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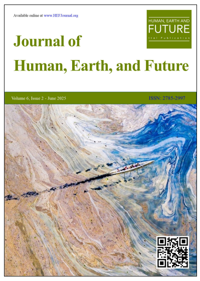

Multifactorial Analysis for the Development of Gluten-Free Sea Buckthorn Snacks

Anar Kabylda [1], Nazym Alzhaxina [1*], Madina Sultanova [1], Askhat Dalabayev [1], Zhansaya Kozhukhova [1]

[1] *Kazakh Scientific Research Institute of Processing and Food Industry LLP, Almaty, 050060, Kazakhstan.*

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Abstract

This study develops an optimal formulation for fortified gluten-free extruded snacks with added sea buckthorn powder, with the objective of expanding the range of products available for both patients with coeliac disease and the healthy population. This required comprehensive physicochemical and organoleptic analyses of raw materials, including rice, buckwheat flour, and sea buckthorn powder, as well as the use of mathematical modelling based on compositional planning to optimise the proportions of the components. The following optimal ratios were identified: 30.10% rice flour, 74.95% buckwheat, and 21.95% sea buckthorn powder, which ensured increased nutritional value and improved the organoleptic properties of the final product. These results demonstrate the possibility of using the developed formulation in industry, reducing dependence on imported analogues, and expanding the range of gluten-free products. The novelty of this study lies in its scientifically based approach to the creation of highly nutritious snacks with antioxidant and vitamin functions through the use of mathematical modelling and complex analysis of raw materials, which contributes to the development of technologies and the strengthening of domestic production. Further value of this study lies in its development of a scientifically based approach to the creation of gluten-free products with high nutritional value. The results obtained contribute to the advancement of gluten-free production and the expansion of the range of functional foods. The practical significance of the results obtained includes the possibility of using the developed recipe in production, which will solve the problem of providing coeliac patients with gluten-free products and overcome consumer dependence on the purchase of expensive imported analogues, while simultaneously developing the processing industry of Kazakhstan.

Keywords: Gluten-Free Snacks; Coeliac Disease; Rice Flour; Buckwheat Flour; Sea Buckthorn Powder; Process Optimisation.

1. Introduction

The snack industry is actively developing products for individuals with gluten intolerance. Considering the wide range of imported analogues, the products developed must meet modern physico-chemical and organoleptic characteristics in order to ensure high quality while meeting the specific needs of consumers [1].

Gluten is a protein found in wheat, rye, and barley. There are three main forms of gluten intolerance, the most common of which is coeliac disease, a chronic disease in which gluten damages the mucous membrane of the small

* Corresponding author: nazjomka1@mail.ru

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intestine. This vegetable protein, which is commonly present in cereal crops, has a toxic effect on intestinal villi, leading to impaired nutrient absorption [2, 3]. Another form of gluten intolerance is gluten allergy, wherein the immune system reacts to gluten as an allergen. Recently, scientists have identified another type of gluten sensitivity, namely non-coeliac, non-allergic intolerance. According to previous research, the number of people suffering from this type of sensitivity significantly exceeds the number of patients with coeliac disease [4, 5].

Coeliac disease is an autoimmune disease characterised by chronic inflammation in genetically predisposed individuals (i.e., individuals with weakened mucous membranes in the small intestine) and is associated with exposure to gluten through oral consumption [6, 7]. Gluten is a group of similar proteins called glutelins and prolamins, which are found together with starch in the seeds of cereal plants like wheat, barley, and rye. In recent years, significant advancements have been made in understanding the diagnosis, pathogenesis, and natural course of this disease. The number of identified cases is constantly growing, including among elderly patients, which indicates significant changes in the detectability of this condition [8].

The main reason behind the increasing number of coeliac disease diagnoses worldwide is the availability of highly sensitive and specific screening methods that identify risk groups [9–12]. Research indicates that global dietary changes, including the spread of modified versions of the Mediterranean diet with excessive gluten intake (up to 20 g/day), may have contributed to an increase in the incidence of coeliac disease [10, 11]. In addition, qualitative changes in gluten itself may increase the incidence of the disease. The production of new grain varieties focused on technological rather than nutritional factors may have played a role in the recent increase in coeliac disease diagnoses [11, 12]. However, these hypotheses have not yet been scientifically verified, and the exact reasons for the increased frequency of coeliac disease diagnoses remain unknown.

The prevalence of coeliac disease also varies by region. It is considered one of the most common autoimmune diseases, affecting approximately 0.5–1% of the population, except in regions where gluten consumption is minimal, such as in sub-Saharan Africa and Japan [12–18].

Epidemiological data indicate that similar outbreaks of morbidity are observed for other autoimmune diseases in the Western Hemisphere [19], which suggests the possible influence of additional factors besides gluten. Notably, women are two to three times as likely to suffer from this disease as men. Furthermore, many cases of coeliac disease remain undiagnosed, as they may not be specific or are poorly expressed [20].

Coeliac disease can be accompanied both by digestive system disorders and various extra-intestinal symptoms. These include pathologies of the liver, pancreas, and thyroid glands; diabetes mellitus; osteoporosis; dermatological problems (dermatitis, psoriatic arthritis); iron deficiency anaemia; vitamin deficiencies; and various neurological disorders [21].

