“Linseed oil gelled emulsion: a successful fat replacer in dry fermented sausages”

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ABSTRACT

Different levels of animal fat replacement by a high omega-3 content carrageenan gelled emulsion in dry fermented sausages were studied in order to improve their fatty acid composition. Percentages of fat replacement were 26.3 % (SUB1), 32.8 % (SUB2) and 39.5 % (SUB3). α-linolenic acid (ALA) content increased up to 1.81, 2.19 and 2.39 g/100 g (SUB1, SUB2, and SUB3 products) as compared to the Control (0.35 g/100g), implying an increment in polyunsaturated fatty acids (PUFA) supply (up to 10.3 %) and reductions in omega-6/ omega-3 ratio (75, 82 and 84 %, respectively). Peroxides and TBARs values were not affected ($P > 0.05$) by the fat modification and a slight low formation of volatile aldehydes derived from lipid oxidation was detected. Fat replacement did not cause relevant modifications on the instrumental color properties and no sensory differences ($P > 0.05$) were found between Control and SUB2 products (32.8 %) for taste and juiciness, pointing out the viability of this formulation for human consumption.

Key words: dry fermented sausage, gelled emulsion, fat replacer, carrageenan, omega-3 content.

1. INTRODUCTION

Nutrition is an important modifiable determinant of chronic diseases, and changes in the diet have strong effects on health throughout life (WHO, 2003). In response to this fact, dietary guidelines are periodically updated according to current knowledge, being fat particularly affected by these recommendations.

Although fermented meat products have been consumed for centuries in many different parts of the world and constitute one of the most important types of food (Toldrá & Hui, 2014), it is well known that the animal fat used in the elaboration of these products contains a higher proportion of saturated fatty acids (SFAs) than polyunsaturated fatty acids (PUFAs) (Muguerza, Ansorena & Astiasarán, 2004). It has been reported that SFA intake is associated with some of the metabolic syndrome's components, and it has been also suggested that the replacement of these fatty acids by PUFAs decreases coronary heart disease (CHD) risk (Skeaff & Miller, 2009). In consequence, research in this area is attempting to improve the fatty acid profile of these products to comply with current health recommendations.

Numerous strategies have been carried out in order to change fat composition in dry fermented sausages, including the use of different non-animal fats (marine and plant sources). Previous studies have reported that the incorporation of different types of oils improves the lipid profile of these products (Ansorena & Astiasarán 2004b; Muguerza et al., 2004; Valencia, Ansorena & Astiasarán, 2006; Jiménez-Colmenero, 2007; García-Íñiguez de Ciriano et al., 2009; García-Íñiguez de Ciriano et al., 2010a; Ruiz-Capillas, Triki, Herrero, Rodríguez-Salas & Jiménez-Colmenero, 2012; Jiménez-Colmenero, Triki, Herrero, Rodríguez-Salas & Ruiz-Capillas, 2013; Triki, Herrero, Rodríguez-Salas, Jiménez-Colmenero & Ruiz-Capillas, 2013).

Most of these studies have been performed using oil in water emulsion (O/W) systems, useful to incorporate components in the lipid phase, (e.g., ω-3 unsaturated fatty acids or antioxidants) with potential health implications in the products. However, stabilization of these emulsions by structural reinforcement is needed to preserve the textural properties of the products (Jiménez-Colmenero et al., 2015). Thus, the use of gelling agents has helped the reformulation processes in mimicking hardness and water holding capacity in different meat products (Marchetti, Andrés & Califano, 2014; Jiménez-Colmenero et al., 2013). In this sense, a gelled O/W emulsion containing 40 % of linseed oil and 1.5 % of kappa-carrageenan was developed by our group (Poyato, Ansorena, Berasategui, Navarro-Blasco & Astiasarán, 2014) as a pork back fat replacer. Its incorporation in fresh and cooked meat products showed nutritional advantages and did not have a negative influence on the sensory properties of the final products at the concentrations used (Poyato et al., 2014; Poyato, Astiasarán, Barriuso & Ansorena, 2015). However, no studies have been performed to test the viability of this gel in dry fermented sausages, which are characterized by a complex physicochemical ripening process.

The objective of this research was to design a technological strategy able to allow the incorporation of a gelled emulsion as a partial fat replacer to improve the lipid content of dry fermented sausages. The technological, nutritional and sensory characteristics, as well as their susceptibility to oxidation were assessed.

2. MATERIAL AND METHODS

2.1. Gelled emulsion preparation

Linseed oil (Naturgreen, Murcia, Spain) was obtained in a local market. The fatty acid profile of the linseed oil used, expressed as g 100 g^{-1} of total FA, was as follows: α-linolenic (58.9), linoleic (15.9), oleic (15.8), palmitic (5.13), stearic (3.06).

Carrageenan (κ-carrageenan) was kindly donated by Cargill (San Sebastián, Spain). Polysorbate 80 was obtained from Sigma-Aldrich Chemical Co. (MO, USA). The gelled emulsion prepared contained 40 % of linseed oil, 1.5 % of carrageenan and 58.5 % of water. Gelled emulsion was prepared according to the method described by Poyato et al. (2014). The oil phase (40 g/100 g emulsion) containing the Polysorbate 80 as surfactant (0.12 g/100 g emulsion) was added to the aqueous phase (that included 1.5 g carrageenan/100 g emulsion) and homogenized. Both phases were previously heated separately to 70 °C. After the homogenization process (16.000 rpm, Ultra-Turrax® T25basic), the emulsions were cooled to room temperature in a sealed flask, allowing the κ-carrageenan to polymerize. The gel was kept overnight under refrigeration (4 °C) until being used.

