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Substituting fat with soy in low-salt dry fermented sausages

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ABSTRACT

This paper investigates whether the partial substitution of fat with soy flakes and the partial substitution of common salt with two commercial blends (Aranca salt™ and Novosal salt™) affected the final dry matter, proximate composition, peroxide index, malondialdehyde values and lactic acid bacteria counts of dry fermented sausages. Six experimental batches were manufactured. Both salt type and fat source affected to the fat amount, the protein content, the collagen content, the peroxide index and the microbial counts and significant interactions between the main effects were found for all those variables. The malondialdehyde values were affected by fat source but not for salt type. In general, soy addition decreased protein content and peroxide values, increased lactic acid bacteria counts and malondialdehyde values and their effect on fat content depended on the used salt.

1. Introduction

In meat products, sensory and microbiological quality depends on the formulation, including salt and fat. Salt is an essential ingredient in cured meat products, such as dry sausages (Spanish chorizo), because it guarantees an adequate texture and flavour and because it controls the growth of pathogenic microorganisms [1]. Excessive consumption of salt is detrimental to human health, but the reduction of salt in dry sausages can be a major problem for the industry because it can cause a loss of quality (shelf life, texture, flavour and consumer acceptability) in addition to causing technological and food security problems [2]. Fats are a fundamental component of the diet, as they provide energy, are part of cell membranes and are the vehicle of fat-soluble vitamins and some hormones. In food, fats provide unctuosity and juiciness and contribute to the texture. However, many people have a negative perception of fats associated with the increase in the number of obese people with an above-average occurrence of diabetes, heart disease and hypertension. Within the framework of this dilemma, the Spanish Ministry of Health and the large food industries signed recently an agreement to gradually reduce the fat, salt and sugar content of food and beverages, in an strategy named as NAOS (http://www.aesan.gob.es/AECOSAN/web/nutricion/seccion/estrategia_naos.htm). Therefore, it is necessary to find tactics that allow the industry to adapt to this new scenario. One possibility to reduce the salt content is to partially replace NaCl with other compounds [3]. A reduction in saturated fat content can be achieved by substituting some of the animal fat with nuts, protein ingredients or carbohydrates [4,5]. Soy protein has been widely used in the meat industry since the 70th of the 20th century [6,7]. Soy has a great amount of protein of good quality, being only deficit in methionine. In addition, its antiatherogenic effects are well described, since it reduces LDL-cholesterol and triglyceride levels and has a high content of oleic and linoleic acid [8,9]. Isolated soy protein has been widely used as a binding agent or as an emulsifier in a matrix to encapsulate oils or other compounds in different meat products [10–12] but there is scarce literature regarding the use of soy protein in dry fermented sausages. Therefore, the aim of the paper was to investigate whether the partial substitution of leg fat with soy and the partial substitution of common salt by other products affected the physicochemical properties of dry fermented sausages.

2. Materials and methods

2.1. Sausage manufacture and sampling

The dry fermented sausages were manufactured in the facilities of Elaborados las Torres (Teruel, Spain). A control batch and 5 experimental batches were manufactured. The control batch and two of the experimental batches were manufactured using lean pork leg (78%) and

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Table 1
The proximate composition (g/100 g) of the lean pork leg, leg fat and soy flakes used in the formulation of the sausages.

	Fat	Saturated fat	Protein	Carbohydrates	Sugars	Fiber	Salt	Water
Lean pork leg	19.0	9.7	19.0	0	0	0	0	62.0
Leg fat	47.1	16.7	12.1	0	0	0	0	40.8
Soy flakes*	9.7	1.6	48.1	30	7.5	16.2	0.008	

* according to product label.

leg fat (20%), named the LEG batches. In the other three experimental batches, lean pork leg (78%), leg fat (13%) and soy flakes (Biogran, S.L., Spain) (1.3%) previously hydrated with tap water (5.3%) were used, and these batches were named SOY. The proximate composition of leg meat and leg fat was analyzed in triplicate with a FoodScan™ NIR instrument (FOSS, Hillerød, Denmark) [13]. The proximate composition of the soy flakes was indicated in the product label. The proximate composition of used lean leg, leg fat and soy flakes is shown in Table 1.

