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The use of linseed oil improves nutritional quality of the lipid fraction of dry-fermented sausages

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Abstract

Improvement of the nutritional quality of the lipid fraction of dry-fermented sausages was achieved by a substitution of one quarter of the amount of pork backfat present in traditional formulations by an emulsion in which linseed oil was included. This improvement was particularly noticeable when 100 mg/kg of butylhydroxytoluene and 100 mg/kg of butylhydroxyanisole were added. P/S ratio increased from 0.4 in the control sausages to 0.6 in the batch with 3.3% linseed oil and to 0.7 in the batch with linseed (3.3%) and antioxidants. The $n-6/n-3$ ratio decreased from 14.1 in control products to 1.7–2.1 in modified products as a consequence of the α-linolenic acid increment. No oxidation problems were detected during the ripening process, with TBA values always lower than 0.23 ppm. Hexanal and nonanal showed the highest values in linseed oil-containing products. Addition of antioxidants avoided the formation of decadienals and other aldehydes from lipid oxidation.

Keywords: Fatty acids; Oxidation; Antioxidants; Chorizo

1. Introduction

The two main parameters currently used to assess nutritional quality of the lipid fraction of food are P/S and $n-6/n-3$ ratios. The P/S ratio is recommended, nowadays, to be above 0.4–0.5 (Enser, 2001; Wood et al., 2004) in order to prevent both, an excess of saturated fatty acids with a negative effect on the LDL cholesterol plasmatic level, and an excess of polyunsaturated fatty acids, some of them being precursors of powerful clotting agents and also being involved in the aetiology of some cancers. The $n-6/n-3$ ratio, which is estimated to be around 15–20 in the current Western diet, should decrease to below 5 or 4 (British Nutrition Foundation, 1992; Wood et al., 2004) to avoid the prothrombotic and proagregatory state induced by a high level of $n-6$ PUFA. Furthermore, a balanced $n-6/n-3$ ratio in the diet is essential for normal growth and development and should lead to decreases in cardiovascular disease and other chronic diseases and should improve mental health (Simopoulos, 1999).

As meat and meat products, are some of the most important sources of dietary fat, modification of the lipid profile of such products, by enhancing $n-3$ polyunsaturated fatty acids, can help to improve the nutritional quality of the occidental diet. Research has been done by feeding animals diets rich in polyunsaturated acids, basically $n-3$. Linseed has been widely used for this purpose, both as seeds (Romans, Johnson, Wulf, Libal, & Costello, 1995; Van Oeckel, Casteels, Warnants, & Boucque, 1997; Specht-Overholt et al., 1997; Matthews, Homer, Thies, & Calder, 2000) and as oil (Fontanillas, Barroeta, Baucells, & Guardiola, 1998; López-Ferrer, Baucells, Barroeta, & Grashorn, 2001; Rey & Morrisey, 2001; D' Arrigo et al., 2002a; D' Arrigo et al., 2002b; Hoz et al., 2003).

It has been observed that, when animals are fed with $n-3$, enriched diets, the oxidation rate of raw meats, including pork meat, increases (López-Bote, Rey, Sanz, Gray, & Buckey, 1997; Nurnberg, Kuchenmeister,

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Nurnberg, Ender, & Hackl, 1999). One of the strategies studied to avoid this problem has been the dietary addition of different amounts of α-tocopheryl acetate (D' Arrigo et al., 2002a, 2002b; Hoz et al., 2003); in some cases, also, synthetic antioxidants, such as BHA, have been used in the animals' feeding (Nam, Lee, Min, & Kang, 1997).

There are only few works dealing with the development of meat products enriched in $n-3$ fatty acids (Leskanich, Matthews, Warkup, Noble, & Hazzledine, 1997; Specht-Overholt et al., 1997; Enser, Richardson, Wood, Gill, & Sheard, 2000; Sheard et al., 2000).

Modification of the ingredients used for the elaboration of dry-fermented sausages, instead of the use of modified meat, has been variously tested. Olive oil has been used as a source of MUFA, yielding technologically viable products without significant changes in PUFA on $n-6/n-3$ ratio (Bloukas, Paneras, & Fournitzis, 1997; Muguerza, Fista, Ansorena, Astiasarán, & Bloukas, 2002). In order to modify the P/S ratio, soy oil was also used in a previous work (Muguerza, Ansorena, & Astiasarán, 2003).

