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Soft-shell clam from the waters of the Kandalaksha Bay: Use for food purposes

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Abstract:
The edible part of the soft-shell clam contains a lot of protein and little fat, which makes it a good source of protein. We studied the chemical composition and nutritional value of the edible part of the soft-shell clam (*Mya arenaria* Linnaeus, 1758) living in the waters of the Kandalaksha Bay. Capillary electrophoresis was used to analyze the amino acid profile for its protein. The protein-water ratio of 17.81% and the protein-water-fat ratio of 17.69% classify soft-shell clams as a medium-protein, low-fat raw material with a high potential for food purposes. It had limiting amino acids (methionine and cysteine totaling 75.93%) and a high content of essential amino acids (valine, histidine, leucine, isoleucine, tyrosine, phenylalanine, and threonine, scoring over 100% each). The biological value of the protein was 72.34%, with a coefficient of difference between amino acid scores of 27.66%. The edible part of the soft-shell clam proved hygienically safe. Nitrosamines, pesticides, polychlorinated biphenyls, and salts of heavy metals (lead, arsenic, cadmium, and mercury) did not exceed the maximum allowable concentrations established for food products. The degree of protein digestibility was 46.8% for the edible part of the soft-shell clam frozen for 6 months at –18°C and slowly air-defrosted. Based on our results, soft-shell clams can be considered an excellent and safe source of high-quality protein and therefore be used in functional food technologies after further studies.

Keywords: Bivalve mollusk, flash freezing, amino acid profile, chemical composition, nutritional value, biological value, protein digestibility

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INTRODUCTION

The Strategy for the development of the Russian Fishery Complex up to 2030 contains several programs for developing aquaculture. One of them aims to cultivate bivalve mollusks and echinoderms, which are steadily in high demand both in the Russian and foreign markets. The main residents of the project are the Russian Far East – Primorsky Krai (90% of production) and the Azov-Black Sea basin (10% of production). Intensive technologies for growing mollusks are expected to collect at least 1.5 tons of biomass per hectare. Russia and its export markets in Southeast Asia are to become the main consumers of mollusks.

Since 2015, the export of fish and seafood from Russia to China, Japan, and South Korea has been growing steadily. These countries traditionally specialize in the fishing and industrial processing of mollusks, which are classified as delicacies. From 2014 to 2018, Russia saw a 62-fold increase in its export of crustaceans to China (from 3.7 to 230.6 million US dollars) and a three-fold increase in its export of mollusks (from 14.0 to 42.7 million US dollars). In 2018 alone, the total export of fish, crustaceans, and mollusks from Russia to China amounted to 1.63 billion US dollars. All the seafood exported to China was grown or caught in the Russian Far East [1].

The growth of interest in bivalve mollusks has been observed in recent years worldwide. Bivalves are primarily an excellent source of vitamin B_{12}, omega-3 fatty acids, choline, iron, selenium, zinc, and other micro- and macronutrients [2], as well as native protein and collagen [3–6].

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Experts from the All-Russian Association of Fishermen (VARPE) and the NTech company have recorded a significant increase in sales of the main types of shellfish. In the first seven months of 2024, retail sales increased by 54.5% in physical terms (25.6% in monetary terms) for frozen scallops, by 50.4% (45%) for squid, and by 2.4% (16.2%) for mussels. In total, the sales of these shellfish grew by 19.11% in physical terms and by 25.18% in monetary terms. This growth is more significant than that of the food market as a whole, which, according to NTech, increased by 19.7 and 7.2%, respectively. VARPE explains the high demand for shellfish by the increased popularity of pan-Asian cuisine among Russians [7].

The most promising species of bivalve mollusks for commercial use today are mollusks that live or grow in the shallow sandy waters of the Sea of Japan, the Sea of Okhotsk, and the Yellow Sea. These seas are the commercial stocks of *Anadara broughtonii*, *Spisula sachali nensis*, *Mactra chinensis*, and other mollusks at depths from 2 to 15 m [8].

