C13

HYDROLYSIS OF THE SLAUGHTER CATTLE BLOOD BY YEAST PROTEASES OF SACCHAROMYCES TYPE

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Possibility to use brewers' yeast biomass as polyenzymatic preparation promoting the hydrolysis of proteins isolated from slaughter animals is demonstrated. Comparative kinetic characteristics of the hydrolysis of the whole blood, its plasma and formed elements are shown, hydrolytic activation energy of blood proteins combined with activated brewers' biomass is determined. The hydrolysis proceeded more intensively if separated proteins of the blood were used. Brewers' yeast biomass may be used not only as enzymes of high substrate specificity, but also as an element for enriching the substrates hydrolyzed with essential aminoacids, isoleucine specially, that permits to intensify 10-30 % biological efficiency of blood proteins. Biochemical tests of food rations proved prospects concerning the apply of the blood hydrolyzed with yeast as food additives for products of general consumption.

Nowadays alimentary protein deficiency makes up 21% of the demand. Specially treated blood received from slaughter animals may serve for making up the protein deficiency.
The purpose of the study was to analyze the complex activity of the yeast biomass which is abundant of enzymes and would be used for enriching food protein hydrolyzates with deficient aminoacids.
The authors used in their study 5% suspension of Saccharomyces Carlsberg yeast of 10 generations with 40% content of active cells combined with the cattle animal blood stabilized with sodium citrate and contained 15% of protein by Lowery.
To activate enzymatic systems the yeast biomass was autolyzed with 2% chloroform added by 50°C in 5 hours. The formol titration was used for controlling the level of hydrolysis by the content of released aminogroups. The blood was fractionated by 2500 rev./min.
Basic constants of the hydrolysis were defined by equations:

$$P/t = V_{max} - Kt \quad (1)$$

$$\ln V = \ln V_{max} - K_i t \quad (2)$$

$$K_m = V_{max} / K_i \quad (3)$$

where P - quantity of aminoacids released, mg-eq/min.ml,
t - time of hydrolysis, minutes,
V_{max} - maximum rate of hydrolysis, mg-eq/min.ml,
K - numerical constant of the hydrolysis,
K_i - intensity constant of the hydrolysis, min^{-1},
K_m - Michaelis efficient constant, mg-eq/ml.

Activation energy of the hydrolysis was estimated by the standard Arrhenius equation.
Using conventional methodology contents of water, ash, nitrogen, carbohydrates, B group vitamines, as well as the solubility were determined. The analysis of aminoacids was carried out in accordance with the standard program in Eppendorf - Biotronic analyzer LC 3000 (Germany).
Curves of the hydrolysis of the whole blood by yeast biomass, after 5-hours activation of enzymatic systems (activation process is not shown), were plotted in Fig.1.
Curves of the hydrolysis were of complicated S-profile, particularly specific for the hydrolysis of formed elements of the blood by 50°C and 55°C. The higher yield of aminogroups released was observed, when hydrolyzing plasma proteins.
For quantity estimation of hydrolytic ability of yeast proteases the linearizing of hydrolysis curves in coordinates of **P/t** = func.(f) was carried out. In practice all curves may be shown in two linear dependences reflected two main stages of hydrolysis process - "quick" and "slow" (Fig.2).
Macrokinetic constants of these stages and the activation energy of the process equal to 100-105kJ/mol may be estimated by equations (1), (2), (3), and Arrhenius equation.
Kinetic data received proved the opportunity to use the yeast for hydrolyzing protein-peptide substrates of different types. It is attractive that while combining the enzymatic hydrolysis of the blood with the yeast biomass autolysis, you would receive hydrolyzates with enriched content of aminoacids.
For this purpose, 1l of the whole blood was added to 1l of the yeast biomass autolyzed by 50°C in 5 hours; then non-hydrolyzed protein and yeast cellular membranes were isolated by centrifuging the mix during 30 min. by 2500 rev./min. or by filtering the mix in nutsch-filter through filter-cloth; at last the mix was dried in a spray drier or freeze-drier. Characteristics of the end product with
10-12% of the yield were summarized in Table 1.

Table 1

Characteristic indices of blood hydrolyzates.

Indices	Enzymatic hydrolyzate	Dried blood
Water, %	8.5 ± 0.1	5.2 ± 0.8
Ash, %	16.4 ± 0.8	5.4 ± 0.5
Total nitrogen, %	8.3 ± 0.2	12.1 ± 0.4
Amine nitrogen, %	3.4 ± 0.3	-
Carbohydrates, %	18.4 ± 0.5	-
Solubility, %	88.4 ± 0.8	41.3 ± 0.9
B group Vitamines, mg %	33.6 ± 0.9	2.5 ± 0.3

Aminoacid analysis of whole blood hydrolyzates combined with the yeast biomass demonstrated that hydrolyzates occured to be enriched with various aminoacids including such essential ones as valine, isoleucine, methionine, lysine. As a result it approximates the content of hydrolyzates to the content of aminoacids of the natural egg-white (Table 2).

