TITLE

FUNCTIONAL DRY FERMENTED SAUSAGES MANUFACTURED WITH HIGH LEVELS OF N-3 FATTY ACIDS: NUTRITIONAL BENEFITS AND EVALUATION OF OXIDATION.

AUTHORS' NAMES

EDURNE MUGUERZA, DIANA ANSORENA, and ICIAR ASTIASARÁN.[√]

Department of Food Science and Technology, University of Navarra, Faculty of Pharmacy, 31008-Pamplona, Spain.

[√]Corresponding author. Tel.: +34-48-425600 ext 6405; fax: +34-48-425649.

E-mail address: iastiasa@unav.es (I. Astiasarán).

ABSTRACT

Dry fermented sausages were manufactured adding fish oil extracts rich in *n*-3 fatty acids in order to obtain functional products and their nutritional advantages and intensity of oxidation process compared with traditional ones. Modified products were manufactured with 0.53% (batch A) and 1.07% (batch B) of fish oil extract. The amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) per 100g of dry fermented sausages were 0.15g and 0.13g in batch A, 0.33g and 0.26g in batch B, respectively, in contrast with the control products which only showed 0.01g EPA and 0.03g DHA. No significant differences were found in the rest of fatty acids. A decrease in the *n*-6/*n*-3 ratios from 16.14 in the control to 7.78 in batch A and 5.32 in batch B was achieved. TBA values were similar in control (0.31ppm) and in batch A products (0.34ppm) but increase significantly in batch B products (1.22ppm). No statistical differences were observed among batches for the content of cholesterol oxidation products (2.36 to 2.43 μg/g fat) leading to similar percentages of oxidation. 7-Ketocholesterol, considered as an indicator of oxidation was not present in any sample. Values obtained for L* and Hue (arctg b*/a*) were comparable to that of meat products. Although no effect was observed in COP formation and instrumental measurements of colour the high level of *n*-3 fatty acid seems to accelerate significantly the oxidation process.

RUNNING TITLE

Functional *n*-3 fatty acid containing dry fermented sausages

KEYWORDS

Dry fermented sausages, n-3 fatty acids, oxidation, colour, COP.

INTRODUCTION

Coronary heart disease (CHD) is a leading cause of mortality and chronic morbidity throughout the developed and developing world. It is widely accepted that consumption of different types of fatty acids has a role in the aetiology of this disease. Furthermore, available data strongly support the strategy of replacing animal and hydrogenated fat with natural liquid vegetable oils, which contain monounsaturated and polyunsaturated fats, in reducing risk of CHD[1].

Traditional meat products are a source of cholesterol and saturated fatty acids (SFA), subjects of concern from the nutritional point of view, particularly with regard to CHD. Many works have been done in order to obtain healthier meat and meat products, some of them focused on decreasing their fat content, and in order to develop new meat products that could be also considered as functional foods[2]. Last researches pointed out that replacing saturated fat with unsaturated fat is probably more effective in lowering risk of CHD that simply reducing total fat consumption[3]. Vegetable oils with a high content of monounsaturated fatty acids (MUFA) have been used as partial substitutes of pork backfat in low-fat frankfurters and other type of cooked products and in a lesser extent in dry fermented sausages[4-12]*)*. In general, significant differences have been found in the fatty acid profiles and cholesterol content leading to nutritional advantages.

Vegetable oils with high content in polyunsaturated fatty acids (PUFA) have also been used to substitute the fat content in cooked sausages[13-15], detecting in some cases problems related to oxidation processes. One of these problems is the colour deterioration by the oxidation of myoglobin to metmyoglobin which results in a brown, unacceptable discolouration of the meat. Colour deterioration and lipid oxidation may be linked, although the precise mechanisms are still unclear[16]. Furthermore, the oxidation of PUFA would induce cholesterol oxidation[17-18]. It is known that cholesterol oxidation products (COP) are clearly involved in the development of cardiovascular

diseases[19], and are also associated with the development of some biological effects like cytotoxicity, mutagenesis and carcinogenesis[20- 22]. Furthermore, some experiments in human show their ability to be absorbed from the diet[23-24].

In today's Western diets, high intakes of *n*-6 fatty acids lead to undesirable ratios (*n*-6/*n*-3) of about 15/1-16.7/1, if not balanced with an appropriate *n*-3 intake[25]. It is known that diets with high content of *n*-3 fatty acids, which are know to be functional products, may reduce plasma levels of tryglicerides, may decrease the platelet aggregation, may have an antiarritmic effect and a beneficial effect in the endothelial disfunction[26-28]. The risk of hypertension, type 2 diabetes and in some cases renal disease, rheumatoid arthritis, ulcerative colitis, Crohn disease and chronic obstructive pulmonary disease can also be reduced with this type of diets[29-31]. The optimal dose or ratio of *n*-6/*n*-3 varies from 1/1 to 4/1 depending on the disease under consideration[25]. Consequently, taking into account the benefits of a low ratio *n*-6/*n*-3 in reducing risk of many diseases, it seems to be interesting to try to increase the dietary *n*-3 fatty acids intake. It has been suggested that foods that are strategically or naturally enriched in *n*-3 fatty acids can be used to achieve desired biochemical effects without the ingestion of supplements or a change in dietary habit[32].

