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Characterisation of "Catalão" and "Salsichão" Portuguese traditional sausages with salt reduction

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ABSTRACT

The present study evaluated the effect of salt reduction on traditional dry-cured sausages' safety, quality and product acceptance, comprising physicochemical and microbiological parameters, biogenic amines, fatty acids, texture profile and sensory analysis. According to our results, salt content had a major effect on microbiological counts, although not compromising the products' safety. Marked differences were identified regarding biogenic amines, in particular for histamine, tyramine and cadaverine, which were detected in larger amounts in products with 3%. Moreover, significant differences in the fatty acids profile have also been found, but only in less abundant components such as linoleic, lauric and heneicosanoic acids. Texture profile analysis of low-salt products, revealed a decrease in hardness and chewiness, along with an increase in adhesiveness values. Sensory evaluations revealed that despite the less intense aroma, products with 3% salt, had a more balanced salt perception. Our results suggest that salt content may be reduced to 50% in dry-cured products, with the obvious health-related advantages.

1. Introduction

Sodium chloride has a number of technological benefits, which make it an essential ingredient for food industry and meat industry in particular. Due to its antimicrobial properties, it inhibits both the growth of the microbiota involved in products' spoilage and pathogens that might pose consumers' health at risk (Slobodan & Vesna, 2011). Furthermore, it also has the ability to enhance meat products' texture, colour and taste (Aaslyng, Vestergaard, & Koch, 2014; Corral, Salvador, & Flores, 2013; Tobin, O'Sullivan, Hamill, & Kerry, 2013).

The correlation between excessive salt consumption and high blood pressure has been repeatedly demonstrated over the years (MacGregor & de Wardener, 2002). Hypertension in its turn increases the risk of developing cardiovascular diseases, which is known to be the leading cause of global death (WHO, 2011). A similar scenario has been observed in Portugal, where cardiovascular diseases are among the primary causes of morbidity, mortality and disability (Ribeiro, Furtado, & Pereira, 2013), as a result of several risk behaviours, namely dietary habits. Furthermore, salt in processed meats is considered an important risk factor for stomach cancer (Key et al., 2004). In face of its negative impact on public health, recommendations have been made in order to reduce salt consumption (Bibbins-Domingo et al., 2010; EC, 2012; WHO, 2002). Bibbins-Domingo et al. (2010) predicted the effect of dietary salt reduction on the development of cardiovascular diseases, and estimated that even moderate reductions could substantially diminish coronary heart disease, strokes and the annual number of deaths. For this reason, meat products are frequently associated with high salt levels, making them unappealing from the nutritional standpoint, leading to an increasing pressure over meat industry to reduce the amounts of added salt.

Despite the health benefits associated to salt reduction, some issues on products' safety and sensory properties can arise (Benedini, Parolari, Toscani, & Virgili, 2012). Regarding sensory properties, products' colour is affected by the reduction of salt content, where a paler colour is usually observed (Aaslyng et al., 2014; Tobin et al., 2013). Moreover, a less

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salty taste, as well as a weaker characteristic flavour, has also been reported (Corral et al., 2013; Ruusunen & Puolanne, 2005). In Italian salami, salt reduction induced a significant increase in lipid oxidation (Zanardi, Ghidini, Conter, & Ianieri, 2010). On the other hand, from the technological standpoint, small amounts of salt influence meat proteins' ability to retain water, therefore affecting texture, as well as the binding capacity of meat and fat (Desmond, 2006). Moreover, microbial loads are prone to increase, compromising products' stability, due to the lack of the salt's antibacterial effect. The released bacterial decarboxylases can remove the amino acids carboxyl group, leading to the accumulation of biogenic amines, which are often associated to consumers' adverse reactions (Alvarez & Moreno-Arribas, 2014).

In Portugal, the production of dry-cured sausages is a deeply rooted practice, often related with cultural practices that are characteristic of each region. For this reason, apart from food safety issues, a reduction in salt content must be carefully evaluated in order to prevent products' mischaracterisation.