2.2 Dry fermented sausages formulation and processing

Fresh lean pork meat and fresh pork back fat were used as raw materials: these were obtained from a local processor. Lean pork meat was trimmed of fat and pork back fat was separated of adhering skin. They were kept frozen until use (-20 ºC).

Four different formulations of dry fermented sausages (6 kg per formulation) were manufactured in a pilot plant according to the general procedure described by Muguerza, Gimeno, Ansorena, Bloukas & Astiasarán (2001). The Control was made using 75 % lean pork meat and 25 % pork back fat. The other three formulations (SUB1, SUB2 and SUB3) were produced with a substitution of 26.3, 32.8 and 39.5 % of pork back fat, respectively, by the gelled emulsion. The substitution levels tested were based on a previous study of our research group, which concluded that sausages with 25 % of substitution with pre-emulsified olive oil in O/W systems were acceptable from the sensorial point of view (Muguerza et al., 2001). Calculations were needed to provide the same content of oil in the gelled emulsion. The amount of pork back fat and

gelled emulsion used in each formulation is shown in Table 1. The formulations also included the following common ingredients per kilogram of meat mixture: 26 g of sodium chloride, 30 g red pepper, 15 g dextrin, 10 g lactose, 12 g powdered milk, 5 g dextrose, 0.5 g sodium ascorbate, 10 g sodium caseinate, 3 g garlic, 2 g polyphosphates, 3 g curavi (a mixture of NaCl, preservatives E-250, E-252 and antioxidant E-331) and 0.15 g Ponceau 4R (E-124). 200 mg/kg of butylated hydroxyanisole (BHA) were also included in all formulations.

Two technological trials were performed for the processing of dry fermented sausages, that differed on the moment of the incorporation of the gel to the rest of ingredients. In both cases the gelled emulsion was added cut into 1x1 cm cubes. In the first trial, the gel was incorporated with the rest of the ingredients into the mincer (chopping step that lasted 50 sec), obtaining a meat matrix that was subsequently mixed in a vacuum mixer (blending step that lasted 65 sec). In the second trial, all the ingredients except for the gel, were chopped in the mincer, and the gel was incorporated after the chopping step, in the vacuum mixer (blending step). This was performed to observe the possible differences on the final appearance of the dry fermented sausages. In both cases, after blending, the prepared sausage mixture was stuffed into artificial casings (60 mm diameter) of collagen material (Viscofán, Cáseda, Spain). Sausages were fermented and ripened for 30 days in specific conditions (Muguerza et al., 2001) in a drying chamber (STA model W 80XDHG-VEH Noáin, Spain). Once ripening was finished, sausages were stored under vacuum in refrigeration conditions (4 ºC), until analysis. As it will be discussed later, results of the first technological trial were not satisfactory, so only one replicate of this experiment was done. The appearance of products in the second technological trial was adequate and hence, the products were elaborated following this technological procedure. The experimental design was carried out in triplicate per each

type of formulation (C, SUB1, SUB2 and SUB3). Several sausages from each replicate and formulation were homogenized to obtain a representative sample for analysis. For each parameter, the number of measurements made in the homogenates is indicated below. Data shown in tables are means and standard error of the three replicates.

2.3 Technological and nutritional analysis

pH was measured directly in the sausage with a pH-meter (micropH 2000) using a needle electrode (model pH electrode 52 31, Crison Instruments SA, Barcelona, Spain). Its evolution was controlled during the entire ripening period. Fat, moisture, protein and ash content were analyzed using official methods (AOAC 2002a, 2002b, 2002c, 2002d) in the ripened products. For each type of formulation, three measurements were done per each of the triplicate batches (n = 9). Carbohydrates were calculated by difference. The method of Folch, Lees & Stanley (1957) was used for the extraction of fat.

Fatty acid profile was determined in the lipid extracts by gas chromatography (Ansorena, Echarte, Ollé & Astiasarán, 2013). Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAME) (AOAC 2002e). The methylated sample was injected in the gas chromatograph. The gas chromatograph available was Perkin-Elmer Clarus 500 equipped with a capillary column SPTM – 2560 (100 m x 0.25 mm x 0.2 μm) and flame ionization detection. The injector was set at 250 °C and the detector temperature was set at 260 °C. The temperature of the column oven was established at 175 °C for 10 minutes increasing up to 200 °C at a pace of 10 °C/min, followed by an increase up to 220 °C at a pace of 4 °C/min and finally maintained at that temperature for 15 minutes. The gas for the flame ionization detector was compressed synthetic gas (O_2-N_2) mixed with hydrogen at a pressure of 20.5 psi. Hydrogen was used as a carrier gas (mobile phase).

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 and by spiking the sample with each standard individually. The quantification of individual fatty acids was based on the internal standard method, using methyl hepadecanoate. For each type of formulation, the value for each individual fatty acid was calculated as the average of four measurements per each of the triplicate batches (n = 12). After the quantification of the individual fatty acids, the sums of saturated, SFA, (caprilic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic and lignoceric acid), monounsaturated, MUFA, (palmitoleic, oleic, vaccenic, erucic, nervonic and eicosenoic acid) polyunsaturated, PUFA, (ω-3: α-linolenic, eicosadienoic, eicosatrienoic, docosapentaenoic, docosahexaenoic acid; ω-6: linoleic, γ-linoleic, arachidonic, docosapentaenoic acid) and *trans*, (*t*-palmitioleic, elaidic, *t*-linoleic, *c*,*t*-linoleic, *t*,*c*-linoleic and brassidic acid) were calculated, as well as PUFA/SFA, (PUFA+MUFA)/SFA and ω-6/ ω-3 ratios.

