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Study of the shelf life of a dry fermented sausage "salchichon" made from raw material enriched in monounsaturated and polyunsaturated fatty acids and stored under modified atmospheres

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Abstract

The microbiological, physico-chemical and sensory properties of salchichon with high unsaturated fat content, packed under vacuum and 20/80% CO_2/N_2 modified atmosphere, were evaluated to determine its quality changes during storage under refrigeration. These sausages were manufactured with pork meat and pork backfat obtained from pigs fed with three different diets (control diet-CO, high oleic diet-HO and high linoleic diet-HL). In general, few significant differences were found in counts of different groups of microorganism between the three types of sausages and no difference between the packaging methods. A reduction in pH values was observed during storage and no great differences were determined by storage period on water activity (a_w). Both parameters (pH and a_w) presented similar results to those found in different Spanish sausages and other European dry fermented products. The sensory results denoted that sensory quality gradually decreased during storage under both packaging conditions (vacuum and 20/80% CO_2/N_2), so it is not advisable to store longer than 150 days. On the other hand, fermented sausages with high content of unsaturated fatty acids had similar sensory properties to those of conventional sausages, and even a comparable sensory stability. In conclusion, the results showed healthier salchichons (HO and HL) similar to the traditional (CO) one could be manufactured and stored under refrigeration after slicing for a reasonable period, but the advantage of the gas mixture packaging (20/80% CO_2/N_2) versus vacuum packaging was not clear.

Keywords: Vacuum packaging; Gas packaging; Fermented sausage; Salchichon; Fatty acid composition; Instrumental texture; Shelf life

1. Introduction

Salchichon is one of the most important dry-cured sausages produced in Spain. The basic ingredients of salchichon are lean pork, pork backfat, salt and spices. After manufacturing, the salchichon is subjected to a preservation treatment with the aim of increasing its shelf life and avoiding problems such as contamination, decolouration and rancidity. Nowadays, due to recent changes in shopping and consuming habits, packaged sliced meat products are an important retail selling method. Therefore, the problem of safe preservation in the meat industry has become more complex as today's products require longer shelf life and greater assurance of protection from microbial spoilage.

Moreover, pork meat has often been blamed for being too high in fat, especially in saturated fat. Saturated fatty acids are well known to raise total and low-density lipoprotein (LDL) cholesterol. Changes in meat fat composition, mainly a reduction of saturated fatty acids together with an increase of mono and polyunsaturated fatty acids, have been persistently recommended by nutritionists for the positive effects that such changes appear to have on the serum balance

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between low and high density lipoproteins and on blood cholesterol levels. The interest in improving the nutritional quality of food by means of manipulation of the fatty acid composition of animal feeds has also been used to improve the fatty acid profile of carcass fat in pigs (Morgan, Noble, Cocchi, & McCartney, 1992; Vanoeckel & Boucque, 1992) and of meat products (Bosi et al., 2000; Hoz, D'Arrigo, Cambero, & Ordóñez, 2004). Besides, it is possible to increase the proportion of unsaturated fats by incorporation of vegetable oils into the meat product (Bloukas, Paneras, & Fournitzis, 1996; Muguerza, Gimeno, Ansorena, Bloukas, & Astiasarán, 2001). However, a relatively highly unsaturated fat in dry fermented sausages can increase lipid oxidation process. In order to prevent it, the sausages would need to be packed under anaerobic conditions. Therefore, industry tries to extend the shelf life of this type of products by storage practices such as modified atmospheres packaging (vacuum and gas mixtures packaging).

Microbiological, chemical and sensory characteristics of typical Mediterranean dry sausages have been extensively studied (Baldini et al., 2000; Comi et al., 2005; Coppola, Mauriello, Aponte, Moschetti, & Villani, 2000; Drosinos et al., 2005; González & Díez, 2002; Lizaso, Chasco, & Beriain, 1999; Moretti et al., 2004; Ruiz Pérez-Cacho, Galán-Soldevilla, León Crespo, & Molina Recio, 2005). Also, studies on the effect of packaging conditions on these characteristics have been carried out (Fernández-Fernández, Rozas-Barrero, Romero-Rodríguez, & Vázquez-Odériz, 1997; Fernández-Fernández, Romero-Rodríguez, & Vázquez-Odériz, 2001; Fernández-Fernández, Vázquez-Odériz, & Romero-Rodríguez, 2002; Yen, Brown, Dick, & Acton, 1988; Zanardi, Dorigoni, Badiani, & Chizzolini, 2002). Moreover, all recent publications about the modification of sausage lipid composition are primarily focused on the nutritional and chemical aspects throughout production and ripening of these meat products (Bryhni, Kjos, Ofstad, & Hunt, 2002; Muguerza, Fista, Ansorena, Astiasarán, & Bloukas, 2002; Severini, De Pilli, & Baiano, 2003; Valencia, Ansorena, & Astiasarán, 2006a; Warnants, Van Oeckel, & Boucqué, 1998) and a limited number of papers have dealt with the link between fatty acid modifications and product shelf life (Ansorena & Astiasarán, 2004; Harms, Fuhrmann, Nowak, Wenzel, & Sallmann, 2003; Sheard et al., 2000; Valencia, Ansorena, & Astiasarán, 2006b).

