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OPTIMIZATION OF MULTI-COMPONENT FOOD MATRICES IN COMBINED COOKIE TECHNOLOGY USING MICROALGAE PROTEIN ISOLATE FOR MILITARY NUTRITION

This study addresses a critical scientific and practical challenge: the development of balanced-composition flour confectionery products tailored for military and health-oriented nutrition. The object of the study is the technology of combined sandwich-type cookies, optimized for protein, fat, and carbohydrate content through the integration of highly processed alternative raw materials.

The technological core is based on the application of a high-protein microalgae isolate (Euglena gracilis), obtained via the pH-shift processing method with a protein concentration of 95 - 96%. The use of a two-stage emulsion dough kneading process has been scientifically justified; this method enables the regulation of the gluten network development during isolate incorporation, ensuring mass plasticity with a moisture content of 18 - 20%. For the filling, a "protein-water-polysaccharide" system was developed based on albumin whey paste and freeze-dried apple powder, which serves as a natural structural stabilizer and water-binding agent.

Using Response Surface Methodology (RSM) through an Orthogonal Central Composite Design (OCCD), the optimal concentrations of the formulation components were established: microalgae isolate at 7 - 9% by flour weight; whey paste at 25–30%, and freeze-dried filler at 3–4% by filling weight. It was demonstrated that this ratio ensures a synergistic effect, forming a stable emulsion structure and neutralizing the specific algal aftertaste.

The developed product is characterized by high biological value: the protein content is 16.2 g/100 g (a 2.5-fold increase compared to the control), and the P: F: C ratio is optimized to 1:0.8:3.2. Microbiological studies established that no growth of osmophilic microorganisms or molds occurred during storage, allowing for a predicted shelf life of up to 6 months without the use of synthetic preservatives.

Keywords: *Euglena gracilis microalgae protein isolate, optimization, military nutrition, albumin whey paste, freeze-dried powders, combined cookies, biological value.*

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ОПТИМІЗАЦІЯ БАГАТОКОМПОНЕНТНИХ ХАРЧОВИХ МАТРИЦЬ У ТЕХНОЛОГІЇ КОМБІНОВАНОГО ПЕЧИВА ІЗ ВИКОРИСТАННЯМ ІЗОЛЯТУ БІЛКА МІКРОВОДОРОСТЕЙ ДЛЯ ВІЙСЬКОВОГО ХАРЧУВАННЯ

Робота присвячена вирішенню актуальної науково-практичної задачі - створенню борошняних кондитерських виробів збалансованого складу для військового та оздоровчого харчування. Об'єктом дослідження є технологія комбінованого печива типу «сендвіч», оптимізованого за вмістом білків, жирів та вуглеводів з використанням продуктів глибокої переробки альтернативної сировини.

В основу технології покладено використання високобілкового ізоляту мікроводоростей (Euglena gracilis), отриманого методом pH-shift обробки з концентрацією білка 95-96 %. Науково обґрунтовано застосування двостадійного емульсійного способу замісу тіста, що дозволяє регулювати розвиток клейковинного каркасу при введенні ізоляту та забезпечувати пластичність маси (вологість 18-20 %). Для начинки розроблено систему «білок-вода-полісахарид» на основі альбумінної сироваткової пасти та сублімованого порошку яблука, який виступає природним стабілізатором структури та вологоутримуючим агентом.

За допомогою методології поверхні відгуку (ОЦКП) встановлено оптимальні кількості рецептурних компонентів: ізолят мікроводоростей - 7-9 % до маси борошна; сироваткова паста - 25–30 % та сублімований наповнювач - 3-4 % до маси начинки. Доведено, що таке співвідношення забезпечує синергетичний ефект, формуючи стійку емульсійну структуру та нівелюючи специфічний водоростевий присмак.

Розроблений виріб характеризується високою біологічною цінністю: вміст білка становить 16,2 г/100 г (збільшення у 2,5 раза порівняно з контролем), а співвідношення Б:Ж:В оптимізовано до рівня 1:0,8:3,2. В ході мікробіологічних досліджень встановлено, що в процесі зберігання не виявлено розвитку осмофільних мікроорганізмів та пліснявих грибів, що дозволяє прогнозувати термін зберігання продукту до 6 місяців без використання синтетичних консервантів.

