TITLE: Evaluation of the oxidation during the shelf life of dry fermented sausages elaborated with olive oil and antioxidants, and stored in different packing conditions.

AUTHORS: Diana Ansorena (*) and Iciar Astiasarán

Affiliations: Departamento de Bromatología, Tecnología de Alimentos y Toxicología Facultad de Farmacia, Universidad de Navarra, 31080-Pamplona, Spain.
Phone: 948-425600. Fax 948-425649. E-Mail: dansorena@unav.es

*To whom correspondence should be addressed

ABSTRACT

Dry fermented sausages produced by a partial substitution of pork backfat with preemulsified olive oil were manufactured and stored (2 and 5 months) using different packing conditions (aerobic/ vacuum piece/ vacuum slices) in order to evaluate the intensity of the oxidation process. Also the effect of the addition of BHT and BHA to one of the modified batches was studied. Addition of olive oil, especially with antioxidants, was more effective than using vacuum storing methods in avoiding lipid oxidation during storage. After 5 months of storage at 4ºC, the combination of the increase in oleic acid and the preservation of PUFA by the antioxidant activity of the olive oil emulsion and antioxidants (when added), lead to better MUFA+PUFA/SFA ratios in olive oil containing sausages (1.90-1.98g/100g fatty acids) and particularly in antioxidants containing sausages (2.02-2.16g/100g) than in control ones (1.72g/100g). Vacuum packaging of the piece was the best method to minimise formation of lipid oxidation volatile compounds.

KEYWORDS: fatty acids, vacuum packaging, aldehydes, BHA and BHT.

7

1. Introduction

The interest in improving the nutritional quality of food by means of modifications of the lipid fraction through increasing the monounsaturated fatty acids fraction (MUFA) has lead to the development of a patent in which olive oil is included with lean meat and other ingredients as a substitute for animal fat in the manufacture of meat products (Domazakis, 2002). Furthermore, benefits other than nutritional have been attributed to olive oil. *In vivo* experiments have shown the protective effect of olive oil against LDL oxidation and against oxidative stress in human cells (Leenen et al., 2002; Manna et al., 2002).

The use of olive oil as a partial substitute for pork backfat in dry fermented sausages has been studied in relation to the possibilities of using different amounts of oil and also to the evaluation of the characteristics of the final product (Bloukas, Paneras & Fournitzis, 1997; Muguerza, Gimeno, Ansorena, Bloukas & Astiasarán, 2001; Muguerza, Fista, Ansorena, Astiasarán & Bloukas, 2002; Severini, De Pilli & Baiano, 2003; Muguerza, Ansorena, Bloukas & Astiasarán; 2003). The nutritional benefits achieved by this type of technological approach deal with the modification of the lipid profile of the sausages, increasing the MUFA and polyunsaturated fatty acid (PUFA) fractions and also decreasing the cholesterol content. In particular, when a 25% substitution was assayed in chorizo de Pamplona, the MUFA fraction increased from 14.07g/100g sausage in the control product to 16.26g/100g in the modified one, whereas the PUFA fraction increased from 3.68g/100g to 4.87g/100g sausage (Muguerza et al., 2002). MUFA and mainly PUFA are known to have hypocholesterolemic activity, being also inversely associated with risk of coronary heart disease, whereas the saturated fatty acid fraction (SFA) is positively associated with the development of CHD (Hu et al., 1997; Kris-Etherton & Yu, 1997).

Retail storage of dry fermented sausages is usually done in aerobic conditions, the product being exposed to oxygen and, in consequence, to a potential oxidation process. Dry fermented sausages being relatively high fat foodstuffs, lipid oxidation can damage their sensorial properties, by generation of degradation compounds such as n-alkenals and dienals, which are associated to rancid taste and odour. Oxidation can also affect the nutritional value of food by decomposition of vitamins, unsaturated essential fatty acids or can even give rise to toxic compounds. Furthermore, besides lipid oxidation, an excessive dehydration can also occur using traditional storage conditions, leading to economic losses because of the weight losses. Consequently, industrial trends are to try to extend the shelf life of this type of product by adopting other storage practices. Vacuum packaging has been proved an effective storage method to reduce oxidation in meat (Kerry, Buckley, Morrisey, O´Sullivan & Lynch, 1998) and in cooked pork sausages (Jo, Ahn & Byun, 2002) and it is being introduced as a commercial way for retail selling of dry fermented sausages (Fernández-Fernández, Rozas-Barrero, Romero-Rodríguez & Vázquez-Odériz, 1997; Fernández-Fernández, Vázquez-Odériz & Romero-Rodríguez, 2002).

Also addition of synthetic or natural antioxidants has been proposed to minimise lipid oxidation in sausages and other meat products, synthetic ones in most cases being more effective than natural ones (Gray, Gomaa & Buckley, 1996; McCarthy, Kerry, Kerry, Lynch & Buckley, 2001; Formanek, Kerry, Higgins, Buckey, Morrisey & Farkas, 2001; Tieko, Guaraldo, Azevedo & Beserra, 2003; Mielnik, Aaby & Skrede, 2003). Even butylhydroxytoluene (BHT) has been studied when incorporated in food packaging to be released during storage of products (Labuza & Breene, 1989).

