Title:

Stability of linseed oil and antioxidants containing dry fermented sausages: study of the lipid fraction during different storage conditions.

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Abstract

Different packaging conditions (aerobic, vacuum and modified atmosphere) were evaluated in order to study the stability of the lipid fraction of dry fermented sausages manufacturated with a partial substitution of pork backfat by linseed oil and antioxidants. After 5 months of storage, α-linolenic acid was better preserved by vacuum and MAP (7.32 and 7.74g/100g fatty acids, respectively) than in aerobic conditions (6.15g/100g fatty acids), without significant differences to values obtained after 2 months of storage for this acid. At the end of the storage, n-6/n-3 fraction in sausages with linseed oil was in all cases lower than 3, in contrast to values obtained for control products that were all higher than 15. Better PUFA/SFA ratios were also observed in modified sausages (0.6-0.7g/100g fatty acids) than control ones (0.3-0.4g/100g fatty acids). No signs of lipid oxidation measured by TBARs and peroxides were detected for modified sausages regardless the packaging system used (TBARs values lower than 0.25ppm and peroxides lower than 4meqO$_2$/kg), pointing at a high effectiveness of the antioxidants. Furthermore, vacuum and MAP prevented 2,4 decadienal formation. Nutritional benefits of linseed oil and antioxidants containing products were maintained after 5 months of storage.

1. Introduction

Reformulation of meat derivatives is one of the strategies that has been studied in order to develop new products with nutritional benefits and also additional functional characteristics. In the case of cured products, new formulations make possible healthier dry fermented sausages by minimizing two of their major drawbacks: high salt content and the presence of saturated fatty acids (SFA) and cholesterol (Muguerza, Gimeno, Ansorena, & Astiasarán, 2004a).

Regarding the lipid fraction, reformulation has been used to reduce the fat content in cooked meat products (Paneras, Bloukas & Papadima, 1996; Grigelmo-Miguel, Abadías-Serós & Martín-Belloso, 1999; Jiménez Colmenero, 2000) and in fermented sausages (Bloukas, Paneras & Fournitzis, 1997; Mendoza, García, Casas & Selgas, 2001; García, Dominguez, Galvez, Casas & Selgas, 2002). Furthermore, reformulation by replacing the animal fat by vegetable oils has been recognised as an interesting way to improve the fatty acid profile of dry fermented sausages. Olive oil was the first vegetable fat used for that purpose because of its high proportion of the monounsaturated oleic acid. Bloukas, Paneras and Fournitzis (1997) found that 20% of pork backfat could be replaced by olive oil in the form of pre-emulsified fat in Greek fermented sausages. A substitution of a 25% of pork backfat with pre-emulsified olive oil in Chorizo de Pamplona, a traditional Spanish fermented sausage, was achieved by Muguerza, Gimeno, Ansorena, Bloukas and Astiasarán (2001). Severini, De Pilli and Baiano (2003) obtained the best results with a 33.3% of substitution with olive oil in salami. Kaayardi and Gök (2004) concluded that a 40% of replacement of beef fat with olive oil had a positive effect on sensory quality of Soudjouks, a popular Turkish dry fermented sausage. These substitutions gave rise to nutritional advantages in relation to cholesterol reduction and improvement of fatty acid profile by increasing

monounsaturated fatty acids (MUFA). Concerning the enrichment on polyunsaturated fatty acids (PUFA), several works are focussed on the development of meat products enriched in n-3 fatty acids obtained from feeding animal diets rich in α-linolenic acid and elaborating meat derivatives with this raw matterial (Leskanich, Matthews, Warkup, Noble & Hazzledine, 1997; Enser, Richardson, Wood, Gill & Sheard, 2000; Sheard, Enser, Wood, Nute, Gill & Richardson, 2000; Hoz, D'Arrigo, Cambero & Ordóñez, 2004; Santos, Ordóñez, Cambero, D'Arrigo & Hoz, 2004). Other experiments have increased the PUFA fraction by substituting pork backfat with seed oils, obtaining increments of the PUFA/SFA ratio when soy oil was used (Muguerza, Ansorena & Astiasarán, 2003), or by the addition of fish oil extracts, improving the n-6/n-3 ratio (Muguerza, Ansorena & Astiasarán, 2004b).

