*Article*

Obtaining and Study of Peptide Compositions Based on Hydrolysates of Collagen-Containing Fish Raw Materials

**Abstract:** Experimental studies of fish cutting waste-scales and skin were carried out, their general biochemical composition was studied, a high content of collagen was established, and elastin was noted which accounted for 76–86% of the protein mass. Processes for the hydrolysis of secondary fish raw materials have been developed: fish scales and skin. Technological schemes have been developed and the influence of the conditions of thermal, enzymatic, enzymatic-thermal, electro- chemical hydrolysis on the amino acid composition and molecular weight distribution (MWD) and antioxidant activity of peptides and proteins in the obtained hydrolysates has been studied. It has been established that the enzymatic and enzymatic-thermal method of hydrolysis of fish scales using the enzyme Alcalase 2.5 L and the electrochemical hydrolysis of the skin of cod, trout and herring made it possible to obtain protein hydrolysates with a protein content of 80–90%. At the same time, 91–98% of enzymatic hydrolysates from scales and 62%, 74% and 82.5% of electrochemically obtained hydrolysates from the skin of trout, herring, cod, respectively, account for the share of low-molecular peptides with a molecular weight of less than 10 kDa. The prospects of their use in functional foods and oil-containing products are noted.

**Keywords:** fish processing waste; hydrolysis methods; protein hydrolysates; molecular weight characteristics; antioxidant activity

# Introduction

The fish processing industry generates more than 60% of its by-products as waste. These large volumes of fishery by-products will create serious pollution and disposal problems in both developed and developing countries. One of the directions in the development of the fishing industry is the development of resource-saving technologies and technologies for deep complex processing of raw materials, including the use of secondary resources. This refers to the European zero waste strategy, which supports all three generally accepted goals of sustainable development: economic well-being, environmental protection, and social well-being [[1](#_bookmark8)].

Waste from fish cutting (heads, ridges, skin, scales, and fins) is a promising source of bioactive peptides. This CFRM (Collagen-containing Fish Raw Materials) consists of 60–80% organic matter of collagen protein and related proteins (procollagen, ossein, scleroprotein, mucoidglucoprotein, myosin, actin, and myogen). In addition to proteins, this CFRM includes neutral fats and phospholipids, inorganic forms of calcium and phosphorus, magnesium, sodium, potassium salts, etc., the extraction of which makes it possible to obtain food additives for specialized products [[2](#_bookmark9)–[5](#_bookmark10)].

At fish processing enterprises in Russia, CFRM is currently virtually not processed. At best, fodder flour, feed for fur farms, or agricultural fertilizers obtain from it, while its

valuable biopotential is not fully utilized. It has been proven that marine collagen (achthyocollagen) in a hydrolyzed state stimulates the synthesis of its own collagen, accelerates the restoration of tissues of the musculoskeletal system, improves joint mobility, reduces pain, and slows down the process of destruction of cartilaginous tissue [[6](#_bookmark11)–[9](#_bookmark13)]. The products of deep hydrolysis of achthyocollagen have an ergogenic effect, are antioxidants and antiseptics, have immunomodulatory, hypotensive, regenerative, reproductive functions, and have a cytotoxic effect on some cancer cell lines [[10](#_bookmark14)–[12](#_bookmark15)]. The opioid properties of marine oligopeptides, the functions of renin inhibitor, platelet activating factor of acetyl hydrolase, prolyl endopeptidase, α-amylase, anticoagulant activity, and induction effect have been defined. The applicability of active peptides of marine origin for the prevention of hypertension and infectious diseases, lowering the level of cholesterol in the blood, as well as fierce as functional food ingredients in the composition of specialized, functional, and personalized products, pharmaceuticals and cosmetics has been shown. They are also in demand in the composition of feed for animals and fish, microbiological media, building ten sides, and textile materials [[6](#_bookmark11),[7](#_bookmark12),[13](#_bookmark16)–[17](#_bookmark17)].

It is known that Collagen is a high molecular weight protein, has a molecular weight of about 300 kDa, a diameter of polypeptide chains of about 14–15 A and an approximate length of 2800 A. However, for the body to assimilate collagen, its destruction is necessary. Hydrolyzed collagen, obtained from native collagen, which is found in the scales, bones, skin and connective tissue of fish, consists of peptides with a low molecular weight, due to which it is easily absorbed and distributed in the human body. The quality of hydrolyzed collagen depends on the average molecular size of its fractions, which vary depending on the method used to extract it. The molecular weight distribution of hydrol- ysed collagen peptides typically ranges from 3–100 kDa. However, many researchers note that only low molecular weight collagen peptides (di- and tripeptides), especially those that have C-terminal Pro or Hyp residues, have shown various bioactivities, including immunomodulatory, antibacterial, antioxidant, ACE inhibitory properties, etc. [[18](#_bookmark18)].

