



# Specialized hypocholesterolemic foods: Ingredients, technology, effects

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## Abstract:

**Introduction.** Overweight and obesity are leading risk factors for metabolic syndrome (MS). From 20 to 35% of Russian people have this condition, depending on their age. MS is a precursor of cardiovascular disease, diabetes mellitus, diabetic nephropathy, and non-alcoholic steatohepatitis. Specialized foods (SFs) with hypocholesterolemic effects are an important component of the diet therapy for MS patients. Creating local SFs to optimize the nutritional status of MS patients and prevent related diseases is a highly promising area of research. The aim of our study was to develop the formulation and technology of SFs and evaluate their effectiveness in MS treatment.

**Study objects and methods.** The objects of the study were food ingredients and SFs. Safety indicators and micronutrient contents were determined by standard methods, whereas nutritional and energy values and amino acid contents were determined by calculation.

**Results and discussion.** Based on medical requirements, we selected functional ingredients and developed a formulation and technology of SFs with an optimized protein, fat, and carbohydrate composition. The formulation included essential micronutrients and biologically active substances with a desirable physiological effect. Clinical trials involved 15 MS patients aged from 27 to 59. For two weeks, they had a low-calorie standard diet with one serving of SFs in the form of a drink instead of a second breakfast. The patients showed a significant improvement in anthropometric indicators. Blood serum tests revealed decreased contents of total cholesterol (by 16.9%), low-density lipoprotein cholesterol (by 15.3%), and triglycerides (by 27.9%).

**Conclusion.** We developed technical specifications and produced a pilot batch of SFs. The trials showed an improvement of lipid metabolism in the MS patients who were taking SFs as part of their diet therapy.

**Keywords:** Metabolic syndrome, specialized food, food ingredients, diet therapy

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## INTRODUCTION

The key factors leading to metabolic syndrome are increased visceral fat and decreased sensitivity of peripheral tissues to insulin resulting in compensatory hyperinsulinemia. These conditions are associated with disorders of carbohydrate, lipid, and purine metabolism and arterial hypertension. External contributors to metabolic syndrome include perinatal development, nutrition structure, level of physical activity, bad habits, stress, and others [1, 2]. Genetic factors also play a role [3]. Almost all metabolic syndrome conditions are risk factors for cardiovascular diseases, and a combination of them significantly increases the risk of

their development. Metabolic syndrome is a precursor of socially significant diseases such as type 2 diabetes, diabetic nephropathy, non-alcoholic steatohepatitis, etc. [4].

According to the International Diabetes Federation (IDF), abdominal obesity is the key criterion for metabolic syndrome diagnosis, whereas arterial hypertension and lipid and carbohydrate metabolism disorders are additional criteria [5].

In Russia, the first unified criteria for metabolic syndrome diagnosis were proposed by the Russian Society of Cardiology (RSC) in 2008 and revised in 2009. They consider the central (abdominal) type of obesity to be the main component of metabolic

syndrome. It is diagnosed at a waist circumference of over 80 cm for women and over 94 cm for men. Other metabolic syndrome criteria include high blood pressure ( $> 130/95$  mm Hg), high triglycerides ( $> 1.7$  mmol/L), high low-density lipoprotein cholesterol ( $> 3.0$  mmol/L), low high-density lipoprotein cholesterol ( $< 1.0$  mmol/L for men;  $< 1.2$  mmol/L for women), fasting hyperglycaemia (fasting plasma glucose  $\geq 6.1$  mmol/L), and impaired glucose tolerance (plasma glucose 2 h after glucose loading within 7.8–11.1 mmol/L). Patients with central obesity and two additional criteria are diagnosed with metabolic syndrome [6].

World statistics for metabolic syndrome depend on the diagnostic criteria. According to numerous studies conducted in various countries, metabolic syndrome is diagnosed in 10–30% of the world adult population. The metabolic syndrome rate in Russia varies from 20 to 35%, depending on the age group (higher in old age). It is 2.5 times as common in women as it is in men [7].

Obesity is a leading risk factor for diet-related diseases, including metabolic syndrome [8]. Over the past three decades, the world rate of overweight and obesity has grown by 30% among adults and by 50% among children. By 2025, 40% of men and 50% of women will be obese [9]. According to the World Health Organization (WHO), overweight and obesity lead to type 2 diabetes (44–57% of cases), coronary heart disease (17–23%), arterial hypertension (17%), gallstone disease (30%), osteoarthritis (14%), malignant neoplasms (11%), as well as impaired reproductive function [10–12].

