



Composite flour production and assessment of the safety quality of gluten-free bread

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Abstract

Nutrition has the great importance on the human feeling. Moreover, there are some pathologies that can be cured only together with feeding correction. So, current article deals with the cause of chronic celiac disease which causes atrophy of the intestinal mucosa and, as a consequence, impairs proper digestion and absorption of nutrients. The therapy for this disease consists of an exclusive and strict lifelong gluten-free diet. A few years ago it was believed to be a rare disease. Today, however, doctors come to disappointing conclusions: the number of celiac disease sufferers is increasing year by year. Wheat starch, amaranth, and chickpea flour are considered to be promising ingredients to replace wheat flour in baked goods recipes. These culinary components are gluten-free and have a rich chemical composition. The current manuscript presents an experimentally verified formulation for the production of gluten-free bread. The verified composition consists of 50% wheat starch, 25% amaranth flour, and 25% chickpea flour. This composition allows getting bread with good quality proprieties which makes ill people's life quality style better.

Keywords: celiac disease; gluten-free product; composite flour; bread; diet.

Practical Application: The usage of composite gluten-free flour in the production of gluten-free bread will allow not only to extend the range but also to produce products with improved physical and chemical and also organoleptic parameters. It allows for increasing bread's nutritional and biological value, which is especially important for people following a gluten-free diet.

1 Introduction

Please use Food safety and quality issues are relevant for all countries, regardless of the maturity of their market economy. Good nutrition is one of the most important determinants of public health it contributes to the prevention of various diseases, prolongs people's life and improves their performance (Rasheva et al., 2019).

As early as 1888 the English physician S. Gee was the first who described the clinical picture of celiac disease (CD) in a child suffering from severe chronic diarrhea, accompanied by emaciation and abdominal enlargement (Lebwohl & Rubio-Tapia, 2021).

CD is an immune-mediated, genetically caused, life-long disorder due to intolerance of specific cereal proteins (dietary gluten). The symptom complex of the CD is caused by atrophy of the villi of the small intestine mucosa and the associated syndrome of impaired intestinal absorption has been conducted. This pathology triggers by consuming cereals like wheat, barley, rye, and sometimes oat (Lebwohl & Rubio-Tapia, 2021; Taşkin & Savlak, 2021). Clinical manifestations of CD are highly variable. But typical symptoms are diarrhea, lymphedema, and steatorrhea. Skin manifestations such as alopecia areata (focal), chronic urticaria, atopic dermatitis also can take place. Psoriasis and vitiligo are diseases that have a pathogenetic link to CD. Such clinical manifestations are most frequently detected in young children. At this age, severe clinical manifestations are more often observed, which lead to protein-energy deficiencies

and delayed physical development. Moreover, children with CD are characterized by poor weight gain, delayed physical development, and in some cases overweight and obesity may be detected (Zhuchkov & Kotlyarov, 2017). Other less common symptoms associated with CD are acute electrolyte disturbances, hypotension, lethargy, and recurrent intestinal intussusceptions (Revnova & Shapovalova, 2015). CD may be asymptomatic, so that it remains undiagnosed in a number of people. Extra intestinal manifestations of CD in children may include liver involvement with a moderate increase in the liver enzymes ALT and AST (no more than in 2-3 times). After a gluten-free diet for 4-8 months, transaminases level is normalized. However, in a small number of patients elevated transaminases level can persist despite strict adherence to the diet. In such cases, other liver diseases, especially autoimmune hepatitis associated with CD, should be excluded (Gurova, 2019; Villanacci et al., 2020). Iron deficiency anemia is detected in 12-69% of children with CD with mucosal atrophy phenomena. Isolated growth delay can be detected in 10% of children with complaints of poor growth rates.

