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EFFECT OF SELECTED PHYSICO-CHEMICAL FACTORS ON BALTIC HERRING MEAT HYDROLYSIS RATE AND PROTEOLYTIC ENZYME ACTIVITY

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Key words: Baltic herring, proteolytic enzyme, protein autohydrolysis, papin, cathepsin, B-500 protease.

The effect of some physico-chemical factors on the rate of Baltic herring meat protein autohydrolysis and hydrolysis due to papain and B-500 protease from *Bacillus subtilis* is reported.

The effect of physico-chemical factors on the rate of protein hydrolysis, occurring with the participation of both endogenous proteolytic enzymes of the material and added enzymatic preparations, is very important in fish processing technologies based on enzymatic processes, and especially in the manufacture of fermented products. Such products in a liquid, paste or whole-fish form have been known for a long time, notably in countries of south-east Asia and in Japan [1, 2, 4, 16]. This kind of food enter into a food market rather slowly which is doubtless due to the character of and the difficulties connected with the steering of biological processes during its industrial production. The most important process contitioning the correct sensory properties of these products is the partial decomposition of the material's proteins by proteolytic enzymes. Hence, knowledge about the dynamics of enzymatic changes of fish muscle proteins is one of the key factors ensuring correct fermentation.

The aim of this work was to determine the effect of selected physico-chemical factors on the activity of proteolytic enzymes and hydrolysis rate in Baltic herring meat proteins.

MATERIAL AND METHODS

Baltic herring (*Clupea harengus membrans L.*) caught in July 1979 was used in the study. Frozen whole fish was kept in cold store at -20°C. The studies commenced after four months of the material's storage in a frozen state. The analyses were performed on 50 separate batches (ca 2 kg each) of fish which were defrosted in a cold room at 0-4°C for 16 h. Next, a part of the defrosted material was broken up in a laboratory meat grinder after previous filleting; the remainder was ground whole. The resultant two kinds of minced meats were the basic study material.

Hydrolysis was carried out in three repetitions according to a previously described method [6] using HCl and NaOH solutions to maintain the desired pH. The variable parameters of hydrolysis were temperature (17, 30, 40, 45, 50, 55, 60 and 70C), time (2, 4, 6, 12, 24 and 48 h) and pH (3.1-9.8). Additions of NaCl as a conserving agent amounted to 5, 10, 15, 20 and 25%. In the study of the effect of pH and temperature on hydrolysis intensity a 2-h heating period was applied. The effect of incubation time was studied at optimal pH and temperature, i.e. in the case of minced muscle meat at pH 4.2 and temperature of 50°C, and in the case of minced meat from whole fish — at pH 3.7 and 50°C. The proteolytic enzyme activity and hydrolysis rate were assessed on the basis of amino nitrogen increments in heated samples (amino nitrogen content in studied sample minus its content in control), amino nitrogen being assayed by the method of Pope and Stevens [11].

The possibilities of increasing the hydrolysis rate of meat proteins were studied using papain (Merck, Federal Republic of Germany) and B-500 protease from *Bacillus subtilis* (Société Rapidase, France).

Total protein content was assayed on a Kjel-Foss Automatic 16210 apparatus (Denmark). The contents of fat, water and ash in the studied material were determined by standard analytical methods [8].

RESULTS AND DISCUSSION

Table shows the results of the assay of the basic chemical composition of the studied material.

The first stage of analysis consisted in determining the pH and temperature at which the proteolytic anzymes of Baltic herring exhibit max-

Table. Chemical composition of raw fish in %%

Total protein	Water	Fat	Residue
16.33	68.75	10.39	3.20

imum activity. The highest activity of muscle proteinases was found to occur at pH 4.2 (Fig. 1). The optimal pH of hydrolysis of minced herring muscle meat proteinases is almost identical with that of the meat of Atlantic hake, Pacific hake and sardine [5] and with the pH of maximum activity of partly purified cathepsin of cod muscles, but differs conside-

