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Fat Reduction and Replacement in Dry-Cured Fermented Sausage by Using High Pressure Processing Meat as Fat Replacer and Olive Oil

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The present paper describes the modification of the lipid fraction of dry-cured fermented sausage through fat reduction (35%) and fat replacement of animal fat with olive oil (up to 10%). High pressure processing (HPP) treated meat was employed as a novel fat replacer to reduce the fat content and as a new strategy to enable a stable incorporation of olive oil in dry-cured fermented sausages. Chemical (proximate composition and fatty acid profile), physical (water retention, structure formation and colour) and sensorial (appearance, texture and flavour) properties were evaluated. It is concluded that 35% of fat reduction is possible without reduction of consumer acceptability. Moreover, the addition of HPP-treated meat as a fat replacer resulted in good mimic of the fat particles together with good physical and sensory properties. Therefore, it resulted in an effective and clean alternative (no added-additives) for fat reduction. However, the incorporation of olive oil either by direct addition (4.3% oil) or within a HPP-created protein network (10% oil) resulted in unacceptable products since the oil was not properly retained inside the sausage matrix. Further studies are needed to find processing strategies that permit a stable incorporation of liquid plant oils to dry-cured fermented sausage for the development of healthier and more sustainable dry-cured fermented meat products.

INTRODUCTION

High fat consumption in western industrialized countries is linked to high blood pressure and other cardiovascular diseases. Cardiovascular disease (CVD) is the main cause of death in the EU [Allender *et al.*, 2008, 2012]. Cancer is the second leading cause of death [WHO, 2012]. Both diseases, CDV and cancer, are related to excess fat intake and imbalanced lipid profile of the diet among other factors. Therefore, the reduction of fat intake and the consumption of foods with healthy lipid profile is a very important determinant to improve eating habits and correct the imbalances [Allender *et al.*, 2008, 2012].

Different approaches such as reducing fat content in meats during production, fat removal from meat during processing and replacement with non-fat components can be applied to reduce the fat content in meat products [Jimenez-Colmenero, 2000]. As well as different strategies to improve the lipid profiles by incorporating plant oils such as direct addition of liquid oil, pre-emulsion of oil, encapsulation of oil and solid fats can be applied to different meat products (fresh, cooked, fermented) [Jimenez-Colmenero, 2007]. In this sense, olive oil has been associated with a decrease in overall and cardiovascular mortality [Trichopoulou *et al.*, 2003], what is more, cumulative evidence suggests that olive oil may have a profound influence on health specially on cardiovascular risk factors [Huang & Sumpio, 2008; Covas *et al.*, 2009]. In meat products fat contributes to flavour, texture, mouth feel, juiciness and overall sensory appreciation of the product. Therefore, any fat reduction or replacement by incorporation of plant oils can affect the acceptability of the products [Ventanas *et al.*, 2010; Olivares *et al.*, 2011].

Moreover, the production of fat-reduced and fat-replaced dry-cured fermented sausages presents some challenges regarding processing troubles such as proper drying, binding and retention of the incorporated liquid oils. The granulated fat in fermented sausages has important technological functions. It helps to loosen the sausage mixture and this aids the continuous release of moisture from the inner layers of the product; a process absolutely necessary for a proper fermentation and flavour/aroma development. The retention of added liquid oils is also a problem due to the relative high temperatures during the fermentation/drying process. For these reasons, dry fermented sausages are the most difficult meat products as far as fat reduction and replacement is concerned [Muguerza *et al.*, 2002; Wirth, 1988]. The use of novel technologies such as high pressure processing (HPP) can offer possibilities not attainable so far [Hugas *et al.*, 2002].

A possible solution for fat reduction in dry-cured fermented sausages is to use proper fat replacers. A method for producing reduced-fat foods such as meat products and sausage

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TABLE 1. Dry-cured fermented sausage formulations and experimental design.

Batches	Code	Ingredients				Total fat (%)	Fat reduction (%)
		Lean meat (%)	Back fat (%)	HPP meat (%)	Olive oil (%)		
Control	C	80.0	20.0	0.0	0.0	24.4	0
Fat reduction	F-RED	90.4	9.6	0.0	0.0	15.9	35
Fat reduction and replacement	F-RED-REP	90.9	4.8	0.0	4.3	15.8	35
Fat reduction with HPP meat	F-RED-HPP	80.0	9.6	10.4	0.0	15.8	35
Fat replacement with HPP meat and oil	F-REP-HPP	61.2	8.6	20.2	10.0	25.8	0

products, on the basis of a sausage meat made from ground meat, optionally a water addition, and the addition of salt, spices, auxiliary substances, and additives has been described [Kortschack *et al.*, 2014]. According to the invention, animal tissue, in particular lean meat, treated with high pressure and/or skin treated with high pressure is fed to the mixture cut to produce the sausage meat as an at least partial fat substitute, whereby the protein content of the mixture is increased and the structure of the product is appealing and not similar to reduced-fat products.