Concerning meat, dietary administration of fish meals or fish oils as a good source of *n*–3 fatty acids to different species of animals (pork, lamb, chicken) has been carried out[33-39]. The direct addition of fish oil to meat products as a way to increase their *n*-3 supply has scarcely been investigated. Park et al.[40] used fish oil at a concentration of 5% in pork frankfurter type sausages, obtaining unacceptable products due to their undesirable fish flavour. No papers have been found in relation to the incorporation of fish oil as a source of *n*-3 fatty acids to dry fermented sausages.

The aim of this work was to study the potential nutritional advantages derived from the addition of different amounts of fish oil extracts to dry fermented sausages analyzing

the repercussion over the intensity of oxidation process through COP formation and colour evaluation of the products. It has to be stated up that from the economical point of view the addition of a fish oil extract rich in *n*-3 fatty acids would not be viable in this type of foodstuffs. Furthermore, the study did not include sensory analysis because this type of extracts, even when employed in small amounts could contribute to off odours, so flavour and taste of the modified products would be probably affected. However, these experiments could be an easy approach to study the potential consequences of the fatty acid profile changes in raw matter got through another ways as animal production practices.

EXPERIMENTAL

Sausage manufacture.

Chorizo de Pamplona, a type of traditional Spanish fermented sausage, was manufactured following the procedure described by Muguerza et al.[11]. Three batches of fermented sausages, about 5 Kg each, were prepared using 75% pork meat and 25% pork backfat. Amounts of 0.53g/100g and 1.07g/100g of a fish oil extract (Omega-3 "700" SOLGAR VITAMINS LTD, UK)were added to batch A and B, respectively. No fish oil extract was added to the control batch.

Chemical analysis.

Moisture was determined according to the Association of Official Analytical Chemist method (AOAC)[41]. Total fat was determined by an extraction technique with petroleum ether according to AOAC[42.] Protein was analyzed according to the Kjeldahl method[43]. Factor 6.25 was used for conversion of nitrogen to crude protein. Peroxide value was determined using method AOAC[44].

The method of Folch et al.[45] was used for the extraction of lipids. Fatty acid composition of fish oil extract and dry fermented sausages manufactured were determined by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters[46]. A Perkin-Elmer Autosystem XL gas chromatograph fitted with a capillary column SP^{TM}-2560 (100 m x 0.25 mm x 0.2 μm) and flame ionization detection was used. The temperature of both the injection port and detector was 220ºC. the oven temperature was programmed to increase from 165 to 220ºC at a rate of 4ºC/min. The carrier gas was hydrogen, 20. psi. The sample size was 0.5 μl. The quantification of individual fatty acids was based on heptadecanoic acid methyl ester (Sigma, St. Louis, MO, USA) as internal standard.

Cholesterol content was analyzed by gas chromatography with previous extraction according to Kovacs et al.[47]. A Perkin-Elmer Autosystem gas chromatograph equipped with an HP1 column (30 m x 0.25 mm x 0.1 μm) was used. The oven temperature was 265ºC. The temperature of both the injection port and detector was 285ºC. The sample

size was 0.5 μl. Cholesterol was identified by comparing its relative and absolute retention times with those of cholestane (sigma) as an internal standar. A Perkin-Elmer Turbochrom programme was used for quantification.

TBA value was determined according to Tarladgis et al.[48] with modifications by Tarladgis et al.[49].

COP: Extraction of lipids and saponification. Saponification was made according to the method of Guardiola et al.[50]. Approximately 1g of fat was added to a flask containing 1M KOH in methanol and kept at room temperature during 22 h to complete the cold saponification. The unsaponificable material was extracted with diethyl ether. The whole organic extract was washed with water and filtered through anhydrous sodium sulfate. Then it was recovered in a round-bottom flask, and the solvent was evaporated using a rotatory vacuum evaporator at 30ºC. A purification with silica cartridges and a derivatization to obtain the trimethyl silyl ethers was performed. Oxysterols content was determined by a Hewlett-Packard 6980 GC with 5973 Mass selective Detector. GC-MS analyses were performed in HP-5MS column (30m X 250um X 0.25 um). Helium was used as the carrier gas (1ml/minute) and the chromatographic conditions were as follows: initial column temperature at 80ºC, held for 1 min and programmed to 250ºC at a rate of 10ºC/min and final column temperature of 280ºC at a rate of 4ºC/min and held for 20 min. The injector temperature was 250ºC and the inlet pressure was 23.2 psig; mass range, m/z=50/550 amu; solvent delay is 20 minutes.

Instrumental measures.

