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# High-temperature hydrolysis as a method for complex processing of sprat (Sprattus sprattus balticus) by-products

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Abstract

The aim of the study was to justify the com-plex processing of smoked sprat heads using the method of high temperature thermal hydrolysis. The new technology was tested with fatty (24%) and medium fatty (13%) raw materials using high temperatures in the range of 130-160 °C. As a result, the hydrolyzed organic mass was separated into fat, protein, mineralprotein and protein-fat emulsion fractions. Freeze-drying of the protein fraction and convective drying of the protein-mineral fraction resulted in food additives containing, respectively, low molecular weight water-soluble peptides and high molecular weight insoluble proteins and minerals. A rational method of hydrolysis is substantiated, including preliminary separation of fat in fatty raw materials and its enzymatic-thermal treatment. The optimal values of temperature and duration of hydrolysis in reactor are substantiated. Under these conditions, proteins are extracted most deeply from sprat raw materials, and the content of low molecular weight peptides in the protein freeze-dried hydrolysate powder is more than 80%. A comprehensive technology has been developed for the production of protein, fat and protein-mineral food additives from sprat processing by-products.

Keywords: Smoked sprat heads ; Thermal hydrolysis ; Enzymatic-thermal hydrolysis ; Protein supplement

## 1. Introduction

Fish and its processed products are an important supplier of food components necessary for human beings. Significant amounts of fish and fish by-products are promising raw materials for protein, food additives and products. Due to the chemical composition, rheological, technological and food properties, fish processing byproducts are widely used in the production of various valuable products and food additives [1-3]. Important field of the modern biotechnology is the production of food and biologically active peptides from various sources. Functional peptides and hydrolysis products have found industrial and commercial applications in the production of pharmaceuticals and nutraceuticals. The properties of peptides and their effects on the immune, cardiovascular, nervous and gastrointestinal systems is a fast-developing research field now [4].

Fermentation is an important way to process and preserve fish. This process gives the product unique taste and texture, and also helps to increase nutritional value and improve functional properties [5]. Further research on fermented fish products and by-products is crucial not only for the food industry, but also for human health. [6-9]. Thanks to the development of enzymatic technologies for protein recovery and modification, it is possible to produce a wide range of food ingredients and industrial products [10, 11]. There are some research that identify the conditions of fermentation on the qualitative and quantitative composition of enzymatic hydrolysis products with the participation of endo- and exoenzymes [12].

Enzymatic peptides from fish products can be valuable immunostimulants. In vitro and in vivo studies have shown that certain peptide fractions in fish protein hydrolysates can stimulate a non-specific immune system [13].

There are 11 fish processing plants producing canned sprats in oil from Baltic sprats and herring in the Kaliningrad region. These plants generate from 2 to 8 tons per day of accumulated smoked fish heads that are not processed or sold. It is impossible to produce feed products from them, therefore, these by-products represent a serious eco-nomic and environmental problem for canning and smoking industries. Currently, smoked fish by-products are disposed as solid household waste. This causes significant eco-nomic and environmental losses. During aqueous extraction difficultly soluble high molecular weight collagen proteins must be converted into low molecular weight peptides, which is possible by exposure to high temperatures in the aquatic environment. In this case, fat cells are destructed, fat is released and localized in the upper part of the system. Mineral substances form a sedimentary fraction together with the unsplit proteins. The subsequent separation of the hydrolyzed system into fractions formed during thermal hydrolysis allows to generate valuable food compositions [14].

The aim of the work was to substantiate the complex processing of smoked sprat heads by high-temperature hydrolysis to produce food additives of protein, lipid, mineralized and mixed organic composition. For this, it was necessary to select the most rational method of hydrolysis and justify its specific modes for maximum extraction, primarily of proteins.

