Contribution of Microbial and Meat Endogenous Enzymes to the Lipolysis of Dry Fermented Sausages

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The role of the starter culture and meat endogenous enzymes in the lipolysis of dry fermented sausages has been studied. Five batches of sausages were manufactured. The control batch was made with aseptic ingredients without microbial inoculation. The other four experimental batches were manufactured with the aseptic ingredients inoculated with *Lactobacillus plantarum* 4045, *Staphylococcus* sp., *L. plantarum* 4045 and *Micrococcus*-12, or *L. plantarum* 4045 and *Staphylococcus* sp. Their effects on pH, a_w, long chain free fatty acid and short chain fatty acid contents and compositions were studied. Sausages inoculated with *L. plantarum* 4045 had the lowest pH as a result of carbohydrate fermentation. However, release of free fatty acids was greater in the inoculated sausages than in the aseptic one. Lipolysis, measured as total content of long chain free fatty acids, occurred not only in the inoculated batches but also in the control, which could suggest that the meat endogenous lipases play an important role in this process. Lipolysis produced an increased release of linoleic and oleic acids. Changes were observed in the short chain fatty acid fraction, but acetic acid was always the major fatty acid.

Keywords: *Dry fermented sausages; ripening; lipolysis; starter cultures; endogenous enzymes*

INTRODUCTION

A large number of biochemical reactions affecting carbohydrates, proteins, and lipids as main substrates occur during the fermentation and ripening of dry fermented sausages. Fermentation of carbohydrates is due to lactic acid bacteria yielding mainly lactic acid, accumulation of which causes a drop in pH to values approaching the isoelectric point of meat proteins. Proteolytic phenomena bring about the partial fragmentation of proteins and lead to the release of nonproteic nitrogen compounds which affect the pH and flavor. Lipids are the major component of dry fermented sausages, ranging from 25% to 55% of crude matter. Lipids are affected by lipolytic and oxidative phenomena which result in the release of fatty acids, carbonyl compounds, and other low molecular weight substances which are very important to the flavor of the final product (Cantoni et al., 1966; Demeyer et al., 1974).

The microbial flora that are established and that participate in the phenomena of fermentation and ripening of sausages proceed from the environment and the equipment used in the manufacture of products made using more traditional methods and from starter cultures in sausage products produced using more modern technology. It is not an easy task to determine the participation of the different bacterial groups in the reactions associated with ripening since the microbial flora are diverse and the effects of the different ingredients and also those produced by the different oxidative reactions must be taken into account. In dry fermented sausages, lipolytic activities have been attributed mainly to microbial lipases (Demeyer et al., 1974; Palumbo and Smith, 1977). Selgas et al. (1986) showed the ability of the genus *Micrococcus* to hydrolyze triglycerides from long chain fatty acids, the type most abundant in meat products, although this ability was dependent on the species studied. Lactobacilli also produce both intra- and extracellular lipases. The activity of these substances was first demonstrated in lactobacilli isolated from cheese (Stadhouders, 1974) and, later, in bacteria isolated from sausages (Sanz et al., 1988), with similar results. The substrates preferentially attacked by the extracellular lipases of these bacteria are monoglycerides, diglycerides, and triglycerides with short chain fatty acids whereas intracellular enzymes have very little effect on triglycerides. No activity was observed in either case on triglycerides with fatty acids of more than six carbon atoms. On the other hand, some authors (Wallach, 1968; Ferrer and Arboix, 1986) have demonstrated the existence of significant lipolytic activity at the beginning of fermentation due to lipases of the muscular and adipose tissue. García et al. (1992) observed a similar increase of free fatty acids in the batches of dry fermented sausages inoculated with lactobacilli and/or micrococci as that observed in those produced by an aseptic method. This implies that the release of fatty acids could, at least partially, be due to the endogenous lipases of the meat. This occurs in other meat products such as cured hams (Hayman and Acton, 1978; Paleari et al., 1991; Motilva et al., 1993a,b), in which lipolytic activity has been attributed to endogenous enzymes since only low levels are reached by microorganisms inside the ham (Francisco et al., 1981). In conclusion, the role played by endogenous lipolytic enzymes and those from microorganisms in the lipolytic reactions occurring during ripening of dry fermented sausages has not yet been clarified. The present work is an attempt to determine the source of these enzymes during the ripening of dry fermented sausage.