For colour measurement, samples were sliced and covered with a polyethylene film, with pressure to obtain a uniform, bubble-free surface. A UV/VIS Perkin-Elmer Lambda 5 spectrophotometer was used to obtain the reflectance spectra from 400 to 700 nm using an integrating sphere. Colour coordinates were obtained with the conditions established by Ansorena et al.[51] (CIE L* a* b* system, angle 10º, illuminant D65). L*, a* and b* parameters indicate lightness, redness and yellowness, respectively. Chroma and Hue were calculated. Each sample was measured at four locations on the slice surface of the dry fermented sausages.

Statistical analysis.

Four samples from each batch were analyzed. Each parameter was determined four times in each sample. Data shown in the tables are the means, standard deviation and coefficient of variation. Analysis of variance (ANOVA) and a Tuckey's b posteriori test were used to determine significant differences ($p \leq 0,05$) among the different types of sausages. Software used was SPSS version 9.0. (© 1998, SPSS inc. Chicago, Illinois).

RESULTS AND DISCUSION

The composition of the extract employed is shown in table 1. Batch A was manufactured adding 5.3g of oil extract by kg of raw mixture and Batch B was manufactured with 10.7 g of fish oil extract per kg of raw mixture. Taking into account the profile of fish oil extract the added amounts of the extract supplied 0.25g/100g raw mixture of eicosapentaenoic acid (EPA) and 0.14g/100g of docosahexaenoic acid (DHA) in batch A and 0.51g/100g EPA and 0.29g/100g DHA in batch B.

The small amount of oil extract added to batches A and B did not increase, as it was expected, the fat content of final products. Table 2 shows that no significant differences were found between the fat content of control and sample B. In the case of batch A, the lower amount of fat compared to the control and sample B is explained by the higher moisture content of those products. No differences were appreciated in the protein content.

Fatty acid profiles of the three types of sausages are shown in table 3. The only significant differences between modified and control products were observed in the percentages of EPA and DHA. The high amounts of those fatty acids in the fish oil extract added (table 1) lead to those increments. Batches A samples increased 10.7 folds the percentage of EPA and 4.9 folds the percentage of DHA in relation to control samples. Batches B samples increased 21.5 folds the percentage of EPA and 9.54 folds the percentage of DHA. The amounts of both *n*-3 fatty acids refered to 100g of dry fermented sausage were 0.01g EPA and 0.03g DHA for control products, 0.15g EPA and 0.13g DHA for batch A and 0.33g EPA and 0.26g DHA for batch B. The supply of *n*-3 by salmon, which is the richest food in *n*-3 among the usually consumed species is 0.89g EPA/100g and 1.12g DHA/100g.

There are still not enough data to determine RDAs for these important fatty acids. A group of scientist have stablished that the adequate intake (AI) for adults was 0.22g/day

for both EPA and DHA in a 2000kcal/diet[25]. Taking into account these values, 50 g of modified sausages, which is the average eaten portion of dry fermented sausages[52], would cover approximately 34% and 30% of the AI for DHA and EPA, respectively in the case of batch A, and 75% and 59% of the AI in the case of batch B. So it can be considered that modified products could contribute in a significant way to the nowadays established dietary intake of EPA and DHA.

As a consequence of the similar amount in the rest of the fatty acids the sum of SFA, MUFA and PUFA scarcely differed between control and modified products. PUFA/SFA ratio did neither show big differences among products, being in the three cases around 0.5-0.6. However, the increase in *n*-3 fatty acids led to an important decrease in the ratio *n*-6/*n*-3. Whereas control sausage showed a ratio of 16.14, modified batches fell to values of 7.78 and 5.32, closer to the considered optimum.

Some studies suggest that the *n*-6/*n*-3 ratio may not be as important as the absolute amount of *n*-3 or *n*-6 fatty acids in the diet because both fatty acids can influence the atherosclerotic process through multiple biological mechanisms, being the prostanoid pathway only one of them[1]. In modified products, the absolute amounts of *n*-3 were much higher (1.12g/100g of total fatty acid in control, 2.03g/100g of total fatty acid in batch A and 3.21g/100g of total fatty acid in batch B) and the *n*-6/*n*-3 were much lower.

The sum of *trans* fatty acids ranged between 1.07 and 1.22g/100g of total fatty acid without showed higher values. None of them showed significant differences among the different products.

