Research Article

Received: 31 July 2019 Revised: 2 September 2019 Accepted article published: 12 September 2019

(wileyonlinelibrary.com) DOI 10.1002/jsfa.10025

Evaluation of linseed oil oleogels to partially replace pork backfat in fermented sausages

Daniel Franco,[a] Artur J Martins,[b,c] María López-Pedrouso,[d]
Miguel A Cerqueira,[c] Laura Purriños,[a] Lorenzo M Pastrana,[c]
António A Vicente,[b] Carlos Zapata[d] and José M Lorenzo[a*]

Abstract

BACKGROUND: Nowadays, fat replacement in meat products is a matter of concern in the meat industry. The objective of this study was to evaluate the replacement of pork backfat with two oleogels of linseed in dry-cured sausages.

RESULTS: Five batches of dry-cured sausages were prepared with two oleogels, a mixture of γ-oryzanol and β-sitosterol (SO) and beeswax (B), at two levels of replacement (20% and 40%) (SO-20, SO-40, B-20, and B-40, respectively) and a control batch. The fatty acid profile improved in terms of nutrition: the polyunsaturated fatty acid / saturated fatty acid (PUFA/SFA) and n-6/n-3 ratio was about 1.41 and 0.93 for the higher levels of replacement, SO-40 and B-40, respectively. Quality parameters such as pH and color also changed with the inclusion of oleogels, resulting in changes in the sensory quality.

CONCLUSION: Oleogels based on linseed enabled the replacement of pork backfat in fermented sausages. Depending on the level of fat substitution, such oleogels could replace fat in dry-cured sausages at the industrial level.

Keywords: sterols; beeswax; textural properties; fatty acid; oleogels, n-6/n-3 ratio

INTRODUCTION

Fermented sausages are popular meat products because of their convenience and their flavor / taste. However, they have a high fat content (around 32%).[1–3] To improve the nutritional quality, most studies have focused on lowering or replacing the fat using different ingredients such as inulin,[4] cellulose gel,[5] konjac gel,[6,7] or boiled quinoa.[8] However, fat is a crucial ingredient because it improves the flavor, texture, and juiciness of dry-cured sausages. Minced fat also contributes to the release of water, promoting fermentation during the curing process.[2,3,9]

Consumer needs regarding diets and healthier lifestyles have been changing over the past few years and fats play a key role.[10] To address this concern, the meat industry has spotlighted lower fat formulations in its products.[11–13] Thus, the reformulation of meat products must achieve a reduction of overall amount of fat and an improvement in the fatty acid (FA) profile.[14–17] However, direct replacement by vegetable oils is quite difficult due to the intrinsic and unique characteristics of animal fats such as texture, mouth feel, and juiciness.[18,19] Indeed, the textural features of meat products change with respect to original product as reported by Barbut *et al.*,[19] who showed a firmer and more rubbery comminute beef product when beef fat was replaced directly with canola oil. To solve these issues, the structuring of vegetable oils using the incorporation of edible structured gels has received much attention in recent years.[20–22]

Edible oleogels, based on healthy oils, are developing as a novel structuring method to reduce the negative effects of *trans* and saturated FAs. An oleogel is composed of an organic liquid trapped in a three-dimensional thermo-reversible network, in which the oleogelator plays a key role.[23] In edible oils, such as sunflower oil, mixtures of plant sterols (γ-oryzanol and β-sitosterol) have recently been investigated, showing excellent potential.[24–26] Specifically, this mixture forms complex supramolecular structures such as tubules, which support gelled structures with a self-assembly system.[27,28] One of the great advantages of the plant sterols is that they have a blood-cholesterol-lowering effect, which is beneficial to human health.[29] Another alternative oleogelator is beeswax, which is mainly composed of hydrocarbons, free fatty acids, esters of fatty acids, and fatty alcohol diesters. This oleogelator has previously been tested in meat products such as pâtés[30] and burgers.[31] However, there has been little information about how oleogels could be used in meat products or about their effects on meat quality and sensory analysis.

The aim of this study was to assess the potential replacement of pork backfat by linseed oleogel using two different oleogelators

---

[*] *Correspondence to: JM Lorenzo, Centro Tecnológico de la Carne de Galicia, Rúa Galicia N° 4, Parque Tecnológico de Galicia, San Cibrán das Viñas, 32900 Ourense, Spain. E-mail: jmlorenzo@ceteca.net*

[a] *Centro Tecnológico de la Carne de Galicia, Rúa Galicia N° 4, Parque Tecnológico de Galicia, Ourense, Spain*

[b] *Centre of Biological Engineering, University of Minho, Braga, Portugal*

[c] *International Iberian Nanotechnology Laboratory, Braga, Portugal*

[d] *Department of Zoology, Genetics and Physical Anthropology, University of Santiago de Compostela, Santiago de Compostela, Spain*

(a mixture of γ-oryzanol and β-sitosterol and beeswax) and different substitution levels, and to investigate its effect on the main quality characteristics (color, texture, and sensory features) of dry-cured sausages.

MATERIALS AND METHODS

Oleogels elaboration

Two oleogels that contain beeswax and a mixture with 60:40 (w/w) γ-oryzanol and β-sitosterol in linseed oil were prepared. In both cases, a commercial linseed oil, Vitaquell®, with 72% polyunsaturated (~55% α-linoleic), 19% monounsaturated, and 9% saturated FAs was used as the oil phase. γ-Oryzanol and β-sitosterol were purchased from Oryza Co. (Tokyo, Japan) and Sigma-Aldrich (Paris, France) respectively. Both oleogels were prepared using 8% (w/w) of oleogels in linseed oil stirring at 80 °C until solubilization (at least 30 min) as explained elsewhere.[32,33]