The growing global demand for mollusks has promoted long-term studies of the lifestyle and production of soft-shell clams (*Mya arenaria* Linnaeus, 1758) that live in coastal sea waters and bays (Kandalaksha and Kola) adjacent to the Kola Peninsula [9–11]. According to these studies, unused reserves of this bivalve mollusk in the Far North of Russia have potential for industrial production and processing.

Dredging is the main method of extracting soft-shell clams, while freezing is the most common industrial method of their processing [12]. Bivalves – fresh or defrosted – are used to make a wide range of culinary products [13].

Today, amateur and industrial production, as well as the protection, of soft-shell clams is not regulated by the government. This increases the availability and economic attractiveness of fishing in Russia's Northern Fishery Basin.

The soft-shell clam (*Mya arenaria*), also known as "sand shell", is a bivalve mollusk that burrows into the ground to a depth of 10 m [12, 14].

Over the last 300–700 years, the species of *Mya arenaria* has re-explored the territories that its ancestors inhabited many millions of years ago. According to scientists, its dominance in the infauna of local water bodies has not affected their endemics [14]. Soft-shell clams inhabit the silty-sandy soils of the littoral of the subarctic and arctic regions of the Atlantic Ocean, including the waters of the Barents and White Seas. In the White Sea, the mollusks were found in the waters of the Onega, Dvina, and Kandalaksha bays. They prefer clean fast-flowing reservoirs, which leads to their increased population and facilitates the counting.

Soft-shell clams can withstand water temperatures from –2 to +28°C, with optimal values from +6 to +14°C. They can also withstand water salinity ranging from 25 to 35‰, with an endurance level of 5‰. They are resistant to anaerobic environmental conditions and able to survive significant oxygen deficiency for a long time.

Potential soft-shell clam fishing areas on the Kola Peninsula are the waters of the White Sea in the Gulf of Chupa of the Kandalaksha Bay, which has a high density of mollusk settlements [12]. The length of mollusks caught in this area ranges from 43 to 74 mm. Their mass ranges from 11 to 44 g, with the edible part accounting for 16–20% of the mass. 100 g of the edible part contains 13.66–16.90% of protein, up to 0.6% fat, (83.08 ± 0.40)% water, and (1.52 ± 0.005)% mineral substances (ash). Nitrogenous compounds in the edible part of the mollusk are represented by nitrogen-terminal amino acids and volatile bases, the mass fractions of which are 6 and 85 mg %, respectively [12].

Despite the abundance of data on the functional and technological properties of bivalves, as well as their composition [2–4, 12, 15], research is lacking on the hygienic safety and nutritional value of soft-shell clams living in the waters of the Kandalaksha Bay, which are of scientific and commercial value.

We aimed to study the indicators of nutritional value and hygienic safety of the soft-shell clam bivalve mollusk (*Mya arenaria*) living in the waters of the Kandalaksha Bay to justify its use for food purposes.

To achieve this aim, we sought to:

– investigate the sensory characteristics of soft-shell clams exposed to flash freezing for preservation;
– explore the chemical composition and nutritional value of soft-shell clams;
– evaluate the functional and technological properties of the mollusk using the protein-water ratio (PWR, %) and the protein-water-fat ratio (PWFR, %) coefficients to identify the most promising processing methods;
– study the amino acid composition of the soft-shell clam protein, including the mass fraction of essential amino acids (EAA), and calculate its biological value indicators (EAA scores, EAA rationality coefficient, amino acid composition rationality coefficient, EAA score difference (*ΔDAS*), EAA score difference coefficient (CDAS), biological value, and EAA comparable redundancy);
– experimentally determine the degree of digestibility of the protein in the edible part of the mollusk;
– examine the hygienic safety of the edible part of the soft-shell clam against the Technical Regulation *On the Safety of Fish and Fish Products* (TR EAEU 040/2016);
– make conclusions on the expediency of food use of the soft-shell clam inhabiting the Kandalaksha Bay.

STUDY OBJECTS AND METHODS

We studied bivalve soft-shell clams (*Mya arenaria* Linnaeus, 1758) living in the Gulf of Chupa of the Kandalaksha Bay (Murmansk Region, Russia). The mollusks were sampled in July 2015 and August 2018. Seasonal changes were not considered in the study.

Chemicals and reagents. All the chemicals used in the study were of analytical grade, produced by Russian chemical enterprises, and met the state standards of the Russian Federation.

Sample preparation. Soft-shell clam bivalve mollusks (*Mya arenaria*), 43 to 74 mm and 11 to 48 g (Fig. 1),



Figure 1 *Mya arenaria* Linnaeus, 1758 (compiled by the authors)

were collected during the expedition. Immediately after the collection, the alive soft-shell clams (about 7 kg) were frozen by the contact method to a temperature in the center of the mollusk to be –18 ± 1°C. They were transported to the laboratory and stored at the same temperature [12].

Before research, the mollusks were thawed in the air at a temperature not exceeding +8°C to a temperature in the center of the mollusk to be –1°C [12]. Then, the mollusks were cleaned manually, and the free liquid was removed. The edible part of the mollusks (about 20% of the total weight) was collected to study the chemical composition, hygienic safety, and protein digestibility. The samples were ground using a micro grinder or blender immediately prior to analysis. The frozen mollusks were stored for a maximum of 8 months.

Proximate composition analysis. The chemical composition of the edible part of the mollusk was determined using standard methods according to State Standard 7636-85. The mass fraction of water was determined by drying a crushed sample at 105°C to constant weight. The mass fraction of fat was determined by weight extraction in a Soxhlet extractor. The mass fraction of ash was obtained by mineralizing a pre-ashed sample at 600°C. The total and non-protein nitrogen contents were determined by the Kjeldahl method modified in terms of sample mineralization with concentrated sulfuric acid. For this, we used a Pro-Nitro A setup and a Selekta Bloc Digest apparatus. Crude protein was determined by multiplying the amount of total nitrogen by a factor of 5.80, which is used to determine total crude protein in jellyfish [3]. Native protein was found by multiplying the difference between total and non-protein nitrogen by 5.8. The obtained concentrations were expressed in terms of wet and dry weight. The protein-water ratio (PWR, %) and protein-water-fat ratio (PWFR, %) were calculated using Eq. (1), (2), respectively.

$$\mathrm{PWR} = \frac{P}{W} \times 100 \quad (1)$$

$$\mathrm{PWFR} = \frac{P}{W + F} \times 100 \quad (2)$$

where P is the mass fraction of protein, %; W is the mass fraction of water, %, where F is the mass fraction of fat, %.

Amino acid analysis. The amino acid composition of the edible part of the mollusk was determined by capillary electrophoresis using the Kapel system according to State Standard R 55569-2013. All determinations were made in triplicate. The amino acid content (threonine, cysteine + methionine, valine, phenylalanine + tyrosine, isoleucine + leucine, lysine) was expressed in g per 100 g of product and in g per 100 g of protein.

Amino acids. Capillary electrophoresis was used to define the mass fractions of proteinogenic amino acids in the form of phenylisothiocarbamyl derivatives – the total content (free and bound forms) of individual amino acids. Since during decomposition of a sample, asparagine and glutamine were hydrolyzed to aspartic and glutamic acids, respectively, their contents were the total contents of these acids with the respective amides. Since leucine and isoleucine were not separated under analysis conditions, we determined their sum.

The Kapel capillary electrophoresis system with a high voltage source of positive polarity was equipped with: a quartz capillary of 75 cm long and 50 μm in inner diameter, a photometric or spectrophotometric detector capable of measuring at 250 to 260 nm, and a computer with Elforan software that registers IT processing of electrophoregrams. The Kapel system is registered in the State Register of Measuring Instruments. Table 1 shows the ranges of measured concentrations of amino acids.

Amino acid scores. Contents of essential amino acids (EAA) in the soft-shell clam protein were measured by calculating the EAA C_j score, a unit fraction or % (Eq. (3)). The calculation followed the World Health Organization (WHO) reference model of amino acid requirements for children of preschool and school age (3–10 years old) and adults [9].

$$C_j = \frac{A_j}{A_{reference}} \quad (3)$$

where A_j is the EAA content in 100 g of protein, g; $A_{referens}$ is the EAA content in 100 g of native protein according to FAO/WHO, g.

