STUDY OF THE PROCESS OF ACID HYDROLYSIS OF KERATIN-CONTAINING RAW MATERIALS AS A METHOD FOR MANUFACTURE OF FOOD ADDITIVES

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For the last years researchers showed a rather greater interest for obtaining hydrolyzed keratin-containing raw materials in connection with their wide use in microbiological and perfumery industries.

As hydrolyzing agents, acid and alkali water solutions more often are used as well as enzymes of the different origin with a keratinase activity [1]. In spite of numerous publications devoted the methods for producing hydrolyzed proteins, their characteristics and methods for their fractionation [2,3], there is almost no information of the kinetics of hydrolysis of keratin-containing raw materials and the process of releasing free amino acids in the course of degradation of peptide bonds what allows to approach a problem of the directed hydrolysis and production of hydrolyzed proteins with pre-determined properties. In this report an attempt is made to more detailed solution of the problem above-mentioned.

As objects for investigations feather residues with protein content of 86,8 % were chosen.

As a hydrolyzing agent water solutions of sulphuric acid with concentrations of 20 and 25 %mass were used.

100 mg of comminuted feather residues were placed into a 30 ml ampule and 15 ml of 20-25 %mass sulphuric acid were added. Then inert gas was blown through this ampule for 2-3 minutes and after all it was placed in thermostat for hydrolysis at a temperature of 100, 110 and 120 °C. In every 30 minutes the ampule was taken out, air-cooled and unsealed. The hydrolysate was filtered; the total content of amino groups released was determined in it by the formol-titration method according to Zerensen [4], the content of free amino acids was determined with an amino acid analyzer (Eppendorf-Biotronic) according to the standard program.

Kinetic estimation of curves was carried out by the methods of mathematical statistics according to formulas [5]:

$$\ln ([P]_\infty - [P]) = \ln [P]_\infty - kt \quad (1)$$

$$\ln ([P]_\infty / ([P]_\infty - [P])) = kt \quad (2)$$

where [P] is a concentraion of a reaction product at the moment t,

$[P]_\infty$ is a concentration of a reaction product after the reaction being completed.

An activation energy of the process was calculated according to Arrenius's equation [5].

Macrokinetic constants of the accumulation of free amino groups during hydrolysis and activation energy are tabulated (Table 1).

Table 1. Macrokinetic characteristics of hydrolysis of feather residues with solutions of sulphuric acid

Concentration of H_2SO_4, %	Temperature of the process, °C	Constant of rate (k), min^{-1}	Activation energy, kJ/mol
20	100	0,00290 +/- 0,00007	51,37 +/- 2,20
	110	0,00466 +/- 0,00005	
	120	0,00692 +/- 0,00008	
25	100	0,00394 +/- 0,00010	72,36 +/- 3,62
	110	0,00677 +/- 0,00002	
	120	0,01330 +/- 0,00002	

The accumulation of 12 from 17 amino acids during hydrolysis can be also described by a reaction of the pseudo-first order what allowed to calculate kinetic constants for them (Tables 2,3).

Table 2. Effective constants (k_{eff}) of accumulation rate of free amino acids and their yield (x) during hydrolysis of feather residues with 20 % sulphuric acid

Amino acid	T = 100 °C		T = 110 °C		T = 120 °C	
	$k_{eff} * 10^3$ min^{-1}	x *) %	$k_{eff} * 10^3$ min^{-1}	x *) %	$k_{eff} * 10^3$ min^{-1}	x *) %
Aspartic acid	4,39 ± 0,15	56	7,28 ± 0,21	76	11,73 ± 0,17	89
Threonine	0,96 ± 0,06	18	2,45 ± 0,07	34	3,64 ± 0,15	55
Glutamic acid	1,34 ± 0,04	23	3,45 ± 0,15	46	5,48 ± 0,16	59
Glycine	3,58 ± 0,09	53	6,57 ± 0,20	74	9,47 ± 0,20	84
Alanine	2,28 ± 0,08	35	4,05 ± 0,25	56	7,73 ± 0,38	78
Leucine	1,36 ± 0,07	30	2,80 ± 0,07	39	3,60 ± 0,03	51
Isoleucine	1,08 ± 0,03	17	1,44± 0,04	24	1,78 ± 0,04	37
Phenylalanine	6,87 ± 0,34	67	7,65 ± 0,14	76	12,70 ± 0,60	89
Tyrosine	6,85 ± 0,22	69	8,99 ± 0,33	79	12,00 ± 0,20	91
Valine	1,49 ± 0,02	29	1,80 ± 0,02	33	2,46 ± 0,05	38
Lysine	0,90 ± 0,02	21	1,65 ± 0,02	33	2,59 ± 0,04	46
Arginine	1,21 ± 0,02	18	-	-	2,93 ± 0,07	41

*) values of amino acid yields are given after 5 h hydrolysis

Table 3. Effective constants (k_{eff}) of accumulation rate of free amino acids and their yield (x) during hydrolysis of feather residues with 25 % sulphuric acid

