Проведено дослідження по розробці технології борошна нуту збагаченого селеном. Розроблена технологія дасть змогу надходження органічних форм мікроелементу дефіцит якого спостерігається у 17 % населення світу.

В результаті дослідження встановлено, що на ступінь акумуляції селену має вплив вміст білка у нативному зерні. Раціонально використовувати розчини для пророщення, які є носіями 75 мкг селену. 95...99 % селену в пророслому зерні акумулюється у сім'ядолі, у білковій фракції. Під час пророщення зерна амінокислотний склад значно збільшується. Вміст лейцину, лізину, аргініну, та триптофану збільшується на 87, 76, 80 % і 55 % відповідно. Основу замінюваних амінокислот складають аспарагінова та глютамінова кислоти та їх аміди, на частку яких в не пророщених зернах нуту припадає 67 %, а в пророщених – 70 %.

Розроблена технологічна схема виробництва борошна нуту відрізняться від контрольної тим, що миття та дезінфекція зерна нуту проводиться водним розчином лимонної кислоти (pH 3,5...4,0). Після чого зерна пророщують у розчині $NaHSeO_3$ протягом 48 годин.

Розроблене борошно за органолептичними показниками має світло-жовтий колір, властивий борошну нуту запах, смак без гіркоти та кислуватих присмаків. За фізико-хімічними показниками відмінності від контролю спостерігаються за масовою часткою вологи – на 1 % менше від контрольного зразка, та масовою часткою жиру, яка зменшується на 2 %. На 0,5 % збільшується масова частка загальної золи та масова частка клітковини. За вмістом ртуті, миш'яку, свинцю розроблене борошно нуту не перевищує допустимі для вживання людини рівні, не містить кадмію та має менший вміст міді на 1 мг/г, ніж допустимий рівень. За кількістю мезофільних аеробних та факультативно-анаеробних мікроорганізмів, пліснявих грибів та дріжджів розроблене борошно нуту є безпечними для вживання. Не містять бактерії групи кишкових паличок та патогенних мікроорганізмів бактерій роду Salmonella.

Проведений комплекс досліджень дає підстави стверджувати, що розроблене борошно нуту є носієм 52 мкг селену у біодоступній органічній формі, чим забезпечує 65 % добової потреби в селені для дорослої здорової людини

Ключові слова: борошно нуту, амінокислотний склад, селен, пророщення, мікроелементи, зерно, нут, метод інверсійної вольтамперометрії

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DEVISING A TECHNOLOGY FOR MAKING FLOUR FROM CHICKPEA ENRICHED WITH SELENIUM

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1. Introduction

The modern science about nutrition, nutritiology, shows that the growth, development, preservation of health, maintenance of high workability, the body's ability to resist infectious diseases and other factors of the environment require physiologically sound nutrition [1]. Special attention must be paid to the shortage of microelements [2]. Selenium is an indispensable microelement in human nutrition, which is the catalyst of biochemical processes and participates in the synthesis and metabolism of hormones. It acts as an agent that promotes detoxification, participates in the formation of erythrocytes, reduces the evolution of hormone-dependent tumors [3]. Selenium deficiency is observed in 17 % of the world population [4]. One way to overcome the deficit of selenium is to develop the culinary foods and rations enriched with the organic forms of selenium, which could be implemented at restaurant establishments, in sanatoriums, preventoriums, hospitals [5].

One of the favorite formulation ingredients in the meals of Slavic peoples is flour [6]. Flour is enriched in 30 % of countries including the United States, Canada, Belgium [7]. In 2020, Ukrainian flour manufacturers would be obliged to add vitamins and minerals to their products [8]. It is rational while devising a technology of enriched flour, to use, as a raw material, legumes, namely chickpea [9]. The plant protein that is part of chickpea grains is capable of accumulating and bio transforming the inorganic forms of selenium, thereby creating its organic forms during steeping in the process of germination [10]. The biological synthesis of the organic forms of selenium, when compared to other methods, requires less energy and economic costs, it is environmentally safe and eliminates the possibility to form harmful by-products [11].

The development of a technology for making legume flour from sprouted grains enriched with micro-elements may include the use of solutions of mineral salts [12]. Sodium hydroselenite ($NaHSeO_3$) is a carrier of 0.52 µg of selenium per gram of a substance [13].

Devising new technologies, involving the enrichment with selenium, is an important task resolving which would solve the important social issue of preserving the nation's health, maintaining high performance, the body's ability to withstand the diseases and other factors in the environment.

2. Literature review and problem statement

The studies reported by institutes of food point to the problem related to a low level of the microelements introduced to the human body with food products [14]. Analysis of literary data shows that a promising direction for improving nutritional value and flour enrichment is the germination of grains [11, 15].

