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EFFECT OF PRODUCTION PROCESS AND STORAGE ON THE ACTIVITY OF TISSUE PEPTIDASES IN FERMENTED SAUSAGE

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Key words: catepsin activity, trypsin-like peptidase activity, fermented sausage, smoking of sausage.

Two complexes of tissue peptidase hydrolases are active in fermented sausage, namely cathepsins and trypsin-like peptidase. Smoking and prolonged storage increases their specific activity, the effect being due to smoking temperature and, during storage, the concentration of hydrogen ions and water content. The latter two factors activate mainly trypsin-like peptidase.

The quality of meat products, shelf like included, is determined first of all by the inactivation of the enzymatic system.

Fermented sausage is an example of meat products in which proteins and enzymes are not denatured with heat. Their production consists in utilizing the effect of enzymes of selected microorganisms giving preplanned desired qualitative properties, including a suitable set of chemical carriers of taste and odour, and determinats of shelf life.

The essence of modern fermented sausage technology is the control of the activity of enzymes, chiefly of bacterial enzymes, and little attention is paid to the other part of the enzymatic system of these products, namely to meat tissue enzymes, an example of which are cathepsins.

The available scientific information about endopeptidases of meat raw material as well as preliminary experimental data on their specific activity justify the need for a systematic analysis of the effect of fermented sausage manufacture and of the subsequent storage on the behaviour of (at least) their cathepsins and trypsin-like peptide hydrolase.

ORGANIZATION OF EXPERIMENTS

The reported experiment was organized according to the assumptions made during the analysis of dynamics of carbohydrates fermentation in fermented sausage and of technological possibilities of controlling the process [6, 7]. Hence, we produced a firm, finely ground fermented dry sausage of the cervelat type using the same raw materials, identical temperatures and times during production and storage, as well as an analogous schedule of analyses. The entire research cycle lasted two years during which the production and analysis cycles were repeated four time. The only variable element in these cycles was the quality of raw material (meat, among others) used in the production of the fermented sausage.

The period of experimental observation for each production cycle was always 46 days (six days od production and 40 days of postproduction storage). There were always performed qualitative analyses of sausage stuffing (F), the sausage during production (after production seasoning, D_p, and smoking, W_p), and of the sausage during postproduction storage (after 10, 20 and 40 days: 10_s, 20_s and 40_s). The basis for every qualitative evaluation were determinations of:

(ii) the water content, by extraction with xylene in a "Cha-Ka" apparatus [1];

(ii) hydrogen ion concentration, with a dagger electrode of an LBS-66 type pH-meter;

(iii) specific activity of cathepsins, spectrophotometrically [3, 5];

(iv) specific activity of trypsin-like peptidase, spectrophotometrically by Anson's method [2, 4].

The specific activity of both peptidases was determined in buffer extract. Analytical samples of disintegrated sausage (about 10 g) were homogenized in 2% solution of potassium chloride (30 ml and 2 drops of Tween 80, t = 10 min in ice bath, blade homogenizer). The homogenized analytical weighed portions were placed in acetate buffer (0.1 M, pH = = 5.5) and left in a refrigerator (t = 30 mm, T = 4°C) for gravitational separation of fat. The separated fatty fraction was then removed by twice-repeated filtering through a fluted filter. Between filterings the weighed portions were stored in the low-temperature conditions that were used previously.

In view of the fixed water content and the known technological addition of salt to the sausage stuffing, there were no analytical determinations of sodium chloride levels either in the manufactured or in the stored sausage. Since the studied salt at no time crystallized on the sausage bar surface, the concentration of its solution in the water contained in the product could always be calculated from the equation

$$K_s = \frac{100 S_f}{W_w}$$

It was assumed in this calculation that all of the water in the sausage, irrespective of its bounding, retains invariable solvent properties. The error resulting from this assumption is small. The meaning of the applied symbols is as follows: S_f — technological addition of salt to the sausage stuffing (% of its mass), W_w — analytically determined water content in the studied sausage (% of its mass), K_s — concentration of salt water solution in this sausage (% of the mass of contained water).

In all, 176 analytical and 44 partly analytical experimental observations were made, which gave a total of 220 observations for the entire experiment (Table 1). The observations were objectivized statistically by variance and corelation analysis.

DISCUSSION OF RESULTS

The results of experimental observations of four repetitions of the production and storage cycles of fermented sausage can be briefly reported as follows:

1. Despite all the usual efforts made in practice, it was impossible to fully uniformize the raw material for production in the range of the studied properties (Table 1). In the separate repetitions of the cycles there were considerable differences not only in the specific activity of the studied enzymes but also in all the other determinations made in the sausage stuffing.

2. The specific activity of both the tissue peptide hydrolases in the sausage stuffing was of the order of several or a dozen or so hundredths of a unit; the activity of trypsin-like peptidase was always higher than of cathepsins. Although the differences are not statistically significant, they are noteworthy from the point of view of changes occurring during the production and storage of sausages *).