In Russia, the overweight and obesity rates have seen a significant growth in the last decade, reaching 60% and 24% among adults and 20% and 5.65% among children, respectively [13–15].

An integrated approach to metabolic syndrome treatment involves combining pharmacological agents and dietetic nutrition. Metabolic syndrome patients should have a low-calorie diet (1200 kcal for women and 1500 kcal for men). The amounts of fats, carbohydrates, and proteins should not exceed 25%, 55%, and 20% of the daily calorie intake, respectively [1, 2, 4]. The main objective is to lower the risk of developing MS-related diseases by reducing body weight and increasing tissue sensitivity to insulin [1].

Metabolic syndrome treatment can be made more effective by enriching the diet with specialized foods that have an improved chemical composition. These foods contain functional ingredients and biologically active substances that meet modern safety requirements and have hypolipidemic and hypoglycaemic effects. As a result, they supply the patient's body with nutrients, including essential polyunsaturated fatty acids, vitamins, macro- and microelements.

Taking into account the biological role of food proteins and their beneficial effect on lipid and carbohydrate metabolism, it is advisable to use ingredients containing complete, easily digestible

proteins. Milk whey proteins have a balanced amino acid composition and a high biological value. Compared to other proteins of animal and plant origin, they have a higher content of essential amino acids (lysine, tryptophan, methionine, threonine) and branched-chain amino acids (valine, leucine and isoleucine), which are involved in synthesizing muscle protein [16, 17].

Among plant proteins, soy proteins have been traditionally used in diet correction and prevention of lipid metabolism disorders and related diseases. They are isolated from unmodified soy with modern water extraction technology. This technology preserves native amino acids and active isoflavones while removing proteolytic enzyme inhibitors, lectins, urease, lipoxygenase, and some other compounds. The highly purified soy isolates contain over 80% protein, are easily digestible, have a balanced amino acid composition, as well as hypocholesterolemic and antiatherogenic effects [18–22].

According to modern scientific literature, the soy protein hypocholesterolemic effect can be explained by cholesterol interacting with peptide fractions in the small intestine. Peptide fractions are formed during protein digestion in the gastrointestinal tract. This interaction impairs the micellar solubility of cholesterol and its absorption, changes the enterohepatic circulation of bile acids, and thus lowers cholesterol in the liver and reduces the expression of certain genes of lipid transport protein mediators [23, 24]. Soy protein has a high content of glutamine, an amino acid necessary for glutathione to form. Glutamine protects cells from damage by free radicals and plays an important role in the functioning of the immune system [25]. Soy protein has a limited content of three essential amino acids: threonine, methionine, and cysteine. Therefore, it is advisable to use it in combination with milk whey protein, whose amino acid composition is closest to that of the "ideal" protein.

Fats are an important supplier of energy in the diet and a source of sterols, fat-soluble vitamins. However, excessive consumption of fats contributes to the development of metabolic syndrome and related complications. General recommendations for treating lipid metabolism disorders are to reduce total cholesterol and saturated fatty acids, while increasing the proportion of monounsaturated fatty acids to 10–15% and polyunsaturated fatty acids to 7–9% of the total caloric intake [26–29]. In recent years, scientists have taken greater interest in the functional role of monounsaturated fatty acids in the human body. According to literature, they reduce the level of atherogenic low density lipoproteins and free radical oxidation in the body, as well as prevent insulin resistance [30–32].

Recent studies confirm the importance of omega-3 polyunsaturated fatty acids in the treatment of lipid metabolism disorders. They also have a beneficial

effect on the lipid profile of blood and reduce the risk of developing cardiovascular diseases [33–35].

The carbohydrate profile should be modified by excluding mono- and disaccharides, which cause a sharp increase in blood glucose, and by introducing slowly digested and absorbed carbohydrates, which cause a gradual increase in postprandial glycemia.

