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COMPARATIVE CHARACTERISTIC OF HYDROLYTIC ENZYMES OF THE BARENTS SEA INTRODUCED CRABS *CHIONOECETES OPILIO* AND *PARALITHODES CAMTSCHATICUS*

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Nowadays one of the key problems of the fish-processing industry in Russia is the development of new fishery objects and their rational use. Due to the increase of snow crab abundance in the Barents Sea, it is possible to predict the potential of this species for the industrial fishing. According to the data obtained by PINRO scientists, in 2009, the total stock of snow crab in the Barents Sea was more than 10 million individuals (Pavlov, 2010).

For several decades, hepatopancreas of the red king crab (*Paralithodes camtschaticus)* has been successfully used for production of complex enzymatic preparations applied in medicine and cosmetology, and also in food and microbiological branches of industry in order to obtain the protein hydrolyzates (Klimova et al., 1990; Mukhin and Novikov, 2001). The snow crab (*Chionoecetes opilio*) hepatopancreas, which has been insufficiently studied in this respect, is also of interest.

The objects of research were the enzymatic preparations derived from hepatopancreas of crustaceans *Paralithodes camtschaticus* and *Chionoecetes opilio*, caught in the different areas of the Barents Sea in 2008–2009.

To obtain enzymatic preparations (EP) the comminuted hepatopancreas was processed with acetone and n-butanol in order to remove lipids and low-molecular compounds (Sakharov et al., 1988).

The fractional composition of proteins in samples was determined by the method of low pressure gel-chromatography using «Pharmacia LKB Biotechnology» equipment. Sephadex G-100 Superfine was used as a stationary phase in a column (1,6x70 sm), 0.15 n NaCl (pH 7) – as an eluent buffer. The protein fractions were registered applying photometry at 280 nm (the optical path length – 2 mm). The molecular weight of proteins (MW) was determined using the calibration curves built after the run of the proteins with known MW through the column: thyroglobulin (670 kDa), g-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) as well as vitamin B12 (1,35 kDa) (Laurent and Killander, 1964).

Proteolytic activity was estimated using the Anson's method**,** by the hydrolysis of 1% sodium caseinate solution (Alekseenko, 1968). The temperature and pH values at which the protease activity was maximum were also determined.

Exochitinase activity was calculated through the release of N-acetylglucosamine (GlcNAc) generated by the chitin hydrolysis, the content of GlcNAc in a hydrolyzate solution was determined by the reaction with 4-dimethylaminobenzaldehyde (Decleire et al., 1996).

In the enzymatic preparations obtained from hepatopancreas of two crab species, *Paralithodes camtschaticus* and *Chionoecetes opilio,* chitinolythic and proteolytic activity has been determined. Seasonal dependence of protease activity of these two crustacean species has been found (Fig. 1). Thus, the activity level of EP in winter-spring period exceeded that one in summer-autumn for both crabs. The obtained results agree with the data of other researchers (Nemova, 1996; Mukhin and Novikov, 2002) and indicate the influence of seasonal rhythms on the activity of proteases. It may be caused by both the feeding pattern and the effect of the environment abiotic factors.

Considerable seasonal fluctuations of proteolytic activity in hepatopancreas of crabs indicate high adaptive abilities of the latter ones.

The molecular-weight composition of proteins in obtained EP for both crab species is quite similar (Fig. 2, Table). The high-molecular fraction makes up a considerable part of the total number of proteins in both samples.