Protein digestibility. The digestibility index of the soft-shell clam protein was determined by the accelerated Jaramillo method. The biological value of protein is characterized, among other things, by the degree of digestion in the human body. The rate at which proteins are attacked by gastrointestinal enzymes (such as pepsin and trypsin) characterizes the degree of protein digestion. The degree of protein digestion (DPD, %) was calculated using the Eq. (4):

$$\mathrm{DPD} = \frac{N_{digested}}{N_{total}} \times 100 \quad (4)$$

where $N_{digested}$ is the amount of nitrogen in the digested part of the protein; N_{total} is the total amount of nitrogen in the protein.

The amount of nitrogen in the digested part of the protein was calculated as a difference between total

Table 1 Amino acid concentrations measured by the Kapel capillary electrophoresis system

Amino acid	Measurement range, mg/dm³	Amino acid	Measurement range, mg/dm³
Alanine (Ala)	0.5–150	Leucine + Isoleucine (Leu+Ile)	0.5–150
Arginine (Arg)	0.5–250	Lysine (Lys)	0.5–100
Asparagine (Asn)	1.0–50	Methionine (Met)	0.4–50
Aspartic acid (Asp)	0.5–50	Proline (Pro)	0.25–500
Valine (Val)	0.4–150	Serine (Ser)	0.3–300
Histidine (His)	0.5–50	Threonine (Thr)	0.5–50
Glycine (Gly)	0.2–50	Tryptophan (Trp)	1.0–50
Glutamine (Gln)	0.5–50	Tyrosine (Tyr)	1.0–150
Glutamic acid (Glu)	1.0–50	Phenylalanine (Phe)	1.0–150

All determinations were performed in triplicate. The amino acid contents were expressed in g per 100 g of product and in g per 100 g of protein

nitrogen in the product and nitrogen in the undigested part of the protein according to the Eq. (5):

$$\text{DPD} = \frac{N_{total} - N_{residual}}{N_{total}} \times 100 \quad (5)$$

where $N_{residual}$ is the content of the residual (undigested) part of the protein.

The content of total and residual (undigested) nitrogen was determined using the accelerated Jaramillo method. For this, a sample of the test product was mineralized in a metal sleeve when heated with a mixture of caustic and sodium acetate. After that, the released ammonia was quantitatively absorbed by a 0.1 N solution of sulfuric acid. The acid that was not bound by ammonium hydroxide at the end of mineralization was determined by back titration with a 0.1 N sodium hydroxide solution in the presence of an indicator.

The amount of residual nitrogen was calculated by the Eq. (6):

$$N_{residual} = \frac{(A - a) \times 1.4}{H \times 1000} \times 100 \quad (6)$$

where A is the volume of 0.1 N sulfuric acid solution in a receiving glass, mL; a is the amount of 0.1 N sodium hydroxide solution used for titration of 0.1 N sulfuric acid solution, mL; 1.4 is the conversion factor for nitrogen; and H is the weight of the sample under analysis.

Hygienic safety indicators. The hygienic safety indicators were determined at the State Regional Center of Standardization, Metrology and Testing in Murmansk Region, the Test Performance Center for Industrial Production of Crude Products and Raw Materials (Murmansk, Russia). Frozen mollusks (–18°C in the center) with a total mass of 1 kg were sampled according to Method M-02-1009-2008 (Sampling Act dated November 19, 2018). They were analyzed for mass concentrations of toxic compounds whose maximum content is regulated in accordance with the Technical Regulation *On the Safety of Fish and Fish Products* (TR EAEU 040/2016) and Technical regulation *On food safety* (TR CU 021/2011) by standardized methods:

– lead and cadmium (mg/kg of product) were measured by atomic absorption according to the guidelines for control State Standard 30178-96;
– arsenic (mg/kg of product) was determined by atomic absorption by electrothermal atomization according to method M-02-1009-2008;
– mercury (mg/kg of product) was measured by destruction of a sample with a mixture of nitric and sulfuric acids, followed by precipitation of mercury with copper iodide, and colorimetric determination in the form of copper tetraiodomercurate against a standard scale according to State Standard 26927-86;
– nitrosamines (the sum of nitrosdimethylamine NDMA and nitrosdiethylamine NDEA, mg/kg of product) were determined by the fluorimetric chemiluminescent method according to MUK 4.1.011-93; and
– pesticides (polychlorinated biphenyls, mg/kg) were measured by capillary gas-liquid chromatography with an electron capture detector according to the methodological guidelines MUK 4.1.1023-01.