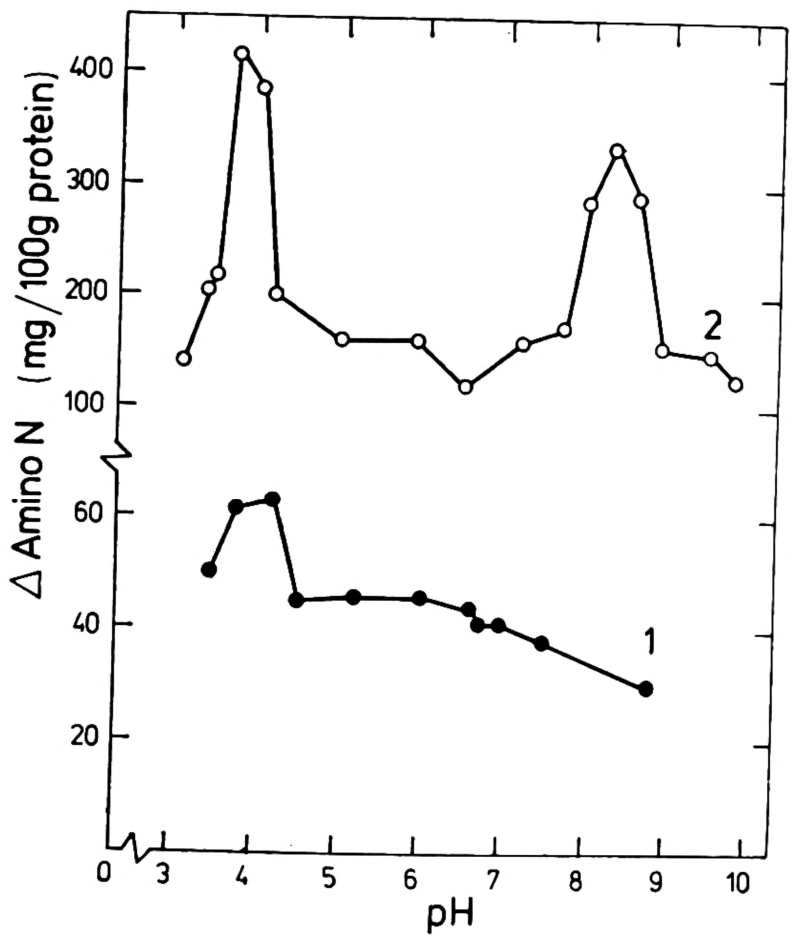

Fig. 1. Effect of pH on amino nitrogen increase during herring minced meat autohydrolysis at 40°C; 1 — fillets, 2 — whole fish

rably from the optimal pH value of purified cathepsin of cod spleen [13] and of proteinase of tunny muscles [7]. Two optimal pH values were found for the hydrolysis of proteins from whole fish, namely 3.7 and 8.3, the amino nitrogen increments being, however, by ca 24% higher in the acidic range than in the basic one. An exceptionally high activity of proteolytic enzymes of this material was found in the pH range 3.1-3.7. The minimum hydrolysis rate of minced whole-fish meat was observed at pH 6.5; the hydrolysis in this case was nevertheless more intense than the proteolysis of minced muscle meat at optimal pH. The optimal values of pH are indicative of the composition of the herring's proteolytic enzyme complex. The muscle tissue contains mainly cathepsin-type enzymes which, according to Szenderiuk [14], exhibit optimal activity at pH 4-5. The proteinases of the fish's internal organs include in addition to cathepsins mainly enzymes of the pepsin-type which are optimally active at pH 1.5-3.5 and also trypsin-type anzymes with optimum activity at pH 7.8-9.5. The probable reason for the stabilization of enzyme activity at pH

higher than 4.5 that was determined in herring muscles was the diffusion of proteinases from internal organs to abdominal and dorsal muscles caused by the storage of fish in a non-eviscerated form [12]. The demonstrated higher rate of hydrolysis of proteins from whole fish in the acidic range is in Baltic herring most probably due to the activity of enzymes of the group of internal-organ pepsin and cathepsins which is higher than the activity of trypsin. In the case of hydrolysis of proteins of other fish, on the other hand, the rate of hydrolysis is higher in the basic medium [3, 15]. Herring is best hydrolysed at pH 3.7 which is evidenced by the more intense hydrolysis at this pH than at pH 8.3 and by the fact that the activity of the trypsin-type proteolytic enzymes undergoes considerable variation in the biological cycle of fish [14].

