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SPECIFIC ACTIVITY OF TISSUE PEPTIDASES IN FERMENTED SAUSAGES MANUFACTURED FROM HOT MEAT

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Changes in specific activity of cathepsins and trypsin-like tissue peptidase were analysed during the production and 40-day storage of cervelat-type fermented sausage manufactured from hot meat. The specific activity of cathepsins in such a sausage is on the average 30% higher than in similar sausages produced from meat refrigerated for 7 days. The specific activity of the other peptidase does not differ depending on the degree of autolysis of the meat raw material. The direction of specific activity changes of the two enzymes in both kinds of the studied sausage is the same, corresponding also to changes in other experimental cycles. The activity of the enzymes is stimulated by the increasing content of common salt, or rather of its natural admixtures. The increasing concentration of salts is due to the decreasing water content in the stored sausages.

A well known and practically applied technological recommendation in the production of fermented sausages advises that their mass be as speedily as possible considerably acidified (pH $\leq$ 5.4) with acid products of lactic fermentation [6]. This arrests the development of proteolytic bacteria and protects sausage protein against putrefaction. In subsequent production stages and during storage of finished products the development of these bacteria is prevanted by other inhibiting agents, mainly by proceeding drying [7].

The mentioned biophysical changes in fermented sausage merely slow down the activity of microbial peptide hydrolases. However, there are at least two other tissue peptidase complexes that are also active, namely cathepsins and trypsin-like peptidase. Their activity depends in a statistically significant manner on the initial quality of the meat raw material and, secondarily, on the production stage and on the duration of storage

of finished fermented sausage [10]. The effect of both the latter factors is effectively modified, but not eliminated, by e.g. the salting of sausage meat [9] or covering the sausage with a protective layer after manufacture [8].

The dependence of tissue peptide hydrolases activity in fermented sausages on raw material prompts the search for its causes in, among others, meat autolysis following slaughter. All the series of experimental fermented sausages in which this activity was determined to date were made exclusively of raw materials in fairly advanced stages of autolysis ($T \approx 5°C$, $t = 7$ days). And the differences between hot and chilled meat as regards usefulness in e.g. the production of cooked sausages are well known [7].

The above facts justify the experimental analysis of the changes in activity of the mentioned two tissue peptidases in fermented sausages manufactured from hot meat.

MATERIAL AND METHODS

MATERIAL

A firm finely ground fermented dry sausage of the cervelat type was used in the experiments. It contained 40% of lean and tendonless pork meat, 40% of similar beef and 20% of pork fat. A part of this meat was processed within 2-3 h after slaughter, and the rest was kept in a refrigerator ($T \approx 5°C$) for 7 days. The sausages were always spiced with, among others, a 3% addition of common salt. All sausage bars ($\phi = 6$ cm) were encased in artificial protein skins, and after maturing ($t = 4$ days, $15 \leq T \leq 17°C$, $85 \leq \varkappa \leq 95\%$) were smoked in cold smoke ($t = 2$ days, $20 \leq T \leq 22°C$) and then stored for 40 days ($14 \leq T \leq 15°C$, $85 \leq \varkappa \leq 90\%$).

METHODS

The experiments were performed with sausage mat, sausage after production maturing and smoking, and after 10, 20 and 40 days of storage. The bar cross-section was divided into three parts (layers extending for 1/3 of radius length); the central and outer layers were always taken for analysis.

Analytical weighed portions (about 10 g each) were taken from the central and outer bar layers, 30 cm^3 of 2% KCl solution and two drops of Tween 80 were added, and the samples were homogenized for 10 min in a water bath. 0.1 M acetate buffer (pH = 5.5) was added to the homogenized sample, and after careful mixing the fat fraction was separated

from it by sedimentation (T = 4°C, t = 30 min) and twice filltered through a filter paper. Between filterings the sample was stored in a refrigerator (T = 4°C, t = 30 min). The filtrate contained complexes of both peptidases. The specific activity of enzymes was determined spectrophoto-

Table 1. Specific activity of cathepsins and trypsin-like peptidase in fermented sausages (10^{-4}U).

