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OPTIMAL CONDITIONS FOR AUTOLYSIS OF JACK FISH MUSCLES *TRACHURUS TRACHURUS* (L.)

OPTYMALNE WARUNKI AUTOLIZY MIĘŚNI OSTROBOKA *TRACHURUS TRACHURUS* (L.)

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The autolysis of jack fish muscles was investigated under various conditions of pH and temperature. The intensity of autolysis was determined according to increase of amino nitrogen denoted by formalin titration.

INTRODUCTION

This work is a continuation of investigations performed on activity of proteolitic fish enzymes. In previous works (Gurgul, et al. 1968; Fik, 1972) were performed the investigations on isolation, separation and activity of proteinases of jack fish muscles - ; examined also was the activity of muscle cathepsins of some marine fish to denaturated hemoglobine. Even, the reactions of isolated enzyme lead in every case towards full understanding and explanation on autolysis of fish muscles, this however does not neceserly reflects its part in multi enzyme system of muscles in whele. Therefore, beyond the experiments on isolation, separation and activity of proteinases in relation to hemoglobine, the proteolysis of fish muscles only were also investigated.

There is very little information on enzymology of fish muscle proteolysis. The problem had been dealt mainly by Russian scientists (Šenderiuk, 1963,1964, 1965; Ertel a. Repina, 1969). Šenderiuk (1963) who investigated optimal conditions for proteolysis of spratt tissue and ascertained highest activity of proteinases at pH 3.6. They also proved that the content of free aminoacids increases 20-40 times during the autolysis. An addition to spratt minced meat of proteolysis products, water and iones Ca^{2+}, Mg^{2+}, did not influence the dynamics of autolysis. Ertel and Repina (1969) observed very high activity of proteinases in autolysis of asiatic halibut meat.

The autolysis process of jack fish muscle under various pH and temperature conditions was investigated in this work.

METHOD

M a t e r i a l. Used for investigations was a jack fish - Trachurus trachurus, caught at N.W. African shelf in February 1970 and frozen as whole. The fish was collected from refrigerating chamber where remained stored in temperature -20°C. The investigations were carried out after one month from catching and freezing.

P r e p a r a t i o n o f m i n c e d m e a t f o r a n a l y s i s. The samples for analysis were prepared each time out of 10 fishes. After defreezing in room temperature, the fish was gutted, filletted and its muscles minced. All these operations were made in refrigerating chamber under temperature 0-4°C. For analysis, small samples were taken directly after preparation and thorough mixing of minced meat.

M e t h o d. The autolysis was performed by incubation under defined temperature of exactly weighed 1 g of ground muscle with 10 ml of 0.2 m acetate buffer of suitable pH containing 0.5% of toluen. After determined period of incubations the proteolysis was interrupted by addition to particular incubating mixture of 10 ml of 5% trichloroacetate acid. After thorough mixing, this was left for 30 minutes for separations of proteins. Thenafter the mixture were filtered through dry filter and amino nitrogen was determined in filtrate. Control samples were prepared by direct adding to incubating mixture 10 ml of 5% trichloroacetate acid.

Amino nitrogen was determined by Sörensen method. Total and protein nitrogen was determined acc. to K j e l d a h l method by oxidization of samples in concentrated H_2SO_4 with addition of selenium mixture. The content of water was determined by drying in temperature 105°C and fat content - by Soxhlet's method.

The ash was determined by oxidization of examined sample above burner and by calcination in oven under temperature 700°C. All experiments were performed 6 times.

RESULTS

Chemical composition of material used for investigations is presented in Table 1. The results of investigations on determination of optimal conditions for jac fish muscle autolysis are presented in Table 2 and 3 and on Fig.1 and 2.