In a previous paper, our research group studied the lipid modifications undergone through the ripening process of dry fermented sausages made with a partial substitution of pork backfat with linseed oil, with and without the addition of butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) as antioxidants (Ansorena & Astiasarán, 2004a). At the end of ripening, sausages with linseed oil plus antioxidants showed the lowest values for SFA and the highest concentrations of PUFA in comparison to the other two batches (control and linseed), together with a marked decrease in the n-6/n-3 ratio (from 14 to 2). As a consequence of the change in the fatty acid profiles, improvement of nutritional quality was achieved. However, in that paper it was also concluded that more research was needed to evaluate the lipid oxidation during the shelf life of these sausages with a high PUFA content.

Effectively, the enrichment of dry fermented sausages in unsaturated fatty acids might induce a higher susceptibility to lipid oxidation, which is one of the main factors limiting the quality and acceptability of meats and derivatives (Morrissey, Sheehy,

Galvin, Kerry & Buckley, 1998). It can also affect the nutritional value of products due to potential changes in the fatty acid profile. Different strategies can be adopted to minimize oxidation either by limiting contact with oxygen and/or by using antioxidants. Zanardi, Dorigoni, Badiani and Chizzolini (2002) concluded that 100% Nitrogen was more efficient than vacuum packaging in controlling fatty acid oxidation of sliced Milano-type sausages. Ansorena and Astiasarán (2004b) showed that vacuum packaging of olive oil enriched sausages was a good method to minimise formation of lipid oxidation products.

Consequently, the objective of this paper was to follow the modifications taking place in the lipid fraction of dry fermented sausages, made with a partial substitution of pork backfat by linseed oil and antioxidants, during 5 months of storage and evaluate their stability under different packaging conditions: aerobic, vacuum and modified atmosphere packaging (MAP).

2. Materials and Methods

2.1 Sausage formulation and processing.

Chorizo de Pamplona, a type of traditional Spanish dry-fermented sausage, was produced according to the procedure described by Muguerza et al. (2001).

Three batches of fermented sausages, about 5 kg each, were prepared. The control batch was produced using 75% lean pork meat and 25% pork backfat. The following ingredients per kilogram of meat mixture were added to the formulation: NaCl 26g , red pepper 30g, dextrin 15g, lactose 10g, powdered milk 12g, dextrose 5g, sodium ascorbate 0.5g, sodium caseinate 10g, garlic 3g, polyphosphates 2g, curavi (a mixture of NaCl with preservatives E-250, E-252 and the antioxidant E-331) 3g, ponceau 4R (E-124) 0.15g. The starter culture was a mixture of *Lactobacillus plantarum* L115 (50%) and *Staphylococcus carnosus* M72 (50%) added at a dose of 10^6-10^7 cfu/kg of mixture.

The modified batch was made by a substituting of 25% of the pork backfat with pre-emulsified linseed oil. The emulsion was prepared by mixing for two minutes eight parts of hot water (50ºC) with one part of isolated soy protein and then with ten parts of linseed oil for another three minutes (Hoogenkamp, 1989a,b). This emulsion was added to the rest of ingredients. 100 mg/kg of butylhydroxytoluene (BHT) and 100 mg/kg of butylhydroxyanisole (BHA) were added as antioxidants. The fatty acid profile of the linseed oil, expressed as g/100g of fatty acids, was as follows: myristic (0.11), palmitic (6.28), t-palmitoleic (0.18), palmitoleic (0.18), stearic (3.91), oleic (24.5), linoleic (18.4), linolenic (46.3), eicosapentaenoic (0.11) and docosahexaenoic (0.07).

The sausages were fermented in a drying chamber (STA model W 80XDHG-VEH Noain, Spain) at 22-23ºC and 90-100% relative humidity (RH) for 24h, 19.5-20.5ºC and 80-90% RH for 24h, 16.5-17.5ºC and 80-90% RH for 24h. Then the sausages were dried for 7 days at 14-15ºC and 74-86% RH, until the end of ripening. At the end of the

ripening process, control and modified sausages were stored at 4ºC in three different conditions: 1/3 pieces were aerobically packed, other third were vacuum packed and finally other third were packed under modified atmosphere (MAP) (80% N_2 and 20% CO_2). A seven layer film was used for packaging: a polyethylene of medium linear density, polyethylene of medium density, polyamide modified, ethylene-vinylidene alcohol alloy, polyamide modified, polyethylene of linear low density and 4.5% ethylene-vinylidene acetate (Super7E2, thickness of 140 µm, Vaessen-Schomaker industrial, S.A.). Analysis was carried out after 2 and 5 months of storage.

2.2 Chemical analysis.

The method of Folch, Lees and Stanley (1957) was used for the extraction of lipids from sausages. Fatty acids were determined in the lipid extract by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (AOAC, 2002a). 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 at 165ºC for 70 min and increased to 220ºC at a rate of 4ºC/min, which was held for 35 min. The carrier gas was hydrogen, and the pressure was 20.5 psi. Split flow was 160cm/s. The identification of the fatty acid methyl esters was 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 each standard individually. The quantification of individual fatty acids was based on the internal standard method, using heptadecanoic acid methyl ester (Sigma, St. Louis, MO, USA).

Nutritional quality was evaluated using the PUFA/SFA ratio (linoleic+α-linolenic+arachidonic+eicosapentaenoic+docosahexaenoic)/(lauric+myristic+palmitic+

stearic+arachidic) and the n-6/n-3 ratio (linoleic+arachidonic)/(α-linolenic+ eicosapentaenoic+docosahexaenoic) which is relevant to the competition for synthesis of longer-chain PUFA between each series.

Peroxide value was determined using the official method of AOAC (AOAC, 2002b). TBARs value was determined according to Tarladgis, Watts, Younathan and Dugan (1960) with modifications by Tarladgis, Pearson and Dugan (1964). Results are shown in mg malonaldehyde/kg sample (ppm).

2.3 Volatile compounds from lipid oxidation

Likens-Nickerson Extraction. Twenty five grams 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. Both solvent and sample mixture were heated to 70ºC and boiling temperature, 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. The volatile compounds were analysed in an HP 6890 GC system (Hewlett-Packard) 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; mass range, 33-350 amu; 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 and, in addition in some cases, by comparison of their retention time with those of standard compounds. The Kovats indices were also calculated according to the method of Tranchant (1982) and were compared with available literature data (Kondjoyan & Berdagué, 1996). Only peaks related to lipid oxidation are shown. Area 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 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 analyzed from each type of dry fermented sausage. Each parameter was determined four times in each sample. Means and standard deviations are shown in the tables. Results were analyzed by a two-way ANOVA (sausage type and storage conditions). When F values of interaction were significant ($p < 0.05$), one-way ANOVA were applied independently for these 2 factors and means were compared by a Tukey's b posteriori test. 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. Student´s t-test was used to determine significant differences ($p < 0.05$) between the different times of storage (2 and 5 months).All statistical analyses were performed using SPSS version 11.0. software package (© 1998, SPSS inc. Chicago, Illinois).

3. Results and discussion

Based on the results of Ansorena and Astiasarán (2004a), control and sausages with added linseed oil and antioxidants were investigated to study of their stability against oxidation and the evaluation of their nutritional quality during their shelf-life stored at different packaging conditions.

A two-way ANOVA was carried out in order to determine the influence of the sausage formulation and the type of packaging in the fatty acid profile after 2 and 5 months of storage (tables 1 and 2). Particular attention was paid to the contents of linoleic and α-linolenic acids, because they are much more abundant in linseed oil (18.4% and 46.3%, respectively) than in pork backfat of traditionally fed pigs (4.87% and 0.41%), and also due to their high oxidation susceptibility: linolenate is 2.4 times more reactive than linoleate, and linoleate is 40 times more reactive than oleate (Frankel, 1998). No statistical interaction was found between sausage formulation and type of packaging for 7 of the 15 analysed fatty acids after 2 months of storage, and for 9 fatty acids after 5 months, so general conclusions about the influence of each factor can be drawn for these acids.

After 2 months, linseed containing sausages maintained lower values for lauric, stearic, palmitoleic, oleic and t-palmitoleic acids in comparison to control sausages and, as expected, higher values for linoleic acid. No influence of the packaging conditions in any of the two types of sausages was noticed for these fatty acids, as no significant differences were found among vacuum, MAP or aerobic storage. Fatty acids affected by the statistical interaction showed a variable behaviour. No clear effect of formulation or packaging was observed for myristic, arachidic, arachidonic, eicosapentaenoic (EPA), docosahexaenoic (DHA) or t-linoleic, which were all at very low amounts in all cases. Palmitic acid showed slightly higher values in control products, being affected by MAP

only in the case of linseed products. α-linolenic acid obviously was significantly higher in linseed products (5.73-7.33g/100g fatty acids) than in control ones (0.6-0.77g/100g fatty acids) as a consequence of the use of linseed oil. In relation to the different packaging conditions, whereas this acid did not show differences in control sausages, in linseed containing products α-linolenic acid was better preserved by vacuum (6.57g/100g fatty acids) and MAP (7.33g/100g fatty acids), than in aerobic conditions (5.73g/100g fatty acids).