3. The period of production and subsequent storage of sausage brings about a statistically highly significant amplification of the specific activity of the tissue enzymes. However, the changes occurring in fermented sausage during this period are secondarily conditioned, in a statistically significant manner, by the initial quality of the raw material (Tables 2 and 3). Every cycle of fermented sausage manufacture is thus a unique biochemical system, statistically different from the other cycles as regards the range of changes of the activity of tissue peptidase hydrolases. Neither

*) The specific activity of pronase, an example of bacterial peptide hydrolase of fermented sausage, is about 1000 times lower than that of tissue peptidases. This proportion is not altered by correct production and storage. This observation is in agreement with the previously determined quantitative stability of proteolytic of fermented sausage [11].

bacteria populations during the technological process and the postproduction storage

Table 1. Variability of the specific activity of tissue peptidases and selected properties of the mass of experimental fermented sausage during production and storage

Production and storage	Layer	Repetition of production cycle (variability of quality of meast raw material)																			
		I					II					III					IV				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
F	W	130	409	56.15	5.34	5.73	421	755	52.62	5.70	5.88	125	786	51.26	5.85	5.92	113	1210	54.17	5.54	5.85
	Z	130	409	56.15	5.34	5.73	421	755	52.62	5.70	5.88	125	786	51.26	5.85	5.92	113	1210	54.77	5.54	5.85
D_p	W	1032	841	54.18	5.54	5.98	428	390	52.82	5.68	5.13	122	838	51.16	5.86	5.75	324	610	51.00	5.88	5.82
	Z	942	660	41.75	7.19	5.90	460	350	47.85	6.27	5.53	210	1093	44.66	6.72	5.70	616	1151	46.83	6.41	5.87
W_p	W	333	578	54.06	5.55	5.95	636	683	52.00	5.77	5.53	304	955	53.64	5.59	5.75	227	979	53.67	5.59	5.92
	Z	444	658	38.53	7.79	5.73	955	1005	43.28	6.93	5.40	726	1016	38.84	7.72	5.92	186	1130	41.10	7.30	5.87
10_s	W	342	499	50.30	5.96	6.10	476	502	50.88	5.90	5.58	1022	878	47.85	6.26	5.72	429	860	49.92	6.01	5.57
	Z	424	1111	32.61	9.20	6.03	338	487	38.91	7.71	5.23	142	957	32.05	9.36	5.92	215	625	34.66	8.66	5.97
20_s	W	636	1311	45.92	6.53	6.08	2457	1438	46.55	6.44	5.38	733	886	39.10	7.67	5.72	1559	804	46.26	6.49	5.65
	Z	433	1278	28.04	10.70	6.08	1135	1230	26.47	11.33	5.28	200	1296	22.35	13.42	5.77	2167	866	24.17	12.41	5.75
40_s	W	1688	1351	40.52	7.40	6.13	2196	1697	39.02	7.69	5.40	774	1651	32.33	9.28	5.71	440	1066	37.14	8.08	5.67
	Z	604	1237	20.53	14.61	6.15	1450	1184	21.00	14.29	5.40	967	1375	18.66	16.08	5.80	566	1321	20.15	14.89	5.67

Periods of production and storage: F — stuffing; D_p — after production seasoning; W_p — after smoking; 10_s, 20_s, 40_s — after 10, 20 and 40 days of postproduction storage, respectively

Layers: W — inside layer, Z — outside layer

Tests for changes: A — specific activity of cathepsins (10^{-3} J), B — specific activity of trypsin-like peptidase(10^{-3}), C — water content (% of sausage mass), D — concentration of salt solution in water of sausage (calculated with the assumption that the entire water content retains solvent properties, expressed in per cent), E — concentration of hydrogen ions (as pH)

Table 2. Analysis of variance of specific activity of cathepsins in experimental fermented sausage

Variance factor	Sum of squares	Degrees of freedom	Mean square	F-Snedecor value		
				calculated	$\alpha = 0.05$	$\alpha = 0.01$
T	15 493 730	47	—	—	—	—
A	1 618 411	3	539 470	5.009	8.70	—
B	6 071 761	5	1 214 352	11.275**	—	9.72
C	164 760	1	184 760	1.715	—	—
AB	5 068 915	15	337 928	3.137*	2.42	3.52
AC	310 358	3	103 453	0.961	—	—
BC	623 931	5	124 786	1.159	—	—
Error	1 615 594	15	107 706	1.000	—	—

T — general variability
A — quality of meat raw material (repetitions of production cycles)
B — processes of production and storage
C — layers

Table 3. Analysis of variance of the specific activity of trypsin-like peptide in experimenta fermented sausage

Variance factor	Sum of squares	Degrees of freedom	Mean square	F-Snedecor value		
				calculated	values from tables	
					$\alpha = 0.05$	$\alpha = 0.01$
T	5 289 701	47	—	—	—	—
A	305 042	3	101 681	2.730	—	—
B	2 576 953	5	515 391	13.836**	—	9.72
C	94 785	1	94 785	2.545	—	—
AB	1 685 241	15	112 349	3.066*	2.41	3.52
AC	35 042	3	11 681	0.314	—	—
BC	35 896	5	6 779	0.184	—	—
Error	—	15	37 249	1.000	—	—

Denotations as in Table 2

of studied layers inside the sausage bar display statistically significant differences.

4. During production and at the beginning of postproduction storage the direction of changes of the specific activity of the studied tissue enzymes is rather unclear (Fig. 1). The range of oscillation of these direction changes is due to differences in the quality of raw material used in the manufacture of fermented sausage (Figs 2 and 4).

