Food products with ingredients rich in ω-3 polyunsaturated fat acids

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Abstract. The paper shows the possibility of enriching various food products with polyunsaturated fat acids of the ω-3 group (ω-3 PUFA). A method is proposed for using a microwave-treated cod liver as the basis for a number of products, including culinary ones. Cod liver oil separated during microwave treatment is also used as a source of ω-3 PUFA. The series of products includes culinary products: salad and zrazy, as well as meat and fish sausages produced without the use of sodium nitrite as a color stabilizer. The results of experimental studies in the development of technology and the selection of the optimal composition of various products with a microwave treated cod liver (salad and zrazy, meat and fish sausages) are presented. It was found that fish oil separated during microwave treatment of cod liver can be used as an oxidizing baking improver, and glutathione from yeast - as a reducing agent. According to the results of the experiments, the most acceptable food formulations were selected.

1. Introduction
One of the important problems in the development of functional food products is their enrichment with fat acids (PUFAs), in particular, with ω-3 [1]. ω-3 PUFAs have a very high nutritional value, have a therapeutic effect in relation to a number of diseases, especially cardiovascular diseases [2]. Fish [3] is a traditional and very common source of ω-3 PUFAs.

However, traditional methods of processing a cod liver have several disadvantages. Firstly, it is very difficult to use frozen cod liver to produce high-quality products. Secondly, the use of very fat liver and frozen raw materials for canned foods leads to a large amount of free fat in the jar. The authors propose to use not a raw cod liver for food, but a semi-finished product from cod liver, which is obtained using microwave processing. It is also advisable to use fish oil separated during such processing. This method of treating a cod liver [4] allows for a longer storage of microwave-treated cod liver (microwave) in the frozen state and prevents the separation of fat during further processing.

The enrichment of a number of combined food products in ω-3 PUFAs by incorporating the selected cod liver oil into the microwave oven can be of particular interest. The use of fish raw materials in sausages producing technology is not a completely new method, but it seems very promising. Unlike traditional meat sausages, meat and fish products allow the use of very valuable fish raw materials, and have more traditional sensory characteristics compared to fish products [5]. Also the use of microwave semi-finished products for the preparation of various salads and zrazy is interesting.
Another area of research includes the use of cod liver oil as a bread improver. The quality of the final product greatly depends on the quality of the main raw material - wheat flour, whose properties are not always constant. Bakery improvers are necessary to provide certain characteristics (rheological, colloidal, sensory) for dough and bakery products. They allow the production of bread and bakery products of stable quality even with unstable quality of flour. One of the widespread groups of bakery agents includes oxidizing and reducing agents. Fats and oils, including extracted fish oil, are oxidative improvers. To achieve the best technological effect, the integrated use of both oxidizing and reducing agents is practiced [6].

Glutathione was selected as a reducing agent. This is a tripeptide containing a cysteine residue with a —SH group. It can break disulfide bonds in a gluten molecule, changing the structural and mechanical characteristics of the dough. Yeast can be used as a source of glutathione. It is also advisable to use accelerated kneading of the dough and reduce the time of its fermentation [7].

2. Materials and methods

2.1. Materials
Microwave treated cod liver (Gadus Morhua), as well as cod liver oil isolated during cooking, were used as a source of ω-3-PUFA. The microwave oven treated cod liver was frozen and stored at a temperature of minus 18 °C. Fish oil extracted during microwave processing was purified by sedimentation and decantation methods. These semi-finished products were used to produce new types of food. Other raw materials (meat, flour, salt, spices, etc.) were obtained at the local market.

2.2. Research and processing methods
The Kjeldahl method [8] uses the SelectaBlocDigest and SelectaPro-Nitro (Spain) modules. The resulting nitrogen content was converted to raw protein (P) according to the following formula:

\[ P = N \times K_c \]

where: \( N \) – total nitrogen content; \( K_c \) – conversion coefficient.

The lipid content in samples of raw materials, fish protein isolate (FPI) and food products made from them (canned food) was determined using a SelectaDET / GRAS extractor (Spain) according to the Soxhlet method.

The composition of lipid fat acids was determined by high performance liquid chromatography (HPLC) with Agilent 1100 (USA) after saponification of lipids with an alcohol solution of binomial KOH and pre-column derivatization using bromophenacyl bromide and triethylamine [9].

