Effect of Nucleic Acids on the Quality Indicators of Functional Fish Products

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Abstract. There have been proposed rational technology of combined fish-grain and fish-vegetable masses for functional nutrition. As the objects of research were selected minced raw fish fillet, minced boiled fish fillet and industrial frozen minced fillet from horse mackerel (Trachurus Trachurus L.). The studies have been carried out in accordance with the experimental design, in which the variable factors were the heat treatment time (10–15 minutes) and the temperature of treatment (82–98 °C). It was found that the organoleptic characteristics of boiled mince from raw fillet of horse mackerel are significantly inferior to the organoleptic characteristics of minced meat from boiled fillet of horse mackerel. When comparing the data for ice cream and freshly prepared minced horse mackerel, it was found that the loss of nucleic acids in industrial minced meat is 4.6–13.1% higher (T = 82 ± 2 and 98 ± 2 °C), the loss of dry matter is higher by 11.3–5.8%, mass - by 8.4–14.6%. The content of the total amount of nucleic acids in industrial minced meat is 11.1% lower than in freshly prepared minced meat. Obviously, in the process of preparing products for dietary nutrition, it is preferable to use minced meat from boiled fish fillets.

1. Introduction

In recent years, new functional food ingredients have been substantiated and characterized by nutrition researchers in many countries [1]. One such class of functional substances are nucleic acids (NA), which are used in many reactions having critical to the survival of individuals [2, 3, 4]. The modern concept of healthy eating sees food safety taking into account the pharmacological and toxicological effects of physiologically active food ingredients on the human body [5]. Improving of research methods allows rating the nutritional profile and develop personalized food recommendations for an individual. For an adequate assessment of the individual nutritional profile, standardized indicators are used, including the protein content in the daily diet [6].

The body's demand for proteins depends on age, sex, physiological state, climatic conditions, intensity of physical work performed, etc. [6]. Both lack and excess of protein in food is negative to the man body. Moreover, the consequences of over use of protein intake can be more expressed than with down intake of fats and carbohydrates. An excess of animal proteins is accompanied by an increased intake of nucleic acids into the man body.
According modern scientific concepts nucleic acids are the high molecular weight compounds with a proven physiological effect of the specific synthesis of biopolymers in the body [7]. In addition, they promote the exchange of purines – uric acid. Uric acid salts are deposited in joints and other tissues, contributing to the development of gout. In case of impaired purine metabolism, there are offered diets with the exclusion or restriction of foods rich in purines, including: meat, meat byproducts, legumes, mushrooms, fish, etc. And on the contrary, they include a lot of products from vegetables and fruits [8].

Nowadays, one of many promising directions of functional products is the improvement of the technology of combined minced products from low-value pelagic fish species, including horse mackerel.

In dietary nutrition, along with the correction of the food set, it is recommended to apply additional technological processing of high-protein raw materials in order to reduce purine bases. So, when boiling meat, up to 50% of total content of purines goes into broth, therefore, in a low-protein diet, boiled meat is used and broths are excluded [8, 9]. In studies on the use of fish as a food product, there is lack of information on special technological methods that allow the use of most pelagic fish in dietary nutrition.

1.1. Aim and tasks of the work

To solve the questions posed, it is necessary to study the influence of culinary and technological methods on the formation of indicators of the quality of fish products.

The aim of the work was to study the effect of nucleic acids preceding the formation of purine bases on the formation of quality and safety indicators for fish products for specialized purposes for functional nutrition.

To achieve this aim, the following tasks were solved:
- investigation of the influence of culinary processing techniques on the change in the chemical composition, structural and mechanical properties of low-value fish varieties, the taste of finished products from them;
- development of formulations and technology of fish products with a low content of nucleic acids.

1.2. Theoretical substantiation

Extractives nitrogenous substances, which include nucleic acids and their derivatives, purine bases, have a local and general irritant effect: by stimulating the stomach glands and the digestive function of the pancreas, they promote better absorption of food, primarily proteins and fats. At the same time, these substances directly or indirectly stimulate the nervous system, which, as a rule, adversely affects the course of many diseases of the circulatory system, nervous system, gastrointestinal tract and kidneys. In addition, purine bases are directly related to metabolic processes, the violation of which is manifested by a delay in the body of uric acid and the deposition of its salts in tissues. In particular, gout is almost always the result of a violation of the metabolism of purine substances. Therefore, all strict diets are characterized by a low content, and in some cases by the absence of soups and sauces based on meat, fish and mushrooms broths, as well as fried and stewed meat and fish dishes [8, 9, 10].

