



Chia and oat emulsion gels as new animal fat replacers and healthy bioactive sources in fresh sausage formulation

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ABSTRACT

This paper examines the effect of emulsion gels (EG) prepared with chia (CEG) and oats (OEG) used as animal fat replacers in reduced-fat fresh sausages (*longaniza*) (LRF) during chilled storage. Reduced-fat samples were reformulated with CEG and OEG, (LRF/CEG and LRF/OEG respectively). Normal (LNF/P) and reduced-fat (LRF/P) (all-pork-fat) sausages were used as controls. Nutritional composition and microbiological, technological and sensory characteristics of sausages were evaluated. The presence of an EG affected ($P < 0.05$) the concentrations of some minerals and amino acids in sausages. CEG improved MUFA and PUFA contents. Cooking loss was lower ($P < 0.05$) in LRF/CEG and LRF/OEG than in the controls. Of all the reduced-fat samples, Kramer shear force values (KSF) were highest ($P < 0.05$) in the ones containing an EG. KSF generally increased ($P < 0.05$) over storage in all samples. The microbial count was significantly affected by the use of CEG. Sensory properties were affected by the incorporation of an EG, but all sausages were judged acceptable.

1. Introduction

Fresh pork sausages, such as “*Longanizas*” (a popular type of product in Spain), are an excellent source of valuable nutrients normally present in meat products (proteins, iron, vitamins, etc.). However, this type of products has been associated with some negative health concerns regarding high fat content (over 27%), unhealthy fatty acid profile, high energy value and Na contents (EFSA, 2010; McAfee et al., 2010).

Structuring oils to create a plastic fat which retains solid-like properties, while possessing a healthier fatty acid profile, has recently been proposed as a new possibility to improve fat content while avoiding undesirable quality changes in the final reformulated meat product (Jiménez-Colmenero et al., 2015). In this regard solid-like lipid materials such as oil bulking systems or structured emulsions, particularly oil-in-water emulsion gels (EG), have been used in healthier lipid meat product reformulation processes (Ruiz-Capillas et al., 2013; Herrero et al., 2014; Poyato et al., 2014; Jiménez-Colmenero et al., 2015; Alejandro et al., 2016). But despite the fact that the use of EGs is potentially especially suitable for improving fat content in fresh meat products (patties, fresh sausages, etc.), where the appearance and structure of fat replacers are more important than in finally comminuted products, there has been very little research. Poyato et al. (2015) reported the use of a sunflower-oil gelled emulsion based on carrageenan and Polysorbate 80 as a pork backfat replacer in fresh meat product (patties) formulation.

An interesting approach to the development of EGs as animal fat replacers would be the use of some plant-based-ingredients which are also sources of healthy fatty acids, fibre, minerals, antioxidants or vitamins, among others. In this regard, chia (*Salvia hispanica*, L.) flour or oat (*Avena sativa*, L.) bran have been used both for their healthier compounds [α -linolenic acid (ALA), insoluble fibre, β -glucans, antioxidants or minerals, etc.]. Moreover, the useful technological properties (gelling capacity, emulsifying activity, fat/water binding capacity, etc.) of these ingredients (Arendt & Zannini, 2013; Coorey et al., 2014; Jiang et al., 2015) could be of considerable assistance in developing structured emulsions, particularly emulsion gels (Pintado, Herrero, et al., 2015a; Pintado et al., 2017). EGs prepared with chia have been used to replace animal fat in frankfurters. These reformulation processes enhance the nutritional composition of the products without affecting their technological properties (Pintado, Ruiz-Capillas, et al., 2015b; Pintado et al., 2016). While oats have been added directly in the formulation of fresh product to overcome fat reduction limitations (Yilmaz & Daglioglu, 2003; Pinero et al., 2008; Angiolillo et al., 2015), as far as the authors know there have been no studies on the preparation and use of oat emulsion gels.

The aim of the present work was to evaluate the effect of using oil-in-water emulsion gels (EG), prepared with olive oil and chia flour or oat bran as animal fat replacers, in terms of nutritional composition, technological properties, and microbiological and sensory characteristics of reduced-fat fresh sausages (*longaniza* type) during chilled

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storage (18 days at 2 °C). These structured EGs, with solid-lipid functionality, have been designed for reformulated fresh sausages, as delivery systems for different healthier bioactive plant compounds (from olive oil, chia flour and oat bran) present in the EGs. Normal- and reduced-fat (all-pork-fat) fresh sausages were used as controls.