Dry-cured sausage elaboration

Five different batches of dry-cured 'Salchichón' sausages were formulated in the pilot plant of the Meat Technology Center of Galicia (Ourense, Spain). A control batch, two batches with β-sitosterol and γ-oryzanol (SO) as oleogelators in linseed oil, and another two batches in beeswax (B) as an oleogelator in linseed oil were made. In the control batch, the pork sausage recipe included lean pork meat (70%), pork backfat (18%), water (8%) and additive (4%). In the other four batches, the replacement level of pork backfat by linseed oleogel was 20% (SO-20, B-20) and 40% (SO-40, B-40). Additive '542 Salchichón' from Laboratorios Ceylamix (Valencia, Spain) was composed, in unknown proportions, of sugar (lactose and sucrose), salt, dextrin, spices (black and white pepper and nutmeg), milk protein, monosodium glutamate (E-621), phosphates (E-450, E-451), sodium erythorbate (E-316), potassium nitrate (E-252), and coloring (E-120). No starter culture was added. The lean was ground through a 12 mm diameter mincing plate; meanwhile, the pork backfat (in a frozen state) and linseed oleogel were ground through an 8 mm diameter mincing plate and vacuum minced with '542 Salchichón' additive at 0.8 bar using an industrial mixer (Fuerpla, Mod. AO-85, Valencia, Spain) for 3 min. The meat mixture was maintained at 3–5 °C for 24 h and then it was stuffed into collagen casings with a diameter of 55–60 mm and a length of 40 cm (Fibran, S.A., Girona, Spain). Sausages were fermented for 2 days at 20 °C and 80–85% of relative humidity, and then transferred into a drying-ripening chamber where they were kept for 49 additional days at 12 °C and 75%–80% relative humidity. Sausages were made in triplicate and 10 dry-cured 'Salchichon' sausages were used per replicate. Samples were taken at the end of the ripening for subsequent analysis. To prepare the samples for analysis, after removing and discarding the outer casing of each dry-cured sausage unit, the edible part was ground in a Moulinette mincer (Moulinex/Swan Holding Ltd, Birmingham, UK) until a homogeneous mass was obtained.

Physicochemical analysis

Quality parameters: pH, color, and proximate composition

The pH was measured using a portable pH meter (Hanna Instruments, Eibar, Spain) equipped with a penetration probe. The color determination of sausages from fresh-cut cross-sections was assessed at three different points with a portable colorimeter (Konica Minolta CM-600d, Osaka, Japan), with a pulsed xenon arc lamp filtered to illuminant D65 lighting conditions, 0° viewing angle geometry, and 8 mm aperture size. Color was reported according to CIELAB space[34]: lightness, (L*); redness, (a*); yellowness, (b*), with the next settings machine (pulsed xenon arc lamp, angle of 0° viewing angle geometry and aperture size of 8 mm). Hue (Hab) and chroma (C*) were calculated from the a* and b* values according to Eqns 1 and 2:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \tag{1}$$

$$h_{ab} = acr\tan\frac{b^*}{a^*} \tag{2}$$

Moisture, protein, fat, and ash were assessed following the ISO recommended standards.[35–37] Total fat was extracted according to the AOCS Official Procedure,[38] while carbohydrate contents were estimated by difference.

Fatty acid profile

Total fat was extracted from a 12.5 g sample and 50 mg was utilized to determine the FA profile. Total FAs were transesterified following the procedure reported in 2015 by Dominguez *et al.*[39] The separation and quantification of the fatty acid methyl esters (FAMEs) was performed using a gas chromatograph (GC-Agilent 6890 N; Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a flame ionization detector following the chromatographic conditions described by Dominguez *et al.*[39] Data of FAME profiles were expressed in grams per 100 g of fat.

Texture profile analysis

Sausage pieces of 1 × 1 × 2.5 cm (height × width × length) were compressed at a crosshead speed of 3.33 mm s^{-1} in a texture analyzer (TA.XTplus, Stable Micro Systems, Vienna Court, UK). The following parameters were recorded: hardness, cohesiveness, springiness, gumminess, chewiness, and adhesiveness by compressing to 80% using a compression probe with 19.85 cm^2 of surface contact. The probe waited for 2 s between the first and second compressions.

Sensory analysis

A pilot consumer test was performed by 28 panelists recruited from personnel of the Meat Technology Center. The testing was carried out in a room equipped with individual tasting booths under white light according to ISO procedures.[40] Water and bread without salt were available for rinsing the mouth between samples. Slices (1.5 mm) were obtained using a commercial slicing machine and were immediately served to the panelists. The five samples were presented to participants following a balanced order for each of them.[41] Panelists evaluated the overall acceptability of each sample using a seven-point hedonic scale, varying from (1) 'liked very much' to (7) 'disliked very much'.[42]

Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics version 23 software package (SPSS, Chicago, IL, USA). A total of 50 dry-cured sausages (ten samples per each batch × five batches) were analyzed for different quality traits. The effect of inclusion of two oleogels at two levels (SO-20, SO-40, B-20 and B-40) was evaluated employing a mixed-model ANOVA and these traits were set as dependent variables, oleogel concentration (0, 20 and 40%) as a fixed effect, and replicate as a random effect. The pairwise differences between least-square means were examined using

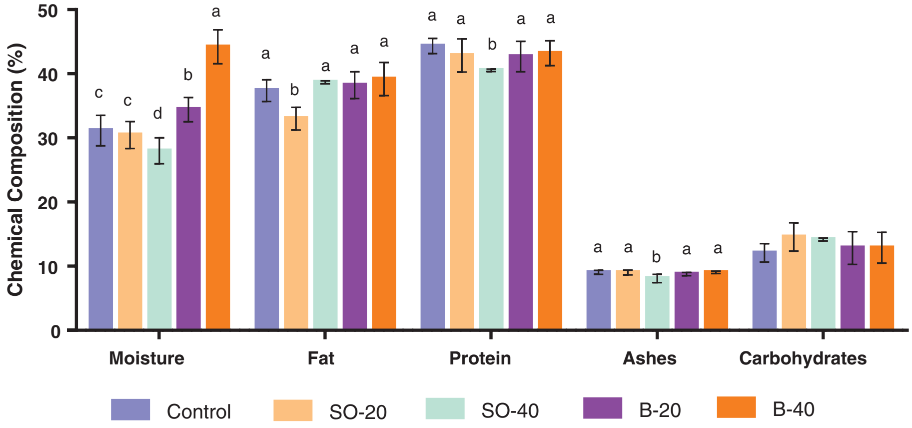

Figure 1. Chemical composition (fat, protein and carbohydrates calculated on dried basis), expressed in percentage of dry-cured sausages elaborated with sterols (γ-oryzanol and β-sitosterol, SO) and beeswax (B) at two levels of fat replacement (20% and 40%).