In addition, regarding fat replacement with plant oils, different studies have used gel-like structures containing oils trapped inside them in order to incorporate oils to meat products [Triki *et al.*, 2013]. It is possible to create tri-dimensional networks or gel structures by using the denaturation ability of HPP treatments. A new strategy based on a pre-emulsion of oil and meat further submitted to HPP to create a tri-dimensional network that retains the oil may be a suitable alternative for a clean (no added-additives) and stable incorporation of oils to dry-cured fermented sausages.

In the present study, new strategies based on HPP treatment are tested for fat-reduction and fat-replacement in dry-cured fermented sausages. A novel fat replacer, HPP-treated meat, is assayed to reduce the fat content (35%). A novel strategy based on retention of oil within a HPP-created structure is tested for the incorporation of olive oil (10%). Chemical (proximate composition and fatty acid profile), physical (water retention, structure formation and colour) and sensorial (appearance, texture and flavour) properties are evaluated in order to develop feasible processing alternatives for fat reduction and fat replacement with plant oils in dry-cured fermented sausages.

MATERIALS AND METHODS

Sausage formulation and processing

The dry-cured fermented sausages consisted of pork lean meat and back fat mixed with curing salt (2.6%) containing sodium nitrite (0.0095%), sodium ascorbate (0.05%), dextrose (3.0%), starter cultures (*Staphylococcus carnosus* and *Lactobacillus sakei*) and white fine pepper (0.2%) (Frutarom, Emmerich, Germany). The different formulations are shown in Table 1. The processing consisted of cutting, mixing, stuffing, fermentation and drying. The meat and fat was minced in a grinder (Mado Primus MEW 603, 1.45 P/kW, Wuppertal, Germany) and subsequently mixed with the curing salts, sugars and starter cultures. After that, the mixture was stuffed (Vemag DP3 Typ 138, Verden, Germany) in industrial permeable casings (cellulose type, 30 mm calibre, Viscofan, Spain). The stuffed sausages were placed in the maturation chamber for fermentation and drying. A standard fermentation/drying was applied (temperature ranged 26–18°C and humidity ranged 96–80%). The process was monitored by measuring pH and weight loss regularly (every 1–2 days). The entire process lasted 15 days after which samples were vacuum packaged and stored at 4°C. The pH value for all batches was 5.1±0.1. The dry-cured fermented sausages were assessed by chemical, physical and sensory analysis.

Experimental design

Dry-cured fermented sausages were formulated to improve the fat fraction by reducing the back fat and increasing the lean meat and by replacing the back fat with virgin olive oil (Table 1). A total of five recipes were elaborated: a control formulation (C), a fat reduced formulation (F-RED) with just less back fat and more lean trimmings, a fat reduced and fat replaced formulation (F-RED-REP) with less back fat, more lean trimmings and some direct addition of olive oil, and two formulations using high pressure processing (HPP): a fat-reduced sausage with HPP-treated meat as a fat replacer (F-RED-HPP) and a fat-replaced sausage with a HPP-created gel that enables the incorporation of olive oil (F-REP-HPP) (Table 1).

FIGURE 1. Picture of HPP meat as a novel fat replacer for meat products.

Virgin olive oil was obtained from local supermarket. HPP-meat was obtained by treating pork lean meat trimmings with HPP for 5 min at 600 MPa at room temperature. Subsequently, the HPP-treated meat was frozen overnight prior to cut it in a bowl cutter (Kilia Vacuum Bowl Cutter 5000 Express, Neumünster, Germany) in order to create the fat-alike particles (Figure 1), which were then incorporated to the meat batter and mixed prior to stuffing. More detailed information about the application can be found in the patent by Kortschack *et al.* [2014]. HPP-created gel with oil added was prepared by making a pre-emulsion, meat and oil (2:1), using a cutter (Kilia Vacuum Bowl Cutter 5000 Express, 7.5 P/kW, Neumünster, Germany) and pouring the olive oil gently. Subsequently, this emulsion was treated by HPP (5 min, 600 MPa, room temperature). The virgin olive oil and these novel ingredients, HPP-treated meat and HPP-treated oil in meat emulsion, were added at the mixing stage prior to the stuffing.