Penetration strength was used to evaluate structural and mechanical characteristics. To obtain this characteristic, FoodChecker (Japan) was used with a spherical indenter (diameter 8 mm) immersed to a depth of 10 mm at a constant speed and at a temperature of 20 °C.

The acid number of the oil was determined after fat extraction using a mixture of chloroform and ethyl alcohol by titration with 0.1 M sodium hydroxide and phenolphthalein. The fat peroxide number was determined by titration of iodine displaced from potassium iodide by peroxides and hydroperoxides of sodium thiosulfate [10].

The water retaining capacity and stability of the minced meat emulsion was determined using the Salavatulina method, which includes the determination of water and fat losses, as well as the characteristics of the emulsion after heating the minced meat in a jar [11].

The porosity of bakery products was determined by determining the mass and volume. The amount of raw gluten was determined using dough (made from flour and water), rinsing with cold water until the starch is completely removed. The porosity of bakery products was determined using the Zhuravlev device, the water content was determined by drying, and the amount of raw gluten was determined after washing the dough ball [12].

Organoleptic methods were carried out using rating scales [13]. A generalized sensory score (%) was calculated by this method.
Experimental design methods (in particular, the central composite rotating design) were used in this study. The selection and evaluation of the regression equation and regression analysis as a whole were performed using OakdaleDatafit 9.1.

3. Results and discussion

3.1. Production of culinary products based on semi-finished liver products

In order to identify the most relevant culinary products with a microwave-treated cod liver, separate marketing studies were conducted using the questionnaire method. The results of these studies showed that buyers may be interested in the following culinary products with a microwave-treated cod liver: salad (34% of the total number of respondents) and zrazy (23%). Thus, it was decided to develop salad and zrazy compositions from the microwave-treated cod liver. The central rotatable compositional plan was used to select a composition that was close to the optimal. The following factors were selected: dosage of cod liver and onion for salad, as well as the proportion of cod liver, potato and eggs for zrazy. The design and results of experiments to optimize salads and zrazy are given in tables 1 and 2, respectively.

Table 1. Plan and results of the experiment for a salad with microwave treated cod liver.

| N | Microwave treated cod liver, % (X₁) | Green onion, % (X₂) | Salt, % | Rise, % | Chicken egg, % | Quality level, Y, % |
|---|-----------------------------------|---------------------|--------|---------|----------------|---------------------|
| 1 | 40                                | 4                   | 1      | 24.44   | 30.56          | 67.53               |
| 2 | 50                                | 4                   | 1      | 20      | 25             | 60.76               |
| 3 | 40                                | 10                  | 1      | 21.78   | 27.23          | 77.5                |
| 4 | 50                                | 10                  | 1      | 17.33   | 21.67          | 53.53               |
| 5 | 45                                | 7                   | 1      | 20.89   | 26.12          | 73.58               |
| 6 | 38                                | 7                   | 1      | 23.99   | 30.01          | 89.76               |
| 7 | 52                                | 7                   | 1      | 17.78   | 22.23          | 82.68               |
| 8 | 45                                | 2.8                 | 1      | 22.76   | 28.44          | 85.55               |
| 9 | 45                                | 11.2                | 1      | 19.02   | 23.78          | 86.18               |

The results of table 1 do not show the high adequacy of the regression analysis due to the use of subjective sensory assessment as an answer. As a result of regression analysis, the following equation is obtained:

\[ Y = a + b \cdot X_1 + c \cdot X_1^2 + d \cdot X_2^2 + e \cdot X_1^3 + f \cdot X_2 + g \cdot X_2^3 \]  

(2)

Where \( a = 140000; b = -12600; c = 427; d = -6.40; e = 0.0358; f = -9.60; g = 0.696 \) – regression coefficients.

The Fisher criterion was 5.22, this is not enough to say both about the adequacy and inadequacy of the regression equation. Data analysis does not allow finding the optimal value of this equation in the factor space, but some results at the boundary of this factor space can be considered close to optimal. In particular, it was found that the highest sensory score belongs to salad sample No. 6.
The results of the analysis of table 2 allowed to obtain the following regression equation:

$$Y = a + \frac{b}{X_1} + \frac{c}{X_1^2} + d \cdot \ln X_2 + e \cdot \ln X_2^2 + f \cdot \ln X_2^3 + g \cdot \ln X_2^4$$

where $a=4640; b = 120.5; c=-21.92; d=7673; e=4584; f=1191; g=113.7$ – regression coefficients.