Tukey's test at a level of 5%. Pearson's linear correlations were also calculated with a coefficient at 5% of significance.

XLSTAT-Sensory version 2018 (Addinsoft SARL, Paris, France) software was used to analyze all the sensory data. Overall acceptability data from the evaluation was analyzed by means of linear mixed model. Oleogels and the replacement level of pork backfat were specified as fixed effects while panelist was specified as a random effect. A significance level of 5% was considered in the analysis and when the effect was significant, significant differences were calculated using Tukey's test.

RESULTS AND DISCUSSION

Chemical composition, pH, and color parameters of dry-cured sausages formulated with oleogels

The proximate chemical composition of dry-cured sausages is displayed in Fig. 1. The statistical analysis showed significant differences for moisture, fat, and protein especially in the case of SO batch. Nevertheless, numerical values among batches were very similar. Moisture content was significantly different among batches ($P < 0.001$) ranging from 27.99 to 44.20%. Interestingly, the water content in the samples decreased with the amount of β-sitosterol and oryzanol oleogel in contrast to the samples with beeswax oleogel. Thus, both of these batches exhibited different types of drying speed from the control batch. The highest water content (44.20%) was determined in SO-40 indicating a slower drying process of dry-cured sausage. These data would suggest that beeswax oleogel could generate a barrier, avoiding water loss during the drying process, in comparison with the control batch, reaching a final water content of 31.14%. On the other hand, batch SO-40 lost significantly ($P < 0.05$) more water than the control batch (27.99 versus 31.14%), suggesting a faster drying process. Interestingly, these variations in the final moisture content among batches will influence textural and sensory parameters. Differences were also found in the fat and protein content in SO batches. Dry-cured sausage forms heterogeneous samples due to the rough minced ingredients, which slightly change the composition of each sausage sample. Differences in water content modified the other chemical components (fat, protein and carbohydrates), on dry matter. The fat content ranged from 33.01% to 39.19% resulting in lower values than other traditional sausages.[43] It has been reported that the amount of fat reduces the sausage's weight loss during the process in dry fermented sausages.[6] Fat reduction or replacement could affect meat product aroma but it also has positive implications for health.[44–46] The protein values ranged from 40.54 to 44.33% resulting in the lowest value ($P < 0.05$) in the batch SO-40, but the difference was not very important.

The pH values among batches were significantly ($P < 0.0001$) affected by oleogelator type and its concentrations as shown in Table 1. The final pH was lower in dry-cured sausages containing beeswax as an oleogelator than those with SO or the controls. It seems possible that these results are due to the acidity of the beeswax mixture compared to pork backfat. This fact could also have important implications for the development of safety products inhibiting the growth of microorganisms.

Color parameters (L*, a* and b*) showed significant differences for all the batches (Table 1). Luminosity (L*) was influenced by the type of oleogelator (SO and B) and its replacement level

Table 1. Effects of oleogelator type (SO and B) and fat replacement (20 and 40%) on pH and color parameters of dry-cured 'Salchichón' sausage, elaborated with linseed oleogel

	Control	SO-20	SO-40	B-20	B-40	*P* value	SEM
pH	5.26 ± 0.05^{a}	5.17 ± 0.05^{b}	5.27 ± 0.04^{a}	5.08 ± 0.08^{c}	4.85 ± 0.05^{d}	<0.0001	0.02
L	44.10 ± 2.10^{c}	45.04 ± 2.24^{ab}	42.98 ± 1.99^{c}	46.64 ± 2.64^{b}	54.68 ± 1.82^{a}	<0.0001	0.68
a*	13.32 ± 1.56^{b}	13.29 ± 1.16^{b}	12.77 ± 1.56^{b}	14.96 ± 0.63^{a}	15.01 ± 0.95^{a}	<0.0001	0.21
b*	9.59 ± 0.84^{c}	9.43 ± 0.70^{c}	12.06 ± 1.80^{b}	11.76 ± 0.62^{b}	15.58 ± 0.89^{a}	<0.0001	0.36
Croma	16.46 ± 1.24^{d}	16.30 ± 1.26^{cd}	17.60 ± 2.08^{c}	19.07 ± 0.74^{b}	21.64 ± 1.15^{a}	<0.0001	0.33
Hue	35.93 ± 4.41^{b}	35.41 ± 1.88^{b}	43.31 ± 3.78^{a}	38.11 ± 3.75^{b}	46.02 ± 1.68^{a}	<0.0001	0.75

$^{a\text{-}d}$ Mean values in the same row not followed by a common letter differ significantly ($P < 0.05$).

Table 2. Effects of oleogelator type (SO and B) and fat replacement (20 and 40%) on textural characteristics of dry-cured 'Salchichón' sausage, elaborated with linseed oleoge

	Control	SO-20	SO-40	B-20	B-40	*P* value	SEM
Hardness	26.97 ± 3.40^{a}	28.12 ± 3.34^{a}	25.88 ± 2.85^{a}	16.48 ± 1.71^{b}	5.85 ± 0.39^{c}	<0.0001	1.27
Springiness	0.47 ± 0.06^{b}	0.52 ± 0.03^{ab}	0.50 ± 0.08^{ab}	0.53 ± 0.02^{a}	0.55 ± 0.01^{a}	0.034	0.007
Cohesiveness	0.38 ± 0.01^{b}	0.36 ± 0.01^{b}	0.38 ± 0.03^{b}	0.36 ± 0.007^{b}	0.42 ± 0.002^{a}	<0.0001	0.004
Gumminess	10.50 ± 1.31^{a}	10.49 ± 0.85^{a}	10.03 ± 1.04^{a}	5.97 ± 0.58^{b}	2.47 ± 0.05^{c}	<0.0001	0.49
Chewiness	5.03 ± 1.01^{a}	5.35 ± 0.71^{a}	4.54 ± 0.73^{b}	3.09 ± 0.43^{c}	1.35 ± 0.08^{d}	<0.0001	0.23

$^{a\text{-}d}$ Mean values in the same row not followed by a common number differ significantly ($P < 0.05$).

