PROTEOLYTIC ACTIVITY OF SQUID PROTEINS

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Formation of the current market of food products satisfying various demands is impossible without development and introduction of underused but nutritive valuable industry objects into processing.

The promising raw material but little used in the fishery industry are Commander squid (Berryteuthis magister) and Humboldt squid (Dosidicus gigas). Lack of recommendations on the fishing period, types of cutting and manufacturing of squid leads to the situation that the product yield might not exceed 30% (of frozen raw material) when processing squid, that is the main cause of underuse of this type of raw material. According to the data from literature such low yield values can be explained by high proteolytic activity of squid enzyme systems.

Data on the chemical composition show that squid is a protein product with low fat content. Results of the studies on amino-acid protein composition and fatty acid lipid composition of squid muscular tissue show its high biological value. It all makes squid a promising product for children's food.

Conclusion to the interconnections between proteinase activity, squid fishing period and types of cutting will be made on the basis of the received data on the integrated study of squid. It will permit to develop scientifically based recommendations on the terms of manufacturing of squid which provide producing the goods of guaranteed quality with high customer properties, met all requirements including those for children's food products.

The objects of the present study were the following samples of Commander squid – males and females of three age group: young species, species of prespawning season and species of spawning season, and also sample of Humboldt squid mantle.

The squid mantle weighing 20–30 gram was thoroughly chopped then 50 ml of buffer (0.05 M Трис-HCl, pH 8.0, containing 10mM NaCl) was poured over, repeatedly homogenized and kept while agitation for 2 hours at the temperature of 7^{o}C. Further the samples were centrifugated for 25 minutes at 3000 rpm; supernatant fluid was sampled and filtered for producing homogeneous solutions, herein after referred to as extracts.

The determine proteolytic activity the following chromogenic substrates were applied: Bz-Arg-pNa (BApNa); Z-Ala-Phe-Arg-pNa (Arg); Suc-Ala-Pro-Phe-pNa (Phe). 500 mcl of buffer (100mM Tris-HCl, pH=7.5), 400 mcl of water and 10 mcl of protein extract solution were introduced into test tubes to carry out enzymatic reaction.

After preliminary incubation of extracts in thermostat for 1 minute at 37^{o}C, 10 mcl of substrate was added, then the extracts were kept for 10 minutes more.

Further the reaction was stopped by introducing 80 mcl of 50% solution of trichloracetic acid into all test tubes (except check test tubes). Final volume of reaction mixture was 1 ml.

Optical density of the reaction mixture was measured at the wave length of 405nm against the relevant control. At the same time the unit of activity was considered to be such amount of enzyme which decomposes 1 micromole of substrate in one minute at the said conditions. We consider specific activity to be a unit of enzyme activity referred to 1 mg of protein in the sample.

The specific activity was evaluated by the formula:

Specific activity, mole/mg*min = $A_{405}/\varepsilon*c*t$, where

A_{405} – absorption at the wave length of 405 nm;

ε – extinction coefficient, equal to 8900 $M^{-1}*cm^{-1}$;

c – protein concentration, mg/ml;

t – reaction time, min.

Electrophoresis was carried out in denaturant non-reducing condition (with 0.1% solution of sodium dodecylbenzenesulfonate, SDS) in plates of polyacrylamide gel (PAAG) with separating 12% PAAG and stacking 4% according to the method of Lammy.

The method of denaturing electrophoresis was applied when carrying out zymography, the gel was copolymerized with gelatin in concentration of 0.05% at the stage of preparation. Set of standard protein-markers of the known molecular mass was used as markers both at the electrophoresis and zymography.

The gel was colored with solution of Kumassi R-250. After electrophoresis the gel with gelatin was immediately washed in 2.5% Triton X-100 (30 minutes) and water (3x10 minutes) then it was kept in tris buffer to carry out enzymatic reaction at the various pH for 1 hour at 37^0C. Further it was colored with the solution of Kumassi R-250, at the same time, the colorless areas were appeared at the places of gelatin hydrolysis (substrate) as a result of decomposition of gelatin by enzymes.