After 5 months of storage, the saturated fatty acids lauric, myristic, palmitic, stearic and palmitoleic maintained their lower amount in the modified sausages, and linoleic acid was still higher than in control products (p<0.01). No significant differences between formulations were found for oleic acid nor among packaging systems. In contrast to what it was found at 2 months, packaging affected linoleic acid concentration, which was protected both by vacuum and MAP systems, showing higher values in comparison to aerobic packaging in control and also in modified sausages.

The behaviour of α-linolenic acid was similar to that at 2 months, with a significant interaction between both factors and a significant protective effect of vacuum and MAP, only in linseed added products. Furthermore, a student´s t-test revealed no significant differences (p>0.05) between 2 and 5 months for α-linolenic in linseed added sausages. Comparing these α-linolenic values to those detected by Ansorena and Astiasarán (2004a) at the end of the ripening of control (0.92g/100g fatty acids) and linseed containing sausages (8.57g/100g fatty acids), certain loss of α-linolenic was noticed during storage. In particular, losses of α-linolenic acid in control sausages varied from 18.4% in MAP conditions to 50% in aerobically packed products. Losses in linseed containing sausages were proportionally lower (9.2% to 27.7%). This finding led us to

conclude that BHT and BHA were effective in preventing α-linolenic oxidation, even when this acid was present in high amounts.

These modifications in the fatty acid profiles in terms of the different types of fatty acids and nutritionally relevant ratios are shown in tables 3 and 4. The SFA and MUFA fractions were higher in control sausages than in modified ones after 2 and 5 months, not being affected in linseed products by the system of packaging used. The PUFA fraction was affected by the use of linseed oil. More differences among packaging systems were detected at 5 months than at 2 months. After 5 months of storage the PUFA/SFA ratio in linseed containing products decreased in the order MAP>vacuum>aerobic. However, no differences were detected among packaging systems for the n-6/n-3 fraction in these products, with values in all cases below 3.

High PUFA levels contribute to a more healthy meat, however it is important to be aware of the possible reduced storage stability and problems connected to fat oxidation (Bryhni, Kjos, Ofstad & Hunt, 2002). Hoz et al. (2004) carried out an experiment where dry fermented sausages were made from meat obtained from pigs fed diets rich in linseed oil with and without tocopherol as antioxidant. Levels of α-linolenic acid reached 6.25g/100g fatty acids in that work. These authors found that, after 4 months of vacuum storage, TBARs values of control sausages (without linseed enrichment) were 2.41ppm, those elaborated with linseed enriched diets reached 3.83ppm and linseed plus tocopherol containing diets maintained TBARs values on 0.82ppm. In our experiment, with α-linolenic values around 6.5g/100g fatty acids, storage of control sausages under vacuum or MAP kept TBARs values similar to that observed for final product (0.08ppm) at the two periods of analysis (tables 5 and 6). Values were in all cases lower than 0.13 ppm, which indicated the efficiency of these packaging systems to control oxidation. However, aerobic storage increased TBARs values with time (p<0.01)

reaching 2.12ppm, value that, in olive oil containing sausages caused rancid notes (Bloukas et al., 1997). Despite the higher content of polyunsaturated fatty acids in the linseed containing sausages at the end of ripening, which a priori can induce oxidation, the presence of the antioxidants preserved sausages from increasing TBARs. In the modified formulation, after 5 months even under aerobic conditions TBARs did not even reach 0.5ppm, low if 1ppm is the limit to detect rancidity (Kusmider, Sebranek, Lonergan & Honeymanhttp, 2002). Similar observations were found for peroxide values, with low values for all batches except for aerobically packed control sausages. The analysis of lipid oxidation volatile compounds showed that the combination of using vacuum or MAP and antioxidants in the linseed containing sausage formulation prevented the formation of most of the analyzed compounds. No peaks for heptanal, octenal or 2,4-decadienal were detected, which is important for sensory aspects, as these compounds are known by their low threshold and off-odour. Only small amounts of hexanal, a typical oxidation indicator from linoleic acid, and nonanal, derived from oleic acid oxidation, were detected. Aerobic storage of linseed containing sausages showed higher values for every compound, as expected. Comparing these data with those obtained by Ansorena and Astiasarán (2004b) in olive oil containing products, also aerobically packed during 2 and 5 months, a higher volatiles amount was detected for linseed sausages, particularly in the decadienal content, pointing at a higher oxidation susceptibility of PUFA in comparison to MUFA.