The potential enhancement of the oxidation process as a consequence of the addition of the fish oil extract was analyzed through the parameters shown in table 4. None of the three analysed batches showed detectable peroxides content. In relation to TBA, different values were found depending on the percentage of fish oil extract added. TBA values for the control samples and samples with the lowest fish oil content (A) were

0.31ppm and 0.34ppm, respectively. These values were lower than 0.5ppm, considered as the threshold value for the appearance of rancidity off flavors in fresh pork[53-54]. However, the batch with the highest content of fish oil (B) showed 4 folds higher values of TBA (1.22ppm). With regard to TBA values and sensory evaluation, a great variability of results have been found in the literature for PUFA's enriched meat and meat products. Leskanich et al.[34] analyzing organoleptic acceptability of fresh sausages manufactured with meat of pigs fed with diets 1% fish oil (giving products containing around 0.50-0.52 g/100 fat of EPA and DHA) found that it was not different from control products, although TBARS were slightly higher. In pork meat of pigs fed with linseed enriched diets, levels of EPA and DHA in muscle were 0.7g/100g and 0.4g/100g, respectively, without showing oxidation problems[55]. In cooked lamb meat with increased levels of long chain *n*-3 fatty acids by means of feeding with a 1.5 % fish oil diet no rancid taste or unpleasant sensory notes were detected[37]. Frankfurter type sausages manufactured with meat from animals fed with a diet rich in PUFA and fish oil (0.4%) resulted in products with undesirable sensory notes due to rancidity, with a TBA value of 1.9[36].

Actually one of the main problems related to lipid oxidation in foods is the formation of COP. Li et al.[56] showed that the formation of COP was accelerated by the PUFA content present in lipids. Despite the higher oxidation process detected in batch B, no statistical differences were observed among the results for the content of COP (table 4). The most abundant COP in all samples was β-epoxycholesterol, followed by cholestanetriol, found as one of the most cytotoxic. 7-Ketocholesterol, considered to be a good indicator of oxidation[57-58] and α-epoxycholesterol were not present in any sample.

Total COP ranged from 2.36μg/g fat in the control products to 2.43μg/g fat for batch B. Taking into account the cholesterol content of the samples the percentage of oxidation

ranged from 0.08 to 0.09 without significant differences between them. These values were in agreement with those found by Zanardi et al.[59] for salami Milano, also another type of dry fermented sausage, in which the percentage of cholesterol oxidation ranged from 0.04 to 0.12. This product showed a very large range of total COP amount (1.42-16.59 ppm)[60]. In dry-cured ham with 12-14 months of aging total COP ranged from 2.8 to 5.8 ppm [61]. Comparing this amounts with those found in other type of foods as cooked turkey, and cooked beef[62] in which total COP can reach values of 93.1 and 32.7, respectively, it can be affirmed that the formation of COP in the control and modified sausages were low.

Table 5 shows the results of colour parameters for every analyzed product. Values obtained for L* ranged between 50.21-45.98, all of them within the normal range for this type of sausages (46.87-54.29)[63]. Values for Hue (arctg b*/a*) were comprised between 36.97 and 38.54. This results were comparable with those obtained by Ghiretti et al.[64] to Mortadella in which Hue values were comprised between 35.03 to 38.76. Zanardi et al.[65] found a correlation of 0.890 ($p<0.05$) between brown scores and TBAR's, in our case no correlations were found between TBA and any of the colour parameters.

CONCLUSION

An increment of the nutritional and functional value of dry fermented sausages was achieved through the addition of a fish oil extract rich in *n*-3 fatty acids. This addition to dry fermented sausages decreased significantly the *n*-6/*n*-3 ratio leading to closer values to the considered optimum. However, samples with the highest oil extract addition showed a great increase of TBA values indicating that oxidation process could be a problem during their shelf life. This consideration should be have into account when trying to increase *n*-3 fatty acids in raw meat production for the industry manufacturing.

AKNOWLEDGEMENTS

We thank PIUNA, Gobierno de Navarra (Departamento de Industria) and Ministerio de Ciencia y Tecnología and Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica I+D+I (project AGL 2001-0965) for their contribution to the financial support of this work.

REFERENCES

1. Hu F, The balance between *n*-6 and *n*–3 fatty acids and the risk of coronary heart disease. *Nutr* **17**: 741-742 (2001).

2. Jiménez-Colmenero F, Carballo J, Cofrades S, Healthier meat and meat products: their role as functional foods. *Meat Sci* **59**: 5-13 (2001).

3. Hu F.B, Manson JE, Willet WC, Types of dietary fat and risk of coronary heat disease: A critical review. *J Am Coll Nutr* **20**: 5-19 (2001).

4. Marquez EJ, Ahmed EM, West RL, Johnson DD, Emulsion stability and sensory quality of beef frankfurters produced at different fat or peanut oil levels. *J Food Sci* **54**: 867-870, 873. (1989).

5. Park J, Rhee KS, Ziprin YA, Low-fat frankfurters with elevated levels of water and oleic acid. *J Food Sci* **55**: 871-874 (1990).

6. Riendau L, Charcuterie allégée sur base de pré-émulsion D' Huile Végétale Essai: Sauccisse fumée porc et boeuf. *V.P.C.* **11**: 56-60 (1990).

7. Bloukas JG, Paneras ED, Substituting olive oil for pork backfat affects quality of low-fat frankfurters. *J Food Sci* **58**: 705-709 (1993).