MATERIALS AND METHODS

Preparation of Sausages. Bovine *M. semitendinosus*, porcine *M. longissimus dorsi*, and lard (6−8 cm thickness) from Iberian pig were aseptically obtained as described by Ordóñez et al. (1989). The muscle exterior surface was sterilized by searing. Then, the burnt tissues were removed down to a

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depth of about 3 mm using sterile instruments. Likewise, the skin of the Iberian pig lard (thickness around 10 cm) was burnt and flakes of internal fat (from a deep of about 5 cm) were removed. Solutions of glucose and lactose were sterilized by filtering (0.45 mm); phosphates, NaCl, nitrates, and nitrites were autoclaved and then concentrated by lyophilization. All operations of sausage manufacture were performed in a laminar flow cabinet (Telstar model CE A), and the operators used sterile surgery masks and gloves. The meat and fat were comminuted in a manual grinder (diameter of the plate holes, 5 mm) previously sterilized by autoclaving. The ingredients were aseptically mixed to give the final composition (%): pork, 40; beef, 40; fat, 13; glucose, 1.5; lactose 0.5; phosphates, 0.3; NaCl, 2.5; KNO_3, 0.02; $NaNO_2$, 0.01; sterilized water was used to dissolve the lyophilized ingredients.

The following batches were prepared.

Batch C: control (sterile ingredients and aseptic manipulation without inoculation).

Batch L: As batch C, but inoculated with *L. plantarum* 4045.

Batch S: Inoculated with an "in vitro" lipolytic strain of genus *Staphylococcus*, kindly given by Dr. M. L. García from Faculty of Veterinary Sciece (León, Spain).

Batch L+M: Inoculated with *L. plantarum* 4045 and *Micrococcus*-12, an "in vitro" lipolytic strain, both isolated from Spanish dry fermented sausages (Sanz et al., 1988; Selgas et al., 1988).

Batch L+S: Inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

BHI (Brain Heart Infusion) and MRS (de Man, Rogosa, Sharpe) broths (Oxoid, Unipath Ltd., Basingstoke, Hampshire, U.K.) were used, respectively, for *Micrococcaceae* and lactobacilli revitalization. One mL of a bacterial suspension (absorbance at 600 nm = 1.0, ca. 10^7 cfu/mL) washed twice and resuspended in sterile physiological saline solution was inoculated into the corresponding batch. The final mixture was then aseptically stuffed into artificial casing soaked (48 h) in sterile 15% (w/v) saline solution and washed just before use in plenty of sterile distilled water. After this treatment the bacterial load of the casing was lower than 10^2 cfu/cm^2. The casings were, likewise, aseptically stuffed with a manual grinder in which the cut plate was replaced by an appropriate sterile funnel. The weight of each batch was about 2.5 kg distributed in 10 sausages of 250 g. They were ripened in a laboratory ripening cabinet (Kowell model CC3AFY) programmed for 48 h at 22 °C and a relative humidity (RH) of 90%, followed by 48 days at 12 °C and 85% RH. Samples were taken at different times of ripening.

Microbial Analyses. Total viable counts (TVC) were determined on PCA (plate count agar) (Oxoid) and *Micrococcaceae* on MSA (mannitol salt agar) (Oxoid), both incubated at 32 °C for 2 days. Lactobacilli were enumerated on double-layer MRS agar (Oxoid) at pH 5.6 and incubated for 4 days at 32 °C.

Chemical Analyses. Measurement of pH was done in a homogenate prepared with an aliquot of sausage (1.5 g) and distilled water (10 mL), using a Crison 2001 pH meter (Crison Instruments S.A., Barcelona). Dry matter was determined by drying at 110 °C to constant weight. A Decagon CX1 dew point hygrometer (Decagon Devices Inc., Pullman, WA) was used to measure the water activity (a_w) at 25 °C.

Lipids were extracted and purified according to the method of Hanson and Olley (1963). Total lipids were gravimetrically determined. All lipid extracts were kept at −20 °C until analysis. The free fatty acid content on the lipid extract was determined by thin-layer chromatography (TLC). In order to do this, an aliquot of the lipid extract was dissolved in chloroform (200 mg/mL) and applied to a 0.25 mm silica gel G-60 plates (Merck, Darmstadt). Plates were developed with petroleum ether/diethyl ether/acetic acid (80/20/1) (v/v/v). A spray of 0.05% $FeCl_3 \cdot 6H_2O$ solution in a mixture of water/acetic acid/sulfuric acid (90/5/5) (v/v/v) (Lowry, 1968), followed by heating in an oven at 120 °C for 20 min, was used to visualize all lipid fractions. Oleic acid (Sigma Chemical Co., St. Louis, MO) was used as reference standard. Fatty acids were quantified by densitometry in a Shimadzu CS-9000 densitometer (Shimadzu Corporation, Kyoto) at 390 nm using the calibration curve for the standard employed. To analyze the fatty acid composition of the free fatty acid (FFA) fraction, an aliquot (1 g) of the lipid extract was dissolved in 2.5 mL of a 95% ethanol/diethyl ether (1/1) (v/v) mixture, and the sodium salts were formed with 5 N NaOH. These salts were extracted from the mixture by washing twice with chloroform/water (1/1) (v/v) and centrifuging at 1500*g* for 10 min in a Sorvall RC-5B centrifuge (Du Pont Instruments, Newtown, CT). The aqueous phase was saturated with NaCl and acidified with HCl to pH 2. The FFA were then extracted by washing with diethyl ether and taken to dryness in a rotatory evaporator. The FFA methyl esters were formed as described by Schlenk and Gellerman (1960) and analyzed with a Konik KNK-3000-HRGC gas chromatograph (Konik Instruments S.A., Barcelona) equipped with a J&W Scientific capillary collum (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) coated with DB-225 on fused silica. Analysis was performed using an initial isothermic period (150 °C, 2 min); thereafter the temperature was increased to 210 °C at a rate of 4 °C/min, and finally an isothermic period (210 °C, 15 min) was established. The fatty acids were identified by comparison with the retention times of authentic standards (Sigma) and were quantified by peak area measurement.

For short chain fatty acid determination, samples of sausage (15 g) were homogenized with 45 mL of distilled water. As internal standard, a 0.6% (w/v) of hexanoic acid aqueous solution was added. The homogenate was centrifuged at 10000*g* for 10 min, and the resulting pellet was re-extracted with an additional volume of water (20 mL) and recentrifuged. The two supernatants were combined, and the volume was recorded. Proteins were precipitated, and sodium salts were formed by adding 25% $ZnSO_4$ and 5 N NaOH (final pH of 10) followed by heating in a boiling water bath. To remove protein precipitate, the mixture was filtered through Whatman No. 54 paper. The filtrate was lyophilized, redissolved in a small volume of distilled water, and acidified with 5 N HCl to pH 2 for extraction into ethyl ether. Immediately after extraction, ethyl ether extracts were analyzed on a Konik KNK-3000-HRGC gas chromatograph equipped with a Phase Sep semi-capillary column (30 m × 0.53 mm) (Alltech Associates, Milford, MA) packed with DB-FFAP (1 mm) on fused silica. Analysis was performed under isothermal conditions (120 °C). Short chain fatty acid identification was made by comparing the retention times with those of authentic standards (BDH) and quantified by peak area measurement against the normalized internal standard.

Due to the complexity of the aseptic sausage elaboration, results are only the mean of two different experiments carried out with different ingredients but the same formulation and starters. Microbial and chemical analyses were done in duplicate.

RESULTS AND DISCUSSION

Microbial Flora. The microbial changes throughout ripening are shown in Figure 1.

In the control batch (C), sausages produced aseptically (Figure 1C), the counts on day 0 of total flora and lactic and micrococcaceae bacteria were less than 10^3 cfu/g and after the ripening stage were close to 10^4 cfu/g and even lower. The maximum total flora count was almost 10^5 cfu/g representing almost 3−4 logarithmic units less than the value reached in conventional sausages (Selgas et al., 1988; Domínguez et al., 1989) and in inoculated batches.