The most valuable are biologically active peptides—tissue-specific low-molecular fragments of protein molecules. It has been proven that low molecular weight peptides with a molecular weight (MW) of less than 10 kDa have pronounced pharmacological activity, which makes them attractive components for use in specialized nutrition [[5](#_bookmark10)]. Low molecular weight peptides, depending on their nature, have hormonal action, analgesic, antitumor, and anti-inflammatory effects, regulate the higher nervous activity, blood pressure, biochemical processes associated with the mechanisms of memory, fear, rage, vascular tone, and others [[19](#_bookmark19)–[21](#_bookmark20)].

Like our results, Chi reported that peptide fractions with low molecular weight have more donating electron/hydrogen peptides that can react with free radicals to produce stable products. In addition, other studies in mackerel hydrolyzed collagen, skin squid collagen hydrolysate and salmon collagen, reported that peptide fractions with molecular weights of *≤*5 kDa presented from 20% to 70% inhibition of DPPH at 5 mg/mL.

Collagen from aquatic organisms is especially promising for obtaining low molecular weight collagen; it attracts the attention of the research community due to its relatively low molecular weight, biocompatibility, low immunogenicity, and the absence of ethnic and religious restrictions. Obviously, low molecular weight collagen is promising for use in medicine, pharmacology as a dietary supplement and in the food industry. Collagen hydrolysates are widely used to increase the moisture-binding and water-retaining capacity of meat and fish raw materials, to increase the content of free amino acids in food products and their digestibility by the human body, in connection with this, it is important to improve the technologies for the hydrolysis of collagen-containing raw materials. This determines its use as a protein filler in the production of meat and fish products, combined products [[22](#_bookmark21)]. With proper organization of processing, the profitability of the resulting products may exceed the profitability from the sale of the aquatic organisms themselves [[23](#_bookmark22)]. Thus, the problem of processing secondary raw materials of hydrobionts is becoming an increasingly important task for the coming years.

The relevance of improving existing and developing new technologies for obtaining low molecular weight collagen is obvious. Marine collagens can be processed using the same processing techniques as mammalian collagens. However, due to some differences in their properties, new technologies may require some tweaking to further improve the properties of the resulting collagen hydrolysates [[24](#_bookmark23)].

Nagai and Suzuki used skin, bones, and fins to produce type 1 collagen using butyl alcohol as an extraction solvent. The yield of collagen was 51.4%, 42.3% and 5.2%, respectively [[25](#_bookmark24)].

In another Nagai [[26](#_bookmark25)] study, cuttlefish skin was extracted with acetic acid, yielding 2% collagen on a dry weight basis. It follows from this that the nature of the solvent significantly affects the yield of collagen.

Liu reported the extraction of collagen from the fins, scales, skin, bones, swim bladders of bighead carp using acetic acid. The collagen content was 5.1%, 2.7%, 60.3%, 2.9%, 59%, respectively [39].

The yield of collagen obtained from various skin sources varies depending on the extraction methods used [[27](#_bookmark26)].

There are several publications describing the properties of fish skin collagen hydrolysates, and even fewer studies have been conducted on the characterization of hydrolysates derived from pepsin-soluble collagen of marine origin.

It is obvious that the development of a technology for the hydrolysis of collagen- containing fish processing waste, which makes it possible to obtain a product with a given MWD, a valuable amino acid composition, a controlled degree of hydrolysis without harmful impurities and a negative impact on the environment, is an important fundamental and applied task.

The purpose of this research was to obtain low molecular weight collagen hydrolysates by various methods from fish processing waste to study and compare the MMP and the properties of these hydrolysates and dietary supplements obtained using them.

# Materials and Methods

* 1. *Materials*

The experiments were carried out at the Faculty of Biotechnology (Biotech) of ITMO University (electrochemical hydrolysis) and at the Department of Food Biotechnology of the Kaliningrad State Technical University. In the research laboratory Altlandsberg GmbH, Germany, R&D department of ANiMOKS GmbH Berlin, Germany

Research objects were skins of slightly salted herring (waste from the production of preserves from Atlantic herring at Baltiyskiy Bereg OOO TD (St. Petersburg, Russia); Atlantic cod skin (waste from skinning fillets, Eco Fish OOO (Murmansk, Russia); Rainbow trout skin (waste from skinning fillets, Rybstandart OOO (the village of Naziya, Leningrad region, Russia); sardine and sardinella scales (waste from the production of sardines canned in oil, fish canning complex of Ros Con OAO, Pionersky, Kaliningrad region, Russia).

* 1. *Methods*
     1. Electrochemical Method for Obtaining Hydrolysates

As a hydrolyzing agent, electrochemically prepared catalysts (catholytes) were used, which have an increased extraction and hydrolysis ability compared to alkaline solutions due to the presence of reducing agents in the form of dissolved hydrogen, and its radicals together with hydroxyl ions.

Catalysts were obtained by electrolysis of low-mineralized aqueous media during unipolar treatment on standard electrolysis plants of the STEL brand (10N-120–01, model 80-03) manufactured by NPO Ekran (Moscow, Russia).

The raw material is crushed to a size of 3–5 *×* 10*−*3 m, mixed with a catholyte solution with pH 12.2 *±* 0.5 and Eh 920 *±* 50 mV, with a relaxation time after electrolysis of 5*−*10 min with hydromodule 1:3–1:6 in a reactor with a stirrer, followed by heating to 80 *±* 5 *◦*C and thermostating in a reactor with a stirrer for 30–40 min and divided into fat and protein

fractions. After separation of the fat fraction, the remaining mixture is centrifuged in a laboratory centrifuge at 3500 rpm. within 10 min. The mineral precipitate, which is the undissolved parts of the raw material after centrifugation, is dried in a fluidized bed on a laboratory dryer.

* + 1. Electrochemical Method for Obtaining Hydrolysates

Fish scale hydrolysates were obtained by ferment lysis and thermohydraulic in water using the Alcalase 2.5 L enzyme (activity 2.5 AU/g), as well as thermally in the controlled hydroautoclave (thermohydrolysis (t = 1–1.5 h, T = 130–140 *◦*C, p = 0.25 MPa, pH 7.0). The hydrolyzed mass was separated by centrifugation into three fractions: protein, lipid, and mineral-protein. The lipid and mineral-protein fractions were sent to feed and/or technical purposes. Water-soluble protein fraction was freeze-dried on a Martin Christ Alpha1-2 LDplus freeze dryer to obtain a peptide additive. The protein-mineral composition was dried by convection at a temperature of 70–75 *◦*C [[10](#_bookmark14),[28](#_bookmark27),[29](#_bookmark28)].

* + 1. Physical and Chemical Research Methods

The studies used standard, generally accepted organoleptic and physicochemical methods; protein—by the Kjeldahl method on the Kjeltek device, ash—by burning in a muffle at 650 *◦*C, fat—by the Soxhlet method, etc. Amino acid (AA) composition of proteins was determined by ion exchange chromatography in the laboratory of UBF GmbH (Altlandsberg, Germany).

The antioxidant activity of scale peptides was assessed by the ability of the extracts to inactivate DPPH radicals [[30](#_bookmark29)].

The antioxidant activity of scale peptides was assessed by the spectrophotometric method according to the ability of the extracts to inactivate DPPH radicals. The method is based on the interaction of antioxidants with stable chromogen radical 2,2-diphenyl-1- picrylhydrazyl (DPPH). The DPPH working solution was used as a control sample, which was prepared by diluting the DPPH standard solution with ethanol (5 *×* 104 M) in ethanol at a ratio of 1:10. To 5 mL of the working solution of DPPH, 50 µL of the studied solutions of peptides in ethanol were added, mixed, and the optical density of the solution was

recorded after 30 min at a wavelength of 517 nm.

Antiradical activity (AA) was determined by the formula:

AA (%) = [(ADPPH *−* Apep)/ADPPH] *×* 100% (1)

where:

ADPPH—optical density of the DPPH working solution Apep—optical density of the peptide solution

The antioxidant activity of scale peptides was assessed by the ability of extracts to inactivate DPPH radicals.

The determination of the molecular weight distribution (MWD) of the components of skin hydrolysis was carried out by gel chromatography based on the volumetric exclusion of molecules of different molecular weights.

Sample preparation included centrifugation (centrifuge 5415C/Eppendorf, Brinkmann instruments, Inc. Cantiage Road, Westbury, NY, USA) for 5 min, 16,100*× g*, followed by 20-fold dilution with a mobile phase (see below for the composition).

Analysis conditions: peristaltic pump Varioperpex 12000 (LKB, Sweden); UV detector (CarloErba, Milano, Italy), 220 nm—for peptides; mobile phase 0.3% (NH4)2SO4, 35–45 mL/h; the volume of the injected sample is 100 µL (sample probe loop).

The following columns were used:

* 16 *×* 170 mm SepharoseCL-4B (Pharmacia Fine Chemicals Co., Stockholm, Sweden)—for molecular weights from 30 kDa to 5 MDa;
* 16 *×* 255 mm SephadexG-75 (Pharmacia Fine Chemicals Co., Sweden)—for molecular weights from 3 kDa to 70 kDa;
* 9 *×* 372 mm SephadexG-15 (Pharmacia Fine Chemicals Co., Sweden)—for molecular weights from 0 to 1500 Da.