Chemical characterization

Chemical composition

Water content was analysed according to method ASU L06.00–1 and ashes according to method ASU L06.00–4. Salt (NaCl) was analysed with German official method ASU L07.00–5/1 (LFGB, 2011). Chloride according to method ASU L06.00–5. Fat content was determined according to German official method "Caviezel" (ASU L06.00–06 and DGF C-III 19) and protein content was determined with method ASU L06.00–7. Hydroxyproline was analysed with method ASU L06.00–8 and used for determination of connective tissue free lean meat content. Nitrates and nitrites were determined with method ASU L08.00–14. Water, ashes and salts amounts, and fat and protein contents are shown in Table 2 and 5, respectively. The following parameters were very similar among batches and ranged as follows: hydroxyproline 0.25±0.01 (g/100 g), connective tissue 2.01±0.11 (g/100), nitrates <10 (mg/kg) and nitrites <10 (mg/kg). The values are the means of duplicate measurements from two different samples exposed to the same treatment.

Lipid oxidation

The extent of lipid oxidation was measured by peroxide value and thiobarbituric acid reactive substances (TBARS). Peroxides were measured using a titrimetry procedure based on PV 320 method and expressed in mEq/kg fat. TBARs were measured using the method described by Jensen *et al*. [1995]. TBARs were expressed as mg malondialdehyde/kg meat. The values are the means of duplicate measurements from two different samples exposed to the same treatment.

Fatty acids analysis

Fatty acid profile is obtained by methylation of fatty acids followed by gas chromatography analysis. The method is according to DGF C-VI 11d [1986]. The values are the means of duplicate measurements from two different samples exposed to the same treatment.

Microbial growth

Aerobic mesophilic bacteria (AB) and enterobacteria (EB) were determined using standard procedures of plate count. After homogenization, samples for AB and EB were serially diluted, plated on solid media, lyngby-IA and violet red bile glucose agar (Oxoid Ltd. UK), and incubated at 30°C for 3 days and 37°C for 24 h, respectively, before counting the colony forming units (cfu/g).

Physical characterization

Texture

Rectangular blocks (2 cm height × 2 cm width) were cut out from the dry-cured fermented sausages. Cut resistance of the fermented sausage was determined with a texture analyser (type TA-XT2, Stable Micro Systems Ltd., UK). Measuring settings were: test speed (2 mm/s) and test distance (30.0 mm). Force resistance of the fermented sausage against the razor blade was recorded during lowering the blade. Maximum cutting force was registered in Newtons (N). Two samples per batch were measured ten times.

Water activity

The water activity (a_w) was determined in a water activity system (Rotronic Gmbh, Ettlingen, Germany) following manufacturer's instructions. Two samples were measured in duplicate per each batch.

Confocal laser scanning microscopy (CLSM)

Protein components were stained by fluorescein isothiocyanate (FITC) (Thermo Fisher Scientific Inc., IL, USA) (absorption 492 nm; emission 520 nm) resulting in green colour, and lipids were marked by Nile Red (Sigma Aldrich, Co., St Luis, MO, USA) (absorption 554 nm; emission 606 nm) resulting red colour in the micrographs. Two μL FITC solution were put on a microscope slide, then a fermented sausage slice (1 mm thick) was positioned on it and Nil Red was applied on the top of the samples. Diffusion time of fluorescing dyes was 12 h at 4°C. CLSM Nikon ECLIPSE E 600 (Nikon Corporation, Japan) was used for structure characterization of dry-cured fermented sausages.

Colour

Colour measurements of parameters (L*. a*, b* values) were carried out. Two samples were taken and measured ten times under same environmental conditions using the Minolta spectrophotometer CM 700d (Konica Minolta GmbH, Germany) and the standard light D65. The results were compared with the control using the index $\Delta E_{ab}{}^*$ which is calculated using Equation 1:

$$\Delta E_{ab}{}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* + a_1^*)^2 + (b_2^* + b_1^*)^2}$$

where, L^*_2 means brightness of sample and L^*_1 brightness of control, a^*_2 means redness of sample and a^*_1 redness of control and b^*_2 means yellowness of sample and b^*_1 yellowness of control.

The results of $\Delta E_{ab}{}^*$ are interpreted as follows: 0–1 means no colour difference between control and sample, 1–2 means that there is only a slight colour difference (trained eye),

FIGURE 2. Visual appearance of fat-reduced and fat-replaced dry-cured fermented sausages.

TABLE 2. Effect of fat reduction and replacement with olive oil on water retention and texture parameters of dry-cured fermented sausages (mean ± standard error) (n=2).