The total flora counts in batch L (Figure 1L) and the lactobacilli counts were similar to those recorded for conventional sausages (Sanz et al., 1988; Selgas et al., 1988; Samelis et al., 1993). Counts started at around 10^7 cfu/g in both cases and increased rapidly during the first few days of ripening (corresponding to the fermen-

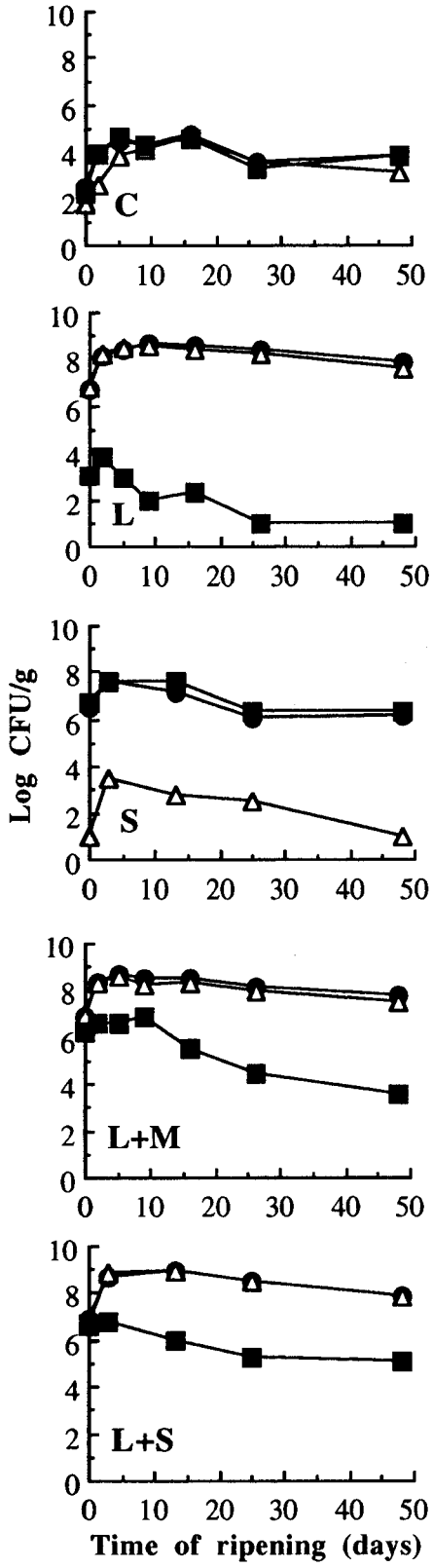

Figure 1. Changes in total viable (●), lactobacilli (△), and *Micrococcaceae* (■) counts during the ripening of experimental dry fermented sausages. (C) Control, aseptic batch without inoculation; (L) aseptic batch inoculated with *Lactobacillus plantarum* 4045; (S) aseptic batch inoculated with *Staphylococcus* sp.; (L+M) aseptic batch inoculated with *L. plantarum* 4045 and *Micrococcus*-12; (L+S) aseptic batch inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

tation stage). leveling off toward the end of the process. On the contrary, microorganisms cultivated in the MSA medium reached maximum levels of around 10^4 cfu/g toward the second day gradually decreasing to negligible values which were in all cases lower than those recorded in conventional sausages. In the latter, initial levels of micrococcaceae bacteria were similar to the lactobacilli and increased moderately during the first few days of ripening, leveling off at around 10^6 cfu/g (Sanz et al., 1988; Selgas et al., 1988; Domínguez et al., 1989).

Batch S inoculated with *Staphylococcus* sp. (Figure 1S) reached maximum levels from 2−6 days of around 10^8 cfu/g, clearly higher than the levels of Micrococcaceae recorded in conventional sausages, in which the maximum values recorded did not exceed 10^6 cfu/g. However, clearly the absence of microbiotic competition, from lactobacilli, which cause a decreased pH in normal conditions, led to increased staphylococcus growth. The maximum lactobacilli count was less than 10^4 cfu/g, 4 logarithmic units less than that recorded during the ripening process in conventional sausages (Lücke, 1987; Sanz et al., 1988).

