International Journal of Engineering and Advanced Technology (IJEAT)
ISSN: 2249-8958 (Online), Volume-8 Issue-4, April 2019

Biotechnology of specialized product for sports nutrition

Natalya Chernopolskaya, Natalya Gavrilova, Maksim Rebezov, Irina Dolmatova, Tatiana Zaitseva, Yuliya Somova, Maria Babaeva, Evgeny Ponomarev, Olga Voskanyan

Abstract*: The aim of the work is the development of biocomponents and biotechnology of specialized product for sports nutrition using them. The type and composition of the components of the corrective composition of the product, their modification by full or partial hydrolysis of whey proteins and immobilization of the association of lactic acid and bifidobacteria in a gel of biopolymers in the presence of a prebiotic is scientifically substantiated. The process of obtaining whey protein concentrate is presented. The optimal amount of the enzyme for the process of incomplete hydrolysis and its parameters were experimentally determined: temperature (48 ± 2) ° C, time (5.0 ± 0.5) h, mass concentration of the enzyme (1.0 ± 0.1)%, active acidity (6.30 ± 0.05) units pH, degree of hydrolysis of whey proteins (48.0 ± 1.8)%. Fructose and maltodextrin were selected as components for correcting the carbohydrate composition of the product, creatine monohydrate was used to regulate the process of building muscle. To determine the composition of the normalized mixture of the product, the chemical composition of the components was studied, an information matrix of the chemical composition of the normalized mixture was developed, balance equations were compiled and entered into mathematical systems. The process of immobilization of lactic acid bacteria (Lactobacillus acidophilus, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. diacetilactis, Streptococcus thermophilus, Bifidobacterium bifidum, Bifidobacterium longum) in the gel "biopolymer - protein" carrageenan - gelatin, taken in the ratio 2: 1 and fermentation of the normalized mixture using immobilized cultures were studied. Biotechnology of the specialized product for sports nutrition was developed.

***Index Terms*: fermentation, probiotics, whey proteins, biotechnology, sports nutrition.**

Manuscript published on 30 April 2019.
***** Correspondence Author (s)
Natalya Chernopolskaya*, Omsk State Agrarian University named after P.A. Stolypin, Omsk, Russia
Natalya Gavrilova, Omsk State Agrarian University named after P.A. Stolypin, Omsk, Russia
Maksim Rebezov, Ural State Agrarian University, Yekaterinburg, Russia; V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia; K.G. Razumovsky Moscow State University of technologies and management (the First Cossack University), Moscow, Russia
Irina Dolmatova, Nosov Magnitogorsk State Technical University, Magnitogorsk, Russia
Tatiana Zaitseva, Nosov Magnitogorsk State Technical University, Magnitogorsk, Russia
Yuliya Somova, Nosov Magnitogorsk State Technical University, Magnitogorsk, Russia
Maria Babaeva, K.G. Razumovsky Moscow State University of technologies and management (the First Cossack University), Moscow, Russia
Evgeny Ponomarev, K.G. Razumovsky Moscow State University of technologies and management (the First Cossack University), Moscow, Russia
Olga Voskanyan, K.G. Razumovsky Moscow State University of technologies and management (the First Cossack University), Moscow, Russia

I. INTRODUCTION

Nutrition is an important element in the training of athletes, both professionals and amateurs. The main task of nutrition is the optimal and timely replenishment of energy costs, as well as plastic and biologically active substances that are actively consumed in the process of intensive muscular activity, so nutrition of athletes should not only be balanced in the amount of nutrients in the diet, but also have differentiated quantitative characteristic depending on the sport and the stage of training of athletes.

For athletes, when assessing daily energy needs, the basal metabolic rate (BMR) is important [1, 2]. Physically active activity can change the rate of basal metabolism, both quantitatively and qualitatively, by the ratio of energetic oxidation substrates (carbohydrates, fats and, in extreme cases, proteins) [3]. In this case, an important role is played by the duration and intensity of training [4]. The result of the nutritional support strategy for training strength and endurance is most often the reorientation of the athlete's metabolic resources [5]. In particular, by including branched chain amino acids in the diet of sports nutrition [6].