2. Materials and methods

2.1. Oil-in-water emulsions gel preparation

Two different oil-in-water (O/W) emulsion gels (EGs) were formulated as animal fat replacers. Chia EG (CEG) was prepared with 20% chia (*Salvia hispanica* L.) flour (Primaria Premium Raw Materials, S. L. Valencia, Spain), 20% olive oil (Carbonell Virgen Extra, SOS Cuétara S.A., Madrid, Spain), 58% water and 2% of a gelling agent based on alginate. An oat bran EG (OEG) was prepared with 20% oat bran (OatWell® 22%, Zeus Química, S.A. Barcelona, Spain), 20% olive oil, 58% water and 2% of the alginate-based gelling agent. The gelling agent was formulated with 0.73% sodium alginate (Tradissimo, TRADES S.A., Barcelona, Spain), 0.73% calcium sulphate and 0.54% sodium pyrophosphate (Panreac Química, S.A. Madrid, Spain). According to the suppliers, the composition of the chia and oat materials used was: chia flour with 22% protein, 31.3% fat (of which 19% α -linolenic fatty acid), 5.1% carbohydrates, and 30.2% total fibre content (mainly insoluble fibre); oat bran with 20% protein, 5% fat, 20% carbohydrates and 44% total fibre content (of which 22% β -glucans); and olive oil with 14.87% saturated fatty acids (SFA), 75.32% monounsaturated fatty acids (MUFA) and 8.97% polyunsaturated fatty acids (PUFA) (Delgado-Pando et al., 2010).

Both EGs were prepared according to (Pintado, Ruiz-Capillas, et al., 2015b; Pintado et al., 2017). Briefly, for each type of EG, first chia flour or oat bran was mixed in a homogenizer (Thermomix TM 31, Vorwerk-España M.S.L., S.C, Madrid, Spain) with water (30 s, approx. 5600 rpm), then the gelling agent was added and mixed (15 s, approx. 5600 rpm). The final mixture was mixed at approx. 5600 rpm with gradual addition of the appropriate amount of olive oil. Finally, each sample was placed in metal containers under pressure to compact it and prevent air bubbles, and stored in a chilled room at 2 °C for 20 h until use.

2.2. Experimental design and manufacture of fresh sausage

Fresh sausages (“longanizas”) were designed to both reduce fat content and enhance the presence of healthy fatty acids (MUFAs, PUFAs and especially ALAs) and other healthy bioactive compounds (minerals, insoluble fibre, β -glucans, etc.) supplied by olive oil, chia flour or oat bran. Such reformulated products could therefore qualify for labelling with some health claims.

Fresh post-rigor meat (30 kg; mixture of biceps femoris, semimembranosus, semitendinosus, gracilis and adductor M) (20.76% protein, 1.72% fat) and pork backfat (6 kg) (0.52% protein, 94.61% fat), each from different animals, were obtained from a local market on different days. The meat was trimmed of visible fat and connective tissue. Lots of approximately 500 g were vacuum packed, frozen and stored at –20 °C until use.

Four different fresh sausage formulations were prepared (Table 1) and each formulation was replicated three times. Two were formulated with all pork backfat (P) as reference: one with normal fat content (~29%, LNF/P) and the other with reduced fat content (~10%, LRF/P), the latter prepared by replacing 75% of pork backfat with added water. Additionally, two different reduced-fat (~10%) fresh sausages were formulated partially replacing pork back fat with CEG (LRF/CEG) or OEG (LRF/OEG) (Table 1).

Briefly for the preparation of these sausages the meat and pork backfat packages were thawed (~18 h at 2 °C) before use. These materials, and previously-prepared chia or oat emulsion gels (CEG and

Table 1
Formulation (%) of fresh sausages.

Samples ^a	Meat	Pork back fat	CEG	OEG	Water
LNF/P	60.0	29.0			7.0
LRF/P	60.0	7.25			28.75
LRF/CEG	60.0	3.0	27.0		6.0
LRF/OEG	60.0	3.0		27.0	6.0

^a Normal fat (LNF/P) and reduced-fat (LRF/P) fresh sausages (longaniza) formulated with pork backfat (P). Reduced-fat sausages reformulated by partially replacing pork backfat: with chia emulsion gel (CEG) denominated LRF/CEG and with oat emulsion gel (OEG) denominated LRF/OEG. All the samples contain 4% of commercial seasoning preparation for fresh sausages.

OEG), were minced to a particle size of 6 mm (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy). According to each formulation (Table 1), for all fresh sausages the appropriate ingredients and 4% of a commercial seasoning preparation containing an appropriate combination of authorized preservatives substances and antioxidants [common salt, corn starch, dextrose, cinnamon essential oil, olive oil, trisodium citrate (E-331iii), preservatives (sodium sulphite E-221, potassium metabisulphite E-224), antioxidant (ascorbate sodium E-301), colorant], (AVI, ANVISA, Madrid, Spain), were placed in a mixer (MAINCA, Granollers, Barcelona, Spain) and homogenized for a total of 4 min. The mixture was kept at 4 °C for 2 h and then manual stuffed into 22 mm-diameter natural lamb casings (Type C-20/22 Julio Criado Gómez S.A., Spain). Samples were hand-linked to 10 ± 2 cm and the resulting strings of fresh sausages were stored at 2 °C overnight. After that, the sausages were placed on expanded polystyrene (EPS) trays (Type 89 white SPT—Linpac Packaging Pravia, S.A. N R.G.S., Spain), covered with oxygen-permeable cling film (LINPAC Plastics, Pontivy, France) in aerobic conditions and kept at 2 °C. Samples from each batch were analysed at days 0, 4, 7, 13 and 18 to monitor the effect of storage on quality characteristics. The casing was first removed for all analyses.