The number of extractives nitrogenous substances from the muscle tissue of meat and fish during cooking depends not only on their content, but also on a number of technological factors — the cooking temperature, the ratio of the product to water, and the degree of product grinding. Abramova J. I. et al. noted that the loss of nucleic acids during heat treatment of hake, cod, pollock had was 36–44 %, and the loss of the acid-soluble fraction, consisting of intermediate products of synthesis and decomposition of nucleic acids, 67–69 % [11].

Data on the influence of technological factors: temperature, the ratio of the product and water, the degree of grinding of the product on the loss of nucleic acids during the heat treatment of pelagic fish were not found in the literature.
2. Objects and methods of researching

In accordance with the aim as the objects of research were selected: the frozen horse mackerel – GOST 32366–2013 Frozen fish. Technical conditions; the frozen horse mackerel fillets – GOST 3948–2016 Frozen fish fillets. Technical conditions; the frozen food mince made from horse mackerel – GOST R 55505–2013 Frozen food minced fish. Technical conditions; semi-finished products and finished culinary products prepared on the basis of developed recipes. As additional raw materials, there were used cereals, vegetables, etc.

Preparation of the samples for laboratory research was carried out in accordance with GOST R 54607.1–2011 Experimental and control samples were prepared from one batch of raw materials.

Determination of moisture content and dry matter content was carried out by drying the samples to constant weight at 105 °C in accordance with GOST R 54607.2–2012.

The total content of nucleic acids (NA) was determined by the method described by Spirin A. S. [12]. The modification of the spectrometric method is based on the extraction of nucleic acids from biological material with hot perchloric acid, followed by the determination of the absorption of extracts in the ultraviolet region of the spectrum at wavelengths λ=270 nm and λ=290 nm, the thickness of the working layer of the cuvette is 10 mm on an SF-56 spectrophotometer.

Determination of the fractional composition of nucleic acids, as well as the acid-soluble fraction was carried out by the spectrophotometric method Tsaney-Markov modified by Berdyshev–Galkin on an SF-56 spectrophotometer, at wavelengths λ=270 nm and λ=290 nm, the thickness of the working layer of the cell is 10 mm.

The amount of acid-soluble fraction (mg%) was calculated using the formula:

\[ K = \frac{(E_{270} - E_{290}) \cdot V}{0.194 \cdot W} \cdot 10.3 \]  

\[ RNA = \frac{(E_{270} - E_{290}) \cdot V}{0.193 \cdot W} \cdot 10.5 \]  

\[ DNA = \frac{(E_{270} - E_{290}) \cdot V}{0.186 \cdot W} \cdot 10.1 \]  

where \( K \) is the content of the acid-soluble fraction of NA (mg%), \( E_{270} \) and \( E_{290} \) are optical density of the solution at wavelengths of 270 and 290 nm, \( V \) is the volume of the extract (ml), \( W \) is the weight (mg).

The determination of \( \alpha \)-amine nitrogen was carried out by the spectrophotometric method of Dieter and Kaiser with ninhydrin on an SF-56 spectrophotometer, at a wavelength of \( \lambda = 580 \) nm, a working layer thickness of the cuvette 10 mm.

The loss of nutrients during heat treatment was determined according to the generally accepted method of Skurikhin I. M. [13].

Organoleptic assessment was carried out on a five-point scale, taking into account the weighting factors [14, 15].

3. The results and discussion

In dietary nutrition, pre-boiling is used to reduce the content of nitrogenous extractive compounds in meat and fish products. While frying of fish soluble substances are extracted in smaller quantities, since the bulk of the moisture released by muscle proteins during denaturation evaporates, and the substances dissolved in it remain in the product. Adding in a small amount of liquid, stewing and steaming occupy an intermediate position in terms of the amount of extractives extracted from the product. The largest amount of soluble substances is extracted from the muscle tissue of fish during the boiling.

The important technological parameters of the cooking of fish minced products are: degree of grinding of filets, weight, size, thickness of pieces, the amount of added water, temperature and duration of heat treatment. In this regard, at the first stage of the study, the effect of preliminary
grinding on the technological and physicochemical indicators of the quality of the samples was studied. For this, two test samples were prepared: sample 1—firstly the fish filet was coarsely ground and after that it was boiled (particle size 0.2 cm); sample 2—pieces of filet without skin and bones (weight of pieces 18±2 g) boiled and subsequent ground (particle size 0.2 cm). Both samples were cooked in 0.5 % aqueous NaCl solution, hydromodule 4, temperature 98 °C, cooking time 15 min.

