Effect of the use of entire male fat in the production of reduced salt fermented sausages

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Abstract

The effect of the use of entire male fat and salt reduction in dry fermented sausages were evaluated. Four different sausage formulations were manufactured with back fat from gilt or entire male and two different salt contents. The physicochemical parameters, sensory characteristics, texture, lipid composition, volatile compounds and boar taint compounds were analysed. The use of entire male fat produced the highest weight losses producing high hardness and chewiness while salt reduction produced a decrease in hardness. Entire male sausages had the lowest oxidation values due to the low content of C18:2n6 while salt reduction did not affect the oxidation process. Boar taint odour was due to the presence of androstenone and skatole but entire male fat sausages had different generation of volatile compounds. The presence of androstenone was perceived by consumers as abnormal odours but also other sausage characteristics such as texture (high hardness) and oxidation were detected due to the different chemical composition of entire males versus gilts.

Keywords: fermented sausages; salt reduction; boar taint; volatile compounds; aroma.

1. Introduction

Castration of male piglets is a common practise used to reduce boar taint among others effects such as the increase in fat proportion of carcasses and reduction of aggressive and sexual behaviour (Babol & Squires, 1995). However, current animal welfare and the European Declaration to stop surgical castration by the end of 2018 (European Declaration on Alternatives to Surgical Castration of Pigs, 2010) will raise the use of uncastrated males. In addition, EU regulation (regulation EC No 854/2004, 2004) indicates that meat must be declared unfit for human consumption if it presents organoleptic anomalies, in particular a pronounced sexual odour. Therefore, meat industry needs to know about consumers' perception of boar taint since the use of boar meat may have economic impact.

Boar taint is especially perceived when boar meat is cooked and it is due to the presence of two substances, androstenone and skatole (Babol & Squires, 1995). Due to their hydrophobicity, both compounds are accumulated in the adipose tissue evoking urine and faecal, bitter or manure odours. Although, other compounds have also been reported to contribute to boar taint odour at a lesser extent such as androtenols (Brennan, Shand, Fenton, Nichols, & Aherne, 1986), indole (García-Regueiro & Diaz, 1989), 4-phenyl-3-buten-2-one (Rius Sole & García-Regueiro, 2001), phenolic compounds (Patterson, 1967) or aldehydes and short chain fatty acids (Rius, Hortós, & García-Regueiro, 2005).

Boar taint is related to sexual maturity as androstenone (5 α -androst-16-ene-3-one) is a steroid synthesized in boar testes while skatole (3-methyl indole) is a metabolite of the amino acid L-tryptophan produced in the large intestine of pigs (Zamaratskaia, Babol, Andersson, & Lundström, 2004). Skatole concentration depends on the availability of tryptophan and composition and activity of intestinal bacteria. Therefore, skatole concentration can be reduced through the addition in the diet of certain carbohydrates (inulin, raw potato starch, etc), enzymes (Øverland, Kjos, Fauske, Teige, & Sørum, 2011) or sorbent material (Jen & Squires, 2011). However, androstenone concentration cannot be easily reduced; the only way known so far is through genetic selection or manipulation; unless immunocastration is carried out. A vaccine called ImprovacTM is actually available but, lower drip loss and darker meat than entire male have been reported meaning a lower propensity to develop pale, soft and exudative meat (Lundström, Matthews, & Haugen, 2009). Nevertheless, consumer perception of boar taint odour should be taken into account, as 99% of consumers are sensitive to skatole (Weiler, Fischer, Kemmer, Dobrowolski, & Claus, 1997) whereas a high number of consumers are anosmic to androstenone. Generally, a higher

proportion of women are more sensitive than men as androstenone perception is genetically regulated (Lundström et al , 2009).

Several consumer studies evaluated the acceptability of meat from entire male and related to androstenone and skatole levels. Usually these studies were performed in raw or cooked meat being boar taint easily detectable during hot consumption (Babol & Squires, 1995). Regarding meat products, the loss of aroma and taste due to boar taint was more noticeable in cooking than drying and curing processes (Bañón, Costa, Gil, & Garrido, 2003a). In contrast, few studies deal with dry fermented sausages. Stolzenbach, Lindahl, Lundström, Chen, & Byrne (2009) studied the effect of boar fat, starter culture and smoke on the sensory quality of Swedish fermented sausages, concluding that the smoking process could mask the odour perception of boar taint as others authors previously reported (Lunde, Egelandsdal, Choinski, Mielnik, Flåtten, & Kubberød, 2008). Smoking practise in dry fermented sausages is common in northern Europe while less common in Mediterranean area where the products are mainly cured and ripened for longer time (Flores, 1997). In a recent study, Meier-Dinkel, Sharifi, Frieden, Tholen, Fischer, Wicke, & Mörlein (2013) determined the effect of label information on overall liking in non-smoke fermented sausages made with pork or boar meat. These authors concluded that label information did not affect the hedonic scores but the sausages made with lower androstenone levels were liked significantly better. In summary, cold consumption, added ingredients or the long ripening time that occurs during manufacturing of dry fermented sausages may be the reasons to partially mask boar taint. But the actual trend to reduce salt content in meat products (Commission European, 2008) affects the generation of volatile compounds and consequently flavour perception in dry fermented sausages (Corral, Salvador, & Flores, 2013). However, nothing is known about the effect of the use of entire male fat in dry fermented sausages and the changes in odour perception due to salt reduction. Furthermore, the purpose of this study was to evaluate the dry fermented sausages quality manufactured with entire male fat and reduced salt content.

2. Material and methods

2.1. Dry fermented sausages preparation and sampling

Three replicates of the experiment were carried out. For each replicate, four formulations (6 kg/formulation and a total of 24 kg/replicate) of slow fermented sausages were manufactured with back fat from gilt or entire male and different salt contents. The pork's ham lean and fat (bellies boneless and skinless) from twelve different animals per sex were purchased from a local producer (Incarlopsa, Spain) and

delivered to IATA-CSIC for processing. Four different fermented sausage formulations were processed at the IATA-CSIC: control formulation with back fat from gilt (C), formulation with entire male back fat (MC) and both formulations with 27 g/kg salt content. Then the same two formulations, gilt back fat (RS) and entire male back fat (MRS), were processed but reduced in 25 % salt content using KCl as substitute.

For each of three replications, the ground meat, the lean pork and the pork backfat were ground through a 10 mm diameter mincing plate and vacuum minced with the following additives (g/kg): sodium chloride (27), lactose (20); dextrin (20); glucose (7); sodium ascorbate (0.5); sodium nitrite (0.15); potassium nitrate (0.15). Pork back fat from entire male and gilt were previously chopped and mixed separately due to variations in androstenone and skatole contents. Also, a commercial starter culture (0.1) SP318 TEXEL SA-301 was added (Danisco, Cultor, Madrid, Spain) containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus* and *Staphylococcus carnosus*. The meat mixture was maintained at 3-5°C for 24h and then, was stuffed into 95 mm diameter collagen casings (Fibran, S.A., Girona, Spain). The final weight of each sausage was 700 g. The sausages were dried for 60 days at 10°C and 70-85 % relative humidity (RH). Temperature and RH of the ripening chambers were continuously recorded. In order to control the ripening process, two sausages from each replicate were weighed almost every day to control weight losses. Also, one sausage from each formulation was used to control the pH by introducing a pH meter HI 99163 (Hanna Instruments Inc., Hoonsocket, USA) into the centre of the sausage as described by ISO 2917 (1999).