The Fisher test was 53.9, which confirms the adequacy of the regression equation with a confidence probability of 0.95. The response surface is shown in Figure 1.

Analysis (3) showed that the optimum is achieved at $X_1 = 0.364; X_2 = 0.0757$. This corresponds to the following dosage of the ingredient per 1 kg of zrazy: cod liver - 253 g; egg - 53 g; potatoes - 695 g. In addition, it should be noted that fairly good sensory results were found in sample No. 9 (table 2). Thus, it was managed to find the most acceptable composition of salad and the optimal composition of zrazy.

3.2. Production of baked goods with cod liver oil

A marketing survey was conducted using questionnaires to determine the most preferred types of bakery products and types of the most desirable additives. The survey results showed that consumers almost equally prefer traditional types of bread: shaped (baked in the form) and hearth. The analysis showed that seaweed (kelp) (68% of the total number of respondents) and fish lipid-based additives (27% of the total number of respondents) were selected by the respondents to be included in the bread recipe from the entire list of the proposed additives produced from marine hydrobionts. Consumers also choose the addition of grain and seeds, for example, oats, flax seeds, sunflower seeds (73% of the total number of respondents) and dietary fiber, for example, bran (56% of the total number of respondents).

Studies have been conducted on the use of cod liver oil in the production of white bread. Cod liver oil was used as an oxidizing agent for baking bread in the last stage of dough kneading. Glutathione was selected as a reducing agent for bakery products. Inactivated yeast cells were used as a source of glutathione; they were added in the first kneading step.

A series of experiments was conducted on baking bread samples with the addition of cod liver oil and glutathione. The best characteristics were obtained when using a dosage of oil - 1% by weight of flour. Table 3 shows the results of studying the characteristics of gluten, and table 4 shows the properties of the samples of the finished product.

**Table 3. The results of the study of gluten of wheat flour with additives.**

| №  | Components                        | Gluten mass, g | The yield of gluten,% | Gluten color        | Elasticity | Elongation, L, cm |
|----|----------------------------------|----------------|-----------------------|---------------------|------------|-------------------|
| 1  | Control Flour + Water            | 9.2            | 24.2                  | light coloured      | elastic    | 18                |
| 2  | Flour + water + cod liver oil (1%) | 9.05           | 23.7                  | light coloured      | elastic    | 10                |
Flour + water + cod liver oil (1%) + inactivated yeast

Table 4. Results of a tasting evaluation of test baking bread samples.

| №  | Fat dosage, %* | The intensity of the fat smell ** | Organoleptic quality level |
|----|----------------|----------------------------------|---------------------------|
| 1  | 0              | 0.25                             | 67.1                      |
| 2  | 1              | 0.94                             | 96.6                      |
| 3  | 1.5            | 1.69                             | 84.4                      |
| 4  | 2              | 4.06                             | 84.2                      |

*– % to the mass of flour.
**– The intensity of the smell of fat was evaluated on the following scale:
0 – absent,
1 - subtle, positive or neutral,
2– low, positive or neutral,
3 - low, negative,
4 - medium, negative,
5 - strong, negative.

Formulations of bakery products from wheat flour with the addition of seaweed, bran and milk thistle were developed to expand the range of bakery products. Bran is a rich source of dietary fiber that helps regulate bowel function. Seaweed are a source of iodine, it also contains natural sorbents (alginites) and sterols, which can have a positive effect on the human body. Milk thistle has both dietary fiber and flavonolignans.

Samples of wheat bread with such additives were prepared and evaluated; the results are shown in table 5.