It was found that the minced fish prepared from sample 1 had a dense consistency, uneven color, low adhesive ability to form culinary products from it (soufflés, puddings, rolls and etc.). Minced fish prepared from sample 2 had a uniform consistency, uniform color, according to its technological properties, it turned out to be acceptable for molding culinary products. Physicochemical and organoleptic characteristics of two samples of minced horse mackerel are given in table 1.

### Table 1. Physicochemical and organoleptic characteristics of two samples of minced horse mackerel.

|                  | Moisture (%) | Loss of dry matter (%) | Mass loss (%) | Loss of α-amin nitrogen in broth (%) | Nucleic acids (%) | Loss of nucleic acids (%) | Organoleptic evaluation |
|------------------|--------------|------------------------|---------------|-------------------------------------|------------------|--------------------------|------------------------|
| Sample 1        | 69.1±0.2     | 25.0±0.6               | 33.9±0.5      | 39.1±1.2                            | 256.5±3.7        | 37.9±1.4                 | 3.5                    |
| Sample 2        | 68.8±0.2     | 20.2±0.5               | 27.7±0.6      | 34.9±1.1                            | 324.8±2.0        | 27.0±1.4                 | 4.1                    |

The data in table 1 show that during heat treatment in sample 2, compared to sample 1, the loss of dry substances was 6 % less, while the loss of nitrogenous extractive substances and nucleic acids (NA) was 4 and 10 % less, respectively.

To optimize of technological parameters for reducing of content of NA there were studied the next parameters: moisture content of the samples (various parts of the horse mackerel: skin and fillet – muscle tissue), loss of dry matter (Loss of DM), content of nucleic acids with acid-soluble fraction (NA with ASF), loss of nucleic acids with acid-soluble fraction (Loss of NA with ASF), content of acid-soluble fraction (ASF), loss of acid-soluble fraction (Loss of ASF), content of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The dynamics of the indicators is shown in table 2.

### Table 2. Effect of boiling on physical and chemical parameters in various parts of the horse mackerel.

|                  | Moisture (%) | Loss of DM (%) | NA with ASF (mg%) | Loss of NA with ASF (%) | ASF (%) | Loss of ASF (%) (mg%) | RNA DNA (mg%) |
|------------------|--------------|----------------|------------------|-------------------------|---------|------------------------|--------------|
| Skin raw         | 65.1±2.7     | 1808.3±15.0    | 1543.3±12.5      | 476.0±0.9               | 754.6±1.3  | 150.9±1.8               | 1250.9±2781.4±113.0 |
| Skin boiled      | 78.5±0.2     | 14.6          | 1543.3±12.5      | 754.6±1.3               | 701.9±11.086.8±0.2  | 142.9±1.3               | 155.9±11.1   |
| Filet raw        | 74.4±0.1     | 20.4          | 1543.3±12.5      | 754.6±1.3               | 142.9±1.3               | 155.9±11.1               | 155.9±11.1   |
| Filet without skin, boiled | 70.4±0.1 | 20.6          | 1543.3±12.5      | 754.6±1.3               | 142.9±1.3               | 155.9±11.1               | 155.9±11.1   |
| Filet with skin, boiled | 71.6±0.4 | 19.8          | 521.7±1.2        | 22.43                  | 145.4±2.238.5          | 159.7±1.7               | 216.6±2.0   |

It can be seen from table 2 that horse mackerel contains relatively high content of NA relative to fish of other families [13].

