Methods for obtaining the feed additives from the starfish of the sea of Japan

E.V. Shadrina, S.N. Maksimova*, D.V. Poleshchuk
Far Eastern State Technical Fisheries University, Russia, 690087, Vladivostok, Lugovaya str., 52B
Corresponding author * : maxsvet61@mail.ru

Abstract. The results of scientific studies on the justification of the use of starfish of the Sea of Japan, Patiria pectinifera and Eustaertias echinosoma, in the technology of feed products are presented. The bio-toxicity level of the studied objects was evaluated, its dependence on the season was determined. Two methods for the production of feed additives from starfish were defined, taking into account the bio-toxicity of starfish and the destination of finished products. The method of enzymatic hydrolysis (using the proteolytic enzymes) was recommended for non-toxic raw materials for obtaining the biologically valuable protein-mineral, protein and mineral feed additives. The method of combined hydrolysis (enzymatic in combination with thermal) allows the use of raw materials, characterized as "biotoxic", with the aim of obtaining the safe protein and mineral feed additives. Technological parameters of the enzymatic (type and activity of the enzyme preparation, temperature, duration of hydrolysis and pH of the medium) and thermal (temperature, duration, pressure) hydrolysis methods are scientifically substantiated. Developed methods for the production of feed additives from starfish of the Sea of Japan provide feed additives that differ in chemical composition and purpose.

Keywords: starfish, technology, hydrolysis, enzymatic, thermal, feed additives, safety, biological value.

1. Introduction

Starfish are benthic organisms that live at depths from the water's edge to the abyssalus, not commercial species, but playing an important role in natural ecosystems [1-3]. They can influence on the number of their victims: mollusks, polychaete worms, balanus, etc., eating them in such quantity that they change the composition of the bottom marine fauna.

Starfish are varied in methods of nutrition, movement. According to the shape of the body, they can be flattened, convex, rounded without rays, with varying degrees of development of the skeleton and its derivatives. At the same time, most of the starfish are predators, feeding mainly on bivalves, as well as gastropods (snails), crustaceans, sea urchins, coral polyps. Starfish often form large clusters in areas of the seabed with an abundance of food. In oyster and mussel farms, they can be harmful, having a significant impact on the development of marine farms [4-9] (Koji Miyoshi et.al., 2018; Brun, E., 1968; Barbeau MA et al., 1998; Anne P. St-Pierre, et al., 2018; JJ Capelle, et al., 2017, Julia Calderwood et al., 2016).

In total, there are about 1,500 species of starfish, of which about 170 species and subspecies inhabit the Far Eastern seas of Russia [10] (Galyshcheva et al., 2009).

Due to its geographical location, the Sea of Japan off the coast of the Primorsky Territory is characterized by significant temperature differences in the summer and winter periods, especially in the littoral zone. In winter, the surface water layer cools down to minus 2°C, and in summer it warms...
up to plus 25°C and above, this is why two types of starfish, such as Patiria pectinifera and Evasterias echinosoma, are most common here.

Due to the development of marine culture in the Far East (both in Russia and abroad), these predatory echinoderms accumulate in large numbers in the territories of marine gardens.

According to the data obtained by Yu.A. Galysheva the accumulations of starfish in Vostok Bay averaged 3.2±2.2 ind/m² for A. pectinifera and 0.7±0.9 ind/m² for A. Amurensis [10] (Galysheva et al., 2009). According to research, the number of starfish in the reservoirs is more than 4 times the number of scallop by September. With an average starfish body weight of 3-5 g, their biomass per 1 hectare of the plantation reached 1.8 tons. Because the shell of the collector limits the movement of echinoderms, they are the main food for scallops and mussels.

The rich chemical composition of starfish allows them to be considered as a potential raw material for the production of biologically active substances and other chemical compounds: proteins, lipids, macro- and microelements, fatty acids, BAS [11-16] (Imamichi, et al., 2013; Shah Amrapali M., et al., 2008; Kakiuchi Mari., Et al., 2002; Choi DH., Et al, 1999; Ishii T., et al, 2006).

