KINETIC PATTERNS OF FERMENTATIVE HYDROLYSIS OF WASTE ANIMAL PRODUCTS

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Rational use of the waste protein materials as obtained in meat processing plants is an important problem connected with the necessity of utilization of animal waste products and potentials for their transformation into aminoacid mixtures and lower peptides used as nutritive additives. The process of transformation of protein materials can be accomplished by the method of fermentation hydrolysis [1].

Such hydrolysis takes place under sufficiently mild conditions and makes it possible at a maximum degree to preserve labile aminoacids. Therefore it seemed interesting to evaluate kinetically the process of degradation of animal proteins under conditions of release of individual aminoacids.

Purpose of investigations

Study of the process of fermentative hydrolysis of protein substrates of animal origin in the process of obtaining of nutritive aminoacid mixtures for feeding, food and microbiological industries.

Object and methods of investigations

Defatted ground meat and bone of pork (GMB) with the content of protein 15,4%, being the waste of the dressing department of OAO "Meat plant Ramensky" was used as an object of investigations.

A water suspension of comminuted pancreas of pigs (SPP) was used as an enzymic preparation. The enzymic complex SPP was activated by incubating at 45^0C during two hours after which the proteolytic activity of the preparation was 9000 E/g.

Aminoacid profile of hydrolysates was analyzed on an aminoacid analyzer LC 3000 of "Eppendorf-Biotronic" (FRG) with the use of automatic program Winpeak [2].

Results and discussion

To study kinetic peculiarities of fermentative hydrolysis of defatted ground meat-bone pork the dependencies of accumulation of nitrogen of aminogroups from time at 40, 45, 50, 55^0C were determined. Linearization of kinetic curves of hydrolysis according to the method [3] allowed to calculate maximum velocities V_{max} of the reaction of hydrolysis of the protein substrate for "quick" and "slow" stages of hydrolysis reaction, as well as the constants of intensity of hydrolysis K_i and the constant of Michaelis K_M. Activation energy E_a of the process was calculated according to the equation of Arrhenius (4). The calculated kinetic characteristics are presented in Table 1.

From Table 1 it can be seen, that the highest degree of protein conversion was achieved at 45^0C. Effective time of hydrolysis was 2,25 hours. Temperature increase higher than 50^0C led to decrease of release of free nitrogen of aminogroups, which most probably was connected with a temperature inactivation of enzymic complex.

To study the mechanism of hydrolysis in more detail the kinetics of accumulation of each of the aminoacids being determined was studied because the kinetics of accumulation of individual aminoacids is little studied and there are almost no data about it in literature (5-7).

Similar to accumulation of aminoacids aminogroups nitrogen and lower peptides, the kinetic curves of release of different aminoacids after their linearization can also be conventionally approximated by two rectilinear areas characterizing "quick" and "slow" stages of the process.

Using the above approach we succeeded in calculating macrokinetic constants at different temperatures of hydrolysis process (Table 2). It should be noted that the proposed kinetic model of the process allows to describe adequately the patterns of accumulation of the most of aminoacids.

Analysis of data obtained shows that the accumulation of arginine, lysine, leucin and tyrosine proceeded most intensively during the whole time period studied. Similarly, though somewhat less intensively took place the release of phenylalanine, histidine and valine. Maximum yield was observed for the indicated acids. Thus, for histidine, arginine and tyrosine after 7 hours the yield was 48-77%, and for valine, lysine, leucin and phenylalanine – 25-38%.

On the contrary, serin, alanine and aspartic acid were forming in the process of hydrolysis largely during a quick stage. In this case the accumulation of aspartic acid at the slow stage was almost stopped. Glycine was formed during hydrolysis both at the quick and slow stages, however the velocity of its accumulation was very low. As a result the yield of aspartic acid and glycine did not exceed 2%.

Activation energy for valine, threonine, tyrosine, arginine, histidine and phenylalanine was rather large that will suggest a large number of proteases present in the studied preparation (SPP) which split a large number of peptide bonds, as formed by the indicated aminoacids and require for this purpose large energy consumption [3].

