Yeast inoculation as a strategy to improve the physico-chemical and sensory properties of reduced salt fermented sausages produced with entire male fat

Sara Corral, Carmela Belloch, José Javier López-Díez, Ana Salvador, Mónica Flores*

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) Avda. Agustín Escardino 7, 46980 Paterna, Valencia, Spain

*Corresponding author. Tel.: +34 96 3900022; fax: +34 96 3636301
E-mail address: mflores@iata.csic.es (M. Flores).
Abstract

Yeast inoculation of dry fermented sausages manufactured with entire male fat was evaluated as a strategy to improve sausage quality. Four different formulations with entire male/gilt back fat and inoculated/non-inoculated with *Debaryomyces hansenii* were manufactured. The use of entire male back fat produced the highest weight losses, hardness and chewiness in dry sausages. Consumers clearly distinguished samples according to drying time and *D. hansenii* inoculation while the use of entire/gilt back fat was not highly perceived. The presence of androstenone and skatole was close to their sensory thresholds. Androstenone was not degraded during the process but skatole was affected by yeast inoculation. *D. hansenii* growth on the surface regulated water release during ripening, reduced hardness and chewiness in entire male sausages and resulted with similar texture to gilt sausages. Yeast inoculation inhibited lipid oxidation providing fruity odours and less oxidized fatty sausages in the sensory analysis. The effectiveness of yeast to mask boar taint was demonstrated by sensory analysis.

Keywords: dry fermented sausages; boar taint; yeast; *D. hansenii*; entire male; sensory.
1. Introduction

Boar taint is an off odour/flavour found in pork from entire male pigs described as urine, sweat and faecal (Lundström, Matthews, & Haugen, 2009). Androstenone and skatole are mainly associated to the development of boar taint, even though other compounds such as androstenols and other tryptophan derivatives have been suggested to contribute at lesser extent to boar taint (Babol & Squires, 1995). In order to avoid the incidence of boar taint among other disadvantages (Corral, Salvador, & Flores, 2016), surgical castration of male piglets has been used extensively. However, the European Union declaration in animal welfare indicates to voluntary stop surgical castration by 2018 (European Declaration on Alternatives to Surgical Castration of Pigs, 2010). Recently, Borrisser-Pairó et al. (2016) studied the prevalence of boar taint in Spanish pig population indicating that about 10% of carcasses were above the high threshold of androstenone and skatole being an important issue for pig industry.

A high number of sensory studies dealt with the acceptability of pork from entire males and related it to androstenone and skatole levels (Font-i-Furnols, 2012). However, variations in sensory results were due to many factors including the methodology used for sensory assessment, meat preparation procedures or consumer characteristics, such as smell sensitivity or culinary habits (Bonneau, 1998). Most of them concluded that boar taint is easily perceived upon cooking (Font-i-Furnols, 2012). On the other hand, consumer acceptability may be improved when boar meat is used for processing i.e. cooked hams and dry sausages, due to the cold temperature used during consumption of these products that minimize odour release and the use of spices that mask unpleasant odours (Desmoulin, Bonneau, Frouin, & Bidard, 1982). Although boar meat with high androstenone levels still produced unpleasant products. Therefore, several authors indicated the use of neck chops marinated with liquid smoke or oregano extracts in processed meat reporting a noticeably reduction of boar taint perception (Lunde, Egelanddal, Choinski, Mielnik, Flåtten, & Kubberød, 2008). In 1974, Walstra suggested the use of untained meat to dilute taint odour, such as 25 % fat from boars in meat products, which are consumed cold, while if their consumption is warm only between 6-12% boar fat was tolerable. In dry cured products, several authors reported that the curing and drying process was not enough to mask boar taint perception when entire meat was used in dry cured ham (Bañón, Costa, Gil, & Garrido, 2003; Bañón, Gil, & Garrido, 2003; Diestre, Oliver, Gispert, Arpa, & Arnau, 1990; Skrlep et al., 2016) and in dry fermented sausages (Corral et al., 2016).

Thus, other strategies should be considered for dry meat products. In this regard, Stolzenbach, Lindahl, Lundström, Chen, & Byrne (2009) pointed out the smoking process to mask boar taint in Swedish fermented sausages however, this
process is not commonly performed in the Mediterranean area (Flores, 1997). In fact, previous studies in Mediterranean type dry fermented sausages manufactured with entire male back fat and reduced salt content indicated the presence of differences not only in aroma perception but also in physicochemical characteristics (Corral et al., 2016). This study concluded that the use of entire male back fat affected consumer perception by the presence of abnormal odours being this effect higher than the one produced by salt reduction. In addition, the entire male sausages resulted with a high hardness and low oxidation values mainly due to differences in fat composition between entire male and gilt back fat. Therefore, it is necessary to look for alternatives that not only counteract the boar taint perception but also take into account the physicochemical differences.

