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Impact of salt reduction on biogenic amines, fatty acids, microbiota, texture and sensory profile in traditional blood dry-cured sausages

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ABSTRACT

Meat industry needs to reduce salt in their products due to health issues. The present study evaluated the effect of salt reduction from 6% to 3% in two Portuguese traditional blood dry-cured sausages. Physico-chemical and microbiological parameters, biogenic amines, fatty acids and texture profiles and sensory panel evaluations were considered. Differences due to salt reduction were perceptible in a faint decline of water activity, which slightly favoured microbial growth. Total biogenic amines content ranged from 88.86 to 796.68 mg kg^{-1} fresh matter, with higher amounts, particularly of cadaverine, histamine and tyramine, in low-salt products. Still, histamine and other vasoactive amines remained at low levels, thus not affecting consumers' health. Regarding fatty acids, no significant differences were observed due to salt. However, texture profile analysis revealed lower resilience and cohesiveness in low-salt products, although no textural changes were observed by the sensory panel. Nevertheless, low-salt sausages were clearly preferred by panellists.

1. Introduction

Health issues such as cardiovascular disease are often due to dietary habits. From an epidemiological standpoint, cardiovascular diseases have a substantial incidence, affecting a significant part of the world population, which according to the World Health Organisation (WHO) is one of the main causes of death (2015).

Among other factors, an excessive salt consumption is pointed out as a major risk factor, due to its direct relation with blood pressure disturbances (Ruusunen & Puolanne, 2005), that can lead to hypertension issues. Although the recommended salt intake ranges from 3.7 to 5.8 g per day (Bibbins-Domingo et al., 2010), it is estimated that this might be much higher, from 8 to 10 g (Desmond, 2006). For this reason, WHO recommendations are to reduce salt consumption and its negative impact on consumers' health (http://ec.europa.eu/health/nutrition_physical_activity/high_level_group/nutrition_salt_en.htm). Recent studies have suggested that reducing 3 g per day in salt intake could represent a decrease in the number of annual deaths between 44,000 and 92,000, as well as a saving about 10–24 billion dollars in health care per year, in the U. S. alone (Bibbins-Domingo et al., 2010). Whether by adding less salt during food gastronomic preparation or avoiding processed food containing high salt levels, consumers' concerns have resulted on a generalised trend to lower salt consumption (Guardia, Guerrero, Gelabert, Gou, & Arnau, 2006).

In some countries, meat and meat products can account for over 20% of the salt dietary intake (Desmond, 2006; Guardia et al., 2006). Its effects in reducing water activity and antimicrobial activity, increasing water retention capacity (thus enabling protein solubilisation) and decreasing the activity of some enzymes, are the main reasons, which make salt essential for the meat industry

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(Desmond, 2006). From a sensory perspective, salt enhances the products' texture, as well as the characteristic salty taste associated to meat products.

For this reason, production of low-salt content dry-cured sausages must assure not only safe and stable products, but also that consumers' acceptability is not affected. In previous studies, lower salty taste and less intensive colour were perceived when less salt was used (Corral, Salvador, & Flores, 2013; Zanardi, Ghidini, Conter, & Ianieri, 2010). On the other hand, due to its oxidative properties, different salt contents can also lead to changes in lipid oxidation profile. Another problem related to salt reduction concerns the increased microbial decarboxylase activity that can lead to higher levels of biogenic amines (Roseiro, Santos, Sol, Silva, & Fernandes, 2006; Suzzi & Gardini, 2003). There are two main reasons why the presence of biogenic amines is related to meat and meat products' quality and safety. On one hand, biogenic amines (such as tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine and tyramine) arise as a consequence of microbial growth. For this reason they are often used as good chemical indicators of products' hygienic quality (Stadnik & Dolatowski, 2010). On the other hand, the biological activity of biogenic amines (mainly histamine, tyramine and tryptamine) has also been related to the occurrence of adverse reactions in the cardiovascular and neurological systems, making their ingestion an important health concern (Suzzi & Gardini, 2003).

In Mediterranean countries, including Portugal, Spain and Italy, traditional dry-cured sausages are widely appreciated, frequently still being manufactured in small processing units, according to traditional practices specific for each geographic area (Elias & Carrascosa, 2010; Moretti et al., 2004; Olivares, Navarro, Salvador, & Flores, 2010). In Portugal, Alentejo is one of the most important regions for dry-cured sausages, where products like "Chouriço Preto" and "Paio Preto" stand out for their unique characteristics, which result from the use of Alentejano pig breed meat, the addition of blood (an important sensitive ingredient) and the manufacture know-how.