The objective of this work was to evaluate the effectiveness of different storage conditions in controlling the lipid oxidation process of dry fermented sausages

manufactured with partial replacement of pork backfat with olive oil. The addition of antioxidants in combination with different storage conditions was also studied.

2. Material and Methods

2.1 Sausage preparation

Chorizo de Pamplona, a type of traditional Spanish fermented sausage, was manufactured following the procedure described by Muguerza et al. (2002). Three batches of fermented sausages, about 5 Kg each, were prepared. Raw material used for the three batches were purchased at the same time in the supermarket to minimise differences due to their origin. They were stored at -20ºC until they were used for the making sausages. A traditional formulation of 75% pork meat and 25% pork backfat was used as a control sausage. 25 % of the pork backfat was substituted by preemulsified olive oil in the other two batches. The emulsion was made according to the procedure described by Hoogenkamp (1989). Eight parts of hot water were mixed for 2min with one part of isolated soy protein, and the mixture was emulsified with 10 parts of olive oil for 3min. The olive oil used was a mixture of virgin olive oil and refined olive oil, and showed the fatty acid profile given in table 1.

100ppm of butylhydroxytoluene (BHT) and 100ppm of butylhydroxyanisole (BHA) were added as antioxidants to one of the batches that included olive oil in the formulation.

Sausages were ripened for 30 days under conditions described by Muguerza et al. (2002) and stored at 4ºC as follows: 1/3 of sausages from of each batch was individually put as a whole piece into plastic bags in aerobical conditions (Aerobically packed pieces), 1/3 was individually vacuum packed (Vacuum packed pieces) and 1/3 was sliced and vacuum packed (Vacuum packed slices). Packaging was carried out in bags of polyamide/polyethylene 90 μm (Corsan). Slices were not aerobically stored because of their short shelf life. Samples were kept under refrigeration (4º) until their analysis (2 and 5 months of storage).

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 from sausages after two and five months of storage by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (AOAC, 2002a). Fatty acid composition was calculated according to Muguerza et al. (2002) Thiobarbituric acid (TBA) value was determined in the initial mixture, after ripening and during storage (2 and 5 months), according to the method used by Tarladgis, Watts, Younathan and Dugan (1960) with modifications of Tarladgis, Pearson and Dugan (1964) and Zipser and Watts (1962). Peroxides were determined according to the AOAC method (AOACb).

2.3 Determination of volatile lipid oxidation compounds

Volatiles were obtained using the Likens-Nickerson method of 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 also 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 70ºC and boiling temperature, respectively, maintaining these conditions for 2 h. After cooling to ambient temperature, the dichloromethane was collected and dried over anhydrous Na_2SO_4. Two distillations per batch of sausage were carried out. Analysis was made in the initial mixture of ingredients, after ripening and during storage.

The volatile compounds were analysed in an 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 X 250 μm X 0.25 μm nominal HP-5MS). The carrier gas was He (1ml/min), and the chromatographic conditions were as follows: initial oven temperature was maintained during 10 min at 40ºC and subsequently programmed from 40 to 120ºC at a rate of 3ºC/min and at a rate of 10ºC/min from 120 to 250ºC, at which it was held for another 5 min, injector temperature, 250ºC, transfer line temperature, 280ºC, ion source temperature, 230ºC, scan speed, 4.49 scan/sec, mass range, 33-350 amu (atomic mass units), solvent delay, 3 min, electron impact at 70 eV. Identification of the peaks was based on 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 time 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 peaks related to lipid oxidation are shown. Area of peaks was 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 peak to that of dodecane (i.s.), and the results were expressed as nanograms of dodecane per gram of dry matter.

2.4 Data Analysis.

Four samples were analysed from each type of sausage and packing condition tested. Each parameter was determined four times in each sample. In tables, mean values are shown. Data analysis was carried out with an SPSS 9.0 program (© 1998, SPSS inc. Chicago, version 9.0. Illinois). A two-way ANOVA test was carried out in order to determine significant differences among sausages depending on the type of formulations and type of storage. Significant interactions were found so independent

one way ANOVA tests were carried out on each variable. Student t test was carried out to compare for each batch results after 2 and 5 months of storage. Principal component analysis was carried out in order to evaluate the influence of the analysed parameters on the total variability found. Varimax rotation was applied in order to maximise the variance in each loading vector.

3. Results and discussion

Significant interactions were found between the type of sausage formulation and the packing system for every analysed parameter ($p<0.05$), so comparison of data was done by means of one way ANOVA carried out separately on formulation and packaging. Table 2 and table 3 show the fatty acid profile of sausages after 2 and 5 months of storage, respectively. Different patterns were observed depending on the type of sausage formulation and conditions of packaging. With regard to the saturated fraction, control sausages showed values higher than 36 g/100 g fatty acids for all packaging conditions and the two times of storage. However, olive oil containing sausages and, above all, those with antioxidants added showed lower values of SFA compared to the control, both at 2 and 5 months of storage ($p> 0.05$). These differences were mainly attributed to the differences found for stearic acid, followed by the major saturated fatty acid, palmitic acid, that was quantitatively less affected by the substitution of pork backfat by olive oil. Except for a slight decrease in vacuum (piece and sliced) control sausages, no differences were found along storage for this SFA fraction when comparing 2 and 5 months. Also small differences were detected comparing different storage systems (aerobically packed piece, vacuum packed piece and vacuum packed slices) for the same type of sausage formulation, giving rise to variations of only around 0.5g/100g fatty acids among batches.