Within each of the 12 batches (3 x 2 x 2) produced approximately 8 sausages were obtained in each batch. Two sausages from each batch were randomly chosen at day 0 and the end of ripening (60 d). In each sausage, colour analyses were done and then, 150 g of the sausage were minced and used for moisture, water activity (A_w) and pH analysis. The remaining minced sausage was vacuum packed and frozen at -20°C for subsequent physicochemical analyses (TBARS, lipid, protein, ion content and fatty acids analyses). From sausages obtained at 60 d, several slices (1 cm thickness) were taken wrapped in aluminium foil, vacuum packaged and stored at -80 °C for volatile analyses. Finally, from each formulation, 2 sausages from each replicate (2 sausages x 3 replicates) were vacuum packed, stored at 4°C and used for texture and sensory tests in less than 3-4 days.

2.2. Physicochemical analysis

The measurement of pH, a_w , weight losses, colour (CIELab L^* , a^* and b^*), moisture, protein and fat was carried out as described by Corral et al. (2013). The ion content was determined by ion chromatography (Corral et al., 2013).

Texture profile analysis (TPA) was performed using TA-Xt.plus Texture Analyzer with Texture Exponent software (version 2.0.7.0 Stable Microsystems, Godalming, UK). At the end of the process, two slices per sausage (3.5 cm diameter and 1.5 cm thick) were compressed twice to 50% of their original height as described by Olivares, Navarro, Salvador & Flores (2010). TPA curves were obtained and the following parameters calculated: hardness, adhesiveness, springiness, cohesiveness and chewiness.

2.3. Lipid profile, lipolysis and lipid autooxidation analysis

Lipid profile was determined by means of total fatty acids which were methylated as described by Berry, Wade, & Cevallos (1965). Fatty acids methyl esters (FAME) were analysed in an Agilent HP 7890B gas chromatograph (GC) equipped with a flame ionisation detector (FID) as described by Corral, Salvador, Belloch, & Flores (2015). For quantification, response factors of the standards respect to an internal standard (C21:0) were calculated using the standard fatty acid methyl ester solution (FAME mix, Sigma-Aldrich, Germany). The results were expressed as percentage of total fatty acids identified.

Lipolysis was tested by analysis of free fatty acids (FFA) released throughout ripening process. FFAs were methylated as described by Olivares, Navarro, & Flores, (2011) and analysed in a GC-FID (Corral et al., 2015). The results were expressed as mg of fatty acid/100 mg of dry fermented sausage in dry matter.

Lipid autooxidation was measured by the thiobarbituric acid reactive substances (TBARS) method according to Corral et al. (2013). The results were expressed as mg malonaldehyde (MDA)/kg in dry matter.

2.4. Volatile compounds analysis

2.4.1. Profile and quantification of total volatile compounds

The analysis of volatile compounds in the headspace (HS) of dry fermented sausage was performed by SPME-GC-MS as described Corral et al. (2015). Five grams of the minced sausage was weighted into a 20 ml HS vial sealed with a PTFE faced silicone septum and 0.75 mg of BHT was added. The vial was equilibrated at 37°C for 30min and then, SPME fibre (CAR/PDMS) was exposed to the HS during 2h at 37°C. The volatile compounds were desorbed in port injection of GC/MS (HP 7890A/5975C) (Hewlett Packard, Palo Alto, CA) for 5min at 240°C (in splitless mode)

and equipped with a Gerstel MPS2 multipurpose sampler (Gerstel, Germany). The volatile compounds were separated using a DB-624 capillary column (J&W Scientific, Agilent Technologies, USA) and identified by comparison with mass spectra from the library database (Nist'05), Kovats retention index (Kovats, 1965) and by comparison with authentic standards. The identified volatile compounds were quantified in SCAN mode using either total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale. The results were expressed as abundance units (AU) 10^{-6} .

2.4.2. Analysis of boar taint compounds

The boar taint compounds analysed were androstenone, skatole, indole and androstenol (Sigma-Aldrich, Germany) according to Meier-Dinkel et al. (2013) with slight modifications. Twenty five grams of sausage sample were minced and heated at 100°C for 20 min in an oven to obtain the liquid fat. Then, 7-ethylindole (0.52 ng) and androstanol (800 ng) (Sigma-Aldrich, Germany) as internal standards were added to 75 mg of the separated liquid fat and mixed in a vortex for 30 s. Subsequently, the fat was extracted with 1 ml methanol, mixed in a vortex and stored at 60°C for 30 min. Then, the methanolic supernatant was separated by centrifugation (12000 rpm for 10 min at -5°C). This supernatant was transferred into a headspace vial and evaporated to dryness by a gentle stream of nitrogen. Finally, the vial was sealed and analysed by HS-SPME-GC/MS using DVB/PDMS fiber (Supelco, Bellefonte, PA, USA). The vial was equilibrated at 100°C for 5 min and then, the SPME fibre was exposed to the HS during 30 min at 100°C. The volatile compounds were desorbed in the injection port of GC/MS (GC Agilent 7890, 5975C MS) for 20 min at 270°C (in splitless mode). The volatile compounds were separated using a HP-5 capillary column (30m, 250µm i.d., and 0.25µm film thickness) (J&W Scientific, Agilent Technologies, USA). The GC oven temperature program began at 50°C and ramped at 6°C/min to 140°C, and held at this temperature for 5 min, then the temperature was ramped at 5°C/min to 240°C and held for 5 min with a total run time of 45 min. The identified volatile compounds were quantified in SIM mode using specific m/z ions; 117 for indole, 130 for skatole and 7-ethyl-indole (IS), 241 for androstenol, 243 for androstanol (IS) and 272 for androstenone. Calibration curves for indole, skatole and androstenone relative to the internal standard were obtained using sunflower oil as matrix. Limits of detection (LOD) and quantification (LOQ) were calculated from a blank vial (containing sunflower oil) plus three and ten times the standard deviation of four blank replicates, respectively. Six measurements were made in each sausage (36 analyses per formulation). The peak areas were compared to their respective internal standard and expressed as ng of compound per g of melted fat.

2.5. Sensory analysis

The assessors were selected based on their sensitivity to androstenone and skatole odours as the perception vary among individuals (Meier-Dinkel, et al., 2013). Among 120 consumers that participated in a consumer acceptability test of fermented sausages manufactured with boar back fat, 22 consumers were selected based on its ability to describe the presence of abnormal odours. A Free choice profile (FCP) (Williams & Langron, 1984) was performed with this group of 22 consumers. The selection of this methodology was based on its no demand for a training stage and the ability to perform individual sessions. In fact, the main advantage of FCP over conventional profiling is that an agreed complex and scientifically vocabulary need not to be defined and untrained consumers may be used as panelists. In FCP each consumer develops an individual list of terms to describe the samples and the consumers must be able to detect differences between samples and verbally describe and quantify the perceived attributes (Oreskovich, Klein, & Sutherland, 1991). To obtain the individual descriptors a Repertory Grid Methodology (RGM) was used. RGM involves triadic or dyadic comparisons of products in one-on-one interviews in which consumers elicit their own set of “constructs” to describe similarities and differences between particular products (Russell & Cox, 2003).

