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THE INFLUENCE OF VARIOUS FACTORS OF THE GERMINATION PROCESS OF GRAINS AND LEGUME CROPS

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Abstract

The research aims: to investigate and optimize the processes of grain swelling up and subsequent germination, and to that end, to test the unique, weak radon mineral waters of Georgia as well as to provide qualitative and quantitative evaluation of some bioactive compounds accumulated during the germination process. In the process of grain germination, weak radon mineral water dramatically accelerated the processes of grain swelling and subsequent germination. At the same time, it significantly reduced the calorie content of cereals and increased the quantitative content of macro-, microelements, and vitamins.

Keywords: *cereal and leguminous crops; germination; germination process optimization; Georgian weak radon mineral water*

Introduction

At the modern stage, the use of plant raw materials in food production is a very relevant, important, and much-discussed scientific problem. From this perspective, germied grain and its extracts are essential to ensure the high biological value of the expected final product (Wigmore, 2021; Yıldırım et al., 2023; Criste et al., 2023). Enzymes that appear after the grain has germied, break down complex storage substances into simpler ones (amino acids, fatty acids, simple sugars) (Tahereh et al., 2023; Lucas-Aguirre et al., 2023). At this time, the amount of vitamins synthesized by the germ increases many times. Because of that, the germied grain contains a biologically active protein complex, peptides, free amino acids, soluble sugars, soluble dietary fiber, biogenic macro- and microelements, vitamins, phytohormones, and other useful nutritional components (Ali et al., 2017; Hernández Cortés, 2022; Zilić et al., 2014).

The use of germied grains and their extracts is one of the promising areas for food industry development. The germied grain flour allows food developers to adjust the quality of food products and enrich them with dietary fibers, vitamins, and minerals (Şenlik et al., 2021; Cagla Kayisoglu et al., 2024).

Regular consumption of germis as a food supplement has a therapeutic effect: it helps clean out toxins from the body and effectively digest food, normalizes the acid-alkaline balance, improves metabolism, increases immunity, and slows down the aging process. In addition, the germis of each culture have a specific set of vitamins and trace elements, and their use contributes to the prevention of certain diseases (Lei et al., 2017).

Germied grains are an ancient health remedy that has been known for over 5,000 years. This healing food has an amazing ability to relieve a person from many diseases at once, preventing their return. True health is what this gift of nature gives us (Ebrar Altıkardes et al., 2024). The special value of germied grain lies in the fact that it is the only „living" food. Their inclusion in the diet is an opportunity for a person to use the whole living organism as food, which has all the natural biological properties and is in the phase of maximum vital activity. Under natural conditions, germinating seeds tighten with all their might during the first few days to form a root as quickly as possible, gain a foothold in the soil, and bring the first leaves to the sun. It is in this short period that a person should use them to get strength and health from such an extraordinary product.

The importance of germied grain became known relatively recently, and experimental research in this direction in Georgia is scarce and mostly limited to our research and a few publications.

Grain germination is a process in which many biochemical events occur, so the grain must be optimally compatible with internal and external factors during germination (Zhang et al., 2023; Tahereh Najib et al., 2023). The physiology of germination involves very complex procedures. Endogenous and exogenous factors play an important role in seed germination. Some endogenous factors are phytohormones and endosperm degradation. Exogenous factors are environmental impacts such as light and temperature (Carrera-Castaño et al., 2020).

The analysis of the patent materials searched in the direction of methods of grain germination and intensification of these processes showed that mostly the processes of grain softening and germination are accelerated by chemical compounds, enzyme preparations, ultrasound and thermo-alkaline hydrolysis, acoustic influence, etc. (Wang et al., 2020; Xiang et al., 2023; Lidan Dong et al., 2024).

Even though as a result of numerous studies, a certain increase in biologically active substances was achieved in the process of grain germination, the duration of the technological process was reduced on average by only 5–20%, compared to classical methods. It is worth noting the fact that the process of grain softening and germination is still quite long, which threatens the microbiological safety (contamination) of the intermediate and target final product, which is unacceptable for food production.

It is estimated that more than 50% of the energy expended in the growth of grains is used to break down the hard coat of the grain, which is usually multi-layered. After germination, the germi emerges from the seed coat (González Carretero et al., 2017).