(20% and 40%). A negative correlation was also found between L* and pH as well as between L* and moisture ($r = -0.875$ and $r = -0.853$ respectively; $P < 0.01$). These findings indicated that dry-cured sausages became darker due to an increase in the final pH. Thus, the replacement of pork backfat with linseed oil gelificated with beeswax strongly increased luminosity. Similar results were reported in other studies in which the replacement of pork backfat by olive oil in fermented sausages provided lighter colored sausages.[13,47–49] However, Stajić *et al.*[50] reported that a higher level of animal fat substitution with pretreated linseed oil did not influence L* values. The redness (a*) values were unaffected by SO oleogelators; however, the beeswax produced an increase in redness, resulting in an average value of 14.98. Finally, as expected, yellowness (b*) was strongly affected ($P < 0.0001$) by replacement of pork fat with linseed oil due to the yellow color of the linseed oil and beeswax, with increments of 25.75% and 62.45% for SO-40 and B-40, respectively, with respect to the control batch. These findings are consistent with another study, which reported that a fat replacement of 10–20% by olive oil resulted in a lighter color and yellowness in fermented sausages.[43] Stajić *et al.*[50] also noticed that animal-fat reduction led to a significant ($P < 0.05$) increase in b* values in all modified frankfurters. These changes to the color in the fermented sausages may influence consumer acceptability, resulting in a SO oleogelator more similar to control sausages in terms of final color.

Textural parameters of dry-cured sausages formulated with oleogels

Table 2 shows the effects of linseed olegels composed of γ-oryzanol and β-sitosterol (SO) and beeswax (B) on texture profile analysis of dry-cured sausages. The effect of beeswax oleogel was stronger than γ-oryzanol and β-sitosterol, resulting in significant differences in nearly all textural parameters (hardness, cohesiveness, gumminess, and chewiness). The textural attributes are affected by the drying process, the fermentation process, and the type of oleogel,[51] which can increase firmness or reduce it as in the present study. However, differences in hardness and chewiness may be due to differences in water content among batches. In agreement with other studies about fermented sausages produced in Spain,[52] Italy,[53] and Turkey,[54] a significant and negative correlation between pH and water content was found ($r = -0.908$, $P < 0.01$). On the other hand, significant ($P < 0.01$) moderate correlations between fat content and textural properties (hardness $r = -0.389$, chewiness $r = -0.429$ and cohesiveness $r = 0.397$) were found. These results agree with other studies which reported that low-fat sausages showed the highest values in hardness and chewiness.[55,56] Dry-cured sausages containing beeswax oleogelator (B-20 and B-40) were significantly lessl hard than control sausages, and those elaborated with a mixture of sterols.

There were strong correlations between moisture content and hardness, gumminess, and chewiness ($r = -0.885, -0.871, -0.773$, respectively; $P < 0.01$). These findings are consistent with those of Lorenzo *et al.*[52] for dry-cured 'chorizo' sausage. Thus, water content and pH play a vital role in textural parameters of dry-cured sausages with oleogels SO and B in linseed oil.

Fatty acid profile of dry-cured sausages formulated with oleogels

The FA composition of dry-cured sausages made with different oleogels (SO and B) in linseed oil using two levels of replacement (20% and 40%) is shown in Table 3. The partial replacement of pork backfat with oleogels had a significant effect on fatty acids with the exception of linoleic acid. As expected, the type of oleogelator and the level of replacement led to significant differences in the lipid profile of dry-cured sausages.

In general, the most abundant fatty acids were monounsaturated fatty acids (MUFAs), followed by saturated fatty acids (SFAs), and polyunsaturated fatty acids (PUFAs), with the exception of B-40 in which PUFA was the majority fraction. Oleic acid was the most predominant FA (32.01–39.84%) followed by palmitic acid (16.32–23.06%) with the exception of B-40 and SO-40 batches, because linolenic acid (19.09–19.94%) and linoleic acid (16.63–17.84%) were more abundant than palmitic acid. The main FAs detected were oleic, palmitic, linoleic, stearic, and linolenic acids, listed in descending order, in agreement with other studies of fermented sausages.[48,57] The amount of linseed oil replacing the pork backfat reduced the SFA content from 35.88 g/100 g obtained in control sausages to 31.85 and 28.78 g/100 g for SO and B batches, respectively. The greatest reductions were achieved for SO-40 and B-40 with SFA reductions of 21.75% and 28.65%, respectively. Other authors have reported important reductions in SFA content when replacing pork backfat with olive oil,[48,58] and linseed oil[50,59] in fermented sausages. The most abundant SFAs were palmitic and stearic acids in all batches, and a marked decrease in palmitic and stearic content was achieved in the highest level of replacement. This outcome is appealing for developing healthy meat products to reduce SFA in the human diet, to avoid increments in LDL-cholesterol and consequently atherogenic and hypercholesterolemic effects.[60] As consequence of replacement, the PUFA fraction and n-3 PUFA content were clearly higher in sausages with linseed oil due to the higher amount of α-linolenic acid. For this reason, PUFA/SFA and n-6/n-3 ratios were increased and reduced, respectively. Specifically, control sausages had the lowest PUFA/SFA ratio (0.64) and the higher n-6/n-3 (10.33); meanwhile a 40% replacement of pork backfat by linseed oleogel modified these ratios significantly ($P < 0.05$) to 1.34 and 1.78, for PUFA/SFA ratio in SO-40 and B-40 treatments, respectively and

