Collagen Complex: A Specialized Fermented Protein-Based Product Enriched With Silicon and Its Promoters

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ABSTRACT
The relevance of this study is driven by the necessity to develop novel, highly effective collagen-based formulations for the nutritional support of connective tissue. The objective of the research was to provide a scientific rationale for the formulation and comprehensive efficacy evaluation of the specialized dietary supplement (DS) Anagran "Collagen Complex," derived from a fermented protein substrate. It was established that the developed "Metacollagen Silicon+" substance is characterized by a high content of specific amino acids: glycine (23.32%), proline (14.11%), and hydroxy-L-proline (12.41%). The molecular weight of the peptides does not exceed 10 kDa, which ensures enhanced bioavailability. Clinical trials demonstrated that daily consumption of a 10-gram dose over 60 days leads to a significant improvement in biochemical markers. The efficacy of the developed product for nutritional support of connective tissue during high physical stress, in the post-traumatic period, and for the prevention of age-related changes in individuals over 35 years of age has been scientifically substantiated and confirmed.
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INTRODUCTION

The continuous pursuit of innovative and highly effective collagen-based formulations is a cornerstone in the development of specialized nutritional products, therapeutic agents, and advanced cosmetic applications [1-7]. These formulations are primarily aimed at enhancing the regeneration and functional capacity of connective tissues, bone structures, and epithelial layers, which are vital for overall health, structural integrity, and physiological performance. The intrinsic biological role of collagen as the most abundant protein in mammals, forming the structural scaffold for various tissues, underscores the importance of understanding and optimizing its utilization in dietary supplements and medical preparations. However, challenges remain in achieving optimal bioavailability and targeted delivery of collagen to specific tissues, necessitating research into advanced processing techniques and synergistic ingredient combinations. This study addresses this need by focusing on the scientific substantiation of the formulation and a comprehensive efficacy evaluation of a specialized dietary supplement, Anagran "Collagen Complex." This product is derived from a unique fermented protein substrate, "Metacollagen Silicon+," engineered to maximize the benefits of collagen peptides, with the strategic incorporation of silicon and other synergistic promoters to enhance its restorative and protective properties for connective tissue.

MATERIALS AND METHODS

The primary focus of this research was the specialized dietary supplement product Anagran "Collagen Complex." The core active component of this product is the proprietary fermented protein substance designated as "Metacollagen Silicon+." This substance is produced through a controlled enzymatic hydrolysis process applied to bovine gelatin [8-14].

Production of "Metacollagen Silicon+":

Enzymatic Hydrolysis: Bovine gelatin was subjected to enzymatic hydrolysis using a specific fungal alkaline protease, Protosyme C (5000 U/g). The selection of this enzyme was based on its demonstrated efficiency in breaking down collagen into peptides with optimal molecular weight and amino acid profiles for bioavailability.

Silicon Fortification: During the hydrolysis process, the substance was further enriched with a natural mineral water source characterized by a high concentration of silicic acid, specifically an orthosilicic acid content of mg/L. This step aims to integrate bioavailable silicon directly into the collagen matrix, enhancing its functional properties.

Formulation of the Anagran "Collagen Complex" Dietary Supplement:

The final dietary supplement formulation was developed based on the "Metacollagen Silicon+" substance and a carefully selected array of synergistic promoters designed to enhance collagen utilization and support connective tissue health. The complete formulation is detailed in **Table 1**. Key components beyond the collagen peptides include:

A complex ("Aminocidin") derived from whey protein peptides and amino acids, featuring metabiotics from Lactococcus lactis cremoris and Lactococcus lactis diacetylactis, along with lactic acid. This component is intended to complement the amino acid profile of collagen and positively influence gut microflora, thereby aiding protein absorption.

* Collagen Promoters: A range of specific nutrients chosen for their known synergistic effects with collagen:
  * Silicic acids (derived from the mineral water)
  * Ascorbic Acid (Sodium Ascorbate 98%)
  * Methylsulfonylmethane (MSM 98%)
  * Hyaluronic Acid (Medium-chain 95%)
  * Vitamin K2 (Menaquinone)
  * Boron (as Sodium Tetraborate)
  * Cholecalciferol (Vitamin D3)
  * Biotin (Vitamin H 99%)
  * Mountain Altai Mumiyo (a complex of trace elements including Potassium, Iodine, Copper, Manganese, Iron, Magnesium, Calcium, and Phosphorus).

A comprehensive suite of analytical techniques was employed to characterize the developed substance and product:

Amino Acid Composition Analysis: High-Performance Liquid Chromatography (HPLC) was utilized to determine the precise amino acid profile of the "Metacollagen Silicon+" substance. This analysis provided quantitative data on the presence of both essential and non-essential amino acids, with particular attention to glycine, proline, and hydroxy-L-proline, critical for collagen stability.

Peptide Molecular Weight Distribution: Techniques (presumably Gel Permeation Chromatography or similar) were employed to determine the molecular weight distribution of the collagen peptides, confirming that the majority do not exceed 10 kDa, a threshold associated with enhanced bioavailability.

