grain, in particular, the content of macronutrients and micronutrients in it.

The purpose of the work is to research the chemical composition and nutritional value of bioactivated buckwheat grain. For this purpose, it is necessary to attain the following objectives:

– to study the content of macronutrients in bioactivated buckwheat grain;

– to estimate potential biological value of bioactivated buckwheat grain protein;

– to study the content of micronutrients in bioactivated buckwheat grain.

Research materials and methods

The reference sample used in the research was first-grade buckwheat groats (not steamed), of the botanical variety Kupava. They are whole or slightly cracked buckwheat kernels that do not pass through a sieve plate with oblong holes sized 1.6x20mm. These kernels are cream-coloured with a greenish hue and have the following characteristics: humidity 12.0%, proportion of sound kernels 99.4%. Buckwheat kernels, in a layer as thick as 20cm, were placed into plastic containers with holes along the entire perimeter. The containers were placed in a tank with water (hydromodulus 1:3), where the grain was steeped at (18±2)°C for 18–24 hours without special lighting. The water in the tank was changed for fresh every 5–6 hours. For better aeration, the grains were stirred, with the air-rest 120 minutes. Then the buckwheat kernels were germinated for up to 24 hours at 18±2°C, with periodic aeration (every 2–3 hours for 5 minutes) to ensure oxygen access and prevent caking. When necessary, buckwheat grains were watered to maintain the humidity 39–40%. During the whole bioactivation cycle, the degree of physiological development of buckwheat grains was visually evaluated. At the end of bioactivation, the humidity of the grain was (39.2±1.0)%, the size of the sprouts was (2.0±0.5)mm, and the endosperm of the grains became loose.

The research was conducted at the Department of Commodity Research and Product Examination of Belarus State Economic University and at the Scientific and Practical Centre for Foodstuffs of the National Academy of Sciences of Belarus. The substances under study, methods, and equipment used in the research are presented in Table 1.

Protein quality of bioactivated buckwheat grain was evaluated by comparing its amino acid composition with that of “ideal” protein by calculating the amino acid score (protein quality) (P_i, %) by the formula

$$P_i = \frac{A_i}{A_i^0} \times 100 \ , \qquad (1)$$

where A_i – the content of the i-th essential amino acid in the protein of the sample analysed, mg/100 g of protein; A_i^0 – content of the i-th essential amino acid in the ideal protein (reference), mg/100 g of protein.

The excess of essential amino acids not used for plastic needs was determined by the coefficient of difference of the amino acid scores (K_p, %) according to the formula

$$K_p = \frac{\sum \Delta P}{n} , \qquad (2)$$

where ΔP – difference of the amino acid score for each essential amino acid as compared to one of the most deficient amino acids;

n – number of essential amino acids.

The coefficient Kp shows the average excess of the amino acid score of essential amino acids in comparison with the lowest score of any essential amino acid, because the excess amount of essential amino acids is not used for plastic purposes.

The value of Kp allowed estimating the potential biological value (comparable redundancy coefficient) (B, %) of the product according to the formula

$$B = 100 - K_P \, . \qquad (3)$$

If a given protein contains all essential amino acids in the necessary proportions, the potential biological value of this protein is 100.

The results were statistically processed using Microsoft Excel.

Table 1 – Research methods and equipment for determining the nutrient content in bioactivated buckwheat grain

