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Fish processing

ACTIVITY OF MUSCULAR CATHEPSINS OF SOME MARINE FISHES
AKTYWNOŚĆ KATEPSYN MIĘŚNIOWYCH NIEKTÓRYCH RYB MORSKICH

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The activity of muscular cathepsins was investigated at seven species of marine fish in 1% KCl extracts. The activity was determined by Anson's method in author's modification with 5% solution of denaturated hemoglobin as substrate.

INTRODUCTION

The fish and its products are subject to quick decomposition processes which diminish the quality and consequently make them not edible for consumption. Much attention have been paid to problems of fish spoilage. Some scientists underlined in their works the importance of enzymes as factors which distinctly contribute towards decomposition process (Siebert, 1962; Makinodan, et al., 1969; Sendriuk, 1969). Quick decomposition of muscles causes the losses of various chemical substances, primarily of protein and fat. The causes of this may generally be divided into endo- and egzogenic factors. In contrary to egzogenic factors which have been more investigated, the endogenic factors are not yet recognized sufficiently. Frequently the autolysis itself can hardly be separated from decaying process. The decomposition originating as autolysis changes over to normal decaying process. Initial changes are caused by endogenic enzymes which prepare the substrate for bacteria; generally such bacteria is not capable to attack the non-affected protein substances. Thus, the activity of endogenic enzymes make the road for bacteria processes and consequently lead to quick spoilage of fish. According to Bramstedt, et al. (1961), decomposition of cod stored in ice comprised two main stages. In first stage, the changes were caused by activity of own enzymes during first six days of storage. The further changes were caused by bacteria and finally are contributed to activity of both factors simultaneously.

The proteolytic enzymes play very important part in changes of fish material not only during the storage but also in many technological processes. It is therefore very important to recognize the activity of muscle proteinases. Further investigations in this field may contribute towards modifications of methods for protection of fish.

METHOD

Material. Used for investigations were the following fish species: cod - Gadus morrhua L., herring - Clupea harengus membras L., plaice - Platessa platessa (L.), brill - Scophthalmus maximus (L.) from Baltic Sea, hake - Merluccius merluccius L., jack fish - Trachurus trachurus (L.), mackerel colias - Scomber japonicus colias Gmelin from N.W. African shelf. The fish was stored under temperature -25°C and the period of storage from catching and freezing until commencement of investigations did not exceed one month.

Preparation of enzyme extract. After defreezing, the fish was gutted and filleted. Attention was paid not to bring into direct contact the tissues with skin and intestines. The fillets were minced in sterile meat grinder. The enzyme extracts were made by homogenizing the minced tissues with chilled 1% KCl solution in ratio 1:2.5 during 5 minutes at 220 r.p.s. and by centrifuging in separator with cooling during 15 minutes at 4000 g. All operations, except defreezing were performed in refrigerating chamber of temperature $0-4^{\circ}\text{C}$.

Method. The activity of proteolytic muscle extracts was determined by A n s o n / B e r g m a y e r ' s 1965) method in author's modifications. 5% solution of denaturated hemoglobin was used as substrate. The samples for incubation comprised 2 ml substrate of 4.4 pH, 3 ml of 0.05 m citrate buffer of the same pH value and 1 ml of muscle extract. The mixtures were incubated during 20 minutes under tempr. 30°C . Thus, the determination of activity was based on initial speed of reactions. Each time the control sample was prepared and its value was deducted from the results of examined sample. The specific activity was expressed in micromoles of tyrosine min/g mg of protein and calculated for one hour to increase its index value.

The protein was determined colorimetrically by biuret method (G o r n a l l, et al. 1949), with application of crystalline albumin of ox serum. All tests were repeated 5 times.

RESULTS

The results of investigations performed on activity of muscle cathepsin of some sea fishes are presented in Table 1. The results prove that within fish groups of Baltic Sea, most active cathepsin contained the muscles of brill. Among the examined fish of African fishing grounds, most active were the endogenic enzymes of hake muscles. Considering that the fish resides in various sea environments, it is apparent that the proteinases of pelagic fish are less active than of fish residing in deeper waters. Particularly active cathepsin were noted in muscles of brill and plaice.