The MUFA fraction was obviously affected by the use of olive oil, and in some cases, by the additional presence of the antioxidants. At the end of the storage period (5 months), this fraction ranged between 48.54 and 49.85g/100g fatty acids for the antioxidant containing sausages, higher than values found for olive oil containing sausages (47.71-48.43g/100g fatty acids) and for control products (44.59-45.38 g/100g fatty acids). These results pointed at an effective protection of antioxidants from oleic

acid degradation, regardless the packing system used. The type of storage did not affect oleic acid concentration in control and olive oil containing sausages. It only revealed significant differences for sausages with antioxidants, where vacuum and sliced products showed lower oleic acid percentage than the piece aerobically packed. Comparing 2 and 5 months of storage, no differences were observed for oleic acid in any batch, except for control sausages aerobically packed, who decreased its concentration during the storage ($p<0.05$), probably due to oxidation processes.

Concerning the PUFA fraction, taking into account the content of linoleic acid in pork backfat and olive oil, the modification of the formulation is not expected to change the concentration of this acid in the modified sausages. However, due to its susceptibility to oxidation, after 2 months of storage higher values were detected in sausages with olive oil and also with antioxidants for sausages packed as pieces, pointing to an antioxidant activity of the olive emulsion oil and obviously, of the BHT and BHA added. No clear results were found after 5 months of storage for this acid. Muguerza et al. (2003) also detected a lower oxidation status in Greek fermented sausages elaborated with olive oil. Comparing the packing systems after 2 months of storage, no differences were found among the batches for linoleic acid. After 5 months, vacuum packaging (both in piece sausages and in sliced ones) seemed to protect linoleic acid from degradation, with higher values than in aerobically packed samples in all cases except for sausages with olive oil, that did not show statistical differences. Amounts obtained for trans fatty acids and omega 3 fatty acids were low with regard to the rest of fatty acids, and they were hardly affected by different packaging, use of olive oil, addition of antioxidants and time.

In general, slight changes in the fatty acid profile were detected for the lipid fractions comparing sausages with different types of storage, but significant differences were

observed among sausages with different formulations. This was also reflected in the MUFA+PUFA/SFA ratios obtained for each batch. At the end of the storage this fraction was 1.72 for control sausages, it ranged between 1.90-1.98 for sausages with olive oil and between 2.02-2.16 for sausages with olive oil and antioxidants. So, unsaturated fatty acids remained more stable with olive oil, and especially with antioxidants added, maintaining with the storage the nutritional benefits detected in the ready to eat sausages (Muguerza et al., 2001).

A tendency towards a lower oxidation level was observed in Milano salami elaborated with oleic acid and vitamin E enriched meat (Zanardi, Novelli & Ghiretti, 2000*).* Severini et al. (2003) found higher TBA values in formulations of salami with olive oil (5, 7.5 and 10%) than in the basic formulation during the drying process. However, after 30 days of storage, sausages with 10 and 7.5% olive oil showed lower TBA values than control. Results obtained in our work for the TBA values in the mixture, after ripening and during storage are reported in figure 1. The highest TBA value was found after 2 months of storage for control sausages aerobically packed (2.26 ppm). This TBA level gave rise to rancid notes in a similar dry fermented sausage elaborated with olive oil (Bloukas et al., 1997). After 5 months of storage, the value for control sausages aerobically packed decreased to 0.63ppm; a value that was expected to be higher. According to some authors, a decrease in TBA values during storage could be attributed to reaction of malondialdehyde with proteins and sugars (Janero, 1990). Together with aerobically stored olive oil containing sausages, control sausages aerobically packed were the only batches that showed TBA values higher than 0.5 ppm. In all formulations, both vacuum packaging and the presence of the antioxidants preserved samples from rising TBA, keeping in low, without significant differences among batches. Analysis of

peroxides (data not shown) revealed also low values for these samples (<2 meq O_2 /Kg), whereas those that showed high TBA results, had peroxide values above 40 meq O_2/Kg.

The results of the analysis of volatile compounds associated with oxidation processes are presented in table 4. Ansorena, Zapelena, Astiasarán & Bello (1998) reported higher values for these selected compounds in dry fermented sausages that suffered a remarkable lipolysis after the addition of a lipase, and an intense oxidation process. In this work, control sausages always showed the highest concentration for all compounds for every storage method and period of analysis. Sausages with olive oil and those including antioxidants did not show differences between them at 2 months, except for hexanal, penthylfurane and nonanal, that had lower concentration on sausages with only olive oil, than in those with antioxidants. However, at 5 months, the antioxidant activity of BHT and BHA became more evident, giving rise to the lowest aldehyde concentration among the 3 types of sausages for every storage system. Furthermore, in some cases these values were even lower than at 2 months: hexanal in aerobically packed samples ($p<0.01$) and nonanal in vacuum-packed samples ($p<0.05$).