Period of production and storage	Part of sausage	Rep. of exp.	Sausages manufactured from:			
			hot meat (H)		cold meat (Cd)	
			cathepsins	trypsin-like peptidase	cathepsins	trypsin-like pepsidase
M		I	506	405	130	433
		II	277	755	421	797
R	C	I	138	841	1032	476
		II	1054	390	428	842
	O	I	100	660	942	402
		II	784	350	460	558
S	C	I	745	578	333	911
		II	546	683	444	474
	O	I	883	658	636	603
		II	1050	1005	955	541
S-10	C	I	514	499	342	348
		II	933	502	424	575
	O	I	517	1111	476	1425
		II	709	487	338	698
S-20	C	I	531	1311	636	370
		II	1125	1438	2457	441
	O	I	825	1278	433	798
		II	1130	1230	1133	528
S-40	C	I	648	1351	1688	1219
		II	5538	1257	2196	1506
	O	I	1195	1697	604	1294
		II	4187	1184	1450	1096

H — hot meat
Cd — cold meat
M — meat
R — after ripening
S — after smoking
S-10, S-20, S-40 — after 10, 20 and 40 days of storage
C — central part of sausage
O — outer part of sausage

metrically by Iodice's method [3] using ninhydrin as indicator according to the procedure of Moore and Stein [5]. The activity of trypsin-like peptidase was determined by Anson's method [2, 4]. The data were treated by analysis of variance and correlation analyses.

RESULTS AND DISCUSSION

The specific activity of cathepsins and trypsin-like peptidase in fermented sausages made from hot meat varies and is different from that observed in the control part of experimental material (Table 1). During the production of both kinds of sausages this variability is less polarized than during the subsequent storage. The two studied peptidases behave rather differently, although their specific activity increases with the increase of storage time (Figs 1 and 2).

The specific activity of cathepsins is conditioned by the variable quality of meat raw material, by production stage, and by the storage of fer-

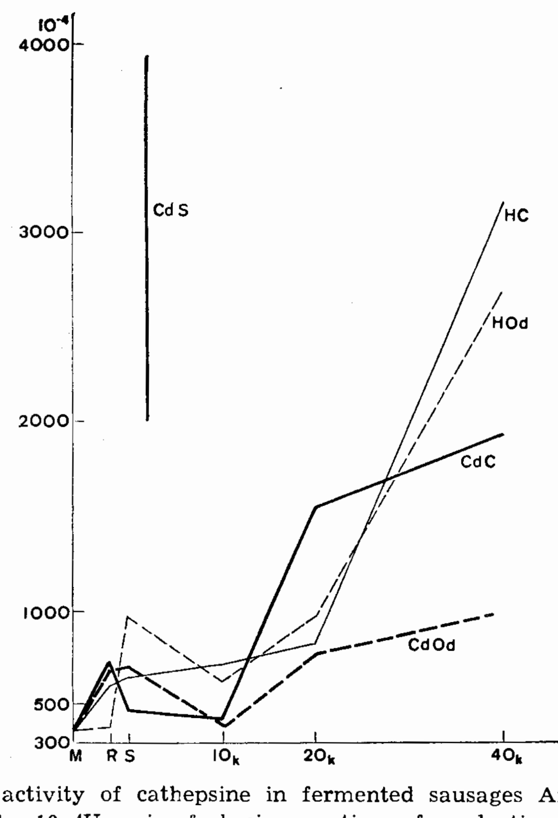

Fig. 1. Specific activity of cathepsine in fermented sausages Axis of ordinates = specific activity, 10^{-4}U, axis of abscissae = time of production and storage, days; — central part of sausages, — — — outer part of sausages. Another indications as in table 1