Biochemical Markers of Blood and Bone Metabolism: Under controlled clinical conditions, specific biochemical parameters in blood and indicators of bone metabolism were assessed to evaluate the product's efficacy.

Clinical Trials:

Study Design: Clinical trials were conducted at the Scientific and Clinical Center for Hormonal Health "ProfMed" LLC in Tomsk, Russia. The study involved individuals receiving the specialized product as part of their dietary regimen.

Dosage and Duration: Participants consumed a daily dose of 10 grams of Anagran "Collagen Complex" (equivalent to 2.5-3 teaspoons dissolved in 200 cm^3 of drinking water) for a period of 60 days (2 months). Each serving contained 7 grams of bovine collagen peptides.

Target Population: The study included patients with conditions such as enzyme deficiency, insulin resistance, and osteopenia.

Outcome Measures: The primary outcome measures involved the monitoring and analysis of blood biochemical parameters and key markers of bone metabolism. The aim was to assess the impact of the supplement on endogenous collagen synthesis, mineral metabolism, and overall connective tissue health.

RESULTS AND DISCUSSION.

A formulation for the specialized product, a dietary supplement under the brand name "Anagran Collagen Complex," was developed based on fermented bovine gelatin, specifically the "Metacollagen Silicon+" protein substance and its promoters **(Table 1)**. A fungal alkaline protease, Protosyme C (5000 U/g), was selected as the enzyme. Natural mineral water with an orthosilicic acid content of 75.7±11.3 mg/L served as the silicon source.

Table 1. Formulation of the Dietary Supplement Anagran "Collagen Complex"

№	Component Name	Content, mg / 10g of product
1	Metacollagen Silicon +	7000 (collagen peptides)
2	Aminocidin	931.2
3	Methylsulfonylmethane 98%	500
4	Sodium Ascorbate 98%	67,9
5	Medium-chain Hyaluronic Acid 95%	50
6	Vitamin K2 (Menaquinone)	0,12
7	Boron (Sodium tetraborate)	2
8	Mountain Altai Mumiyo (microelement complex)	20
9	Cholecalciferol (D3)	0.005 (200 IU)
10	Biotin 99%	0,05

Animal-derived collagen is physiological and more effective for the human body, as it exhibits high affinity for connective tissue and does not require additional restructuring of amino acid chains during metabolic processes in the liver [15-22].

The amino acid composition of the biotechnological substance "Metacollagen Silicon+" was analyzed using high-performance liquid chromatography **(Figure 1)**.

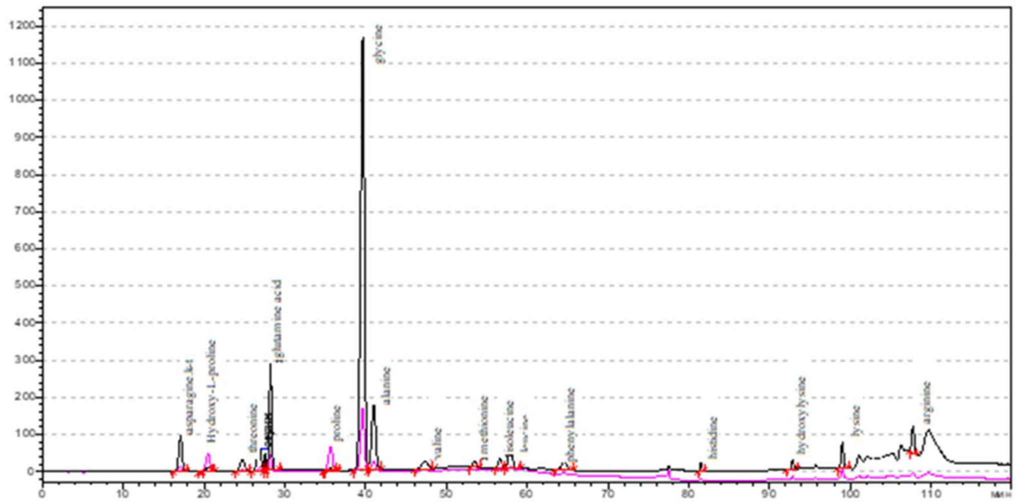

Figure 1. Chromatogram of the amino acid composition of "Metacollagen Silicon +"

The amino acid content is as follows, mg/g (% of total sum): Aspartic acid – 59.06±0.35 (5.91); Hydroxy-L-proline – 124.11±1.55 (12.41); Threonine - 18.41±0.04 (1.84); Serine – 33.57±0.37 (3.36); Glutamic acid - 106.55 ± 0.52 (10.66); Proline - 141.15 ± 2.00 (14.11); Glycine - 233.20 ± 1.90 (23.32); Alanine - 93.67 ± 0.64 (9.37); Valine - 22.99 ± 0.13 (2.30); Methionine - 7.02 ± 0.08 (0.70); Isoleucine - 15.80 ± 0.12 (1.58); Leucine - 31.53 ± 0.07 (3.15); Phenylalanine - 19.61 ± 0.07 (1.96); Histidine - 6.58 ± 0.28 (0.66); Hydroxylysine - 10.34 ± 0.28 (1.03); Lysine - 38.47 ± 1.32 (3.85); Arginine - 67.20 ± 0.83 (6.72). The substance includes seven of the eight essential amino acids: leucine, isoleucine, valine, lysine, methionine, threonine, and phenylalanine. More than 50% of the amino acids in "Metacollagen Silicon +" consist of the total of glycine, proline, and hydroxyproline, which ensure the stability of the primary and secondary structure of collagen.

