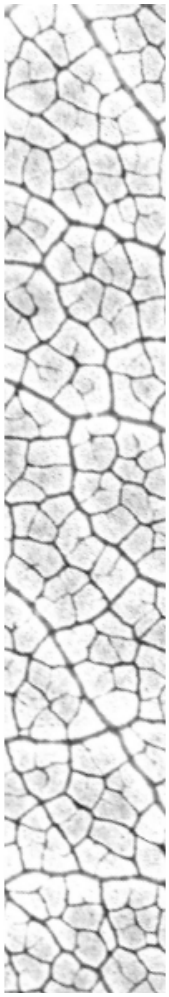

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Physical and chemical profile and food safety of gluten free bread

Botagoz Kulushtayeva [1*], Gulnur Nurymkhan [1], Nina Burakovskaya [2], Maxim Shadrin [2], Tatyana Smirnova [3], Oksana Sagina [3], Marina Mirgorodskaya [3], Sergey Smirnov [4]

[1] Shakarim State University of Semey, KAZAKHSTAN
[2] Omsk State Technical University, Omsk, RUSSIA
[3] K.G. Razumovsky Moscow State University of Technologies and Management (the First Cossack University), Moscow, RUSSIA
[4] Russian State Agrarian Correspondence University, Balashikha, RUSSIA
*Corresponding author: Botagoz Kulushtayeva

Abstract
The article presents the physical and chemical profile of gluten free bread, comprising gluten free composite flour, eggs, dry yeast, salt and water. The composite flour consists of 50% wheat starch, 25% amaranth and 25% chickpea flour. The composite flour production scheme and gluten free bread process flow diagram are designed. The gluten free bread is characterized by its higher weight, volume and porosity values as compared with wheat bread. Developed gluten free bread does not contain toxic elements, mycotoxins. The concentration of cesium-137 and strontium-90 is in low quantity and far less than regulation standards.

Keywords: gluten, flour, bread, amaranth, chickpea, safety, toxic elements

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INTRODUCTION

The development of the production of food products, enriched with essential nutrients, specialized functional foods, as well as dietary (therapeutic and prophylactic) is one of the priorities of the state policy in the field of healthy nutrition (Shansharova and Abdraimova 2011). Nowadays, the production of specialized food products is rapidly growing, including food products that are free from certain ingredients. These ingredients (allergens, certain types of proteins, oligosaccharides, polysaccharides, etc.) are not recommended for certain medical reasons. Taking into account the success of nutrigenomics and nutrigenetics, the trend towards individualization of diets will increase, which will lead to an increase in the market of specialized foods. Gluten-free foods are one of the segments of this market (Hamer 2005).

In the past few years, a gluten-free diet has been widespread dramatically in popularity. Gluten-free foods are considered equivalent to healthy eating, along with low-sugar and low-carbohydrate foods (Kulushtayeva et al. 2019). Gluten-free foods, in fact, can bring great benefits when it comes to health. Many people have gluten intolerance or hypersensitivity to it, which can cause many negative side effects, from digestive problems to mood changes. Eating foods containing gluten is dangerous for patients with celiac disease (Padalino et al. 2017).

There are several types of gluten-free flour, one of them is amaranth flour. It is important to note that amaranth flour and products from it have a prophylactic effect on many systems of the body: they reduce cholesterol levels, improve the condition of the arteries, reduce the risk of cardiovascular and oncological diseases, promote slag removal (Derkanosova et al. 2018, Zvyagin et al. 2015). The special value of amaranth is the presence in its composition of a substance such as squalene, which has a strong antioxidant effect.

Next gluten-free flour is chickpea flour, which is the basis for the production of Indian sweets, thick soups, fritters and other original dishes (Tertychnaya et al. 2010, Timofeev 2014). Being a source of potassium, zinc, calcium and protein, chickpea also contains a lot of soluble dietary fiber, which are necessary for health and proper life of human. It is proved that the introduction of chickpea flour in human diet improves the digestive processes. **Table 1** presents the chemical composition of different types of flour.

