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Meat Science, Vol. 46, No. 1, 115–128, 1997

PII: S0309-1740(97)00013-2 0309-1740/97 $17.00+0.00

Proteolysis in Dry Fermented Sausages: The Effect of Selected Exogenous Proteases

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(Received 10 October 1995; revised version received 31 December 1995; accepted 2 February 1996)

ABSTRACT

The effect of three commercial proteases (pronase E from Streptomyces griseus, *aspartyl proteinase from* Aspergillus oryzae *and papain) on protein breakdown and the sensory characteristics of dry fermented sausages was investigated. Water soluble, non-protein, 5% phosphotungstic acid soluble, 5% sulphosalicylic acid soluble and total volatile basic nitrogen contents increased during fermentation, stabilizing later until the end of ripening (26th day). Nitrogen values were always greater in the aspartyl proteinase added batch in comparison with the other protease added batches. Total free amino acid changes showed a similar pattern to those observed for the 5% sulphosalicylic acid soluble nitrogen. The electrophoretic studies demonstrated that proteolysis of high molecular weight myofibrillar and sarcoplasmic proteins was more prominent in protease added batches. It was especially intensive in papain one. The dominant amino acids at the end of ripening were similar in all batches. Tyramine and histamine increased throughout ripening. No significant differences in sensory properties were found between control and pronase E and papain added batches, but they were significantly different ($p < 0.01$) from the sausages containing aspartyl proteinase, due to an excessive softening. The effect of exogenous enzyme addition on the flavour potentiation of dry fermented sausage is discussed.* © *1997 Elsevier Science Ltd*

INTRODUCTION

Protein breakdown during dry sausage ripening yields polypeptides, peptides, free amino acids, etc. These reactions are catalysed by endogenous enzymes, such as cathepsins (Toldrá *et al.*, 1992) and trypsin-like peptidases (Pezacki and Pezacka, 1986), as well as proteases produced by micro-organisms involved in the ripening process, mainly those of Micrococcaceae (Guo and Chen, 1991; Selgas *et al.*, 1993), but also moulds (Geisen *et al.*, 1992) and yeasts (Woods and Kinsella, 1980) in those dry sausages in which they are present. Compounds resulting from protein breakdown and those generated from amino acids transformation are involved in the flavour development in dry fermented sausages.

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As ripened sausage manufacturing involves a high cost of storage until a suitable matured state is reached, a shortening of this period would be convenient. To reach this goal, some attempts have recently been made in dry fermented sausages using proteases from *Lactobacillus* spp (Næs *et al.*, 1991, 1992, 1995) and lipases (Fernández *et al.*, 1995*a,b*; Zalacain *et al.*, 1995). The authors of the present work have also applied this approach to a Spanish dry fermented sausage (salchichón) by using pronase E from *Streptomyces griseus* (Díaz *et al.*, 1993), aspartyl proteinase from *Aspergillus oryzae* (Díaz *et al.*, 1992) and papain from *Carica papaya* (Díaz *et al.*, 1996). Pronase E and aspartyl proteinase have previously been used in accelerated cheese ripening (Law and Wigmore, 1982), while papain is widely used as a meat tenderizer. In the previous experiments only the sausages with 600 units of pronase E added showed better sensory properties than the control batch (Díaz *et al.*, 1993). When higher amounts of proteinases were added, an excessive softening of sausages appeared. It was concluded, in general, that new investigations were needed in order to adjust the doses of the enzymes to be added. The present work reports the results of a comparative study on the effect of the addition of the three proteases mentioned above on the proteolysis in dry fermented sausages. The enzyme doses added were selected according to the results of the above mentioned works.

MATERIALS AND METHODS

Sausage preparation and sampling

Dry fermented sausages were manufactured in an experimental plant of a local factory. The composition of sausages was (%w/w): pork (56), beef (12), lard (25), dextrose (0·8), lactose (1·0), dextrine (1·8), salt (2·5), sodium glutamate (0·25), nitrates (0·0085), nitrites (0·0065), black pepper (0·14) and sodium ascorbate (0·046). Ingredients were mixed in a cutter, with particle size reduction to about 3 mm. Sausage mixture was divided in four batches (2 kg each). Each protease (from Sigma Chemical Co., St Louis, Mo, USA) was added to each respective batch at the following concentrations: 300 enzyme units of pronase E, 100 units of aspartyl proteinase and 500 units of papain. Batches were named as 300PRO, 100ASP and 500PAP, respectively. One proteolytic unit represented the amount of enzyme that produced an increase of 1 unit in the absorbance at 440 nm per hr, using azocasein (Sigma) as substrate (0·8% in Tris-HCl buffer 0·2M, pH 6·5). The fourth batch was the control, to which no enzymes were added.