Batches#	Water (g/100 g)	Ashes (g/100 g)	Salt (g/100 g)	Drying loss (g/100 g)	Water activity (a_w)	Cutting force (N)
C	36.6±0.3^{a}	5.2±0.1^{a}	4.0±0.1^{a}	36.1±0.9^{b}	0.90±0.14^{a}	13.1±1.8^{b}
F-RED	38.8±0.4^{a}	6.2±0.1^{b}	4.5±0.1^{b}	45.6±0.2^{c}	0.89±0.14^{a}	16.7±0.7^{c}
F-RED-REP	42.4±0.1^{ab}	5.5±0.1^{a}	4.1±0.1^{a}	38.8±0.3^{b}	0.91±0.05^{ab}	8.5±0.2^{a}
F-RED-HPP	38.4±0.2^{a}	6.0±0.1^{ab}	4.4±0.1^{ab}	42.3±1.0^{c}	0.89±0.14^{a}	13.2±0.1^{b}
F-REP-HPP	44.1±1.0^{b}	5.2±0.1^{a}	4.1±0.1^{a}	27.8±0.6^{a}	0.92±0.07^{ab}	8.6±1.2^{a}

Different superscripts within the same column mean significant difference at *P-value* < 0.05. #: Batches are defined in Table 1.

2–4 stands for a detectable colour difference, 4–5 for an intensive colour difference and >5 for a very intensive colour difference.

Sensory analysis

The control (C) and the two dry-cured fermented sausages that had proper structure, F-RED and F-RED-HPP, were sensory evaluated. A Friedman test was carried out to determine which samples were more appreciated by consumer regarding different sensory attributes. The sensory trials were carried out in a room with cabinets with controlled illumination at room temperature. Slices of 5 mm thickness of dry-cured fermented sausages were offered to the panellists in small white plastic cups numbered with random numbers of three digits after 17 days of storage under refrigeration (4°C). The panellists were asked to evaluate the appearance, smell, taste and texture in order to rank the three samples (C, F-RED and F-RED-HPP) in a scale from 1= "Dislike" and 3 = "Like". The sensorial analysis was interpreted using the Friedman test [ISO 8587, 1988 (E)]. Basically, the sum of the scores for each of samples and for each attribute is subtracted from the sum of the scores of the sample with the lowest score. This difference was compared to values according to 5% and 1% of significance.

A second sensory test, a triangle test, was carried out to ascertain differences between the reference dry-cured fermented sausage (C) and the fat-reduced formulations (F-RED and F-RED-HPP). In a triangle test, two samples are the same and one is different. Different random numbers were given to all samples. Three triangle tests: 1) C to F-RED, 2) C to F-RED-HPP and 3) F-RED to F-RED-HPP were performed regarding differences in flavour and texture.

Data analysis

The results were analysed by a one-way analysis of variance (ANOVA) using the software (Past online version 2.17b, Hammer & Harper) in order to discriminate the significant differences.

RESULTS AND DISCUSSION

The dry-cured fermented sausages can be visualized in Figure 2. Generally speaking, the fat-reduced dry-cured fermented sausages, F-RED and F-RED-HPP, had proper structure and good appearance, whereas the fat-replaced (with added olive oil) dry-cured fermented sausages, F-RED-REP and F-REP-HPP, showed improper binding of the matrix, oil dripping and were unacceptable (Figure 2). In addition, F-RED and F-RED-HPP were slightly dryer than the control (C) due to the higher proportion of lean meat, which was dried during the processing (Table 2). In contrast, F-RED-REP and F-REP-HPP had higher moisture due to the fact that the added-oil impeded a normal water evaporation during the drying-ripening process. This effect is in agreement with Muguerza *et al.* [2002] and Yildiz-Turp & Serdaroglu [2008] who also observed higher moisture in dry fermented sausage manufactured with added oil. The dry-cured fermented sausages with HPP-treated meat added (F-RED-HPP) had similar appearance to the control (Figure 2) but 25% less fat content. The HPP-treated meat was a good fat replacer as it simulated adequately the appearance of the fat particles in the dry-cured fermented sausage slice (Figure 2).

