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Dietary product based on sea urchin caviar and *Sardinops melanostictus* fat

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ABSTRACT

Various biologically active substances are present in the caviar (roe) of sea urchins, which cause a positive effect on the human body and are used as part of dietary supplements for the prevention and treatment of many diseases. Caviar food products are represented by a small assortment. This paper presents the findings of research on the development of caviar products with pronounced dietary properties based on sea urchin caviar and *Sardinops melanostictus* fat. Sardine oil is a rich source of omega-3 fatty acids. The developed caviar products contain phospholipids in the amount of 7.5–9.2 g/100 g and polyunsaturated fatty acids of the omega-3 family in the amount of 6.53–10.05 g/100 g. During 6 months of storage at a temperature from –2°C to –6°C, the quality and safety indicators of products did not change. Caviar products have been recommended for therapeutic and preventive dietary nutrition for cardiovascular diseases.

1. INTRODUCTION

Sea urchin caviar is a rich source of various biologically active substances and is of particular value in the diet of the population [1,2]. It contains full proteins and sulfated polysaccharides [3], a large set of fat-soluble and water-soluble vitamins, macronutrients, and trace elements [4] of pigments, carotenoids [5], and naphthoquinones [6]. Lipids of sea urchin caviar contain up to 58.0% polyunsaturated fatty acid (PUFA), including the omega-3 family, up to 38.0% of the total fatty acids. When sea urchin caviar was included in the diet, various medicinal properties were noted: Antioxidant [7,8], antitumor [9,10], anti-inflammatory [11], antimicrobial [12], and others [13,14].

Various biologically active food additives and medicines were obtained based on sea urchin caviar [15]. At present, the range of food products from sea urchin caviar is not large. It includes frozen products or caviar derivatives (oils, sauces, and pastes), in which the mass fraction of caviar is 50.0–70.0% lower than in natural caviar products, which makes them more affordable for the population [16]. The composition of these products includes vegetable oil, which is dominated by omega-6 PUFAs, but no omega-3 PUFAs.

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Omega-3 fatty acids are essential for humans since their bodies do not synthesize them in sufficient quantities but only receive them with food [17]. The physiological role of omega-3 fatty acids and the mechanisms of their pharmacological action have been determined by experimental studies [18,19]. It has been established that omega-3 fatty acids are precursors of a wide range of different lipid mediators. They regulate metabolic pathways and inflammatory responses, which determine a wide range of their positive effects on the human body [20-23]. The most pronounced therapeutic and prophylactic effect on the human body is exerted by PUFAs of the omega-3 family, in particular, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids [24-26]. In this regard, fats with a high content of EPA and DHA are used in the production of dietary products, biologically active additives, and pharmaceuticals.

Fish oil is a rich source of omega-3 fatty acids [27]. The use of fish oil with a high content of PUFAs of the omega-3 family in caviar products will significantly increase the dietary value of the products. The purpose of this work is to substantiate and develop a dietary product with a high content of omega-3 PUFAs and phospholipids based on sea urchin caviar and fish oil.

2. MATERIALS AND METHODS

2.1. Examined Material

The main component for obtaining a dietary product was sea urchin roe *Strongylocentrotus nudus* and *Strongylocentrotus intermedius*, which are actively fished in the Sea of Japan.

An additional component in the dietary product was fish oil obtained from *Sardinops melanostictus* sardine. In sardine lipids, the PUFA content reaches 35.0–40.0% of the total fatty acids, including the omega-3 family, 22.0–32.0% [28].

2.2. Study of Organoleptic Properties

Sensory evaluation of products included the determination of external signs and organoleptic properties.

2.3. Determination of the Mass Fraction of Water

The water content of urchin roe was measured by drying in a laboratory oven to a constant weight at 105°C. Ground samples of 2.0 g in weight were put into a clean dried weighing bottle and dried in a laboratory oven at a temperature of 105°C to the point of constant weight. The first weighing was carried out after 3 h, while the following ones were carried out every 30 min. If the difference between the two weightings did not exceed 0.001 g, the mass obtained was considered to be constant. The mass fraction of water (%) (Separations 2021, 8, 174) three of nine was calculated by determining the difference between the sample weight before and after drying.

2.4. Determination of the Mass Fraction of Proteins

The protein content of the samples was determined by Kjeldahl's method [29], using the automatic Kjeltec Auto Analyzer 2300 (Hoganas, Sweden). The protein content in the samples (%) was then calculated by multiplying the total nitrogen content by the nitrogen-to-protein conversion factor (6.25).

2.5. Definitions of Amino Acids

The amino acid profile of the protein was determined using the amino acid analyzer "Hitachi L-8800" (Tokyo, Japan). The amino acid score was calculated by determining the ratio of each essential amino acid in the protein investigated to the amount in the FAO/WHO amino acid standard.

