

Article

Nutritional Value of Sea Urchin Roe (*Strongylocentrotidae*)—Study of Composition and Storage Conditions

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Abstract: Although the roe of sea urchins inhabiting the Far Eastern seas possesses many healing properties and may be used as a dietary product, a reduction and deterioration in its nutritional quality during storage occurs. Therefore, in order to make sea urchin products widely accessible to the world population, it is very important to have appropriate technology to keep the roe from spoiling. To store sea urchin roe for a long time, methods of pre-processing sea urchin gonads before freezing were tested. In terms of preserving organoleptic properties and nutritional quality, the most adequate procedure consists of a short period (20 or 30 s) of heat (boiling water) treatment of sea urchin roe after removal from the shell. This procedure results in an inactivation of enzymes that catalyze the hydrolytic processes of lipids and proteins during storage. After blanching and cooling, the roe was packed, frozen and kept at a temperature of $-18\text{ }^{\circ}\text{C}$ and $-25\text{ }^{\circ}\text{C}$. The quality of sea urchin roe did not change during storage at the temperature of $-18\text{ }^{\circ}\text{C}$ for 6 months, and at the temperature of $-25\text{ }^{\circ}\text{C}$ for 10 months.

Keywords: sea urchins; roe; caviar; storage; quality



Citation: Matveeva, V.A.; Shulgina, L.V.; Prikhodko, Y.V.; Shulgin, Y.P.; Madej, K.; Piekoszewski, W. Nutritional Value of Sea Urchin Roe (*Strongylocentrotidae*)—Study of Composition and Storage Conditions. *Separations* **2021**, *8*, 174. <https://doi.org/10.3390/separations8100174>

Academic Editor: Paraskevas D. Tzanavaras

Received: 16 July 2021

Accepted: 21 September 2021

Published: 7 October 2021

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1. Introduction

Sea urchins of the *Strongylocentrotidae* family are widespread seabed animals with a specific round shape of the outer skeleton, which is covered with numerous needles [1]. Stocks of sea urchins in the Far Eastern seas cause intensive fishing, with the recommended capture level being about 8500 tons annually. In recent years, measures have been developed in the region to maintain the stability of sea urchin stocks by establishing farms for their breeding.

Sea urchins are of great importance as sources of roe, the yield of which ranges from 6.0% to 20.0%, depending on their biological state [2]. Sea urchin roe has high food and biological value, and is rich in various biologically active substances [2–4], which display antioxidant effects [5,6], and antitumor [7,8], anti-inflammatory [9], geroprotective [10], antimicrobial [11] and other medicinal properties [12,13]. Due to this, a number of dietary supplements and pharmaceuticals recommended for the prevention and treatment of various pathological conditions of the human body have been developed on the basis of sea urchin roe [10,14,15].

Despite the considerable reserves of sea urchins, food products made from them are practically unavailable. The main reason for this is the reduction and deterioration in urchin roe quality during storage. The change in the quality of the urchin roe is due to the high activity of the enzymes found in it. Protein proteolysis, lipid hydrolysis and

oxidation glycogen, phosphocreatine and ATP, and the formation of melanoidin pigments occur under their influence [4,16]. The structure and consistency of salted or frozen roe products are impaired within a short period of time; the flavour and colour characteristics change, and an unpleasant odour and bitter taste appear [17]. Valuable roe products are inaccessible to the general population due to the unavailability of proper technologies for sea urchin roe processing, which would ensure its quality and stability during storage.

The aim of the study was to evaluate the effect of low-temperature storage with appropriate pretreatment on the composition of sea urchin caviar.

2. Materials and Methods

2.1. Examined Material

Black (*Strongylocentrotus nudus*) and grey (*Strongylocentrotus intermedius*) sea urchins, which are actively fished in the Sea of Japan, were studied.

2.2. Initial Procedure for Preparing Sea Urchins for Study

After being collected, sea urchins were put into boxes and covered with finely crushed ice in an amount no less than 50% relative to the raw mass of sea urchins. They were then kept in these conditions until roe extraction out of the shell occurred at ambient air temperature (no higher than 10 °C) for no longer than three days. The urchins' shells were cut into two parts by special scissors, and the roe was extracted and put into a bath of 2.4% salt solution. The roe was kept in the salt solution for further processing for no longer than 2 h, during which time it maintained its external characteristics.