Among the risk factors for athletes' health and impaired homeostasis, there may be insufficient vitamin and mineral provision of the body, resulting in metabolic disorders, reduced efficiency and professional reliability [7, 8]. Galstyan A.G., Elikov A.V., as well as Rakhmanov R.S., Gruzdeva A.E. et al. carried out studies of the antioxidant status of athletes with the inclusion of concentrated food products manufactured using cryogenic technology into the diet [9, 10]. The results obtained confirm the opinion of Zychowska M. et al. [11] that the state of the anti-oxidant system of the body is an important indicator of health and athletic performance. To increase the athlete's body resistance to the performance of physical loads, the prospect of using food products made from plant extracts rich in natural antioxidants in the diets of athletes employed in various sports was determined [12].

Erdman A., Travis D.T., Burke M. report that at present sports nutrition is a dynamically developing field of science and practice, since the performance of an athlete is enhanced by a well-planned nutrition strategy [13]. In this process, the use of a specialized sports nutrition acquires a special meaning [14, 15, 16].

Based on the results of their own research, scientists Lavrinenko S.V., Nikityuk D.

B., Litvinova F. B. et al. report that modern research in the field of nutriciology and the scientific development of specialized food products (SFP) are necessary to improve athletic performance, prevent occupational diseases, and preserve the health of athletes [17, 18].

The question of the metabolic role of proteins, fats and carbohydrates under intense physical loads has attracted the attention of scientists for more than 150 years. Modern researchers V.M. Vorobyova, L.N. Shatnyuk et al. found that the optimal ratio of these substances in the diets of athletes is 1: 0.8: 4, in percentage terms, this corresponds to 15:24:61 (by calorie content) and differs somewhat from the balanced formula of a smaller proportion of fat (1: 1: 4 or 14:30:56), which is explained by the peculiarities of performing sports exercises in which the oxidation of carbohydrates occurs at a lower cost of oxygen [19,20].

The most significant in the formulations of products for sports nutrition are protein, for the use of which there are only general recommendations that take into account the amount of daily protein intake and the intensity of physical activity of an athlete.

In the development of specialized protein products that are available in powder, tablet or capsulated form, which contain more than 50% protein and are intended primarily to power athletes of power and speed-strength sports. For the preparation of protein cocktails, manufacturers recommend using water, skimmed milk or juice.

Concentrates and isolates of whey proteins, sodium and calcium caseinates, skimmed milk powder, dried milk whey, skimmed soy flour, soy protein isolates and other concentrates are used as protein sources.

Abby Thompson, Mike Boland, and Harjinder Singh summarize the results of numerous studies on milk proteins and highlight their special biological value in human nutrition [21].

Paul J. Moughan reports that milk proteins play an important role in the development of the production of functional foods, since they contain a large number of essential amino acids - ideal components for specialized foods. Certain amino acids play an important physiological role (for example, tryptophan regulates muscle building), as a result of which they are used in sports nutrition. Milk proteins as sources of biologically active peptides are also essential for normalization of the gastrointestinal tract [22, 23].

A. Tepel, describing milk proteins, notes that traditional “generic” notions, caseins and whey proteins, work for macroproteins. The use of the plural in these names is due to the heterogeneity of these substances.

Caseins at pH 4.6 and 20 ° C are an insoluble part of milk proteins. All caseins are phosphoproteins.

Whey proteins at pH 4.6 and 20 ° C are “soluble” milk proteins. A common characteristic is the presence of cysteine in their composition and the formation of intramolecular disulfide bonds in the primary structure.

Among the whey proteins, the main components are β-lactoglobulin, accounting for 56% of their total mass, and α-lactalbumin, which accounts for 21%. Serum albumin (7%), immunoglobulins (14%) and lactoferrin (2%) are present in smaller quantities. Along with the whey proteins themselves, whey contains proteosepeptone fractions and glycomacropeptides, split off as a result of rennet enzyme exposure to casein.