Nutrients	Research method and equipment
Protein	The nitrogen content was determined according to ISO 5983-2:2009 on an automatic Kjeldahl decomposition unit Turbotherm with a distiller Vapodest. The protein content was calculated by multiplying the nitrogen content value by the factor k=5.53 [23]
Amino acid composition	High-performance liquid chromatography (HPLC) according to the standard MVI.MN 1363-2000 "Method for determination of amino acids in food products by high-performance liquid chromatography" using a liquid chromatograph Agilent 1200
Lipids	The fat content was determined on a fat analyser Soxterm by the Soxhlet method, which consists in extracting fat from a product with a solvent (diethyl ether), followed by removing the solvent, drying, and weighing the fat extracted.
Starch	The starch content was determined according to GOST 10845 by the polarimetric method, which consists in dissolving the starch contained in the product in a hot diluted hydrochloric acid solution, followed by precipitation and filtration of dissolved protein substances and measuring the angle of optical rotation of the starch solution.
Total sugar	The total sugars (per inverted sugar) were determined by permanganatometry according to GOST 8756.13. The method is based on the ability of carbonyl groups of sugars to reduce copper oxide (II) in an alkaline environment to copper oxide (I). When dissolved with ferric ammonium sulphate, the copper oxide (I) formed oxidises to copper oxide (II) and reduces iron (III) to iron (II), the amount of which is determined by titration with a solution of potassium permanganate
Fibre	The raw fibre content was determined according to ISO 6865:2000 with a fibre analyser Fibretherm FT 12
Minerals	The method of atomic-emission spectroscopy with inductively coupled plasma according to MUK 4.1.1482-2003 "Determination of the content of chemical elements in diagnosed biosubstrates, multivitamin preparations with microelements, bioactive food supplements, and raw materials for their production by atomic emission spectroscopy with inductively coupled argon plasma" using an atomic emission spectrometer Optima 2100 DV
vitamin B1 (thiamine)	High-performance liquid chromatography (HPLC) according to GOST EN14122-2013 using a liquid chromatograph Agilent 1200
vitamin B2 (riboflavin)	High-performance liquid chromatography (HPLC) according to GOST EN14152-2013 using a liquid chromatograph Agilent 1200
vitamin B5 (pantothenic acid)	Gas chromatography according to MVI.MN 3008-2008 "Methodology for determination of the mass fraction of pantothenic acid in specialised food products and dietary supplements" using a gas chromatograph Trace 1310 GC
vitamin B6 (pyridoxine)	High-performance liquid chromatography (HPLC) according to GOST EN14164-2013 using a liquid chromatograph Agilent 1200
vitamin C (ascorbic acid)	High-performance liquid chromatography (HPLC) according to GOST 34151. The method is based on extracting vitamin C from a sample with a solution of metaphosphoric acid, with further reduction of *L*(+)-dehydroascorbic acid to *L*(+)-ascorbic acid and determination of total *L*(+)-ascorbic acid using high-performance liquid chromatography (HPLC) with spectrophotometric detection at 265nm.
vitamin E (tocopherols)	High-performance liquid chromatography (HPLC) according to GOST EN12822-2014 using a liquid chromatograph ACELLA LC with a diode-array detector and mass spectrometers

Results of the research and their discussion

Sprouting, in particular, bioactivation, is widely used to increase the nutritional value of cereal grains. This complex biological process involving many biochemical reactions leads to changes in the nutritional value and sensory properties of grain. Bioactivated buckwheat grain under study had a pleasant sweetish taste and a delicate nutty aftertaste. Table 2 shows the content of macronutrients in bioactivated buckwheat grains. The content of proteins, lipids, and carbohydrates in the reference sample and in the bioactivated grains with a sprout up to 2.5mm long is lower as compared with the amounts described in [5]. This is obviously determined by the botanical variety of buckwheat and the germination conditions. 100g of bioactivated buckwheat grains can satisfy, on average, the daily requirement in protein by 8.7%, in lipids by 0.5%, in carbohydrates by 10% due to the starch content and by 1.7% due to the sugar content. The energy source in bioactivated buckwheat grain is carbohydrates, most significantly starch, which, along with simple sugars, is well absorbed by the human body (Table 2). Because of bioactivation, the complex polysaccharide of starch is decomposed and the content of sugars increases by 12 times compared with the reference sample. As a result, the taste of bioactivated grain becomes sweetish. The increased content of sugars is also confirmed in previous publications [5,13].