The protease addition, sausages preparation and ripening conditions were the same as those previously reported (Díaz *et al.*, 1993). Sausages were ripened for 26 days and samples (about 200 g) of each batch were taken at various times (0, 2, 5, 15 and 26 days) during ripening. After aseptically removing the casing, a portion (10 g) was inmediately taken for microbial analysis. The remainder was used for chemical analyses as described below. All analyses were made in duplicate.

Microbial analyses

Total viable, Micrococcaceae and lactic acid bacteria were enumerated as previously reported (Díaz *et al.*, 1993).

Chemical analyses

Water activity, pH and selected nitrogen fractions, which included water soluble (WSN), non-protein (NPN), phosphotungstic acid (PTN), sulphosalycilic acid (SSN) and total

volatile basic (TVBN) nitrogens, were determined as previously described (Díaz *et al.*, 1993).

Sarcoplasmic protein extracts were prepared according to Toldrá *et al.* (1993). Four grams of sausage meat were homogenized for 2 min in 40 ml of 0·03M potassium phosphate, pH 5·0. The resulting extract was centrifuged for 20 min at 10 000 g and 4°C. The supernatant was collected. The pellet was re-extracted in the same conditions and the new pellet was homogenized in 8M urea containing 1% (w/v) β-mercaptoethanol for 2 min. The extract was centrifuged again in the same conditions. The supernatants containing the myofibrillar and sarcoplasmic proteins were dialysed exhaustively against distilled water and lyophilised. Afterwards, they were analysed by PAGE using a 'Phast-System' electrophoresis equipment (Pharmacia LKB, Uppsala, Sweden). Sodium dodecyl sulphate (SDS)-PAGE was performed on 20% homogeneous gels in accordance with the manufacturer's instructions. The electrophoretograms were run with standards (Sigma) of known molecular weight [α-lactalbumin (14·2 kDa), trypsin inhibitor (20·1 kDa), trypsinogen (24 kDa), carbonic anhydrase (29 kDa), ovalbumin (45 kDa), phosphorylase B (97·4 kDa), β-galactosidase (116 kDa) and myosin (205 kDa)]. Gels were stained with Coomasie Brilliant Blue G-250 and the intensity of the bands was measured at 610 nm in a Shimadzu CS-9000 densitometer (Shimadzu Corporation, Kyoto, Japan).

For free amino acids (FAA) determination, a portion of SSN was used. The analysis of these compounds was made as previously reported (Díaz *et al.*, 1993). Amine determination was carried out as described by Ordóñez *et al.* (1991) from a portion (10 g) of sausage sample.

Sensory analysis

At the end of ripening, samples of the four batches were assessed by a panel composed of at least 18 trained members. A triangle test was used, according to the International Standards Organization (I.S.O.) (TC 34/SC 12 Regulation). In this case, each panellist judged four of the possible combinations and only assessed two different sausage samples per session. Panelists were asked about the global characteristics of the samples. In this way, an attempt to establish the possible differences among the four samples was made.

Samples were also examined by panelists to judge the colour, appearance, texture and flavour according to a hedonic scale from 1 (very bad) to 10 (very good). The overall quality was calculated according to the following expression, which had been formerly developed in our Department from the opinion of regular consumers (about 40 people):

Overall quality = (colour and appearance × 0·1) + (texture × 0·25) + (flavour × 0·65).

Results were statistically treated by applying the ANOVA variance analysis, using the Statview program (Abacus Concepts Inc.) running in an Apple Macintosh LC Computer.

RESULTS AND DISCUSION

Microbial flora

No effect of proteases on the microbial changes during ripening was observed (data not shown). These results were similar to those previously obtained in sausages with added pronase E (Díaz *et al.*, 1993), aspartyl proteinase (Díaz *et al.*, 1992) and papain (Díaz *et al.*, 1996).