Other manifestations include enamel hypoplasia, recurrent aphthous stomatitis, reduced bone mineral density, osteoporosis, osteomalacia, dental enamel defects and various types of anaemia, arthritis or arthralgia. Some children on a gluten-free diet have improved bone mineralisation, while in others (especially during puberty) it may not recover (Revnova & Shapovalova,

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2015; Kayar & Dertli, 2019). Gluten intolerance is not confined only to CD, but can cause food allergies in young children, with manifestations ranging from skin rashes to chronic diarrhoea. For example, the organisation of school meals provides the development and implementation of diets mainly for healthy children, but there is a problem with the organisation of meals for children with hereditary diseases such as phenylketonuria, cystic fibrosis and CD. The development of gluten-free products and their inclusion in patients' diets is an urgent issue and requires further study.

The significant increase in the incidence of autism spectrum disorders (ASD) among the child population in recent years, the lack of efficacy of traditional treatment approaches, and the search for new therapies have led to renewed interest in supplementing therapeutic techniques with dietary therapies (Bavykina et al., 2017, 2019). The use of gluten-free (GFD) and casein-free (CFD) diets in ASD is not officially regulated by international protocols for the management of patients. Although a number of studies confirm the effectiveness of diet therapy in correcting neuropsychiatric status and gastroenterological abnormalities (Adams et al., 2018; Rubenstein et al., 2018).

The evidence for the therapeutic value of diets is limited and inconclusive. Diet therapy can be used if a food allergy or gluten (casein) intolerance is diagnosed. Some children with autism spectrum disorder have gluten and casein intolerance (Nadpara et al., 2022). However, other authors declare that a gluten-free diet can be introduced in patients with no intolerance to get a therapeutic effect in some pathologies (Taşkin & Savlak, 2021). So, before prescribing nutritional therapy, patient should be assessed to clarify the nature of the intolerance and choose the best dietary therapy. The results suggest that many patients with autism suffer from increased absorption of exorphins formed in the intestine due to incomplete digestion of gluten and casein (Adams et al., 2018; Rubenstein et al., 2018; Bavykina et al., 2019).

There is important data on the relationship between gluten disease, fertility and pregnancy. Patients who do not monitor the progression of CD have a higher rate of miscarriages. They have delayed fetal growth and a lower birth weight of the child. CD is diagnosed in 1-19% of infertile men. Researchers have identified signs of hypogonadism, sexual dysfunction and sperm quality disorders in patients with CD. And these symptoms disappeared after the introduction and adherence to a gluten-free diet (Novikova & Lapin, 2018).

A strict dietary treatment excluding cereals such as wheat, rye, barley and oats remains the main approach in the treatment of CD, leading to the disappearance or reduction of manifestations of the disease (Khmelevskaya et al., 2019). These crops contain up to 80% spare gluten proteins in the endosperm of cereal grains. The major components of gluten are prolamines (wheat gliadin, rye secalin, barley hordein and oat avenin), which make up 5-50% of total protein and are soluble in 60-80% ethanol solution, and glutelins, soluble in 0.1-0.2% alkaline solutions. Barley takes the second place after wheat in terms of toxicity. In the biotechnology and food industry, Tanner Gregory and John Howitt Crispin Alexander's invention is known to produce food or beverage based on barley grain without toxic hordeine, or by removing most or all of the genes encoding toxic hordeine

sequences, using genetically engineered modification. Among the cereal family, oat is the only crop that still generates debate on toxicity for patients with CD (Tiunov et al., 2018b).

The fact that gluten-free diets are very popular around the world makes it difficult to diagnose. More and more people exclude wheat from their diet on their own, without medical advice. However, a strict gluten-free diet can lead to the following adverse effects (Lavrova et al., 2021). First of all, it is a deficiency in a number of nutrients (thiamine, folate, magnesium, calcium, and iron) and dietary fiber. The other effect is excess weight gain due to the hypercaloric nature of many commercially available gluten-free products. And in last, the increased monetary costs due to the higher cost of gluten-free products.

Hence, this type of diet therapy must be controlled and therapeutically justified. The diet should be high in protein, high in fat and rich in minerals (particularly calcium) and vitamins (Tiunov et al., 2018b).

The Association of European Celiac Societies (AOECS) has worked hard to promote the purity of gluten-free products. Thanks to this association, even minimal quantities of gluten are labelled obligatorily, with a special marking in the form of a crossed spike, which must necessarily appear on the label or the inscription "gluten free" (Tiunov et al., 2018a).

