Influence of the Simultaneous Addition of the Protease Flavourzyme and the Lipase Novozym 677BG on Dry Fermented Sausage Compounds Extracted by SDE and Analyzed by GC-MS

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A dry fermented sausage (chorizo de Pamplona) was elaborated with the simultaneous addition of a lipase (Novozym 677BG) and a protease (Flavourzyme) and ripened during 21 days, in contrast to the control without enzymes and ripened during 35 days. Faster and more intense lipolytic and proteolytic activities were observed in the modified sausage, despite its shorter maturation time. At the end of the ripening, a determination of the profile of compounds extracted by simultaneous distillation−extraction with dichloromethane was carried out. The total amounts of extracted compounds (expressed in milligrams of dodecane per gram of dry matter) were 2.5 in the sausage with enzymes and 1.9 in the control. The chemical groups showing increments due to the use of enzymes were esters (103.5% increment) and acids (87% increment) in both cases due to the greater presence of long-chain fatty acid products. However, development of substances originated from further degradation process of amino acids and free fatty acids did not seem to have taken place.

Keywords: *Dry fermented sausages; enzymes; SDE profile*

INTRODUCTION

The use of lipolytic and proteolytic enzymes in dry fermented sausage could increase the development of volatile compounds through a greater synthesis of their precursors. The improvement of sensorial quality of these products using enzymes requires the determination of volatile compounds so that it could be possible to evaluate their role in the development of higher quality and better acceptability of products (García Regueiro and Díaz, 1995).

Addition of proteases and lipases to dry fermented sausages to shorten the ripening time has been used since the early 1990s (Ordoñez et al., 1998). It has been well stablished that this strategy leads to many changes in the lipidic and nitrogen fractions, depending on the kind of enzyme and the dose employed, with certain repercussions in the sensorial properties of the products (Díaz et al., 1996, 1997; Fernández et al., 1995; Zalacain et al., 1997a−c; Zapelena et al., 1998, 1999). Furthermore, when a lipase and a protease were added simultaneously to the manufacture of chorizo de Pamplona, a typical Spanish sausage, significant increases in some amino acids and some free fatty acids were found, but no differences were detected in the sensory quality, compared with the control, except for a slight softening (Ansorena et al., 1998).

Compounds resulting from protein breakdown and lipolysis and those originated by their transformation into volatile and sapid substances play an important role in the flavor of dry fermented sausages. However, not many papers focus attention on the determination of the influence of the use of enzymes on the volatile profile of the sausages. Hagen et al. (1996) identified 45 different compounds in a model salami (without spices and without smoking) and detected a significant effect on 9 of them in those treated with a protease from *Lactobacillus paracasei* subsp. *paracasei* (NCDO 151 proteinase). Maturity flavor was more pronounced in sausages with NCDO 151 proteinase added, even if the ripening time was shorter.

Bruna et al. (1998) tested the simultaneous use of a protease (Pronase E) and a mold extract (from *Penicillium olsonii*) to potentiate the dry fermented sausage flavor. They found similar volatile compound patterns for all elaborated batches, although those with the protease and the mold extract showed the highest concentration, followed by those with only the protease. No effects were found on odor, rancid flavor, and flavor intensity.

The objective of this work was to evaluate the modification on the development of the compounds extracted by simultaneous distillation−extraction (SDE) in a dry fermented sausage, as a consequence of the proteolytic and lipolytic activity caused by the simultaneous addition of a lipase from *Thermomyces lanuginosus* (Novozym 677BG) and a commercial mixture of exo- and endoproteases from *Aspergillus oryzae* (Flavourzyme).

MATERIAL AND METHODS

Sausage Elaboration. Sausages containing lipase and protease and a control sausage (without enzymes) were made in a pilot plant. The lipase was Novozym 677BG, a microbial enzyme from *T. lanuginosus* (Novo Nordisk A/S), and the protease was Flavorzyme (Novo Nordisk A/S), a mixture of proteases with both exo- and endopeptidase activities, obtained by the controlled fermentation of *A. oryzae.* A mixture of *Lactobacillus plantarum* L115 (10%) and *Staphylococcus carnosus* M72 (90%) from Lacto-Labo (TEXEL) was used as starter culture at 10^6-10^7 colony-forming units (cfu)/g.