The optimal hydrolysis rate of proteins of both kinds of minced meats was found to occur at 50°C (Fig. 2). The raising of temperature to 70°C reduced hydrolysis rate more severely in whole-fish minced meat than in minced muscle meat. It thus seems probable that the proteolytic en-

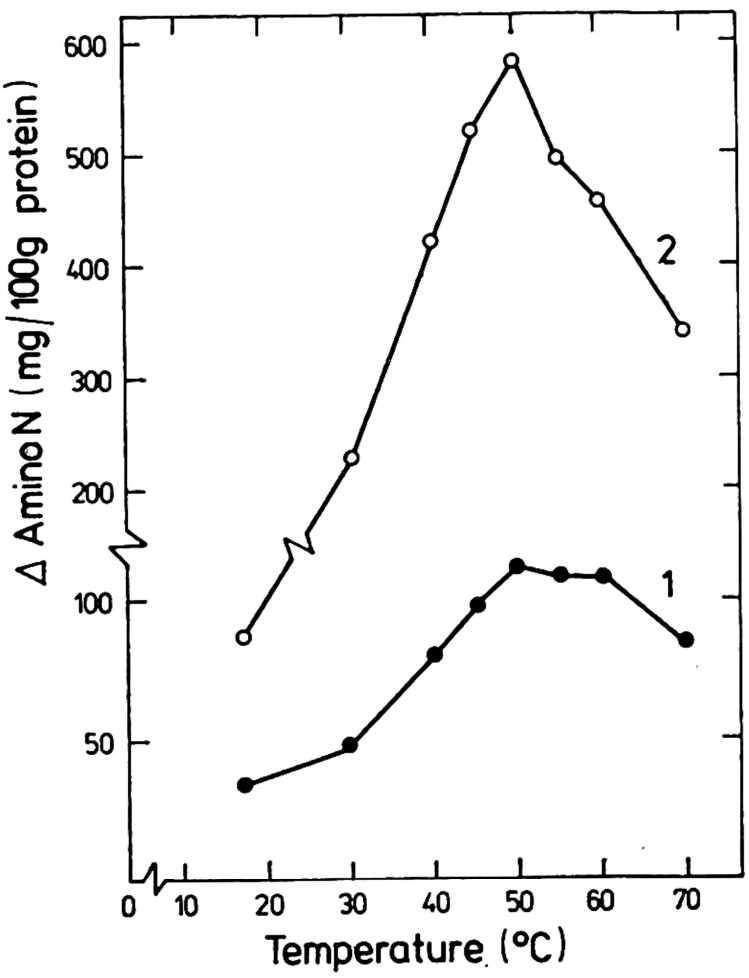

Fig. 2. Effect of temperature on amino nitrogen increase during herring minced meat autohydrolysis at optimal pH; 1 — fillets (pH 4.2), 2 — whole fish (pH 3.7)

zymes of the herring's internal organs and alimentary tract are more vulnerable to heat denaturation than are muscle cathepsins.

The effect of NaCl on the intensity of herring protein hydrolysis was examined. The experiments were performed in optimal pH and tempera-

ture conditions using minced whole fish (Fig. 3). It was found that an addition of NaCl seriously affects the hydrolysis rate, the effect depending on the concentration of salt and on proteolysis time. Each of the applied NaCl concentrations reduced hydrolysis rate, with the most severe inhibition occurring at 15, 20 and 25% NaCl. The inhibitory effect of salt decreased with the increase of hydrolysis time. A similar effect of