2.6. Determination of the Fractional Composition of Lipids

Lipids were extracted from the samples by the Bligh and Dyer method [30]. To study the fractional composition of lipids (triglycerides, phospholipids, sterols, etc.), a thin-layer chromatography method was used (analytical plates "Sorbfil," Sorbpolymer, Krasnodar, Russia) in a system of solvents hexane/diethyl ether/acetic acid, 70:30:2 (by volume) as the eluent. For the development of chromatograms, a 10% alcohol solution of phosphomolybdic acid was used followed by heating the plates at 110°C. Individual classes of lipids were identified by comparison with the standard compounds applied to the plate. Image software v.1.47 (National Institute of Health, Bethesda, MD, USA) was used for quantification.

2.7. Determination of the Composition of Fatty Acids

The composition of fatty acids in the form of methyl esters was determined by gas-liquid chromatography with a GC-2010 Plus chromatograph (Shimadzu, Japan) with a flame ionization detector. Column: Zebron™ Phase ZB-FFAP 50 m × 0.32 mm. Evaporator and detector temperatures were 250°C and 200°C, respectively. The thermostat temperature was programmed from 100° C to 185°C at a rate of 6–8°C/min and then maintained at 185°C until the end of the analysis. Fatty acid methyl ethers were identified by carbon number value [29] and using certified reference materials; a set of fatty acid methyl esters from Siemens (Darmstadt, Germany) was used as the internal standard.

2.8. Determination of the Mass Fraction of Carbohydrates

Determination of free carbohydrates was carried out by high-performance liquid chromatography with an electrochemical detector using a Dionex CarboPac PA20. Separation of aqueous extracts in samples was performed on column (3 × 150 mm) with an AminoTrap guard column (3 × 30 mm) manufactured by Dionex (Germering, Germany), using an eluent 10 mm solution. The identification of carbohydrates was carried out using internal standards of arabinose, glucose, ribose, mannose, galactose, fructose, xylose, sucrose, and lactose from Supelco (Burlington, MA, USA). The concentration was calculated by the peak area relative to the calibration dependence obtained in the analysis of standard solutions of carbohydrates.

2.9. Determination of the Mass Fraction of Minerals

The total mineral content was determined by the ratio of the sample weight difference before and after combustion to the original sample weight mass.

2.10. Statistics

The computed statistical parameters included the mean values and the standard mean error (n = 5). The results were subjected to statistical analysis using Microsoft Excel 2003 and Statistica 6.0 ($P < 0.05$).

3. RESULTS

To obtain the product, sea urchin caviar and additional components were used according to the composition indicated in Table 1. Three versions of recipes for caviar products were prepared (1, 2, and 3), which differed in the ratio of the main components.

The mass fraction of proteins in the used sea urchin caviar was 13.8 ± 0.8%, fat 6.9 ± 1.2%, carbohydrates 2.7 ± 0.5%, and mineral substances 2.2 ± 0.2%. The share of PUFAs was 35.78% of the total fatty acids, including the omega-3 family, 23.7%, and the amount of DHA and EPA, 16.28%.

The separation of fat from the minced *S. melanostictus* was carried out by the thermal method [31]. In the resulting fat, the phospholipid content was 13.1% of the total lipids. The share of PUFA was 37.4% of the total fatty acids. The bulk of PUFAs was omega-3 fatty acids (30.3% of all fatty acids), and the sum of DHA and EPA was 23.9%.

To obtain a flavored oil, ground turmeric was used. It was kept at room temperature for 48 h in refined sunflower oil in a ratio of 2:100. After infusion of the mixture, the liquid part was separated from the sediment and used to obtain a caviar product. Dietary additive "betulin" was used to reduce the activity of oxidative, hydrolytic, and microbial processes. Food supplement "betulin" is a dry extract of birch bark. The main natural substance in this supplement is pentacyclic triterpene alcohol. Betulin is insoluble in water; it forms a suspension with oils

Table 1: Table of composition of caviar products.

Components	Content (% of net mass) in caviar product		
	Variant 1	Variant 2	Variant 3
Sea urchin caviar	55.0	60.0	68.0
Sardine fat	22.0	18.6	15.0
Flavored oil	20.1	18.5	14.3
Emulsifier	0.8	0.8	0.6
Food supplement "betulin"		0.1	
Edible salt		2.0	

and fats. In fat and oil products, it exhibits a pronounced antioxidant and antimicrobial effect, which leads to an increase in their shelf life [32]. Betulin has a positive effect on the human body and is used as a dietary supplement. The recommended level of consumption of betulin for humans is 40–80 mg per day [33].