The obtained results show that the highest protein content is observed in the extract produced from commander squid of spawning season (male) as well as in the extract of humboldt squid. The data can indicate that tissues of different species destruct in the different way depending on the squid type, its gender and maturity stage.

When determining of activity of proteinases of trypsin and chemotrypsin type by the method of electrophoresis, zymograms and also when applying low-molecular substrates (pH ranging from 4,0 to 8,0) it was found that all the extracts were active on the most available substrates – BapNa, on which all further studies were carried out.

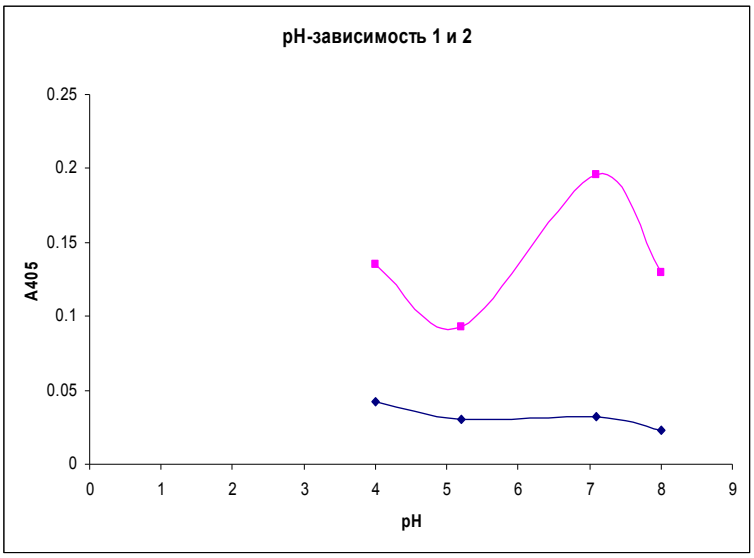

Fig. 1. pH-dependence of proteolytic protein activity of the squid of prespawning season (■ – ♀, ▼ – ♂)

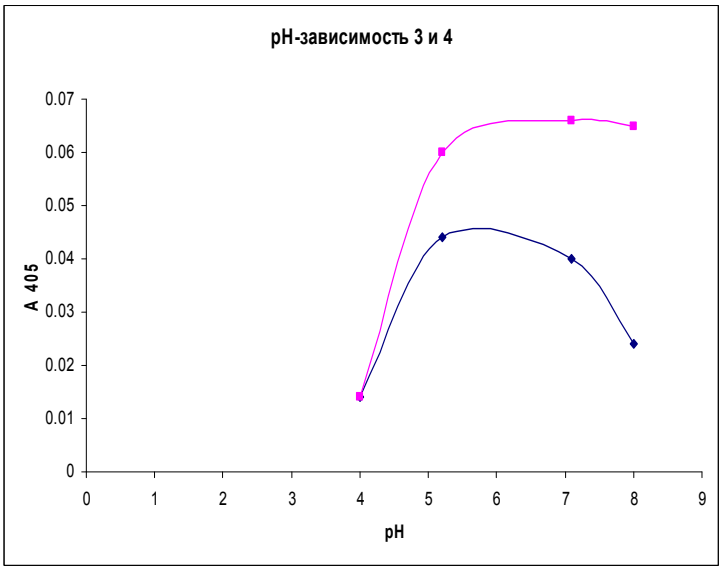

Figure 2. pH-dependence of proteolytic protein activity of the young squid (■ – ♀, ▼ – ♂)

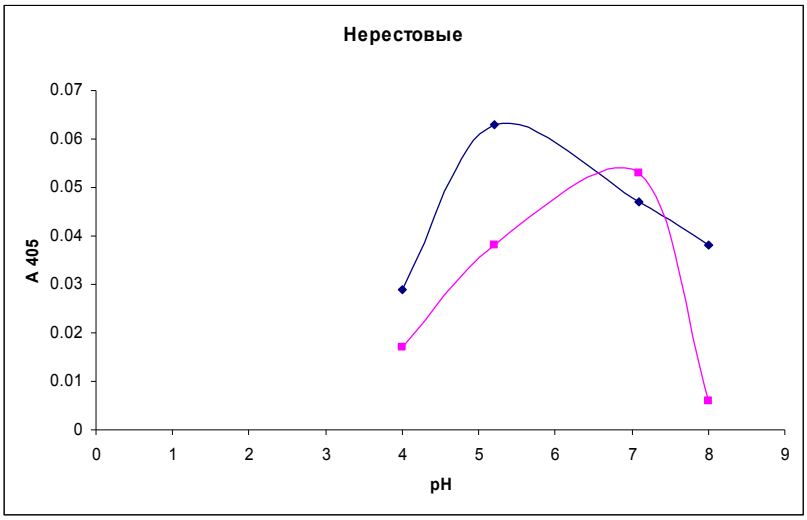

Fig. 3. pH-dependence of proteolytic protein activity of the squid of spawning season (■ – ♀, ▼ – ♂)

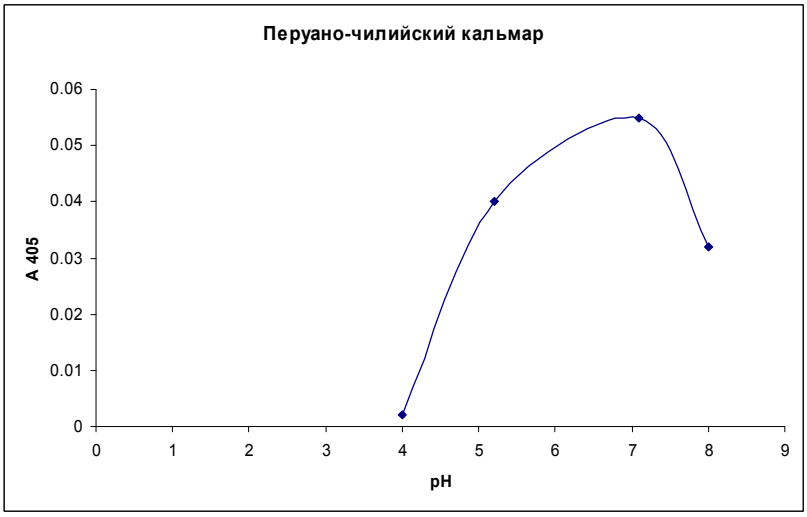

Figure 4. pH-dependence of proteolytic protein activity of Humboldt squid

As it is evident from the results presented in Figures 1–4, regardless of the gender, the young squids have protein-enzymes in mantle showing proteolytic activity in a wide range of pH values – from 4.5 to 8.0 (Fig. 2). In prespawning maturity stage pH-optimum for activity of the extracts of squid female specimen is 7.5 and 4.0 (Fig. 1), and pH-optimum of proteolytic enzyme activity of male squid specimen is shifted in the acidic region 4.0. The proteinase pH-optimum of female specimen of spawning period is 7.5, and male specimen – 5.2 (Fig. 3). pH optimum for the activity of the extracts from a sample of the mantle Humboldt squid is 6,0–7,0.

The enzymes of all extracts in the range of 55–60 kDa show proteolytic activity. At the same time the enzymatic activity depending on pH values are not appeared. Probably it is connected with the fact that proteases with different pH-optimum values have the same or sufficiently close molecular masses.

When analyzing pH-stability of squid proteinases the trend of loss of activity is seen during incubation with increase in pH, which persists regardless of the pH-optimum of the protease activity and the squid type. If the pH value (at which the enzymes were preincubated) coincided with its pH-optimum, then about 20–25% of enzyme activity was remained.

Conclusions

The specific activity of squid proteases increases from young species to prespawning maturity stage species at pH 4.0, and then decreases significantly to the spawning stage species. At pH = 8.0, this activity is similar for young and pre-spawning species, but in the extracts of spawning squid also reduced in 2.5–3 times.

Upon squid maturing the pH-optimum of proteinases varies over a wide range – from 5.0 to 8.0 for the young species, in a narrow range – for the squids of spawning maturity stage, distinguishing by the gender: the pH-optimum of females – 7.5, males – 5.2.

By the method of zymogram it is found that chiefly enzymes with molecular mass of 55–60 kDa have gelatinous activity.

The study of the stability of squid proteinases at different pH values was carried out and a quite rapid loss of activity when increasing pH value to the alkaline region was shown.

PALE ARCTIC COMMON TAIMEN *HUCHO TAIMEN* WITHIN THE REPUBLIC OF SAKHA (YAKUT) – SPAWNING BIOLOGY AND ARTIFICIAL REPRODUCTION

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There are two species of taimen – common taimen *Hucho taimen* (Pallas, 1773) and Sakhalin one *H. perryi* (Brevoort, 1856), inhabiting rivers of the Russian Federation. Common taimen (or taimen) is one of the largest members of family Salmonidae that reaches 2.1 m in length, 105 kg in weight, and 60 years old in age. Its valid Latin name is *Hucho taimen* (Pallas, 1773) which has undergone five revisions made by P.S. Pallas himself. As a result this fish periodically changed its generic, specific or sub specific nomenclature. Therefore, the junior synonym is considered to be *Salmo fluviatilis*, the senior one – *Hucho hucho taimen,* other synonyms are *Salmo taimen* and *Salvelinus taimen* (Froese, Pauly, 2010).

In Russia, taimen is known under different local names: Russian – krasulya, len', talmen, Yakut – beal, Evenki – chonkchur (Sivtseva, Mikodina, 2009); in the English literature it is met as Siberian taimen and Siberian salmon. This species is an object of commercial fishing, game fishing and amateur one (Kirillov F.N., 1972; Kirillov A.F., 2002, 2009; Kirillov A.F. et al., 2007; Sidorov, Tyaptirgyanov, 2004) and aquaculture (Zelyonkin, Fedorova, 1997; Korablina, Ivanova, 2001; Mikodina, Lyubaev, 2005; Kouřil et al., 2009). Being a predator, taimen has delicacy meat and red caviar.

In the territory of the Republic of Sakha (Yakut) as part of Pale Arctic, taimen inhabits the rivers of the Arctic Ocean Basin running to the Laptev Sea. These are large rivers of different length – Undyulyung (*syn* Yundyulyun) R. (414 km), Omoloy R. (593 km), Yana R. (906 km), Anabar R. (939 km), Olenyok R. (2292 km), Lena R. (4400 km), and also south Lena River tributaries: left – Vilyui R. (2650 km), and right – Aldan R. (2273 km). Taimen habitats in Pale Arctic of the Yakut are extremely severe. For instance, the lower Lena R. with its tributaries is located not only beyond the North Polar Circle, but it is also found within the permafrost zone.

In Russia, taimen is included in the Red List Data of the Republic of Altay and Altay territory, Krasnoyarsk territory, Republic of Tyva, and Republic of Buryatiya. Its stock in the Yakut water systems steadily decreases, though it has not reached the critical level yet. In this connection, in this Republic, unlike other Russian Federation territories, it has not included in the Regional Red List Data yet. The stock reduction of taimen in the Yakut water systems is proved by the dynamics of catches (excepting the Great Patriotic War of 1941–1945): in 1940 – 25 tons, 1943 – 179 t, 1945 – 71 t, 1950 – 27 t, 1960 – 26 t, 1970 – 9 t, 1980 – 16 t, 1990 – 10 t, 2000 – 3 t. Since 1999, commercial fishing of taimen in the Republic is banned, and its fishery is allowed only as a by-fishing (10%) (see Sidorov, Tyaptirgyanov, 2004). According to the official statistics, in the zero years of XXI century its catches did not exceed 6 t. Thus, in 2006 the catches of taimen were 5 t, in 2007 – 3.9 t, in 2008 – 5.7 t, in 2009 – 5.98 t. By July, 2010 it has been caught 26.2 t, the Total Allowable Catch (TAC) for 2011 is estimated to be 28 t. Slightly less than half the TAC is allocated for recreational requirements, including the Lena R. – 8.5 t, in the Anabar and Olenyok Rs. together – 2.1 t.