The structure formation of dry-cured fermented sausages is determined by a complex association of dynamic processes such as mincing, mixing, salt content, stability of emulsion, acidification following fermentation and concomitant drying. All these stages impact to each other to obtain a proper product and thus it is important to discuss salts content, water evaporation and texture on one approach. Salts and water, drying loss, water activity (a_w) and cutting force are then shown in Table 2. F-RED and F-RED-HPP had

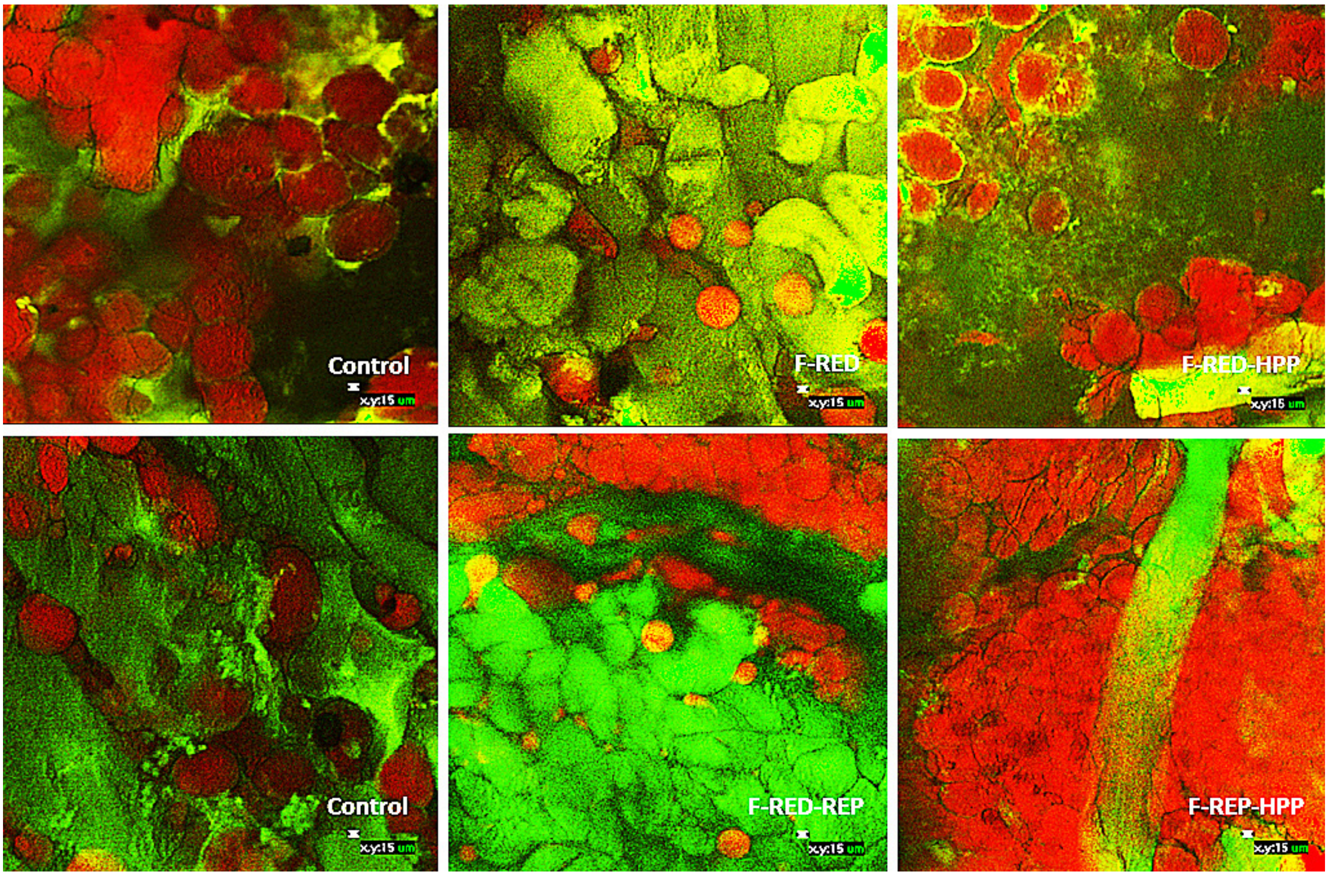

FIGURE 3. Confocal laser scanning microscopy (CLSM) images of fat-reduced and fat-replaced dry-cured fermented sausages.