2.4 Lipid oxidation analysis

In order to assess the oxidation status of the dry fermented sausages, peroxides, TBARs and volatile aldehydes formed were measured.

Peroxide Index (PI) was analysed at 510 nm following the method of Shanta & Decker (1994) with modifications. Briefly, an aliquot of sample (corresponding to approximately 10 mg of fat) was transferred to a tube. The residue was dissolved in 5 mL of a mixture Butanol:Methanol (2:1). $SCNNH_4$ (30 % in distilled water, 25 µL) was added and tubes were vortexed for 4 s. Then, a solution of $FeCl_2$ (36 mM in HCl, 25 µL) was added and tubes were vortexed. After 15 min, absorbance was measured at 510 nm (FLUOStar Omega spectrofluorometric analyzer, BMG Labtechnologies, Offenburg, Germany). A calibration curve with Iron (III) Chloride was used for

165 quantification ($y = 5.787x + 0.0322$; $R^2 = 1$). Results were expressed as mg ROOH / kg
166 sample.
167 TBARs value was determined at 532 nm according to Tarladgis, Watts, Younathan &
168 Dugan (1960) with modifications by Tarladgis, Pearson & Jun (1964). Results were
169 expressed in mg malonaldehyde MDA/kg sample.
170 The determination of the volatile aldehydes, hexanal, heptanal and nonanal, was
171 carried out by the HS-SPME–GC–MS method. Sample (2 g) was put into a 25 mL vial
172 and capped with a rubber cap. After a period of sample heating (30 min at 60 ºC), a
173 fiber coated with DVB/CAR/PDMS (Divinylbenzene/ Carboxen/
174 Polydimethylsiloxane, 50/30 μm film thickness, Supelco) was inserted into the
175 headspace of the sample and maintained for 45 min at 60 ºC for adsorption of volatile
176 compounds. The fiber was desorbed for 15 min in the injection port of a gas
177 chromatograph model HP 6890 Series (Hewlett Packard), equipped with a HP Mass
178 Selective Detector 5973. A fused-silica capillary column (30 m long × 0.25 mm inner
179 diameter × 0.25 μm film thickness, from Agilent Technologies), coated with a non-
180 polar stationary phase (HP-5MS, 5 % phenyl methyl siloxane) was used. The
181 operating conditions were as follows: the oven temperature was set initially at 42
182 °C (5 min hold), increased to 120 °C at 3 °C/min and to 250 °C at 10 °C/min (5
183 min hold); the temperatures of the ion source and the quadrupole mass analyzer
184 were kept at 230 °C and 150 °C, respectively. Helium was used as carrier gas at 1
185 mL/min; injector and detector temperatures were held at 250 °C and 280 °C,
186 respectively. Mass spectra were recorded at 70 eV; using scan mode (Range: 33-
187 350 atomic mass unit). Before performing every extraction, cleanness of the fiber was
188 checked by running a blank and confirming the absence of peaks in the
189 chromatogram. Identification of the peaks was based on comparison of their mass

190 spectra with the spectra of a commercial library (Wiley 275.L, Mass Spectral
191 Database) and, by comparison of their retention times with those of standard
192 compounds. For semi-quantitative purposes, area of peaks was measured by
193 integration of the total ion current of the spectra. When overlapping occurred, the
194 calculation of the total area of a compound was based on the integration of a single ion
195 and taking into account the relative ratio in which this ion is present in that compound.
196 Results were expressed as area/sample weight (g) x 10^3.
197 Results for peroxides and TBARs were calculated, for each type of formulation, as the
198 average of four measurements per each of the triplicate batches (n = 12). For volatile
199 aldehydes, for each type of formulation two measurements were done per each of the
200 triplicate batches (n = 6).

201 **2.5 Instrumental color**

202 Instrumental color measurement was performed with a digital colorimeter
203 (Chromameter-2 CR-200, Minolta, Osaka, Japan). The homogenized sausage mixture
204 was put into a plate of 1 cm height. They were covered with a polyethylene film with
205 pressure to obtain a uniform, bubble-free surface. Color coordinates were obtained
206 using the CIE *L*a*b** system, angle 10 °, illuminant D65. *L**, ***a**** and *b** parameters
207 indicate lightness, redness and yellowness, respectively. For each type of formulation,
208 eight measurements were done per each of the triplicate batches (n = 24).

209 Chroma angle ($Chroma = \sqrt{a^{*2} + b^{*2}}$), Hue angle ($Hue = \arctan\left(\frac{b^*}{a^*}\right)$) and the

210 Euclidean distance ($\Delta E = \sqrt{\left(L_c^* - L_m^*\right)^2 + \left(a_c^* - a_m^*\right)^2 + \left(b_c^* - b_m^*\right)^2}$) were calculated for
211 comparison between control (c) and modified products (m).