## 2. Materials and methods

Experiments were carried out at the Center for Advanced Protein Utilization Technologies of the Kaliningrad State Technical University. The process of high temperature hydrolysis was realized in an autoclave at adjustable temperatures from 130 to 160 °C. In total, 23 experiments were realized with fish processing by-products (sprattus sprattus balticus) from «RosKon» and «Za Rodinu» canning factories. The raw materials differed in catch seasons (autumn-winter and spring sprats) and chemical composition (fat content from 13 to 24%). The optimal parameters of temperature and duration of hydrolysis were substantiated, as well as the rationality of preliminary defatting before hydrolysis and fermentation. For this, the degree of protein extraction from raw materials and the purity of the obtained compositions were analyzed. During the experiments, the heads of smoked sprats in the amount of 1,5 kg after grinding were mixed with warm water (70-80 °C) in a ratio of 1:1. When the fat content in the mixture was more than 20% in the obtained fish suspension, the released fat was removed after centrifuga-tion at a speed of 3900 rpm. The remaining partially defatted sprat pulp was loaded into the reactor. This equipment is a 5 litre autoclave with a stirrer equipped with an automatic heating/cooling system. The product processing temperature was from 130 to 160 °C with pressure levels from 1,5 to 6,0 bar and total duration 60 to 170 minutes. In some experiments, the raw materials after defatting were treated with proteolytic enzymes (Alcalase and/or Protosubtilin G3X, 0.25-0.5% related to the weight of the system («raw material + water»). After fermentation, the mixture was processed in the reactor and thermal hydrolysis was carried out.

The mixture discharged from the reactor after cooling up to 60 °C was centri-fuged at 3900 rpm and separated by decantation into fat (upper), protein (middle) and mineralized (sedimentary) fractions. In some experiments emulsion layer formed, which was separated from the fat fraction and examined. The protein fraction was concentrat-ed in UL - 2000E rotary evaporator to a dry matter content of 15% and sublimated in Martin Christ Alpha1-2 LDplus laboratory freeze-dryer at condenser temperature of -55 °C. The sedimentary mass was dried by convection in laboratory oven at 60 °C. The choice of a freeze-dried method of dehydration of protein hydrolysates is due to the need to preserve the nature of the formed water-soluble products of protein hydrolysis (peptides, amino acids), which determine their high biological value. The sediment fraction containing high molecular weight insoluble proteins and minerals was dried in a traditional (convection) way.

Thus, four types of natural organic compositions were produced from the heads of smoked sprats using the hightemperature hydrolysis method. The compositions were used as food additives in various food products. The purity of the obtained fractions and the completeness of the extraction of organic substances were estimated by the chemical composition of the fractions using operational material balances for protein, fat and minerals. The determination of the mass fractions of water, proteins, fat, minerals was carried out according to the Russian analytical standard GOST 7636. Statistical processing of experimental data was carried out using standard techniques at a 95% confidence level. The tables show the average data from three repeated measurements.

## 3. Results and discussion

The main research results are presented by the indicators of mass yields of organic fractions (protein, fat, protein-mineral) from sprat raw materials, as well as data on their chemical composition. It was found that during the hydrolysis of sprat raw materials of medium fat content (13,3 – 18,8%), the amount of aqueous extract with dissolved organic substances ranges from 42,6 to 60,6% of the mass of raw materials and water (76,3-124,6% of the mass raw materials). The amount of this extract increases with increasing hydrolysis temperature from 130 °C to 160 °C. When processing raw materials with higher fat content (20,2 – 24,5%), the amount of the extract decreases to 36,4-43,5% of the total mass of raw materials and water. At the same time, a stable emulsion (protein-water-water-fat mixture) appears in the hydrolyzed mass, which practically does not separate into fat and protein hydrolysate. The mass of the wet protein-mineral sediment formed after filtration of the hydrolyzed system varies from 64,0% to 94,8% of the raw material weight. Moreover, upon hydrolysis of more fatty raw materials, the sludge mass turns out to be greater (84,2-94,8% of the raw material weight) than upon hydrolysis of raw materials with lower fat content (64,0-73,5%). In the experiments with preliminary separation of fat with minced raw materials, the mass of the precipitated fraction decreases to 60,764,0% of the raw material weight, regardless of its fat content. In the case of preliminary fermentation of the raw material with proteases, the mass of the precipitate after thermal hydrolysis falls to 30,3%. This feature is important during processing of raw materials with high fat content, since fat prevents the deep fractionation of raw materials.