Table 1

Activity of muscle cathepsins of some marine fishes

Species	Content of protein in mg/ml of extract	Activity	
		μM of tyrosine/hour/ml of extract	μM of tyrosine/hour/mg of protein
Cod	13.5	1.755	0.130
Plaice	9.5	2.660	0.280
Mackerel colias	26.0	2.106	0.081
Hake	16.0	1.904	0.119
Jack fish	15.0	0.960	0.064
Brill	11.0	5.060	0.460
Herring	20.0	1.060	0.053

The changes appearing during storage at 2°C of freshly prepared muscle extracts of three fish species were also subjected to examinations. Free tyrosine and proteolytic activity were determined in stored extract. The results of this test are presented in Table 2 and 3. The ascertained increase of free tyrosine proves to autolysis of stored extracts. The proteolytic activity increased also in storage of extracts. Such increase was probably due to extract autolysis, better liberation of cathepsin and due to partial denaturation of non-active proteins. Denaturated proteins are more open to influence of enzymatic decomposition. It is highly probable that certain part play also here the bacteria enzymes. The extracts partially purified did not demonstrate any increase of proteolytic activity under storage conditions.

The activity of muscle cathepsin of examined frozen fish is higher than the activity of proteinases of mammals meat, and of muscle cathepsin of some fresh fishes. According to Siebert (1957, 1958), fresh tissues of sea fish demonstrated also higher catheptic activity than corresponding tissues of mammals. In comparisons to mammals, 1 g of fish fresh tissue contains 5 times more cathepsins in liver, 6 times more in spleen and 10 times more in muscles. The explanation of such high activities in fish tissues may coincide with fact that in both cases the activities were determined in temperatures near to temperature of mammals body. The fish average physiological

Table 2

Content of acid soluble tyrosine in fresh and stored extracts of fish muscle

Species	Fresh extract	Stored extract		Percent changes in relation to fresh extract
	μg of tyrosine/ml	storage period in days at $+2^\circ\text{C}$	μg of tyrosine/ml	
Hake	50	3	54	+8
Brill	42	6	52	+24
Herring	40	2	44	+10

Table 3

Changes of proteolytic activity of crude fish muscle extracts during storage at $+2^\circ\text{C}$

Species	Protein content in mg/ml extract	Storage period in days	Cathepsin activity in μM of tyrosine/hour/ml of extract	Changes of activity in %
Brill	11.0	0	5.060	-
	11.0	10	7.130	+41
Hake	16.0	0	1.904	-
	16.0	5	3.046	+60
Herring	20.0	0	1.060	-
	20.0	2	1.325	+25

temperature is by 30°C lower and in living conditions act in their cells only about 0.1 of activity measured in temperatures above 30°C . In case of frozen fish, high proteolytic activity of muscles may be caused by liberations of cathepsins from lysosome particles damaged during freezing and defreezing. The results obtained in this investigations on high activity of cathepsins in muscle of frozen fish are in compliance with data of Gould (1965), who ascertained about 2 time increase in activity malateoxydoreductase directly after freezing of fish tissue. Also Šenderiuk (1965) reports on higher increase of aminonitrogen during proteolysis of spratt frozen in comparison with fresh.

Probably the enzymes of fish from cooler waters posses higher activity than the species of warm water. This is supported by the results obtained in the present investigations. Generally, the activity of muscle enzymes of

Baltic fish was higher than activity of muscle cathepsins of African fish. Only herrings possessed lower activity than proteinases of African fish. It is worth noting that mackerel colias commonly considered as one of most spoiling fish possesses the catheptic activity in muscle only by 26% higher than jack fish. It is possible that the proteins of this fish are more open to denaturation in freezing process. Probably in mackerel autolysis, important part played also the proteinases of internal organs. This was suggested by report of Š e n d e r i u k (1969), who, while investigating the proteolysis of internal organs ascertained highest proteolytic activity in internal organs of mackerel. Also S i e b e r t (1957) assigns certain part in fish decomposition to internal proteinases.