The aim of the present work was to determine the influence of a high content of unsaturated fatty acids on salchichon characteristics and to establish the advisable period of storage under refrigeration when the sausage was sliced and packed under vacuum and 20% CO_2/80% N_2 modified atmosphere for retail sale.

2. Materials and methods

2.1. Raw material

The raw meat was obtained from pigs growing in the "Centro de Pruebas de Porcino" (Instituto Tecnológico Agrario de Castilla y León, Hontalbilla, Spain). Pigs (Large White × Pietrain) × (Large White × Landrace) were fed with a conventional pig diet from 19 to 70 kg live weight. Then, pigs were divided into three groups for providing the experimental diets. The three diets were formulated with the same ingredients except for the fat source:

(1) Control (CO): consisted of maize, barley, wheat, and soybean principally.
(2) High oleic (HO): consisted of maize, barley, wheat, soybean and sunflower oil.
(3) High linoleic (HL): consisted of maize, barley, wheat, soybean and soya oil.

The ingredients, chemical composition and fatty acid profile of these experimental diets are shown in Table 1 (data provided by "Centro de Pruebas de Porcino"). All pigs were fed *ad libitum* with the experimental diets. Animals, having reached a live weight of 125 kg, were stunned and slaughtered at a local slaughterhouse. The meat and backfat were obtained from carcases, after chilling overnight at 1 ± 1 °C. The backfat and the meat from pigs fed with different diets were used to prepare each batch of sausage. Raw material (lean and fat) used for the three batches (CO, HO and HL) was stored separately at −20 °C until they were used for making sausages.

2.2. Sausage formulation and processing

All the sausages were manufactured the same day, using the same technology and according to a traditional formulation, which consisted of 75% pork meat and 25% pork backfat. Lean pork meat and pork backfat were minced (P-32 FUERPLA, Valencia, Spain) to a particle size of about 8 mm and subsequently mixed in a vacuum mixer (A-85 FUERPLA, Valencia, Spain) with the following common ingredients per kilogram of meat mixture: 25 g sodium chloride, 5 g dextrose, 4 g white wine, 3 g ground black pepper, 1.5 g sucrose, 1 g GDL (Glucono D-Lactone), 1 g polyphosphates, 1 g ground white pepper, 1 g nutmeg, 0.45 g sodium ascorbate, 0.15 g sodium nitrite, 0.10 g potassium nitrate. This sausage mixture was stuffed into natural casings (62–65 mm ∅). The sausages were fermented in a drying chamber (Hermekit, Cenfrio, Spain) at 15 °C and 90–100% relative humidity (RH) for 18 h, 22–23 °C and 90% RH for 48 h, at 14–15 °C and 80–90% RH for 10 days. Then the RH was slowly reduced to 75% until the end of the ripening process (a total of 28 days). At that time, sausages were packed and stored at 6 °C as indicated below.

2.3. Packaging and storage of samples

Manufactured sausages, two pieces randomly selected for each batch, were sliced at 1 mm thickness and 100 g of slices were placed in polystyrene trays. Besides, two slices of salchichon, 1.5 cm thick were put on trays to carry out

Table 1
Ingredients, calculated metabolizable energy and chemical composition of the three experimental diets: control (CO), high oleic (HO) and high linoleic (HL)

	Control diet (CO)	High oleic diet (HO)	High linoleic diet (HL)
Ingredients (%)			
Maize	20	20	20
Barley	27.8	23.8	23.8
Wheat	25.7	25.7	25.7
Soybean	23.3	23.3	23.3
Soya oil	–	–	4
Sunflower oil	–	4	–
Sodium chloride	0.3	0.3	0.3
Calcium carbonate	0.9	0.9	0.9
Bicalcium phosphate	1.4	1.4	1.4
L-Lisine	0.18	0.18	0.18
Calculated energy (kcal/kg)	2214.74	2442.54	2450.94
Chemical analysis			
Dry matter (%)	88.5	88.1	89
Crude protein (%DM)	17.9	17.7	18.6
Crude fat (%DM)	2	5.4	5.5
Crude fiber (%DM)	4	3.3	3.3
α-Tocopherol (μg/g)	9.62	9.89	8.02
Fatty acid composition (%)			
16:0 (palmitic)	13.84	8.59	12.42
18:0 (stearic)	5.01	3.33	3.52
18:1 (oleic)	27.39	54.96	22.01
18:2 (linoleic)	44.98	28.49	52.95
18:3 (linolenic)	4.87	1.69	5.90

instrumental texture measurements. Then, the trays were packed under:

(a) Vacuum: trays were packed with plastic bags (polyamide/polyethylene with an oxygen transmission rate of 30–40 $cm^3/m^2/24$ h/bar at 23 °C and 50% RH and a water vapour transmission rate of 2.5 $g/m^2/24$ h at 23 °C and 50% RH, supplied by WK Thomas España S.L., Rubí, Spain) which were subjected to vacuum and sealed using a packer (mod. EVT-7-TD Tecnotrip, Barcelona, Spain).
(b) Gas mixture: trays were evacuated and flushed with a selected gas mixture, 20/80% CO_2/N_2, (Carburos Metálicos S.A., Barcelona, Spain). Then, they were closed by heat-sealing with a packer (mod. TSB-100 Tecnotrip, Barcelona, Spain) with a high barrier film (with an oxygen transmission rate of 5 $cm^3/m^2/24$ h/bar at 23 °C and 50% RH and a water vapour transmission rate of 19 $g/m^2/24$ h at 23 °C and 90% RH supplied by BEMIS EUROPE, L'Hospitalet de Llobregat, Spain). Packages had a headspace volume ratio of 1:1 (MØller et al., 2003). The gas content of each pack and the residual O_2 were controlled using a gas analyzer1450 B3 Servomex (Aries, Madrid, Spain).

All samples were stored at 6 °C until their analysis. Samples of all treatments were analysed at 0, 15, 30, 60, 90, 150 and 210 days of storage.

The whole experiment was replicated twice.

2.4. Determination of fatty acids

Fatty acids were determined on the lipid extract from ripened sausages. The Bligh and Dyer (1959) method was used for the lipid extraction. The fatty acid composition was determined by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (Morrison & Smith, 1964). A Perkin–Elmer Autosystem XL gas chromatograph fitted with a capillary column Omegawax 320 (30 m × 0.32 mm i.d. and 0.25 μm film thickness) and flame ionization detection was used. The temperature of both the injection port and the detector was 260 °C. The carrier gas was helium, 11 psi. The sample volume was 0.5 μl. Fatty acids methyl esters were identified by comparison with standards run previously alone or together with samples. Fatty acids methyl esters were expressed as percentage of total methyl ester content.

2.5. Microbial analysis

Ten grams of each sample were taken aseptically and homogenised with 90 ml of tryptone water (Scharlau, Spain) for 2 min in a sterile plastic bag in a PK 400 Masticator. Serial decimal dilutions were made in sterile tryptone water and duplicate 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread onto total count and selective agar plates.

The microbiological analyses made on the samples were: aerobic mesophilic bacteria determined on 3 M Petrifilm

Aerobic Count Plate (Bioser, Barcelona, Spain) incubated at 30 °C for 48 h, psychrotrophic bacteria on Plate Count Agar (Scharlau, Spain) incubated at 7 °C for 10 days, anaerobic bacteria on Schaedler Agar (Scharlau, Spain) overlayed with 5 ml of the same medium and incubated at 37 °C for 48 h, enterobacteria on 3 M Petrifilm Enterobacteriaceae Count Plate (Bioser, Barcelona, Spain) incubated at 37 °C for 24 h, enterococci on Slanetz Bartley Agar (Scharlau, Spain) incubated at 37 °C for 48 h, pseudomonads on Pseudomonads Agar (Oxoid, Spain) supplemented with Cetrimide, Fucidine and Cephaloridine (CFC, Oxoid, Spain) incubated at 30 °C for 48 h, lactic acid bacteria (LAB) on MRS Agar (Scharlau, Spain) incubated anaerobically in 6% CO_2, at 30 °C for 72 h, Micrococcaceae on MSA (Scharlau, Spain) incubated at 37 °C for 48 h and yeasts and moulds on 3 M Petrifilm Yeast and Mold Count Plate (Bioser, Barcelona, Spain) incubated at 25 °C for 5 days.

For experimental purposes, the detection limit of the above mentioned techniques was 10 cfu/g except for enterococci, pseudomonads, LAB and Micrococcaceae whose limit was 10^2 cfu/g.

2.6. pH and a_w analyses

pH was measured by blending 10 g of product with 10 ml of distilled water for 2 min. A pH meter 507 (Crison, Barcelona, Spain) equipped with a glass electrode was used for this measurement. Water activity (a_w) was measured by CX2 AQUA LAB equipment (Decagon, Washington, USA).

2.7. Instrumental texture measurement

Instrumental texture profile analysis (TPA) (Breene, 1975) was performed with a texture analyzer TA-XT2 (Stable Micro Systems, Haslemere, UK). The Texture Expert, version 1.20 (Spanish), computer program by Stable Micro Systems was used for data collection and calculations. Six cubes of salchichon ($1 \times 1 \times 1$ cm) were compressed twice with a cylindrical probe of 1 cm diameter, at 1 mm/s speed and the level of compression was 60% of the thickness of the sample. The test was always accomplished in an air-conditioned room at 21 ± 1 °C and samples were tempered for 1 h before texture analysis. The parameters determined from the force–time curves were hardness, springiness, cohesiveness and chewiness. Hardness was defined by peak force during first compression cycle and expressed in gram. Springiness was defined as a ratio of time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reserval. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Chewiness was obtained by multiplying hardness, springiness and cohesiveness and expressed in gram.

