

Changes in the content of biologically active substances during germination of cereal and legume grains

Irma Berulava¹, Maria Silagadze¹,
Volodymyr Kovbasa², George Pkhakadze³

1 – Akaki Tsereteli State University, Kutaisi, Georgia

2 – National University of Food Technologies, Kiev, Ukraine

3 – Agricultural University of Georgia, Tbilisi, Georgia

Abstract

Keywords:

Cereals
Legumes
Germination
Total phenols
Antioxidant
activity

Article history:

Received
15.07.2024
Received in
revised form
20.11.2024
Accepted
31.03.2025

Corresponding author:

Maria Silagadze
E-mail:
mariasilagadze@
yahoo.com

DOI:

10.24263/2304-
974X-2025-14-
1-9

Introduction. The research aims to study the changes in the content of bioactive compounds accumulated during the swelling and subsequent germination of cereal and legume grains, as well as to provide their qualitative and quantitative assessment.

Materials and methods. Wheat, green lentil, pea, and soybean grains swollen in drinking or in a weak radonic mineral water and germinated. The content of amino acids was determined by chromatography; fats by Soxhlet method; total phenols by Folin-Ciocalteu; total flavonoids by spectral and antioxidant activity by DPPH methods.

Results and discussion. The amount of amino acids during the grain germination process increased. The obtained results indicate the positive effect of mineral water on the accumulation of amino acids. Particularly good results were observed for soybean and pea samples. The change in the total amount of fats (%) during the process of grain swelling and germination was determined. An increase in the amount of fat was observed in all samples, especially in samples swollen in mineral water and subsequently germinated. The best results were observed for pea (increased by 1.47 times) and lentil (increased by 1.23 times) grains. Based on the study of phenolic compounds, it was found that the amount of total phenols in germinated grains increased compared to the original ones, except pea grains, where a slight decrease was observed. The amounts of total phenols changed significantly in grains germinated after swelling in weak radonic mineral water, compared to grains germinated after swelling in drinking water. The best results were shown by wheat, soybean, and green lentil grains. As for the content of flavonoids, their increase was found in the all test samples, especially in grains germinated after swelling in mineral water. Based on the comparative analysis of the flavonoid content, soybean (1.80 mg/g) and green lentil (1.29 mg/g) grains were considered the best, followed by peas (1.02 mg/g). A similar result was observed for the content of phenolic acids, mg/g: green lentils, 1.61; soybeans, 1.52; peas, 0.85. It was established that when using mineral water for swelling, the antioxidant activity of the test samples increased? %: soybeans by 45.8; peas by 43.6; green lentils by 40.87%. It was also shown that the grains germinated after swelling in mineral water possess high antioxidant activity, %: soybeans, 64.51; peas, 46.63; green lentils, 42.5.

Conclusions. The use of weak radonic mineral water has a positive effect on activation of the grain swelling and germination, as well as accumulation of biologically active substances in them.

Introduction

The grains of traditional cereals, legumes, and pseudocereals (quinoa, amaranth, buckwheat) significantly contribute to a balanced human diet worldwide (Perri et al., 2020). Large-scale studies conducted in recent years have established that the use of these raw materials provides enrichment of the body with a full set of biologically active substances (Alvarez-Jubete et al., 2010), and the efficiency of absorption is significantly increased by using germinated grains (Ali and Elozeiri, 2017; Khatun et al., 2023) and extracts derived from them (Jeong et al., 2019).

It is known enzymes produced during the germination process break down complex reserve substances into simpler ones. At this time, the amount of biologically active compounds synthesized by the germ increases. Germinated grains contain biologically active protein complexes, peptides, free amino acids, soluble sugars, soluble dietary fiber, biogenic macro- and microelements, vitamins, phytohormones, and other useful food components (Nkhata et al., 2019; Rodrigues, 2010; Stabnikova et al., 2019; 2022).

Due to the significant changes in nutritional and physicochemical properties of grains after the activation of dormant enzymes, germination has been recognized as a method for improving the nutritional quality of whole grains (Donkor et al., 2012). In addition, it has also been proven that the germination process even leads to changes in the structure of the microbiota in several types of cereals (Landry et al., 2018, Owolabi et al., 2020).