In this sense, yeast may be an alternative to mask boar taint odour as they are involved in different biochemical mechanisms releasing aroma compounds (Flores, Corral, Cano-García, Salvador, & Belloch, 2015). Yeast has oxygen-scavenging and lipolytic activities that delay rancidity and further catabolize products of fermentation, such as lactate increasing the pH and contributing to the development of less tangy and more aromatic sausages (Hammes & Knauf, 1994; Gori, Mortensen, Arneborg, & Jespersen, 2007). In this sense, yeast can modulate water release during sausage drying and also regulate the oxidation process. The aroma potential of yeast isolated from natural fermented sausages was recently evaluated (Cano-García, Flores, & Belloch, 2013) in addition to its ability to enhance the aroma in reformulated dry fermented sausages (Corral, Salvador, Belloch, & Flores, 2014, 2015). However, nothing is known about the ability of *D. hansenii* yeast to counteract boar taint perception or masked the effect produced in tenderness and oxidation by the use of entire back fat. Therefore, the aim of the present study was to evaluate the ability of *D. hansenii* yeast to improve the quality of dry fermented sausages with reduced salt content and manufactured with entire male back fat.

2. Material and methods

2.1. Preparation of yeast inoculum

Yeast strain (*Debaryomyces hansenii* P2) previously isolated from naturally fermented sausages (Cano-García et al., 2013) was used for the inoculation of dry fermented sausages. Yeast was cultivated on GPY medium (2% glucose, 0.5% peptone, 0.5% yeast extract) and the grown cells washed with sterile saline solution (0.9% NaCl) and centrifuged (7000 rpm for 10 minutes at 4°C) to remove the culture medium. The collected cells were prepared to a concentration of $10^8$ cells/ml. The concentrated yeast cells were cold stored until inoculation.
2.2. Dry fermented sausages and sampling

In order to test the effect of fat type (gilt vs entire male) and yeast inoculation, four different formulations of dry fermented sausages were manufactured (6 kg/formulation). Formulation with back fat from gilt (HRS), formulation with entire male back fat (MRS) and the same two formulations, gilt back fat (HRS+Y) and entire male back fat (MRS+Y) inoculated with \textit{D. hansenii} yeast. Three replicates of the experiment were carried out. Pork’s lean and back fat (bellies boneless and skinless) from 12 different animals per sex were purchased from a local producer (Incarlopsa, Spain) and delivered to IATA-CSIC for processing.

For each of three replications, lean (50 % lean pork meat) and fat (50% pork back fat from gilt or entire male) was ground through a 10mm diameter mincing plate and vacuum minced with the following ingredients: 20 g/Kg lactose, 20 g/kg dextrin, 7 g/kg glucose, 20.3 g/kg sodium chloride (NaCl), 6.7 g/kg potassium chloride (KCl), 0.5 g/kg sodium ascorbate, 0.15 g/kg sodium nitrite and 0.15 g/kg potassium nitrate. Also, a commercial starter culture (0.1 g/kg) SP318 TEXEL SA-301 was added (Danisco, Cultor, Madrid, Spain) containing \textit{Lactobacillus sakei, Pediococcus pentosaceus, Staphylococcus xylosus} and \textit{Staphylococcus carnosus}. Pork back fat from entire male and gilt were previously chopped and mixed due to variations in androstenone and skatole contents to achieve and homogeneous mass. Appropriate volumes of yeast strain \textit{D. hansenii} P2 (Cano-García et al., 2013) suspension were added to the inoculated formulations (HRS+Y and MRS+Y) at final concentration of 5x10^6 cells/g. The mixture of each formulation was kept at 3-5ºC for 24h and then, was stuffed into 95 mm diameter collagen casings (Fibran, S.A., Girona, Spain) being the final weight of each sausage approximately 500 g. The sausages were dried for 63 days at 10ºC and 70-85 % relative humidity (RH). In order to control the ripening process, weight losses and pH were measured almost every day.

A total of 12 batches (3 x 2 x 2) were produced and approximately 12 sausages were obtained in each batch. Two sausages from each batch were randomly chosen at different ripening times (0, 43 and 63 d) as long ripening times are related to consumer acceptance and flavour development (Olivares, Navarro, & Flores, 2011). In each sausage, sausage colour was measured, a sliced of approximately 20 g were taken for microbial analyses and 100 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 -20ºC for subsequent physicochemical analyses (TBARS, lipid, protein). From sausages collected at 43 and 63 d, a slice (1 cm thickness) was taken wrapped in aluminium foil, vacuum packaged and stored at -80 ºC for boar taint analysis. Finally at 43 d and 63 d of ripening, 2 sausages from each batch were
vacuum packaged and stored at 4°C and used for texture and sensory analysis in less than 3-4 days.

