CJES
Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Development of protein bars for functional nutrition

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ABSTRACT

This study investigates protein bars made with fermented soy protein isolate, designed to meet daily protein needs for various groups like children and athletes. These ready-to-eat bars offer extended shelf life and enhanced nutrition, aligning with global healthy eating trends. The research details the bars' chemical composition and nutritional value, including amino acid profiles, mineral content, and vitamins, assessing their ability to meet recommended protein intake, particularly for primary school children. It also evaluates the impact of combining wheat flour and plant-based protein on product quality. Findings show these bars can enrich diets and promote health due to their nutritional and functional benefits. With an energy value of 369-386 kcal per 100g, a 50g portion provides 184-193 kcal, or 9-10% of a 2000 kcal daily diet. The study's practical contribution lies in demonstrating the potential of fermented soy protein isolate in high-protein bar production, supported by the developed formulation and processing technology.

Keywords: Plant protein, Nutritional value, Protein bars, School nutrition.
Article type: Research Article.

INTRODUCTION

Ensuring adequate nutrition for the population, in which dietary protein constitutes a key component, remains a persistent global challenge. Proteins are essential macromolecules responsible for maintaining the structure and function of living organisms (Sá *et al.* 2020). The development of innovative technologies for producing protein-rich foods from plant-based raw materials represents a major contemporary approach to expanding food resources and improving dietary patterns. According to estimates by the Food and Agriculture Organization (FAO), the global population is expected to exceed 9 billion by 2050 (Liang *et al.* 2020). Consequently, global food demand is projected to increase by approximately 70%. This trend necessitates the identification of alternative food sources capable of meeting the growing nutritional needs of humanity. Soybeans represent a promising raw material for the development of functional and specialized food products due to their high nutritional value and well-balanced amino acid composition (Unicef 2024). The modern healthy food industry increasingly focuses on the development of dietary (therapeutic and preventive) products. Such products must comply with stringent safety and efficacy standards while providing essential nutrients and energy. Importantly, they should contribute to health improvement and reduce the risk of diet-related diseases. To achieve these objectives, functional food ingredients (FFI) are incorporated into product formulations. The selection of functional ingredients requires a strong scientific rationale. It is necessary to develop biotechnological approaches for creating functional ingredients enriched with macro- and micronutrients responsible for the clinical efficacy of food products. This demands in-depth research and innovative strategies in food product development. According to global rankings of per capita protein consumption, individuals in developed countries such as Iceland (~141 g/day) and the United States (~114 g/day) consume significantly more protein compared to those in developing or least developed countries, where average intake is approximately 46 g/day (Yankovskaya & Dunchenko 2025; Contreras-Pacheco *et al.* 2025). An analysis of statistical data on the consumption of major food products in the Republic of Kazakhstan, including meat, fish and seafood, milk and dairy products, and eggs, reveals insufficient protein intake in several regions. Particularly low consumption levels have been reported in Shymkent and the Kyzylorda region, where protein intake is considered inadequate. In Mangystau, West Kazakhstan, Almaty, and Turkestan regions, the situation is relatively better; however, protein consumption remains at the borderline between insufficient and adequate levels. These findings are consistent with data on food security potential presented in projections for food security up to 2050 (Guo *et al.* 2023). A variety of protein-containing products, including protein bars, are currently available on the market to address protein deficiency. However, domestic products are predominantly fruit- or cereal-based bars, whereas imported products more commonly contain milk proteins. In this context, the development of technology for producing protein bars based on alternative protein sources is of particular relevance. Therefore, research aimed at developing such technology is highly relevant.