Table 2

Aminoacid content of animal blood hydrolyzates

Aminoacids	Enzymatic hydrolyzate, combined with yeast proteases, g/100g	Egg-white, g/100g of protein (standard)
Ala	8.9 ± 0.2	6.7
Arg	5.5 ± 0.3	2.4
Asp	7.5 ± 0.7	7.0
Val	7.9 ± 0.2	7.9
Gly	4.5 ± 0.2	3.5
Glu	12.9 ± 0.8	12.4
His	3.8 ± 0.2	2.4
Ile	4.8 ± 0.3	6.6
Leu	12.4 ± 0.6	8.8
Lys	2.2 ± 0.1	6.4
Met	2.3 ± 0.1	3.1
Pro	4.7 ± 0.2	4.2
Ser	8.6 ± 0.3	8.4
Thr	4.7 ± 0.4	5.0
Trp	1.3 ± 0.1	1.7
Tyr	4.6 ± 0.2	4.3
Phe	6.4 ± 0.2	5.8
Cys	1.8 ± 0.1	2.3

Indices summarized in Tables 1 and 2 showed that hydrolyzates received in the process of destruction of amido bonds of blood proteins under the action of yeast enzymes occured to be enriched not only by essential aminoacids, but also by vitamines of B group. Actually it became evident that the end product had the higher nutritive value than the very blood.

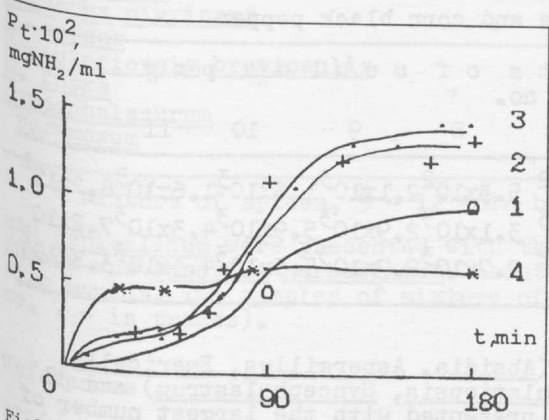
Rats were used for preliminary tests of food rations. The whole blood, blood partially hydrolyzed with yeast, and blood completely hydrolyzed were added to rations. Results showed that those rations which contained partially hydrolyzed blood proteins combined with yeast biomass enzymes had the highest efficiency. Their biological value was 12-20% higher (Table 3).

Table 3

Biological value of cattle animal blood preparations

Blood preparation	Gain in weight of rats, g/day	Consumption of nitrogen, g/day	Efficiency coefficient of nitrogen	Net utilization of protein
Whole blood (dried)	0.34 ± 0.08	0.09 ± 0.02	3.8 ± 0.70	34.7 ± 1.8
Enzymatic hydrolyzate	0.61 ± 0.09	0.11 ± 0.01	5.4 ± 0.64	41.0 ± 2.6
Blood hydrolyzate (after deep hydrolysis)	0.54 ± 0.03	0.12 ± 0.01	4.3 ± 0.60	36.8 ± 3.3

On the basis of data received the residual brewer's yeast may be recommended not only as a source of proteinases with extensive substrate specificity, but as the mean for enriching poor protein hydrolyzates with deficient aminoacids and vitamines in the food additives industry.



Figl. Accumulation of products of whole blood hydrolysis by yeast autolyzate versus the time by different temperatures (°C):
1 - 40°C, 2 - 50°C, 3 - 55°C, 4 - 60°C

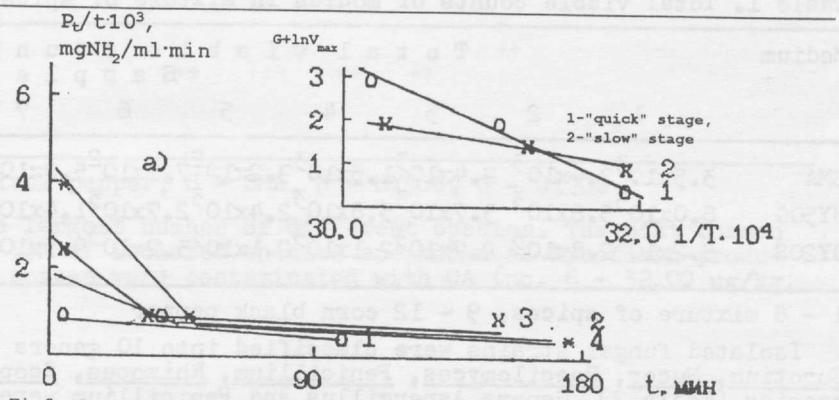


Fig2. Determination of maximum apparent rates (à) and activation energy of the whole blood proteins hydrolysis by the yeast autolyzate.
1 - 40°C, 2 - 50°C, 3 - 55°C, 4 - 60°C.