2.3. Microbial analysis

Sausage samples were aseptically removed of collagen casings and homogenized with sterile saline solution (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 min. Decimal dilutions were prepared in sterile saline solution. Lactic acid bacteria and *Staphylococci* population were determined by spread plating on MRS agar anaerobically (Scharlau Chemie SA, Barcelona, Spain) and Mannitol Salt Agar (Scharlau Chemie SA, Barcelona, Spain), respectively. Both mediums were incubated at 37 °C for 2 days. Yeast count was obtained in Rose Bengal Agar with chloramphenicol (RBA) (Conda SA, Madrid, Spain) at 28°C for 3 days. Fifteen yeast colonies were isolated from each formulation and replicate at 0, 43 and 63 d of the ripening process and subjected to molecular characterization by minisatellite PCR amplification using the M13 primer as described by Cano-García et al. (2013).

2.4. Chemical analysis

The measurement of pH, $a_w$, weight losses, colour (CIELab $L^*$, $a^*$, $b^*$), moisture, protein and fat was performed as described by Corral, Salvador, & Flores (2013). 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.

2.5. Texture profile analysis

Texture profile analysis (TPA) was carried out using TA-XT.plus Texture Analyzer with Texture Exponent software (version 2.0.7.0 Stable Microsystems, Godalming, UK). At 43 and 63 d of the ripening process, three different slices (3.5 cm diameter and 1.5 cm thick) of two sausages from each formulation and replicate 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.

2.6. Analysis of boar taint compounds

The boar taint compounds, androstenone, skatole and indole, were analysed as described by Corral et al. (2016). Briefly, sausage fat was separated from the sausage by heating at 100°C. Melted fat (75 mg) was extracted with 1 ml methanol, centrifuged and the methanolic supernatant transferred into a headspace vial and finally, evaporated by a gentle stream of nitrogen. Internal standards, 7-ethylindole and androstanol were added to the melted fat. The boar taint compounds were extracted by SPME-GC/MS using DVB/PDMS fiber (Supelco, Bellefonte, PA, USA). The SPME extraction and GC/MS conditions were described by Corral et al. (2016). The
quantification of boar taint compounds was carried out by comparing their peak areas with that of the internal standards added. The results were expressed as ng of compound/g melted fat.

2.7. Sensory analysis

A group of 25 consumers, 21 women and 4 men with ages ranging from 25 to 46 years old, were chosen based on its ability to describe the presence of abnormal odours and took part in a Free Choice Profile (FCP) analysis as previously reported (Corral et al., 2016). These assessors participated in a previous consumer acceptability test of fermented sausages manufactured with boar back fat where 120 consumers participated. In the first session, the terms used by each consumer describing the differences among sausages were generated by Repertory Grid Method (RGM). The samples were presented in triads and each consumer described the similarities and differences among samples within each triad in their own terms. This method was repeated until all samples were tried. Consumers evaluated the appearance, taste, texture and flavour of dry fermented sausages.

Sensory differences among sausage formulations were analysed by FCP (Williams, & Langron, 1984). In a standardized test room with separate booths, each consumer evaluated his own list of terms by rating the intensity for each sample using 10 cm unstructured scale with the extremes “Not perceived” and “Intense”. 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. The presentation order was balanced to avoid a serving order effect.

2.8. Statistical analysis

Data were analysed using Generalized Linear Model (GML) procedure of statistical software (XLSTAT 2011, v5.01, Addinsoft, Barcelona, Spain). The model included fat type and yeast inoculation as fixed effects and replicates as random effects. 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.

3. Results and discussion

3.1. Physicochemical analysis

Least squares means of physicochemical parameters and microbial counts at 43 and 63 d of processing are shown in Table 1. Weight losses of 33-37 and 40-46% were reached at 43 and 63d, respectively (Table 1). The use of entire male back fat in
dry fermented sausages produced the highest weight losses at both ripening times since entire male back fat had less fat content than the one from gilts and therefore their water content was the highest (Babol & Squieres, 1995, Corral et al., 2016). This fact was observed in the chemical composition of the manufactured sausages just after stuffing into casings at 0 days (Table 1 supplementary material), as sausages manufactured from entire male (MRS and MRS+Y) had less fat content and higher moisture and protein contents than HRS and HRS+Y formulations, also the differences in protein and fat contents were maintained at the two ripening times (Table 1). Nevertheless, moisture was affected by the inoculation of *D. hansenii* (MRS+Y and HRS+Y formulations) as the highest moisture was observed at 63 d as a consequence of yeast growth on sausage surface that regulated water release throughout the drying process. At the two drying times, yeast inoculation produced a slight increase in pH due to the consumption of organic acids by yeast such as lactic acid (Durá, Flores, & Toldrá, 2004), and also a high a_w value explained by the yeast growth on surface as previously mentioned.

