RESEARCH PAPER

Biocomplex with Modified Collagen Substance and Metabiotic Whey Protein Hydrolysateu

Poznyakovsky Valeriy Mikhailovich[1], Kurkina Yulia Yurievna[2], Avstrievsky Alexander Nikolaevich[3], Tokhiriyon Boisjoni[4*]

[1]Department of Hygiene Kemerovo State Medical University of the Ministry of Health of the Russian Federation, Kemerovo, Russia.
[2]Higher Engineering School of Agrobiotechnology "Agrobiotech" (HES A), Department of Agrobiotechnologies, Tomsk, Russia.
[3]Art Life Company, Tomsk, Russia.
*[4]*Department of Management, Entrepreneurship and Engineering, Ural State University of Economics, Yekaterinburg, Russia.*

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ABSTRACT
This article presents the development and scientific substantiation of a new biologically active supplement (BAS) "Collagen 5000 Multivitamins," designed as a vitamin-collagen biocomplex for the nutritional support of connective and epithelial tissues. The authors detail a unique formulation centered around "Metacollagen Silicon+," a short-chain animal-derived collagen with low molecular weight, which significantly enhances absorption and bioavailability compared to standard hydrolyzed collagen. A key innovation is the integration of natural silicon sourced from the "Chazhemto" mineral waters of Western Siberia, which acts as a collagen promoter to improve tissue structural integrity. The study describes the synergistic roles of 13 essential vitamins and minerals, such as Ascorbic Acid, Biotin, and a full B-vitamin complex, which facilitate endogenous collagen biosynthesis and provide antioxidant protection. The technological process is highlighted by an enzymatic hydrolysis method (confirmed by RF Patent No. 2827228) conducted under mild physiological conditions without pH correction, ensuring the preservation of bioactive properties. Quality control parameters, including amino acid profiles (L-glycine, L-proline, L-oxyproline) and micronutrient stability, were verified over a 27-month storage period. The resulting product is positioned as a functional tool for preventing premature aging of the skin, vascular systems, and osteoarticular apparatus, produced in compliance with ISO 9001, 22000, and GMP standards.

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INTRODUCTION

Biotechnological production is becoming increasingly vital in the development of specialized products with systemic action. New large-scale technological projects are being designed for the metabolic correction of dysfunctional states and maladaptation syndromes in modern humans. One of these key research areas involves biotechnological collagen substances which, together with promoters, form the basis of high-efficacy biocomplexes for health-preserving food systems [1-15].

Collagen has a profound evolutionary history, having fulfilled vital protein functions in highly organized animals and humans for millions of years-forming the skeleton, protecting cells, tissues, organs, and the body as a whole-providing significant advantages in the complex struggle for survival [16-30].

MATERIALS AND METHODS

A new form of biologically active supplement (BAS) "Collagen 5000 Multivitamins" has been developed, A high-efficacy dietary biocomplex has been developed in the form of a collagen concentrate enriched with silicon, hyaluronic acid, and methylsulfonylmethane (MSM). This product acts as a universal molecular building block for food systems, renewing and supporting essential body structures: connective and bone tissues, mucous membranes, integuments, synovial fluids, intercellular spaces, and more.

The formulation utilizes animal-derived "metacollagen" obtained through the enzymatic hydrolysis of food-grade bovine gelatin (180-110 Bloom, type B) or bone gelatin (240 Bloom). These sources are considered the most physiologically compatible and effective for humans due to their affinity with connective tissue, requiring no extensive restructuring of amino acid chains during metabolism. The hydrolysis of native collagen is conducted under mild temperatures and pH levels close to physiological values. The metacollagen composition includes a blend of types I, II, and III. Type I accounts for 90% of total collagen and is found in tendons, organs, and bones; Type II is a constituent of the cartilage in knees, shoulders, and other joints; Type III is the primary type of reticular fiber cartilage, typically occurring alongside Type I.

Based on market analysis and the characterization of microbial proteases [5-7], a fungal alkaline protease (Protozym S, 5000 U/g) was utilized as the enzyme.

The biotechnological substance "Metacollagen Silicon+" is a product of the enzymatic hydrolysis of native collagen containing 1000 amino acid residues, of which 20% are proline and hydroxyproline, 10% are alanine, and the remainder consists of other amino acids, with glycine appearing as every third residue. Collagen is the unique protein characterized by its hydroxyproline content.