Water activity (a_w), moisture and pH

Changes in water activity and moisture (data not shown) followed the typical trend in these products reported by other authors (Baumgartner *et al.*, 1980; Stiebing and Rödel, 1988). The a_w values decreased from 0·96 (initial level) to 0·86 in the control batch, and to 0·87–0·88 in protease-added batches. The moisture was approximately 3% lower in the control batch than in the other batches.

The pH values showed a similar pattern in all batches (data not shown). They decreased sharply during fermentation from an initial value of 6·1 to about 5·0 at the 5th day of ripening. Afterwards, the pH stabilized until the end of the experiment. These results are in agreement with those reported by Mendoza *et al.* (1983) and García de Fernando and Fox (1991) in other dry fermented sausages, and with the former experiments in which low concentrations of these proteases were added (Díaz *et al.*, 1992, 1993, 1996).

Nitrogen fractions

Figure 1 shows the changes in WSN, NPN and PTN, respectively, during the ripening of control, pronase E, aspartyl proteinase and papain-added batches. As occurred in previous works (Díaz *et al.*, 1992, 1993, 1996), these fractions achieved higher values in protease-added batches than those of the control.

The aspartyl proteinase-added batch always showed greater values of these fractions than the other proteinase-added batches, which reached similar values. This effect may be attributed to the pH, more favorable to the activity of the aspartyl proteinase, which develops its optimum activity at pH values between three and five (Belitz and Grosch, 1987), very close to the pH of experimental sausages.

A continous increase of SSN values were observed in all protease-added sausages (Fig. 2), while the control batch levels showed a trend to stabilize. The levels of TVBN (Fig. 2) showed a trend to increase until the end of ripening. 100ASP batch levels were not as different as in the other nitrogen fractions, and the 300PRO and 500PAP batches showed very similar values. Levels in the control batch were slightly higher than those reported by Langner *et al.* (1972) and Lois *et al.* (1987) for conventional sausages.

Table 1 shows the densitometric areas of SDS-PAGE electrophoresis of myofibrilar proteins of dry fermented sausages. Some bands were identified according to their molecular weights, determined by comparison with standards. Heavy myosin chain (band A), M-protein (B), C-protein (C), α-actinin (E), β-actinin (F), actin (I), tropomyosin (J) and troponin I (N) were detected in the control batch along the ripening. No bands, detected at the beginning of the ripening, disappeared in the control batch during the process, and some new bands of low molecular weights were observed after fermentation (bands V, X, Y and AC). In general, greater myofibrillar protein changes have been reported in other dry sausages. Verplaetse *et al.* (1989) detected an increase of 75·9% in the concentration of polypeptides below 36 kDa during ripening, while myosin heavy chain decreased by 49% and actin and troponin T by 30%. Similar results have been reported by Garriga *et al.* (1988) and García de Fernando and Fox (1991) in several meat proteins during the ripening of dry sausages.

Protease-added batches showed a higher degradation of myofibrillar proteins. It was especially pronounced in the papain batch, in which proteins over 26 kDa were not detected after fermentation. Several new bands were observed in this batch (N, O, X, Y, Z, AB and AC). Some of them (N, O, Z and AB) were not detected in the other batches. This pattern was similar to that previously reported (Díaz *et al.*, 1996) when 800 units of papain were added. The aspartyl proteinase and pronase E batches showed a roughly similar proteolytic breakdown pattern, although some differences were observed. These