The objective of this work was to evaluate the lipid modifications undergone in dry-fermented sausages, during the ripening process, when pre-emulsified linseed oil was used in the formulation, mainly focussing attention on the changes in the P/S and $n-6/n-3$ ratios. Furthermore, the effect of the incorporation of antioxidants was tested, in order to prevent a detrimental oxidation process.

2. Materials and methods

2.1. Sausage preparation

Chorizo de Pamplona, a type of traditional Spanish dry-fermented sausage, was elaborated according to the procedure described by Muguerza, Gimeno, Ansorena, Bloukas, and Astiasarán (2001). Three batches of fermented sausages, about 5 kg each, were prepared. The control sausage was based on a traditional formulation of 75% pork meat and 25% pork backfat. In the other two batches, 25% of the total pork backfat was substituted by pre-emulsified linseed oil with soy protein. The emulsion was made according to the procedure described by Hoogenkamp (1989). Eight parts of hot water were mixed, for 2 min, with one part of isolated soy protein, and the mixture was emulsified with 10 parts of olive oil for 3 min. 100 mg/kg of butylhydroxytoluene (BHT) and 100 mg/kg of butylhydroxyanisole (BHA) were added as antioxidants in one of the batches that, included linseed oil in the formulation. The percentages of meat and fat sources are detailed in Table 1. The fatty acid profile of the linseed oil, expressed at g/100 g of fatty acids, was as follows: myristic (0.11), palmitic (6.28), palmitelaidic (0.18), palmitoleic (0.18), stearic (3.91), oleic (24.5), linoleic (18.4), linolenic (46.3), eicosapentaenoic (0.11) and docosahexaenoic (0.07).

Table 1
Percentages of meat and fat sources and presence of antioxidants in the three batches of dry-fermented sausages elaborated

	Control (%)	Linseed (%)	Linseed + antioxidants
Lean meat	75	75	75%
Pork backfat	25	18.75	18.75%
Linseed oil	–	3.3	3.3%
BHT + BHA	–	–	100 + 100 mg/kg

Analyses of parameters were carried out in the initial mixture (day 0) and after 3, 15 and 30 days of ripening. Three replications of the experiment were carried out.

2.2. Chemical analysis

Extraction of lipids was carried out using a chloroform/methanol mixture (Folch, Lees, & Stanley, 1957). Fatty acids were determined in the lipid extracted by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (AOAC, 2002a, Chap. 41). A Perkin–Elmer Autosystem XL gas chromatograph fitted with a capillary column SP™-2560 (100 m × 0.25 mm × 0.2 μm) and flame ionization detection was used. The temperature of both the injection port and detector was 220 °C. The oven temperature was programmed to increase from 170 to 200 °C at a rate of 0.2 °C/min. The carrier gas was hydrogen, 20.5 psi. The sample size was 0.5 μl. The identification of the fatty acid methyl esters was done by comparison of the retention times of the peaks in the sample with those of standard pure compounds (Sigma, St. Louis, MO, USA) and by spiking the sample with those compounds. The quantification of individual fatty acids was based on the internal standard method, using heptadecanoic acid methyl ester (Sigma, St. Louis, MO, USA).

Thiobarbituric acid (TBA) value was determined according to the method used by Tarladgdis, Watts, Younathan, and Dugan (1960) with modifications of Tarladgdis, Pearson, and Dugan (1964) and Zipser and Wats (1962). Peroxides were determined according to the AOAC method (2002b, Chap. 41).

2.3. Determination of lipid oxidation compounds

2.3.1. Likens–Nickerson extraction

25 g of frozen sausage were 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), was attached to a modified Likens–Nickerson apparatus; 5 ml of dichloromethane were also added to fill the apparatus solvent return loop. Solvent and sample mixture were heated to 70 °C and boiling tem-

perature, respectively, maintaining these conditions for 2 h. After cooling to ambient temperature, the extract of dichloromethane was collected and dried over anhydrous Na_2SO_4. Two distillations per batch of sausage were carried out.