5. After 40 days of storage, the specific activity of cathepsins in these products is 4.5-8.8 (slightly over 7 the average) times higher than in the

sausage stuffing. During this same period the specific activity of trypsin-like peptide hydrolase increases by 100-320%, i.e. by 200% on the average.

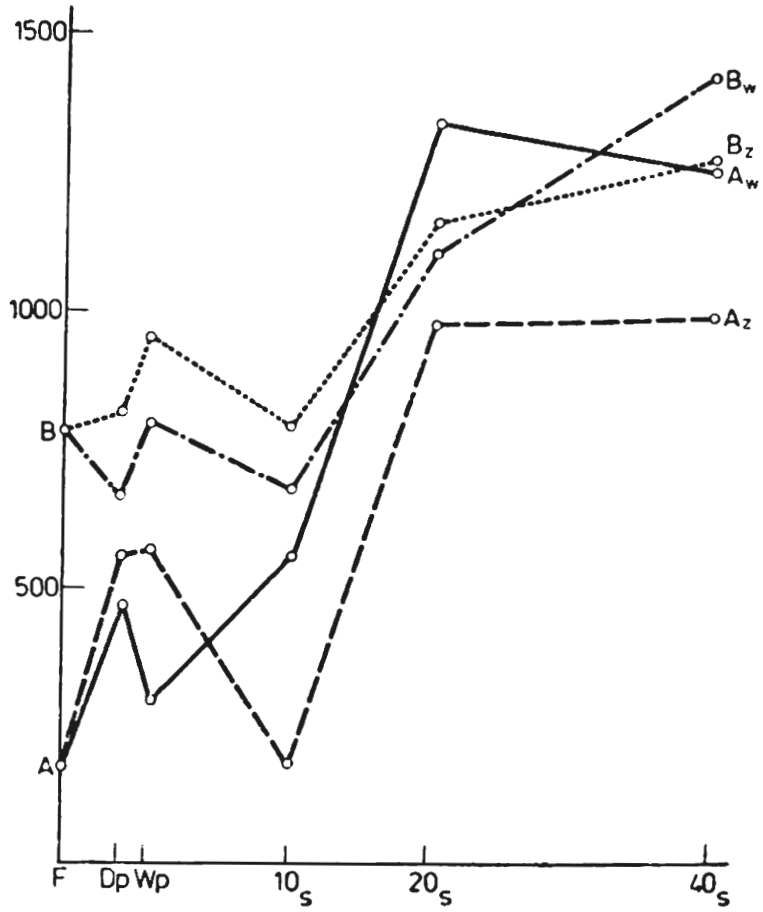

Fig. 1. Effect of the production process and storage on the specific activity of tissue peptidases in fermented sausage (mean values from four repetitions); y-axis — specific activity of tissue peptidases (10^{-3} J), x-axis — time of production and storage (days); A_x, A_z — cathepsins in inside and outside layers of sausage bar, respectively; B_w, B_z — trypsin-like peptidase in layers inside and outside layers of sausage bar, respectively; for explanation of F... 40_s see Table 1

6. The highest specific activity of cathepsins is most often found in fermented sausage after 20 days of postproduction storage (Figs 1 and 2). There are cases, however, when the activity increases throughout the 40-day period of fermented sausage storage (Fig. 2, production cycle repetition 1). The initial quality of the raw material used in production also affects the variation of specific activity of the other tissue peptidase during storage (Fig. 3). However, in most cases, after 40 days of storage the period of increase of the specific activity of this peptidase is still not over (Fig. 1).

7. The dynamics of specific activity changes of the two enzymes studied is not identical during production and during the storage of fermented sausage. In most coses it is particularly intense in two periods, namely during smoking and between the second and third week after the end of smoking (Fig. 4). The initial quality of raw material differentiates this feature of both tissue peptide hydrolases, too (Fig. 5). Cathepsins

