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## ASSESSMENT OF THE CONTENT OF FLAVONOIDS IN GREEN BUCKWHEAT FLOUR

The object of research is green buckwheat flour (GBF) from various brands, including Ecorod, Ecoorganic, Ms. Tally, Ecosmak, and Ahimsa, which is considered a promising raw material for functional food production. One of the main challenges in studying GBF is the insufficient amount of data on the quantitative content of individual flavonoid compounds, particularly quercetin and rutin, as well as the lack of systematic comparison of these indicators between different manufacturers. This limits the possibilities for the targeted use of GBF as a source of biologically active substances for the development of products with enhanced antioxidant activity.

The study applied absorption spectrophotometry based on the formation of chelate complexes with aluminum chloride ( $AlCl_3$ ), which allowed the determination of total flavonoid content and subsequent calculation of quercetin and rutin equivalents. This approach enables a quantitative assessment of the bioactive potential of the raw materials under study and reveals variability depending on the brand.

The obtained results indicate a significant concentration of flavonoids in GBF: quercetin content ranged from 0.8 to 2.6 mg/100 g dry matter (DM), and rutin content ranged from 1.62 to 5.25 mg/100 g DM. This can be explained by the fact that green buckwheat, unlike heat-treated grain, preserves natural enzyme activity and its phytochemical complex, with flavonoids playing a leading role.

The applied approach provides a more reasonable evaluation of the functional value of green buckwheat grain and flour, which is of direct practical importance for the food industry. The results confirm the potential of GBF as a gluten-free ingredient for functional food products aimed at reducing oxidative stress and preventing chronic non-communicable diseases.

**Keywords:** green buckwheat, green buckwheat flour, flavonoids, rutin, quercetin, functional nutrition.

Received: 18.07.2025

Received in revised form: 14.09.2025

Accepted: 06.10.2025

Published: 30.10.2025

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### How to cite

Sereda, O., Demydova, Y., Koshel, O., Ponomarenko, V., Voronin, O., Kuznietsova, I., Zaichuk, L. (2025). Assessment of the content of flavonoids in green buckwheat flour. *Technology Audit and Production Reserves*, 5 (3 (85)), 29–34. <https://doi.org/10.15587/2706-5448.2025.341092>

### 1. Introduction

The intensification of anthropogenic impact on the environment, including chemical, physical and biological pollution, as well as the increase in the level of chronic psycho-emotional stress among the population, lead to a systemic load on the human body, stimulating the compensatory activity of various physiological systems. One of the effective ways to optimize health is the rationalization of nutrition with the inclusion of special or functional products in the diet. The key factor in the effectiveness of such products is the qualitative and quantitative composition of functional ingredients, which directly affect the regulation of physiological functions and biochemical reactions of the body.

According to modern scientific ideas, more than 20,000 different food compounds of natural (plant, animal or microbial) origin are necessary for the full functioning of the human body.

Within the framework of the modern concept of functional and personalized nutrition, products with high biological value occupy an important place. It is based on the principles of preventive medicine and the application of nutraceutical approaches. The focus is on bioactive compounds of natural origin, in particular flavonoids. They are considered as potential modulators of metabolic homeostasis and risk factors for chronic non-communicable diseases.

Despite their relatively low content in food products, flavonoids are of great importance for human health. They play a key role in the

prevention of the so-called "diseases of civilization". Among them are metabolic syndrome, type 2 diabetes, atherosclerosis, neurodegenerative conditions (including Alzheimer's disease), hypertension, obesity and some forms of oncopathologies. Such effects are explained by the antioxidant, anti-inflammatory, capillary-stabilizing and antimutagenic activity of these compounds.

Flavonoids and other phenolic compounds belong to secondary metabolites of plants and are characterized by the presence of an aromatic ring with at least one hydroxyl group [1]. Flavonoids are divided into different subgroups depending on the carbon atom of the C ring connected to the B ring and the degree of unsaturation and oxidation of the C ring. Flavonoids and other related phenolic substances are natural chemicals in plants [2]. Their antioxidant activity has been extensively studied and is believed to be related to their number and/or chemical structure, such as the arrangement of hydroxyl groups [3]. Due to the high reactivity of phenolic hydroxyl groups and carbonyl functionality, flavonoids are actively involved in the regulation of numerous metabolic processes in the human body [4]. They exhibit P-vitamin activity and also have effects on anti-inflammatory, antioxidant, antihyperglycemic, anticancer, hepatoprotective, choleric, diuretic, antidiabetic, hypotensive and anticarcinogenic reactions in the body [5].