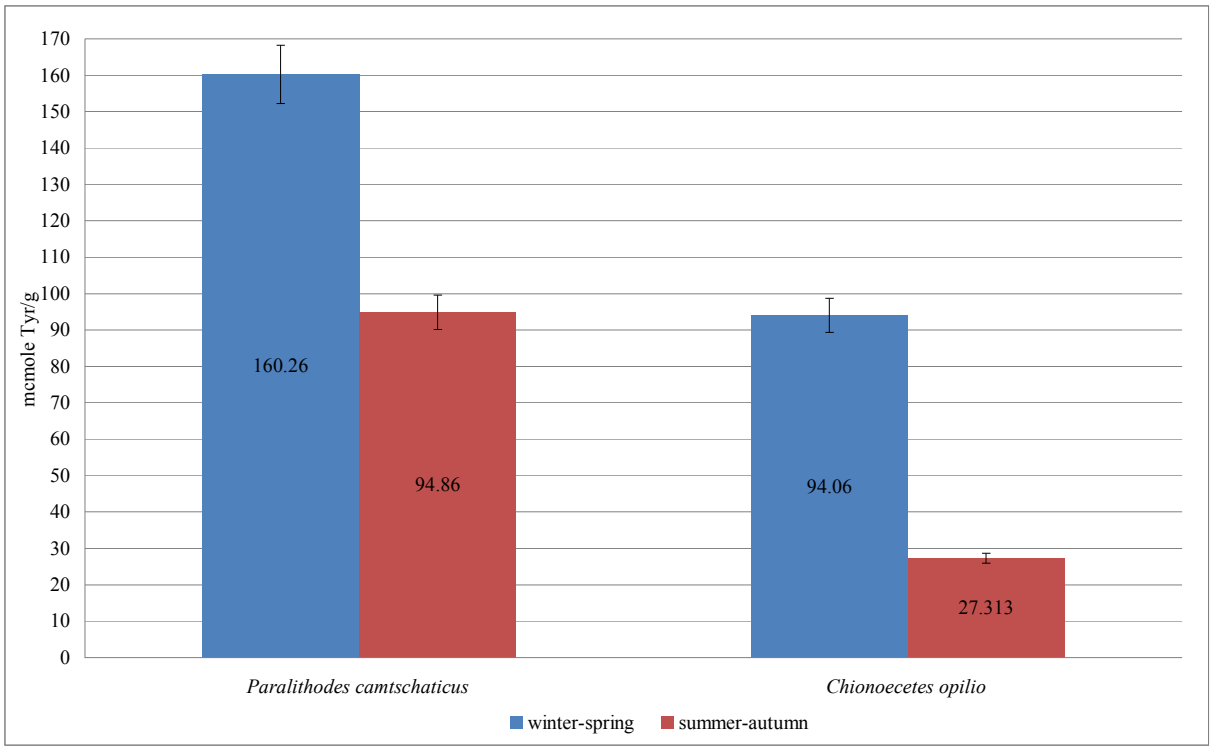

Fig. 1. Seasonal variations of the proteolytic activity of EP, derived from snow and red king crabs

