ENZYMIC ACTIVITY OF PANCREAS

Berdutina A.V., Nekludov A.D., Mitaleva S.I.
All-Russian Meat Research Institute, Talalikhina 26, 109316, Moscow, Russia
Baer N.A., Dubina V.I., "Ramensky" Meat-packing Plant

The pancreas is known to contain a number of enzymes that are usually isolated as the enzymic complex under the name of pancreatin. It is widely used in medicine and food industry because of the broad substrate specifity and ability to hydrolyze proteins, fats, and polysaccharides of various chemical composition (1).
Enzymes were usually separated and refined by means of multistage and expensive process which sometimes provokes the decrease of enzymic activity. In this connection, in recent years works concerning the usage of biomass, containing enzymic preparations for fermentative hydrolysis, but not individual preparatious appeared (2,3).
In spite of the fact that such a method allowed to obtain cheap enzymic preparations, it was rarely applied in practical conditions for receiving enzymic complex of slaughter animal pancreas. In that way, an attempt was made to use such a complex for hydrolyzing blood proteins. Some positive results were got (3).

PURPOSES

The purpose of the study was to analyze the enzymic complex of the pancreas and to define conditions for protein hydrolysis.

MATERIALS AND METHODS

In the study a pig pancreas was used. The proteolytic activity of its enzymes was estimated by Anson modified method (4). The found values of proteolytic activity of different animals pancreas samples, depending on the quantity of added water (hydromudulus) are presented in Table 1.
For all the samples the maximum proteolytic activity was observed if the ground pancreas was diluted with water. It result was revealed that to obtain enzymic preparations with maximum proteolytic activity, it was advisable to add 0.5 l distilled water and 50-70 ml ethyl alcohol preservative to 1 kg of ground pancreas chilled to $5^0C \pm 2^0C$, and to mix it thoroughly ($5^0C \pm 2^0C$) during 30-45 mins. The obtained macerate can be stored at the same temperature during 7-10 days without loss of proteolytic activity.
Studies carried out showed, that the enzymic activity of the pancreas did not actually change for 2-3 days at the room temperature, whereas the activity of native enzymes decreased significantly quicker. While analyzing indices of thermal stability, it was revealed that the proteolytic activity of enzymes was actually stopped at temprature higher than 65 ^0C .

Table 2. Values of activation constants and E_a of pancreatic enzymic complex

Temperature, ^0C	Activation of pancreatic enzymic complex		Inactivation of pancreatic enzymic complex	
	Activation constant $K_a \cdot 10^2$, min^{-1}	E_a, kJ/mol	Inactivation constant, $K_{in} \cdot 10^2$, min^{-1}	E_a, kJ/mol
45	2.74 ± 0,08		1.44 ± 0.02	
50	3.38 ± 0,11	55.15 ± 0,86	1.96 ± 0.03	60.24 ± 0.32
55	5.19 ± 0,13		2.98 ± 0.04	

Dependence of the proteolytic activity of pancreatic enzymic complex on pH of the medium is shown in Fig.4. According to published data (1), optimal pH value of separated and refined pancreas enzymic complex (pancreatin) was 8. However, pancreas macerate (Pic.4) showed the higher proteolytic activity at pH 7,0; it can suggest that enzymes were in immobilized state.

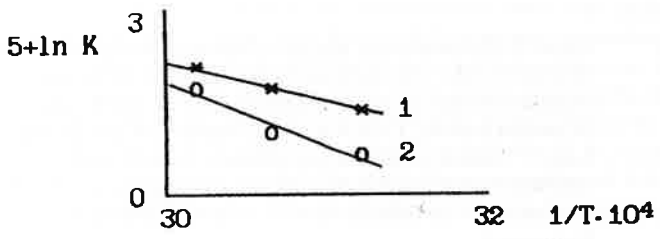

Fig.3. Estimating of the activation energy (1) and activation energy of the process of inactivation inactivation (2) of pancreatic enzymes.

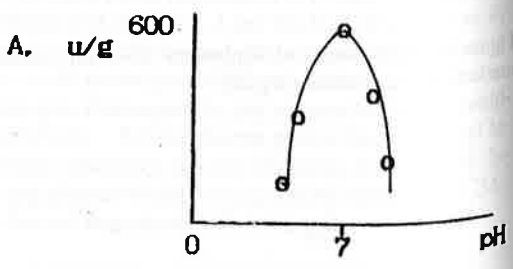

Fig.4. Activity of enzymic preparation made of ground pancreas against medium pH: x/PA (proteolytic activity), unit/g.