Recently, an increase in the processing of whey and the large-scale introduction of membrane separation technologies have caused great interest in the use of whey proteins [24]. Gavrilova N.B, Moliboga E.A., Chernopolskaya N.L. et al. developed technologies for specialized nutritional products for athletes using whey proteins [25, 26]. In accordance with the recommendations of the European Nutrition Scientific Committee (Scientific Committee on Food of European Commission), all food products for athletes are divided into 4 categories:

- Category A - carbohydrate-rich energy foods;
- category B - carbohydrate electrolyte solutions;
- category C - proteins and protein components;
- category D - biologically active food supplements (essential nutrients, other food components). The most common are proteins and protein components [25].

The purpose of the study is the development of biotechnology of specialized product for sports nutrition using them.

II. MATERIALS AND METHODS

The following study objects were used in the work: the” Pancreatin-LekT” proteolytic enzyme, the association of lactic acid bacteria and the bifidobacteria Lactobacillus acidophilus (Pa), Lactobacillus lactis subsp. cremoris (K), Lactis lactis subsp. diacetylactis (D), Streptococcus thermophilus (St), bifidobacterium bifidum and bifidobacterium longum (BF) in the composition of the lyophilized polytype concentrate BK-Altai-LS-Bifi, bacterial formula PaKDStBF.

The repetition of experiments is fivefold. The results were subjected to statistical processing using the methods of correlation and regression analysis using standard “MathCAD - 14 Professional” software packages.

To obtain reliable and complete characteristics of the objects under study, modern research methods were applied.

The active acidity (pH) was determined by a potentiometric method using the IPL-101 ionometer (Russia) according to GOST 32892–2014.

For microbiological studies, certified methods of performing measurements in accordance with GOST ISO7218-2015 were used: - limiting dilution method using nutrient media - sterile skimmed milk and agar with hydrolyzed skimmed milk; - limiting dilution method using the Blourock environment. A microbiological box with the TENCAN purification system (China) was used for research.

Organoleptic evaluation of the finished products was performed by the method of blind degustation, developed on the basis of GOST 28283-2015.

The amount of protein in the hydrolyzate was determined on the analyzer "RapidNCube" (Germany); molecular weight and protein concentration with the help of the cell for electrophoresis “PROTEANIIxi”, qualitative and quantitative composition of amino acids on an automated AAA analyzer AAA - 339 (Czech Republic).

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III. RESULTS AND DISCUSSION

Achieving the goal set in the work is connected with conducting analytical and experimental studies on the scientific substantiation of the type and composition of the components of the composition of the product, modifying them with modern methods, including the use of full or partial hydrolysis of whey proteins and immobilization of the association of lactic acid and bifidobacteria in gel biopolymers in the presence of prebiotic, which increases the degree of assimilation of all nutrients by the athlete's body [26]. The whey protein concentrate was prepared as follows: cheese whey was separated at a temperature of 35 ... 40 ° C to separate fat and casein dust; pasteurized at a temperature of 72 ... 75 ° C during 15 ... 20 s and cooled to 50 ... 55 ° C [27].

Whey protein concentrate was obtained by whey ultrafiltration at a continuous installation using filters with pore size (40 ± 4) microns. Then it was concentrated to a dry matter concentration of (50.0 ± 2.0)% and hydrolyzed by a complex enzyme consisting of lipase, trypsin, chymotrypsin and alfa-amylase. The optimal amount of enzyme for the process of incomplete hydrolysis and its parameters was experimentally determined:

- temperature of hydrolysis process (48 ± 2) ° C;
- hydrolysis process time (5.0 ± 0.5) h;
- mass concentration of the enzyme (1.0 ± 0.1)%;
- active acidity of the hydrolyzate (6.30 ± 0.05) units. pH;
- degree of hydrolysis of whey proteins (48.0 ± 1.8)%.

Organoleptic characteristics, chemical composition and biological value are determined in the hydrolyzate. The results are presented in table 1. Mass fraction of dry substances in the hydrolyzate (50 ± 2)%, including proteins (9.2 ± 0.2)%, carbohydrates (40.0 ± 0.5)%.