To stabilize the structure of the product, a food additive E471 was used as an emulsifier. Egg roe and prepared additional components were placed in a mixer, homogenized at a speed of 2400 rpm for 7 min until a thin homogeneous mass was formed. The resulting mixture was packed into cans made of polymeric materials with a capacity of 25–50 cm^3.

Samples of caviar products were stored at temperatures ranging from –2°C to –6°C. Monthly studies of the indicators of product quality and safety were conducted. The content of nutrients in caviar products is shown in Table 2. The composition of lipids in caviar products is shown in Table 3.

The results of studying the composition and amount of fatty acids in lipids of caviar products are shown in Tables 4 and 5.

4. DISCUSSION

The produced samples of caviar products belong to the group of emulsion products. They represented a finely ground homogeneous plastic mass of orange color, characterized by a pleasant caviar taste and a weak spicy smell.

In terms of protein content, caviar products of different variants [Table 2] insignificantly differed. The presence of proteins in products is due only to the caviar component. Proteins have been balanced in terms of essential amino acids. There were no limiting amino acids.

The fat content in caviar products, depending on the variant, ranged from 34.4% to 46.0% of net weight. The study of the lipid component showed that the main class is triacylglycerides, the content of which reached 70.18–72.34% of the total lipids [Table 3]. The proportion of phospholipids in the fat component was 20.16–21.71%, depending on the product variant. Their content ranged from 7.5 to 9.2 g/100 g of caviar products. The highest content of phospholipids was noted in the product sample, the original formulation of which contained 68.0% sea urchin roe. It is known that phospholipids have anti-inflammatory and cardioprotective properties [34]. They have a positive effect on lipid

Table 2: Table of general chemical composition of caviar product.

Components	Content (%) in caviar product		
	Variant 1	Variant 2	Variant 3
Water	43.5±1.5	46.5±2.0	51.2±2.5
Protein	7.7±0.6	8.2±0.9	9.8±1.2
Fat	46.0±0.5	42.5±0.5	34.4±0.4
Carbohydrates	1.4±0.2	1.7±0 2	1.9±0.3
Mineral substances	1.2±0.1	1.3±0.1	1.5±0.2

Table 3: Table of composition of lipids of caviar products.

Lipid classes	Content (% of total lipids) in caviar product		
	Variant 1	Variant 2	Variant 3
Triacylglycerides	72.34	71.03	70.18
Phospholipids	20.16	20.74	21.71
Mono- and di-acylglycerides	1.77	2.02	2.23
Free fatty acids	2.75	2.63	2.48
Sterols	2.24	2.32	2.33
Sterol esters	0.24	0.60	0.57

Table 4: Table of composition of fatty acids in lipids of caviar products % of the total amount of fatty acids.

Fatty acids	The content in the caviar product (% of the total amount of fatty acids)		
	Variant 1	Variant 2	Variant 3
12:0	0.52	0.52	0.56
13:0	0.03	0.02	0.02
14:0	9.36	1.60	10.82
15:0	0.30	0.28	0.18
16:0	10.23	10.34	10.53
17:0	0.24	0.18	0.14
18:0	2.2	2.23	2.93
20:0	0.47	0.50	0.55
14:1 *n*-9	0.9	0.10	0.11
14:1 *n*-5	1.17	1.28	1.45
16:1 *n*-7	5.32	5.71	5.29
16:1 *n*-5	2.23	2.31	2.60
17:1 *n*-8	0.3	0.28	0.26
18:1 *n*-11	0.17	0.19	0.21
18:1 *n*-9	7.58	8.16	8.27
18:1 *n*-7	2.35	2.33	2.09
18:1 *n*-5	0.31	0.31	0.33
19:1 *n*-9	0.07	0.07	0.08
20:1 *n*-11	3.95	4.58	4.86
20:1 *n*-9	3.97	4.77	5.31
20:1 *n*-7	0.83	0,9	1.01
20:1 *n*-5	1.22	1.33	1.51
22:1 *n*-11	0.77	0.65	0.56
22:1 *n*-9	0.81	0.87	0.95
24:1 *n*-9	0.13	0.11	0.09
16:2 *n*-4	0.36	0.31	0.28
16:4 *n*-1	0.64	0.58	0.52
18:2 *n*-9	0.32	0.34	0.39
18:2 *n*-6	14.65	14.02	13.29
18:2 *n*-4	0.07	0.06	0.04
18:3 *n*-6	0.12	0.11	0.11
18:3 *n*-3	0.66	0.69	0.75
18:4 *n*-3	3.00	2.29	2.47
18:4 *n*-1	0.07	0.06	0.04
20:2 *n*-6	0.96	0.90	0.85
20:3 *n*-9	0.52	0.56	0.64
20:3 *n*-6	0.36	0.39	0.43
20:3 *n*-3	0.61	0.66	0.75
20:4 *n*-6	3.59	2.89	2.78
20:4 *n*-3	1.93	1.40	1.03
20:5 *n*-3	7.99	7.87	7.03
21:5 *n*-3	0.10	0,08	0.07
22:5 *n*-3	1.36	1.35	1.37
22:6 *n*-3	6.45	6.34	5.62

Table 5: Table of content of various groups of fatty acids in caviar products.

