KINETIC AND BIOLOGICAL CHARACTERISTICS OF BLOOD PROTEIN HYDROLYZATES OF SLAUGHTER ANIMALS AND THEIR MIXES WITH SOYA ISOLATES

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The blood is known to be a low efficient food protein additive due to its low content of indispensable amino acids - isoleucine and methionine. To increase its biological efficiency the blood protein are usually combined with other vegetable or animal proteins having a high content, as least, of one of the above-mentioned indispensable amino acids. Milk proteins or soya isolates are most often used for this purpose (Faivishevsky, Voinov, 1986).

Obviosly, the kinetic parameters of hydrolysis and the biological indices of such mixes will differ from usual proteins. To test such an assumption a hydrolysis was carried out of 1% solutions of the whole blood and its main proteins (albumin and globin), as well as of 1% suspension of soya isolate and its mix with whole blood by pancreatin at temperatures 40-60°C, pH 8,0 and weight ratio of enzyme to substrate 1:50.

The macrokinetic constants of hydrolysis were determined by formulae (Ivashov and Nekludov, 1992):

$$P/\tau = V_{max} - k\tau \qquad (1)$$

$$\text{Ln } V = \ln V_{max} - k_i\tau \qquad (2)$$

$$K_m = \frac{V_{max}}{K_i} \qquad (3)$$

where:

P - yield of hydrolysis products, mg-equiv. of liberating amino group (NH_2)/ml;
V - rate of hydrolysis, mg-equiv. NH_2/min;
V_{max} - effective maximum rate of hydrolysis, mg-equiv. NH_2/min;
K - dimensionless constant of proportionality;
K_i - effective constant of hydrolysis process intensity, min^{-1};
K_m - effective constant of Michaelis, mg-equiv. NH_2/ml.

The number of amino groups, liberating in the course of the hydrolysis, was determined by a formol titration according to Zerensen (Gouben-Veil, 1963).

The electrophoretic separation of hydrolyzates samples was carried out on vertical electrophoresis apparatus in a gel layer with the thickness 0.8 mm and size 10 x 10 cm in a tris-glycine buffer with strength of the current 36 mA and voltage 210 V during 2,5 hours. Electrophoregrams scanning was carried out on densitometer of the firm "Hirschmann".

Fig. 1 shows the curves of the hydrolysis of proteins of the whole blood, albumin and globin, and of the proteins of soya isolates and their mixes with blood proteins. As can be seen from the figure, the hydrolysis process finishes practically 40-60 min after the beginning of the process, the greater increase of the liberated amino groups is observed in case of using globin as a substrate.

As can be seen from the densitograms in Fig. 2 the hydrolyzates of the whole blood have 65-75% unhydrolyzed initial protein, and there appear new protein fractions with a molecular mass 30-50 kD and 13-18 kD. A similar picture can be observed for other protein hydrolyzates. This points to the fact that hydrolysis stops not due to splitting of the whole substrate, but due to inhibition of hydrolysis by the products of reaction. Thus, one can conclude that under the usual conditions the extent of enzymatic conversion of proteins by pancreatin reaches on the average 20-30%.

Fig. 3 shows the linearization of the curves of the hydrolysis in the system of reversed coordinates. Practically all the curves of the hydrolysis can be, by convention, presented as two macrosteps: the rapid and slow ones, the kinetic constants of which, calculated according to formulae (1)-(3) and the equation of Arrhenius are presented in Table 1.

As can be seen from the Table, the kinetic constants of the hydrolysis are greatly dependent on the chosen substrate. The individual proteins - albumin and globin - hydrolyse most intensively, while the mixes of the proteins - both in the case of blood and the soya isolate - hydrolyze worse.

Table 2 shows the data about biological efficiency of blood hydrolyzates and their mixes with soya isolates. From these data it follows that mixing the blood with soya isolate and subsequent hydrolysis of the obtained mix leads to obtaining the hydrolyzates, the biological value of which is 40-50% more than that of the hydrolyzates of blood.

References

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Table 1. Kinetic constants of hydrolysis of protein substrates by pancreatin at 50°C.

Table 2. Indices of biolodical value of blood hydrolyzates and their mixes with soya isolate.

Fig. 1. Dependence of yield of hydrolysis products of protein substrates by pancreatin from time at different temperatures: 1 - 40; 2 - 50; 3 - 60°C.

Fig. 2. Densitograms of blood protein before (a) and after (b) hydrolysis.

Fig. 3. Graphic determination of maximum apparent rates (a) and energy of activation (b) of hydrolysis process of the whole blood by pancreatin.