These amino acids contribute to the effective synthesis of endogenous collagen and regenerative processes in various pathological conditions.

The peptide size in "Metacollagen Silicon +" was determined to be less than 10 kDa. Compared to other peptides of this size, they possess superior bioavailability and efficacy [23].

The product contains the substance "Aminocidin" (whey protein peptides and amino acids, metabiotic LC-Lc-Cr (L. lactis cremoris), metabiotic LC-Lc-Dl (L. lactis diacetylactis), and lactic acid). Aminocidin" is derived from whey protein via fermentation with probiotic microbial strains and complements the amino acid score of the collagen. It contains metabiotics that favorably affect the microflora and promote protein absorption.

In addition to biotechnological substances, the collagen complex includes collagen promoters—components that work synergistically with it: silicic acids, Vitamin C, methylsulfonylmethane, hyaluronic acid, Vitamin K2, boron, cholecalciferol (D3), biotin (Vitamin H), and "stone oil" (Mountain Altai Mumiyo) as a source of macro- and microelements — potassium, iodine, copper, manganese, iron, magnesium, calcium, and phosphorus [15].

One serving of the specialized product (10g) Anagran "Collagen Complex" contains 7g of biologically complete bovine collagen peptides. Collagen promoters within the biocomplex increase its physiological utilization efficiency in the body. Without them, collagen efficiency is negligible, and it cannot provide targeted support to connective tissue. These nutrients serve as cofactors for enzymes that ensure bond formation in collagen and the metabolism of connective tissue, promoting the synthesis of collagen and elastin [23]. From this perspective, Anagran "Collagen Complex" functions as an integrated metabolic system.

Silicic acids occupy a specific place in the biocomplex. It has been established that silicon compounds in bioavailable form (ortho- and metasilicic acids) are necessary for the normal functioning of epithelial and connective tissues, providing them with strength, elasticity, and impermeability. These properties, due to the presence of silicon, are important not only for the skin but also for blood vessels, where silicon is primarily concentrated in elastin and, to a lesser extent, in collagen. In atherosclerosis, the silicon content in connective tissue decreases. This leads to a loss of elasticity in arterial walls due to a drop in elastin levels. Simultaneously, wall permeability increases, allowing "bad" lipids to penetrate the plasma and deposit inside blood vessels as atherosclerotic plaques. Similar changes occur during aging; thus, atherosclerosis is prevalent among the elderly, often resulting in hypertension, heart attack, or stroke.

Silicon compounds actively participate in the growth of hair and nails; deposited silicon chemically cross-links keratin macromolecules, increasing their resistance to liquids [24-30]. One of the simplest diagnostic signs of silicon deficiency is brittle nails. This phenomenon is noted in pulmonary diseases, in chronically ill and weak children, where the silicon content in nails drops by 30-50% [31].

Animal experiments have shown that silicon increases the rate of bone mineralization and calcification alongside Vitamin D. The metabolism of silicon and calcium is closely linked. For instance, aging is associated with an imbalance between these elements — a decrease in silicon and an increase in calcium in connective tissues. The introduction of silicon into the diet promotes calcification and the healing of bone tissue [32-38]. For maximal silicon absorption by bone tissue, Vitamin K is required, which plays a vital role in bone mineralization through the carboxylation of osteocalcin. Its deficiency may hinder the inclusion of silicon in metabolic processes [39].

Clinical evidence of the biocomplex's effectiveness was obtained by including it in the diet of patients with enzyme deficiency, insulin resistance, and osteopenia at a dose of 10g (2.5-3 teaspoons) dissolved in 200 cm^3 of drinking water. One portion contains 7g of full-value bovine collagen peptides. The duration of the course was 2 months. Blood biochemical parameters and bone metabolism markers were studied. It was found that consuming the specialized product compensates for essential amino acid deficiencies, increases levels of protein, calcium, phosphorus, and alkaline phosphatase, optimizes cellular enzyme reactions, and improves the functioning of APUD system signaling peptides, ensuring the activation of bone tissue metabolism [40-47].

CONCLUSIONS

The developed biocomplex can be recommended for: increased physical exertion; sports, physical education, fitness, and tourism; restoration and improvement of joint mobility; strengthening ligaments and reducing pain; postpartum recovery; recovery nutrition after surgery; the post-traumatic period; burn disease and other wound healing processes; and age-related changes in individuals over 35 years old.

The product is manufactured at the "Artlife" company facilities, certified under the international standard ISO 22000:2018 and GMP rules - Codex Alimentarius (RCP-1 (1969), Rev. 5 (2020)), and is successfully sold in the consumer market, allowing it to be positioned as an innovative product.

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