Germination is a simple, inexpensive, and environmentally friendly method for producing plant-based foods with functional properties. It enhances the nutritional and medicinal properties of plant-based foods by reducing antinutrients and increasing the accumulation of antioxidants, flavonoids, phenolic acids, and vitamins, thereby increasing the value of the grains (Carrera-Castaño et al., 2020; Onopriichuk et al., 2022; Onopriichuk et al., 2024; Rahman et al., 2023).

Various studies have shown that germination leads to increased bioavailability of minerals in the seeds (Oghbaei and Prakash, 2020; Pjak et al., 2019), and besides, germination is considered a desirable process because it has the function of reducing the amount of antinutrients in the grains (e.g., phytic acid, which combines with various minerals to form phytates) (Atudorei et al., 2020; Chinma et al., 2020; Ghavidel and Prakash, 2007).

Based on numerous studies of the germination process of cereal and legume grains, germinated grain semi-finished products are widely used in various sectors of the food industry (bakery, confectionery, dairy, and meat products) (Atudorei et al., 2021; Eker et al., 2020), especially in the form of powders and extracts, to improve their nutritional profile or sensory and quality characteristics. Germinated grains (malt) have been used as the main raw materials in beer production for many years. Grains of various cereals and legumes, as well as many non-traditional raw materials, have been proposed for the preparation of malt. It was established that the germination process improves the bioactive properties of grains, which are important for use as raw materials in the food industry.

Analysis of the materials searched for germination methods and intensification of the mentioned processes revealed that the processes of grain swelling and germination are mostly accelerated by means of chemical compounds (Dufková, 2019), enzyme preparations (Kurmanbayeva et al., 2023), ultrasonic and thermo-alkaline hydrolysis (Luo et al., 2022; Xia et al., 2017), acoustic effects (Guimarães et al., 2020), and weak radonic mineral water (Berulava et al., 2024).

Although numerous studies demonstrated a certain increase in the content of biologically active substances during grain germination. It is noteworthy that the processes of grain swelling and germination still remain quite long, which poses a threat to the

microbiological safety (contamination) of the intermediate and target final product, which is unacceptable for food production. The duration of this technological process has been reduced on average by 5-20% compared to classical methods.

The present research aims to study the dynamics of changes in the content of bioactive compounds accumulated during the pre-processing (swelling) and subsequent germination process of some cereal and legume grains using Georgia's unique, weak radonic chloride-hydrocarbonate-sulfate mineral water, as well as to provide their qualitative and quantitative assessment.

Material and methods

Materials

The objects of the research were cereals grown in western Georgia – wheat and legumes – green lentils, peas and soybeans. Grains swollen in drinking water; grains swollen in weak radonic chloride–hydrocarbonate–sulfate mineral water sustradone (3–7.5 Mache units; or 40–100 BAC), chlorine–hydrocarbonate–sulfate, sodium–magnesium–calcium mineral water (with total mineralization of 0.7–0.8 g/l), as well as grains swollen after germination in drinking water, grain germinated after swelling in mineral water.

Swelling and germination of cereal and legume grains

The raw materials for germination were selected according to the following principle: the raw materials had to be local (Georgia), low–glycemic, agglutinative, or low–gluten, with a high protein content. Initially, foreign impurities and damaged grains were removed from the selected grains, washed well with running water, and only then was soaked in water for germination. During the experiments, each grain was taken in an amount of 20 g, and 100 ml of water was added. Swelling was carried out both in drinking water and in the weak radonic chloride–hydrocarbonate–sulfate mineral water of the Tskaltubo resort (instead of ordinary drinking water). The samples were placed in a thermostat and kept for 10 hours at a temperature of 22–40°C. During the swelling process, changes were determined in the reaction medium pH and grain mass (swelling ability). It should be noted that no significant changes in pH and grain mass were observed because of germination in drinking water. It was determined that the use of mineral water significantly accelerated this process and reduced its time by 50–60% (4–5 hours) compared to traditional, drinking water germination. At the next stage, those grains were germinated that were swollen in both drinking and mineral water, for which an automated germinator was used. Germination was carried out at different temperatures in the range of 20–38°C. The duration of germination was 48 hours. The high temperature regime was not used in order to preserve the natural properties of the main useful substances. Based on the conducted studies, the optimal regimes were established: for the swelling process in weak radonic mineral water, the duration was 3–6 hours at the temperature 22–40 °C, and for the germination process the duration for different grains ranged from 8 to 18 hours, at the temperature 28–33°C (Berulava et al., 2024).