Paper [16] describes findings from a study into the influence of flour made from sprouted lentils that were used to enrich bread with protein. And a sea cabbage powder to enrich bread with iodine. The authors demonstrate a positive influence exerted by raw components on the rheological characteristics of dough and the increase in the biological value of finished products. They, however, did not establish how the process of grain germination affected its amino acid composition. It is known from [17] that the germination of grain is accompanied by a change in the amino acid composition due to the creation of a nutrient in the grain for the growth of shoots.

Study [18] investigated the possibility of making flour from the sprouted grains the legume variety *Phaseolus aureus*. The authors established a change in the amino acid composition towards the increased amount of essential amino acids, namely asparagine, and noted the high hydrophilicity of flour in meat minces. They found the optimum concentrations when making sausage products; however, the proposed technology is not a carrier of microelements.

The expediency of using mineral salts was scientifically substantiated in order to enrich with microelements [19].

The lupine grain is sprouted in a solution of potassium iodate – the production technology includes grain washing, its alternating water soaking, germination, drying, grinding. The flour made by the devised technology is the carrier of iodine (30 µg/100 g) and has an elevated protein content [20]. It was proven that the use of sodium hydroselenite ($NaHSeO_3$) when germinating soybean grains in the technology of selenium-containing malt improves the quality of bakery products by increasing the lipoxygenase enzyme and increasing the protein content [21]. The technique for obtaining selenium-containing malt includes the washing of grains, their alternating air-water soaking, germination, and drying, which differs in that the steeping of grain is carried out in a solution of selenium with a concentration from 1.7 to 3.2 µg/ml to the content of selenium in malt from 15 to 28 µg/g of dry grain [22].

An analysis of the scientific literature data about the experience of enriching chickpea flour with selenium established that the flour is enriched at the stage of grain growing. The technique is characterized by the fact that the grain, before planting in the soil, is moistened with a solution of sodium selenite with a concentration of 20 µg per 1 m^2. It is accompanied by the simultaneous application of fertilizer into the soil with a concentration of 18.7±0.01 mg/m^2 [23]. The disadvantage of a given technique is the complexity and duration of the process; the large consumption of sodium selenite is economically impractical. It is more rational to enrich chickpea flour with selenium during the process of germination.

There is a technique for enriching chickpea flour with microelements whereby sea food salt is dissolved in distilled water in the ratio 2:1; the obtained solution is used for sprouting the grains of chickpea until the formation of sprouts whose length is 1...2 mm. The sprouted grains are dried to a humidity of 12...14 %. They are ground to a particle size of 1 mm. It is shown that the chickpea flour made in line with the devised technology has an elevated content of macro- and micro-elements [24].

There are scientific studies into the development of a chickpea flour enrichment technology whose scientific basis is the germination of grains in the aqueous extract of *Laminaria japonica* [24]. The proposed technique makes it possible to produce flour, enriched in selenium, with high consumer properties.

However, there are the unresolved issues related to determining the content of a mass fraction of selenium, which is biotransformed into the grain and anatomical parts of the sprouted grains. It was not established how a protein content in grain influenced the degree of microelements accumulation. The reason for this might be associated with difficulties in the course of the experiment, related to the complexity of determining the mass fraction of microelements, which, as indicated in [25], are rather unstable compounds capable of oxidation, transformation, evaporation.

By analyzing the literary data on current approaches to determining the content of selenium in food products, it was found that there are many techniques to determine the mass fraction of selenium in different environmental objects. The most accurate and modern method for determining the selenium content is the method of mass spectrometry with inductively connected plasma [26]. However, the use of this method requires expensive imported equipment.

For medical purposes, the kinetic cerium-arsenide method is widely used for the determination of selenium in the urine [27], which is considered to be quite reliable. This method could also be used to determine iodine in some foods, such as processed cheese.

To determine selenium in beverages, a method of atomic absorption spectrophotometry with electron-thermal atomization was proposed [28]. A given method allows the determination of up to 10 µg of selenium in 100 ml of the sample. This method is used not only to control the selenium content in the enriched products but also to study a microelement during storage.

Each of the above methods was designed for a specific product group and is not suitable for controlling the selenium mass share in other products for a variety of reasons, in particular, due to the lack of sensitivity and the presence of defective substances.

A rather universal method with good reproducibility and accuracy is a polarographic method using the voltammetric analyzer "Ecotest VA" made in Russia. The method was tested on different foods: bakery and confectionery, cheese and dairy products, as well as sausage samples. The sensitivity of this method is quite high; it is 0.02 μg/kg [29].

The analog of "Ecotest VA" is the AVA-3 voltammetric analyzer, designed by Burevisnik Research and Production Company (St. Petersburg, Russia). A significant advantage of the AVA-3 analyzer is that it allows the selenium to be detected not only in different types of foodstuffs but also in feeds, medicines, biological objects (blood, urine). Measurement of selenium occurs in the aqueous medium of a mineralized sample, which is quite optimal, given the ability of selenium to form volatile compounds. The analyzer operation is completely focused on a personal computer [30].