2.8. Sensory evaluation

Sensory evaluation was carried out on salchichon slices after each storage time by an experienced eight-member sensory panel. Eight training sessions were held to familiarise the judges with the attributes to evaluate and the scale to use. Evaluation was always done regarding initial characteristics of the fresh product prior to packing. Colour, odour, taste, hardness, juiciness and overall acceptability were scored on a five-point hedonic scale as follow: 5 = excellent, 4 = good, 3 = acceptable, 2 = fair and 1 = unacceptable (Kotzekidou & Bloukas, 1996). Moreover, when the overall acceptability score was less than 3 the product was considered expired and when scores lower than 3 were obtained, the reason had to be stated.

2.9. Statistical analysis

Data obtained for fat content and fatty acid composition were statistically analysed using one-way analysis of variance (ANOVA). Data collected for microbiology, pH, a_w, texture and sensory attributes were statistically analysed by a three-factor factorial arrangement. The factors were the three salchichon types (control, high oleic and high linoleic), the two packaging methods (vacuum and 20% CO_2/80% N_2) and the storage time (0, 15, 30, 60, 90, 150 and 210 days). The data were analyzed by analysis of variance (ANOVA). When main effects were significant, the means were separated by Fisher's least significant difference test at 1% level ($LSD_{0.01}$). The level of significance $P < 0.01$ was used for all comparisons and will be used for the remainder of this discussion. Data analyses were conducted using the statistical package Statgraphics Plus 5.0.

3. Results and discussion

3.1. Fatty acid composition

The fatty acid composition of the three salchichon types is shown in Table 2. In general, significant differences were observed between the group CO and those with modified fat composition. In relation to the saturated fatty acid (SFA) fraction, CO salchichon showed the highest values and that difference was mainly attributed to the significant differences found on myristic, palmitic and arachidic acids. Concerning the monounsaturated fatty acid (MUFA) fraction, the HO group showed higher values than values found for HL and CO salchichon. The oleic acid was responsible of this fact. The polyunsaturated fatty acid (PUFA) fraction was higher in the HL group due to the higher supply of unsaturated fatty acids from soya oil included in the pigs diet. With these fatty acid profiles, modified products (HO and HL) showed better ratios than control products (CO) from a nutritional point of view. MUFA + PUFA/SFA and PUFA/SFA, which are considered beneficial in relation to blood lipid profile (Valencia et al., 2006b) were higher in modified products than in control ones. The ratio of

Table 2
Fat (% dry matter) and fatty acid (% of total fatty acids) composition of the different sausage types: control (CO), high oleic (HO) and high linoleic (HL)

	Sausage mixture		
	CO	HO	HL
Fat content (% dry matter)	51.43 ± 0.65^{c}	46.90 ± 1.10^{a}	48.39 ± 0.22^{b}
Fatty acid composition (%)			
Lauric C12:0	0.12 ± 0.02^{a}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}
Myristic C14:0	1.66 ± 0.12^{b}	1.42 ± 0.01^{a}	1.43 ± 0.08^{a}
Palmitic C16:0	25.71 ± 0.48^{c}	23.53 ± 0.16^{a}	24.22 ± 0.16^{b}
Palmitoleic C16:1	2.33 ± 0.09^{b}	2.27 ± 0.01^{b}	1.95 ± 0.05^{a}
Margaric C17:0	0.18 ± 0.01^{a}	0.19 ± 0.02^{a}	0.20 ± 0.01^{a}
Margaroleic C17:1	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}	0.14 ± 0.01^{a}
Stearic C18:0	14.12 ± 0.15^{b}	12.54 ± 0.34^{a}	13.96 ± 0.17^{b}
Oleic C18:1	40.71 ± 0.54^{b}	43.61 ± 0.13^{c}	38.50 ± 0.38^{a}
Linoleic C18:2	12.82 ± 0.43^{a}	14.08 ± 0.57^{b}	16.94 ± 0.34^{c}
Linolenic C18:3	1.03 ± 0.06^{a}	1.11 ± 0.04^{a}	1.40 ± 0.05^{b}
Arachidic C20:0	0.28 ± 0.02^{c}	0.21 ± 0.00^{a}	0.25 ± 0.02^{b}
Gadoleic C20:1	0.88 ± 0.07^{a}	0.80 ± 0.02^{a}	0.90 ± 0.14^{a}
$\sum$ SFA	42.08 ± 0.69^{c}	38.00 ± 0.53^{a}	40.17 ± 0.286^{b}
$\sum$ MUFA	44.07 ± 0.56^{b}	46.83 ± 0.15^{c}	41.49 ± 0.34^{a}
$\sum$ PUFA	13.85 ± 0.49^{a}	15.19 ± 0.61^{b}	18.34 ± 0.38^{c}
PUFA + MUFA/SFA	1.38^{a}	1.63^{c}	1.49^{b}
PUFA/SFA	0.33^{a}	0.40^{b}	0.47^{c}

Results are expressed as means ± standard deviations.
[a–c] Averages with different letters in the same row are different ($p < 0.01$).