The molecular weight distribution of peptide hydrolysates of fish scales was assessed by mass spectrometry when separating them into fractions by high performance liquid chromatography, HPLC/Phenomenex (Yarra 3uSEC-200) in the laboratory of ANiMOX (Berlin, Adlershof, Germany).

Statistical data processing was performed using the Microsoft Office 2010 and Mathcad 2000 Professional software packages at a 95% confidence level.

# Results

Experimental data on the chemical (Table [1](#_bookmark0)) and amino acid (Table [2](#_bookmark1)) composition of the scales and skin of fish indicates the feasibility of using the protein biopotential of these types of waste to obtain hydrolyzed protein products.

**Table 1.** Chemical composition of bone and casing waste from fish butchering, in %.

herring

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Object Name Waste Name** | **Water** | **Fat** | **Protein**  **N Total** *×* **5.62 m** | **Ash** | **Total Nitrogen** |
| Sardines Scales | 72.9 *±* 0.5 | 8.4 *±* 0.2 | 15.3 *±* 0.75 | 3.3 *±* 2.25 | 2.7 *±* 0.5 |
| Sardinella Scales | 66.7 *±* 0.5 | 9.5 *±* 0.2 | 20.2 *±* 0.75 | 3.6 *±* 2.25 | 3.6 *±* 0.5 |
| Lightly salted Skin | 57.4 *±* 0.6 | 19.6 *±* 0.2 | 19.2 *±* 1.0 | 5.4 *±* 2.00 | 3.4 *±* 0.5 |
| Cod Skin | 69.3 *±* 0.6 | 1.2 *±* 0.2 | 20.0 *±* 1.0 | 6.6 *±* 2.00 | 3.5 *±* 0.5 |
| Trout Skin | 57.2 *±* 0.6 | 15.2 *±* 0.2 | 22.5 *±* 1.0 | 5.1 *±* 2.00 | 4.0 *±* 0.5 |

**Table 2.** Amino acid composition of bone and casing waste from fish butchering, in %.

**Average Mass Fraction of AA on Dry Matter**

**Sardines Scales Sardinella Scales Herring Skin Cod Skin Rainbow Trout Skin**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino Acid** | **g/100 g** | **g/100 g** | **g/100 g** | **g/100 g** | **g/100 g** | **g/100 g g/100 g g/100 g g/100 g**  **Protein g/100 g** | | | | |
|  | **Protein** | **Scales** | **Protein** | **Scales** | **Protein** | **Skin** | **Skin** |  | **Protein** | **Skin** |
| Aalanine | 5.84 | 3.31 | 11.20 | 5.60 | 11.4 | 5.91 | 12.4 | 3.84 | 12.10 | 6.41 |
| Arginine | 7.23 | 4.10 | 7.90 | 4.00 | 8.00 | 3.58 | 9.20 | 2.85 | 11.20 | 5.93 |
| Asparagine | 0.00 | 0.00 | 0.10 | 0.10 | 6.9 | 3.00 | 1.56 | 0.48 | 6.22 | 3.22 |
| Aspartic acid | 6.05 | 3.43 | 4.90 | 2.50 | 2.10 | 1.09 | 2.23 | 0.69 | 2.14 | 1.13 |
| Carnosine | 0.00 | 0.00 | 0.10 | 0.01 | - |  | - |  | - |  |
| Citrulline | 0.02 | 0.01 | 0.00 | 0.00 | - |  | - |  | - |  |
| Cystine | 0.21 | 0.12 | 0.00 | 0.00 | - |  | - |  | - |  |
| Glutamine | 0.04 | 0.02 | 0.80 | 0.40 | 3.31 | 1.71 | 3.80 | 1.18 | 4.73 | 2.50 |
| Glutamic acid | 9.29 | 5.27 | 8.50 | 4.30 | 11.5 | 5.98 | 10.63 | 3.29 | 10.24 | 5.43 |
| Glycine | 12.57 | 7.13 | 26.00 | 13.1 | 6.50 | 3.38 | 6.20 | 1.92 | 5.90 | 3.12 |
| Histidine | 1.66 | 0.94 | 1.20 | 0.60 | 2.82 | 1.45 | 2.29 | 0.71 | 2.33 | 1.20 |
| Hydroxyproline | 9.45 | 4.99 | 10.70 | 4.40 | 4.40 | 2.28 | 3.72 | 1.15 | 3.90 | 2.07 |
| Leucine (n) | 3.65 | 2.07 | 2.70 | 1.30 | 1.85 | 0.96 | 2.10 | 0.65 | 1.90 | 1.01 |