Meat was minced and trimmed with a mincer (Tecmaq Alimat 130/2, Barcelona, Spain) fitted with a grille with 6 mm diameter vents. For each combination, 3 subsets were obtained: SALT (2% NaCl), ARANCA (2% Aranca salt™) or NOVOSAL (2% Novosal salt™). Aranca salt™ is a blend of KCl, $C_6FeK_4N_6$, and $Na_4Fe(CN)_6$, whereas Novosal salt™ is composed of 50% NaCl and 50% KCl, $C_6H_5K_3O_7$, $MgSO_4$, $MgCO_3$ and $CaCO_3$. Thereafter, for each subset, 500 g of typical commercial additives (garlic powder and paprika) was added and mixed for 3 min with a blender (Fuerpla, Valencia, Spain).

All batches were kept at 2 °C for 24 h, stuffed (REX RVF 327REX Technologie GmbH & Co KG, Thalgau, Austria) into 34–36 mm diameter natural pig casings (Vicente Gallent, Valencia, Spain), and then immersed in a water bath containing a suspension of *Penicillium candida* (Lallemand Inc., Montreal, Canada). A sample of five sausages per batch was collected at this point to measure the initial dry matter and other five to measure the initial lactic acid bacteria counts of the different batches. Thereafter, the other sausages (around 20 pieces for each batch) were weighed and hung to dry at 75% relative humidity and 3 °C, without heat or forced air for approximately two months, until the weight losses were of 30%. Weekly, five sausages from each batch were weighed and checked for optimal drying. Once the sausages reached the specific final weight loss (30%), all sausages from each batch were weighed, cut into 2 portions, vacuum packed (MCOEX material, Coimbra Pack, S.L., Spain) and randomly assigned to the different analyses. The following analyses were performed: proximate composition (dry matter, protein, fat, reducing sugar and collagen), peroxide index, thiobarbituric acid reactive substances (TBARS) and lactic acid bacteria counts.

2.2. Analytical procedures

To calculate the dry matter samples were dried in an oven (Memmert UFP 600, Schwabach, Germany) at 105 °C until constant weight and calculated as the percentage of initial sample weight [14].

Fat, protein and reducing sugars contents was analyzed according to Spanish Official Methods [15]. Protein content was determined by Kjeldahl method. A factor of 6.25 was used to convert total nitrogen in protein content. Fat quantification was made by Soxhlet extraction. Sugar content was determined by Luff-Schoorl procedure. Collagen and hydroxyproline contents were assessed following the method by Bonnet and Kopp [16] with a coefficient of 8 to convert hydroxyproline in collagen. Proximal composition data are expressed as g/100 g of dry matter. The peroxide value was measured following Aguirrezábal, et al. [17]. The results were expressed as meq of oxygen/kg of sample. Thiobarbituric acid reactive substances (TBARS) was used according to the method of Ripoll, et al. [18]. TBARS values are expressed as milligrams of MDA per kilogram of muscle.

For microbiological analysis, an internal procedure based on ISO

Table 2
The means, standard deviations and *p* values of the main effects (salt type and fat source) for weight losses and initial and final dry matter percentages (expressed ad percentages by weight), for the fat, protein, reducing sugar and collagen contents (expressed as g/100 g of dry matter) and for the peroxide index and TBARS values in the dry fermented sausages produced with different salt type (common salt, Aranca® and Novosal®) and fat source (fat leg or partial replacement by soy).