Literature review and problem statement

Recent advances in plant-based and alternative protein sources, along with emerging approaches to ingredient selection and processing technologies, have created new opportunities for the development of food products with improved nutritional profiles. Thus, the choice of protein source affects not only the nutritional value of the product (including total protein content and amino acid composition), but also consumer acceptability and overall production costs. The global demand for convenient, nutrient-dense foods has driven significant growth in the high-protein bar market. These products serve multiple functions, including meal replacements for athletes, post-exercise recovery aids, and convenient snacks for busy consumers seeking healthier alternatives to traditional confectionery products (Dhiman *et al.* 2023). High-protein bars typically contain 15-30% protein by weight and are formulated to provide sustained energy while supporting muscle protein synthesis and recovery. Previous studies have demonstrated that the use of dairy proteins, particularly whey protein concentrate, contributes to the development of high-protein products (Kinsella & Morr 1984). Whey proteins are considered complete proteins and are especially rich in branched-chain amino acids (BCAAs), making them highly suitable for sports nutrition applications (Kerksick *et al.* 2017). Milk protein concentrates offer additional technological advantages due to their water-binding capacity and ability to improve bar texture (Banach *et al.* 2014). However, their application in high-moisture systems may present technological challenges (Banach *et al.* 2014). Despite these advantages, whey protein production is relatively costly and technologically demanding. Furthermore, animal-derived proteins, including dairy proteins, are associated with several limitations, such as allergenicity, dietary restrictions (e.g., for vegetarian and vegan consumers), and sustainability concerns. In addition, dairy proteins may contribute to undesirable aftertaste and texture changes during storage, particularly in products with elevated moisture content (Alcorta *et al.* 2021). Plant-based proteins have attracted considerable attention as sustainable and hypoallergenic alternatives to dairy proteins (Day 2013). Soy, pea, rice, and sunflower proteins are among the most extensively studied sources for high-protein bar formulations (González-Pérez & Vereijken 2007). These proteins offer several advantages, including lower environmental impact, suitability for plant-based diets, and diverse functional properties (Małecki *et al.* 2020). However, they generally exhibit lower protein digestibility-corrected amino acid score (PDCAAS) values compared to animal proteins and may require complementary blending to achieve a balanced amino acid profile (Schaafsma 2000). Pea protein has emerged as a particularly promising ingredient due to its favorable amino acid composition, high lysine content, and relatively neutral flavor profile (Małecki *et al.* 2020). Studies have shown that pea protein can be successfully incorporated into high-protein bars with acceptable sensory properties when properly formulated (Philipp *et al.* 2017). Rice protein, although limited in lysine, offers hypoallergenic characteristics and can be combined with complementary protein sources to improve overall nutritional quality. Sunflower protein represents an underutilized source with potential applications in protein bars derived from oil-processing by-products (González-Pérez & Vereijken 2007; Małecki *et al.* 2020). However, optimization of extraction and processing methods is required to enhance its functional properties and reduce off-flavors. Current formulations of high-protein bars face several interconnected challenges that complicate product development. First, the choice of protein source critically influences not only nutritional quality but also functional properties, sensory characteristics, and cost. Dairy proteins – particularly whey protein concentrate and milk protein concentrate – dominate commercial formulations due to their superior amino acid profiles and techno-functional properties. However, these sources are limited by allergenicity, dietary restrictions for vegan consumers, and sustainability concerns. Plant proteins have been widely investigated as alternatives, with pea, soy, rice, and sunflower proteins showing significant potential. Nevertheless, plant proteins typically exhibit lower digestibility, incomplete amino acid profiles (necessitating complementary blending), and complex sensory attributes, such as "beany" or bitter flavors. Emerging protein sources, including microalgae, demonstrate exceptional nutritional potential (40-70% protein content and the presence of bioactive compounds), but face consumer acceptance barriers related to color and taste (Małecki *et al.* 2020). Second, formulation technology must account for complex interactions between proteins, binding agents, moisture content, and functional ingredients. Texture hardening during storage remains a persistent quality issue, driven by protein-protein interactions, moisture migration, and Maillard reactions. Control of water activity is critical for both microbial stability and textural properties; however, optimal ranges (typically ~0.60-0.75) vary depending on the protein source (Szydłowska *et al.* 2020). Third, although functional enrichment through antioxidants, dietary fibers, and other bioactive compounds offers added value, their incorporation introduces additional technological challenges. Antioxidant-rich ingredients may affect color and flavor, dietary fibers influence texture and water-binding capacity, and interactions among multiple functional components may lead to unpredictable effects on overall product quality. The existing literature predominantly focuses on individual aspects, such as comparisons of protein sources, texture characterization, or the incorporation of functional ingredients. However, there is a lack of systematic studies addressing the integrated optimization of formulation parameters to achieve a balance between nutritional, technological, and sensory properties. At the same time, unresolved challenges remain regarding the utilization of protein sources derived from Kazakhstan-based raw materials. These limitations are primarily associated with the absence of established processing technologies for certain protein types within the country and their relatively high production costs, which constrain the feasibility of their industrial application. Global data analysis indicates that legumes, particularly soybeans, represent one of the most promising protein sources for human nutrition. In terms of total protein production among agricultural crops, soy ranks second (62.7 million tons), following wheat (71 million tons). However, while approximately 74% of wheat protein is utilized for food purposes, soybean protein utilization does not exceed 10%, highlighting its significant potential as a global protein resource (Crops 2023). The market for plant-based food proteins includes a wide range of traditional products derived from whole soybeans, which are recognized for their health benefits. Advances in food processing technologies have enabled the production of soy-based ingredients with improved sensory properties, leading to the development of new generations of soy proteins with enhanced techno-functional characteristics and expanded applications in food systems. Soy protein, derived from *Glycine max*, is the most widely used plant-based substitute for animal protein. Its biological value is comparable to that of animal proteins, with a digestibility of approximately 95%. Unlike animal proteins, it does not contribute to uric acid formation, which makes it particularly suitable for use in specialized and pediatric nutrition products (Simms 2023). The global market for soy protein ingredients is rapidly expanding, with products available in the form of isolates, concentrates, textured proteins, and various soy flours and grits (Guo *et al.* 2023). In turn, the soybean market in the Republic of Kazakhstan is demonstrating dynamic growth, driven by increasing interest in alternative protein sources and sustainable agriculture. In 2024, soybean cultivation in Kazakhstan exceeded 200 000 hectares, with a gross harvest of approximately 260 000 tons. The primary export markets for Kazakhstani soybeans include Sweden and Uzbekistan (Flo *et al.* 2025). Soybeans represent one of the most protein-rich plant-based raw materials, containing up to 50 g of protein per 100 g, which exceeds the protein content of meat by approximately twofold. Soybeans are extensively processed into a wide range of products, including tofu, soy milk, and soy flour. Soy-derived ingredients, such as defatted soy flour, protein concentrates, isolates, and textured soy products, are increasingly utilized across various sectors of the food industry, particularly in meat processing. Soy protein isolates and concentrates, as well as textured soy protein (commonly referred to as soy meat), are comparable to animal proteins in terms of overall biological value. Technologies for incorporating soy-based ingredients into dairy, bakery, fat-and-oil, and other food products have been widely developed (Guo *et al.* 2025). Soy proteins constitute a high-quality source of complete protein nutrition due to their content of essential amino acids and biologically active macronutrients. In addition, they contain dietary fiber, polyunsaturated fatty acids, oligosaccharides, and carbohydrates. Soy proteins are highly digestible and have been associated with various health benefits, including reduced risks of cardiovascular diseases, lowered levels of low-density lipoprotein