Studies by American scientists on the assessment of daily flavonoid intake among adults (over 19 years of age) showed that the average level of their intake is approximately 227–230 mg per day. The largest share is

made up of flavan-3-ols (about 80%), flavonols – 8%, flavanones – 6%, anthocyanidins – 5%, and flavones and isoflavones – 1% each [6]. It is worth noting that individual levels of flavonoid intake can vary significantly depending on the characteristics of the diet. In the digestive tract, flavonoids undergo metabolic transformations with the formation of conjugated metabolites or simplified phenolic compounds, which are characterized by increased bioavailability and better absorption. Flavonoids are found in many products of plant origin, as well as in some fungi and even microorganisms. Flavonoids accumulate mainly in flowers, leaves, and less often in stems, roots, rhizomes, and fruits. Their content depends on the phase of vegetation, sunlight intensity, altitude, and other factors [4].

Recently, buckwheat has been highlighted among alternative plant-based products containing flavonoids. It is known that buckwheat is a pseudocereal cereal, which is distinguished by its nutritional profile and adaptability, attracting attention not only due to its grains, but also due to its processing products. It is believed that pseudocereal grains can be processed, like cereals, into flour and then into secondary bakery products, but, unlike cereals, they do not belong to the grass class [7]. Buckwheat is considered a crop rich in compounds with antioxidant, antimicrobial and anti-inflammatory properties, such as phenolic acids and flavonoids (mainly rutin) [8]. In addition, in its composition, it contains a significant amount of dietary fiber, important minerals and vitamins, functioning as an indispensable ingredient in various culinary contexts. It is a vital link in solving the problem of nutrient deficiencies and increasing food security [9]. The presence of slowly digestible proteins and starches in buckwheat emphasizes the nutritional value of its processed products [10].

Green buckwheat is a buckwheat kernel obtained by peeling without the use of heat treatment, which allows preserving natural enzymes, in particular polyphenol oxidase and glucosidase, and also ensures the ability of the grain to germinate. This opens up opportunities for increasing the flavonoid content by germination, fermentation or soft drying. The product is characterized by a high protein content (approximately 15%), which includes all eight essential amino acids, which determines its high biological value among plant protein sources. The antioxidant activity of green buckwheat is up to 155 mg/100 g, which determines its significant potential in the prevention of oxidative stress [11].

The grain is also a source of vitamins, in particular thiamine, riboflavin, nicotinic acid and tocopherol, the content of which significantly exceeds similar indicators for traditional cereals. The mineral profile includes significant concentrations of iron (6–10 mg/100 g), zinc (2–3 mg/100 g) and selenium (2–5 µg/100 g), which increases its value in the context of micronutrient support of the body [9].

Green buckwheat flour retains a rich content of tannins and organic acids (oxalic, citric, malic, gallic, caffeic, chlorogenic, pyrocatechin and maleic), which contributes to its increased bioactivity. Its energy value is 280–320 kcal/100 g, which provides prolonged satiety without overloading the digestive system. The flour contains a complex of B vitamins (B1, B2, B6, B9), as well as vitamins E and PP, combined with phosphorus, which support neurogenesis and the functioning of nervous tissue. Among macroelements, potassium, magnesium, and phosphorus are of leading importance, and among microelements, iron, copper, zinc, chromium, molybdenum, and manganese, which comprehensively enhances the physiological value of the product [10].

Quercetin is a member of the flavonol class and is one of the major polyphenolic compounds of plant origin. It is widely distributed in nature and is found in numerous foods, including green buckwheat, green tea, cranberries, apples, onions, asparagus, radish leaves, blueberries, broccoli, and coriander. In the natural environment, quercetin exists in various forms, the most common of which are its glycoside and ester derivatives: quercetin-3-O-glycoside, quercetin-3-sulfate, quercetin-3-glucuronide, and quercetin-3'-methyl ester.

The pharmacological properties of quercetin cover a wide range of actions: it exhibits pronounced antioxidant, anti-inflammatory, cardioprotective, antiviral, and antibacterial activity. Experimental and clinical studies have shown its potential benefits in the treatment and prevention of cardiovascular diseases, cancer, diabetes, neurodegenerative disorders, allergies, bronchial asthma, peptic ulcer, osteoporosis, arthritis, and eye diseases [12].

Rutin is a natural flavonoid glycoside that is a widespread secondary metabolite of plants. In the scientific and technical literature, it is also referred to as rutoside, quercetin-3-O-rutoside, sophorine, or vitamin P. From a chemical point of view, rutin is called: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  6)- $\beta$ -D-glucopyranosyloxy]-4H-chromen-4-one. It is a yellowish crystalline powder with a molar mass of 610.521 g/mol. The compound is poorly soluble in water, but is soluble in pyridine. The ionization constant (pKa) is 6.17 and the melting point is about 125°C [13].

Rutin is one of the most promising natural compounds, demonstrating a wide range of pharmacological activities due to various molecular mechanisms of action. It shows therapeutic potential in the treatment of a number of pathological conditions, including varicose veins, hemorrhoidal diseases and internal bleeding [14]. In addition, numerous studies confirm its antioxidant, antihypertensive, antidiabetic, anti-inflammatory and cardioprotective properties [15]. Since rutin, together with other phenolic compounds, is an active component of many natural foods and beverages (including apples, grapes, citrus fruits, black tea), it is considered an important nutraceutical molecule with potential health benefits [16].

The biological activity of rutin is largely due to the presence of four hydroxyl groups and a residue of the disaccharide rutinose attached at the C-3 position. It is believed that the rutinose fragment increases the functional activity of the molecule, probably due to an increase in the number of reactive centers [17]. At the same time, the low solubility of rutin in an aqueous environment limits its bioavailability and practical use, which has led to interest in structural modification of the molecule. One approach is to introduce additional functional groups, in particular carboxyl, to the sugar component in order to increase water solubility [18].

Buckwheat grains are characterized by a high content of biologically active flavonoids, among which the most significant are quercetin and rutin. Such a phytochemical composition allows to consider buckwheat as a functional food product capable of exerting a preventive or even therapeutic effect on the body. Buckwheat flour contains significant amounts of catechins, anthocyanins, isoquercetin, and myricetin, compounds that have antioxidant, antihypertensive, anti-inflammatory, and anticancer properties, and also contribute to maintaining glucose homeostasis, reducing the manifestations of diabetes, and regulating lipid metabolism, preventing the development of atherosclerosis [19].

Flavonoids, in particular quercetin and its glycoside form, rutin, are among the main polyphenolic components of buckwheat (*Fagopyrum esculentum Moench*). Their content depends on the variety, growing conditions, fertilizer use, climatic factors, and the method of processing the raw material.

Despite significant scientific interest in the biological activity of flavonoids, studies on determining their quantitative composition in green buckwheat flour and assessing the impact of this raw material on the functional properties of food products remain insufficiently systematized. There is a lack of experimental data that would confirm the feasibility of using green buckwheat flour as an effective source of flavonoid compounds for the formation of functional products.

*The aim of the research* was to determine the quercetin content in green buckwheat flour of different brands, convert the obtained data into rutin content, and assess the potential of this raw material as a source of biologically active substances for the production of functional food products.

## 2. Materials and Methods

### 2.1. Research materials

The object of research is green buckwheat flour (GBF) of various trademarks "Ecorod", TM "Ecoorganic", TM "Ms. Tally", TM "Ecosmak", TM "Ahimsa", which is considered a promising raw material for the production of functional food products.

The study used five samples of GBF flour provided by different manufacturers represented on the Ukrainian market:

- sample 1 – TM "Ecorod" (Kyiv, Ukraine);
- sample 2 – TM "Ecoorganic" (Kyiv, Ukraine);
- sample 3 – TM "Ms. Tally" (Poltava region, Ukraine);
- sample 4 – TM "Ecosmak" (Berezan, Ukraine);
- sample 5 – TM "Ahimsa" (Dnipro, Ukraine).

Flour of all brands is made by grinding green buckwheat grains that have not been subjected to heat treatment (not steamed or roasted), which corresponds to the classification as "green buckwheat". Manufacturers note that the products do not contain gluten, artificial colors, flavors, preservatives, monosodium glutamate, dairy ingredients and other allergens. This makes the flour suitable for use in functional and gluten-free food formulations. Samples were stored in airtight containers in a dark, dry room at a temperature of  $(20 \pm 2)^\circ\text{C}$  until analytical studies were conducted.

### 2.2. Research methods

The determination of total flavonoid content, in particular rutin and quercetin, in green buckwheat flour samples was carried out using absorption spectrophotometry based on the formation of a chelate complex of flavonoids with aluminum ions. This method is widely used due to its sensitivity, simplicity and the ability to assess the total flavonoid profile in the form of rutin or quercetin equivalents.