Taking the abovementioned issues into account, the present study evaluated the effect of a 50% reduction in salt content in two Portuguese traditional products: "Catalão" and "Salsichão", without depreciating the products' sensory characteristics. For that purpose, physicochemical and microbiological parameters, biogenic amines, fatty acids (FA) profiles and texture profile analysis (TPA) were assessed as function of the amount of added salt. Sensory evaluation was also performed in order to determine the impact of salt reduction on consumers' acceptability.

2. Materials and methods

2.1. Dry-cured sausages processing and sampling procedures

Meat obtained from hybrid Iberian × Duroc pigs, was manually cut into large pieces and then mechanically minced (5.0 mm × 5.0 mm) and mixed with minced (5.0 mm × 5.0 mm) backfat (5%), white wine (3,5%), sodium chloride (NaCl), black pepper (*Piper nigrum L.*) (0.15%), white pepper (0.15%), cumin (*Cuminum cyminum L.*) (0.10%), disodium diphosphate (0.04%), pentasodium triphosphate (0.04%), $NaNO_3$ (0.003%) and KNO_2 (0.003%). Nitrates and nitrites have been added in the form of the commercial additive NITROS 5/5 (Formulab, Portugal). Two meat batters were processed differing only in salt content: low-salt (3% NaCl) and regular-salt (6% NaCl).

The batters were left for two days under refrigeration at 5 °C and 90% relative humidity for maturation purposes.

Salted natural casings were desalted as follows: casings were washed with running water and maintained in 3% acetic acid (*v*/v) for 1 h. After draining, washed casings may be stored at 7 °C for a maximum of 48 h. Immediately before stuffing, casings were rehydrated in 2% acetic acid (*v*/v) at room temperature for 30 min and then for another 45 min in water.

Each meat batter was then divided and stuffed into cleaned natural casings with 36–38 mm (pig small intestine) for "Catalão" and 50–55 mm (pig large intestine, rectum) for "Salsichão". Each sausage (end-product) weighted around 150 g for "Catalão" and around 300 g for "Salsichão".

Chemical and microbiological characteristics of raw meat, salt (only microbiological parameters) and casings have been studied before (Elias & Carrascosa, 2010).

The drying process took place in an environmental controlled chamber (7 °C and 80–85% relative humidity) until a 35% weight loss was reached, which took about 18 days or 35 days for "Catalão" and "Salsichão", respectively.

Three independent batches were processed, with duplicate samples being collected at three different ripening stages: 0% (meat batter, immediately before stuffing), 20% (half-cured product) and 35% weight loss (end-product). Sausages were immediately analysed for physicochemical and microbiological parameters, TPA and sensory evaluation, whereas samples for determining biogenic amines and FA profiles were immediately frozen and kept at −20 °C until they were analysed. Textural and sensory analyses were performed only for end-products.

2.2. Physicochemical parameters

Dry-cured sausages salt content was confirmed following the analytical protocol described in ISO 1841-2 (1996). After the casings were removed, pH was determined using a Crison 507 pH-meter (Barcelona, Spain) according to procedures described in ISO 2917 (1999) and water activity (a_w) measured with a hygrometer (Hygroskop Rotronic DT, Zurich, Switzerland) equipped with a WA-40 probe at 25 °C.