The data on the yields of the dried hydrolysis products obtained from sprat raw materials for different hydrolysis methods is presented in Table 1. In search of rational methods of hydrolysis, the maximum yield of the protein fraction, its organoleptic characteristics, and the minimum amount of emulsion in the hydrolyzed system were taken into account. It was found that a stable emulsion is formed during the processing of fat-ty raw materials (fat content of more than 20%) in experiments without preliminary separation of fat (marked as Table 1, experiments 5, 6, 11, 12, 15).

Table 1: Mass yields of various fractions of organic substances in dried state processed in different methods of hydrolysis of sprat raw materials

| No. | Hydrolysis type | Hydrolysis parameters: Temperature (°C) / duration (min) (ratio of "raw materials + water" 1:1) | Protein freeze-dried water-soluble fraction: Yield, % in relation to wet fraction weight | Protein freeze-dried water-soluble fraction: Yield, % in relation to raw materials weight | Protein-water-fat emulsion: Yield, % of raw materials | Protein-mineral sediment: Yield, % in relation to wet fraction weight | Protein-mineral sediment: Yield, % in relation to raw materials weight |
|---|---|---|---|---|---|---|---|
| 1 | Thermal hydrolysis, without preliminary defatting | 130 / 85 | 7,6 | 6,91 | 0,12 | 31,2 | 22,0 |
| 2 | | 135 / 75 | 5,0 | 4,09 | 0,17 | 30,6 | 24,8 |
| 3 | | 130 / 85 | 5,3 | 5,11 | 0,21 | 29,4 | 23,7 |
| 4 | | 160 / 170 | 8,9 | 10,0 | 1,87 | * | * |
| 5 | | 130 / 80 | 5,4 | 4,7 | 3,16 | 30,7 | 26,4 |
| 6 | | 130 / 80 | 5,5 | 4,1 | 2,95 | 28,3 | 26,8 |
| 7 | | 130 / 75 | 5,4 | 4,4 | 0,35 | 28,6 | 27,2 |
| 8 | | 130 / 75 | 5,3 | 4,9 | 0,19 | 29,9 | 20,3 |
| 9 | | 130 / 75 | 5,0 | 5,5 | 0,13 | 31,3 | 20,9 |
| 10 | | 130 / 80 | 5,0 | 5,7 | 0,42 | 32,3 | 20,7 |
| 11 | | 130 / 75 | 7,8 | 6,3 | 5,68 | 39,1 | 35,1 |
| 12 | | 130 / 75 | 7,5 | 5,4 | 4,12 | 35,9 | 34,4 |
| 13 | | 130 / 85 | 7,9 | 6,7 | 2,46 | 34,9 | 33,1 |
| 14 | | 130 / 75 | 8,6 | 6,6 | 0,69 | 38,4 | 34,0 |
| 15 | | 130 / 90 | 5,1 | 4,1 | 2,32 | 40,2 | 32,5 |
| 16 | | 130 / 85 | 7,0 | 5,3 | 0,96 | 32,8 | 31,1 |
| 17 | Thermal hydrolysis, with preliminary defatting | 130 / 70 | 5,6 | 4,7 | 1,31 | 31,3 | 24,6 |
| 18 | | 130 / 75 | 5,4 | 4,6 | 0,22 | 33,0 | 25,5 |
| 19 | | 130 / 80 | 5,2 | 5,3 | 0,27 | 31,3 | 21,8 |
| 20 | | 130 / 75 | 6,7 | 6,9 | 0,37 | 32,9 | 22,6 |
| 21 | | 130 / 90 | 7,3 | 6,4 | 0,16 | 37,3 | 29,0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 22 | Preliminary defatting, enzymatic hydrolysis (protosubtilin, 0,5%), Thermal hydrolysis | 130 / 75 | 8,4 | 9,7 | 0,48 | 36,0 | 20,7 |
| 23 | Preliminary defatting, enzymatic hydrolysis (protosubtilin, 0,25%), Thermal hydrolysis | 130 / 60 | 8,5 | 9,8 | 1,12 | 30,7 | 22,8 |

* Samples were under food quality

Table 1 shows that the yield of freeze-dried protein hydrolysates ranges from 5,5 to 9,8% by weight of the processed smoked sprat raw materials. The highest yield of 10,0% was obtained with thermal hydrolysis with temperature of 160 °C for 170 minutes. However, at the same time, the sedimentary fraction turned out to be unacceptable in terms of organoleptic quality indicators (dark burnt mass with specific ammonia odor); therefore, this regime was declared unacceptable.