*Sample preparation and extraction of flavonoids:* For the extraction of flavonoid compounds, 70% ethanol was used as an extractant. An accurate weight of the ground flour sample ( $1.00 \pm 0.01$  g) was transferred to a 100 ml flat-bottomed round-bottomed flask, to which 50 ml of 70% ethanol (volume fraction) was added. The mixture was boiled in a water bath under reflux for 60 minutes with periodic stirring to improve mass transfer. After the first extraction, the supernatant was decanted or filtered through a paper filter. The extraction procedure was repeated three times, each time with a new portion of 70% ethanol, to ensure complete extraction of flavonoids. The combined extracts were collected in a 100 ml volumetric flask and the volume was adjusted to the mark with 70% ethanol. The resulting combined extract was cooled to room temperature and stored in a dark place until analysis (no more than 24 hours).

*Preparation of test and compensation solutions:* For quantitative analysis, an aliquot of the extract (5.00 ml) was taken and transferred to a 25 ml volumetric flask. 3.00 ml of 3% aqueous aluminum chloride solution ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ) was added to the flask, after which the volume was adjusted to the mark with 70% ethanol. The mixture was thoroughly mixed and kept at room temperature in the dark for 30 minutes to complete the formation of the chelate complex.

The compensation (control) solution was prepared similarly, but without adding aluminum chloride solution – only 5.00 ml of the original extract was added to the flask and brought to the mark with 70% ethanol. This solution served as a background control sample during measurements.

*Spectrophotometric measurements:* The absorption spectra of the solutions were measured on a Cary 50 UV-Vis spectrophotometer (Agilent Technologies, USA), using quartz cuvettes with an optical path length of 10 mm. The optical density of the test solution was measured against the compensation one at a wavelength of 415 nm.

*Construction of a calibration curve and calculation of the results:* Calibration curves were constructed using standard solutions of quer-

etin in 70% ethanol in the concentration range from 1 to 25  $\mu\text{g}/\text{ml}$ . For each point, the absorbance was measured under the above conditions and a graph of "optical density – concentration" was constructed. The linear calibration equation had the form

$$A = a \cdot C + b, \quad (1)$$

where  $A$  – the measured optical density;  $C$  – the flavonoid concentration ( $\mu\text{g}/\text{ml}$ );  $a$  – the slope (derivative of the curve);  $b$  – the free term (usually close to zero).

The results were given in mg per 100 g of absolutely dry matter of the sample.

To conduct a comparative analysis of the flavonoid content in green buckwheat flour (GBF) samples, the amount of quercetin ( $\text{C}_{15}\text{H}_{10}\text{O}_7$ ) was converted into its glycoside form – rutin ( $\text{C}_{27}\text{H}_{30}\text{O}_{16}$ ), which is the main flavonol in buckwheat products [20]. Rutin is a rutinose of quercetin, i. e. a compound of the aglycone of quercetin with the disaccharide rutinose [21].

The conversion was carried out taking into account the molecular masses of the substances: the molecular mass of quercetin is 302.24 g/mol, and rutin is 610.52 g/mol. Thus, to reduce the amount of quercetin to the rutin equivalent, the formula

$$\begin{aligned} \text{Rutin content} &= \text{Quercetin content} \cdot \frac{610.52}{302.24} = \\ &= \text{Quercetin content} \cdot 2.02. \end{aligned} \quad (2)$$

It should be noted that this conversion is conditional, since the samples contain both free quercetin and its glycoside forms, and their ratio is determined by the varietal characteristics and grain processing conditions. The use of the rutin equivalent allows to unify the results and simplify their comparison with the literature data.

## 3. Results and Discussion

As a result of studying the content of flavonoid compounds in green buckwheat flour (GBF) samples obtained from different manufacturers, significant variability in the concentrations of quercetin and rutin was established. In particular, the content of quercetin in five samples ranged from 0.8 to 2.6 mg/100 g of dry matter (DM), and the content of rutin – from 1.62 to 5.25 mg/100 g DM (Fig. 1).