The purpose of this study is to develop the technological scheme of production of composite flour and gluten free bread from amaranth and chickpea flour

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Table 1. Chemical composition of different types of flour

Flour type	Moisture, %	Protein, %	Fat, %	Carbohydrates, %	Fibers, %	Ash, %
Wheat flour	14.0	10.3	1.1	70.6	3.5	0.5
Gluten-free flour						
Amaranth flour	14.0	9.5	3.9	67.8	1.1	2.8
Buckwheat flour	9.0	13.6	1.2	71.9	2.8	1.5
Corn flour	14.0	7.2	1.5	72.1	4.4	0.8
Chickpea Flour	14.0	20.1	4.3	48.4	10.2	3.0
Rice flour	9.0	7.4	0.6	80.2	2.3	0.5
Soybean flour	9.0	43.0	8.0	22.0	14.1	5.3
Lentil flour	7.6	21.3	0.6	48.5	2.3	2.3

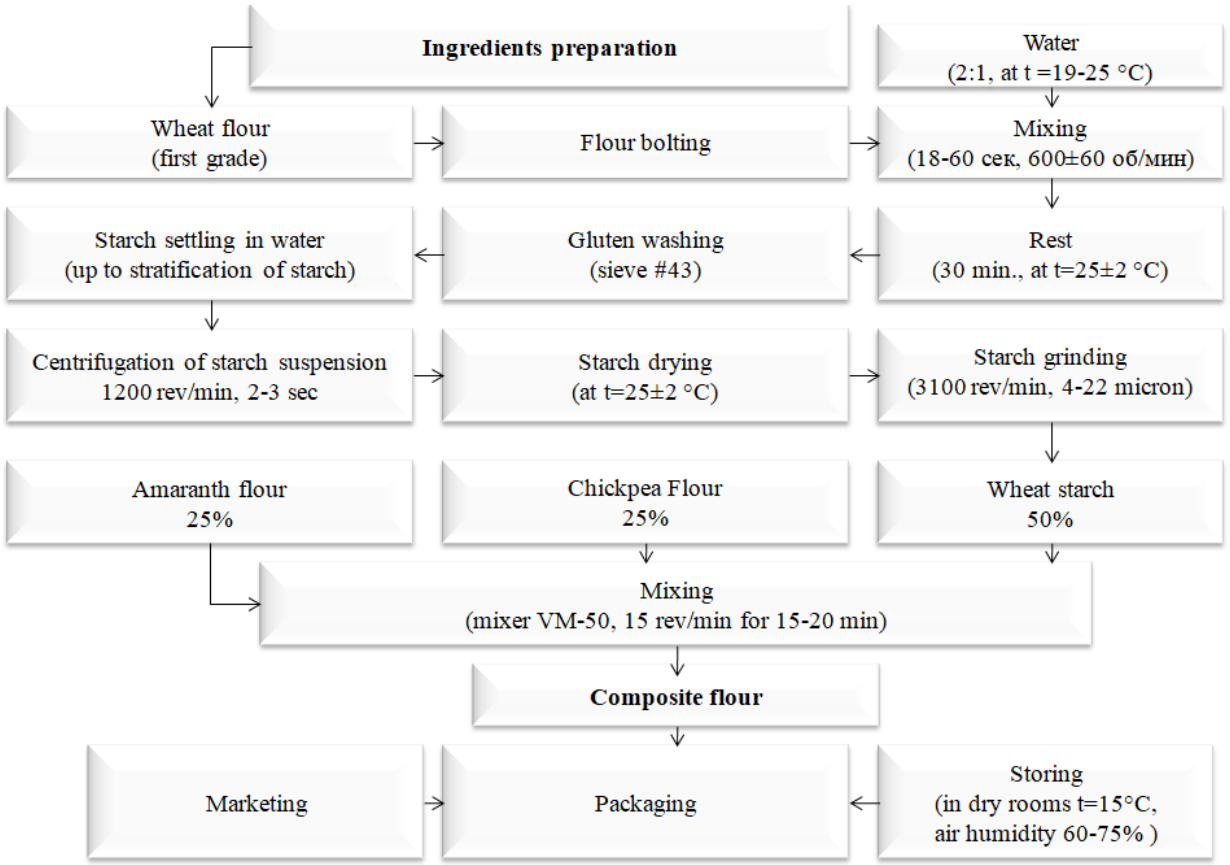

Fig. 1. Composite flour production scheme

and determine its physical and chemical profile and food safety.

MATERIALS AND METHODS

Based on the technological scheme for producing gluten-free composite flour, the first task is to obtain wheat starch from wheat flour. Wheat flour was sifted, then mixed with water and kneaded. After storing for 30 minutes, at a temperature of 25 ± 2 ° C, the gluten was washed off by using silk sieve #43.