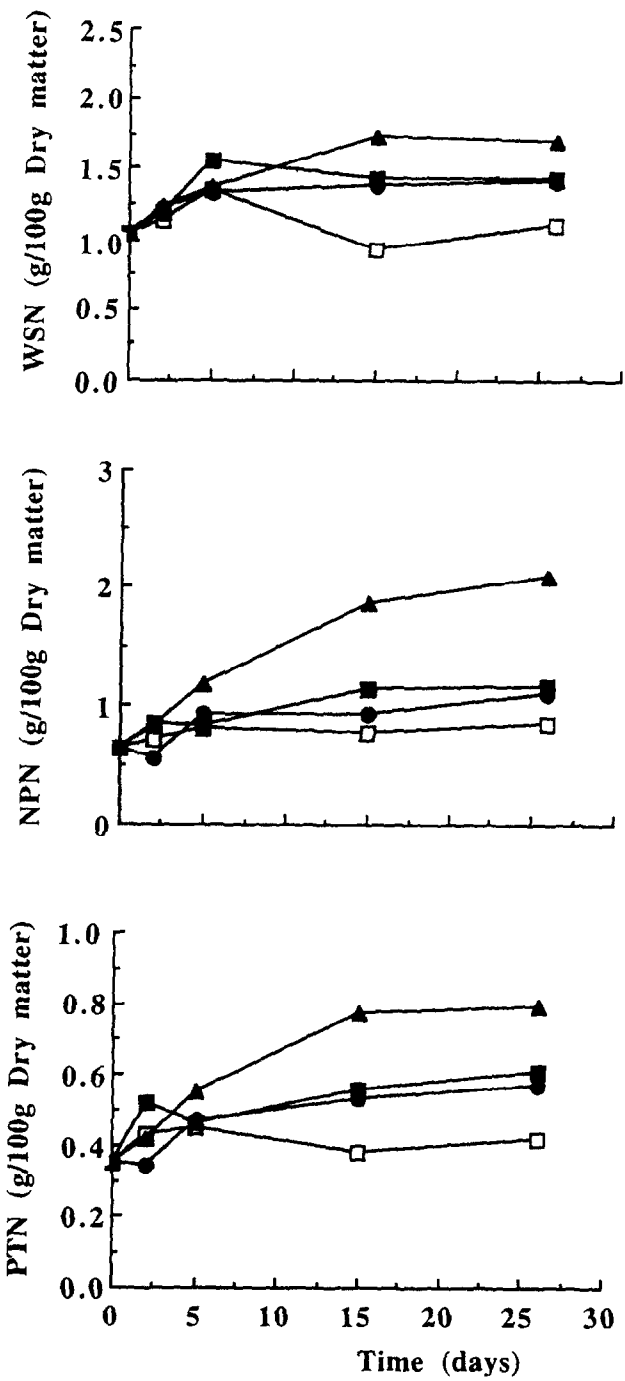

Fig. 1. Effect of the addition of proteases (□ Control; ■ pronase E (300 U.); ▲ aspartyl proteinase (100 U.); ● papain (500 U.) on the changes in water soluble (WSN), non-protein (NPN) and 5% phosphotungstic acid soluble (PTN) nitrogens during the ripening of experimental dry fermented sausages.

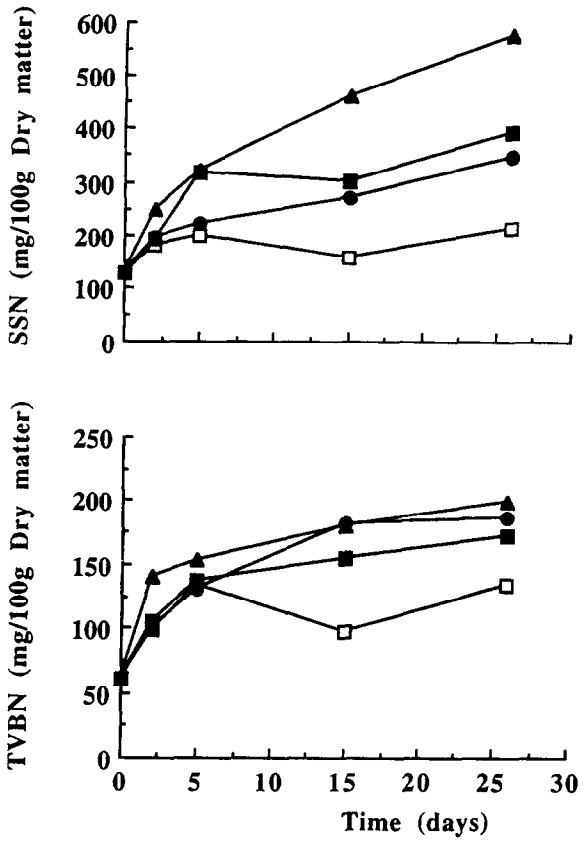

Fig. 2. Effect of the addition of proteases (□ Control; ■ pronase E (300 U.); ▲ aspartyl proteinase (100 U.); ● papain (500 U.) on the changes in 5% sulphosalicylic acid soluble (SSN) and total volatile basic (TVBN) nitrogens during the ripening of experimental dry fermented sausages.

affected the proteins B and C, which were not found after fermentation in the aspartyl proteinase batch, probably yielding the new 120 kDa band (D). Likewise, in this batch other peptidic fractions were also observed (J, S, T and X), which did not occur in the pronase E batch.