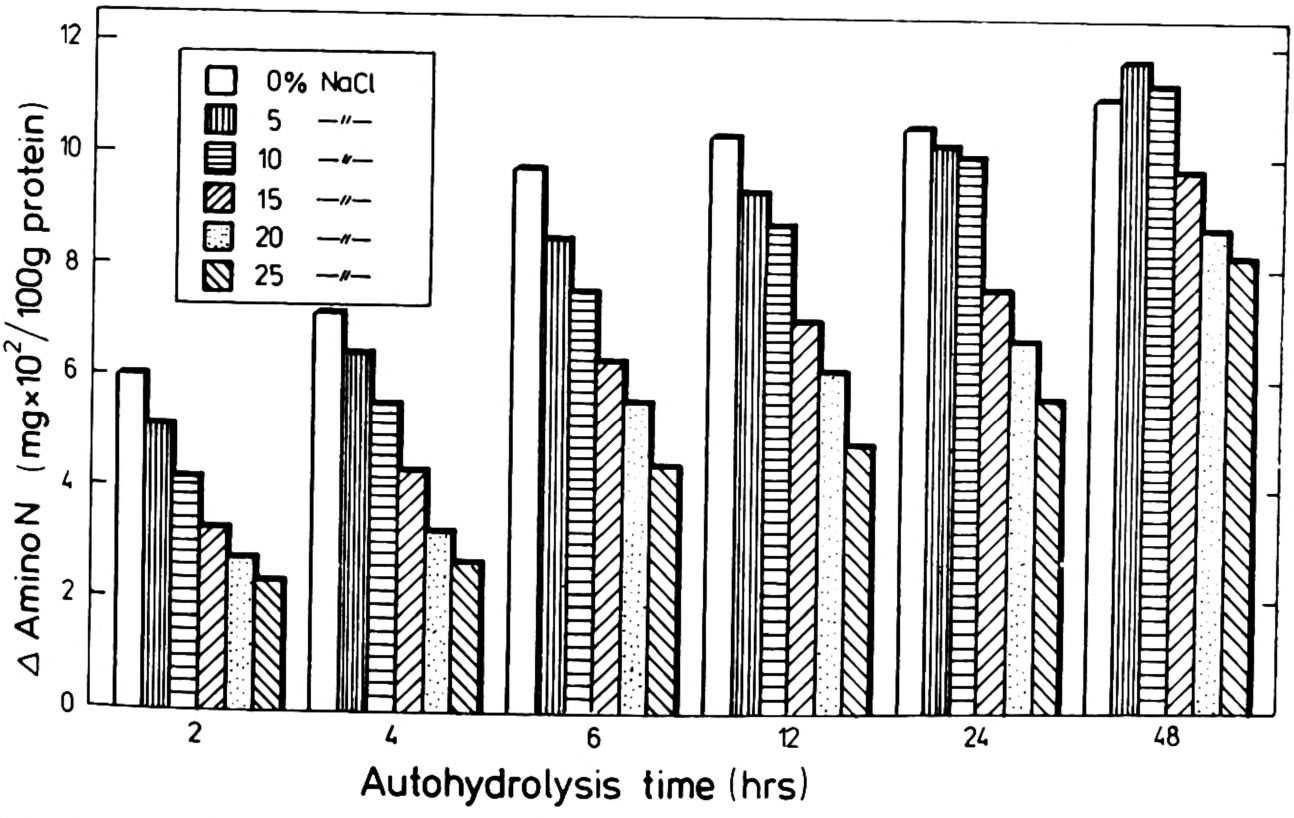

Fig. 3. Effect of NaCl addition on liberation of amino nitrogen during autohydrolysis of herring minced meat (from whole fish) at pH 3.7 and 50°C

NaCl on the activity of herring muscle enzymes was demonstrated by Podeszewski and Jasińska [10], and the same dependence was observed in the case of other fish species [9, 15]. The inhibition of fish material hydrolysis in such conditions is most probably due to a partial denaturation of enzymes. This fact should be borne in mind during the processing of fish aimed at obtaining fermented products.