Methods

Determination of dry matter content. The amount of dry matter was determined using a RADWAG MA 50r. (Finland) thermometer, where drying was carried out under the influence of infrared rays (Berulava et al., 2024).

Determination of the amount of amino acids. Amino acids were studied by high-performance liquid chromatography HPLC-UV, RI; UPLC-PDA, MS method (Waters, UPLC Acquity, QDa Detectore). For the separation of compounds, a chromatographic column Acquity UPLC BEN C18, 1.7 m, solvent system: 0.3% formic acid (solvent A) and acetonitrile (solvent B) was used. Gradient-solvent B: 0 – 20 min, 5–16%; 20–28 min, 16–40%; 28–32 min, 40–47%; 32–36 min, 70–99%; 36–45 min, 99% and 45–46 min, 99–5%. Injection: 10 µL. Prior to chromatography, samples and eluents were filtered through 0.45 µm pore filters.

Determination of the amount of fats. The Soxhlet method was used to obtain fat. ISO 14156:2001 | IDF 172:2001 (Short Note No. 348/2019).

Determination of total phenols by the Folin-Ciocalteu method (in terms of gallic acid). The identification of phenolic compounds was carried out by ultra-high-performance (pressure) liquid chromatography UPLC-PDA, MS method (Waters, UPLC Acquity, QDa Detectore). For the separation of compounds, a chromatographic column Acquity UPLC BEN C18, 1.7 m, solvent system: 0.3% formic acid (solvent A) and acetonitrile (solvent B) were used. Gradient-solvent B: 0 – 20 min, 5–16%; 20–28 min, 16–40%; 28–32 min, 40–47%; 32–36 min, 70–99%; 36–45 min, 99% and 45–46 min, 99–5%. Injection: 10 µL. Prior to chromatography, samples and eluents were filtered through a 0.45 µm pore filter. The sample taken for analysis was extracted with 80%–ethyl alcohol. 1 ml of the total extract volume was placed in a 25–ml volumetric flask, then 5 ml of distilled water and 1 ml of Folin–Ciocalteu reagent were added, and the solution was kept for 8 minutes at room temperature, then 10 ml of 7% sodium carbonate solution was added and made up to volume with distilled water, mixed well, and kept in the dark at room temperature for 60 minutes to stabilize the reaction. The determination was performed at 750 nm using a 1 cm thick cuvette using 1 ml of 80%–ethyl alcohol as a control and the analysis was performed in the same sequence, recalculating the data obtained because of the determination on the calibration curve of gallic acid.

The total phenol content is calculated according to the formula:

$$X = (D \cdot K \cdot V \cdot F) \cdot 1000 / m, \quad (1)$$

where X is the total phenol content, mg/kg; D is an optical density; K is a gallic acid conversion factor; F is a dilution factor; V is the total extract volume, ml; m is the mass of raw material taken for extraction, g.

Quantitative determination of total flavonoids by spectral method using the AlCl₃ reagent. The sample taken for analysis was extracted with 80%–ethyl alcohol at a temperature of 70–75 °C. 1 ml of the total extract volume was placed in a 10-ml test tube, then 5 ml of H₂O was added, 0.3 ml of 5 % NaNO₂ was left for 5 minutes, then 0.3 ml of 10% AlCl₃ was added and left for 6 minutes, then 2 ml of 1N NaOH was added, and the determination was carried out at 510 nm. 1 ml of the corresponding extractant was taken as a control and undergone the same process. The data obtained as a result of the determination were recalculated on a rutin calibration curve. The total flavonoid content was calculated by the formula:

$$X = (D \cdot K \cdot V \cdot F) \cdot 1000 / m, \quad (2)$$

where D is an optical density; K is a rutin conversion factor; F is a dilution factor; V is the total extract volume, ml; m is the mass of raw material taken for extraction, g.