Ключові слова: *альтернативні білки, Euglena gracilis, оптимізація, військові раціони, сиркова паста, сублімовані порошки, печиво, нульовий голод.*

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Problem statement in general form and its connection with important scientific or practical tasks

The modern paradigm of food science is aimed at transforming traditional mass-consumption products into functional products. Flour confectionery, in particular cookies, occupy one of the leading positions in the consumer basket, but their nutrient profile often does not meet the requirements of a healthy diet [1]. Typical recipes are characterized by an excessive content of easily digestible carbohydrates and saturated fats with an acute deficiency of complete protein, dietary fiber and micronutrients [2]. According to WHO, such an imbalance is a risk factor for the development of metabolic syndrome and nutritionally dependent diseases [3]. In this regard, the development of technologies for enriching confectionery products with alternative sources of protein and reducing their glycemic index is an urgent scientific and practical task [4].

The global trend towards alternative food sources «Future Foods» stimulates the search for new protein resources that would combine high biological value and environmental sustainability of production [5]. Among non-traditional raw materials, microalgae (in particular, *Euglena gracilis*), which are recognized as one of the most promising sources of protein of the 21st century. Unlike traditional agricultural crops, microalgae biomass contains 50–70% protein with a full spectrum of essential amino acids, as well as unique bioactive pigments and vitamins [6].

To meet the needs of military nutrition, the dough base recipe requires additional enrichment with high-quality protein, which contributes to the rapid restoration of physical resources. A promising innovative step is the use of microalgae protein isolate *Euglena gracilis*. Its introduction into the dough significantly increases the overall biological value of the product due to essential amino acids and immunomodulatory properties. β - glucans, and also plays an important technological role. Thanks to interactions with wheat flour proteins are improving elastic-plastic properties masses that guarantees stable quality and balanced nutrient profile final product. [7].

Analysis of recent research and publications

To eliminate technological defects of native raw materials, the use of deep processing, in particular the pH-shift treatment method (acid-alkaline extraction) [8] is promising. The essence of the method is to transfer proteins into a soluble state at extreme pH values (alkaline pH 10-12 or acidic pH 2-3) [9]. This leads to partial unfolding of the protein globule and exposure of hidden hydrophobic groups. The next stage is precipitation at the isoelectric point (pH 4.5), during which the protein refolds into a “molten globule” structure [10]. Studies confirm that microalgae protein isolates obtained by the pH-shift method have a protein purity of 95-96%, as well as significantly improved functional properties: increased solubility due to a change in the surface charge of molecules and emulsifying ability, which provides a fine-pore structure of cookies [11]. In addition, the extraction process allows the removal of low-molecular compounds responsible for bitterness and specific odor [12].

The key hypothesis of this study is to achieve a synergistic effect when combining plant (microalgae) and animal (whey) proteins [13]. At the same time, whey proteins (albumins and globulins) contain an excess of methionine and cysteine, but have a relatively lower content of arginine and phenylalanine [14]. Combining microalgae protein isolate and albumin paste allows for the implementation of the principle of mutual enrichment (complementarity bringing the amino acid profile of the finished product closer to the “ideal protein” according to the FAO/WHO scale [15].

Secondly, there is a technological synergy. In the food matrix of sandwich cookies, the interaction of different biopolymers determines the texture. Microalgae isolate due to its high-water absorption capacity, ensures the density of the dough base and prevents its brittleness [16]. At the same time, the whey proteins of the filling, when interacting with polysaccharides (freeze-dried fruits), form stable gel-like structures [17]. This binary protection prevents moisture migration from the filling into the dough, preserving the crispy properties of the cookies during the shelf life [18].

The weak point of sandwich cookies is the filling, which traditionally consists of fat and powdered sugar [19]. An innovative approach is the use of freeze-dried (lyophilized) fruits. The sublimation technology allows you to preserve up to 95–97% of the vitamins and anthocyanins of the starting material [20]. Powders of freeze-dried apples or strawberries, due to their high content of native pectin, act as natural stabilizers of the filling structure [21]. When introduced into a wet medium of whey paste (cold mixing technology), they bind free moisture, allowing you to exclude added sugar and synthetic thickeners [22].