In this regard, conducting research on the evaluation of the techno-chemical characteristics and methods of industrial processing of starfish with the preservation of their raw potential is relevant and promising.

2. Materials and Methods

Starfish, the most common in the Sea of Japan, were the main research material: Patiria pectinifera and Evasterias echinosoma.

Starfish were harvested in the North Khasansky Bay of the Primorsky Territory in the spring-summer-autumn periods in a live, cooled, frozen form.

The following enzyme preparations were also used as materials for research: protosubtilin G3x (120 PU/g); collagenase (165 PU/g); protamex (400 PU/g); alcalase 2.5 L (2.5 AU-A/g (Anson units)).

The determination of protein substances was done by oxidation of an organic substance by burning it in sulfuric acid in the presence of a catalyst, distilling the resulting ammonia with steam, trapping it with a solution of sulfuric acid and determining the nitrogen content by titration.

The mass fraction of fat was determined by the extraction method in the Soxhlet apparatus. The method is based on the extraction of fat with an organic solvent from a dry sample and the determination of its mass by weighing [17].

The mass fraction of water was determined by evaporating it from the sample under study during heat treatment at a temperature of 100–105°C and determining the change in mass by weighing it.

Mineral substances were determined by removing the organic substances from the analyzed sample by burning and determining the resulting ash by weighing.

The amino acid composition of the proteins was determined on a Hitachi L-8800 aminoanalyzer by liquid chromatography in accordance with the instruction manual for the device.

The number of mesophilic aerobic and facultative anaerobic microorganisms was determined by sowing the test sample into the nutrient medium, incubating the crops, counting all the visible colonies grown.

The degree of toxicity was estimated using the accelerated biotesting method of the object under study by assessing the toxicity of the biological test system (ciliates) Tetrahymena pyriformis, which included a 48-hour exposure of the samples with a population of ciliates. During this time, changes in the vital activity of organisms were recorded and the threshold of toxic action was estimated according to the degree of their death.

3. Results and Discussion

To determine the rational methods of industrial processing of starfish and the intended purpose of the finished product, the overall chemical composition of the raw material (starfish entirely and its individual parts of the body) was studied (Tables 1, 2).
Table 1. - The total chemical composition of starfish, %

| Starfish                  | Water  | Protein (total nitrogen) | Lipids | Mineral substances | Nitrogen-free extractives |
|---------------------------|--------|--------------------------|--------|-------------------|--------------------------|
| *Evasterias echinosoma*   | 73.2-70.16 | 10.76-11.90 | 0.70-1.75 | 12.64-13.20 | 2.70-3.99                |
| *Patiria pectinifera*     | 68.1-58.52 | 8.03-9.02  | 0.30-1.17  | 22.3-28.04  | 1.27-3.25                |

The study of the total chemical composition of starfish showed that they contain protein from 8.03 to 11.90%, the amount of mineral substances varies from 12.64 to 28.04%.

The results of the study of the total chemical composition of different body parts of starfish are shown in Table 2.

Table 2.- Chemical composition of different parts of starfish, %

| Sample          | Water/dry substances | Protein (total nitrogen) | Lipids  | Mineral substances |
|-----------------|----------------------|--------------------------|---------|--------------------|
| *Evasterias echinosoma* |          |                   |         |                    |
| Cover tissue    | 71.90 / 28.10        | 9.50 (1,520)            | 0.88    | 17.07              |
| Viscera         | 74.75 / 25.35        | 14.34 (2,295)           | 6.11    | 1.91               |
| Gonads          | 83.80 / 16.30        | 11.59 (1,855)           | 1.86    | 2.39               |
| *Patiria pectinifera* |          |                   |         |                    |
| Cover tissue    | 56.74 / 43.31        | 9.66 (1,545)            | 0.77    | 32.04              |
| Viscera         | 80.60 / 19.40        | 10.25 (1,640)           | 1.35    | 7.67               |
| Gonads          | 81.25 / 18.81        | 13.15 (2,105)           | 3.80    | 1.32               |

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Experimental data indicate that amount of dry substances in *Evasterias echinosoma* is 15% less than in *Patiria pectinifera*, which is associated with increased mineralization of Patiria cover tissue. The dry substances amount in the guts and caviar of starfish does not differ significantly.