2.3.2. Analysis

The volatile compounds were analyzed in a HP 6890 GC system (Hewlett–Packard, Palo Alto, USA) 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 × 0.25 μm nominal HP-5MS). The carrier gas was He (1 ml/min), and the chromatographic conditions were as follows: initial oven temperature was maintained during 10 min at 40 °C and subsequently programmed from 40 to 120 °C at a rate of 3 °C/min and at a rate of 10 °C/min from 120 to 250 °C, at which it was held for another 5 min; injector temperature was 250 °C, transfer line temperature, 280 °C, ion source temperature, 230 °C, scan speed, 4.49 scan/s, mass range, 33–350 amu (atomic mass units), solvent delay, 3 min, electron impact at 70 eV. Identification of the peaks was based on the comparison of their mass spectra with the spectra of the Wiley library (HPCHEM Wiley 275 6th Ed.) and, in some cases, a comparison of their retention times with those of standard compounds was also carried out. The Kovats indices were also calculated, according to the method of Tranchant (1982), and were compared with available literature data (Kondojoyan & Berdagué, 1996). Only compounds related to lipid oxidation are shown. Areas of peaks were measured by integration of the total ion current of the spectra or by calculation of the total area, based on integration of a single ion. Semiquantitative determination of the volatile compounds was based on the ratio of their peaks to that of dodecane (i.s.), and the results were expressed as nanogrammes of dodecane per gramme of dry matter.

2.4. Data analysis

For each replication of the experiment, three samples were analyzed from each type of sausage and period of analysis. Each parameter was determined four times in each sample. In the Tables, mean values are shown. An ANOVA test was carried out in order to determine significant differences among sausages, depending on the type of formulation. Data analysis was carried out with an SPSS 9.0 programme (© 1998, SPSS Inc. Chicago, version 9.0. IL).

3. Results and discussion

The analysis of the total lipid content at the end of the ripening process showed values of 33.7% for the control sausage, 31.8% for the linseed oil-containing batch and 29.7% for the sausages with linseed oil and antioxidants. The fatty acid profiles of the products, expressed as g/100 g fatty acids, are presented in Table 2. It can be seen, that the values obtained along the ripening process, for every type of sausage, did not show noticeable changes on the different days of analysis. Consequently, the desiccation process did no affect the total fatty acid profile. Differences shown in this table among batches are due to the differences in the raw matters used in control and modified products. The greatest difference was observed for α-linolenic acid that, at the end of the ripening, increased from 0.92 g/100 g fatty acids in control to 7.99 g/100 g fatty acids in modified products without antioxidants. Enser et al. (2000), analyzing the fatty acid composition of different tissues and meat products from pork fed linseed, obtained increments for α-linolenic of 1.36-fold in relation to sausages elaborated from control raw materials. Those authors also found some significant increases in EPA. In linseed-containing products, eicosapentaenoic acid (EPA) showed slight amounts during the first steps of maturation, but this acid disappeared at the end of the ripening. In a previous paper, soy oil was used in the same concentration (Muguerza et al., 2003), leading to increments for myristic, palmitic, palmitoleic, oleic acids and especially linoleic acid. No differences were found for EPA or DHA. Nam et al. (1997) found that arachidonic acid (C20:4, $n-6$) content decreased significantly with increased feeding of linseed oil in poultry meat. In our work, arachidonic acid was not detected in any sample. Those authors also found that the MUFA fraction was not affected by the diet.

Analyzing the effect of the addition of antioxidants, higher values for linoleic and α-linolenic acids were detected in antioxidant-containing sausages, than in linseed sausages from the 15th day of ripening. These results indicated that the presence of antioxidants could reduce the oxidation of these fatty acids, which are the most susceptible to oxidation. Different compounds with antioxidant properties have been used to avoid the oxidation of PUFAs. Van Ruth, Shaker, and Morrisey (2001) showed the efficiency of methanolic extracts of soybean seeds for inhibiting linseed oxidation. Lauridsen, Nielsen, Henckel, and Sorensen (1999) observed a slight decrease of $n-6$ PUFA in fats when pigs were fed supplemented α-tocopheryl acetate and rapeseed oil. However, Monahan et al. (1992) and López-Bote et al. (1997) did not find any effect of α-tocopheryl acetate supplementation on the fatty acid composition in muscle. Rey et al. (2001) explained the differences found for the effect of vitamin E by the fatty acid compositions of different fat sources in animal feeding for the different experiments. Evidently phospholipids in muscle cell membranes are the main lipids susceptible to oxidation in meat (Pikul, Leszczynski, & Kummerow, 1984;

