Assessment of biotechnological potential of king crab 
(P.camtschaticus) peeling waste

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Abstract. The article presents the results of the study of the biotechnological and biogenic potential of king crab (P. camtschaticus) peeling waste, obtained during the production of boiled-frozen limbs. The waste used was a tadhopper shell and a conditionally protein portion (gills and insides) of the crab. It has been experimentally established that the proportion of the shell is about 60-65% of the weight of the waste, the proportion of the conditional protein-lipid part varies within the range of 30-35%. The waste contains proteins (13.37 ± 0,05%), lipids (2.68 ± 0,1%), minerals (8.14 ± 0,25%) and carbohydrates (4.22 ± 0,05%). Biological value of wastes is confirmed by data of amino acid composition of proteins, content of macro- and microelements and determination of relative biological value. Obtained results confirm high potential of king crab peeling waste as raw material for biologically valuable products.

1. Introduction

Now more and more attention is paid to the use of waste processing of food raw materials. For their rational use, it is necessary to study the chemical composition, primarily the composition of proteins and amino acids [1, 2].

At present, during crab fishing and processing in marine application according to the current technological instructions, crab peeling wastes include: carapace, abdomen, gills, membrane, insides (including liver). According to the data of different sources, the share of waste from crab processing can be up to 80% [3, 4].

Tentatively, the waste is represented by three fractions: chitin (shell of the head and limbs), protein (abdomen) and lipid (insides). In the crab food production, most often boiled-frozen limbs, there are carapace, abdomen and insides after cutting. All three fractions (chitin, protein and lipid) are included in this waste.

In most cases, these wastes are not sorted, accumulated or recycled, but disposed of by dumping into the sea. In the event that a large amount of waste is dumped at one site, the sanitary condition of the water area may deteriorate. Therefore, the problem of crab waste reclamation is urgent and up-to-date [5].

Among the Far Eastern crabs, the most widely known is king crab (P. camtschaticus). It belongs to craboids (Lithodidae), as it has four pairs of walking limbs, the fifth is hidden and is under the
pantsuit. The habitat is the Bering Sea, the Okhotsk Sea, the Sea of Japan, the Barents Sea, the west coast of North America from the area of Cape Barrow to the archipelago of Queen Charlota in the south [6-8].

The body of king crab consists of a carapace and a poorly developed belly. The inner bone skeleton at the king crab is absent, with the entire body covered with a hard panty. Carapace, which covers the head and chest, serves as an external skeleton and protects the internal organs. The carapace includes chitin [9].

The size and weight of the crab depends on the species, age and gender. The king crab at the age of 2-3 years has the shell of the head-chest - 2-2.5 cm wide, at the age of 9-10 years - 10-12 cm, and by 17-18 years it reaches 19-20 cm.

To solve the problem of rational processing of crab, you can conduct analytical and experimental studies of king crab peeling waste in order to obtain biologically valuable products from it.

2. The purpose of the study
The main problem is the rational and complex use of the raw material of the water origin. The high percentage of peeling waste is the peculiarity of processing. The aim of this research is the determination of biogenic and biotechnology potential of king crab peeling waste for biologically valuable products.

3. The object of the study
The object of the study was a king crab peeling waste; crab was caught in North-Okhotsk subzone and subzobne Pnymorye.

4. Materials and methods
The main research material was the frozen king crab (*P.camtschaticus*) peeling waste, caught in the Sea of Japan. The composition of the waste included the carapace, gills, insides (including liver). The ratio of different parts of the king crab (*P.camtschaticus*) body was determined according to the guidelines of VNIRO.

Preliminary preparation of samples for the research was grinding the carapace, gills, insides and mixing them thoroughly.

The study of the samples chemical composition was carried out according to the standard method according to GOST 7636-85.

The frozen portions of the crab were hydrolyzed for 22 h at 110 ± 1 °C with 6 M HCl in sealed glass tubes filled with nitrogen. Following hydrolysis, 1 mL of hydrolyzate was withdrawn and evaporated to dryness under vacuum at 45 °C to remove HCl. The hydrolyzate was dissolved in 1 mL of sodium citrate buffer (pH 2.2), and then the samples were analyzed by a Hitachi L8800 Automatic Amino Acid Analyzer (Hitachi, Tokyo, Japan). The identity and quantity of each amino acid was assessed by comparison with the retention time and peak area of standard (Sigma).