Today the creation of specialized products for the diets of patients with CD is actively practiced (Tiunov et al., 2018a; Conte et al., 2019; Desnilasari et al., 2019). An important parameter in the development of a corrective diet is the sparing principle of the gastrointestinal tract (both mechanical and chemical). Foods and dishes that increase fermentation and have an adverse effect on liver function, as well as all kinds of allergens, including lactose, are excluded. Gluten content in food for people with food intolerance should not exceed 100 mg (1 mg/100 g of food) otherwise there may be a relapse of the disease (Khmelevskaya et al., 2019).

The technology of gluten-free products production has several problematic points. Gluten (gluten base) gives a porous structure and makes the crumb elastic. During dough fermentation, the gases retain the gluten, causing it to increase in volume several times. Therefore, gluten-free products turn out flat and with an undeveloped crumb (Stoin et al., 2021). Rice, corn, buckwheat, millet, sorghum and amaranth flours can be used to produce gluten-free bread. But, soya flour has a characteristic bean odor and products made from this type of flour in its pure form are prone to quick hardening. People with a food allergy to gluten also often suffer from lactose intolerance (Khmelevskaya et al., 2019; Bavykina et al., 2019; Bresciani et al., 2021). In this case, the technology for making bread involves only the proofing of the dough (there is no fermentation process). The products are characterised by an unleavened taste and a poorly expressed aroma. To improve the quality of gluten-free bread, leavening agents are used (Myhonik & Hetman, 2019).

The problem of getting optimal organoleptic, and rheological properties of specialized flour products when wheat flour is excluded from the formulation, development, and introduction of these products to the market are relevant scientific goals (Kapustina, 2020).

2 Materials and methods

The authors carried out work on the development of gluten-free composite gluten-free flour technology (Table 1), enriched with protein, dietary fiber, B vitamins, macro- and microelements, which will improve the quality of products made from this flour by organoleptic and physical and chemical parameters, increase the shelf life of products, expand the range of bakery products.

Methods, proposed by Demirkesen et al. (2010), were used to investigate the chemical composition. X-ray spectral analysis has found great practical application in various industries in the determination of many elements in various materials.

X-ray spectral qualitative and quantitative analyses are based on the dependence of the intensities of the spectrum lines of certain elements on the content in the samples under analysis. The interaction of primary and secondary radiation with the sample leads to dependence of the analytical line intensity both on the content of the element to be determined and on the chemical composition of the sample to be analyzed (Feng et al., 2021). This method has its several preferences like as minimal needs for the sample preparation, no destruction of the sample, it has a high spatial resolution, and the possibility of multiple measurements of the elements (Célia et al., 2022). The intensity of the analytical line of the secondary $I_{a_{2,i}}$ spectrum is written as Equation 1:

$$I_{a_{2,i}} = f \cdot (C_x \cdot a_i \cdot b_i) \quad (1)$$

where C_x is the content of the element to be determined in the sample; a_i , b_i are parameters, the size of which is determined by various factors related to the physical features of the analyzed material (Fetisov, 2020).

2.1 Objects of the study

The object of the study is gluten-free bread. Several types of bread were made up. They were examined for qualitative indicators, both organoleptic (by the tasting committee on a five-point scale) and physicochemical. During organoleptic evaluation the basic qualitative indicators such as appearance, colour on the cut, smell, taste, consistency of the product were established. To determine the humidity of the bread an accelerated standard method was used. It is based on drying to constant weight of the bread crumb weight (Gosudarstvennyye Standarty State Standard, 1997).

The middle of the bakery was cut out (70 g) and the crust and the underbread layer about 1 cm thick were cut off. The crumb

was quickly crushed with a knife and mixed. Two 5 g portions weighed to the nearest 0.01 g were placed in pre-weighed beakers (or chemical cups) and transferred to a desiccator ($t=140-145^\circ\text{C}$). Exposure time was 50 min, $t=130\pm 2^\circ\text{C}$. After the elapsed time, the beakers were removed, covered with caps and cooled in a desiccator (or in the air) for 10-15 min. Then the beakers were weighed and the bread moisture content was calculated in percentage (Equation 2):

$$W = 100 \cdot (m - m_1) / m \quad (2)$$

where m is the mass of raw crumb; m_1 is a mass of the dry substance of the bread.