The creation of multicomponent food systems containing protein isolates and structured fillings is a complex technological task. Changing the concentration of one component nonlinearly affects rheology and organoleptic [23]. Empirical formulation selection in such cases is ineffective. Modern methodology involves the use of orthogonal plans, which allows finding the optimum region between the maximum biological value and consumer characteristics [24].

Despite the existence of studies on the separate use of microalgae protein isolates or whey processing products, a comprehensive technology for creating combined cookies, where the protein base of the dough is also optimized (microalgae protein isolate) *Euglena gracilis*), and functional filling, for military food needs, has not been sufficiently researched.

Formulation of the article’s objectives

The aim of the work is to optimize multicomponent food matrices in the technology of combined cookies in terms of the content of proteins, fats and carbohydrates at maximum values of organoleptic assessment using microalgae protein isolate (*Euglena gracilis*) and albumin paste for military food.

Presentation of the main research material

Technology for manufacturing combined cookies. The defining stage of the work was the scientific substantiation of the technological parameters for obtaining and preparing functional ingredients, in particular, high-protein isolate of microalgae protein (*Euglena gracilis). The use of innovative* pH-shift processing technology (acid-base extraction) allowed to achieve a protein purity of 95-96% in terms of dry matter. The mechanism of this modification is based on the

controlled unfolding of the globular structure of proteins in an alkaline environment with subsequent refolding during precipitation at the isoelectric point. Such processing provided a significant increase in the functional and technological properties of the isolate, in particular its emulsifying ability and solubility, and also allowed to eliminate the specific aftertaste, which is a critical factor for use in confectionery products of a delicate flavor range.

The formation of the structure of the dough matrix for sandwich cookies required taking into account complex colloidal processes in a multicomponent dispersed system. The introduction of high-protein isolate significantly changes the water absorption capacity of flour, therefore, to prevent the formation of a tight gluten structure and ensure the friability of the finished product, a two-stage emulsion mixing method was used. At the first stage, the fat phase was dispersed with a sugar substitute and salt until a stable foam was formed, which ensured aeration of the mass and the creation of crystallization centers for future porosity. At the second stage, a protein-polysaccharide mixture of isolate and flour was introduced. microalgae protein isolate (*Euglena gracilis*), having a high hydration capacity, competes with gluten proteins for available moisture, which allows regulating the development of the gluten framework and obtaining a plastic dough with a moisture content of 18–20%. Heat treatment of semi-finished products in a convection oven at a temperature of 175±5 °C for 12-15 minutes ensured complete denaturation of proteins, fixation of the porous structure and achievement of a final moisture content of 4-5%, which guarantees the microbiological stability of the product.

In parallel, a technology for preparing the filling based on the emulsion system "protein-water-polysaccharide" was developed. Homogenization of unsweetened whey paste (albumin mass) with freeze-dried apple powder (dispersion 50-100 μm) at a temperature of 20-25 °C allowed to create a structure with high viscosity and plasticity. Freeze-dried apples, due to the high content of native pectin, play the role of an effective moisture-retaining agent. This creates a thermodynamic barrier to moisture migration into the baked base, maintaining its crispy properties throughout its shelf life, and also prevents syneresis of the filling.

Mathematical modeling and optimization of the formulation. In order to find the extremum of the response function (maximum organoleptic assessment with a balanced composition), the response surface methodology was applied. A second-order orthogonal central compositional design (OCCD) was used for four factors (n=4). This allowed us to assess nonlinear effects and interfactor interactions. The input variables (factors) were coded in the range from - α to + α (where α = 1.414 for orthogonality) and are given in Table 1.

Table 1

Experimental design matrix and levels of factor variation

Factors (Xi)	Factor name and dimension	-α (-1.414)	-1 (lower)	0 (basic)	+1 (top)	+α (+1.414)
x_1	Microalgae protein isolate (*Euglena gracilis*), % by weight of flour	3.0	5.0	7.0	9.0	11.0
x_2	Fat component, g	10.0	12.5	15.0	17.5	20.0
x_3	Whey paste, % by weight of filling	15.0	20.0	25.0	30.0	35.0
x_4	Sublimated powder, % by weight of filling	1.0	2.0	3.0	4.0	5.0

As a result of computer processing of experimental data (using the Statistica 12 application software package), second-order mathematical models were obtained that adequately describe the influence of the studied factors on the quality indicators of cookies (Table 2).