212 **2.6 Sensory test**

213 The sensory tests were performed in accordance with Spanish guidelines for triangle
214 tests (Norma UNE-EN ISO 4120: 2004) using dry fermented products elaborated in the
215 three batches. Two triangle tests were done in two separate sessions to determine the
216 existence of perceptible sensory differences in the attributes most susceptible to
217 modification after the reformulation (odor, taste and juiciness). The two triangle tests
218 were; (1) comparison between Control and SUB3 (39.5 %) products, and (2)
219 comparison between Control and SUB2 (32.8 %) products. The panel that carried out
220 both tests consisted on 21 semi-trained judges, which regularly participate in sensory
221 analyses on different types of meat products. They also have experience with the
222 technical aspects of the methodology of a triangle test. In each session, the evaluation
223 was conducted as follows: three samples, of which two were identical, were offered to
224 each panelist. The samples were presented sliced (about 2 mm thick), served at room
225 temperature on a white plate, and coded using three-digit numbers chosen randomly.
226 The sessions were carried out in normalized testing booths and under controlled red
227 light to neutralize possible differences in color or appearance of the samples. The 21
228 judges were asked to smell and taste the samples individually and instructed to indicate,
229 for each attribute, which sample differed from the others. The tests included a section in
230 which panelists were asked about the reason, if any, of the differences found among
231 samples, or panelists could also describe any particular note detected in the sensory
232 evaluation. Water and neutral crackers were served to the panelists to rinse the mouth
233 between the samples. Correct and incorrect replies were recorded in each session.
234 Results were compared with tables of minimum number of correct responses required
235 for significance in a triangle testing for difference (Norma UNE-EN ISO 4120: 2004).

2.7 Statistical analysis

The entire trial was performed in triplicate. For each formulation (Control, SUB1, SUB2 and SUB3), results were expressed as mean and standard error of the results obtained from the three independent batches. The difference between mean of values was determined using one-way analysis of variance (ANOVA) and multiple comparisons of means were done using Bonferroni Post Hoc procedure to evaluate the statistical significance ($P \leq 0.05$) among formulations. Formulation was assigned as fixed factor. Statistical analysis of the sensory test was done according to the instructions of the norm UNE-EN ISO 4120: 2004. The statistical analysis of data was done using the STATA/IC 12.1 program (StataCorp LP, Texas, USA). Significance level of $P \leq 0.05$ was used for all evaluations.

3. RESULTS AND DISCUSSION

3.1 Technological aspects

The incorporation of the gel into the dry fermented sausage formulation implied a technological modification to the conventional processing procedure that included the following steps: chopping, blending, stuffing and ripening. A first trial was performed applying the traditional technological process, in which the addition of the ingredients (including the gel) was done during the chopping step. The obtained products at the end of the ripening process showed a slight deficiency in the appearance of the slices, differing from the traditional products. In particular, it was not possible to clearly differentiate the gel portions from the meat matrix due to their disintegration (data not shown). The second trial was performed in order to solve this technological problem. In this case, the addition of the gel was done during the blending step, and this was the adequate way to obtain a final product in which the gel maintained its consistence and

resembled the appearance of the pork back fat. Therefore, the rest of the experimental design was performed with this technological modification.

The pH evolution of the products is shown in Figure 1. Initial pH values ranging from 5.76 to 5.88 ($P > 0.05$) decreased quickly during the first four days. At the end of the ripening period, the four types of products presented similar pH values of 5.17-5.21 ($P > 0.05$). No effect of the fat replacement was noticed on the pH evolution of the sausages, that remained in standard values for this type of products.

3.2 Chemical composition

General composition analysis showed a slight increase in moisture content of reformulated products as compared to the Control (Table 2). This finding was accompanied by a decrease in the fat content, when the gel was used as fat replacer. Fat content of the Control sausage was 30.80 % while fat levels in reformulated products were 28.92, 26.49 and 25.20 % for SUB1, SUB2 and SUB3. These changes represented a fat reduction about 6, 14 and 18 %, respectively. Values for ash and protein were similar for all products ($P > 0.05$). As a consequence of the reformulation and lower fat content, energy value slightly decreased, from 1740 to 1566 kJ per 100 g product. The reductions achieved in these three products (SUB1, SUB2 and SUB3) were 4, 8 and 10 % of energy, respectively, compared with the Control product. Regarding the improvement in the fatty acid profile (Table 3), pork fat replacement by the gel increased ($P < 0.05$) the α-linolenic fatty acid (ALA) content in modified sausages. Linseed oil contributed mainly with the supply of this fatty acid, as it could be expected attending to its lipid profile. ALA was present in 1.81, 2.19 and 2.39 g/100 g of SUB1, SUB2 and SUB3 products, respectively, in contrast to what was found in Control products (0.35 g/100 g product). The use of the gel also contributed to decrease ($P < 0.05$) the ω-6 fatty acid linoleic, in a dose-dependent manner. These values, together

285 with an increase ($P < 0.05$) in ω-3 content, led to a very relevant decrease in the ω-6/ ω-
286 3 ratio, which was reduced ($P < 0.05$) between fourfold and sixfold in the reformulated
287 products (from 10.20 to 2.52, 1.87 and 1.62, respectively). Similar values in the ω-6/ ω-
288 3 ratio (2.1; 1.95) have been found in previous studies for modified products using a soy
289 protein emulsion based on linseed oil as pork fat replacer (Ansorena & Astiasarán,
290 2004a; García-Íñiguez et al., 2010b). Improvements in PUFA/SFA and
291 (PUFA+MUFA)/SFA ratios were observed due to the significant decrease in SFA
292 content as a consequence of the addition of the gel and also due to the higher PUFA
293 content ($P < 0.05$) in modified products.
294 The amount of *trans* fatty acids in these type of products were very low (0.04 to 0.10
295 %), as expected, due to the low content of these fatty acids in the raw materials used
296 (pork back fat and linseed oil).

