Technology for obtaining essential fatty acids and protein hydrolyzate from by-products of slightly salted herring production

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Abstract. The technology for the rational use of collagen-containing waste, namely the skin from cutting slightly salted herring, is presented. The technology for obtaining oil containing polyunsaturated fatty acids and collagen hydrolyzate from by-products of production of preserves from slightly salted herring was developed. The rational parameters of hydrolysis in gentle conditions using electrochemically obtained catholytes have been determined and substantiated, which ensures waste-free processing of raw materials and the reduction of oxidized nutrients. The cryoconcentration method was used to concentrate essential acids. We studied the phase transitions of the obtained lipids in the temperature range from + 15 °C to minus 40 °C in a calcium chloride environment using a low-temperature refrigeration unit. The properties of 5 lipid fractions formed during lipid phase transitions have been identified and studied. The technology was applied to fatty raw materials - skin from cutting slightly salted herring into fillets. The physical and chemical properties of raw materials and finished products have been investigated. The mass yields of fish oil and collagen hydrolyzate have been determined. Comparative analysis of fatty acid composition of fish skin before and after electrochemical treatment has been carried out.

1. Introduction
Currently, in the Russian food industry, preserves made from low-salt herring are traditionally popular among the population, since the product has a high nutritional value, taste characteristics and an affordable price. Fish of the herring family have a number of advantages. One of them is “non-aquaculture” origin and non-contamination with technogenic impurities, which is due to the specific nutrition of this fish species mainly by plankton. Also, the fish oil of the herring family is characterized by a valuable fatty acid composition; it contains about 15% of omega-3 fatty acids. For the production of preserves, it is customary to use fish with a high fat content, since the latter contributes to the intensification of fermentation processes during salting [1]. Fish of the herring family are characterized by significant fluctuations in fat content depending on the time of catch. In the manufacture of preserves, about 30% of secondary by-products are formed. Depending on the processing method (manual or mechanical), the amount of skin obtained varies from 7 to 12%. Currently, the industrial enterprises do
not use technologies for the recycling of this waste, which leads to environmental pollution and a decrease in the profitability of production [2].

However, during salting, the quality of fat deteriorates due to enzymatic and oxidative reactions. There are thermal, enzymatic, acid-base methods [3] for extracting fish oil from raw materials, but they either do not provide the desired mass yield of the target product, or significantly reduce its quality due to hydrolysis and oxidation. We know the electrochemical technology of extracting raw material nutrients, which ensures the recovery of previously oxidized forms of nutrients due to the presence of reducing properties in the extractants.

Also, fish skin is rich in collagen [4], the main advantage of which is the development of kosher and halal products. Collagen from cattle and porcine sources can pose a serious risk to human health, as some diseases are transmitted through food, such as the outbreak of bovine spongiform encephalopathy and foot and mouth disease, so marine collagen can provide the consumer with safe food, without any fear of such diseases [5].

In addition, the fish industry can use fish waste to produce cheap collagen. The processing industry uses about 25% of the total weight of fish, while the remaining 75% is treated as waste. Converting waste to collagen can help avoid environmental problems associated with fish waste and products that generate added value that increase the economic return of the fish processing industry [6]. Fish collagen is absorbed into the body up to 1.5 times more efficiently and has a higher bioavailability than pork and bovine collagen [7].

Fish protein hydrolysates (FPH) are produced by enzymatic or chemical processing of fish protein and are considered to be an effective approach to using unrecognized fish biomass in edible protein products rather than as animal feed or fertilizer. Peptides obtained by enzymatic hydrolysis of fish proteins with well-defined molecular weight ranges, adapted for superior functionality and interesting bioactivities, are in high demand [8]. They also support a high content of essential amino acids and better absorption. Hydrolysates with a low degree of hydrolysis show better functional properties, while a high degree of hydrolysis leads to higher bioactive peptides with low molecular weight. Moreover, protein recovery is a major factor to be considered when converting a protein to its hydrolyzate form from an economic point of view. In addition, fish protein hydrolysates contain higher protein content, ranging from 60% to 90%, demonstrating its potential use as protein supplements for human nutrition [9]. Therefore, FPH can successfully replace functional compounds such as sodium caseinate and BSA used in food formulation, cosmetic and pharmaceutical industries.

This article presents the technology for obtaining essential fatty acids and protein hydrolyzate from the wastes of the production of slightly salted herring.