A very important factor of the hydrolysis process is the degree of fat extraction from sprat raw materials, which ranges from 4,2% (at 130 °C) to 14,5% (at 160 °C) in relation to the raw materials weight. Longer thermal hydrolysis duration deteriorates the fat quality which is partially hydrolyzed and oxidized. Therefore, fat should be extracted in hot water from minced raw materials with subsequent centrifugation for preserving its quality.

The chemical composition of the produced protein products is given in Table 2. Thus, the protein content in freeze-dried protein hydrolysates ranges from 59,2 to 88,4%, while in the undissolved state in the dried proteinmineral sediments protein content ranges from 50,2 to 65,8% of the product weight. Findings on the rational parameters for protein extraction using high-temperature hydrolysis of sprat by-products can be made from the material balances of the main sub-stances in operations. Figure 1 and Table 2 illustrate the main operations of the technology and the quantitative results of hydrolysis experiments. The results show that using high-temperature thermal hydrolysis at 130 °C allows to isolate in the form of a freeze-dried hydrolysate from 11,3 to 43,8% of the protein contained in the raw material. With temperature increase to 160 °C up to 46,3% of the protein contained in the raw materials is extracted to the hydrolysate, which is significantly higher than average values, but its quality deteriorates significantly (color, taste, odor etc.). The amount of protein remaining in dry protein-mineral sediments is significant from 50,2 to 65,8%.

Table 2: Chemical composition of freeze-dried protein hydrolysates and protein-mineral sediments from sprat by-products from various hydrolysis tests, % of the mass

| Nr. | Freeze-dried protein hydrolysates | | | | | Dried protein-mineral sediments | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water | Dry matter | Fat | Ash | Protein | Water | Dry matter | Fat | Ash | Protein |
| 1 | 2,9 | 97,1 | 30,3 | 6,7 | 60,1 | 3,6 | 96,4 | 20,2 | 19,1 | 57,2 |
| 2 | 4,1 | 96,0 | 4,0 | 12,0 | 80,0 | 3,6 | 98,4 | 17,3 | 19,7 | 59,5 |
| 3 | 6,2 | 93,8 | 8,1 | 11,5 | 74,2 | 3,4 | 96,6 | 20,2 | 17,5 | 59,0 |
| 4 | 5,7 | 94,3 | 1,0 | 5,0 | 88,4 | 4,6 | 95,5 | 15,8 | 21,7 | 57,9 |
| 5 | 6,4 | 93,6 | 4,9 | 9,8 | 78,9 | 4,9 | 95,1 | 10,3 | 19,0 | 65,8 |
| 6 | 6,8 | 93,9 | 4,4 | 8,3 | 80,3 | 4,4 | 95,5 | 16,7 | 18,7 | 60,2 |
| 7 | 5,1 | 94,9 | 3,2 | 9,8 | 81,9 | 5,4 | 94,5 | 16,8 | 19,2 | 58,5 |
| 8 | 6,9 | 93,1 | 2,5 | 12,7 | 77,8 | 4,0 | 96,0 | 20,6 | 15,6 | 59,8 |
| 9 | 8,8 | 91,2 | 1,4 | 13,9 | 75,9 | 4,3 | 95,7 | 18,1 | 18,5 | 59,1 |
| 10 | 6,6 | 93,4 | 3,2 | 14,7 | 76,0 | 4,4 | 95,7 | 17,7 | 20,0 | 57,9 |
| 11 | 9,0 | 91,0 | 7,0 | 13,5 | 70,6 | 4,0 | 96,1 | 24,4 | 18,3 | 51,3 |
| 12 | 7,6 | 92,4 | 0,9 | 14,4 | 77,1 | 4,8 | 95,2 | 22,0 | 19,0 | 54,2 |
| 13 | 7,9 | 92,1 | 1,4 | 13,4 | 77,3 | 4,0 | 96,0 | 20,9 | 20,0 | 55,1 |
| 14 | 7,5 | 90,5 | 16,2 | 13,8 | 62,5 | 20,2 | 79,8 | 17,0 | 12,7 | 50,2 |
| 15 | 9,0 | 91,1 | 21,4 | 10,5 | 59,2 | 3,0 | 97,1 | 23,8 | 17,1 | 56,1 |
| 16 | 14,4 | 85,6 | 3,9 | 11,5 | 70,2 | 6,8 | 93,2 | 18,0 | 18,1 | 57,2 |
| 17 | 7,2 | 92,8 | 2,4 | 9,3 | 81,0 | 2,2 | 97,8 | 16,0 | 19,7 | 62,0 |
| 18 | 5,1 | 94,9 | 5,8 | 9,0 | 84,4 | 4,4 | 95,6 | 16,2 | 18,9 | 60,5 |
| 19 | 7,5 | 92,5 | 2,4 | 14,0 | 76,1 | 4,6 | 95,4 | 17,1 | 17,8 | 60,6 |
| 20 | 6,6 | 93,4 | 3,4 | 9,8 | 80,2 | 6,7 | 93,3 | 16,4 | 17,7 | 59,3 |
| 21 | 6,3 | 93,7 | 8,3 | 13,3 | 72,1 | 5,6 | 94,4 | 14,3 | 21,2 | 59,0 |
| 22 | 6,7 | 93,4 | 2,4 | 7,8 | 83,2 | 3,8 | 96,4 | 19,9 | 23,5 | 53,0 |
| 23 | 6,7 | 93,3 | 2,0 | 8,6 | 82,7 | 6,9 | 93,2 | 14,7 | 24,0 | 54,5 |