2.3. Sea Urchin Gonad Pretreatment Methods for Long-Term Storage

For the purpose of long-term storage of sea urchin caviar, several methods for the pretreatment of sea urchin gonads before freezing were tested:

Method 1—the roe was placed in a saline solution (6% NaCl), cooled to 15 °C and kept for 30 min. It was then laid out in a strip and packed in trays, frozen and stored at a temperature of minus 18 °C and minus 25 °C.

Method 2—samples were prepared as in Method 1, and then packed in trays with a preservative (sorbic acid in an amount of 0.1% of the total weight was added to the caviar), to be frozen and stored at a temperature of minus 18 °C and minus 25 °C.

Method 3—sea urchin roe extracted from ovaries was blanched in boiling water for 20–30 s in special trays; after cooling, the caviar was packed in other trays, frozen and stored at a temperature of minus 18 °C and minus 25 °C.

2.4. Sensory Evaluation of Roe

Sensory evaluation was carried out by a panel of five selected assessors trained according to the international standard [18]. Each sample was initially assessed independently by the panelists, followed by a discussion of sensory characteristics in order to reach a consensus. Sensory evaluation of the roe involved the identification of external features and organoleptic properties. External features were identified after defrosting the roe and unpacking the product; colour, pigmentation or spots, and sediments were determined. When evaluating organoleptic properties, the flavour, taste and consistency of the product were established. Furthermore, the extent to which the flavour and taste typical for roe were maintained was determined.

2.5. Water Content Determination

The water content of urchin roe was measured by drying in a laboratory oven to a constant weight at 105 °C. In brief, 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; the following ones were carried out every 30 min. If the difference between two weightings did not exceed 0.001 g, the mass obtained was considered to be constant. The mass fraction of water (%)

was calculated by determining the difference between the sample weight before and after drying.

2.6. Determination of Bioactive Compounds

The protein content of the samples was determined by Kjeldahl's method [19], using the automatic Kjeltex Auto Analyser 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).

The amino acid profile of the protein was determined by 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.

Lipids were extracted from the samples by the Bligh and Dyer method [20]. 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 a 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.

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 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 esters were identified by carbon number value [19] and by using certified reference materials; a set of fatty acid methyl esters from Siemens (Darmstadt, Germany) was used as the internal standard. Total mineral content was determined by the ratio of the sample weight difference before and after combustion to the original sample weight mass.

Determination of free carbohydrates was carried out by HPLC with an electrochemical detector using a Dionex CarboPac PA20. A 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.

The product's relative biological value was determined by the bio-testing method using *Tetrahymena pyriformis infusoria* as a test object [21].

2.7. Statistics

The computed statistical parameters included the mean values and the standard mean error ($n = 5$).

The results were subjected to statistical analysis (analysis of variance with Turkey post-variance test) using Microsoft Excel 2003 and Statistica 6.0 ($p < 0.05$).

3. Results and Discussion

Depending on the sea urchin species, the roe extracted from the shell differed in colour features.

The roe of one species of sea urchin from the same harvest was usually of different shades of the same colour (different shades of orange, red, grey, etc.). Grey sea urchins roe was mainly a yellow, orange, or yellow-grey colour, whereas that of black sea urchins

was from light yellow and light orange up to grey-brown hues. The immature roe of sea urchins was sordid-black or purplish. The colour of the roe depends on the fat content and pigments such as carotenoids, β -carotene and echinenone, naphthoquinones, melanin, as well as lipofuscin, which is sometimes found in the urchins' roe [1,22].

To keep sea urchin roe for a long time, three methods (described in Section 2.3) of sea urchin gonad pre-processing were tried before freezing. Then, the roe was thawed at a temperature of 2–6 °C for 8–10 h. The caviar of sea urchins, which had been prepared according to Methods 1 and 2, changed its organoleptic properties after 30 days of freezing storage. The roe and sediment developed a liquid/fluid consistency, the characteristic sweetish taste decreased, and bitterness appeared.

The most effective procedure was ovary blanching in boiling water for 20 or 30 s. Short-time heat treatment may have resulted in the inactivation of enzymes that catalyze the hydrolytic processes of lipids and proteins during storage. Under the influence of high temperature, fibrous connective proteins of the ovaries' surface film, and the roe layer contiguous to it, coagulated. This helped to conserve moisture inside the ovary and prevent roe fluidity under freezing storage conditions. After blanching and cooling, the roe was packed, frozen and kept at a temperature of minus 18 °C and minus 25 °C.