2.3. Composition and energy content

2.3.1. Proximate analysis and energy value

Moisture and ash contents were determined in triplicate according to AOAC (2005). Fat content was evaluated in triplicate following the method of Bligh & Dyer (1959). Protein was measured in quadruplicate with a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St Joseph, MI. USA). Total dietary fibre (TDF) was estimated from chia and oat bran composition according to supplier data. Energy content was calculated based on 9 kcal/g for fat; 4 kcal/g for protein and carbohydrate and 2 kcal/g for dietary fibre (European Union (EU), 2011).

2.3.2. Mineral content

For mineral content determination, freeze-dried samples (Lyophilizer Telstar-Cryodos Equipment, Tarrasa, Spain) were prepared by acid digestion (Pintado, Herrero, et al., 2015a). The minerals were quantified on a ContrAA 700 High-Resolution Continuum Source spectrophotometer (Analytik Jena AG, Jena, Germany) equipped with a Xenon short-arc lamp (GLE, Berlin, Germany). Three determinations were carried out per sample to measure Mg, Na, K, Zn, Fe, and Mn and results were expressed as mg/100 g product.

2.3.3. Amino acid profile

Amino acid content was determined and measured using ninhydrin derivative reagent and separated by means of cation-exchange chromatography, using a Biochron 20 automatic amino acid analyzer (Amersham Pharmacia Biotech. Biocom, Uppsala, Sweden) following the methodology described in Serrano et al. (2005).

2.3.4. Fatty acid profile

Fatty acid contents were determined (in triplicate) by saponification

and bimethylation as described by Lee et al. (2012), using C13:0 as internal standard. Fatty acid methyl ester (FAME) was analysed by gas chromatography on an Agilent gas chromatograph (Model 7820A, CA-USA) fitted with a GC-7 Agilent HP-88 capillary column (60 m × 250 µm × 0.2 µm), and a flame ionization detector was used. Injector and detector temperatures were 250 and 260 °C respectively. The temperature profile of the oven was 125 °C, increasing by 8 °C/min to 145 °C (held for 26 min) and 2 °C/min to 220 °C (held for 5 min). Fatty acids were identified by comparison of the retention times with the standard of fatty acids (FAME) (47015-U Supelco PUFA No.2 Animal Source, Sigma-Aldrich Co., St. Louis, MO, USA) and quantified as reported by Salcedo-Sandoval et al. (2014). Fatty acids were expressed as g of FAME/100 g product.

2.4. Technological properties

2.4.1. pH determination

pH values were determined over chilled storage in quadruplicate using a 827 Metrohm pH-meter (MetrohmAG, Switzerland) at room temperature on homogenates (ratio of 1:10 w/v of sample/distilled water).

2.4.2. Water and fat binding properties

Water and fat binding properties of fresh sausages associated with the thermal process were determined according to Carballo et al. (1995). Briefly, total fluid release (TFR), water release (WR) and fat release (FR) were measured after heating around 20 g of sample (placed in containers of 27 mm diameter) at 70 °C for 30 min in a water bath. TFR was expressed as % of initial sample weight. WR was determined as weight loss after heating (16 h/105 °C in a drying oven) and expressed as % of initial sample weight. FR was calculated as the difference between TFR and WR. Three determinations for each sample were carried out during storage.

2.4.3. Texture analysis

Kramer shear force (KSF) was performed during chilled storage using a miniature Kramer (HDP/MK05) cell with a 5-bladed head to perform a shearing test. Kramer shear tests were carried out on 2 cm sections per treatment group (previously weighed) at room temperature. A 25 kg load cell was used. Force was exerted to a compression distance of 25 mm at 0.8 mm/s crosshead speed using a TA-XT.plus Texture Analyzer (Texture Technologies Corp. Scarsdale, NY). KSF values were calculated as the maximum force per g of sample (N/g). Measurements were carried out five times.

2.5. Microbiological analyses

Microbiological analysis of fresh sausages was carried out during chilled storage as follows: 10 g of each sample was aseptically taken and placed in a sterile plastic bag with 90 mL of buffered peptone water (Panreac, Darmstadt, Germany). The sample was homogenized for 2 min in a stomacher blender (Stomacher Colworth 400, Seward, London, UK), then appropriate decimal dilutions were plated or spread on the following media: Plate Count Agar (PCA) (Panreac, Darmstadt, Germany) for the total viable counts (TVC), incubated at 37 °C for 48 h; De Man, Rogosa, Sharpe Agar (MRS) (Merck, Darmstadt, Germany) for lactic acid bacteria (LAB), incubated at 37 °C for 48 h, and Violet Red Bile Glucose Agar (VRBG) (Panreac, Darmstadt, Germany) with a double layer for *Enterobacteriaceae* incubated at 37 °C for 24 h. All microbial counts were converted to logarithms of colony-forming units per gram (Log cfu/g).