Lipids are essential in nutrition as an energy-related and structural material (they enter the composition of cell walls). They are also involved in the metabolism of other nutrients, for example in absorption of vitamins A and D. During bioactivation, lipids and carbohydrates are decomposed to support germ growth, which reduces their content: by 2.3 times the lipid content and by 1.2 times the starch content. That is why the lipid content in bioactivated buckwheat grain is low (Table 2). The research [7] also finds that as the sprouting time increases, the content of sugars increases, too, while the level of lipids decreases.

Dietary fibre is an important component of bioactivated grains. In Fan Zhu's research described in the article [27], it is found that dietary fibre of buckwheat contains bioactive substances, but its content decreases significantly when the grain is hulled. The most important dietary fibre of bioactivated buckwheat grain is cellular tissue often referred to as fibre. Its content increases by 68% as compared with the reference sample, amounts to 6.5% of the daily requirement (Table 2), and can have a positive effect on human health [27, 28]. Dietary fibre is almost indigestible and has no calorific value. Passing through the human gastrointestinal tract, fibre improves peristalsis and normalises intestinal microflora.

A specific feature of buckwheat proteins is their high biological value as compared with other cereal grains [18]. Nutritionally, proteins are important primarily because of their contribution to biological and calorific value. The bioactivated buckwheat grain variety under study is close to sprouted grains of other crops by its protein content [6].

During bioactivation of buckwheat grain, the protein substances contained in it are hydrolysed by proteolytic enzymes to amino acids and peptides, which are used for the construction of new sprout cells and the proper metabolism [5]. In the research [5], it was concluded that when buckwheat was sprouted for 72 hours, its protein content increased by 7%. However, in our research, bioactivation of buckwheat grain led to almost no change in the protein content. This is probably because the process of protein hydrolysis (proteolysis) is in its very beginning. The research has found that bioactivated buckwheat grain contains all the essential amino acids necessary for a person's development, which makes up 30.5% of the total amount of amino acids (Table 3). Among the nonessential amino acids, there is a high content of glutamic acid, what is also described in [26].

It has also been found that bioactivated buckwheat grains are high in phenylalanine and tyrosine (30%), leucine (20%), lysine (12%), and valine (12%) in relation to the total content of essential amino acids, which is consistent with the other scientists' research [28]. The amino acid score has been calculated, and it has been established that the limiting amino acid was threonine (31.7%), and there was an excess of tryptophan (162.3%) and phenylalanine and tyrosine taken together (150.6%).

The quality of protein is determined by its biological value and digestibility. Biological value depends on the content and proportion of essential amino acids in proteins and reflects how well the amino acid composition of protein meets the needs of the human body. The proteins of bioactivated buckwheat grain are incomplete in their amino acid composition and do not contain enough threonine.

There is another fact for qualitative assessment of protein: the lower the coefficient of differences of the amino acid score *Kd* is (ideally, it should tend to zero), the better is the balance of essential amino acids and the more rationally the human body can use them. For bioactivated buckwheat, the coefficient of differences of the amino acid score (*Kd*=54.75%) and the potential biological value of protein (45.25%) have been calculated. Protein of bioactivated buckwheat grain has been found to be of average biological activity, since the potential biological value differs from 100. In general, the balance of essential amino acids in bioactivated buckwheat grain was at an average level, which is also consistent with the research [26].

Table 2 – Protein, fat, and carbohydrate content in buckwheat grain (P=0.05)

Parameters	Buckwheat grain before bioactivation (reference) on a dry basis	Bioactivated buckwheat grain at actual moisture content/on a dry basis	Daily requirement for group I [24, 25] (18–59 years old, women/men)
Protein, g (*n*=10)	9.40±0.06	5.67±0.02/ 9.32±0.05	58/72
Lipids, g (*n*=4)	1.21±0.1	0.32±0.01/ 0.53±0.1	60/81
Carbohydrates, g starch (*n*=4)	60.70±0.1	31.84±0.6/ 52.37±0.6	257/358
total sugars (*n*=4)	0.69±0.4	5.10±0.3 8.39±0.3	
Dietary fibre, g (*n*=11)	1.25±0.2	1.30±0.2 2.10±0.1	20