2.3. Microbiological analyses

Microbial analyses were performed following the analytical protocols described by Laranjo et al. (2015). For that purpose, 10 g of each sample were diluted into 90 mL of peptone water (BDH Prolabo), decimal dilutions prepared, pour-plated and incubated as follows: mesophilic bacteria in Tryptone Glucose Extract (TGE) Agar (Scharlau) at 30 °C for 48 h; lactic acid bacteria (LAB) in de Man, Rogosa and Sharpe (MRS) Agar (Scharlau) at 30 °C for 48 h under anaerobic conditions in an AnaeroJar (Oxoid) using an AnaeroGen sachet (Oxoid); enterobacteria in Violet Red Bile Glucose Agar (VRBG) (Biokar) at 30 °C for 48 h; enterococci in Slanetz and Bartley Agar (Biokar) at 37 °C for 48 h, staphylococci in Mannitol Salt Agar (MSA) (Biokar) at 37 °C for 48 h; yeasts and moulds in Rose Bengal Chloramphenicol (RBC) (Scharlau) at 25 °C for 48 h. *Campylobacter* spp. enumeration was performed with 10 g of sample homogenised in 90 mL of supplemented Nutrient Broth (Oxoid) and inoculated in *Campylobacter* Blood Free Selective Medium Agar (LABM). Plates were incubated for 48 h at 41.5 °C under microaerophilic conditions in an AnaeroJar (Oxoid) using a GENBox microaer sachet (bioMérieux). For *E. coli* counts 10 g of the sample were homogenised in Tryptose Phosphate Broth (Oxoid) and an aliquot was inoculated in Tergitol 7 (Biokar) supplemented with triphenyltetrazolium chloride (TTC) (Biokar). Incubation was carried out 44 °C for a 24 h period. All microbiological analyses were carried out in triplicate and results expressed in log CFU/g.

For *Salmonella* spp. detection a pre-enrichment was performed homogenising 25 g of sample in 225 mL of peptone water (Scharlau) and incubated at 37 °C for 18 h. After this selective enrichment step, the resulting cell suspension was inoculated both in Rappaport Vassiliadis Broth (Scharlau) and in Muller-Kauffmann Tetrathionate (MKTT) Broth (Scharlau) supplemented with iodine solution and Brilliant Green-Novobiocin (Scharlau). After a 24 h incubation period, at 42.5 °C and 37 °C, respectively, both cultures were inoculated by surface streaking cultures in Xylose Lysine Deoxycholate (XLD) Agar (Scharlau) and Hektoen Enteric Agar (Scharlau) and finally incubated at 37 °C for 24 h. *Salmonella* spp. presence was assessed by the growth of typical colonies, their subsequent isolation and identification being performed according to ISO 6579 (2002).

2.4. Biogenic amines profile

The analytical protocol described by Roseiro, Santos, Sol, Silva, and Fernandes (2006) was followed for biogenic amines extraction and quantification. Four grams of previously homogenised samples were extracted with perchloric acid aqueous solution (0.4 M) and extracts were centrifuged (10 min at 800*g*). The supernatant was filtered and the resulting pellet extracted once more. Supernatants were combined, the internal standard (1,7-diaminoheptane) was added and final volume adjusted to 50 mL. Biogenic amines derivatization was carried out using dansyl chloride in alkaline medium. The unreacted dansyl chloride was then removed with ammonia and filtered through an Acrodisc membrane 25 mm GHP, GF 0.45 μm (Gelman Sciences, Inc.). An aliquot of 20 μL of the biogenic amines extract was injected for

chromatographic separation. The HPLC system was composed by an Alliance Separation Module 2695 (Waters, Milford, MA), coupled to a Dual λ UV/Vis Detector 2487 (Waters, Milford, MA) set for 254 nm wavelength. Chromatographic separation was carried out with a Spherisorb 5 μm ODS2 column with 4.0 × 125 mm (Waters, Germany) column and a gradient elution program combining aqueous ammonium acetate solution 0.1 M as solvent A (Panreac, Barcelona, Spain) and acetonitrile as solvent B (Panreac, Barcelona, Spain). The gradient began at 50% and finished at 90% acetonitrile in 19 min with a 10 min equilibration step before the next analysis.