Next, the starch dissolved in water was settled in the tank before separation. The water was drained, and the starch suspension was centrifuged and washed until pure starch was obtained. The starch was dried at room temperature 25±2 °C. The yield of starch from 1 kg of flour was 50%. Next, the dried starch was ground in a laboratory mill.

As can be seen, from the scheme (**Fig. 1**), the gluten-free amaranth and chickpea flour were added to the wheat starch. The composition was mixed for homogeneous distribution of ingredients. The gluten-free composite flour was packed on a packaging machine and stored in dry rooms, at a relative humidity of 60-75%, the room temperature should be no more than 15 °C.

The ingredients, such as, gluten-free composite flour, salt, water, yeast, eggs are used for making bread. The technological scheme of making bread consists of the following stages: ingredients preparation, dough kneading, forming, proving, during which the volume of dough increases due to fermentation, baking (**Fig. 2**).

The most important and long stage of making bread is dough making. According to the recipe, the

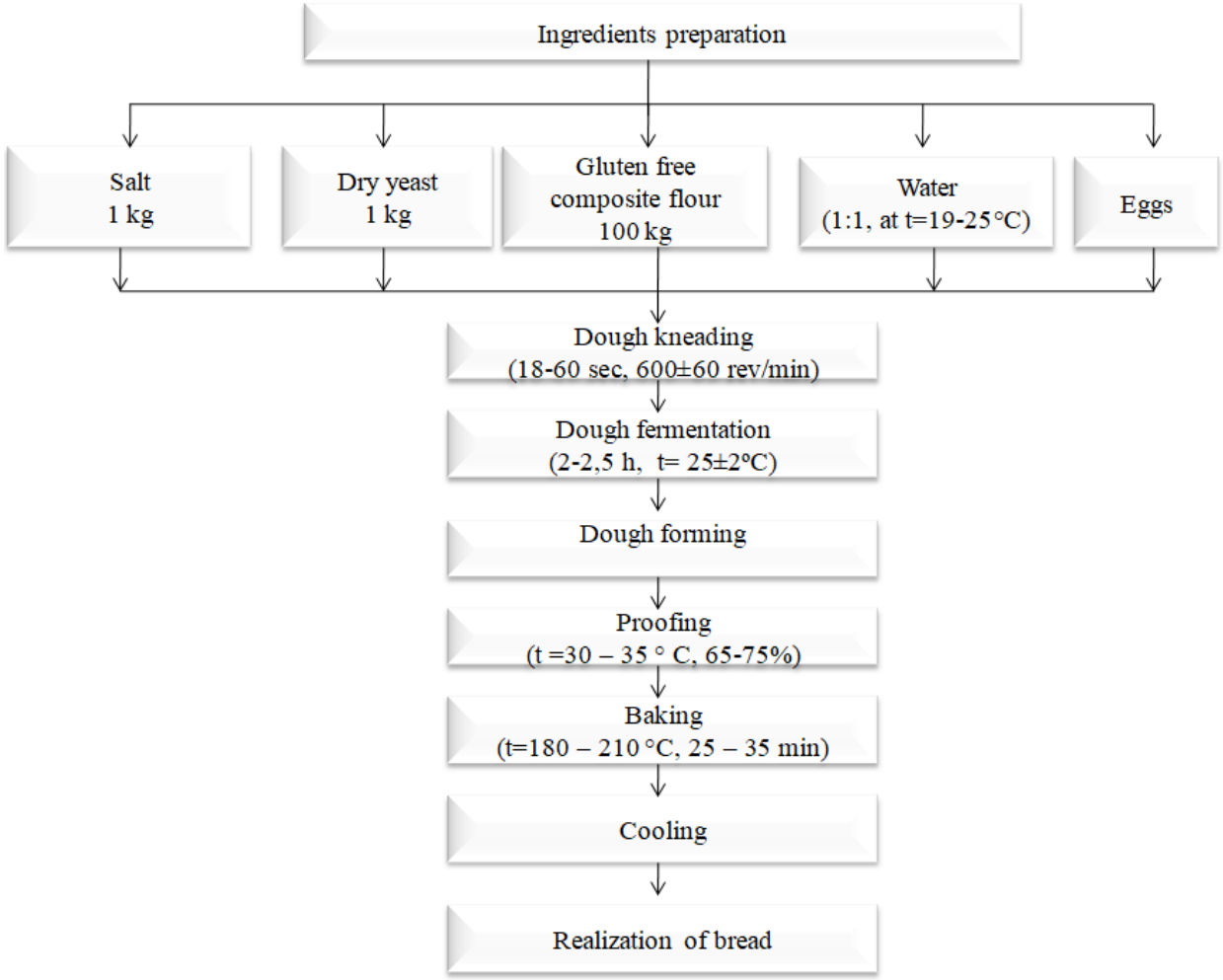

Fig. 2. Gluten free bread process flow diagram

ingredients are thoroughly kneaded and fermented for 2.5 to 3 hours. Then, the fermented dough is formed.