The only confirmed method for treating coeliac disease is a strict gluten-free diet. i.e., a dietary regime that completely eliminates the consumption of gluten found in wheat, rye, barley, oats, and their hybrid varieties, regardless of their form or presence in products [22–24]. In recent years, new therapeutic approaches capable of providing an alternative to diet therapy have also been extensively studied. A gluten-free diet improves quality of life, restores intestinal tissue, and prevents the development of refractory coeliac disease, osteoporosis, bowel cancer, and neurological disorders [25]. However, this diet also has significant disadvantages in terms of its nutritional value, technological characteristics, and organoleptic properties. In particular, gluten-free products contain insufficient amounts of protein, fibre, iron, B vitamins, and folate, with high fat and carbohydrate content [26]. Consequently, current research is focused on increasing the nutritional value of gluten-free foods, especially bakery products, by using alternative cereals that are rich in nutrients, such as quinoa and amaranth. The role of functional ingredients, including hydrocolloids, proteins, and natural food additives, is also being actively studied to improve the physico-chemical, technological, and sensory characteristics of gluten-free products [27].

According to the Ministry of Health of the Republic of Kazakhstan, over the past three years, there has been an increase in patients with coeliac disease in Kazakhstan across all age groups.

The geographical scope of the analysis is presented in Figures 1 and 2 and includes almost the entire territory of the Republic of Kazakhstan. The data were recorded during 2022–2024 in the cities of Astana, Almaty, and Shymkent and in 11 regional centres.

Figure 1. Geographical coverage of this study, including all 17 regions and 3 cities of significance of the Republic of Kazakhstan (2022–2024)

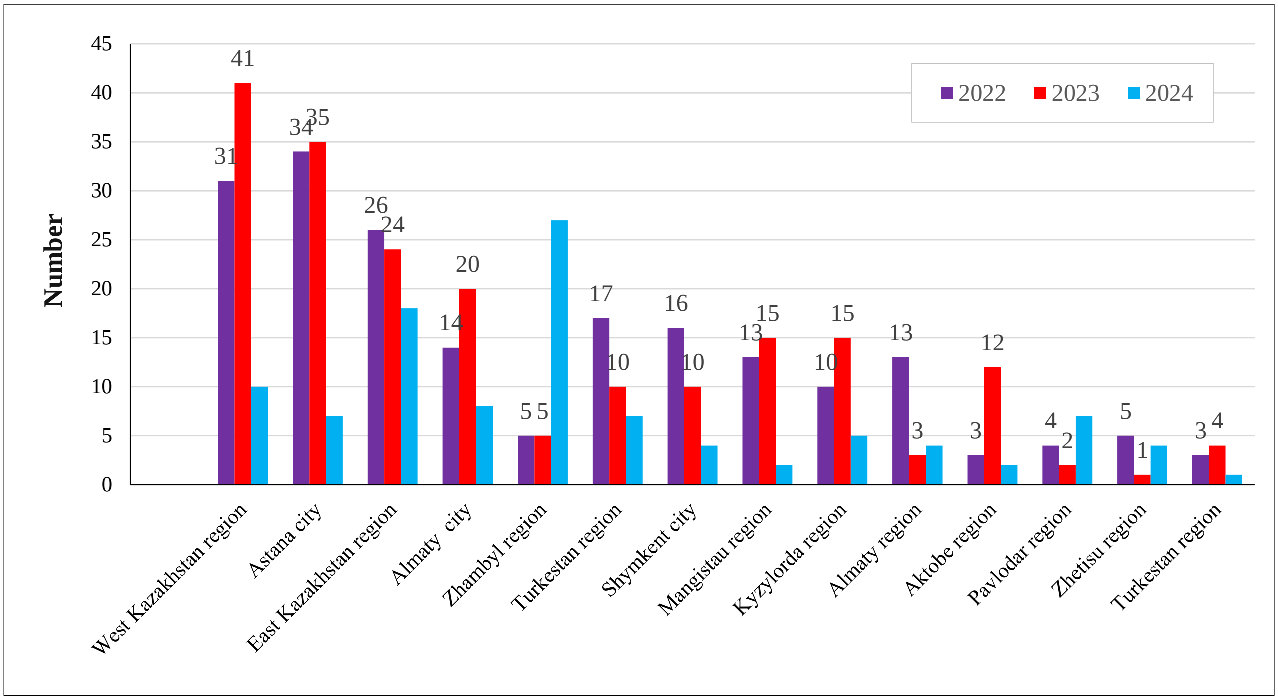

Figure 2. Number of patients registered at the dispensary

The data shown in Figure 1 indicate significant regional differences in the number of patients. The highest increase was observed in 2024 in the Zhambyl region, where the number of patients peaked at 27. A relatively high level of patient registration was observed in the West and East Kazakhstan regions, with persistently high indicators throughout the period of data collection. Notably, in most regions, there was a moderate decrease in the number of patients by 2024, which may indicate an improvement (or *vice versa*, a deterioration) in the availability and quality of medical diagnostics and more effective disease prevention measures.

In summary, these diagrams emphasise the regional specifics of morbidity and the need for the development of differentiated approaches to healthcare management in various regions of Kazakhstan.

Currently, there is a tendency to use non-traditional grains and new ingredients for cereal production. Simultaneously, scientific research in the field of multigrain and fibre use is gaining momentum [28–32]. Gluten-free food products available on the market are significantly more expensive than conventional products. This can create a financial burden on patients with coeliac disease, especially in light of annual increases in food prices.

Moreover, gluten-free diets may not be balanced owing to their low nutritional value. Saturni and Ferretti showed that coeliac patients suffered from nutritional deficiencies (dietary fibre, minerals, protein, and vitamins) that negatively affected their health [33]. This highlights the need for the development of gluten-free foods that are both affordable and nutrient-rich to ensure that individuals with coeliac disease can lead healthy lifestyles [34].

Snacks are small portions of convenient, light, and easy-to-prepare foods that can be consumed between meals. Snacks include foods that help to maintain energy levels, saturate the body with nutrients, and normalise metabolism throughout the day [35, 36].