the highest content of ashes and salts since they had more lean meat and consequently had also higher weight losses, due to the drying process, than dry-cured fermented sausages with added oil (F-RED-REP and F-REP-HPP). F-RED-REP and F-REP-HPP resulted in an unacceptable structure formation and also a limited water removal from the product leading to a product with higher moisture (Figure 2 and Table 2) [Yildiz-Turp & Serdaroglu, 2008]. These results were in agreement with the fewer weight losses, which are due to insufficient drying, and the highest values of a_w observed (Table 2). The samples containing added oil, F-RED-REP and F-REP-HPP, had slightly higher a_w values than the rest of batches (Table 2). Regarding the structure formation, which can be assessed by measuring the cutting force of the product, it can be observed that the oil-added dry-cured fermented sausages, F-RED-REP and F-REP-HPP, did not have a proper texture or structure [Yildiz-Turp & Serdaroglu, 2008]. They had values of cutting force of 8.5–8.6 N in comparison to 13.1 N for the control. In contrast, the sample F-RED was tougher (16.7 N) likely due to the higher amount of lean meat and consequently of weight loss during the drying process (Table 2). However, the F-RED-HPP had a similar texture to the control, 13.2 and 13.1 N, respectively. It seems that despite the higher amount of lean meat in this formulation, the HPP-treated meat added as fat replacer limited the water evaporation that resulted in a higher moisture and a better texture, less tough, and then more similar characteristic to the reference product (C), which is very important for the sensory acceptability of the product.

A closer look at the product structure is obtained by CLSM analysis. The CLSM snapshot can be observed in Figure 3, where proteins displayed green colour and lipids were red. Fermented sausage is a poly-dispersed system consisting of a continuous phase (water, soluble proteins, ions), a dispersed liquid phase (fat particles) and a dispersed solid phase (non-solvated muscle fibre particles, connective tissue, spices). The addition of oil produces agglomeration of oil phases which leads to an unstable emulsion and improper binding of the product (Figure 2 and 3).

The colour parameters, L*, a*, b* values are shown in Table 3. The L* value was lower in F-RED and F-RED-REP than in C, F-RED-HPP and F-RED-HPP samples. It is well-known that HPP treatment increases L* value through protein denaturation [Bajovic *et al.*, 2013]. There were no significant difference in relation to colour parameter a*, which is related to redness. While b* value, yellowness, was increased in F-REP-HPP, as this sample contained the higher amount of olive oil (10%). Overall, colour appearance is always a very determinant quality trait to consider in product development. The use of HPP can lead to an increase of L* value, which is a desired attribute as for the HPP-meat to be a good fat replacer and simulates the fat particles in the slice of the product. The incorporation of higher amount of lean meat can increase a* value, and the addition of oil can increase b* value. A balanced colour is important determinant of consumer acceptability of meat products such as Bologna type cooked sausage [Beiloune *et al.*, 2014]. The ΔE serves to evaluate the overall change in colour (Figure 4). ΔE is between 3–5 for

TABLE 3. Effect of fat reduction and replacement with olive oil on colour of dry-cured fermented sausages (mean ± standard error) (n=2).

Batches[#]	Colour		
	L*	a*	b*
C	52.9±1.1[c]	9.8±0.9[a]	5.51±0.72[ab]
F-RED	48.4±1.6[b]	9.0±0.7[a]	4.7±0.9[a]
F-RED-REP	45.2±2.4[a]	10.1±0.9[a]	5.6±0.6[ab]
F-RED-HPP	52.0±1.5[c]	11.1±1.1[a]	5.5±0.9[ab]
F-REP-HPP	54.6±1.8[c]	9.6±1.1[a]	7.1±0.7[b]

Different superscripts within the same column mean significant difference at *P-value* < 0.05. [#]: Batches are defined in Table 1.

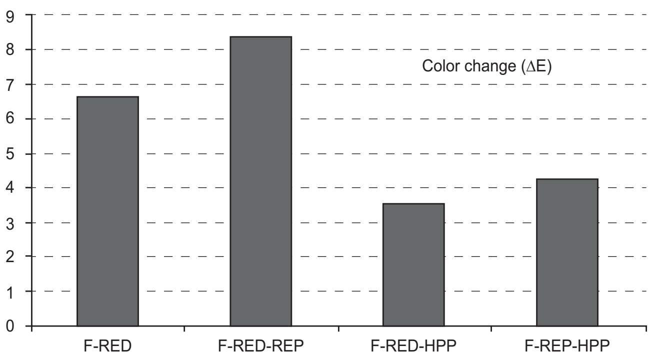

FIGURE 4. Effect of fat reduction and replacement with olive oil on overall colour change compared to control (ΔE) of dry-cured fermented sausages.

F-RED-HPP and F-REP-HPP and >5 for F-RED and F--RED-REP, which means an intense and very intense colour difference in relation to the control (Figure 3). This can also be observed in Figure 2. These colour changes observed are due to the different amount of lean meat (red) and fatty tissue (white) and the addition of olive oil (yellow).

