Effect of the addition of pancreatic lipase on the lipolysis during the ripening of dry fermented sausages.

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SUMMARY

The effect of the addition of pancreatic lipase at two levels (40 and 60 units) on the lipolysis of dry fermented sausages has been studied. In the lipase-added batches, the level of total free fatty acids was higher (about 3-fold) than that of the control. At the end of the ripening, the individual free fatty acids (C-14:0, C-16:0, C-16:1, C-18:0, C-18:1, C-18:2 and C-18:3) of the lipase batches reached higher values (2.1 to 5.37-fold) than those of the control. Organolleptically, no differences were observed between lipase batches and the control. It seems to be necessary to add a higher concentration of enzyme.

INTRODUCTION

During the ripening of dry fermented sausages, the product losses 20-40 % of their original weight and lipids and proteins are attacked by enzymes from meat and bacteria. It is in this step when the product adquires the characteristic texture and flavour. The specific flavour of a given dry sausage is due to many compounds. Some of them are added to the sausages with the ingredients (e.g. spices), other are formed without the participation of microorganisms (e.g. by autooxidation reactions) and other are generated as a result of microbial activities on carbohydrates, lipids and proteins.

The acceleration of lipolysis by the addition of either animal or microbial lipases has been succesfully applied to the relative strong-flavoured cheeses (Law, 1984), such as some Italian hard cheeses (Moskowitz, 1980) or blue cheeses (Jolly and Kosikowski, 1975). This treatment has the combined effect of increasing the typical fatty acid flavour and catalysing more rapid liberation of precursors of short-chain flavour compounds (Law, 1984). It has been also reported (Demeyer et al., 1974) that insaturated free fatty acids are more easily breakdown than in the esterified form by autooxidation processes. Therefore, it has been hypothetized that the addition of lipases to dry sausage would yield a higher concentration of free fatty acids than in conventional sausages, which will be available for further transformations. The result would be a higher accumulation of flavouring compounds. This approach has not been proved in dry sausages.The investigations carried out on this respect are reported in the present work.

MATERIALS AND METHODS

Samples and sampling. Two batches of dry fermented sausages were made by adding 40 and 60 units of pancreatic lipase (Sigma). One unit is defined as the quantity of lipase that hydrolizes 1 mequivalent of fatty acid from olive oil in 1 hour at 37ºC and pH of 7.7. As control, an additional batch without adding enzyme was manufactured . The initial sausage mixture contained (% w/w): pork (55), beef (12), lard (25), glucose, lactose and dextrine (3.5), NaCl (2.5%), nitrates and nitrites (0.15), ascorbate (0.046), glutamate (0.25), black pepper (0.14). Sausages were ripened in a KOWELL model CC-3-1cabinet. Fermentation was carried out at 22ºC and 90% of relative humidity (RH) for 20 hours. Thereafter, temperature and RH were gradually decreased to reach 12ºC and 70% RH in 10 days. These conditions were kept until the end of the ripening (14 days). Samples were taken at different times.

Analysis. For monitoring the microbial changes, total viable organisms (TVC), lactobacilli and Micrococcaceae were enumerated on Plate Count Agar (Oxoid), on double layer MRS (Oxoid) at pH 5.7 and MSA (Oxoid) media, respectively. A Crison model Digit - 501 pHmeter was used to measure the pH. Water activity (a_w) was measured with a Decagon CX1 apparatus. Dry matter was determined according to the AOAC (24.002) method.

Lipids were extracted as described by Hanson and Olley (1963) from a homogenate of sausages. Separation of lipids was performed by thin layer chromatography (TLC) on 0.25 mm silica gel G-60 plates developed with petroleum ether/diethyl ether/acetic acid (80/20/1) (v/v/v). Triolein, diolein, monolein, oleic acid and cholesterol (all from Sigma) were used as reference standards. A solution of 0.05% $FeCl_3 \cdot 6\,H_2O$ in a mixture of water/acetic acid/sulfuric acid (90/5/5) (v/v/v) (Lowry, 1968) was used to visualize all lipid classes, and to assist in the identification of the different apolar lipids the Dudzinski (1962), Schiff-periodate (Shaw, 1968) and Lowry (1968) reagents were employed. The apolar lipids were determined by densitometry in a Shimadzu model CS-9000 densitometer, using the same standards than those employed in TLC analysis.

Free fatty acids (FFA) were analyzed by gas-liquid chromatography (GLC). From a sample of the lipid extract dissolved in 95% ethanol the sodium salts (pH = 10.0) were formed with methanolic NaOH (0.1 N). The sodium salts were extracted from the mixture by washing twice with chloroform/water (1/1) (v/v). The aqueous phase was saturated with NaCl, the pH brought to 2.0 and the FFA extracted with diethyl ether. FFA were taken to dryness in a rotatory evaporator. FFA methyl esters were formed as described by Schlenk and Gellerman (1960). Fatty acid methyl esters were analyzed with a Perkin-Elmer model GC 8420 chromatograph equipped with a capillar column (30m x 0.25 mmi.d.) J. & W. Scientific packed with DB-225 on fused silica. The analysis were performed according to a program in which an initial isotherm period (180ºC, 2 min.) was established and thereafter by using a temperature gradient from 180ºC to 220ºC at an increasing rate of 4ºC/min., remaining at 220ºC for an additional 15 min. period. The identification of different fatty acid methyl esters was made by comparison with authentic standards (Sigma).