The amount of protein in the cover tissue of *Evasterias echinosoma* and *Patiria pectinifera* is almost the same (9.5 - 9.7%). In the insides of sea stars, the amount of protein is different and ranges from 10.25 to 14.34%. The value of this indicator in caviar of starfish is comparable with the protein content in the entrails and is 11.6 - 13.1%.

The amount of lipids in the cover tissue of stars is insignificant (0.77 - 0.88%). The amount of lipids in the insides of *Evasterias echinosoma* is 6.1%, which is 4.5 times higher than in the insides of *Patiria pectinifera*. The amount of lipids in the caviar of *Evasterias echinosoma* is two times less (1.9%) than in *Patiria* (3.8%).

Thus, from the conducted research, the dependence of the chemical composition of both the whole star and its individual tissues on its species is observed. This is connected, in our opinion, with the difference in the degree of mineralization of the cover tissues of starfish and with their biological state, first of all, with maturity.

Studies have shown that the proteins of both starfish contain all the essential amino acids. Their total amount in the cover tissues of the stars is 36.4 - 39.4% of the value of amino acids, in the insides of *Patiria pectinifera* and *Evasterias echinosoma* - 40.3 and 44.3%, respectively. The amount of essential amino acids in caviar of starfish is 40.0 - 41.0%.

The amount of mineral substances in the cover tissue of *Evasterias echinosoma* is almost two times lower (17.07%) than in the tissue of *Patiria pectinifera* (32.04%). The amount of mineral substances in the insides of starfish is also noticeably different, but their number is low (in *Evasterias echinosoma* - 1.91%, and in *Patiria* - 6.67%). The amount of mineral substances in the starfish caviar is insignificant (1.32 - 2.39%).

The amount of macro- and microelements in the studied samples of starfish is presented in Table 3. *Patiria pectinifera*
Table 3. The amount of macro- and microelements, mg/kg (in raw tissue)

| Sample          | P     | Na    | Ca     | K      | Mg     | Mn | Fe   | Zn    | Cu   | Ni   |
|-----------------|-------|-------|--------|--------|--------|----|------|-------|------|------|
| Evasterias echinosoma |       |       |        |        |        |    |      |       |      |      |
| Cover tissue    | 1332,2| 276,8 | 2009,0 | 83,15  | 116,07 | 5,32| 63,78| 55,21 | 18,66| 0,11 |
| Viscera         | 152,9 | 190,1 | 0,8    | 95,05  | 18,37  | 0,28| 112,8 | 36,73 | 5,43 | 0,08 |
| Gonads          | 180,1 | 235,5 | 0,64   | 106,42 | 24,55  | 0,58| 16,83| 20,07 | 1,91 | 0,09 |
| Patiria pectinifera |       |       |        |        |        |    |      |       |      |      |
| Cover tissue    | 775,8 | 194,3 | 2049,1 | 48,43  | 226,54 | 37,66| 67,25| 11,34 | 9,98 | 0,07 |
| Viscera         | 101,2 | 161,4 | 3,1    | 124,0  | 1,36   | 0,19| 216,9| 41,42 | 3,95 | 0,08 |
| Gonads          | 92,8  | 231,9 | 36,2   | 130,74 | 45,0   | 42,6| 71,69| 22,69 | 3,14 | 0,09 |

The peculiarity of the covering fabric of Evasterias echinosoma is a higher phosphorus content, which is almost twice the amount of this element in the cover part of Patiria pectinifera, which in turn is richer in magnesium than in Evasterias echinosoma, almost twice [17]. The data on calcium amount for two samples is of the same order. Compared with the covering tissue, the internal organs of both types of stars have a higher amount of potassium and iron. The sodium amount in all parts of the body of stars is approximately the same.