PUFA/SFA amounted 0.40 and 0.47 for HO and HL salchichon respectively. These values are according to the dietary guidelines of the COMA (1984) who recommends a PFA/SFA ratio of 0.45.

3.2. Microbial results

The results of the microbiological analysis are presented in Table 3. Salchichon type significantly affected the microbial numbers except for enterobacteria, pseudomonad and yeast and mould counts. The lowest counts were found on CO salchichon. The differences observed between salchichon groups could be due probably to two reasons: the solubilisation of CO_2 in the product and the antibacterial activity of fatty acids. Previous research (Devlieghere & Debevere, 2000) has indeed demonstrated that the concentration of dissolved CO_2 in the water-phase of a food determines the growth inhibition of microorganisms in a modified atmosphere. When CO_2 is dissolved in the fat-phase of a food, part of the CO_2 in the gas-phase will be consumed and thus less will be left to dissolve in the water-phase of the food which results in a lower CO_2-concentration in the water-phase in fatty foods. The solubility of CO_2 in the fat-phase is probably more dependent on how liquid the fat phase is at each temperature. Sivertsvik, Rosnes, and Jeksrud (2004) observed a higher solubility of CO_2 in unsaturated fat than in saturated fat, probably due to the liquidity of the fat (the melting point of saturated fatty acids is higher than the melting point of unsaturated fatty acids). Considering that counts from CO sausage were lower when it was packed with a gas mixture including CO_2 than when it was vacuum packed, the higher solubility of CO_2 in water-phase of this sausage (with the highest content in saturated fat) could be a reasonable explanation for higher microorganism growth inhibition observed. However, further investigations about the CO_2 solubility in fat-phase with different fatty acids will have to be done.

On the other hand, Branen, Davidson, and Katz (1980) found that for saturated fatty acids, hydrophobic groups had the greatest influence on antibacterial activity. Besides, Ouattara, Simard, Holley, Piette, and Bégin (1997) studied the antibacterial activity of fatty acids against meat spoilage organisms and found that lauric and palmitoleic acids exhibited a great inhibitory effect. According to the results shown in Table 2, the CO salchichon had a higher percent of saturated fatty acids and palmitoleic acid than HO and HL salchichon. This fact could contribute to the lowest counts observed in the CO samples. However, the Ouattara et al. (1997) investigation was carried out with inoculated petri plates and the extrapolation of these results to meat systems must be done with caution.

Packaging method had no significant effect on counts of different microorganism groups, except for yeast and mould counts that were lower in gas mixture (20/80% CO_2/N_2) packaging.

The counts of different microbial groups obtained after 28 days of ripening (0 days of storage) were in agreement with those obtained by other authors in other traditional fermented sausages (Comi et al., 2005; Drosinos et al., 2005; Lizaso et al., 1999) and showed that the lactic acid bacteria constituted the major microflora of the sausages. Due to the good adaptation of lactic acid bacteria to the meat environment and their faster growth rates during fermentation

Table 3
Effect of salchichon type (CO: control, HO: high oleic and HL: high linoleic) packaging method and storage time on microbiological parameters of salchichon stored at 6 °C

	Mesophilic aerobic bacteria	Anaerobic bacteria	Psychrotrophs	Enterobacteria	Pseudomonads	Enterococci	Lactic acid bacteria	Micrococcaceae	Yeasts and moulds
Salchichon type[A]									
CO	8.19[a]	7.77[a]	7.93[a]	1.18	2.21	4.20[b]	8.05[a]	3.38[a]	2.69
HO	8.44[b]	7.97[b]	7.97[a]	1.12	2.26	3.94[a]	8.25[b]	3.58[ab]	2.91
HL	8.42[b]	8.01[b]	8.29[b]	ND	2.19	3.83[a]	8.30[b]	3.49[b]	3.02
$LSD_{0.01}$	0.12	0.15	0.30	0.10	0.13	0.13	0.12	0.18	0.36
Packaging method[B]									
Vacuum	8.35	7.90	8.01	1.10	2.22	3.98	8.19	3.52	3.00[b]
20% CO_2/80% N_2	8.36	7.98	8.11	1.09	2.22	4.00	8.21	3.45	2.56[a]
$LSD_{0.01}$	0.10	0.13	0.25	0.08	0.10	0.11	0.10	0.15	0.30
Storage time[C]									
0	8.44[bc]	8.00[cd]	8.30[bc]	1.65[b]	3.48[b]	4.40[c]	7.90[a]	3.78[d]	5.22[e]
15	8.15[a]	7.51[a]	7.28[a]	1.03[a]	2.05[a]	3.92[a]	8.40[cd]	3.36[ab]	4.66[d]
30	8.46[bc]	8.25[e]	8.07[b]	ND	ND	3.99[ab]	8.35[c]	3.42[abc]	3.20[c]
60	8.38[b]	8.16[de]	8.03[b]	ND	ND	3.81[a]	8.23[bc]	3.21[a]	1.94[b]
90	8.58[c]	8.14[de]	8.55[c]	ND	ND	4.14[b]	8.54[d]	3.68[cd]	1.83[b]
150	8.34[b]	7.77[bc]	8.28[bc]	ND	ND	3.88[a]	8.13[b]	3.56[bcd]	1.42[ab]
210	8.09[a]	7.57[ab]	7.91[b]	ND	ND	3.78[a]	7.84[a]	3.37[ab]	1.16[a]
$LSD_{0.01}$	0.18	0.23	0.46	0.15	0.19	0.21	0.18	0.28	0.55