The sensory analysis was carried out with a tasting pannel (18 members) which performed two tests: a triangle test, according to the I.S.O. TC 34/sc 12 standard and a preference test, by evaluating the different qualities between batches .

RESULTS AND DISCUSSION

Fig. 1 shows the microbial, a_w and pH changes during the ripening of the control batch. The lipase-added batches showed a similar pattern (data not shown). The changes of the flora during ripening was quite similar to those observed in other sausages (Palumbo and Smith, 1977; Sanz et al., 1988; Selgas et al., 1988) i. e., the number of colonies developed on MSA medium stabilized between 10^6-10^7 c.f.u./g and lactic acid bacteria increase rapidly during the first days after which numbers of these organisms remain constant at levels of 10^8-10^9 c.f.u./g. The pH and the a_w progresively decreased to achieve steady values of 5.1 and 0.89, respectively. The behaviour of these parameters was similar to that observed by other authors (Sanz et al., 1988).

TLC revealed the presence of 8-9 spots in the lipid extracts obtained from all batches. According to their Rfs and behaviour with general and specific reagents, six of them were characterized as monoglycerides (Rf=0.01), diglycerides (Rf=0.12), free cholesterol (Rf=0.17), free fatty acids (Rf=0.26), triglycerides (Rf=0.80) and hydrocarbons plus cholesteryl esters (Rf=0.96). These substances are the same as those reported in meat from several animals (Chang-Haan and Yeon-Hee, 1982). Two or three spots with Rfs amid those of the FFA and cholesterol were not identified. In this TLC system, the movility of monoglycerides was very low and, therefore, they were quantified together with polar lipids, which remained in the origin.

During ripening of sausages, no clear changes in the levels of triglycerides were observed in the control batch but a trend to decrease was found in the lipase-added batches (Fig. 2), which was higher in the batch manufactured with 60 lipase units . However, a clear increase of mono- (it was assumed that phospholipids content do not change during ripening) and diglycerides (Fig. 3) and all FFA (Fig. 4 and 5) was observed in all batches. As expected, this increase was higher in the lipase-added batches. Data from 40 lipase units batch are not shown but the FFA changes during ripening showed a similar pattern. The differences affected to the levels of FFA, which were higher in the 60 lipase units batch.

From Fig. 4 and 5 it may be deduced that the C-18:1 was the fatty acid reaching the highest concentrations. It accounted for 35-40% of the total FFA at the end of the ripening although the highest increase, in relation with the control batch, was observed in the C-14:0 (4-fold and 5.37-fold for the 40 and 60 lipase units batches, respectively). The increases observed for the other FFA are shown in the table 1.

TABLA 1. Concentration of different free fatty acids (mg/100g D.M.) in experimental sausages after 14 days of ripening.

Fatty acid	Batches			Ratio of	
	Control	40 units	60 units	40/Control	60/Control
C-14:0	11.0	44.0	59.1	4.00	5.37
C-16:0	152.2	442.3	442.7	2.90	2.91
C-16:1	27.2	81.4	95.8	2.99	3.52
C-18:0	75.5	217.4	250.7	2.88	3.32
C-18:1	250.4	816.1	903.6	3.26	3.61
C-18:2	169.5	356.0	405.6	2.10	2.39
C-18:3	40.0	87.5	117.4	2.18	2.93
Total FFA	725.8	2044.7	2274.9	2.82	3.13

At the end of the ripening, the total FFA reached (g/100g D.M.) concentrations of 0.72 (control), 2.05 (40 units) and 2.28 (60 units). The values of the control batch were similar than those reported by other authors in different types of fermented sausages (Cerise et al., 1973; Bianchi et al., 1985 and Domínguez and Zumalacárregui, 1991). Despite of the higher concentrations of total FFA observed in lipase-added batches in relation to the control one, the values reached in lipase-added batches were also similar to those reported in other Spanish (Domínguez and Zumalacárregui, 1991) and Hungarian (Nagy et al., 1989) dry sausages. Therefore, the effect of lipase was not as efficient as expected. The sensory analysis support this result since no significative differences were observed between the control and the lipase-added batches.

In conclusion, it seems to be necessary to add higher quantities of lipase than those used in this work. To check the effectiveness of the lipase to enhance the flavour of sausages it is also necessary to study the carbonyls formation from free fatty acids. Researchs are now being realized.