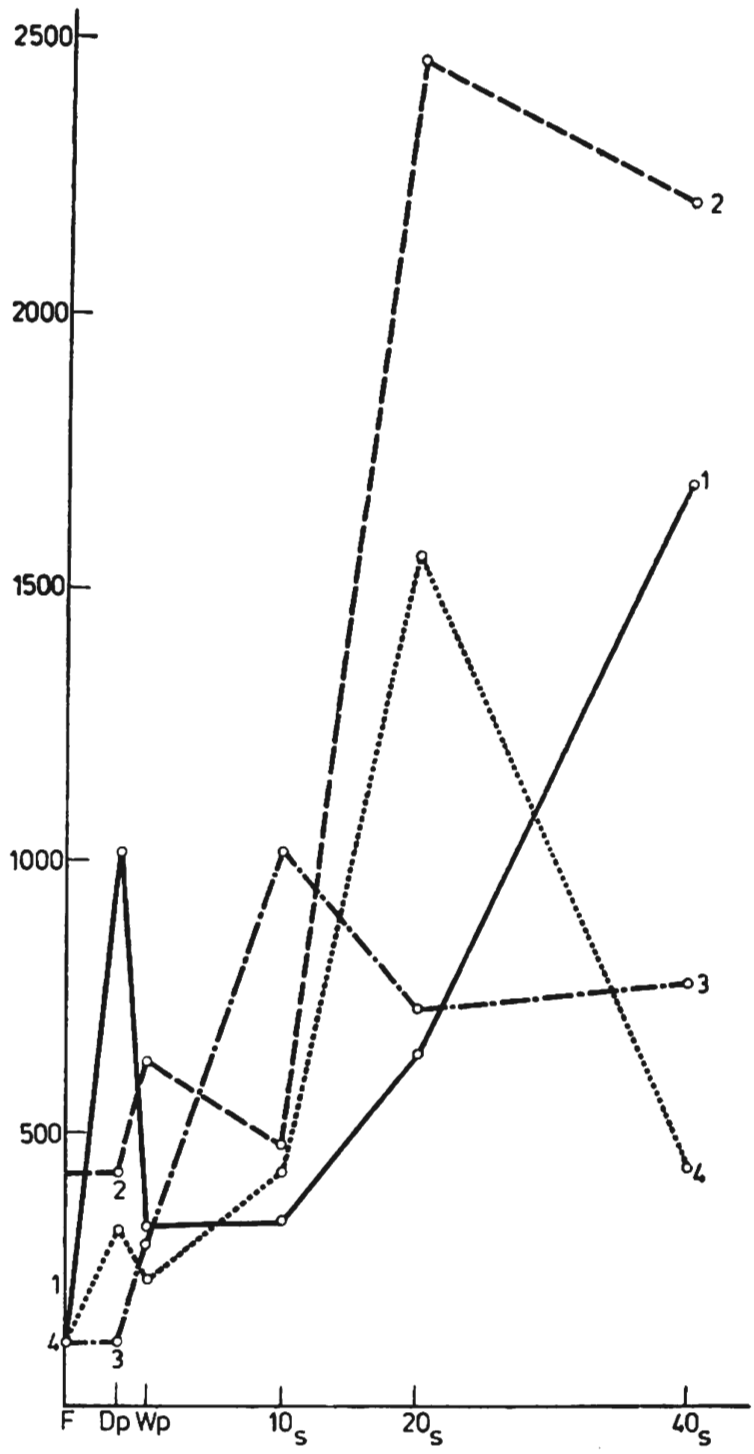

Fig. 2. Effect of the production process and time of storage on the specific activity of cathepsins in the inside layer of experimental fermented sausage bars; y-axis — specific activity of cathepsins (10^{-3} J); x-axis — time of production and storage (days); 1, 2, 3, 4 — subsequent repetitions of the production cycle; for explanation of F ... 40_s see Table 1

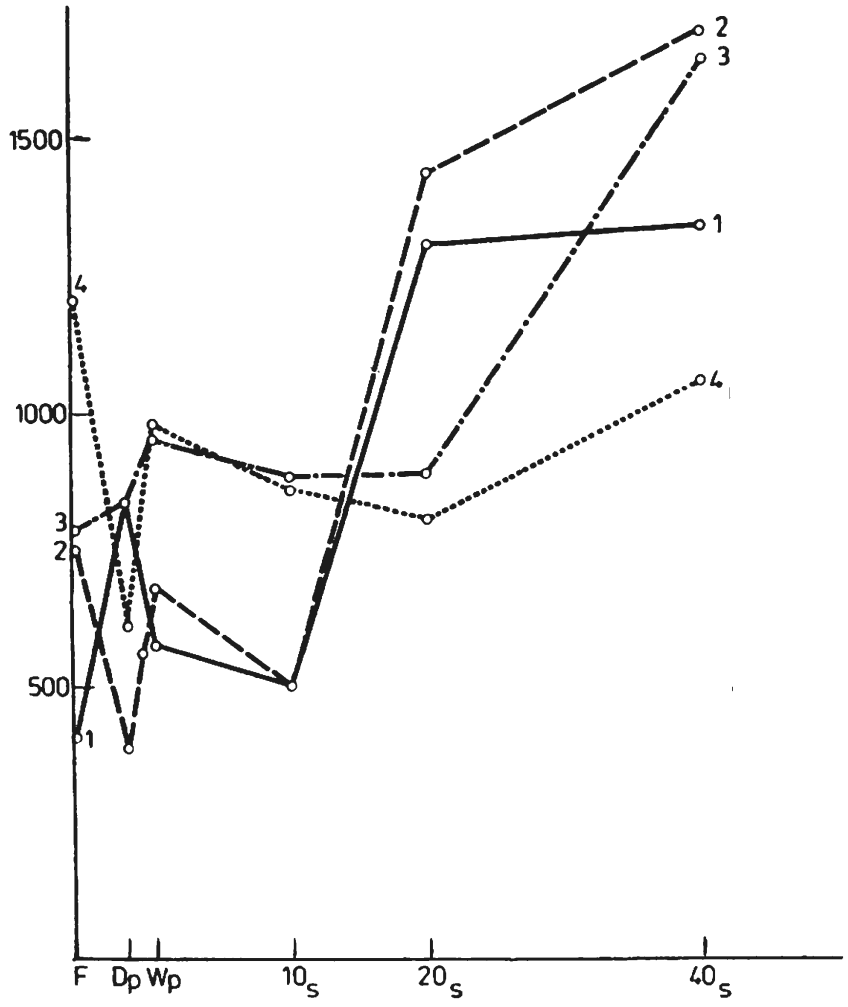

Fig. 3. Effect of the production process and time of storage on the specific activity of trypsin-like peptidase in the inside layer of experimental fermented sausage bar; y-axis — specific activity of trypsin-like peptidase (10^{-3} J); other denotations as in Fig. 2