This indicates the complexity of the hydrolysis of these proteins, consisting mainly of collagen proteins, and relatively low degree of its transition to a soluble state under the influence of high temperatures. Experiments show that with sprat by-products preliminary defatting does not significantly affect the degree of protein extraction from raw materials.

Table 3 shows that from 100 kg of sprat by-products 10,33 kg of protein hydrolysate powder, 8,69 kg of fat, 1,73 kg of protein-fat emulsion and 17,02 kg of proteinmineral dry supplement can be produced.

Technological scheme of the integrated processing of smoked sprat heads according to the most rational hydrolysis regime is shown in Figure 1.

The final products of hydrolysis (protein, proteinmineral and fat) are characterized by valuable chemical composition (Table 2). They are recommended for use as food additives. They have pleasant organoleptic properties. The additives are safe in the content of harmful substances and meet the safety requirements for food additives (Technical Regulation of the Eurasian Customs Union TR TS 021/2011). Analysis of the content of benzo(a)pyrene showed its presence in all additives below the permissible level (0.001 mg/kg). It is rational to introduce these additives into the composition of some food products as sources of low molecular weight peptides and collagen proteins, fat and minerals. The protein supplement was positively tested in protein bars, fat in mayonnaise and oil filling in natural canned mackerel. Protein-mineral supplement was used as a part of canned fish paste. Original crackers and biscuits were produced using liquid protein-fat emulsion. The results of the experiments indicate the economic profitability of the developed method for the processing of sprat by-products due to the low cost of raw materials, relative simplicity and complexity of processing.

Table 3: Yield of semi-finished products, the final hydrolysis products and their chemical composition during the enzymatic thermal hydrolysis of sprat waste