The tryptophan content was determined in a separate analysis. The weighed samples were hydrolyzed in 5 N NaOH containing 5% SnCl2 (w/v) for 20 h at 110 °C [8]. After hydrolysis, the hydrolyzate was neutralized with 6 N HCl and centrifuged, and then the supernatant was analyzed by a Hitachi L8800 Automatic Amino Acid Analyzer. The identity and quantity of tryptophan was assessed by comparison with the retention time and peak area of standard (Sigma). All determinations were performed in triplicate. Amino acid ((threonine (Thr), tryptophan (Trp), cysteine + methionine (Cys+ Met), valine (Val), phenylalanine + tryptophan (Phe+ Tyr), isoleucine (Ile), leucine (Leu), lysine (Lys)) contents were expressed as protein content g / 100 g.

Amino acid score. Essential amino acid score was calculated with respect to the FAO/WHO reference of amino acid requirement pattern for the preschool and school children (3–10 years old) and adults as follows:

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\text{Amino acid score} = \frac{\text{Amino acid in a sample}}{\text{Reference amino acid}} \times 100
\]
Determination of elements. Mineral contents in the dried crab portions were determined by atomic absorption spectrometry using a spectrometer AA-7000 (Shimadzu, Japan) with the system of double atomization (flame and electrothermal). Iron(Fe), zinc(Zn), copper(Cu), manganese(Mn), nickel(Ni), molybdenum(Mo), aluminium(Al), cadmium(Cd), plumbum(Pb) and chromium (Cr) were determined using graphite cuvette and deuterium background corrector lamp according to the method of AOAC. The content of mercury was determined by flameless atomic absorption using the analyzer of mercury «Hg-1» («Hiranuma», Japan).

Determination of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) in the dried clam portions uses air-acetylene flame at fuel flow rate of 50 L h⁻¹ according to the method of AOAC Method 999.11.

5. Discussion of the results

The main task is to determine the crab size and mass characteristics, including king crab (P.camtschaticus) while studying the biogenic and biotechnological potential of king crab peeling waste for industrial processing.

The width of king crab (P.camtschaticus) carapace varied within 12-24 cm, which is consistent with the results of previously conducted studies [10]. Since the size of the carapace differed significantly, the samples were divided into size groups, as shown in table 1. In the formation of waste samples, only the cephalothorax was considered, from which waste was extracted: the shell (together with the membrane), gills, and insides (including the liver). The results of the studies of the arithmetic mean values of the five parallel definitions are presented in table 1.

| Table 1. The mass fraction of king crab (P.camtschaticus) peeling waste, %. |
|-------------------------------|------------------|------------------|------------------|
| Type of peeling waste | Width carapace, cm |
| | 12-16 | 16-20 | 20-24 |
| Carapace | 62.20 | 59.90 | 65.30 |
| Gills | 11.80 | 12.30 | 5.30 |
| Insides | 25.90 | 27.80 | 29.30 |

While studying the mass fraction of king crab waste, it was found that the shell was noticeably predominant among them, averaging from 60 to 65% of all peeling waste. The proportion of the conditionally protein-lipid part, including the gills and insides, varied between 30-35%.

Because of absent sorting peeling waste in the conditions of fishing and production of food crab products, it was decided to combine all the waste into a single common sample, called «mixed peeling waste».

Further research was aimed at assessing the biogenic and biotechnological potential of «mixed peeling waste». In the course of research, we noted that about 65% of king crab samples have a carapace size from 16 to 20 cm. Based on the data obtained, it was decided to use individuals of those size groups in which the number of samples is predominant in future studies.

The results of total chemical composition of «mixed peeling waste» are presented in table 2.

| Table 2. The total chemical composition of the king crab (P.camtschaticus) «mixed peeling waste». |
|-------------------------------|------------------|------------------|------------------|------------------|
| Sample | Dry matter | Protein | Mass fraction, % | Mineral substance | Carbohydrates |
| | | | | | |
| Peeling waste | 28.41± 0.31 | 13.37 ± 0.05 | 2.68 ± 0.1 | 8.14 ± 0.25 | 4.22± 0.05 |

The obtained data show that «mixed peeling waste» is characterized by a high content of dry substances – 28.41%, consisting mainly of proteins (13.37%) and minerals (8.14%), which together make up 76% of the mass of all waste.
When studying the protein component of «mixed peeling waste», the full range of amino acids was determined (table 3).