Sausage colour was measured and both factors, yeast inoculation and the type of back fat used, affected colour parameters (Table 1). The use of entire male back fat resulted in a decrease in L* parameter at 43 d probably due to its higher moisture content compared to gilt back fat (Babol & Squiere, 1995). Even though no differences were detected on L* parameter at 63 d, the yellowness (b*) was the lowest in MRS formulation at 43 and 63 d probably as a result of its lowest fat content.

*D. hansenii* inoculation clearly showed an antioxidant effect on sausages as observed by the lowest TBARS values of inoculated formulations. Growth of moulds or yeast on sausage surface has shown a protective role against lipid oxidation (Hammes & Knauf, 1994). This result agrees with a previous report by Corral et al. (2015) in dry fermented sausages with reduced salt and fat contents and also inoculated with the same strain of *D. hansenii*.

The results of the texture TPA analysis are shown in Table 2. The use of entire male back fat and yeast inoculation showed an effect mainly on hardness and chewiness at both times. Additionally at 63 d, yeast inoculation affected the springiness and cohesiveness. MRS formulation presented the highest values of hardness and consequently chewiness at 43 and 63 d due to its lower fat content (Babol & Squires, 1995; Corral et al., 2016; Olivares et al., 2010, Mora-Gallego, Guardia, Serra, Gou & Arnau, 2016). Once again, the yeast role on water release and consequently on drying was observed by a higher hardness in MRS than MRS+Y formulation at both ripening times; although, this effect was also observed in gilts formulations (HRS and HRS+Y) but only at 63d. The same trend was observed by Corral et al. (2016).
3.2. Microbial analysis

Despite having added the same starter concentration, the formulation showed slight differences in bacterial counts at 0 days (Table 1 supplementary material). The yeast inoculated formulations showed the highest LAB and Staphylococci counts in meat mixture which resulted in high bacterial counts after 63 d of process. Although high LAB counts were found in yeast inoculated formulations this fact did not result in low pH values. In contrast, the yeast inoculated formulations had significantly high pH values (at 43 and 63 d) due to the consumption of organic acids by yeast (Table 1) (Gori, Mortensen, Arneborg, & Jespersen, 2007).

Regarding yeast counts, the inoculated formulations showed the highest yeast counts at 0, 43 and 63 d although the non-inoculated formulations had also high yeast counts at the two drying times. Microsatellite M13 patterns revealed that D. hansenii P2 was the only yeast recovered from inoculated formulations, whereas in the non-inoculated formulations a mix of isolates consisting of indigenous yeasts as well as D. hansenii P2 was observed (Figure 1 Supplementary Material). Only two yeast colonies different from the inoculated P2 were recovered from non-inoculated formulations at the initial time. At drying times 43 and 63 d a mix of indigenous plus inoculated yeasts was observed in non-inoculated formulations. The percentage of D. hansenii P2 in non-inoculated formulations was higher in HRS than in MRS formulation at 43 d, although at 63 d these percentages were more similar. The most probable origin of the indigenous yeasts could be non-sterile material or surfaces used for pork meat and fat manipulation, whereas the presence of D. hansenii P2 in non-inoculated formulations is probably due to cross-contamination between formulations by air circulation inside the drying chamber (Nielsen, Jacobsen, Jespersen, Koch, & Arneborg, 2008). Moreover, the sampling process used does not separate between sausage surface and internal; however, in the inoculated sausages yeasts are homogeneously distributed in the internal and surface during sausage preparation, whereas in non-inoculated sausages isolated yeasts are primarily concentrated on the surface which is the initial point of contamination (Giaouris et al., 2014). The different yeast count and distribution of D. hansenii P2 between non-inoculated and inoculated sausages would explain the important differences in physico-chemical parameters observed between them. Finally, our results indicate that D. hansenii inoculation did not affect the growth of LAB or Staphylococcus as previously observed by Corral et al. (2014).  

3.3. Boar taint analysis

Levels of boar taint compounds in sausages’ melted fat at 43 and 63 d were analysed by SPME-GC-MS and results are shown in Figure 1. Clearly, the formulations
manufactured with entire male back fat presented high levels of androstenone (383-885 ng/g melted fat) while this steroid was not present in those manufactured with gilt back fat. This finding confirms the sexual origin of androstenone as observed previously (Corral et al., 2016). The androstenone differences found between formulations and drying times may be due to the high variability detected among sausages and replicates although care was taken to achieve a homogeneous mass by previously chopping and mixing the pork back fat (Corral et al., 2016). On the other hand, indole and skatole compounds were present in melted fat from entire male as well as from gilt as they are formed from L-tryptophan degradation in the large intestine of pigs (Zamaratskaia & Squires, 2009). During fermented sausage processing, the highest levels of indole were detected in MRS+Y and HRS formulations at both ripening times. However, skatole concentrations were affected by yeast metabolism as observed at 63 d where inoculated formulations showed the highest concentrations.