Measurement of temperature in the shell. The temperature in the thickness of the mollusk, including frozen ones, was measured using a high–precision, ultrafast TESTO 735-1 electronic thermometer (Table 2) registered in the Russian State Register of Measuring Instruments (Fig. 2).

Statistical analysis. Experimental results were expressed as mean values with standard deviation (n = 3). The least squares method was used to calculate the standard deviation.

RESULTS AND DISCUSSION

Table 3 shows the sensory properties of the soft-shell clams stored for eight months at –(18 ± 1)°C in a polymer non-hermetic container. The test samples were air-defrosted immediately before analysis.

Table 2 Characteristics of measuring instruments

Characteristic	TESTO 735-1 electronic thermometer	K/T probe (primary converter to the electronic thermometer)
Controlled temperature range, °C	from –20 up to 800	from –200 before 1370
Temperature sensitivity limit, °C	0.05	0.10
Measurement error	± 0.2°C, or ± 0.3%	± 0.3°C



Figure 2 Testo 735-1 electronic thermometer (compiled by the authors)

Table 3 Sensory properties of the frozen bivalve mollusk *Mya arenaria* after air defrosting

Indicator	Description
Appearance	The shells are intact, without contamination; no change after defrosting
Color	The shells are from milky white to grayish white; the body is from transparent pinkish white to slightly nontransparent grayish white
Consistency of the edible part	Juicy, tender
Taste	The taste is characteristic of fresh mollusk, slightly iodine; no off-flavors
Mass fraction of mineral impurities (sand, silt, etc.), %	Not more than 0.1 per total mass of the mollusk

Table 4 demonstrates the mass fractions of the main nutrients in the composition of the mollusk tissues. The protein-water ratio and the protein-water-fat ratio calculated to determine the preferred processing method for raw soft-shell clams were 17.81% and 17.69%, respectively. Figure 3 shows the amino acid profile of the protein in the edible part of the soft-shell clam. The hygienic safety indicators for the mollusk are shown in Figs. 4–8.

As can be seen in Figs. 4–8, the values of the hygienic safety indicators in the edible part of the mollusk were 6.5–87.0 times lower than the established limits.

Table 4 Chemical composition of the frozen bivalve mollusk *Mya arenaria* after air defrosting

Indicator	Result in g per 100 g of the edible part
Water	83.08 ± 0.40
Protein	13.66 ± 0.05
Fat	0.60 ± 0.02
Carbohydrate	1.16
Ash (minerals)	1.520 ± 0.005
Mass fraction of total nitrogen (TN), %	2.425 ± 0.550
Mass fraction of non-protein nitrogen (NPN), %	0.240 ± 0.110
Crude protein (CP), %	14.07 ± 3.17

Carbohydrate data are mean ± standard deviation (n = 3). The content of carbohydrates, %, was calculated according to the Eq.: Carbohydrate = 100 – (moisture + protein + fat + ash)

Our study demonstrated good preservation of frozen bivalve soft-shell clams. After 6 months of storage at –(18 ± 1)°C, the mollusks showed excellent sensory properties after air defrosting (Table 3). They had no noticeable or quality-reducing changes in appearance, color, flavor, and consistency, which is especially important for food purposes.

The edible part of the mollusk had a high protein content (Table 4). Our data were consistent with those in other studies. For example, Chinensis and Broughtonii [8] reported a protein content of 12.22 to 15.82 g in 100 g of the edible part of bivalve mollusks commercially harvested in the Sea of Japan.

Due to the low (less than 1%) fat content, soft-shell clams can be used for dietetic and functional products. Their protein-water ratio and protein-water-fat ratio coefficients classify them as medium-protein, low-fat raw material. The best ways to process them for food purposes are cooking, freezing, or manufacturing sterilized multi-component canned foods (including those with vegetables, cereals, etc.).