[a–e] Means within the same column and the same main effect with different superscript letters are different ($p < 0.01$).
[A] Each number represents the average value of each parameter for all samples with the same salchichon type.
[B] Each number represents the average value of each parameter for all samples with the same packaging method.
[C] Each number represents the average value of each parameter for all samples with the same storage time.

and sausage ripening, they become the dominant microflora (Drosinos et al., 2005).

The storage time affected all microbial flora studied in this work, and all decreased with storage except for LAB, which underwent an initial increment. Besides, a significant interaction between type of sausage and storage time was found for LAB (Table 6), since decreasing along storage was faster and counts at 210 days were lower in salchichon control than in the other two types. Aerobe, anaerobe, psychrotroph, enterococci and Micrococcaceae decreases were slow; remaining nearly constant for up to 90-150 days. In general, the stability found for the counts during storage time, could be explained taking into account that packaging in anoxic environments retards microbial growth and delays spoilage due to slow proliferation of bacteria capable of tolerating anaerobic conditions (Martínez, Djenane, Cilla, Beltrán, & Roncalés, 2006). Enterobacteria and pseudomonad counts were subjected to a significant inhibition and values under the detection limit were found after 15 days of storage, probably due to the strong competitive effect of lactic acid bacteria on the rest of the endogenous flora. Lactic acid bacteria suppress the growth of Gram-negative bacteria by producing organic acids and various antibacterial metabolic products (Daeschel, 1989; Holzapfel, Guisen, & Schillinger, 1995; Weber, 1994). Finally, yeast and mould counts were progressively eliminated during storage, and their numbers decreased to near the detection limit at the end of storage. The anoxic packaging and the CO_2 solubility could explain these observations.

3.3. pH and a_W results

Salchichon type and storage time affected the pH values (Table 4). The initial pH of sausages was 5.09. This low pH is normal for a fermented sausage, considering that organic acids, mainly lactic, are present in these type of sausages as a result of carbohydrate breakdown during fermentation, and the final pH for these products drops below 5.3 (Bloukas et al., 1996; Muguerza et al., 2002). Regarding Table 6, the factor with the highest effect on pH was the storage time, and the pH values significantly decreased throughout storage. The reduction of pH during storage with modified atmosphere packaging was also reported in other fermented products like Pastrami by Laleye, Lee, Simard, Carmichael, and Holley (1984). The decrease in pH values during the refrigerated storage was attributed to the activity of lactobacilli and the dissolution of CO_2 into meat product, however in this case no effect on pH of type of packaging was observed. Therefore, CO_2 dissolved into the meat product did not determine acidification. Moreover, a significant interaction was found between packaging method and storage time (Table 6), due to slightly different change of pH in salchichon packed under vacuum, reaching a lower pH at 210 days.

The results of water activity (a_w) analysis (Table 4) showed that there were significant differences between a_w of the three types of sausages, but these values were within the range obtained by other authors in Spanish (Hoz et al., 2004) and in other European dry fermented sausages (Zanardi et al., 2002). Packaging method had no

Table 4
Effect of salchichon type (CO: control, HO: high oleic and HL: high linoleic) packaging method and storage time on physicochemical and texture parameters of salchichon stored at 6 °C

	pH	a_w	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)
Salchichon type[A]						
CO	5.00^{ab}	0.862^{a}	6029.35^{b}	0.55^{a}	0.43^{b}	1485.48^{b}
HO	5.01^{b}	0.876^{c}	4905.13^{a}	0.59^{b}	0.44^{b}	1270.77^{a}
HL	4.98^{a}	0.869^{b}	5347.33^{a}	0.56^{a}	0.41^{a}	1264.18^{a}
$LSD_{0.01}$	0.02	0.005	448.03	0.01	0.02	135.79
Packaging method[B]						
Vacuum	4.99	0.870	5470.12	0.57	0.43	1340.44
20% CO_2/80% N_2	5.00	0.869	5384.55	0.56	0.43	1339.84
$LSD_{0.01}$	0.02	0.004	365.81	0.01	0.02	110.87
Storage time[C]						
0	5.09^{d}	0.865^{a}	4980.75^{a}	0.54^{a}	0.41^{a}	1107.68^{a}
15	5.05^{c}	0.867^{a}	4850.25^{a}	0.53^{a}	0.43^{abc}	1133.74^{a}
30	5.05^{c}	0.875^{b}	5157.72^{a}	0.57^{b}	0.41^{ab}	1263.27^{a}
60	4.97^{b}	0.872^{ab}	5061.16^{a}	0.58^{b}	0.41^{ab}	1241.21^{a}
90	4.99^{b}	0.871^{ab}	5527.97^{ab}	0.59^{b}	0.45^{c}	1470.73^{b}
150	4.99^{b}	0.869^{ab}	6204.83^{b}	0.57^{b}	0.44^{bc}	1583.16^{b}
210	4.84^{a}	0.865^{a}	6208.65^{b}	0.58^{b}	0.44^{bc}	1581.20^{b}
$LSD_{0.01}$	0.03	0.008	684.37	0.02	0.03	207.43