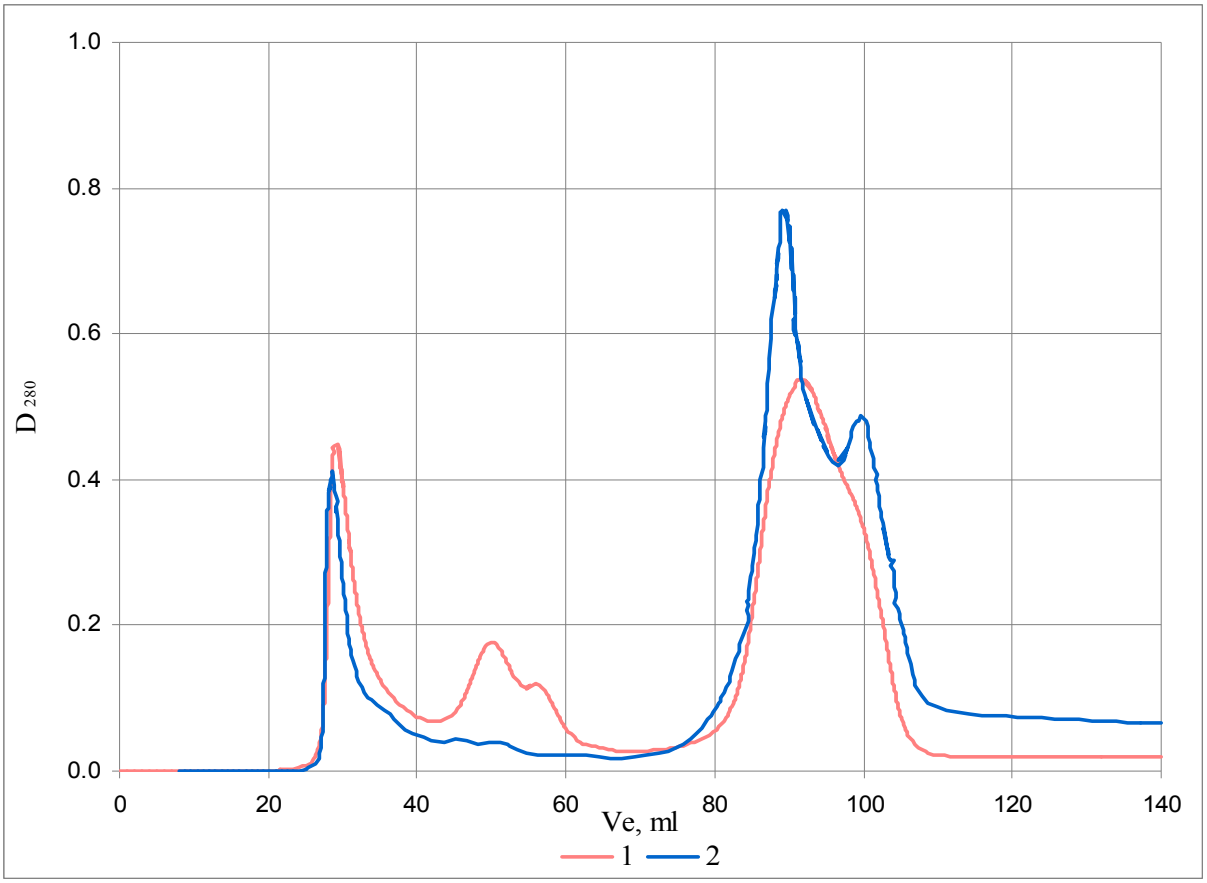

Fig. 2. The elution profile of proteins of hepatopancreas EP: 1 – *Paralithodes camtchaticus*; 2 – *Chionoecetes opilio*. Column Sephadex G-100 Superfine (1.6x70 sm), elution speed 12 ml/hour

Table. Fractional composition of proteins in studied EP

The source of EP	Protein fractions with MW (kDa),%		
	<4	10–70	>100
The red king crab *Paralithodes camtchaticus*	68.12±0.3	16.09±0.1	15.79±0.1
The snow crab *Chionoecetes opilio*	82.47±0.4	1.38±0.1	16.15±0.1

According to the available data, the protein fraction with MW from 10 to 40 kDa provides the basic proteolytic activity of proteinase preparations from the red king crab hepatopancreas (Mukhin and Novikov, 2002). The percentage of proteins with intermediate MW in EP obtained from the red king crab hepatopancreas is 10 times more than that one in the snow crab EP. Nevertheless, the proteotytic activity values are of the same order of magnitude in summer-autumn, and differ less then twice in winter-spring. Then, it is possible to assume that the specific activity of intermediate protein fractions of snow crab hepatopancreas EP is higher. Therefore, with the appropriate EP processing it is possible to predict higher activity of proteinases in it.

A considerable portion of low-molecular fractions in the examined samples can be explained by the drawbacks of the method to derive EP applying which a certain amount of low-molecular ballast substances is not removed from the preparation. On the other hand, some proteins with higher MW can get to the last fraction, due to the insufficiently complete chromatographic division caused by the interaction of the carrier and the examined sample.

When determining the conditions (pH, temperature) for the maximal EP hydrolytic activity the following data have been obtained: the activity peaks were observed at pH of 4.0 and 7.0, the maximum activity was noted at 50 °C. At the temperature of 5–10 °C the EP activity was minor. These data are similar to the characteristics obtained in researches on the proteolytic activity of EP from hepatopancreas of the red king crab (Mukhin and Novikov, 2002). Thereby, the hydrolysis for the industrial purposes can be carried out without the change of conditions for both EPs.