The final result is expressed as the arithmetic average of the two determinations.

Porosity (P), characterizing the property of bread, academic performance (more or less) (Baturina et al., 2013), was calculated by the Formula 3:

$$P = 100 \cdot (V - V_1) / V \quad (3)$$

where V is the volume of the cut out crumb; V_1 is the volume of a well pressed crumbles crumb.

To determine the acidity, small pieces of bread crumb were cut out and a 5 g portion was weighed with an accuracy of 0.01 g on the scale. After thorough grinding it was transferred to a 150 mL dry beaker and 70 mL distilled water was added in parts. In the first step, 20 mL of the taken water was poured into the beaker with the bread and the crumb was rubbed with a glass rod to obtain a homogeneous mass, and then the remaining water was added. The mixture was left at room temperature, and then the liquid layer was poured into a dry beaker. A pipette was used to take 20 mL of settled liquid (without sediment), 2-3 drops of 1% alcohol solution of phenolphthalein was added and titrated with 0.1 n sodium hydroxide solution from a burette until the slight pink colouring appeared without disappearing within a minute. The acidity of the bread was calculated according to Formula 4:

$$X = \frac{V \cdot V_1 \cdot a}{10m \cdot V_2} \cdot K \quad (4)$$

where V is the volume of solution of molar concentration 0.1 mol/dm³ of sodium hydroxide or potassium hydroxide consumed during titration of the test solution, cm³; V_1 is the volume of distilled water taken for acid extraction from the test product, cm³;

Table 1. The composite flour included the following components, mass %.

Flour component	Percentage content	Component property
Wheat starch	70	Improves quality of flour products, their porosity, volume, consistency and delays of bread firming (Zhang et al., 2021).
Amaranth flour	19-21	Contains more than 20% protein, essential amino acids, fat included 50% omega-6 polyunsaturated fatty acid, has a significant amount of vitamins E, A, B1, B2, B4, C, D (Belkin, 2019).
Chickpea flour	9-11	Has low glycemic index and contains protein high level and dietary fibre, unsaturated and saturated fatty acids, B, A, K, PP, E, C vitamins; beta-carotene, Mn, K, Na, Mg, Se, Ca, Zn, Cl, Fe, I, P, S, Mo, Pb, V, Si, Ti, and Co (Baturina et al., 2013; Koseoglu & Celikel, 2022).

a is a conversion factor per 100 g of sample; K is a correction factor to convert the used sodium hydroxide or potassium hydroxide solution to a solution of the exact molar concentration of 0.1 mol/dm^3 ; 0.1 is a coefficient of conversion of sodium hydroxide or potassium hydroxide solution with a molar concentration of 0.1 mol/dm^3 to 1.0 mol/dm^3 ; m is a weight of the portion, g; V_2 is the volume of the test solution taken for titration, cm^3 .

2.2 Preparation of samples

In order to determine the optimum dosage of raw materials, 4 formulations were made

- 1) The first sample: wheat starch - 80%; chickpea and amaranth flour of 10% for each;
- 2) The second sample: wheat starch - 70%, amaranth and chickpea flour of 15% for each;
- 3) The third sample: wheat starch - 60%, amaranth and chickpea flour of 20% for each;
- 4) The fourth sample: wheat starch - 50%; chickpea and amaranth flour of 25% of each.

3 Results and discussion

We have developed composite flours in different formulations. The common characteristic of all three components of the composite flours is the absence of gluten in their composition. Literature data prove, the cruel adherence to a gluten-free diet leads to a rehabilitation of the structural function of the intestinal villi in 80% of patients (Khmelevskaya et al., 2019).

We have carried out studies to produce a gluten-free product. Wheat starch, amaranth and chickpea flour were chosen as raw materials. These crops do not contain gluten in the form in which it causes CD.