In batches L+M and L+S inoculated with lactobacilli and *Micrococcaceae* (Figure 1, L+M and L+S), the changes observed for the different microbial groups corresponded with the results described for conventional sausages by several authors (Palumbo and Smith, 1977; Selgas et al., 1986; Sanz et al., 1988). This behavior was only to be expected since these two batches were the only ones to contain the usual flora.

Due to microbial activities, the experimental sausages were of two different colors. Those related to *Micrococcaceae* (S, L+M, and L+S) were a typical deep pink color reflecting the reduction of nitrates to nitrites and the formation of the nitrosylmyoglobin. The other two sausage batches (C and L), without inoculation of *Micrococcaceae*, were a brown color indicating no nitrate and nitrite reduction. The absence of the pink color in batches C and L means that the contaminating microbial population was not large enough to affect sausage ripening and that its effects were probably negligible.

pH. The pH of the mixture of meat, lard, and other ingredients to be used for sausage manufacture (day 0) was similar in all cases (around 5.9). After casing, two clearly different trends were observed depending on the microorganism inoculated. On the one hand, after the initial value of 5.9, the pH of batches inoculated with lactobacilli (L, L+M, and L+S) decreased sharply (until 5.2) during the first 10 days of the process. After this time they leveled off and even began to increase slightly until the end of the ripening period, achieving final values around 5.4. These results are within the wide interval of values recorded for this type of product that ranges from 4.5, cited by Genigeorgis et al. (1986) for American salami and also by Sanz et al. (1988) for Spanish sausages, to values greater than 6.0 in Italian and Hungarian salamis (Graner et al., 1983; Incze, 1987; Nagy et al., 1989) and in Vich "salchichón" (Ferrer and Arboix, 1986). These differences mainly depend on the type and amount of sugar added, on the kind of microbial flora, and on the ripening conditions, etc. (Burgos, 1981). The other trend in pH was that observed for batches C and S. The lactobacilli count in these batches remained around 10^4 cfu/g or less and was not able to produce the usual decrease in pH.

Moisture, Water Activity (a_w), and Fat Content. The water content after sausage manufacture was about 63% of the fresh product and decreased gradually and constantly throughout ripening, reaching a final value of around 25−30%. Similar final values to those recorded in this work were also described by Incze

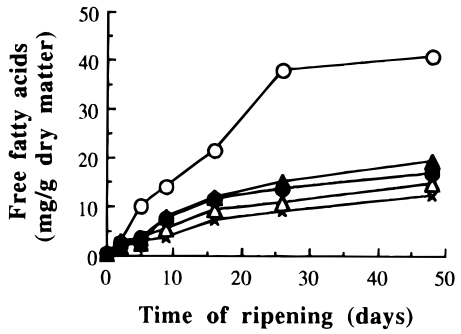

Figure 2. Changes in the free fatty acid content during the ripening of experimental dry fermented sausages. (×) control, aseptic batch without inoculation; (△) aseptic batch inoculated with *L. plantarum* 4045; (○) aseptic batch inoculated with *Staphylococcus* sp.; (▲) aseptic batch inoculated with *L. plantarum* 4045 and *Micrococcus*-12; (●) aseptic batch inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

(1987) and Astiasarán et al. (1990) in different sausages. The a_w (0.97 at day 0 of ripening) decreased concomitantly with loss in water content, reaching final values of 0.85−0.86 which are normal for sausages of this type (Palumbo et al., 1976; Ziegler et al., 1987).

All sausages showed a similar fat content of about 53% of dry matter. Similar results have been reported (Samelis et al., 1993) in different dry fermented sausages.

Free Fatty Acids (FFA). Figure 2 shows the changes in the total FFA content during ripening. In all batches an increase of these substances was observed throughout the ripening process. The lowest value at the end of the ripening period was reached in the control batch (12.0 mg/g DM), followed by batches L (14.7 mg/g DM), L+S (16.9 mg/g DM), and L+M (19.3 mg/g DM) although no important differences were found between these batches, e.g. the value recorded in batch L+M was only about 1.6-fold higher than that recorded in batch C. However, the final value reached by the batch inoculated with only staphylococci (40.7 mg/g DM) was clearly higher (3-fold) than that of the control batch at the end of ripening.

These are very interesting results because they determine the source of lipolytic activity in dry fermented sausages. Both *Micrococcaceae* strains were selected "in vitro" according to their lipolytic activity. Therefore, one could expect this activity to be reflected in the sausages, when these were used as starter cultures. However, this was only the case when the staphylococci was inoculated alone but not when either staphylococci or micrococci were inoculated together with lactobacilli (batches L+S and L+M). In the first case, staphylococci reached levels close to 10^8 cfu/g and the pH 5.9 while both this parameter (5.1−5.3) and the *Micrococcaceae* load (10^6−10^4 cfu/g) were lower in the other two batches. One may, therefore, deduce that in conventional batches, the lipolytic activity from *Micrococcaceae* is not completely developed. This may be due to the increase in acidity produced by *Lactobacilli* growth, which inhibits the growth of *Micrococcaceae* or their lipolytic activity.

On the other hand, when both lactobacilli and *Micrococcaceae* were inoculated, about 70% of lipolytic activity, calculated from final values of FFA recorded in the two batches (L+S and L+M) and those of the control batch, is due to meat tissue lipases, confirming previous claims by other authors for both dry fermented sausages (García et al., 1992; Montel et al., 1993; Molly et al., 1996) and dry ham (Motilva et al., 1993a,b). It can be also concluded that it is not necessary to make any extra effort, as many authors do, to isolate *Micrococcaceae* strains on the basis of their lipolytic activity to be used as starter cultures because the primary lipolysis is provided by endogenous enzymes. Therefore, the main role of *Micrococcaceae* is that of the reduction of nitrates and nitrites, which does not interfere with these bacteria collaborating in some biochemical phenomena, other than primary lipolysis, occurring throughout the ripening.

Table 1. Changes in the Long Chain Free Fatty Acids (wt %) during the Ripening of Experimental Dry Fermented Sausages

		fatty acid					
batch[a]	day	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2
C	0	1.77	28.51	1.67	16.56	43.53	7.57
	5	1.55	24.41	1.74	14.36	49.53	8.05
	26	1.58	27.20	1.67	13.96	46.54	8.58
	48	1.40	19.70	1.82	13.29	52.48	10.87
L	0	1.77	28.51	1.67	16.56	43.53	7.57
	5	1.80	26.25	1.85	16.21	45.36	8.37
	26	1.44	23.58	1.80	14.12	50.03	8.64
	48	1.81	26.87	1.98	13.62	46.34	8.95
S	0	1.77	28.51	1.67	16.56	43.53	7.57
	5	1.63	28.46	1.41	14.26	47.01	7.15
	26	1.52	26.15	1.58	14.85	45.96	9.87
	48	1.91	24.91	1.87	15.76	45.87	9.61
L+M	0	1.77	28.51	1.67	16.56	43.53	7.57
	5	2.54	27.00	1.58	16.97	44.42	7.16
	26	1.71	25.21	1.70	13.94	48.22	8.81
	48	1.54	24.25	1.78	14.30	48.24	9.45
L+S	0	1.77	28.51	1.67	16.56	43.53	7.57
	5	1.71	28.01	1.77	14.85	45.79	7.22
	26	2.01	27.14	1.92	12.87	48.74	7.21
	48	1.82	26.92	1.79	14.41	45.22	9.73

[a] C, control, aseptic batch without inoculation; L, aseptic batch inoculated with *L. plantarum* 4045; S, aseptic batch inoculated with *Staphylococcus* sp.; L+M, aseptic batch inoculated with *L. plantarum* 4045 and *Micrococcus*-12; L+S, aseptic batch inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

To analyze the individual fatty acids released during ripening, only fatty acids comprising more than 1% were considered. These were myristic (C-14:0), palmitic (C-16:0), palmitoleic (C-16:1), stearic (C-18:0), oleic (C-18:1), and linoleic (C-18:2). The relative concentrations of these fatty acids at the beginning of the experiment (day 0), the end of the fermentation phase (day 5), and in the middle and at the end of the ripening phase (days 26 and 48) are shown in Table 1. Data from days 2, 9, and 16 of ripening are not shown because in all cases values were similar to those shown in Table 1. Oleic acid was the predominant fatty acid present in all the samples analyzed, present at levels between 42% and 55%, followed by palmitic acid (20−30%), stearic acid (10−17%), and linoleic acid (7−11%). Palmitoleic and myristic acids were the fatty acids present in the smallest proportion reaching values lower than 3%. These results are similar to those recorded by other authors (Domínguez and Zumalacárregui, 1991; Astiasarán et al., 1993; Beriain et al., 1993) in different kinds of dry fermented sausages.