It is known that the germination process is significantly influenced by active acidity pH, oxidation-reduction potential, the degree of mineralization, and other factors. Therefore, the key issue of the presented research is the investigation and optimization of the germination process of raw materials selected for germination. To that end, the use of the unique natural mineral waters of Georgia of different compositions and mineralization in the process of grain germination is relevant. This study was conducted for the first time, and it is innovative.

The present study aims: to investigate and optimize the processes of grain pre-treatment (swelling up) and subsequent germination and evaluate the effectiveness of these processes, and to that end, to test the mineral waters of Georgia with unique, different chemical composition and mineralization, and to determine the optimal regimes of the said processes under the influence of various environmental factors, as well as to provide qualitative and quantitative evaluation of some bioactive compounds accumulated during the germination process.

Materials and Methods

Research objects were – cereals: wheat (Triticum), flax (Linum usitatissimum), leguminous crops: peas plants (Pisum), soybeans (Glycine), green lentils (Lens), broad beans (Vicia faba); weak radon water of Tskaltubo resort; softened (swelled up) cereals and fruits of leguminous crops; germied grains and legumes

These raw materials were grown in Georgia, in peasant holdings and farms, and were purchased from LLC "Chemi Maragi".

Modern standard and modified research methods were used to perform scheduled work.

Sample mass was determined using an electronic digital analytical balance model SF-40 ˚C (Toms, Qilin, China);

We softened grain in a POL-EKO thermostat to create optimal conditions in chemical cups. Grain germination was carried out in an automated germier DY4102(LCD display)-OYSIR. In the process of grain softening, the pH of the reaction was determined using a pH meter – "Mettler Toledo".

We determined overall chemical composition of raw and germied grains, in particular the content of dry matter, protein, fat, carbohydrates, and ash. We also studied the microstructure of dry, softened and germied grain on the OMAX digital microscope.

To determine the quality of raw materials, the determination of proteins, fats, carbohydrates, vitamins, and mineral substances was carried out in accordance with the recommendations of the Codex Alimentarius (http://www.fao.org/3/i2085e/i2085e00.pdf) using the latest colorimetric, spectral and chromatographic methods. The total amount of proteins was determined by the Kjeldahl method, while the fat content – by the Soxhlet method.

We performed mathematical processing of experimental data according to the results of tre-fivefold repetitions of tests using statistics and regression analysis methods.

These studies were conducted in the laboratories of the Department of Food Technology at Akaki Tsereteli State University (Kutaisi, Georgia). The study of vitamins and mineral substances of cereals was conducted at the research base of the Western Georgia Regional Center of Chromatography of the Faculty of Natural Sciences and Health at the Batumi Shota Rustaveli State University.