Bread is baked in special ovens at 180–220 °C, for 20–25 minutes. Baked bread is placed in trays and cooled for 2-2.5 hours and packaged.

Determination of Bread Porosity

Bread porosity is determined by using the Zhuravlev device which consists of the following parts:

a) a metal cylinder with an inner diameter of 3 cm and a pointed edge on one side;

b) wooden sleeve;

c) a wooden or metal tray with a transverse wall and a slot with 1.5 cm deep positioned at a distance of 3.8 cm from the wall.

A slice of bread with a width of at least 7 - 8 cm is cut from the center of the bread. From the crumb of the piece at a distance of at least 1 cm from the bread heel, the device is dredged with a cylinder. The sharp edge of the cylinder is pre-lubricated with vegetable oil. The cylinder is penetrated into a piece of crumb by rotation. The cylinder filled with the crumb is placed on the tray so that its rim fits snugly into the slot on the tray. Then the bread crumb is pushed out of the cylinder with a wooden sleeve for 1 cm and cut off at the edge of the cylinder with a sharp knife. A cut off slice of crumb is removed. The crumb left in the cylinder is pushed out to the wall of the tray and also cut off at the edge of the cylinder. To determine the porosity of wheat bread, 3 cylindrical cavities of 27 cm^3 each are made. The cut-off slices of the crumb are weighed with an accuracy of 0.01 g (National Standard GOST 5669-96).

The bread porosity is calculated by Equation 1:

$$x = \frac{\left(V - \frac{G}{p}\right)}{V} \times 100 \quad (1)$$

where: V — total volume of cut-off crumbs, cm^3; G — weight of cut-off crumb, g; p — density of bread.

Determination of Acidity

Bread acidity depends on the acidity of the flour from which it is baked. In addition, during the fermentation of the dough, lactic and acetic acids are also formed. The presence of organic acids in bread gives a taste and dietary value.

Table 1. Physical and chemical characteristics of bread

Bread	Weight, g	Volume, cm³	Bread porosity, %	Bread acidity, ⁰
Wheat bread	110	140	36.6	2.2
Gluten-free bread	115	150	37.35	2.2

1. To determine the acidity, 25 g of crushed crumb is placed in a dry wide neck bottle (500 ml) with a well-fitted stopper.
2. A volumetric flask (250 ml) is filled with distilled water and ¼ is poured into a bottle with crumb.
3. The crumb is mixed with glass rod with a rubber into smooth homogenous paste, periodically adding distilled water.
4. The bottle is closed with the stopper and shaked vigorously for 2 minutes, then let rest for 10 minutes.
5. After this, the bottle is shaken again and left for 8 minutes.
6. The pooled top layer of liquid is poured into a dry beaker through gauze. Then 50 ml of liquid is pipetted into a 100 ml flask, 3 drops of phenolphthalein are added and then titrated with 0.1 N sodium hydroxide solution until slightly rosy coloring (Skuratovskaya 2001).

Acidity (X) is calculated according to Equation 2:

$$X = \frac{V \cdot V_1 \cdot \alpha}{10m \cdot V_2} \cdot K \quad (2)$$

V – volume of 0,1 mole/dm³ molarity of sodium hydroxide solution or potassium hydroxine, used during the titration of the test solution, cm³;

V_1 – volume of distilled water required for acid extraction from the tested sample, cm³;

α - conversion factor for 100 g of sample;

K - coefficient of correction of sodium hydroxide or potassium hydroxine to the 0.1 mole/dm³ solution

1/10 - coefficient of correction of sodium hydroxide or potassium hydroxine of 0.1 mole/dm³ molarity to 1.0 mole/dm³.

m- weight of sample, g;

V_2 - volume of test sample, cm³.