Amino acid	T = 100 °C		T = 110 °C		T = 120 °C	
	$k_{eff} * 10^3$ min^{-1}	x *) %	$k_{eff} * 10^3$ min^{-1}	x *) %	$k_{eff} * 10^3$ min^{-1}	x *) %
1	2	3	4	5	6	7
Aspartic acid	4,92 ± 0,08	64	9,08 ± 0,13	83	16,30 ± 0,43	96
Threonine	1,80 ± 0,07	27	4,72 ± 0,22	53	12,70 ± 0,89	89
Glutamic acid	2,54 ± 0,07	36	5,44 ± 0,16	61	12,54 ± 0,71	94
Glycine	4,99 ± 0,11	69	10,58 ± 0,34	89	23,57 ± 1,57	98
Alanine	3,01 ± 0,10	49	6,31 ± 0,26	73	15,25 ± 0,68	96
Leucine	1,64 ± 0,02	28	3,94 ± 0,08	53	8,19 ± 0,16	81
Isoleucine	0,52 ± 0,02	15	1,51± 0,02	34	3,15 ± 0,06	56
Phenylalanine	6,22 ± 0,25	72	12,00 ± 0,22	89	18,40 ± 0,28	97
Tyrosine	5,46 ± 0,16	62	7,56 ± 0,25	73	8,72 ± 0,27	85
Valine	1,42 ± 0,02	33	1,73 ± 0,02	39	2,89 ± 0,07	53
Lysine	1,31 ± 0,02	27	4,00 ± 0,07	50	5,34 ± 0,07	66
Arginine	1,71 ± 0,09	31	3,95± 0,01	53	6,26 ± 0,05	67

*) values of amino acid yields are given after 5 h hydrolysis

Unfortunately, our attempt to determine effective constants of accumulation rates for histidine, serine, proline and sulphur-containing amino acids has failed since a part of them was undergoing an oxidative destruction during hydrolysis.

As is obvious from tables, the quantity of amino acids being released is increased with increasing the concentration of sulphuric acid and temperature of hydrolysis, the different amino acids being released with a different rate. The accumulation of aspartic and glutamic acids, glycine, alanine and phenylalanine occurs the most quickly. Their yield after 5 h hydrolysis at a temperature of 120 °C reached 80-95 %. Isoleucine and leucine are released from polypeptide links rather more difficult. It was shown that the yield of isoleucine was 40-50 %, leucine - 55-70 %. The accumulation of lysine, arginine and valine was the slowest one.

Some amino acids during hydrolysis are undergoing an oxidative destruction. As is obvious from Fig.1, histidine begins to destruct already in 3,5-4 hours, the degree of its destruction reached 80 % in 24 hours. This effect becomes apparent the more strongly, the more strictly are conditions of hydrolysis.

The same relationship was observed in case of serine whose destruction degree was 30-35 %.

It was established that a practically total destruction of methionine occurred in three hours of hydrolysis.

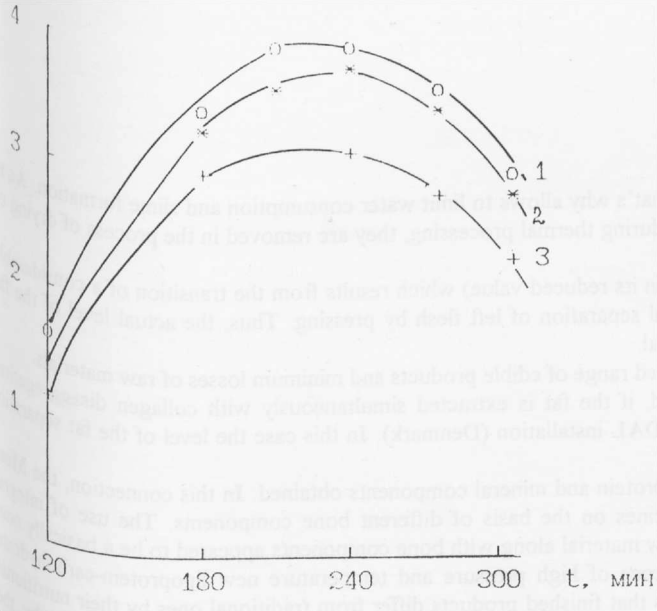

Fig.3. Relationship between the rate of histidine accumulation and the time of hydrolysis (concentration of sulphuric acid is 25% mass) 1: T=100 °C; 2: T=110 °C; 3; T=120 °C

Summarizing the data obtained, it can be noted that making conditions of hydrolysis more stronger leads to losses of such essential amino acids as methionine, serine and histidine. Therefore, as the most acceptable conditions of hydrolysis which can ensure a rather rapid accumulation of amino acids with minimum losses the following ones were chosen: temperature 110 °C, concentration of sulphuric acid 20 %, time of the process - 4,5-5 h. Hydrolysates produced have a high content of a number of essential amino acids and can be used as protein additives in the food and meat processing industries.

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