Many factors affect the production of skatole and L-tryptophan metabolites, indole or indole-3-acetic acid, in pig intestine such as the availability of tryptophan, red-ox potential, and production of intermediate metabolites being pig diet a determinant factor in the production of indole/skatole ratio (Deslandes, Gariépy, & Houde, 2001). Many bacteria are capable of producing indole and indole acetic acid such as Lactobacillus but few produce skatole (Deslandes et al., 2001). This is the case of Clostridium scatologenes who can degrade tryptophan directly to skatole (Whitfield, 1998). In case of yeasts, few is known about their role in the degradation or generation of skatole or L-tryptophan metabolites and the studies have been focused on deodorization of waste (Yan, Liu, Yuan, Liao, & Li, 2013) or on the production of off-odours in wine (Hoenicke, Borchert, Gru, & Simat, 2002, Maslow, Jeromel, Herjavec, Jagatić Korenika, Mihaljević, & Plavša, 2011). Moreover, further studies should take into account the interaction of bacteria and yeast metabolisms, as this could be an important factor in the differences observed among formulations of dry fermented sausages.

### 3.4. Sensory analysis

A free choice profile analysis (FCP) on the manufactured fermented sausages was performed. This methodology was recently used in dry sausages manufactured with reduced salt and entire male meat demonstrating that the highest differences among samples were explained by the use of different back fat types (entire male vs gilt) (Corral et al., 2016). In conventional descriptive sensory analysis information on the spontaneous sensations that actually occur when a product is eaten is lost (Varela & Ares, 2012). So to avoid these disadvantages and obtain more direct information
about the sensations that consumer perceives while eating, Free Choice Profile (FCP) can be used with the Repertory Grid (RG) method as a previous step. FCP differs from conventional profiling in that each consumer develops an individual list of terms to describe the samples rather than having a common scorecard. It remains similar in that the consumers must be able to detect differences between samples and verbally describe and quantify the perceived attributes (Oreskovich, Klein, & Sutherland, 1991).

Figure 2A shows the descriptors used by each assessor in different colours while the two dimensions GPA obtained and the main mentioned descriptors explained by each dimension are listed next to each dimension in Figure 2B. The total variance explained by the two dimensions was 65.5%. Dimension 1 accounted for 39.2% of the variance and was mainly related to odour terms. On the right side of the plot, terms like acid, fruity, sweet appeared characterizing the inoculated yeast formulations at both ripening times (43 and 63 d). While on the left side, terms like cured, rancid odour and umami and tasty characterized the non-inoculated yeast formulations (43 and 63 d). On the other hand, dimension 2 accounted for 26.3% of the variance, positively related to dry texture terms and negatively related to juicy texture. On the top of the plot, terms like hardness, firm texture, salty, metallic or bitter taste characterized the samples at 63 d. While on the bottom of the plot, terms like juicy, oily appearance, bright, exudate characterized samples at 43 d.

Therefore, the samples appeared distributed along dimension 1 according to yeast inoculation and along dimension 2 according to drying time; however, no clear effect was observed regarding the use of entire male fat. Consumers were able to perceived fruity or sweet odours mainly produced by *D. hansenii* in inoculated formulations. These samples were described as white fat and not as rancid odour, these terms are in accordance to the low TBARS values observed in these yeast inoculated formulations (Table 1) (Corral et al., 2015). Moreover, the non-inoculated yeast sausages (MRS and HRS formulations) presented strong flavour terms such as umami, tasty or cured and rancid odours. This fact is probably the result of the highest lipid oxidation values that were perceived by untrained consumers as strong flavours. In addition, terms like oily texture, fat % or fatty taste were placed on the right side probably related to the presence in the sausages of gilt back fat (HRS formulation) that were characterized by the highest fat content.

As a consequence of the long drying time, consumer perception in term of texture was in agreement to the TPA analysis, as 63 d sausages were perceived more hardness, firm texture and fat/lean cohesiveness than 43 d sausages. As well as, the longest drying time increased the salty, metallic or bitter taste due to low moisture content and the use of KCl as substitute of NaCl. Although, the short drying time
provided juicy or bright sausages. Generally, consumer’s preference is based on long ripening time and high fat contents (Olivares et al., 2010) associated with the development of specific aroma compounds. Regarding salt reduction and substitution with KCl, all formulations were reduced in salt and the KCl bitterness was most appreciated at longer times (63d). Similar results were reported by Mora-Gallego et al., (2016) who confirmed that consumers acceptability is not affected neither the bitterness when salt reductions of 0.5% using KCl were done in dry sausages although this result was obtained in small calibre dry sausages with shorter drying times.

Regarding boar taint perception, animal odour was mainly perceived in formulations at 43d (MRS and MRS+Y formulations) since drying time and cured aroma developed could have masked this odour (Bañón et al., 2003). The androstenone level in MRS and MRS+Y formulations were above its threshold (0.5-1 µg/g) while skatole levels (143-345 ng/g) were close to its threshold of 200-250 ng/g (Font-i-Furnols, 2012). It is not clear which compound, androstenone or skatole, produces the highest impact on boar taint odour (Babol & Squieres, 1995). Androstenone seems to have a greater influence on boar flavour while skatole on boar odour explained by their polarity (Meier-Dinkel, Gertheiss, Müller Wesoly, & Mörlein, 2015). Androstenone (non-polar and mostly fat soluble) is retained in the fat matrix being more stable and longer lasting flavour whereas skatole (water and fat soluble) is more easily released (Lundström et al., 2009). In summary, both compounds would be responsible for the abnormal odour terms described however, the variability explained by the panel during the FCP analysis would not be due to the presence of this animal odour as no clear distinction was detected between HRS and MRS samples. According to our results, consumers clearly distinguished samples according to drying time and \textit{D. hansenii} inoculation while the use of entire/gilt back fat was not highly perceived. The sensory analysis demonstrates the effectiveness of yeast inoculation to mask boar taint perception although sausages with high levels of boar taint compounds could still evoke abnormal odours.

4. Conclusion

\textit{D. hansenii} inoculation and long ripening times are able to compensate and, in some instances, improve the negative effects produced by the use of entire male back fat in dry fermented sausage processing. Moisture content and $a_w$, hardness, and lipid oxidation were apparently controlled by yeast inoculation. \textit{D. hansenii} growth on the surface regulated the water release during ripening, reduced hardness and chewiness in entire male back fat sausages and resulted with a similar texture to sausages manufactured with gilt back fat. In addition, the inhibition of the lipid oxidation in yeast
inoculated sausages was observed by the low TBARS values, presence of descriptors such as white fat and fatty taste together with the development of fruity odour notes. In terms of sensory analysis, the long ripening and yeast inoculation masked boar taint flavour and provided fruity, and less oxidized fatty sausages.

Acknowledgements

Financial support from AGL 2012-38884-C02-01 from MINECO (Spain) and FEDER funds are fully acknowledged. The authors are grateful to S. M. Bascuas for technical assistance.

References


FIGURE LEGENDS

Figure 1. Concentration of boar taint compounds (ng/g melted fat, mean ± sem) in reduced salt fermented sausages at A) 43 days and B) 63 days of drying manufactured with: entire male back fat (MRS); entire male back fat and inoculated with \textit{D. hansenii} (MRS+Y); gilt back fat (HRS) and gilt back fat and inoculated with \textit{D. hansenii}. Different letters in the same group and time indicate significant differences among formulations at P<0.05.

Figure 2. A) Correlation of the GPA analysis of the free choice profile data. B) Two dimension GPA plot of the differences among reduced salt dry fermented sausages manufactured with back fat (from gilt or entire male) and \textit{D. hansenii} inoculation. 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 1 Supplementary data. Electrophoretic patterns of minisatellite M13 PCR amplification of strains isolated at 0 d, 43 and 63 d. In each photograph: first and last lanes represent “M” 100 pb ladder (Invitrogen, Carlsbad, CA, USA) followed by 15 isolated strains in sausage formulations. Sausage formulations manufactured with: entire male back fat (MRS) and gilt back fat (HRS) and the corresponding formulations inoculated with \textit{D. hansenii}. 
**Table 1.** Effect of back fat (from gilt or entire male) and *D. hansenii* inoculation on physicochemical parameters and microbial counts in dry fermented sausages at different drying times (43 and 63 days).

