Article

Prospects of Red King Crab Hepatopancreas Processing: Fundamental and Applied Biochemistry

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Abstract: Since the early 1980s, a large number of research works on enzymes from the red king crab hepatopancreas have been conducted. These studies have been relevant both from a fundamental point of view for studying the enzymes of marine organisms and in terms of the rational management of nature to obtain new and valuable products from the processing of crab fishing waste. Most of these works were performed by Russian scientists due to the area and amount of waste of red king crab processing in Russia (or the Soviet Union). However, the close phylogenetic kinship and the similar ecological niches of commercial crab species and the production scale of the catch provide the bases for the successful transfer of experience in the processing of red king crab hepatopancreas to other commercial crab species mined worldwide. This review describes the value of recycled commercial crab species, discusses processing problems, and suggests possible solutions to these problems. The main emphasis is placed on the enzymes of the hepatopancreas as the most highly salubrious product of waste processed from red king crab fishing.

Keywords: marine fisheries; aquatic organisms; brachyura; anomura; commercial crab species; red king crab; Kamchatka crab; processing waste; hepatopancreas; waste recycling; enzymes; proteases; hyaluronidase

1. Crab Processing Waste

The global growth in consumer needs for food products based on commercial species of marine organisms (fish, crabs, squids, etc.) has stimulated fishers to increase production. However, the inability to increase the catch volume endlessly and related waste recycling problems have forced the consideration of advanced processing of secondary raw materials to obtain new and commercially valuable products. Worldwide, crabs are a favorite catch of fishers due to the high price of their meat. The main northern commercial species of crab include the following:

- Red king crab (Paralithodes camtschaticus)
- Blue king crab (Paralithodes platypus)
- Spiny brown king crab (Paralithodes brevipes)
- Golden king crab (Lithodes aequispinu)
- Opilio snow crab (Chionoecetes opilio)
- Tanner snow crab (Chionoecetes bairdi)
- Triangle tanner crab (Chionoecetes angulatus)
- Red snow crab (Chionoecetes japonicus)
- Chinese mitten crab (Eriocheir sinensis)
- Hair crab (Erimacrus isenbeckii)
The first four species belong to the infraorder of half-tailed or fake crabs (Anomura) of the suborder Pleocyemata of the order Decapoda. The rest are of the infraorder of true crabs (Brachyura) (Fig. 1).

All crabs have a massive cephalothorax (covered with carapace from above), a flat abdomen, and are bent under the cephalothorax. Brachyura representatives move with the help of four pairs of pectoral legs, and the fifth pair of limbs are claws. A specific feature of the Anomura is the asymmetric structure of the body (the right claw is larger than the left) and the presence of only three pairs of walking legs (one of the five pairs is hidden under the carapace and is used for the regular cleaning of the gills).

Red king crab, or Kamchatka crab, is famous around the world. In recent decades, the catch of this crab by Russian fishers has reached 15,000–20,000 tonnes per year. The original habitat of the red king crab ranges from Karaginsky Island off the east coast of Kamchatka and Shelikhov Bay in the Sea of Okhotsk to Hokkaido Island and the Korea Strait. The crab is also found on the west coast of North America from Cape Barrow to the Queen Charlotte Archipelago in the south [1,2].

Red king crab was successfully introduced to the Barents Sea in the 1960s and 1970s. Optimal temperature conditions, the absence of natural predators, and a sufficient amount of food led to the spread of acclimatized red king crab from the coast of the Kola Peninsula to Norway and north
to Svalbard (Fig. 2). Individuals of this king crab population are larger, grow faster, and mature earlier than individuals of the far-eastern population [3]. The rapid growth of the northern king crab population is an environmental problem [3,4]. The growth rate of the resource potential of this population made it possible to open commercial fishing in Norway in 2002 and in Russia in 2004.