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Table 1. Evolution of Content (Milligrams of Tyr per Gram of Dry Matter) in Total α-NH_2 Groups during the Ripening Time[a]

	mixing	3 days	9 days	15 days	21 days	35 days
control	25.51 ± 0.43a	33.15 ± 1.10b	38.66 ± 2.97c	40.57 ± 2.19d	43.57 ± 1.74e	46.63 ± 1.50e
enzymes	25.51 ± 0.43a	44.33 ± 3.71b	49.77 ± 1.12c	54.56 ± 2.73d	58.02 ± 3.73e	
SL	ns	**	***	***	** (**)	

[a] Student *t* test: SL (significance level); ns, not significant, $p > 0.05$; **, $p < 0.01$; ***, $p < 0.001$; (**) between control sausage with 35 days and sausages with enzymes with 21 days. ANOVA: within the same row different letters denote significant differences among phases of analysis ($p < 0.05$).

Table 2. Evolution of Acidity Value (Grams of Oleic Acid per 100 g of Fat) during Ripening Time[a]

	mixing	3 days	9 days	15 days	21 days	35 days
control	2.10 ± 0.10a	3.19 ± 0.07b	3.93 ± 0.14c	4.75 ± 0.10d	5.50 ± 0.13e	5.67 ± 0.05e
enzymes	2.10 ± 0.10a	3.95 ± 0.09b	5.95 ± 0.19c	7.44 ± 0.09d	9.61 ± 0.20e	
SL	ns	***	***	***	*** (***)	

[a] Student *t* test: SL (significance level); ns, not significant, $p > 0.05$; ***, $p < 0.001$; (***) between control sausage with 35 days and sausages with enzymes with 21 days. ANOVA: within the same row different letters denote significant differences among phases of analysis ($p < 0.05$).

The two types of sausages were made with a standard formulation comprising lean pork meat (70%), pork back fat (30%), red pepper (30 g/kg), NaCl (28 g/kg), dextrin (15 g/kg), powdered milk (12 g/kg), lactose (10 g/kg), sodium caseinate (10 g/kg), dextrose (5 g/kg), garlic (3 g/kg), polyphosphates (2 g/kg), Curavi (a mixture of nitrate and nitrite; 0.3 g/kg), and Ponceau 4R (E-124; 0.3 g/kg).

Lean pork meat and fat back pork were minced in a cutter to a particle size of ~3 mm (this small particle size is a characteristic of chorizo de Pamplona, a Spanish type of dry fermented sausage). Subsequently, all ingredients and the starter culture were added and mixed in a vacuum kneading machine. The mixture was divided into two batches of 7 kg each. Lipase (0.500 LU/g of mixture) and protease (0.02 LAPU/g of mixture) were added to batch 1, and no enzymes were added to batch 2 (control). After the initial fermentation in a ripening cabinet [24 h at 24 °C and 100% relative humidity (RH), 24 h at 22 °C and 85% RH, 24 h at 20 °C and 80% RH], sausages were transferred to different drying chambers for ripening. Control was maintained during 35 days until a weight loss of 33% and modified sausage was held during 21 days. Four batches of each type of sausage were made.

Analytical Methods. *Proteolysis and Lipolysis Measurements.* Acidity values (grams of oleic acid per 100 g of fat) were determined using International Standard ISO 1740 (ISO, 1980). Total free α-NH_2-N groups were extracted with citrate buffer, and trichloroacetic acid (TCA) was added to precipitate the proteins. They were subsequently analyzed through a ninhydrin colorimetric method using tyrosine as the standard (Massi, 1963).

Likens−Nickerson Extraction. Twenty-five grams of frozen sausage was ground and placed in a 250 mL flask with 100 mL of water. A second flask with 5 mL of dichloromethane and 150 μg of dodecane (i.s.) was also attached to a modified Likens−Nickerson apparatus. Five milliliters of dichloromethane was also added to fill the apparatus solvent return loop. Both solvent and sample mixture were heated to 70 °C and boiling temperature, respectively, maintaining these conditions during 2 h. After cooling to ambient temperature, the extract of dichloromethane was collected and dried over anhydrous Na_2SO_4. Three distillations per batch of sausage were carried out.