Relatively high catheptic activity of fish muscle, beyond special functions of muscle cathepsins, should probably be assigned to individual physiologic features of fish. Considerable reconstruction of muscle proteins towards sex organs is taking place at certain fishes before spawning. According to available informations on synthesis of new protein for building of gonad protein, the desintegration of muscle protein is taking place and this forms the source of amino acids. Such disintegration of fish muscle occurs due to presence of muscle cathepsins as sole proteinases of relatively high activity. It is possible that such peculiarities in life cycle of fish are the basis for tissue richness in proteolytic enzymes. Thus the muscle cathepsin of fish possess not only final functions in autolysis but their primary duty is to maintain economically the proteins which are so indispensable for life of this animals.

For a technologists, most important is the participation of proteolytic enzymes in autolysis of fish muscle. It seems to be logical that described in this work activity of proteinases is sufficient for explanation of quick disintegration of muscle proteins, particularly under assumption that meat of mammals possesses 0.1 of this activity only. It was also calculated that the activity of cathepsin contained in 1 g of fresh fish meat is sufficient to disintegrate 290 mg of protein in temp. about 40°C during 24 hours and this is nearly twice of the amount possessed by 1 g of fish muscle. However such values of decomposition are not attainable in practice; the values cited indicate clearly that in general quick spoilage of fish may be assigned to the activity of proteolytic endogenic enzymes.

CONCLUSIONS

1. Muscle cathepsins of pelagic fish possess lower activity than muscle proteinases of fish residing in deeper waters.
2. Proteolytic activity of crude extracts should be determined directly after preparation - such extracts can not be stored.

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AKTYWNOŚĆ KATEPSYN MIĘŚNIOWYCH NIEKTÓRYCH RYB MORSKICH

S t r e s z c z e n i e

Zbadano aktywność katepsyn mięśniowych w ekstraktach 1% roztworu KCl następujących gatunków ryb: dorsza, śledzia, gładzicy, skarpia z Morza Bałtyckiego oraz morszczuka, ostroboka i makreli kolas z wód północno-zachodniego szelfu Afryki. Aktywność proteolityczną oznaczano za pomocą metody Ansona w modyfikacji własnej.

Badania wykazały wysoką aktywność proteinaz mięśniowych ryb mrożonych. Ogólnie aktywność katepsyn mięśniowych ryb bałtyckich, z wyjątkiem śledzia, była wyższa niż ryb z wód afrykańskich. Składowane ekstrakty mięśniowe w temperaturze +2°C wykazywały przyrost wolnej tyrozyny oraz wzrost aktywności proteolitycznej.

АКТИВНОСТЬ МЫШЕЧНЫХ КАТЕПСИНОВ В ЭКСТРАКТАХ НЕКОТОРЫХ МОРСКИХ РЫБ

Резюме

Исследована активность мышечных катепсинов в экстрактах 1% КСД следующих рыб: трески - *Gadus morrhua* L., сельди - *Clupea harengus* membras L., морской камбалы - *Platessa platessa* (L.) и тюрбо - *Scophthalmus maximus* (L.) из Балтийского моря, а также мерлузы - *Merlucius merlucius* (L.), ставриды - *Trachurus trachurus* (L.) и макрели - *Scomber Japonicus colias* (Gmelin) из вод северо-западного шельфа Африки. Активность определяли по методу Ансона в собственной модификации, применяя в качестве субстрата 5% раствор денатурированного гемоглобина. Установлено, что катепсины мышц балтийских рыб за исключением сельди имеют более высокую активность, чем рыбы из африканских районов лова. Самую высокую протеолитическую активность проявили мышцы тюрбо и морской камбалы, самую низкую - мышцы сельди. Исследовано также хранение свежеприготовленных мышечных экстрактов при температуре +2°C. Во время хранения установлено увеличение свободного тирозина и увеличение протеолитической активности экстрактов.

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