The amount of fatty acids	Content in caviar product					
	Variant 1		Variant 2		Variant 3	
	%*	г/100 г**	%	г/100 г	%	г/100 г
Saturated	23.35	10.62	24.67	10.41	25.73	8.85
Monounsaturated	32.08	14.6	33,.95	14.33	34.98	12.03
PUFA	43.76	19.91	40.9	17.26	38.46	13.23
n-6	19.68	8.95	18.31	7.73	17.46	6.0
n-3	22.10	10.05	20.68	8.73	19.09	6,.53
EPA+DHA	14.44	6.57	14.21	6.0	12.65	4.35

Designation: *Percentage of the total amount of fatty acids; **g/100 g of product, PUFA: Polyunsaturated fatty acid, EPA: Eicosapentaenoic, DHA: Docosahexaenoic

metabolism in the human body and are used for diseases of the liver and cardiovascular system [35,36]. It is known that the required level of daily consumption of phospholipids for humans is 7 g [33]. In this regard, caviar products weighing 50 g will provide the human body with these valuable compounds by 53.0–65.0% or more. Products with a net weight of 25.0 g will provide the human body with phospholipids by 26.0–32.0% of the recommended daily intake. The developed caviar products represent an additional source of essential phospholipids.

The study's results of individual fatty acids in lipids of caviar products [Table 4] showed that, in all variants products, the group of PUFAs prevailed [Table 5].

The fraction of saturated fatty acids in lipids of products was the smallest in terms of quantity; their content was 8.85–10.62 g/100 g of product. This group was dominated by palmitic (16:0) and myristic (14:0) acids. The fraction of monounsaturated fatty acids was significantly higher (32.08–34.98% of the total fatty acids), and the content was 12.03–14.6 g/100 g of food. This group was dominated by oleic (18:1 *n*-9) and palmitoleic (16:1 *n*-7) acids. The source of oleic acid is the main component of vegetable oils; in the lipids of marine organisms, it is practically not detected or is present in insignificant quantities.

The amount of PUFA in the lipids of caviar products was 38.46–43.76% of the total amount of fatty acids. The amount of PUFA in the lipids of caviar products was 38.46– 43.76% of the total amount of fatty acids, the main part of them (19.09-22.1%) is represented by omega-3 fatty acids, among which EPA predominated (20:5 *n*-3) and DHA (22:6 *n*-3). The content of PUFAs of the omega-3 family was 6.53–10.05 g/100 g of caviar products, and the sum of EPA and DHA was 4.35–6.57 g. The higher the mass fraction of fish oil in the composition of caviar products, the higher the content PUFA of the omega-3 family. The recommended daily intake of omega-3 fatty acids for humans is at least 3 g, and EPA + DHA is 0.8–1.5 g [33,37,38].

Most clinical studies have shown that the consumption of fatty acids of the omega-3 family in such concentrations leads to a decrease in the risk of cardiovascular, neoplastic, and other diseases, as well as a decrease in mortality from various causes [39-41]. The authors noted that a daily intake of more than 1 g of omega-3 PUFAs (especially EPA and DHA) is already effective in reducing the risk of myocardial infarction and cardiac death [42,43]. A higher effect is achieved by patients using EPA of more than 1 g/day in the secondary prevention of cardiovascular diseases [44,45].

In this regard, caviar products in a 25 g package will fully satisfy the daily requirement of the human body for PUFAs of the omega-3 family. During storage for 6 months at a temperature from –2°C to –6°C, the quality and safety indicators of caviar products did not change.

5. CONCLUSION

The technology and formulation of products based on sea urchin caviar and *S. melanostictus* fat with a high content of phospholipids and PUFAs of the omega-3 family have been developed.

The content of phospholipids in caviar products is 7.5–9.2 g/100 g, and PUFA of the omega-3 family is 6.53–10.05 g/100 g, including EPA and DHA of 4.35–6.57 g/100 g. The main source of phospholipids in the composition of products is sea urchin caviar in the amount of 55.0–68.0% and the source of PUFAs of the omega-3 family is the fat of *S. melanostictus* in the amount of 15.0–22.0%.

The developed caviar products meet the requirements of specialized products for dietary therapeutic and prophylactic nutrition for diseases of the cardiovascular system and liver.

6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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