In the first session, Repertory Grid Method (RGM) was used to generate an individual set of terms for each assessor. For this purpose, sets of three samples were presented to each participant who described the similarities and differences among sausages within each triad in their own terms. The order of triad presentation was balanced among the consumers (Gains, 1994). One slice of each sausage (4mm thickness) was randomly labelled with three digit codes and presented on a small white plate at room temperature. Water and unsalted bread was provided to clean the palate between samples. Consumers were asked to generate terms concerning the appearance, flavour, taste and texture of the samples. Then, sensory differences among samples were evaluated using FCP. For each consumer, a table with his/her own list of terms was provided and were asked to rate the intensity of each term for each sample using 10 cm unstructured scale with the extremes “Not perceived” and “Intense”. Each consumer evaluated the four samples in a standardized test room with separate booths. The samples were presented following a balanced design to avoid a serving order effect.

2.6. Statistical analysis

Data were analysed using the Generalized Linear Model (GLM) procedure of statistical software (XLSTAT 2011, v5.01, Addinsoft, Barcelona, Spain). The data was

analysed using the linear mixed model and included fat type and salt reduction as fixed effects, and replicates as random effect. When significant effect of the treatment group was detected ($P<0.05$), least squares means (LSM) were compared using Tukey test. For sensory data, a Generalized Procrustes Analysis (GPA) was applied to the Free Choice Profile data. In addition, principal component analysis (PCA) was performed to evaluate the relationships among instrumental variables (physic-chemical parameters, texture, free fatty acids (SFA, MUFA, PUFA), TBARS, boar taint compounds and volatile compounds) and formulations.

3. Results

3.1. Physicochemical analysis

The effect of back fat type (from gilt or entire male) and salt reduction on sausage physicochemical parameters at the end of process (60 d) are shown in Table 1. At the end of the ripening process, 36-39% weight losses were reached and water activity (a_w) decreased from 0.963-0.966 to 0.874-0.883. In addition, pH decreased from 5.8 to 4.6-4.8 values with small differences among formulations. These parameters, pH, a_w and weight losses, were affected by salt reduction while back fat type only affected pH and weight losses. The highest weight losses were found in formulations manufactured with entire male back fat (MC and MRS). Also salt reduction produced high a_w values. Respect to chemical composition (moisture, fat and protein) no significant differences were obtained among formulations (Table 1). As expected, significant differences were found in ion content as salt reduced treatments (RS and MRS) had the lowest Na^+ and Cl^- content and the highest K^+ content. Therefore, a salt reduction of approximately 30 % was achieved producing a healthy dry fermented sausage.

In order to evaluate differences in appearance, sausage colour was measured obtaining L^* , a^* and b^* coordinates (Table 1). Only yellowness b^* coordinate was affected by back fat type and salt reduction.

According to texture profile analysis, the use of back fat type and salt reduction had an effect on hardness and consequently on chewiness (Table 2). The use of entire male back fat produced the highest hardness and chewiness. In addition, a reduction of the same parameters was observed in reduce salt formulation (RS). Nevertheless, the use of entire male back fat in fermented sausages also decreased the cohesiveness.

3.2. Fatty acid analysis and oxidation

The total fatty acid profile of dry fermented sausages at 0 days of processing was analysed and few differences were found (Table 3). Total saturated monounsaturated and polyunsaturated fatty acids were not affected by back fat type neither salt reduction. Only, high percentages of C15:0 and C17:0 and low C18:2n6 and C20:2n6 were found in entire male back fat formulations (MC and MRS).

The free fatty acids were analysed. At the beginning of the process (0 d) no differences in total FFA concentrations (data not shown) were observed among formulations. An increase from an initial free fatty acid concentration of 389-604 mg/100g to a final (60 d) 1670-2045 mg/100g (Table 4) was observed due to the ripening process. The generated free SFA and MUFA at the end of process (60 d, Table 4) showed differences among formulations. Several FFAs were affected by back fat type or salt reduction (Table 4). The interaction of the effects of back fat type and salt reduction affected n6 PUFA free fatty acids and indeed the total PUFA concentrations as observed by a significant higher concentration in RS than MRS formulations explained by the highest abundant of free C18:2n6. The MC formulation showed the highest significant concentration of several FFA such as C12:0, C15:0, C17:0, C14:1; C16:1, C17:1, C18:1, C18:3n3, C20:3n6, C22:4n6 while the RS formulation had the highest content of C20:1n9 and C18:2n6.

The oxidation index TBARS was clearly different among formulations, formulations manufactured with entire male back fat had the lowest TBARS values and this value was not affected by the salt reduction.

3.3. Volatile compounds

A total of 98 volatile compounds were identified in dry fermented sausages while three of them (1-octene, 2-methyl-2-propanol and 3-ethyl benzaldehyde) were tentatively identified (Table 5). The identified volatile compounds were classified according to the most likely origin (Ordoñez, Hierro, Bruna, & De La Hoz, 1999) (Table 5). The most abundant group of compounds was those derived from lipid autooxidation (60-69%) and carbohydrate fermentation (16-21%) reactions, since hexanal and acetic acid represented 31-38% and 12-15% of the total area, respectively (Table 5). The use of entire male back fat in sausage manufacture showed a significant effect on volatile compounds decreasing those generated from the lipid autooxidation reactions and increasing those from carbohydrate fermentation and esterase activity. On the other hand, the effect of salt reduction was only observed in compounds generated from carbohydrate fermentation (Table 5).

Lipid autooxidation mainly originates aldehydes among others compounds such as alkanes, ketones, acids and alcohols. In general, the use of entire male back fat

brought a decrease of volatile compounds from lipid autooxidation in agreement with the reduced TBARS values detected in these formulations (MC and MRS, Tables 4 and 5). These formulations had the lowest abundance of 1-propanol, 2-methylfuran, pentanal, 1-pentanol, hexanal, 1-hexanol, pentanoic acid, hexanoic acid while only 2-propanol and octanoic acid had the highest abundance. On the other hand, salt reduction produced an increase of volatile compounds from lipid autooxidation in salt reduced formulations from gilts (RS, Table 5) such as octanal, decanal, 1-hexanol and 2-heptanal.

Both back fat type and salt reduction barely affected the generation of compounds from lipid β -oxidation reactions (Table 5). Only, 1-penten-3-ol and 2-hexanone were found in a higher abundance in RS formulation. By contrast, the compounds generated from carbohydrate fermentation by microbial reactions were affected by back fat type and salt reduction. The use of entire male fat produced an increase in the abundance of acetaldehyde, ethanol, acetic acid and 2,3-butanedione in MC and MRS formulations (Table 5), while salt reduction did not show a clear effect. In the formulations processed with gilt back fat, only a significant decrease of butanoic acid was detected in RS formulation while in entire male back fat formulations, an increase of ethanol and 2-butanone were observed in MRS.

Back fat type and salt reduction did not show an effect on total volatile compounds originated from amino acid degradation. However, individual effects were observed, while the use of entire male back fat produced the highest abundance of 2-methylpropanal, methylpyrazine, and methional, gilt back fat produced benzene and dimethyl disulfide. On the other hand, salt reduction produced an increase of dimethyl sulfide, methional, benzaldehyde and benzeneacetaldehyde while only a decrease of dimethyl trisulfide was observed.

The generation of ester compounds by microbial activity was affected by the use of different back fat types. In this sense, an increase of ester compounds were observed in formulations manufactured with entire male back fat especially for ethyl acetate and, to a lesser extent, ethyl butanoate. In contrast, salt reduction only produced a significant reduction of methyl acetate in RS and MRS formulations.