**Table 2.** *Cont.*

**Average Mass Fraction of AA on Dry Matter**

**Sardines Scales Sardinella Scales Herring Skin Cod Skin Rainbow Trout Skin**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino Acid** | **g/100 g Protein** | **g/100 g Scales** | **g/100 g Protein** | **g/100 g Scales** | **g/100 g Protein** | **g/100 g Skin** | **g/100 g Protein Skin** | **g/100 g** | **g/100 g Protein** | **g/100 g Skin** |
| Isoleucine (n) | 2.12 | 1.21 | 1.00 | 0.50 | - |  | - |  | - |  |
| Lysine (n) | 2.72 | 1.54 | 4.00 | 2.00 | 0.70 | 0.36 | 0.35 | 0.11 | 0.39 | 0.21 |
| Methionine (n) | 2.84 | 1.61 | 0.01 | 0.01 | 5.32 | 2.70 | 5.10 | 1.58 | 5.60 | 2.97 |
| Ornithine | 0.07 | 0.04 | 0.00 | 0.00 | 3.80 | 1.98 | 3.11 | 0.96 | 3.34 | 1.77 |
| Phenylalanine (n) | 2.75 | 1.56 | 2.20 | 1.10 | - |  | - |  | - |  |
| Proline | 9.42 | 5.34 | 11.70 | 5.90 | 1.61 | 0.83 | 1.62 | 0.50 | 1.50 | 0.79 |
| Serine | 4.00 | 2.27 | 2.90 | 1.50 | 2.51 | 1.30 | 2.30 | 0.71 | 2.11 | 1.12 |
| Taurine | 0.10 | 0.06 | 0.00 | 0.00 | 3.90 | 2.03 | 3.40 | 1.05 | 3.42 | 1.81 |
| Threonine (n) | 1.22 | 1.69 | 2.10 | 1.10 | 6.50 | 3.38 | 6.20 | 1.92 | 5.90 | 3.12 |
| Tyrosine | 1.53 | 0.87 | 0.60 | 0.30 | 2.82 | 1.45 | 2.29 | 0.71 | 2.33 | 1.20 |
| Valine (n) | 2.05 | 1.16 | 1.50 | 0.80 | 4.40 | 2.28 | 3.72 | 1.15 | 3.90 | 2.07 |

From the data in Table [1](#_bookmark0) it follows that the waste: fish skin and scales contain a significant amount of protein (15–23%), fat (9–18%) and minerals (3–5%), which indicates their high nutritional and biological value.

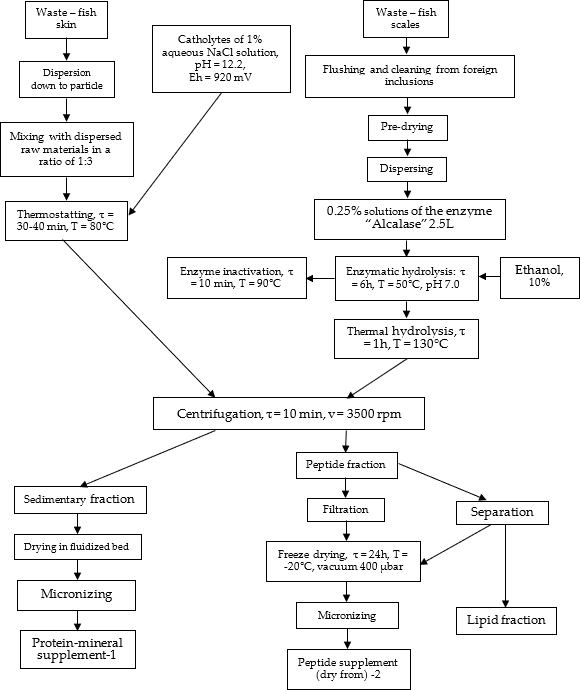
From Table [2](#_bookmark1) it follows that the raw materials contain the greatest amount of amino acids characteristic of collagen: glycine, proline and hydroxyproline, which proves the collagen nature of the main proteins. The total content of collagen and elastin of the scales is 76.26–86.35% of the mass of proteins, and the skin is 79.21–88.32%.

Peptide hydrolysates from scales were prepared in three ways: thermal, enzymatic with the use of the Alcalase 2.5 L enzyme preparation, and enzymatic-thermal were investigated. Skin hydrolysates were prepared using electro-chemical derived catholytes. The resulting polypeptide compositions were used to obtain dietary supplements of peptide and peptide-mineral nature. The technological scheme is shown in Figure [1](#_bookmark2).

The key stages of the technology for the manufacture of peptide additives are: preparation of a homogenized mixture from dispersed collagen-containing raw materials (CFRM) and water, electrochemical preparation of a catholyte-catalyst and enzyme solution, temperature control of the mixture at a given temperature, separation of the hydrolyzed suspension into three fractions (lipid, peptide and protein-mineral), purification of obtaining peptide fractions from lipid (fat) and non-hydrolyzed components, and freeze-drying of enzymatically obtained peptides and their dispersion to a powder state—BAA-2 and drying of electrochemically obtained hydrolysates together with a mineral residue in a fluidized bed—BAA-1.