The high silicon content in the substance is ensured by the use of silicon-mineral therapeutic water, extracted from a well 850 meters deep in the Western Siberia region (Chazhemto resort, Tomsk region). The orthosilicic acid content in one liter of water is 75.7±11.3 mg, showing a natural affinity for human organs and tissues. The soluble form of silicon ensures active metabolism within the gastrointestinal tract. As collagen promoters, silicon compounds are essential for the normal functioning of connective and epithelial tissues, providing elasticity, impermeability, and structural strength.

In addition to silicon, the water contains other beneficial elements: potassium, magnesium, calcium, iron, iodine, sulfates, and bicarbonates. This natural bio-resource is critical not only for the skin but also for blood vessels. It has been shown that in atherosclerosis, silicon levels decrease significantly, leading to the disappearance of elastin. Along with the loss of arterial wall elasticity, permeability increases, allowing lipids to penetrate the plasma and deposit on vessel walls as atherosclerotic plaques. Similar changes are characteristic of the aging process, which explains the high prevalence of atherosclerosis among the elderly.

RESULTS AND DISCUSSION

The formulation of the developed dietary supplement **(Table 1)** and the characteristics of its ingredients are presented below.

Table 1. Formulation of the Dietary Supplement "Collagen Concentrate with Silicon, Hyaluronic Acid, and MSM"

No.	Component Name	Content per 1 dose, mg	% of RDI[1]/ AI[2]
1	Raw material for food production "Metacollagen Silicon+" (hydrolyzed collagen, silicon-mineral water, potassium sorbate, sodium benzoate) Metasilicic acids	10,000 0.35	-
2	"Aminocidin" (metabiotic LC-Lc-Cr (L. lactis cremoris), metabiotic LC-Lc-Dl (L. lactis diacetylactis), maltodextrin (carrier E459), lactic acid (acidity regulator E270))	9,000	-
3	Methylsulfonylmethane (MSM)	500	-
4	Sodium ascorbate Vitamin C	67.9 60	100[1]
5	Hyaluronic acid Hyaluronic acid	55 50	100[2]
6	K2 2000 ppm CWS K2 (menaquinone)	60.0 0.12	100[2]
7	Complex food additive "Boron-complex" (sodium borate, gum arabic (stabilizer E414)) Boron	17.7 2	100[2]
8	Altai Mountains Shilajit (Stone Oil) (food salt series "Stone Oil", magnesium citrate, zinc citrate trihydrate, maltodextrin)	20	
9	Cholecalciferol 100 SD/S Vitamin D3	2 0.005 (200 IU)	100[1]
10	Vitamin H (Biotin)	0.05	100[1]
Excipients			
11	Purified water	3,657.85	-
12	Potassium sorbate (preservative)	22.5	-
13	Sodium benzoate (preservative)	22.5	-
14	Nisin (preservative)	2.5	-
15	Lactic acid 80% (acidity regulator) Lactic acid	312 250	-
16	Instangum BA (stabilizer)	1,250	-
Total		25,000	-

[1] RDI – Recommended Daily Intake according to Annex 2 of TR CU 022/2011.
[2] AI – Adequate Intake according to Annex 5 of the Uniform Sanitary-Epidemiological and Hygienic Requirements of the EAEU.
Does not exceed the upper tolerable intake level.

Ingredient characteristics

Silicon compounds are actively involved in the growth of hair and nails by forming cross-links within keratin macromolecules, ensuring resistance to environmental factors. The most straightforward diagnostic sign of silicon deficiency is nail brittleness.

The biotechnological substance "Metabiotic LC-Lc-Cr, LC-Lc-Dl" with whey protein hydrolysate is a protein hydrolysate fermented by Lactococcus lactis subsp. diacetylactis and Lactococcus lactis cremoris. These microorganisms possess the genetic capacity to break down whey proteins into highly bioavailable peptides due to their affinity with human proteins. This ingredient enhances the protein value of the product through peptide enrichment and contains bacterial metabolites such as short-chain fatty acids (acetate, propionate, butyrate, lactate), vitamins, amino acids, and enzymes that support proper

intestinal function. Furthermore, the bacteria process the lactose from the protein hydrolysate, rendering this peptide source lactose-free [66-72].

Collagen hydrolysates are utilized for medical purposes in the form of energy supplements, geriatric products, and therapeutic diets. They are indicated for patients with digestive, absorption, and amino acid metabolism disorders, as well as those with malnutrition related to trauma, burns, oncology, and hepatic encephalopathy. They also serve as agents to improve the regeneration of tendons and joints in professional athletes.