At the next stage of the study, there was studied the dynamics of α-amine nitrogen and the NA content in the horse mackerel samples under different cooking modes: 45–50 °C (collagen of fish muscle tissue is not resistant to hydrothermal effects, its denaturation begins at 40 °C); 75–80 °C (the content of water-soluble proteins decreases sharply); 95–98 °C (deep denaturation changes in muscle and connective tissue proteins take place) [16]. Cooking samples weighing 15–20 g, 15 mm thick was carried out in distilled water (the ratio of the product to water 1: 4) for 15 min, table 3.
Table 3. Dynamics of losses of soluble nitrogenous extractive substances depending on the temperature of heat treatment of test samples of horse mackerel fillets.

| Temperature of heat treatment (°C) | Loss of DM (%) | Mass loss (%) | Loss of α-amine nitrogen in broth (%) | Loss of NA (%) |
|-----------------------------------|----------------|---------------|--------------------------------------|----------------|
| 45–50                             | 10.3±0.4       | 8.2±0.3       | 24.2±1.3                             | 9.4±0.6        |
| 75–80                             | 19.9±0.5       | 22.1±0.4      | 31.9±1.5                             | 15.7±0.7       |
| 90–95                             | 20.9±0.4       | 27.2±0.4      | 34.9±1.4                             | 15.6±0.7       |

As you can see from the table 3, at a temperature of the liquid cooking medium of 45–50 °C compared with a temperature of 75–98 °C, the loss of dry matter was two times less, the loss of NA was 1.7 times less, and the loss of α-amine nitrogen was also lower. In connection with the above, further studies were carried out at a temperature of 75–80 °C.

To determine the duration of the heat treatment, providing a significant decrease in the content of NA in the horse mackerel samples, they were heated until they reached a temperature of 77±2 °C in the center of the pieces and kept at intervals of 5, 10, 15, and 20 min. The results are shown in the figure.

Figure 1. Dynamics of losses of soluble nitrogenous extractives depending on the duration of heat treatment (5, 10, 15, 20 min) of horse mackerel fillet samples.

As can be seen from the figure, the duration of the heat treatment of horse mackerel samples of 10–20 min compared to the 5-minute treatment leads to an increase in the loss of α-amine nitrogen in the broth by 1.7–2.1 times, and the loss of NA increases by 2.0–2.3 times.

It is known that to increase the water-holding capacity and enrich products with functional food ingredients the technology of combined minced fish issues uses fillers such as seaweed [17], emulsions based on vegetable oil, vegetables and cereal flour [18, 19], sodium alginate [20]. These recommendations are not possible to transfer to minced meat prepared from cooked fish, due to changes in their structural and mechanical properties. In addition, the choice of cereals should be based not only on their technological properties, especially on their binding capacity, but also on the content of nucleic acids [21, 22]. For this purpose, to choose of cereals, the content of nucleic acids in the following cereals was investigated: millet, corn, flour from barley and rice, as well as in viscous porridge from barley and rice (hydromodule 3.7).

Analysis of the data obtained showed that according to the content of nucleic acids cereals and flour from them can be arranged in decreasing order, figure 2.
The content of nucleic acids in cooked cereals was significantly lower (5.6–5.7 times) than in raw ones, the losses during cooking were 19.9–24.3 %, which can be explained by partial destruction of nucleic acids during prolonged heat treatment (from 1 to 2 hours depending on species of cereal).

Recently, a scientific justification has appeared for the recommendation of using mashed vegetable masses as fillers in minced fish products [18]. At the same time, along with an increase in the yield, enrichment of dishes, they play the role of structure-forming agents, increasing the binding and forming properties of semi-finished products and finished products [19, 20, 21]. At the same time, the question of their technological properties in minced meat from boiled fish, the possible amount in formulations, as well as their ability to reduce the content of nitrogenous extractives, including nucleic acids, remains open. In this regard, the content of nucleic acids in vegetables and losses during heat treatment were investigated.

The objects of research were: carrots, potatoes, rutabagas, turnips, beets, white cabbage, cauliflower, onions. As a result of the study, differences in the content of nucleic acids in different vegetables were noted. So, according to their content, it can be arranged in decreasing order, figure 3.

During heat treatment, the loss of nucleic acids in vegetables ranged from 29 to 40 %. The loss of nucleic acids during heat treatment is partly due to the mechanism of destruction of cellular structures, the subsequent transition to liquid [23, 24].

4. Conclusion
The work investigated the content of the nucleic acids and the acid-soluble fraction in horse mackerel, determined the losses under different cooking modes.

It was found that the optimal mode of heat treatment, from the point of view of the minimum content of nucleic acids, is boiling fish at a temperature of 98.2 °C, for 10 minutes at a hydromodule 4.

When preparing dietary culinary products from fish for diseases associated with impaired purine metabolism, it is recommended to use minced boiled skinless and boneless fillets of horse mackerel and as fillers—viscous porridge from cereals or flour from it: barley or rice, as well as vegetables: carrots or turnips.

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