A previous study, using a different kind of sausages, has already highlighted that salt content may be significantly reduced without compromising food safety nor depreciating quality (Laranjo et al., 2016). Therefore, the present work intended to evaluate the effect of lower salt contents in these sausages regarding microbial, sensory and texture characteristics, as well as on fatty acids and biogenic amines profile throughout the ripening process.

2. Materials and methods

2.1. Sausage technology and sampling procedures

Three independent batches (30 kg of meat batter each) of "Chouriço Preto" (CP) and "Paio Preto" (PP) were produced in a local factory using commercial hybrid Iberian x Duroc pig breed meat.

Meat was initially cut into pieces measuring about 25 mm and then mechanically minced and mixed with salt, red pepper (*Capsicum annuum* L.) paste (2.5% w/w), water reconstituted (1:2 (w/v)) dried blood powder (4% v/w), garlic (*Allium sativum* L.) paste (1% w/w), disodium diphosphate (0.03% w/w), pentasodium triphosphate (0.03% w/w), nitrate (0.003% w/w) and nitrite (0.003% w/w). Nitrates and nitrites have been added in the form of the commercial additive NITROS 5/5 (Formulab, Portugal). Since red pepper and garlic pastes both have in its original composition 17% salt, salt was added to the mixture, so that final concentration in end-product was 3 and 6%.

The meat mixture was let to rest under refrigeration at 5 °C and 90% relative humidity for a period of 48 h, after which it was divided in two sub-batters (of approximately 15 kg each) and stuffed in natural casings obtained from pig intestine. Two different diameters were used: 36–38 mm from small intestine for CP (tied in a horseshoe shape) and 50–55 mm from large intestine (rectum) for PP.

After stuffing, sausages were kept in a smoking room with smoke generated from oak wood (*Quercus ilex* L.) during 48 h, with temperatures ranging from 18 to 24 °C and 30–60% relative humidity. After this smoking stage, sausages were dried under controlled conditions in cure chambers at 9 °C and 80–85% relative humidity. On average, the drying stage took about 14 or 30 days for CP and PP, respectively, until 35% weight losses were reached.

Three samples were collected at three different ripening stages: after stuffing, at 20% weight loss and end-product (35% weight loss). All samples were analysed for pH, water activity (a_w), microbiological parameters and biogenic amines content. End-products were also analysed for their fatty acids, texture profile and sensory attributes.

2.2. Physicochemical analyses

For pH assessment, sausages casings were removed and values measured with a Crison 507 (Barcelona, Spain) pH-meter following the procedures described in ISO 2917 (1999). Water activity was determined with a hygrometer (Hygroskop Rotronic DT, Zurich, Switzerland) equipped with a WA-40 probe at 25 °C. Salt content of end-products was confirmed through determination of chlorides according to the Volhard method as described in ISO 1841-1 (1996).

2.3. Microbiological analyses

Microbiological analyses were carried out according the analytical protocols described by Laranjo et al. (2015). Briefly, decimal dilution series were prepared in buffered peptone water (Scharlau, Spain), plated and incubated as follows: mesophiles in Tryptone Glucose Extract (TGE) Agar (Scharlau, Spain) at 30 °C for 48 h; lactic acid bacteria (LAB) in de Man, Rogosa and Sharpe (MRS) Agar (Scharlau, Spain) at 30 °C for 48 h under anaerobic conditions in an AnaeroJar (Oxoid, UK) using an AnaeroGen sachet (Oxoid, UK); enterobacteria in Violet Red Bile Glucose (VRBG) Agar (Biokar, France) at 30 °C for 48 h; enterococci in Slanetz and Bartley Agar (Biokar, France) at 37 °C for 48 h; staphylococci in Mannitol Salt Agar (MSA) (Biokar, France) at 37 °C for 48 h; yeasts and moulds in Rose Bengal Chloramphenicol Agar (Scharlau, Spain) at 25 °C for 5 days.

Enumeration of *Campylobacter* spp. was performed according to the ISO 10272-2 (2006). *Escherichia coli* counts followed the procedures described in ISO 16649-2 (2012a). *Listeria monocytogenes* enumeration was performed according to standard procedures described in ISO 11290-2 (2014). Detection of *Salmonella* spp. was performed according to the ISO 6579 (2002).

All microbiological analyses were performed in triplicate and the results expressed in log cfu g^{-1}, while for *Salmonella* spp. the presence/absence was denoted by the growth of typical colonies with isolation and biochemical identification.

2.4. Biogenic amine analysis

Biogenic amines quantification was performed according to the experimental protocol described by Roseiro et al. (2006).

Four grams of sample previously homogenized were extracted with 40 mL perchloric acid aqueous solution (0.4 M). The extract was then centrifuged for 10 min at 800*g* and the supernatant was filtered. Resulting pellet was extracted once more and supernatants were combined. Internal standard (1,7-diaminoheptane)