The following were selected as key feedback functions:

1. Y_1 - Comprehensive organoleptic assessment (points, max. 20).
2. Y_2 – Balanced in terms of protein, fat, and carbohydrate content.

Table 2

Mathematical regression models and their statistical characteristics

Feedback function	Regression equation (in coded variables)	Statistical characteristics (R_2, p- value)
Y_1 - Organoleptic evaluation (points)	$Y1 = 19{,}42 + 0{,}45x_1 + 0{,}38x_3 - 1{,}42x_1^2$-0.95 x_3^2+$0.88x_3x_4$	$R^2 = 0{,}94$; F ross> F tabl P<0.05
Y_2 - Balanced in terms of protein, fat, and carbohydrate content	$Y2 = 12{,}80 + 2{,}35x_1 - 0{,}60x_2 + 1{,}95x_1^2$+$0.75x_1x_3$	$R^2 = 0{,}91$; F ross> F tabl P<0.05

Analysis and interpretation of the obtained models. The statistical significance test of the obtained equations using the Fisher exact test (F-test) confirmed their adequacy to the experimental data with a confidence level of 95%. High values of the coefficient of determination (R_2 >0.91) indicate that the variation of the selected factors explains 91-94% of the change in the initial parameters of the quality of the finished product.

The equation for Y_1 is characterized by a high value of the free term (b_0 = 19.42), which indicates the achievement of high taste properties in the center of the experimental design. The greatest influence on the formation of the organoleptic profile is exerted by quadratic effects. The negative sign at the quadratic coefficient of the content of microalgae protein isolate (-1.42x_1^2) mathematically describes the parabolic nature of the dependence. This means that when

the concentration of the isolate increases to a certain level (7-9 %), the quality increases, but a further increase in the dosage leads to a sharp decline in the assessment due to the appearance of a specific algae flavor and deterioration of the texture.

Research on the nutritional and biological value of the optimized product. Based on the solution of the optimization problem, a recipe for combined cookies for special forces (military personnel) was developed. A comparative analysis of the nutrient profile (Table 3) demonstrates significant advantages of the developed product.

Table 3

Comparative characteristics of the nutrient composition (per 100 g of product)

Indicator	Control sample (traditional sand)	Optimized sample (with microalgae protein isolate)
Proteins, g	6.5	16.2
Fats, g	29.0	13.5
Carbohydrates, g	68.0	52.0
Correlation B: F: C	1:4.5:10.5	1:0.8:3.2

Comparative analysis of the nutrient composition demonstrates significant advantages of the developed combined cookies over traditional analogues. The protein content increases by 2.5 times (up to 16.2 g/100 g), while the fat content is reduced to 13.5 g, and carbohydrates - to 52.0 g, which provides a balanced ratio of B:F:C at the level of 1:0.8:3.2.

Study of organoleptic indicators of combined cookies. Based on the solution of the multi-criteria optimization problem, a pilot batch of combined cookies was produced for in-depth product evaluation (Fig. 1).