Table 5. Physico-chemical characteristics of wheat bread with additives.

| Indicator           | Wheat bread with cod liver oil and inactivated yeast | Wheat bread with added cod liver oil, inactivated yeast and sea kale | Wheat bread with added cod liver oil, inactivated yeast and bran | Wheat bread with added cod liver oil, inactivated yeast and milk thistle meal |
|---------------------|-----------------------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------|--------------------------------------------------------------------------|
| Humidity, W, %      | 33.0                                                | 33.5                                                                | 32.0                                                            | 32.0                                                                    |
| Porosity, P, %      | 76.0                                                | 63.0                                                                | 61.0                                                            | 67.0                                                                    |
| Acidity, X, degrees | 3.0                                                 | 2.9                                                                 | 3.3                                                             | 3.1                                                                     |
| Quality level, %    | 93.0                                                | 92.3                                                                | 87.2                                                            | 84.0                                                                    |

Thus, a sufficiently high-quality product can be achieved by adding seaweed additives. The use of bran also allows getting a high-quality product.

3.3. Implementation of FPI and microwave treated cod liver for the production of meat and sausages

The production of meat and sausages is one of the promising areas for the use of semi-finished products from cod liver. Previous studies have led to the development of technology for meat and fish sausages with microwave, which has been optimized for the main ingredients [4].

In addition to balancing the main ingredients, it is very important to find the dosage of additional components that affect the yield of the finished product, structural and mechanical characteristics, and color. So, the next stage in the development of the composition was the optimization of additional ingredients.

Guar gum (food polysaccharide-derived hydrocolloid) is used as a thickener and emulsifier to provide the most appropriate structural and mechanical characteristics. It was decided to abandon the use of tomato paste as a dye, but use fermented rice to obtain a slightly pink color.
An objective characteristic of the color of the finished product is its additive color model (RGB). Using this model instead of sensing color enhances the accuracy of the experiment. But the RGB model includes 3 parameters that change the range from 0 to 255, so it is reasonable to determine the total square deviation from the optimal task. A subsequent selection of colors was made, and the most acceptable colors for sausages were determined using an expert method. The results show that the optimal RGB color is 248.170.168.

Thus, a central composite design of a two-factor experiment can be developed. The generalized optimization parameter includes 5 separate parameters: \( Y_1 \) - water-holding ability; \( Y_2 \) - fat capacity; \( Y_3 \) is the stability of the emulsion; \( Y_{R4} \) – relative structural and mechanical characteristic calculated according to (4):

\[
Y_{R4} = \exp\left(-\frac{Y_1 - Y_{opt1}}{\Delta Y_1}\right)
\]

Where \( Y_1 \) – penetration force, kPa;
\( Y_{opt1} \) – optimal penetration force, kPa (36.1);
\( \Delta Y_1 \) – critical change in penetration index, above which a significant effect on consistency is observed, kPa (5).

\( Y_5 \) – deviation from the optimal color, determined by the formula

\[
Y_5 = 100 - \frac{100}{R^2 + G^2 + B^2} Y_1^2 \exp(Y_1 - Y_{opt1})^2
\]

where \( K_1, K_2, K_3, K_4, K_5 \) – significance factors, respectively equal to 0.2; 0.2; 0.1; 0.1; 0.4.

The plan of the two-factor experiment and the results are shown in table 6.

### Table 6. Plan and results of processing the experimental data on auxiliary components.

| № | \( X_1 \) | \( X_2 \) | \( Y_1 \) | \( Y_2 \) | \( Y_3 \) | \( Y_{R4} \) | \( Y_5 \) |
|---|---|---|---|---|---|---|---|
| 1 | 0.2 | 0.1 | 60.73 | 12.19 | 97.63 | 95.65 | 83.99 | 67.51 |
| 2 | 0.2 | 0.5 | 60.64 | 12.40 | 97.41 | 97.81 | 79.49 | 65.93 |
| 3 | 0.4 | 0.1 | 61.64 | 14.46 | 97.49 | 94.44 | 84.33 | 68.14 |
| 4 | 0.4 | 0.5 | 59.96 | 14.14 | 97.18 | 91.99 | 80.75 | 66.44 |
| 5 | 0.3 | 0.3 | 60.84 | 13.62 | 97.60 | 98.31 | 83.25 | 67.78 |
| 6 | 0.16 | 0.3 | 60.50 | 13.29 | 97.46 | 95.94 | 85.06 | 68.12 |
| 7 | 0.44 | 0.3 | 63.57 | 12.97 | 97.98 | 89.70 | 85.33 | 68.21 |
| 8 | 0.3 | 0.02 | 61.84 | 12.37 | 97.59 | 99.00 | 80.32 | 66.63 |
| 9 | 0.3 | 0.58 | 61.76 | 13.18 | 97.63 | 98.66 | 78.46 | 66.00 |
| 10 | 0 | 0 | 57.27 | 11.12 | 96.13 | 92.78 | 79.01 | 64.17 |