A principal component analysis including lipid oxidation parameters and those fatty acids that accounted for more than 0.5mg/100g fatty acids was performed. The first two components explained 88% of the total variance, with the following distribution (46% for component 1 and 42% for component 2). Figure 1 shows the plot of both the coefficients of the rotated matrix and the different types of samples analyzed in this

study. Component 1 was characterized by high values for oxidation parameters (2,4-decadienal=0.973 and t,t,2,4-decadienal=0.969) and negative loading values for linoleic and α-linolenic acids. Component 2 was however characterized by high values for saturated fatty acids (stearic=0.963; myristic=0.945; palmitic=0.936) and also negative values for linoleic and α-linolenic acids. In consequence, the distribution of the samples clearly separated control products, higher in SFA, from modified ones, which were located in the negative values zone for factor 2. In relation to the packaging conditions, only aerobically packaged control sausages were separated from the rest of samples, as no clear distinction was detected among packaging conditions in modified sausages. A similar result was obtained in an experiment carried out by partial substitution of pork backfat by olive oil in dry fermented sausages, where it was concluded that sausage formulation had a greater influence than vacuum and aerobic storage conditions on the oxidation process (Ansorena & Astiasarán, 2004b). In that paper MAP was not evaluated.

It can be concluded that sausages elaborated with a partial substitution of pork backfat by an emulsion containing linseed oil plus antioxidants and showing relevant nutritional benefits with regard to traditional products maintained their stability against oxidation during 5 months in refrigeration, especially under vacuum or MAP. Storage during 5 months affected the fatty acid profile of sausages with linseed oil in comparison those products analysed just at the end of the ripening process, but they still were considerably better than control sausages from the nutritional point of view. No relevant differences were found between vacuum and MAP in controlling the lipid oxidation process.

Acknowledgements

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Figure 1. Plot of the principal component analysis carried out with lipid oxidation parameters and those fatty acids that accounted for more than 0.5mg/100g fatty acids.
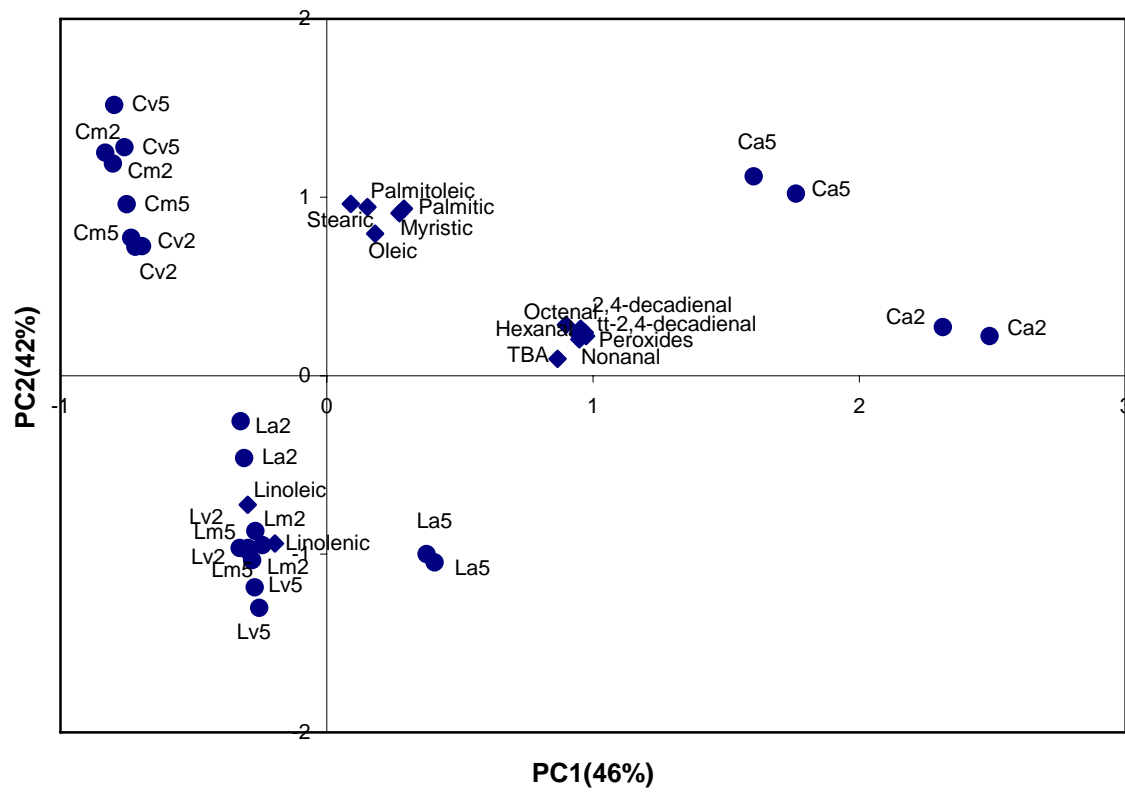
Ca: control sausage aerobic; Cv: control sausage vacuum; Cm: control sausage MAP, La: linseed sausage aerobic; Lv: linseed sausage vaucum; Lm: linseed sausage MAP (2 and 5 are the storage months in each case).

