11 Determination of the amino acid score and the biological value of fermented sausages manufactured using starter cultures

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The application of microbial cultures in the different technologies of fermented meat products manufacture goes back a long way. The action of the microorganisms is manifested in inducing complex biological, chemical and physical processes which determine the colour, flavour, and the storage stability of the meat products (Pezacki, 1965, 1970; Santa Ichwan, 1969; Buyanov, 1973; Scharner, 1973; Schiefer and Schone, 1980).

It was found (Sippach, 1982) that microbial culture application results in intensifying production processes (Modic et al., 1982) and improving quality indices. All this determines the interest of investigators also in the preservation or the improvement of the nutritive value of finished products (Rogov et al., 1981; Pokrovsky, 1975).

The estimations of biological value from the chemical and the biological points of view are criteria of the nutritive value of the products. The biological value of proteins is expressed in relative values, i.e., in comparison with analogical indices obtained using standard proteins, such as egg white and cow milk protein (Vysotsky, 1976; FAO/WHO 1973). The estimate of protein from the chemical point of view is a determination of its amino acid composition, the ratio of essential to total amino acids, and the estimation of amino acid numbers.

The present work aims at the study of the amino acid composition and the biological value, from the chemical point of view, of two fermented sausage products manufactured using starter cultures.

Materials and Methods

Analyses were made of samples: initial raw materials, and finished product, of two fermented sausage items, I and II. Item I was made of non-graded beef, lean pork, and backfat; item II consisted of non-graded beef and semi-fat pork. A freeze-dried microbial preparation containing micrococci and lactobacilli was introduced in part of the products. The preparation had been made at the Meat Technology Research Institute, Sofia. Another part of the products manufactured without a microbial preparation served as control. Initial ripening was effected at 25 °C and a relative air humidity of 95% for 48 h; afterwards drying took place at 15 °C and gradually decreasing humidity.

The production cycle was considered completed in about 14 days on reaching a water content of 40-42%.

Protein was determined by Kjeldahl's method, according to Bulgarian State Standard (BDS) 9374-82, fat content by Soxhlet, BDS 8549-74.

An acid hydrolysis of proteins was conducted using 6N hydrochloric acid at 110 °C for 22 hours, and deproteinization with alcohol.

The levels of the individual amino acids were determined using a Hitachi KLA-3 automatic amino acid analyser by the method of Spackman, Stein and Moore, 1958.

A high performance liquid chromatograph LJ-2, 'P. Elmer', was used for the alkaline hydrolysis of the proteins with a subsequent liquid chromatography determination of the essential amino acid tryptophan, and also F-detection with a spectrophotometer of the same company. The programming of analyses and the reading-off of results was done using a Sigma-10 microprocessor after Vries et al. (1980).

Amino acid numbers were determined by the chemical score method (Vysotsky et al., 1976; Nesterin and Skurikhin, 1979).

The energy content of the products was estimated as the sum of the energies of protein and fats by the formula:

$$E_{MJ} = (\% \text{ protein} \times 17) + (\% \text{ fat} \times 38) \times 10$$

The ratio A/E; P, protein energy, %; and E, the energy of pure proteins, %, were determined after Dvorak and Vognarová (1970).

Results and Discussion

The results of the amino acid analysis of Product I, initial raw materials, controls (without preparation), and experimental variants (manufactured using microbial cultures), expressed in g/100 g of product and in g/100 g of protein, are shown in Table 1.

The total amino acids per 100 g of product correspond to the total protein determined. No significant differences can be observed in the individual values for the essential amino acids estimated in relation to protein, what is confirmed by the amino acid numbers (the amino acid scores) estimated, which have approximately the same values (Table 3).

Small differences can be observed in the non-essential amino acids. E.g., the amino acids proline, hydroxyproline and glycine are lower in the control compared to the initial raw material. But since the contents of non-essential amino acids are not of a decisive importance for the biological value of protein and, further, the differences manifested here are not great, their interpretation would only have a theoretical significance.

The quality of protein in the samples analysed was good (from the chemical point of view), which was demonstrated by the absence of limiting amino acids (of a low amino acid number, below 100) and by the high per cent contents of essential amino acids.

Table 1. Amino acid composition of fermented sausages: Product I.

Amino acid	Initial sausage meat		No preparation (Control)		With preparation	
	g/100 g product	g/100 g protein	g/100 g product	g/100 g protein	g/100 g product	g/100 g protein
Total protein, %		15,70		22,40		22,10
Isoleucine	0,68	4,33	1,02	4,54	1,04	4,70
Leucine	1,28	8,17	1,83	8,18	1,83	8,28
Lysine	1,28	8,17	1,92	8,57	1,89	8,54
Methonine	0,40	2,56	0,62	2,75	0,61	2,76
Cystine	0,17	1,08	0,19	0,84	0,20	0,92
Threonine	0,71	4,53	1,07	4,76	0,99	4,50
Tryptophan	0,20	1,28	0,31	1,40	0,30	1,38
Tyrosine	0,53	3,35	0,79	3,53	0,79	3,58
Phenylalanine	0,65	4,13	0,93	4,15	0,93	4,19
Valine	0,79	5,02	1,12	4,99	1,13	5,11
Hydroxyproline	0,12	0,79	0,09	0,39	0,19	0,87
Alanine	0,91	5,81	1,34	5,99	1,27	5,73
Arginine	0,97	6,20	1,44	6,44	1,47	6,65
Aspartic acid	1,54	9,84	2,18	9,75	2,10	9,51
Histidine	0,57	3,64	0,89	3,98	0,81	3,68
Glycine	0,83	5,31	1,07	4,76	1,10	4,96
Glutamic acid	2,63	16,73	3,69	16,47	3,57	16,16
Proline	0,77	4,92	0,98	4,37	1,03	4,65
Serine	0,65	4,13	0,93	4,15	0,85	3,83
Total	15,68		22,41		22,10	
Essential/Total amino acids, %	42,62		43,71		43,96	

According to data of FAO/WHO (1973), a quality protein is the one that contains 36 g of essential amino acids per 100 g of protein.