| Raw materials and hydrolysis products | Yield of products, % raw material | Chemical composition, % Water | Dry matter | Protein | Ash | Fat |
|---|---|---|---|---|---|---|
| Hydrolysis mixture: Smoked sprat heads + water, relation 1 : 1 | 100 | 81,8 | 18,2 | 9,0 | 2,6 | 6,6 |
| Primary products of hydrolysis of sprat raw materials: Wet protein-mineral sediment | 47,67 | 64,4 | 35,6 | 20,1 | 9,2 | 6,3 |
| Liquid protein hydrolysate | 118,67 | 92,0 | 8,0 | 7,4 | 0,6 | 0 |
| Fat | 8,69 | 0 | 100,0 | 0 | 0 | 100,0 |
| Protein-fat emulsion | 1,73 | 51,4 | 48,6 | 6,8 | 0,8 | 41,0 |
| Final products of the hydrolysis of sprat raw materials: Freeze-dried protein hydrolysates | 10,33 | 6,7 | 93,3 | 82,7 | 8,6 | 2,0 |
| Dried protein-mineral sediments | 17,02 | 3,4 | 96,6 | 54,5 | 24,0 | 18,1 |
| Protein-fat emulsion | 1,73 | 75,9 | 24,1 | 10,0 | 0 | 14,1 |
| Fat | 8,69 | 0 | 100,0 | 0 | 0 | 100,0 |

Mode: preliminary enzymatic hydrolysis, Alcalase 2.5L 0,25%, 2 hours at 60°C, preliminary defat-ting; thermal hydrolysis 60 min, T=130 ° C

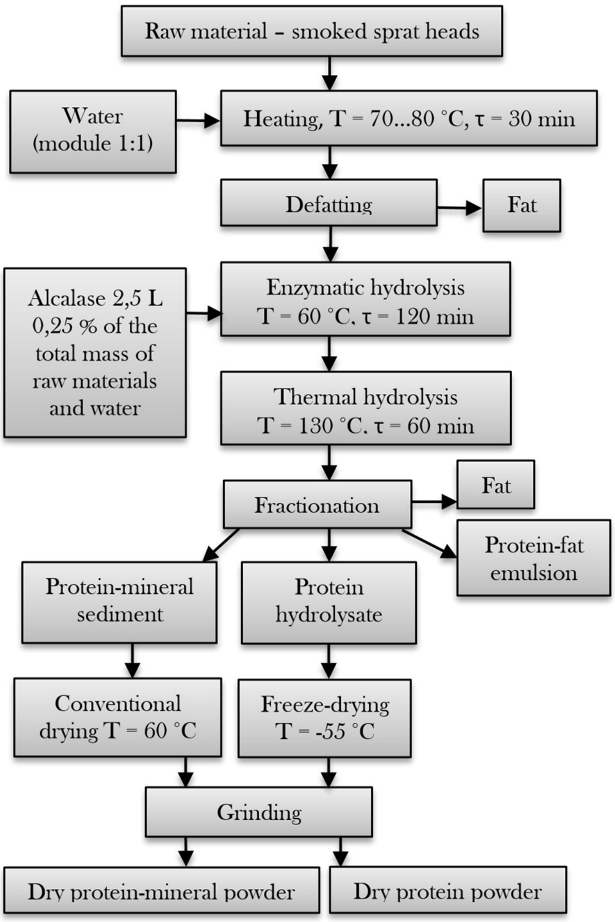


Figure 1. Technological scheme of the integrated processing of sprat by-products according to the rational regime of hydrolysis

## 4. Conclusion

High-temperature hydrolysis is a rational method for the complex processing of sprat by-products. Valuable nutritional supplements of protein, protein-mineral and fat composition can be produced using this method. Rational method of hydrolysis is substantiated, including preliminary defatting of raw materials, treatment with Alcalase enzyme and thermal hydrolysis in reactor at temperature of 130 ºC for 60 minutes. This method allows the maximum fractionation of raw materials into organic components, to obtain maximum performance for the extraction of water-soluble protein and fat yield. With optimal parameters the yield of sublimated protein hydrolysate is 8,7% of the mass of raw materials with protein content of 82,7%. The degree of protein extraction into the hydrolysate is 48,0% of the initial protein content in raw material. The yield of the protein-mineral fraction is 17,02 % with protein content of 54,5% in the dried additive and 24,0% of mineral substances. Moreover, the obtained protein, fatty, protein-mineral and protein-fatty emulsion compositions meet the requirements of food additives. They have pleasant organoleptic characteristics and are safe. The hydrolysis products have been tested positively as food additives in various products. The established rational parameters for the processing of sprat by-products make it possible to recommend them to fish canning and smoking plants to obtain valuable food additives and generate additional profit.

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