The average exochitinase activity for the red king crab is 2.16 mg of GlcNAc/ml, for the snow crab – 2.03 GlcNAc/ml, i.e. the given values are quite similar. According to the data obtained by the other researchers, the enzymes responsible for exochitinase activity, have a slightly higher MW than proteases, but their MW falls within the same range (10–70 kDa) (Rysakova, 2008). Then, in compliance with the data obtained during the fractionation of EPs (Figure 2), it is possible to speak about higher specific exochitinase activity of the snow crab hepatopancreas EP.

During the carried out researches some characteristics of *Chionoecetes opilio* hydrolases have been determined, and they turned to be similar to those ones of *Paralithodes camtchaticus* as a whole. The obtained data allow speaking about the prospects of utilization of *Chionoecetes opilio* hepatopancreas as an accessible raw material for the commercial purposes in the given area. Obviously, the data available at the moment are not enough to make the concrete recommendations, but, taking into account high adaptive abilities of the snow crab and its increasing abundance, it seems to be necessary to continue researches in this direction.

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SHELF LIVES OF CANNED FOOD "NATURAL PACIFIC SAURY"

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Saury fish is an important item in the realm of fishing. Products from this fish at the Russian market are being in much demand; particularly, the demand for canned goods from saury has grown because they are inexpensive and available for all strata of the population.

The canned food is produced under State standard (GOST) No. 7452–97 from fresh, cooled and frozen glazed saury that has been stored for no more than 2 months. The shelf life of such canned food is 2 years while the American and European manufacturers' analogs have generally the shelf life of five years and over [5].

The aim of this work is to substantiate shelf lives of the canned food "Natural Pacific Saury" having high quality and nutritive value.

As the object of research, a batch of canned fish produced at the Preobrazhenskaya Base of Trawling Fleet, JSC, from frozen saury fish gazed with sea water and stored for 2 months at –25°C was employed.

The canned fish quality was characterized in terms of microbiological, organoleptic, and physicochemical characteristics as well as nutritive value.

Microbiological studies were carried out pursuant to "Hygienic Requirements for Safety and Nutritive Value of Foodstuff" and methodological instructive regulations on the determination of foodstuff shelf lives.

The contents of nonprotein nitrogen and volatile base nitrogen as well as acid-degree value and peroxide number were determined according to State standard (GOST) No. 76–36–85; the amino acid composition of proteins was determined on an AAA-835 Hitachi amino analyzer; the fraction composition of lipids was studied by HPLC; the lipid fraction was analyzed by GLC on a Shimadzu 16A gas chromatograph. Parameters responsible for the variation of protein substances and lipids were determined in the dense portion, liquid (broth) and middle samples of the canned fish.

The organoleptic component was appraised by the method of rating.

The results of the microbiological studies showed that the canned food "Natural Pacific Saury" complied with the industrial sterility requirements over a period of 3.5 years.

The content of nonprotein nitrogen in the canned fish after the fabrication thereof varies from 0.16–0.23% in the dense portion, 0.32–0.38% in the middle sample up to 0.92–0.96% in the broth. After 2 years of storage, the nonprotein nitrogen content amounted to 0.37% and by the 3d year it became 0.52% (in the middle sample); in the dense portion it was 0.28% after 2 years and 0.41% after 3 years; in the broth it constituted 0.92% after 2 years and 0.98% after 3 years. When the storage time is increased by 1 year, the nonprotein nitrogen content rises by approx. 30% in the middle sample and dense portion and by 6% in the broth.

The nonprotein nitrogen accumulation by the end of 3.5 years of storage is to a greater extent characteristic for the dense and middle samples than for the broth.

The content of volatile base nitrogen after 2 years is 43 mg% in the middle sample and 48 mg% by the 3d year; 36 mg% in the dense portion and 42 mg% by the 3d year; 60 mg% in the broth and 66 mg% by the 3d year, that is, the volatile base nitrogen content increases by 10, 14 and 9%, respectively.