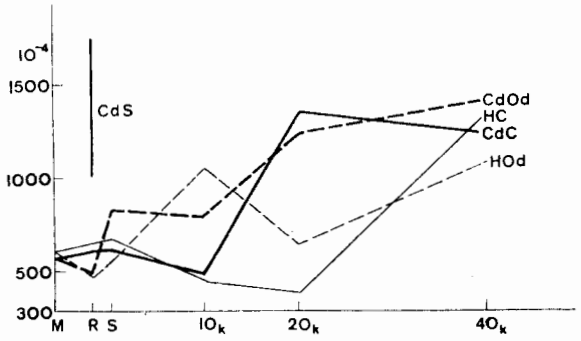

Fig. 2. Specific activity of trypsin-like peptidase in fermented sausages. Indicationes how figure 1

mented sausage. As for the other peptidase, the list of affecting factors is much longer and the statistical significance thereof is greater. Included among them is the stage of the meat's post-slaughter autolysis. The initial meat quality, on the other hand, has a highly statistically significant effect on the specific activity of this peptidase in interaction with the remaining three technological factors.

The activity of the discussed tissue peptidases is different in hot than in cold meat. During several days of cold storage the cathepsina loss, on the average, 30% of their initial activity, but in the separate repetitions of the experimental cycle the direction of these changes was quite the

Table 2. Analysis of variance specific activity of the tissue peptidases in fermented sausages

Sources of variance	Degree of freedom	F-value caltulated for:		F-value from table	
		cathepsins	trypsin-like peptidase	$\alpha = 0.05$	$\alpha = 0.01$
A	1	2.520	49.941		
B	1	1.165	12.271		
P	5	6.961	147.867	5.05	10.97
D	1	8.044	1.959		
AP	5	2.297	48.008		10.97
BP	5	1.153	18.320		
BD	1	1.169	27.931		
PD	5	3.832	20.211		10.97
ABP	5	0.342	9.980	5.05	
APD	5	2.991	17.088		10.97
BPD	5	0.736	16.699		10.97
Error	5	—	—	—	—
Total	47	—	—	—	—

A — extend/of meat autolysis
B — part of sausage cross-section
P — stage of production process or storage
D — variation of meat quality (in repeated experiments)

opposite. No great differences were found in the corresponding activity of trypsin-like peptidase. We may at most speak of a systematic albeit modest increase of this enzyme's activity during raw material refrigeration.

Regardless of the raw material used in the production of fermented sausage, the specific activity of both the situated peptidases increases in them during production and storage. The greatest activity increment is always during storage, especially towards the end of the studied period. This is in agreement with earlier observations [8, 9]. The increment of the activity is different in the two tissue peptide hydrolases (greater in cathepsins), in meat raw material in different stages of post-slaughter autolysis (greater in hot meat), and in the various layers of the sausage bar (greater in the inner layers; cf. Fig. 3) *). The differentiated intensity