Determination of total antioxidant activity by the DPPH method. Antioxidant activity was determined (using the stable radical of 2,2-diphenyl-1-picryl hydrazyl) by the

DPPH method. For the determination of total antioxidant activity, reactions occurring by the radical mechanism between a specific, colored radical and an extract with antioxidant activity were used, where the change in the optical density of the solution was determined spectrophotometrically, and the total antioxidant activity of both a specific substance and compounds was evaluated. DPPH – ($C_{18}H_{12}N_5O_6$ $M=394.33$) is a stable free radical with a maximum absorption at 515 – 517 nm, the purple-violet coloration of the methanol extract changes to light yellow upon reduction. To determine antioxidant activity – radical bonding ability, 3 ml of DPPH reagent (0.1 mM DPPH – 0.004 g/100 ml of ethyl alcohol) was added to 1 ml of the analyzed extract, and 30 minutes later, the optical density of the test sample was determined spectrophotometrically at 515 nm. The reference solution was a DPPH reagent added to 96%-ethyl alcohol. Antioxidant activity was calculated by 50% inhibition of stable free radicals (DPPH) using the following formula:

$$In \% = AC - AS/AC \cdot 100, \quad (3)$$

where In % is the inhibition of 0.1 mM DPPH (within 40-60%); AC is the absorption of a 0.1 mM a DPPH alcoholic solution, and AS is the absorption of the test extract. The antioxidant activity of 1 mg of sample was calculated using the following formula:

$$C = m/V \cdot F \cdot 50/In, \quad (4)$$

where C is a sample's mg, which inhibits 0.1 mM DPPH by 50%; m is a mass of the sample taken in milligrams; V is a volume of the test extract (ml); F is the dilution factor; inhibition of In % – 0.1 mM DPPH (within 40-60%); 50 % is a calculated inhibition.

Statistical analysis

All measurements were conducted in triplicate. Values were presented as the mean $\pm$ standard deviation.

Results and discussion

Determination of dry matter content in the grains

In order to germinate the grain, at the initial stage of the research, the dry matter content of wheat, soybean, pea, and green lentil grains was determined in the original grain and at different stages of the swelling process – in the grains swollen in drinking water, in grains germinated after swelling in drinking water, in grains swollen in mineral water, and in grains germinated after swelling in mineral water. Figure 1 illustrates the results obtained.

As the diagrams illustrate, the amount of dry matter in the selected original grains is almost the same and varies within 90.22–91.67%. During the swelling process, the amount of dry matter in grains swollen in drinking water decreased as follows: for wheat by 31.14%; for soybeans by 49.27%; for peas by 43.01%; for green lentils by 52.01%; and in grains swollen in mineral water, the results were as follows: for wheat by 27.47%; for soybeans by 52.57%; for peas by 39.69%; for green lentils by 47.79%. During the germination, the dry matter content in the grains germinated after swelling in drinking water decreased as follows: for wheat by 43.79%; for soybean by 51.12%; for chickpea by 48.39%; for green lentils by 57.54%; as for the grains germinated after swelling in mineral water, the results were as follows: for wheat by 41.68%; for soybean by 53.43%; for pea by 53.8%; for green lentils by 56.81%. The best results were observed for soybeans and green lentils.

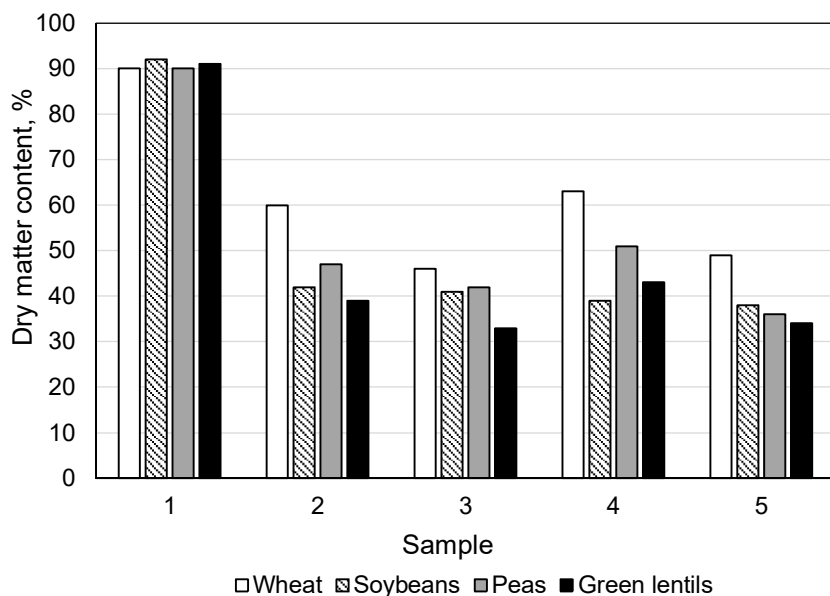


Figure 1. Dry matter content in the grains, %:

- 1 – Original grain;
- 2 – Swollen in drinking water;
- 3 – Germinated after swelling in drinking water;
- 4 – Swollen in mineral water;
- 5 – Germinated after swelling in mineral water.