When using the above procedure, irrespective of the species of sea urchin, the roe was characterized by a cucumber flavor and had a pleasant slightly salted sweetish taste and fine texture. However, the roe of the grey sea urchin had a more intense sweetish taste than that of the black species. Moisture ranged from 73% to 75.7% in grey to black sea urchins, respectively, which was similar to that observed in Turkish sea urchins, where it was 78.36–80.93% [23]. To evaluate the nutritional value of the studied sea urchin species under the used storage conditions, the energy value and chemical composition of sea urchin roe after freezing were also determined. As is shown in Table 1, grey and black sea urchin roe is classified as a moderately protein-containing product of lower caloric value; these results were similar to those obtained for the Turkish sea urchin [23]. The fat content of the grey sea urchin roe turned out to be as much as 15.8% higher than that of black sea urchin, which is likely to have had an influence on its brilliant colour. The carbohydrate content of grey sea urchins can be as much as 40% higher than that of black ones, which is responsible for the sweeter flavor of the former's roe. Dincer and Cakli [23] showed a similar carbohydrate content (about 2%) in the Turkish sea urchin.

Table 1. The nutrient content and energy value of sea urchin roe.

| Components | Per 100 g of Raw Sea Urchins Roe | |
|--------------------|----------------------------------|-----------------|
| | <i>S. intermedius</i> | <i>S. nudus</i> |
| Water, g | 73.0 ± 3.2 | 75.7 ± 2.7 |
| Protein, g | 13.9 ± 1.1 | 13.8 ± 0.7 |
| Fat, g | 7.3 ± 1.2 | 6.3 ± 1.5 |
| Carbohydrates, g | 3.5 ± 0.7 | 2.1 ± 0.4 |
| Minerals, g | 2.3 ± 0.2 | 2.1 ± 0.2 |
| Energy value, kcal | 117.3–153.3 | 102.4–138.2 |

In our study, an analysis of findings relating to the amino acid composition of sea urchin roe proteins showed that it was characterized by a high content of essential amino acids in a ratio near to the FAO/WHO standard protein scale (Table 2). The proportion of free amino acids was $3.2 \pm 1.1\%$ in the grey sea urchins' roe and $2.9 \pm 0.8\%$ in the black (of the total quantity of amino acids). The dominant amino acids of this group were glutamine and asparagine, glycine, alanine and arginine, which are involved in the development of the flavour properties of the sea urchin roe. Similar results were presented by Cuevas-Acuña et al. [24].

Table 2. Amino acid composition of sea urchin roe proteins.

| Amino Acids | Amino Acid Standard FAO/WHO g/100 g Protein | Sea Urchins Roe | | | |
|----------------|---|-----------------------|-------|-----------------|-------|
| | | <i>S. intermedius</i> | | <i>S. nudus</i> | |
| | | A | S | A | S |
| Leu | 7.0 | 7.1 ± 0.5 | 101.4 | 7.0 ± 0.6 | 100.0 |
| Phe + Tyr | 6.0 | 7.2 ± 0.3 | 120.0 | 7.8 ± 0.8 | 130.0 |
| Lys | 5.5 | 6.9 ± 0.3 | 125.4 | 6.2 ± 0.5 | 112.7 |
| Val | 5.0 | 5.8 ± 0.6 | 116.0 | 5.5 ± 0.6 | 110.0 |
| Ile | 4.0 | 4.7 ± 0.4 | 117.5 | 4.6 ± 0.5 | 115.0 |
| Thr | 4.0 | 6.6 ± 0.4 | 165.0 | 6.9 ± 0.3 | 172.5 |
| Met + Cys | 3.5 | 4.6 ± 0.3 | 131.4 | 4.8 ± 0.6 | 137.1 |
| Σ essential | 35.0 | 42.9 ± 3.1 | | 42.8 ± 3.6 | |
| Ala | | 5.7 ± 0.6 | | 5.8 ± 0.4 | |
| Arg | | 5.5 ± 0.4 | | 5.2 ± 0.3 | |
| Asp | | 11.6 ± 0.7 | | 12.1 ± 0.5 | |
| His | | 7.3 ± 0.5 | | 6.8 ± 0.3 | |
| Gly | | 4.2 ± 0.1 | | 3.8 ± 0.2 | |
| Glu | | 13.2 ± 0.6 | | 12.9 ± 0.7 | |
| Pro | | 2.9 ± 0.2 | | 3.3 ± 0.1 | |
| Ser | | 5.2 ± 0.3 | | 5.4 ± 0.4 | |
| Σ nonessential | | 55.6 ± 3.0 | | 55.3 ± 3.4 | |