Hexanal content has been frequently used as a marker for lipid oxidation. At the end of ripening its concentration ranged between 91 ng dodecane/g dm (for the antioxidant containing sausages) and 133 (for control sausages). During the first 2 months of storage it increased to values between 195 and 11789, depending on the packing conditions and formulation of batches. Vacuum packaging of the piece kept hexanal formation to low levels, particularly for olive oil sausages (195 and 203 ng dodecane/g dm, with and without antioxidants respectively). A slight increase of this compound compared to the end of ripening was observed after 5 months of storage in sliced vacuum packed samples (up to 5585 ng dodecane/g dm), whereas this increment was more intense in aerobically stored sausages (up to 12345 ng dodecane/g dm).

Total volatile content decreased for all types of sausages in the following order: aerobically packed piece>vaccuum packed slices>vaccum packed piece. These differences were not always detectable with TBA and peroxides results.

The antioxidant activity found in the modified sausages shown in this work can be attributed to a combination of different factors: the use of soy protein for the emulsification of the oil, which is known to have antioxidant properties (Rominj, Cuppett, Zeece, Parhurst & Lee, 1991; Wu & Brewer, 1994; Peña-Ramos & Xiong, 2003), the presence of tocopherols in the olive oil, and obviously to the use of BHA and BHT. In a previous work with Greek fermented sausages, the antioxidant properties of olive oil and the soy protein were explained (Muguerza et al., 2003).

Principal component analysis was carried out including the main fatty acids potentially subjected to oxidation (oleic, linoleic and linolenic acids), volatile compounds, TBA and peroxide values, in order to elucidate the influence of the type of sausage and packing system on the variability found among samples. Two components explained the 79% of the total variability. The first component, that explained the 70% of the variance was defined (with loadings >0.8) especially by peroxides, aldehydes and oleic acid. Figure 2 represented samples on the Varimax rotated scores separated by their type of formulation. A clear difference according to both components was observed between control sausages and modified ones. However, if the samples were represented by the type of storage (fig 3), no clear distinction among samples was detected. It could be concluded that the sausage formulation had greater influence that the storage conditions on the oxidation process of dry fermented sausages made with a partial substitution of pork backfat with olive oil and with the addition or not of antioxidants.

Acknowledgements

Authors are grateful to D. Luis Jáuregui and to D. Pablo Garísoain for their contribution to the analysis of the samples. This work has been carried out with the financial support of the project AGL 2001-0965 from the Ministerio de Ciencia y Tecnología (Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica I+D+I) and the Programa Ramón y Cajal.

REFERENCES

Ansorena, D., Zapelena, M.J., Astiasarán, I. & Bello, J. (1998). Addition of Palatase M (Lipase from *Rhizomucor miehei*) to dry fermented sausages: effect over lipolysis and study of the further oxidation process by GC-MS. *Journal of Agricultural and Food Chemistry*, *46*(8), 3244-3248.

AOAC. 2002a. Methyl esters of fatty acids in oils and fats 969.33 (Chap 41, pp 19-20). In Official methods of analysis, (17th ed) Gaithersburg, Maryland: Association of Official Analytical Chemist.

AOAC. 2002b. Determination of peroxide content. 965.33. (Chap 41, pp 12). In Official methods of analysis (17th ed) Gaithersburg, Maryland: Association of Official Analytical Chemists.

Bloukas, J.G., Paneras, E.D. & Fournitzis, G.C. (1997). Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science,* 45, 133-144.

Domazakis, E. Patent. PTC Int. Appl. WO 2002065860 A1 29 Aug 2002, 7pp.

Fernández-Fernández, E., Rozas-Barrero, J., Romero-Rodríguez, M.A. & Vázquez-Odériz, M.L. (1997). Changes in the physicochemical properties and organoleptic quality of Galician chorizos during curing and after vacuum packing. *Food Chemistry*, *60*(4), 555-558.

Fernández-Fernández, E., Vázquez- Odériz, M.L. & Romero-Rodríguez, M.A. (2002). Sensory characteristics of Galician chorizo sausage packed under vacuum and under modified atmospheres. *Meat Science, 62*, 69-71.

Folch, J., Lees, M. & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497-509.

Formanek, Z., Kerry, J.P., Higgins, F.M., Buckey, D.J., Morrisey, P.A. & Farkas, J. (2001). Addition of synthetic and natural antioxidants to α-tocopheryl acetate supplemented beef patties: effects of antioxidants and packaging on lipid oxidation. *Meat Science, 58*, 337-341.

Gray, J.J., Gomaa, E.A. & Buckey, D.J. (1996). Oxidative quality and shelf life of meats. *Meat Science*, 43(Suppl). S111-S123.

Hoogenkamp, H.W. (1989). Low-fat and low-cholesterol sausages. *Fleischerei, 40* (10), III-IV.

Hu, F.B., Stampfer, M.J., Manson, J.E., Rimm, E., Colditz, G.A., Rosner, B.A., Hennekens, C.H. & Willett, W.C. (1997). Dietary fat and risk of coronary heart disease in women. *New England Journal of Medicine, 337*, 1491-1499.