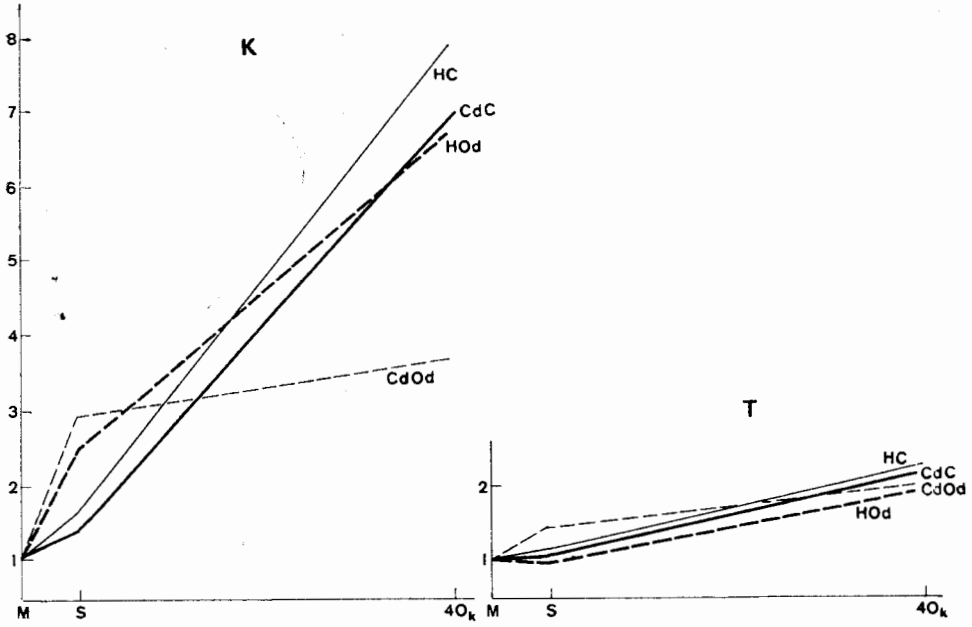

Fig. 3. Dynamics of the change of specific tissue peptidases activity during production and storage of fermented sausages. Axis of ordinates = relative changeability of enzymes specific activity, axis of absissae = time of production and keeping, days; K — specific activity of cathepsines, T — specific activity of trypsin similar peptidase. Another indications as in table 1

*) The dynamics of specific actvity changes in the two peptidases was calculated as follows

$$y = y_p + y_k,$$

$$y_p = \frac{\bar{x}_s + \bar{x}_M}{\bar{x}_M},$$

$$y_k = \frac{\bar{x}_{40} - \bar{x}_s}{\bar{x}_M}$$

of changes is illustrated by the variation coefficients (Table 3) which indicate that the variability of specific activity of cathepsins active in the experimental fermented sausages is on the average twice higher than of the other peptidase, 1.5 times higher in sausages from hot meat than is sausages from matured raw material; in the inner and outer bar layers the variability is practically the same. The activity of tissue trypsin-like peptidase is in all these cases quite equal. Hence, in favourable conditions, the cathepsins may be a more important cause of unwonted phenomena in fermented sausages (autolysis) than trypsin-like peptidase.

Given the sizeable range of statistical confidendce, the indicated properties may be regarded as only tendencies. However, when the experiments are more numerous the tendencies may be statistically significant. In the present state of research we may nevertheless point to two characteristic phenomena:

— greater susceptibility of cathepsins to environmental stimuli which may include the post-slaughter autolysis of meat (Fig. 1); and

— greater overall resistivity of trypsin-like peptidase, or mutual neutralization of the numerous stimuli acting upon it, something indicated by analysis of variance (Table 2, Fig. 2).

The most crucial role among the three potential enzymatic stimulants (or inhibitors) is apparently played by water content in fermented sausage [8, 9]. The dependence is inversely proportional, which is surprising at first glance. As we know, however, the progressing dehydration of the sausage mass in accompanied by an increasing concentration of bivalent ions of calcium and magnesium. There is also a greater amount of reducing radicals (e.g. SH), and more destruction of cell structures (mitochondria and lysosomes) with the resultant liberation of enzymes from inactive complexes. It is also possible that changing environmental conditions activate the various cathepsins of this complex in a specific order.

Multiple regression analysis with the choice of the best subset of independent variables proved that hydrogen ion concentration does not determine in a statistically significant manner the specific activity of the two tissue peptide hydrolases active in fermented sausages. This is in full agreement with the previous statements. Among the independent variables, most significant statistically, which determine this activity regardless of the stage of the meat's post-slaughter autolysis, is common salt concentration, and in particular cases the water content (Table 4).

where:

y_p — relative increase of specific activity during production,
y_s — specific activity increase during storage,
y — total specific activity change during the period of observation,
$\bar{x}_M$ — arithmetical mean of specific activity in sausage-meat,
$\bar{x}_s$ — arithmetical mean of specific activity immediately after smoking,
$\bar{x}_{40}$ — arithmetical mean of specific activity in sausage after 40 days of storage.