(LDL) cholesterol, obesity, and type 2 diabetes. The use of soy protein isolates in food systems enables the formulation of products with protein contents exceeding 90%, while also minimizing undesirable beany flavors through deodorization processes. Particular attention has been paid to the development of peptide-based ingredients derived from enzymatic hydrolysis of food proteins, which allows modification of amino acid composition and enhancement of functional properties. Currently, there is a growing trend toward the development of food products with improved consumer characteristics and functional ingredients. This trend is largely driven by the increasing prevalence of non-communicable diseases, risk factors of which include excessive consumption of simple sugars beyond recommended dietary levels (Stanhope 2022). Plant-based raw materials play a critical role in the development of healthy foods due to their diverse macro- and micronutrient composition, making them a valuable resource for the production of natural and high-quality products (Schaafsma 2000; Alcorta *et al.* 2021; Simms 2023; Yankovskaya & Dunchenko 2025). Contemporary nutritional trends are oriented toward the production of specialized functional foods designed for different population groups (children, athletes, students, etc.), which are ready-to-eat, have extended shelf life, and exhibit enhanced nutritional value. Among ready-to-eat food products, protein bars occupy a leading position in the market. These products are typically composite systems consisting of a cereal or plant-based matrix (flakes, puffed or rolled grains), nuts, dried fruits, seeds, flavoring agents, and fortifying ingredients (such as sprouted or whole grains and bran), formed into a confectionery-type product (Childs *et al.* 2007). A key characteristic of protein bars is that they require no preparation and can serve as convenient "on-the-go" snacks. Despite their relatively small size and mass (50-60 g), they contain a high concentration of biologically active compounds. Protein bars are characterized by elevated protein content, supplemented with carbohydrates (including dietary fiber), vitamins, and minerals. They are designed to maintain physiologically adequate levels of macro- and micronutrients and to support rapid recovery after physical exertion. A defining feature of these products is their high protein content. Various raw materials are used as protein sources in bar formulations, including whey protein concentrate, egg protein, hydrolyzed soy protein, milk protein concentrates, and intact legume proteins (Perović *et al.* 2021). The most common approach to increasing protein content is the incorporation of protein concentrates or isolates. Protein concentrates are typically derived from processed milk whey and contain approximately 50-70% protein, whereas protein isolates represent highly purified protein fractions of animal or plant origin with protein contents of at least 90%. In 1993, the World Health Organization introduced the protein digestibility-corrected amino acid score (PDCAAS), a metric used to evaluate protein quality based on human amino acid requirements and digestibility. The PDCAAS value ranges from 0 to 1, with 1 representing the highest quality. Soy protein isolate has a PDCAAS value of 1, indicating excellent digestibility and amino acid balance (Yankovskaya & Dunchenko 2025). Due to their favorable amino acid composition, high digestibility, and nutritional value, soy protein isolates represent an optimal ingredient for incorporation into protein-based confectionery products. The primary objective of including such ingredients in formulations is to enhance protein digestibility and ensure nutritional balance. Protein bars are widely consumed across all age groups. Various additives are used to enhance their nutritional value, including fresh and dried fruits, nuts, plant-derived ingredients, dairy components, seeds, oilseed by-products, grains, and candied fruits. A wide range of components can be incorporated to develop functional products, including amino acid complexes, vitamin-mineral premixes, water-acid systems (e.g., citric acid), invert syrup, molasses, and honey. The production of ready-to-eat bars involves various technological processes, such as hydrothermal and mechanical treatment, thermoplastic extrusion, high-temperature micronization, barothermal processing, boiling, baking, drying, and pressing. These methods enable the production of products with controlled safety and quality parameters, although some loss of heat-sensitive nutrients may occur due to high-temperature processing (Ribeiro-Santos *et al.* 2015). To achieve the desired bar structure, different types of syrups are used as binding agents, including sucrose syrup, invert sugar syrup (commonly used in sports nutrition), glucose-fructose syrup, and maltose syrup. Fructose syrup is often used in dietary formulations. The appropriate selection of syrup consistency ensures effective binding of dry ingredients, resulting in a plastic, easily formable mass that is neither excessively hard nor sticky. Consequently, the final product exhibits a relatively high energy density (Ribeiro-Santos *et al.* 2015). Products with modified peptide and amino acid compositions are intended for individuals with metabolic disorders, digestive issues, allergies, as well as for enteral nutrition and sports applications. Recent clinical and experimental studies have demonstrated the potential of enzymatic hydrolysates of soy protein isolates to improve lipid metabolism and mitigate complications associated with metabolic syndrome. However, both globally and in the Republic of Kazakhstan, the production of such enzymatic hydrolysates remains limited, restricting their use as functional food ingredients in preventive and therapeutic nutrition products (Taillie 2003). Therefore, the lack of studies investigating the use of fermented soy protein isolate in the development of protein bars highlights the relevance and necessity of further research in this area.

Aim and objectives of the study

The aim of this study was to evaluate the effect of fermented soy protein isolate on the chemical composition, quality, and nutritional value of protein bars. This approach provides a basis for the development of formulations and processing technology for high-nutritional-value protein bars derived from Kazakhstan-based raw materials characterized by adaptability to changing climatic conditions. To achieve this aim, the following objectives were defined:

To investigate the organoleptic, physicochemical, and microbiological properties of fermented soy protein isolate as a formulation ingredient in protein bars;

To evaluate the quality parameters of the developed protein bars;

To assess the contribution of the developed products to meet the daily protein requirements of primary school-aged children;

To examine the combined effect of wheat flour content and plant protein (soy isolate) concentration on the sensory properties of the developed bars.

MATERIALS AND METHODS

This study investigated the quality of protein bars made with fermented soy protein isolate. The bars were developed to provide a stable protein source, aligning with global trends in healthy eating. Sensory appeal and consumer acceptance were prioritized. Consumer preferences were assessed by an expert panel using organoleptic analysis, while technological factors like packaging and storage were excluded. All ingredients were used within regulatory limits and presumed safe. A control bar (C) was made with wheat flour. Three experimental samples were developed with different levels of fermented soy protein isolate added relative to flour mass: sample №1: 10%; sample №2: 15%; sample №3: 30%. The formulations of control and experimental samples are presented below.

Table 1. Formulation of control and high-protein bars

Name of raw materials and unit of measurement	Quantity of raw materials in the samples			
	Control	**№1**	**№2**	**№3**
Wheat flour (g)	240	216	204	168
Cane sugar (g)	120	100	120	120
Butter (g)	180	180	220	180
Salt (g)	–	1	1	1
Egg (pcs.)	–	–	–	1
Plant protein (soy isolate; g)	–	24 (10%)	36 (15%)	72 (30%)

The organoleptic and physicochemical quality parameters of high-protein bars were determined. Moisture content was determined in accordance with GOST 9404, and ash content according to GOST 27494.

The content of vitamins B_1, B_2, PP, C, and E was determined in accordance with GOST 29138, GOST 29139, and GOST 29140.