Analysis of Volatile Compounds. The volatile compounds were analyzed in an HP 6890 GC system (Hewlett-Packard) coupled to a 5973 mass selective detector (Hewlett-Packard). A total of 1 μL of the extract was injected into the GC, equipped with a capillary column (30 m × 250 μm i.d. × 0.25 μm film thickness, HP-5MS). The carrier gas was He (1 mL/min), and the chromatographic conditions were as follows: initial oven temperature was maintained during 10 min at 40 °C and subsequently programmed from 40 to 120 °C at a rate of 3 °C/min and at a rate of 10 °C/min from 120 to 250 °C, at which it was held for another 5 min; injector temperature, 250 °C; mass range, 33−350 amu; solvent delay, 4 min; electron impact at 70 eV.

Identification of the peaks was based on comparison of their mass spectra with the spectra of the Wiley library and, in addition in some cases, by comparison of their retention time with those of standard compounds. The Kovats indices were also calculated according to the method of Tranchant (1982) and were compared with available literature data (Kondjoyan and Berdagué, 1996). Only known peaks are shown. Areas of peaks were measured by integration of the total ion current of the spectra or by calculation of the total area based on integration of a single ion. Semiquantitative determination of the volatile compounds was based on the ratio of their peak to that of dodecane (i.s.), and the results were expresed as nanograms of dodecane per gram of dry matter.

Data Analysis. Data analysis was carried out with an SPSS program. Values for acidity and total free α-NH_2-N groups are the mean of 8 determinations (2 batches of product per type of sausage and 4 determinations per batch were carried out). Values for volatile profiles are the mean of 12 determinations (3 distillations per batch of sausage and 2 injections per distillation were carried out). A Student *t* test was used to determine significant differences between the two types of sausages for every studied parameter in each phase. An analysis of variance (ANOVA) was used to determine differences among phases for every type of sausage along the ripening process.

DISCUSSION

A previous work with these two enzymes was carried out to select the most suitable dose to be subsequently employed simultaneously in the elaboration of chorizo de Pamplona. The enzymes were tested individually. Three different concentrations of Flavourzyme were assayed (0.07, 1, and 2.8 LAPU/g). Sensory analysis of these products revealed serious texture defects, suggesting that a lower dose of these enzyme should be used (Ansorena et al., 1997). The dose chosen was 0.02 LAPU/g. Novozym 677BG was also tested at three different concentrations (0.5, 1.5, and 3 LU/g); all of them produced an increase in the acidity value (Ansorena et al., 1997). The lowest dose was chosen for this experiment (0.5 LU/g).

The lipolytic and proteolytic activities of the enzymes at the chosen doses were confirmed by the measure of the acidity values and the content of total free α-NH_2 groups, respectively. By the third day of ripening differences in the proteolytic activity were found be-