Table 2
Total fatty acids along ripening process for the three types of sausages (g/100 g fatty acids)

	Control				Linseed				Linseed + antioxidants			
Days	0	3	15	30	0	3	15	30	0	3	15	30
SFA												
Lauric	0.13a	0.13a	0.13b	0.13a	0.12a	0.11a	0.11a	0.12a	0.17b	0.16a	0. 12a	0.13a
Myristic	1.58c	1.57c	1.55b	1.55b	1.24a	1.24a	1.25a	1.33a	1.41b	1.41b	1. 31a	1.35a
Palmitic	25.2b	25.1b	24.9b	24.8b	21.8a	21.9b	21.9a	22.1a	21.2a	21.1a	20.6a	21.5a
Stearic	14.5c	14.5c	14.2c	14.3c	13.0b	12.9b	12.5b	12.7b	11.9a	11.6a	11.5a	11.4a
Arachidic	0.21a	0.22a	0.15a	0.15a	0.21a	0.21a	0.22b	0.24b	0.19a	0.19a	0. 20ab	0.17a
Behenic	0	0a	0.42a	0.31a	0	0a	0.28a	0.27a	0	0.35b	0.24a	0.25a
MUFA												
Palmitoleic	2.75b	2.72b	2.64b	2.66b	2.32a	2.31a	2.37a	2.37a	2.40a	2. 39a	2.44ab	2.42a
Oleic	39.6c	39.8b	39.8a	40.0b	37.4b	38.4ab	37.7a	37.4a	37.1a	37.1a	37.3a	36.7a
PUFA												
Linoleic	14.2a	14.2a	14.2a	14.2a	14.2a	14.2a	14.4a	14.7a	16.4b	16.4b	16.8b	16.7b
α-Linolenic	0.88a	0.88a	0.88a	0.93a	7.95b	7.81b	7.87b	8.03b	7. 91b	8.30b	8.87c	8.57c
Arachidonic	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
EPA	0a	0a	0a	0a	0.7b	0.07b	0.05b	0a	0a	0a	0a	0a
DHA	0.13b	0.13a	0.13a	0.13b	0.11b	0.10a	0.28a	0.10a	0.04a	0.34a	0.09a	0. 07a
TRANS												
t-Palmitoleic	0.46c	0.45a	0.45c	0.44c	0.35a	0.34a	0.33a	0.33a	0.40b	0. 24a	0.39b	0.40b
Elaidic	0.18a	0.19a	0.31a	0.22a	0.26b	0.27b	0.23a	0.20a	0.33b	0.33b	0. 22a	0.16a
t-Linoleic	0.10a	0.09a	0.14a	0.15a	0.10a	0.10a	0.12a	0.12a	0.12a	0.12a	0. 09a	0.16a
Brassidic	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a

For each parameter and time of analysis, different letters denote significant differences among types of sausages. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Buckley et al., 1989). In our case, no problems have been found in relation to the oxidation process during ripening, may be because, the enrichment of $n-3$ PUFA was achieved basically by the addition of triglycerides, whereas, the enrichment by dietary treatment modified both triglycerides and phospholipids of meat.

The mentioned fatty acid modifications gave rise to some differences in the total fatty acids fractions, SFA, MUFA and PUFA, supplied by the product (Table 3). Decreases in Σ SFA and Σ MUFA are observed in both modified products, reaching the lowest amounts in those with antioxidants. Comparing these sums with those shown by sausages with 25% substitution of pork back fat with soy oil (Muguerza et al., 2003) it is clear that, referred to the respective control, the decreases for both fractions were lower when linseed oil was used. Also, the increase of the PUFA fraction was lower for linseed oil-added products. However, sausages with linseed oil plus antioxidants showed the highest decrease in SFA and the highest increase of PUFA. The P/S ratio rose significantly in modified sausages with linseed oil and, in the case of sausages with antioxidants, was nearly twice the value found for the control. It has to be taken into account that the increase in this ratio in relation to control, is basically due to the increase in α-linolenic acid. Effectively, the $n-6/n-3$ ratio decreased from 13.5 in control products to 1.80–1.93 in modified products as a consequence of the α-linolenic acid increment. Evidently the dietary $n-6/n-3$ ratio can influence the pig muscle $n-6/n-3$ ratio of polar and neutral lipids (Hogberg, Pickova, Andersson, & Lundstrom, 2003). When modifications of this ratio are obtained by feeding pigs with diets containing linseed, $n-6/n-3$ ratios of meats are very diverse. Some authors found values around 3.5–4.5 in muscle (Leskanich et al., 1997; Rey et al., 2001), 1.34 in subcutaneous fat (D' Arrigo et al., 2002a, 2002b) and 4.9–4.6 in sausages elaborated from raw modified meats (Leskanich et al., 1997; Enser et al., 2000).