	SALT		ARANCA		NOVOSAL		P values		
NaCl percentage into the mix*	2%		0%		50%				
	Leg	Soy	Leg	Soy	Leg	Soy	Salt type (S)	Fat source (F)	SxF
Weight losses (% weight) ($n = 20$/batch)	29.8 ± 1.71	30.3 ± 1.86	28.7 ± 1.65	28.9 ± 1.30	29.6 ± 2.90	31.3 ± 2.30	0.754	0.687	0.168
Initial dry matter (% weight) ($n = 5$/batch)	40.3 ± 0.07	44.9 ± 2.47	45.3 ± 0.78	44.4 ± 2.19	43.9 ± 0.92	43.0 ± 0.35	0.164	0.306	0.056
Final dry matter (% weight) ($n = 5$/batch)	62.6 ± 1.06	68.6 ± 0.84	63.5 ± 0.85	79.9 ± 5.45	69.5 ± 0.56	74.2 ± 15.77	0.394	0.062	0.460
Fat (g/100 g of dry matter) ($n = 5$)	45.9 ± 1.35 c	50.6 ± 0.68 b	54.0 ± 1.39 a	45.6 ± 1.48 c	50.8 ± 0.77 b	43.8 ± 0.89 d	0.050	0.002	<0.001
Protein (g/100 g of dry matter) ($n = 5$)	46.0 ± 0.98 a	41.3 ± 1.69 b	40.9 ± 0.75 b	32.1 ± 1.52 d	38.8 ± 0.74 c	38.6 ± 0.62 c	<0.001	<0.001	0.012
Reducing sugar (g/100 g of dry matter) (n = 5)	0.7 ± 0.20	0.8 ± 0.40	0.3 ± 0.23	0.5 ± 0.09	0.8 ± 0.19	0.6 ± 0.34	0.154	0.872	0.546
Collagen (g/100 g of dry matter) (n = 5)	2.7 ± 0.16 a	2.7 ± 0.40 a	2.8 ± 0.23 a	2.7 ± 0.26 a	2.7 ± 0.18 a	2.3 ± 0.40 b	0.003	0.001	0.001
Collagen/protein ratio	5.8 ± 0.39 cd	6.6 ± 0.84 bc	6.8 ± 0.55 b	8.5 ± 0.96 a	6.9 ± 0.58 b	5.0 ± 0.40 d	0.001	0.587	<0.001
Peroxides (meq O_2/kg)	3.27 ± 1.36 a	0.23 ± 0.11 b	0.34 ± 0.20 b	0.35 ± 0.14 b	3.44 ± 1.22 a	0.17 ± 0.09 b	0.010	<0.001	0.011
TBARS (mg/kg)	0.39 ± 0.024 bc	0.52 ± 0.060 a	0.37 ± 0.028 c	0.49 ± 0.023 a	0.40 ± 0.051 bc	0.45 ± 0.034 ab	0.419	<0.001	0.190

a,b.- different letters indicates differences between batches ($p < 0.05$).
* In all batches, 2% *w*/w was added. Following producers' labels, Aranca has not NaCl and Novosal has 50% of NaCl.

15214:1998 [19] was used. A 10 g sample of sausage was aseptically weighted in a sterile plastic bag. Subsequently samples were homogenized with 90 ml of a sterile solution of 0.1% (*w/v*) peptone water (Oxoid, Unipath, Basingtoke, UK), for 2 min at 20–25 °C in a Masticator blender (IULInstruments, Barcelona, Spain), thus making a 1/10 dilution. Serial 10-fold dilutions were prepared by mixing 1 ml of the previous dilution with 9 ml of 0.1% (*w/v*) sterile peptone water. For lactic acid bacteria enumeration, 100 ml samples of similar 10-fold dilutions were plated on Man, Rogosa and Sharpe (MRS) agar. After incubation at 35 °C for 3 days or 30 °C for 5 days in an aerobic atmosphere supplemented with 5% carbon dioxide, the cfu/g of sample was determined, and the results are expressed as log cfu/g.

2.3. Statistical analysis

For the statistical analysis of the results, XLStat 17.03 software was used. Normal distribution of data was checked by a Kolmogorov-Smirnov test and then, a multivariate analysis (GLM) was performed with salt type and fat source as the main effects. The least squares mean (LSM) was separated using Duncan's *t*-test with a $p < 0.05$ significance level.

3. Results and discussion

The mean values for weight loss and dry matter are shown in Table 2. The end of drying was considered 30% depletion. The current dry matter results are in accordance with those described by most authors [20–23]. The mean initial dry matter was 43.1% for batches manufactured with fat from the leg (from now, LEG) and 44.1% for batches manufactured with soy (from now, SOY). At the end of ripening, LEG batches presented a mean dry matter of 65.2%, whereas SOY batches had a mean value of 74.2%. Nevertheless, no differences in initial or in final dry matter were observed between batches, indicating that all the batches dried properly [11].