Table 3. Results of Profiles of Compounds Extracted by SDE

			control		Flavourzyme + Novozym		
RI[a]	RID[b]	compound	mean	SD	mean	SD	signif[c]
		acids					
		2-methylpropanoic acid	790.89	86.85	948.17	117.20	ns
		butanoic acid	5213.28	434.40	2823.04	399.77	***
848	B	3-methylbutanoic acid	15422.93	1006.43	9726.66	1878.19	**
858	B	2-methylbutanoic acid	4757.94	264.47	4023.00	488.18	*
999	A	hexanoic acid	12809.47	1473.03	9359.68	2239.98	*
1188	A	octanoic acid	49202.69	6216.80	69621.44	13001.22	*
1386	A	decanoic acid	144008.96	18366.92	225711.84	36031.19	**
1572	A	dodecanoic acid	37490.09	5559.30	51319.10	10622.04	ns
1774	A	tetradecanoic acid	100904.45	15857.18	211425.31	17746.43	***
1871	C	pentadecanoic acid	2458.02	987.88	4256.43	191.86	*
1957	A	9-hexadecenoic acid	59729.99	8031.46	126429.93	8638.42	***
1983	A	hexadecanoic acid	439392.08	68022.44	912943.22	78847.86	***
2113	A	9,12-octadecadienoic acid	4444.02	894.21	9174.81	686.61	***
2123	A	9-octadecenoic acid	737.89	180.41	1981.49	98.61	***
2177	A	octadecanoic acid	348.43	32.93	643.88	48.13	***
		subtotal	877711.12 46.36%		1640387.97 64.40%		
		alcohols					
		?-methyl-2-butenol	1031.34	695.44	nd		
850	C	2-furanmethanol	1393.39	115.93	813.19	89.45	***
1100	A	linalool	3964.62	320.68	4669.42	497.45	ns
1112	C	phenylethyl alcohol	4845.85	483.92	4409.43	254.91	ns
		subtotal	11235.20 0.59%		9892.04 0.39%		
		aldehydes					
804	A	hexanal	1697.55	86.20	2755.94	392.37	**
828	B	2-furancarboxaldehyde	9190.02	600.25	9069.23	477.89	ns
959	B	benzaldehyde	2007.79	268.84	3066.31	771.71	*
1040	B	phenylacetaldehyde	28653.54	2167.02	37858.84	3079.30	**
1106	B	nonanal	3599.00	510.42	3249.29	587.99	ns
1615	B	tetradecanal	227.34	152.40	200.49	24.63	ns
1713	B	pentadecanal	7019.42	917.00	5523.84	703.83	*
1830	B	hexadecanal	390769.80	60332.30	269281.35	24277.26	*
2009	B	octadecenal	70600.09	7597.90	58793.00	3941.26	**
2032	C	octadecanal	72181.41	4847.92	69428.66	4449.33	ns
		subtotal	585945.96 30.95%		459226.93 18.03%		
		ketones					
1094	C	2-nonanone	1338.61	352.02	nd		
1460	B	geranyl acetone	4519.12	356.70	4486.80	939.59	ns
1491	B	*β*-ionone	4419.22	246.48	3405.08	376.84	**
1537	C	5,6,7,7-a-tetrahydro-4,4,7a-trimethyl-2(4*H*)-benzofuranone	3660.02	195.43	2522.82	217.99	***
1696	B	2-pentadecanone	15330.39	1119.67	8860.68	739.88	***
1746	C	9*H*-fluoren-9-one	2518.08	216.36	2429.03	119.74	ns
		subtotal	31785.43 1.68%		21704.41 0.85%		
		esters					
1003	B	hexanoic acid ethyl ester	nd		1257.30	143.86	
1128	A	octanoic acid methyl ester	1366.13	68.86	2027.19	102.37	***
1328	A	decanoic acid methyl ester	5777.45	216.43	7446.08	1125.26	*
1398	B	decanoic acid ethyl ester	7640.57	448.22	11084.48	1771.10	**
1531	A	dodecanoic acid methyl ester	463.98	5.07	565.48	42.66	**
1597	B	dodecanoic acid ethyl ester	1843.61	243.27	3037.03	320.58	**
1725	A	tetradecanoic acid methyl ester	5060.54	754.20	9838.12	1203.70	**
1805	B	tetradecanoic acid ethyl ester	3884.81	401.55	9903.22	1168.94	***
1843	C	tetradecanoic acid isopropyl ester	6169.06	552.24	nd		
1938	A	hexadecanoic acid methyl ester	16764.60	1295.68	27717.68	1469.06	***
2005	C	hexadecanoic acid ethyl ester	16999.61	1501.16	43302.82	3581.44	***
2101	A	9,12-octadecadienoic methyl ester	14366.96	683.74	28429.16	2372.57	***
2107	A	9-octadecenoic methyl ester	10363.59	858.67	29997.56	3787.84	***
2134	A	octadecanoic methyl ester	nd		9617.38	1040.41	
2207	B	octadecanoic ethyl ester	nd		394.83	60.18	
		subtotal	90700.91 4.79%		184618.35 7.25%		