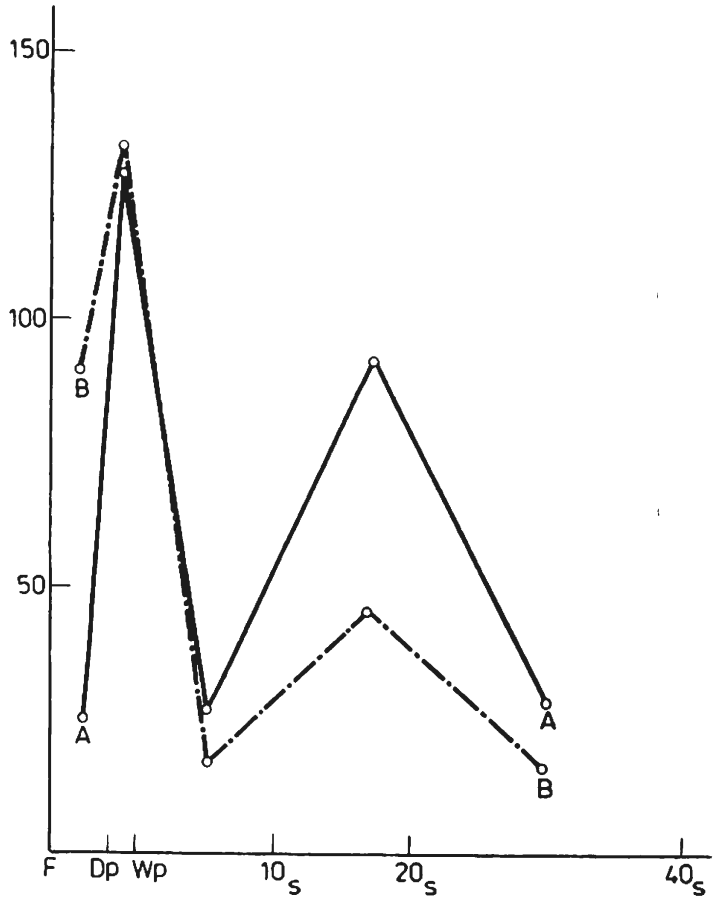

Fig. 4. Dynamics of diurnal changes of specific activity of tissue peptidases in the inside layer of experimental sausage bar (mean values from four repetitions); y-axis — dynamics of diurnal of specific activity (J 10^{-3} d^{-1}), x-axis — time of production and storage; d — days; A — cathepsins; B — trypsin-like peptidase