Summing up, we can conclude that the most universal method with good reproducibility and accuracy, which could be used for detecting selenium in such a food product as chickpea flour, is an inversion voltammetry method.

Since the detailed data on resolving the above-mentioned issues are lacking, it is necessary to deepen and expand research in this field.

3. The aim and objectives of the study

The aim of this study is to devise a technology for producing chickpea flour enriched with selenium. This would make it possible to develop new products, culinary meals, food rations that could neutralize the selenium deficiency conditions experienced by people and preserve the health of the nation.

To accomplish the aim, the following tasks have been set:

– to study the content of the mass fraction of selenium in the soaked chickpea grains with a different protein content depending on the concentration of selenium in the solution and the soaking time;

– to investigate the distribution of selenium in the anatomical parts of sprouted grain;

– to examine the dependence of change in the amino acid composition of chickpea flour made from native grains and sprouted in a solution of sodium hydroselenite;

– to devise a technological protocol for making chickpea flour enriched with selenium;

– to investigate the organoleptic, physical-chemical, microbiological quality indicators of the developed chickpea flour enriched with selenium.

4. Materials and methods to study the development of a chickpea flour technology and to examine quality indicators for the developed products

4. 1. Materials to study the mass fraction of selenium in chickpea grains

In the course of our research into the content of a mass fraction of selenium in the soaked chickpea grains we used the early-ripe chickpea variety "Krasnokutskyy 195", with a protein content of 22.92 %, a germination period of 95...105 days; and the medium-ripe chickpea variety "Yugo-Vostok", with a protein content of 15.95 %, a germination period of 115...125 days, the harvest of 2018 from the farm "Agrotek" in the city of Kyiv (Ukraine).

The characteristics of steeping solutions are given in Table 1.

Table 1

Characteristics of steeping solutions (per 1,000 cm^3 of H_2O)

No. of entry	Selenium content in solutions (1 g $NaHSeO_3$ is a carrier of 0.52 μg/g of selenium)			
1	Content of $NaHSeO_3$, g	10.4	26	39
2	Content of selenium, μg	20	50	75

The prepared solutions could be used for 72 hours, the specified period is followed by the appearance of a vitreous film at the solutions' surfaces and the emergence of an unpleasant odor.

4. 2. Methods to study the content of the mass fraction of selenium in the grain and the anatomic parts of a sprouted grain

The mass fraction of selenium in the grain, cotyledons, shoots was determined using the voltammetric analyzer "AVA-3", which is equipped with an indicator electrode, an auxiliary electrode, an electrode of the type comparison.

The method of selenium detection is based on the electrochemical reduction of Se (IV) to Se (0); the registration of an analytical signal in the sweep stage is the result of electrochemical reaction Se (0) to Se (IV). We determined the mass concentration of selenium in the examined sample using a method of standard additions. The sample batch was treated with a solution of potassium hydroxide, burned at the electric stove, then, by using the system for microwave ashing "PHOENIX" (Daewoo, China). The resulting ash was mixed in water, neutralized to a pH of 4...6, and centrifuged. The resulting mass was introduced to an electrochemical cup with a background solution and performed measurements. Based on the results, we calculated the mass share of selenium.

4. 3. Methods to study the amino acid composition of chickpea flour

We analyzed the amino acid composition of the examined samples by the method of ion exchange and liquid chromatography at the amino acid analyzer AAA T-339M (Czech Republic) and the liquid chromatograph LC-20 (TM Shimadzu, Japan).

The batches weighing 0.3 g were poured with 10 cm^3 of distilled water and 10 cm^3 of concentrated hydrochloric acid. The samples were placed into a dry-heat chamber with a temperature of 130 °C for 8 hours. Then we filtered it through the filter and washed with distilled water. The resulting solution was poured into a porcelain cup and evaporated at an electric stove to a volume of 0.5...1.0 ml. We measured the pH (optimal – 2.2±0.02 units). The resulting sample was poured through a membrane filter with a diameter of 0.45 μm. It was injected into a chromatographic ion-exchange column of the analyzer AAA T-339 M. The analysis was conducted automatically and lasted for 115 minutes. Upon completion of the analysis, the acquired chromatogram was decoded and we calculated the areas of peaks for each amino acid. Tryptophan at the acidic hydrolysis of protein is almost completely decomposed, so we determined it the liquid chromatograph LC-20 by TM Shimadzu. The sample was subjected to alkaline hydrolysis (NaOH at 100 °C, 16...18 h, in the presence of 5 % tin chloride). The hydrolysate, after neutralization by a mixture of citric and hydrochloric acids (to prevent gelatinating), was analyzed at an amino acid analyzer.