Scientists around the world are conducting research into new types of raw materials, taking into account local characteristics [37, 38]. For example, the effect of extrusion on the functional properties of rice- and chickpea-based snacks with the addition of passion fruit peel powder has been studied [39]. In another study, gluten-free snacks based on different rice varieties were developed, and their physical, physico-chemical, and sensory properties were assessed [40]. An approach to optimise the recipe for gluten-free muffins based on lentil flour using the mixture design method has also been proposed [41]. Some studies focus on the influence of additives, such as vegetable purees, on the textural and technological parameters of extruded snacks [42]. Recipes using cassava starch and vegetable cake have also been studied, in which the physical and technological properties of finished products were assessed [43].

In the development of dry breakfast cereal recipes, multivariate optimisation methods are widely used to obtain an optimal and mathematically sound mixture composition [44, 45]. However, the application of such methods in the development of gluten-free extruded snacks, especially those enriched with vitamins and protein due to local raw materials, remains limited. Despite significant progress in the development of gluten-free products, issues related to the use of local raw materials, such as buckwheat flour and sea buckthorn powder, which have high protein, vitamin, and antioxidant contents, remain unresolved. In addition, recipe optimisation methods, such as Scheffe's mix design, have seen limited use in the development of gluten-free extruded snacks.

Although research is being conducted in Kazakhstan on the development of gluten-free products, it mainly focuses on bakery products and pasta. At present, no studies have addressed the development of extruded gluten-free snacks using local raw materials, such as buckwheat flour and sea buckthorn powder, coupled with mathematical optimisation methods. The present study aims to fill these knowledge gaps by presenting the physico-chemical characteristics of the selected raw materials and optimising the recipe for a fortified gluten-free snack using multivariate variance analysis according to the Scheffe method.

1.1. Relevance

This work aims to expand the range of gluten-free products, which is vital considering the growing interest in healthy eating and the need for functional foods. The development of gluten-free snacks with improved organoleptic and nutritional characteristics will contribute to improving the diets of individuals with gluten intolerance, as well as those wishing to adopt a healthy lifestyle. This study also explores the possibility of using fruit and berry powders to create fortified gluten-free snacks with improved functional properties.

1.2. Purpose and objectives

The purpose of this research is to examine the properties of different types of gluten-free flour used as the base for the mixture, and to optimise the compositional parameters that influence the formulation of fortified extruded gluten-free snacks containing sea buckthorn powder. This will make it possible to obtain products using gluten-free ingredients while expanding the range of fortified products for improving the quality of life of individuals with coeliac disease.

This study comprises the following tasks:

- Examining the physico-chemical and organoleptic characteristics of rice and buckwheat flour and sea buckthorn powder;
- Optimising the parameters of the mixture using multifactorial analysis to further create a formulation of fortified extruded gluten-free snacks.

2. Material and Methods

The general scheme of the study is presented in Figure 3.

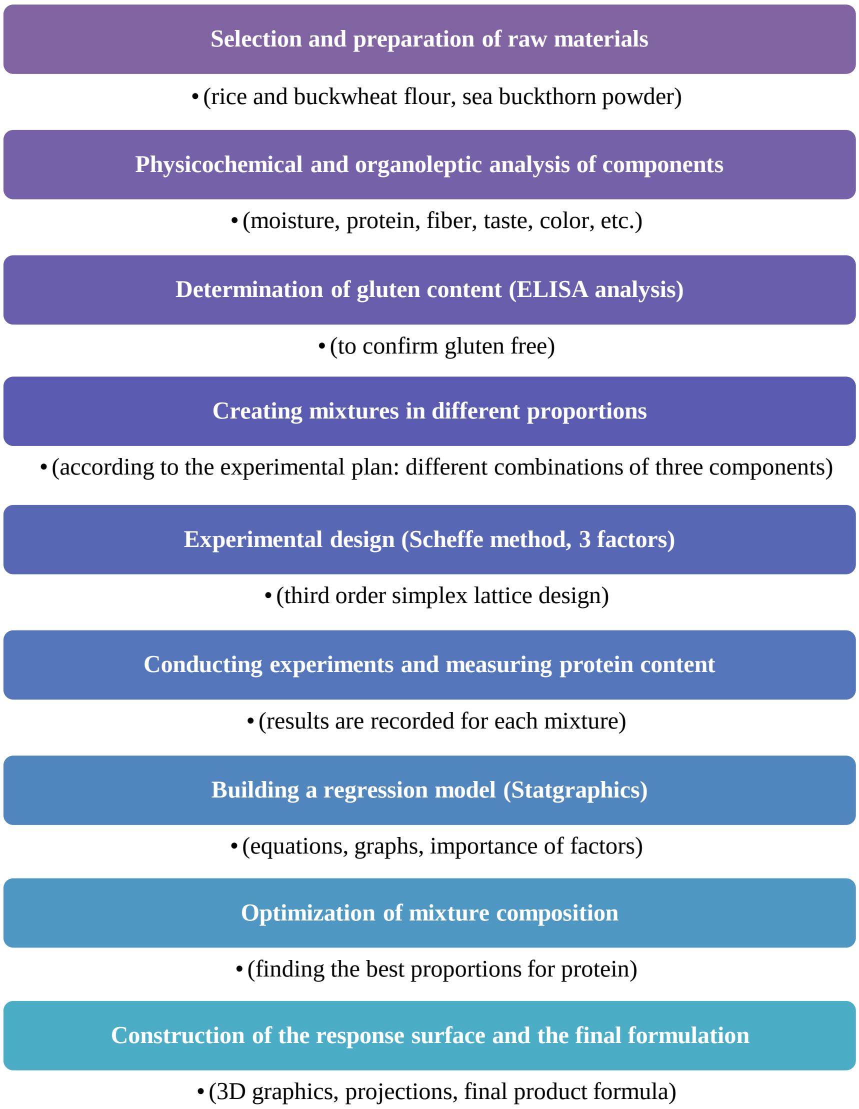

Figure 3. Block diagram showing the methodological stages of development and optimisation of the recipe for fortified gluten-free snacks