![Red king crab native and invasive distribution](image)

**Figure 2.** Red king crab range.

More than half of the red king crab catch is waste. In Russia, the crab catch is often processed immediately on a fishing vessel. One of the methods for processing the crab on a ship is as follows: the just-caught crab is placed on a hook; the limbs are additionally cleaned, boiled, and frozen; and the broken carapace with entrails is dumped immediately into the sea as a waste product. On average, this waste can account for 50% of the catch mass [5,6]. The mass fraction of carapace from these wastes is approximately 60%; the rest comprises the entrails (including the digestive organ, the hepatopancreas) [2]. In terms of protein content, these wastes are identical to crab meat, and even superior in terms of the content of minerals, lipids, and carbohydrates. Crab flour can be obtained as animal feed from crab processing waste. In addition, the crab shell is an excellent source of raw materials for the production of chitin and chitosan, which can be used to meet the demands of the food industry and medicine [6].

### 2. Crab Hepatopancreas

The hepatopancreas is an organ of the digestive system that functions as both the liver and pancreas [7]. In red king crab, the hepatopancreas makes up about 90% of the intestines of the carapace and 5–10% of the total weight of the animal [8]. Due to digestion and crab diets, the Decapoda hepatopancreas secretes a wide variety of highly active enzymes.

Under the action of the digestive enzymes of the hepatopancreas, food is broken down into easily digestible substances. The hepatopancreas of crabs of the family Lithodidae (infraorder Anomura, for example, red king crab) consists of a brown mass of fragile liver tubules and occupies most of the body cavity. The integrity of these tubules is easily destroyed, even with a slight mechanical impact, and the enzymes enter the body cavity, where they start the process of autolysis. The hepatopancreas of real crabs (Brachyura, for example, crab-opilio) is a shapeless orange-brown mass [1].

### 3. Red King Crab Hepatopancreas Enzyme Use

The hepatopancreas of the digestive system of commercial crabs is a valuable source of a complex of enzymes with various activities: collagenolytic, proteolytic, hyaluronidase, lipase, nuclease, etc.
The complex of proteolytic enzymes of the red king crab hepatopancreas is of interest in various industries. For example, the prospect of using an hepatopancreas enzyme preparation in the hydrolysis of soy protein was recently highlighted [9]. A red king crab hepatopancreas enzyme preparation was successfully used to separate eggs from the connective tissue of ovaries of commercial fish [10], to hydrolyze minced fish meat to obtain a dietary food ingredient [11], to hydrolyze crustacean processing waste products to obtain components for microbiological nutrient media [12], and to isolate chondroitin sulfate from marine wastes, namely from tissues of marine aquatic organisms [13]. Based on collagenolytic proteinases, wound healing and wound cleansing preparations [14–16], including wound dressings [17,18], have been designed.

4. Hepatopancreas Recycling Technologies

Many technologies are used to process the hepatopancreas of commercial crab species to obtain enzymatic preparations. Most technologies were developed for the hepatopancreas of king crab, but some were transferred to the processing of the hepatopancreas from other commercial species (for example, snow crab and blue crab) [19,20]. Most often, the initial raw material is the hepatopancreas, which is homogenized in salt buffers or by osmotic shock under hypotonic conditions (excess distilled water). The integrity of the hepatopancreas tissue is easily destroyed during the freezing/thawing process; therefore, no considerable efforts are required for mechanical homogenization. However, rarely, a colloid mill can be used [21]. Sometimes, triton X-100 or sodium dodecyl sulfate (SDS) is added to the homogenization buffer [19,22]. The homogenate is incubated at room temperature for several hours. Under such conditions, cell autolysis occurs, which increases the level of protein extraction; however, this can lead to the inactivation of target enzymes. A significant problem in further processing is caused by lipids. Ballast substances and lipids are removed from the homogenate by centrifugation or flocculation followed by chitosan filtration [20–24] or immediately by filtration through the hollow fiber [25,26]. The filtrate is concentrated by ultrafiltration with hollow fibers with a pore size of 15–30 kDa and then dried by freeze-drying or spray-drying. The choice of hollow fibers with a specific pore size (considering the variation in real pore sizes) should be based on the known molecular weights of the target enzymes (Tables 1 and 2). To obtain more purified preparations before drying, proteins are precipitated with ammonium sulfate and/or tert-butanol, and then ion exchange, hydrophobic, or affinity chromatography is performed [27–30]. In the simplest case, after homogenization, proteins are precipitated with an excess of cooled acetone (“acetone powder”) [31]. This method is not suitable for scaling, since the use of a large volume of acetone is unsafe for both humans and the environment. Most of the developed technologies have been successfully tested on tens and hundreds of kilograms of hepatopancreas as raw materials [19–21,23–26]. The yield of dry final substance in such protocols varies from 0.6% to 1.3% based on the feedstock [23–26], and the collagenolytic activity is 500–3,000 units Mandl/mg (the substrate is collagen type I, at 37 °C) [25,26], even reaching 11,000 Mandl/mg (the substrate is collagen type III, at 42 °C) [19], whereas the amount of protein in dry matter is 80–98% [19,23,25,26].