Determination of the amino acid and fat content in the grains

The amino acid composition of proteins was determined in the test samples on a mg/g glycine basis, and the change in the total amount of fats (%) during the process of grain swelling and germination was also determined. The results are presented in Table 1 and 2.

Results from the analysis indicate that at all stages of the grain germination process, there is an increase in the number of amino acids compared to the original grain. The number of amino acids in wheat grains germinated after swelling in drinking water increased by 1.69 times, in soybean grains by 2.8 times, in pea grains by 4.26 times, and in green lentils by 1.75 times. In grains germinated after swelling in mineral water, the figures are as follows: in wheat by 2.8 times, in soybeans by 2.77 times, in peas by 5.06 times, in green lentils by 1.69 times. The results obtained indicate the positive effect of mineral water on the accumulation of amino acids. Particularly good effects were observed in soybean and pea samples. As for the change in fat, an increase was observed in all samples, especially in samples germinated after swelling in mineral water. The best results were achieved with peas (increased by 1.47 times) and lentils (increased by 1.23 times).

Table 1

Amino acid content in the grains

Grains	Amino acids, on a mg/g glycine basis
Wheat	
Original grain	0.75±0.01
Swollen in drinking water	1.18±0.01
Germinated after swelling in drinking water	1.27±0.02
Swollen in mineral water	0.96±0.03
Germinated after swelling in mineral water	2.11±0.02
Soybeans	
Original grain	6.66±0.04
Swollen in drinking water	10.75±0.02
Germinated after swelling in drinking water	18.66±0.03
Swollen in mineral water	12.42±0.02
Germinated after swelling in mineral water	18.48±0.01
Peas	
Original grain	3.64±0.01
Swollen in drinking water	14.92±0.03
Germinated after swelling in drinking water	15.52±0.01
Swollen in mineral water	17.76±0.04
Germinated after swelling in mineral water	18.41±0.01
Green lentils	
Original grain	12.87±0.02
Swollen in drinking water	14.00±0.01
Germinated after swelling in drinking water	22.52±0.01
Swollen in mineral water	16.73±0.03
Germinated after swelling in mineral water	21.82±0.02

Table 2

Fat content in the grains, %

Treatment	Cereals			
	Soybean	Wheat	Green lentils	Peas
Germinated after swelling in mineral water	18.03	2.26	2.30	2.03
Swollen in mineral water	18.66	2.08	2.90	1.61
Germinated after swelling in drinking water	17.11	2.27	2.17	1.87
Swollen in drinking water	15.03	2.02	1.48	1.56
Original grain	16.53	2.25	1.86	1.37

Changes of the total phenolic contents during the germination of the grains

The swelling and germination processes had a significant impact on the amount of total phenols in all grains (Table 3).

Table 3

Total phenolic content in the grains

Grains	TPC, mg/g*	PCA, mg/g**	TFC, mg/g***
Wheat			
Original grain	1.25±0.01	0.41±0.02	0.82±0.02
Swollen in drinking water	3.27±0.03	1.82±0.01	1.23±0.01
Germinated after swelling in drinking water	3.61±0.04	1.57±0.03	1.83±0.03
Swollen in mineral water	2.86±0.02	1.43±0.04	1.24±0.05
Germinated after swelling in mineral water	4.47±0.01	1.94±0.05	1.67±0.06
Soybeans			
Original grain	1.30±0.02	0.71±0.01	0.53±0.01
Swollen in drinking water	3.78±0.03	1.60±0.03	1.28±0.04
Germinated after swelling in drinking water	3.20±0.04	1.51±0.02	1.50±0.02
Swollen in mineral water	4.00±0.01	2.10±0.04	1.84±0.02
Germinated after swelling in mineral water	3.55±0.05	1.52±0.02	1.80±0.06
Peas			
Original grain	1.47±0.02	0.82±0.02	0.46±0.01
Swollen in drinking water	1.43±0.03	0.67±0.04	0.75±0.05
Germinated after swelling in drinking water	1.49±0.02	0.79±0.02	0.67±0.04
Swollen in mineral water	1.71±0.05	0.69±0.06	0.94±0.07
Germinated after swelling in mineral water	2.01±0.07	0.85±0.05	1.02±0.06
Green lentils			
Original grain	1.19±0.02	0.70±0.04	0.31±0.06
Swollen in drinking water	2.73±0.04	0.98±0.03	1.64±0.03
Germinated after swelling in drinking water	2.89±0.01	1.49±0.01	1.20±0.01
Swollen in mineral water	3.15±0.03	1.67±0.02	1.33±0.02
Germinated after swelling in mineral water	3.08±0.01	1.61±0.02	1.29±0.04