<table>
<thead>
<tr>
<th></th>
<th>43 days</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>63 days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRS</td>
<td>MRS+Y</td>
<td>HRS</td>
<td>HRS+Y</td>
<td>RMSE</td>
<td>P&lt;sub&gt;T&lt;/sub&gt;</td>
<td>P&lt;sub&gt;Y&lt;/sub&gt;</td>
<td>P&lt;sub&gt;FxY&lt;/sub&gt;</td>
<td>MRS</td>
<td>MRS+Y</td>
<td>HRS</td>
</tr>
<tr>
<td>pH</td>
<td>4.68 b</td>
<td>5.07 a</td>
<td>4.84 ab</td>
<td>5.07 a</td>
<td>0.20 ns</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>4.89 bc</td>
<td>4.95 a</td>
<td>4.87 c</td>
</tr>
<tr>
<td>Aw&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.907</td>
<td>0.915</td>
<td>0.906</td>
<td>0.914</td>
<td>0.006 ns</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>0.846 b</td>
<td>0.874 a</td>
<td>0.853 b</td>
</tr>
<tr>
<td>Weight losses (%)</td>
<td>35.50 b</td>
<td>33.46 c</td>
<td>32.48 d</td>
<td>32.48 d</td>
<td>0.56 ***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>45.93 a</td>
<td>43.62 b</td>
<td>41.65 c</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>24.02 b</td>
<td>23.54 b</td>
<td>29.20 a</td>
<td>30.76 a</td>
<td>1.83 ***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>27.48 b</td>
<td>28.42 b</td>
<td>32.12 a</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>27.24 ab</td>
<td>27.92 a</td>
<td>25.02 bc</td>
<td>23.57 ab</td>
<td>1.59 ***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>31.59 a</td>
<td>31.62 a</td>
<td>29.09 b</td>
</tr>
<tr>
<td>L*</td>
<td>50.97 b</td>
<td>53.93 a</td>
<td>53.74 a</td>
<td>55.09 a</td>
<td>1.53 **</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>46.53</td>
<td>48.60</td>
<td>49.74</td>
</tr>
<tr>
<td>a*</td>
<td>18.25</td>
<td>18.15</td>
<td>18.33</td>
<td>18.43</td>
<td>0.55 ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>16.86</td>
<td>17.65</td>
<td>17.37</td>
</tr>
<tr>
<td>b*</td>
<td>6.63 c</td>
<td>7.20 ab</td>
<td>7.05 b</td>
<td>7.46 a</td>
<td>0.27 **</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>6.03 b</td>
<td>6.50 ab</td>
<td>6.52 a</td>
</tr>
<tr>
<td>TBARS (mg/kg d.m.)</td>
<td>1.32 b</td>
<td>0.40 b</td>
<td>2.26 a</td>
<td>0.47 b</td>
<td>0.52 **</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>1.96 b</td>
<td>0.41 c</td>
<td>2.92 a</td>
</tr>
<tr>
<td>LAB (log cfu/g d.m.)</td>
<td>7.99 b</td>
<td>8.08 b</td>
<td>7.96 b</td>
<td>8.25 a</td>
<td>0.09 *</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>7.91 ab</td>
<td>8.13 a</td>
<td>7.89 b</td>
</tr>
<tr>
<td>Staphylococci (log cfu/g d.m.)</td>
<td>5.08 a</td>
<td>4.78 b</td>
<td>4.54 b</td>
<td>4.88 ab</td>
<td>0.18 **</td>
<td>ns</td>
<td>***</td>
<td>5.26 b</td>
<td>5.41 ab</td>
<td>4.89 b</td>
<td>5.89 a</td>
</tr>
<tr>
<td>Yeast (log cfu/g d.m.)</td>
<td>4.62 b</td>
<td>6.51 a</td>
<td>3.86 b</td>
<td>6.56 a</td>
<td>0.27 ns</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>5.27 b</td>
<td>6.19 a</td>
<td>5.32 b</td>
</tr>
</tbody>
</table>

MRS: sausages manufactured with entire male back fat; MRS+Y: sausages manufactured with entire male back fat and inoculated with *D. hansenii*; HRS: sausages manufactured with gilt back fat; HRS+Y: sausages manufactured with gilt back fat and inoculated with *D. hansenii*.

<sup>1</sup>RMSE: root mean square error.

<sup>2</sup>P<sub>T</sub>: P value of type of fat effect, P<sub>Y</sub>: P value of *D. hansenii* inoculation effect, P<sub>FxY</sub>: P value of interaction between type of fat and *D. hansenii* inoculation effects. ***: P<0.001; **: P<0.01; *: P<0.05; ns: P>0.05. Different letters in the same row at each processing time indicate significant differences among batches.

<sup>3</sup>Aw. Water activity, TBARS: Thiobarbituric acid reactive substances, LAB: Lactic acid bacteria.
Table 2. Effect of back fat (from gilt or entire male) and *D. hansenii* inoculation on texture parameters in dry fermented sausages at different drying times (43 and 60 days).

<table>
<thead>
<tr>
<th></th>
<th>43 days</th>
<th>63 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRS</td>
<td>MRS+Y</td>
</tr>
<tr>
<td>Hardness (N)(^3)</td>
<td>99.97 a</td>
<td>79.07 b</td>
</tr>
<tr>
<td>Adhesiveness (N.s)</td>
<td>-1.83 b</td>
<td>-1.12 b</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.69 b</td>
<td>0.72 b</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.65 a</td>
<td>0.67 ab</td>
</tr>
<tr>
<td>Chewiness (N)</td>
<td>45.04 b</td>
<td>38.19 ab</td>
</tr>
</tbody>
</table>

MRS: sausages manufactured with entire male back fat; MRS+Y: sausages manufactured with entire male back fat and inoculated with *D. hansenii*; HRS: sausages manufactured with gilt back fat, HRS+Y: sausages manufactured with gilt back fat and inoculated with *D. hansenii*.