The aroma of dry-fermented sausages is greatly influenced by volatile compounds resulting from the oxidation of the lipid fraction during ripening (Mateo & Zumalacárregui, 1996; Edwards, Ordóñez, Dainty, Hierro, & Hoz, 1999; Ansorena, Astiasarán, & Bello,

Table 3
Lipid fractions (g/100 g fatty acids) and ratios of nutritional interest obtained for the dry-fermented sausages ripened for 30 days

	Control	Linseed	Linseed + antioxidants
Σ SFA	41.7	36.4	34.8
Σ MUFA	42.3	40.7	39.5
Σ PUFA	15.3	22.3	24.3
P/S	0.4	0.6	0.7
$n-6/n-3$	14.1	1.7	2.1

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

P/S, Polyunsaturated fatty acids/saturated fatty acids.

Table 4
Lipid oxidation parameters of the control and modified sausages at the end of the ripening process

	Control	Linseed	Linseed + antioxidants
TBA (mg malonaldehyde/kg sample)	0.08a	0.23b	0.08a
Peroxides (meq O_2 /kg fat)	2a	0.83a	0a
Hexanal (ng dodecane/g dm)	226.5a	309b	219a
Octenal (ng dodecane/g dm)	60b	66.5b	33.5a
Nonanal (ng dodecane/g dm)	292a	592b	314a
Tt2,4-decadienal (ng dodecane/g dm)	25.5b	22.5b	0a
2,4-decadienal (ng dodecane/g dm)	105c	91.5b	0a

Different letters in the same row denote significant differences among types of sausages. dm, dry matter.

2000). Lipid oxidation parameters and some of the most common volatile lipid-derived compounds are gathered in Table 4. TBA and peroxides showed very low values in the three analyzed products. Modified sausages without antioxidants showed a significantly higher value for TBA than did the control. Also, hexanal (from oxidation of linoleic) and nonanal (from oxidation of oleic acid) were in significantly higher amounts for modified sausages without antioxidants, these results being in agreement with TBA values. Different results were found (in relation to the effect of enrichment of meats with $n-3$ PUFA) for degree of lipid oxidation and development of volatile compounds. No effects were found on flavour on, in general, on sensorial meat quality (Enser et al., 2000; Sheard et al., 2000; Leskanich et al., 1997; Melton, 1990). On the other hand, Elmore, Mottram, Enser, and Wood (1999, 2000), studying the effects of α-linolenic acid enrichment on the volatile compounds of cooked beef and lamb, found that there were significant increases in some of the lipid oxidation products. These authors concluded that autoxidation appeared to be prompted by increased levels of PUFAs. Octenal and 2,4-decadienal did not show significant differences between control and modified products without antioxidants. The addition of antioxidants maintained low values for all compounds, and especially avoided the formation of dienals, characterized by their off-flavour. It is notable, however, that the concentrations of these compounds in control and linseed-containing sausages were not considered high, being within the range of commercial sausages (Ansorena, Gimeno, Astiasarán, & Bello, 2001). Although no sensorial evaluation was carried out by a trained panel on the analyzed products, no differences were observed in appearance and odour among the three batches.

In conclusion, the results obtained in this work indicated, that the addition of linseed oil to the formulation of dry-fermented sausages has a relevant influence on the nutritional quality of the products, without substantially modifying the flavour and oxidation status of the ready-to-eat products. However, more research is needed to substantiate evolution of the lipid oxidation process during the shelf life of linseed-containing sausages.

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