As a result of processing, the following regression equation was obtained:

\[
Y = 64.31 + 14.59 \cdot X_1 - 21.82 \cdot X_1^2 + 13.30 \cdot X_2 - 26.81 \cdot X_2^2
\]

The Fisher criterion for this equation is 11.12, the probability of inadequacy is 0.01. All regression coefficients are significant at a confidence level of at least 0.99. The response surface is shown in Figure 2.

Using the differential method, a local maximum was found. The optimal values of the factors: \( X_1 = 0.33 \), \( X_2 = 0.25 \).

The optimal composition of meat and fish sausages are shown in table 7.
Physical and chemical experiments were conducted to prove the functional properties of the finished products. The results are shown in table 8. Thus, due to additives, monounsaturated acids predominate in sausages, in addition, a large amount of \( \omega-3 \) PUFA is present. A low amount of \( \omega-6 \) PUFA can be offset by vegetable oils.

**Figure 2.** Response surface to optimize additional ingredients.

**Table 7.** The optimal formulation of meat and sausages for the main and auxiliary components.

| The main raw material, kg |       |
|--------------------------|-------|
| Lean pork veined         | 70.20 |
| Cod liver microwave-blanced | 25.71 |
| FPI of blue whiting      | 2.09  |
| Chicken eggs             | 2.00  |
| Potato starch            | 3.00  |

| Spices and materials, kg |
|--------------------------|
| Table salt               | 2.50  |
| Granulated sugar         | 0.10  |
| White pepper powder      | 0.25  |
| Black pepper powder      | 0.25  |
| Fermented rice           | 0.25  |
| Guar gum (E412)          | 0.33  |
| Ice-water mixture        | 15.00 |

**Table 8.** The results of physico-chemical tests of meat and fish sausages.

| Indicator                          | Results |
|------------------------------------|---------|
| Moisture content, %                | 63.36   |
| Mass fraction of protein, %        | 16.14   |
| Mass fraction of fat, %            | 15.85   |
| Mass fraction of sodium chloride, %| 2.52    |
| Mass fraction of ash, %            | 2.82    |
| The acid number of fat product, mg KOH/g of fat | 2.09 |
| Penetration number, kPa            | 59.33   |

| Fatty acid composition, % of total fat acids |
|---------------------------------------------|
| Capric (C10:0)                              | 0.42   |
| Lauric (C12:0)                              | 0.86   |
| Myristine (C14:0)                          | 5.99   |
| Myristolein (C14:1)                        | 0.60   |
| Pentadecane (C15:0)                        | 0.57   |
| Palmitic (C16:0)                           | 22.48  |
| Palmitoleic (C16:1)                        | 6.19   |
| Margarine (C17:0)                          | 0.71   |
Stearin (C18:0) 7.72
Oleic (C18:1) 30.84
Linoleic (C18:2) 4.13
Linolenic (C18:3) 1.55
Octadecatetraenoic (C18:4) 8.86
Gondoin (C20:1) 0.1
Eicosadiene (C20:2) 0.34
Eicosatrienic (C20:3) 0.07
Arachidonic (C20:4) 0.29
Eicosapentaenoic (C20:5) 0.25
Erucic (C22:1) 0.29
Docosapentaenoic (C22:5) 3.26
Docosahexaenoic (C22:6) 4.54

4. Conclusions
Technologies have been developed for the preparation of culinary products from a microwave-treated cod liver. The most acceptable salad composition with cod liver was not found. The optimal ratio of ingredients for zrazy with cod liver was determined.

It was proved that the use of a combination of cod liver oil and glutathione as bakery products improves both the structural, mechanical and sensory characteristics of bakery products. The addition of components such as seaweed, bran and milk thistle can in several ways increase the nutritional value of the product and satisfy consumer demand for baked goods with natural additives.

The technology for processing meat and fish sausages with RBI and microwave-treated cod liver has been developed, the optimal composition has been found and justified. The experimental samples are under the study.

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