The shelf life of a product can be evaluated by chemical and microbial deterioration. There were no differences among batches neither in the extent of lipid oxidation (*i.e.* peroxides and TBARs) nor in the microbial growth (*i.e.* aerobic mesophilic bacteria and enterobacteria) of dry-cured fermented sausages at day 15 of storage (Table 4). Therefore, these dry-cured fermented sausages are oxidative stable and microbially safe for human consumption.

In relation to protein and fat contents, those were according to ingredient percentages (*i.e.* lean meat and olive oil addition and back fat removal) in the formulations (Table 1 and 5). Fat reduced formulations, F-RED and F-RED-HPP, had the highest protein contents, while the fat replaced formulations with the highest amount of oil (10%), F-REP-HPP, had the lowest protein content and the highest fat content (Table 5). Energy (kcal/100 g) followed an order according to fat and protein content. Overall, it was achieved a 17–22% reduction of caloric content for the fat-reduced formulations (F-RED, F-RED-REP and F-RED-HPP), and 12.5% for the fat-replaced formulation (F-REP-HPP) (Table 5). It must be noted that the replaced formulations with added oil, F-RED-REP and F-REP-HPP, were technologically non-acceptable. Therefore technological alternatives must be developed to guarantee a proper incorporation of liquid oils in dry-cured fermented sausages.

Several nutrition organisations recommend on our diet not only a certain limitation of fat intake but also a balance between saturated and unsaturated fatty acids [FAO/WHO Expert Consultation, 2008]. According to our results, it is confirmed that the lipid nutritional index PUFA + MUFA/SFA is slightly improved by the addition of olive oil (Table 6). However the improvement is clearly insufficient to achieve the nutritional recommendations of PUFA/SFA≥0.50 and (PUFA+MUFA)/SFA≥2.00. It must be noted that fat consumption must be evaluated on an entire diet basis and not on individual foods. Nevertheless, a fat reduction of 35% is achieved without diminishing the quality and acceptability of the product. A fact which is very relevant to reduce the fat intake and allows the label of the product as a 'reduced-in' according to EU regulations [European Union 2006 and 2012]. In addition recently, it has been described that the healthy properties of fat are associated not only with amount of PUFA and MUFA, but to some extent might be related to the amount of stearic acid. Despite the fact, that it belongs to the SFA, it lowers the LDL cholesterol level, is neutral with respect to HDL cholesterol, and tends to lower the ratio of total to HDL cholesterol [Hunter *et al.*, 2010].

The sensory analysis is always a crucial step whether the strategies could be successfully transferred to industry. The Friedman test identified a trend regarding the order of preference for the samples: F-RED-HPP > F-RED > C, although the differences were not statistically significant.

TABLE 4. Effect of fat reduction and replacement with olive oil on shelf life measured as lipid oxidation and microbial growth of dry-cured fermented sausages at day 15 of storage (mean ± standard error) (n=2).

Batches[#]	Lipid oxidation		Microbial growth	
	Peroxide value (mEq/kg fat)	TBARs value (mg MDA/kg)	Aerobic mesophilic (cfu/g)	Enterobacteria (cfu/g)
C	<1[##]	0.06±0.00[a]	6.70 ± 0.42 x 10^7 [a]	<10[##]
F-RED	<1	0.08±0.01[a]	7.65 ± 0.78 x 10^7 [a]	<10
F-RED-REP	<1	0.07±0.00[a]	7.05 ± 0.49 x 10^7 [a]	<10
F-RED-HPP	<1	0.09±0.01[a]	6.75 ± 0.77 x 10^7 [a]	<10
F-REP-HPP	<1	0.07±0.00[a]	6.90 ± 0.14 x 10^7 [a]	<10

Different superscripts within the same column mean significant difference at *P-value* < 0.05. [#]: Batches are defined in Table 1. [##]: Detection limit.

TABLE 5. Effect of fat reduction and replacement with olive oil on protein, fat and energy content of dry-cured fermented sausages (mean ± standard error) (n=2).

Batches#	Fat	Protein	Energy		
			Fat	Protein	Total
	(g/100 g)		(kcal/100 g)		
C	29.6±0.5[b]	27.1±0.5[b]	266.2±4.5[b]	108.4±2.1[b]	374.6±6.6[b]
F-RED	19.5±0.6[a]	34.5±0.3[c]	175.5±5.4[a]	137.8±1.1[c]	313.3±6.5[a]
F-RED-REP	21.8±0.2[a]	29.3±0.4[b]	196.0±2.0[a]	117.3±1.7[b]	313.3±3.7[a]
F-RED-HPP	22.6±0.5[a]	32.3±0.5[c]	203.2±4.3[a]	129.1±1.8[c]	332.3±6.1[a]
F-REP-HPP	28.9±0.6[b]	22.3±1.2[a]	260.3±5.2[b]	89.3±4.7[a]	349.6±9.9[ab]

Different superscripts within the same column mean significant difference at *P-value* < 0.05; #: Batches are defined in Table 1.