Table 3. Multiple regression of specific tissue peptidases activity in fermented sausages

Enzyme	Place of activity	Influence of the most essential independent variable						Verifying test	N° of equation
		most essential independent variable	director of influence	push	direction	regression coefficient of correlation	determ.		
Cathepsins	HC	x_1	+	-10221.51	3407.91	0.6079	36.95	0.0360	1
	H	x_1	+	-1033.95	429.89	0.6333	40.11	0.0270	2
	CDC	x_1	+	-3322.83	1216.23	0.8174	66.82	0.0012	3
	CD	x_2	−	1936.26	-30.10	0.5525	30.53	0.0625	4
Trypsinlike peptidase	HC	x_1	+	-850.20	455.19	0.8747	76.51	0.0027	5
	H	x_1	+	144.53	130.13	0.5989	35.87	0.0396	6
	CdC	x_2	−	3722.93	-58.29	0.8061	64.98	0.0015	7
	Cd	x_1	+	-96.55	205.36	0.8678	75.30	0.0003	8

x_1 — content of domestic salt (% of sausages weight),
x_2 — content of water (% of sausages weight),
+ — directly proportional dependence,
- — inversely proportional dependence.
The other notations as in Table 1.

Table 4. Critical content of water of experimental fermented sausages

Water (% of weight)	Sausages manufacture from			
	hot meat		cold meat	
	part of sausage			
	central	outer	central	outer
Total content (mean) in meat and fat mixture	57.85	57.85	54.39	54.39
Total content (mean) in sausages after 40 days of storage	41.14	24.41	39.77	20.77
Hydration	5.20	5.20	4.55	4.55

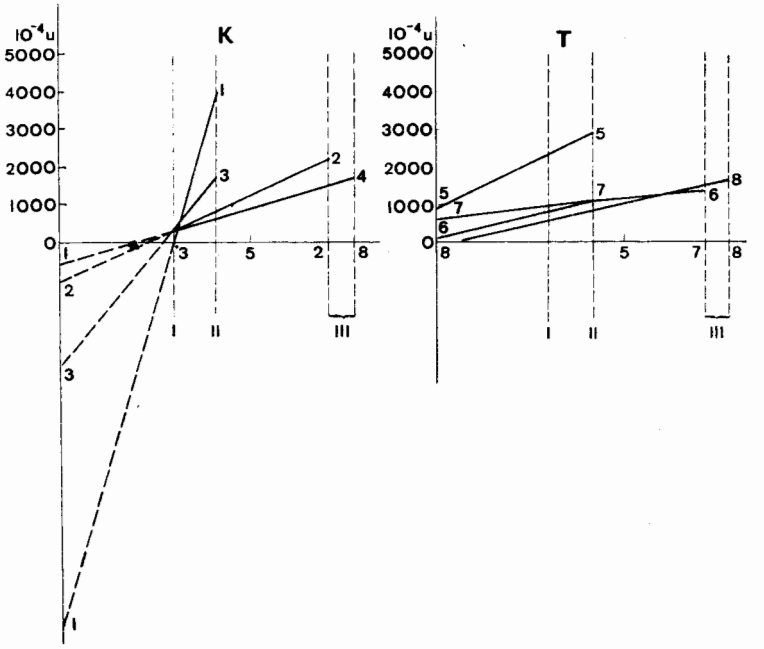

Fig. 4. Mathematical models of changeability dynamics of specific tissue peptidases activity during production and storage of fermented sausages; K — specific activity of cathepsins, T — specific activity of trypsin like peptidase. Axis of ordinates = = relative changebility of enzymes specific activity, 10^{-4}U.
Axis of abscissae = content of common salt, % of sausages weigh 1 ... 8 — numer of equations in table 4; I — content of common salt in meat and fat mixture, II — content of common salt central part of sausage after 40 days of storage, III — content of common salt in outher part of sausage after 40 days of storage; — — — —theoretical part of mathematical model, ———— — technological profitable part of mathematical model.

With the exception of one case the regression is always very significant statistically (Table 4, Eq. 4). The increase of salt concentration in the sausage bat due to dehydration is accompanied by an increase of specific activity of cathepsins in both the studied bar layers. This increase is

greater in sausages from hot meat than in those made from meat refrigerated for 7 days (Table 4, Eqs 1 and 3, and Eqs 2 and 4). This regularity is not observed in the other studied tissue peptidase.