Table 1. Total fatty acids (g/100g of fatty acids) of control and linseed oil containing sausages after 2 months of storage.

	Control sausage			Linseed oil sausage			Sausage	Pack	PxS
	Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP			
Lauric	0.12 (0.00)	0.11 (0.01)	0.11 (0.00)	0.10 (0.01)	0.10 (0.00)	0.10 (0.01)	***	ns	ns
Myristic	1.41cd (0.03)	1.41cd (0.04)	1.47d (0.08)	1.33bc (0.06)	1.24ab (0.05)	1.19a (0.09)	-	-	*
Palmitic	25.46c (0.72)	24.93c (0.52)	25.13c (0.39)	23.29b (0.60)	22.68b (0.69)	21.29a (0.53)	-	-	*
Stearic	14.85 (0.85)	15.43 (0.48)	15.39 (0.22)	12.29 (0.33)	12.64 (0.37)	12.06 (0.24)	***	ns	ns
Arachidic	0.18c (0.02)	0.16bc (0.02)	0.13b (0.02)	0.00a (0.00)	0.16bc (0.03)	0.14bc (0.01)	-	-	***
Palmitoleic	2.52 (0.03)	2.48 (0.07)	2.51 (0.03)	2.28 (0.10)	2.19 (0.03)	2.26 (0.06)	***	ns	ns
Oleic	40.91 (0.40)	40.96 (1.51)	41.23 (0.55)	39.06 (0.98)	38.18 (1.27)	38.31 (0.77)	***	ns	ns
Linoleic	12.51 (0.43)	12.80 (0.66)	12.25 (0.34)	15.14 (0.62)	15.41 (0.74)	16.07 (0.30)	***	ns	ns
α-Linolenic	0.77a (0.03)	0.60a (0.04)	0.65a (0.04)	5.73bc (0.29)	6.57c (0.47)	7.33d (0.10)	-	-	***
Arachidonic	0.26ab (0.03)	0.31bc (0.01)	0.25ab (0.04)	0.20a (0.02)	0.33c (0.05)	0.34c (0.02)	-	-	**
EPA	0.05b (0.00)	0.00a (0.00)	0.06b (0.01)	0.00a (0.00)	0.00a (0.00)	0.06b (0.00)	-	-	***
DHA	0.09b (0.01)	0.10b (0.01)	0.09b (0.01)	0.00a (0.00)	0.00a (0.00)	0.12b (0.02)	-	-	***
t-Palmitoleic	0.40 (0.01)	0.40 (0.01)	0.42 (0.02)	0.35 (0.02)	0.34 (0.02)	0.35 (0.01)	***	ns	ns
Elaidic	0.18 (0.00)	0.18 (0.02)	0.18 (0.01)	0.18 (0.00)	0.15 (0.02)	0.28 (0.12)	ns	ns	ns
t-linoleic	0.12b (0.02)	0.11b (0.03)	0.12b (0.02)	0.00a (0.00)	0.00a (0.00)	0.12b (0.02)	-	-	***

Results are expressed as mean (standard deviation). n=16. PxS: packaging system x sausage; Values in the same row bearing different letters are significantly different (p< 0.05) ns: Not significant; P>0.05, ***P<0.001. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid.

Table 2. Total fatty acids (g/100g of fatty acids) of control and linseed oil containing sausages after 5 months of storage.

	Control sausage			Linseed oil sausage			Sausage	Pack	PxS
	Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP			
Lauric	0.12 (0.01)	0.12 (0.01)	0.12 (0.01)	0.11 (0.01)	0.10 (0.01)	0.11 (0.00)	**	ns	ns
Myristic	1.52 (0.07)	1.43 (0.07)	1.46 (0.06)	1.24 (0.03)	1.18 (0.10)	1.23 (0.10)	***	ns	ns
Palmitic	25.58 (1.10)	24.61 (1.78)	24.95 (0.34)	22.07 (0.66)	21.48 (1.20)	21.32 (0.58)	***	ns	ns
Stearic	15.32 (0.56)	15.12 (1.27)	15.65 (0.15)	12.69 (0.33)	12.53 (0.41)	12.05 (0.59)	***	ns	ns
Arachidic	0.13ª (0.01)	0.13a (0.02)	0.22b (0.03)	0.11a (0.01)	0.12a (0.00)	0.12a (0.01)	-	-	***
Palmitoleic	2.57 (0.11)	2.47 (0.19)	2.49 (0.07)	2.31 (0.09)	2.21 (0.11)	2.29 (0.07)	***	ns	ns
Oleic	40.80 (1.38)	40.20 (3.91)	39.60 (0.59)	39.41 (1.75)	37.90 (1.44)	37.94 (1.88)	ns	ns	ns
Linoleic	12.40 (0.42)	14.09 (0.84)	13.19 (0.25)	14.74 (0.87)	15.83 (0.70)	16.02 (0.72)	***	**	ns
α-Linolenic	0.47a (0.03)	0.66a (0.06)	0.75a (0.02)	6.15b (0.48)	7.32c (0.48)	7.74c (0.46)	-	-	**
Arachidonic	0.29 (0.03)	0.41 (0.03)	0.39 (0.02)	0.25 (0.03)	0.41 (0.01)	0.36 (0.03)	ns	***	ns
EPA	0.00a (0.00)	0.00a (0.00)	0.05b (0.00)	0.05b (0.00)	0.05b (0.01)	0.05b (0.01)	-	-	***
DHA	0.08b (0.00)	0.12c (0.01)	0.09b (0.00)	0.07a (0.10)	0.11c (0.01)	0.12c (0.01)	-	-	***
t-Palmitoleic	0.42 (0.02)	0.40 (0.03)	0.40 (0.02)	0.37 (0.02)	0.35 (0.01)	0.36 (0.01)	***	ns	ns
Elaidic	0.22a (0.13)	0.34b (0.02)	0.50c (0.09)	0.32b (0.01)	0.31b (0.03)	0.17a (0.02)	-	-	***
t-linoleic	0.10ab (0.01)	0.12bc (0.01)	0.13c (0.01)	0.10ab (0.01)	0.08a (0.00)	0.11bc (0.01)	-	-	**

Results are expressed as mean (standard deviation). n=16. PxS: packaging system x sausage; Values in the same row bearing different letters are significantly different (p< 0.05) ns: Not significant; P>0.05, **P<0.01, ***P<0.001. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid.

Table 3. Fatty acid categories (g/100g fatty acids) and ratios with nutritional relevance in control and linseed oil containing sausages after 2 months of storage.

	Control sausage			Linseed oil sausage			Sausage	Pack	PxS
	Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP			
ΣSFA	41.93c (1.15)	42.05c (0.97)	42.24c (0.27)	37.03b (0.91)	36.82b (0.71)	34.79a (0.47)	-	-	*
ΣMUFA	43.27 (0.43)	43.44 (1.57)	43.74 (0.57)	41.35 (1.08)	40.37 (1.29)	40.58 (0.74)	***	ns	ns
ΣPUFA	13.68a (0.45)	13.81a (0.70)	13.29a (0.42)	21.08b (0.89)	22.31b (1.23)	23.89c (0.39)	-	-	**
PUFA/SFA	0.33a (0.01)	0.33a (0.01)	0.31a (0.01)	0.57b (0.01)	0.61b (0.04)	0.69c (0.02)	-	-	***
MUFA+PUFA/ SFA	1.36a (0.04)	1.36a (0.03)	1.35a (0.02)	1.68b (0.18)	1.70b (0.09)	1.85c (0.05)	-	-	**
n-6/n-3	13.94b (0.38)	18.80d (0.99)	15.76c (0.72)	2.68a (0.03)	2.40a (0.08)	2.19a (0.03)	-	-	***

Results are expressed as mean (standard deviation). n=16. PxS: packaging system x sausage; Values in the same row bearing different letters are significantly different (p< 0.05) ns: Not significant; P>0.05, ***P<0.001. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 4. Fatty acid categories (g/100g fatty acids) and ratios with nutritional relevance in control and linseed oil containing sausages after 5 months of storage.