Table 3 – Amino acid composition of bioactivated buckwheat grain (g per 100 g, *n*=3, *P*<0.05)

Parameters	Bioactivated	Amino acid sample	Amino acid

	buckwheat grain	(FAO/WHO)	score, %
Essential amino acids	1.6867	36	
including:			
valine	0.1983	5.0	70.0
isoleucine	0.1442	4.0	64.0
leucine	0.3416	7.0	86.0
lysine	0.2018	5.5	64.7
methionine + cystine	0.1244	3.5	62.3
threonine	0.0720	4.0	31.7
tryptophan	0.0920	1.0	162.3
phenylalanine + tyrosine	0.5124	6.0	150.6
Nonessential amino acids	3.8369		
including:			
alanine	0.5084		
arginine	0.4665		
asparagic acid	0.4642		
histidine	0.0223		
glycine	0.3608		
glutamic acid	1.0972		
proline	0.6231		
serine	0.2944		
Total amino acids	5.5236		

To ensure human health, the content of minerals in the diet must be maintained at a level that meets a person’s physiological needs. The need for essential minerals varies from a few micrograms to almost one gram a day [24]. However, the chemical composition of buckwheat grain, in particular, its content of minerals, is influenced by many different factors: genetic factors, growing conditions, agronomic features, and others. Minerals have no energy value, but without them, people’s life is impossible, because they participate in water-salt and acid-base metabolism, which are important processes in the body. Unlike vitamins and amino acids, minerals do not decompose when acted upon by high temperatures, oxidants, acids, alkalis, and other factors [30]. The most important factor affecting the mineral content is steeping and sprouting grain. During sprouting, grain absorbs mineral elements contained in the liquid and, consequently, their amount changes [31]. Also, during germination, minerals become absorbable by the body [5,19,31]. The content of minerals in bioactivated buckwheat grain compared with the reference sample is shown in Fig. 1 and 2.

The mineral content in bioactivated buckwheat grain is not high enough to satisfy fully human requirements. Of the recommended daily allowance of mineral substances [24], 100g of bioactivated buckwheat grain provide 6.7% of potassium, 15.4% of magnesium, 4.2% of calcium, 14.2% of zinc, 24% of copper, 18.9% of iron for women and 34% for men, 48.5% of manganese. It should be noted, though, thatbioactivated buckwheat grain is higher in potassium (277mg per 100g), calcium (69.7mg per 100g), manganese (1595.4mcg per 100g), copper (394mcg per 100g), and zinc (2796mcg per 100g) as compared with the reference sample.

The content of vitamins in bioactivated buckwheat grain has been investigated in different batches, and Table 4 shows its minimum and maximum values. Vitamins, like minerals, are of particular importance in human nutrition, since they participate in many important enzymatic reactions. An increase in water-soluble vitamins (B_1, B_2, B_5, B_6, C) and a decrease in vitamin E were recorded during bioactivation, which is obviously due to biochemical processes in the course of sprouting. The content of vitamin B_1 (thiamine) in bioactivated buckwheat grain provides 23.0% of the daily requirement, which is consistent with the information in [29]. The content of other B vitamins ranges from 4.6% to 8.0% of the daily requirement. Vegetable products are commonly considered to be the main source of vitamin C. However, in bioactivated buckwheat grain, the vitamin C content is 1.45–3.32mg per 100g. Obviously, bioactivated buckwheat grain cannot be a source of vitamin C. Vitamin E is found in bioactivated buckwheat grain in several isomeric forms: α-, β-, γ-, δ-tocopherols. Most vitamin E is contained as a sum of β- and γ -tocopherol, which is consistent with the research [13].

Conclusion

The most important source of macronutrients that bioactivated buckwheat grain provides the human body with are carbohydrates (31.84±0.6g per 100g) and proteins (5.67±0.02g per 100g). Also, bioactivated buckwheat grain contains all essential amino acids. Among nonessential amino acids, there are large amounts of glutamic acid (1.0972g per 100g). Proteins of bioactivated buckwheat grain are incomplete by their amino acid composition and contain not enough threonine.