[a–e] Means within the same column and the same main effect with different superscript letters are different ($p < 0.01$).
[A] Each number represents the average value of each parameter for all samples with the same salchichon type.
[B] Each number represents the average value of each parameter for all samples with the same packaging method.
[C] Each number represents the average value of each parameter for all samples with the same storage time.

Table 5
Effect of salchichon type (CO: control, HO: high oleic and HL: high linoleic) packaging method and storage time on sensory parameters of salchichon stored at 6 °C. Values rated on a 5 point scale (5 = excellent, 4 = good, 3 = acceptable, 2 = fair and 1 = unacceptable)

	Colour	Odour	Taste	Hardness	Juiciness	Acceptability
Salchichon type[A]						
CO	4.4	4.2	4.1	4.2^{a}	4.3	4.2
HO	4.6	4.2	4.2	4.4^{b}	4.4	4.3
HL	4.5	4.2	4.1	4.2^{ab}	4.3	4.2
$LSD_{0.01}$	0.15	0.16	0.18	0.16	0.15	0.13
Packaging method[B]						
Vacuum	4.5	4.2	4.1	4.2	4.3	4.3
20% CO_2/80% N_2	4.5	4.2	4.1	4.2	4.3	4.3
$LSD_{0.01}$	0.12	0.13	0.15	0.13	0.13	0.10
Storage time[C]						
0	4.9^{d}	4.7^{d}	4.7^{d}	4.8^{d}	4.9^{e}	4.8^{f}
15	4.9^{d}	4.7^{d}	4.7^{d}	4.8^{d}	4.9^{de}	4.8^{ef}
30	4.8^{d}	4.6^{d}	4.6^{d}	4.5^{c}	4.7^{cd}	4.6^{e}
60	4.5^{c}	4.3^{c}	4.2^{c}	4.3^{c}	4.4^{c}	4.4^{d}
90	4.2^{b}	3.9^{b}	3.9^{c}	4.0^{b}	4.1^{b}	4.1^{c}
150	4.4^{bc}	3.9^{b}	3.6^{b}	3.9^{b}	4.1^{b}	3.9^{b}
210	3.8^{a}	3.4^{a}	3.2^{a}	3.5^{a}	3.3^{a}	3.4^{a}
$LSD_{0.01}$	0.22	0.24	0.27	0.25	0.23	0.19

[a–f] Means within the same column and the same main effect with different superscript letters are different ($p < 0.01$).
[A] Each number represents the average value of each parameter for all samples with the same salchichon type.
[B] Each number represents the average value of each parameter for all samples with the same packaging method.
[C] Each number represents the average value of each parameter for all samples with the same storage time.

effect on a_w values, while the storage time significantly affected them. However, if change of a_w is considered, it could be concluded that the a_w during storage hardly changed with respect to water activity at the packaging time.

3.4. Instrumental texture

Table 4 shows the results of instrumental texture parameters. No significant differences in the results of the TPA were found between packaging methods; however the sal-

Table 6
Analysis of variance on the effect of salchichon type, packaging method and storage time on different salchichon attributes (*F*-values for independent variables and interactions)