4. 4. Methods for devising the technological protocols of chickpea flour production

During the development of a technological protocol for chickpea flour enriched with selenium, we selected a technological protocol for the production of chickpea flour from sprouted grains as control. It includes the sorting and cleaning chickpea grains from impurities. Water and air treatment over 42...52 hours. Drying at a temperature of 50...75 °C to humidity 8...10 %. Drying at a temperature of 100...115 °C, to humidity 6...8 %, peeling, separation of shoots and roots, grinding to pass through a silk sieve No. 35.

4. 5. Methods to study the organoleptic, physical-chemical, microbiological quality indicators of the developed chickpea flour enriched with selenium

We determined the organoleptic indicators such as color, smell, taste in line with DSTU 7662.

The physical-chemical parameters were determined according to the following procedures:

– the mass share of moisture was determined using a portable electronic moisture meter, whose principle of operation is based on measuring the capacitance and high frequency, together with compression and automatic temperature compensation. “Super Matic”, (Foss Electric, Denmark), “Brabender” drying chamber (Rotex, Poland), the vacuum-thermal device “OVZ-1” (Rotex, Poland), based on the method described in DSTU 7621;

– the mass share of fat was determined based on the content of fat in a fat-free residue, according to the “Rushkovskiy” method, described in DSTU 7458;

– the mass fraction of crude protein was determined at a device from the system “Kjeltec Auto 1030 Analyzer” (“Falling Number”, Sweden), according to the methodology described in DSTU 7169;

– the mass fraction of total ash was determined by a method of ashing, the used accelerator was nitric acid. A sample of chickpea flour was ashed by roasting at free access of air. Carbon, hydrogen, nitrogen and partially oxygen are evaporated, leaving only minerals in the form of oxidative compounds. According to the method described in GOST 13979.6;

– the mass fraction of fiber was determined by treating a batch of chickpea flour with 1.2 % of sulfuric acid in one liter of distilled water. We injected 7 ml of concentrated sulfuric acid with a density of 1.8 g/cm^3 and added water to the solution to a tag; – 2.5 % solution of caustic sodium. We dissolved alkali based on the ratio of 30 g per 1 liter of distilled water. The concentration of the caustic sodium solution was determined as follows: 2.5 % solution of caustic sodium is 0.64 N solution; –ethyl alcohol, 96 %; –diethyl ether;

– the mass share of gluten was determined at the device “Glutomatic” (“Falling Number”, Sweden), according to the methodology described in DSTU ISO 21415-1:2009;

– the content of toxic elements, namely: the content of lead, cadmium, copper, zinc, was determined according to the procedures described in DSTU 31262. Mercury content – in accordance with MU 5178; arsenic content – in accordance with GOST 30178;

– the microbiological indicators such as the number of mesophilic aerobic and facultative anaerobic microorganisms were determined in line with a procedure described in DSTU 8446. The bacteria of the group *Escherichia coli* were determined according to DSTU ISO 4832. The content of pathogenic microorganisms, bacteria of the genus *Salmonella* were determined according to the procedure described in DSTU 12824. Mold fungi and yeast content were determined according to the procedures described in DSTU 8447.

5. Results of studying the technology for producing chickpea flour enriched with selenium and examining quality indicators of the resulting products

5. 1. Studying the content of a mass fraction of selenium in chickpea grains

The results of our experimental study are given in Table 2.

Table 2

Content of the mass fraction of selenium in the soaked chickpea grains with a different protein content, depending on the concentration of selenium in the solution and the soaking time

No. of entry	Chickpea variety	Protein content, %	Selenium concentration, µg/g				
			0	20	50	75	Organoleptic indicators, points $X^1/X^2/X^3$
Selenium content in sprouted grain, 12 hours, µg/g							
1	Krasnokutskyy 195	22.92	–	9	15	24	5/5/5
2	Yugo-Vostok	15.95	–	6	12	19	5/5/5
Selenium content in sprouted grain, 24 hours, µg/g							
1	Krasnokutskyy 195	22.92	–	13	21	32	5/5/5
2	Yugo-Vostok	15.95	–	10	18	28	5/5/5
Selenium content in sprouted grain, 48 hours, µg/g							
1	Krasnokutskyy 195	22.92	–	22	35	52	5/5/5
2	Yugo-Vostok	15.95	–	17	29	49	5/5/5
Selenium content in sprouted grain, 72 hours of steeping, µg/g							
1	Krasnokutskyy 195	22.92	–	31	39	55	4/3/3
2	Yugo-Vostok	15.95	–	24	32	50	4/3/2

Note: organoleptic indicators: X^1 – 12 h of steeping; X^2 – 24 h of steeping; X^3 – 48 h of steeping. Values of organoleptic indicators in points: 1 – very poor, not to be used, 90 % of the grains are blackened, rotten; 2 – ≤70 % of grains are spoiled, blackened, rotten; 3 – ≤30 % of grains are spoiled, blackened; 4 – ≥10 % of grains are spoiled; 5 – grains are suitable for the manufacture of flour

Fig. 1 shows a change in the organoleptic indicators of flour depending on the time of soaking and the concentration of sodium hydroselenite in the solution for steeping.