3.4. Boar taint compounds

The analysis of boar taint compounds was analysed in fermented sausages at the end of the process (60d). The levels of boar taint compounds in the fat extracted from the fermented sausages are shown in Figure 1. The compounds detected and quantified were indole, skatole, and androstenone while androstenol was not detected in sausages. All the concentrations detected were above the detection limits obtained

with the SPME-GC-MS method (LOD: 0.1ng/g for indole, 6.8 ng/g for skatole and 76.2 ng/g for androstenone). The use of gilt back fat proved the absence of androstenone levels in the sausages as this hormone was not detected in C and RS formulations. Concerning skatole and indole, both compounds were detected in the four formulations although both were significantly higher in entire male sausages (MC and MRS) than in gilts sausages (RS and C). About the effect of salt reduction, no effect was observed on indole and skatole although a high androstenone level was detected in MRS sausages.

3.5. Sensory analysis

The sensory panel consisted of 14 women and 8 men that performed a free choice profile analysis. The result of the free choice profile analysis is shown in Figure 2A that shows the descriptors used by each assessor (in different colour) while Figure 2B shows two dimensions GPA obtained and the list of main descriptors mentioned which explained each dimension. The total variance explained by the two dimensions was 77.5%. Dimension 1 accounted for 51.82% of the variance and was mainly related to taste and odour terms. As observed, the samples appeared distributed along dimension 1 according to the type of fat used. On the left side of the plot, odour terms like acid, cured, animal, stable and urine odour, fetid, sweat, ammonia were placed which characterized MRS and MC sausages manufactured with entire male back fat. In addition, the consumers also perceived these samples harder than gilt fat formulations as it was observed in texture profile analysis (Table 2). On the right side of the plot, terms as juicy, dry texture, fat odour and yellow fat characterized sausages manufactured with gilt back fat (C and RS) which could be in accordance to TBARS analysis, since the highest lipid oxidation was observed in the C and RS formulations (Table 4).

Dimension 2 accounted for 25.63% of the variance and was related to taste and texture terms. In this dimension, the samples appeared distributed according to the salt content. The unreduced salt sausages (MC and C) placed in the negative part of the Y axis, are related to salty taste, fibrous texture, colour, fat/lean cohesiveness while salt reduced sausages (RS and MRS) appeared in the positive part of the Y axis. These samples were related to fat percentage, gritty, colour uniformity, oily appearance and texture and bitter taste. As expected, the consumers perceived control formulations (MC and C) more salty than salt reduced formulations (MRS and RS) which were perceived with bitter taste probably due to KCl addition. In addition, MRS and RS sausages were perceived as fatty using different terms like fat percentage, oily appearance and oily texture.

In order to determine the relationship of the instrumental variables with boar taint perception, a principal component analysis was done using the following parameters; chemical composition, pH, ion content, volatile compounds (classified per origin group), boar taint compounds and texture parameters (Figure 3). Two principal components were able to explain 59 % of the total variability observed. The most important variable was PC1 as it explained 41 % of the variability and was related to the back fat type used while the PC2 only explained 18% of the variability and was related to salt reduction. Treatments manufactured with entire male back fat appeared in the positive part of PC1 and were related to the presence of boar taint compounds, weight losses, hardness and chewiness and volatiles produced from esterase activity and carbohydrate fermentation. While sausages manufactured with gilt back fat appeared on the negative part and were related to TBARS, PUFA, and volatiles generated from lipid oxidation reactions. However, PC2 differentiated samples produced with different salt contents; samples with reduced salt content appeared in the negative part and were related to the presence of K⁺ and a_w. Therefore, samples with reduced salt content were differentiated but the highest variability was explained by the use of different back fat type as previously observed in the consumer sensory analysis.

4. Discussion

All the studies regarding the use of entire male in the manufacture of dry cured meat products has been focused on boar taint perception (Bañón et al., 2003a, b; Stolzenbach et al., 2009) and consumer acceptability (Meier-Dinkel et al., 2013) but none of them on other physicochemical characteristics. Moreover, the changes in sausage quality and odour perception due to salt reduction must be taken into consideration (Corral et al., 2013, 2015).

Thus, the results of this study demonstrated that both factors (back fat type and salt reduction) affect the quality of fermented sausages. On the one hand, the lowest fat content of entire male back fat was responsible for the highest weight losses in entire male sausages (MC and MRS) although, this difference was not perceived in the final fat sausage content (Table 1). Concerning colour, the effect of back fat type on sausage colour parameters was not clear. Although several studies reported a slightly darker colour in entire male meat (Babol & Squires, 1995), there are not studies in dry fermented sausages. On the other hand, salt reduction produced sausages with high a_w values (Corral et al., 2013) and redness (a*), probably due to the salt reduction effect on *Micrococcaceae*. This microorganism is responsible for colour development in fermented sausages through its nitrate reductase activity (Ibañez, Quintanilla, Irigoyen,

García-Jalón, Astiasarán, & Bello, 1995); but other authors have not observed any effect on colour parameters (Corral et al., 2013). Regarding yellowness (b^*) the lowest values in entire male sausages can be related to the lowest TBARS values as the consequence of oxidative reactions is the formation of coloured polymers in fat exhibiting yellow to orange colours (Ruiz et al., 2002).

Evaluation of the sausage texture revealed the highest hardness and chewiness in entire male sausages probably due to the highest water, protein and less lipid content of this back fat than castrates and gilts (Babol & Squires, 1995). But the differences in fatty acid composition were minimal and the texture differences observed would be probably related to the highest weight losses. In addition, a reduction of the same parameters was observed in reduced salt formulations (RS and MRS) (Gou, Guerrero, Gelabert, & Arnau, 1996) as a result of a high muscle protein breakdown due to NaCl inhibition of the proteolytic process (Toldrá & Flores, 1998). Nevertheless, this effect was only observed in chewiness in a previous study where low reductions (16 %) were carried out (Corral et al., 2013).

The main effect of castration was reported in lean meat and intramuscular fat percentage although, regarding the adipose tissue, entire males had lower SFA and higher PUFA concentrations than castrates (Pauly, Luginbühl, Ampuero, & Bee, 2012). These changes may affect product quality during fermented sausage processing. The difference in fatty acid composition is less pronounced when comparing entire male/gilts than entire male/castrated (Babol & Squires, 1995). This is in line with findings about the absence of differences in total SFA and PUFA content among dry sausage formulations; although the most abundant PUFA fatty acid (C18:2n6) was detected at the lowest content in entire male sausages (Table 3).

Regarding the use of back fat type in dry fermented sausage processing, there are not studies dealing with the lipolysis process. Our study showed that back fat type and salt reduction affected n6 PUFA free fatty acids and indeed the total free PUFA concentration. Recent studies in fermented sausages indicated a lipolysis increase by salt reduction as a consequence of the lack of inhibition of lipolytic enzymes (Corral et al., 2013, 2015). The same trend was exclusively observed in sausages manufactured with gilt back fat (RS) (Table 4). Even though the differences in the generation of free fatty acids were not clear, sausage lipid oxidation was analysed. The entire male sausages (MC and MRS) had the lowest oxidation values probably related to the low PUFA profile found in these dry fermented sausages (Table 3) especially, in the low content of C18:2n6 fatty acid.