2.6. Sensory analysis

A 20 member sensory panel was selected from among staff members who are familiar with this kind of products and terminology. Samples

(at day 2 after preparation) were cooked at 210 °C for 2 min in a contact electric grill (Princess classic multigrill type 2321, The Netherlands) then cut into 2 cm sections, placed on plates and served to the panelists. These were asked to evaluate the following parameters on a hedonic scale from 0 (dislike) to 10 (like): colour, odour, flavour, texture and general acceptability.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed to evaluate the statistical significance ($P < 0.05$) of the effect of sample formulation and two-way ANOVA as a function of formulation and storage time using the SPSS program (v.22, IBM SPSS Inc.; Chicago, IL, USA). Formulation and storage time was assigned as fixed effects and replication as a random effect. The entire experimental design was followed in triplicate and differences between replicates were not significant ($P < 0.05$). The values in the tables are given in terms of mean values and standard error of the mean. Least squares differences (LSD) were used for comparison of mean values among formulations and Tukey's HSD test to identify significant differences ($P < 0.05$) between formulations and storage time.

3. Results and discussion

3.1. Composition and energy content

3.1.1. Proximate analysis and energy value

Proximate analysis of products, generally close to the target levels, showed some significant differences between formulations (Table 2). While normal fat samples (LNF/P) had the lowest ($P < 0.05$) moisture content, all-pork reduced fat sausage (LRF/P) showed the highest ($P < 0.05$) (Table 2). These results are consistent with the fact that the pork backfat was partially replaced by water in the case of LRF/P or by the corresponding EG in LRF/CEG or LRF/OEG (Table 1). No differences were observed in protein and ash contents irrespective of formulation (Table 2). However, protein provided > 20% of the energy value.

As expected, LNF/P registered the highest ($P < 0.05$) fat content, while the rest of the reduced-fat samples had similar fat levels ($P > 0.05$) (Table 2), reaching fat reductions of between 62 and 66%. Additionally, sausages containing chia flour or oat bran EG (CEG and OEG) registered TDF values of approximately 1.6 and 2.4% respectively (Table 2).

The reduced-fat samples registered lower (50.8–56.4%) energy values than normal-fat sausage.

3.1.2. Mineral content

The mineral content of sausages can be important for purposes of nutrition and health. The mineral content of all-animal-fat samples (LNF/P and LRF/P) was comparable to that of similar products (Moreiras et al., 2013). In general, the concentration of minerals increased ($P < 0.05$) when either emulsion gel was used in the product formulation (Table 2). Products containing chia (LRF/CEG) displayed the highest ($P < 0.05$) Ca and Mg contents, while those containing oat (LRF/OEG) showed the highest ($P < 0.05$) Fe and Mn contents.

3.1.3. Amino acid profile

The amino acid profiles in these samples were affected by the formulation. The LNF/P and LRF/P products showed the typical profile of meat products with all meat protein (Ayo et al., 2007). On the other hand, the use of chia and oat EG in the product's formulation affected ($P < 0.05$) the concentration of some amino acids. In this regard, the product made with chia (LRF/CEG) contained higher ($P < 0.05$) proportions of aspartic acid (3.4%), serine (4.0%), glutamic acid (3.3%) and arginine (4.1%), while the presence of oat (LRF/OEG) increased the levels of serine (3.1%), glutamic acid (3.7%) and methionine (18.9%)

Table 2
Proximate analysis (%), energy value (kcal/100 g of product) and mineral content (mg/100 g product) of fresh sausages.

Parameters	Samples ^a			
	LNF/P	LRF/P	LRF/CEG	LRF/OEG
Proximate analysis				
Moisture	52.17 ± 0.16 ^a	73.75 ± 0.31 ^c	66.58 ± 0.77 ^b	66.34 ± 0.81 ^b
Protein	14.03 ± 0.72 ^a	12.88 ± 0.62 ^a	13.63 ± 0.91 ^a	12.58 ± 0.83 ^a
Fat	29.87 ± 1.67 ^b	10.01 ± 1.25 ^a	11.36 ± 0.31 ^a	10.62 ± 0.36 ^a
Total dietary fibre			1.63	2.38
Ash	2.85 ± 0.18 ^a	2.71 ± 0.04 ^a	3.17 ± 0.07 ^a	3.58 ± 0.71 ^a
Energy value	324.95	141.61	160.02	150.66
Mineral content				
Sodium	981 ± 23.5 ^{ab}	963.7 ± 19.5 ^a	1051.3 ± 14.1 ^b	1040.8 ± 21.0 ^b
Potassium	282.9 ± 13.6 ^a	312.5 ± 14.8 ^{ab}	343.7 ± 16.9 ^b	322.9 ± 8.3 ^{ab}
Magnesium	19.1 ± 0.9 ^a	18.8 ± 0.5 ^a	37.7 ± 2.0 ^c	33.6 ± 0.7 ^b
Calcium	6.8 ± 0.6 ^a	7.6 ± 0.3 ^a	112.9 ± 1.5 ^c	80.9 ± 1.8 ^b
Iron	0.54 ± 0.0 ^a	0.58 ± 0.1 ^a	1.02 ± 0.1 ^b	1.22 ± 0.0 ^c
Manganese	0.10 ± 0.0 ^a	0.10 ± 0.0 ^a	0.29 ± 0.0 ^b	0.78 ± 0.0 ^c
Zinc	1.20 ± 0.0 ^a	1.33 ± 0.0 ^a	1.69 ± 0.1 ^b	1.74 ± 0.1 ^b
Phosphorus	111.3 ± 10.6 ^a	121.0 ± 2.8 ^b	191.7 ± 3.2 ^c	185.7 ± 3.7 ^c