The molecular weight distribution of hydrolysates obtained by enzymatic and thermal methods from sardine and sardinella scales was studied by gel permeation chromatography. The results are presented in Figure [2](#_bookmark3) and Table [3](#_bookmark4).

The Figure [2](#_bookmark3) and Table [3](#_bookmark4) show that it follows from them that the fraction obtained by the enzymatic-thermal hydrolysis method has the highest content of hydrolyzed protein substances (83.9–85.2%). The largest amount of low molecular weight peptides with a molecular weight of less than 10 kDa is found in hydrolysates of the enzymatic hydrolysis method (89.6–91.7%).

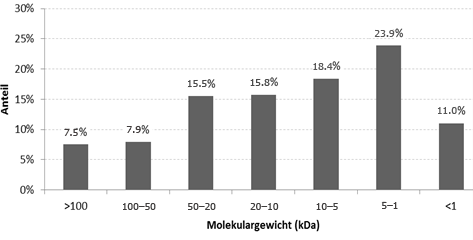
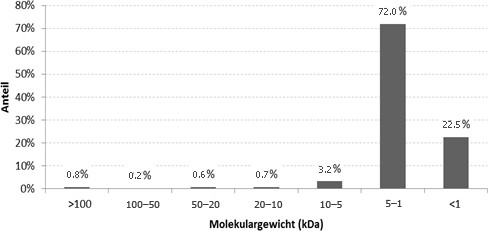


**Figure 1.** Technological scheme of enzymatic-thermal and electrochemical hydrolysis of fish skin and scales and production of peptide (2) and peptide-mineral (1) bioadditives based on them.

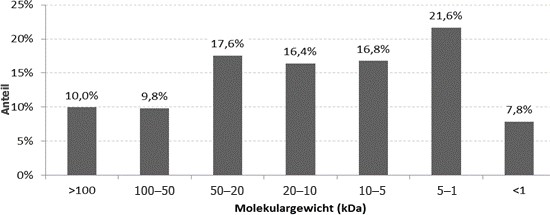
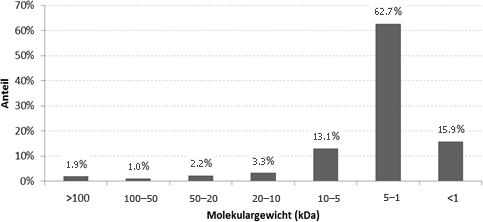
This potentially indicates the presence of biological activity in the peptides of these compositions. It is important that the resulting peptide compositions contain more than 80% of molecules with a molecular weight of less than 10 kDa.

The study of the molecular weight distribution of peptide hydrolysates from the skin obtained using electrochemically obtained catalysts—catholytes was also carried out using gel chromatography. Figure [3](#_bookmark5) shows the output curves of gel chromatography of peptides of skin hydrolysates obtained on the Sephadex G-75 carriers used-for molecular weights of 3 kDa to 70 kDa and Sephadex G-15—for molecular weights from 0 to 1500 Da. In all graphs, the abscissa is the output volume, ml; the ordinate is photoabsorption at 220 nm.

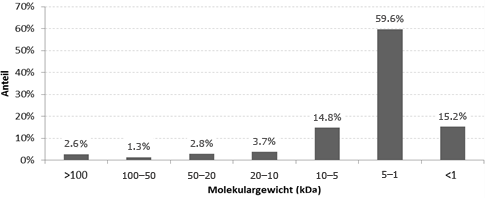
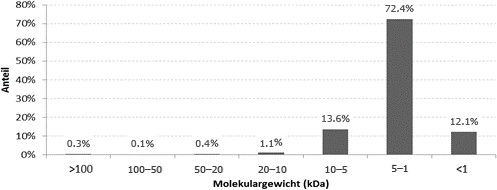
Table [4](#_bookmark6) and histograms in Figure [4](#_bookmark7) show the quantitative results of calculations of the component composition of collagen hydrolysates depending on the molecular weight.

(**a**) (**b**)



(**c**) (**d**)

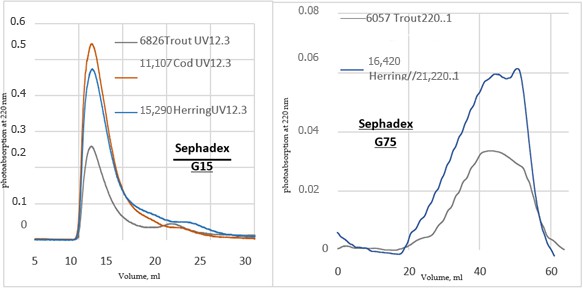
(**e**) (**f**)

**Figure 2.** Molecular weight distribution of peptides of the protein fraction obtained by various methods of hydrolysis of sardine (**a**–**c**) and sardinella scales (**d**–**f**): (**a**) thermal; (**b**) enzymatic; (**c**) enzymatic- thermal; (**d**) thermal; (**e**) enzymatic; (**f**) enzymatic-thermal.