2. Materials and methods

The material for the study was skin waste from skinning fillets of Atlantic herring Clupea harengus. Producer was Faroe Islands, plant - FO229, catch area - FAO227, size range - 4-7 pcs / kg, catch period - October 2020. Since herring is one of the most widespread objects of aquatic fishing, which accounts for up to 30% of the catch, and preserves from light-salting herring are a popular product in the northwest region. A medium-sized enterprise processes up to 25 tons of herring fillets per day, and skin waste accounts for up to 16% (4-5 tons per day), which shows the feasibility of developing a technology for processing this particular raw material.

The skin waste was formed when salting herring in the traditional way - wet salting in accordance with the technological scheme (Figure 1).
The collection of herring skin was carried out mechanically by means of a vacuum system. We mounted to the skinning machine a branch pipe of the vacuum pipeline, into which the removed skin enters and then moves to the place of waste collection - plastic vat containers.
The catholyte was obtained in industrial electrolyzers on STEL-type installation. At all stages, the pH and redox potential values were monitored using a stationary pH meter (Edge HI 2002-02). Temperature control during thermostating was carried out with a built-in thermometer with feedback.

Moisture and ash content for fish raw materials, iodine and acid number for lipids were determined in accordance with GOST 7636-85 (Fish, marine mammals, invertebrates and products of their processing. Methods of analysis).

The analysis of the qualitative composition of the obtained fish oil samples was carried out on gas chromatography-mass spectrometer GCMC-TQ 8040 (Shimadzu) in the full ion current mode and in the scanning mode for individual ions (SIM). The temperature of the ion source is 200 °C, the interface is 250 °C, and the mass scanning is in the range m/z = 45-500. In the analysis of fatty acids, we used their precolumn derivatization with a KOH solution in methanol to obtain methyl esters of carboxylic acids. Separation of the resulting derivatives was carried out on an Rxi-5SiMs capillary chromatographic column (30m x 0.25 mm x 0.25 µm). The carrier gas is helium, the gas flow rate is 1.03 ml / min. Split / splitless mode was used (splitless 1 min, then split 10: 1). Injector temperature 220 °C. Temperature programming mode: the initial isothermal section is 50 °C for 1 min, then the column temperature rises to 250 °C (10 °C / min), the final isothermal section is 250 °C for 10 minutes. The total chromatography time was 35 min.

3. Results and Discussions

It is known that the wastes from cutting Atlantic herring, like the fish themselves, are characterized by a valuable chemical composition and a high fat content (Table 1), which indicates the expediency of using them as a raw material source for obtaining a biologically active substance (BAS) of a lipoid nature [10]. Collagen-containing waste from lightly salted herring is one of the most promising sources for obtaining fish oil and protein hydrolysates, since, due to the popularity of this type of product, the amount of this waste is large and they are not used in agriculture due to the presence of salt in them. At the same time, this disadvantage is an advantage in electrical processing technologies. Also, the promising use of these wastes is due to the partial hydrolysis of raw materials during the salting process, which facilitates the extraction of nutrients from the protein matrix [11]. If in the oil isolated from the carcass of fresh fish, amine nitrogen is almost completely absent, then in the oil isolated from the carcasses of salted fish, amine nitrogen is found in a rather significant amount. Its accumulation proceeds in proportion to the time, which suggests the formation of compounds from the breakdown products of proteins and oil and their dissolution in oil. Control over maturation (loss of raw taste and smell by fish, acquisition of the appropriate taste and aroma - "bouquet") can be carried out by observing the distribution of oil in the fish meat. In fresh and salted immature fish, oil is found either in the cells of muscle tissue or in the subcutaneous tissue in the form of isolated drops; in fish, in which the processes of protein breakdown and the structure of muscle tissue have changed, oil permeates its entire mass - a continuous film of oil is visible on the cut. This indicator is very characteristic of mature fish.

It was found that the oil content of fresh Atlantic herring is 11% higher than that of medium-salted Atlantic herring, and the moisture content is by 1.7% lower. The protein content in both types of herring is approximately the same (Table 2).

To quantitatively evaluate the skinning process and determine the mass yield of the waste part, the main industrial semi-finished product was used - Atlantic herring fillet doubled on the skin. For the experiment, 10 batches of fillets were taken, 10 pieces each (Table 3). Skinning was done by means of machine. After skinning, a visual assessment of the waste was made, as well as the weighing of the resulting amount of herring skin.