Table 3. Effects of oleogelator type (SO and B) and fat replacement (20 and 40%) on the fatty acid profile of dry-cured ‘Salchichón’ sausage, elaborated with linseed oleogel

	Control	SO-20	SO-40	B-20	B-40	*P* value	SEM
C14:0	1.32 ± 0.13^{a}	1.30 ± 0.18^{a}	0.94 ± 0.09^{c}	1.14 ± 0.06^{b}	0.87 ± 0.22^{c}	<0.0001	0.03
C16:0	23.06 ± 1.91^{a}	22.99 ± 2.64^{a}	17.28 ± 1.33^{c}	20.47 ± 0.85^{b}	16.32 ± 3.67^{c}	<0.0001	0.51
C16:1n7	2.46 ± 0.25^{a}	2.41 ± 0.34^{ab}	1.76 ± 0.17^{c}	2.17 ± 0.12^{b}	1.63 ± 0.42^{c}	<0.0001	0.06
C17:0	0.42 ± 0.04^{a}	0.41 ± 0.04^{a}	0.31 ± 0.01^{c}	0.36 ± 0.01^{b}	0.27 ± 0.05^{d}	<0.0001	0.01
C17:1n7	0.32 ± 0.03^{a}	0.32 ± 0.04^{a}	0.24 ± 0.02^{b}	0.29 ± 0.01^{a}	0.22 ± 0.05^{b}	<0.0001	0.007
C18:0	10.68 ± 0.72^{a}	10.55 ± 0.77^{a}	8.67 ± 0.34^{c}	9.65 ± 0.24^{b}	7.79 ± 1.12^{d}	<0.0001	0.19
C18:1n7t	0.26 ± 0.02^{a}	0.27 ± 0.02^{a}	0.25 ± 0.02^{ab}	0.23 ± 0.01^{b}	0.19 ± 0.03^{c}	<0.0001	0.005
C18:1n9c	$39.84 \pm \pm 2.41^{a}$	39.84 ± 3.81^{a}	34.25 ± 2.09^{bc}	37.36 ± 1.39^{ab}	32.01 ± 2.92^{c}	<0.0001	0.50
C18:1n7c	2.89 ± 0.22^{a}	2.86 ± 0.31^{a}	2.31 ± 0.15^{b}	2.68 ± 0.10^{a}	2.17 ± 0.47^{b}	<0.0001	0.05
C18:2n6c	17.01 ± 0.82	17.58 ± 1.80	17.84 ± 1.12	16.79 ± 0.82	16.63 ± 2.06	0.874	0.30
C20:0	0.12 ± 0.005^{b}	0.12 ± 0.007^{b}	0.13 ± 0.004^{a}	0.14 ± 0.008^{a}	0.13 ± 0.009^{a}	0.004	0.001
C18:3n3	4.22 ± 2.55^{a}	6.64 ± 2.73^{a}	19.09 ± 1.55^{a}	10.19 ± 0.88^{b}	19.94 ± 2.06^{a}	<0.0001	0.77
C20:2n6	0.65 ± 0.07^{a}	0.63 ± 0.07^{a}	0.46 ± 0.03^{c}	0.57 ± 0.01^{b}	0.41 ± 0.08^{c}	<0.0001	0.01
C20:3n6	0.16 ± 0.01^{a}	0.16 ± 0.02^{a}	0.12 ± 0.008^{b}	0.14 ± 0.008^{a}	0.12 ± 0.02^{b}	<0.0001	0.003
C20:4n6	0.58 ± 0.05^{a}	0.56 ± 0.07^{a}	0.46 ± 0.02^{b}	0.54 ± 0.03^{a}	0.48 ± 0.11^{b}	0.0001	0.11
C22:5n3	0.13 ± 0.01^{a}	0.13 ± 0.01^{a}	0.10 ± 0.006^{b}	0.12 ± 0.004^{a}	0.10 ± 0.02^{b}	<0.0001	0.002
SFA	35.88 ± 2.83^{a}	35.64 ± 3.65^{a}	28.07 ± 1.83^{c}	31.97 ± 1.13^{b}	25.60 ± 3.10^{c}	<0.0001	0.64
MUFA	46.09 ± 2.96^{a}	46.39 ± 3.51^{a}	39.62 ± 2.48^{bc}	43.03 ± 1.75^{ab}	36.46 ± 4.05^{c}	<0.0001	0.75
PUFA	22.88 ± 3.89^{c}	26.08 ± 4.44^{bc}	37.79 ± 2.48^{a}	28.55 ± 1.51^{b}	38.27 ± 5.45^{a}	<0.0001	0.99
n-3	4.35 ± 3.70^{c}	6.93 ± 3.72^{bc}	19.21 ± 1.58^{a}	10.15 ± 0.83^{b}	20.04 ± 4.08^{a}	<0.0001	0.79
n-6	18.42 ± 0.93	19.03 ± 1.93	18.48 ± 1.18	18.11 ± 0.99	18.13 ± 2.39	0.906	0.22
n-6/n-3	10.33 ± 3.65^{a}	6.12 ± 2.63^{b}	0.96 ± 0.06^{c}	1.75 ± 0.06^{c}	0.90 ± 0.03^{c}	<0.0001	0.55
PUFA/SFA	0.64 ± 0.18^{d}	0.74 ± 0.18^{d}	1.34 ± 0.08^{b}	0.89 ± 0.02^{c}	1.48 ± 0.07^{a}	<0.0001	0.05
AI	0.41 ± 0.04^{a}	0.39 ± 0.04^{a}	0.28 ± 0.01^{c}	0.35 ± 0.004^{b}	0.26 ± 0.004^{c}	<0.0001	0.009
TI	0.83 ± 0.24^{a}	0.70 ± 0.23^{a}	0.31 ± 0.02^{c}	0.50 ± 0.01^{b}	0.28 ± 0.01^{c}	<0.0001	0.59

$^{a\text{-}d}$ Mean values in the same row not followed by a common number differ significantly ($P < 0.05$).