In our experiments, as far as the levels of essential amino acids were concerned, we obtained 43,31 g for product I, control, and 43,96 g for the experimental variants, respectively. Total amino acids in initial mixtures were 15,68 g/100 g of product, and in the controls and in experimental variants the values were 22,41 g/100 g of product and 22,10 g/100 g of product, respectively. The % ratios of essential to total amino acids were 42,62%, 43,71%, and 43,96% in initial raw material, the control and the experimental samples, respectively.

Table 2 presents the results of the amino acid analysis of Product II, including initial raw material, control and experimental variants.

Here, too, the total amino acid content related to 100 g of product was the lowest in the initial raw material (14,58 g), followed

Table 2. Amino acid composition of fermented sausages: Product II.

Amino acid	Initial sausage meat		Without preparation (Control)		With preparation	
	g/100 g product	g/100 g protein	g/100 g product	g/100 g protein	g/100 g product	g/100 g protein
Total protein, %		14,60		20,50		18,70
Isoleucine	0,63	4,32	0,92	4,50	0,90	4,82
Leucine	1,28	8,40	1,72	8,40	1,53	8,21
Lysine	1,25	8,57	1,82	8,86	1,61	8,61
Methionine	0,37	2,54	0,54	2,61	0,52	2,78
Cystine	0,17	1,19	0,24	1,17	0,14	0,75
Threonine	0,67	4,58	0,95	4,63	0,89	4,76
Tryptophan	0,20	1,36	0,31	1,50	0,24	1,28
Tyrosine	0,48	3,31	0,69	3,39	0,65	3,48
Phenylalanine	0,58	3,99	0,85	4,17	0,76	4,06
Valine	0,77	5,26	1,03	5,02	0,94	5,03
Hydroxyproline	0,12	0,85	0,11	0,52	0,14	0,75
Alanine	0,84	5,76	1,22	5,93	1,10	5,89
Arginine	0,89	6,11	1,30	6,32	1,22	6,53
Aspartic acid	1,36	9,33	2,02	9,83	1,78	9,52
Histidine	0,53	3,65	0,80	3,91	0,75	4,01
Glycine	0,72	4,92	0,95	4,63	0,89	10,76
Glutamic acid	2,30	15,78	3,27	15,96	3,04	4,76
Proline	0,89	6,11	0,93	4,56	0,84	4,49
Serine	0,58	3,99	0,84	4,10	0,75	
Total	14,58		20,51		18,69	
Essential/total amino acids, %	43,52		44,25		43,80	

by that in experimental (18,58 g) and control (20,58 g) variants. No differences were observed in the essential amino acid levels determined in relation to 100 g of protein, except the small reduction of cystine in control and experimental groups. But the amino acid number estimated (Table 3) is 100 even with that reduction, which is a good characteristics and allows not to be interpreted. There are no limiting amino acids in any of the three groups.

larger deviations are observed in the non-essential amino acids. Lowered values were found for hydroxyproline and proline in control and experimental variants of products compared to the initial raw materials.

The absence of limiting amino acids and the high values of essential amino acids and the total amino acids characterize the good quality of the protein in both fermented sausage products. On the basis of results it could be stated that the applied technologies using a microbial preparation do not affect protein quality. The data of ours coincide with the results of Rogov et al.

Table 3. Amino acid numbers of fermented sausages

Essential amino acids	Product I			Product II		
	Initial sausage meat	No preparation (Control)	With preparation	Initial sausage meat	No preparation (Control)	With preparation
Isoleucine	108	114	117	108	113	121
Leucine	117	117	118	120	120	117
Lysine	149	156	155	156	161	157
Cystine	104	103	105	107	108	100
Threonine	113	119	113	115	116	119
Tryptophan	128	140	138	136	150	126
Phenylalanine	125	128	130	122	126	126
Valine	100	100	102	105	100	101

Table 4. Indices characterizing the biological value of fermented sausages (after Dvorak & Vognarová, 1970)

Period of ageing, days	E_{MJ}		P (%)		A/E		E (%)	
	Contr.	Exp.	Contr.	Exp.	Contr.	Exp.	Contr.	Exp.
Product I								
1	12009	11596	22,8	24,6	80,9	80,9	18,61	20,23
18	19464	19337	19,6	19,4	84,7	80,0	16,60	15,52
Product II								
1	14039	14559	18,5	18,6	80,3	80,3	14,86	14,94
18	19535	19519	17,7	16,3	83,3	80,0	14,74	13,05

show that there are no changes in amino acid composition during the ageing process of fermented products manufactured with starter cultures.

The results obtained by us with respect to the energy content of the products, the % protein energy and the energy obtained from pure proteins are shown in Table 4.

The small differences observed in the indices investigated can go without interpretation since their values are high in general.

The toral amino acid content, the amino acid scores, the ratio of essential to total amino acids, and also the energy content, determine the tested fermented products manufactured using a microbial preparation, to be products of a high biological value.

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