Table 1. Proteolytic activity of enzymic preparations received from slaughter pig pancreas

Value of Hydro-modulus	Proteolytic activity in sample					
	unit/ml			unit/g		
	first	second	third	first	second	third
7.50	1095	980	1012	6083	5444	5622
5.00	1325	1268	1184	7934	7593	7090
2.50	2283	2216	2190	7983	7748	7657
1.50	3708	3692	3484	9270	9230	8710
1.00	5350	5460	4798	10700	10920	9596
0.50	8188	7972	7553	12406	12079	11443
0.25	7865	6772	6459	9831	8465	8073

Activation and inactivation constants of the enzymic complex were estimated after proper holding time of 1 % water suspension of the ground pancreas by 45 ^{0}...55 ^{0}C.

Analysis of obtained data (Fig.1) showed that the maximum activity of enzymes was obtained at 50 ^{0}C after 1,5 hour and remained stable during 2 hrs. The increase or decrease of temperature led to the decrease of the enzymic activity, and the thermal stability of the enzymic complex decreased, when the temperature increased.

Anamorphoses of the temperature dependence in the process of activation and inactivation of pancreating enzymic complex (Fig.2) allowed to estimate activation and inactivation constants (Tab.2), and plotting of corresponding Arrhenius dependences on InK-1T coordinates (Fig.3) allowed to estimate the values of activation energy during the inactivation process of the protealytic enzymic complex.

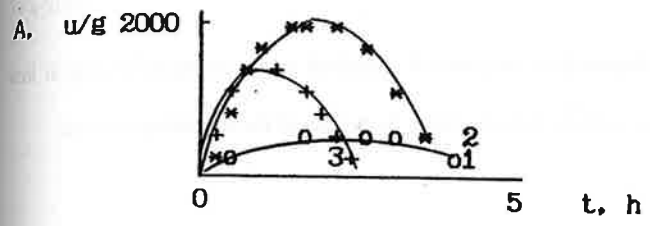

Fig.1. Proteolytic activity of enzymes in the pancreas against holding time at various temperatures , ^{0}C : 1 - 45; 2 - 50; 3 - 55
1 - PA (proteolytic activity), unit/g; 2 - time, hours

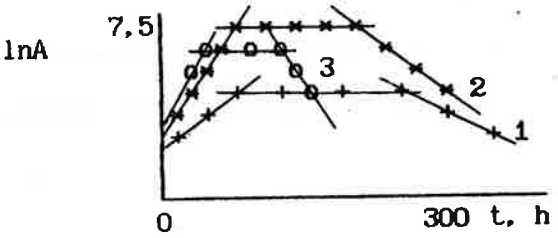

Fig.2. Logarithmic dependence of proteolytic activity (PA) of enzymic complex against time at different temperatures, ^{0}C: 1 - 45; 2 - 50; 3 - 55.

CONCLUSION

The conditions for achieving maximum activity at 50 ^{0}C found in the investigations of proteolytic activity of the enzymes of pancreas, allowed to determine the conditions for carrying out the enzymatic hydrolysis of secondary protein. Thus, the enzymic hydrolysis of 30 % water suspension of meat and bone ground material by 2 mass% pancreas preparation allowed to obtain enzymic hydrolysate with 20 % protein conversion at pH 7 during 3 hrs. Optimazing the process of hydrolysis and applying specific procedures of this process, will allow to obtain the high yield of enzymic hydrolysates in presence of pancreas enzymes.

REFERENCES

1. Mashkovsky M.D. Medicines. 12 ed.//M. Medicine. 1993. V.2, P.63
2. Nekludov A.D., Fedorova N.E. et. al. Enzymic profile of autolyzing Sacchromces yeasts. //Applied biochemistry and microbiology. 1993. V.29, No 5, P. 734-743
3. Ivashov V.I. et al. Concerning some macrokinetic laws of protein proteolysis. //Proceedings of RAS. 1992. V.322. No 5. P. 989-993
4. Rukhlyadeva A.P., Polygalina G.V. Methods for estimating the activity of hydrolytic enzymes. //M. Light and Food Industry. 1981. P. 179-184