2.1. Investigation of the Quality Characteristics of Raw Materials

Rice flour (Kyzylorda region) and buckwheat (Almaty region) produced in the Republic of Kazakhstan and dried sea buckthorn powder of the Altay variety (Almaty region) were used to manufacture the extruded gluten-free snack mixture. The gluten content in flour was determined via an enzyme immunoassay using a sandwich ELISA (RIDASCREEN®FAST Gliadin) in accordance with FLOUR 4.1.2880-11. This analysis was used to quantify contamination with wheat prolamins (gliadin) in raw foods, such as in various types of flour (buckwheat, rice), and in processed foods, including ready meals, noodles, sausages, baked goods, ice cream, and beverages [46].

The sandwich ELISA results were obtained using a 96-well MultiskanTM FC flatbed photometer equipped with a built-in shaker. The analyser measured the wavelength in the 450 nm range. This device is highly reliable and durable, easy to operate, and quick to set up. The physico-chemical and organoleptic characteristics of the rice and buckwheat flour were studied in accordance with GOST 31645-2012. The physico-chemical and organoleptic characteristics of sea buckthorn berries were studied in accordance with GOST R 59661-2021, GOST 24027.2-80, and GOST 34130-2017. Subsequently, the sea buckthorn berries were dried using an air-cooled freeze dryer (Alpha 1-2 LDplus) in accordance with GOST 31372-2010. The air dryer was used to remove water from the raw material before it was ground. The dried samples were crushed using a mixer, mortar, homogeniser, and crusher until a homogeneous mass was obtained according to GOST 26671-2014.

2.2. Optimisation of Mixture for Fortified Extruded Gluten-Free Snack

The use of optimisation methods for mixture design is related to the methodology of the response surface, whereby the formed product is a mixture of components whose proportions are determined using mathematical models of varying complexity (linear, quadratic, or cubic) [47, 48]. The use of mixture design is relatively rare in food science; however, there are examples of its use to optimise various processes, such as in determining the composition of the mobile phase in chromatographic studies [49], acid mixtures for decomposition procedures [50], extraction solutions for sampling suspensions [51], and organic solvents for isolating target compounds [52]. The optimisation process was carried out according to the Scheffe simplex-lattice method for processing experimental data, which is also used in developing the composition of mixtures to create formulations. In this study, to optimise the formulation of a vitaminised extruded gluten-free snack, the effect of different proportions of rice, buckwheat flour, and sea buckthorn powder was studied.

3. Results

3.1. Investigation of the Quality Characteristics of Raw Materials

The gluten content of the selected flour samples was analysed (Table 1) to determine the level of gluten contamination. Wheat flour of the highest grade with a gluten content of 29% was used as a control sample.

Table 1. Analysis of gluten content in flour from cereals and legumes

No.	Flour	Repeat 1	Repeat 2	Average value	Gliadin, mg/kg	Gluten, mg/kg	Result
1	Wheat (control)	3.060	2.268	2.664	>70	>130	+
2	Rice	2.015	0.909	1.462	0.30	0.60	−
3	Buckwheat	1.255	1.050	1.1525	0.34	0.68	−

Note: *(+) – positive; (−) – negative.*

As shown in Table 1, wheat gluten is a more heterogeneous protein fraction than gliadin. For each polypeptide glutenin chain, there are two disulphide bonds with neighbouring gliadin chains. When wheat was tested for gluten, its gliadin level was greater than 70 mg/kg, which exceeds the sensitivity of the determination method; thus, these data indicate that the gluten content in wheat exceeds the permissible limit. In contrast, the gluten content in rice and buckwheat flour does not exceed the permissible level for gluten-free products because the gluten-forming glutelins of grain crops have a linear structure, unlike the gluten of crops, which are unable to form gluten.

Only the wheat flour returned a positive gluten test, whereas the other samples either did not contain gluten or contained less than 3.30 mg/kg of gluten. Notably, rice (0.60 mg/kg) and buckwheat (0.68 mg/kg) flour contained minimal amounts of gluten, making them ideal for individuals with coeliac disease. The organoleptic and physico-chemical properties of the analysed rice flour sample were analysed, the results of which are shown in Table 2.

Table 2. Organoleptic and physico-chemical properties of rice flour

No.	Indicators	GOST 31645-2012	Sample
1	Visual features	A uniform, crumbly mass with small fragments of shells	Loose mass
2	Colour	White with cream or yellowish shades	White
3	Smell	Typical for such flour, odourless, neither mouldy nor musty	Pronounced aroma of fresh rice flour, without other odours
4	Taste	Typical for such flour, without sourness, bitterness or foreign tastes	Neutral, without unnecessary flavours
5	Presence of mineral impurities	There should be no crunch when chewing flour	There is no crunch when chewing flour
6	Pest infestation	Not allowed	Not detected
7	Moisture content, %	Not more than 12.0	9.74
8	Protein content, %	Not more than 7.4	4.72
9	Ash content, %	-	1.26
10	Fat content, %	-	1.1
11	Fiber content, %	-	3.4
12	Starch content, %	79.1	66.8
13	Acidity, degrees	Not more than 2.0	0.3

Table 2 shows that the rice flour sample was white in colour with a creamy tint and the characteristic odour of rice flour, without extraneous taste and odour, which meets the requirements of GOST 31645-2012. The mass fraction of moisture (9.74%), protein content (4.72%), fat content (1.1%), mass fraction of total ash (1.26%), and starch content (66.8%) also meet the requirements of GOST 31645-2012. The qualitative characteristics of buckwheat flour were analysed, the results of which are presented in Table 3.