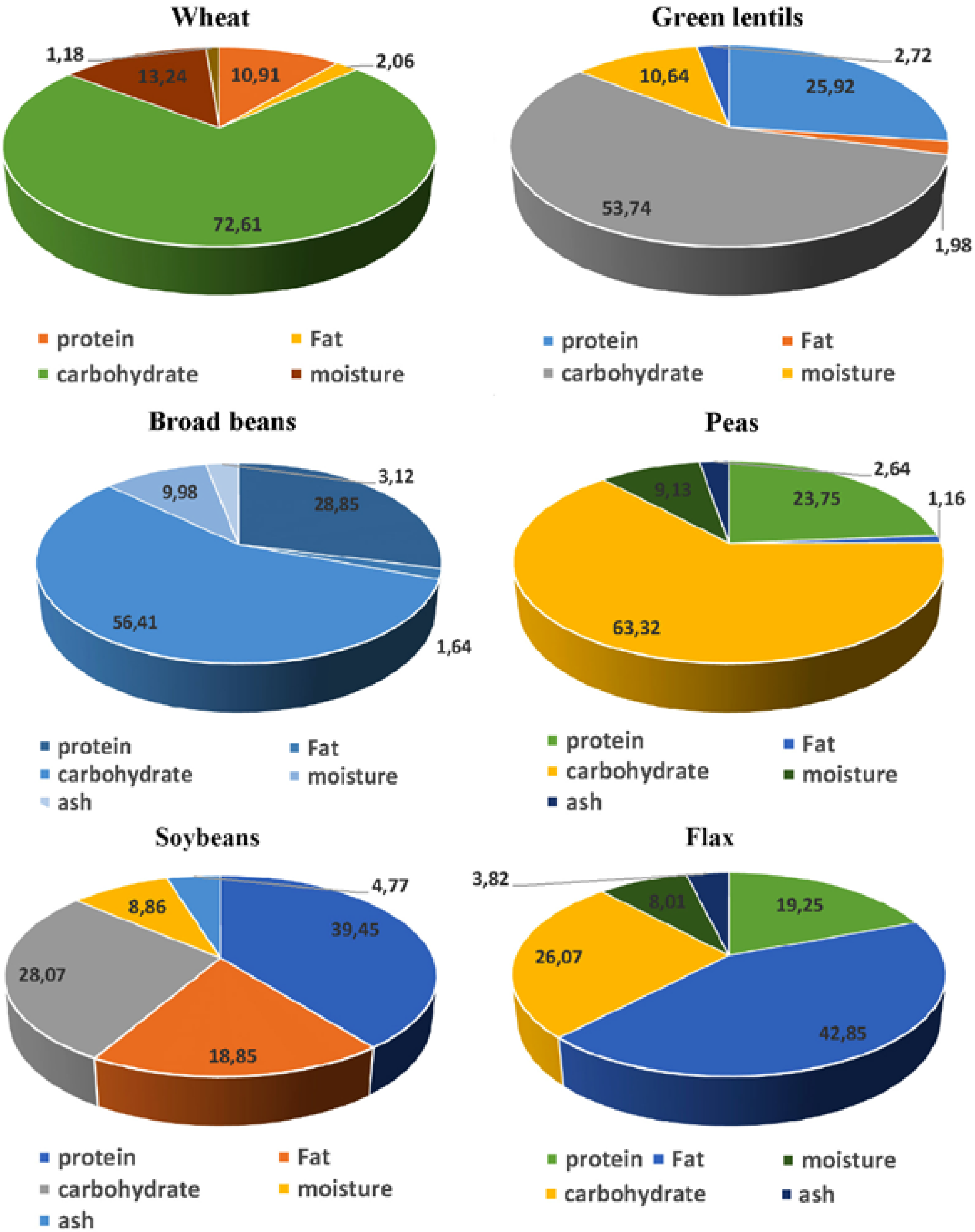
Results and Discussion

At the beginning of the experiment, we selected the raw materials, where we were guided by the following principle: raw materials should be local (Georgia), low-glycemic, gluten-free or low-gluten, with a high protein content, since the work was aimed at the development of indigenous plant resources (Georgia), and their rational use and the use of the final product (germied grain) in the food industry as an additive, with the prospect of having to use them in prophylactic and functional food, by bringing the final products to the condition of the commercialization process. The raw materials we selected for germination are shown in Figure 1.

Figure 1. *Local raw materials selected for research*

Figure 1. *Local raw materials selected for research (continues)*

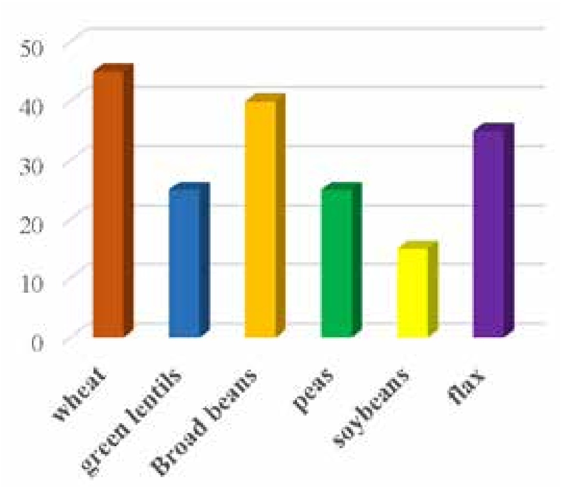
Figure 2. *Chemical composition of raw materials*