The basic conditions for enzymes obtained with the mentioned technologies include the maintenance of a neutral pH of the enzyme solution; at pH values below 5.5 and above 9.5, the activity of the proteolytic complex and its individual components dramatically decreases. At pH values below 3 and above 10.5, parts of the enzymes are irreversibly inactivated [23,32].

5. Enzymes of the Red King Crab Hepatopancreas

5.1. Proteolytic Enzymes

Currently, 10 proteolytic enzymes of hepatopancreas—collagenolytic serine proteinase PC (PC—Paralithodes camtschaticus), collagenolytic serine proteinase PC 2, trypsin-like proteinase A, chymotrypsin-like proteinase C, aminopeptidase PC, carboxypeptidase PC, trypsin PC, elastase, cathepsin L, and metalloproteinase—have been described in the literature in detail (Table 1). Most
of these are small proteins up to 30 kDa (based on the denaturing gel electrophoresis data) with an isoelectric point (pI) of 2.5–4.1. The optimal working conditions for these enzymes include a neutral pH and temperature range from 25 to 55 °C. The collagenolytic serine proteinases are of particular interest due to their ability to degrade native collagen of types I–III. Most likely, these two enzymes are the basis of the active substance in existing wound healing and wound cleaning preparations. Collagenolytic activity has also been assigned to other hepatopancreas proteinases. However, the temperature of the reaction mixture in these studies was often 42 °C, at which native collagen partially denatures; therefore, the measured activity may refer not to true collagenase but to gelatinase activity. Despite the large number of scientific works devoted to hepatopancreas enzymes, the complete nucleotide sequence of mRNA is known only for collagenolytic serine proteinase PC, trypsin PC, cathepsin L, and metalloproteinase.

5.2. Nucleases and Other Enzymes of Hepatopancreas

The red king crab hepatopancreas is a source of enzymes such as nucleases (Tab. 2). Ca$^{2+}$- and Mg$^{2+}$-dependent DNase, which is characterized by high thermal stability, has been studied in detail: the enzyme is thermostable after 3 h of incubation at 60 °C, whereas incubation for 30 min at 100 °C resulted in only 7% loss of activity [49]. Based on the circular dichroism (CD) spectrum, the protein appears to be a compact globule and consists mainly of β-layers (75%) [50]. The pI value is 4 and the optimal reaction temperature range is 50–60 °C [51].

This DNase has a pronounced specificity for the secondary structure of DNA and predominantly cleaves double-stranded substrates (DNA and RNA–DNA duplexes, while the RNA remains intact). The minimum duplex size for cleavage by DNase is 9 bp; the enzyme does not hydrolyze RNA [51]. The DNase cleaves phosphodiester bonds with the formation of 5′-phosphate and 3′-OH terminal groups. Ca$^{2+}$ and Mg$^{2+}$ together have a positive synergistic effect on the rate of the hydrolysis reaction. The unique properties of DNase enable it to be used effectively for the rapid analysis of single nucleotide polymorphisms and the normalization of nucleic acid libraries [52–55]. In the hepatopancreas of the king crab, other nucleases (RNases) and phosphoesterases were found (Tab. 2).

The enzyme preparation from the hepatopancreas contains a number of other enzymatic activities, which allows this enzyme complex to be used for the depolymerization of β-glycosidic bonds in chitosan [59–61]. Lipase activity has also been observed [23]; however, other activities of hepatopancreas enzymes have not been sufficiently studied in comparison with proteolytic and nuclease activities.