The breakdown of sarcoplamic proteins during ripening is shown in Table 2. In the control batch, a degradation pattern similar to that previously reported (Díaz *et al.*, 1996) was observed. The three proteinases assayed provoked a greater degradation than that of the control batch. No sarcoplasmic proteins over 40 kDa were detected after fermentation. Once again, the highest degradation was observed in the papain batch.

From these results, it may be deduced that each enzyme shows a different activity with meat proteins. However, despite the intensive protein degradation when papain was added, an excessive softening in the sausages was not observed and their sensorial properties were similar to those of the control batch (see below). This effect was probably due to the fact that papain is more active on myofibrillar proteins than against connective tissue, (here mainly collagen) (Wilson *et al.*, 1992). Aspartyl proteinase caused a remarkable softening of sausages, which could be partially due to the solubility of the protein fragments arising from the enzyme activity.

The concentrations of free amino acids in experimental sausages are presented in Table 3. Overall, the results were similar to those previously obtained (Díaz *et al.*, 1992, 1993, 1996). In relation to the control, Asp, Asn + Ser and Trp were the amino acids in which the highest increases during ripening were observed in the three experimental batches, although other amino acids (e.g. Met in batches 100ASP and 500PAP, Lys in batches 300PRO and 100ASP and Thr in 300PRO batch) also showed important increases. The total free amino acids recorded at the end of the ripening were about 2, 3, 4, and 6-fold higher than that of the initial value in the control, 500PAP, 300PRO and 100ASP batches, respectively. These results are consistent with the changes found in both myofibrillar (Table 1) and sarcoplasmic (Table 2) proteins. In both tables, it may be observed that the greatest accumulation of polypeptides between 8 and 12 kDa occurred in the 500PAP batch. This means that aspartyl proteinase and pronase E were the most efficient enzymes in releasing amino acids.

Table 4 shows the amine concentration of experimental dry fermented sausages. No important changes were observed during ripening for 2-phenylethylamine, spermidine and spermine. Tryptamine only showed a slight increase in protease added batches, at the same time that Trp decreased (Table 3). However, a clear increase was detected in putrescine + histamine and tyramine. Although the presence of putrescine in dry fermented sausages has been reported (Dierick *et al.*, 1974; Vandekerckhove, 1977), lactic acid bacteria seem to be unable to produce diamines (Dainty *et al.*, 1986; Edwards *et al.*, 1987), while these compounds are generated by *Pseudomonas* spp and *Enterobacteriaceae* (Slemr, 1981). These organisms may be present in raw meat but they decrease quickly during ripening (Lücke *et al.*, 1984). Although putrescine could be produced by the metabolic activity of other micro-organisms, such as Gram-positive bacilli, which appear in late stages of ripening (Palumbo *et al.*, 1976; Selgas *et al.*, 1988), moulds or yeasts, the data for putrescine plus histamine probably relate only to the histamine content. The values were higher in protease-added batches, especially in the 100ASP batch. This fact is in agreement with the high level of histidine observed in these sausages (Table 3). Histamine production depends primarily on the concentration of lactic acid bacteria in dry fermented sausages (Tschabrun *et al.*, 1990). Tyramine concentration increased in all batches throughout ripening. Although intermediate values were higher in protease-added batches, as occurred when greater amounts of enzymes were used (Díaz *et al.*, 1992, 1993, 1996), final levels were very similar to those showed by the control batch.

Sensory properties

Pronase and papain-added batches did not differ from the control batch in the triangle test. Nevertheless, significant differences ($p < 0.01$) were found when the aspartyl proteinase-added batch was compared to the other three batches (data not shown), due to an excessive softening. Table 5 shows the effect of the addition of the three enzymes on some sensory characteristics. The overall quality of pronase E and papain batches were scored at values close to those of the control.

The aspartyl proteinase batch showed a remarkable softening, obviously due to excessive proteolysis. However, although appearance and texture scores were judged by the panel as similar to those of batches with higher amounts of this enzyme (Díaz *et al.*, 1992), the results in flavour evaluation were different. When higher amounts of aspatyl proteinase were added (Díaz *et al.*, 1992) a better flavour was obtained, probably due to the levels of protein fragments formed.

In an attempt to either accelerate the ripening or potentiate the flavour of dry fermented sausages, we have explored the addition of pronase E from *Streptomyces griseus* (Díaz *et al.*, 1993), aspartyl proteinase from *Aspergillus oryzae* (Díaz *et al.*, 1992), papain from

TABLE 1
Changes in Myofibrillar Proteins during Dry Sausage Ripening

		Batch											
		Control			*Aspartyl proteinase 100 U.**			*Pronase E 300 U.*			*Papain 500 U.*		
Protein	*Estimated MW (kDa)*	*0†*	*2*	*26*	*0*	*2*	*26*	*0*	*2*	*26*	*0*	*2*	*26*
A	204	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+ + +	+ + + +		
B	160	+ +	+ +	+	+ +			+ +	+	+	+ +		
C	135	+ +	+ +	+ + +	+ +			+ +	+ +	+ +	+ +		
D	120					+ + +	+ + +						
E	100	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+ +	+ +	+ + +		
F	75	+ +	+ + +	+ + +	+ +	+ +	+ +	+ +	+	+ +	+ +		
G	57	+	+	+ +	+	+	+	+	+	+	+		
H	41	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+ + +	+ + + +	+ + +	+ +	+ + + +		
I	37	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+	+ +	+ + +		
J	33					+ +	+ +						
K	28	+	+	+	+	+	+	+	+	+	+		
L	26	+ + +	+ + + +	+ + +	+ + +	+ +	+ +	+ + +	+	+ +	+ + +	+ +	+ +
M	24	+	+	+	+	+ +	+ +	+	+	+ +	+	+ +	+ +
N	23											+	+
O	22											+	+
P	21	+ + +	+ + + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+	+ + +		
R	19	+ +	+ + +	+ +	+ +	+	+	+ +	+	+	+ +		
S	18					+	+						
T	17					+	+						
U	16	+	+ +	+ +	+	+ +	+ +	+	+	+	+	+	+
V	15		+	+ +									
X	14		+	+ +		+ +	+ +					+ +	+ +
Y	12		+	+ +								+ +	+ +
Z	11											+ + +	+ + +
AB	10											+ + + +	+ + + +
AC	9		+	+ +								+ + + +	+ + + +

Densitometric electronic integration area: + 500–1000; + + 1000–2000; + + + 2000–5000; + + + + > 5000.
*For unit definition, see text.
†Times in days.

TABLE 2
Changes in Sarcoplasmic Proteins during Dry Sausage Ripening

		Batch											
		Control			*Aspartyl proteinase 100 U.**			*Pronase E 300 U.*			*Papain 500 U.*		
Protein	*Estimated MW (kDa)*	*0*†	*2*	*26*	*0*	*2*	*26*	*0*	*2*	*26*	*0*	*2*	*26*
1	204	+ +	+	+	+ +			+ +			+ +		
2	100	+ +	+ +		+ +			+ +			+ +		
3	84	+	+		+			+			+		
4	69	+ +	+ + +	+ + + +	+ +			+ +			+ +		
5	61												
6	57	+ + +	+ + +	+ +	+ + +			+ + +			+ + +		
7	49			+ +									
8	44	+ +	+ +		+ +			+ +			+ +		
9	40	+ + + +	+ + + +		+ + + +			+ + + +	+		+ + + +		
10	38			+ + + +									
11	36	+ +	+ + + +	+ + + +	+ +	+ +	+ +	+ +	+		+ +		
12	28	+	+ +	+ +	+	+ +	+ +	+	+ +		+		
13	25	+	+ + +	+ +	+	+ + +	+ + +	+	+ +		+		
14	24	+ +	+ + +	+ +	+ +			+ +	+ +		+ +		
15	22		+ +	+									
16	21								+				
17	18	+ +	+ +	+	+ +	+	+	+ +	+		+ +		
18	17.5					+	+		+	+			
19	17								+	+			
20	16			+ + +		+ +	+ +		+ + +	+ + +		+	+
21	15					+ + +	+ + +					+	+
22	14											+ +	+ +
23	13	+	+ +	+ + +	+	+ +	+ +	+	+ + +	+ + +	+		
24	12					+ + +	+ + +		+ + +	+ + + +			
25	11			+ +		+ +	+ +		+ +	+ + + +		+ + + +	+ + + +
26	10			+ +		+ +	+ +					+ + + +	+ + + +
27	9									+ + + +		+ + + +	+ + + +
28	8			+ +								+ + + +	+ + + +

Densitometric electronic integration area: + 500–1000; + + 1000–2000; + + + 2000–5000; + + + + > 5000.
*For unit definition, see text.

TABLE 3
Changes in the Free Amino Acid Contents (mg $100\,g^{-1}$ dry matter) during Dry Sausage Ripening

Amino acid	*Batch*																
	Control					*Pronase E 300 units**				*Aspartyl proteinase 100 units*				*Papain 500 units*			
	0†	*2*	*5*	*15*	*26*	*2*	*5*	*15*	*26*	*2*	*5*	*15*	*26*	*2*	*5*	*15*	*26*
Asp	Tr	24	Tr	45	49	21	28	94	149	43	70	200	229	15	117	121	125
Glu	148	332	260	268	318	266	243	371	696	312	354	808	967	204	279	388	441
Hydroxyproline	ND	ND	ND	Tr	Tr	Tr	ND	ND	ND	ND	ND	ND	Tr	Tr	ND	Tr	Tr
Asn + Ser	21	34	29	35	62	59	63	115	162	75	85	208	259	37	46	78	114
Gly + Gln	96	115	91	78	105	127	123	174	211	137	128	284	320	104	116	162	202
His	229	361	260	227	346	277	269	390	483	306	270	557	630	236	248	378	385
Thr	20	36	28	52	91	45	57	145	234	80	112	265	321	31	54	92	149
Ala	81	148	127	112	209	157	191	317	410	187	191	486	620	124	130	239	282
Pro	46	93	78	73	118	105	113	163	253	101	90	210	282	73	89	128	167
Arg	28	ND	ND	19	Tr	ND	ND	Tr	61	Tr	20	44	98	ND	ND	Tr	45
Cys	51	Tr	40	29	44	66	Tr	29	46	43	Tr	49	54	Tr	36	52	63
Tyr	18	Tr	Tr	Tr	Tr	7	16	23	29	18	25	47	56	12	15	23	22
Val	32	62	57	65	108	95	122	214	274	109	153	365	428	64	88	144	192
Met	18	25	19	23	31	27	29	74	49	52	62	170	179	21	27	53	69
Ile	20	39	39	47	79	70	92	156	185	102	125	268	306	42	58	83	129
Leu	36	94	94	101	178	140	194	319	359	211	254	592	637	98	119	183	284
Phe	19	38	48	52	86	71	93	157	191	104	125	310	312	47	61	106	147
Trp	36	37	9	10	8	38	11	24	26	65	36	53	34	22	24	31	24
Lys	80	121	99	88	169	171	186	324	462	258	283	735	804	99	119	186	315

Tr, trace amounts;ND, not detected.
*For unit definition, see text.
†Times in days.

TABLE 4
Changes in Amine Contents (mg $100\,g^{-1}$ dry matter) during Dry Sausage Ripening