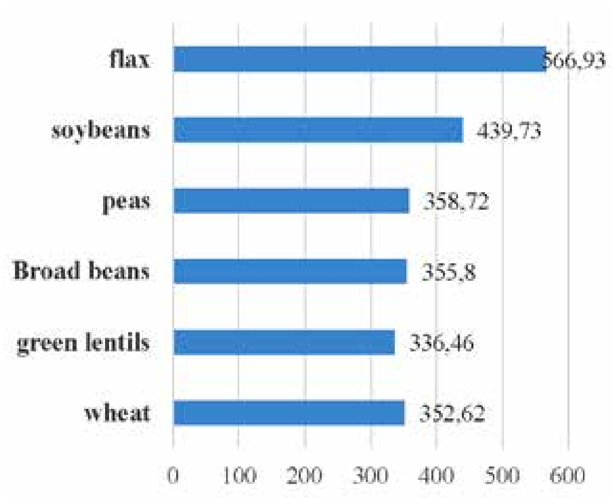
After selecting the raw materials for germination, we studied their basic chemical composition. The amount of moisture, proteins, fats, carbohydrates, and ash in the grain was determined. We calculated their energy value. The results of the study are shown in Figure 2. Figure 3 illustrates the main characteristics of the selected raw materials: glycemic index, thousand grains weight and caloric content of raw materials.

The results of the present research demonstrate that that lentils have a low energy value, followed by wheat, broad beans and peas plants, which have almost the same energy value. The caloric content of soybean and flax is significantly higher than other studied raw materials, in particular, on average, soybean – 20–40% and flax – 38–41%. According to the glycemic index, soybeans have the lowest values, followed by lentils and peas plants. The magnitudes of wheat, flax, and broad beans are above them, but can also be considered low-glycemic.

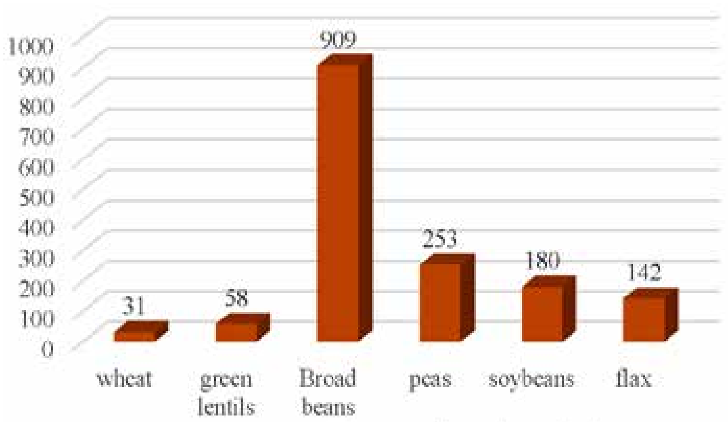
Figure 3. *Characteristics of selected raw materials*



Glycemic index of raw materials



Calorie content of raw materials, kCal



Thousand grain weight, g

The process of grain germination consists of two main stages – softening to swell it up, and then germination of swelled grain. Grain germination actually starts from the moment it absorbs water and is completed after the formation of the germi from the germ (Hernández Cortés, 2022). It is known that physiological processes in living cells depend on the presence of water, and that germination is impossible if water is not absorbed from the environment. Absorption of water in the grain leads to the initiation of various physical and chemical processes, resulting in germination.

Grain germination was carried out as follows: we removed extraneous matter and damaged grains from the seed, washed it well with running water, soaked it in water to remove "dead" grains, then they floated to the surface. Then we washed the grain again, and only after that we soaked it in water to swell it up. During the work, we used the traditional method (Wigmore, 2021). During the tests, we took 20 g of each grain, and added water in a fivefold amount. In order to optimize the processes of grain germination, we carried out germination both in drinking water and in the weak radon chloride-hydrocarbonate-sulfate mineral water from the resort of Tskaltubo (instead of ordinary drinking water). We conducted the study of the influence of mineral waters on the process of grain germination for the first time.