Intensive sweeteners of natural or synthetic origin and sugar substitutes from the polyol family (xylitol, sorbitol, maltitol, lactitol, isomaltitol, and erythritol) are widely used in the food industry to form the sensory profile characteristic of traditional sweet products, in particular drinks. Intensive sweeteners (such as aspartame, saccharin, cyclamates, potassium acesulfame, sucralose, etc.) are sweeter than sugar dozens or even hundreds of times. However, they barely cause any hyperglycemic or insulinemic effect. Polyols, which are polyhydric alcohols in chemical structure, have a lower calorie content and sweetness rate than sucrose (except xylitol with a sweetness rate of one). Polyols cause a more gradual increase in postprandial glycemia compared to carbohydrates. They do not require insulin for absorption, which makes them suitable for in low-calorie and diabetic foods. Mixing sweeteners often produces a synergistic effect, which makes it possible to achieve a sweetness profile close to sucrose [36, 37]. Excessive intake of sweeteners can have an adverse effect on the gastrointestinal tract causing increased bowel sounds, a feeling of bloating or heaviness, and diarrhea. Therefore, some polyols have upper permissible levels of daily intake, for example 40 g for xylitol and sorbitol, 45 g for erythritol, and 3 g for mannitol [38].

Although dietary fiber is not an essential nutrient, its deficiency is a risk factor for many diseases. Dietary fiber is known to normalize the motor-evacuation function of the large intestine and have a prebiotic effect. Mostly soluble dietary fibers (alginates, pectin, inulin,  $\beta$ -glucans, gum arabic, some hemicelluloses, and modified celluloses) have a beneficial effect on lipid and carbohydrate metabolism. Their hypocholesterolemic effect is due to their ability to bind and excrete bile acids and slow down cholesterol absorption in the small intestine. They also reduce lipids absorption by increasing their excretion and inhibit cholesterol synthesis in the liver caused by the formation of short-chain fatty acids during dietary fiber fermentation in the large intestine. The hypoglycemic effect of soluble dietary fibers is caused by slowing gastric emptying, decreasing availability of starch for digestive enzymes, and reducing glucose absorption in the small intestine. As a result, dietary fibers lower postprandial glycemia [39, 40].

Minerals and vitamins are essential food components that perform important physiological functions in the body. There is a problem of micronutrient deficiency

in Russia, which is a risk factor for many nutrition-related diseases. Therefore, it is advisable that metabolic syndrome patients enrich their diet with vitamins (groups B, C, E, A, D, beta-carotene), minerals (potassium, magnesium, calcium), and trace elements (chromium, zinc) [41, 42].

Trivalent chromium (Cr) is vital for normal carbohydrate metabolism in humans and animals [43]. Chromium stimulates glucose delivery into cells, inducing genes of intracellular signalling systems. There is evidence of direct interaction of chromium with insulin. In particular, it interacts with its dimers, thus stabilizing the hormone structure or enhancing its binding to the receptor [44]. The biochemical and physiological effects of zinc in mammals are determined by its ability to regulate the chronic inflammatory status by reducing inflammatory cytokines, reduce the effects of oxidative stress, and participate in lipid and carbohydrate metabolism.

Zinc deficiency can be an important risk factor for type 2 diabetes. Plasma zinc levels are inversely correlated with glycated hemoglobin levels in diabetes [45]. Zinc improves glucose metabolism and insulin sensitivity in diabetics. It plays an important role in the synthesis, deposition, and secretion of insulin in pancreatic  $\beta$ -cells. Zinc deficiency has a negative effect on insulin sensitivity and glucose tolerance [46–49]. In addition, zinc stimulates glycolysis, inhibits gluconeogenesis, and is involved in glucose transport in adipocytes [50]. The metabolic effect of zinc in obesity is associated with its impact on adipokines, hormones of adipose tissue (interleukin 6, tumour necrosis factor, leptin, adiponectin, and others) [51–53]. In particular, experimental studies show that an adequate level of zinc in adipose tissue is important for the normal functioning of adipocytes and leptin synthesis [52]. Complexes of chromium and zinc with enzymatic hydrolysates of various food proteins can be effectively used to obtain new food sources of these trace elements in an organically bound and highly bioavailable form. Using such complexes in human nutrition is physiologically justified [54].

The Russian market of dietetic foods for the prevention and treatment of nutrition-related diseases (including metabolic syndrome) is quite limited. This situation creates a need for studies aimed to develop new foods that meet modern safety and clinical efficacy requirements.

Powdered specialized foods are most suitable for a clinical setting. They can be used to make drinks and cocktails or added to ready-made cereals and dairy products (kefir, fermented baked milk, yogurt, and curdled milk). In addition, dry products are easy to transport and store, are microbiologically stable, and have a long shelf life. Their production technology ensures a wide range of products with various sensory profiles.

In connection with the above, our study aimed to develop and evaluate the clinical efficacy of specialized foods intended for dietetic treatment of lipid metabolism disorders in metabolic syndrome patients.