Table 1. Chemical composition of fresh and slightly salted Atlantic herring.
Table 2. Herring skin yield.

| Weighed portion          | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | Total |
|--------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| Fillet weight, g         | 2396| 2311| 2256| 2336| 2412| 2358| 2285| 2322| 2425| 2317| 23418 |
| Skin weight, g           | 193 | 220 | 205 | 227 | 214 | 186 | 183 | 196 | 256 | 228 | 21108 |
| Skin waste part, %       | 8.1 | 9.5 | 9.1 | 9.7 | 8.9 | 7.9 | 8.0 | 8.4 | 10.6| 9.8 | 9.0   |

The average mass yield of waste in the form of skin is 8 to 11 percent. After the machined skinning process, a large amount of meat and dorsal fin remains on the skin (Fig. 2).

Figure 2. Components of the waste part.

Table 3. Mass yield of waste part components.
The final output of the dispersed waste part is on average 6.5% of the mass of fillets taken for processing. These data can be used to calculate the productivity and equipment required for a herring skin processing line in the future.

In terms of physical and chemical parameters, the semi-finished product is close to the indicators of the original semi-finished product - herring fillet on the skin.

Salt content was 3.8-4.2%, acidity - 0.17-0.23% for vinegar essence (70%), pH = 5.8-6.1%.

| Weighed portion | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | Total |
|-----------------|----|----|----|----|----|----|----|----|----|----|-------|
| Waste part initial weight, g | 193 | 220 | 205 | 227 | 214 | 186 | 183 | 196 | 256 | 228 | 2108 |
| Skin weight, g | 60.3 | 65.4 | 73 | 75 | 68 | 66 | 59 | 53 | 86 | 80 | 685.7 |
| Skin yield, g | 31.2 | 29.7 | 35.6 | 33 | 31.8 | 35.5 | 32.2 | 27 | 33.6 | 35.1 | 32.5 |
| Mass of meat, g | 82.2 | 96 | 88 | 93 | 86 | 72 | 70 | 83 | 96 | 93 | 859.2 |
| Meat yield, g | 42.6 | 43.6 | 42.9 | 41 | 40.2 | 38.7 | 38.3 | 42.3 | 37.5 | 40.8 | 40.8 |
| Mass of fin, g | 22 | 27 | 30 | 27 | 22 | 25 | 20 | 27 | 22 | 20 | 242 |
| Fin yield, g | 11.4 | 12.3 | 14.6 | 11.9 | 10.3 | 13.4 | 10.9 | 11.2 | 10.5 | 8.8 | 11.5 |
| Total weight after separation, g | 164.5 | 188.4 | 191 | 195 | 176 | 163 | 149 | 158 | 209 | 193 | 1786.9 |
| Total yield, g | 85.2 | 85.6 | 93.2 | 85.9 | 82.2 | 87.6 | 81.4 | 80.6 | 81.6 | 84.6 | 84.8 |
| Weight loss for separation, % | 14.8 | 14.4 | 6.8 | 14.1 | 17.8 | 12.4 | 18.6 | 19.4 | 18.4 | 15.4 | 15.1 |
| Mass of dispersed skin, g | 143.7 | 156.3 | 162 | 155 | 148 | 152 | 157.3 | 140 | 143 | 156 | 1513.3 |
| Dispersed semi-finished product yield from herring fillet, % | 6 | 6.8 | 7.2 | 6.6 | 6.1 | 6.4 | 6.9 | 6 | 5.9 | 6.7 | 6.5 |
The complex processing of by-products from the production of herring fillet preserves was carried out electrochemically, followed by the release of lipids from protein solutions. For the complete dissolution of protein, the optimal parameters of the raw material processing were selected: voltage, current, hydronic modulus, time and temperature of suspension heating in stirred reactor.

To obtain a protein-lipid emulsion from collagen-containing wastes remaining in the production of preserves from slightly salted herring, with the subsequent separation of oil from it, rich in saturated omega-3-fatty acids, vitamins and phospholipids, the raw material was hydrolyzed in stirred reactor in catholyte medium, obtained by electrochemical method and possessing alkaline and reducing properties. In the process of electrochemical processing, proteins, polypeptides, lipids went into solution in the form of an emulsion, and the mineral precipitate. Due to the reducing properties of catholyte, gentle conditions and mild processing modes, 95-97% of high-quality refined lipids passed from the raw material into the solution.