Janero, D.R. (1990). Malonaldehyde and thiobarbituric acid reactivity as diagnostics indices of lipid peroxidation and peroxidative tissue injury. *Free Radicals and Biological Medicine, 9*, 515-540.

Jo, C., Ahn, D.U. & Byun, M.W. (2002). Irradiation-induced oxidative changes and production of volatile compounds in sausages prepared with vitamin E-enriched commercial soybean oil. *Food Chemistry, 76*, 299-305.

Kerry, J.P., Buckey, D.J., Morrisey, P.A., O´Sullivan, K. & Lynch, P.B. (1998). Endogenous and exogenous α-tocopherol supplementation: effects on lipid stability (TBARS) and warmed-over flavour (WOF) in porcine M. Longissimus dorsi roasts held in aerobic and vacuum packs. *Food Research International*, *31*(3), 221-216.

Kondojoyan, N. & Berdagué, J.L. (1996). *A compilation of relative retention indices for the analysis of aromatic compounds.* Laboratoire Flaveur (INRA). Theix, France.

Kris-Ehterton, P. & Yu, S. (1997). Individual fatty aids on plasma lipids and lipoproteins: human studies. *American Journal of Clinical Nutrition, 65*(Suppl): 1628S-1664S.

Labuza, T.P. & Breene, W. (1989). Applications of active packaging for improvement of shelf-life and nutritional quality of fresh and extended shelf-life foods. *Journal of Food Processing and Preservation, 13*, 1-69.

Leenen, R., Roodenburg, A.J.C., Vissiers, M.N., Schuurbiers, J.A.E., Van Putte, K.P.A., Wiseman, S.A. & Van de Put, F.H.M.M. (2002). Supplementation of plasma with olive oil phenols and extracts: influence on LDL oxidation. *Journal of Agricultural and Food Chemistry, 50*(5), 1290-1297.

Manna, C., Dángelo, S., Migliardi, V., Loffredi, E., Mazzoni, O., Morrica, P., Galletti, P. & Zappia, V. (2002). Protective effect of the phenolic fraction from virgin olive oil against oxidative stress in human cells. *Journal of Agricultural and Food Chemistry, 50*(22), 6521-6526.

McCarthy, T.L., Kerry, J.P., Kerry, J.F., Lynch, P.B. & Buckey, D.J. (2001). Evaluation of the antioxidant potential of natural food/plant extracts compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Science, 57*, 45-52.

Mielnik, M., Aaby, K. & Skrede, G. Commercial antioxidants control lipid oxidation in mechanically deboned turkey meat. (in press). *Meat Science.*

Muguerza, E., Fista, G., Ansorena, D., Astiasarán, I. & Bloukas, J.G. (2002). Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science, 61*, 397-404.

Muguerza, E., Gimeno, O., Ansorena, D., Bloukas, J.G. & Astiasarán, I. (2001). 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 Science, 59*, 251-258.

Muguerza, E., Ansorena, D., Bloukas, J.G. & Astiasarán, I. (2003). Effect of fat level and partial replacement of pork backfat with olive oil on the lipid fraction and volatile compounds of Greek dry fermented sausages. *Journal of Food Science 68*(4), 1531-1536.

Peña-Ramos, A.E. & Xiong, Y.L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science, 64*, 259-263.

Romijn, A., Cuppett, Z.L., Zeece, M.G., Parkhurst, A.M. & Lee, M.L. (1991). Impact of soy protein isolates and specific fractions on rancidity development in a cooked, refrigerated beef system. *Journal of Food Science, 56*, 188-190.

Severini, C., De Pilli, T. & Baiano, A. (2003). Partial substitution of pork backfat with extra-virgin olive oil in "salami" products: effects on chemical, physical and sensorial quality. *Meat Science, 64*(3), 323-331.

Tarladgis, B.G., Pearson, A.M. & Dugan, L.L. Jr. (1964). 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. *Journal of the Science of Food and Agriculture, 15*, 602-607.

Tarladgis, B.G., Watts, B.M., Younathan, M.T. & Dugan, L.R. Jr. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of American Oil Chemistry Society*, *37*, 44-48.

Tieko, R., Guaraldo, L.A., Azevedo, M.A. & Beserra, F.J. (2003). Oxidative stability of fermented meat goat sausage with different levels of natural antioxidant. *Meat Science*, *63*, 43-49.

Tranchant, J. (1982). *Manuel pratique de chromatographie en phase gazeuse.* Paris: Masson. Pp-301-337.