The flavour developed during the ripening of fermented sausages is highly related to the lipolysis process. Thus, the study of volatile compounds was performed

by using SPME-GC-MS although comparisons are limited due to differences in extraction techniques (Corral et al., 2015). All compounds reported have been previously detected in dry fermented sausages except 3-ethylbenzaldehyde (Stahnke, 2002). For the first time, this study demonstrated that the use of entire male back fat significantly produced a decrease of volatile compounds coming from lipid autooxidation reactions while those generated from carbohydrate fermentation and esterase activity increased (Table 5). This result is in contrast to those reported by Rius et al. (2005) who studied the production of volatile compounds from heated entire male back fat, relating the boar taint odour not only to the presence of androstenone and skatole but also to the formation of short chain aldehydes and fatty acids that produced undesirable aromas (rancid, fatty, mouldy). On the other hand, the effect of salt reduction in volatile compounds generated in fermented sausages produced the increase of those compounds derived from lipid autooxidation, carbohydrate fermentation and amino acid degradation (Table 5) as also reported recently Corral et al. (2013, 2015). Although there are scarce studies dealing with the effect of entire males and salt reduction, a recent study (Škrlep, Čandek-Potokar, Batorek Lukač, Prevolnik Povše, Pugliese, Labussière, & Flores, 2016) has shown an effect on the use of entire males in dry cured ham production, although few significant effects were detected due to the use of entire males or immunocastrates (IC). Nevertheless, the authors reported a higher content of ester compounds, methyl acetate and hexanoate, in entire males than IC dry cured hams. This is in accordance to our results that also showed an increase of esters in entire male sausages however, differences between dry cured ham and sausage processing makes this result difficult to compare.

The presence of boar taint compounds was analysed to evaluate the effect of the use of entire male back fat in sausage manufacture. While only the entire male formulations contained androstenone above its sensory perception threshold (0.5-1.0 µg/g fat) (Walstra et al., 1999), skatole concentration was detected in all sausage formulations above its sensory threshold (0.20-0.25 µg/g fat) (Walstra et al., 1999). These results confirm the sexual origin of androstenone while skatole is originated from tryptophan degradation (Zamaratskaia et al., 2004). Variations in androstenone contents due to salt reduction can be related to the sausage manufacture process since, pork back fat was previously chopped and mixed to achieve a homogeneous mass. However, differences can be present and responsible for the high variability detected among formulations sausages and replicates.

Consumer ability to perceive androstenone odour is decisive to detect boar taint odour (Meier-Dinkel et al., 2013) therefore, an initial panelist screening was performed for the free choice profile analysis. From this sensory analysis (FCP), it was observed

that the effect of fat type was higher than the produced by salt reduction. Consumers perceived a high hardness, low yellowness related to white fat descriptor, and lowest lipid oxidation in entire male formulations. Furthermore, boar taint odour was perceived by consumers and described as animal, stable or urine odour due to the presence of boar taint compounds above their threshold (Walstra et al., 1999). The formulation with reduced salt MRS was characterized with the highest concentration of androstenone (Figure 1) so, this would explain the sensory terms related to boar taint (animal, urine and stable) detected by consumers. But regarding skatole concentrations, the four formulations contained it although at the lowest level in gilts (C and RS, Figure 1). This means that skatole would affect the perception of consumers at a lesser extent than the presence of androstenone (Stolzenbach et al., 2009). By contrast, salt reduction effect was less perceived and detected by an increase of a_w and consequently decreased in hardness, and presence of bitter taste associated to KCl use as NaCl substitute. Therefore, the presence of boar taint compounds in fermented sausages can be perceived by general consumers describing the presence of abnormal odours but also other sausage characteristics such as texture and oxidation are affected due to the different chemical composition (mainly fat) of entire males versus gilts.

In general, different alternatives have been proposed in meat products to counteract these changes. But these alternatives were only focused on boar taint perception and none of them pay attention to fat composition and oxidation. In addition, while smoking had a positive effect to limit boar taint perception (Walstra, 1974), the use of starter cultures was insufficient to mask it (Stolzenbach et al., 2009). On the other hand, boar taint odour has been perceived in long ripened meat products from Mediterranean area such as dry cured ham (Bañón et al. 2003a, b). These types of products are characterized by low use of smoking treatments and this strategy would not be feasible to limit its perception. Generally, it is assumed that the aroma developed during long curing processes would mask boar taint perception (Bañón et al., 2003) but as shown in the present study, other characteristics, highest hardness and lowest oxidation, were perceived. So further research is necessary to look for other strategies to limit boar taint perception but also they may counteract the physicochemical changes observed.

5. Conclusion

Both factors (back fat type and salt reduction) affected the quality of dry fermented sausages. Consumers perceived a higher effect of fat type than the produced by salt reduction and these differences were also detected by the physicochemical characteristics measured. Meanwhile salt reduction increased a_w and

decreased hardness, the sausages were described by consumers as gritty, oily appearance, oily texture and bitter taste. In contrast, the presence of boar taint compounds in fermented sausages was perceived by general consumers describing the presence of abnormal odours but also other sausage characteristics such as texture (high hardness) and oxidation, were detected due to the different chemical composition (mainly fat) of entire males versus gilts.

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FIGURE LEGENDS

Figure 1. Levels of boar taint compounds (mean $\pm$ sem) in fermented sausages (60d) manufactured with gilt back fat (C), salt reduced sausages manufactured with gilt back fat (RS); sausages manufactured with entire male back fat (MC); salt reduced sausages manufactured with entire male back fat (MRS). Different letters in the same group indicate significant differences at $P < 0.05$ among formulations.

Figure 2. A) Correlation of the GPA analysis of the flash profiling data. B) Two dimension GPA plot of the differences among dry fermented sausages (60d) manufactured with salt reduction and back fat (from gilt or entire male). The main descriptors correlated with the first two dimensions of the average space are listed on the boxes and the number of times that the descriptor was mentioned.

Figure 3. Loadings of the first two principal components (PC1-PC2) of sausage formulations (◇), and instrumental variables (●).

Table 1. Effect of salt reduction and back fat (from gilt or entire male) on physicochemical parameters of dry fermented sausages at 60 d.

	C	RS	MC	MRS	RMSE	P_f^1	P_s	P_{fs}
Moisture (%)	38.9	38.3	38.6	37.5	1.33	ns	ns	ns
Fat (%)	26.9	29.6	28.4	28.7	1.83	ns	ns	ns
Protein (%)	28.5	28.5	29.1	30.1	1.56	ns	ns	ns
Na ⁺ (mg/g d.m.)	31.5 a	21.4 b	31.2 a	21.4 b	1.20	ns	***	ns
K ⁺ (mg/g d.m.)	9.0 c	15.9 b	8.4 c	17.1 a	0.72	ns	***	**
Cl ⁻ (mg/g d.m.)	38.5 a	32.5 c	36.1 b	32.2 c	1.56	*	***	ns
pH	4.8 a	4.7 b	4.6 c	4.7 b	0.019	***	***	***
a _w	0.874 b	0.883 a	0.877 ab	0.881 ab	0.007	ns	*	ns
Weight losses (%)	37.2 b	36.6 b	39.4 a	38.8 a	0.52	***	*	ns
L*	48.4	48.2	49.4	49.8	1.57	ns	ns	ns
a*	17.9	18.9	17.1	17.8	0.98	ns	ns	ns
b*	7.1 a	7.1 a	6.2 b	6.9 a	0.42	**	*	*

C: sausages manufactured with gilt back fat, RS: salt reduced sausages manufactured with gilt back fat; MC: sausages manufactured with entire male back fat; MRS: salt reduced sausages manufactured with entire male back fat; SEM: standard error of the mean.

¹ P_f : P value of the type of fat effect, P_s : P value of salt content effect, P_{fs} : P value of interaction between type of fat and salt content effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: $p > 0.05$.

Different letters in the same row indicate significant differences among batches.

Table 2. Effect of salt reduction and back fat (from gilt or entire male) on texture parameters in dry fermented sausages at 60 d.

	C	RS	MC	MRS	RMSE	P_f^1	P_s	P_{fs}
Hardness (N)	135.24 b	116.98 c	175.94 a	163.22 a	13.42	***	***	ns
Adhesiveness(N.s)	-3.20	-3.08	-3.28	-3.26	0.52	ns	ns	ns
Springiness	0.64	0.63	0.59	0.59	0.06	ns	ns	ns
Cohesiveness	0.65 a	0.64 a	0.61 b	0.62 ab	0.02	***	ns	ns
Chewiness (N)	56.51 ab	46.91 b	63.25 a	59.55 a	9.78	**	ns	ns

C: sausages manufactured with gilt back fat, RS: salt reduced sausages manufactured with gilt back fat; MC: sausages manufactured with entire male back fat; MRS: salt reduced sausages manufactured with entire male back fat. SEM: standard error of the mean.

¹ P_f : P value of the type of fat effect, P_s : P value of salt content effect, P_{fs} : P value of interaction between type of fat and salt content effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: $p > 0.05$.

Different letters in the same row indicate significant differences among batches.

Table 3. Effect of salt reduction and back fat (from gilt or entire male) on total fatty acid profile (%) in dry fermented sausages at 0 d.

Fatty acids	C	RS	MC	MRS	RMSE ¹	P _f ²	P _s	P _{fxs}
C12:0	0.09 b	0.09 ab	0.09 ab	0.10 a	0.004	*	ns	ns
C14:0	1.42	1.44	1.40	1.37	0.060	ns	ns	ns
C15:0	0.06 b	0.07 ab	0.09 a	0.08 ab	0.010	*	ns	ns
C16:0	22.88	22.39	23.23	23.67	0.981	ns	ns	ns
C17:0	0.33 c	0.39 bc	0.54 a	0.48 ab	0.067	**	ns	ns
C18:0	12.11	10.96	12.54	13.41	1.500	ns	ns	ns
C20:0	0.03	0.03	0.04	0.03	0.010	ns	ns	ns
SFA	36.92	35.36	37.92	39.14	2.423	ns	ns	ns
C14:1	0.04	0.03	0.05	0.04	0.014	ns	ns	ns
C16:1	2.51	2.71	2.86	2.74	0.196	ns	ns	ns
C17:1	0.09	0.11	0.15	0.12	0.026	ns	ns	ns
C18:1	40.46	41.63	41.14	39.99	1.983	ns	ns	ns
C20:1 n9	1.21	1.20	1.02	0.97	0.163	ns	ns	ns
MUFA	44.31	45.68	45.28	43.89	2.182	ns	ns	ns
C18:2n6	15.54 a	15.66 a	13.30 b	13.79 b	1.347	*	ns	ns
C18:3 n3	0.75	0.78	0.78	0.72	0.049	ns	ns	ns
C18:3 n6	0.06	0.06	0.05	0.06	0.009	ns	ns	ns
C20:2 n6	0.61 a	0.61 a	0.52 b	0.52 b	0.042	**	ns	ns
C20:3 n6	0.05 b	0.06 ab	0.07 a	0.05 ab	0.011	ns	ns	*
C20:4 n6	1.06	1.17	1.17	1.06	0.111	ns	ns	ns
C22:4 n6	0.23	0.24	0.27	0.25	0.042	ns	ns	ns
n6	17.53	17.64	15.19	15.73	1.631	ns	ns	ns
C20:5 n3	0.08	0.08	0.11	0.09	0.032	ns	ns	ns
C22:5 n3	0.35	0.40	0.41	0.37	0.068	ns	ns	ns
C22:6 n3	0.06	0.06	0.08	0.06	0.007	ns	ns	ns
n3	1.23	1.32	1.61	1.24	0.187	ns	ns	ns
PUFA	18.77	18.96	16.79	16.97	1.618	ns	ns	ns

C: sausages manufactured with gilt back fat, RS: salt reduced sausages manufactured with gilt back fat; MC: sausages manufactured with entire male back fat; MRS: salt reduced sausages manufactured with entire male back fat.

¹SEM: standard error of the mean.

²P_f: P value of the type of fat effect, P_s: P value of salt content effect, P_{fxs}: P value of interaction between type of fat and salt content effects. ***: P<0.001; **: P<0.01; *: P<0.05; ns: p>0.05.

Different letters in the same row indicate significant differences among batches.

Table 4. Effect of salt reduction and back fat (from gilt or entire male) on free fatty acids (FFA, mg/100g) and TBARS in dry fermented sausages at 60 d.

Free fatty acids	C	RS	MC	MRS	RMSE ¹	P _f ²	P _s	P _{fxs}
C12:0	1.67 bc	1.78 ab	1.92 a	1.58 c	0.16	ns	*	*
C14:0	18.88	22.38	24.31	19.30	2.89	ns	ns	**
C15:0	1.09 b	1.06 b	1.91 a	1.22 b	0.17	**	**	*
C16:0	248.12	290.65	307.69	246.07	39.75	ns	ns	ns
C17:0	4.04 c	4.76 bc	7.09 a	5.33 b	0.68	**	**	*
C18:0	129.61	148.41	153.77	135.25	14.22	ns	ns	ns
C20:0	0.82	0.91	0.93	0.78	0.09	ns	ns	ns
SFA	404.58 b	470.33 ab	497.45 a	409.53 b	56.65	ns	ns	**
C14:1	0.44 b	0.40 b	0.77 a	0.47 b	0.09	*	*	*
C16:1	40.15 b	44.86 b	57.90 a	46.44 b	6.05	*	ns	*
C17:1	4.57 c	4.91 bc	8.38 a	5.47 b	0.70	***	*	**
C18:1	674.18 b	763.77 b	885.57 a	729.86 b	97.39	**	ns	ns
C20:1 n9	28.75 b	35.72 a	29.41 b	25.05 b	2.76	**	ns	**
MUFA	748.09 b	849.78 b	982.03 a	807.29 b	107.79	**	ns	ns
C18:2n6	403.80 ab	472.54 a	437.76 ab	370.40 b	37.75	ns	ns	*
C18:3 n3	20.24 b	22.28 b	26.82 a	23.50 ab	2.41	*	ns	ns
C18:3 n6	0.52	0.56	0.59	0.56	0.09	ns	ns	ns
C20:2 n6	22.10	25.49	24.30	22.06	1.99	ns	ns	ns
C20:3 n6	2.32 b	2.55 b	2.93 a	2.57 b	0.22	*	ns	ns
C20:4 n6	38.80	41.04	40.13	36.20	3.77	ns	ns	ns
C22:4 n6	7.29 b	7.69 ab	8.49 a	7.13 b	0.75	ns	*	ns
n6	474.83 bc	549.87 a	514.20 ab	438.92 c	43.78	ns	ns	*
C20:5 n3	3.91	4.13	4.23	3.87	0.41	ns	ns	ns
C22:5 n3	15.95	16.33	18.54	16.12	2.06	ns	ns	ns
C22:6 n3	2.05	2.14	1.90	1.78	0.31	ns	ns	ns
n3	42.15	44.89	51.49	45.27	4.57	ns	ns	*
PUFA	516.98 bc	594.76 a	565.68 ab	484.19 c	47.92	ns	ns	*
Total FFA	1669.65	1914.87	2045.16	1701.01	209.17	ns	ns	ns
TBARS	6.24 a	6.24 a	3.42 b	3.57 b	1.09	***	ns	ns

C: sausages manufactured with gilt back fat, RS: salt reduced sausages manufactured with gilt back fat; MC: sausages manufactured with entire male back fat; MRS: salt reduced sausages manufactured with entire male back fat.

¹SEM: standard error of the mean.

²P_f: P value of the type of fat effect, P_s: P value of salt content effect, P_{fxs}: P value of interaction between type of fat and salt content effects. ***: P<0.001; **: P<0.01; *: P<0.05; ns: p>0.05.

Different letters in the same row indicate significant differences among batches.

1 **Table 5.** Effect of salt reduction and back fat (from gilt or entire male) on volatile compounds (expressed as AU x 10⁻⁶) extracted by HS-SPME in the
2 headspace of dry fermented sausages (60d).

Compound	Fermented sausage batches ¹														
	LRI ²	LRI ² standard	RI ³	C		RS		MC		MRS		RMSE ⁴	P ⁵ _f	P _s	P _{fxs}
Lipid autooxidation				2935.09	ab	3152.00	a	2597.89	b	2363.33	b	201.2	*	ns	ns
Pentane	501	500	a	94.05		89.71		78.14		82.19		17.99	ns	ns	ns
Propanal	524	521	a	9.21	b	20.31	a	22.95	a	10.27	b	1.18	ns	ns	***
2-Propanol (45) ⁶	540	537	a	2.00	bc	1.49	c	2.46	ab	3.18	a	0.49	**	ns	ns
Hexane	601	600	a	13.39		10.03		11.76		13.41		3.41	ns	ns	ns
1-Propanol	612	611	a	3.15	a	3.12	a	1.49	b	1.52	b	0.24	***	ns	ns
2-Methylfuran	615	615	a	1.55	ab	1.81	a	1.19	b	1.18	b	0.22	**	ns	ns
Butanal	622	620	a	5.17		8.25		6.83		5.74		1.96	ns	ns	ns
Tetrahydrofuran	643	642	a	1.90	a	0.38	bc	0.26	c	0.47	b	0.07	***	***	***
1-Heptene	692	696	a	4.86		4.17		3.94		2.96		0.89	ns	ns	ns
Heptane (71) ⁶	700	700	a	21.14		15.29		14.11		18.98		5.28	ns	ns	ns
2-Ethylfuran	719	719	a	21.68	a	15.91	ab	19.57	a	11.80	b	3.68	ns	*	ns
Pentanal (44) ⁶	737	736	a	57.32	a	61.20	a	48.42	ab	35.36	b	9.94	*	ns	ns
1-Octene	795		b	22.04	a	18.79	ab	19.93	a	15.86	b	1.70	ns	*	ns
Octane	800	800	a	260.24		264.64		249.38		236.80		58.50	ns	ns	ns
Propanoic acid (74) ⁶	804	806	a	1.04		1.12		1.11		0.99		0.11	ns	ns	ns
2-Pentenal (E) (55) ⁶	805	804	a	0.73		0.63		0.87		0.92		0.15	ns	ns	ns
1-Pentanol (55) ⁶	826	823	a	4.30	ab	5.70	a	2.33	b	2.80	b	0.90	**	ns	ns
Hexanal	842	839	a	1618.69	ab	1744.89	a	1432.75	bc	1244.22	c	211.76	*	ns	ns
Nonane	900	900	a	25.76		24.42		25.09		21.70		2.26	ns	ns	ns
2-Hexenal (E)	904	903	a	40.21		38.60		35.15		32.58		5.91	ns	ns	ns
2-Butylfuran	908	909	a	26.38		27.17		26.11		24.67		1.73	ns	ns	ns
1-Hexanol	922	921	a	117.87	b	187.22	a	38.23	c	38.90	c	10.81	***	**	*
Heptanal (44) ⁶	939	939	a	9.89		11.82		11.38		10.02		2.30	ns	ns	ns
2,4-Hexadienal (E,E)	968	968	a	22.75		21.00		21.83		16.14		4.34	ns	ns	ns
Pentanoic acid (60) ⁶	973	978	a	1.05	b	1.41	a	1.10	b	0.87	b	0.14	*	ns	**
Decane	1000	1000	a	38.30		37.39		33.51		36.62		3.02	ns	ns	ns
2-Pentylfuran (81) ⁶	1010	1009	a	11.85		16.42		7.60		8.40		3.66	ns	ns	ns
2-Heptenal (Z) (41) ⁶	1011	1008	a	1.32	bc	5.34	a	1.70	b	0.96	c	0.26	***	***	***
1-Heptanol (70) ⁶	1023	1021	a	0.80	a	0.68	ab	0.87	a	0.46	b	0.15	ns	*	ns

Octanal (43) ⁶	1048	1044	a	5.18	b	8.28	a	5.14	b	5.74	ab	1.17	ns	*	ns
Hexanoic acid (60) ⁶	1075	1075	a	11.39	ab	15.07	a	11.31	ab	8.88	b	2.12	*	ns	*
2,4-Heptadienal (E,E) (81) ⁶	1077	1076	a	0.21		0.26		0.44		0.30		0.13	ns	ns	ns
Undecane	1100	1100	a	35.02		41.47		41.60		38.94		5.98	ns	ns	ns
2-Octenal (E) (41) ⁶	1115	1113	a	1.72		2.37		1.66		1.04		0.84	ns	ns	ns
Nonanal	1149	1148	a	88.89		89.11		77.92		87.01		20.07	ns	ns	ns
Heptanoic acid	1165	1166	a	31.30		21.01		24.89		26.57		5.17	ns	ns	ns
Dodecane	1200	1200	a	53.52	a	39.10	ab	43.86	ab	31.07	b	8.10	ns	*	ns
2-Nonenal (Z)	1222	1218	a	62.53		60.55		60.84		57.70		8.05	ns	ns	ns
Decanal	1257	1253	a	39.89	b	60.74	a	53.83	ab	56.61	a	7.91	ns	*	ns
Octanoic acid (60) ⁶	1264	1264	a	0.94	c	1.04	bc	1.37	a	1.23	ab	0.14	**	ns	ns
2,4-Nonadienal (E,E)	1287	1287	a	42.82		42.59		43.10		44.68		3.65	ns	ns	ns
Tridecane	1300	1300	a	73.15		75.68		61.64		66.71		14.13	ns	ns	ns
2,4-Decadienal (E,E) (81) ⁶	1392	1392	a	0.26		0.49		0.42		0.30		0.16	ns	ns	ns
Decanoic acid	1438	1451	a	49.65		55.32		49.80		56.56		6.15	ns	ns	ns
Bacterial metabolism															
Lipid β oxidation				226.83		214.80		212.57		202.03		14.50	ns	ns	ns
2-Pentanone	733	731	a	13.26		9.03		9.13		9.60		2.50	ns	ns	ns
1-Penten-3-ol (57) ⁶	740	737	a	3.48	b	5.22	a	3.20	b	2.84	b	0.73	*	ns	*
2,3-Pentanedione	743	740	a	13.62		14.94		19.73		16.52		2.58	ns	ns	ns
2-hexanone	835	835	a	25.27	a	25.04	a	15.95	b	15.58	b	4.15	**	ns	ns
2-Heptanone	933	933	a	60.82		50.60		56.08		48.68		7.31	ns	ns	ns
1-Octen-3-ol (57) ⁶	1031	1028	a	6.40		9.01		5.04		5.94		1.37	ns	ns	ns
3-Octen-2-one	1094	1094	a	38.22		44.33		44.52		39.20		8.40	ns	ns	ns
2-Nonanone	1140	1139	a	65.77	a	56.63	b	58.90	b	63.67	ab	3.38	ns	ns	*
Carbohydrate fermentation				693.94	c	737.45	b	826.54	a	844.65	a	34.25	***	*	ns
Acetaldehyde		466	a	7.36	b	9.14	ab	10.16	ab	12.85	a	2.09	*	ns	ns
Ethanol	507	507	a	63.04	bc	52.05	c	84.52	b	118.60	a	13.17	***	ns	*
Acetone (43) ⁶	529	527	a	14.23		11.26		18.57		15.18		3.49	ns	ns	ns
2,3-Butanedione (43) ⁶	626	624	a	0.38	bc	0.32	c	0.45	ab	0.49	a	0.05	**	ns	ns
2-Butanone	630	629	a	13.39	b	17.65	b	16.21	b	23.66	a	3.07	*	*	ns
Acetic acid	715	714	a	502.52	b	568.77	ab	593.65	a	589.55	a	46.84	*	ns	ns
3-Hydroxy-2-butanone	780	783	a	18.26		23.29		24.33		21.14		6.39	ns	ns	ns
Butanoic acid	888	891	a	74.77	a	54.97	b	78.65	a	63.19	a	8.95	ns	**	ns
Amino acid degradation				203.90		209.40		205.81		201.38		11.65	ns	ns	ns
Methanethiol		472	a	12.42	a	5.92	b	5.28	b	5.62	b	2.40	*	ns	ns