Note: *TPC, total phenols, mg/g, in terms of chlorogenic acid; **PCA, phenol-carbonic acids, mg/g, in terms of gallic acid; TFC, total flavonoid content, mg/g, on a quercetin basis.

The total phenolic content of germinated grains generally increased compared to the original grain, except for pea grains, where a slight decrease was observed. The total phenolic content of grains germinated after swelling in weak radonic mineral water was significantly changed compared to that of grains germinated after swelling in drinking water. The best results were shown by wheat, soybean, and green lentil grains. Peas showed the lowest content. It is noteworthy that these indicators changed insignificantly within 10 hours after germination, decreasing in pea samples. The prolonged germination time significantly increased the total phenolic content. Kruma et al. (2016) and Kim et al. (2016) studied the germination process of wheat, oats, barley, and rye grains using drinking water. The content of total phenols during the germination process was studied. It was shown that an increase in total phenols was observed in oat samples and a decrease in rye samples. According to (Tiana et al., 2011), lentils have the highest amount of total phenols compared to soybeans, beans, and peas. Compared to the results of the research of these scientists, in our studies, soybeans are considered the best in terms of phenolic content, followed by green lentils and finally peas.

As for the content of flavonoids, their increase was observed in all the test samples, especially in the grains germinated after swelling in mineral water. Based on the comparative analysis, the best were soybean (1.80 mg/g) and green lentil (1.29 mg/g) grains, followed by peas (1.02 mg/g). A similar result was observed in the content of phenolic acids: green lentils, 1.61 mg/g; soybeans, 1.52 mg/g; peas, 0.85 mg/g.

Antioxidant activity of the grains

It is known that antioxidant activity is closely related to the content of phenols (Khang et al., 2016; Xu et al., 2009). All the studied samples showed a significant amount of total phenols and more or less effective antioxidant activity. Antioxidant activity was assessed by the DPPH method, and the results are illustrated in Figure 2.

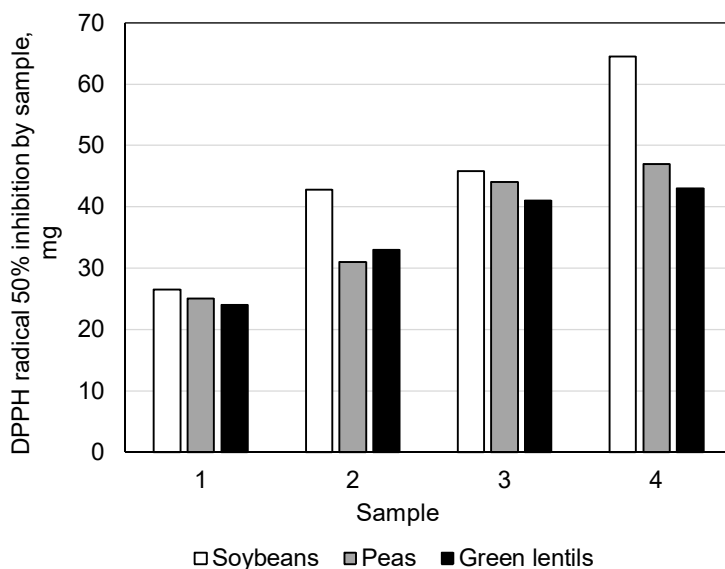


Figure 2. Antioxidant activity of the grains:
1 – Original grain;
2 – Swollen in drinking water;
3 – Germinated after swelling in drinking water;
4 – Swollen in mineral water

It was determined that the antioxidant activity of the studied samples increased when swelling using mineral water (soybean 45.8%, peas 43.6%, green lentils 40.87%). It was also shown, according to the samples, that the grains germinated after swelling in mineral water are distinguished by high antioxidant activity (soybean 64.51%, peas 46.63%, green lentils 42.5%) compared to the grains germinated after swelling in drinking water. The high antioxidant activity of swollen and germinated grains and legumes has been confirmed by studies by other scientists research works (Khang et al., 2016; Şenlik et al., 2023).