Table 3 (Continued)

			control		Flavourzyme + Novozym		
RI[a]	RID[b]	compound	mean	SD	mean	SD	signif[c]
		sulfur compounds					
848	B	3,3′ -thiobis(1-propene)	260.52	33.21	276.90	38.61	ns
907	B	3-(methylthio)propanal (methional)	4402.61	794.89	5688.34	1274.03	ns
912	C	methyl 2-propenyl disulfide	603.88	48.16	500.35	146.84	ns
1075	C	di-2-propenyl disulfide	8450.60	600.70	7567.63	1188.21	ns
		subtotal	13717.61		14033.23		
			0.72%		0.55%		
		aromatic hydrocarbons					
		toluene	579.30	10.64	496.81	66.07	*
858	B	xylene	961.19	54.27	988.67	89.50	ns
989	B	trimethyl-105/120-benzene (B)	3647.80	330.68	2760.92	440.23	*
1017	B	methyl-?-methylethylbenzene (?-cymene)	1221.85	191.49	nd		
1175	C	naphthalene	1709.89	50.80	1965.29	110.16	**
1285	C	methylnaphthalene	1755.95	233.71	1923.23	228.32	ns
1301	C	methylnaphthalene	nd		1765.30	399.65	
1446	C	acenaphtene	2528.90	146.87	1978.73	424.45	ns
1565	D	trimethylnaphthalene	4619.46	327.09	4543.70	818.72	ns
1584	C	9*H*-fluorene	2963.51	304.61	2936.36	385.37	ns
1589	D	trimethylnaphthalene	4088.47	181.01	3434.46	790.06	ns
		subtotal	24076.31		22793.46		
			1.27%		0.89%		
		terpenes					
930	B	α-pinene	2686.58	320.96	1568.90	39.17	***
971	A	sabinene	6552.83	491.54	3257.62	121.48	***
1001	B	3-carene	6032.82	1160.66	2660.30	389.85	**
1022	A	limonene	6458.40	659.42	4209.47	361.84	**
		subtotal	21730.63		11696.31		
			1.15%		0.46%		
		phenols					
987	B	phenol	16272.43	1116.01	10906.67	502.47	***
1056	B	4-methylphenol (*p*-cresol)	11905.88	716.40	9842.39	531.95	**
1079	B	2-methylphenol (*o*-cresol)	30749.18	2112.69	22504.83	948.67	***
1087	B	guaiacol (2-methoxyphenol)	5030.07	297.57	3712.68	474.90	**
1141	D	ethylphenol	1548.14	165.22	1502.63	64.46	ns
1149	D	2-dimethylphenol	5533.52	151.79	4789.95	457.62	*
1151	D	2-dimethylphenol	5994.92	358.01	4689.92	283.79	**
1168	D	ethylphenol	5611.27	424.73	3416.12	209.15	***
1170	D	2-dimethylphenol	14007.86	2097.99	9681.41	437.80	**
1178	D	2-dimethylphenol	4335.60	476.71	4006.70	466.03	ns
1190	D	2-methoxy-4-methylphenol	12689.04	1101.45	10630.04	1330.06	ns
1192	D	3-dimethylphenol	5156.99	582.89	3772.87	403.61	**
1219	C	4-vinylphenol	10006.65	547.65	7434.16	885.80	**
1238	D	methylethylphenol	2529.32	134.15	1990.15	167.44	**
1244	D	dimethoxyphenol	4490.48	354.51	3249.14	318.21	**
1259	D	?-methyl-?-ethylphenol	3592.82	366.38	3798.71	210.10	ns
1265	D	trimethylphenol	1608.56	149.87	1582.47	192.08	ns
1270	D	trimethylphenol	1885.12	182.55	1515.51	81.48	*
1277	C	4-ethyl-2-methoxyphenol	11259.57	1184.91	7581.67	1146.94	**
1311	B	4-vinyl-2-methoxyphenol	14729.11	1037.38	13650.01	2038.70	ns
1355	C	2,6-dimethoxyphenol	14711.02	1775.20	8837.54	1720.11	**
1361	B	eugenol	1608.54	152.84	1308.14	89.87	*
1371	C	2-methoxy-4-propylphenol	4492.82	555.19	3125.60	367.02	**
1454	C	isoeugenol	1829.35	156.57	1357.12	198.37	*
		subtotal	191578.25		144886.42		
			10.12%		5.69%		
		nitrogen compounds					
1063	B	2-acetylpyrrole (1058)	1786.82	148.13	1660.41	298.46	ns
1086	C	tetramethylpyrazine	4369.79	169.77	4148.27	668.96	ns
		subtotal	6156.61		5808.68		
			0.33%		0.23%		
		others					
806	B	tetrachloroethylene	2106.32	152.60	1387.77	189.21	***
1315	C	1,2,3-trimethoxybenzene	2477.84	81.92	2340.44	259.13	ns
1407	C	1,2,3-trimethoxy-5-methylbenzene	5617.37	482.32			**
1515	C	dibenzofuran	6534.71	342.66	5182.51	883.25	*
1533	D	2,3,5-trimethoxytoluene ??	8963.66	1296.35	5821.33	729.06	**
1797	C	anthracene	8286.51	907.66	9119.20	1078.15	ns
		subtotal	33986.42		23851.26		
			1.80%		0.94%		

