OPTIMIZATION OF THE TECHNOLOGY OF MANUFACTURING DRY ENZYMIC BLOOD HYDROLYZATE

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INTRODUCTION

It is well known that the degradation of blood proteins is performed in different ways using different methods. One of those already implemented and applied frequently in world practice, is degradation by proteolytic enzymes. Interest towards that method gave rise to a number of studies on the optimum parameters affecting the hydrolysis process, but the published results were obtained under labotatory conditions, in most cases (1, 2, 3). For this reason, any data on industrial trials might be useful to specialists.

The purpose of this work was to judge the expedience of applying some technology and equipment decisions in the industrial manufacture of enzymic blood hydrolyzate in Bulgaria.

MATERIALS AND METHODS

Fresh pork blood was obtained in the slaughter-house in the non-stabilized form and was transferred, by pumping, to a specialized treatment shop. Blood was diluted with water in an autoclave and, at 55°C and pH 8,5, was treated with Alkaline protease B-79. Throughout the hydrolysis process, pH was kept within the range of 8,3-8,7. Proteinaceous nitrogen in the finished dry product was determined by Kjeldal's method, and the content of -amino nitrogen, by Sörensen's method. Liquid hydrolyzate concentration was done, under laboratory conditions, using the Minilab equipment made by DDS, Denmark, with type OO-TsA-95 membranes, Bulgarian make, at a pressure of 5 MPa. The drying of the concentrate was done using a spray dryer of a capacity of 230 l of evaporable moisture per hour, with an inlet temperature of 290°C and an outlet one of 90-100°C. The analysis of samples of hydrolyzate obtained by enzymic or sulphuric acid hydrolysis, using gel chromatography on Bio Gel P-2 with automatic recording of absorbance at 280 nm, was done under the following conditions: a column of 0,9x x 13,5 cm of Bio Gel P-2 superfine; 0,1 AcOH buffer; pH 3,0; 5% solutions, samples of 100 µl; rate, 6 ml/h; paper rate, 2 mm/min.

RESULTS AND DISCUSSION

Fig. 1 shows, graphically, the dependence of the degree of hydrolysis on the duration of the process. It is evident from the diagram that hydrolysis was fast during the first 60 min and DH value reached 25%. Afterwards, hydrolysis degree decreased and, at 20 h, reached 36%. It is logical, under the circumstances, to proceed to the inactivation of the enzyme after 120 min and to the further treatment of the substrate. Industrial production, however, offers a surprise. It lies in the data on the yield of dry product depending on the duration of the hydrolysis process and the DH characteristics (Table 1).

The data in the Table indicate that DH increased slowly with time. Substrate concentration, however, increased rapidly after the 8th hour and, at 20 hours, 82% of the dry substance of the blood processed was in the liquid hydrolyzate. On drying the liquid product hydrolyzed for 2 hours, dry substance losses were considerable: some 40%, while on drying following hydro-