### STUDY OBJECTS AND METHODS

The following ingredients were used to develop specialized foods for metabolic syndrome patients:

- Supro Plus 221 D IP soy protein isolate with 80% protein (Solae, USA);
- Lacprodan 80 whey protein concentrate with 80% protein (Arla Foods Ingredients SF, Argentina);
- MD1925 QS maltodextrin with 18.9% dextrose equivalent (DE) (Syril, France);
- Cegepal 03-C microencapsulated rapeseed oil with 68% fat (BASF Personal Care and Nutrition GmbH, Germany);
- Crystalline maltitol with 99.5% main component (Shandong Lujian Biological Technology Co., LTD, China);
- Genu DZ citrus pectin with 58–62% esterification (CP Kelco Germany GmbH, Germany);
- Life, DHA S17-P100 docosahexaenoic acid (DSM Nutritional Products Europe Ltd, Switzerland);
- Karnipur Crystallin L-carnitine with 99% main component (Lonza Ltd, Switzerland);
- EM28304 vitamin premix (DSM Nutritional Products Europe Ltd, Switzerland): vitamins A, D<sub>3</sub>, E, K<sub>1</sub>, C, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, PP, calcium D-pantothenate, folic acid, biotin, maltodextrin;
- 2-aqueous lactic acid magnesium (PURAC biochem B.V., Spain);
- Carbonic calcium (Mineraria Sacilese S.P.A, Italy);
- Potassium citrate 3-substituted monohydrate (V.A.G. Chemie GmbH, Germany);
- Zinc chloride (analytic grade, State Standard 4529-78<sup>1</sup>);
- 6-aqueous chrome chloride (analytic grade, State Standard 4473-78<sup>11</sup>);
- Sodium hydroxide (analytic grade, State Standard 4328-77<sup>111</sup>);
- Apple natural food flavoring (Givaudan Schweiz AG, Switzerland);
- Apricot natural food flavoring (Givaudan Schweiz AG, Switzerland);
- Stevilia E mixture of sweeteners: erythritol (E968), stevia extract (E960) (TU 9197-002-49929776-13 (Aspasvit, Russia); and
- Powdered beta-carotene dye (DSM Nutritional Products Europe Ltd., Switzerland).

All the ingredients met safety requirements established by the Technical Regulations of the Customs

Union, namely 021/2011<sup>IV</sup>, 033/2013<sup>V</sup>, and 029/2012<sup>VI</sup>. The food additives were used within the amounts established in Technical Regulations 029/2012.

The specialized food physicochemical parameters were determined by standard methods, namely:

- moisture mass fraction: according to State Standard 29246-91<sup>VII</sup>;
- vitamin A: according to State Standard R 54635-2011<sup>VIII</sup>;
- vitamin E: according to State Standard R 54634-2011<sup>IX</sup>;
- vitamins C, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, minerals (calcium, magnesium, potassium, chromium, zinc), mono- and disaccharides, L-carnitine: according to Regulation 4.1.1672-03<sup>X</sup>;
- water activity: by a mirror-cooled dew point sensor on an AquaLab 4TE analyser (Decagon Devices, USA);
- amino acid composition of the milk and soy protein component: by calculation using the manufacturers' specifications;
- nutritional and energy values, percentage of average daily requirement for nutrients and energy: by calculation using the handbook on chemical composition and caloric content of food ingredients, taking into account recommended daily intake of nutrients and energy according to Technical Regulations 022/2011<sup>XI</sup>, the Uniform Sanitary Epidemiological and Hygienic Requirements for the Goods Subject to Sanitary and Epidemiological Supervision (Control), as well as manufacturers' specifications [55].

### RESULTS AND DISCUSSION

Producing specialized foods that meet the biomedical requirements for metabolic syndrome patients involves selecting ingredients with a desirable chemical composition and hypocholesterolemic effect.

<sup>IV</sup> TR TS 021/2011. Tekhnicheskiiy reglament Tamozhennogo soyuza "O bezopasnosti pishchevoy produktsii" [TR CU 021/2011. Technical regulations of the Customs Union "On food safety"]. 2011.

<sup>V</sup> TR TS 033/2013. Tekhnicheskiiy reglament Tamozhennogo soyuza "O bezopasnosti moloka i molochnoy produktsii" [TR CU 033/2013. Technical regulations of the Customs Union "On milk and dairy products safety"]. 2013. 107 p.