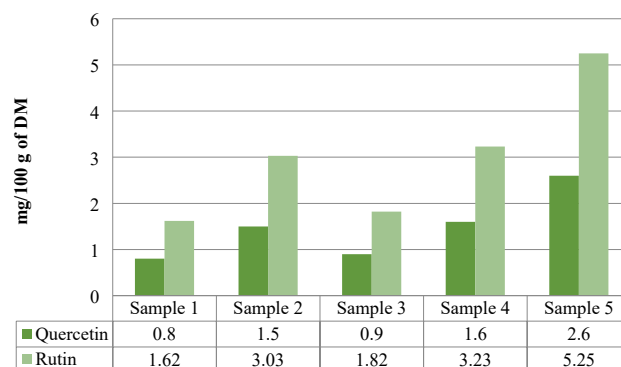


Fig. 1. Flavonoid content in GBF samples

Among the studied samples, sample 5 had the highest content of flavonoid compounds (7.85 mg/100 g of DM), exceeding sample 4 by 62.5% and control sample 1 by more than three times, which indicates the potentially high biological value of this raw material for further use in functional products.

The lowest flavonoid content was recorded in samples 1 and 3, where the concentration did not exceed 2.42 mg/100 g of DM and

4.83 mg/100 g of DM, which may indicate the influence of certain technological or raw material factors that caused partial degradation of flavonoid compounds.

As indicated in the documentation for the samples, flour is made from unprocessed grain. However, even slight heating during drying or cleaning of the grain can lead to partial degradation of polyphenolic compounds. It is possible that in samples with lower flavonoid content, the raw material was processed at temperatures that did not fully preserve the phenolic profile.

The main amount of quercetin and rutin is localized in the outer layers of the grain (pericarp and seed coat). Samples containing a higher percentage of whole grain fraction, as a rule, have a higher level of bioactive compounds. On the contrary, excessive cleaning of the grain before milling can significantly reduce the total flavonoid content. Long-term storage, contact with oxygen, exposure to light or moisture can also cause oxidation of flavonoids and a decrease in their concentration.

The obtained results of the quantitative determination of quercetin in green buckwheat flour samples are generally consistent with the literature data and confirm the bioactive potential of this raw material for functional nutrition. The established concentrations of flavonoid compounds indicate that even a small addition of such flour to the diet can provide a significant replenishment of the antioxidant effect in the body.

In the literature, it is reported that the average rutin content in green buckwheat ranges from 2–5 mg/100 g of DM, which is fully consistent with the results of our study. For example, [22] recorded a rutin level of about 3.8 mg/100 g of DM in sprouted buckwheat flour, which corresponds to the values obtained by us (sample 2, 4, 5). Similarly, [23] reported quercetin concentrations in the range of 0.6–2.1 mg/100 g of DM, which also correlates with our data (above 0.8 to 2.6 mg/100 g).

Other studies highlight the pharmacological activity of flavonoids: flavonoids significantly reduce the level of oxidative stress *in vivo* and contribute to improving glycemic control. In particular, [24] found that adding green buckwheat extracts to the diet of animals with metabolic syndrome reduced glucose, triglycerides, and inflammatory markers. Studies [25] showed that rutin inhibits neuronal apoptosis in Alzheimer's disease model systems, confirming its neuroprotective properties.

According to studies [22], the quercetin content in green buckwheat ranged from 1.2 to 2.8 mg/100 g of DM. Another study [26] reported quercetin concentrations in the range of 0.5–2.3 mg/100 g of DM in different buckwheat genotypes.

Sprouted green buckwheat is particularly promising. A study [27] noted an increase in the content of quercetin and rutin due to the activation of enzymatic systems during germination. This allows to consider sprouted grains or fermented buckwheat products as raw materials for functional products with a targeted bioactive profile.

Also, in [28] it was demonstrated that buckwheat processing products have the ability to inhibit lipid peroxidation and exhibit high antioxidant activity compared to other pseudocereals. In contrast, [29] showed that heat treatment of buckwheat at temperatures above 80°C significantly reduces the level of flavonoids, especially quercetin, which emphasizes the importance of optimizing technological regimes to preserve the bioactive composition.

Thus, the variability of the flavonoid composition of the samples, revealed in our study, can be explained both by the varietal characteristics of the raw material and differences in the technology of its processing. This confirms that green buckwheat is a promising source of quercetin and rutin, and the bioactive composition largely depends on the technological process and origin of the raw material. The highest content of flavonoids was found in sample 5 (Ahimsa). This may indicate differences in the grinding technology. Presumably, the outer layers of the grain are better preserved. This result emphasizes the feasibility of using this sample in further studies. It is worth involving a wider range of producers, genotypes and growing conditions.