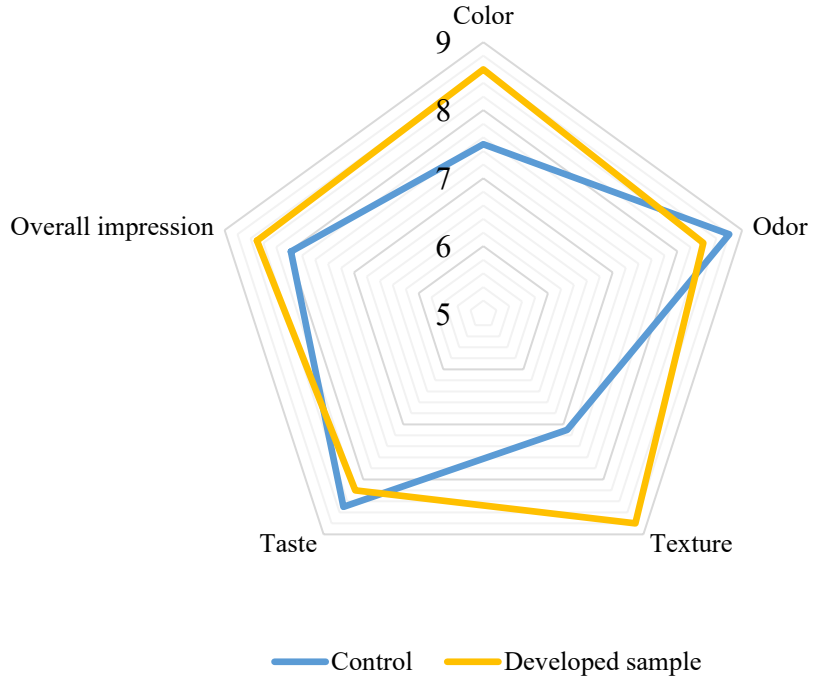

Fig. 1. Organoleptic evaluation of developed cookies

Tasting evaluation by profile analysis method showed that the developed product has a correct geometric shape without deformations, and its surface is characterized by uniformity and the absence of cracks, which indicates the complete relaxation of internal stresses in the protein-carbohydrate network of the dough. The color of the cookies is golden-beige with a slight olive tint at the break, due to the presence of natural pigments of microalgae protein isolate. The texture of the base is defined as crumbly, but resistant to mechanical loads during transportation, and biting is accompanied by a characteristic crunch. The filling demonstrates plasticity and a delicate creamy consistency without signs of syneresis. The taste profile is balanced: light fruit acidity and milky notes effectively mask the specific aftertaste of microalgae protein isolate, and the absence of cloying sweetness positively correlates with the concept of healthy food products.

Microbiological stability and shelf-life studies. During microbiological studies, it was found that no development of osmophilic microorganisms was detected during storage, which allows predicting a shelf life of the product of up to 6 months without the use of synthetic preservatives.

To experimentally confirm the predicted shelf life, the sanitary and hygienic indicators of the experimental batch of cookies were monitored for 6 months of storage in a sealed polypropylene film package (temperature 18±3°C, relative humidity $\omega < 75\%$). The dynamics of microbiological indicators are given in Table 4.

Table 4

Dynamics of microbiological quality indicators of combined cookies during storage

Indicator name	Permissible levels (according to DSTU/TU)	Freshly produced product (0 months)	After 3 months of storage	After 6 months of storage
MAFAM, CFU/g, not more	5×10^4	1.5×10^2	4.2×10^2	2.8×10^3
Coliforms, in 0.1 g	Not allowed	Not detected	Not detected	Not detected
Molds, CFU/g, no more	1×10^2	< 10	1.5×10^1	4.5×10^1
Yeasts, CFU/g, no more	5×10^1	Not detected	< 10	2.0×10^1
Pathogenic microorganisms (*Salmonella*), in 25 g	Not allowed	Not detected	Not detected	Not detected
S. aureus, in 0.1 g	Not allowed	Not detected	Not detected	Not detected

As the table data show, throughout the entire period under study, the microbiological indicators of the developed cookies did not exceed the regulated norms. A slight increase in the number of mesophilic bacteria (MAFAM) at the 6th month of storage remains within the permissible range (an order of magnitude below the critical threshold). The absence of mold and yeast growth above the norm confirms the effectiveness of reducing water activity due to the binding of moisture by the biopolymers of the filling - pectin of sublimated apples and proteins of whey paste. This allows us to guarantee a shelf life of the product of 6 months without the use of synthetic preservatives.

Conclusions of this research and prospects for further research in this direction

The developed technology is an effective tool for the formation of crisis -resistant food systems, offering an effective way to valorize the latest proteins in extreme consumption conditions.

Introduction of microalgae protein isolate *Euglena gracilis* (purity 95-96%), obtained through innovative pH-shift extraction, made it possible to create a functional flour matrix that meets the high requirements of military nutrition.

Mathematical modeling confirmed the synergy of the recipe components: the integration of 7-9% algae isolate, 25-30% whey paste and 3-4% freeze-dried fruit guarantees maximum sensory characteristics of the product.

The product demonstrates outstanding nutritional indicators (2.5 - fold increase in protein content to 16.2 g/100 g; B:F:V = 1:0.8:3.2), which helps to overcome nutrient deficiencies during increased physical exertion.

The created biopolymer filling system reliably blocks moisture migration, ensuring microbiological safety (absence of pathogens and mold) and a stable shelf life of up to 6 months without artificial preservatives. The projected shelf life of the product without synthetic preservatives is up to 6 months.

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