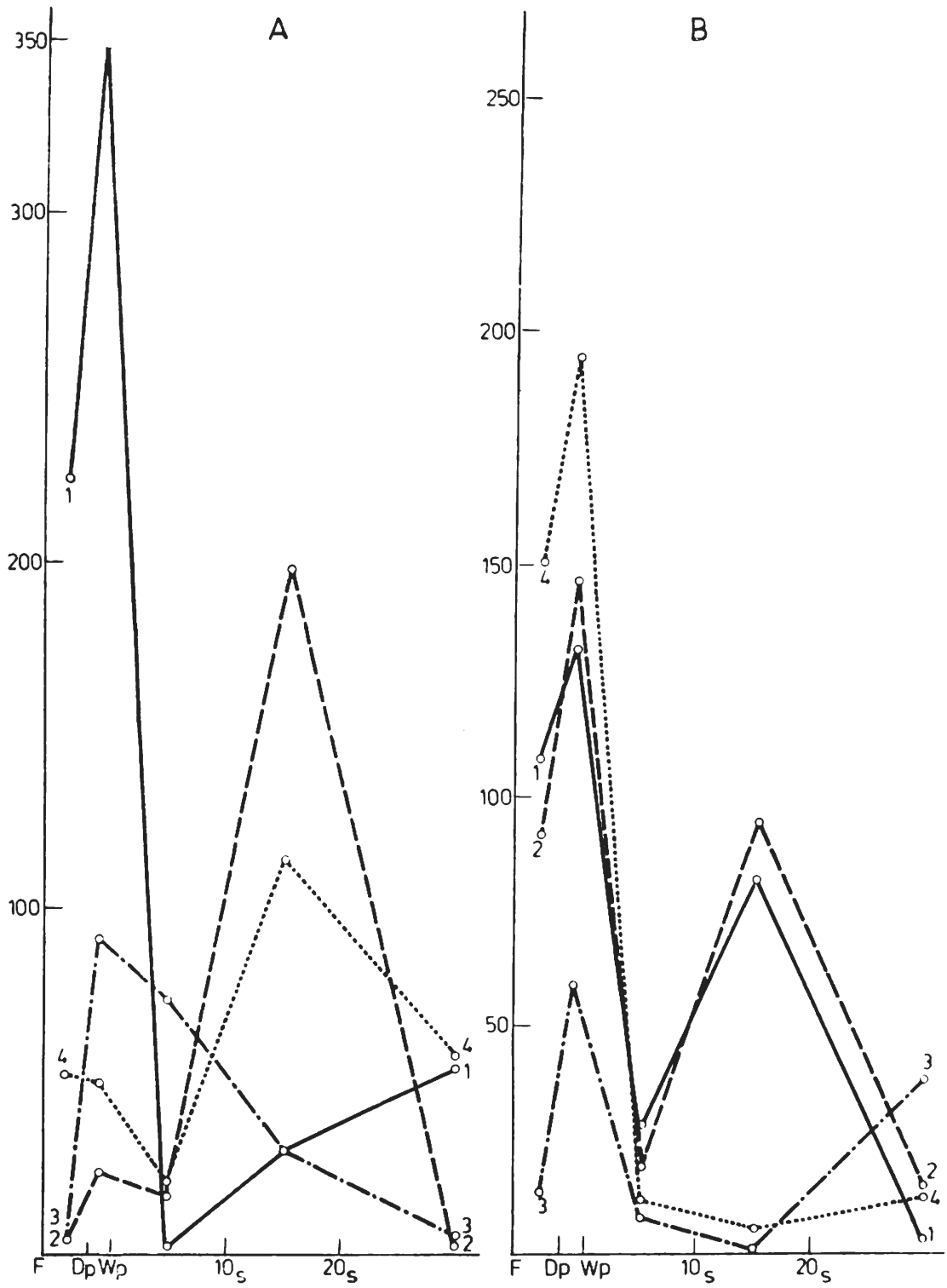

Fig. 5. Dynamics of diurnal changes of the specific activity of cathepsins (A) and trypsin-like peptidase (B) in the inside layer of experimental fermented sausage bar; y-axis — dynamics of diurnal changes of specific activity (J 10^{-3} d^{-1}); A — cathepsins; B — trypsin-like peptidase; d — day; other denotations as in Fig. 2

appear to be more susceptible to chemical and physical changes in fermented sausage than trypsin-like peptidase.

8. The causes of the presented phenomena must of course be sought among factors acting as stimulants or inhibitors of the effectivity of enzymes in general. In the case in hand, we must mention first of all heat energy, water, salt (sodium chloride) and hydrogen ions (Table 1).

Raw meat products are exposed to the highest temperature during smoking ($18°C \leqslant T \leqslant 20°C$). The specific activity of both studied tissue

enzymatic systems increases most rapidly in this particular stage of sausage manufacture (Fig. 4).

The analysis of individual effects of each of the three remaining factors gives no such straighforward results (Figs 6 and 7). The analysis of multiple corelation of the effect of meat raw material quality, characterized by water and salt content as well as ion density, did not confirm their effect on the specific activity of fermented sausage cathepsins. A statistically significant effect of hydrogen ion concentration and of