Determination of Rheological Properties

The rheological properties of the bread dough is determined by alveograph in accordance with the National Standard GOST 28795 (ISO 5530). The summary of test method consists in measuring and recording the changes in pressure inside a bubble in a test sample of a certain thickness. Alveograph is used to study the rheological characteristics of a non-fermenting dough. These dough properties are evaluated according to the alveograph curves.

The samples of dough kneaded are prepared by kneading flour and 2.5% aqueous solution of salt. The ratio of flour and salt solution is taken of 250 g of flour with a moisture content of 14.4% and 127.7 ml of saline solution. The dough should have a temperature of 25 °C. The dough kneading in the alveograph kneader lasts 8 minutes, after which the dough is pushed out by a special device through the kneader's outlet orifice onto the receiving plate. Formed standard-sized dough disks are kept in the alveograph thermostat at 25 °C. The alveograph test is performed after 26 minutes of kneading beginning.

Table 2. Bread's crumb characteristics

Indicator	Description
Baking rate	Well baked, dry, elastic
Dough mixing	Without lumps and unmixed ingredients
Porosity	Without holes and tighten
Taste	Sweety
Flavor	Meet the requirements for bread

Microbiological evaluation of the gluten free bread was performed according to the National Standard GOST 10444.15-94 "Determination of the number of mesophilic aerobic and facultative anaerobic microorganisms"; GOST 31747-2012 "Determination of the coliform bacterium group (coli-form)", GOST 10444.12201 "Mold determination", MU 2142-80 "Determination of pesticides (α, β, γ - isomers), dichlorodiphenyltrichloroethane". Gas chromatograph "Crystallux-4000M" was used to quantify these indicators.

Determination of Toxic Elements

The concentration of lead was determined according to National Standard GOST 26932-86; cadmium by GOST 26933-86; mercury by GOST 26927-86 and arsenic by GOST 26930-86. The samples were analyzed using the "Atomic – Absorption Spectrometer Quantum Z.ETA" equipment.

RESULTS AND DISCUSSION

Important physical and chemical indicators of bread quality are moisture, porosity and acidity. High humidity reduces the nutritional value of bread, impairs its taste and reduces the shelf life. The higher the grade of flour, the lower the rate of moisture of the bread. Acidity affects the taste properties of bread. Not enough or too sour bread is unpleasant to taste. The higher the porosity of the product, the longer they retain their freshness and are better absorbed by the body. Well-aerated bread with uniform fine thin-walled porosity is better impregnated with digestive juices and therefore more fully digested (Kazakov and Karpilenko 2005, Yeliseeva 1987).

Physical and chemical characteristics of gluten free bread are presented in **Table 1**. The gluten free bread comparing with wheat bread has more weight and volume. The bread porosity was also higher.

Dough preparation is one of the most important operations in the bread production process. The quality of the bread depends on the properties of the dough.

Table 3. Effect of composite flour on rheological properties of dough

Sample	Indicators		
	Flour strength	Elasticity, (p) 1mm	Ratio p/l
Control (wheat flour dough)	259	87	0.95
Composite flour dough	194	30	1.56

Table 4. Concentration of toxic elements in gluten-free composite flour

Toxic element	Allowable concentration by regulation standards	Concentration in gluten-free composite flour
Lead	0.5	Not detected
Arsenic	0.2	Not detected
Cadmium	0.1	Not detected
Mercury	0.03	Not detected

Table 5. Concentration of mycotoxins in gluten-free composite flour

Mycotoxin	Allowable concentration by regulation standards	Concentration in gluten-free composite flour
Aflotoxin B1	0.005	Not detected
Deoxynivalenol	0.7	Not detected
T-2 toxin	0.1	Not detected
Zearalenone	0.2	Not detected
Ochratoxin A	0.005	Not detected

Table 6. Microbiological evaluation of gluten-free bread

Indicator	Allowable concentration by regulation standards	Concentration in gluten-free composite bread
Potato bacillus		Not detected
Total viable count, CFU/G	$1*10^3$	$1*10^3$
Escherichia coli group bacteria – CGB in 1.0 g	Prohibited	Not detected
Fungi, CFU/G	50	15

The physical, chemical and biochemical processes are directly affect to dough development time during the kneading (Krivosheev et al. 2018).

Studies of the physical properties of the dough showed that the introduction of composite flour with different dosages influence on the properties of flour, which is very important for the process of proofing and baking bread. Indicators of the structural-mechanical properties of the dough with composite flour are given in **Table 3**.