8. Bloukas JG, Paneras ED, Fournitzis GC, Sodium lactate and protective culture effects on quality characteristics and shelf- life of low-fat frankfurters produced with olive oil. *Meat Sci* **45**: 223-238 (1997a.)

9. Pappa IC, Bloukas JC, Arvanitoyannis IS, Optimization of salt, olive oil and pectin level for low-fat frankfurters produced by replacing pork backfat with olive oil. *Meat Sci* **56**: 81-88 (2000).

10. Bloukas JG, Paneras ED, Fournitzis GC, Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Sci* **45**: 133-144 (1997b).
11. Muguerza E, Gimeno O, Ansorena D, Bloukas JG, Astiasarán I, Effect of replacing pork backfat with pre-emulsified olive oil on lipid fraction and sensory quality of Chorizo de Pamplona-a traditional Spanish fermented sausage. *Meat Sci* **59**: 251-258 (2001).
12. Muguerza E, Fista G, Ansorena D, Astiasarán I, Bloukas JG, Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Sci.* **61**: 397-404 (2002).
13. Paneras ED, Bloukas JC, Vegetables oils replace pork backfat for low-fat frankfurters. *J Food Sci* **59**: 725-733 (1994).
14. Papavergou EJ, Ambrosiadis JA, Psomas J, Storage stability of cooked sausages containing vegetable oils. *Zeitschrift fur lebensmittel-Untersuchung und-forschung*, **200**: 47-51 (1995).
15. Tan SS, Aminah A, Mohd Suria Affandi Y, Atil O, Babji AS, Chemical, physical and sensory properties of chicken frankfurters substituted with palm fats. *International J Food Sci Nutr* **52**: 91-98 (2001).
16. Renerre M, Review: factors involved in the discolouration of beef meat. *International J Food Sci Technol* **25**: 613-630 (1990).
17. Kortytowski W, Bachowski GJ, Girotti AW, Photoperoxidation of cholesterol in homogeneous solution, isolated membranes and cells: comparison of the 5α- and 6β-hydroperoxides as indicators of single oxygen intermediacy. *Photochem. Photobiol* **56**: 1-8 (1992).
18. Osada K, Kodama T, Cui L, Yamad K, Sugano M, Levels and formation of oxidized cholesterols in processed marine foods. *J Agric Food Chem* **41**: 1893-1989 (1993).

19. Smith LL, Review of progress in sterol oxidations: 1987-1995. *Lipids* **31**: 433-487 (1996).
20. Guardiola F, Codony R, Addis PB, Rafecas M, Boatella J, Biological effects of oxysterol: current status. *Food Chem Toxicol* **34**: 193-211 (1996).
21. Kubow S, Toxicity of dietary lipid peroxidation products. *Trends Food Sci Technol* **1**: 67-70 **(**1990).
22. Sevanian A, Peterson AR, The cytotoxic and mutagenic properties of cholesterol oxidation products. *Food Chem Toxicol* **24**: 1103-1110 (1986).
23. Emanuel HA, Hassel CA, Addis PB, Bergmann SD, Zavoral JH, Plasma cholesterol oxidation products (oxysterols) in human subjects fed a meal rich in oxysterols. *J Food Sci* **56**: 843-847 (1991).
24. Linseisen J, Wolfram G, Absorption of cholesterol oxidation products from ordinary foodstuff in humans**.** *Ann Nutr Metab* **42**: 221.230 (1998).
25. Simopoulos AP, The importance of the ratio o omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother* **56**: 365-379 (2002).
26. Harris W, Fish oils and plasma lipid & and lipoprotein metabolism in humans: a critical review. *J lipid Res* **30**: 785-807 (1989).
27. Von Shacky C, N-3 fatty acids and the prevention of coronary atherosclerosis. *Am. J Clin Nutr* **71**: 224-227 (2000).
28. Kang JX, Leaf A, Prevention of fatal cardiac arrhytmias by polyunsaturated fatty acids. *Am J Clin Nutr* **71**: 202-207 (2000).
29. Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J, Spur BW, Robinson DR, Corey EJ, Lewis RA, Austen KF, Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophyl and monocyte leukotriene generation and neutrophyl function. *N Engl J Med* **312**: 1217-1224 (1985).