In order to speed up the hydrolysis of proteins from whole fish, papain and B-500 protease was applied. The hydrolysis with additions of the two enzymes was performed with different NaCl concentrations and at natural PH of the material (pH 6.5) since it is close to the optimal pH of both preparations [5]. The results of the experiments are given in Figs 4 and 5.

In the course of a 2-h hydrolysis of samples with papain a partial inhibition of the activity of this enzyme was found in all the applied NaCl concentrations, the greatest inhibition bein caused by salt in concentrations of 20 and 25% (Fig. 4). The inhibitory effect of salt on the enzyme's activity decreased with the increase of incubation time and depended not only on the amount of NaCl but also on the concentration of the enzyme. In

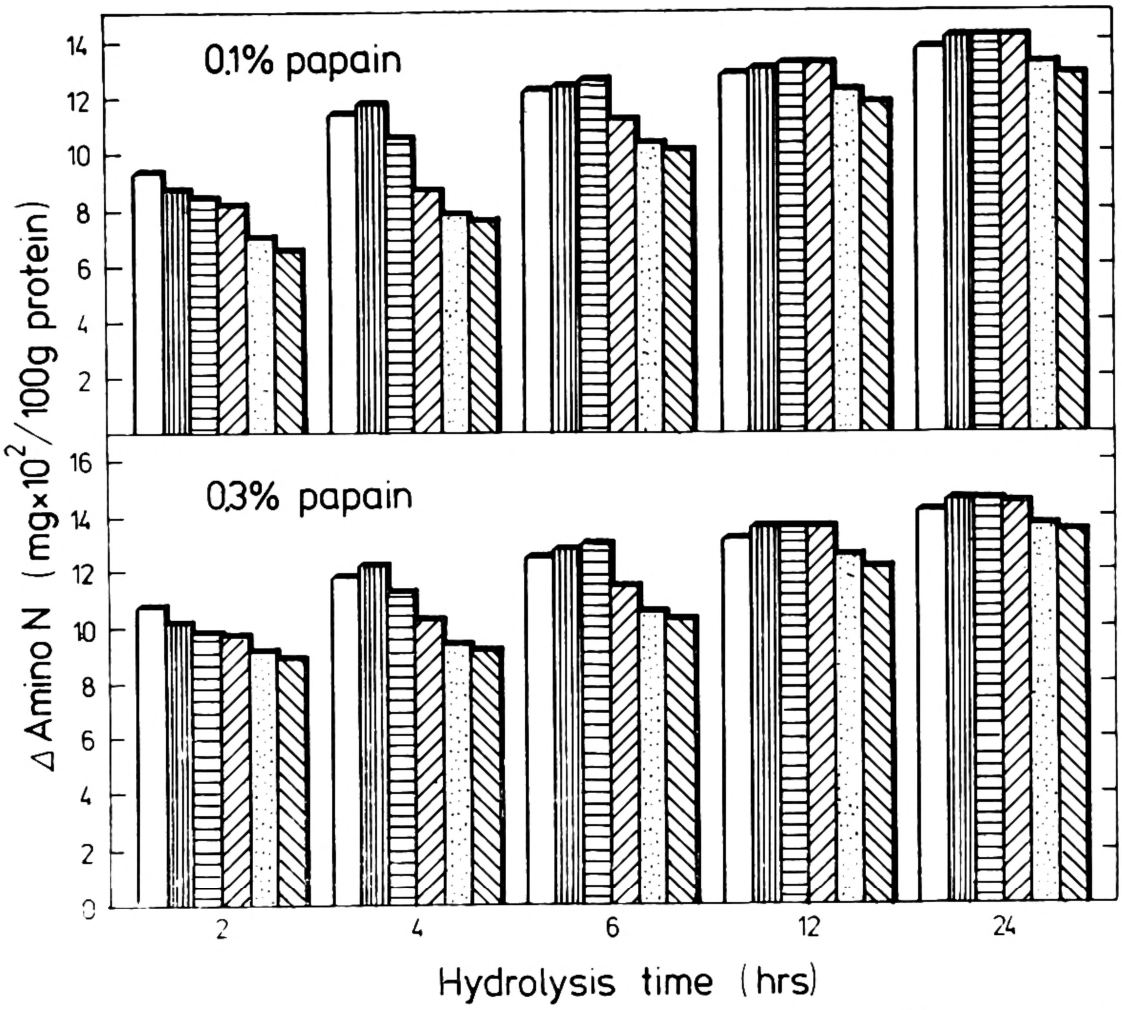

Fig. 4. Effect of NaCl concentration on hydrolysis rate of herring minced meat (from whole fish) at pH 6.5 and 50°C after addition of papain; denotations as in Fig. 3