The elastic properties of the dough deteriorate when adding composite flour, in the case of amaranth and chickpea flour, wheat starch, as indicated by the indicator of elasticity to extensibility (p/l). Therefore, when kneading dough with the addition of amaranth and chickpea flour, as well as wheat starch, intensive mixing is necessary.

Determination of Toxic Elements, Mycotoxins in Gluten-free Composite Flour and Bread

Heavy metals represent an extensive and highly toxicological group of substances. Usually 4 elements are considered: Hg (mercury), Pb (lead), Cd (cadmium), As (arsenic). High level of toxic elements in body leads to intoxication, and the deficiency of many elements in food and water can lead to severe diseases (Assenova et al. 2016). The concentration of toxic elements in the studied flour was not detected (**Table 4**). According to research results, they showed their safety and allowing expanding the range of gluten-free foods.

Mycotoxins are toxic substances produced by mold fungus under certain conditions. Mycotoxins can be found in products affected by mold, which are visible to the naked eye, and also in a substart where the mold is not visible or dead (O'Sullivan 2005). Therefore, in appearance quality product may contain hazardous substances for the human body. The maximum allowable concentrations of mycotoxins of each group in different raw materials are regulated by norms and standards. The results of the study of the content of mycotoxins are presented in **Table 5**.

As can be seen from the **Table 5**, the content of mycotoxins was not detected. Mycotoxins develop cancer, genetic disorders in next generations, has a negative effect on the liver, kidneys, nervous system, can cause inflammation of the skin (Kryukov and Popova 2012).

Microbiological Evaluation of Gluten-free Bread

Microbiological evaluation of gluten-free bread was carried out for detection of the presence of bacteria, yeast, mold fungi and pathogens of potato bacillus (Bacillus subtilis), which are not killed during bread baking (Chernaya et al. 2017). **Table 6** shows that the introduction of composite gluten-free flour into the bread recipe does not lead to contamination of the baked products with spore bacteria, since they have not been identified for the entire period of observation of the colonies on the seeded media.

Food product safety assessment is determined by absence of coliform bacteria, the majority of pathogenic microorganisms. In terms of microbiological indicators, bread should correspond to the established indicators of total viable count, coliform bacteria and fungi. According to **Table 6**, it can be concluded that using of gluten-free composite flour in the production of bread does not affect

Table 7. Concentration of toxic elements in gluten-free bread

Toxic element	Allowable concentration by regulation standards	Concentration in gluten-free bread
Lead	0.5	Not detected
Arsenic	0.1	Not detected
Cadmium	0.015	Not detected
Mercury	0.2	Not detected

Table 8. Concentration of radionuclides in gluten-free bread

Radionuclides	Allowable concentration by regulation standards	Concentration in gluten-free bread
Cesium – 137	40	5.9
Strontium – 90	20	4.7

the microbiological characteristics of the finished product.

Determination of Toxic Elements and Radionuclides in Gluten Free Bread

The toxicity level of gluten free bread was evaluated by the content of toxic elements. Excess amount of heavy metals in foods leads to serious diseases in the human body. The concentration of lead, cadmium, mercury and arsenic, as they are considered dangerous for the organism as a whole, is presented in **Table 7**.

As a result of the study of bread, the content of heavy metals was not detecte. Poisoning of the body with the above elements is accompanied by constipation and nausea, malaise, problems with the heart and pallor of the skin, anemia, problems with the nervous system and many other serious diseases (Duruibe et al. 2007).

The radionuclides concentration in food products lead to internal exposure, which is much more dangerous for humans than external exposure. Eating foods with a high content of radionuclides have a detrimental effect on all living cells, become the cause of oncological processes, the genetic code is also destroyed, which causes serious genetic diseases (Duyssembaev et al. 2017, Kakimov et al. 2016).

Thus, the table shows that the content of radionuclides does not exceed the values of regulation standards. The higher doses of radionuclides in human body lead to disruption of the normal metabolic functions of the body, the integrity of blood vessels, which leads to bleeding, reduced immunity, the body's exposure to infectious diseases, excites cancer cells.

CONCLUSION

The results of this investigation address matters related to improving quality, increasing the nutritional value and expanding the range of gluten-free products. On the basis of the research conducted, a gluten-free bread formulation was developed, containing 50% wheat starch, 25% amaranth and 25% chickpea flour. The developed recipe allows to expand the range of gluten-free food products of domestic production and to ensure the preventive orientation of products, excluding allergic reactions caused by wheat protein.

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