	Batch																
	Control					*Pronase E 300 units**				*Aspartyl proteinase 100 units*				*Papain 500 units*			
Amine	*0*†	*2*	*5*	*15*	*26*	*2*	*5*	*15*	*26*	*2*	*5*	*15*	*26*	*2*	*5*	*15*	*26*
Triptamine	5·5	6·1	5·1	4·9	3·3	5·9	5·7	7·9	7·8	4·6	3·7	9·8	9·1	3·0	7·1	7·6	7·5
2-Phenylethylamine	ND	Tr	Tr	Tr	1·4	2·7	Tr	2·1	2·6	Tr	Tr	2·3	6·4	Tr	ND	Tr	1·8
Putrescine + Histamine	ND	25·6	41·4	44·1	79·6	60·7	84·9	141·1	156·1	60·5	108·8	234·0	241·6	43·6	56·0	86·9	94·8
Spermidine	Tr	Tr	2·7	Tr	3·5	Tr	Tr	3·1	2·9	Tr	2·9	3·0	Tr	2·8	Tr	2·3	Tr
Spermine	9·8	Tr	11·9	5·2	14·6	6·9	11·5	12·1	9·2	10·6	15·3	9·3	10·6	12·6	10·5	12·3	7·5
Tyramine	ND	8·1	9·3	10·2	14·4	17·1	22·7	22·8	17·4	2·5	18·1	19·3	17·8	10·7	14·9	14·4	13·0
Cadaverine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Tr, trace amounts;ND, not detected.
*For unit definition, see text.
†Times in days.

TABLE 5
Effect of Proteases on Selected Organoleptic Characteristics of Experimental Dry Fermented Sausages after 26 days of Ripening (0–10 scale)

Batch	*Protease units**	*Colour and appearance*	*Texture*	*Flavour*	*Overall quality*†
Control	0	7·5a	7·1a	7·6a	7·5a
Pronase E	300	7·4a	7·0a	7·4a	7·3a
Aspartyl proteinase	100	4·1b	3·2b	4·9b	4·4b
Papain	500	7·2a	6·7a	7·5a	7·3a

a,b,c: different letters in each column means significant difference at $p < 0 \cdot 01$.
*For unit definition, see text.
†Overall quality = (colour and appearance × 0·1) + (texture × 0·25) + (flavour × 0·65).

Carica papaya (Díaz *et al.*, 1996) and pancreatic lipase (Fernández *et al.*, 1995*a,b*). The results demonstrate that it is possible to accelerate both the proteolysis and lipolysis phenomena. When proteinases were added, final products ranged from sausages in which, in comparison with conventional types, no important chemical changes (NPN, PTN, SSN, TVBN and free amino acids and amines) were observed (if a low amount of enzymes were added), to final products in which great increases in the above-mentioned nitrogen fractions were produced (if a high level of enzymes were added) and, in some batches, the enzyme provoked an excessive softness (spreadable texture). Similarly, when pancreatic lipase was added, a range of increased levels of free fatty acids was obtained according to the amounts of enzyme added from normal (similar to that observed in conventional dry sausages) to a very high level of free fatty acid in the product, in which an 'oily exudate' was observed.

These results mean that it is possible to accelerate the proteolysis and lipolysis phenomena by the addition of the corresponding enzyme in the appropriate concentration. However, the sensory analysis demonstrated that, in some cases, only a slight increase in the flavour was obtained, i.e. it is possible to obtain very drastic degradations of proteins and fat which, in turn, produce spectacular increases of the degradation products (amino acids and fatty acids, respectively) without these effects resulting in a noticeable increase in the flavour. Thus, it seems to be that the addition of proteinases and lipases alone is not useful in shortening the ripening time. In our opinion, it is necessary to have a long period of ripening in order to allow the transformation of free amino acids and fatty acids through microbial (oxidative deaminations, decarboxylations, etc.) and/or chemical (Strecker and Maillard reactions, autoxidations of fat, etc.) ways to yield aromatic compounds (aldehydes, ketones, lactones, alcohols, esters, etc.). These have been proved to be the main compounds responsible for the flavour of dry fermented sausages (Edwards *et al.*, 1991).

Thus, to shorten the ripening of sausages, the addition of proteinases and lipases may be useful for providing substrates for transformation into aromatic compounds. Therefore, it is necessary, besides the addition of proteinases and lipases, to create conditions (or to add either an efficient starter or other kinds of enzymes) so that the above-mentioned volatiles may be formed, in a shorter time than usual, from free amino acids and fatty acids generated by the enzymes.

ACKNOWLEDGEMENTS

This work was supported by the Comision Interministerial de Investigación Científica y Técnica (CICYT), project ALI 88/0005 and ALI92-0461. O.D. and M.F. were beneficiaries of grants from Formación de Personal Investigador from the Ministerio de Educación y Ciencia. We thank Industrias CABO for graciously providing the ingredients and manufacturing the experimental sausages.

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