Research has noted the chemotactic activity of short peptides (Pro-Hyp and Pro-Hyp-Gly) toward human fibroblasts, peripheral blood neutrophils, and monocytes. Additionally, collagen hydrolysate peptides exhibit antihypertensive and antioxidant activities, specifically inhibiting the angiotensin-converting enzyme (ACE), which regulates blood pressure. The intake of collagen hydrolysate stimulates the synthesis of extracellular matrix macromolecules by chondrocytes, reducing pain in patients with osteoporosis and osteoarthritis.

Fermented whey protein hydrolysate and collagen peptides act as sources of amino acids essential for cellular nutrition and tissue renewal. This is particularly crucial during cellular cascades involving the release of stress hormones, inflammatory mediators (cytokines), and significant intracellular energy expenditure, which can lead to protein-energy malnutrition.

Alongside biotechnological substances, the concentrate contains other collagen promoters (including silicon) that exhibit synergistic properties: methylsulfonylmethane, cholecalciferol, hyaluronic acid, vitamins C, K2, biotin, and "stone oil" (shilajit) as a source of macro- and microelements (phosphorus, potassium, calcium, iodine, magnesium, copper, iron, and manganese). These promoters ensure the functional efficiency of collagen and elastin, providing targeted support for connective tissue [61-65].

Nutritional support in the form of this bioconcentrate is vital for strengthening the immune system, as 80% of immune cells are localized in the gastrointestinal tract. These include plasma cells, small lymphocytes, and T- and B-cells that synthesize immunoglobulins responsible for local mucosal immunity. Imbalances in gut bacteria can impair mineral absorption and lead to systemic depletion [51-60].

Disruptions in intestinal homeostasis-caused by inflammation, irritants, or toxins-can lead to "Leaky Gut Syndrome." This condition allows pathogens, toxins, and antigens to enter the bloodstream, triggering inflammatory and autoimmune responses that underlie many diseases, such as arthritis, lupus, type I diabetes, and multiple sclerosis.

Silicon, similar to Vitamin D, enhances the calcification and mineralization of bone tissue. A shift in the balance between silicon and calcium leads to metabolic disorders and functional impairment [46-50].

Vitamin K2 (menaquinone) is involved in the carboxylation of osteocalcin, playing a critical role in bone mineralization. It serves as a coenzyme for the γ-carboxylation of glutamic acid residues in functional proteins (including prothrombin and osteocalcin). It also acts as a factor in thrombosis prevention by activating coagulation processes in the liver and the extrahepatic endothelial anticoagulant protein S [37-45].

Boron is an essential component for enzymatic reactions involving steroid hormones, magnesium, Vitamin D, and calcium. It helps reduce inflammation and improves blood lipid profiles and neuronal function. Boron deficiency is linked to decreased brain activity and impaired attention. In bone marrow cell cultures, increased boron levels have been shown to significantly elevate Type I collagen and osteocalcin levels.

Cholecalciferol (Vitamin D3) is the natural form of the vitamin synthesized in the skin under UV light. It facilitates the absorption of phosphates and calcium in the intestines and their reabsorption in the renal tubules. It is essential for bone calcification and parathyroid function. It acts as an anti-rachitic factor and is particularly important for menopausal women to prevent osteoporosis [31-36].

Methylsulfonylmethane (MSM) is an organosulfur compound with anti-inflammatory properties. It is used to strengthen joints and ligaments by providing bioavailable sulfur for protein synthesis.

Ascorbic Acid (Vitamin C) is crucial for the maturation of elastin and collagen. It controls the hydroxylation of prolyl and lysyl residues via proline hydroxylase. A deficiency leads to "loose" collagen, characteristic of scurvy, resulting in bleeding and tooth loss.

Hyaluronic Acid maintains water balance in cells and the extracellular space, ensuring skin elasticity and smoothness. It aids in restoring joint cartilage and alleviating arthritic pain.

Biotin (Vitamin H), specifically the natural d(+)-biotin, is essential for skin health, preventing hair loss or greying, and supporting the energy supply to nerve cells.

Altai Mountains Stone Oil (Shilajit/White Mumiyo). This substance contains 49 chemical elements, including "organogenic" (life-forming) elements. Its effective and multifaceted influence on the body is attributed to its unique composition and high concentration of biologically active substances. Archaeologists have discovered traces of stone oil in the Altai, Pamir, and Sayan mountains dating back more than 400 million years.