It was established that the content of selenium in flour made from sprouted grains after 12 hours of steeping, for the chickpea variety “Krasnokutskyy 195”, increases to 9, 15, 24 μg/g, in the solutions with a concentration of selenium of 20, 50, 75 μg, respectively. For the chickpea variety "Yugo-Vostok", one observes an increase of 6; 12 19 μg/g, in the solutions with a concentration of selenium of 20, 50, 75 μg, respectively. The tendency to an increase in the content of selenium in flour made from steeped grains is observed in all examined samples, which were steeped for 24, 48, 72 hours; however, when steeping during 72 hours with a concentration of selenium of 20 μg and above, there is a deterioration of the organoleptic characteristics of grains – there appear spoiled, blackened grains that affect the color of the resulting flour (Fig. 1, *d*).

a *b* *c* *d* *e*

Fig. 1. Change in the organoleptic indicators of flour made from sprouted grains of chickpea depending on the time of steeping and the concentration of sodium hydroselenite in the solution for steeping: *a* – steeped for 12 hours, mass share of selenium is 24 μg/g; *b* – steeped for 12 hours, in an aqueous solution (control); *c* – steeped for 24 hours, mass share of selenium is 32 micrograms/g; *d* – steeped for 48 hours, mass share of selenium is 52 μg/g; *e* – steeped for 72 hours, mass share od selenium is 55 μg/g

5. 2. Studying selenium distribution in the anatomical parts of sprouted grain

The issue, which characterizes the effectiveness of this development, is to determine the degree of selenium localization in a protein fraction; this is important because the organic compounds of selenium – selenium-containing amino acids have the greatest bioavailability and degree of retention in the human body. Therefore, we examined selenium distribution in the anatomical parts of the sprouted grain. The study results are given in Table 3.

Table 3

The distribution of selenium in the anatomical parts of the sprouted grain (steeped for 48 hours, the $NaHSeO_3$ concentration was 39 g/1,000 cm^3 H_2O), chickpea variety "Krasnokutskyy 195"

No. of entry	Anatomical part of the sprouted chickpea grain	Selenium content, μg/g		
		Control	Krasno-kutskyy 195	Yugo-Vostok
1	Cotyledons	traces	51±0.2	42±0.3
2	Shoots	–	4±0.3	7±0.3
3	Sprout-ed grain (whole)	traces	55±02	49±0.3

The cotyledons in grains of the chickpea variety "Krasnokutskyy 195" and "Yugo-Vostok" accumulate 51 and 42 μg/g selenium, while the sprouts have only 4 and 7 μg/g, which indicates the biotransformation of microelement into the protein fraction, possibly into the organic form.

5. 3. Studying the dependence of change in the amino acid composition of chickpea flour

One of the most important indicators that characterize the biological value of chickpea flour is its amino acid composition. We examined the dependence of change in the amino acid composition of flour made from the chickpea variety "Krasnokutskyy 195", obtained from the native grain, sprouted in an aqueous solution and sprouted in a solution of $NaHSeO_3$ for 48 hours, with the concentration of $NaHSeO_3$ of 39 g/1,000 cm^3 H_2O.

The study results are given in Table 4.

The content of leucine, lysine, arginine, and tryptophan increases by 87, 76, 80 %, and 55 %, respectively. The base of the substituted amino acids are aspartic and glutamic acids and their amides, whose share in the non-germinated chickpea grains is 67 %, and in the grains sprouted for 48 hours – 70 %. The total content of amino acids in the flour made from the grains germinated in the sodium hydroselenite solutions increases by 58 μg/g of dry substances compared to flour made from the grains sprouted in an aqueous solution.

Table 4

Dependence of change in the amino acid composition of chickpea flour made from the native grains and sprouted in a solution of sodium hydroselenite for 48 hours

No. of entry	Amino acids	Chickpea grains' content, μg/g dry substances		
Essential amino acids:		Native grain	Sprouted in an aqueous solution	Sprouted in 39 g $NaHSeO_3$/1,000 cm^3 H_2O
1	Valine	14.3	23.2	28.7
2	Isoleucine	10.5	19.3	21.5
3	Leucine	20.3	34.5	38.0
4	Tyrosine+phenylalanine	22.7	42.1	44.7
5	Lysine	21.7	34.4	38.2
6	Methionine	2.7	4.1	5.3
7	Threonine	8.0	11.9	16.9
8	Tryptophan	3.6	4.2	5.6
9	Arginine	17.2	26.7	31.0
10	Histidine	7.4	13.2	16.7
Non-essential amino acids				
11	Cysteine	1.8	2.5	3,7
12	Alanine	10.9	19.0	22,2
13	Aspartic acid+ asparagine	30.2	59.1	67,6
14	Glycine	9.6	13.2	15,0
15	Glutamic acid+ glutamine	43.0	82.0	87,2
16	Serine	13.1	20.6	25,7
Total content of amino acids		237.0	410.0	468.0

5. 4. Methods to develop a technological protocol for producing chickpea flour enriched with selenium

When developing a technological protocol for the production of chickpea flour enriched with selenium, the chosen control was a technological protocol for producing the sprouted chickpea flour.

The technological protocol for producing chickpea flour enriched with selenium is shown in Fig. 2.

The devised technological protocol differs from the control one by that the washing and disinfection of chickpea grains involve an aqueous solution of citric acid (pH 3.5...4.0). Chickpea grains contain about 2 % of phytic acid [31], which prevents the absorption of mineral substances; in order to increase the absorption of selenium, chickpea was preliminary washed in an acidic environment to inactivate the phytic acid. The enzyme phytase, formed during steeping, neutralizes phytic acid. Chickpea is also subjected to the process of removing an unwanted "nut" odor.

Another difference from the control technology is steeping the chickpea grains in a solution of $NaHSeO_3$ (the concentration of selenium in a solution is 20...75 μg/g for up to 48 hours. The devised technological protocol for producing chickpea flour enriched with selenium does not require any specialized technological equipment.

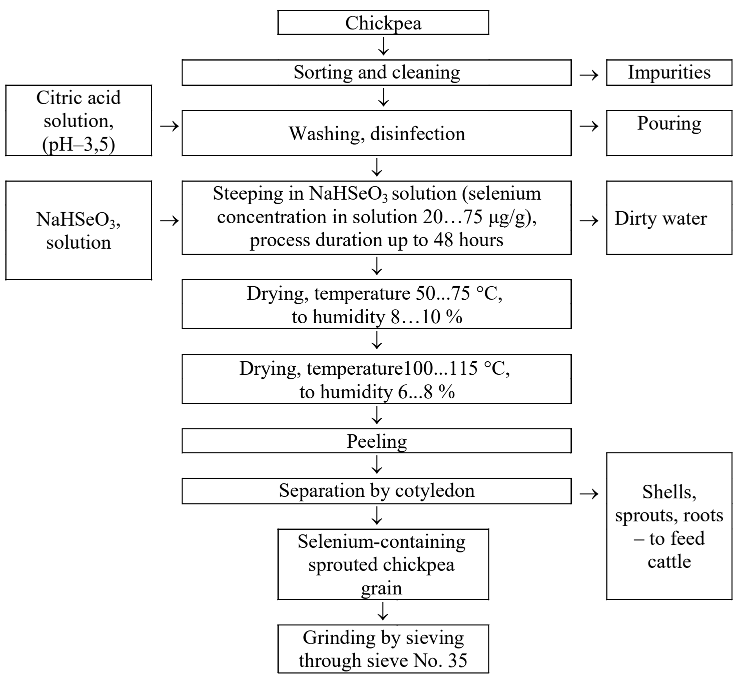

Fig. 2. Technological protocol for producing chickpea flour enriched with selenium

5. 5. Studying the organoleptic, physical-chemical, and microbiological quality indicators of the chickpea flour enriched with selenium

The results of studying the organoleptic parameters of the chickpea flour enriched with selenium are given in Table 5.

Data in Table 5 show that the developed chickpea flour has a light-yellow color, the smell that is characteristic of chickpea flour, the taste without bitterness and sour flavors. In terms of color, the experimental sample is different from the control sample, which has a lighter-creamy color (Fig. 1, *b*, *d*); however, according to the normative and technical documents for chickpea flour (DSTU 2209-93), such deviations are allowed.

Table 5

Organoleptic indicators of chickpea flour enriched with selenium

Indicator	Permissible norm based on ND	Control	Experiment	Compliance with norms
Color	from light-yellow to creamy	creamy	light-yellow	positive
Smell	inherent in chickpea flour without foreign smells	no foreign smells	no foreign smells	positive
Taste	no bean aftertaste, no bitterness, not sour	without bitterness, no sour and foreign flavors	without bitterness, no sour and foreign flavors	positive

Note: ND – normative documents

The physical-chemical indicators of the developed chickpea flour are given in Table 6.