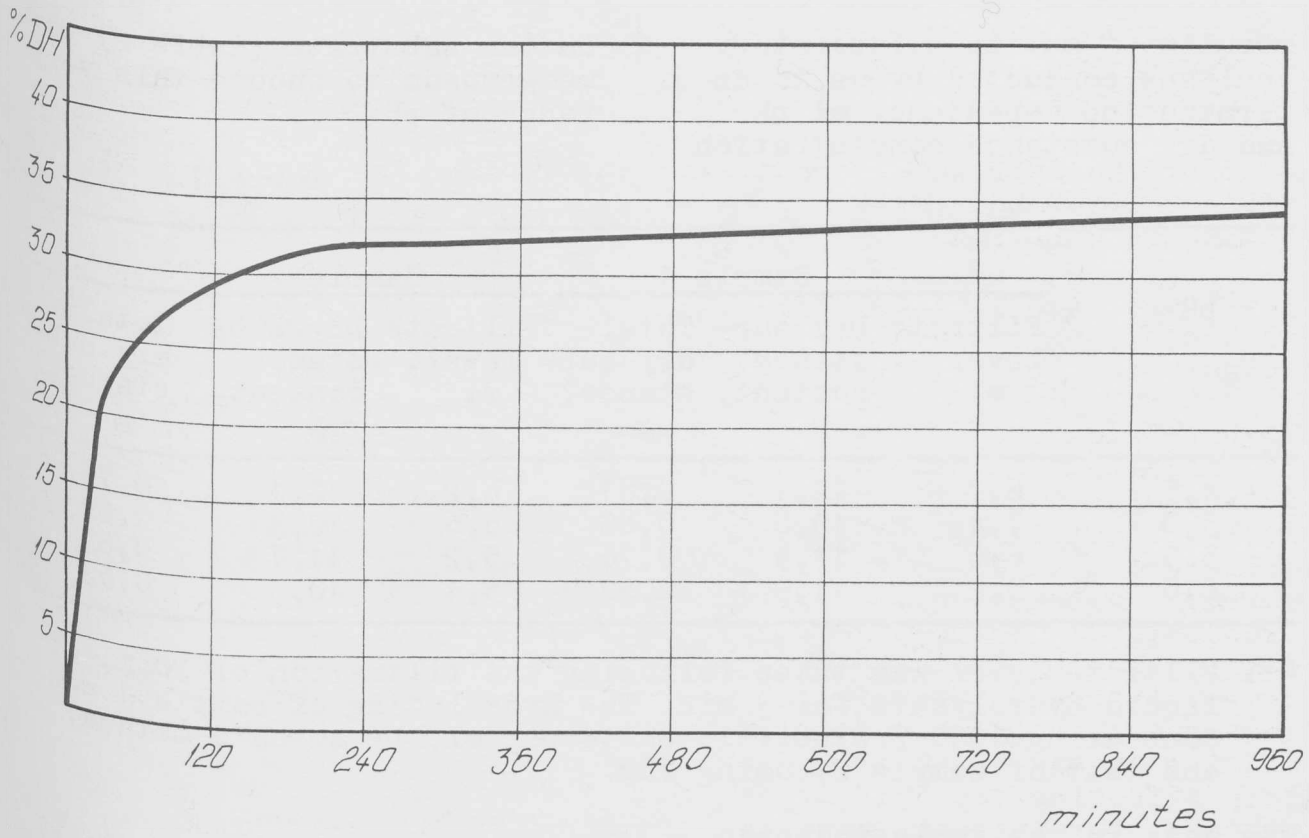


Fig. 1

Table 1.

Hydrolysis time, h	2	4	8	12	16	20
Degree of hydrolysis, %	28	32	33	34	35	36
Dry substance content in the liquid hydrolyzate, % of the dry substance of processed blood	66	67	69	79	81	82
Dry hydrolyzate yield, % of the dry substance of processed blood	39	42	46	63	67	67
Dry product losses upon drying, %	40	36	34	20	18	18

lysis for 12-20 h, they were twice lower: 20 or 18%, respectively. A probable explanation is that, with the higher degree of hydrolysis (of over 33% in this case), the mass of dried particles increased and, following a change in their velocity in the cyclone, they dropped in kinetic energy and precipitated rapidly. There may also be some other causes the nature of which may be found on relevant studies. In this case, it is the practical results that are important for us, i.e., it is of economic advantage that the process lasts some 20 h. It should be noted here that, prior to the filtration of the liquid hydrolyzate, pH of the medium was adjusted to 4,5-5,0. The

results of multiple laboratory analyses conducted by us to determine the dependence of pH and dry substance concentration in the substrate (Table 2) gave us grounds to choose this very range of pH.

Table 2.

pH	Sample 1			Sample 2		
	Filtrate level, ml	Dry substance content, %	Total dry substance, g	Filtrate level, ml	Dry substance content, %	Total dry substance, g
4,5	6,7	12,1	0,81	6,2	12,1	0,75
5,0	7,4	10,8	0,80	5,6	12,3	0,69
5,5	6,6	11,5	0,76	5,2	11,7	0,61
6,0	5,2	11,9	0,62	5,1	10,3	0,53

NB. Filtrate level was taken following the filtration of 20 ml of liquid hydrolyzate for 3 min. The hydrolyzing of sample 1 was done using 1000 proteolytic units per ml, by Anson's method, and that of sample 2, using 750.

The data in the table indicate that the contents of total dry substance (g) was the highest at pH values within the range of 4,5-5,0.

The processing of liquid hydrolyzate by reverse osmosis yielded a factor of protein concentration of 2,1, and a volumetric factor of concentration, 2,79.

Fig. 2 shows changes in the permeability of the membranes in the course of the process. The graph shows that, immediately after the start of the equipment, the level of permeated material began to decrease slowly. While it was 100 $l/h.m^2$ initially, in 30 min it decreased twofold.

Fig. 3 shows the graphs of two samples of hydrolyzates (enzymic and sulphuric acid ones), analysed using gel chromatography on Bio Gel P-2 with automatic recording of absorbance at 280 nm. This method is used to determine the distribution of the peptides obtained based on molecular mass. It is well known that, with gel chromatography on Bio Gel P-2, the pattern obtained does not correspond to actual distribution,

