Specialized diet based on modified collagen-containing fish tissue

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Abstract. The study addresses the issue of complex processing of collagen-containing fish raw materials, and expansion of the range of specialized fish products of osteotropic action such as biocrips and protein bars. Small Baltic bream and heads of Baltic sprat, by-product of canned sprat production, were used as raw materials. The raw materials were pre-modified by high-temperature hydrolysis to break down collagen-containing connective tissues and soften bone tissues. Hydrolysis was performed in a thermoreactor in an aqueous medium at 120-40 °C, under pressure of 0.15 to 0.20 MPa within 60–90 minutes. Heat-treated raw materials were divided into fractions, which were used in specialized product processing. Chemical composition of raw materials and finished products was investigated. The technology of biocrips based on thermally modified muscle tissues of Baltic bream and bone supplement from its bones has been proposed. The formulation and technology of molded bars based on sublimated water-soluble protein fractions and a protein-mineral supplement made from heat-treated heads of Baltic sprat are substantiated. The functionality of the products in terms of the mineral content is shown. Recommendations for the use of specialized products for groups of people with deficiency of calcium, phosphorus, for sports and gerodietetic nutrition are elaborated.

1. Introduction
One of the key factors in maintaining public health is nutrition that regularly provides the human body with sufficient energy and vital nutrients. The priority of nutrition for the population health is indicated by the order of the Government of the Russian Federation No. 1364-r of June 29, 2016 On approval of the Strategy for Improving the Quality of Food Products in the Russian Federation until 2030. The modern market of specialized products adequate to the specific nutritional status of elderly and old people, athletes and people with active lifestyle suggests fermented milk, bakery, fish and other types of products. Specialized food products largely exhibit high content of functional ingredients and can be referred to as functional products. Systematic use of specialized functional food products helps prevent the deficiency of valuable bioactive substances responsible for functioning of certain body organs or systems [1].

Another important task of food production is implementation of waste-free technologies for processing natural resources of rationally used secondary raw materials [2].

A promising but underutilized source of raw materials for specialized sports and gerodietetic nutrition are low-value small bony fish and secondary raw materials left after fish cutting (heads,
ridges, scales). Along with high-quality proteins and lipids, this raw material contains minerals, nucleotides, glucosamines and other compounds that exhibit not only plastic but also pharmacological activity [3]. Proteins of this raw material are the constituents of vital organs and tissues, they make complexes with mineral, lipid and nucleic materials, contain all essential amino acids, and therefore demonstrate valuable biopotentia [4]. Modern biotechnological practice focuses on isolation of valuable bioactive peptides, calcium, phosphorus and other osteotropic substances from tissues of secondary collagen-containing fish raw materials [5].

An individual needs to consume bioavailable collagen daily to activate regeneration processes of elastic tissues in joints, ligaments, blood vessels and other organs. One of the techniques to extract collagen and increase its bioavailability in collagen-containing fish raw materials is its thermal modification. Under high controlled temperature, collagen tissues are hydrolyzed to low-molecular-weight proteins and peptides, which are highly digestible [6, 7]. Heat-treated collagen-containing fish raw material is further fractionated to separate water-soluble proteins, lipids, insoluble minerals and high-molecular-weight proteins. This processing makes it possible to fully use the biopotential of raw materials to obtain valuable specialized products to strengthen the human musculoskeletal system.

Development of specialized products with digestible collagen and their use in diets of people of older age groups and athletes to strengthen the musculoskeletal system is a highly relevant challenge for modern nutritional science. Structured snack foods enriched with beneficial biologically active substances, such as biocrips and bars that represent a concentrated source of plastic, energetic and biologically active substances, are gaining popularity. The advantage of these products is that they are ready-to-eat, easy-to-use, and exhibit storage stability under normal conditions.

The aim of the study is to substantiate a fundamental technological solution for manufacturing specialized food using thermally modified collagen-containing fish tissues to maintain stability of the supporting and integumentary tissues of an individual, which concerns athletes and elderly people.