Table 3. Organoleptic and physico-chemical properties of buckwheat flour

No.	Indicators	GOST 31645- 2012	Sample No. 3
1	Visual features	Homogeneous, crumbly mass with small fragments of shells	Homogeneous, crumbly mass
2	Colour	Cream, light beige and beige with a grey tint	Creamy
3	Smell	Characteristic of this type of flour: odourless, not musty or mouldy	Odourless, fresh, not musty or mouldy
4	Taste	Inherent to this type of flour: not sour, not bitter, without foreign tastes	Neutral, without unnecessary flavours
5	Presence of mineral impurities	Crunching during chewing is not allowed	There is no crunch when chewing
6	Pest infestation	Not allowed	Not detected
7	Moisture content, %	Not more than 12.0	9.2
8	Protein content, %	Not more than 13.6	6.96
9	Ash content, %	-	2.7
10	Fat content, %	-	2.1
11	Fiber content, %	-	7.8
12	Starch content, %	70.2	59.5
13	Acidity, degrees	Not more than 6.0	1.8

As shown in Table 3, the buckwheat flour sample had a creamy colour and a smell characteristic of this type of flour, without any extraneous taste or odour, which meets the organoleptic requirements of GOST 31645-2012. The physico-chemical properties of the buckwheat flour sample indicate that it not only meets the requirements of GOST 31645-2012 but also has a 1.47 times higher protein content (6.96%), twice the fat and ash content (2.1% and 2.7%, respectively), and a lesser starch content (59.5%) than the rice flour sample. Thus, the buckwheat flour has advantages over rice flour and is capable of supplementing it, making it a good component for the snack mixture.

Sea buckthorn – a berry that grows across Kazakhstan – is a good source of vitamins and was chosen as the fortifying component in the snack mixture.

Table 4. Qualitative characteristics of sea buckthorn powder

No.	Indicators	Sample		Control methods
	Organoleptic properties			
1	Visual features	Fine granular hygroscopic powder		GOST R 59661-2021
2	Colour	Different shades of brown		
3	Smell	Aroma of dried sea buckthorn		
4	Taste	Characteristic of dried sea buckthorn		
5	Presence of mineral impurities	No crunch when chewing		
	Physico-chemical properties			
7	Moisture content, %	8.0		GOST 24027.2-80
8	Squirrels, %	1.4		GOST R 59661-2021
9	Fats, %	8.0		
10	Carbohydrates, %	4.1		
11	Fiber, %	10		
12	The content of metal impurities, %	Not more than 0.0003		GOST 34130-2017
13	Vitamins, %	B1	2.1	GOST R 59661-2021
		C	30.4	
		A	28.0	
		PP	2.4	

As shown in Table 4, the sea buckthorn powder sample exhibited different shades of brown colour and a characteristic odour. This meets the organoleptic requirements of GOST R 59661-2021. The physico-chemical properties of the sea buckthorn powder sample – moisture (8.0%), protein (1.4%), fat (8.0%), fibre (10%), vitamin B1 (2.1%), vitamin C (30.4%), vitamin A (28.0%) and vitamin PP (2.4%) – are fully compliant with the requirements of GOST R 59661-2021.

Thus, the samples of rice, buckwheat flour, and sea buckthorn powder are shown to complement each other and can be considered suitable, full-fledged bases for creating a mixture.

3.2. Mixture Optimisation for Fortified Extruded Gluten-Free Snacks

The optimisation process allows us to select quantitative indicators for each component [53, 54]. Calculations were made using the Statgraphics Centurion software package. The experiment was carried out using a simplex-lattice plan of the third order. During the experiment, different formulations of fortified gluten-free snacks made from buckwheat flour, rice flour and sea buckthorn powder were developed. The variable factors in the formulations included the mass fractions of rice flour (x_1), buckwheat flour (x_2) and sea buckthorn powder (x_3), which were varied according to the Scheffe plan of the third order. Other experimental conditions remained unchanged. The results of the experiments allowed the change in protein to be characterised.

The mixing components forming the planning matrix and experimental results are shown in Table 5.

Table 5. Scheffe's plan and experimental results

Experiment numbers	Mass fraction of components						Protein, %
	Encoded values			Natural values			
	x_1	x_2	x_3	Rice flour, g	Buckwheat flour, g	Sea buckthorn powder, g	y
1	1	0	0	100	0	0	4.72
2	2/3	1/3	0	66.6667	33.3333	0	5.60
3	2/3	0	1/3	66.6667	0	33.3333	3.60
4	1/3	2/3	0	33.3333	66.6667	0	6.20
5	1/3	1/3	1/3	33.3333	33.3333	33.3333	6.92
6	1/3	0	2/3	33.3333	0	66.6667	2.50
7	0	1	0	0	100	0	6.96
8	0	2/3	1/3	0	66.6667	33.3333	5.90
9	0	1/3	2/3	0	33.3333	66.6667	3.50
10	0	0	1	0	0	100	1.40

The results presented in Table 5 demonstrate the effect of using different mass fractions of mixture components on the protein content in the finished product. The highest protein content (6.96%) was recorded in the sample containing 100% buckwheat flour. The addition of sea buckthorn powder to the mixture decreased the protein content, likely due to the lower protein content of this component. The lowest protein content (1.40%) was found in the sample containing 100% sea buckthorn powder, highlighting the importance of combining various protein sources to achieve an optimal nutritional composition.