Table 3 (Continued)

			control		Flavourzyme + Novozym		
RI[a]	RID[b]	compound	mean	SD	mean	SD	signif[c]
		alkanes					
900	B	nonane	nd		5688.34	1274.03	
1500		pentadecane + 99	4546.25	311.36	2644.91	447.11	***
		subtotal	4546.25		8333.25		
			0.24%		0.33%		
		total	1893170.69		2547232.3		

[a] RI, retention indices for the DB5 column. [b] RID, reliability of identification, indicated by the following symbols: A, mass spectrum and retention time identical with those of an autentic sample; B, mass spectrum and Kovats index in agreement with the corresponding literature data; C, mass spectrum consistent with spectra reported in the Wiley library data; D, tentative identification by mass spectrum. nd:not detected. [c] Significance: ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Results expressed in nanograms of dodecane per gram of dry matter.

tween the two batchess; the concentration of total free α-NH_2 groups was higher in the sausage with enzymes (Table 1). Between the third and ninth days of ripening, the value for the enzyme-containing sausage was similar to that for the control batch at the end of the maturation. A similar effect was observed for the acidity value, with the lipolytic activity of Novozym being shown also from the third day of ripening (Table 2). At the end of the process both parameters were higher in the modified sausages, despite the shorter maturation time.

Table 3 shows the volatile profile of compounds extracted by SDE and analyzed by GC-MS. A total of 104 different substances were identified between the two types of analyzed sausages, which were grouped into 11 chemical families: phenols (24), acids (15), esters (15), aromatic hydrocarbons (11), aldehydes (10), ketones (6), alcohols (4), sulfur compounds (4), terpenes (4), nitrogen compounds (2), and others (7). Expressed in milligrams of dodecane per gram of dry matter, a total of 2.5 mg was extracted in the modified sausage, and 1.9 mg was the amount extracted in the control. However, the amounts of only two chemical groups was clearly greater in the enzyme-containing sausages: acids and esters.

The most affected fraction by the use of the enzymes was the esters, which increased their concentration by 103.5% in the modified sausages with regard to the control, due to the increment in the long-chain fatty acid esters C_{16} and C_{18}. The contribution of this group to the total area was 4.8% for the control batch and 7.3% for the modified one. This increment in esters could be related to the great lipolytic activity suffered by the enzyme-containing sausages, which led to a greater amount of free fatty acids. These compounds are the substrate for microorganisms to the synthesis of esters. Esters are detected in products with a high concentration of acids and alcohols (Stahnke, 1994). Ethyl esters are formed by enzymatic reaction between acids and ethanol (Hinrichsen and Pedersen, 1995).

Acids were quantitatively the most abundant group in both types of sausages, accounting for 46 and 64% of the total area in the control and enzyme-containing sausages, respectively. This increment (87%) in their concentration was mainly due to the presence of long-chain fatty acids. They do not contribute directly to the flavor of the dry fermented sausages but could be substrates for lesser flavoring compounds of lower threshold value and greater volatility.