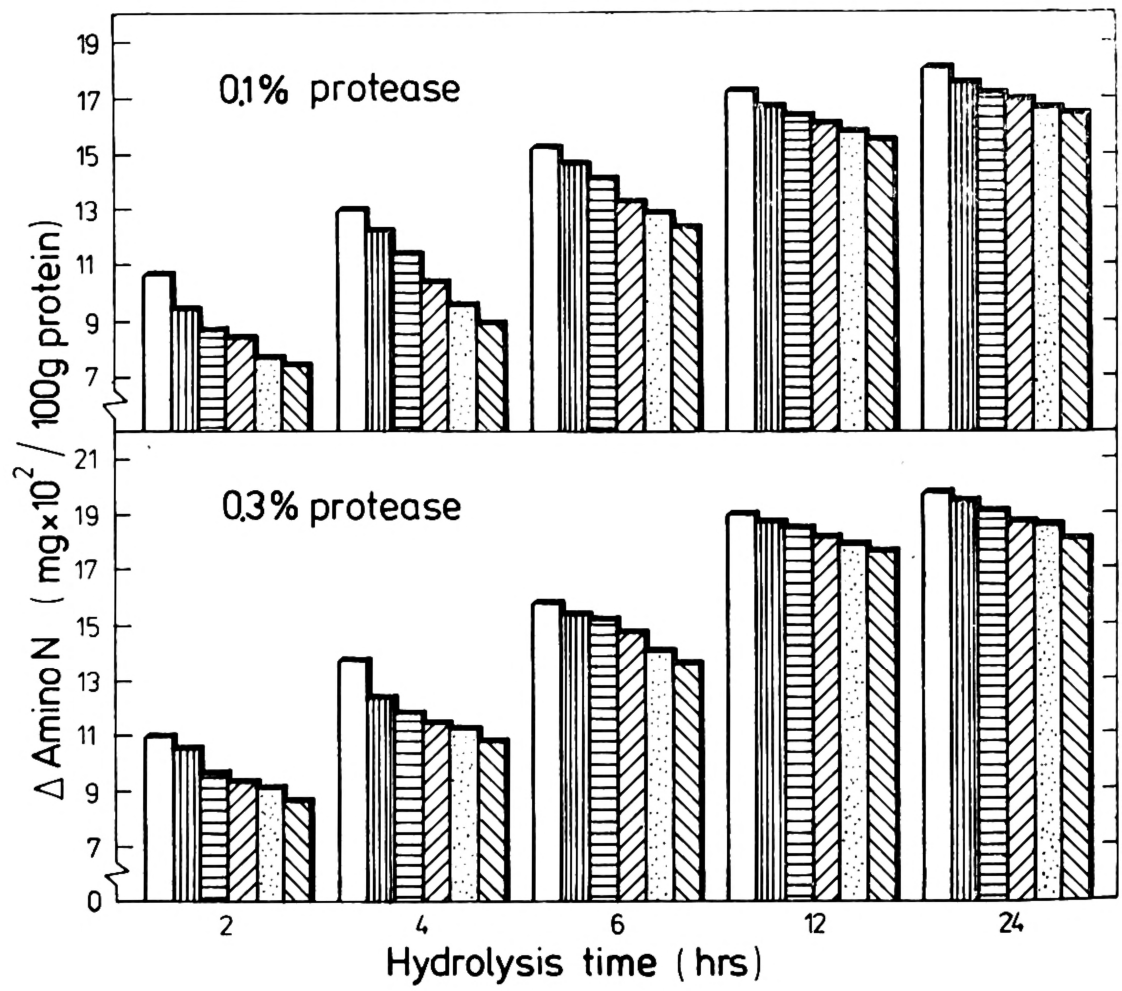

Fig. 5. Effect of NaCl concentration on hydrolysis rate of herring minced meat (from whole fish) at pH 6.5 and 50°C after addition of B-500 protease; denotations as in Fig. 3

the case of hydrolysis of minced herring by B-500 protease the addition of salt inhibited its activity to a slightly high degree than in the case of papain (Fig. 5). Protease demonstrated a higher activity with regard to herring proteins than papain. The highest increments of amino nitrogen were observed in the first 12 h of hydrolysis. The extension of proteolysis to 24 h resulted in an increase of nitrogen increment that was relatively small compared to that after 12 h.

The applied proteolytic enzymes were more resistant to the inactivating effect of NaCl than the herring's endogenous enzymes and increased hydrolysis to different extents. The B-500 protease was more active in liberating amino nitrogen from the proteins than papain, and it is thus the former that should be used to speed up hydrolysis of herrings intended for fermented products.

CONCLUSION

1. The considerably more rapid authydrolysis of minced whole herring than of minced muscle meat indicates that the complex of proteolytic enzymes of internal organs and alimentary tract plays a greater role in the decomposition of this material's proteins than muscle cathepsins.
2. The more intense autohydrolysis of whole herring in acidic pH than in basic pH shows that the total activity of cathepsins and pepsin-type enzymes exceeds in this species that of trypsintype enzymes.
3. The rate of herring protein hydrolysis in natural pH may be increased by adding papain or B-500 protease to the material, with more intense proteolysis achieved with the use of the latter.