Our studies have confirmed the high efficiency of weak radonic mineral water used at the swelling stage in the germination process of grains and legumes, a significant optimization of the technological process of germination, which contributed to reducing the

time of the overall germination process of various grains taken for analysis by 16-24 hours instead of 36-48 hours compared to the traditional method. It should also be noted that the optimization process contributed to achieving better results in the accumulation of biologically active substances in the conditions of a short germination duration compared to the traditional process.

Conclusions

1. The dry matter content of wheat, soybean, pea and green lentil grains was determined in the original grain and at different stages of the germination process – in grains swollen in drinking water, in grains germinated after swelling in drinking water, swollen in weak radonic mineral water, and in grains germinated after swelling in mineral water. It was shown that the amount of dry matter decreases at different stages of the germination process. The best results were obtained for soybean and green lentil grains.
2. The amino acid composition of proteins was determined in the test samples on a mg/g glycine basis. It was established that at all stages of the grain germination process, there is an increase in the amount of amino acids compared to the original grain. After swelling in drinking water, the amount of amino acids in wheat grains germinated increased by 1.69 times, in soybean grains by 2.8 times, in pea grains – by 4.26 times, and in green lentils by 1.75 times. In grains germinated after swelling in mineral water, the figures are as follows: in wheat by 2.8 times, in soybeans by 2.77 times, in peas by 5.06 times, and in green lentils by 1.69 times. The results obtained indicate the positive effect of mineral water on the accumulation of amino acids. Particularly good effects were observed in soybean and pea samples.
3. The change in the total amount of fats (%) during the process of swelling and germination of grains was determined. An increase in the amount of fat was observed in all samples, especially in samples germinated after swelling in mineral water. The best results were observed in peas (increased by 1.47 times) and lentils (increased by 1.23 times).
4. Based on the study of phenolic compounds, it was determined that the amount of total phenols in germinated grains increased compared to the original grain, except for pea grains, where a slight decrease was observed. The amount of total phenols changed significantly in grains germinated after swelling in weak radonic mineral water, compared to grains germinated after swelling in drinking water. The best results were shown by wheat, soybean and green lentil grains. As for the content of flavonoids, their increase was found in all the test samples, especially in the grains germinated after swelling in mineral water. Based on the comparative analysis, soybean (1.80 mg/g) and green lentil (1.29 mg/g) grains were considered the best, followed by peas (1.02 mg/g). A similar result was observed in the content of phenolic acids: green lentils, 1.61 mg/g, soybeans, 1.52 mg/g, peas, 0.85 mg/g.
5. It was established that the antioxidant activity of the test samples increases when swelling in mineral water (soybeans 45.8%, peas 43.6%, green lentils 40.87%). Also, according to the samples, it was shown that grains germinated after swelling in mineral water are distinguished by high antioxidant activity (soybean 64.51%, peas 46.63%, green lentils 42.5%), compared to grains germinated after swelling in drinking water.
6. Based on the analysis of the above results, the use of weak radonic mineral water was considered a priority in terms of optimizing the processes of grain swelling and germination and the accumulation of biologically active substances in these processes.

Acknowledgements. This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [FR-23-3721 Development of innovative technology for production of whey-based functional lactic acid drink].

Chemical studies were conducted at the Regional Chromatographic Center of Western Georgia, Shota Rustaveli Batumi State University, and we express our gratitude to the staff of the center (A. Kalandia, M. Vanidze, I. Japaridze, I. Kartsivadze, and N. Abashidze).

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Cite:

UFJ Style

Berulava I., Silagadze M., Kovbasa V., Pkhakadze G. (2025), Changes in the content of biologically active substances during germination of cereal and legume grains, *Ukrainian Food Journal*, 14(1), pp. 84–96, <https://doi.org/10.24263/2304-974X-2025-14-1-9>

APA Style

Berulava, I., Silagadze, M., Kovbasa, V., & Pkhakadze, G. (2025). Changes in the content of biologically active substances during germination of cereal and legume grains. *Ukrainian Food Journal*, 14(1), 84–96. <https://doi.org/10.24263/2304-974X-2025-14-1-9>