Tskaltubo mineral water is distinguished by its unique properties. The origin of Tskaltubo water is considered the oldest from a geological point of view (100–150 thousand years ago), in the formation of which hydrochemical processes take part. However, it is assumed that the formation of Tskaltubo water takes place at the expense of huge underground static water resources, which rise to the surface of the balneological zone thanks to the hydrostatic pressure of karst waters. According to the mixed hypothesis, chlorine-rich water rises from Middle Jurassic sedimentary rocks, mixes with sulfate water in the upper crust, and is then desalinated by hydrocarbonate water in the Lower Cretaceous limestones. Tskaltubo mineral water is enriched with radon in the bulk sedimentary sand. Tskaltubo's mineral springs have always attracted attention due to their distinctly different properties from other waters. It does not contain toxic elements, its chemical composition is characterized by stability and does not change over time. All ingredients are below the lower limit. This is confirmed by comparing the chemical analyzes carried out during the last 70–80 years. Physico-chemical composition of water: the said mineral water is distinguished by quite solid physico-chemical parameters. It contains noble gas – radon, large amount of nitrogen and helium. It belongs to weak radon (1––2.7 nki/l; or 3–7.5 one trap; or 40–100 bq), chlorine-hydrocarbonate-sulfate, sodium-magnesium-calcium waters, with a total mineralization of 0.7–0.8 g/l. The daily debit of the springs is 13–15 mln. liter. The highly effective action of this is due to its complex composition and peculiar combination of the main components of the saline composition. The natural temperature of water is +33––35 °C. Biologically active trace elements were found in Tskaltubo's mineral springs such as iodine, bromine, manganese, lithium, boron, zinc, strontium, and copper, which play an important role in the vital activity of the body. Gases play an important role: nitrogen, radon, helium, and argon. Mineral water is enriched with radon in bulk sedimentary sand(https://tskaltuboresort.ge/eng/static/37/;ttps://www.georgianholidays.com/attraction/resorts-in-georgia/tskaltubo).

We soaked the grain in drinking and mineral water. The samples were placed in a thermostat and left for 10 hours at a temperature of 22 ÷ 40 °C. In the process of softening, we determined the change in pH and grain mass (swelling capacity). It should be noted that there were no significant changes in pH and grain mass as a result of softening in drinking water. The exception were the grains soaked in Tskaltubo water, where the mass increase started after 2 h and reached a maximum at 4 h and remained practically unchanged for the next 6 h. The best results were shown by soybean, flax, green lentil and broad beans delayed at a temperature 35 °C. The active acidity pH underwent a slight change during 10 hours and after 10 hours it reached: in the case of wheat in water, from 6.31 to 5.82; lentils – from 6.31 to 5.71 md; green peas – from 6.31 to 5.65; broad beans – from 6.31 to 4.98, soybean – up to 5.04, flax – up to 5.61. As for the process of dissolving in Tskaltubo mineral water, under similar conditions, the value of PH changed from the initial 7.10 up to 5.10–5.91.

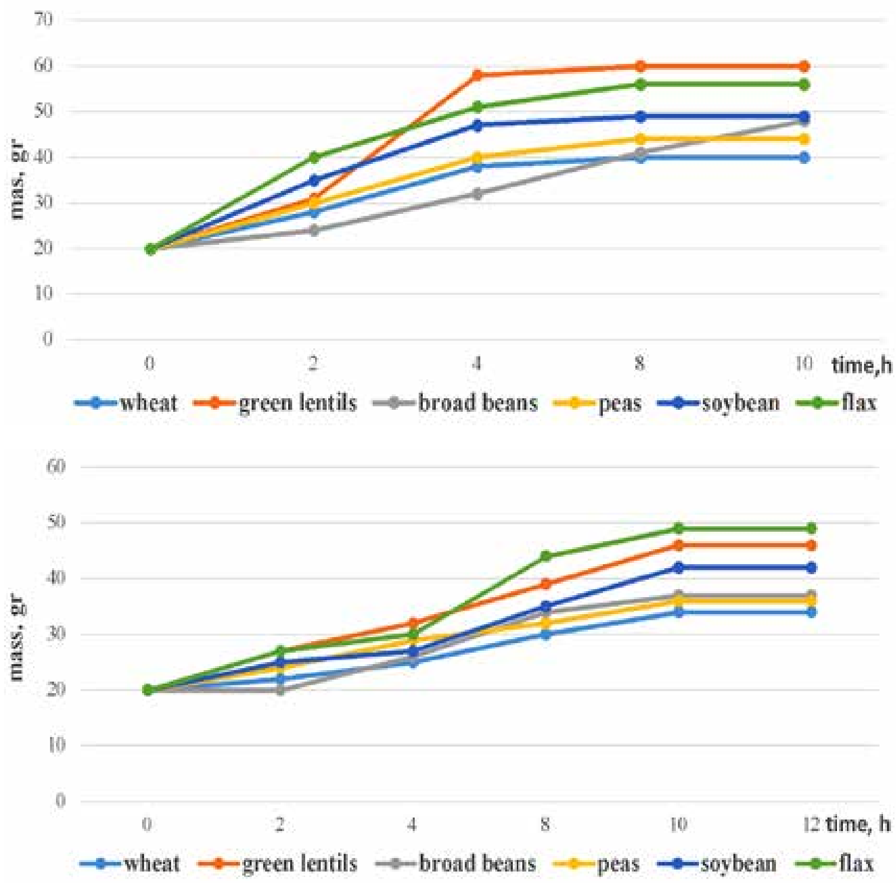
Figure 4 illustrates the dynamics of pH and mass changes in the process of grain softening in drinking water and weak radon mineral water. It can be seen that in the case of grain softening in drinking water, no significant increase in mass was observed within 8 hours, the maximum result was reached only within 10 to 12 hours. As for the process of softening in mineral water, the optimal result was revealed in 4 hours. Green lentils, flax, soybeans were distinguished with the best results, followed by peas and wheat. As for the broad bean, since it did not experience an increase in mass during the mentioned time period, we excluded it from further studies.

The temperature change in the range of 22÷40 °C did not affect the result much. Since our goal was to reduce the duration of the digestion process, it was considered optimal to digest the samples for 3–4 hours, and this process reduced the time by 50–60% (4–5 hours) compared to traditional, potable water digestion. Since only 3 cereals, namely green lentils, soybeans and flax, showed the best results in the Dalbobi Porce, we conducted further studies only on these 3 cereals.

Figure 4. *Dynamics of mass change in the process of softening of selected grains in drinking (1) and mineral water (2) in a thermostat at a temperature of 35 °C*

(1)

(2)



At the next stage, grains were germied in both drinking and mineral water, for which an automated germier was used. Germination was carried out at different temperatures in the range of 20–38 °C. Germination duration was 48 hours. We avoided the high temperature regime in order to preserve the natural properties of the main useful substances. Our goal was to select a high-protein raw material and maintain its properties as much as possible throughout the germination cycle. Germination was carried out up to 2–3 mm in length.

In the process of grain germination, we studied the influence of temperature and duration of germination on its quality. As a result of the experiments, the optimal terms and modes of germination were determined: for the samples soaked in drinking water, the duration of time was – 24 hours for soybeans, 18–20 hours for green lentils, 40–45 hours for flax. Accordingly, the optimal temperature for all samples was 26–28 °C. As for the samples soaked in mineral water, the optimal duration of the germination process was observed as follows: for green lentils and soybeans – 8–12 hours, and for flax – 16–18 hours. Temperature: 28–33 °C. There was no significant change in the duration of the germination process during temperature variation. Figure 5 illustrates the pictures of grains germied in mineral water.

Figure 5. *Germied grains soaked in mineral water*

Green lentils **Flax** **Soybeans**

In the next stage of the research work, we studied the chemical composition of the best germied samples (green lentils, flax, soybean) and calculated the caloric content. Since the selected raw materials in germied form are intended for functional food, we considered it necessary to determine their macro-, microelements and vitamin content. For comparison, the corresponding parameters of raw grain and grain soaked in drinking water were used. The chemical composition, content of vitamins, macro- and microelements of the grain soaked in drinking and mineral water (softened) and subsequently germied are given in Tables 1, 2.