5.3. Hyaluronidase Activity of hepatopancreas Homogenate

Currently, the structure of hyaluronidases of the Malacostraca class has not been studied in sufficient detail. In the open database of UniProt protein sequences, there are only four representatives of this class for which amino acid sequences of hyaluronidases are available: two representative of Decapods, including Whiteleg shrimp (*Litopenaeus vannamei*, UniProt A0A423SH46) and orange mud crab (*Scylla olivacea*, two UniProt proteins A0A0P4VV1 and A0A0N7ZAX3), and two representative of Isopoda, which are pill-bug *Armadillidium vulgare* (only one of two proteins is characterized as UniProt A0A444ST78) and *Armadillidium nasatum* (UniProt A0A5N5TJL6) (Figure 1). Earlier, hyaluronidase activity was found in the complex of enzymes of hepatopancreas homogenate [62].

In our previous work, the kinetics of the hydrolysis of hyaluronic acid in cosmetic fillers with hepatopancreas homogenate was studied for the first time [63]. Using the methods of turbidimetric analysis, atomic force microscopy, and nuclear magnetic resonance spectroscopy, the kinetics of hydrolysis and the structural transformation of hyaluronic acid under the action of homogenate enzymes were investigated. We found that the obtained homogenate has activity comparable to commercially available hyaluronidase preparations. In this work, we demonstrated the possibility of using an enzymatic preparation based on an hepatopancreas homogenate for the treatment of complications after the injection of fillers based on hyaluronic acid. Further studies of the effectiveness...
<table>
<thead>
<tr>
<th>Proteinase</th>
<th>kDa (based on electrophoresis)</th>
<th>Opt. pH</th>
<th>Opt. °C</th>
<th>pI</th>
<th>Substrate specificity and other</th>
</tr>
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<tbody>
<tr>
<td>Collagenolytic serine proteinase PC [33]</td>
<td>29 23.5(^1) [34]</td>
<td>7.5</td>
<td>47–55</td>
<td>3</td>
<td>Preferably hydrolyzes peptide bonds, the carbonyl part of which consists of Arg, Lys, and hydrophobic amino acids. Hydrolyzes native collagen type I even at 4 °C [35]. The mRNA nucleotide sequence was determined [34], European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database: AF461035.</td>
</tr>
<tr>
<td>Collagenolytic serine proteinase PC 2 [36]</td>
<td>25</td>
<td>8.5</td>
<td>38–40</td>
<td>?</td>
<td>Preferably hydrolyzes peptide bonds, the carbonyl part of which consists of positively charged amino acids. Hydrolyzes native collagen types I–III.</td>
</tr>
<tr>
<td>Trypsin-like proteinase A [37]</td>
<td>27 30 [38]</td>
<td>7.9</td>
<td>55 [38]</td>
<td>2.5</td>
<td>Preferably hydrolyzes peptide bonds, the carbonyl part of which consists of Arg and Lys. Proteolytic activity is not inhibited by ethylenediaminetetraacetic acid (EDTA), and partially inhibited by soybean trypsin inhibitor.</td>
</tr>
<tr>
<td>Chymotrypsin -like proteinase C [39]</td>
<td>24</td>
<td>9</td>
<td>55 [38]</td>
<td>2.9 [38]</td>
<td>Preferably hydrolyzes peptide bonds, the carbonyl part of which consists of hydrophobic amino acids (Phe, Val, and Leu). Not inhibited by Tos-Phe-CH(_2)Cl (chymotrypsin inhibitor).</td>
</tr>
<tr>
<td>Aminopeptidase PC [40]</td>
<td>110</td>
<td>6</td>
<td>36–40</td>
<td>4.1</td>
<td>Effectively cleaves N-terminal amino acids: Arg, Lys, Leu, Phe, and Met. Most likely it is a homodimer, Zn-containing enzyme.</td>
</tr>
<tr>
<td>Carboxypeptidase PC [41]</td>
<td>34</td>
<td>6.5</td>
<td>55</td>
<td>3.1</td>
<td>Effectively cleaves C-terminal amino acids: Arg, Lys, Phe, Tyr, Leu, and Ile. The enzyme is inhibited by 0.5 mM Ag(^+), Zn(^{2+}), Cd(^{2+}) and 1 mM EDTA, whereas it is activated by Co(^{2+}) and Ca(^{2+}).</td>
</tr>
<tr>
<td>Trypsin PC [42]</td>
<td>29 23 [43]</td>
<td>7.5–8</td>
<td>55</td>
<td>&lt;2.5</td>
<td>Preferably hydrolyzes peptide bonds, the carbonyl part of which consists of Arg and Lys. The mRNA nucleotide sequence was determined [34], EMBL: AF461036.</td>
</tr>
<tr>
<td>Elastase [44], [45]</td>
<td>28.5</td>
<td>8–8.5</td>
<td>50 [46]</td>
<td>4.5</td>
<td>Hydrolyzes native elastin (inhibited by elastinal), Triton X-100 (1%) and SDS do not affect the elastinase activity. NaCl, MnCl(_2), CdCl(_2) at a concentration of 1–100 mM stimulate elastinase activity, whereas it is inhibited by HgCl(_2) (100 mM).</td>
</tr>
<tr>
<td>Cathepsin L [47]</td>
<td>29 24(^1) [43]</td>
<td>8</td>
<td>25</td>
<td>?</td>
<td>Enzyme has cathepsin activity, hydrolyzes Z-Phe-Arg-pNA substrate. Hydrolyzes collagen types X and VI. HgCl(_2), E64, and leupeptin inhibit cathepsin activity; soybean trypsin inhibitor practically does not suppress activity. The mRNA nucleotide sequence was determined, EMBL: HQ437281</td>
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<tr>
<td>Metalloproteinase [48]</td>
<td>22.2(^1)</td>
<td>8–8.5</td>
<td>45</td>
<td>?</td>
<td>Destroys peptide bonds formed by both acidic and hydrophobic amino acids. Hydrolyzes azocollagen. Proteolytic activity is maintained at 1–3 M NaCl and is inhibited by isopropanol, o-phenanthroline, and EDTA. Zn-containing enzyme. The mRNA nucleotide sequence was determined, EMBL: AF492483</td>
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Table 1. Hepatopancreas proteolytic enzymes. \(^1\) expected molecular weight based on the mRNA nucleotide sequence, \(^2\) mass spectrometry data. PC—Paralithodes camtschaticus.