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Fig. 1

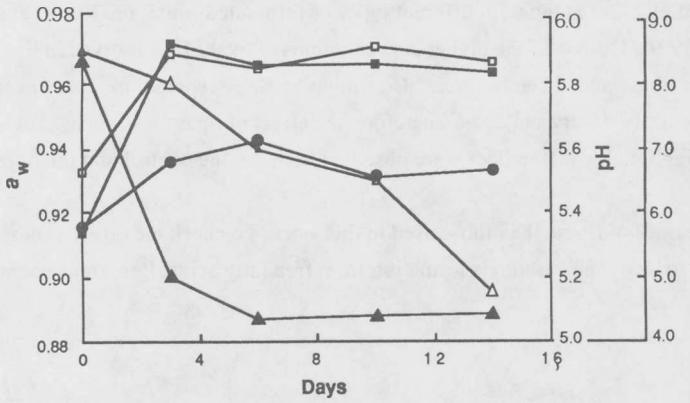


Fig. 1: Changes in microbial flora (□) total viable count; (■) lactobacilli and(●) Micrococcaceae, water activity (△) and pH (▲) during the ripening of the control batch.

Fig. 2: Changes in triglycerides during the ripening of dry fermented sausages added of 40 units (▲) and 60 units (■) of pancreatic lipase and without lipase (□).

Fig. 3: Changes in mono- (dotted lines) and diglycerides (full lines) during the ripening of dry fermented sausages added of 40 units (circles) and 60 units (triangles) of pancreatic lipase and without lipase (squares).

Fig. 2

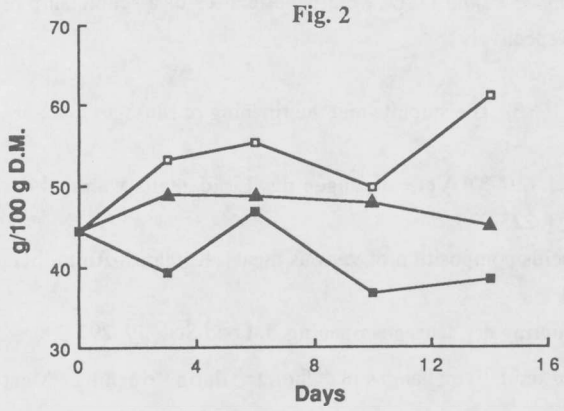
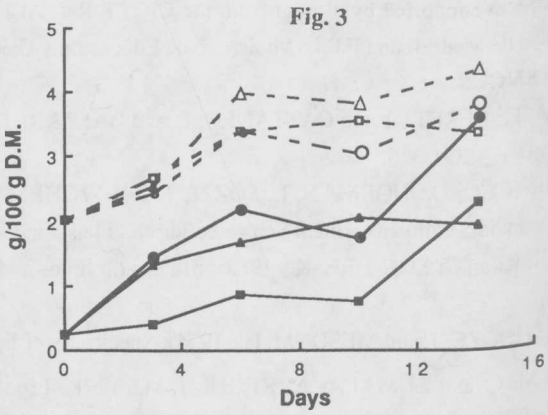


Fig. 3



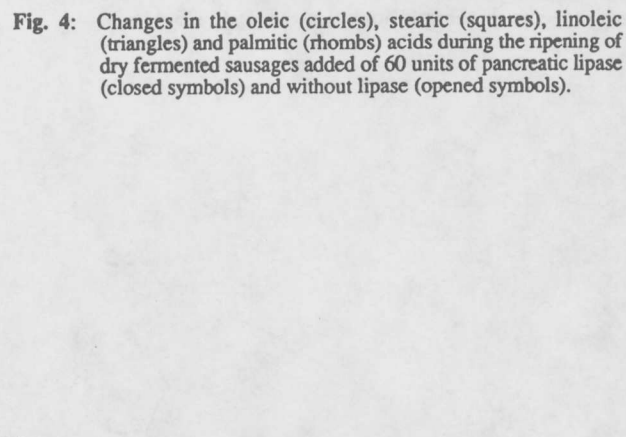


Fig. 4: Changes in the oleic (circles), stearic (squares), linoleic (triangles) and palmitic (rhombs) acids during the ripening of dry fermented sausages added of 60 units of pancreatic lipase (closed symbols) and without lipase (opened symbols).

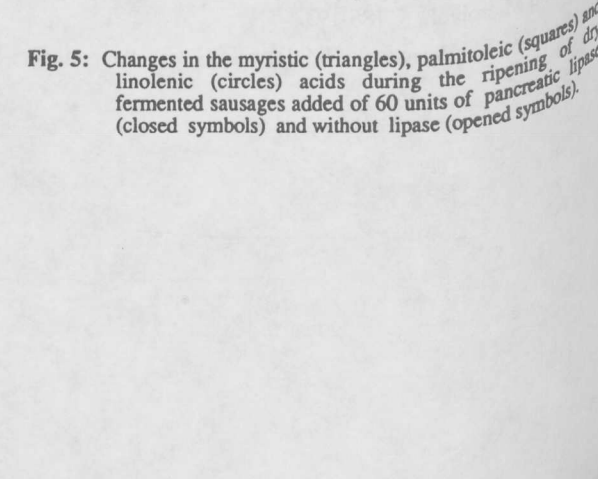


Fig. 5: Changes in the myristic (triangles), palmitoleic (squares) and linolenic (circles) acids during the ripening of dry fermented sausages added of 60 units of pancreatic lipase (closed symbols) and without lipase (opened symbols).