Table 6

Physical-chemical characteristics of chickpea flour enriched with selenium

Indicator	Permissible norm based on ND	Control	Experiment	Compliance with norms
Humidity, %	8.0	7.0	6.0	positive
Fat content, %	15.0	14.0	12.0	positive
Protein content, %	40.00	22.92	39.70	positive
Ash content, %	7.0	4.0	4.5	positive
Gluten content, %	4.5	2.5	3.0	positive
Selenium content, μg/g	80	traces	52	positive

The developed flour differs from the control sample by such indicators as a mass fraction of moisture (1 % less than the control sample) and a mass fraction of fat, which decreases by 2 %. The differences are observed in the indicators for a mass fraction of the total ash and the mass fraction of fiber towards a 0.5 % increase for two indicators.

The developed flour is the carrier of 52 μg/g selenium, which corresponds to 65 % of the daily requirement in this microelement.

Results from determining the content of toxic elements and microbiological indicators in the chickpea flour enriched with selenium are given in Table 7, 8.

As one can see from Table 7, in terms of the mercury, arsenic, and lead content the developed chickpea flour does not exceed the levels allowable to human intake, it does not contain cadmium and it has a smaller content of copper than the permissible level, by 1 mg/g.

Table 7

Content of toxic elements in the chickpea flour enriched with selenium

Indicator	Permissible norm, not exceeding	Actual amount	Compliance with norms
Mercury, ppm	0.02	≥0.02	positive
Arsenic, ppm	0.2	≥0.2	positive
Copper, ppm	10	9	positive
Lead, ppm	0.5	≥0.5	positive
Cadmium, ppm	0.1	–	positive
Zinc, ppm	50.0	50.0	positive

Table 8

Microbiological indicators of the chickpea flour enriched with selenium

Indicator	Permissible norm	Actual amount	Compliance with norms
Number of mesophilic aerobic and facultative anaerobic microorganisms, CFU per 1 g	0.1×10^5	0.1×10^5	positive
Bacteria of the group of *Escherichia coli*	not allowed	not detected	positive
Pathogens, bacteria of the genus *Salmonella*, per 25 g	not allowed	not detected	positive
Mold fungi, CFU per 1 g	0.1×10^2	0.1×10^2	positive
Yeasts, CFU per 1 g	0.1×10^2	0.1×10^2	positive

The results of studying the microbiological indicators of the chickpea flour enriched with selenium (Table 8) allow us to assert that in terms of the number of mesophilic aerobic and facultative anaerobic microorganisms, mold fungi, and yeasts the experimental samples of the developed flour are safe for use. They do not contain bacteria from the group of *Escherichia coli* and pathogens of bacteria of the genus *Salmonella*.

The summary of our study, chapter 5. 5, gives grounds to assert that the developed flour, according to DSTU 2209-93, is compliant, for all quality indicators, with the permissible norms and meets the requirements of normative-technical documents for food chickpea flour.

6. Discussion of results of studying the development of a technology for the chickpea flour enriched with selenium and quality indicators of the enriched flour

Having studied the content of the mass fraction of selenium in soaked chickpeas with different protein content, the concentration of selenium in the solution and the time of soaking . It is established that the rational range of concentrations of sodium hydroselenite in the solution is up to 39 g, soaked for no more than 48 hours, which corresponds to the selenium content of 20...75 µg/g. Increased concentration leads to microbiological spoilage of the grain mass and changes in the color of the flour: there appear the dark inclusions of parts of the blackened grains (Fig. 1, *d*). Chickpea grains are able to accumulate a micro-element whose content depends on the protein content in the native grain. We assume that the solutions of sodium selenite affect the permeability of the membranes of seed cells in chickpea, contribute to the loosening of their shells, which leads to the active diffusion of the ions of selenium from the solution into the inner space of the seed. It is also possible to assume that there is a process of assimilation of the inflow of ions, the result of which is the formation of new bioavailable organic selenium-containing compounds (Table 2).

Having examined the distribution of selenium in the anatomical parts of the sprouted grains, it has been proven that in the sprouted grains of chickpea 95...99 % of selenium is in a cotyledon, possibly in the protein fraction. This indicates a high degree of selenium conversion into an organic form during steeping in a $NaHSeO_3$ solution.

We have investigated the dependence of change in the amino acid composition of the chickpea flour made from native grains and sprouted in a solution of sodium hydroselenite. It allows us to assert that the grains of chickpea contain all essential amino acids and our inferior to perfect protein only in terms of quantitative content. The content of essential amino acids such as valine, isoleucine, tyrosine+phenylalanine, methionine, threonine, histidine in the grains germinated in a solution of sodium hydroselenite for 48 hours increases by almost 2 times (Table 4). We assume the activation of the enzyme system of the grain, which contributed to the change in the amino acid composition and accumulation of selenium; given the high hydrophilic protein capacity, the mass of the steeped grains increased two-fold.