Attributes	Source of variance					
	A	*B*	*C*	*A* × *B*	*A* × *C*	*B* × *C*
Microbiological parameters						
Mesophilic aerobic bacteria	22.27***	0.11^{ns}	15.05***	1.51^{ns}	1.65^{ns}	4.66***
Anaerobic bacteria	12.66***	4.68*	28.29***	3.17*	3.28***	3.30**
Psychrotrophs	20.35***	4.12*	37.57***	16.84***	19.52***	11.32***
Enterobacteria	52.78***	0.41^{ns}	166.71***	0.41^{ns}	45.40***	0.41^{ns}
Pseudomonads	1.02^{ns}	0.00^{ns}	96.19***	0.00^{ns}	0.53^{ns}	0.00^{ns}
Enterococci	28.00***	0.47^{ns}	16.02***	2.08^{ns}	0.94^{ns}	1.80^{ns}
Lactic acid bacteria	21.17***	0.59^{ns}	32.74***	1.82^{ns}	3.69***	2.10^{ns}
Micrococcaceae	5.5**	1.82^{ns}	9.37***	3.21^{*s}	1.51^{ns}	6.72***
Yeasts and moulds	1.43^{ns}	16.78***	126.32***	1.82^{ns}	0.66^{ns}	2.91*
Physico-chemical parameters						
pH	24.02***	5.52*	297.54***	0.40^{ns}	4.67***	70.71***
a_w	33.36***	0.28^{ns}	3.78**	7.26***	3.66***	2.04^{ns}
Texture parameters						
Hardness (g)	22.70***	0.39^{ns}	9.92***	5.19**	1.76^{ns}	1.61^{ns}
Springiness	24.96***	2.75^{ns}	16.48***	4.33*	1.85*	1.39^{ns}
Cohesiveness	7.54***	0.25^{ns}	5.13***	0.86^{ns}	2.34**	0.86^{ns}
Chewiness (g)	11.45***	0.00^{ns}	12.72***	2.87^{ns}	0.86^{ns}	1.01^{ns}
Sensory parameters						
Colour	3.74*	1.42^{ns}	45.71***	0.30^{ns}	1.24^{ns}	1.42^{ns}
Odour	0.36^{ns}	0.13^{ns}	58.57***	0.22^{ns}	2.02*	1.36^{ns}
Taste	1.00^{ns}	0.23^{ns}	64.05***	1.31^{ns}	2.38**	1.41^{ns}
Hardness	4.63*	0.00^{ns}	51.87***	0.10^{ns}	0.68^{ns}	1.85^{ns}
Juiciness	1.80^{ns}	0.02^{ns}	80.73***	1.02^{ns}	1.02^{ns}	1.66^{ns}
Acceptability	3.06*	0.00^{ns}	98.75***	1.01^{ns}	2.34**	0.97^{ns}

A = salchichon type; *B* = packaging method; *C* = Storage time.
ns = not significant.
* Significant at $p < 0.05$.
** Significant at $p < 0.01$.
*** Significant at $p < 0.001$.

chichon type and the storage time affected texture parameters. Control salchichon showed the highest values for hardness and chewiness. Muguerza et al. (2001) found that fermented sausages with incorporation of pre-emulsified olive oil were softer than control sausages. Besides, Warnants et al. (1998) found that salami with high PUFA levels was softer due to an effect of PUFA on the consistency of the backfat.

The hardness, springiness, cohesiveness and chewiness values showed an increase during the whole period of storage, but the mean values were close to those obtained for fermented sausages by other authors (Hoz et al., 2004; Muguerza et al., 2001).

3.5. Sensory results

The sensorial characteristics studied on the three groups of sausages, are reported in Table 5. No differences were found between sausage groups except for hardness, and neither between either atmosphere. These results were unexpected, but of a great practical interest since they demonstrated that fermented sausages with high content of unsaturated fatty acids had similar sensorial properties to those of conventional sausages, and even a comparable sensory stability. The little differences among the evolution of the different products could be due to the natural antioxidants included in the diets (Table 1) that enhanced unsaturated fatty acid stability. However, the storage time had significant effects on sensory parameters that decreased gradually during the whole period of storage. At the beginning of storage, all sausages had a light red colour and a discrete spicy smell and taste, principally of black pepper. Ruiz Pérez-Cacho et al. (2005) determined the main sensory attributes of salchichon and specified that the black pepper odour and aroma is one of the major distinctive characteristics. The panellists did not perceive a rancid taste, in any case. Muguerza et al. (2001) found similar results for other Spanish dry sausages manufactured by replacing pork back fat with pre-emulsified olive oil. Regarding storage time, the judges, in some samples, detected a loss of spicy odour and taste and a higher acid taste, as well as they noticed a slight increase of hardness. These results are according to those observed in the pH and the instrumental hardness measurements. Besides, these results denoted that packaging under both conditions (vacuum and 20/80% CO_2/N_2) did not prevent the changes that occur in dry fermented sausages

during ripening. These results agree to those provided by Fernández-Fernández et al. (2002) for Galician chorizo sausage.

4. Conclusions

It can be concluded that it is possible to manufacture dry fermented sausages with high MUFA or PUFA content, using as raw material fat and meat from pigs fed on diets enriched with either sunflower oil or soya oil. These modifications give rise to nutritional advantages. Besides, the results of this study indicate that the modification of fatty acid percentages and the packaging method did not involve great changes over the microbiological, physical and texture parameters. Storage time was the main factor of modification of sausage characteristics, so a storage time longer than 150 days is not advisable. Focusing on sensory characteristics, the different sliced salchichons packed under vacuum and with 20/80% CO_2/N_2 modified atmosphere, after long storage presented a loss of spicy odour and taste, an increase of acid taste and a slight increase of hardness principally.

Finally, with a view of commercial use, the advantage of the gas mixture packaging (20/80% CO_2/N_2) versus vacuum packaging of different salchichon types was not clear.

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