The technology for the production of composite flour consists of the following processes. Wheat starch, amaranth and chickpea flour are mixed in a mass ratio of 7:2:1. The obtained composite flour is packed in polypropylene bags of 450 g and stored at $+6-10^\circ\text{C}$ for not more than 8 months. The flour's chemical composition determines its nutritional value and baking properties. Starch-containing products are mainly used in production by foreign manufacturers and that goods made from these products have a high cost, and their nutritional value is low (Luppo & de Dekere, 2010; Adams et al., 2018). However, starch is indispensable in the technology of gluten-free flour products production. A neutral taste, specific viscosity, hygroscopicity, high resistance to heat treatment, the ability to stabilise emulsions and a long shelf life are the main properties of wheat starch. One of the most important properties of starch is its ability of its grains to swell in water when the temperature rises, giving a viscous colloidal solution (cluster). The gelling temperature of wheat starch is $60-62^\circ\text{C}$. A distinctive feature of wheat starch is its ability to form clusters, which are stable under heat, agitation and prolonged storage (Belkin, 2019).

Amaranth flour is characterized by its high protein content compared to wheat, rice, and corn. The analysis of the amaranth

flour contents protein from 13.1 to 21.00%. This is too important to get quality gluten-free bread. It is rich in such minerals as Ca, Mg, Cu, Zn, Fe, K, and P as well as biologically active substances like phytosterols and polyphenols, saponins, and squalene. The crude fat content of the amaranth is in the range of 5.6 to 10.9%. Moreover, vitamin C was found in it compared to wheat for example (Gebreil et al., 2020). One more important has to be added. Its proteins and peptides have antimicrobial activity as well as therapeutic anticancer, antioxidant, antihypertensive, and hypocholesterolemic effect (Ramkisson et al., 2020).

Chickpea flour reduces insulin, sugar and cholesterol levels and minimizes the risk of diabetes. It is also good for people with dermatitis, sclerosis, autoimmune diseases, autism, attention deficit disorder and other diseases. Regular consumption of meals made from chickpea flour contributes to cancer prevention (Kapustina, 2020).

Flour mainly consists of proteins and carbohydrates which are the most important its components and defined the properties of the dough and the quality of the products. We have analyzed the chemical composition of the composite flour. Based on the results of the chemical composition study of the composite gluten-free flour, the second formulation was selected from the three formulations (Table 2).

The chemical composition in the flour showed that Na, P, K, Ca, C are in significant amounts.

In case of preparing bread according to the three composite flour formulations, a dosage of 20% amaranth flour and 10% chickpea flour were chosen as the most balanced in terms of the organoleptic characteristics of the bread that was the most balanced in terms of total characteristics. Other samples were found to be unsatisfactory due to changes in flavor, color and aroma as well as a reduction in the porosity of the crumb (Figure 1).

It can be noted that the fourth sample according to the results of physico-chemical and organoleptic indicators can be used as a product for wide consumption, as well as for preventive nutrition. In the first sample not large voids in the crumb were found, according to organoleptic indicators the appearance of the second and third samples did not meet the requirements of the standard. The samples had not correct shape with bursts and large voids.

The weight, volume, porosity and acidity of the finished product were evaluated (Table 3). The porosity of bread refers to the volume of pores in a given volume of crumb, expressed in percent. That is its high or low digestibility and varies from 45 to 76% in different types (Novitskaya & Artemova, 2016). Well loosened bread is better absorbed by the digestive juices and is better digested.

The mass fraction can determine the correctness of the technological process that is the dosage of raw materials, flour

Table 2. The chemical composition of the flour.