The technological scheme for obtaining fish oil and protein hydrolyzate from the skin of slightly salted herring is shown in Figure 3.

![Figure 3. Technological scheme for obtaining fish oil and protein hydrolyzate from the skin of slightly salted herring.](image-url)
Fermented herring skin was dispersed to a particle size of $5 \times 10^{-3}$ m, mixed with catholyte in stirred reactor. The catholyte was a weak saline solution obtained in the cathode chamber of a diaphragm electrolyzer with a pH of not less than 12.2, Eh less than 860 mV in a 1:3 ratio. The mixture was thermostated in stirred reactor for 40 minutes at a temperature of 85 ± 5 °C. After keeping in the reactor, the mixture was centrifuged at 4000 rpm for 15 minutes to remove oil.

At the same time, complete dissolution of the skin, including the fins, was achieved; this effect was obtained under gentle conditions without processing the raw material in an electric field inside the electrolyzer space and a significantly lower hydromodule 1:3. This effect was achieved due to the partial hydrolysis of tissues during salting.

The mass yield of oil from the skin of slightly salted herring was significant - 22-25%, which is close to 90% of the theoretical. The physicochemical and biochemical compositions of the obtained products were investigated: fish oil and collagen hydrolyzate.

Fatty acid composition of salted herring skin oil is presented in Table 4.

| Acid name                        | Content, mg / g | Content, % |
|----------------------------------|-----------------|------------|
| Lauric C₁₂H₂₄O₂                   | 0.7             | 0.07       |
| 9-tetradecene C₁₄H₂₈O             | 0.6             | 0.06       |
| Myristic C₁₄H₂₈O₂                  | 52              | 5.2        |
| Pentadecane C₁₅H₃₂                 | 3               | 0.3        |
| 9-hexadecene C₁₆H₃₆O             | 41              | 4.1        |
| Hexadecanoic (palmitic) C₁₆H₃₂O₂  | 85              | 8.5        |
| Linoleic C₁₈H₃₂O₂                  | 31              | 3.1        |
| Linolenic C₁₈H₃₆O₂                 | 4               | 4          |
| 9-octadecene (oleic) C₁₀H₁₈O       | 53              | 5.3        |
| Stearic C₁₈H₃₆O₂                  | 5               | 0.5        |
| Cis-5,8,11,14,17-Eicosahexaenoic C₂₀H₃₂O₂ | 43       | 4.3        |
| Cis-4,7,10,13,16,19-Docosahexaenoic C₂₂H₄₀O₂ | 33       | 3.3        |
| Docosanoic C₂₂H₄₄O₂                 | 12              | 1.2        |

It was revealed that fish oil, obtained electrochemically from wastes of fermented salted herring (Table 4), contains more than 20% omega-3 polyunsaturated fatty acids of the total fatty acids, but this amount is not enough to meet the daily human need (according to methodological recommendations (MP 2.3.1.2432–08)). Thus, the development of a technology for the concentration of omega-3 polyunsaturated fatty acids is urgent.

Oil from secondary fish raw materials was filtered until a transparent viscous mass without inclusions was obtained and stored at +4 °C. To concentrate the oil containing omega-3 acids, the object of study was cooled. The oil was placed in glass tubes. The process of cooling oil in test tubes was carried out on the installation, which is the container with a solution of calcium chloride, cooled by a low-temperature refrigeration unit. The average cooling and freezing rate is 0.3 °C / s. The temperature inside the sample and in the cooling medium was recorded with thermocouples.

It was found that phase transitions in oil occur intensively at temperatures: -6 °C, -14 °C, -37 °C. Phase transitions are accompanied by the deposition of lipid fractions less saturated with double bonds
After centrifugation, the supernatant lipid fraction enriched in unsaturated fatty acids was additionally cooled.

![Figure 4](image)

**Figure 4.** Dependence of temperature in fish oil on the time of its cooling and freezing.

### 4. Conclusion

As a result of the work, the technology has been developed for obtaining fish oil from fish skin - waste from the production of preserves from fish of the Herring family with a high yield using electrochemically obtained catholytes at a low concentration of hydroxyl ions. The oil was characterized by satisfactory quality characteristics due to the presence of reducing properties in the extractant.

The expediency of using wastes from the production of lightly salted herring preserves - skin as a source of collagen, fish oil, and unsaturated fatty acids - was also presented.

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