<sup>VI</sup> TR TS 029/2012. Tekhnicheskiiy reglament Tamozhennogo soyuza "Trebovaniya bezopasnosti pishchevykh dobavok, aromatizatorov i tekhnologicheskikh vspomogatel'nykh sredstv" [TR CU 029/2012. Technical regulations of the Customs Union "Safety requirements for food additives, flavours and processing aids"]. 2012.

<sup>VII</sup> State Standard 29246-91. Dry canned milk. Methods for determination of moisture. Moscow: Izdatel'stvo standartov; 2001. 6 p.

<sup>VIII</sup> State Standard R 54635-2011. Functional food products. Method of vitamin A determination. Moscow: Standartinform; 2013. 12 p.

<sup>IX</sup> State Standard R 54634-2011. Functional food products. Method of vitamin E determination. Moscow: Standartinform; 2013. 15 p.

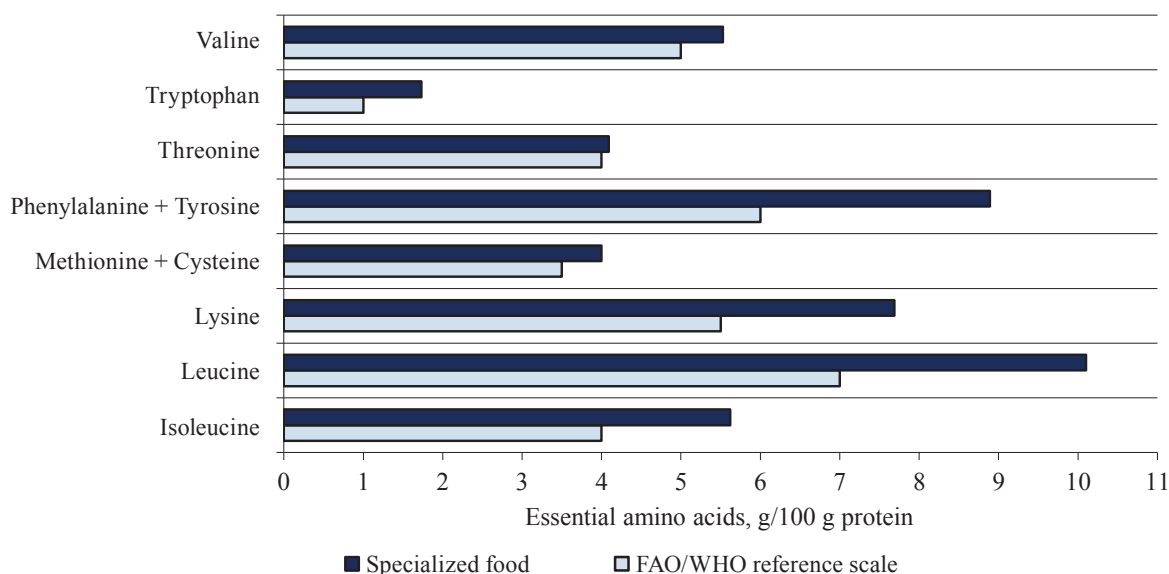
<sup>X</sup> Regulation 4.1.1672-03. Guidelines on quality and safety control methods for biologically active food additives. Moscow: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of Russia; 2004. 240 p.

<sup>XI</sup> TR TS 022/2011. Tekhnicheskiiy reglament Tamozhennogo soyuza "Pishchevaya produktsiya v chasti ee markirovki" [TR CU 022/2011. Technical regulations of the Customs Union "Food labelling"]. 2011. 29 p.

<sup>1</sup> State Standard 4529-78. Reagents. Zinc chloride. Specifications. Moscow: Izdatel'stvo standartov; 1990. 10 p.

<sup>11</sup> State Standard 4473-78. Reagents. Chromic (III) chloride hexahydrate. Specifications. Moscow: Izdatel'stvo standartov; 1992. 15 p.

<sup>111</sup> State Standard 4328-77. Reagents. Sodium hydroxide. Specifications. Moscow: Izdatel'stvo standartov; 2001. 19 p.



**Figure 1** Essential amino acids in the specialized food protein component (compared to a reference scale)

Consumer properties of the product (physicochemical and sensory indicators) and its safety depend primarily on the ingredients, their technological and sensory compatibility.