297 **3.3 Overall nutritional value: nutrition and health claims**

298 According to the Regulation (EC) No. 1924/2006 and Regulation (EU) No. 116/2010,
299 some nutrition claims could be applied to the modified dry fermented sausages (Table
300 4). Regarding the protein, the use of the claim ´high protein´ can be made in all products
301 (in this case, including control), as the energy value provided by protein was, at least, 20
302 % of the total energy value of the product. The use of the claim ´source of omega-3 fatty
303 acids´ can be made when a food supplies more than 0.3 g ALA per 100 g and per 100
304 kcal. This requirement is fulfilled in all modified products (SUB1, SUB2, and SUB3).
305 Moreover, the claim ´high omega-3 fatty acids´ can be applied in SUB3 products, as
306 they contain more than 0.6 g ALA $100g^{-1}$ and 100 $kcal^{-1}$.
307 In addition, according to the Regulation (EU) No 432/2012 about health claims, due to
308 the high protein content of these dry fermented sausages, the health claims ´Protein
309 contributes to the growth of muscle mass´ and ´Protein contributes to the maintenance

of muscle mass and normal bones´ may be attributed to all products. Concerning the ALA content in the modified products, it is allowed to claim that ´ALA contributes to the maintenance of normal blood cholesterol levels; the beneficial effect is obtained with a daily intake of 2 g of ALA´.

Besides nutritional labelling, national and international authorities set recommendations about ALA intake. It has been proposed that an Adequate Intake for α-linolenic acid should cover a 0.5 % of total daily energy value, which would be 1.1 g/day, in a 8360 kJ/ diet (EFSA, 2010; Ros et al., 2015). Taking into account that the recent evaluation at the International Agency for Research on Cancer (IARC, 2015) recommended limiting the consumption of processed meat products to 50 grams per day, this portion (50 g) of SUB1 product would cover 82.3 % of the needs for ALA, whereas for SUB2 and SUB3 products, 100 % of this recommendation would be covered with this serving.

3.4 Lipid oxidation

The oxidation of fatty acids and other lipid compounds during the ripening and storage of dry fermented sausages can give rise to rancidity, which could negatively affect their quality (Muguerza et al., 2001). Peroxides and TBARs were measured at the end of the ripening period. Moreover, the analysis of some volatile compounds typical from lipid oxidation was also carried out (Table 5). Despite the high content of unsaturated fatty acids, more susceptible to oxidation than MUFA and SFA, peroxides and TBARs values showed that lipid oxidation of the reformulated sausages measured by classical methods was not affected ($P > 0.05$) by the fat replacement. Both parameters remained in low levels. Morales, Rios & Aparicio (1997) reported that an appropriate way to detect the beginning of lipid oxidation could be the measurement of hexanal and nonanal. Whereas no significant differences were noticed for hexanal content among formulations, nonanal content was significantly higher ($P < 0.05$) in SUB2 products,

335 and especially in SUB3 products. The sum of total aldehydes was also particularly
336 increased ($P < 0.05$) in SUB3 products, which could possibly be responsible for the
337 strange taste notes reported in this last formulation.

338 **3.5 Color**

339 A first approach to evaluate the consequences of the fat modification over the sensory
340 properties of the products consisted on a color CIELab analysis (Table 6).

341 The control sausage showed lower values ($P < 0.05$) of redness (a^*) and yellowness
342 (b^*) as compared to the reformulated products. As a result, Chroma values were
343 significantly higher ($P < 0.05$), indicating a more intense color and less greyish in the
344 modified products according to the L^* a^* b^* tridimensional plot. However, Hue angle
345 was slightly modified with the reformulation, pointing to a very similar tint of samples,
346 especially when using the CIE L^* a^* b^* system for measurements. In any case, both
347 Chroma and Hue values were within the normal range for this type of product (Gimeno,
348 Ansorena, Astiasarán & Bello, 2000). Other authors (Muguerza et al., 2004; Salazar,
349 García & Selgas, 2009; Utrilla, García-Ruiz & Soriano, 2014) have also observed that
350 when fat content is substituted, the reformulated dry fermented sausages were redder
351 than the Control. Euclidean distance (ΔE) values were calculated respect to the Control
352 products and no significant differences ($P > 0.05$) were found among the different
353 substitution levels.

354 **3.6 Sensory analysis**

355 Applying Norma UNE-EN ISO 4120 criteria, the triangle test (1) between Control and
356 SUB3 products, reported significant differences ($P < 0.05$) in odor and taste, that made
357 these products not acceptable from the sensory standpoint (data not shown). In addition,
358 some panelists reported unpleasant notes when they were asked about the reason of the
359 differences found for the taste of these modified products that could be probably related

with the highest nonanal content observed in these products and the significant increase ($P < 0.05$) of total aldehydes as compared to Control products. On the other hand, results of the triangle test (2) between Control and SUB2 products (Table 7), showed that only 11 panelists identified the correct sample for taste and juiciness attributes, meaning that there were no statistical differences between both compared products for these two sensory attributes ($P > 0.05$). In the case of odor, 13 out of the 21 panelists correctly identified the sample, which could be interpreted as the existence of significant differences ($P < 0.01$) between both products for this attribute. However, none of the panelists detected a negative odor note on these products, so these results allowed us to conclude that reformulated products with a 32.8 % of fat replacement level can be considered acceptable for consumption.