0.96 and 0.90, for n-6/n-3 ration in SO-40 and B-40 treatments, respectively.

The improvement of these two ratios is important for developing healthy meat products because an excessive amount of n-6 PUFA, resulting in higher n-6/n-3 ratios, contributes to several types of pathogenesis, such as cardiovascular diseases.[61] Hence, the average n-6/n-3 ratio of 0.93 in the two batches with higher replacement (SO-40 and B-40), instead of 10.33 presented in the control samples suggest a nutritional benefit, according to European Food Safety Authority recommendations.[60] This finding is in agreement with other studies which reported that the inclusion of linseed oil,[50,59] olive oil[58] or fish oil[62] decreased the n-6/n-3 ratio in different sausages.

Sensory attributes of dry-cured sausages formulated with oleogels

Sensory analysis indicated that there were significant differences in overall acceptability ($P < 0.05$), influenced by oleogelator type and replacement level of pork backfat. Figure 2 shows the overall acceptability results for the batches that were analyzed. This depends on many attributes and their interactions. Panelists detected significant differences ($P < 0.05$) regarding the dry-cured sausages, with overall acceptability scores of 1.86 ± 0.16, 3.07 ± 0.21, 4.71 ± 0.34, 3.21 ± 0.22 and 4.57 ± 0.29 for CO, SO-20, SO-40, B-20 and B-40, respectively. Although the control batch showed more acceptability ($P < 0.05$) than the 20% substitution batches, both formulations scored in the positive part of the hedonic scale used in this study, obtaining a positive acceptance. However, SO-40 and B-40 scored in the negative part of the hedonic scale. Regarding oleogelator type (SO and B), the panelists did not find significant differences in overall acceptability, although both were scored less for acceptability ($P < 0.05$) than the control. This finding is in agreement with data reported by Stajić *et al.*[50] who found that that all modified frankfurters had similar grades to control groups in terms of color, taste, odor, and overall appearance.

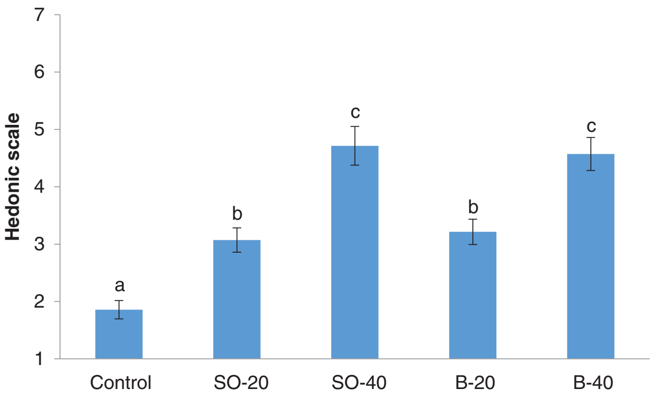

Figure 2. Means and standard errors of the mean (SEM) of the acceptability scores (rated on a seven-point category hedonic scale) of dry-cured sausages elaborated with sterols (γ-oryzanol and β-sitosterol, SO) and beeswax (B) at two levels of fat replacement (20% and 40%).

CONCLUSIONS

Edible oleogels based on mixtures of γ-oryzanol and β-sitosterol and beeswax in linseed oil can replace pork backfat in fermented sausages. One of the issues that emerges from our findings is that the drying process is modified by the incorporation of oleogelator and the level of replacement, affecting textural and sensorial attributes.

Regarding fatty acids, the improvement of PUFA/SFA and n-6/n-3 ratios in sausages with oleogels is particularly important for developing healthy meat products. However, sensorial parameters also change substantially resulting in a clear distinction among products. These healthier dry-cured sausages need to be enhanced with respect to textural parameters to meet consumer demands.

ACKNOWLEDGEMENTS

Artur Martins is recipient of a fellowship supported by doctoral advanced training (project NORTE-69-2015-2015) funded by the European Social Fund under the scope of Norte2020 – Programa Operacional Regional do Norte. Jose M. Lorenzo is member of the HealthyMeat network, funded by CYTED (ref. 119RT0568). Thanks to GAIN (Axencia Galega de Innovación) for supporting this research (grant number IN607A2019/01).

REFERENCES

1 Lorenzo JM, Michinel M, López M and Carballo J, Biochemical characteristics of two Spanish traditional dry-cured sausage varieties: androlla and botillo. *J Food Compos Anal* **13**:809–817 (2000).

2 Gómez M and Lorenzo JM, Effect of fat level on physicochemical, volatile compounds and sensory characteristics of dry-ripened "chorizo" from Celta pig breed. *Meat Sci* **95**:658–666 (2013).

3 Fonseca S, Gómez M, Domínguez R and Lorenzo JM, Physicochemical and sensory properties of Celta dry-ripened "salchichón" as affected by fat content. *Grasas Aceites* **66**:e059 (2015).

4 Mendoza E, García ML, Casas C and Selgas MD, Inulin as fat substitute in low fat, dry fermented sausages. *Meat Sci* **57**:387–393 (2001).

5 Campagnol PCB, dos Santos BA, Wagner R, Terra NN and Rodrigues Pollonio MA, Amorphous cellulose gel as a fat substitute in fermented sausages. *Meat Sci* **90**:36–42 (2012).

6 Ruiz-Capillas C, Triki M, Herrero AM, Rodriguez-Salas L and Jiménez-Colmenero F, Konjac gel as pork backfat replacer in dry fermented sausages: processing and quality characteristics. *Meat Sci* **92**:144–150 (2012).

7 Lorenzo JM, Munekata PES, Pateiro M, Campagnol PCB and Domínguez R, Healthy Spanish salchichón enriched with encapsulated $n-3$ long chain fatty acids in konjac glucomannan matrix. *Food Res Int* **89**:289–295 (2016).