The obtained results on the content of quercetin and rutin in green buckwheat flour are of important practical importance, since these flavonoid compounds have powerful antioxidant activity and positively affect human health. Their presence in food raw materials allows to attribute products made from such raw materials to healthy food products, in particular bakery and pasta products, gluten-free products, food supplements and nutraceuticals. Although an official recommended daily allowance (RDA) for quercetin has not been established, clinical studies usually use from 10 to 1000 mg/day [30]. However, most dietary recommendations mention an effective daily dose of 50–500 mg, especially in metabolic disorders or as antioxidant support [31]. Rutin also does not have a clearly established RDA, but the effective daily dose in nutraceutical studies is 30–100 mg [17]. As a flavonoid, rutin exhibits angioprotective, anti-inflammatory and antioxidant activity, and can be recommended in doses of 30–100 mg/day for preventive action.

Sample 5, when consumed in 100 g of flour, provides up to 15.7% of the daily requirement for rutin. This corresponds to approximately 50 mg per day. The content of quercetin covers 3–5% of the daily requirement. In total, this amounts to up to 7–10% of the daily requirement for flavonoids. Regular consumption of such flour may be beneficial. Additional enrichment of products with concentrated extracts will increase its value.

The results obtained indicate the presence of significant concentrations of flavonoids in green buckwheat flour, which confirms its potential as a source of bioactive compounds. At the same time, it is necessary to take into account the possible degradation of polyphenols during heat treatment, in particular baking of bakery products. This limits the direct use of flour as a source of flavonoids in finished products. A promising direction is the GBF use in gluten-free formulations, products with mild processing or in combination with concentrated extracts to enrich the diet with bioactive substances.

Due to the natural absence of gluten, green buckwheat is one of the key raw materials for the development of gluten-free food products. The presence of quercetin and rutin enhances the functional properties of such products, making them not only safe, but also useful.

Flour or extracts of green buckwheat can be used to create powder concentrates, capsules, tablets, drinks with the declared content of polyphenols. This allows the creation of natural nutraceutical products that can have a preventive effect on many diseases, in particular, oncological pathologies, neurodegenerative diseases, and cardiovascular disorders.

Thus, the results of studies on the quantitative content of quercetin and rutin in GBF samples create a scientific basis for the introduction of this raw material into the production of innovative healthy food products with high biological value.

The results of this research are based on the analysis of a limited number of samples and the conditions of their cultivation and processing. Therefore, the data obtained cannot be fully extrapolated to all types and varieties of grain crops. In addition, the possible influence of external factors (agrotechnical techniques, climatic conditions, storage and processing characteristics) requires additional assessment. To implement the results in practice, expanded studies are necessary involving a larger number of producers, regions and modern control methods.

Further scientific work should be directed to studying the influence of genotypic characteristics of grain crops, various technological schemes of processing and storage conditions on the preservation of biologically active substances. It is also promising to determine the bioavailability of flavonoids in finished food products and develop technological solutions to preserve their maximum quantity during production.

#### 4. Conclusions

As a result of the study of five samples of green buckwheat flour, presented by different manufacturers, it was found that the quercetin



content ranged from 0.8 to 2.6 mg/100 g of dry matter, and the calculated rutin content ranged from 1.62 to 5.25 mg/100 g of dry matter. The maximum values of quercetin and rutin were recorded in sample 5 (TM "Ahimsa"), the minimum – in sample 1 (TM "Ecorod").

The observed variability in flavonoid concentrations is explained by differences in raw materials (varietal characteristics, growing conditions), grain processing and storage technologies. Higher values of quercetin and rutin are characteristic of samples produced from raw materials that have undergone minimal thermal or technological treatment.

The results obtained confirm that green buckwheat flour is an important source of biologically active flavonoids, in particular quercetin and rutin, which have antioxidant, anti-inflammatory and cardioprotective properties. This allows to substantiate the feasibility of using green buckwheat in the production of functional food products, gluten-free products, bakery and pasta products, food additives and nutraceuticals. At the same time, it is necessary to take into account the limitations associated with the GBF use in the composition of finished food products. Thermal treatment, in particular baking at high temperatures, can lead to the degradation of polyphenolic compounds and, accordingly, a decrease in their functional value. Thus, the results of the study confirm the bioactive potential of the raw material itself, but do not give a direct answer regarding the content of flavonoids in finished products.

Further research is planned to expand the sample, study the influence of technological processing (thermal, enzymatic, germination) on the stability of quercetin and rutin, as well as to study their bioavailability in finished food products.

### Conflict of interest

The authors declare that they have no conflict of interest regarding this research, whether financial, personal, authorial or other, which could influence the research and its results presented in this article.

### Financing

The research was conducted without financial support.

### Data availability

The manuscript has no related data.

### Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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