Moisture mass fraction was determined according to GOST 21094;

Carbohydrate mass fraction was determined by the Bertrand method (GOST 25832);

Dietary fiber mass fraction was determined by the gravimetric method (GOST 31675);

Fat mass fraction was determined using a Soxhlet apparatus (GOST 5668);

Protein mass fraction was determined by the Kjeldahl method (GOST 10846);

Ash mass fraction was determined by the incineration method (GOST 10847);

Energy value (kcal) was calculated based on product composition using standard conversion factors: proteins – 4 kcal g^{-1}, fats – 9 kcal g^{-1}, carbohydrates – 4 kcal g^{-1}. Organoleptic characteristics (appearance, color, taste, odor, and texture) were evaluated using a descriptive profile method on a 9-point scale by an expert panel (GOST 25832). Each sample was assessed by at least three independent experts. Toxic elements, including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), were determined by atomic absorption spectrometry (GOST 26932), expressed in mg kg^{-1}. Cesium-137 was determined by gamma spectrometry according to GOST R 54015.

The amino acid composition was analyzed in accordance with GOST R 55569. The calculation of the contribution to the daily protein requirement for different age groups of children was performed based on the protein content in a 50 g portion of the experimental bar sample and the recommended daily protein intake for the respective age group, expressed as a percentage. The evaluation of the effect of wheat flour and plant protein concentrations on the organoleptic characteristics of the product was carried out using a combination of instrumental, analytical, and statistical methods. Experimental design was performed using Response Surface Methodology (RSM) with a quadratic model. The independent variables were:

A: wheat flour content (g 100 g^{-1}),

Y_1: plant protein content (g 100 g^{-1}).

Factor ranges were defined and a grid of values was constructed to generate the response surface. A quadratic regression model was used to describe the three-dimensional response surface:

$Z=b_0+b_1A+b_2Y_1+b_{11}A_2+b_{22}Y_{12}+b_{12}AY_1Z=b_0+b_1A+b_2Y_1+b_{11}A_2+b_{22}Y_{12}+b_{12}AY_1$,

where Z – predicted sensory score. Calculations and visualization were performed using Python (NumPy, Matplotlib libraries).

Statistical analysis included: estimation of regression coefficients; analysis of variance (ANOVA); evaluation of factor significance; determination of optimal formulation parameters. A significance level of $p < 0.05$ was applied.

Results of the study on the development of protein bars for children's nutrition

Evaluating the properties of fermented soy protein isolate as a formulation component for protein bars

The organoleptic, physicochemical, and microbiological characteristics of fermented soy protein isolate (samples №1 and №2) as a formulation ingredient for protein bars are presented in Tables 2-4.

Table 2. Organoleptic characteristics of fermented soy protein isolate

Parameter	Characteristics of fermented soy protein isolate samples	
	№1	**№2**
Appearance	light yellow powder	light yellow powder
Color	creamy	creamy
Odor	odorless	odorless
Taste	neutral	neutral

Table 3. Physicochemical parameters of fermented soy protein isolate

Parameter	Value for fermented isolate samples		
	control (regulatory requirements)	**№1**	**№2**
Moisture, %	Should be sufficiently low to ensure microbiological stability under recommended storage conditions.	5.6 ± 0.01	6.0 ± 0.01
Crude protein content, %	The parameter (N × 6.25) shall be not less than 40% on a dry matter basis, excluding added vitamins, minerals, amino acids, and food additives.	93.9 ± 0.01	93.0 ± 0.01

Table 4. Microbiological parameters of fermented soy protein isolate

Quality parameter	Quality parameter value		
	by regulatory requirements	**Sample №1**	**Sample №2**
Pathogenic microorganisms, including *Salmonella* (in 25 g)	not permitted	not detected	not detected
Total viable count (CFU g^{-1}), max	5×10^4	9×10^1	12×10^1
Coliforms (in 0.1 g)	not permitted	not detected	not detected
Staphilococcus aureus (in 0.1 g)	not permitted	not detected	not detected
Sulfite-reducing clostridia (in 0.1 g)	not permitted	not detected	not detected
Yeasts (CFU g^{-1}), max	100	$<1.0 \times 10^1$	2.0×10^1
Molds (CFU g^{-1}), max	100	2×10^1	4×10^1

In accordance with regulatory requirements, fermented soy protein isolate is evaluated based on microbiological and organoleptic parameters, ensuring product safety and harmlessness. Samples №1 and №2 comply with all regulatory requirements for fermented soy protein isolates. The studied samples of fermented soy protein isolate were used as the main protein raw material in subsequent experiments.

Evaluation of quality parameters of the bars

The results of the quality assessment of bars formulated with fermented soy protein isolate are presented in Tables 5-7.

Table 5. Organoleptic characteristics of high-protein bars

Parameter	Parameter characteristics for the sample			
	GOST 24901 (control)	**№1**	**№2**	**№3**
Appearance (including cross-section)	Regular shape without deformation; minor deviations allowed if not affecting quality. Surface: smooth or patterned, without swelling, indentations, or signs of incomplete mixing	Rectangular bar. Upper surface: rough with punctures and relief, with occasional crumb inclusions. Lower surface: rough, floury with crumb and bran inclusions, relief structure	Rectangular bar. Upper surface: rough with punctures and relief, with occasional crumb inclusions. Lower surface: rough, floury with crumb and bran inclusions, relief structure	Rectangular bar. Upper surface: rough with punctures and relief; slight flouriness and occasional crumb inclusions allowed. Lower surface: rough, floury with crumb and bran inclusions, relief structure
Color	Uniform, characteristic of the product; slightly darker shade allowed on protruding parts or edges	Light brown	Light brown	Light brown
Odor and taste	Characteristic, without foreign odors or off-flavors	Buttery aroma, floury taste	Buttery aroma, floury taste	Buttery aroma, floury taste
Brittleness	Flat shape with brittle, crumbly, uniformly porous structure	Dense, dry texture requiring effort to break	Dense, dry texture requiring effort to break	Dense, dry texture requiring effort to break

Table 6. Comparative analysis of physicochemical parameters of experimental samples.