30. Phillipson BE, Rothrock DW, Connor WE, Harris WS, Illingworth DR, Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertrigliceridemia. *N Engl J Med* **312**: 1210-1216 (1985).
31. Simopoulos A.P. Essential fatty acids in health and chronic disease. *Am J Clin Nutr* **70**: 560-569 (1999).
32. Mantzioris E, Cleland LG, Gibson RA, Neumann MA, Demasi M, James MJ, Biochemical effects of a diet containing foods enriched with n-3 fatty acids. *Am J Clin Nutr* **72**: 42-48 (2000).
33. Overland M, Tangbol O, Haug A, Sundstol E, Effect of fish oil on growth performance, carcass characteristics, sensory parameters and fatty acid composition in pigs. *Acta Agric Scand* **46**: 11-17 (1996).
34. Leskanich CO, Natthews KR, Warkup CC, Noble RC, Hazzledine M, The effect of dietary oil containing (n-3) fatty acids on the fatty acid, physicochemical, and organoleptic characteristics of pig meat and fat. *J Anim Sci* **75**: 673-683 (1997).
35. Lopez-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA, n-3 enrichment of chicken meat.1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. *Poultry Sci* **80**: 741-752 (2001).
36. Bryhni EA, Kjos NP, Ofstad R, Hunt M, Polyunsaturated fat and fish oil in diets for growing-finishing pigs: effects on fatty acid composition and meat, fat, and sausage quality. *Meat Sci* **62**: 1-8 (2002).
37. Ponnampalam EN, Sinclair AJ, Egan AR, Ferrier GR, Leury BJ, Dietary manipulation of muscle long-chain omega-3 and omega-6 fatty acids and sensory properties of lamb meat. *Meat Sci* **60**: 125-132 (2002).
38. Lin JH, Lin YH, Kuo CC, Effect of dietary fish oil on fatty acid composition, lipid oxidation and sensory property of chicken frankfurters during storage. *Meat Sci* **60**: 161-167 (2002).

39. Enser M, Richardson RI, Wood JD, Gill BP, Sheard PR, Feeding linseed to increase the *n*-3 PUFA of pork, fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Sci* **55**:201-212 (2000).

40. Park J, Rhee KS, Keeton, JT, Rhee KC, Properties of low-fat frankfurters containing monounsatured and omega- 3 polyunsaturated oils. *J Food Sci* **54**: 500-504 (1989).

41. AOAC, Determination of moisture content. 950.46. *In Official methods of analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists. (2002a).

42. AOAC, Fat (crude) or ether extract in meat. 960.39. *In Official methods of analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists. (2002b).

43. AOAC, Crude protein in meat. 928.08. *In Official methods of analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists. (2002c).

44. AOAC, Determination of peroxide content. 965.33. *In Official methods of analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists. (2002d).

45. Folch J, Lees M, Stanley GHS, A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**: 497-509 **(**1957).

46. AOAC, Preparation of methyl ester. 969.33. *In official methods of analysis* (17th ed.) Gaithersburg, Maryland: Association of official analytical Chemists. (2002e).

47. Kovacs MIP, Anderson WE, Ackman RG, A simple method for the determination of cholesterol and some plant sterols in fishery-based food products. *J Food Sci* **44**: 1299-1301, 1305. (1979).

48. Tarladgis BG, Watts BM, Younathan MT, Dugan, LR, Jr, A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J Am Oil Chem Soc* **37**: 44-48. (1960).

49. Tarladgis BG, Pearson AM, Dugan LL, Jr, Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. *J Sci Food Agric* **15**: 602-607 (1964).
50. Guardiola FR, Codony PB, Rafecas M, Boatella J, Comparison of three methods for the determination of oxysterols in spray-dried egg. *J chromatogr A* **705**: 289-304. (1995).
51. Ansorena D, De Peña MP, Astiasarán I, Bello J, Colour evaluation of chorizo de Pamplona, a Spanish dry fermented sausage: comparison between the CIE lab* and the Hunter Lab systems with illuminants D65 and C. *Meat Sci* **46**: 313-318 (1997).
52. Muñoz M, Aranceta J, García-Jalón I, *Nutrición Aplicada y dietoterapia*. Ed. EUNSA, Pamplona. (1999).
53. Gray JI, Pearson AM, Rancidity and warmed-over flavor. In AM, Pearson TR Dutson, *Advances in meat research, Restructured meat and poultry products,* Ed by Van Nostrand Reinhold Co. New York, pp. 221-269 (1987).
54. Lanari MC, Schaefer DM, Cassens RG, Scheller KK, Atmosphere blooming time affect colour and lipid stability of frozen beef from steers supplemented with vitamin E. *Meat Sci* **40**: 33-44 (1995).
55. Sheard PR, Enser M, Wood JD, Nute GR, Gill BP, Richardson RI, Shelf life and quality of pork and pork products with raised n-3 PUFA. *Meat Sci* **55**: 213-221 (2000).
56. Li N, Ohshima T, Shozen KI, Ushio H, Koizumi C, Effects of the degree of unsaturation of coexisting triacylglycerols on cholesterol oxidation. *J Am Oil Chem Soc* **71**: 623-627 (1994).
57. Zunin P, Boggia R, Evangelisti F, Identification and quantification of cholesterol oxidation products in canned tuna. *J Am Oil Chem Soc* **78**: 1037-1040 (2001).