In appearance it is a thick, viscous mass. The taste and smell is clean, whey. The color is light cream.

Table 1: Amino acid composition of whey proteins hydrolysate

Amino acid	mg/100g of product
Sum of essential amino acids, including	4273.35
Valine	530.39
Isoleucine	620.20
Leucine	905.00
Lysine	762.95
Methionine	274.20
Threonine	594.07
Tryptophane	96.12
Phenylalanine	490.40
Sum of nonessential amino acids, including	4647.57
Alanine	358.61
Arginine	119.10
Aspartic acid	568.99
Histidine	178.60
Glycine	180.20
Glutamic acid	1555.77
Proline	410.92
Serine	331.44
Tyrosine	184.72
Cystine	752.22
Total	8920.92

The data presented in Table 1 testify to the high biological value of whey hydrolysate proteins, which is confirmed by the results of the calculation of the amino acid scores, shown in Table 2.

Table 2: Amino acid scores of whey hydrolysate

Amino acid	FAO scale		Whey hydrolysate	
	mg/1g	AS	mg/1g	AS
Isoleucine	40	100	69.50	173.76
Leucine	70	100	101.45	144.94
Lysine	55	100	85.53	155.51
Methionine + Cystine	35	100	115.07	328.77
Phenylalanine + Tyrosine	60	100	75.68	126.14
Threonine	40	100	66.60	166.50
Tryptophane	10	100	10.77	107.70
Valine	50	100	59.46	118.92

Fructose and maltodextrin are selected as the components that correct the carbohydrate composition, combining fast and slow carbohydrates, which provide a gradual flow of energy into the athlete's body. To regulate the process of building muscle, a special dietary supplement, creatine monohydrate (KreatinePowder, USA), has been studied. Recommendations for its use include from 4 g to 6 g twice a day for a week and then for the next 4-6 weeks to 4 g per day. By calculation, the amount of creatine in one (one-time) package of a specialized product of 0.5 l (4.5 ± 0.5) g is determined. Since proteins: fats: carbohydrates in a specialized product should be quantitatively referred to as 1: 1: 4, the basic (basic) recipe was calculated from correlation and regression analysis and standard software packages “MathCAD - 14 Professional” [28].

Based on the results of the analysis of the chemical composition of the components, an information matrix has been developed (Table 3).

Table 3: Information matrix of the chemical composition of the normalized mixture

Component	Indexing	Weight percentage, %			
		Dry substance, %	including		
			protein	fat	carbohydrates
Normalized milk	X_1	12,2	3,2	3,2	4,8
Milk cream	X_2	27,2	2,5	20,0	4,0
Carbohydrates: fructose maltodextrin	X_3	99,5	-	-	99,5
Whey hydrolysate	X_4	52,0	9,2	0,6	40,0

To determine the amount of each component, balance equations have been compiled and entered into mathematical systems for determining the chemical composition of the normalized mixture and its compliance with the requirements of sports nutrition products.

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Balance equations:

- for protein $У_1 = X_1 \cdot 0{,}032 + X_2 \cdot 0{,}025 + X_4 \cdot 0{,}092$
- fat $У_2 = X_1 \cdot 0{,}032 + X_2 \cdot 0{,}20 + X_4 \cdot 0{,}006$
- for carbohydrates $У_3 = X_1 \cdot 0{,}048 + X_2 \cdot 0{,}0040 + X_3 \cdot 0{,}995 + X_4 \cdot 0{,}40$
- by mass $У_3 = X_1 + X_2 + X_3 + X_4 = 100$

The component composition of the normalized mixture for the subsequent fermentation process is shown in Table 4. Since creatine is added to the product prior to packaging, its amount in the mixture is not taken into account.

Table 4: Component composition of the normalized mixture of the new product

Components	Amount, kg	Weight percentage, %			
		Dry substance, %	including		
			protein	fat	carbohydrates
Normalized milk	70.5	8.60	2.25	2.25	3.38
Milk cream	7.5	2.04	0.20	1.70	2.66
Carbohydrates: fructose maltodextrin	3.0	3.00	-	-	3.00
Whey hydrolysate	19.0	10.40	1.70	0.15	7.6
Total:	100	24.04	4.15	4.10	16.64

The ratio P: F: C = 1: 1: 4.