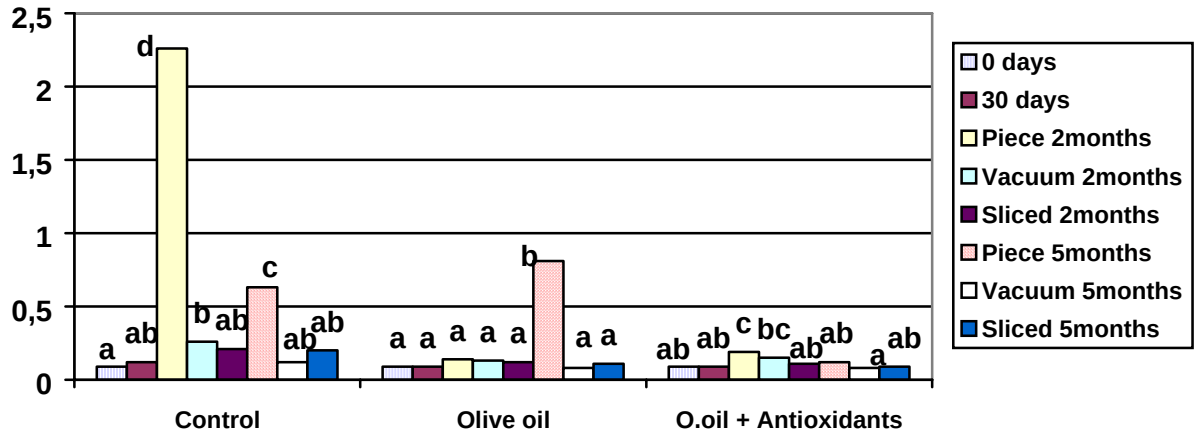
Wu, S.Y. & Brewer, M.S. (1994). Soy protein isolate antioxidant effect on lipid peroxidation of ground beef and microsomal lipids. *Journal of Food Science, 59,* 702-706.

Zanardi, E., Novelli, E. & Ghiretti, G.P. (2000). Oxidative stability of lipids and cholesterol in salame Milano, coppa and Parma ham: dietary supplementation with vitamin E and oleic acid. *Meat Science, 55,* 169-175.

Zipser, M.W. & Wats, B.M. (1962). A modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats. *Food Technology, 16,* 102-107.

FIGURES

Figure 1. TBA values for the three types of sausages along storage under different conditions (ppm).



For each type of sausage formulation, different letters denote significant differences among storage systems and time of analysis ($p<0.05$).

Figure 2. Varimax rotated scores on types of sausages for PC1 and PC2.

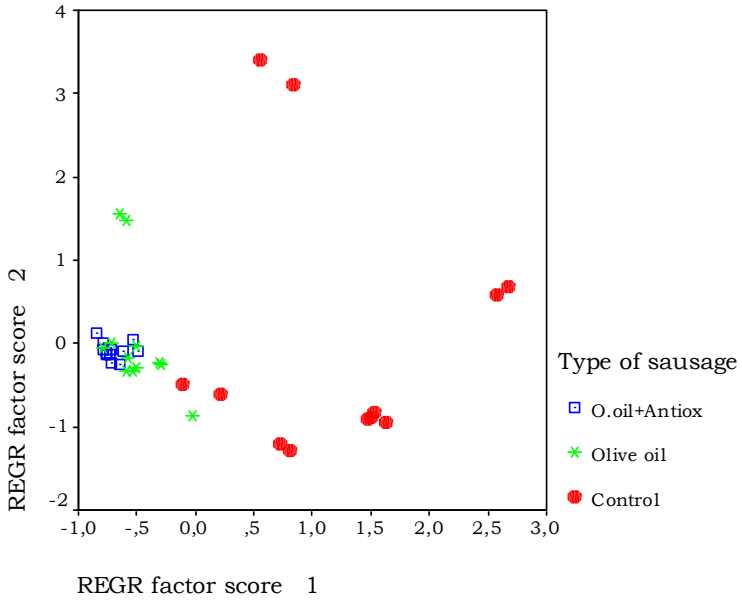
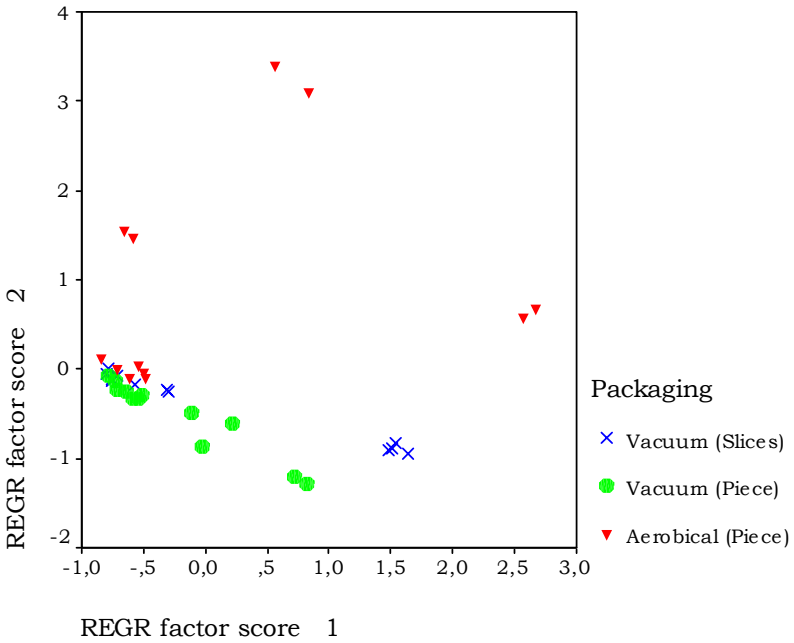


Figure 3. Varimax rotated scores on packaging systems for PC1 and PC2.