58. Penazzi G, Caboni MF, Zunin P, Evangelisti E, Tisconia E, Gallina Toschi T, Lecker G, High performance liquid chromatographic determination of free 7-ketocholesterol in some foods by two different analytical methods. *J Am Oil Chem Soc* **72**:1523-1527 (1995).
59. Zanardi E, Novelli E, Ghiretti GP, Chizzolini R, Oxidative stability of lipids and cholesterol in salame Milano, coppa and Parma ham: dietary supplementation with vitamin E and oleic acid. *Meat Sci* **55**: 169-175 (2000).
60. Novelli E, Zanardi E, Ghiretti GP, Campanini G. Dazzi G, Madarena G, Chizzolini R, Lipid and cholesterol oxidation in frozen stored pork, salame milano and mortadella. *Meat Sci* **48**: 29-40 (1998).
61. Vestergaard CS, Parolari G, Lipid and cholesterol oxidation products in dry-cured ham. *Meat Sci* **52**: 397-401 (1999).
62. Du M, Nam KC, Ahn DU, Cholesterol and lipid oxidation products in cooked meat as affected by raw-meat packaging and irradiation and by cooked-meat packaging and storage time. *J Food Sci* **66**: 1396-1401 (2001).
63. Gimeno O, Ansorena D, Astiasarán I, Bello J, Characterization of chorizo de Pamplona: instrumental measurements of colour and texture. *Meat Sci* **69**: 195-200 (2000).
64. Ghiretti GP, Zanardi E, Novelli E, Campanini G, Dazzi G, Madarena G, Chizollini R, Comparative evaluation of some antioxidants in salame milano and mortadella production. *Meat Sci* **47**: 167-176 (1997).
65. Zanardi, E, Dorigoni, V, Badiani, A; Chizzolini, R. Lipid and colour stability of Milano-type sausages: effect of packing conditions. *Meat Sci.* **2002.** *61*, 7-14.

Table 1. Fatty acid profile of fish oil extract employed (g/100g of total fatty acid)

SFA	MUFA	PUFA
C14:0 Myristic 0.55 (0.03; 4.82)	C16:1 Palmitoleic 3.33 (0.13; 3.80)	C18:2 Linoleic 1.33 (0.06; 4.41)
C16:0 Palmitic 2.15 (0.07; 3.37)	C18:1 Oleic 8.59 (0.28; 3.21)	C18:3 α-Linolenic 0.83 (0.09; 10.49)
C18:0 Stearic 0.81 (0.04; 4.53)		C20:4 Araquidonic 2.13 (0.06; 2.68)
		C20:5 EPA 49.07 (0.40; 0.82)
		C22:6 DHA 28.03 (0.71; 2.52)
TOTAL.....3.51	TOTAL.....11.92	TOTAL.....…….81.39

Values between parenthesis are (standard deviation, SD; coefficient of variation CV).

Table 2. Results for moisture, fat an protein of the different products: Control (traditional formulation); A and B (products manufactured with the fish oil extract).

	Control	**A**	**B**
Moisture (%)	29.77^{a} (0.44; 1.48)	32.75^{b} (0.39; 1.19)	30.03^{a} (0.13; 0.43)
Fat (%)	32.73^{b} (0.89; 2.72)	29.22^{a} (0.62; 2.12)	33.35^{b} (1.04; 3.12)
Protein (%)	26.48^{a} (0.86; 3.25)	26.18^{a} (1.12; 4.28)	26.64^{a} (0.74; 2.78)

Values in the same row bearing different letters are significantly different ($p \leq 0.05$). Values between parenthesis are (standard deviation, SD; coefficient of variation CV).

Table 3. Fatty acid content (g per 100g of total fatty acid) in dry fermented sausages manufactured with traditional formulation (Control) and with the fish oil extract (A and B)