^a For samples denominations, see Table 1. Means ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

as compared with all-meat protein. These results were consistent with the concentration (1.1–1.2%) of chia and oat used during the formulation of the sausages and the amino acid composition of these chia and oat ingredients and the EG with these compounds. Others authors have also reported that chia protein is rich in certain amino acids, chiefly aspartic, arginine and glutamic; the main amino acids reported in oat are lysine and sulphurated amino acids such as methionine (Pomeranz et al., 1971; Olivos-Lugo et al., 2010; Arendt & Zannini, 2013).

The presence of amino acids such as arginine, glutamic acid or methionine has been associated with different health benefits, and therefore the changes associated with the reformulation strategy could help to obtain products with a healthy amino acid profile (Kurowska & Carroll, 1994; Feldman, 2002). Especially noteworthy is the low lysine/arginine ratio in the samples (LRF/CEG) with chia emulsion gel, with effects in reducing atherosclerosis and benefits for heart and blood pressure (Feldman, 2002).

3.1.4. Fatty acid profile

Fatty acid composition was affected by formulation: fat level and source of lipid content (animal fat, olive oil, chia or oat bran) (Table 3).

All-animal-fat products LNF/P and LRF/P registered the highest ($P < 0.05$) SFA content (Table 3), decreasing to levels below 30% of total fatty acid when animal fat was replaced by an EG. Of the reduced-fat samples, sausages containing OEG registered the highest ($P < 0.05$) MUFA content (Table 3), up to almost 60% of total fatty acids. Oleic acid was the most abundant fatty acid in all samples (Table 3), although proportionally higher in the products formulated with plant ingredients (47% for LRF/CEG and 54% for LRF/OEG). LNF/P had the highest linoleic acid content (Table 4), although this was proportionally higher in the products formulated with EG. Sausage containing chia (LRF/CEG) registered the highest ($P < 0.05$) ALA content, accounting for approximately 10% of total fatty acids, between 21 and 18 times higher than in the other reduced-fat samples (LRF/P and LRF/OEG). Similarly, other authors (Pintado, Herrero, et al., 2015a; Souza et al., 2015; Pintado et al., 2016) observed an increase in ALA in meat products reformulated with chia. This effect is consistent with the fact that chia contains high levels (> 60%) of ALA (Ayerza & Coates, 2011; Ixtaina et al., 2011). Several studies have demonstrated the beneficial effect of n-3 PUFAs in the prevention of coronary heart diseases by management of hyperlipidaemia, increase of blood LDL-cholesterol, and others (Dias et al., 2014). Given that the dietary recommendation for total n-3 PUFAs is estimated at between 1.4 and 3 g/day (Garg et al., 2006; EFSA, 2010), LRF/CEG sausage with 1.08 g of ALA/100 g of product

Table 3
Fatty acid profile (g/100 g of product) and nutritional significance ratios of different fresh sausages.

Parameters	Samples ^a			
	LNF/P	LRF/P	LRF/CEG	LRF/OEG
C14:0	0.50 ± 0.00 ^c	0.17 ± 0.02 ^b	0.06 ± 0.00 ^a	0.07 ± 0.01 ^a
C16:0	7.68 ± 0.11 ^c	2.62 ± 0.26 ^b	1.87 ± 0.09 ^a	1.93 ± 0.13 ^a
C18:0	3.91 ± 0.06 ^c	1.39 ± 0.15 ^b	0.86 ± 0.05 ^a	0.83 ± 0.07 ^a
Total SFA	12.31	4.25	2.85	2.90
C16:1	0.74 ± 0.01 ^c	0.26 ± 0.02 ^b	0.17 ± 0.01 ^a	0.20 ± 0.01 ^a
C18:1n9	12.65 ± 0.17 ^c	4.39 ± 0.42 ^a	5.20 ± 0.20 ^b	5.59 ± 0.31 ^b
C 18:1 n7	1.06 ± 0.04 ^b	0.36 ± 0.03 ^a	0.30 ± 0.02 ^a	0.33 ± 0.02 ^a
C20:1n9c	0.32 ± 0.01 ^c	0.11 ± 0.01 ^b	0.07 ± 0.00 ^a	0.07 ± 0.00 ^a
Total MUFA	14.77	5.12	5.73	6.18
C18:2n6	2.04 ± 0.02 ^c	0.82 ± 0.09 ^a	1.25 ± 0.05 ^b	1.10 ± 0.08 ^b
C18:3n3	0.13 ± 0.00 ^b	0.05 ± 0.01 ^a	1.08 ± 0.02 ^c	0.06 ± 0.00 ^a
Total PUFA	2.41	0.98	2.42	1.25
PUFA/SFA	0.20	0.23	0.85	0.43
n - 6/n - 3	17.04	19.03	1.21	18.53

Means ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$). SFA: Saturated fatty acid. MUFA: Monounsaturated fatty acid. PUFA: Polyunsaturated fatty acid.