and safety of hyaluronidase from hepatopancreas on model animals will enable us to develop new drugs for the treatment of complications of filler injections in the near future.
Enzymes | kDa (based on gel-chromatography) | Opt. pH | Known properties
--- | --- | --- | ---
Ca\(^{2+}\)- and Mg\(^{2+}\)-dependent DNase [56] | 53 and 47\(^1\) 42\(^2\) 41.5\(^3\) [51] | 7–8 6.6 [51] | The primary structure was determined. There are two sequences in UniProtKB, Q8I9M9 (2003) and B6ZLK3 (2009), differing by two amino acids.

Alkaline RNase (AlkR) [57] | 19 | 7.2–7.5 | Broad specificity. Poorly hydrolyzes poly(AUC). MgCl\(_2\) at a concentration of 10–50 mM stimulates the activity of enzyme. 0.1 M NaCl inhibits the enzyme activity by 50%.

Acid RNase (AcR and AcR') [57] | 33 and 70 | 5.5 | Does not hydrolyze poly(C) and poly(AUC). MgCl\(_2\) inhibits its activity. 0.25 M NaCl inhibits its activity by 50%. Most probably, these are monomeric and dimeric forms of the same protein.

Two acidic phosphomonoesterases [58] | 80 and 82 | 5.5 | Do not hydrolyze (3',5')cAMP; 1.5 M NaCl inhibits the enzyme activity by 20% (protein 80 kDa); 1.1M NaCl inhibits the enzyme activity by 50% (protein 82 kDa).

Alkaline phosphomonoesterase [58] | 80 | 7.2–7.5 | Does not hydrolyze (3',5')cAMP (cyclic adenosine monophosphate). 0.4 M NaCl inhibits the enzyme activity by 50%.