8 Fernández-Diez A, Caro I, Castro A, Salvá BK, Ramos DD and Mateo J, Partial fat replacement by boiled quinoa on the quality characteristics of a dry-cured sausage. *J Food Sci* **81**:C1891–C1898 (2016).

9 Munekata PES, Domínguez R, Franco D, Bermúdez R, Trindade MA and Lorenzo JM, Effect of natural antioxidants in Spanish salchichón elaborated with encapsulated n-3 long chain fatty acids in konjac glucomannan matrix. *Meat Sci* **124**:54–60 (2017).

10 Marchetti L, Andrés SC and Califano AN, Low-fat meat sausages with fish oil: optimization of milk proteins and carrageenan contents using response surface methodology. *Meat Sci* **96**:1297–1303 (2014).

11 Heck RT, Vendruscolo RG, de Araújo Etchepare M, Cichoski AJ, de Menezes CR, Barin JS *et al.*, Is it possible to produce a low-fat burger with a healthy $n-6/n-3$ PUFA ratio without affecting the technological and sensory properties? *Meat Sci* **130**:16–25 (2017).

12 Leite A, Rodrigues S, Pereira E, Paulos K, Oliveira AF, Lorenzo JM *et al.*, Physicochemical properties, fatty acid profile and sensory characteristics of sheep and goat meat sausages manufactured with different pork fat levels. *Meat Sci* **105**:114–120 (2015).

13 Domínguez R, Pateiro M, Agregán R and Lorenzo JM, Effect of the partial replacement of pork backfat by microencapsulated fish oil or mixed fish and olive oil on the quality of frankfurter type sausage. *J Food Sci Technol* **54**:26–37 (2017).

14 de Carvalho FAL, Lorenzo JM, Pateiro M, Bermúdez R, Purriños L and Trindade MA, Effect of guarana (*Paullinia cupana*) seed and Pitanga (*Eugenia uniflora* L.) leaf extracts on lamb burgers with fat replacement by chia oil emulsion during shelf life storage at 2 °C. *Food Res Int* **125**:108554 (2019).

15 Heck RT, Saldaña E, Lorenzo JM, Correa LP, Fagundes MB, Cichoski AJ *et al.*, Hydrogelled emulsion from chia and linseed oils: a promising strategy to produce low-fat burgers with a healthier lipid profile. *Meat Sci* **156**:174–182 (2019).

16 da Silva SL, Amaral JT, Ribeiro M, Sebastião EE, Vargas C, de Lima Franzen F *et al.*, Fat replacement by oleogel rich in oleic acid and its impact on the technological, nutritional, oxidative, and sensory properties of Bologna-type sausages. *Meat Sci* **149**:141–148 (2019).

17 Franco D, Martins AJ, López-Pedrouso M, Purriños L, Cerqueira MA, Vicente AA *et al.*, Strategy towards replacing pork backfat with a linseed oleogel in frankfurter sausages and its evaluation on physicochemical, nutritional, and sensory characteristics. *Foods* **8**:366 (2019).

18 Pehlivanoğlu H, Demirci M, Toker OS, Konar N, Karasu S and Sagdic O, Oleogels, a promising structured oil for decreasing saturated fatty acid concentrations: production and food-based applications. *Crit Rev Food Sci Nutr* **58**:1330–1341 (2018).

19 Barbut S, Wood J and Marangoni A, Potential use of organogels to replace animal fat in comminuted meat products. *Meat Sci* **122**:155–162 (2016).

20 Barbut S, Wood J and Marangoni A, Quality effects of using organogels in breakfast sausage. *Meat Sci* **122**:84–89 (2016).

21 Martins AJ, Vicente AA, Cunha RL and Cerqueira MA, Edible oleogels: an opportunity for fat replacement in foods. *Food Funct* **9**:758–773 (2018).

22 Agregán R, Barba FJ, Gavahian M, Franco D, Khaneghah AM, Carballo J *et al.*, *Fucus vesiculosus* extracts as natural antioxidants for improvement of physicochemical properties and shelf life of pork patties formulated with oleogels. *J Sci Food Agric* **99**:4561–4570 (2019).

23 Stortz TA, Zetzl AK, Barbut S, Cattaruzza A and Marangoni AG, Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technol* **24**:151–154 (2012).

24 Bot A and Agterof WGM, Structuring of edible oils by mixtures of γ-oryzanol with β-sitosterol or related phytosterols. *J Am Oil Chem Soc* **83**:513–521 (2006).

25 Panagiotopoulou E, Moschakis T and Katsanidis E, Sunflower oil organogels and organogel-in-water emulsions (part II): implementation in frankfurter sausages. *LWT Food Sci Technol* **73**:351–356 (2016).

26 Bot A and Flöter E, Chapter 2 - EdibleEdible oil oleogels based on self-assembled β-sitosterol+γ-oryzanol tubules, edible oleogels, in *Edible Oleogels*, Second edn. AOCS Press, London. pp. 31–63 (2018).

27 Dassanayake LSK, Kodali DR and Ueno S, Formation of oleogels based on edible lipid materials. *Curr Opin Colloid Interface Sci* **16**:432–439 (2011).

28 Patel AR and Dewettinck K, Edible oil structuring: an overview and recent updates. *Food Funct* **7**:20–29 (2016).

29 Sawalha H, Venema P, Bot A, Flöter E, Den AR and Van Der Linden E, The phase behavior of γ-oryzanol and β-sitosterol in edible oil. *J Am Oil Chem Soc* **92**:1651–1659 (2015).

30 Gómez-Estaca J, Herrero AM, Herranz B, Álvarez MD, Jiménez-Colmenero F and Cofrades S, Characterization of ethyl cellulose and beeswax oleogels and their suitability as fat replacers in healthier lipid pâtés development. *Food Hydrocoll* **87**:960–969 (2019).

31 Moghtadaei M, Soltanizadeh N and Goli SAH, Production of sesame oil oleogels based on beeswax and application as partial substitutes of animal fat in beef burger. *Food Res Int* **108**:368–377 (2018).