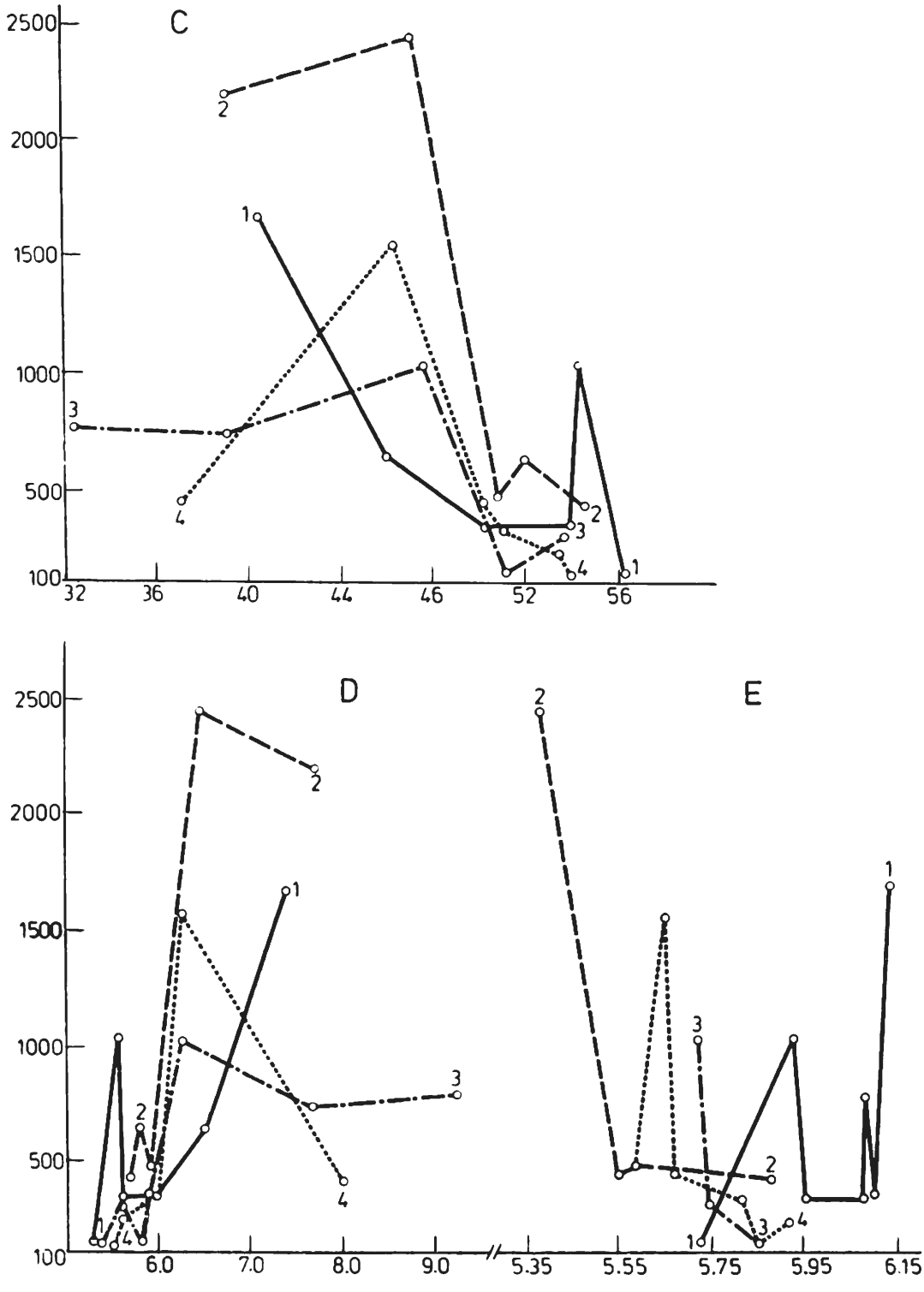

Fig. 6. Effect of water content (C), concentration of water solution of salt in sausage mass (D), and of hydrogen ions (E) on the specific activity of cathepsins in the inside layer of experimental fermented sausage bars; x-axis — C, D and E as in Table 1; remaining denotations as in Fig. 2

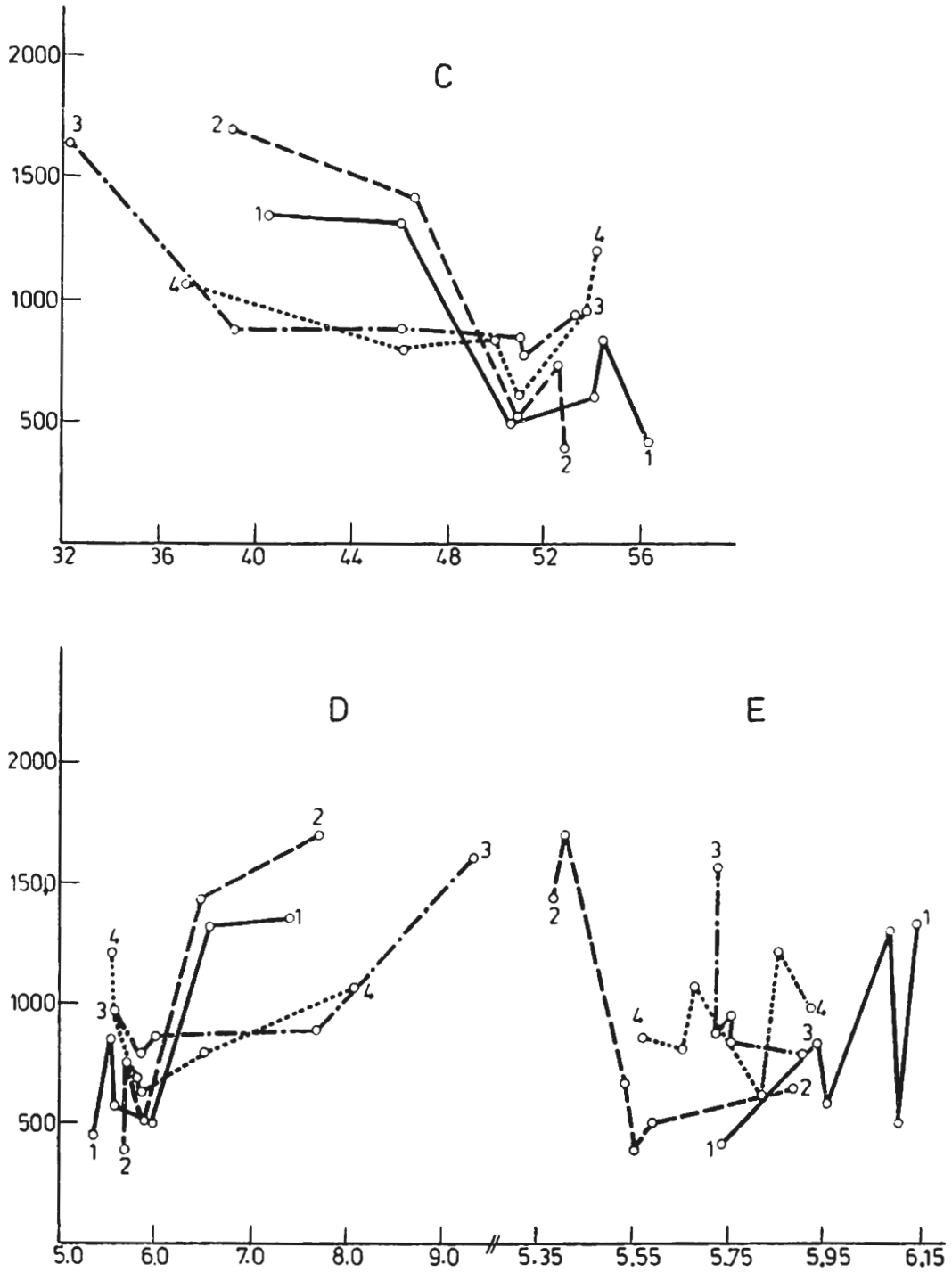

Fig. 7. Effect of water content (C), concentration of water solution of salt in sausage mass (D) and of hydrogen ions (E) on the specific activity of trypsin-like peptidase in the inside layer of experimental fermented sausage bar; x-axis — C, D and E as in Table 1; remaining denotations as in Fig. 2

water content on the specific activity of trypsin-like peptidase was found in two cases only.