Sea urchin roe fat analysis findings showed that it was characterized by neutral and polar lipids. Triglycerides and phospholipids are the main classes (Figure 1). Similar to the studies of Dincer and Cakli [23], we showed the highest content of glycine and alanine in our research, whereas the third most common amino acid was arginine, which the quoted authors did not identify. A more precise comparison is made difficult by the difference in the units in which the amino acid content is given in both studies [25].

The proportion of phospholipids is 25.5–27.1% of the total amount of lipids, which is equivalent to a content of 2.0 g in 100 g of sea urchin roe. Other authors [26] of studies on dry gonads of the same species of sea urchin showed that the content of this class of lipids was almost twice as high. Phosphatidylcholine is the main roe phospholipid (58.9–60.7%). In their studies, phosphatidylcholine also predominated among phospholipids, but its content was about 10% higher than in our studies, although they used dry gonads taken from three species (*Glyptocidaris crenularis*, *Strongylocentrotus intermedius* and *Strongylocentrotus nudus*). Phospholipids are a part of the cell membrane, providing the appropriate cell permeability. They are involved in the metabolic control of cholesterol and prevent its deposition on blood vessel walls, mitigating the risk of atherosclerosis and cardio-vascular system diseases [27]. Sea urchin roe also contain di- and mono-glycerides, free fatty acids, cholesterol and other sterols.

Sea urchin roe lipid analysis showed that the content of saturated fatty acids was 30.2% (of total fatty acid content) in the grey sea urchin roe and 33.5% in the black species (Table 3). Palmitic acid (16:0) was dominant among them, the proportion of which in the saturated fatty acids group was 40.0% in black sea urchin roe, and 50.0% in the grey species. There was also a high content of myristic acid (14:0); its quantity in the black sea urchin roe was twice as much as in grey sea urchin roe. An analysis of the fatty acids of sea urchins from the Sardinian Sea showed a much lower concentration of saturated fatty acids and a higher concentration of polyunsaturated acids [28].

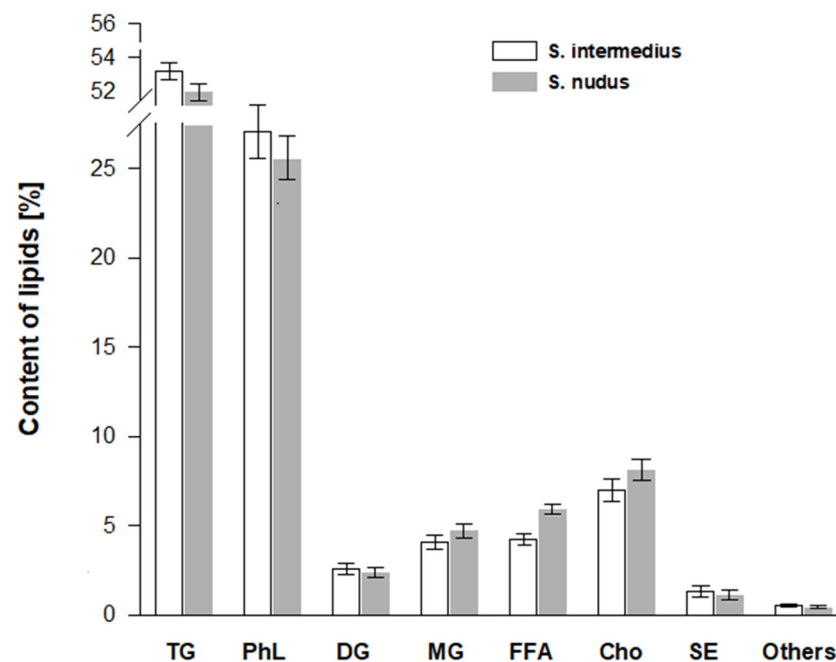


Figure 1. Lipid composition of the roe of sea urchins of the *Strongylocentrotus* genus. Notations: TG—triglycerides, PhL—hospholipids, DG—diglycerides, MG—monoglycerides, FFA—free fatty acids, Cho—cholesterol, SE—sterine ethers.