4. CONCLUSIONS

Dry fermented sausages enriched in α- linolenic acid could be successfully elaborated with an adequate technological process by the incorporation of a gelled emulsion prepared with linseed oil. Final acceptable products (32.8 % of animal fat substitution) had a 26 % of fat, 2.32 g ω-3 FA/100 g product and ω -6/ ω -3 ratio of 1.87. The reformulation process did not cause oxidation problems, and no perceptible differences were reported for taste and juiciness as compared to a traditional product.

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515 **FIGURE CAPTIONS**

516 Figure 1. pH evolution of the different dry fermented sausages as affected by
517 processing.

518 **TABLE CAPTIONS**

519 Table 1. Main ingredients for the 4 types of dry fermented sausages.

520 Table 2. Mean values of general composition and energy values of the different dry
521 fermented sausages.

522 Table 3. Fatty acid profile of the different dry fermented sausages after the ripening
523 period, expressed in grams of fatty acid (FA) per 100 g of product.

524 Table 4. Parameters related to nutrition claims in the different formulations of dry
525 fermented sausages.

526 Table 5. Parameters related to lipid oxidation in the different formulations of dry
527 fermented sausages.

528 Table 6. Mean values for instrumental color CIE *L** *a** *b** evaluation in the different
529 formulations of dry fermented sausages.

530 Table 7. Scores obtained in the triangle test (triangle testing for difference) comparing
531 Control and SUB2 products (Norma UNE-EN ISO 4120: 2004).

Table 1. Main ingredients for the 4 types of dry fermented sausages.

Samples	Fat replaced (%)	Pork meat (g)	Pork back fat (g)	Gelled emulsion (g)
C	0	4500	1500	-
SUB 1	26.3	4500	1105.5	394.5
SUB 2	32.8	4500	1008	492
SUB 3	39.5	4500	907.5	592.5

Amounts calculated for 6 kg of each product.

Table 2. Mean values of general composition and energy values of the different dry fermented sausages.

	CONTROL	SUB 1 (26.3 %)	SUB 2 (32.8 %)	SUB 3 (39.5 %)
Moisture (%)	29.29 (0.08)a	29.27 (0.36)a	30.86 (0.10)b	31.97 (0.11)c
Fat (%)	30.80 (0.32)c	28.92 (0.48)b	26.49 (0.18)b	25.20 (0.34)a
Protein (%)	22.07 (0.56)a	21.81 (0.12)a	22.47 (0.97)a	21.28 (0.04)a
Ash (%)	5.11 (0.46)a	6.12 (0.17)a	6.19 (0.18)a	5.88 (0.55)a
Carbohydrates (%)	12.73	13.88	13.99	15.67
Total energy value (kJ/100g)	1740 (14)c	1677 (18)bc	1601 (22)ab	1566 (12)a
Energy from fat (kJ/100g)	1159 (12)c	1087 (18)b	997 (7)a	948 (11)a
Energy from fat (%)	67 (0.61)b	65 (0.31)ab	62 (0.67)a	61 (0.58)a
Fat reduction (%)	-	6	14	18
Energy value reduction (%)	-	4	8	10

For each parameter, different small letters among percentages of substitution indicate significant differences ($P < 0.05$) based on post hoc Bonferroni test. Standard errors of the mean (SEM) appear in parentheses.

Table 3. Fatty acid profile of the different dry fermented sausages after the ripening period, expressed in grams of fatty acid (FA) per 100 g of product.