2.5. Fatty acids profile

After processing, sausages were minced, lyophilized and stored under refrigeration at 4 °C in glass flasks until further analysis. FA were extracted by accelerated solvent extraction (ASE) means using a 34 mL stainless steel extraction cell (fitted with two cellulose filters) coupled to a Dionex 100 system. For that purpose, 300 mg of the lyophilized sample were blended with 6 g of drying agent (Diatomaceous Earth, Dionex Corporation, California) and loaded into extraction cell. Lipidic fraction was extracted twice by static extraction cycles (5 min each) using a chloroform/methanol (60:40 (*v*/v)) solution (Merck, Darmstadt, Germany) containing 100 mg/L of 2,6-Di-*tert*-butyl-4-methylphenol (BHT), to prevent oxidation, at 100 °C and 12.4 MPa. Solvent was removed using a rotavapor R-114 coupled to a B-480 bath, a Vacobox B-177 and a vacuum controller B-720 (all from Buchi). Solid residue was then suspended in 1 mL chloroform and an aliquot (100 μL) was once more dried under a stream of nitrogen and the residue saponified in the presence of methanolic NaOH 0.5 N solution (70 °C, 15 min). Samples fatty acids esterification was performed using boron-trifluoride-methanol (10 g BF_3/L CH_3OH, Merck-Schuchardt, Germany), according to Morrison and Smith (1964). Quantification of fatty acids methyl esters (FAMEs) was accomplished using a GC system (Hewlett Packard 6890 Series) equipped with split-splitless injector, an auto-sampler, a flame-ionisation detector (FID), an Omegawax 320 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness, Supelco, Bellefonte, PA, USA) and HPChem software (2002). During chromatographic analysis, oven temperature was raised from 140 °C to 240 °C at 4 °C/min rate and injector and detector temperatures were set to 250 and 270 °C, respectively. Helium was used as carrier gas and was flowing through the system at a 1.2 mL/min rate. For peaks identification a 37-component FAME Mix standard (Supelco) was used as reference complemented with the determination of Kovats indexes (data not shown). For each sample, the relative fatty acid composition was quantified.

2.6. Texture profile analysis

TPA was accomplished in accordance to Caine, Aalhus, Best, Dugan, and Jeremiah (2003) and Honikel (1997) procedures. For that purpose, five 1 cm thick slices from three different sausages were used. Double compression cycle tests were carried out at room temperature (20 °C ± 1 °C) using a cylindrical flat-ended plunger (with a diameter of 1.13 cm and an area of 1 cm^2) coupled to a Stable Micro System TA-Hdi (Stable Micro Systems, Godalming, England). Force/time curves obtained for double 50% compressions at 1 mm/s speed, separated for 5 s intervals each were used to determine hardness, adhesiveness, springiness, cohesiveness, resilience and chewiness.

2.7. Sensory evaluation

Ten qualified tasters (five women and five men with ages between 35 and 60 years) trained in accordance with ISO 8586-1 (1993) were selected. Sensory evaluation took place in a room especially prepared for that purpose following the methodology described by ISO 8589 (2012). Thirty minutes prior each session, sausages were sliced (3 mm thick) and three slices were randomly disposed in white dishes. Each dish was identified with a random three digit number. Moreover, neutral water and crackers were also provided so tasters could rinse their mouths between evaluations. Each sample was rated in triplicate. Tasters were asked to rate samples' colour intensity, off colours, aroma intensity, off aromas, hardness, succulence, flavour intensity, off flavours, salt perception and overall acceptability based on a 0 ("minimum perception") to 100 ("maximum perception") quantitative descriptive analysis (QDA®) scale. Salt perception was the exception, where 50% corresponds to the optimum value.

2.8. Statistical analysis

Analyses of variance (ANOVA) for the factors salt content, calibre and weight loss were performed using Statistica™ v.8.0, software from Statsoft (StatSoft Inc., 1984–2007). The factor batch was not considered, since there were no significant differences between batches (data not shown). Pearson's correlation and principal component analysis (PCA) were also carried out. Tukey Honest Significant Difference (HSD) test was used to determine significant differences ($P < 0.05$). