Addition of Palatase M (Lipase from *Rhizomucor miehei*) to Dry Fermented Sausages: Effect over Lipolysis and Study of the Further Oxidation Process by GC−MS

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Three doses [0.150, 0.300, and 1 LU/g (where 1 LU is the amount of enzyme that liberates 1 μmol of butyric acid/min from an emulsified tributyrin substrate at pH 7.0 and 30 °C)] of Palatase (lipase from *Rhizomucor miehei*) were added to a dry fermented sausage formulation to study their effect over the lipid fraction and some sensory properties. The enzyme caused significant increments for all free fatty acids analyzed, which were proportionally lower for linolenic acid and linoleic acid. No differences in rancid odor or taste were observed in the sensory evaluation of sausages elaborated with enzyme with regard to the control. However, an acceleration of the oxidation process after a period of storage under freezing conditions was found in the lipase added sausages. The analysis of volatile profiles by GC−MS showed that aldehydes, which basically originate by autoxidation of unsaturated fatty acids, were 43.59% for control sausage and 64.26, 65.65, and 59.99% for sausages with 0.150 LU/g, 0.300 LU/g, and 1 LU/g of lipase, respectively.

Keywords: *Palatase M; lipolysis; oxidation; aldehydes; volatile profiles*

INTRODUCTION

Aroma and distinctive flavors of fermented sausages are found to be related, at least in part, to the hydrolytic and oxidative changes occurring in the lipid fraction during ripening with production of carbonyl compounds (Demeyer et al., 1974). The acceleration of the hydrolytic process by the addition of microbial lipases is one of the strategies used to shorten the ripening of these products. Fernández et al. (1995), analyzing the effect of pancreatic lipase, concluded that an addition of 60 and 90 units of this enzyme was useful in enhancing flavor and reducing the ripening time. They also found a clear increase of all free fatty acids and carbonyl content, but inconsistent changes were observed in the short-chain fatty acid fraction (C6−C9).

This technology has also been extensively applied in the elaboration of fermented dairy products (Picón et al., 1997). Palatase M 200L has been successfully added to the manufacture of Kasar cheese (Kocak et al., 1996). These authors found that lipase-treated cheeses had higher sensory scores for flavor at the beginning of ripening but developed a rancid note after approximately 30 days.

Development of oxidative off-flavors has long been recognized as a serious problem during the holding or storage of meat products for subsequent consumption (Gray and Pearson, 1994). Brewer et al. (1992), examined changes in volatiles concentration in ground pork during long-term storage and reported an increase in hexanal and 2,4-decadienal during the initial stages (week 13) of storage and a decrease at latter stages (weeks 26 and 39). As a result of the oxidation of food lipids, a wide variety of volatile and nonvolatile compounds are synthesized which may result in rancid odor and taste. They also contribute to reduce the product's shelf life. These changes in quality prevent consumer acceptance of oxidized food products (Kochhar, 1993). Rancidity in uncooked meat is not normally apparent until it has been stored for several months (Gray and Pearson, 1994), but the use of lipolytic and proteolytic enzymes could increase the development of volatile compounds through an increment in the synthesis of precursors of these substances (García Regueiro et al., 1995).

In a previous work carried out in our department (Zalacain et al., 1997), the effect of the addition of 3 amounts (0.075, 0.1, and 0.150 LU/g, where 1 LU is the amount of enzyme that liberates 1 μmol of butyric acid/min from an emulsified tributyrin substrate at pH 7.0 and 30 °C) of Palatase on the ripening of dry fermented sausages was studied. At the assayed doses, this enzyme increased the acidity value as well as the concentration of FFA (except for linoleic), but no rancidity was observed by both chemical and sensory analysis. Although no significant differences were found in the sensory evaluation of odor intensity, acid taste, rancid taste, and overall acceptability, juiciness and pleasant taste were significantly better evaluated in the sausage with lipase than in the control. All these previous data showed that this enzyme could possibly have possitive sensorial effects at higher doses but also that it could give rise to problems related to an acceleration of rancidity.

In this paper, higher amounts of Palatase than those tested by Zalacain et al. (1997) were assayed to check if they had any additional effect over the lipolytic and oxidative processes and over the sensory properties related to them. Furthermore, the analysis of volatile compounds by GC−MS after a period of storage of the sausages under freezing conditions was carried out to

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Table 1. Values of TBA (mg of Malonic Aldehyde/kg of Dry Matter) and Free Fatty Acids (mg/100 g of Fat) Obtained for Control and Enzyme Added Sausages[a]

TBA	control, $2.79 \pm 0.19_a$	0.150 LU/g, $2.87 \pm 0.07_a$	0.300 LU/g, $4.07 \pm 0.22_c$	1 LU/g, $3.68 \pm 0.11_b$
myristic acid	$51.18 \pm 0.67_a$	$115.40 \pm 7.96_b$ (125.47)	$162.90 \pm 4.33_c$ (218.29)	$214.07 \pm 20.74_d$ (318.27)
palmitic acid	$766 \pm 17.46_a$	$1023.51 \pm 28.56_b$ (33.62)	$1596.79 \pm 65.05_c$ (108.46)	$2444.81 \pm 34.51_d$ (219.17)
palmitoleic acid	$96.17 \pm 2.76_a$	$134.19 \pm 2.31_b$ (39.53)	$228.38 \pm 15.01_c$ (137.47)	$344.78 \pm 8.69_d$ (258.51)
stearic acid	$420.89 \pm 15.38_a$	$535.19 \pm 1.74_b$ (27.16)	$810.66 \pm 70.02_c$ (92.61)	$1208.13 \pm 23.43_d$ (187.04)
oleic acid	$1715.82 \pm 38.11_a$	$2093.54 \pm 8.63_a$ (22.01)	$3396.61 \pm 199.17_a$ (97.96)	$5194.23 \pm 45.76_b$ (202.73)
linoleic acid	$1487.22 \pm 40.86_a$	$1595.06 \pm 10.33_a$ (7.25)	$2309.65 \pm 135.94_b$ (55.30)	$2760.22 \pm 22.77_c$ (88.59)
linolenic acid	$117.53 \pm 1.85_a$	$124.58 \pm 0.09_a$ (6.00)	$174.76 \pm 11.49_b$ (48.69)	$218.42 \pm 4.87_c$ (85.84)
total of FFA	4654.81	5621.47	8679.75	12384.66

[a] Values in parentheses are the increments (expressed in percentages) with regard to the control. In the same row, different letters denote significant differences ($p < 0.05$) between doses.

analyze differences in the compounds formed as a consequence of the oxidation process.

MATERIALS AND METHODS

Four different batches of sausages were elaborated in a pilot plant. One of them was the control sausage without enzyme, and the others were elaborated with different amounts of Palatase M 200L: 0.150, 0.300, and 1 LU/g. Palatase M 200L (Novo Nordisk A/S) is a fungal lipase produced through fermentation of a selected strain of *Rhizomucor miehei*. A mixture of *Lactobacilus plantarum* L115 (10%) and *Staphylocous carnosus* M72 (90%) from Lacto-Labo (TEXEL) was used as a starter and supplied at 10^6-10^7 cfu/g. All batches were elaborated with a standard formulation of lean pork meat, 75%; pork back fat, 25%; 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, 3 g/kg; Ponceau 4R (E124); 0.3 g/kg; and sodium ascorbate, 0.5 g/kg.

Lean pork meat and fat back pork were minced in a cutter with a particle size reduction to about 3 mm (this little particle size is a technological characteristic of Chorizo de Pamplona, a Spanish kind 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 four batches. The first one was named "control" and no enzyme was added to it. Amounts of 0.150, 0.300, and 1 LU of enzyme/g of mixture were, respectively, added to the rest of the batches. Two batches per type of sausage were elaborated. After the initial fermentation phase that took place in a laboratory ripening cabinet [24 h at 24 °C and saturation relative humidity (RH), 24 h at 22 °C and 85% RH, 24 h at 20 °C and 80% RH], sausages were transferred into a drying chamber to complete 20 days of ripening (14 °C and 77% RH). At the end of this time, all the products showed pH values included in the range of 5.04−5.10 and percentages of desiccation between 32.5 and 34.9%. Values of FFA, TBA, and sensory evaluation were determined in final product. Samples of each type of products (control and enzyme added sausages) were storaged during 1 year under freezing conditions (−20 °C). After this period, differences in the oxidation process were analyzed by the determination of volatile compounds.

Analytical Methods. Thiobarbituric acid value was determined according to Targladis et al. (1960, 1964). Lipid fraction was extracted from the sausages with a mixture of chloroform and metanol (2:1), according to Bligh and Dryer (1959). FFAs were determined as described by Zalacain et al. (1995) by shaking the lipid extract with an anion-exchange resin (Amberlyst A-26, Sigma Chemical Co.). The resin bound FFAs were methylated directly and the individual acids quantified using as an internal standard heptadecanoic acid by gas chromatography. Determination was carried out using a Perkin-Elmer model Sigma-300/dual FID (oven temperature, 185 °C; detector temperature, 240 °C; injector temperature, 250 °C).

Sensory Evaluation. Quantitative descriptive analysis (QDA) was carried out to compare sensory properties of control and enzyme-containing sausages. Four samples per batch were examined by 10 selected and trained panelists to judge rancid odor, acid odor, rancid taste, and acid taste. A continuous scale between 1 and 9 was used for evaluation. Control sausage was always taken as reference with a score of 5. Value 1.0 corresponded to the lowest intensity for each parameter and value 9.0 to the highest one.

Likens−Nickerson Extraction. A total of 25 g 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 [internal standard (i.s.)] was also attached to a modified Likens−Nickerson apparatus. A total of 5 mL of dichloromethane was also added to fill the apparatus solvent return loop. Both solvent and sample mixture were heated to 70 °C and boiling, respectively, mantaining these conditions during 2 h. After cooling to ambient temperature, the extract of dichloromethane was collected and dried over anhydrous Na_2SO_4. Three destilations per type of sausage were carried out.

Analysis of Volatile Compounds. The volatile compounds were analyzed in a 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 where it was held for another 5 min. Injector temperature, 250 °C; mass range, 30−350 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. Only known peaks are shown. Semiquantitative determination of the volatile compounds was based on the ratio of their peak areas to the peak of dodecane (internal standard), and the results were expresed as micrograms of dodecane per gram of dry matter.

Data Analysis. Data analysis was carried out with SPSS program. Values for FFA and numbers of TBA are the mean of eight determinations (two batches of product per type of sausage and four determinations per batch were carried out). Values for volatile profiles are the mean of six determinations (three destilations per type of sausage and two injections per destilation were carried out). ANOVA was used to determine significant differences ($p < 0.05$) between the four types of sausages for every studied parameter.

DISCUSSION

The addition of Palatase at the three tested doses caused a significant increment of the content of all free fatty acids analyzed in the enzyme added sausages with regard to the control (Table 1). The highest increments (expressed in percentages respect to the control) were observed for myristic, palmitoleic, and palmitic acids, whereas the increment for linoleic and linolenic acids was proportionally lower. Demeyer et al. (1974) found that the rate of lipolysis during dry fermented sausages

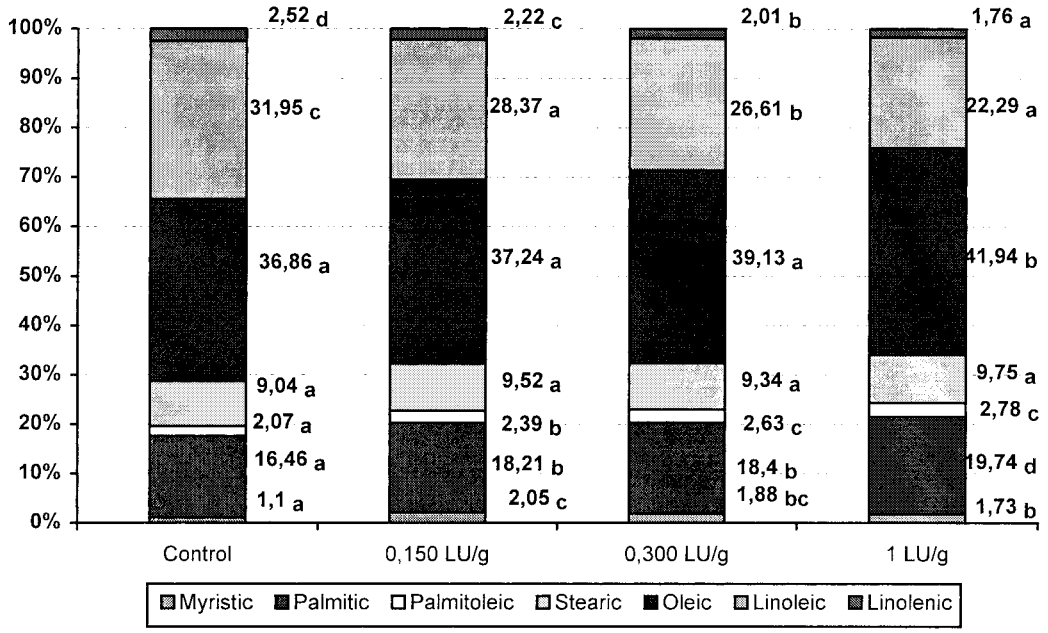

Figure 1. Percentages of each free fatty acid respect to the total content of FFA for control and sausages with enzyme. Different letters denote significant differences ($p < 0.05$) between doses for every fatty acid.

ripening decreased in the order linoleic > oleic > stearic > palmitic acids as a consequence of both positional and structural specificity. Zalacain et al. (1997) using Palatase M did not find an increment in the linoleic release in relation to the control. Fernández et al. (1995) also found that the addition of pancreatic lipase to dry fermented sausages did not lead to an increment of linoleic acid, which may be related to a specificity of the lipase for the fatty acid structure or to the degradation of free linoleic acid by oxidative processes. Figure 1 shows the proportions of each fatty acid with respect to the total content of FFA for control and enzyme added sausages. It can be observed that percentages of linolenic and, especially, linoleic acids decreased as the doses of lipase increased. Whereas the percentage of these two acids for the control sausage was 34.47%, values for 0.150, 0.300, and 1 LU/g were, respectively, 30.59, 28.62, and 24.05%. This lower proportion of polyunsaturated FFA could be explained by the acceleration of the oxidation process caused by the addition of the enzyme.

The oxidation process was evaluated by the TBA analysis. No correlation was found between the TBA values or intensity of the oxidation process and the amount of the enzyme added (Table 1). TBA for the 0.150 LU/g batch did not show significant differences with control, and TBA for 1 LU/g was significantly lower than that of 0.300 LU/g. These results suggested that higher doses of Palatase M did not lead to a higher degree of oxidation during the ripening of the products. Furthermore, no rancid odor or taste were appreciated by panelists in the sensory evaluation of none of the products (Figure 2). Both acid taste and acid odor were not affected by the addition of the enzyme, despite the higher concentration of free fatty acids observed in the enzymes added sausages. These sensory properties have been related to the presence of shorter chain fatty acids (Girard and Bucharles, 1991).

Oxidation process takes place basically in the FFA fractions. Profiles of volatile compounds were obtained by GC−MS after a period of storing sausages under freezing conditions to analyze possible differences in the

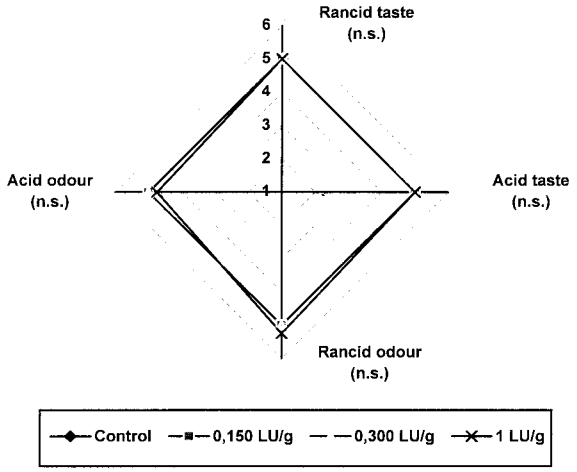

Figure 2. Sensory evaluation: results of QDA carried out in the control sausage and in the sausages with enzyme.

intensity of oxidation process as a consequence of the higher FFA concentration originated by the addition of the enzyme. Table 2 shows a strong increment in the total amount of volatile compounds between the modified sausages and the control. The autoxidation of unsaturated fatty acids leads to many decomposition compounds, which include aliphatic aldehydes, that are the most important volatile breakdown products because they are major contributors to unpleasant odors and flavors in food products (Kochhar, 1993). Content of aldehydes, expressed in micrograms of dodecane, were 46.56 μg for control sausage and 110.58, 136.56, and 187.41 μg for sausages with 0.150, 0.300, and 1 LU/g of enzyme, which represent 43.59, 64.26, 65.65, and 59.99% of the total amount of volatile compounds, respectively.

Johansson et al. (1994), studying the volatile compounds of fermented sausages with different starter cultures, found that approximately 0.65% of the total area was aldehydes derived from lipid oxidation. Stahnke et al. (1994) found that the 11.31% of the total amount of volatile compounds of sausages with *Staphylococcus xylosus* was aldehydes. The higher content of aldehydes in the analyzed samples showed that all of

Table 2. Profiles of Volatile Compounds Obtained by SDE and GC−MS Analysis (Results Expressed in Micrograms of Dodecane per Gram of Dry Matter)

	control		0.150 LU/g		0.300 LU/g		1 LU/g		
	mean[a]	s.d.	mean[a]	s.d.	mean[a]	s.d.	mean[a]	s.d.	I^b
toluene			0.087_a	0.004			0.106_b	0.002	b
1-pentanol			0.329_a	0.012	0.445_b	0.028	0.567_c	0.020	b
octane	0.785_a	0.076	2.288_b	0.099	3.332_c	0.234	4.316_d	0.116	b
hexanal	6.311_a	0.174	18.171_b	0.795	24.007_c	0.672	39.067_d	2.109	a
furanocarboxaldehyde	0.310_a	0.041	0.291_a	0.004	0.394_b	0.015	0.466_c	0.019	b
t-2-hexenal			0.334_a	0.021	0.434_b	0.030	0.667_c	0.026	b
xylene	0.182_c	0.018	0.096_a	0.014	0.126_b	0.017	0.084_a	0.004	b
hexanol							0.252	0.024	b
5-metil-2-hexanone							0.393	0.014	b
nonane							0.227	0.029	b
n-heptanal	0.346_a	0.008	0.698_b	0.027	1.061_c	0.069	1.396_d	0.035	b
metional	0.265_c	0.011	0.136_b	0.013	0.256_c	0.013	0.100_a	0.003	b
disulfide	0.164	0.006							b
2−4-hexadienal							0.093	0.015	b
alpha-pinene	0.235_c	0.011	0.107_a	0.014	0.190_b	0.032	0.230_b	0.024	b
etanone			0.159_a	0.004	0.262_b	0.031	0.376_c	0.019	b
2-heptenal	0.756_a	0.047	1.811_b	0.079	2.301_c	0.103	3.181_d	0.143	b
beta-pinene	0.641_c	0.014	0.371_a	0.006	0.354_a	0.023	0.441_b	0.016	b
1-octen-3-ol	0.246_a	0.028	0.418_b	0.009	0.561_c	0.040	0.997_d	0.014	b
2,3-octanedione			0.384_a	0.021	0.427_a	0.070	0.585_b	0.023	b
furan-2-pentyl	1.026_a	0.056	2.860_b	0.103	3.588_c	0.217	8.616_d	0.150	b
2,4-heptadienal			0.933_a	0.025	1.045_a	0.057	1.430_b	0.133	b
n-octanal			0.713_a	0.031	0.630_a	0.096	0.946_b	0.112	a
delta-3-carene	0.378_b	0.053	0.594_c	0.052	0.251_a	0.005	0.257_a	0.028	b
t,t-2,4-heptadienal	0.361_a	0.023	1.611_b	0.128	1.659_b	0.119	1.790_b	0.110	b
hexanoic acid							0.784	0.033	a
p-cymene			0.150_a	0.005			0.168_a	0.015	b
limonene	0.936_b	0.032	1.185_c	0.015	0.827_a	0.041	0.920_b	0.075	a
benceneacetaldehyde	1.671_a	0.128	2.235_b	0.039	2.821_c	0.042	2.968_c	0.151	b
2-octenal	0.776_a	0.018	1.987_b	0.130	2.660_c	0.115	4.081_d	0.112	b
phenol d5			0.553_a	0.016			1.167_b	0.081	b
3,5-octadien-2-one			0.248_a	0.011	0.315_b	0.009	0.507_c	0.033	b
disulfide di-2-propenyl	1.218_c	0.037	0.523_a	0.025	0.679_b	0.059	0.624_b	0.050	b
tetramethylpyrazine	0.390_c	0.019	0.236_a	0.007	0.283_b	0.010	0.225_a	0.024	b
guaiacol	0.501_d	0.008	0.246_b	0.008	0.311_c	0.015	0.126_a	0.021	b
linalool	0.221_c	0.021	0.156_a	0.018	0.193_b	0.005	0.276_d	0.010	a
nonanal	1.089_a	0.033	2.062_b	0.103	2.792_c	0.158	3.880_d	0.066	b
octanoic acid methyl ester					0.171_a	0.005	0.195_a	0.019	b
t-2-nonenal	0.577_a	0.021	1.619_b	0.085	2.040_c	0.159	3.276_d	0.163	b
octanoic acid	1.811_a	0.361	5.037_b	0.155	5.044_b	0.537	13.138_c	0.147	a
t,t-nona-2,4-dienal	0.420_a	0.006	1.240_b	0.082	1.627_b	0.041	3.671_c	0.485	b
2-decenal	1.313_a	0.012	4.722_b	0.094	6.105_c	0.479	9.082_d	0.185	b
t,t-2,4-decadienal	5.278_a	0.084	19.349_b	0.376	23.270_c	0.541	32.226_d	1.433	b
2,4-decadienal	12.880_a	0.059	37.316_b	0.308	46.034_c	2.009	57.797_d	1.412	b
3-dodecen-1-al	1.317_a	0.029	4.260_b	0.081	5.833_c	0.477	8.150_d	0.151	b
decanoic acid methyl ester			0.200_a	0.013	0.235_b	0.018	0.361_c	0.022	a
decanoic acid	4.659_a	0.516	6.001_b	0.118	5.858_b	0.373	10.067_c	0.164	a
decanoic acid ethyl ester	0.348_a	0.030	0.417_b	0.003	0.532_c	0.023	0.613_d	0.035	b
beta-cariophyllen	0.197_c	0.027	0.122_{ab}	0.003	0.145_b	0.010	0.100_a	0.013	b
2,4-undecadienal			0.194_a	0.011	0.254_b	0.012	0.371_c	0.030	b
geranyl acetone	0.247_{ab}	0.031	0.204_a	0.031	0.301_{bc}	0.027	0.348_c	0.035	b
beta-ionone	0.241_a	0.013	0.253_a	0.003	0.349_c	0.023	0.295_b	0.037	b
decanoic acid propyl ester			0.206_a	0.002	0.224_a	0.021	0.340_b	0.017	b
pentadecane			0.348_a	0.012	0.437_b	0.024	0.484_c	0.021	b
dodecanoic methyl ester			0.128_a	0.011	0.154_b	0.006	0.181_c	0.014	a
2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl			0.145_b	0.007	0.171_a	0.007			b
dodecanoic acid	1.607_a	0.103	1.520_a	0.075	1.611_a	0.085	2.444_b	0.170	a
1-dodecen-1-yne			0.508_a	0.054			0.742_b	0.075	b
dodecanoic ethyl ester			0.398_b	0.018			0.293_a	0.021	b
2,4-dodecadienal							0.282	0.013	b
heptadecene			0.087_a	0.006	0.108_b	0.012	0.202_c	0.011	b
2-pentadecanone	0.764_c	0.026	0.486_a	0.007	0.600_b	0.018	0.488_a	0.007	b
tetradecanoic acid methyl ester	0.123_a	0.008	0.169	0.001	0.175_b	0.007	0.448_c	0.031	a
tetradecanoic acid	4.675_b	0.270	4.158_{ab}	0.105	3.628_a	0.268	8.604_c	0.496	a
tetradecanoic acid ethyl ester	0.247_a	0.034	0.620_b	0.022	0.840_c	0.141	2.155_d	0.098	b
hexadecanal	10.161_c	0.152	6.459_a	0.081	8.607_b	0.691	8.850_b	0.270	b
hexadecanoic acid methyl ester	0.388_a	0.055	0.572_b	0.029	0.449_a	0.045	1.001_c	0.067	a
9-hexadecenoic acid	1.605_a	0.282	2.514_b	0.270	1.961_a	0.148	4.936_c	0.192	a
hexadecanoic acid	13.533_b	1.300	11.934_b	0.218	8.659_a	1.105	26.694_c	2.836	a
hexadecanoic acid ethyl ester	1.013_a	0.110	1.410_b	0.138	1.428_b	0.078	3.403_c	0.271	b
9-octadecenal	1.757_c	0.043	1.195_a	0.074	1.590_b	0.025	1.427_b	0.223	b
octadecanal	1.543_a	0.072	1.639_a	0.066	1.787_a	0.254	2.309_b	0.077	b
linoleic acid methyl ester	0.776_b	0.073	0.517_a	0.091	0.398_a	0.015	1.339_c	0.233	a
oleic acid methyl ester	0.739_b	0.069	0.648_b	0.032	0.359_a	0.043	1.461_c	0.144	a
linoleic+oleic acids	18.030_b	1.852	11.419_a	0.424	22.909_c	3.720	17.318_b	0.785	a
linoleic acid ethyl ester	1.077_b	0.188	0.863_{ab}	0.088	0.749_a	0.087	1.753_c	0.200	b
oleic acid ethyl ester	0.435_a	0.009	1.156_b	0.111	1.139_b	0.025	2.869_c	0.270	b
total	106.795		172.099		208.468		312.373		

[a] Within a row, different letters denote significant differences ($p < 0.05$) between batches. [b] *I*, identification methods. a, mass spectrum and retention time identical to those of an authentic sample. b, mass spectrum compared with Wiley data base.

them have suffered a great oxidation process. Furthermore, the increments observed in the enzyme added sausages showed that the oxidation process had taken place with major intensity in these products. The most abundant aldehydes, which were also the ones that suffered the highest increments with regard to the control, were 2,4-decadienal, hexanal and *t,t*-2,4-decadienal. All of them originated from linoleic acid. The 2,4-decadienals have oily, fatty, deep-fat frying odors, and hexanal has been found to smell of tallow, fat, or oil (Berdagué et al., 1991). The last has been used to monitor lipid oxidation during the storage of meat (Ajuyah et al., 1993). Concentration of high molecular weight aldehydes did not increase with the dose of enzyme, and in some cases, they even decreased. Dirinck et al. (1997) pointed out that these high molecular weight aldehydes could act as precursors of the volatile alkanals and alkenals, and also that, due to their lower volatility, their direct contribution to ham aroma should be of less importance.

No data about the effect of lipases on the volatile profile of dry fermented sausages have been found in the bibliography. Hagen et al. (1996) studied the effect of the NCDO151 proteinase on the concentration of volatile compounds of sausages. They found variation in 9 of the 45 compounds detected, but none of them were aldehydes.

Content of medium chain fatty acids were specially increased in sausages elaborated with the highest dose of enzyme (1 LU/g). They play a significant role in the desirable aroma as well as in the off-flavors of many foodstuffs, and they are produced during the autoxidation of higher fatty acids and their glyceride esters (Kochhar, 1993). Other non-carbonyl compounds originated from oxidation were found: furan-2-pentyl (from autoxidation of linoleic acid, and with buttery smell) and 1-octen-3-ol (smells to mushroom).

Sensory analysis after storage of sausages was not carried out, since off-flavors were easily detected and it could have been unpleasant for panelists. Furthermore, potential toxicity could have been originated by the great oxidation.

It can be concluded that the use of the lipase Palatase M at the three tested doses caused modifications in the FFA profiles of sausages which were not reflected in sensorial properties in the final product. However, by analysis of the volatile profiles by GC−MS of the products stored under freezing conditions, an increment could be detected in the content of aldehydes of enzyme added sausages respect to the control that could mean an acceleration of the further oxidation process in these products.

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LITERATURE CITED

Ajuyah, A. O.; Fenton, T. W.; Hardin, R. T.; Sim, J. S. Measuring lipid oxidation volatiles in meats. *J. Food Sci.* **1993**, *58* (2), 270−273, 277.

Berdagué,J. L.; Denoyer, C.; Le Quéré,J. L.; Semon, E. Volatile components of dry-cured ham. *J. Agric. Food Chem*. **1991**, *39*, 1257−1261.

Bligh, E. G.; Dryer, W. J. A rapid method of total lipid extraction and purification. *Can J. Biochem. Physiol.* **1959**, *37*, 911.

Brewer, M. S.; Ikins, W. G.; Harbers, C. A. Z. TBA values, sensory characteristics, and volatiles in ground pork during long-term frozen storage: Effects of packaging. *J. Food Sci.* **1992**, *57, 558.*

Demeyer, D.; Hoozee, J.; Mesdom, H. Specificity of lipolysis during dry sausage ripening. *J. Food Sci.* **1974**, *39*, 293−296.

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.

Girard, J. P.; Bucharles, C. La acidificación. In *Tecnología de la carne y los productos cárnicos*; Girard, J. P., Ed.; Acribia, SA: Zaragoza, Spain, 1991.

Gray, J. I.; Pearson, A. M. Lipid-derived off-flavours in meat-formation and inhibition. In *Flavour of Meat and Meat Products*; Shahidi, F., Ed.; Blackie Academic and Profesional: London. 1994.

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.

Johansson, G.; Berdagué, J. L.; Larsson, M.; Tran, N.; Borch, E. Lipolysis, proteolysis and formation of volatile components during ripening of a fermented sausage with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter cultures. *Meat Sci.* **1994**, *38*, 203−218.

Kocak, C.; Bitlis, A.; Gursel, A.; Avsar, Y. K. Effect of added fungal lipase on the ripening of Kasar cheese. *Milchwissenschaft* **1996**, *51* (1),13−17.

Kochhar, S. P. Oxidative pathways to the formation of off-flavours. In *Food Taints and Off-Flavours*; Saxby, M. J., Ed.; Blackie Academic and Profesional: London, 1993.

Picón, A.; Medina, M.; Gaya, P. Maduración acelerada del queso. Aplicación en quesos Españoles. *Rev. Esp. Lecheria* **1997**, *82*, 36−44.

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

Targladis, B. G.; Pearson, A. M.; Dugan, L. L., Jr. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. *J. Sci. Food Agric.* **1964**.

Targladis, B. G.; Watts, B. M.; Younathan, M. T.; Dugan, L. R., Jr. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. A. Oil Chem. Soc.* **1960**, *37*, 44−48.

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.* **1997**, *45* (1), 99−105.

Zalacain, I.; Zapelena, M. J.; Astiasarán, I.; Bello, J. Dry fermented sausages elaborated with lipase from *Candida cylindracea*. Comparison with traditional formulations. *Meat Sci.* **1995**, *40*, 55−61.

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