Concentration of some acids from the microbial degradation of ramified amino acids, 2-methylbutanoic acid and 3-methylbutanoic acid, was lower in the sausages with enzyme. These compounds possess very strong, cheesy odors that will be of importance and also may have a positive impact on aroma due to conversion into fruity esters (Stahnke, 1995). No differences were found for 2-methylpropanoic acid. However, the concentration of phenylacetaldehyde, an aldehyde originated from the microbial degradation of phenylalanine and suggested as a proteolysis indicator, was significantly higher in the sausage with enzymes. Berdagué et al. (1993) pointed out that the proteolysis accounted for ~6% of the total compounds extracted with dynamic headspace from an unspiced dry sausage. They concluded that this low percentage of compounds derived from amino acids was due to a restricted proteolysis because of inhibitory physicochemical conditions (low water activity, high salt contents, and low pH values). Our results showed that in the sausages with enzymes there was a significantly greater proteolytic activity. It could be that the shorter ripening time was not enough to allow the development of secondary reactions which would lead to some of the compounds.

Aldehydes content decreased significantly from 31 to 18% due to the lower amounts of pentadecanal, hexadecanal, and octadecanal. As suggested by Dirinck et al. (1997), these high molecular weight aldehydes could act as precursors for the volatile alkanals and alkenals. The higher amount of hexanal found in the modified sausages could support this hypothesis.

García Regueiro et al. (1998) found a higher release of terpenes in dry fermented sausages elaborated with a highly proteolytic strain of *Staphylococus xylosus* LTH 2102. They concluded that the higher degradation of proteins can reduce the interaction of terpenes and proteins. The use of the mixture Flavourzyme and Novozym did not contribute to the increased content of terpenes, which was greater in the control sausage (1.15%) than in the the modified sausage (0.46%).

Sulfur compounds originated from garlic and did not show significant differences, and neither did most of the aromatic hydrocarbons. Phenols showed, in general, greater amounts in the control sausage. Compounds of these chemical groups were not related to the use of exogeous enzymes.

In summary, the addition of the enzymes Novozym and Flavourzyme at the assayed doses led to higher and faster proteolytic and lipolytic activities in the modified sausages with regard to the control, despite their shorter ripening time. Esters and acids were the most affected chemical groups among those compounds extracted by SDE. The greater proteolysis in the sausages with enzymes did not have result in the expected increment

of amino acid derivatives. Other chemical groups were not affected by the addition of the enzymes.

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LITERATURE CITED

Ansorena, D.; Zapelena, M. J.; Astiasarán, I.; Bello, J. Activity of Flavourzyme and Novozym 677BG in the manufacture of Chorizo de Pamplona. In *VII Meeting on Industrial Application of Enzymes*; AIQS: Barcelona, Spain, 1997; p 163.

Ansorena, D.; Zapelena, M. J.; Astiasarán, I.; Bello, J. Simultaneous addition of Palatase M and Protease P to a dry fermented sausage (Chorizo de Pamplona) elaboration: effect over peptidic and lipid fractions. *Meat Sci*. **1998**, *50* (1), 37−44.

Berdagué, J. L.; Monteil, P.; Montel, M. C.; Talon, R. Effects of starter cultures on the formation of flavour compounds in dry sausage. *Meat Sci*. **1993**, *35*, 275−287.

Bruna, J.; Fernández, M.; Hierro, E.; de la Hoz, L.; Ordóñez, J. A. Use of *Penicillium olsonii* extracts to potentiate the dry fermented sausage flavour. In *44th ICOMS*; Barcelona, Spain, 1998; pp 800−801.

Díaz, O.; Fernández, M.; García de Fernando, G. D.; de la Hoz, L.; Ordoñez, J. A. Effect of the addition of Papain on the dry fermented sausage proteolysis. *J. Sci. Food Agric*. **1996**, *71*, 13-21.

Díaz, O.; Fernández, M.; García de Fernando, G. D.; de la Hoz, L.; Ordoñez, J. A. Proteolysis in dry fermented sausages: the effect of selected exogenous proteases. *Meat Sci*. **1997**, *46* (1), 115−128.