T a b l e 1

Chemical composition of investigated material

Total nitrogen %	Protein nitrogen %	Aminonitrogen, mg/g of muscle	Fat %	Water %	Ash %
2.88	2.55	0.80	5.10	75.26	1.51

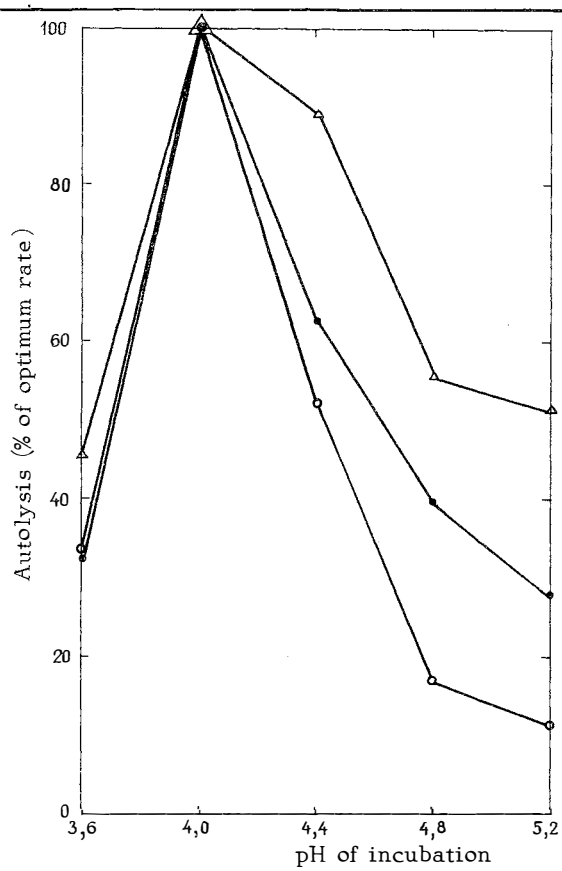

Fig.1. Influence of pH on autolysis of jack fish muscle in temp. 26°C
o ——— o - 2 hrs incubation
● ——— ● - 4 hrs incubation
Δ ——— Δ - 6 hrs incubation

Table 2

Autolysis of jack-fish muscles in temperature 26°C and pH 3.6-5.2

Hours of autolysis	Increase of aminonitrogen in mg/g of muscle				
	Autolysis pH				
	3.6	4.0	4.4	4.8	5.2
2	0.0693	0.2050	0.1071	0.0350	0.0234
4	0.0917	0.2821	0.1765	0.1120	0.0795
6	0.1337	0.2933	0.2605	0.1631	0.1510

Obtained data clearly indicate to relationship of autolysis rate to pH and temperature of environment. The rate attained its maximum within range pH 3.6-4.4 (Tab.2, Fig.1). The change of pH from optimum towards acidity causes higher decrease in activity of proteolytic enzymes than towards the bases direction. Such fact proves important role of the enzymes in autolysis of fish material. Highest activity of jack fish muscle proteinases occurs within narrow range of pH. Small offsetting of pH value towards acidity, changes basically rate or reaction. The optimum pH may result from cooperation of several various factors. It may result from actual and reversible influence on maximum rate of reaction or due to relation of substrate to enzyme. If the activity is examined within wider range of pH, the influence of environment may irrevocably inactivate the enzyme at both sides of optimum. The changes of enzymatic activity in different pH are caused by degree of ionization components. Probably, only one ione form of enzyme is catalytically active.