We have devised a technological protocol for producing chickpea flour enriched with selenium. It has been proven that the proposed technological production scheme does not require any changes in the conventional sequence of stages or any specialized technological equipment. It could be implemented at any enterprise that carries out grain germination.

The organoleptic, physical-chemical, and microbiological quality indicators of the developed chickpea flour enriched with selenium have been examined; it was found that in terms of the organoleptic indicators the developed flour differs by color and has a yellower color compared to control. No bitterness of sour and foreign taste.

According to the physical-chemical indicators, the developed chickpea flour contains 12 % of fat, 39.7 % of protein, 4.5 % of ash, 3 % of fiber at a 6 % moisture content. The content of selenium is 52 µg, which covers 65 % of the daily requirement for selenium (Table 6). The developed flour contains 0.02 ppm of mercury, 0.2 ppm of arsenic, 9 µg/g of copper, 0.5 ppm of lead and 50 ppm of zinc, and is compliant with the permissible norms, which indicates that the content of selenium in chickpea flour does not affect its safety indicators. The microbiological indicators of the developed chickpea flour are within 0.1×10^5 CFU per 1 g of mesophilic aerobic and facultative anaerobic microorganisms, 0.1×10^2 CFU per 1 g of mold fungi and yeasts, which is within a normal range. The developed flour, according to DSTU 2209-93, is compliant with permissible norms for all quality indicators and complies with the requirements of the normative and technical documents regarding food chickpea flour (Table 5–8).

It is advisable to use the devised technology for the production of culinary meals for sanatoriums, preventoriums, hospitals, and restaurant establishments. A healthy person and a person suffering selenium-deficiency could consume 50...100 g of the chickpea flour enriched with selenium, which would satisfy 33...65 % daily requirement for a given microelement.

Increasing the concentration of eating selenium-containing flour can affect the human body in different ways. According to the WHO recommendations, a toxic dose is 900 µg per day (the average daily human need for selenium varies from 70 to 100 µg). Given that the level of safe intake of inorganic selenium is much lower than the level of its organic forms, it is possible to assume safe consumption while increasing the recommended doses. However, clinical studies into the biological efficacy of the developed products are the prospects for further research.

7. Conclusions

1. We have examined the mass fraction of selenium in the steeped chickpea grains with a different protein content depending on the concentration of selenium in the solution and the steeping time. It was found that the degree of accumulation of selenium is affected by the content of protein in the native grain: the greater the protein content the better the accumulation of selenium by a sprouted grain. It is established that it is rational to use solutions that are carriers of 20...75 µg of selenium soaked for up to 48 hours.

2. Our study of selenium distribution in the anatomical parts of the sprouted grain has shown that in the composition of the sprouted chickpea grain 95...99 % of selenium is in a cotyledon, in the protein fraction. This indicates the high degree of selenium conversion into the organic form during steeping in a solution of $NaHSeO_3$.

3. The germination of chickpea grains changes the amino acid composition. The content of essential amino acids, valine, isoleucine, tyrosine+phenylalanine, methionine, threonine, histidine, in the grains germinated in sodium hydroselenite for 48 hours increases by almost 2 times. The content of leucine, lysine, arginine, and tryptophan increases by 87, 76, 80 %, and 55 %, respectively. The base of the substituted amino acids are aspartic and glutamic acids and their amides, whose share in the non-germinated chickpea grains accounts for 67 %, and in the grains germinated in the solutions of sodium hydroselenite for 48 hours – 70 %. The total content of amino acids in the flour made from grains germinated in the solutions of sodium hydroselenite is increased by 58 µg/g of dry substances compared to the flour made from grains germinated in an aqueous solution.

4. The devised technological protocol for producing chickpea flour enriched with selenium does not need to change the conventional sequence of stages or any specialized technological equipment and could be implemented at any enterprise that performs grain germination.

5. The developed flour, in terms of its organoleptic indicators, differs in color and has a yellower color compared with control. No bitterness of sour and foreign taste. By its physical-chemical indicators, it contains 12 % of fat, 39.7 % of protein, 4.5 % of ash, 3 % of fiber at a 6 % humidity. The content of selenium is 52 µg, which covers 65 % of daily demand in selenium. In its composition, the flour contains 0.02 ppm of mercury, 0.2 ppm of arsenic, 9 µg/g of copper, 0.5 ppm of lead and 50 ppm of zinc: these are the permissible norms, indicating that the content of selenium in chickpea flour does not affect its safety indicators. The microbiological indicators are within 0.1×10^5 CFU per 1 g of mesophilic aerobic and facultative anaerobic microorganisms, 0.1×10^2 CFU per 1 g of mold fungi and yeasts, which is within normal limits. The developed flour meets the requirements of normative technical documentation.

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