Table 4 shows that after two weeks of storage at –18°C, the contents of native protein and non-protein nitrogenous compounds were 90 and 10%, respectively.

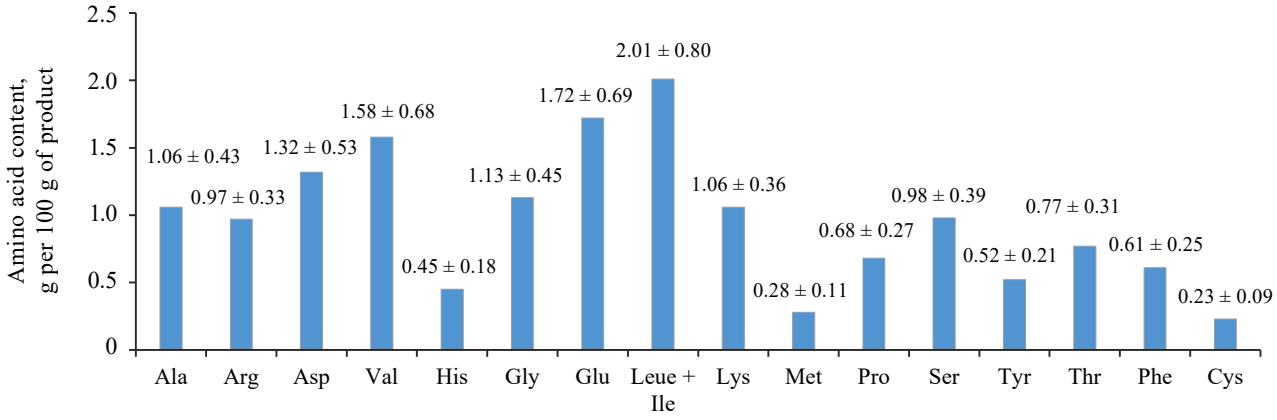


Figure 3 Amino acid profile of the protein in the edible part of the soft-shell clam. Data are mean ± standard deviation (n = 3)

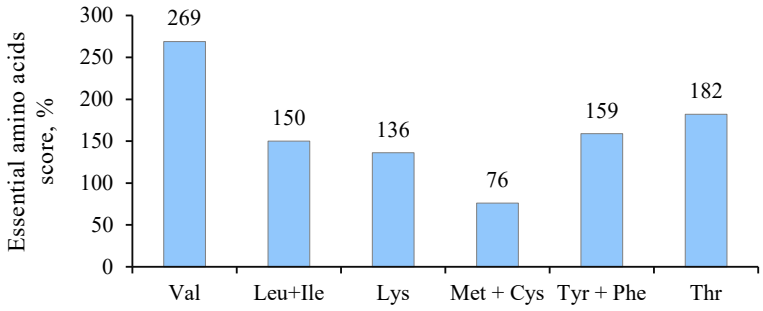


Figure 4 Essential amino acid scores in the protein of the edible part of *Mya arenaria*

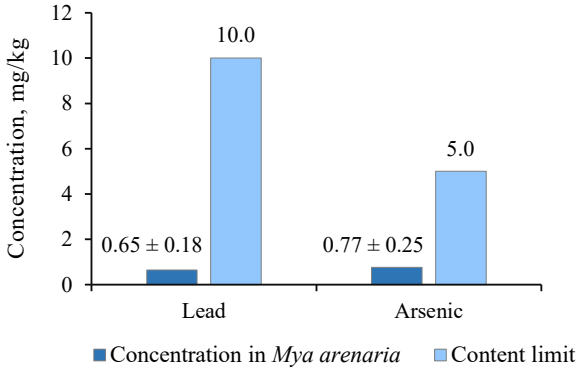


Figure 5 Concentrations of arsenic and lead in the soft-shell clams. Data are mean ± standard deviation (n = 3)