Dimethyl sulfide (62) ⁶	533	532	a	0.06	b	0.08	ab	0.07	b	0.11	a	0.02	ns	**	ns
Carbon disulfide (76) ⁶	538	537	a	6.50	ab	6.10	b	7.26	ab	8.39	a	1.05	*	ns	ns
2-Methyl-2-propanol	568		b	0.38		0.24		0.36		0.34		0.06	ns	ns	ns
2-Methylpropanal	594	590	a	2.45	b	2.73	b	2.86	ab	3.73	a	0.49	*	ns	ns
Benzene	675	672	a	1.94	a	1.85	ab	1.40	ab	1.29	b	0.30	*	ns	ns
3-Methylbutanal	688	687	a	14.95		14.75		16.12		17.69		3.29	ns	ns	ns
2-Methylbutanal (58) ⁶	699	698	a	1.45		1.26		1.40		1.37		0.32	ns	ns	ns
Dimethyl disulfide	772	771	a	11.59	a	12.27	a	10.02	ab	8.96	b	1.20	*	ns	ns
2-Methylpropanoic acid (43) ⁶	859	864	a	0.41		0.37		0.56		0.41		0.08	ns	ns	ns
Methylpyrazine (94) ⁶	860	859	a	0.11	b	0.14	ab	0.19	a	0.18	a	0.04	*	ns	ns
Ethyl benzene	883	881	a	22.22		19.56		20.78		18.27		2.68	ns	ns	ns
2,6-Dimethylpyrazine (108) ⁶	943	943	a	0.13	b	0.18	a	0.20	a	0.20	a	0.03	*	ns	ns
2-Acetyl-1-pyrroline (43) ⁶	964	960	a	0.41	a	0.29	bc	0.22	c	0.34	ab	0.05	ns	ns	*
Methional (48) ⁶	965	964	a	0.50	c	0.74	b	0.75	b	1.08	a	0.07	***	***	ns
Dimethyl trisulfide	1002	1002	a	33.14	a	28.15	ab	30.79	ab	25.31	b	3.36	ns	*	ns
Benzaldehyde	1018	1013	a	48.19	b	55.93	a	51.49	ab	54.64	a	3.21	ns	*	ns
Benzene acetaldehyde (91) ⁶	1108	1104	a	1.42	b	2.43	a	2.15	ab	2.27	a	0.40	ns	*	ns
Phenol (94) ⁶	1110	1108	a	5.22		6.43		6.51		6.28		0.65	ns	ns	ns
3-Ethyl benzaldehyde	1233		b	40.43	b	49.97	a	47.40	a	44.90	ab	3.25	ns	ns	*
Esterase activity				149.11	b	155.36	b	229.79	a	233.49	a	25.39	***	ns	ns
Methyl acetate	551	549	a	22.81	a	13.32	b	23.49	a	12.73	b	2.24	ns	***	ns
Ethyl acetate	635	635	a	49.80	b	54.87	b	134.59	a	148.45	a	28.78	***	ns	ns
Propyl acetate	748	748	a	7.47	b	9.26	a	6.38	b	6.68	b	0.79	**	ns	ns
Ethyl butanoate (71) ⁶	830	829	a	0.71	b	0.45	c	1.15	a	1.01	a	0.12	***	*	ns
Ethyl 2-hydroxypropanoate	866	865	a	40.13		50.51		36.68		40.58		6.18	ns	ns	ns
Ethyl hexanoate (88) ⁶	1030	1026	a	0.40		0.53		0.57		0.48		0.13	ns	ns	ns
Ethyl octanoate	1230	1226	a	27.79		26.43		26.94		23.55		3.93	ns	ns	ns
Unknown or contaminants compounds				114.00		91.73		107.95		106.41		6.73	ns	ns	ns
Pyridine (79) ⁶	787	783	a	0.04	b	0.04	b	0.05	b	0.17	a	0.01	***	***	***
Toluene (91) ⁶	788	790	a	3.20	ab	2.75	b	3.48	a	2.83	ab	0.39	ns	*	ns
p-Xylene	891	890	a	30.22		17.24		30.07		28.82		6.83	ns	ns	ns
o-Xylene	916	915	a	22.86		23.23		19.49		19.35		3.16	ns	ns	ns
Styrene	918	917	a	15.60		16.39		16.70		16.34		1.73	ns	ns	ns
3-Carene (93) ⁶	1023	1019	a	0.27	a	0.19	ab	0.19	ab	0.10	b	0.05	*	*	ns
Butyrolactone (42) ⁶	1022	1021	a	0.81	a	0.54	b	0.84	a	0.56	b	0.11	ns	**	ns
D-Limonene (68) ⁶	1046	1042	a	3.67	a	3.26	ab	2.29	c	2.45	bc	0.49	**	ns	ns

p-Cimene	1051	1047	a	36.91	a	27.69	b	34.28	a	35.17	a	2.51	ns	*	*
Dimethyl sulfone (79) ⁶	1060	1060	a	0.17	b	0.16	b	0.33	a	0.33	a	0.05	***	ns	ns
4-Methyphenol (107) ⁶	1196	1194	a	0.25		0.24		0.22		0.29		0.06	ns	ns	ns

The main groups according to the most likely origin in bold.

¹ C: sausages manufactured with gilt back fat, RS: salt reduced sausages manufactured with gilt back fat; MC: sausages manufactured with entire male back fat; MRS: salt reduced sausages manufactured with entire male back fat.

² Linear retention indices (LRI) of the compounds eluted from the GC-MS using a DB-624 capillary column (J&W Scientific 30 m x 0.25 mm i.d. x 1.4 µm film thickness).

³ Reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, tentatively identification by mass spectrum.

⁴ SEM: standard error of the mean.

⁵ P_f : P value of the type of fat effect, P_s : P value of salt content effect, P_{fxs} : P value of interaction between type of fat and salt content effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: $p > 0.05$. Different letters in the same row indicate significant differences among batches.

⁶ Target ion used to quantify the compound when the peak was not completely resolved.