The mean values for the fat, protein, reducing sugar and collagen contents of the sausages are shown in Table 2. The current results for fat content were higher than the 25–35% reported by most authors [24–26] but in accordance with the 55% reported by Beriain, et al. [27] in several commercial brands of Spanish dry sausages. The current percentage of protein was slightly higher than that reported by other authors in similar products, which ranged from 18 to 40% [21,28,29]. An interaction between the salt type and the fat source was found for the fat content. Then, SALT batches increased the fat content when soy was added, while the opposite occurred with ARANCA and NOVOSAL batches. The protein content was affected by both the type of salt and the fat source with a marked interaction between effects. In all batches, the protein content decreased by the addition of soy, except in NOVOSAL, in which no changes were observed. The increase on fat content in soy-based products have been reported by some authors [30] whereas other authors stated that the soy addition in meat products not always result in differences in fat content [12,31]. Soy protein act as fat encapsulating agent, forming a layer around the fat drops and protecting them. This stabilization effect on the emulsion is related to more hydrophilic-lipophilic groups in the molecule, that increase the protein-lipid and protein-water interactions. Then, the observed and unexpected increase in fat content on SALT-SOY sausages could be due to these protein-lipid interactions [12,32]. Regarding protein, it has been reported that the addition of soy protein to a meat emulsion would increase salt-soluble proteins, as a result of the interaction between soy protein and actomyosin. Lee, Kim, Hwang, Kim, Kim, Kim and Cho [12] found that the increase of protein content in soy-based products depended on the addition percentages, being this increase lower when soy was added at 2% level than when it was added at <2% level. Soy protein are especially sensitive to environmental conditions and tend to unfold and aggregate depending on pH or ionic strength, for example. Then, the microstructure of the new formed protein-network in the emulsion affect to the attributes of soy-based products [31]. Therefore, the effect of the percentage of addition as well the soy protein solubility and protein-other compounds bindings must to be taken into account and investigated in further studies.

There were no differences in reducing sugar content between batches. In general, the reducing sugar content was very low and at the limit of detection of the technique. Results are not surprising since meat has a low amount of carbohydrates and only soy supplied carbohydrates in our formulations.

Finally, regarding collagen content, the effect of substitution was noticeable only in the SOY batches, so NOVOSAL-SOY presented lower collagen content than the others, among which there were no differences. The collagen content results agree with the 1.5–2% reported by other authors [29]. From a practical point of view, these differences could be not really important, especially considering the very poor nutritional value of collagen, since it is deficient in some of the nutritionally essential amino acids, such as isoleucine, phenylalanine / tyrosine, and sulfur amino acids [33].

Spanish regulation [34] stated that the final composition of this kind of sausage should have less than 57% fat, less than 2% reducing sugars, more than 30% protein, a collagen/protein relationship lower than 16% and less than 1% added proteins. All our sausages did fulfil these requirements.

We have not analyzed in this experiment other components (mainly, fiber and carbohydrates other than sugar, such as starches, for example) that could be interesting from a nutritional point of view but were not mandatory from legal requirements. Similarly, the Spanish legislation does not require a maximum or minimum salt content and therefore, in the present experiment, salt content has not been measured. Nevertheless, ARANCA salt does not have any NaCl whereas that Novosal salt is composed of 50% NaCl and considering that the ARANCA batches presented lower fat contents than NOVOSAL batches, the salt ARANCA would seem preferable.

Also thinking about the nutritional value of the new formulations, an estimation of calories content could be indirectly and approximately calculated from the proximal composition data [35]. Then, SALT-LEG have approximately 382 Kcal/100 g whereas SALT-SOY have approximately 435 Kcal/100 g, since as commented, SOY addition resulted in higher fat amounts. On the opposite, ARANCA-LEG have 435 Kcal/100 g whereas ARANCA-SOY have 420 Kcal/100 g and NOVOSAL-LEG have 435 Kcal/100 g whereas NOVOSAL-SOY have 416 Kcal/100 g. That is, the SOY addition can allow to reduce the fat content depending on the used SALT but the reduction in energy content was not always noticeable, since SOY is also an energetic food.

The mean values for the oxidation indexes of the sausages are shown in Table 2. In our study, the peroxide index was affected by the interactions between salt type and fat addition. Then, in the LEG batches, ARANCA presented lower values than SALT and NOVOSAL. Nevertheless, in the SOY batches there was not found differences between batches. The peroxide values of the SALT-LEG and NOVOSAL-SOY batches were similar to those reported by other authors, whereas the others are much lower than expected [11,20]. The peroxide index has been proposed as a measure of the stage of the lipolysis phase since peroxides are the primary components of the oxidation process, but they disappear over time, resulting in other compounds [11]. Some studies pointed out that NaCl could promote the oxidation of meat products due to a disruption of the integrity of the cells, favouring the contact of the oxidizer with the substrates as well as due to a release of iron who acts as a catalyser [36,37]. This could partially explain why the peroxide values of SALT were higher than those of ARANCA in the LEG batches. A peroxide level of 4.0 meqO_2/kg has been identified as the upper limit for acceptable quality in dry sausages [38], and all our formulae were below that threshold.