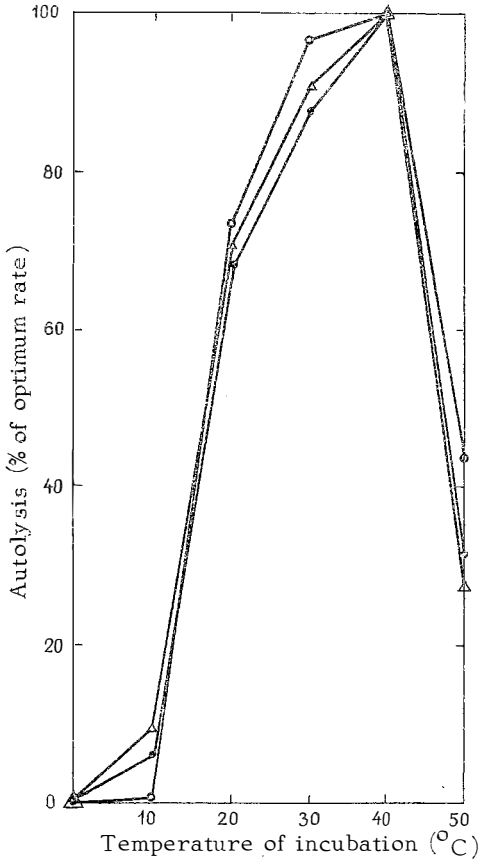

Fig.2. Influence of temperature on autolisis of jack fish in pH 4.0

o ——— o - 2 hrs incubation

● ——— ● - 4 hrs incubation

Δ ——— Δ - 6 hrs incubation

This is supported by fact that catalytic activity occurs within considerably narrow range of pH. Also, the substrate and the complex enzyme-substrate may be subjected to changes in relation to ionizing of their groups which dissociate in relation to pH.

Temperature optimum of enzymes results from increase of catalytic rate in conjunction with raise of temperature and from decrease of protein thermal stability as the temperature increases. Highest proteolytic activity was noted in temperature 40°C (Tab.3, Fig.2). Very high activity of jack fish muscle proteinases was also proved in temperature 30°C. Relatively low activity occured in temperatures 0 and 10°C. Under temperature 20°C, the proteolysis occured with the rate about 70% of optimal conditions. In temperature 50°C occured rapid decrease of autolysis rate which was increasing in relation to extended incubation time.

Table 3

Autolysis of jack fish muscle in pH 4.0 and various temperatures

Hours of autolysis	Increase of aminonitrogen (in mg/g of muscle)					
	Temperature °C					
	0	10	20	30	40	50
2	0.0005	0.0012	0.1680	0.2212	0.2284	0.0996
4	0.0019	0.0230	0.2520	0.3250	0.3712	0.1180
6	0.0024	0.0380	0.2792	0.3580	0.3936	0.1091

CONCLUSIONS

1. Autolysis process of jack fish muscle is most intense in pH 3.6-4.4 and in temp. about 40°C.

2. Smaller decrease of proteolytic enzymes activity at neutralising side is of great importance for autolysis of jack fish muscles.

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OPTYMALNE WARUNKI AUTOLIZY MIĘŚNI OSTROBOKA TRACHURUS TRACHURUS L.

Streszczenie

Zbadano proces autolizy mięśni ostroboka Trachurus trachurus (L.) w różnych warunkach pH i temperatury po 2, 4 i 6 godzinach inkubacji. Intensywność autolizy wyrażono w przyroście azotu aminowego, oznaczonego przy pomocy miareczkowania formolowego. Stwierdzono, że autoliza przebiegała najintensywniej w przedziale pH 3,6-4,4 i w temperaturze ok. 40°C. Zmiana pH od optimum w kierunku kwaśnym powoduje większy spadek aktywności enzymów proteolitycznych niż w kierunku zasadowym. Fakt ten świadczy o dużym znaczeniu enzymów proteolitycznych w rozkładzie mięśni ryb. Wykazano również gwałtowny spadek prędkości autolizy wraz z podwyższeniem temperatury z 40 do 50°C. Spadek ten stawał się wyraźniejszy w miarę przedłużania czasu inkubacji.

ОПТИМАЛЬНЫЕ УСЛОВИЯ АВТОЛИЗА МЫШЦ СТАВРИДЫ TRACHURUS TRACHURUS (L.)

Резюме

Исследован процесс автолиза мышц ставриды Trachurus trachurus (L.) в разных условиях pH и температуры после 2,4 и 6 часов инкубирования. Интенсивность автолиза выражена увеличением аминного азота, определённого при помощи формольного титрования. Установлено, что автолиз протекал наиболее интенсивно в границах pH 3,6 - 4,4 и при температуре около 40°C. Изменение pH от оптимум в кислотном направлении вызывает большее уменьшение активности протеолитических энзимов, чем в щелочном направлении. Этот факт свидетельствует о большом значении протеолитических энзимов в процессе порчи рыбы. Установлено также резкое уменьшение скорости автолиза вместе с увеличением температуры от 40 до 50°C. Это уменьшение становилось более заметным по мере увеличения времени инкубирования.

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