Evaluation of the chemical composition of starfish allows us to characterize them as valuable protein raw materials, rich in macro- and micro-elements, which predetermines the prospects for the use of these echinoderms in the production of feed additives.

Since it is known that starfish are characterized by varying degrees of biotoxicity [19] (Kicha A.A., et al, 2011), this indicator was assessed in Patiria pectinifera and Evasterias echinosoma using the biotesting method of individual anatomical parts of the objects studied. Evaluation of the results and conclusions on the degree of biotoxicity are presented in Table 4. According to the method, the heating was carried out at a temperature of 80°C for 20 minutes. The biotoxicity estimation was differentiated by levels. With a strong and moderate level of toxicity, there are deviations in the development of cells of the test culture of Tetrahy mena pyriformis, characterized by a decrease in motility or fixation of individuals.

Table 4. Estimation of starfish biotoxicity

| Sample          | Sample preparation | Duration of exposure 5 min | 2 h | 24 h | 48 h | Estimation of biotoxicity |
|-----------------|--------------------|---------------------------|-----|------|------|--------------------------|
| Evasterias echinosoma |                   |                           |     |      |      |                          |
| Cover tissue    | Unheated           | 1,5                       | 4,8 | 3,0  | —    | Toxic                    |
|                 | Warmed up          | 2,8                       | 7,3 | 9,0  | 61,3 | Nontoxic                 |
| Viscera         | Unheated           | 3,7                       | 30,0| —    | —    | Moderately toxic         |
|                 | Warmed up          | 6,3                       | 17,3| 45,0 | 42,6 | Nontoxic                 |
| Gonads          | Unheated           | —                         | —   | —    | —    | —                        |
|                 | Warmed up          | 2,0                       | —   | —    | —    | Highly toxic             |
| Patiria pectinifera |                   |                           |     |      |      |                          |
| Cover tissue    | Unheated           | 2,5                       | 2,7 | 82,0 | 76,8 | Moderately toxic         |
|                 | Warmed up          | 2,3                       | 4,7 | 135,0| 170,0| Nontoxic                 |
| Viscera         | Unheated           | —                         | —   | —    | —    | —                        |
|                 | Warmed up          | 2,5                       | 2,6 | —    | —    | Toxic                    |
| Gonads          | Unheated           | —                         | —   | —    | —    | —                        |
|                 | Warmed up          | —                         | —   | —    | —    | Highly toxic             |

α→ν - cell death

The results of the studies have shown that the gonads of both starfish are highly toxic, regardless of the sample preparation. The viscera of the Patiria pectinifera retain toxicity even in the warmed up sample, which is not characteristic of Evasterias echinosoma. Cover tissues of Patiria were assessed as moderately toxic. After thermal treatment, the covering tissues of both starfish were rated as nontoxic [20] (Bogdanov, 2015).
The determined biotoxicity of starfish confirms the literary data and substantiates the expediency of developing a method for obtaining a safe feed product.

Reducing the biotoxicity of certain types of starfish tissues can be achieved by using the special technological methods. The greatest effect under the experimental conditions was achieved using the alcohol extraction and high-temperature treatment, as a result of which the tissues of starfish were rated as non-toxic [20] (Bogdanov, 2015).

As shown by the results of experimental studies, under using the starfish, characterized by a low level of biotoxicity, it is advisable to apply the technology with one-step hydrolysis (enzymatic), in which the finished product is characterized by high biological value and availability of components for the consumer's body.