32 Martins AJ, Cerqueira MA, Fasolin LH, Cunha RL and Vicente AA, Beeswax organogels: influence of gelator concentration and oil type in the gelation process. *Food Res Int* **84**:170–179 (2016).

33 Martins AJ, Cerqueira MA, Pastrana LM, Cunha RL and Vicente AA, Sterol-based oleogels' characterization envisioning food applications. *J Sci Food Agric* **99**:3318–3325 (2019).

34 CIE Recommendations on Uniform Color Spaces, Color-difference equations, and metric color terms. *Color Res Appl* **2**:5–6 (1977).

35 International Organization for Standardization, ISO 1442:1997 - Determination of moisture content.

36 International Organization for Standardization, ISO 937:1978 - Determination of nitrogen content.

37 International Organization for Standardization, ISO 936:1998 - Determination of ash content.

38 AOCS Official Procedure Am 5–04, Rapid determination of oil / fat utilizing high temperature solvent extraction, *Rapid Determ Oil/Fat Util high Temp solvent Extr* (2005).

39 Domínguez R, Crecente S, Borrajo P, Agregán R and Lorenzo JM, Effect of slaughter age on foal carcass traits and meat quality. *Animal* **9**:1713–1720 (2015).

40 International Organization for Standardization, ISO 8589:2007 - Sensory analysis-General guidance for the design of test rooms.

41 Macfie HJ, Bratchell N, Greenhoff K and LV V, Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *J Sens Stud* **4**:129–148 (1989).

42 Meilgaard M, Vance Civille G and Thomas Carr B, in *Sensory Evaluation Techniques*, ed. by Third SET. California, USA (2010).

43 Bloukas JG, Paneras ED and Fournitzis GC, Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Sci* **45**:133–144 (1997).

44 Flores M, Understanding the implications of current health trends on the aroma of wet and dry cured meat products. *Meat Sci* **144**:53–61 (2018).

45 Heck RT, Fagundes MB, Cichoski AJ, de Menezes CR, Barin JS, Lorenzo JM *et al.*, Volatile compounds and sensory profile of burgers with 50% fat replacement by microparticles of chia oil enriched with rosemary. *Meat Sci* **148**:164–170 (2019).

46 Lorenzo JM, Montes R, Purriños L and Franco D, Effect of pork fat addition on the volatile compounds of foal dry-cured sausage. *Meat Sci* **91**:506–512 (2012).

47 Muguerza E, Ansorena D, Bloukas JG and Astiasarán I, Effect of fat level and partial replacement of pork backfat with olive oil on the lipid oxidation and volatile compounds of Greek dry fermented sausages. *J Food Sci* **68**:1531–1536 (2003).

48 Beriain MJ, Gómez I, Petri E, Insausti K and Sarriés MV, The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. *Meat Sci* **88**:189–197 (2011).

49 Domínguez R, Agregán R, Gonçalves A and Lorenzo JM, Effect of fat replacement by olive oil on the physico-chemical properties, fatty acids, cholesterol and tocopherol content of pâté. *Grasas Aceites* **67**:e133 (2016).

50 Stajić S, Stanišić N, Tomasevic I, Djekic I, Ivanović N and Živković D, Use of linseed oil in improving the quality of chicken frankfurters. *J Food Process Preserv* **42**:e13529 (2018).

51 Oh I, Lee JH, Lee HG and Lee S, Feasibility of hydroxypropyl methylcellulose oleogel as an animal fat replacer for meat patties. *Food Res Int* **122**:566–572 (2019).

52 Lorenzo JM, González-Rodríguez RM, Sánchez M, Amado IR and Franco D, Effects of natural (grape seed and chestnut extract) and synthetic antioxidants (buthylatedhydroxytoluene, BHT) on the physical, chemical, microbiological and sensory characteristics of dry cured sausage "chorizo". *Food Res Int* **54**:611–620 (2013).

53 Casaburi A, Aristoy MC, Cavella S, Di Monaco R, Ercolini D, Toldrá F *et al.*, Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (southern Italy) as affected by the use of starter cultures. *Meat Sci* **76**:295–307 (2007).

54 Bozkurt H and Bayram M, Colour and textural attributes of sucuk during ripening. *Meat Sci* **73**:344–350 (2006).

55 Liaros NG, Katsanidis E and Bloukas JG, Effect of the ripening time under vacuum and packaging film permeability on processing and quality characteristics of low-fat fermented sausages. *Meat Sci* **83**:589–598. Elsevier {BV} (2009).

56 Salazar P, García ML and Selgas MD, Short-chain fructooligosaccharides as potential functional ingredient in dry fermented sausages with different fat levels. *Int J Food Sci Technol* **44**:1100–1107 (2009).

57 Muguerza E, Ansorena D and Astiasarán I, Functional dry fermented sausages manufactured with high levels of n-3 fatty acids: nutritional benefits and evaluation of oxidation. *J Sci Food Agric* **84**:1061–1068 (2004).

58 Utrilla MC, García Ruiz A and Soriano A, Effect of partial replacement of pork meat with an olive oil organogel on the physicochemical and sensory quality of dry-ripened venison sausages. *Meat Sci* **97**:575–582 (2014).

59 Valencia I, Ansorena D and Astiasarán I, Stability of linseed oil and antioxidants containing dry fermented sausages: a study of the lipid fraction during different storage conditions. *Meat Sci* **73**:269–277 (2006).

60 Scientific, Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J* **8**:1461 (2010).

61 Williams CD, Whitley BM, Hoyo C, Grant DJ, Iraggi JD, Newman KA *et al.*, A high ratio of dietary n-6/n-3 polyunsaturated fatty acids is associated with increased risk of prostate cancer. *Nutr Res* **31**:1–8 (2011).

62 Valencia I, O'Grady MN, Ansorena D, Astiasarán I and Kerry JP, Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. *Meat Sci* **80**:1046–1054 (2008).