Analyzing the above-mentioned one can suppose that enzymes of pancreas hydrolized in the first place actomyosin, myosin, myoglobin and to a less extent collagen, as demonstrated by a low content in the hydrolyzates of glycine, alanine, aspartic and glutamic acid, serine as well as a very low content of proline, i.e. those aminoacids that are essentially in collagen.

According to the results of study of kinetics it was suggested to carry out the process of fermentative hydrolysis of defatted ground meat and bone at the parameters, as follows: temperature 45^0C, duration 3 hours. Under these conditions the experimental samples of hydrolyzates with the degree of conversion of protein 32% which contain the whole complex of essential and non-essential aminoacids were obtained.

Thus, study of kinetics of release of aminoacids during hydrolysis of meat and bone materials allows to determine the optimum parameters for obtaining aminoacids mixtures for food purposes with a pre-determined composition. The obtained meat and bone hydrolyzates contain all the necessary base aminoacids that allows to use these products in feeding, food and microbiological industries.

References

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Table 1

Kinetic characteristics of the hydrolysis of defatted ground meat and bones by a fermentative complex SPP

Kinetic characteristics	Temperature, ^{0}C ("quick" stage)				Temperature, ^{0}C ("slow" stage)		
	40	45	50	55	40	45	55
$V_{max} \times 10^2$, mgNH_2/ml min^{-1}	1.52±0.10	1.92±0.17	2.32±0.23	2.55±0.12	0.56±0.05	0.68±0.05	0.98±0.08
$K_i \times 10^2$, min^{-1}	1.12±0.02	1.21±0.04	1.65±0.10	1.65±0.02	0.21±0.01	0.22±0.01	0.51±0.02
$K_M \times 10^2$, mgNH_2/ml	1.36±0.11	1.59±0.10	1.41±0.12	1.54±0.11	2.67±0.18	3.09±0.20	1.92±0.13
E_a, kJ/mol	29.83±0.81				32.20±0.04		

Table 2

Kinetic characteristics of the process of accumulation of individual aminoacids at 45^{0}C

Aminoacid	$V_{max} \times 10^3$, g/100 g protein min^{-1}		$K_i \times 10^2$, min^{-1}		$K_M \times 10^2$, g/100 g protein	
	"quick" stage	"slow" stage	"quick" stage	"slow" stage	"quick" stage	"slow" stage
ALA	10.58±0.55	3.40±0.34	1.56±0.02	0.19±0.01	0.68±0.05	1.79±0.14
ARG	30.04±0.33	15.36±0.60	0.82±0.01	0.26±0.01	3.66±0.29	5.91±0.49
ASP	2.97±0.14	0.92±0.09	1.60±0.02	0.27±0.01	0.19±0.01	0.34±0.03
GLY	2.45±0.01	1.13±0.09	0.96±0.01	0.22±0.01	0.26±0.02	0.51±0.04
GLU	9.02±0.09	4.04±0.35	1.05±0.01	0.18±0.01	0.86±0.06	2.24±0.18
HIS	16.94±0.52	8.22±0.55	0.77±0.03	0.26±0.01	2.20±0.18	3.16±0.24
ILEU	8.31±0.01	3.94±0.15	0.97±0.01	0.26±0.01	0.86±0.07	1.52±0.11
LEU	27.80±0.88	12.47±0.96	0.84±0.03	0.24±0.01	3.31±0.27	5.20±0.42
LYS	24.31±0.65	10.43±0.64	1.13±0.01	0.20±0.01	2.15±0.18	5.22±0.40
MET	6.33±0.70	5.22±0.36	1.80±0.20	0.34±0.01	0.35±0.03	1.54±0.12
PHE	16.48±0.01	8.91±0.60	0.59±0.01	0.27±0.01	2.79±0.21	3.30±0.26
SER	7.27±0.15	2.38±0.22	1.43±0.01	0.21±0.01	0.51±0.04	1.13±0.09
THR	5.70±0.10	2.49±0.13	1.10±0.01	0.23±0.01	0.52±0.04	1.08±0.08
TYR	21.65±1.03	10.60±0.72	0.73±0.04	0.28±0.01	2.97±0.22	3.79±0.29
VAL	13.82±0.14	6.44±0.45	1.00±0.01	0.22±0.01	1.38±0.11	2.93±0.22