^a For samples denominations, see Table 1.

(Table 4), can make a very important contribution to dietary intake.

The reformulation process also improves on recommended PUFA/SFA (> 0.4) and n - 6/n - 3 PUFA (< 4) ratios (Wood et al., 2004; Simopoulos, 2008), especially with the presence of chia (Table 3). Several authors have improved these ratios by replacing animal fat with vegetable oil in meat product reformulation (Delgado-Pando et al., 2011; Pintado, Herrero, et al., 2015a; Pintado et al., 2016).

3.1.5. Nutritional and health claims

Sausages produced in the present study could be labelled with some nutrition and health claims according to composition (Table 4) (European Commission, 2006; European Union, 2012).

3.2. Technological properties

3.2.1. Cooking loss

Water and fat binding properties (TFR, FR and WR) were affected ($P < 0.05$) by the formulation but not by chilled storage so Fig. 1 shows only the mean values over the storage period. Sausages made

Table 4

Nutrition and health claims authorized in the reformulated fresh sausages with chia and oat emulsion gels as animal fat replacers according to Regulation (EC) No 1924/2006 and Commission Regulation (EU) No 432/2012.

Samples	Nutrition claims	Conditions applying to them	Health claims
LRF/P LRF/CEG LRF/OEG	High protein	Be made where at least 20% of the energy value of the food is provided by protein	Proteins contribute to: <ul style="list-style-type: none"> - a growth in muscle mass - the maintenance of muscle mass - the maintenance of normal bones Protein is needed for normal growth and development of bone in children
LRF/P LRF/CEG LRF/OEG	Reduced fat	Be made where the reduction in content is at least 30% compared to a similar product	-
LRF/OEG	β -glucans	Be made for food which contains at least 1 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these sources per quantified portion	Beta-glucans contribute to the maintenance of normal blood cholesterol levels
LRF/P LRF/CEG LRF/OEG	Energy reduced	Be made where the energy value is reduced by at least 30%	-
LRF/OEG	Source of manganese	Be made where the product contains at least a significant amount as defined in the Annex to Directive 90/496/EEC (> 15% of 2 mg)	Manganese contributes to: <ul style="list-style-type: none"> - normal energy-yielding metabolism - the maintenance of normal bones - the normal formation of connective tissue - the protection of cells from oxidative stress
LRF/CEG LRF/OEG	Source of zinc	Be made where the product contains at least a significant amount as defined in the Annex to Directive 90/496/EEC (> 15% of 10 mg)	Zinc contributes to: <ul style="list-style-type: none"> - normal metabolism of carbohydrates, macronutrients, fatty acids, vit. A - normal acid-base metabolism - normal cognitive function, DNA synthesis, fertility and reproduction, protein synthesis, - maintenance of normal hair, bones, nails, skin, vision, testosterone levels in blood - the normal function of the immune system - the protection of cells from oxidative stress Zinc has a role in the process of cell division
LRF/CEG	High omega-3 fatty acids	Be made where the product contains at least 0.6 g alpha-linolenic acid (ALA)/100 g of product and per 100 kcal	ALA contributes to the maintenance of normal blood cholesterol levels. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA
LRF/CEG LRF/OEG	High content unsaturated lipid	Be made where at least 70% of the fatty acids present in the product derive from unsaturated fat under the condition that unsaturated fat provides > 20% of energy of the product	Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels [MUFA* and PUFA* are unsaturated fats]

*MUFA: Monounsaturated fatty acid.

*PUFA: Polyunsaturated fatty acid.

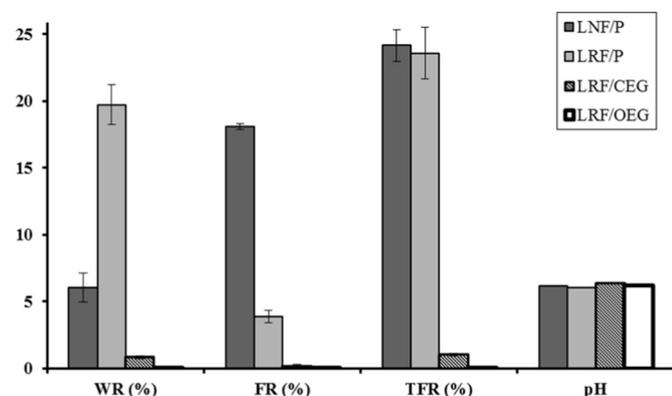


Fig. 1. Cooking loss (%) [TFR (total fluid release); FR (fat release) and WR (water release)] and pH. For samples denominations see Table 1. LRF/OEG registered no cooking loss.

with all-animal-fat had similar ($P > 0.05$) TFR values (around 24%) irrespective of fat content. However, while in LNF/P most of the fluid release (FR) was fat, in reduced-fat sample it was mostly water. On the other hand, TFR was < 1% in sausages with chia emulsion gel (LRF/CEG) and close to zero in sausages with oat emulsion gel (LRF/OEG) (Fig. 1). Previous studies showed non-noticeable release of either chia

or oat olive oil-in-water emulsion gels after heat treatment (Pintado, Ruiz-Capillas, et al., 2015b; Pintado et al., 2017). All these results indicate that fresh sausages reformulated with a chia or oat emulsion gel as fat replacer are very stable to thermal treatment.