Name of the sample	Chemical composition, %
Gluten-free composite flour	Mg-0.62; Na-1.83; P-1.46; K-2.83; Ca-1.10; S-0.70; Al-0.34; Si-0.41; O-33.46; C-57.25

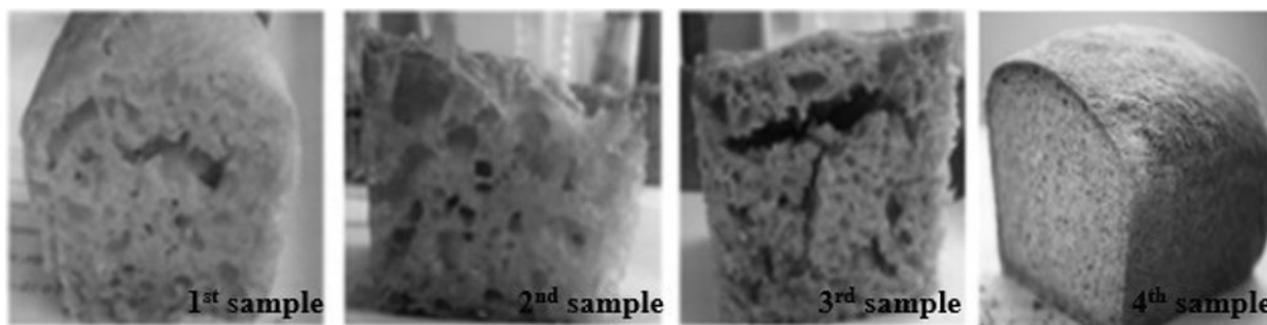


Figure 1. Assessment of crumb porosity in organoleptic evaluation of bread samples.

Table 3. Physic and chemical parameters of prepared bread.

Sample	Weight, g	Volume, cm ³ /g	Porosity, %	Acidity, deg H
First	110	140	37	2.2
Second	110	110	23	2.2
Third	100	85	26	2.2
Fourth	115	150	37	2.2

Table 4. Organoleptic characteristics of bread.

Sample	Appearance	Color	Crumb condition	Porosity	Taste and smell
1 st	The shape is correct, smooth, without big cracks or bursts	Light brown	Baked, elastic, not damp to the touch	Developed, thin-walled with small voids	Inherent to this type of product, with no extraneous taste or smell
2 nd	The shape is not correct, smooth, without big cracks or bursts	Light brown	Baked, elastic, not damp to the touch	Uneven, with large pores in the crumb	Inherent to this type of product, with no extraneous taste or smell
3 rd	The shape is not correct, smooth, without big cracks or bursts	Dark brown	Baked, elastic, not damp to the touch	Developed, thin-walled with large voids	Inherent to this type of product, with no extraneous taste or smell
4 th	The shape is correct, smooth, without big cracks or bursts	Light brown	Baked, elastic, not damp to the touch, with bursts	Developed, thin-walled without voids	Inherent to this type of product, with no extraneous taste or smell

and water. It allows us to determine the energy value of the bread. Reduced moisture content increases the energy value of the bread (Lavrova et al., 2021). Higher moisture content reduces the caloric value and quality of the bread, it is heavy, less digestible, more susceptible to mould and easily deformed.

From the above samples, the 4th sample was selected because it was better than the other samples in terms of organoleptic properties (Table 4). The amaranth flour gives a very pleasant taste. It was praised by tasters at a high level.

Further work in the chosen direction is promising. Thus, the possibility of using processed plant products in the form of citrus dietary fibers, quinoa, montin, chumiza, sago, sorghum or pectin and red chokeberry powder (common or varieties) as part of a gluten-free mixture should be investigated more thoroughly. Banana flour can also be used as a source of plant proteins. The effect of these components on the quality of the

dough and the physical and organoleptic properties of gluten-free bread should be additionally studied (Khmelevskaya et al., 2019; Belyavskaya et al., 2018; Yano, 2019).

4 Conclusions

The gluten-free menu is based on the introduction of dishes and products from a variety of gluten-free raw materials: rice, corn, soya amaranth flour which has a reduced proportion of starch-containing raw materials used to prepare gluten-free products in the diet. However, such products have low nutritional value due to their high starch content and low content of dietary fiber, vitamins and minerals. A formulation for gluten-free bread was developed on the basis of conducted studies. Its composition consisted of 50% wheat starch, 25% amaranth flour and 25% chickpea flour. The developed formulation, along with its international counterparts, makes it possible to expand the range of gluten-free food products.

Conflict of interest

The authors of the manuscript declare there is no conflict of interest in the presented research.

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