Individual analysis of the changes of the content of the different fatty acids (Table 1) reveals that the relative proportion of oleic acid increases in all batches, reaching, in most cases, maximum values between days 9 and 16 (data not shown). After this time percentages decreased although not as low as initial values. This decrease in the percentage of oleic acid toward the end

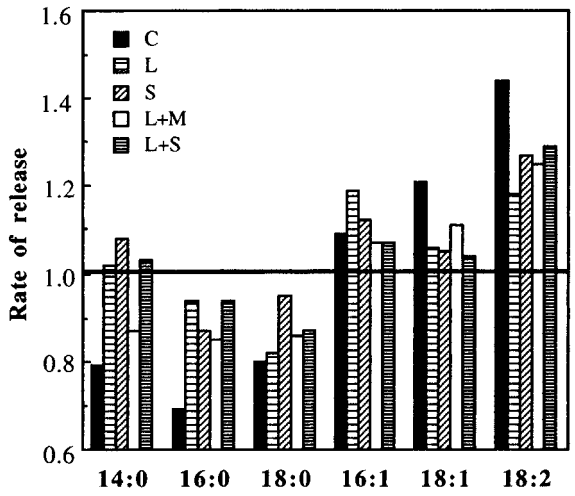

Figure 3. Rate of release of the individual fatty acids from experimental dry fermented sausages. (C) control, aseptic batch without inoculation; (L) aseptic batch inoculated with *L. plantarum* 4045; (S) aseptic batch inoculated with *Staphylococcus* sp.; (L+M) aseptic batch inoculated with *L. plantarum* 4045 and *Micrococcus*-12; (L+S) aseptic batch inoculated with *L. plantarum* 4045 and *Staphylococcus* sp.

of ripening has also been observed by other authors (Cerise et al., 1973; Domínguez and Zumalacárregui, 1991; Astiasarán et al., 1993). In general, the percentage of palmitic acid decreases throughout ripening. This decrease has also been observed in salami by Cerise et al. (1973) and Paleari Bianchi et al. (1985) and in "chorizo" by Domínguez and Zumalacárregui (1991) and Astiasarán et al. (1993). These decreases are concomitant with the increases in others fatty acid percentages mainly the linoleic acid, in which a trend to increase was observed along ripening. Furthermore, oleic acid may be also subjected to autooxidative phenomenom, which may give rise to an additional decrease. Linoleic acid is also involved in this process but, in this case, it may be higher the ratio liberation/oxidation. The trends observed in the two dominant fatty acids (oleic and palmitic) are in accordance with the results obtained in a study on the action of a *Micrococcus* sp. strain on pig fat by Debevere et al. (1976), who also observed an increase in oleic acid and a decrease in palmitic acid with increasing time with which the microorganism was in contact with the fat. No clear tendency was observed with respect to changes in the relative proportions of stearic acid. Nevertheless, this unusual behavior was also observed by other authors (Astiasarán et al., 1993).