The analysis of significance of the effect of the same three properties in the various phases of manufacture and storage of the experimental sausage gave similar results. An effect on the specific activity of cathepsins in the period of their maturing that was close to statistically significant was exerted by water content; a similar effect during smoking was due to hydrogen ion concentration. Let us recall that smoking is the period of greatest intensity of carbohydrate homofermentation and greatest acidification of the sausage mass. The effect of interaction of the

three studied properties of fermented sausage on the specific activity of their cathepsins is always greater than the effect of each property analysed separately.

None of the considered properties had a statistically significant effect on the specific activity of trypsin-like peptidase of fermented sausage in any of the production and storage periods. Their interaction even reduced the effectivity of influence on the specific activity of this peptide hydrolase.

9. The changes of specific activity of both systems of tissue peptidases after production and during storage of fermented sausage take a course that differs considerably from the expected one. Two causes may be seen as responsible for this. Fermented sausage is not a homogeneous, completely identified and static research material that usually serves to identify enzymes and study their properties. Moreover, cathepsins are a complex of monomeric enzymes of fairly diverse properties. In the present state of knowledge it cannot be ruled out that in the course of manufacture and storage of fermented sausage during the changes in chemical properties that accompany these processes, the specific activity of separate monomers of the cathepsin complex changes or actually predominates.

10. Due to its considerable specificity towards denatured protein substrate, typical for trypsin in Anson's reaction, the second of the studied peptidases was described as trypsin-like. This observation, however, is not its full identification, and in view of this there emerge at least two groups of questions, namely:

10.1. What is the structure of this peptide hydrolase: does it also consist of several enzymes or is it e.g. a dimeric or diisoenzymatic enzyme? This question is prompted by the observed two-phase (two-stage) activation of this enzyme by sodium chloride (Fig. 7D) [10].

10.2. To which enzymatic group of peptidase does the considered trypsin-like peptide hydrolase of fermented sausage belong: e.g. to the serine or the cysteine? In other experiments [9] there was observed a considerable increase of the content of free serine and threonine, as well as of cysteine and tyrosine. However, this observation cannot serve as a basis for identification since there is still no adequate information about the effect of bivalent cations (Ca, Mg) and reducing groups (e.g. SH) on the specific activity of trypsin-like peptidase in fermented sausage [10]. Since the manufacture of fermented sausage involves the use of salt which contains admixtures of both cations, the effect of this salt must not be identified with the effect of sodium chloride on the activity of the tissue peptidase system.

CONCLUSIONS

1. There are at least two complexes of tissue peptide hydrolases active in fermented sausage: the cathepsin complex and trypsin-like peptidase, i.e. enzymes typical for meat raw material.

2. The manufacture and storage of fermented sausage changes the specifi activity of both endopeptidases. Postproduction storage is a period of a statistically highly significant increase of this activity.

3. The extent of this activation is, however, statistically significantly modified by the quality of meat raw material used in the manufacture of these sausage. It was proved that the concentration of hydrogen ions and water content has a statistically significant effect on the specific activity of trypsin-like peptidase, and an almost statistically significant effect on the specific activity of cathepsins of fermented sausage.

4. The effectivity of changes of the specific activity of both tissue peptide hydrolases is also affected by the temperature of smoking fermented sausage.

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WPŁYW PROCESU PRODUKCJI I PRZECHOWYWANIA NA AKTYWNOŚĆ TKANKOWYCH PEPTYDAZ WĘDLIN SUROWYCH

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Streszczenie

W wędlinach surowych typu serwolatki przebadano dynamikę zmian aktywności hydrolaz peptydowych. Doświadczenia przeprowadzono w 4 powtórzeniach w ciągu 46 dni (każde), w tym 40 dni przechowywania. Określano zawartość wody, stężenie jonów wodorowych, aktywność katepsyn i peptydazy trypsynopodobnej. Stwierdzono aktywność obydwu kompleksów enzymów (rys. 1-5). Podczas przechowywania wędlin wzrasta aktywność obu endopeptydaz. Rozmiar aktywności enzymatycznej był modyfikowany jakością użytego surowca mięsnego (tab. 1), stężeniem jonów wodorowych oraz zawartością wody (wodochłonnością) w stopniu statystycznie istotnym odnośnie do trypsynopodobnej peptydazy (tab. 3, rys. 7) oraz w bliskim istotnie statystycznie stopniu na aktywność katepsyn (tab. 2, rys. 6). Na efektywność zmian aktywności specyficznej obu tkankowych hydrolaz peptydowych wpływa także temperatura wędzenia wędlin surowych. Przedyskutowano hipotezy prawdopodobnej budowy hydrolazy peptydowej.