	CONTROL	**SUB 1 (26.3 %)**	**SUB 2 (32.8 %)**	**SUB 3 (39.5 %)**
Caprilic C8:0	nd	nd	nd	nd
Capric C10:0	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a
Lauric C12:0	0.02 (0.00)a	0.02 (0.00)a	0.01 (0.00)a	0.01 (0.00)a
Myristic C14:0	0.36 (0.00)c	0.31 (0.01)b	0.26 (0.01)a	0.23 (0.01)a
Palmitic C16:0	6.52 (0.00)d	5.94 (0.02)c	5.20 (0.00)b	4.81 (0.04)a
***t*-palmitoleic C16:1t Δ9t**	0.02 (0.00)a	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a
Palmitoleic C16:1	0.62 (0.00)c	0.55 (0.00)b	0.48 (0.00)ab	0.42 (0.02)a
Stearic C18:0	3.50 (0.00)c	3.26 (0.04)b	2.93 (0.01)a	2.83 (0.06)a
Elaidic C18:1t	0.04 (0.01)ab	0.07 (0.00)b	0.02 (0.00)a	0.02 (0.00)a
Oleic C18:1 (ω-9)	12.42 (0.01)d	10.64 (0.03)c	9.72 (0.01)b	9.24 (0.06)a
Vaccenic C18:1 (ω-7)	1.04 (0.00)c	0.87 (0.00)b	0.79 (0.00)ab	0.72 (0.02)a
***t*-linoleic C18:2Δ9t.12t**	nd	nd	nd	nd
***c,t*-linoleic C18:2Δ9c.12t**	nd	nd	nd	nd
***t,c*-linoleic C18:2Δ9t.12c**	nd	nd	nd	nd
Linoleic C18:2Δ9c.12c	4.98 (0.00)c	4.67 (0.01)b	4.14 (0.00)b	3.87 (0.03)a
Arachidic C20:0	0.05 (0.00)a	0.05 (0.00)a	0.05 (0.00)a	0.04 (0.00)a
γ-linolenic C18:3 (ω-6)	0.02 (0.00)b	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a
Eicosenoic C20:1 (ω-9)	0.24 (0.00)b	0.18 (0.00)a	0.18 (0.00)a	0.17 (0.00)a
α-linolenic C18:3 (ω-3)	0.35 (0.00)a	1.81 (0.01)b	2.19 (0.01)c	2.39 (0.08)d
Behenic C22:0	nd	nd	nd	nd
Brasidic C20:1t Δ13t	0.02 (0.00)a	0.01 (0.00)a	0.02 (0.00)a	0.01 (0.00)a
Erucic C22:1	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a
Eicosatrienoic C20:3 (ω-3)	0.05 (0.00)b	0.04 (0.00)a	0.03 (0.00)a	0.03 (0.00)a
Arachidonic C20:4 (ω-6)	0.17 (0.00)b	0.16 (0.00)a	0.15 (0.00)a	0.12 (0.00)a
Lignoceric C24:0	0.01 (0.00)a	0.01(0.00)a	0.01 (0.00)a	0.01 (0.00)a
Eicosapentaenoic C20:5 (ω-3)	nd	nd	nd	nd
Nervonic C24:1 (ω-9)	nd	0.01 (0.00)a	nd	nd
Docosapentaenoic C22:5 (ω -6)	0.04 (0.00)c	0.03 (0.00)c	0.04 (0.00)b	0.03(0.00)a
Docosapentaenoic C22:5 (ω -3)	0.08 (0.00)b	0.07 (0.00)a	0.07 (0.00)ab	0.06 (0.00)ab
Docosahexaenoic C22:6 (ω-3)	0.02 (0.00)b	0.02 (0.00)a	0.02 (0.00)a	0.02(0.00)ab
SFA	10.45 (0.01)d	9.59 (0.05)c	8.46 (0.00)b	7.93(0.01)a
MUFA	14.34 (0.01)d	12.26 (0.04)c	11.18 (0.01)b	10.55(0.11)a
PUFA	5.92 (0.00)a	6.81 (0.00)b	6.66 (0.01)b	6.53 (0.11)b
ω-3	0.51 (0.00)a	1.93 (0.01)b	2.32 (0.00)c	2.50(0.08)d
ω-6	5.21 (0.00)d	4.88 (0.01)c	4.34 (0.01)b	4.03(0.03)a
ω-6/ω-3	10.20 (0.07)d	2.52 (0.01)c	1.87 (0.00)b	1.62(0.04)a
PUFA/SFA	0.57 (0.00)a	0.71 (0.00)b	0.79 (0.00)b	0.82(0.01)c
PUFA+MUFA/SFA	1.94 (0.00)a	1.99 (0.01)b	2.11 (0.00)c	2.15 (0.00)d
trans	0.08(0.01)b	0.10 (0.01)b	0.05 (0.00)a	0.04 (0.00)a

Standard errors of the mean (SEM) appear in parentheses. Values with different letters among percentages of substitution indicate significant differences (P < 0.05) based on post hoc Bonferroni test. ´nd´ indicate that the fatty acid was not detected in the sample.

Table 4. Parameters related to nutrition claims in the different formulations of dry fermented sausages.

Samples	Energy provided by protein (kcal/100 kcal)	Omega 3- fatty acids	
		(g α-linolenic/100 g)	(g α-linolenic/100 kcal)
CONTROL	21.2[1]	0.35	0.08
SUB1	22.0[1]	1.81	0.45[2]
SUB2	21.6[1]	2.19	0.57[2]
SUB3	20.4[1]	2.39	0.64[2,3]

Each superscript number refers to the nutrition claims listed below. 1´high protein´, 2´source of omega 3- fatty acids´, 3´high omega 3- fatty acids´.

Table 5. Parameters related to lipid oxidation in the different formulations of dry fermented sausages.

	CONTROL	**SUB 1 (26.3 %)**	**SUB 2 (32.8 %)**	**SUB 3 (39.5 %)**
PI	0.36 (0.01)a	0.38 (0.01)a	0.33 (0.01)a	0.34 (0.01)a
TBARS	0.64 (0.03)a	0.59 (0.01)a	0.58 (0.01)a	0.59 (0.02)a
Hexanal	361 (32)a	451 (17)a	536 (47)a	578 (26)a
Heptanal	71 (3)a	132 (9)ab	165 (22)b	149 (4)ab
Nonanal	470 (49)a	520 (49)a	780 (17)b	1187 (18)c
Total aldehydes	902 (23)a	1103 (21)ab	1481 (16)b	1914 (11)c

Peroxides (PI) are expressed in mg ROOH/kg product, TBARs are expressed in mg MDA/kg product and the volatile compounds are expressed by area/sample weight (g) x 10^3. Values with different letters among percentages of substitution indicate significant differences ($P < 0.05$) based on post hoc Bonferroni test. Standard errors of the mean (SEM) appear in parentheses.

Table 6. Mean values for instrumental color CIE L* a* b* evaluation in the different formulations of dry fermented sausages.