Fermentation of the normalized mixture was carried out by the association of lactic acid bacteria consisting of the probiotic Lactobacillus acidophilus and the accompanying lactic acid cultures, contributing to the formation of a dense curd with a pronounced fermented taste of Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. diacetilactis, Streptococcus thermophilus and Bifidobacterium bifidum and Bifidobacterium longum bifidobacteria, which were previously activated on sterile skimmed milk at a temperature of (37 ... 38) ℃ for (1.0 ± 0.2) h and immobilized in a gel "biopolymer - protein" carrageene - gelatin, taken in a ratio of 2: 1, then the resulting mixture was dosed into sterile forms and kept for 15 – 20 minutes to form thin films (membranes). As a prebiotic, a beekeeping product was used - royal jelly, which contains all the substances necessary to increase the vital activity of bifidobacteria: essential amino acids (valine, methionine, tryptophan, etc.); vitamins B1, B2, B3, B6, B12, Bc, C, H, PP, E, folic acid, nicotinic acid and biotin [29, 30]. The amount of royal jelly was determined experimentally by microbiological indicators of the results of immobilization (table 5). In test samples (membranes), the amount of royal jelly varied: in the first experiment 0.1%, in the second experiment 0.2%, in the third experiment 0.3%, in the fourth experiment 0.4%, in the fifth experiment 0.5% . As a control, a prototype (membrane) was used without the addition of royal jelly.

Table 5: Effect of prebiotic amount on membrane microbiological indicators

Sample	Royal jelly amount, %	Lactic acid bacteria count, CFU/cm^3	Bifidobacteria count, CFU/cm^3	Acidophilus bacterium count, CFU/cm^3
Control	0	$8.0 \cdot 10^9$	$4.2 \cdot 10^8$	$7.2 \cdot 10^9$
Experiment 1	0.1	$1.5 \cdot 10^{10}$	$8.9 \cdot 10^8$	$1.0 \cdot 10^{10}$
Experiment 2	0.2	$2.7 \cdot 10^{10}$	$1.0 \cdot 10^9$	$2.3 \cdot 10^{10}$
Experiment 3	0.3	$4.2 \cdot 10^{10}$	$3.2 \cdot 10^9$	$4.0 \cdot 10^{10}$
Experiment 4	0.4	$7.0 \cdot 10^{10}$	$4.0 \cdot 10^9$	$6.8 \cdot 10^{10}$
Experiment 5	0.5	$8.4 \cdot 10^{10}$	$4.5 \cdot 10^9$	$7.5 \cdot 10^{10}$

These microbiological studies (table 5) indicate a high concentration, both of the total number of lactic acid microorganisms, and of probiotics, which meets the regulatory requirements for bacterial concentrates. A rational amount of prebiotic, when immobilizing the studied association of lactic acid cultures and bifidobacteria, is 0.8 - 0.9% of royal jelly from a mixture of biomass.

The components of the normalized mixture: milk, cream, carbohydrates (fructose, maltose) and whey hydrolyzate were mixed at a temperature of (45 ± 5) ° C for 20 ... 25 minutes, then homogenized at a pressure of 10 ... 15 MPa, pasteurized at a temperature (92 ± 2) ° C during 15 ... 20 s, cooled to (38 ... 39) ° C, with stirring, the starter was added in immobilized form. Fermentation was carried out before the formation of a dense curd with a titratable acidity of not more than (90 ... 95) ° T.

The technological process of a specialized product for sports nutrition is implemented in accordance with the following flowchart shown in fig. 1. The assortment of a new product can be expanded by using fruit and berry fillers and vitamin-mineral complexes, which are added to the fermented product or are in a combined package and used by the athlete with the product. Organoleptic characteristics of a new product must comply with the regulatory requirements presented in Table 6.