The technological scheme of the production of feed additives by biomodification of starfish using the enzyme preparations (by method of enzymatic hydrolysis) is presented in Figure 1.

In the case of processing of starfish caught during the spawning period and characterized as “biotoxic”, the technology of feed additives using two-stage combined hydrolysis (enzymatic combined with thermal) was developed and substantiated, since using only enzymatic hydrolysis does not provide detoxification of the raw materials. The combined hydrolysis, due to the presence of harsh heat treatment at 140 °C in the technological process, provides for the detoxification of starfish.

The technological scheme of the production of feed additives using the combined method is presented in Figure 2. This technological scheme differs from the previous one by introducing into the technological process an additional operation “thermal hydrolysis” and the absence of the use of sorbic acid.

Feed additives obtained from starfish in two ways have similar organoleptic characteristics: powder from light cream to brown-gray color with a pleasant sea smell.

The chemical composition of feed additives is significantly different, which determines the purpose of the developed feed products (Table 4).

| The method of obtaining the feed additive | Feed additive | Water | Protein | Lipids | Nitrogen-free extractives | Mineral substances |
|-----------------------------------------|---------------|-------|---------|--------|--------------------------|-------------------|
| Enzymatic                               | PMFA          | 6.90  | 27.41   | 7.4    | 25.28                    | 33.01             |
|                                         | PFA           | 8.97  | 58.35   | 14.92  | 3.47                     | 15.29             |
|                                         | MFA           | 5.73  | 11.94   | 2.82   | 20.99                    | 58.52             |
| Combined                                | CPFA          | 7.60  | 76.40   | 0.50   | -                        | 15.5              |
|                                         | CMFA          | 6.50  | 12.43   | 3.13   | 8.45                     | 69.49             |

As can be seen from the results presented in Table 4, feed additives PFA, CPFA and PMFA are characterized by a high protein content and can be used for the cultivation of poultry, and the feed products MFA, CMFA and PMFA, characterized by a rich mineral composition, are recommended for laying hens.

4. Conclusion

As a result of experimental studies of the technological characteristics of the *Patiria pectinifera* and *Evasterias echinosoma* starfish of the Sea of Japan, the expediency of using these common and reproducible aquatic biological resources as feedstock to obtain feed additives has been substantiated.

Studies of the chemical composition of starfish showed the predominance of albumens in *Patiria pectinifera* and mineral substances in *Evasterias echinosoma*. The quantitative content of proteinaceous (from 8.03 to 11.90%) and mineral substances (from 12.64 to 28.04%) in starfish can be the basis for the production of protein-mineral, protein and mineral feed additives.
Figure 1 - Technological scheme of the production of feed additives from starfish by the method of enzymatic hydrolysis
Figure 2 - Technological scheme of the production of feed additives from starfish by combined method of hydrolysis
To obtain digestible and biologically valuable feed products, biomodification of starfish was applied by enzymatic hydrolysis using the proteolytic enzymes. Based on toxicity studies and methods of detoxification of studied starfish and their individual components, the need to develop two methods for obtaining the feed products from these objects has been substantiated. Since the tissues of starfish in different seasons of their catch were characterized by varying degrees of biotoxicity (from non-toxic to strongly toxic), detoxification was applied using the thermal hydrolysis at 140 °C. Thus, two methods of industrial processing of sea stars have been developed, depending on the level of their toxicity: enzymatic and combined (enzymatic in combination with thermal) hydrolysis. Technological parameters of the enzymatic preparation (type and activity of the enzyme preparation, temperature, hydrolysis duration and pH of the medium) and thermal (temperature, duration, pressure) hydrolysis methods were scientifically grounded. The developed methods for obtaining the feed additives from starfish were differentiated in application both in terms of the level of toxicity of the feedstock and the intended use of safe and biologically valuable ready-made feed products (protein products for growing the poultry and mineral products for laying hens).

Conflicts of Interest: The authors declare no conflict of interests.

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