In the TBARS test (Table 2), soy addition resulted in higher values, independently of the salt type used. The TBARS method measures the malondialdehyde (MDA) concentration, and considering that MDA is the

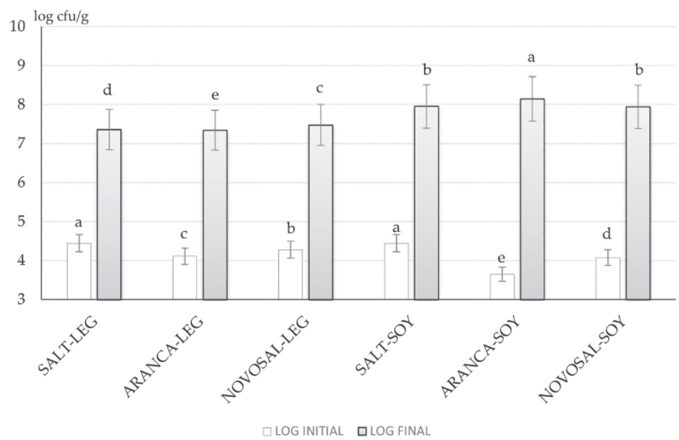

Fig. 1. The means for the lactic acid bacteria count at the beginning ($n = 5$/batch) and at the end ($n = 5$/batch) of the ripening process (log cfu/g) in the dry fermented sausages elaborated with different salt type (common salt, Aranca® and Novosal®) and fat source (fat leg or partial replacement by soy). Bars represents mean standard deviation.
a,b.- differences between batches ($p < 0.05$).

major degradation product of lipid peroxides [39], it could be hypothesized that soy addition promotes the degradation of lipids. Additionally, it should be considered that fatty acids of soy are mainly unsaturated fatty acids, which may enhance this degradation in SOY batches. This is in accordance with other authors who reported that the addition of soy protein isolate in emulsion gels resulted in higher total MUFA and PUFA percentages [40]. Finally, it has been reported [30] that the addition of soy protein to pork sausages influences emulsion stability because soy proteins form an electrically charged layer around fat droplets and promote protein-lipid interactions. Further studies are necessary to elucidate whether these changes in emulsion properties could affect lipids prone to degradation. Regarding the effect of salt type on TBARS values, the current results showed that no differences were found in response to the NaCl proportion on the salt blend (SALT, ARANCA or NOVOSAL). Current TBARS values were similar to those reported by other authors, which were approximately 0.45 mg/kg [5,41].

Fig. 1 shows the change in lactic acid bacteria count during the ripening process. In all the batches, the count increased over time, as expected. Both salt type and fat source affected the lactic acid bacteria counts, with SOY batches presenting higher values than the LEG batches. An interaction was found between main effects, then, at the end of the ripening, ARANCA-LEG batch presented the lowest counts whereas ARANCA-SOY presented the highest ones. Current counts agreed with those reported by most authors [20,42,43]. It has been reported that when NaCl is reduced, the LAB count increases [26] in disagreement with current results. On the other hand, it was reported that the addition of soy protein isolate reduced the Aw [30], which in turn could be resulted in lower bacteria growths. Then, further studies are necessary to elucidate whether the spatial distribution and the way in which water is linked on the emulsion could underlie the increased growth of bacteria in SOY batches compared with LEG batches.

From current experiment, we can conclude that soy addition induced an increase in the fat content of SALT bacthes, while the opposite occurred with ARANCA and NOVOSAL. In general, soy addition resulted in lower protein content, reducing the nutritional quality of the sausages. Soy addition caused higher lactic acid bacteria counts, which could lead to sensory problems. The soy addition resulted in lower peroxide values, maintaining values less than 4.0 meqO_2/kg, which is considered an undesirable threshold. Nevertheless, TBARS values increased when soy was added. Further studies are necessary to elucidate the effect of these substitution strategies on the physicochemical properties of sausages, especially on microbial counts and lipid degradation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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