Parameter	Parameter value for the sample			
	Control	**№1**	**№2**	**№3**
Moisture content (%)	≤10.0	3.89 ± 0.05	7.66 ± 0.05	4.49 ± 0.06
Carbohydrates (%)	60–75	49.9 ± 0.58	47.90 ± 0.71	43.95 ± 0.92
Dietary fiber (%)	not regulated	3.19 ± 0.05	2.85 ± 0.08	2.73 ± 0.09
Fat (%)	2.5–10.0	19.39 ± 0.07	14.46 ± 0.06	18.01 ± 0.08
Protein (%)	7.5–9.0	17.28 ± 0.06	19.39 ± 0.07	23.08 ± 0.08
Ash (%)	≤1.2	6.23 ± 0.05	7.52 ± 0.06	7.69 ± 0.05
Energy value, kcal/100 g	410–450	385,94	369,48	383,22

Table 7. Amino acid composition of high-protein bars (mg/g protein; Sun 2011).

Amino acid	FAO/WHO requirement (primary school children)	Amino acid content in samples (mg g^{-1} protein)		
		№1	**№2**	**№3**
Arginine (conditionally essential)	–	160,0	130,0	130,0
Lysine	75	52,0	48,0	48.0
Tyrosine (non-essential)	–	39,0	64,0	64,0
Phenylalanine	(Phe + Tyr) 34	64,0	38,0	64,0
Histidine	–	39,0	28,0	28,0
Leucine + isoleucine	56 (leucine)	75,0	–	73,0
Methionine (Met + Cys)	–	20,0	13,0	11,0
Valine	41	84,0	48,0	61,0
Proline (non-essential)	–	62,0	64,0	76,0
Threonine	44	50,0	51,0	30,0
Serine (non-essential)	–	63,0	48,0	51,0
Alanine (non-essential)	–	68,0	42,0	48,0
Glycine (non-essential)	–	75,0	64,0	40,0
Total identified amino acids (mg g^{-1})	–	851	–	724
Rate of total protein (%)	–	85,1%	–	72,4%

The comparative amino acid composition of the experimental protein bar samples for children's nutrition is presented in relation to FAO/WHO requirements for primary school children.

Calculation of plant protein intake adequacy for primary school children in Kazakhstan

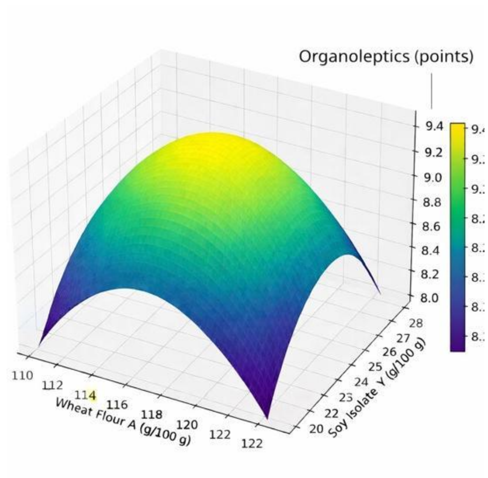
Table 8 presents the calculation of daily protein requirement coverage for different age groups of children based on the protein content in 50 g portions of the experimental samples. The calculation was performed based on the protein content in 50 g of the experimental high-protein bar and the recommended daily protein intake for each specific age group, expressed as a percentage.

Table 8. Coverage of daily protein requirements when consuming one portion of a high-protein bar (50 g)

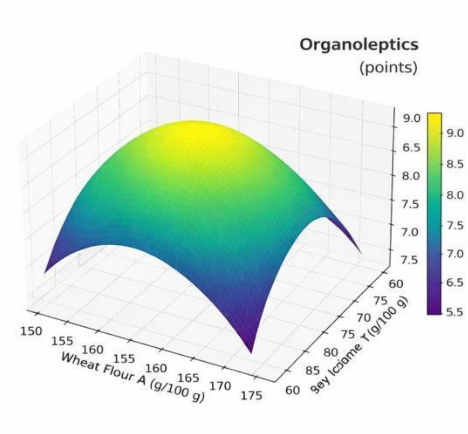
Age group	Daily requirement, (g day^{-1})	Protein requirement coverage when consuming 50 g day^{-1} (%)		
		№1 (8.64 g)	**№2 (9.70 g)**	**№3 (11.54 g)**
4–6 years	19	45.5	51.1	60.7
7–10 years (recommended)	28–34	25.4–30.9	28.5–34.6	33.9–41.2
7–10 years (optimal)	32–38	22.7–27.0	25.5–30.3	30.4–36.1
11–14 years, boys (recommended)	42	20.6	23.1	27.5
11–14 years, boys (optimal)	48–52	16.6–18.0	18.7–20.2	22.2–24.0
11–14 years, boys (active)	55–65	13.3–15.7	14.9–17.6	17.8–21.0
11–14 years, girls (recommended)	41	21.1	23.7	28.1
11–14 years, girls (optimal)	46–50	17.3–18.8	19.4–21.1	23.1–25.1
11–14 years, girls (active)	52–60	14.4–16.6	16.2–18.7	19.2–22.2

Effect of wheat flour and soy isolate content on the organoleptic evaluation of protein bars

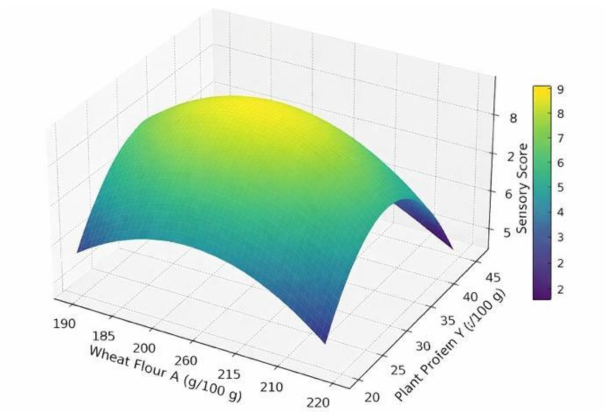
The combined effect of wheat flour content and plant protein – soy isolate concentration on the organoleptic evaluation of the developed high-protein bars is shown in Fig. 1.



a



b



c

Fig. 1. Response surface illustrating the combined effect of wheat flour concentration (A) and soy isolate (Y_1) on the organoleptic characteristics of protein bars: *a* – sample №1; *b* – sample №2; *c* – sample №3.