The estimated effects of the complete protein model are shown in Table 6.

Table 6. Estimated effects of the complete protein model

Original	Sum of squares	Df	Mean-square	Meaning	P-coefficient
218.463	Average	218.463	Linear		
24.1487	2	12.0743	12.92	0.0045	Quadratic
3.03672	3	1.01224	1.15	0.4297	Special cubic
3.26378	1	3.26378	.	40.32	0.0079
Cubic	0.242845	3	0.0809485		
Mistake	7.62446E-14	0	0		
Total	**249.155**	**10**			

An analysis of the estimated effects of the complete protein content model (Table 6) revealed that the main contribution to data variation was the linear effect (sum of squares = 218.463), indicating that the proportions of the mixture components have a significant influence on protein content.

Linear effect: the sum of squares is 24.1487, the degree of freedom (Df) is 2, and the RMS value is 12.743. A high value of the F-criterion (12.92) and low value of the P-coefficient (0.0045) indicate a statistically significant impact of linear effects on protein content. This implies that changing the proportions of the main ingredients (rice flour, buckwheat flour and sea buckthorn powder) has a significant impact on the protein content of the product.

Quadratic effect: the sum of squares for the quadratic term is 3.03672, Df = 3, and the RMS value is 1.01224. The value of the F-criterion (1.15) and high P-coefficient (0.4297) indicate that quadratic effects do not significantly influence protein content, which might suggest a linear dependence.

Special cubic interaction: the sum of squares (3.26378), F-criterion (40.32) and low P-coefficient (0.0079) indicate the significant impact of special cubic effects on the protein content, suggesting the presence of complex interactions between the components of the mixture, and their influence on protein levels.

Cubic effect: the sum of the squares is 0.242845, Df = 3, and the RMS value is 0.0809485, indicating a minor impact of cubic effects.

Error and total sum of squares: the near-absence of error (7.62446E-14) confirms the high accuracy of the model. The total sum of squares (249.155) reflects the total contribution of all factors to the variability of the protein content.

The data analysis revealed that linear and special cubic effects have the greatest impact on protein content, whereas the quadratic and general cubic effects do not have a statistically significant impact. This confirms that changing the proportions of the mixture components significantly impacts the protein content of the product. Therefore, to optimise the mixture, we must consider linear dependencies as well as complex interactions between the components.

Table 7. Results of the complete model

Model	SE	R-Square	Adj. R-Square
Linear	0.966832	78.68	72.59
Quadratic	0.936299	88.57	74.29
Special cubic	0.284514	99.21	97.63
Cubic		100.00	0.00

Table 7 shows the results of fitting different models to the protein data. The average model consists only of a constant. The linear model consists of first-order terms for each component. The quadratic model adds cross products between pairs of components. The special cubic model adds terms that include the products of the three components. The cubic model adds additional third-order terms. Each model is shown with a P-value that verifies whether the model is statistically significant compared to the RMS value for the term below. The development of a technology for extracting amino acids from grain crops of domestic varieties

The standard error of the estimate and the R-squared statistics for each of the models are shown in Table 8.

Table 8. Analysis of variance (ANOVA) for protein

Original	Sum of squares	Df	Mean-square	Meaning	P-coefficient
Special cubic model	30.4492	6	5.07487	62.69	0.0031
Common mistake	0.242845	3	0.0809485		
Total (correspondent)	**30.692**	**9**			

where: R-squared = 99.2088%; R-squared (adjusted for D.F.) = 97.6263%; Standard error est. = 0.284514; Average absolute error = 0.0922854; Durbin–Watson statistics = 3.45933 (P = 0.9946); Residual autocorrelation with a delay of 1 = −0.751945.

As shown in Table 8, information regarding the protein values obtained using the matched model includes:

- The observed protein content (if any);
- The predicted protein value using the matched model; and
- The 95.0% confidence limits for the average response.

3.2.1. Optimisation of the Reaction of the Mixture for Fortified Extruded Gluten-Free Snacks

Reaction optimisations are of key importance, as they describe the conditions under which the maximum protein yield can be achieved. The main points that justify the need for data are shown in Table 9. The optimal value (7.26903) serves as a criterion for developing a successful formulation or technology. This value confirms the result of the optimisation, which is the central point of this study. Without this value, it is impossible to evaluate the effectiveness of the selected approaches.

Table 9. Combination of factor levels

Ratio	Low	High	Optimal
Mass fraction of rice flour	0.0	100.0	30.0999
Mass fraction of buckwheat flour	0.0	100.0	47.9511
Mass fraction of sea buckthorn powder	0.0	100.0	21.9499

Table 9 shows the contribution of each factor (mass fractions of rice flour, buckwheat flour and sea buckthorn powder) to achieving the target result. The presence of low, high and optimal values indicates the range of possible variation of each parameter, as well as the location of the optimal point. This enables us to evaluate the flexibility of the technology, as well as to understand how changes in each of the factors influences the final result.

These data are useful not only theoretically, but also for further industrial implementation. Manufacturers can focus on optimal values for the process parameters. Thus, the dependence of protein on the components of the gluten-free snack mixture can be represented as the mass fractions of the individual ingredients, and the regression equation can be written as follows:

$$y = 30.099x_1 + 47.9511x_2 + 21.9499x_3 \quad (1)$$

Based on the obtained regression equation, a three-dimensional model was constructed, providing a plane that characterises the dependence of protein on the mass fraction of the components of a fortified gluten-free snack mixture. Figures 4 and 5 show graphical representations of the dependency graphs.