Dirinck, P.; Van Opstaele, F.; Vandendriessche, F. Flavour differences between nothern and southern European cured hams. *Food Chem*. **1997**, *59* (4), 511−521.

Fernández, M.; de la Hoz, L.; Díaz, O.; Cambero, M. I.; Ordóñez, J. A. Effect of the addition of pancreatic lipase on the ripening of dry-fermented sausages−Part 2. Free fatty acids, short-chain fatty acids, carbonyls and sensory quality. *Meat Sci*. **1995**, *40*, 351−362.

García-Regueiro, J. A.; Díaz, I. Aplicaciones del análisis de volátiles en productos cárnicos. *Eurocarne* **1995**, *38*, 85−90.

García-Regueiro, J. A.; Rius, M. A.; Hortós, M.; Hugas, M., Díaz, I. Influence of starter cultures on the volatile profile of a Spanish dry fermented sausage (fuet). In *44th ICOMS*; Barcelona, Spain, 1998; pp 802−803.

Hagen, B. F.; Berdagué, J. L.; Holck, A. L.; Naes, H.; Blom, H. Bacterial proteinase reduces maturation time of dry fermented sausages. *J. Food Sci*. **1996**, *61* (5), 1024−1029.

Hinrichsen, L. L.; Pedersen, S. B. Relationship among flavor, volatile compounds, chemical changes, and microflora in Italian-type dry-cured ham during processing. *J. Agric. Food Chem*. **1995**, *43*, 2932−2940.

ISO. Determination of acidity content. ISO 1740-1980. In *International Standards Meat and Meat Products*; International Organization for Standarization: Ginebra, 1980.

Kondjoyan, N.; Berdagué, J. L. *A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds*; Laboratoire Flaveur (INRA): Theix, France, 1996.

Massi, O. La scomposizione proteica nei prosciutti quale indice del processo di maturazione. *Vet. Ital*. **1963**, *14*, 957−964.

Ordóñez, J. A.; Fernández, M.; Hierro, E.; Bruna, J. M.; Herranz, B.; de la Hoz, L. Consideraciones acerca de la maduración acelerada de embutidos crudos curados. *Eurocarne* **1998**, *68*, 53−57.

Stahnke, L. H. Aroma compounds from dried sausages fermented with *Staphylococcus xylosus*. *Meat Sci*. **1994**, *38*, 39−53.

Stahnke, L. H. Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels−Part II. Volatile components. *Meat Sci*. **1995**, *41* (2), 193−209.

Tranchant, J. *Manuel Pratique de Chromatographie en Ohase Gazeuse*; Masson: Paris, France, 1982; pp 301−337.

Zalacain, I.; Zapelena, M. J.; De Peña, M. P.; Astiasarán, I.; Bello, J. Use of lipase from *Rhizomucor miehei* in dry fermented sausages elaboration. Microbial, chemical and sensory analysis. *Meat Sci*. **1997a**, *45* (1), 99−105.

Zalacain, I.; Zapelena, M. J.; De Peña, M. P.; Astiasarán, I.; Bello, J. Lipid fractions of dry fermented sausages change when starter culture and/or *Aspergillus* lipase are added. *J. Food Sci*. **1997b**, *62* (5), 1076−1079.

Zalacain, I.; Zapelena, M. J.; De Peña, M. P.; Astiasarán, I.; Bello, J. Application of Lipozyme 10.000L (from *Rhizomucor miehei*) in dry fermented sausage technology: study in a pilot plant and at the industrial level. *J. Agric. Food Chem*. **1997c**, *45*, 1972−1976.

Zapelena, M. J.; Ansorena, D.; Zalacain, I.; Astiasarán, I.; Bello, J. Dry fermented sausages manufactured with different amounts of commercial proteinases: evolution of total free α-NH_2-N groups and sensory evaluation of the texture. *Meat Sci*. **1998**, *49* (2), 213−221.

Zapelena, M. J.; Astiasarán, I.; Bello, J. Dry fermented sausages made with a protease from *Aspergillus oryzae* and/or a starter culture. *Meat Sci*. **1999**, *52*, 403−409.

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