	CONTROL	**SUB 1 (26.3 %)**	**SUB 2 (32.8 %)**	**SUB 3 (39.5 %)**
L*	46.83 (1.07)a	46.23 (0.30)a	50.21 (0.88)b	47.28 (0.54)ab
a*	17.01 (0.48)a	22.77 (0.87)b	22.00 (0.74)b	23.77 (0.94)b
b*	11.12 (0.53)a	16.66 (0.80)b	14.55 (0.68)b	17.21 (1.06)b
Hue	33.05 (0.57)a	36.11 (0.27)b	33.38 (0.43)a	35.69 (0.67)b
Chroma	20.33 (0.69)a	26.44 (0.29)b	26.38 (0.99)b	29.36 (1.37)b
ΔE	-	10.51 (1.03)a	9.14 (0.34)a	12. 80 (0.76)a

For each parameter, different small letters among percentages of substitution indicate significant differences ($P < 0.05$) based on post hoc Bonferroni test. Standard errors of the mean (SEM) appear in parentheses.

Table 7. Scores obtained in the triangle test (triangle testing for difference) comparing Control and SUB2 products (Norma UNE-EN ISO 4120: 2004).

Control *Vs.* SUB 2 (32.8%)

	Odor	**Taste**	**Juiciness**
Correct replies	13 ($P < 0.01$)	11 (ns)	11 (ns)
Incorrect replies	8	10	10

For n=21 panelists, the number of correct answers to conclude that perceptible differences exist between samples was 12 ($P < 0.05$), 13 ($P < 0.01$) and 15 ($P < 0.001$). ns = not significant.

SUPPLEMENTARY MATERIAL

Linseed oil gelled emulsion: a successful fat replacer in dry fermented sausages.

Marta Alejandre, Candelaria Poyato, Diana Ansorena, Iciar Astiasarán

Figure S1. Picture of the gelled emulsion.

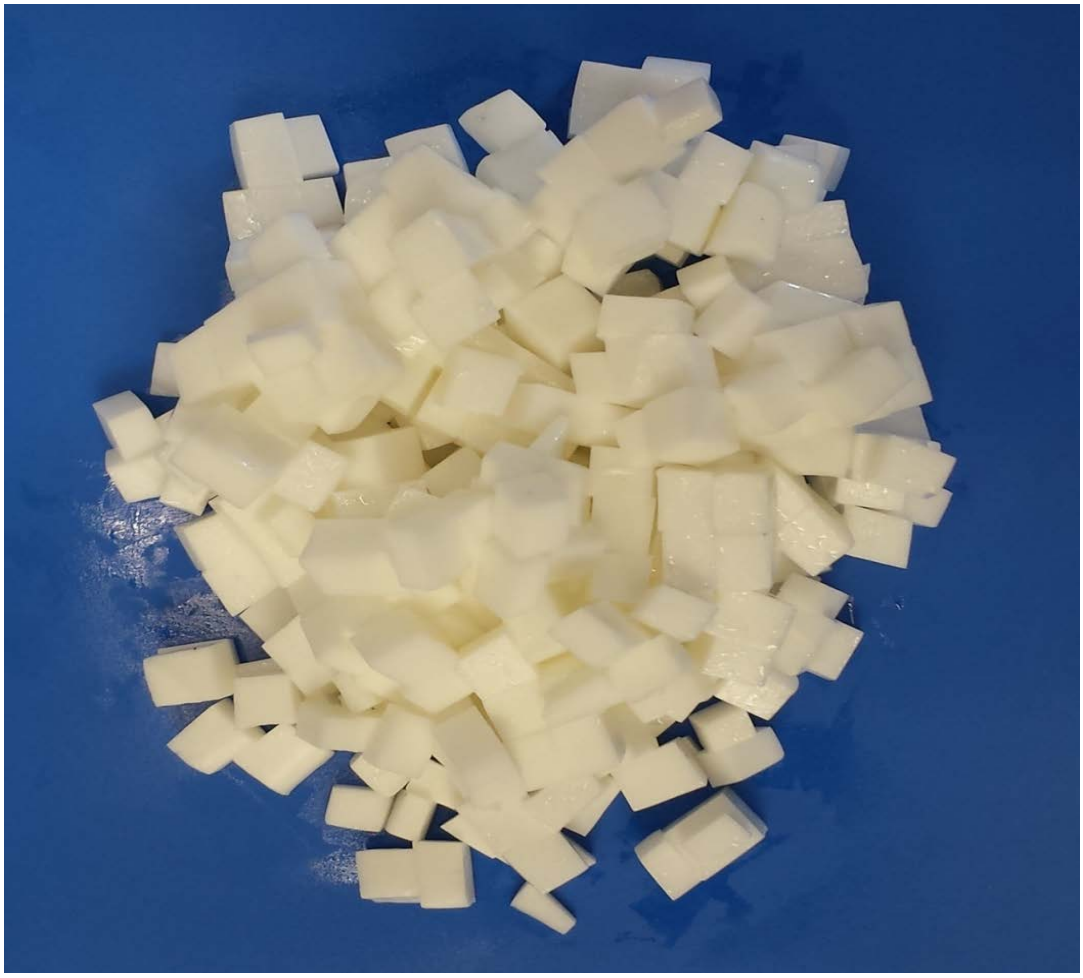

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Figure S2. Pictures of the dry fermented sausages (% pork fat replaced) C: Control (0%) SUB1: Substitution 1 (26.3%). SUB2: Substitution 2 (32.8%). SUB3: Substitution 3 (39.5%).