**Table 3.** The content of interchangeable and irreplaceable amino acids in the «mixed peeling waste» of the king crab (*P.camtschaticus*).

| Amino acids | Protein content g / 100 g | FAO/WHO scale | Amino acid score |
|-------------|--------------------------|---------------|-----------------|
| Thr         | 5.121                    | 4.0           | 128.02          |
| Ile         | 4.143                    | 4.0           | 103.57          |
| Leu         | 6.605                    | 7.0           | 94.35           |
| Val         | 4.441                    | 5.0           | 88.82           |
| Lys         | 6.129                    | 5.5           | 111.43          |
| Cys +Met    | 0.248+0.406              | 3.5           | 18.68           |
| Tyr +Phe    | 4.733+4.308              | 6.0           | 150.68          |

The amount of essential acids

| Sample       | Content, mg/kg |
|--------------|----------------|
| Peeling waste|                |
| Na           | 6228           |
| Ca           | 18202          |
| K            | 2013.9         |
| Mg           | 1340.1         |
| Mn           | 0.15           |
| Fe           | 96.04          |
| Zn           | 60.16          |
| Cu           | 14.89          |

The content of essential amino acids in the king crab (*P.camtschaticus*) peeling waste exceeds the amount of essential amino acids for an ideal protein [10]. The amino acids threonine, lysine, tyrosine and phenylalanine for all samples correspond to the level set by FAO/WHO, the rest are limiting.

As we noted above, most of the peeling waste is shell. The share of minerals in the king crab (*P.camtschaticus*) peeling wastes is to 8.14. To identify the peeling waste mineral components we have carried out studies of macro- and microelements in table 4.

**Table 4.** Content of macro-and microelements of king crab (*P.camtschaticus*) by dry weight.

As can be seen from the results presented in table 4, the king crab peeling waste is dominated by elements such as calcium and sodium. It is also worth noting the high content of potassium and magnesium. The presence of macro and microelements in the samples under study provides a high biological value of «mixed waste». Taking into account the high specific weight of peeling waste, the experimental data obtained are of not only scientific but also practical interest.

For the biological evaluation of king crab (*P.camtschaticus*) peeling waste, an express method was used using the *Tetrahymena pyriformis* infusoria, which was most widely used in research practice.

The results of these studies are shown in figure 1.
Figure 1. The growth curve of the infusoria cells number in the media under study.

It is known that the dependence of the development of microorganisms on environmental conditions can be determined by measuring the growth of biomass over a certain period of time. Thus, based on the results of studies to determine the number of infusoria cells on media containing samples of king crab (*P. camtschaticus*) peeling waste, it was noted that their development over time is subject to a known pattern (figure 1). According to this figure, we can note several successive stages of development in a certain sequence, during which the rate of cell reproduction changes. For samples of muscle tissue and the king crab (*P. camtschaticus*) peeling waste, the period (the plot curve from 24 to 72 hours) is typical for the logarithmic or exponential growth phase. The duration of this phase for the sample muscle tissue of the king crab (*P. camtschaticus*) was 96 h. To the late logarithmic phase, the number of cells reached a maximum, and there was no long-term stationary phase of development, after which came a period of decay characterized by a decrease in number of cells due to depletion of the nutrient medium and accumulation in the products of metabolism of ciliates.

The duration of each phase and the nature of the growth curve characterize the speed of the life processes of the test organism and, as a result, the relative biological value (or digestibility) of the object under study.

To determine the relative biological value of the waste, the muscle tissue of the king crab (*P. camtschaticus*) was used as a comparison sample. The results are presented in table 5.

Table 5. The results of determining the relative biological value of the studied samples.

| Samples                        | The number of infusoria cells in 1 cm$^3$ during the transition to the stationary phase | Relative biological value, % relative to the control sample |
|--------------------------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------|
| King crab (*P. camtschaticus*) peeling waste | 6.8x10$^5$                                                                            | 40.0                                                     |
| Control – king crab (*P. camtschaticus*) tissue | 1.7x10$^6$                                                                            | 100                                                      |
The relative biological value of the studied samples was lower than that of the comparison sample, since the waste contains a large amount of mineral substances, and lipids are also present, which are a less attractive source of nutrients for the infusoria. However, the value of the studied indicator for waste is significant.

6. Conclusion
The obtained data allow us to conclude that from a biological point of view, the «mixed peeling waste» has its own value, which can be used for the production of various types of feed and biological valuable products.

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