	Control sausage			Linseed oil sausage			Sausage	Pack	PxS
	Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP			
ΣSFA	42.67 (1.70)	41.39 (3.11)	42.40 (0.46)	36.22 (1.01)	35.41 (1.66)	34.83 (1.16)	***	ns	ns
ΣMUFA	43.37 (1.47)	42.46 (4.09)	42.10 (0.58)	41.72 (1.84)	40.11 (1.53)	40.23 (1.93)	*	ns	ns
ΣPUFA	13.23 (0.43)	15.28 (0.89)	14.48 (0.27)	21.27 (1.36)	23.72 (1.18)	24.30 (1.20)	***	***	ns
PUFA/SFA	0.31a (0.00)	0.37c (0.01)	0.34b (0.01)	0.59d (0.02)	0.67e (0.02)	0.70f (0.01)	-	-	***
MUFA+PUFA/ SFA	1.33a (0.01)	1.39a (0.02)	1.33a (0.03)	1.70b (0.04)	1.80c (0.04)	1.85c (0.04)	-	-	**
n-6/n-3	23.00d (1.54)	18.49c (1.05)	15.24b (0.42)	2.39a (0.06)	2.17a (0.04)	2.07a (0.05)	-	-	***

Results are expressed as mean (standard deviation). n=16. PxS: packaging system x sausage; Values in the same row bearing different letters are significantly different ($p < 0.05$) ns: Not significant; $P > 0.05$, *$P < 0.05$, ***$P < 0.001$. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 5. Lipid oxidation parameters of control and linseed oil containing sausages after 2 months of storage. TBARs is expressed in ppm, Peroxides in meq O_2/kg and volatiles in ng dodecane/g dry matter.

	Control sausage			Linseed oil sausage			Sausage	Pack	PxS
	Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP			
TBARs	1.79b (0.22)	0.07a (0.01)	0.07a (0.01)	0.25a (0.08)	0.11a (0.02)	0.08a (0.02)	-	-	***
Peroxides	204.99d (1.50)	0.5a (0.00)	0.5a (0.00)	1.53b (0.05)	3.53c (0.15)	2.99c (0.16)	-	-	***
Hexanal	22810b (2615)	142a (22.63)	605a (33.23)	929a (54.45)	159a (15.56)	212a (21.92)	-	-	***
Heptanal	1705b (136.47)	0.00a (0.00)	34a (1.41)	125a (16.26)	0.00a (0.00)	0.00a (0.00)	-	-	***
Octenal	4379b (472.32)	0.00a (0.00)	0.00a (0.00)	165a (32.53)	0.00a (0.00)	0.00a (0.00)	-	-	***
Nonanal	4054b (328.10)	298a (9.90)	433a (25.46)	623a (16.97)	395a (21.92)	586a (31.82)	-	-	***
2,4-decadienal	29565b (2921)	0.00a (0.00)	111a (8.49)	1242a (30.41)	0.00a (0.00)	0.00a (0.00)	-	-	***
tt-2,4-decadienal	8271b (872.6)	0.00a (0.00)	44a (2.83)	242a (24.04)	0.00a (0.00)	0.00a (0.00)	-	-	***

Results are expressed as mean (standard deviation). n=16. PxS: packaging system x sausage; Values in the same row bearing different letters are significantly different ($p < 0.05$) ***$P < 0.001$.

Table 6. Lipid oxidation parameters of control and linseed oil containing sausages after 5 months of storage. TBARs is expressed in ppm, Peroxides in meq O_2/kg and volatiles in ng dodecane/g dry matter.

	Control sausage			Linseed oil sausage			Sausage	Pack	PxS
	Aerobic	Vacuum	MAP	Aerobic	Vacuum	MAP			
TBARs	2.12c (0.24)	0.13a (0.01)	0.08a (0.00)	0.38b (0.06)	0.10a (0.03)	0.08a (0.01)	-	-	***
Peroxides	120.39d (0.68)	1.85b (0.14)	2.08b (0.13)	3.59c (0.25)	1.80b (0.06)	0.5a (0.00)	-	-	***
Hexanal	35394e (173.24)	2053c (36.06)	737b (18.38)	9310d (195.8)	274a (48.10)	973b (140.71)	-	-	***
Heptanal	2075c (141.42)	212a (14.14)	79a (12.02)	756b (4.24)	0.00a (0.00)	0.00a (0.00)	-	-	***
Octenal	4697c (157.68)	217a (7.07)	73a (4.95)	1268b (14.14)	0.00a (0.00)	0.00 (0.00)	-	-	***
Nonanal	4507e (272.24)	825bc (18.38)	478ab (57.27)	1990d (30.41)	358a (2.83)	1166c (29.70)	-	-	***
2,4-decadienal	23019c (1552)	398a (6.36)	157a (7.78)	6561 (581.9)	0.00a (0.00)	0.00a (0.00)	-	-	***
tt-2,4-decadienal	7099c (485.07)	107a (9.19)	35a (3.54)	1679b (139.3)	0.00a (0.00)	0.00a (0.00)	-	-	***

Results are expressed as mean (standard deviation). n=16. PxS: packaging system x sausage; Values in the same row bearing different letters are significantly different (p< 0.05) ***P<0.001.