TABLES

Table 1. Fatty acid profile of the olive oil used in the modified sausages.

Fatty acid	Amount (g/100g fat)
Lauric	0.02
Mirystic	0.12
Palmitic	10.74
Palmitelaidic	0.02
Palmitoleic	0.14
Stearic	2.93
Oleic	71.08
Elaidic	0.10
Linoleic	8.83
Arachidic	0.48
Linolenic	0.67
Behenic	0.23
Eicosapentaenoic	0.53
Docosahexaenoic	0.04

Table 2. Fatty acid profile (g/100g fatty acids) of sausages after 2 months of storage.

	Aerobically Packed Piece			Vacuum Packed Piece			Vacuum Packed Slices		
	C[a]	O[a]	A[a]	C	O	A	C	O	A
Lauric	0.15aA	0.13aA	0.14aA	0.16aA	0.15aB	0.14aA	0.16aA	0.14aAB	0.16aA
Myristic	1.29bAB	1.16aA	1.26abA	1.39aB	1.32aA	1.25aA	1.44bA	1.17aA	1.24aA
Palmitic	22.14bB	20.44aA	20.86aA	22.56bB	22.09abB	20.89aA	22.75aA	20.44bA	20.65bA
Stearic	12.47cAB	11.07bA	9.18aA	12.63cB	11.32bA	9.63aC	12.27cA	11.14cA	9.25aB
Arachidic	0.18aA	0.27bC	0.16aA	0.23aA	0.20aB	0.17aA	0.19aA	0.08aA	0.78bB
Behenic	0.31aB	0.39aB	0.29aA	0.42cB	0.25aA	0.33bA	0.23aA	0.33bAB	0.33bA
SFA	**36.54**	**33.46**	**31.89**	**37.39**	**35.33**	**32.41**	**37.04**	**33.30**	**32.41**
Palmitoleic	2.29bA	2.07aA	2.70cB	2.31aA	2.22aA	2.62bAB	2.36bA	2.09aA	2.60cA
Oleic	43.83aB	46.48bA	46.76bAB	42.64aA	45.40bA	46.42bA	42.32aA	46.33bA	46.77cB
MUFA	**46.12**	**48.55**	**49.46**	**44.95**	**47.62**	**49.04**	**44.68**	**48.42**	**49.37**
Linoleic	15.45aA	15.89bA	16.57cA	15.26aA	15.8bA	16.43cA	16.26aA	16.22aA	16.22aA
Linolenic	0.72aA	0.75aAB	0.95bA	0.67aA	0.66aA	0.89aA	0.77aA	0.89aB	0.86aA
Arachidonic	0aA	0.11cB	0.02bB	0.08bB	0aA	0aA	0aA	0aA	0aA
EPA	0.07aB	0.10aA	0.10aA	0aA	0.09bA	0.11bA	0aA	0.13bA	0.11bA
DHA	0.08aA	0.090aA	0.09aA	0.09aA	0.08aA	0.09aA	0.08aA	0.07[a]A	0.08aA
PUFA	**16.22**	**16.94**	**17.73**	**16.1**	**16.17**	**17.56**	**17.11**	**17.31**	**17.27**
t-Palmitoleic	0.41aA	0.39aA	0.39aA	0.41aA	0.39aA	0.39aA	0.42cA	0.41aA	0.37aA
Elaidic	0.44bA	0.64cB	0.23aA	0.32aA	0.30aA	0.24aA	0.42aA	0.26aA	0.46aB
t-Linoleic	0aA	0aA	0.13bA	0aA	0aA	0.11bA	0aA	0.10bB	0.11bA
Brassidic	0.12bA	0aA	0.14bA	0.27bB	0.14aB	0.14aB	0.28cB	0.14bB	0cA
TRANS	**0.97**	**1.03**	**0.89**	**1**	**0.83**	**0.88**	**1.12**	**0.91**	**0.94**

[a]C: Control sausage, O: Olive oil containing sausages, A: sausages with olive oil and antioxidants.
For each type of storage condition, different lower case letters denote significant differences among types of sausages ($p<0.05$). For each type of sausage, different upper case letters denote significant differences among packaging conditions ($p<0.05$).

Table 3. Fatty acid profile (g/100g fatty acids) of sausages after 5 months of storage.