The above observation is partly in agreement with previous results [9] which showed that the specific activity of cathepsins is inversely proportional to the salt content in a sausage with 4.5 or 7.5% additions of salt to the sausage-meat. The activity of the other peptidase, on the other hand, is directly proportional to the common salt content when 1.5% salt was added to the fermented sausage-meat mass. In the presently reported production cycle 3% of salt was added, and towards the end of the storage period its content in the sausage was up to 4.2% in the central layer and 7.5% in the outer layer.

The presented facts are of course only a tentative explanation of the biomechanism of the given stimulus' effect on the specific activity of tissue peptidases in stored fermented sausages. These mechanisms may be associated not only with the effect of NaCl and its admixtures in common salt but also with the consequences of dehydration, since each salt concentration is inversely proportional to the water content. This dependence is modelled by the function

$$S_1 = \frac{S \cdot X}{X_1} \qquad X_1 = \frac{S_1 \cdot X_2}{X_2'} = \frac{S \cdot W}{X_2},$$

where S = salt content in sausage-meat (%) with water content = X_1 (%); S_1 (%) — salt content in sausage with water content of X_2 (%), and a is the directional coefficient of effect in the approximate equation.

The effect of salt (directional coefficient) on the specific activity of trypsin-like peptidase in hot meat (Table 4, Eq. 5) may thus be described by the function

$$a = 5459.28X_1 + 361.89X_2$$

Transformations of the mathematical models of regression indicate that also in this case the water content may be regarded as the basic stimulant or inhibitor of the activity of both the studied tissue enzymes active in fermented sausages. The previous results differed from ours in a different function describing the dependence, namely our function is curvilinear whereas the other is rectilinear.

CONCLUSIONS

1. The activity of cathepsins in hot meat is greater than in meat that was refrigerated for several days. These differences remain throughout the production period and 40-day storage of fermented sausages. The specific activity of cathepsins in sausages manufactured from hot meat is on the average 30% higher than in sausages from chilled meat.

2. The specific activity of trypsin-like peptidase from hot meat does not differ in a statistically significant way from this activity in sausages from chilled meat.

3. The variability of specific activity of cathepsins, especially in the central layers of hot-meat fermented sausage bars, exceeds several times that of trypsin-like peptidase.

4. The stage of post-slaughter autolysis of the raw material does not change the increase of specific activity of both the enzymes towards the end of experimental storage that was observed in different experimental materials.

5. Among the technological factors responsible for the increase of enzymatic activity of both the peptidases during storage, mention is due to the increasing common salt concentration and the simultaneous water content decrease in fermented sausages. The vectors of both the effects coincide. The increased intensity of enzymatic effectiveness may be most probably regarded as a physico-chemical consequence of dehydration and of the chemical action of catalysts.

6. The studies confirmed our previous observations of the low specific activity of both the tissue peptidases, ranging from a few hundredths to a few tenths of the enzymatic activity unit.

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AKTYWNOŚĆ WŁAŚCIWA PEPTYDAZ TKANKOWYCH W WĘDLINACH SUROWYCH Z MIĘSA NIEWYCHŁODZONEGO

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Streszczenie

Analizowano produkcyjną i przechowalniczą (40 dób) zmienność aktywności właściwej katepsyn oraz tkankowej pepsydazy trypsynopodobnej w wędlinach surowych typu serwolatka, produkowanych z mięsa niewychłodzonego po uboju. Stwierdzono, że aktywność właściwa katepsyn w tak wyprodukowanych wędlinach jest przeciętnie o 30% większa niż w podobnych wędlinach, które wytworzono z mięsa przez 7 dób przechowywanego w chłodni. Aktywność właściwa drugiej pepsydazy nie wykazuje natomist żadnego zróżnicowania, zależnego od zaawansowania autolizy mięsnego surowca tych wędlin. Kierunek przechowalniczych zmian aktywności właściwej obu badanych enzymów w obu badanych odmianach wędlin nie różni się od siebie, ani też od stwierdzonego w innych cyklach doświadczalnych. Ich aktywność stymuluje rosnąca zawartość soli kuchennej, a właściwie jej naturalnych przymieszek. Przyczyną coraz większego stężenia tych soli mineralnych jest jednoczesne zmniejszenie się zawartości wody w przechowywanych wędlinach.