**Table 3.** Chemical composition of various fractions of fish scale hydrolyzate obtained by various hydrolysis methods.

|  |  |  |
| --- | --- | --- |
| **Hydrolysis Methods** | **The Content of Protein Substances in the Sublimated Protein Fraction, % 1** | **The Content of Peptides in the Protein Fraction with MW** *≤* **10 kDa 2** |
|  | Sardine scales |  |
| Thermal | 27.5 | 53.3 |
| Enzymatic | 65.0 | 98.1 |
| Enzymatic-thermal | 83.9 | 91.7 |
|  | Sardinella scales |  |
| Thermal | 27.2 | 46.2 |
| Enzymatic | 55.9 | 97.7 |
| Enzymatic-thermal | 85.2 | 89.6 |

Note: 1—% by weight of the protein fraction; 2—% of the mass of the protein fraction.

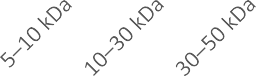
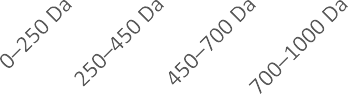


**Figure 3.** Output curves of gel chromatography of peptides of collagen concentrate samples on the carriers used.

**Table 4.** Molecular weight distribution of peptides in collagen samples hydrolysates, wt.% content of fractions.

herring skin

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Molecular 0–250 Da 250–450** | | **450–700** | **700–1000 1–5 kDa 5–10 kDa 10–30 kDa 30–50 kDa >50 kDa** | | | | | |
| **Weight (MW)** | **Da** | **Da** | **Da** |  |  |  |  |  |
| Lightly salted 4.0 | 5.6 | 9.1 | 20.2 | 17.8 | 17.6 | 20.2 | 4.3 | 1.2 |
| Cod skin 9.2 | 9.1 | 8.9 | 18.6 | 20.5 | 16.2 | 15 | 1.8 | 0.7 |
| Trout skin 2.6 | 3.6 | 10 | 21.2 | 9.6 | 15.3 | 28.9 | 6.9 | 1.9 |



% 35

30

25

20

15

10

5

0

Slightly salted herring

Cod Rainbow trout

**Figure 4.** Histogram of the molecular weight distribution of peptides in samples of hydrolysates of fish skin collagen.

There are differences in the nature of sample hydrolysis: trout skin hydrolyzate contains the highest amount of high molecular weight peptides (1.9% of peptides, MW > 50 kDa) with the least amount of low molecular weight peptides (2.6% of peptides, MW from 0 to 250 Da). The opposite picture is observed in the hydrolyzate of low-salted herring. The cod skin hydrolyzate sample occupies an intermediate position between the other two. From the data in Table [3](#_bookmark4) it follows from that the share of peptides with a molecular weight of less than 10 kDa, which have the highest biological activity obtained from trout skin, accounts for 62%, from herring skin—74%, from cod skin—82.5%. At the same time, the share of peptides with a molecular mass of more than 30 kDa accounts for only 5% on average.

The experimental results also agree with the calculations of the hydrolysis degree of collagen hydrolysates, determined by the value of amine nitrogen, which is maxi- mum in herring skin samples (Namin = 2.6%) and minimum in trout skin hydrolysates (Namin = 0.18%).

Thus, under the action of catalysts-catholytes obtained by electrolysis of low-mineralized (1–2%) aqueous solutions, a deep hydrolysis of collagen-containing wastes—fish skin also occurs with the production of peptide hydrolysates containing 65–71% of physiologically active low-molecular peptides with a molecular weight of less than 10 kDa.

Study of antioxidant activity (AA) on the DPPH-radical of low molecular weight fractions of protein hydrolysates with M.M. less than 10 kDa solutions of peptides from sardine scales obtained by the enzymatic-thermal method and electrochemical methods showed the presence of antioxidant efficiency, quantitatively correlated with the concentration of the peptide additive: 1% solution of the peptide was characterized by an AA value of 28.12%, and 5% solution had an AA index of 54.48%. Accordingly, the obtained results make it possible to recommend the use of peptide compositions from collagen-containing fish waste-CFRM as antioxidants in the composition of various fat-containing products.

# Discussion

The developed methods for the hydrolysis of fish waste make it possible to obtain polypeptide hydrolysates with a high yield with a protein content of more than 80% in dry matter. Hydrolysates obtained by enzymatic and enzymatic-thermal methods from sardine and sardinella scales had a fraction of a low–molecular physiologically active fraction of peptides with a molecular weight of less than 10 kDa—98.1%.