Acidic phosphodiesterase [58] | 57 | 4.8–5 | 50% inhibition at 1.4 M NaCl.

Alkaline phosphodiesterase [58] | 51 | 7.2–7.5 | No inhibition up to 1.4 M NaCl is observed.

Table 2. Nucleases and other enzymes of hepatopancreas. 1 denaturing gel chromatography data, 2 denaturing electrophoresis, 3 expected molecular weight based on the mRNA nucleotide sequence.

6. Other Valuable Non-protein Components of the King Crab Hepatopancreas

The crab hepatopancreas is a source of enzymes and other valuable products. For example, a preparation with the properties of an inhibitor of serine proteinases was obtained and characterized from the hepatopancreas, and its effect on the process of human blood plasma coagulation was studied [64]. The tested inhibitor showed an anticoagulant effect that was more pronounced when combined with heparin. Procedures have been developed to obtain an inhibitor both from the raw material [65] and in the recombinant form [66].

The hepatopancreas contains a large amount of fat, which varies from 10% to 26% [8,67,68]. In the study of the fractional composition of crab fat, it was found to contain triglycerides at a rate of up to 55%, as well as polyunsaturated fatty acids, including n-3 (14%–24% of the total of all fatty acids). Hepatopancreas fat does not contain toxic substances and can be used as a food supplement or as an ingredient for cosmetic products.

7. Prospects of Waste Processing from Other Commercial Crab Species

The commercial species of crabs include representatives of the fake (Anomura) and true crabs (Brachyura) of Pleocyemata. Brachyura includes the Opilio snow crab (Chionoecetes opilio), which is also a commercially important catch for marine fisheries. Crabs of this suborder have similar enzymatic activity in their digestive system. For example, the proteolytic activities of enzyme preparations from the hepatopancreas of red king crab and Opilio crab are practically the same [11]. The zymogram showed that the hepatopancreas of the Opilio snow crab contains at least 10 proteolytic enzymes.
The activity of the proteolytic complex was comparable to the commercially available collagenase of the gas bacillus *Clostridium histolyticum* [69,70]. In these works, proteolytic enzymes of the hepatopancreas of the Opilio snow crab, but not the red king crab, were isolated and biochemically characterized, and the amino acid N-terminal sequences of proteolytic enzymes were obtained. The authors noted their mistake in their next work [71]. Unfortunately, these incorrect data were published in the UniProt database. For example, the amino acid sequence UniProtKB-P20734 (COGC_PARCM) actually belongs not to *Paralithodes camtschaticus*, but to *Chionoecetes opilio*. The sequence of UniProtKB-P34153 (COG1_CHIOP) and UniProtKB-P34156 (COG4_CHIOP) is not derived from the hepatopancreas proteins of *Chionoecetes opilio*, but from *Paralithodes camtschaticus*. The rest of the N-terminal sequences of proteolytic enzymes of these two crabs in [69,70] completely coincide in the UniProt database, which once again confirms the biochemical similarity of the digestive systems of the representative of Pleocyemata.

The close phylogenetic kinship and similar ecological niches of the commercial crab species, as well as the industrial scale of the catch, provide grounds for the successful transfer of the experience of the processing of the king crab hepatopancreas to other crab species to obtain new and valuable products. For example, the enzymatic complex of hepatopancreas of the Opilio snow crab was successfully used in the production of protein hydrolysates from cod waste processing, as well as to improve the consistency and juiciness of cod fillets at the stage of the salting of the semi-finished product [11,20,72].

Russia is a leader in terms of the catch level of red king crab, and this catch is increasing every year. Most of this catch comes from fishers in the Russian Far East; however, the total catch of Russia and Norway in the Barents and Norwegian Seas is expected in the near future to equal and even exceed the catch level in the Russian Far East. In the United States (Alaska), red king crab is also caught, but to a lesser extent. Fishers in Alaska have caught 200–400 tonnes of king crab per year from 2013 to 2020 [73]. However, at the same time, the United States catches about 5,000–10,000 tonnes of crabs that are collectively known as king crabs [74]. Other commercial species of crabs are also promising species for advanced processing. For example, Food and Agriculture Organization (FAO) data indicate that the global catch of Opilio snow crab is more than 100,000 tonnes. We confidently state that high catch levels of large commercially significant crabs, and therefore vast amount of waste, provide an opportunity to develop waste processing technologies and include them in the industrial process. The most promising direction of processing should be considered the processing of the hepatopancreas as a source of new and high-margin products.