A three-dimensional response surface plot describing the combined effect of wheat flour (A) and soy isolate (Y_1) concentrations on the organoleptic characteristics of the final product is presented in Fig. 2. The curvature of the surface and the smooth gradients of response variation indicate a significant influence of both main factors (A and

Y_1) as well as their interaction. This confirms the adequacy of the selected quadratic regression model and indicates a synergistic effect of the ingredients determining the final product quality.

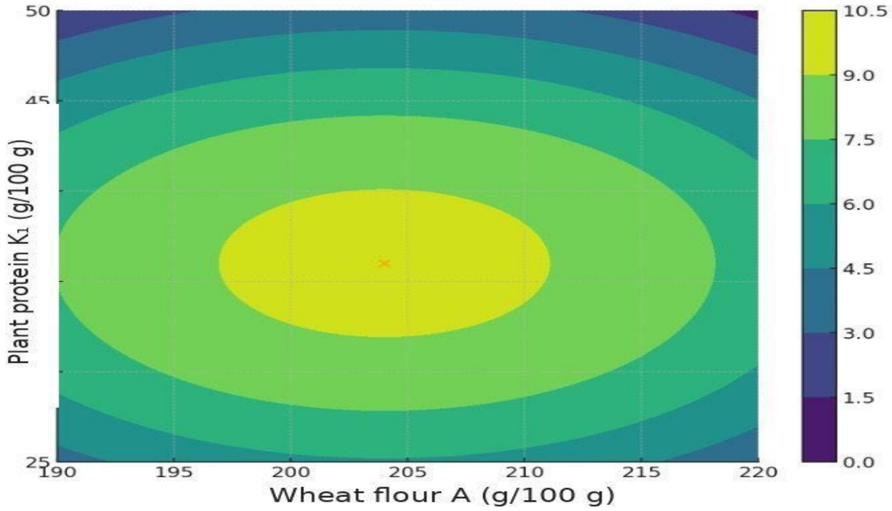


Fig. 2. Effect of wheat flour (A) and plant protein (Y_1) concentrations on the organoleptic characteristics of protein bars.

DISCUSSION

The fermented soy protein isolate samples obtained in this study, in terms of quality characteristics (organoleptic and physicochemical parameters presented in Tables 2 and 3), comply with the requirements specified in the regulatory documents described in Section 4, as well as with the general standard for plant protein products of the Codex Alimentarius (CXS 174-1989). It should be noted that the studied samples of soy protein isolate exhibited a high protein content of 93.0-93.9% on a dry weight basis, confirming their high quality and protein value, as well as their suitability for use in functional nutrition (Kinsella & Morr 1984; Banach *et al.* 2014; Kerksick *et al.* 2017; Simms 2023). The microbiological analysis of fermented soy protein samples (Table 4) demonstrated the absence of pathogenic microorganisms. No coliform bacteria were detected. *Staphylococcus aureus*, sulfite-reducing clostridia, total mesophilic aerobic and facultative anaerobic microorganisms, yeasts, and molds did not exceed the permissible regulatory limits. Thus, the fermented soy isolate used in the study represents a high-protein raw material that complies with regulatory quality requirements and was therefore utilized in further product development. The incorporation of high-protein soy isolate into the bar formulation resulted in products with satisfactory organoleptic characteristics meeting the requirements for this type of product (Table 5). The experimental samples exhibited no deformation, with a slightly rough surface containing inclusions of crumbs and bran, which is considered acceptable. A light brown color, pleasant appearance, buttery aroma, and floury taste were imparted by the fermented soy isolate (Millward 2012; Meinlschmidt *et al.* 2016). Previous studies (Kinsella & Morr 1984; Alcorta *et al.* 2021) have reported challenges related to sensory properties in bars enriched with plant proteins. The fermentation approach applied in the production of soy isolate in this study made it possible to overcome several technological limitations, including sensory and organoleptic issues. The bars exhibited a dense and dry texture requiring effort to break, which is necessary to maintain structural integrity. Soy lipids also act as carriers of flavor compounds and contribute positively to the aftertaste. The results of the physicochemical analysis of three experimental high-protein energy bar samples are presented in Table 6 in comparison with the requirements of GOST 24901 “Cookies. General specifications” (used as a baseline standard for low-moisture confectionery products). The most significant difference between the developed samples and conventional cookies according to GOST 24901 is the protein content. All three samples demonstrated protein levels in the range of 17.28-23.08%, which is 2-3 times higher than the standard protein content for sugar cookies (7.5-9.0%). Sample №3 exhibited the highest protein content (23.08 ± 0.08%), allowing it to be classified as a “high-protein product” according to Technical Regulation TR CU 022/2011 (requirement >20% protein). This level is comparable to professional sports bars and exceeds most commercially available analogues on the market of the Republic of Kazakhstan (15-20%). The calculation of protein intake showed that consumption of a 30 g bar provides 8.64 g of protein (28.8% of daily requirement) for sample №1, 9.70 g (32.3%) for sample №2, and 11.54 g (38.5%) for sample №3. The increase in protein content across samples №1 (17.28%), №2 (19.39%), and №3 (23.08%) reflects variations in the proportion of fermented soy protein isolate in the formulation. Previous studies have investigated the use of plant proteins such as pea, rice, sunflower, and others in the formulation of protein bars (González-Pérez & Vereijken 2007; Day 2013; Małecki *et al.* 2020). However, these products generally exhibited significantly lower protein content compared to those based on animal and soy proteins (Schaafsma 2000; Taillie 2003).