Monounsaturated fatty acids made up as much as 27.6% of the total content of fatty acids in grey sea urchin roe lipids, and as much as 31.2% in the black species. Palmitoleic acid 16:1(n-7) was dominant among monounsaturated fatty acids, with its content in grey sea urchin roe being 25.1%, and 13.6% in the black variety. The proportion of oleic acid 18:1(n-9) reached 16.5% of total fatty acid content in the grey sea urchin roe lipids, whilst in the black species it was 6.8%. Vaccenic 18:1(n-7), gadolenic 20:1(n-11) and 11-eicosenoic acid 20:1(n-9) acids were also determined; their content was no less than 10.0% of the total monounsaturated fatty acid content, irrespective of the sea urchin species. Polyunsaturated fatty acids constituted 35.3–42.1% of the total fatty acid content in the lipids of the frozen sea urchin roe. Arachidonic 20:4(n-6) and eicosapentaenoic 20:5(n-3) acids were dominant in that group, with their proportion in the polyunsaturated fatty acids total content being 24.7% and 15.0%, respectively, in the grey sea urchin, and 24.4% and 16.6%, respectively, in the black species. The ratio of omega-6 (32.2–34.3%) to omega-3 (30.9–33.4%) fatty acids in the polyunsaturated fatty acids group was 1:1 on average, which demonstrates the high value of the sea urchin roe lipids. The results of our research on lipids and fatty acids are difficult to compare with the results of Angioni and Addis [28] due to differences in the reporting of results (concentration content), although there are similar trends.

Table 3. Lipid fatty acid composition of the roe of sea urchins.

| Fatty Acid | Content, % of Total Fatty Acid | |
|--|--------------------------------|---------------------------|
| | <i>S. intermedius</i> | <i>S. nudus</i> |
| 14:0 | 6.65 ± 0.83 | 12.59 ± 2.1 ^a |
| 14:1 | 0.10 ± 0.02 | 0.32 ± 0.06 ^a |
| 15:0-i | 0.53 ± 0.04 | 2.76 ± 0.43 ^a |
| 15:0 | 0.46 ± 0.02 | 0.51 ± 0.06 |
| 16:0-i | 0.24 ± 0.01 | 0.10 ± 0.02 ^a |
| 16:0 | 16.72 ± 2.31 | 13.10 ± 1.42 ^a |
| 16:1(n-11) | - | 0.20 ± 0.04 |
| 16:1(n-7) | 6.94 ± 0.65 | 4.23 ± 0.39 |
| 16:1(n-5) | 1.21 ± 0.13 | 7.74 ± 0.75 ^a |
| 17:0-ai | 1.42 ± 0.21 | 0.10 ± 0.01 ^a |
| 16:2(n-4) | - | 0.23 ± 0.03 |
| 3,7,11,15-tetramethyl hexadecanoic acid (Phytanic) | 0.18 ± 0.01 | 0.27 ± 0.04 ^a |
| 17:0 | 0.16 ± 0.02 | 0.11 ± 0.02 ^a |
| 17:1 | 0.10 ± 0.01 | 0.13 ± 0.03 |
| 16:4(n-1) | - | 0.10 ± 0.02 |
| 18:0 | 1.93 ± 0.13 | 1.21 ± 0.23 ^a |
| 18:1(n-11) | 0.54 ± 0.03 | 0.86 ± 0.10 ^a |
| 18:1(n-9) | 4.57 ± 0.34 | 2.13 ± 0.14 ^a |
| 18:1(n-7) | 3.38 ± 0.28 | 3.71 ± 0.52 |
| 18:1(n-5) | 1.50 ± 0.09 | 0.82 ± 0.11 ^a |
| 19:0-i | 0.15 ± 0.01 | 0.12 ± 0.03 |
| 18:2(n-6) | 1.42 ± 0.21 | 0.72 ± 0.06 ^a |
| 18:2(n-4) | 0.37 ± 0.02 | 0.19 ± 0.03 ^a |
| 18:3(n-6) | - | 0.16 ± 0.02 |
| 18:3(n-3) | 1.96 ± 0.31 | 0.88 ± 0.07 ^a |
| 18:4(n-3) | 3.75 ± 0.44 | 2.29 ± 0.32 |
| 20:0 | 0.22 ± 0.04 | 0.27 ± 0.04 |
| 20:1(n-11) | 3.08 ± 0.36 | 4.91 ± 0.61 ^a |
| 20:1(n-9) | 3.38 ± 0.50 | 3.17 ± 0.27 |
| 20:1(n-7) | 1.05 ± 0.22 | 0.85 ± 0.06 |
| 20:2, Δ5,11 | 4.88 ± 0.51 | 5.17 ± 0.43 |
| 20:2, Δ5,13 | 3.12 ± 0.28 | 2.37 ± 0.30 ^a |
| 20:2(n-6) | 0.98 ± 0.11 | 0.64 ± 0.04 ^a |
| 20:3, Δ5,11,14 | 0.86 ± 0.07 | 1.04 ± 0.18 |
| 20:3(n-6) | 1.21 ± 0.15 | 0.73 ± 0.06 ^a |
| 20:4(n-6) | 10.43 ± 2.0 | 8.62 ± 1.05 |
| 20:3(n-3) | 0.76 ± 0.09 | 0.86 ± 0.07 |
| 20:4(n-3) | 0.22 ± 0.03 | 1.05 ± 0.09 ^a |
| 20:5(n-3) | 6.34 ± 0.66 | 5.85 ± 0.49 |
| 22:1(n-9) | 0.12 ± 0.02 | 0.72 ± 0.08 ^a |
| 22:2, Δ7,13 | 0.34 ± 0.02 | 0.21 ± 0.03 ^a |
| 22:2, Δ7,15 | 2.56 ± 0.34 | 1.17 ± 0.12 ^a |
| 22:6(n-3) | 0.53 ± 0.07 | 1.17 ± 0.16 ^a |
| 24:1(n-9) | 0.11 ± 0.01 | 0.50 ± 0.04 ^a |
| Others | 5.53 ± 0.67 | 5.12 ± 0.71 |