Crab is processed using different approaches. If the caught crab is not boiled, complex waste processing is possible to maximize the yield of new and valuable products. After limb separation, the rest of the body can be completely processed: the carapace can be used for chitosan production, the hepatopancreas for the production of multicomponent enzymatic complexes and specific purified enzymes, the fat from the hepatopancreas for dietary nutrition or biofuel production, and the gills and other internal organs as a feed supplement for birds, fish, and other animals. This approach adopts the principle of non-waste recycling.

The processing methods of the crab hepatopancreas to obtain enzyme preparations can be divided into two strategies: obtaining complexes of various enzymes or the further purification of specific enzymes (or class of enzymes) from this complex. The enzyme complex is appropriate for the treatment of multicomponent substrates where the simultaneous degradation of proteins, nucleic acids, and other polymers into monomers is required, for example, in the production of easily digestible food products from animal and plant tissues. The activity of the enzyme complex in the preparations will differ for the hepatopancreas on a case-by-case basis. In this regard, the technology for using such enzymatic preparations should allow for fluctuations in the activities in different batches of the preparation. The
second strategy, based on the isolation of one enzyme from the complex, has advantages, since the final product can be used to produce a high-margin product (for example, a pharmaceutical product or a reagent for scientific research). For this purpose, it is important to have a simple and scalable method for the purification of a specific enzyme. Notably, all the currently existing technologies for processing the crab hepatopancreas do not meet the specified requirements. The main vector of development in this field could be an approach including several stages of tangential flow filtration and affinity chromatography using a cheap carrier.

All these works discussed in this review consider the processing of a specific type of waste (carapace/hepatopancreas/fat). However, the waste is most often a mixture, which is difficult to sort. To implement the principle of non-waste recycling, it is necessary to develop new approaches to recycling this mixture, which will convert the waste into new valuable products. The relevance of deep processing consists of the sustainable continual increase in catches of fisheries. On the one hand, a growth in catch leads to an increase in waste, which might cause a number of environmental problems. On the other hand, insufficient control of the catch will lead to a decrease in the crab population, which will entail a sharp decline in the economic performance of the industry in the coming years. Thus, all types of processing could reduce the environmental burden, as well as help satisfy the financial appetites of the industry by selling new valuable products.

The main problem with the complex processing of wastes from commercial marine species is the complexity of their collection and storage under sailing conditions in a trawler and delivery onshore for further processing. The solution to this problem could be, for example, the creation of a maximally automated integrated waste processing unit immediately on a fishing vessel, which could eliminate the problem of the time spent by fishermen on the manual removal of an organ, as well as its storage and transportation. Another method of solving this problem is to establish an adequate cost of the hepatopancreas for fishers, allowing a stable supply of raw materials onshore on an industrial scale. The task could be simplified if the non-waste processing of live crabs is organized onshore. Further studies of the hepatopancreas of commercial crab species in fundamental scientific terms and considering its potential applications will increase the value of crab processing waste, possibly leading to a time when this will equal or even exceed the cost of crab meat.
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Klimova, O.; Chebotarev, V. Collagenolytic complex of proteases from the hepatopancreas of the Kamchatka crab: enzymologic activity of the individual components. Biuleten’ eksperimental’ noi biologii i meditsiny 1999, 128, 391–396.


Sakharov, I.; Dzhunkovskayia, A. Elastase from the hepatopancreas of the king crab. Biochemistry (Moscow) 1993, 58, 1445–1453.


57. Menzorova, N.I.; Sibirtsev, J.T.; Rasskazov, V.A. Ribonuclease from the Hepatopancreas of the Red King Crab *Paralithodes camtschatica*. *Applied Biochemistry and Microbiology* 2009, 45, 369–373.