	Control	**A**	**B**
Lauric 12:0	0.14[a] (0.00; 3.24)	0.14[a] (0.01; 6.02)	0.14[a] (0.01; 5.40)
Myristic 14:0	1.34[a] (0.03; 1.89)	1.36[a] (0.04; 3.22)	1.31[a] (0.06; 4.31)
Palmitic 16:0	21.94[a] (0.16; 0.75)	22.17[a] (0.74; 3.35)	21.27[a] (0.39; 1.84)
Stearic 18:0	11.37[a] (0.05; 0.46)	11.26[a] (0.40; 3.56)	11.29[a] (0.07; 0.58)
Arachidic 20:0	0.27[a] (0.01; 4.52)	0.26[a] (0.02; 8.24)	0.30[a] (0.04; 13.82)
Behenic 22:0	0.36[a] (0.02; 5.01)	0.29[a] (0.10 32.95)	0.40[a] (0.06; 15.88)
Σ SFA	35.42	35.48	34.71
Palmitoleic 16:1	2.28[a] (0.01; 0.32)	2.39[a] (0.11; 4.60)	2.21[a] (0.08; 3.62)
Oleic 18:1	41.81[a] (0.20; 0.48)	42.77[a] (1.68; 3.93)	41.26[a] (0.27; 0.64)
Erucic 22:1	0.23[a] (0.00; 0.29)	0.33[a] (0.15; 45.20)	0.31[a] (0.01; 3.41)
Σ MUFA	44.32	45.49	43.78
Linoleic 18:2 *n*-6	17.94[a] (0.13; 0.73)	15.65[a] (3.15; 20.12)	16.93[a] (0.10; 0.59)
α-Linolenic 18:3 *n*-3	0.95[a] (0.07; 7.37)	0.85[a] (0.03; 4.08)	0.87[a] (0.01; 0.90)
Arachidonic 20:4 *n*-6	0.14[a] (0.00; 0.71)	0.14[a] (0.04; 31.26)	0.16[a] (0.01; 4.74)
EPA 20:5 *n*-3	0.06[a] (0.01; 10.24)	0.64[b] (0.03; 5.36)	1.29[c] (0.05; 4.25)
DHA 22:6 *n*-3	0.11[a] (0.01; 7.83)	0.54[b] (0.03; 4.82)	1.05[c] (0.03; 2.58)
Σ *n*-3	1.12	2.03	3.21
Σ *n*-6	18.08	15.79	17.09
Σ PUFA	19.20	17.82	20.30
PUFA/SFA	0.54	0.50	0.58
n-6 / *n*-3	16.14	7.78	5.32
Palmitelaidic *trans* 16:1	0.43[a] (0.00; 0.70)	0.47[a] (0.03; 6.38)	0.44[a] (0.01; 1.24)
Elaidic *trans* 18:1	0.23[a] (0.03; 12.18)	0.23[a] (0.07; 31.92)	0.17[a] (0.03; 17.98)
Linolelaidic *trans* 18:2	0.12[a] (0.01 11.52)	0.16[a] (0.01; 6.86)	0.15[a] (0.03; 22.35)
Brassidic *trans* 22:1	0.29[a] (0.08; 27.45)	0.36[a] (0.13; 36.21)	0.45[a] (0.02; 5.18)
Σ Trans	1.07	1.22	1.21

SFA: saturated fatty acid; **MUFA**: monounsaturated fatty acid; **PUFA**: polyunsaturated fatty acid. **EPA**: Eicosapentaenoic; **DHA**: Docosahexaenoic. Values in the same row bearing different letters are significantly different ($p \leq 0.05$).Values in the parenthesis are (standard deviation, SD; coefficient of variation CV).

Table 4. Cholesterol (mg/100g product), Cholesterol Oxides Products (COP) (μg/g fat) and oxidation parameters of the different products: Control (traditional formulation); A and B (manufactured with the fish oil extract)

	Control	**A**	**B**
Peroxide index (meq O_2/Kg fat)	n.d	n.d	n.d
TBA value (ppm)	0.31[a] (0.01; 3.22)	0.34[a] (0.01; 2.94)	1.22[b] (0.02; 1.64)
7α-Hydroxycholesterol	0.01[a] (0.008; 8.36)	0.03[a] (0.002; 9.71)	0.02[a] (0.002; 12.08)
7β-Hydroxycholesterol	0.24[a] (0.02; 6.90)	0.25[a] (0.02; 9.93)	0.26[a] (0.03; 13.34)
β-Epoxycholesterol	1.38[a] (0.12; 8.46)	1.38[a] (0.09; 6.63)	1.44[a] (0.20; 14.01)
α-Epoxycholesterol	0[a]	0[a]	0[a]
Cholestanetriol	0.58[a] (0.007; 1.15)	0.57[a] (0.001; 0.24)	0.57[a] (0.01; 2.27)
25-Hydroxycholesterol	0.15[a] (0.003; 1.93)	0.14[a] (0.004; 3.01)	0.14[a] (0.01; 8.33)
7-Ketocholesterol	0[a]	0[a]	0[a]
Total COP	***2.36***	***2.37***	***2.43***
Cholesterol	94.56[a] (9.22; 9.75)	90.59[a] (2.13; 2.35)	91.38[a] (3.78; 4.14)
% Oxidation	0.08	0.09	0.08

Values in the same row bearing different letters are significantly different ($p \leq 0.05$). Values in the parenthesis are (standard deviation, SD; coefficient of variation CV) n.d.: non detected

Table 5. Mean values for colour (CIE L*a*b* system) measures in dry fermented sausages manufactured with traditional formulation (Control) and with the fish oil extract (A and B)

	Control	**A**	**B**
L*	46.07[a] (2.77; 6.02)	50.21[b] (1.00; 1.98)	45.98[a] (1.69; 3.67)
a*	14.62[b] (1.09; 7.43)	10.19[a] (1.15; 11.32)	12.50[ab] (1.04; 8.32)
b*	11.65[c] (1.84; 13.97)	7.67[a] (0.47; 6.16)	9.93[b] (1.02; 10.3)
Chroma	18.69	12.75	15.96
Hue	38.54	36.97	38.46

Values in the same row bearing different letters are significantly different ($p \leq 0.05$). Values in the parenthesis are (standard deviation, SD; coefficient of variation CV).