The moisture content (Table 6) shows a progressive increase across the samples: 3.89% (№1), 4.49% (№2), and 7.66% (№3). Although the moisture content in all experimental samples does not exceed the maximum permissible level specified by GOST 24901 (≤10.0%), the difference of 3.77 percentage points between the extreme samples is technologically and sensorially significant. The optimal moisture range for high-protein bars is 8-12% with controlled water activity (AW) in the range of 0.65-0.70%. Sample №3, with a moisture content of 7.66%, approaches the lower boundary of the optimal range, which explains its improved organoleptic characteristics compared to sample №1. The moisture-retention capacity of the fermented soy isolate contributed to the observed moisture content in the experimental bar samples. Previous studies involving whey protein (Alcorta *et al.* 2021) reported that increasing protein content is often associated with challenges related to texture and moisture retention. The use of fermented soy isolate allows these issues to be addressed while also being more cost-effective (Meinlschmidt *et al.* 2016). The fat content varies in the range of 14.46-19.39%, exceeding the standard values for cookies according to GOST 24901 (2.5-10.0%) by 1.5-8 times. This increase is intentional and functionally justified for bars intended for school-aged children. It has previously been noted that soy lipids act as carriers of flavor compounds and enhance aftertaste. Fat content demonstrates an inverse relationship with protein content. In sample №1, fat content is 19.39% and protein content is 17.28% (ratio 1.21:1). In sample №2, fat content is 14.46% and protein content is 19.39% (ratio 0.78:1). In sample №3, fat content is 18.01% and protein content is 23.08% (ratio 0.75:1). Samples №2 and №3 exhibit a more favorable protein-to-fat ratio (1:0.75-0.78), which is close to the recommended range for schoolchildren (1:0.6-0.8). Sample №1, with a ratio of approximately 1:1.12, may be more suitable for children who prefer lower protein intake. The results indicate that fermented soy isolate is an effective raw material for the development of high-protein bars with acceptable sensory properties when properly formulated to achieve balanced nutritional and technological characteristics. The energy value of all samples (369-386 kcal/100 g) falls within the target range for energy bars (350-420 kcal), but is 10-15% lower than that of conventional cookies (410-450 kcal). This reduction is attributed to lower levels of simple carbohydrates (43.95-49.95% vs. 60-75%), a higher proportion of protein (lower caloric density: 4 kcal g^{-1} vs. 9 kcal g^{-1} for fats), and the presence of non-caloric dietary fiber (2.73-3.19%). The carbohydrate content in the developed samples (43.95-49.95%) is substantially lower than in traditional cookies (60-75% according to GOST 24901-89). This reduction of 15-30 percentage points represents a key distinguishing feature and contributes to a lower glycemic index. Sample №3, with the lowest carbohydrate content (43.95%), is of particular interest for athletes (fat mass reduction while maintaining muscle mass), individuals controlling carbohydrate intake, and consumers with prediabetes or type 2 diabetes (upon medical consultation). The presence of dietary fiber in all samples (2.73-3.19%) further contributes to glycemic index reduction by slowing carbohydrate digestion, increasing intestinal chyme viscosity, and modulating glucose absorption.

The ash content in the samples ranges from 6.23% to 7.69%, exceeding the limit specified in GOST 24901-89 (≤1.2%) by 5-6 times. This increase is not a defect but reflects functional enrichment with mineral components.

The high ash content correlates with the use of fermented protein isolate, which contains approximately 3-6% mineral substances. Sample №3 exhibits the highest ash content (7.69%), consistent with its highest protein content (23.08%) and indicating a higher proportion of fermented soy isolate in the formulation. Analysis of the amino acid composition of the protein bars (Table 7) showed that lysine, typically a limiting amino acid in plant proteins, is present in all samples at levels of 48-52 mg g^{-1}, corresponding to 107-116% of FAO/WHO requirements for primary school children (7-10 years). This indicates adequate biological value. Methionine represents a potential limiting amino acid in the amino acid profile. In sample №1, its content is 20.0 mg g^{-1} (125% of the requirement, adequate level). In sample №2, it is 13.0 mg g^{-1} (81%, borderline), and in sample №3, 11.0 mg g^{-1} (69%, insufficient). Methionine deficiency in samples №2 and №3 is typical for formulations with a high proportion of plant proteins. Leucine is a key amino acid for stimulating muscle protein synthesis and is present in samples №1 and №2 at 73-75 mg g^{-1} (combined with isoleucine), with an estimated leucine content of 45-48 mg g^{-1}. A 50 g portion of sample №3 contains 11.54 g protein × 0.045 g leucine/g protein = 0.52 g leucine. This represents only 17-26% of the optimal threshold required to maximally stimulate muscle protein synthesis (2-3 g leucine per intake). To achieve an anabolic effect, consumption of 2-3 bars (100-150 g) would be required (1.04-

1.56 g leucine), or combination with other protein sources (e.g., milk or a protein shake). The high nutritional value and balanced amino acid composition make high-protein bars with soy isolate a valuable food product. Analysis of protein requirements for primary school children (7-10 years) showed that the recommended FAO/WHO intake is 28-34 g day^{-1}, the optimal intake is 32-38 g day^{-1}, and for physically active children 35-42 g day^{-1}. One serving of the developed bar (50 g) provides 25.4-30.9% of the daily requirement for sample №1, 28.5-34.8% for sample №2, and 33.9-41.2% for sample №3.

As shown in Table 8, consumption of one 50 g bar provides:

for primary school children (7-10 years): approximately 25-42% of daily protein requirements, depending on the sample and reference intake;

For children aged 4-6 years: approximately 45-61%, making the bars a significant protein source;

For adolescents aged 11-14 years: a lower proportion (e.g., 20-28% for boys under recommended intake);

For physically active boys aged 11-14 years: 13-21%;

For physically active girls aged 11-14 years: approximately 14-22%, depending on the sample.

Analysis of experimental samples with varying levels of fermented soy isolate demonstrated that sample №3 (30% soy isolate) exhibited the most favorable nutritional characteristics, namely:

Protein content of 23.08%, qualifying the product as a “high-protein product” according to TR CU 022/2011 (>20%);

Optimal protein-to-fat ratio of 1:0.78, corresponding to recommendations for school-aged children (7-10 years);

Minimum carbohydrate content (43.95%), contributing to a lower glycemic index;

Maximum mineral content (ash 7.69%), indicating a high level of micronutrients.

Analysis of amino acid composition (Table 7) demonstrated high biological value of the developed protein bars for schoolchildren (7-10 years):

Lysine (48-52 mg g^{-1} protein): 107-116% of FAO/WHO requirements;

Valine (48-84 mg g^{-1}): 123-215% of requirements;

Threonine (30-51 mg g^{-1}): 130-222% of requirements;

Histidine (28-39 mg g^{-1}): 187-260% of requirements;

Phenylalanine + tyrosine: 168% of requirements.