The Russian market offers a wide range of functional ingredients and biologically active substances that meet modern safety requirements. In addition to satisfying human needs for food and energy, they also have a health-beneficial effect due to their physiological action. The amount of a functional ingredient in the product should be physiologically significant. It means that it should meet the physiological need for it and, at the same time, ensure adequate consumer properties (appearance, taste, aroma, texture, etc.).

A 1:1 ratio of soy protein isolate and milk whey protein was used as a protein component in the specialized food formulation. Lacprodan 80, a whey protein concentrate available on the Russian market, is produced using membrane technology that does not have a denaturing effect on proteins. Whey protein concentrate contains about 80% of complete easily digestible protein with a high amino acid score compared to the FAO/WHO reference scale (1985). Its minerals (mg/100 g) include calcium (365 mg), sodium (246 mg), magnesium (52 mg), and potassium (524 mg) [56]. The low lactose content makes this protein source suitable for people with lactase deficiency.

Supro Plus XT 221D IP, an isolated soy protein used in the specialized food formulation, contains 85% protein, about 3% fat, and 1% carbohydrates. Its minerals (mg/100 g) include calcium (50 mg), phosphorus (900 mg), magnesium (34 mg), potassium (1300 mg), sodium (780 mg), iron (12 mg), and copper (1.6 mg).

This combination of milk and soy proteins allowed us to optimize the amino acid composition of the protein component and ensure a high score of essential amino

acids compared to the FAO/WHO reference scale (1985) (Fig. 1).

The specialized food fat component is a mixture of rapeseed oil microcapsules and docosahexaenoic acid (DHA). A source of monounsaturated and polyunsaturated fatty acids, rapeseed oil contains about 60% oleic acid, 20% linoleic ( $\omega$ -6) acid, and 10%  $\alpha$ -linolenic ( $\omega$ -3) acid. DHA is a powder with a slight fishy odor that contains 17–21.5% of DHA isolated from microalgae.

Maltodextrin, a product of incomplete hydrolysis of corn starch, was used as a source of carbohydrates. These are so-called “complex” carbohydrates with a sweetness rate of 0–0.3 and DE 18.9%.

Maltitol (a polyhydric alcohol obtained by hydrogenating starch-based maltose) and Stevilia E (a mixture of erythritol and stevia extract) were used to form the taste profile of the rehydrated beverage. Maltitol had a sweetness rate of 0.8, and Stevilia E was five times as sweet as sugar.

The widespread use of various types of pectin as a source of soluble dietary fiber is due to the chemical structure of its molecules. Differences in physicochemical properties (solubility, gelling and complexing ability) are determined by the degree of esterification and the molecular weight of pectin molecules. The ability of pectin to dissolve in water and form colloidal systems is important for its use in food production and for its physiological effect on the human body. The FAO/WHO experts recommend pectin for treating cardiovascular diseases, hyperlipidemia, diabetes, impaired glucose tolerance, obesity, and hypomotor dyskinesia of the colon and the gallbladder [37]. Knowing that highly esterified pectins have an increased solubility in water, we used citrus pectin with a 58–62% degree of esterification.

L-carnitine (levocarnitine) is a biologically active substance whose effectiveness is clinically proven. This compound plays a major role in the transport of fatty acids into mitochondria. In clinical practice, L-carnitine is successfully used in treating a wide range of diseases, such as anorexia, chronic fatigue syndrome, cardiovascular pathology, hypoglycemia, male infertility, and kidney disease [57]. The studies conducted by the Clinic of Nutritional Treatment at the Federal Research Centre of Nutrition and Biotechnology demonstrated the effectiveness of L-carnitine in the diet of patients with metabolic disorders accompanied by obesity [58]. According to physiological needs for minor and biologically active food substances, the average L-carnitine requirement for adults is 300 mg/day. The maximum daily intake may reach 900 mg/day [38].

To improve the vitamin status of patients, specialized foods were enriched with vitamins A, D<sub>3</sub>, E, K<sub>1</sub>, C, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, PP, calcium D-pantothenate, folic acid, and biotin in the form of a special water-soluble premix.

In view of the importance of minerals in physiological processes that ensure normal functioning of the body, we used salts rich in magnesium, potassium, and calcium, namely lactic magnesium, potassium citrate, and calcium carbonate. We also used protein-chelate complexes of zinc and chromium ions with peptides of soy protein hydrolysate. The preparation of an enzymatic soy protein hydrolysate is described in [59].