3.2.2. pH determination

The pH values of sausages were affected ($P < 0.05$) by formulation but not ($P > 0.05$) by storage. Fig. 1 shows only the mean values over the storage period. The highest ($P < 0.05$) pH values were registered in the samples containing chia and oat, while values were lowest in all-animal-fat samples. These values are consistent with others reported for fresh sausages (Ruiz-Capillas & Jimenez-Colmenero, 2010; Scapin et al., 2015).

3.2.3. Texture

KSF was affected ($P < 0.05$) by formulation and storage, with interaction ($P < 0.05$) between the two factors (Table 5). In all-animal-fat sausages, KSF decreased significantly with fat reduction. This effect may be related to the fact that pork backfat content was reduced by increasing the proportion of added water while keeping the amount of protein essentially constant (Table 2), consequently making for a softer product (Jiménez-Colmenero et al., 1996). The same behaviour has been reported in similar meat products such as fresh sausages and patties (Pinerio et al., 2008; López-López et al., 2010; Triki et al., 2013).

Table 5

Kramer shear force (KSF) (N/g) of fresh sausages during chilled storage.

Samples ^a	Days of storage (4 ± 2 °C)				
	0	4	7	13	18
LNF/P	1.30 ± 0.12 ^{b12}	1.47 ± 0.03 ^{b12}	1.44 ± 0.15 ^{b2}	1.51 ± 0.14 ^{b2}	1.20 ± 0.06 ^{b1}
LRF/P	1.02 ± 0.06 ^{a1}	1.01 ± 0.11 ^{a1}	1.13 ± 0.12 ^{a1}	1.04 ± 0.12 ^{a1}	1.34 ± 0.14 ^{b2}
LRF/CEG	0.84 ± 0.04 ^{a1}	0.88 ± 0.11 ^{a1}	0.86 ± 0.08 ^{a1}	1.07 ± 0.13 ^{a2}	0.88 ± 0.10 ^{a12}
LRF/OEG	1.27 ± 0.12 ^{b1}	1.27 ± 0.11 ^{b1}	1.33 ± 0.08 ^{b12}	1.51 ± 0.16 ^{b23}	1.57 ± 0.09 ^{c3}

^a For samples denominations see Table 1. Means ± standard deviation. Different letters in the same column for each parameter evaluated and different numbers in the same row indicate significant differences ($P < 0.05$).

In reduced-fat sausages, KSF was similar ($P > 0.05$) in samples reformulated with animal fat (LRF/P) and with chia emulsion gel (LRF/CEG), while reformulated sausages with oat emulsion gel LRF/OEG registered similar ($P > 0.05$) KSF to normal-fat samples (LNF/P). Given that the protein–moisture ratio and lipid contents were similar in samples formulated with an EG, the differences found in KSF between LRF/CEG and LRF/OEG (Table 5) would appear to be due to the presence of oat in the emulsion gel. This textural behaviour could be due to the technological properties of oat, which can act as an emulsifier and stabilizer, providing high emulsion stability as well as acting as a gelling agent (Bohm & Kulicke, 1999; Brummer et al., 2014; Nieto-Nieto et al., 2014). Particularly, it is commonly believed that adding larger amounts of soluble β -glucan, present in oat, improves the firmness of a meat product (Lazaridou & Biliaderis, 2007).

Generally, KSF increased with time in storage, although the magnitude (generally very scant) and time varied with formulation (Table 5). Other authors have noted similar textural behaviour in fresh meat products (Triki et al., 2013; Overholt et al., 2016). (Pintado, Ruiz-Capillas, et al., 2015b; Pintado et al., 2017) reported a significant increase in puncture force and gel strength of chia and oat structured emulsions during chilled storage. This hardening of both emulsion gels could explain the increase of KSF observed in sausages formulated with oat or chia emulsion gel (LRF/CEG and LRF/OEG) during storage. The same textural behaviour was observed in cooked sausages in which a gelled emulsion was used as an animal fat replacer (Poyato et al., 2014; Pintado, Herrero, et al., 2015a; Pintado et al., 2016). During chilled storage hardness was similar between control (formulated with animal fat) and products reformulated with gelled emulsion which suggested that the gelled emulsion ingredient could efficiently maintain the hardness of the original product (Poyato et al., 2014).