A deficiency of methionine was identified in sample №2 (11.0 mg g^{-1}; 32% of the FAO/WHO requirement for children aged 7-10 years), indicating an insufficient level, and in sample №3 (13.0 mg g^{-1}; 38% of the requirement), corresponding to a borderline value. The leucine content ranges from 45 to 48 mg g^{-1} of protein, and a single serving of the bar (50 g) provides only 0.52 g of leucine, corresponding to 17-26% of the optimal threshold (2-3 g) required for maximal stimulation of muscle protein synthesis. To meet the daily leucine requirement for schoolchildren, consumption of 2-3 bars (100-150 g) or combination with additional protein sources is recommended. Thus, the developed high-protein bars can be considered a full-fledged functional product for school-aged children. The developed bars serve as a source of nearly all essential amino acids, micro- and macronutrients, and vitamin E. They exhibit favorable sensory and functional properties at a relatively low production cost. According to the results of the study, the combined effect of wheat flour content and plant protein (soy isolate) concentration on the organoleptic evaluation of the developed bars was described by a three-dimensional response surface (Fig. 1). This surface represents the joint influence of wheat flour content (A, g 100 g^{-1}) and soy isolate content (Y_1, g 100 g^{-1}) on the organoleptic score. The surface exhibits a pronounced parabolic (paraboloid) shape with a distinct maximum, indicating a nonlinear relationship between the response and the studied factors. As shown in Fig. 1, an increase in organoleptic score is observed by elevating soy protein content up to 24 g 100 g^{-1} and wheat flour content of approximately 216 g 100 g^{-1}. In this region, the response surface reaches maximum values (approximately 9.3-9.6 points), indicating an optimal formulation. Deviations from this region – either increasing or decreasing the levels of flour or protein – lead to a decline in organoleptic score, confirming the quadratic nature of the model and the presence of an optimum within the experimental range. The three-dimensional plot presented in Fig. 1 illustrates the response surface describing the combined influence of wheat flour (A) and soy isolate (Y_1) concentrations on the organoleptic characteristics of the finished product. The X and Y axes represent the varying levels of ingredients (150-175 g /100 g^{-1} for wheat flour and 60-85 g 100 g^{-1} for soy isolate), while the Z axis represents the predicted organoleptic score calculated using the quadratic model. The surface exhibits a dome-shaped profile, indicating the presence of an optimum in the central region. The maximum response is observed at A = 162 g 100 g^{-1} and Y_1 = 72 g 100 g^{-1}, corresponding to the highest predicted organoleptic score (approximately 9.5 points). This point is indicated in Fig. 2 by a marker. The model also accounted for constant formulation components, including butter (180 g) and egg (1 unit), which are additionally indicated in Fig. 1. The response surface clearly demonstrates that deviations from the optimal ratio of wheat flour to soy isolate lead to a decrease in organoleptic quality, reflected by a reduction in surface height. This confirms the importance of selecting a balanced composition of raw materials when developing products with desired consumer properties. The three-dimensional plot presented in Fig. 2 further illustrates the response surface characterizing the influence of wheat flour (A) and plant protein (Y_1) concentrations on the organoleptic properties of the developed product. The variation ranges were 190-220 g 100 g^{-1} for wheat flour and 25-50 g 100 g^{-1} for plant protein. The surface exhibits a pronounced parabolic shape, typical for quadratic models used in Response Surface Methodology (RSM). The maximum response is located in the optimal region at A = 204 g 100 g^{-1} and Y_1 = 36 g 100 g^{-1}, where the highest predicted organoleptic score (approximately 9.5 points) is observed. The plot (Fig. 2) clearly shows that both increases and decreases in factor levels relative to the optimum result in a decline in organoleptic score, as reflected by the reduction in surface height. These findings confirm the necessity of optimizing the ratio of raw material components in order to achieve high consumer quality characteristics. Future research directions may include optimization of the amino acid composition of protein bars, enrichment with micro- and macronutrients, and expansion of the flavor range.

CONCLUSION

The fermented soy isolate samples used for the development of protein bars for schoolchildren exhibited moisture contents of 5.6% and 6.0%, and crude protein contents of 94% and 93%, respectively. Microbiological parameters complied with sanitary and hygienic standards. The vitamin E content in protein bars formulated with fermented soy isolate reached 46.11 mg 100 g^{-1}, covering the daily requirement when consuming 50 g of the product. B-group vitamins were also present in amounts providing 7-15% of the recommended daily intake. Toxic elements and radionuclides were not detected in the experimental samples, confirming compliance with the Technical Regulation of the Customs Union TR CU 021/2011. The energy value of the developed bars was 369-386 kcal 100 g^{-1}, which falls within the optimal range for energy products (350-420 kcal). Consumption of one 50 g portion provides 184-193 kcal, corresponding to 9-10% of the daily energy requirement (2000 kcal) for primary school children (7-10 years). The combined effect of wheat flour content and plant protein (soy isolate) concentration on organoleptic evaluation demonstrated a maximum score of approximately 9.5 points for the formulation containing 15% fermented soy isolate.

Conflict of interest

The authors declare that they have no conflict of interest related to this study, whether financial, personal, authorship-related, or otherwise, that could have influenced the research or its results presented in this paper.

Funding

This research was carried out within the framework of financial support provided by the scientific and technical program of the Ministry of Agriculture of the Republic of Kazakhstan BR22883587: “Improvement and development of science-intensive technologies for deep processing of agricultural raw materials to strengthen food security of the Republic of Kazakhstan.”

Data availability

The manuscript has no associated data.

Use of artificial intelligence

Artificial intelligence *(GPT-5.1, OpenAI)* was used to visualize the influence of wheat flour and soy isolate ratios on organoleptic characteristics in Section 5.4 using Response Surface Methodology (RSM) with a quadratic model. The AI-assisted output enabled the calculation and visualization of optimal formulation parameters ensuring the best technological and organoleptic characteristics. Validation of the results obtained using AI tools was based on expert sensory evaluation of protein bars for children and was verified for structural correctness, consistency with defined parameters, and internal coherence.

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