To attain the aim, the following tasks were set: to study the chemical composition of collagen-containing tissues of Baltic bream and sprat; to develop a method for their thermal modification to increase physiological digestibility of tissues; to justify the technological scheme for preparation of specialized multicomponent products using modified fish tissues; to assess the final product quality.

2. Methods

2.1. Scheme, parameters of thermal hydrolysis and quality assessment methods

Experimental studies were conducted in the laboratory of the Department of Food Biotechnology, Kaliningrad State Technical University.

The study flow diagram is shown in Figure 1.

At the first stage, the analysis of scientific and technical and patent literature were analyzed to show the relevance and main area of the study. The study aim and tasks for its achievement were formulated. The second stage of the study included experimental trial of the technology and formulations of specialized products. At the third stage, the quality of finished products was experimentally assessed: the food, biological and energy value was determined; organoleptic and physicochemical evaluation was performed.
The main indicators of nutritional value and physicochemical indicators of the quality of biocrisps and bars were determined using conventional and generally accepted research methods.

The organoleptic characteristics of the finished product quality were assessed using the methods specified in GOST 7631-2008. The energy value of the finished product was assessed using the coefficients from TR CU 022/2011.

Fish raw materials were exposed to high-temperature processing in a thermoreactor (Figure 2) at temperatures of 120–140 °C under pressure of 0.20 MPa for 60–90 minutes. Pre-treated fish raw materials (Baltic bream and sprat heads) were mixed with hot water at 70–80 °C at the ratio of 1:3 and 1:1, respectively. The bream was cut into pieces 10–15 cm wide, and sprat heads were chopped.

After thermal exposure, the bream tissues were manually separated into bone and muscle components and used separately for biocrisps, and the fish bone tissue was ground to a homogeneous mass (powdery state) after hot drying at 120 °C for 60 min. A thermally modified sprat head suspension was divided into fractions: water, fat, and mineral deposit. The water fraction and mineral deposit were dried and used as an ingredient of the bar formulation.

3. Results
Fish products based on meat and bone and secondary raw materials are highly biopotential for creation of specialized osteotropic products based on modified tissues. The average chemical composition of tissues of small Baltic bream and Baltic sprat heads are shown in Table 1.
Table 1. General chemical composition of small Baltic bream and Baltic sprat heads (averaged data), g per 100 g.

|                      | Muscular tissue of Baltic bream | Bone tissue | Heads of Baltic sprat |
|----------------------|---------------------------------|-------------|-----------------------|
| Proteins             | 17.1                            | 29.7        | 13.1                  |
| Fats                 | 4.4                             | 13.8        | 10.3                  |
| Minerals             | 1.1                             | 28.4        | 4.5                   |
| Water                | 77.4                            | 28.1        | 72.1                  |

The meat and bone tissues of bream are of valuable biopotential due to high concentration of bioactive substances of osteotropic action (collagen, amino acids, fatty acids, potassium, calcium, phosphorus, manganese, magnesium, etc.).

The authors’ studies have shown that high temperatures of 120–140 °C destroy the connective tissues of the bream cut into pieces, and after that the muscle tissue can be easily separated from the bone [3, 5]. This tissue modification enables using the muscle and bone mass of fish practically without loss as separate components in the formulation of specialized products.

Another advantage of high-temperature processing of bream tissues is increased fragility of its bones. After exposure to temperatures above 100 °C and pressure of 0.15 to 0.20 MPa, fish bones are significantly softened and can be easily finely ground to prepare a powdery bone mass. This composition is a source of useful minerals (calcium, phosphorus) and collagen protein, and its addition to the muscle tissue increases the biological value of products. It is also known that minced meat and bone fish exhibit an increased radioprotective property. The biological significance of fish bone tissue is also associated with high content of calcium in the digestible form [8]. The foregoing testifies to the prospects for development of new specialized food compositions based on thermally modified bream tissues comparable with dried biocrips.

The studies of high-temperature hydrolysis of Baltic sprat heads in water medium showed that this treatment provides a modified system that consists of three fractions grouped by specific gravity:
- fat fraction (upper), consists of lipids with small admixtures of protein and water;
- water fraction (middle), consists of extracted low-molecular-weight peptides;
- mineral fraction (lower), consists of minerals combined with high-molecular-weight proteins.

The fractions are easily separated by centrifugation and, according to their parameters, can be recommended as part of specialized osteotrophic multicomponent bars (Table 2). The water and mineral fractions are dried and further ground into powder. The properties of the resulting powders make them promising as a complex food supplement and are a source of minerals (potassium and phosphorus) and low-molecular-weight easily digestible peptides. These supplements can improve the quality characteristics of numerous food products. Therefore, the development of the bar formulation supplemented with the prepared supplements is relevant and practically significant.

Table 2. Organoleptic characteristics and mass yields of fractions produced from thermohydrolyzed heads of Baltic sprat (raw material: water ratio is 1:1).

| Characteristic                   | Description                                                                 |
|----------------------------------|-----------------------------------------------------------------------------|
| Water fraction                   | Homogeneous liquid substance, without foreign inclusions, fishy taste and smell, without negative features. |
| Fat fraction                     | Homogeneous liquid substance, medium density, light yellow to light brown, fishy taste and smell, without rancid taste and smell |
| Mineral-protein deposit          | Characteristic of heat-treated fish raw materials, without negative features, fishy taste and smell |

|                         | Mass fraction in the thermally modified system, %: |
|-------------------------|--------------------------------------------------|
| Water fraction (protein) | 69.1                                              |
| Fat fraction            | 5.1                                               |
| Mineral deposit         | 25.8                                              |
The formulation of specialized oat-vegetable bars intended for gerodietetic nutrition was supplemented with 5% of the mass of the protein fraction and mineral deposit at a ratio of 1:1. The bar formulation is presented in Table 3. For bar manufacturing, oatmeal, zucchini and pepper were finely ground, and then fish supplements were added as a source of biologically active substances of osteotropic action, a water-binding agent and a structurant. The structured food mass was held for mass-exchange physicochemical processes, after which the mass was molded in the form of piece bars weighing 20–30 g.

| Table 3. Formulation of bars based on thermally modified tissues of Baltic bream. |
|-----------------------------------------------|-------------------|
| **Ingredients**                             | **g, per 100 g of the finished product** |
| Oatmeal                                      | 35                |
| Zucchini                                     | 35                |
| Sweet pepper                                 | 17                |
| Salt                                         | 3                 |
| Protein / mineral fraction                   | 5/5               |

The resulting bone mass of the Baltic bream exhibited quality indicators (Table 4) that made it a valuable mineral-protein raw material – a source of functional ingredients of osteotropic action (collagen proteins, calcium, phosphorus). The mass is suitable for enriching a variety of structured minced fish products intended to strengthen the human musculoskeletal system. In our studies, the bone mass was added to the biocrips formulation together with other components (Table 5) [3].

| Table 4. Organoleptic and chemical characteristics of food bone mass produced from the bones of thermally modified tissues of Baltic bream. |
|-----------------------------------------------|-------------------|
| **Organoleptic characteristics**             | **Bone mass**     |
| Appearance                                   | Homogeneous mass, without foreign inclusions, |
| Color                                        | Light beige to light brown |
| Smell and taste                               | Characteristic of heat-treated fish raw materials, without bad smell and taste |
| Consistency                                   | Homogeneous |
| Mass fraction,%:                             |                   |
| - water                                      | 29.7              |
| - protein                                    | 28.9              |
| - fat                                        | 13.8              |
| - minerals, including:                       |                   |
| - calcium                                    | 28.4              |
| - phosphorus                                 | 12.3              |
| - calcium                                    | 8.5               |

| Table 5. Formulation of biocrips based on thermally modified tissues of Baltic bream. |
|-----------------------------------------------|-------------------|
| **Ingredients**                              | **per 100 g of the finished product** |
| Table salt                                   | 2.2                |
| Baking powder food (Dr. Oetker)              | 1.5                |
| Wheat flour                                  | 16                 |
| Mineralized bone supplement                  | 4.8                |
| Thermally modified muscle tissues of bream   | 75.5               |

Based on the results of studies, a basic technological scheme for producing specialized food products intended for sports and gerodietetic nutrition (biocrips and bars) was developed (Figure 3). The process begins with preparation and thermal modification of collagen fish tissues (sprat heads,
small bream). Heat-treated tissues are fractionated to separate the protein and mineral fractions, whereas the fat fraction is separated, removed and used separately. After drying and fine grinding, protein and mineral fractions are introduced together with other components into the composition of product formulations, and then the mass is placed into the molds of a designed shape (sticks, rectangles, circles, animals, fish, etc.) and baked to readiness. After cooling, the finished products are packed by piece (bars) or by weight (biocrips), marked and stored until sale. The products can be sold through a specialized network of retail outlets, network marketing, sports clubs, sports sections, or directly to consumers and customers.

The next stage of the study was to assess the quality and safety of the produced biocrips and bars, as well as to substantiate the nutritional value and functionality. An important aspect of this stage was the development of characteristics regulated in the documentation when justifying the product type.

**Figure 3. Technological scheme for manufacturing specialized biocrips and bars based on thermally modified fish tissues.**
Therefore, the organoleptic characteristics were analyzed by qualified specialists through tasting, energy-chemical parameters were calculated, and the content of biologically active components was determined [9–11]. The results are summarized in Tables 6–9.

Table 6. Organoleptic parameters of finished biocrips and bars.

| Parameter | Characteristic |
|-----------|----------------|
| **Biocrips** | |
| Taste | pleasant, fishy, moderately salty, without foreign aftertaste, no taste of bone mass |
| Smell | bready, pleasant, without foreign odors |
| Color | golden brown |
| Consistency | dense, medium hard, easy to bite and chew |
| Appearance | a variety of solid shapes, 0.5 cm thick |
| **Bars** | |
| Taste | pleasant, light fishy, balanced with flavor of the components |
| Smell | light fishy, pleasant, balanced with smell of the components |
| Color | light brown to brown |
| Consistency | dense, homogeneous, baked |
| Appearance | molded plates of 10*3 cm, with visual inclusions of the formulation components |

Table 7. Energy-chemical quality characteristics of finished biocrips and bars.

| Product name | Mass fraction, % | Protein | Fat | Carbohydrates | Energy value, kcal |
|--------------|------------------|---------|-----|---------------|-------------------|
| Biocrips     |                  | 14.7    | 3.6 | 11.6          | 128               |
| Bars         |                  | 5.4     | 2.2 | 21            | 126               |

Table 8. Estimated functionality of biocrips according to the degree of satisfaction of the amount of functional ingredients with physiological norms.

| Component | Content, per 100 g | Recommended physiological daily norm (MP 2.3.1.2432-08), g/100g | % Satisfactory physiol. norms | Functionality |
|-----------|--------------------|---------------------------------------------------------------|--------------------------------|---------------|
| Water     | 72.5               | -                                                             | -                             | -             |
| Protein   | 14.7               | 80                                                            | 18.25                         | -             |
| Fats      | 3.6                | 80                                                            | 1.48                          | -             |
| Carbohydrates | 4.1         | 320                                                           | 3.46                          | -             |
| Minerals: Potassium, mg | 5.1            | 298                                                           | 11.9                          | -             |
| Calcium, mg | 436           | 1200                                                          | 36.3                          | +             |
| Component          | Content per 100 g | Recommended physiological daily norm (MP 2.3.1.2432-08), g / 100g | % Satisfactory physiol. norms | Functionality |
|--------------------|-------------------|---------------------------------------------------------------|-------------------------------|---------------|
| Water, g           | 66.7              | -                                                            | -                             | -             |
| Protein, g         | 5.4               | 80                                                           | 6.75                          |               |
| Fats, g            | 2.2               | 80                                                           | 2.75                          |               |
| Carbohydrates, g   | 21                | 320                                                          | 6.56                          | +             |
| including dietary fibers | 4.7          | 25                                                           | 18.8                          | +             |
| Minerals, mg:      | 4.7               |                                                               |                               |               |
| Potassium, mg      | 382.5             | 2500                                                         | 15.3                          | +             |
| Magnesium, mg      | 64                | 400                                                          | 16                            | +             |
| Phosphorus, mg     | 144               | 800                                                          | 18                            | +             |
| Manganese, mg      | 1.32              | 2                                                            | 66                            | +             |

Table 9. Estimated functionality of bars according to the degree of satisfaction of the amount of functional ingredients with physiological norms.

Analysis of the data provided in Table 8 shows that the produced biocrips are functional products in terms of the content of mineral functional ingredients; 100 g of products (recommended norm) consumed will satisfy the daily human need for calcium by 36.3% and for phosphorus by 27.5%.

Analysis of the data provided in Table 9 shows that the produced bars are functional products in terms of the content of dietary fiber, potassium, magnesium, phosphorus and manganese; 100 g of products (recommended daily norm) consumed will satisfy the physiological need of a person for these substances by 18.8%, 15.3%, 16%, 18% and 66%, respectively (GOST R 52349-2005).

Functional minerals in the developed products (biocripts and bars) perform important functions in the body:

Potassium is an essential component of intracellular fluid and intercellular substances which supports acid-base balance. Due to transmission of nerve impulses, it regulates the work of the myocardium and other muscle tissues and participates in synthesis of proteins and glycogen.

Calcium is one of the main components of the bone tissue involved in formation of teeth, blood coagulation processes, and transmission of nerve impulses to muscles. It also supports functioning of the nervous system.

Phosphorus is an element of organic compounds which participates in conversion of energy, formation of bones and teeth, and formation of proteins, fats and carbohydrates. It supports functioning of the brain, nervous system, and cardiovascular system.

Magnesium is an integral part of the intracellular fluid which participates in formation of the bone tissue, and teeth, and protein synthesis. It transmits nerve impulses to muscle tissues to maintain the central nervous system control and supports functioning of cell membranes.
Manganese is involved in protein and energy metabolism. It promotes the release of energy value from food, participates in synthesis of thyroxine (the main hormone of the thyroid gland), conversion of sugar, formation and oxidation of glucose, and in absorption of calcium, phosphorus and iron. It also regulates the amount of cholesterol in the blood.

The results of the experimental studies show that biocrips and bars produced using the components of modified fish tissues are suitable for use in the diet of the general population to strengthen the musculoskeletal system, which is important for sports and gerodietetic nutrition.

4. Conclusion
The studies show the perspectiveness of using thermally modified collagen tissues of fish raw materials as part of specialized osteotropic diet. The fractions of hydrolyzed meat and bone mass produced from Baltic bream and Baltic sprat heads can be used as a basis to manufacture structured products such as biocrips and bars. After hot drying, molded multicomponent masses with flavoring and structure-forming agents become organoleptically pleasant products which are easy-to-use and functionally provide the body with bioactive substances for nourishing the skin, joints and bones with calcium, phosphorus, potassium, magnesium, manganese, as well as collagen amino acids. The developed products are recommended for use by all groups of people to maintain stability of the musculoskeletal system, which is in demand among athletes and people who lead an active lifestyle. These products will be useful in the diet of elderly people who suffer from osteoporosis and arthrosis as a source of substances involved in regeneration of bone and integumentary tissues.

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