Figure 3 shows the rate of release of each individual fatty acid. Values show the ratio of the percentage of each fatty acid on the day 48 to that on day 0 of ripening (i.e., % free 18:2 at 48th day of ripening/% free 18:2 at day 0 of ripening). Values higher than 1.0 indicate a higher release rate producing an increase of the percentage of fatty acid given in Table 1. When the values of Figure 3 are compared, a hierarchy appears for the release of fatty acids with 18:2 > 18:1 ~ 16:1 > 14:0 > 16:0 ~ 18:0. In general, the inoculated batches showed a similar rate of fatty acid release but the aseptic one was clearly different with a higher release of 18:2 and 18:1. This fact indicates that both microbial and meat endogenous enzymes preferentially hydrolyze the outer fatty acid of the triglyceride molecule as previously described (Alford et al., 1971; Kilara, 1985) or have a preference for the polar lipid fraction (mainly phospholipids) since it has higher levels of polyunsaturated fatty acids than the apolar one (Foegeding et al., 1996). Positions *sn*1 and *sn*3 are the most frequently occupied by unsaturated fatty acids; nearly 30% of the dominant octadecenoic acids (oleic and linoleic) are esterified in the *sn*1 position and about 50−60% of the same fatty acids appear in *sn*3 (Christie and Moore, 1970). The higher C-18:2 and C-18:1 release rate in the aseptic batch probably indicates that meat enzymes have a greater specificity for the *sn*3 position or polar lipids than microbial enzymes. Similar results as those recorded in the experimental batches have been reported by Molly et al. (1996). These authors showed that polyunsaturated fatty acids are released from the polar lipid fraction, and their specific release is higher than for monounsaturated and saturated fatty acids although it seems that the pattern of lipolysis is similar when endogenous and microbial effects are compared.

Short Chain Fatty Acids. The levels of short chain fatty acids generated during the ripening period were low. This is a consequence of the lack of adequate substrate since meat and adipose tissue glycerides are esterified to long chain fatty acids (Leseigneur-Meynier and Gandemer, 1991).

Acetic acid was the major short chain fatty acid. After an initial acetic acid content of about 10 mg/100 g DM a sharp increase (reaching about 30 mg/100 g DM) was observed in batches inoculated with lactobacilli during the fermentation phase (day 5). It can, therefore, be deduced, as many authors have previously claimed (Dainty et al., 1979; Kandler, 1983; Demeyer et al., 1986), that acetic acid is formed mainly by carbohydrate fermentation. A decrease (until 8−10 mg/100 g DM) in the level of acetic acid was recorded in these batches probably due to its participation in other chemical reactions. On the other hand, when lactobacilli were not inoculated (batches C and S) there was no change in the acetic acid content throughout the ripening process, which may suggest that the microorganism load was not high enough to ferment carbohydrates or was unable to do it. Under anaerobic growth conditions staphylococci may ferment glucose producing lactic acid as the main product and acetate and pyruvate in lower amounts (Kloos et al., 1992) which could explain the small amount of acetic acid found in batch S.

The level of *n*-butyric acid decreased constantly during ripening from about 1.3 to 0.5 mg/100 g DM in all batches. Dainty et al. (1979) observed that, in systems in which lactic acid bacteria develop great activity, such as vacuum-packed meats, accumulation of *n*-butyric acid is more closely associated with the lipases from meat than with microbial activity. In dry fermented sausages *n*-butyric acid is, therefore, probably formed from the activity of meat endogenous lipases on short chain fatty acid glycerides during the fermentation phase when pH and temperature conditions are optimum. Because the appropiate substrates are present in very low amounts (Leseigneur-Meynier and Gandemer, 1991), no more *n*-butyric acid is generated along ripening and, in contrast with vacuum-packed meats, the initial levels progressively decrease, which may be due to a migration, together with the water, to the surface during the dehydration occurring along ripening. Once on the sausage surface the *n*-butyric acid may be either volatilized or used by molds growing in the surface.

With respect to analysis of the remaining short chain fatty acids no consistent patterns were obtained. Propionic acid ranged from 0.22 to 0.65, isobutyric from 0.03

to 0.26, *n*-valeric from 0.01 to 0.21, and isovaleric from 0.15 to 1.0 mg/100 g DM.

Conclusion. Results of the present study seem to indicate that lipolysis from meat endogenous enzymes in dry fermented sausages accounts for more than 60% of total free fatty acid release with a hierarchy of the rate of fatty acid release: 18:2 > 18:1 ~ 16:1 > 14:0 > 16:0 ~ 18:0. When lipolysis is due to endogenous enzymes, the rate of release of C-18:2 and C-18:1 is higher than that produced by microbial enzymes.

ABBREVIATIONS USED

a_w, water activity; BHI, brain heart infusion broth; DM, dry matter; FFA, free fatty acids; MRS, de Man, Rogosa, Sharpe broth or agar; MSA, mannitol salt agar; PCA, plate count agar; RH, relative humidity, TLC, thin-layer chromatography; TVC, total viable counts.

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