Thus, the specialized food formulation included the following ingredients based on the requirements for metabolic syndrome dietary therapy: whey protein concentrate, soy protein isolate, microencapsulated rapeseed oil, maltodextrin, docosahexaenoic acid, maltitol, pectin, potassium citrate, magnesium lactic acid, calcium carbonate, a mixture of sweeteners (stevia extract, erythritol), vitamin premix (vitamins A, E, C, D<sub>3</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, PP, folic acid, pantothenic acid, K<sub>1</sub>, biotin), L-carnitine, organic sources of zinc and chromium, beta-carotene dye, and natural flavoring agents.

The specialized food technology included the following main stages: enzymatic hydrolysis of soy protein isolate, obtaining protein-chelate complexes of zinc and chromium, preparing a mixture of minor ingredients (pre-mix), obtaining specialized foods, packaging, and labelling.

The protein-chelate zinc complex was obtained by mixing a pre-prepared 10% aqueous solution of enzymatic soy protein hydrolysate and a 25% aqueous solution of zinc chloride in the ratio of 10:1, adding a solution of sodium hydroxide to reach pH 7.0–7.1, and then thermostating for 60 min at room temperature with constant stirring. To remove sediment and mechanical impurities, the resulting solution was microfiltered in a tangential flow with a pore diameter of under 5.0 µm. Those zinc ions which were not related to the peptide-amino acid matrix were removed by nanofiltration. The filtrate was pasteurized at 75°C for 30 s and freeze-dried.

The protein-chelate chromium complex was obtained by mixing a 10% aqueous solution of enzymatic hydrolysis of soy protein isolate and a 10% aqueous solution of chromium chloride in the ratio of 100:1. The nanofiltration stage was excluded from the process. The obtained protein-chelate complexes were ground in a knife mill and sieved through a sieve with a 1.0 mm mesh diameter.

The protein-chelate complexes were fine powders with the following characteristics: beige color, 2.3% moisture, specific smell, bitter-salty taste, and high solubility in water. Zinc complex had a zinc content of 46.1 mg/g and chromium complex had a chromium content of 4.7 mg/g.

The main objective of dry mixing is to achieve uniform distribution of minor ingredients in the product. We used the technology of phased mixing, taking into account the 1:10<sup>-2</sup>–1:10<sup>-3</sup> ratio of the main ingredients (sources of proteins, fats, and carbohydrates) and micro additives (macro- and microelements, vitamins, and biologically active substances).

At the first stage, a premix was obtained using docosahexaenoic acid, calcium carbonate, flavoring agents, vitamin premix, beta-carotene, protein-chelate chromium complexes of zinc and chromium, L-carnitine, and 10% of the formulated amount of microencapsulated rapeseed oil for more even distribution. The ingredients were mixed in a turbulent mixer at 40 rpm for 35 min. At the second stage, the resulting premix was mixed with the rest of the ingredients at 40 rpm for 30 min. The fill factor of the mixing chamber was 0.7. Using a complex trajectory of mixing under the influence of gravity with a specified multidirectional spatial movement of the mixing chamber minimized the negative effect of centrifugal forces and prevented so-called “dead zones” and heating of the product. Direct filling batchers were used to package 30 g portions of the finished product in film bags.

The above technology allowed us to obtain a homogeneous powdery mixture with evenly distributed minor ingredients, which ensured a recommended intake of all the nutrients with every portion of the product. With a moisture content of 3.35 ± 0.04% and a water activity indicator ( $A_w$ ) of 0.2304 ± 0.0009, the specialized food is a low-moisture product. This characteristic ensures the stability of its properties, as well as of quality and safety indicators throughout its shelf life.

Powdered specialized foods can be added to ready-made cereals, desserts, and fermented dairy products. When rehydrated, they can be used as a drink or a cocktail. For this, the contents of a package (30 g) must be poured into a glass and stirred vigorously with 100–150 mL of hot water (60–80°C) until the product is homogeneous, or beaten in a blender. The amount of water can vary, depending on the desired consistency. One serving (30 g) is recommended per day.

**Table 1** Contents of specialized food nutrients per 100 g, one serving and % of the average daily requirement (ADR)

Nutrient	100 g	Serving (30 g)	% of ADR (30 g)*
Protein, incl.	20.9	6.3	8
animal protein, g	11.7	3.5	
plant protein, g	9.2	2.8	
Fat, incl.	14.3	4.3	5
oleic acid	10.6	3.2	21**
(monounsaturated), g			
docosahexaenoic ( $\omega$ -3) acid, mg	600	180	26**
$\alpha$ -linolenic ( $\omega$ -3) acid, mg	1770	530	76**
Carbohydrates, incl.	28.4	8.5	2
lactose, g	1.14	0.34	
Soluble dietary fiber (pectin), g	4.5	1.35	68*
Maltitol, g	9.5	2.85	
L-carnitine, mg	360	108	36**
Minerals			
Calcium, mg	1075	323	32
Magnesium, mg	406	122	30
Potassium, mg	2270	680	19
Zinc, mg	13.0	3.9	26
Chromium, $\mu$ g	57.0	17	34**
Vitamins			
C, mg	196	59	100
B <sub>1</sub> , mg	2.23	0.54	39
B <sub>2</sub> , mg	1.98	0.67	42
B <sub>6</sub> , mg	2.2	0.66	33
B <sub>12</sub> , $\mu$ g	3.0	0.9	90
PP, mg	18.0	5.4	30
Folic Acid, $\mu$ g	700	210	105
Pantothenic acid, mg	5.4	1.62	27
A, $\mu$ g	800	240	30
D <sub>3</sub> , $\mu$ g	10.0	3.0	60
E, mg	12.1	3.63	36
K <sub>1</sub> , $\mu$ g	90.0	27.0	23**
Biotin, $\mu$ g	30.0	9.0	18**
Energy value/calorie content, kJ/kcal	1500/358	450/107	4

\* Technical Regulations of the Customs Union 022/2011

\*\* Uniform Sanitary Epidemiological and Hygienic Requirements for the Goods Subject to Sanitary and Epidemiological Supervision (Control)

Table 1 shows the contents of food components and biologically active substances in 100 g of the specialized food and its 30 g serving, as well as a percentage of the average daily requirement for macro- and micronutrients in one serving.

The content of macro- and micronutrients in one specialized food serving meets the medical recommendations for metabolic syndrome treatment.

Based on our studies, we developed Technical Specifications 10.86.10-007-01897222-2018 “Specialized food for dietetic preventive and dietetic therapeutic

nutrition – an instant drink”. Valetok Prodimpeks, a Russian research and production company produced a pilot batch of specialized foods. The sanitary-chemical and microbiological tests confirmed the products’ compliance with the current regulatory requirements established by Technological Regulations of the Customs Union 021/2011 and 027/2012<sup>xii</sup>.

The clinical efficacy of specialized foods was assessed by the Department of Metabolic Diseases at the Federal Research Centre of Nutrition and Biotechnology. The study involved 15 metabolic syndrome patients aged 27 to 59. For two weeks, they had a 1500 kcal hypocaloric standard diet with one specialized food drink instead of a second breakfast. During the treatment, the patients showed a decrease in body weight, body mass index, waist volume, and body fat mass by an average of 3.6, 3.9, 3.9, and 4.4%, respectively. Their blood serum tests featured a decrease of 16.9% in total cholesterol, 15.3% in low-density lipoprotein cholesterol, and 27.9% in triglycerides, compared to the initial level.

## CONCLUSION

Based on the requirements of modern nutritional science, we developed a formulation of specialized foods for metabolic syndrome patients, including ingredients and biologically active substances with a hypolipidemic effect. Our technology ensures uniform distribution of minor ingredients and, therefore, a desirable content of nutrients in each serving of the product according to medical and biological requirements. Further, we developed Technical Specifications 10.86.10-007-01897222-2018 “Specialized food for dietetic preventive and dietetic therapeutic nutrition – an instant drink”. The clinical trials of a pilot batch of specialized foods within a standard hypocaloric diet showed their effectiveness for metabolic syndrome patients.

## CONTRIBUTION

Concept development – A.A. Kochetkova, V.K. Mazo; data collection and processing, writing a manuscript – V.M. Vorobyeva, I.S. Vorobyeva, Kh.Kh. Sharafetdinov, S.N. Zorin; text editing – A.A. Kochetkova, V.K. Mazo.

## CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

<sup>xii</sup> TR TS 027/2012. Tekhnicheskiy reglament Tamozhennogo soyuza “O bezopasnosti otdel'nykh vidov spetsializirovannoy pishchevoy produktsii, v tom chisle dieticheskogo lechebnogo i dieticheskogo profilakticheskogo pitaniya” [TR CU 027/2012. Technical regulations of the Customs Union “On the safety of certain types of specialized foods, including dietetic therapeutic and dietetic preventative nutrition”]. 2012. 26 p.

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