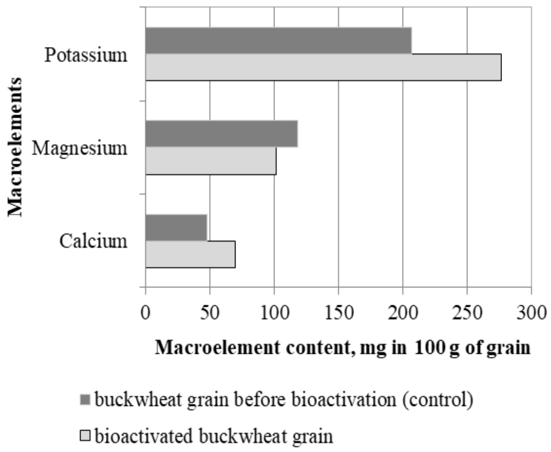

Fig. 1. Content of macroelements

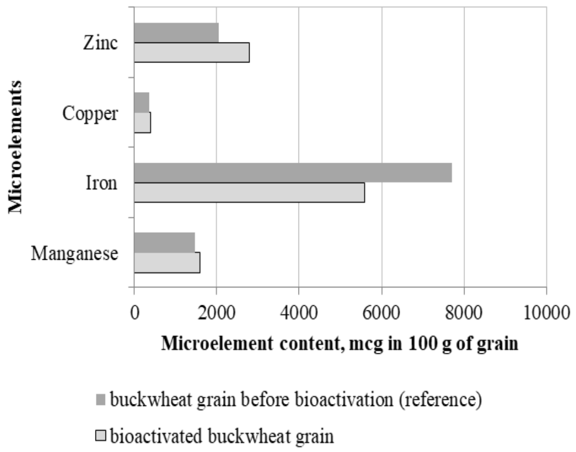

Fig. 2. Content of microelements

Table 4 – Vitamin content in buckwheat grain (in 100 g of grain, n=2, P=0.05)

Parameters	Buckwheat grain before bioactivation (reference) on a dry basis	Bioactivated buckwheat grain at actual moisture content (min–max) / on a dry basis	Daily requirement for group I (18–59 years old) [24]	% of daily requirement
Vitamin B_1 (thiamine), mg	0.36	0.31–0.38 / 0.57	1.5	23.0
Vitamin B_2 (riboflavin), mg	0.085	0.077–0.088 / 0.13	1.8	4.6
Vitamin B_5 (pantothenic acid), mg	0.58	0.40 / 0.66	5.0	8.0
Vitamin B_6 (pyridoxine), mg	0.101	0.089–0.090 / 0.148	2.0	4.5
Vitamin C, mg	0.95	1.45–3.32 / 2.71	90.0	2.7
Vitamin E, mg Including:	4.45	1.452–2.356 / 3.13	15.0	12.7
α-tocopherol	0.15	0.060–0.105 / 0.13		
β-tocopherol and γ-tocopherol	4.10	1.316–2.145 / 2.85		
δ-tocopherol	0.20	0.076–0.106 / 0.15		

The difference coefficient of the amino acid score is 54.75%, and the potential biological value of protein is 45.25%. 100g of bioactivated buckwheat grain provide 48.5% of the daily requirement of manganese, 18.9% and 34.0% of iron (for women and men respectively), 24% of copper, 15.4% of magnesium, about 23.0% of vitamin B_1, and 12.7% of vitamin E. Bioactivated buckwheat grain also contains fibre (an average of 6.5% of the daily requirement). Consequently, bioactivated buckwheat grain, due to the above-listed substances, has nutritional and health benefits and can be used to create products with certain medicinal effects. These experimental results on bioactivated buckwheat grain can complete the databases of chemical composition of food products. The information can also be used for non-commercial communication (in particular, when bioactivated buckwheat grain is consumed as food) an in the development of food products with bioactivated grain added. These data, though, cannot be used for other purposes, such as product labelling.

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