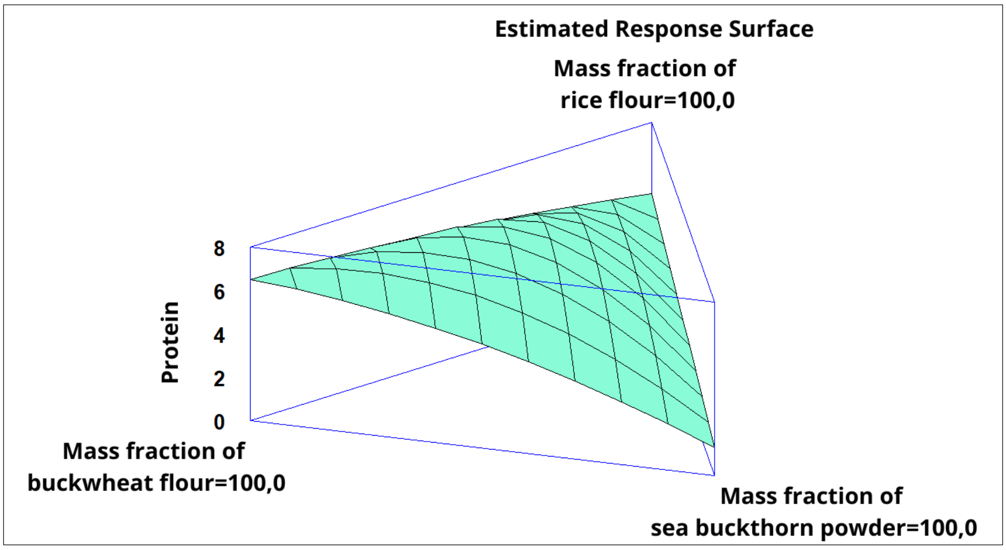

Figure 4. Response surface of the output parameter and protein dependence on the mass fraction of components

As shown in Figure 4, an analysis of the behaviour of the received response surface showed that the optimal protein zone for fortified extruded gluten-free snacks requires the following mass fractions: 30.099% rice flour, 47.9511% buckwheat flour and 21.9499% sea buckthorn powder.

Figure 5 shows projections of the response surface representing the dependence of the protein content on the mass fractions of rice flour, buckwheat flour and sea buckthorn powder. The graph indicates that the maximum protein content (approximately 7.2–8.0, red zone) is observed when the mass fraction of buckwheat flour is the highest (approximately

47.95%), the mass fraction of rice flour is moderate (approximately 30.1%), and the mass fraction of sea buckthorn powder is the lowest (approximately 21.95%). This area coincides with the optimal values shown in Table 9.

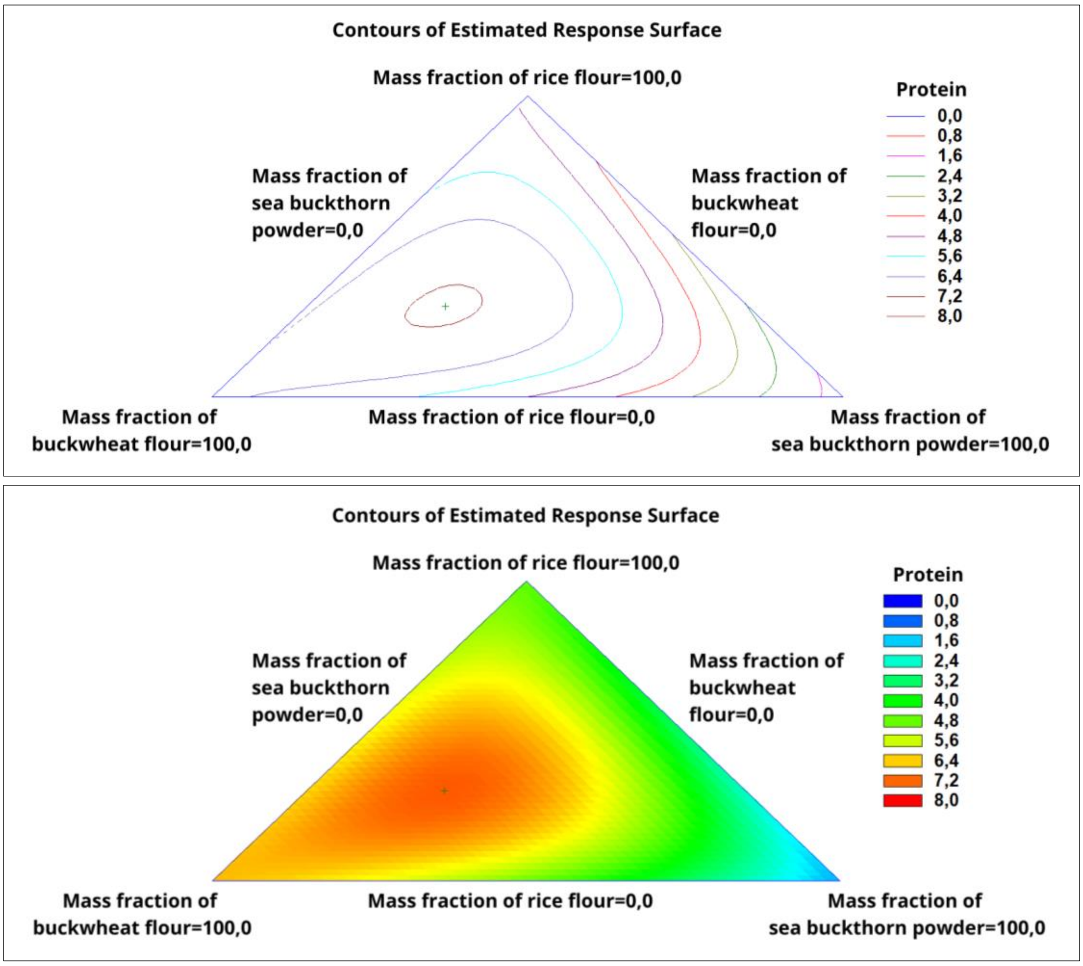

Figure 5. Projections of the cross-sections of the response surface, characterising the dependence of the protein on the mass fraction of the components