Table 6: Organoleptic characteristics of a specialized product

Indicator	Characteristic
Consistency	Homogeneous, pasty
Taste and smell	Sour-milk, without foreign tastes and odors. Flavor of the filler is allowed.
Colour	From white to cream. A slight shade of filler color is allowed.

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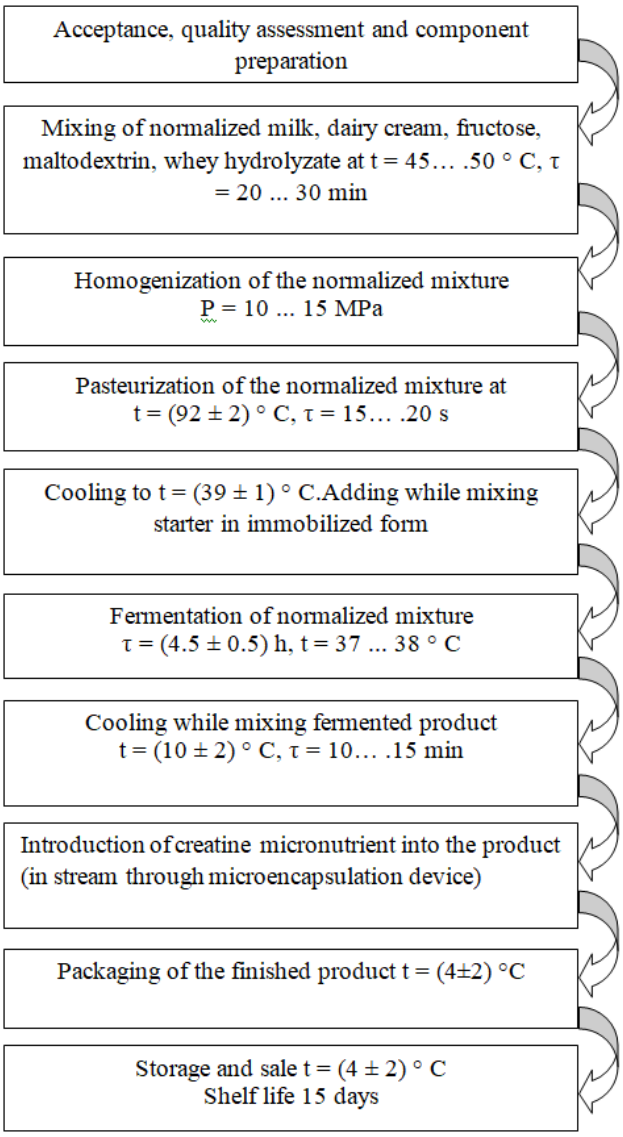

Fig. 1: Block diagram of biotechnology of a specialized product for sports nutrition

Physical and chemical indicators of a specialized product for sports nutrition are presented in table 7, microbiological - in table 8.

Table 7: Physical and chemical indicators of a specialized product

Indicator	Value
Fat, %, not less	4,0
Protein, %, not less	4,0
Acidity, °T, no more	4,0…4,5
Temperature, ° C.	4±2
Phosphatase	absent

Table 8: Microbiological indicators of a specialized product

Indicator	Value
Lactic acid microorganisms, CFU/cm^3, not less	$1 \cdot 10^8$
Probiotic microorganisms: Lactobacillusacidophilus, CFU/cm^3, not less	$1 \cdot 10^8$ $1 \cdot 10^7$
Bifidobacteria, CFU/cm^3, not less	

Also, the manufacturer of specialized products provides and ensures their safety in the content of potentially hazardous substances and microbiological indicators.

IV. CONCLUSION

Theoretical analysis and experimental studies allowed to scientifically substantiate and practically develop the component composition and biotechnological parameters of the production of a specialized product, whose component composition meets the regulatory requirements of sports nutrition products according to the protein: fat: carbohydrate ratio, like 1: 1: 4. The product is also enriched with essential amino acids, peptides through the use of whey hydrolysate and probiotics, which increases its digestibility and normalizes the athlete's gastrointestinal microflora.

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