Table 1. *Chemical composition of germied grain*

Nutrients	Nutrients, in equivalent to 100 g, g					
	Germied grain, (swelled up in drinking water)			Germied grain, (swelled up in mineral water)		
	Green lentils	Flux	Soybean	Green lentils	Flux	Soybean
Protein	8.7	7.6	13.2	9.0	8.02	13.0
Fat	1.72	17.7	6.9	2.0	21.1	7.0
Carbohydrate	23.4	12.8	8.8	221.	13.01	9.0
Water	65.1	60.01	67.1	65.0	559.	67.0
Ash	1.1	19.	3.4	2.0	2.0	3.0
Calorie content, kcal	143.9	240.9	150.1	154.4	274.02	151.0

As shown in Table 1, as a result of germination the grains soaked in drinking water for 13–45 hours, the caloric value decreased by 2.2–2.9 times compared to the raw grain's caloric value, while the caloric content of the grains soaked in mineral water for 8–18 hours decreased by 2.06 ÷ 2.9 times. Using mineral water, this result was achieved in an average of 27 hours less time.

Table 2 clearly indicates that the use of mineral water significantly increased the content of macro-, microelements and vitamins, which led to a high biological value of germied grains. Of particular note is the fact that vitamin C was practically absent in raw green lentils and soybeans. Vitamin C in the grains soaked in drinking water and subsequently germied was recorded for green lentils – 31.2 mg%, for soybeans – 21.6 mg%. With the use of mineral water, these values increased further and amounted to 41.2 mg% and 31.7 mg%, respectively.

Table 2. *The content of macro-, microelements and vitamins in germied grain (mg%)*

Components	Amount of components, mg%								
	Raw grain			Germied grain, (swelled up in drinking water)			Germied grain, (swelled up in mineral water)		
	Green lentils	Flux	Soybean	Green lentils	Flux	Soybean	Green lentils	Flux	Soybean
Vitamins									
B1, mg	0.56	1.78	0,89	0.75	1.72	0.92	0.96	1.76	1.28
B2, mg	0.19	0.25	0,18	0.22	0.35	0.33	0.52	0.58	0.51
B5, mg	–	1.08	2.05	1.10	0.56	0.67	1.45	0.62	0.71
B6, mg	–	0.37	0.92	0.36	0.18	0.39	0.51	1.09	1.12
B9, mkg	–	78.0	188.0	–	45	231,0	–	70.0	315.0
Vitamin C, mg	–	0.69	–	31.2	0.32	21.6	41.2	0.49	31.7
Macro-elements									
Calcium, mg	85	247	348	51	102	157	62	118	171
Magnesium, mg	78	390	228	70	159	184	97	225	247
Sodium, mg	60	33	7	23	18	48	33	26	52
Potassium, mg	658	789	1640	615	326	1086	687	456	1201
Phosphorus, mg	278	650	608	329	243	414	558	487	652
Microelements									
Iron, mg	11.8	5.82	10.1	12.8	2.26	10.8	14.6	4.16	12.1
Zinc, mg	2.6	4.48	2.5	–	1.78	4.0	3.01	20.8	5.3
Copper, mkg	645	1180	516	716	506	590	742	614	698
Manganese, mg	1.2	2.48	2.8	2.1	1.23	2.2	2.8	17.9	2.7

The obtained results provide the basis for continuing fundamental research to create a complete picture of the dynamics of the accumulation of biologically active compounds in the process of grain germination.

Conclusions

1. A study of the grain swelling process was conducted. It was determined that the use of mineral water significantly accelerated the said process and reduced its time by 50–60% (4–5 hours) compared to traditional drinking water. Based on the obtained results, only 3 cereals were considered as priority, particularly green lentils, soybeans and flax.

2. The optimal modes and parameters of the germination process were determined: the duration of germination for samples soaked in drinking water was 24 hours for soybeans, 18–20 hours for green lentils, 40–45 hours for flax. Accordingly, the optimal temperature for all samples was 26–28 °C. As for the samples soaked in mineral water, the optimal duration of the germination process was observed as follows: for green lentils and soybeans – 8–12 hours, and for flax – 16–18 hours, and the temperature: 28–33 °C.

3. It was determined that as a result of the use of weak radon mineral water in the process of grain germination, the caloric content of grain was significantly reduced and the content of macro-, microelements and vitamins increased. It was observed that vitamin C was practically absent in raw green lentils and soybeans. Vitamin C in the grains soaked in drinking water and subsequently germied was recorded for green lentils – 31.2 mg%, for soybeans – 21.6 mg%. With the use of mineral water, these values increased further and amounted to 41.2 mg% and 31.7 mg%, respectively.

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