As the mass fraction of rice flour increases (i.e. moves towards the upper corner), the protein content decreases (transition from red to blue). Similarly, as the mass fraction of sea buckthorn powder increases (i.e. moved to the right corner), the protein content decreases. This indicates that buckwheat flour is the main factor influencing protein content. The graph shows the interaction between the three components. The optimal value is achieved in the balanced zone, and not at the extreme values of any one of the factors. A significant decrease in protein is observed if any one of the components dominates (values at the boundaries of the triangle).

Projection analysis enables us to adjust the formulation to achieve the desired protein content. The three components can be balanced to create products with specified nutritional characteristics. The resulting graph visualises the influence of the composition of the mixture on the protein content, confirming that the maximum result is achieved with the optimal ratios of the components indicated in Table 9. This helps to demonstrate the effectiveness of the proposed optimisation model.

Thus, by applying the developed mathematical model, we determined the optimal formulation of the fortified gluten-free snack mixture based on the results obtained.

4. Discussion

Optimisation of the composition of the mixture showed that the best characteristics are achieved with a rice flour content of 30.10%, a buckwheat flour content of 47.95%, and a sea buckthorn powder content of 21.95%. Sensory tests confirmed the excellent taste qualities of the resulting product, and physico-chemical analysis demonstrated optimal texture and density. The addition of sea buckthorn powder contributed to an increase in the vitamin and antioxidant content, which is consistent with the data presented in studies of the functional properties of sea buckthorn [55, 56].

A comparative analysis using existing gluten-free products revealed that the proposed formulation has several advantageous characteristics, including nutritional value and taste. Previous studies have reported insufficient nutritional value in traditional gluten-free products owing to their low protein and dietary fibre content [57, 58]. The addition of buckwheat flour improved the amino acid profile of the product, whereas sea buckthorn, owing to its high vitamin C and carotenoid contents, exhibited increased antioxidant properties [59-61].

A comparison with other studies on extruded gluten-free products indicates that the use of rice and buckwheat flour in certain proportions improves the textural properties of the product. Numerous studies [62, 63] have shown that increased buckwheat flour content causes an increase in density and crispness; this was also observed in the present study. However, the addition of sea buckthorn powder provided a balance between the hardness and porosity of the product, making it more attractive to consumers.

Our results confirm the possibility of optimising the parameters of a mixture for an extruded gluten-free snack with high nutritional value. Using mathematical modelling, we determined the optimal proportions of ingredients that provide improved physico-chemical and organoleptic properties. A similar approach has previously been successfully applied in the optimisation of bakery products [64, 65] and extrusion snacks [66, 67], confirming its effectiveness for developing gluten-free products.

Additionally, the developed formulation can be successfully produced at scale, and could thus contribute to the expansion of the availability of gluten-free products and the development of the food processing industry in Kazakhstan. A promising area for further research involves assessing the impact of extrusion process parameters on the quality of the final product and its storage [68-70].

5. Conclusion

This study successfully optimised the recipe for vitaminised extruded gluten-free snacks based on rice and buckwheat flour with the addition of sea buckthorn powder, intended for the diet of people suffering from gluten intolerance. A comprehensive physico-chemical and sensory analysis of the raw materials confirmed its high quality and functional suitability for use in gluten-free products.

The use of simplex lattice planning and mathematical modelling methods ensured an appropriate selection of the mixture composition. The optimal ratio of components was determined to include rice flour (30.10%), buckwheat flour (47.95%) and sea buckthorn powder (21.95%); this was established based on response graphs and confirmed using experimental data. The resulting product is characterised by balanced organoleptic indicators, improved texture and increased nutritional value.

The addition of sea buckthorn powder contributed to the enrichment of snacks with vitamins, natural antioxidants and dietary fibre, allowing us to classify the developed product as functional. A comparative analysis with existing analogues on the market showed competitiveness in a number of key indicators, including amino acids and vitamin composition.

The scientific novelty of this work lies in its integration of mathematical experimental planning in the development of gluten-free products, which is rarely used in domestic practice. The practical significance of the study lies in the possibility of introducing the developed recipe into production, thereby expanding the range of high-quality food products for people with coeliac disease and adherents of functional nutrition.

Prospects for further research include studying the influence of extrusion parameters and storage conditions on the stability, biological value and consumer properties of the product during long-term storage.

6. Declarations

6.1. Author Contributions

Conceptualisation, A.K. and N.A.; methodology, N.A.; software, M.S.; validation, Zh.K.; formal analysis, Zh.K.; investigation, Zh.K.; resources, A.K. and A.D.; data curation, A.K. and A.D.; writing—original draft preparation, A.K. and A.D.; writing—review and editing, N.A. and M.S.; visualization, N.A. and M.S.; supervision, N.A. and M.S.; project administration, N.A.; funding acquisition, N.A. All authors have read and agreed to the published version of the manuscript.

6.2. Data Availability Statement

The data presented in this study are available in the article.

6.3. Funding

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6.5. Institutional Review Board Statement

Not applicable.

6.6. Informed Consent Statement

Not applicable.

6.7. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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