	Aerobically Packed Piece			Vacuum Packed Piece			Vacuum Packed Slices		
	C[a]	O[a]	A[a]	C	O	A	C	O	A
Lauric	0.13aA	0.13aB	0.12aA	0.15c	0.12aA	0.13bAB	0.13aA	0.14aB	0.14aA
Myristic	1.33bA	1.15aA	1.18aA	1.35cA	1.16bA	1.26aB	1.32bA	1.17aA	1.27bB
Palmitic	21.94bA	21.50abA	20.44aA	22.06cA	20.580bA	21.00aB	21.91cA	20.35aA	20.95bB
Stearic	12.24cA	10.84bA	9.13aA	12.32cA	11.71bB	9.65aB	12.52cA	11.46bAB	9.53aB
Arachidic	0.08aA	0.17abA	0.21bA	0.20aG	0.24aA	0.21aA	0.14aAB	0.24bA	0.15abA
Behenic	0.46aA	0.35aA	0.36aAA	0.29aA	0.27aA	0.29aA	0.35aA	0.37aA	0.22aA
SFA	**36.68**	**33.14**	**31.41**	**36.37**	**34.08**	**32.54**	**36.38**	**33.73**	**32.26**
Palmitoleic	2.32abA	2.22aB	2.47bA	2.33bA	2.02aA	2.66cB	2.33bA	2.11aAB	2.64cB
Oleic	43.06aA	46.21bA	47.38cC	42.27aA	45.69bA	45.88cA	42.50aA	46.03bA	46.58cB
MUFA	**45.38**	**48.43**	**49.85**	**44.59**	**47.71**	**48.54**	**44.82**	**48.14**	**49.22**
Linoleic	16.17aA	15.55aA	16.22aA	17.12cB	16.05aA	16.47bB	16.85bB	16.03aA	16.59bB
Linolenic	0.68aA	0.73aA	0.88bA	0.90aB	0.90aB	0.93aA	0.85aB	0.87aB	0.95aA
Arachidonic	0aA	0aA	0aA	0aA	0aA	0aA	0aA	0aA	0aA
EPA	0.10aB	0.11aA	0.37aA	0aA	0.12bA	0.10bA	0.06aAB	0.10aA	0.11aA
DHA	0.10aA	0.9aA	0.38aA	0.17aA	0.15aA	0.11aA	0.14aA	0.12aA	0.11aA
PUFA	**17.05**	**17.29**	**17.85**	**18.19**	**17.22**	**17.61**	**17.9**	**17.12**	**17.76**
t-Palmitoleic	0.42aA	0.40abA	0.37aA	0.40bA	0.40bA	0.38aB	0.43bA	0.41bA	0.37aA
Elaidic	0.30aB	0.26aA	0.21aA	0.21aA	0.26aA	0.21aA	0.23aA	0.27aA	0.22aA
t-Linoleic	0.14aB	0.12aA	0.13aB	0.14aB	0.13aA	0.16aB	0.08aA	0.12aA	0.09aA
Brassidic	0.49aA	0.12aA	0.09aB	0.05aA	0.17bB	0.14bC	0.14bA	0.15bAB	0aA
TRANS	**1.35**	**0.9**	**0.8**	**0.8**	**0.96**	**0.89**	**0.88**	**0.95**	**0.68**

[a]C: Control sausage, O: Olive oil containing sausages, A: sausages with olive oil and antioxidants.
For each type of storage condition, different lower case letters denote significant differences among types of sausages ($p<0.05$). For each type of sausage, different upper case letters denote significant differences among packaging conditions ($p<0.05$).

Table 4. Volatile compounds from oxidation (ng dodecane/g dry matter).

		Aerobically Packed Piece			Vacuum Packed Piece			Vacuum Packed Slices		
	Mo	[a]C	[a]O	[a]A	C	O	A	C	O	A
Hexanal	2	11789cC	866aC	1360bB	1333bA	203aA	195aA	5585cB	511bB	257aB
	5	12365cC	2799bC	466aB	1309bA	179aA	115aA	6914cB	1216bB	208aA
Heptanal	2	810bC	160aC	139aB	184bA	29aA	46aA	475bB	75aB	94aAB
	5	1177bB	278aC	111aB	321bA	62aA	48aA	484cA	139bB	56aA
Pentylfurane	2	2831cC	83aC	493bB	467cA	0aA	106bA	2003bB	45aB	140aA
	5	3606cC	1069bC	308aC	550cA	105bA	79aA	2261cB	571bB	164aB
Octenal	2	1614bC	137aC	273aB	219bA	0aA	0aA	874bB	80aB	0aA
	5	1993cC	457bC	103aC	260cA	43bA	0aA	1110cB	223bB	65aB
Nonanal	2	2020cC	526aA	928bC	651bA	498aA	409aA	1368bB	559aA	706aB
	5	2800cC	875bC	569aB	1758cB	388bA	348aA	1245cA	674bB	318aA
Tt2,4-decadienal	2	4813bC	65aB	69aB	218bA	0aA	5aA	3053bB	30aAB	12aA
	5	5313cC	1372bC	15aC	482bA	6aA	5aA	2958cB	362bB	9aB
2,4-decadienal	2	13153bC	262aB	230aB	723bA	0aA	8aA	8029bB	111aA	23aA
	5	17529cC	4216bC	41aC	1738bA	16aA	9aA	8079cB	1403bB	22aB
Total	2	37030	2099	3492	3795	730	769	21387	1411	1232
	5	44783	11066	1613	4835	799	640	23051	4588	842

[a]C: Control sausage, O: Olive oil containing sausages, A: sausages with olive oil and antioxidants. Mo: months Lower case letters denote significant differences among type of sausage for every phase and type of packaging ($p<0.05$). Upper case letters denote significant differences among packing conditions for every phase and type of sausage ($p<0.05$).