\(^1\)RMSE: root mean square error.

\(^2\)P\(_f\): *P* value of type of fat effect, P\(_Y\): *P* value of *D. hansenii* inoculation effect, P\(_{fY}\): *P* value of interaction between type of fat and *D. hansenii* inoculation effects. ***: *P*<0.001; **: *P*<0.01; *: *P*<0.05; ns: *P*>0.05. Different letters in the same row at each processing time indicate significant differences among batches.

\(^3\) N: Newtons, N.s. Newtons per second.
Figure 2

A) Colour (21), Cured odour (8), Rancid odour (6), Umami taste (6), Fibrous texture (5), Tasty (2)

B) Hardness (22), salty taste (15), firm texture (18), acid taste (11), fat/lean cohesiveness (9), metallic taste (7), bitter taste (6), cheesy (5).

Colour (21)
Cured odour (8)
Rancid odour (6)
Umami taste (6)
Fibrous texture (5)
Tasty (2)

Oily texture (14)
Acid odour (13)
Fruity odour (7)
Chewy (6)
Fat % (6)
White fat (6)
Sweet odour (3)

Juicy (12), bright (7), oily appearance (7), animal odour (7), exudate (4).
Figure suplementary

Non inoculated Yeast

Yeast Inoculated

Entire Male (MRS) Gilt (HRS)

Initial

43d

63d

Entire Male (MRS)

Gilt (HRS)
Table 1. Supplementary material. Effect of back fat (from gilt or entire male) and *D. hansenii* inoculation on physicochemical parameters and microbial counts in dry fermented sausages at 0 days.

<table>
<thead>
<tr>
<th></th>
<th>MRS</th>
<th>MRS+Y</th>
<th>HRS</th>
<th>HRS+Y</th>
<th>RMSE</th>
<th>Pf</th>
<th>Py</th>
<th>PfxY</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.78 b</td>
<td>5.82 ab</td>
<td>5.84 a</td>
<td>5.82 a</td>
<td>0.04</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>aw</td>
<td>0.961 a</td>
<td>0.960 ab</td>
<td>0.958 a</td>
<td>0.958 a</td>
<td>0.002</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>62.98 a</td>
<td>62.79 ab</td>
<td>61.23 ab</td>
<td>60.11 b</td>
<td>1.67</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>16.71 b</td>
<td>17.04 b</td>
<td>20.03 a</td>
<td>20.47 a</td>
<td>1.14</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>18.44 ab</td>
<td>18.51 a</td>
<td>17.98 ab</td>
<td>17.85 b</td>
<td>0.53</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>L*</td>
<td>58.98 b</td>
<td>59.29 b</td>
<td>60.73 a</td>
<td>61.57 a</td>
<td>2.84</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>a*</td>
<td>14.34 a</td>
<td>14.18 a</td>
<td>12.18 b</td>
<td>14.04 a</td>
<td>1.03</td>
<td>***</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>b*</td>
<td>11.35 b</td>
<td>11.81 ab</td>
<td>11.34 b</td>
<td>12.22 a</td>
<td>0.51</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>TBARS (mg/kg d.m.)</td>
<td>0.42 a</td>
<td>0.34 ab</td>
<td>0.39 a</td>
<td>0.26 b</td>
<td>0.07</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>LAB (log cfu/g d.m.)</td>
<td>5.75 bc</td>
<td>6.02 a</td>
<td>5.65 c</td>
<td>5.90 ab</td>
<td>0.12</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>Staphylococci (log cfu/g d.m.)</td>
<td>5.51 b</td>
<td>5.72 a</td>
<td>5.24 c</td>
<td>5.56 b</td>
<td>0.09</td>
<td>***</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>Yeast (log cfu/g d.m.)</td>
<td>0.36 b</td>
<td>0.19 a</td>
<td>0.35 b</td>
<td>0.16 a</td>
<td>0.62</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
</tr>
</tbody>
</table>

MRS: sausages manufactured with entire male back fat; MRS+Y: sausages manufactured with entire male back fat and inoculated with *D. hansenii*; HRS: sausages manufactured with gilt back fat, HRS+Y: sausages manufactured with gilt back fat and inoculated *D. hansenii*.

1RMSE: root mean square error.

2Pf: P value of the type of fat effect, Py: P value of *D.hansenii* inoculation effect, PfxS: P value of interaction between type of fat and *D. hansenii* inoculation 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.