Molecular weight plays an important role in their antioxidant activity. For example, lower molecular weight fractions of collagen hydrolyzate isolated from Spanish mackerel skin showed higher radical scavenging capacity, while higher molecular weight fractions showed higher emulsifying properties, making these fractions potential antioxidants. Lower molecular weight peptides from salmon can be used as a reducing agent and free radical scavenger in oxidation processes, as well as an antioxidant with the effect of protecting DNA from cell damage caused by oxidation [[31](#_bookmark30),[32](#_bookmark31)].

Hydrolysates obtained from salmon, cod and lightly salted herring using electro-chemicals of the obtained catholyte contained this fraction in an amount of only 75%.

The difference in the degree of hydrolysis can be due not only to the peculiarities of the production methods, but also to the different protein composition of the raw materials [[33](#_bookmark32)–[37](#_bookmark33)]. In addition, the raw material source- fish skin, is more tonnage than scales [[38](#_bookmark34)].

Recently, collagen hydrolysates have gained wide acceptance for promising health benefits, such as improved bone density [[39](#_bookmark35)], reduction of joint pain [[40](#_bookmark36)], blood pressure [[41](#_bookmark37)] and may prevent atherosclerosis [[42](#_bookmark38)]. It is known that low-molecular-weight fish collagen has antioxidant properties associated with its unique sequence of glycine-proline-alanine components; it has characteristics similar to pork hydrolyzed collagen, and can also be considered as an alternative to mammalian collagen for use in food products [[43](#_bookmark39),[44](#_bookmark40)].

An important advantage of using fish derivatives is the fact that both of these products, com- pared with bovine derivatives, do not pose a risk of transmission of spongiform encephalopathy. Fish bones can be used to produce calcium. In bones, it is contained in the most digestible and biocompatible form of the human body-hydroxyapatite. Important proper- ties of hydroxyapatite are associated with its thermodynamic stability at physiological pH values [[45](#_bookmark41)]. In addition, they contain vitamins, macro- and microelements (in particular D, B2, B6, PP, Mg, F, Mn, Cu), which contribute to better absorption of calcium by the body.

Combining the mineral component with hydrolyzed collagen is advisable.

Dietary supplements based on hydrolysates of fish scales and skin are recommended for use in formulations of specialized functional nutrition products [[2](#_bookmark9)]. Given the high collagen content, hydrolysates can be used to correct metabolic disorders in the synthesis and regeneration of collagen tissues in the body [[46](#_bookmark42)–[51](#_bookmark43)]. The antioxidant activity of the obtained hydrolysates was established in the work.

The antioxidant activity of the obtained hydrolysates was established in the work. The introduction of the obtained peptide additives with antioxidant activity into fat-containing products (mayonnaises, creams, sauces) will increase the potential antioxidant stability of their lipid fraction [[52](#_bookmark44)–[55](#_bookmark45)]. Low molecular weight peptides can also potentially be used as emulsifiers in fat-containing food systems in the form of polyelectrolytes with a positive (amino group) and negative (carboxyl group) charge [[56](#_bookmark46)].

# Conclusions

It has been shown that collagen-containing waste from fish butchering—skin and scales have a valuable chemical composition and are a promising source for obtaining protein hydrolysates rich in low-molecular biologically active peptide, mainly collagen fraction less than 10 kDa. The chemical and amino acid composition of collagen-containing wastes—sardine and sardinella scales and skin of trout, cod and lightly salted herring was studied, the presence of a large amount (up to 22%) of collagen-containing proteins in them was established. The protein components of hydrolysates of sardine and sardine scales obtained by enzymatic hydrolysis using the enzyme Alcalase 2.5 L and the enzymatic-thermal method and hydrolysates of skin of trout, cod and lightly salted herring obtained using an electrochemically obtained hydrolysis catalyst-catholyte were studied, obtained by electrochemical method. These methods make it possible to obtain a protein material with a protein content of 83.9–85.2%. The antioxidant activity of the obtained hydrolysates was investigated, and it was found that their antioxidant index is more than 54%. The proportion of low-molecular physiologically active peptides with a molecular weight of 316 less than 10 kDa in them is 91.7–98.1% for enzymatic and 65.3–75% for hydrolysates obtained using catholyte, respectively. A technological scheme is proposed for the production of hydrolysates from collagen-containing fish waste by enzymatic, enzymatic-thermal methods and using catholyte obtained by electrochemical method and preparation with their use of dry forms of biologically active additives of peptide and peptide-mineral nature. Considering the presence of a large amount of collagen peptides in the obtained biologically active supplements, it is recommended to use them as part functional foods for athletes, people with musculoskeletal system problems and cardiovascular diseases. The antioxidant activity of collagen polypeptides allows them to be used as antioxidant additives in the food and pharmaceutical industries, especially in the composition of fat-containing products and preparations.

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