



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

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## 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.

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## 1. Introduction

Boar taint is an off odour/flavour found in pork from entire male pigs described as urine, sweat and faecal (Lundstöröm, 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 to 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, Borrissier-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 et al., 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 and 12% boar fat was tolerable (Walstra, 1974). 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; Škrlep 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, and 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

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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 min 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 *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 10 mm 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 *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus* and *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 *D. hansenii* P2 (Cano-García et al., 2013) suspension were added to the inoculated formulations (HRS + Y and MRS + Y) at final

concentration of  $5 \times 10^6$  cells/g. The mixture of each formulation was kept at 3–5 °C for 24 h 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 10 °C 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 ( $3 \times 2 \times 2$ ) 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 –20 °C 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 °C 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 <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 (CIE Lab L\*, a\*, b\*), moisture, protein and fat was performed as described by Corral, Salvador, and 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, and 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

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