The quality and safety indicators of the developed biocomplex were studied following 27 months of storage at temperatures ranging from 0 to 25°C and air humidity not exceeding 60%.

The product is packaged in sachet, stick, or zip-lock bags of 25 g, or in bottles/jars ranging from 25 to 1000 g.

The regulated quality parameters are presented in **Table 2**.

Table 2. Regulated Organoleptic and Physicochemical Quality Indicators of the Dietary Supplement

Parameter	Characteristic
Color	From cream to brown
Appearance	Liquid; a slight precipitate settles
Flavor and Aroma	Specific, sweet and sour with a whey aftertaste
Content per 25 grams	-
Vitamin C, mg	60 (48 – 72)
Vitamin K2, mcg	120 (96 – 144)

Vitamin H (Biotin), mcg	50 (35 – 65)
Hyaluronic acid, mg	50 (40 – 60)
Boron, mg	2.0 (1.6 – 2.4)
Methylsulfonylmethane (MSM), mg	Not less than 500
Glycine*, mg per 25 grams	Not less than 1050
Proline* content, mg	Not less than 820
Oxyproline (Hydroxyproline)*, mg	Not less than 500
Potassium sorbate, %	Not more than 0.1
Sodium benzoate, %	Not more than 0.1

* The parameter is regulated by the formulation dosage; the quantitative content is verified in the substance during the incoming quality control of raw materials.

Table 3 presents the nutritional value of the dietary supplement. The intake of 25 grams (the recommended daily dose) ensures the guaranteed supply of essential micronutrients to the body.

Table 3. Nutritional Value of Collagen Concentrate with Silicon, Hyaluronic Acid, and MSM

Nutrient	g per 25 g (1 serving)	g per 100 g*	% of RDI[1] / AI[2] per 25 g serving
Proteins, g	4.84	19.36	6.45[1]
Fats, g	0.00	0.00	0
Carbohydrates, g	0.03	0.11	0.01[1]
Organic acids, g	0.25	1.0	-
Soluble dietary fiber, g	1.25	5	62.5[2]
Alanine, g	0.42	1.69	6.41[2]
Arginine, g	0.24	0.95	3.89[2]
Aspartic acid + Asparagine, g	0.26	1.05	2.14[2]
Methionine + Cysteine/Cystine, g	0.02	0.08	1.06[2]
Glutamic acid + Glutamine, g	0.46	1.84	3.27[2]
Glycine, g	1.05	4.18	29.86[2]
Histidine, g	0.02	0.10	1.14[2]
Isoleucine (BCAA), g	0.07	0.27	3.33[2]
Leucine (BCAA), g	0.13	0.51	2.79[2]
Lysine, g	0.13	0.51	3.13[2]
Phenylalanine + Tyrosine, g	0.08	0.32	1.84[2]
Proline, g	0.82	3.27	18.16[2]
Serine, g	0.14	0.55	1.66[2]
Threonine, g	0.08	0.30	3.17[2]
Valine (BCAA), g	0.10	0.42	4.18[2]
Hydroxyproline, g	0.71	2.83	-
Hyaluronic acid, mg	50	-	100[2]
Boron, mg	2	-	100[2]
Vitamin C, mg	60	-	100[1]
Vitamin K2, mg	0.12	-	100[2]
Vitamin H (Biotin), mg	0.05	-	100[1]
Vitamin D3, mg	0.005	-	100[1]
Energy value, kcal	20.4	67.9	-
Energy value, kJ	85	284	-

Directions for use: Take 25 g (the contents of one bottle, sachet, stick, or zip-bag) once daily with a meal, having previously dissolved it in ½ cup of drinking water (100 cm³). Shake well before use.

Based on the functional properties of the ingredients, the following applications are recommended:

Increased physical exertion: During sports, fitness, or tourism; for recovery and improved joint mobility, strengthening of ligaments, and pain reduction.

Physiological support: During the postpartum period, for burn and wound healing processes, and recovery after surgeries or injuries.

Age-related skin changes: To improve elasticity, hydration, color, and structure, and to smooth fine wrinkles. Strengthening

and prevention of nail plate peeling. Hair protection against loss and excessive oiliness.

CONCLUSION

The novelty of the formulation and the production technology is confirmed by a Russian Federation patent. The product has been tested and implemented in manufacturing at the biotechnological cluster enterprises of the "ArtLife" company (Tomsk) in accordance with the requirements of international ISO 9001, 22000, and GMP standards. This guarantees the stability of quality characteristics and demand in the healthy nutrition consumer market.

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