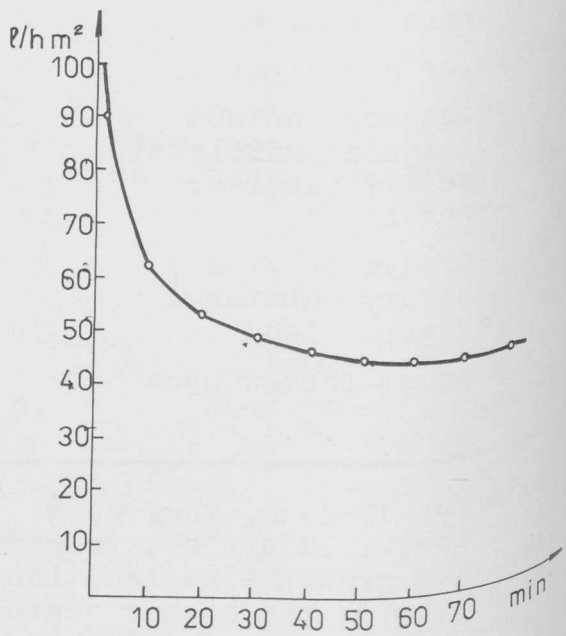
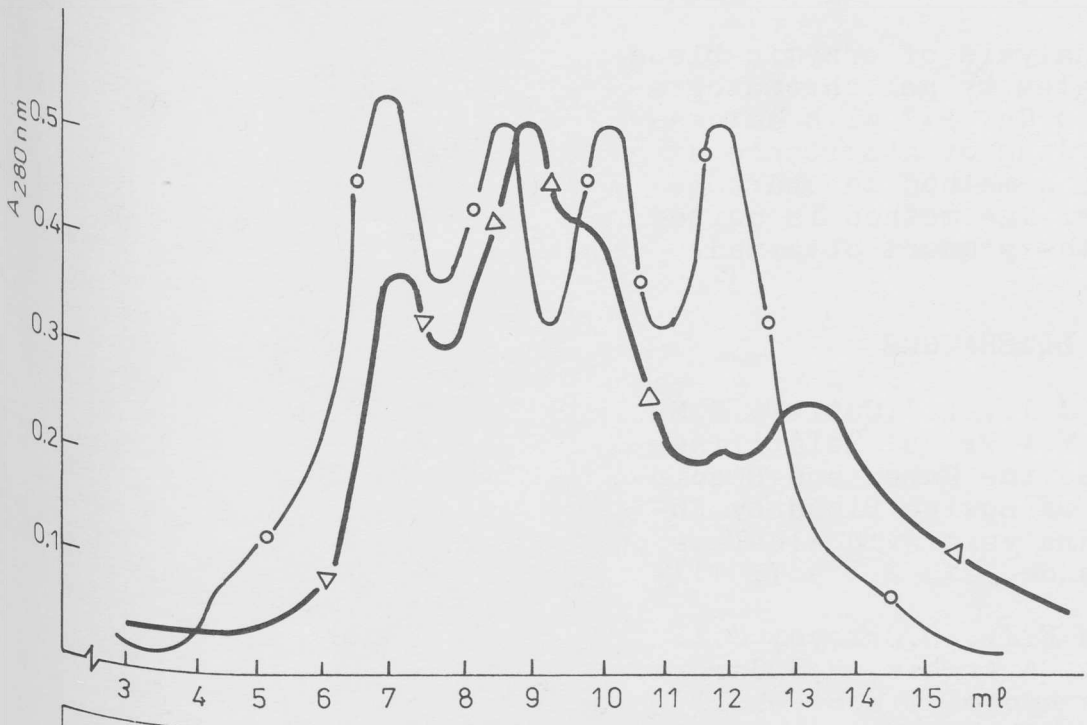


Fig. 2



Indices	Enzimic hydrolyzate —o—	Sulphuric acid hydrolyzate —Δ—
Total protein	82,78	86,86
α amino nitrogen	4,88	8,12
DH	36,40	58,46

Fig. 3

which is accounted for by the absorption of aromatic amino acids and their short peptides on the matrix of the carrier gel. As a result of that, the amino acids tyrosine and tryptophan and their short peptides lag rather far behind the other amino acids and peptides. This, on its part, allows a unique characterization of each protein hydrolyzate, with a degree of hydrolysis of 10-40%. In fact, the diagram obtained at 280 nm represents a 'finger-print' of the relevant product and may serve for its control and standardization. The curves in the figure indicate that the peak at elution volume of 12 ml in the sulphuric acid hydrolyzate is slightly outlined (tryptophan is almost entirely destroyed) and reduced more than twice compared to its content in the enzymic blood hydrolyzate.

CONCLUSIONS

1. On industrial scale, it is advantageous from the economic point of view to conduct enzymic hydrolysis under the conditions stated for 12-20 h. This contributes to increasing the yield of finished product as a result of the increased dry substance content in the liquid hydrolyzate and to reducing drying losses.
2. The optimum pH value of liquid hydrolyzate prior to filtration constitutes 4,5-5,0. At the said pH, dry substance content in the substrate is the highest.
3. Reverse osmosis is a suitable method to concentrate enzymic blood hydrolyzate.

4. The analysis of enzymic blood hydrolyzates by gel chromatography on Bio Gel P-2 with automatic recording of absorbance at 280 nm is a method to characterize them. The method is suited also to the product obtained.

LITERATURE

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