TABLE 6. Effect of fat reduction and replacement with olive oil on fat unsaturation and lipid nutritional indexes of dry-cured fermented sausages (mean ± standard error) (n=2).

Batches#	Fat unsaturation##			Lipid nutritional indexes	
	SFA	MUFA	PUFA	[(PUFA+MUFA)/SFA]	[PUFA/SFA]
	(g/100 g)				
C	17.0±0.1[f]	9.2±0.1[b]	3.4±0.1[b]	0.74	0.20
F-RED	10.3±0.1[a]	6.5±0.1[a]	2.7±0.1[a]	0.90	0.26
F-RED-REP	11.2±0.1[b]	8.2±0.1[b]	2.3±0.1[a]	0.94	0.21
F-RED-HPP	12.9±0.2[c]	7.1±0.1[a]	2.6±0.1[a]	0.75	0.20
F-REP-HPP	14.3±0.2[d]	11.5±0.1[c]	3.2±0.1[b]	1.03	0.22
Recommendation	-###	-	-	≥ 2.00	≥ 0.50

#: Batches are defined in Table 1. ##: SFA; saturated fatty acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids. ###: Total fat intake must be 20–35 % of energy intake (E). SFA is recommended to be 10 % of E, PUFA 6–11 % of E and MUFA is calculated by difference [FAO/WHO Expert Consultation, 2008].

Only the attribute taste of the sample F-RED-HPP was statistically preferred in comparison to the control sample (C) (95% of significance). The triangle test revealed that panellists were not able to differentiate C, control, from F-RED--HPP, fat-reduced with HPP-meat added. However, the panellists were able to differentiate between C and F-RED (99% of significance), and between F-RED and F-RED-HPP (99% of significance) for both sensory parameters, texture and flavour. Overall, F-RED was tougher than C and F-RED-HPP, whereas F-RED-HPP cannot be distinguished from C neither in texture nor in flavour appreciation, which is very important to maximise consumer acceptability in relation to the reference product, C. The results indicated that it was possible to reduce the fat content (35%) without affecting the sensory appreciation (F-RED). The use of HPP-meat made the product even more appreciated (F-RED-HPP).

Fat reduction of meat products is possible without causing a major impact in terms of quality, acceptability and price [Jiménez Colmenero, 2000; Beiloune *et al.*, 2014]. Moreover, the use of plant oils instead of animal fat not only results in a healthier product but also a more sustainable product (*i.e.* by the lower carbon foot print involved in the use of oils from vegetable sources). Different authors have emphasized the inclusion of plant oils in the formulations of healthier meat products [Jiménez-Colmenero, 2007; Beiloune *et al.*, 2014]. In addition, these products will be accompanied with a reduction of meat consumption and contribute to the establishment of more efficient meat production systems for a sustainable world [Vinnari, 2008]. As the global meat demand increases it will be expected a higher use of protein and fat from vegetable origins. It is technologically easier to incorporate liquid oils in fresh and cooked meat products than in dry-cured fermented products. Nevertheless, different strategies for the stable incorporation of liquid oils to dry-cured fermented sausages such as pre-emulsion with proteins, formation of tridimensional networks and encapsulation has been proposed [Jimenez-Colmenero 2007; Yildiz-Turp & Serdaroglu, 2008; Herrero *et al.*, 2011]. In the future, it is forecasted that more technological alternatives will have to be developed in order to enable the addition of liquid plant oils to dry-cured fermented sausages while ensuring a proper structure formation, oil retention, water removal and after all sensory acceptability.

CONCLUSIONS

It is concluded that a fat reduction of 35 % in dry-cured fermented sausages is possible without reduction of product acceptability by consumer. The addition of HPP-treated meat as fat replacer resulted in proper structure, visual aspect, fat reduction and sensory acceptability. Therefore, it is a feasible and clean label alternative to deliver fat reduction in dry-cured fermented sausages. However, incorporation of olive oil resulted in an unacceptable product because of olive oil was not retained within the sausage matrix. Further investigations are needed to find processing strategies that permit a stable incorporation of liquid vegetable oils to dry-cured fermented sausage for the development of healthier and more sustainable meat products.

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