^a statistical significant differences between species $p < 0.5$.

Sea urchin roe bio testing using *Tetrahymena pyriformis* infusoria [20] as an indicator showed that the specific biological value was $102.0 \pm 9.0\%$ against casein (standard protein). This is the presence of digestible proteins in the eggs of sea urchins, together with the number and the ratio of essential amino acids. The high values of this index show the protein-exchanging intensification in the living body. In our opinion, this is due to the above-mentioned properties of sea urchin roe proteins together with the high content of polyunsaturated fatty acids, in combination with a lower proportion of neutral fats.

Frozen sea urchin roe was kept at a temperature of minus 18 °C and minus 25 °C. Its organoleptic properties remained unchanged for six months when stored at the temperature of minus 18 °C, and for 10 months at the temperature of minus 25 °C.

4. Conclusions

Technology was developed for the long-term storage of frozen sea urchin roe for healthy and dietary meals. Short-time heat treatment of raw sea urchin roe before freezing allowed us to reduce the hydrolytic processes acting on lipids and proteins, decrease the loss of moisture inside ovaries, prevent the development of a fluid consistency and maintain their quality. To evaluate the nutritional value of the studied sea urchin species under the applied storage conditions, appropriate sensory and water content evaluations, as well as analysis of chemical composition, were performed.

Frozen sea urchin roe that has undergone previous short-time heat treatment may be stored without undesirable changes in nutritional value, even for 10 months at a temperature of minus 25 °C. Thus, products from sea urchin roe may be recommended as an additional source of essential amino acids, polyunsaturated fatty acids and phospholipids due to their dietary/nutritional value.

The conducted research confirmed the differences in the chemical composition between particular species of sea urchin.

Author Contributions: Conceptualization, W.P.; Formal analysis, V.A.M. and Y.P.S.; Project administration, Y.V.P.; Writing—original draft, L.V.S.; Writing—review & editing, K.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work has been supported by the Russian Science foundation (project # 14-50-00034).

Conflicts of Interest: All contributing authors have no conflict of interest to declare.

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