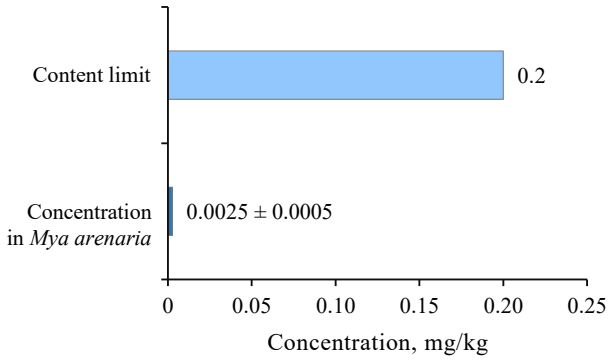


Figure 6 Concentrations of mercury in the soft-shell clams. Data are mean ± standard deviation (n = 3)

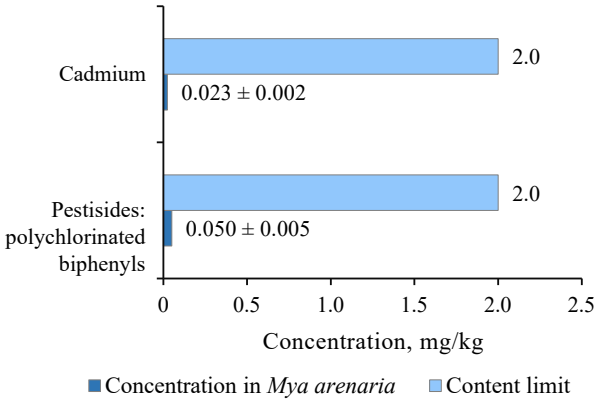


Figure 7 Concentrations of cadmium and pesticides in the soft-shell clams. Data are mean ± standard deviation (n = 3)

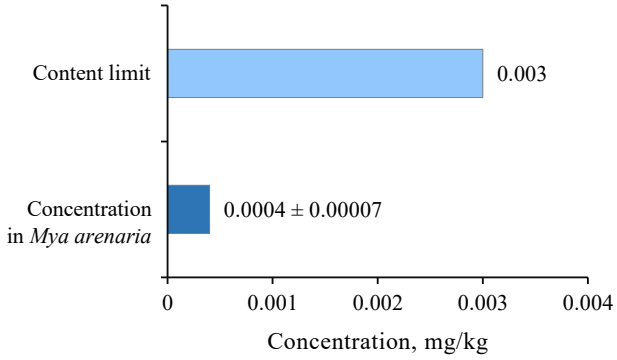


Figure 8 Concentrations of nitrosamines in the soft-shell clams. Data are mean ± standard deviation (n = 3)

This ratio of protein and non-protein nitrogen is conventional for many aquatic organisms and can indicate freshness and high quality of frozen raw materials – mollusk.

The amino acid profile shows the biological value of protein in the mollusk. The protein in its edible part contains essential amino acids and histidine, with the exception of tryptophan which was not determined. Among the essential amino acids, the pair "methionine + cysteine" (76%) was the limiting one. This result was consistent with that in the study of bivalve mollusks in the Sea of Japan (73%) [8].

The soft-shell clam protein may be considered defective in the absence of information about the tryptophan content.

The digestibility of the protein in the edible part that has not undergone thermal cooking was at a fairly high level of 50%.

An analysis of the hygienic safety of the consumed part of the mollusk revealed full compliance with the established standards. In the samples, none of the tested compounds reached the maximum permissible level. Moreover, the concentration of the detected contaminants was significantly lower than the established limits.

CONCLUSION

The edible part of *Mya arenaria* Linnaeus, 1758 is hydrated, high in valuable protein, and low in fat. The protein content per its dry weight is more than 80%. The protein of the soft-shell clam contains essential amino acids, except for tryptophan, the content of which was not determined. The dominant essential amino acids are glutamic acid, leucine + isoleucine, and valine. *Mya arenaria* living in the waters of the Kandalaksha Bay of the White Sea can be used as raw material for food production.

The biological value of shellfish products can be rised by combining them with other proteins rich in tryptophan.

CONTRIBUTION

All the authors were involved in the experimentation, data collection, management, and writing of the paper, as well as its reading and approval, prior to submission.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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