Title: Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae *Schizochytrium sp*.: influence on nutritional properties, sensorial quality and oxidation stability.

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A**bstract**

Significant enrichment of dry fermented sausages in n-3 polyunsaturated fatty acids (PUFA) was achieved by incorporating algae oil from *Schizochytrium sp* into an emulsion that partially substituted pork backfat from the traditional formulation. Two different levels of substitution were initially tested, both including BHA and BHT as antioxidants: (15% and 25%). A triangular test showed that products with 25% substitution were not sensorially acceptable. Sausages with 15% substitution supplied 1.30g/100g product of docosahexaenoic acid (DHA), with an interesting n-6/n-3 ratio of 2.62. No signs of oxidation were detected at the end of the ripening process, with low values of TBARs (<0.2 mg/kg), peroxides (<2meqO_2/kg fat) and volatile aldehydes. Storage of these sausages under vacuum during 30 days totally guaranteed their stability, whereas after 90 days certain degree of oxidation was detected, only by means of aldehydes analysis. Hexanal, nonanal and dienals increased, whereas no increment in TBARs or peroxides were observed. Aerobical storage of these sausages was not viable due to a high oxidation susceptibility of the new formulation, despite the use of antioxidants.

Key words: DHA, functional ingredient, novel food, n-3 PUFA, lipid, storage.

Running Head: n-3 enriched sausages with algae oil

1. Introduction

Studies suggest that long chain n-3 polyunsaturated fatty acids (PUFAs) support good cardiovascular health, may have a beneficial effect in several forms of cancer and also in diseases with an immunoinflammatory component, and they play a role in the brain (Ruxton, Calder, Reed & Simpson, 2005). Brain consists of 60% structural fat, with docosahexahenoic acid (DHA) as the most abundant fatty acid in the grey matter and furthermore it is involved in the eye retina development. DHA can not be synthesized *de novo* in human and it is only poorly synthesized via elongation and desaturation ~~of its~~ from the essential fatty acid precursor α-linolenic acid (Pawlosky, Hibbeln, Novotny & Salem, 2001). Natural sources of long chain n-3 PUFA are fish oils, marine protists, dinoflagellates and microalgae (Ward & Singh, 2005). Lovegrove, Brooks, Murphy, Gould and Williams (1997) concluded that enriched manufactured foods, supplying a mean daily intake of 1.4g eicosapentaenoic acid + docosahexaenoic acid, were a feasible vehicle for increasing n-3 PUFA intake, leading to increases in fasting HDL-cholesterol concentration.

Research and development teams in meat industry have made great efforts during the last decades to improve the nutritional profile of meat products. In relation to the quality of fat, some works have been done to decrease the total saturated fatty acids (SFAs) and/or to increase the monounsaturated fatty acids (MUFAs) and PUFAs, using vegetable oils as partial substitutes of the conventional fat from animal origin (Muguerza, Gimeno, Ansorena & Astiasarán, 2004a). Some efforts have also been made trying to develop meat products with significant amounts of long chain n-3 PUFA. The fortification of meat products with n-3 PUFA can be performed in two ways: by feeding animals used for food production with n-3 PUFA to generate n-3 enriched eggs, meat and milk; or by direct addition of n-3 PUFA sources as ingredients during processing.

The use of fish oil, although efficient from the nutritional point of view, has given rise to some sensory problems (Elmore et al., 2005; Park, Keeton & Rhee, 1989; Muguerza, Ansorena & Astiasarán, 2004b).

Algae oil is a significant source of long chain n-3 PUFA. In fact, microalgae have been explored as potential sources of functional ingredients (Herrero, Cifuentes & Ibañez, 2006) and concerning the lipid fraction, they are the initial producers of DHA in the marine food chain. They have been used as feed supplements to enrich poultry and milk products (Abril, Barclay & Abril, 2000; Papadopoulos, Goulas, Apostolaki & Abril, 2002) and were considered safe as a supplement in swine feed (Abril, Garret, Zeller, Sander & Mast, 2003). In particular, the oil from the microalgae *Schizochytrium sp*., supplying at least 32% of DHA, has been allowed as a novel food ingredient (2003/427/CE). According to this Decision of the European Union, this oil can be used at different concentrations as a DHA source in different products and analogues, spreadable fat and dressings, breakfast cereals, food supplements, dietary foods for special medical purposes and foods intended for use in energy-restricted diets weigh reduction.

Algae oil shows additional benefits as long PUFA supplier over fish oils, including low taste intensity and off-odor problems. In any case, incorporation of n-3 PUFA into food systems is potentially problematic due their susceptibility to suffer oxidation. Lipid oxidation is a major reason for food spoilage, where the molecular oxygen can attack unsaturated fatty acid, developing undesirable off-flavour products and untasty flavour. Synthetic antioxidants, such as butylhydroxianisol (BHA) and butylhydroxitoluene (BHT) have been commonly used to control the formation of free radicals and prevent lipid oxidation in meat products (Ansorena & Astiasarán, 2004a; Muhammet & Mükerrem, 2005; Sebranek, Sewalt, Robbins & Houser, 2005).

The aim of this work was to develop dry-fermented meat products using algae oil as functional ingredient, selecting the most suitable amount to be added, on basis of nutritional and sensory aspects. The impact on the stability of these products during their storage under different conditions (aerobic and vacuum packaging) has also been studied.

2. Materials and methods

Sausage formulation and processing.

Three batches of dry fermented sausages (Chorizo de Pamplona), about 9 kg each, were prepared according to the procedure described by Muguerza, Gimeno, Ansorena, Bloukas and Astiasarán (2001). Control batch was produced using 75% lean pork meat and 25% pork backfat. Modified batches were prepared by substituting a 25% and 15% respectively of pork backfat by pre-emulsified algae oil DHASCO®-S. (Market Biosciences Corporation, Columbia, USA).

The emulsion was prepared by mixing, for two minutes, eight parts of hot water (50ºC) with one part of isolated solid soy protein, and then with ten parts of algae oil for another three minutes (Hoogenkamp, 1989ab). Analysis of the fatty acid profile and cholesterol content of the algae oil was carried out in our laboratory. Results expressed as g/100g of fatty acids, were as follows: lauric (0.32), myristic (9.09), palmitic (22.86), t-palmitoleic (0.07), palmitoleic (0.21), stearic (0.57), elaidic (0.01), oleic (1.11), vaccenic (0.13), linoleic (0.46), γ-linolenic (0.22), α-linolenic (0.09), behenic (0.03), brassidic (0.41), erucic (1.71), arachidonic (0.51), eicosapentaenoic (1.25), docosapentaenoic n-6 (15.44) and docosahexaenoic (42.41). Algae oil contained 154mg cholesterol/100g.

The following ingredients per kilogram of meat mixture were added to the three formulations: NaCl 26g, red pepper 30g, dextrin 15g, lactose 10g, milk powder 12g, dextrose 5g, sodium ascorbate 0.5g, sodium caseinate 10g , garlic 3g , polyphosphates 2g, curing agents (a mixture of NaCl, preservatives E-250 -sodium nitrite-, E-252 – potassium nitrate-and antioxidant E-331-sodium citrate-) 3g, ponceau 4R (E-124) 0.15g. The starter culture used was a mixture of *Lactobacillus plantarum* L115 (50%) and *Staphylococcus carnosus* M72 (50%) to each 10^6-10^7 cfu/kg of mixture. 100mg/kg of

butylhydroxytoluene (BHT) and 100mg/kg of butylhydroxyanisole (BHA) were added as antioxidants in two the experimental batches.

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, only control and modified sausages with 15% of substitution with algae oil were stored at 4ºC. Each type of sausage was divided in two groups: one group of products was aerobically packed and the other group was vacuum packed (Ramon Seri: VP Mod: 45, Pamplona, Spain) using bags of polyamide/polyethylene 90μm (Corsan, Pamplona, Spain). Analysis was carried out after 30 and 90 days of storage at 4ºC.

Chemical analysis.

Moisture was determined according to the Association of Official Analytical Chemists (AOAC, 2002a). Total fat was determined by an extraction with petroleum ether according to the (AOAC, 20). The method of Folch, Lees and Stanley (1957) was used for the extraction of lipids. Fatty acids were determined in the lipid extract by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters, which were finally solved in hexane (AOAC, 2002c). A Perkin-Elmer Clarus 500 gas chromatograph (PE, Shelton, CT, USA) fitted with a capillary column SP^{TM}-2560 (100m x 0.25mm x 0.2μm) and flame ionization detection was used. The temperature of the injection port was 250ºC and of the detector was 260ºC. The oven temperature was programmed at 175ºC during 10min and increased to 200ºC at a rate of 10ºC/min, then increased to 220ºC at a rate of 4ºC/min, which was held for 15min. The carrier gas was hydrogen, and the pressure was 20.5psi. Split ratio was 120:1. The

identification of the fatty acid methyl esters was done by comparison of the retention times of the peaks in the sample with those of standard pure compounds (Sigma, St. Louis, MO, USA) and by spiking the sample with each standard compound individually. The quantification of individual fatty acids was based on the internal standard method, using heptadecanoic acid methyl ester (Sigma, St. Louis, MO, USA). 1ml of the internal standard solution (7mg/ml) was added to 1ml of the fatty acid methyl ester hexane solution, just previously to be injected. Peroxide value was determined using the official method of the AOAC (2002d). 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.

Cholesterol content was analyzed by gas chromatography with previous extraction with hexane according to Kovacs, Anderson and Ackman (1979). Derivatization to obtain the trimethyl silyl ethers of cholesterol was performed. A Perkin-Elmer Autosystem XL gas chromatograph equipped with an HP1 column (30m x 0.25mm x 0.1μm) was used. The oven temperature was 265ºC. The temperature of both the injection port and detector was 285ºC. Cholesterol was identified by comparing its retention time with that of a standard (Sigma, ST. Louis, MO, USA) and quantification was done by using pure cholestane (Sigma, St. Louis, MO, USA) as an internal standard, which was added to the sample as a solution (2mg/ml), previously to the extraction procedure. A Perkin-Elmer Turbochrom programme was used for quantification.

Volatile compounds

A Likens-Nickerson extraction using dicloromethane was carried out according to the method described by Ansorena, Zapelena, Astiasarán and Bello (1998). 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. 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.

Analysis. The volatile compounds were analyzed in a HP 6890 GC system (Hewlett-Packard, Palo Alto, USA) coupled to a 5973 mass selective detector (Hewlett-Packard). A total of 1 μl of the extract was injected into the GC, equipped with a capillary column (30 m 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 the comparison of their mass spectra with the spectra of the Wiley library (HPCHEM Wiley 275 6th Ed.) and, in some cases, a comparison of their retention 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 (Kondjoyan & Berdagué, 1996). Only compounds 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.

Sensory analysis

Sensory analysis was performed by a fully trained analytical taste panel of 20 members. A discrimination method (triangular test) was applied to determine the existence of perceptible sensorial differences in appearance, odor and taste between the control sausage and the sausages with different percentages of fat substitution by algae oil. Samples were presented sliced, on a white plate, at room temperature. Each panelist was presented with three samples simultaneously, of which two of them were identical. Each taster was asked to identify the different sample. The number of correct responses given by panellists were determined and data shown in the table corresponded to the mean value obtained for each type of product.

According to the Norma UNE 87-006-92 (1992), for a 20 member panel, the difference between samples was significant (it means, panellists were able to identify the different sample) if the number of correct answers was 11 ($p<0.05$), 13 ($p<0.01$) and 14 ($p<0.001$).

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 one-way ANOVA. When F values were significant ($p<0.05$), means were compared by a Tukey's b posteriori test. Pearson correlation test was applied among oxidation parameters. The multivariate method principal component analysis (PCA) was also carried out making possible to identify the most important directions of variability in a multivariate data matrix and to present the results in graphical plots. In these plots, samples close together have similar

characteristics and variables close together are positively correlated. Varimax rotation was applied in order to maximise the variance in each loading vector. All statistical analyses were performed using SPSS version 13.0 software package (© 2004, SPSS inc. Chicago, Illinois).

3. Results and discussion

Control and modified formulations with different percentages of algae oil showed some differences in the moisture content at the end of the ripening process, despite using the same technological conditions for all products. These differences, although not too high, were probably the cause, together with the intrinsic differences due to the raw material, of the significant differences found for the amount of total fat (table 1). In fact, pork backfat used in control sausages is partially substituted by an emulsion containing oil, water and soy protein. Effectively, products with a 25% substitution of pork backfat by algae oil showed nearly 4% less fat than control ones. However, products with a 15% substitution did not show significant differences in the fat content in relation to control ones, showing much lower differences in moisture.

Cholesterol is an important compound from the nutritional and healthy point of view. When vegetable oils have been used as partial substitutes of pork backfat in dry fermented sausages, different results had been found. Muguerza, Ansorena and Astiasarán (2003) using soy oil (25% substitution) found only slight decreases in the cholesterol amount (92.96mg/100g product in control sausages, 87.71mg/100g in sausages prepared with 25% of substitution). Using olive oil, also at a 25% substitution level, cholesterol was reduced from 94.24 mg/100g in control, to 82.06 mg/100g in olive oil containing sausages (Muguerza et al., 2001). It has to be noted that algae oil contains 154mg cholesterol/100g product and pork backfat has only 70mg/100g product. So the use of algae oil in the formulations of fermented sausages might increase their cholesterol content. However, no statistical differences were found for this parameter among the three elaborated batches ($p<0.05$). This finding means an advantage compared to the use of other long chain n-3 PUFA in dry fermented sausages, such as deodorized fish oil (Valencia, Ansorena & Astiasarán, 2006a)**.** In that

work, a significant increase in the amount of cholesterol was found in the modified products (134mg cholesterol/100g product) in relation to control sausages (102mg cholesterol/100g product).

Analysis of the fatty acid profiles of the three types of products were done at the end of ripening process (table 2). Although significant differences were found for every saturated fatty acid between control and modified products, only when a 25% of substitution was tested, the total saturated fatty acid fraction showed a significant decrease (from 38.58g/100g in control to 35.66g/100g in 25% algae oil product). Greater changes were observed for MUFA and PUFA fractions. A significant decrease was observed for oleic acid, giving rise to a decrease of MUFA from 46.37g/100g fatty acids in control products to 41.98 and 42.28 g/100g fatty acids in modified products. On the contrary, PUFA increased from 14.98 to 19.01 and 21.91g/100g fatty acids in 15% and 25% modified products, respectively. These increments were directly related to the increments found for the contents of EPA n-3, DPA n-6 and basically DHA n-3. For all of them it can be seen that the higher the algae oil amount used in the formulation, the higher the long chain PUFA content in modified sausages. In 15% algae oil products, total n-3 reached 5.26g/100g fatty acids, whereas total n-6 did not change significantly from the value found for the control. In 25% algae oil products, the total n-3 was 6.54g/100g fatty acids and also a significant increase for total n-6 was observed. Considering these data from the nutritional point of view, modified batches with 15% and 25% algae oil, supplied 1.34 and 1.49g/100g sausage of EPA+DHA, respectively. These values are notably higher than the minimum adequate daily intake of 0.65g for the sum of these two fatty acids established by Simopoulos, Leaf and Salem (1999). Valencia et al. (2006a) using deodorized fish oil (25% substitution), developed sausages in which the total amount of EPA+DHA only reached 1.10g/100g product, with a n-6/n-

3 ratio of 2.97. In this work, lower n-6/n-3 ratios were achieved, being 2.62 and 2.35 in modified products. This fact meant a clear benefit compared to control products, that showed a n-6/n-3 ratio of 9.41. When linseed oil was used as a substitute of pork backfat, PUFA/SFA and n-6/n-3 ratios were around 0.6 and 2, respectively (Ansorena & Astiasarán, 2004b). However, in those products, the amounts of long chain n-3 PUFA (EPA and DHA) were almost zero.

All these beneficial changes in the lipid fraction achieved by increasing the PUFA content could make the products more susceptible to oxidation. Although n-3 PUFA are flavourless, they easily undergo radical oxidation, forming objectionable off-flavor compounds (Frankel, 1998). In consequence, it is important to control the intensity of this process in the products. Table 3 shows the results obtained for the traditionally used parameters to measure the intensity of the primary and secondary oxidation and also the content of specific volatile compounds formed as a consequence of lipid oxidation.

At the end of ripening, no signs of oxidation increment in modified products with regard to control sausages were observed in any of the parameters used. TBARs showed values lower than 0.2mg/kg and peroxides did not reach 2meq O_2/kg fat, pointing at the stability of products at the end of ripening process. Also, none of the dienals, typical oxidation products from long chain PUFA characterized by their rancid odour, were found in any of the elaborated batches. The use of BHA+BHT in addition to the ascorbate added to the traditional formulation in order to guarantee their stability, gave also good results in previous papers in which studies on the lipid fraction of dry fermented sausages were performed (Ansorena et al., 2004a).

When working on the nutritional improvement of traditional products by changing raw materials, it is of great importance to maintain the typical sensorial characteristics in order to ensure their commercial success. A sensory triangular test was carried out to

evaluate whether the panel members were able to distinguish between control and modified products (table 4). Results confirmed that appearance and odour did not show statistical differences between control and modified products for most of the panel members. 85-90% of the panellists indicated that control and modified products were similar in appearance, and 70-75% found that they were similar in odour. In relation to taste, different results were observed depending on the concentration of algae oil in the modified products. 90% of the panellists indicated no differences between control and 15% substitution products. However, 80% of the panel was able to distinguish the sausages with 25% algae oil from the control. These results determined that products with 25% algae oil substitution were not viable from the sensory point of view, due to a special taste, not typical from chorizo de Pamplona, detected by panellists.

As a consequence of the no viability of formulations with 25% algae oil substitution, the second part of the research was only carried out with 15% algae oil products. Samples were kept during 30 and 90 days in aerobic and vacuum conditions in order to analyze the stability of the lipid fraction with a high presence long chain PUFA n-3.

In previous papers it had been noted that oxidation can sometimes induce small changes in food lipid profiles depending on the intensity of the process. Certain loss in α-linolenic acid was detected in linseed enriched sausages after 5 months of storage, particularly in aerobic conditions, still maintaining better nutritional properties than traditional sausages (Valencia, Ansorena & Astiasarán, 2006b). In this work, the fatty acid profile did not show relevant changes during storage in any of the conditions tested for both types of products (table 5 and 6). Sausages elaborated with algae oil at 15% substitution level and antioxidants did not modify their lipid profile during 90 days of storage, regardless the packaging system used. These results pointed out the stability of the fatty acids in this conditions and let us to conclude about the maintenance of the

nutritional benefit of algae oil containing sausages during storage. Data found for TBARs and peroxides gave some interesting results (table 7). Control sausages did not show any significant increment in TBARs during storage ($p<0.05$) and peroxides were not detected. However, modified products showed a high peroxide value and a clear increment of TBARs in presence of oxygen at 90 days of storage, reaching a value of 1.93 mg/kg. It has to be noted that substitution of pork backfat in dry fermented sausages with linseed oil and olive oil at level of 25%, both using antioxidants, did not induce an increase in TBARs during storage in aerobic conditions (Ansorena et al., 2004a; Valencia et al., 2006b).

When the amounts of volatile aldehydes were analyzed, more information about the evolution of oxidation was obtained. Both for control and modified products after 30 days of storage in vacuum conditions, only slight differences were found in the profile of these compounds in relation to final product. When the storage was done aerobically, modified products showed a significant increment for 8 of the 10 analyzed volatiles whereas only 2 increased in control ones. This indicated a higher susceptibility of modified products to oxidation, which was only detected in aerobic conditions. When the storage was prolonged to 90 days, control sausages started showing higher values for hexanal and nonanal in aerobic conditions, considered normal for this type of products (Ansorena et al., 2004a). In the case of modified products the signs of oxidation were evident for aerobic storage conditions being total aldehydes content nearly 9 fold the value observed at the end of ripening. Samples corresponding to vacuum storage also showed significant amounts of hexanal and three analyzed dienals, pointing out a certain oxidation degree not detected by traditional parameters. Nevertheless, Pearson correlation test reported high statistically significant correlations between TBARs and peroxides values and volatile aldehydes derived from unsaturated

fatty acids oxidation. Every aldehyde correlated better with peroxides than with TBARs, with the highest value for tt-2,4-heptadienal ($r=0.991$; $p<0.01$), except for t2-heptenal, that gave the highest value ($r=0.995$; $p<0.01$) for the correlation with TBARs. The two decadienals gave, for both TBARs and peroxides, significant correlations higher than 0.96.

A principal component analysis with varimax rotation (PCA) was performed with results from fatty acid profiles and oxidation parameters as variables. Fig. 1 shows both the loading vectors of PC1 (first principal component) versus PC2 (second principal component) for these parameters in a bidimensional plot, and the score vectors for the different samples. The analysis showed that about 46.78% of the total variation was explained by PC1 and the 32.83% by the PC2, concluding that almost 80% of the total variance in the variables was condensed into two new variables (PCs). The first PC was defined by high positive values for long chain fatty acids, typical from algae oil, (0.97 for DHA also for DPA n-6) and low values for oleic acid (-0.98). PC2 was defined by high positive values for the oxidation parameters, particularly the two decadienals (0.97). The score plot revealed a clear separation of sausages according to the ingredients used in the formulation. Products including algae oil, with high long chain fatty acids content, were located on the right side of the plot, with positive values for PC1, whereas control products had all negative values on PC1. Regarding their storage conditions, no clear distinction was appreciated for control sausages, all located close together in the plot, pointing at a great stability of products during storage regardless the packaging system used. However, some differences were detected for modified products. Aerobically stored samples, and particularly during longer period of time (90 days) were clearly separated from vacuum treated ones and products analyzed just at the end of ripening (final products). Algae oil containing sausages stored under vacuum

showed a slightly different behaviour. Sausages after 30 days of storage hardly differed from final products, whereas as it can be expected by the previous commented results found for volatile compounds, after 90 days of storage they had slightly lower scores for PC1 and higher for PC2. This finding led us to conclude that despite the use of antioxidants, a good storage system of algae oil containing sausages becomes of great importance, and confirms the need of controlling a potential oxidation, even at conditions of absence of oxygen.

In summary, the results reported in the present research showed that algae oil can be used as a functional ingredient in dry fermented sausages in a limited amount. The obtained products had good sensorial quality, showed better n-6/n-3 ratio than traditional sausages, and supplied a relevant amount of DHA. Their stability to the oxidation should be guaranteed with the use of antioxidants and the storage under vacuum conditions.

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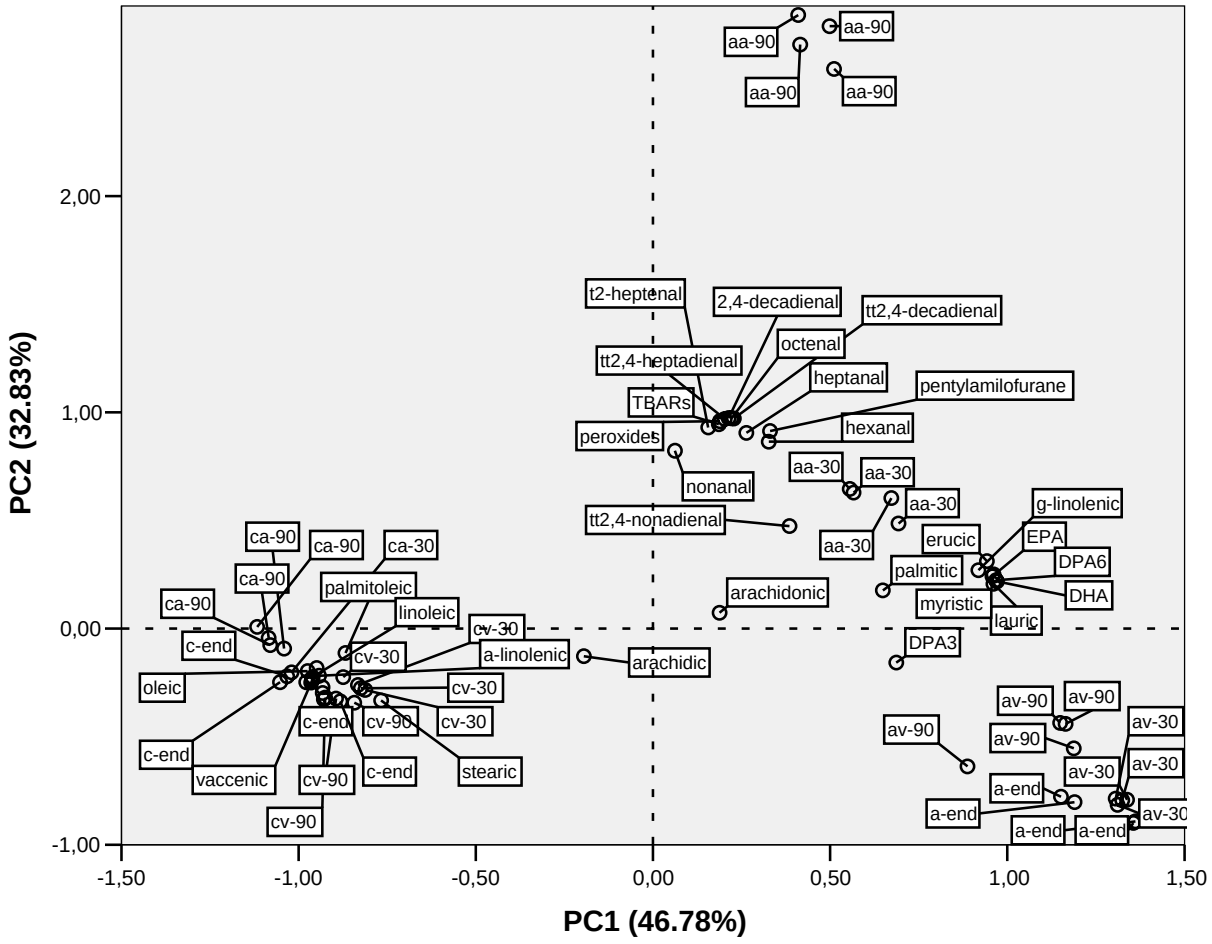
Figure 1. Plot of parameters and representation of the control and modified samples according to the factors of the rotated matrix the Principal Component Analysis.



c-end: Control sausage at the end of ripening
ca: control sausage aerobically packed (30 and 90 mean the days of storage)
cv: control sausage vacuum packed (30 and 90 mean the days of storage)
a-end: Algae oil containing sausage at the end of ripening
aa: Algae oil containing sausage aerobically packed (30 and 90 mean the days of storage)
av: Algae oil containing sausage vacuum packed (30 and 90 mean the days of storage)

Table 1. Results of moisture, fat and cholesterol in control and modified sausages at the end of the ripening process.

	Control	15% algae oil	25% algae oil
Moisture (%)	29.26 ± 0.04b	28.62 ± 0.49a	33.33 ± 0.30c
Fat (%)	32.89 ± 0.31b	32.52 ± 0.45b	28.10 ± 0.29a
Cholesterol (mg/100g product)	89.48 ± 3.61a	82.72 ± 5.98a	90.75 ± 4.94a

Results are expressed as mean ± standard deviations. n=16. Values in the same row bearing different letters are significantly different ($p < 0.05$).

Table 2. Total fatty acids (g/100g of fatty acids) and ratios with nutritional relevance in control and modified sausages at the end of the ripening period.

	Control	**15% algae oil**	**25% algae oil**
Lauric C12:0	0.09 ± 0.00a	0.12 ± 0.00b	0.11 ± 0.00b
Myristic C14:0	1.24 ± 0.01a	1.95 ± 0.01b	2.15 ± 0.05c
Palmitic C16:0	24.36 ± 0.02b	24.87 ± 0.05b	22.75 ± 0.61a
Stearic C18:0	12.80 ± 0.13c	11.93 ± 0.01b	10.57 ± 0.33a
Arachidic C20:0	0.09 ± 0.00c	0.07 ± 0.00b	0.05 ± 0.00a
∑SFA	38.58 ± 0.11b	38.93 ± 0.06b	35.66 ± 1.00a
Palmitoleic C16:1	2.10 ± 0.02c	2.03 ± 0.01b	1.95 ± 0.04a
Oleic C18:1(n-9)	40.60 ± 0.05b	36.49 ± 0.14a	36.40 ± 0.87a
Vaccenic C18:1(n-7)	2.82 ± 0.00b	2.59 ± 0.00a	2.87 ± 0.07b
Eicosenoic C20:1(n-9)	0.86 ± 0.00c	0.75 ± 0.01a	0.80 ± 0.02b
Erucic C22:1	0.00 ± 0.00a	0.12 ± 0.00b	0.18 ± 0.00b
∑MUFA	46.37 ± 0.07b	41.98 ± 0.15a	42.28 ± 1.01a
Linoleic C18:2(n-6)	13.03 ± 0.05c	11.85 ± 0.02a	12.50 ± 0.34b
α-Linolenic C18:3(n-3)	0.97 ± 0.02b	0.86 ± 0.01a	0.87 ± 0.02a
γ-Linolenic C18:3(n-6)	0.03 ± 0.00a	0.05 ± 0.00b	0.06 ± 0.00c
Arachidonic C20:4 (n-6)	0.38 ± 0.00a	0.40 ± 0.01ab	0.42 ± 0.02b
Eicosadienoic C20:2 (n-3)	0.00a	0.00a	0.56 ± 0.01b
Eicosapentaenoic C22:5(n-3)	0.03 ± 0.00a	0.13 ± 0.00b	0.16 ± 0.00c
Eicosatrienoic C20:3 (n-3)	0.12 ± 0.00b	0.09 ± 0.00a	0.12 ± 0.00b
Docosapentaenoic 22:5 (n-6)	0.09 ± 0.00a	1.46 ± 0.05b	1.82 ± 0.06c
Docosapentaenoic 22:5 (n-3)	0.22 ± 0.00a	0.25 ± 0.00c	0.24 ± 0.00b
Docosahexaenoic C22:6(n-3)	0.10 ± 0.00a	3.92 ± 0.16a	5.16 ± 0.19c
∑n-3	1.44 ± 0.02 a	5.26 ± 0.16b	6.54 ± 0.23c
∑n-6	13.54 ± 0.05a	13.75 ± 0.05a	15.36 ± 0.42b
∑PUFA	14.98 ± 0.04a	19.01 ± 0.21b	21.91 ± 0.66c
t-Palmitoleic C16:1t	0.00a	0.02 ± 0.00b	0.06 ± 0.00c
t-Linoleic C18:2t	0.00	0.00	0.00
Elaidic C18:1t	0.07 ± 0.00b	0.06 ± 0.00a	0.08 ± 0.00c
Brassidic C20:1t	0.00a	0.00a	0.01 ± 0.00b
∑TRANS	0.07 ± 0.00a	0.08 ± 00b	0.15 ± 0.00c
PUFA/SFA	0.39 ± 0.00a	0.49 ± 0.01b	0.61 ± 0.00c
MUFA+PUFA/SFA	1.59 ± 0.01b	1.57 ± 0.00a	1.80 ± 0.00c
n-6/n-3	9.41 ± 0.18c	2.62 ± 0.07b	2.35 ± 0.02a

Results are expressed as mean ± standard deviations. n=16. Values in the same row bearing different letters are significantly different ($p < 0.05$).

Table 3. Lipid oxidation parameters in control and modified sausages at the end of the ripening period. Peroxides is expressed in meq O_2/kg fat, TBARs in mg/kg, and volatiles in ng dodecane/g dry matter.

	Control	15% algae oil	25% algae oil
Peroxides	0.00a	0.00a	1.23 ± 0.08b
TBARs	0.13 ± 0.01b	0.16 ± 0.01c	0.08 ± 0.00a
Hexanal	560 ± 51a	805 ± 41b	512 ± 38a
Heptanal	156 ± 9a	157 ± 5a	159 ± 7a
t-2-heptenal	0.00	0.00	0.00
2-pentylamilofurane	97 ± 5a	286 ± 1c	186 ± 11b
tt-2,4-heptadienal	0.00	0.00	0.00
Octenal	0.00	0.00	0.00
Nonanal	1021 ± 46b	781 ± 35a	852 ± 82a
tt-2,4-nonadienal	0.00	0.00	0.00
tt-2,4-decadienal	0.00	0.00	0.00
2,4-decadienal	0.00	0.00	0.00

Results are expressed as mean ± standard deviations. n=16. Values in the same row bearing different letters are significantly different ($p < 0.05$).

Table 4. Sensory scores of triangular test for control sausages vs. modified sausages at the end of the ripening period.

Control vs 25%algae oil	**Appearance *n* (% within group)**	**Odor *n* (% within group)**	**Taste *n* (% within group)**
Correct replies	2 (10%) ns	6 (30%) ns	16 (80%) ***
Incorrect replies	18 (90%)	14 (70%)	4 (20%)
Total	20 (100%)	20 (100%)	20 (100%)
Control vs 15%algae oil			
Correct replies	3 (15%) ns	5 (25%) ns	6 (10%) ns
Incorrect replies	17 (85%)	15 (75%)	14 (90%)
Total	20 (100%)	20 (100%)	20 (100%)

For n=20, the difference between samples was significant if the number of correct answers was 11 ($p<0.05$), 13 ($p<0.01$) and 14 ($p<0.001$; ***). Ns: not significant.

Table 5. Total fatty acids (g/100g of fatty acids) and ratios with nutritional relevance in **control sausages**.

	Final product	30 days aerobic	30 days vacuum	90 days aerobic	90 days vacuum
Lauric C12:0	0.09 ± 0.00b	0.09 ± 0.00b	0.09 ± 0.00b	0.09 ± 0.00b	0.08 ± 0.00a
Myristic C14:0	1.24 ± 0.01ab	1.23 ± 0.01ab	1.24 ± 0.00ab	1.26 ± 0.00b	1.22 ± 0.02a
Palmitic C16:0	24.36 ± 0.02c	24.17 ± 0.06a	24.35 ± 0.03c	24.29 ± 002bc	24.23 ± 0.04ab
Stearic C18:0	12.80 ± 0.13b	12.57 ± 0.02a	12.83 ± 0.01b	12.50 ± 0.01a	13.12 ± 0.01b
Arachidic C20:0	0.09 ± 0.00a	0.10 ± 0.00a	0.10 ± 0.00a	0.09 ± 0.00a	0.12 ± 0.00b
∑SFA	38.57 ± 0.11b	38.15 ± 0.05a	38.60 ± 0.04b	38.23 ± 0.03a	38.78 ± 0.06c
Palmitoleic C16:1	2.09 ± 0.02a	2.11 ± 0.01a	2.10 ± 0.00a	2.14 ± 0.00b	2.09 ± 0.00a
Oleic C18:1(n-9)	40.60 ± 0.05c	40.51 ± 0.12c	40.00 ± 0.03a	40.50 ± 0.02c	40.20 ± 0.07b
Vaccenic C18:1(n-7)	2.82 ± 0.00b	2.83 ± 0.00bc	2.79 ± 0.00a	2.82 ± 0.00b	2.83 ± 0.00c
Eicosenoic C20:1(n-9)	0.86 ± 0.00bc	0.85 ± 0.00bc	0.82 ± 0.02a	0.84 ± 0.00b	0.86 ± 0.00c
Erucic C22:1	0.00	0.00	0.00	0.00	0.00
∑MUFA	46.37 ± 0.07c	46.30 ± 0.11c	45.71 ± 0.03a	46.30 ± 0.02c	45.98 ± 0.08b
Linoleic C18:2(n-6)	13.04 ± 0.05a	12.98 ± 0.04a	12.99 ± 0.04a	13.19 ± 0.01b	12.97 ± 0.09a
α-Linolenic C18:3(n-3)	0.97 ± 0.02bc	0.95 ± 0.00ab	0.95 ± 0.00a	0.98 ± 0.00c	0.96 ± 0.00ab
γ-Linolenic C18:3(n-6)	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a	0.03 ± 0.00a
Arachidonic C20:4 (n-6)	0.38 ± 0.00a	0.40 ± 0.00b	0.42 ± 0.00c	0.40 ± 0.00b	0.44 ± 0.00d
Eicosapentaenoic C22:5(n-3)	0.03 ± 0.00a	0.04 ± 0.00b	0.04 ± 0.00b	0.04 ± 0.00b	0.04 ± 0.00b
Eicosatrienoic C20:3 (n-3)	0.12 ± 0.00a	0.11 ± 0.01a	0.12 ± 0.00a	0.12 ± 0.00a	0.12 ± 0.00a
Docosapentaenoic 22:5 (n-6)	0.09 ± 0.00d	0.00 ± 0.00a	0.08 ± 0.00c	0.08 ± 0.00c	0.07 ± 0.00b
Docosapentaenoic 22:5 (n-3)	0.22 ± 0.00a	0.22 ± 0.00a	0.22 ± 0.00a	0.21 ± 0.01a	0.21 ± 0.00a
Docosahexaenoic C22:6(n-3)	0.10 ± 0.01a	0.09 ± 0.01a	0.10 ± 0.00a	0.09 ± 0.00a	0.10 ± 0.00a
∑n-3	1.44 ± 0.02a	1.41 ± 0.02a	1.42 ± 0.01a	1.44 ± 0.01a	1.43 ± 0.00a
∑n-6	13.54 ± 0.05b	13.41 ± 0.04a	13.54 ± 0.04b	13.71 ± 0.02c	13.51 ± 0.06b
∑PUFA	14.98 ± 0.04b	14.82 ± 0.05a	14.96 ± 0.04b	15.15 ± 0.01c	14.96 ± 0.01b
t-Palmitoleic C16:1t	0.00a	0.41 ± 0.00	0.42 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
t-Linoleic C18:2t	0.00	0.00	0.00	0.00	0.00
Elaidic C18:1t	0.02 ± 0.00a	0.30 ± 0.02c	0.29 ± 0.02c	0.29 ± 0.02c	0.25 ± 0.02b
Brassidic C20:1t	0.00a	0.01 ± 0.00b	0.00a	0.01 ± 0.00b	0.01 ± 0.00b
∑TRANS	0.02 ± 0.00a	0.72 ± 0.02d	0.73 ± 0.02d	0.32 ± 0.02c	0.28 ± 0.03b
PUFA/SFA	0.39 ± 0.00b	0.38 ± 0.00a	0.39 ± 0.00b	0.40 ± 0.00c	0.38 ± 0.00a
MUFA+PUFA/SFA	1.59 ± 0.01b	1.60 ± 0.00c	1.57 ± 0.00a	1.61 ± 0.00c	1.57 ± 0.00a
n-6/n-3	9.41 ± 0.18a	9.48 ± 0.08a	9.51 ± 0.06a	9.53 ± 0.10a	9.43 ± 0.06a

Results are expressed as mean ± standard deviations. n=16. Values in the same row bearing different letters are significantly different ($p < 0.05$).

Table 6. Total fatty acids (g/100g of fatty acids) and ratios with nutritional relevance in **modified sausages (15% substitution)**.

	Final product	30 days aerobic	30 days vacuum	90 days aerobic	90 days vacuum
Lauric C12:0	0.12 ± 0.00b	0.10 ± 0.00a	0.11 ± 0.00ab	0.11 ± 0.00ab	0.11 ± 0.00ab
Myristic C14:0	1.95 ± 0.01b	1.86 ± 0.01a	1.94 ± 0.00b	1.97± 0.03b	1.94 ± 0.03b
Palmitic C16:0	24.87 ± 0.05a	24.70 ± 0.03a	25.10 ± 0.01a	24.83 ± 0.09a	24.28 ± 0.84a
Stearic C18:0	11.93 ± 0.01ab	11.85 ± 0.01a	12.25 ± 0.01c	12.01 ± 0.06b	12.37 ± 0.13d
Arachidic C20:0	0.07 ± 0.01a	0.07 ± 0.01a	0.10 ± 0.00b	0.11 ± 0.01b	0.11 ± 0.00b
∑SFA	38.93 ± 0.06ab	38.59 ± 0.04a	39.47 ± 0.01b	39.03 ± 0.06ab	38.80 ± 0.69a
Palmitoleic C16:1	2.03 ± 0.01a	2.06 ± 0.00a	2.00 ± 0.00a	2.03 ± 0.06a	2.03 ± 0.03a
Oleic C18:1(n-9)	36.49 ± 0.14b	36.97 ± 0.02c	35.63 ± 0.02a	36.31 ± 0.04b	36.29 ± 0.42b
Vaccenic C18:1(n-7)	2.59 ± 0.00bc	2.62 ± 0.10c	2.53 ± 0.00a	2.56 ± 0.02b	2.60 ± 0.03bc
Eicosenoic C20:1(n-9)	0.75 ± 0.01b	0.76 ± 0.00bc	0.73 ± 0.01a	0.76 ± 0.00bc	0.77 ± 0.01c
Erucic C22:1	0.12 ± 0.00b	0.09 ± 0.00a	0.11 ± 0.00b	0.13 ± 0.01c	0.11 ± 0.03b
∑MUFA	41.98 ± 0.15b	42.50 ± 0.02c	41.00 ± 0.03a	41.79 ± 0.09b	41.80 ± 0.48b
Linoleic C18:2(n-6)	11.85 ± 0.02ab	11.81 ± 0.01a	11.80 ± 0.00a	11.88 ± 0.08ab	11.97 ± 0.15b
α-Linolenic C18:3(n-3)	0.86 ± 0.00a	0.86 ± 0.01a	0.85 ± 0.00a	0.86 ± 0.00a	0.89 ± 0.02b
γ-Linolenic C18:3(n-6)	0.05 ± 0.00a	0.05 ± 0.00a	0.05 ± 0.00a	0.05 ± 0.00a	0.05 ± 0.00a
Arachidonic C20:4 (n-6)	0.40 ± 0.01b	0.38 ± 0.00a	0.43 ± 0.00c	0.43 ± 0.00c	0.43 ± 0.00c
Eicosapentaenoic C22:5(n-3)	0.13 ± 0.00b	0.11 ± 0.00a	0.13 ± 0.00b	0.14 ± 0.01b	0.14 ± 0.00b
Eicosatrienoic C20:3 (n-3)	0.09 ± 0.00ab	0.09 ± 0.00ab	0.08 ± 0.00a	0.10 ± 0.09b	0.09 ± 0.00ab
Docosapentaenoic 22:5 (n-6)	1.46 ± 0.05b	1.29 ± 0.01a	1.43 ± 0.01b	1.43 ± 0.01b	1.43 ± 0.02b
Docosapentaenoic 22:5 (n-3)	0.25 ± 0.00c	0.24 ± 0.01bc	0.23 ± 0.03a	0.22 ± 0.01a	0.23 ± 0.02ab
Docosahexaenoic C22:6(n-3)	3.92 ± 0.16b	3.44 ± 0.01a	3.87 ± 0.03b	3.82 ± 0.01b	3.86 ± 0.07b
∑n-3	5.26 ± 0.16b	4.75 ± 0.01a	5.17 ± 0.02b	5.14 ± 0.04b	5.21 ± 0.07b
∑n-6	13.75 ± 0.05ab	13.53 ± 0.01a	13.72 ± 0.01b	13.79 ± 0.07bc	13.89 ± 0.16c
∑PUFA	19.01 ± 0.21b	18.28 ± 0.02a	18.89 ± 0.03b	18.93 ± 0.03b	19.10 ± 0.17b
t-Palmitoleic C16:1t	0.02 ± 0.00b	0.36 ± 0.00d	0.34 ± 0.00c	0.01 ± 0.00a	0.01 ± 0.00a
t-Linoleic C18:2t	0.00a	0.00a	0.02 ± 0.00b	0.01 ± 0.00a	0.02 ± 0.00b
Elaidic C18:1t	0.06 ± 0.00a	0.27 ± 0.01c	0.25 ± 0.01b	0.22 ± 0.01b	0.27 ± 0.02c
Brassidic C:20:1t	0.00	0.00	0.00	0.00	0.00
∑TRANS	0.08 ± 0.00a	0.63 ± 0.01e	0.61 ± 0.01d	0.25 ± 0.01b	0.30 ± 0.02c
PUFA/SFA	0.49 ± 0.01ab	0.47 ± 0.00a	0.48 ± 0.00ab	0.49 ± 0.00ab	0.50 ± 0.01b
MUFA+PUFA/SFA	1.57 ± 0.00b	1.57 ± 0.00b	1.52 ± 0.00a	1.56 ± 0.00b	1.57 ± 0.05b
n-6/n-3	2.62 ± 0.07a	2.85 ± 0.01b	2.65 ± 0.01a	2.68 ± 0.03a	2.66 ± 0.03a

Results are expressed as mean ± standard deviations. n=16. Values in the same row bearing different letters are significantly different ($p < 0.05$).

Table 7. Lipid oxidation parameters in **control sausages** and in **modified sausages (15% substitution)** during storage of sausages**.** Peroxides is expressed in meq O_2/kg fat, TBARs in mg/kg, and volatiles in ng dodecane/g dry matter.

		Final product	30 days aerobic	30 days vacuum	90 days aerobic	90 days vacuum
Peroxides	Control	0.00	0.00	0.00	0.00	0.00
	Modified	0.00a	1.39 ± 0.06b	0.00a	8.67 ± 0.9c	0.00a
	Student-t	-	***	-	***	-
TBARs	Control	0.13 ± 0.01a	0.12 ± 0.00a	0.14 ± 0.02a	0.16 ± 0.01a	0.16 ± 0.01a
	Modified	0.16 ± 0.01a	0.28 ± 0.00b	0.14 ± 0.02a	1.93 ± 0.06c	0.19 ± 0.01a
	Student-t	**	***	ns	***	*
Hexanal	Control	560 ± 51ab	660 ± 48bc	758 ± 12c	1286 ± 99d	483 ± 36a
	Modified	805 ± 41a	4153 ± 75c	689 ± 44a	5006 ± 523d	1787 ± 78b
	Student-t	**	***	*	***	***
Heptanal	Control	165 ± 9a	214 ± 10b	258 ± 12b	247 ± 6b	172 ± 1a
	Modified	157 ± 5a	811 ± 2c	183 ± 7a	1052 ± 30d	348 ± 7b
	Student-t	ns	***	***	***	***
t-2-heptenal	Control	0.00	0.00	0.00	0.00	0.00
	Modified	0.00a	0.00a	0.00a	740 ± 29b	0.00a
	Student-t	-	-	-	***	-
2-pentilamilofurane	Control	97 ± 5a	150 ± 4b	177 ± 6b	358 ± 1c	148 ± 1b
	Modified	286 ± 1b	1630 ± 26d	216 ± 12a	2535 ± 6d	603 ± 3c
	Student-t	***	***	***	***	***
tt-2,4-heptadienal	Control	0.00	0.00	0.00	0.00	0.00
	Modified	0.00a	422 ± 12b	0.00a	1776 ± 152c	0.00a
	Student-t	-	***	-	***	-
Octenal	Control	0.00a	0.00a	0.00a	31 ± 3c	18 ± 4b
	Modified	0.00a	158 ± 5c	0.00a	523 ± 18d	53 ± 2b
	Student-t	-	***	-	***	***
Nonanal	Control	1021 ± 46a	1033 ± 40b	1194 ± 40c	1180 ± 11c	1100 ± 10b
	Modified	781 ± 35a	1773 ± 130c	770 ± 67a	1904 ± 25c	1440 ± 65b
	Student-t	***	***	***	***	-
tt-2,4-nonadienal	Control	0.00	0.00	0.00	0.00	0.00
	Modified	0.00a	0.00a	0.00a	254 ± 14b	310 ± 56b
	Student-t	-	-	-	***	***
tt-2,4-decadienal	Control	0.00a	0.00a	0.00a	38 ± 3c	13 ± 1b
	Modified	0.00a	367 ± 29c	0.00a	1021 ± 7d	59 ± 3b
	Student-t	-	***		***	***
2,4-decadienal	Control	0.00a	0.00a	0.00a	145 ± 9c	37 ± 2b
	Modified	0.00a	906 ± 57c	0.00a	3188 ± 54d	179 ± 6b
	Student-t	-	***		***	***

Results are expressed as mean ± standard deviations. n=16. Values in the same row bearing different letters are significantly different ($p< 0.05$). Student-t test: - (not done); ns (not significant $p>0.05$); * ($p<0.05$); *** ($p<0.001$).