4. The endogenous proteolytic enzymes of herring are more susceptible to the inactivating effect of NaCl than papain and B-500 protease.

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WPŁYW NIEKTÓRYCH CZYNNIKÓW FIZYKOCHEMICZNYCH NA AKTYWNOŚĆ ENZYMÓW PROTEOLITYCZNYCH I SZYBKOŚĆ HYDROLIZY BIAŁEK MIĘSA ŚLEDZIA BAŁTYCKIEGO

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Streszczenie

Badano wpływ niektórych czynników fizykochemicznych (pH, temperatura, czas inkubacji, dodatek NaCl) na aktywność enzymów proteolitycznych i szybkość hydrolizy białek mięsa śledzia bałtyckiego.

Stwierdzono maksimum aktywności proteinaz mięśniowych w pH 4,2 a proteinaz rozdrobnionej ryby całej w pH 3,7 i 8,3 z tym, że hydroliza w pH 3,7 była intensywniejsza niż w pH 8,3 (rys. 1). W obu przypadkach największą aktywność endogennych enzymów proteolitycznych wykazano w temp. 50°C (rys. 2). Stosowany w charakterze konserwanta chlorek sodu w stężeniach do 10 g na 100 g rozdrobnionej ryby całej powodował nieznaczne hamowanie aktywności enzymów tego surowca po krótkiej hydrolizie i wpływał na nie stymulująco po długim czasie hydrolizy (rys. 3). Większe stężenia NaCl znacznie zmniejszały szybkość hydrolizy białek.

W celu zwiększenia szybkości hydrolizy białek ryby całej zastosowano papainę (rys. 4) i proteazę B-500 (rys. 5), z których ta ostatnia, pomimo większego inaktywującego wpływu chlorku sodu, była aktywniejsza niż papaina. Oba preparaty enzymatyczne charakteryzowały się mniejszą podatnością na inaktywujący wpływ NaCl, aniżeli endogenne enzymy proteolityczne surowca. Do hydrolizy śledzia w warunkach przemysłowych spośród tych dwóch preparatów lepiej nadaje się proteaza B-500.