3.3. Microbiological analysis

Fresh sausages are highly perishable products and a favourable medium for the growth of microorganisms. The initial levels of TVC were lower than 4.5 Log cfu/g and the highest ($P < 0.05$) levels were observed in the sausages formulated with chia emulsion gel (LRF/CEG); also, it was in this sample that the highest levels of *Enterobacteriaceae* (3.25 Log cfu/g) were observed. The LAB counts were similar ($P > 0.05$) in all sausages. These initial microbiological counts were lower in all samples than others found in similar meat products (Ruiz-Capillas & Jimenez-Colmenero, 2010; Triki et al., 2013).

During chilled storage there was a significant increase in the TVC and LAB in the normal-fat sausages (LNF/P) and sausages formulated with chia emulsion gel (LRF/CEG). In this last sample, levels of TVC were higher ($P < 0.05$) than 6 Log cfu/g after day 13 of storage, associated with higher ($P < 0.05$) levels of LAB and *Enterobacteriaceae*. In all samples, *Enterobacteriaceae* counts decreased (< 2 Log cfu/g) over storage. In general, lower levels of microorganisms were observed than reported by other authors in fresh sausages during chilled storage (Georgantelis et al., 2007; do Amaral et al., 2015). These low levels could be associated with the commercial seasoning preparation used for the fresh sausage (Ruiz-Capillas & Jimenez-Colmenero, 2010; Triki

et al., 2013). However, the microbial count was significantly affected by the use of CEG in the formulation, while the use of OEG showed no effect compared with the LRF/P.

3.4. Sensory evaluation

Normal and reduced all-animal fat sausages (LNF/P and LRF/P) generally scored significantly higher than the rest for all the sensory parameters considered except for odour, where scores were similar ($P > 0.05$) for all samples (Fig. 2). Additionally, texture scores were similar ($P > 0.05$) for LNF/P, LRF/P and LRF/CEG, while colour scores were similar ($P > 0.05$) for LNF/P, LRF/P and LRF/OEG (Fig. 2). Flavour scores were similar ($P > 0.05$) for LRF/P and LRF/OEG and lowest ($P < 0.05$) for LRF/CEG (Fig. 2). The samples containing an emulsion gel (LRF/CEG and LRF/OEG) scored worse for general acceptability, but all these samples were judged acceptable (> 5) by the panellists (Fig. 2). Other authors have reported no significant differences in sensory properties (odour, colour, taste, hardness, juiciness and fattiness) for other fresh meat products (burger patties) reformulated with a polyunsaturated gelled emulsion as pork back-fat replacer (Poyato et al., 2015).

4. Conclusions

Chia or oat emulsion gels as animal fat replacers may be novel sources of nutritional and healthy components in frequently-consumed meat products such as fresh sausages (*longaniza* type) that produce no detrimental changes in their sensory and technological properties.

Sausages reformulated with these emulsion gels could be labelled with different nutrition and health claims depending on chia or oat incorporation. Both emulsion gels with chia and oat, entail a reduction in fat and energy content, and so these products could qualify for “reduced fat content” and “energy-reduced” claims and significantly improve fat content in line with nutritional recommendations, given their low levels of SFAs and high levels of MUFAs. These reformulation processes also improve on the recommendations for PUFA/SFA and $n - 6/n - 3$ PUFA ratios. In particular, sausages with chia emulsion gel have also significantly improved ALA content. Additionally,

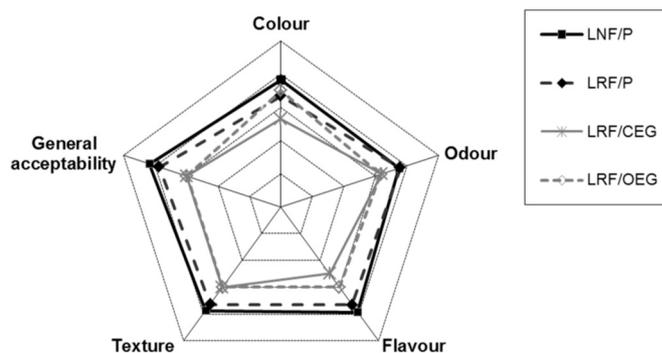


Fig. 2. Sensory evaluation of fresh sausages. For sample denominations see Table 1.

sausages reformulated with chia emulsion gel significantly improve Ca and Mg concentrations, while samples with oat improve Fe and Mn contents. Amino acids such as aspartic acid, serine, glutamic acid and arginine increase in sausages containing chia emulsion gel, and serin, glutamic acid and methionine in samples containing oat emulsion gel.

These novel reformulation strategies also affect certain technological properties such as water- and fat- binding properties and texture depending on whether a chia or oat emulsion gel is used. The use of these fat replacers generally reduced cooking loss, while increasing shear force only in samples containing oat emulsion gel. Although these reformulation processes entailed changes in sensory characteristics of the products, they were judged to be acceptable and of adequate microbiological quality (day 0). Generally, chilled storage had little effect on the technological characteristics of the sausages excepting textural alterations in terms of increased hardness in all samples, and the microbial count (TVC) was only significantly affected by the use of CEG in the formulation after 13 days.

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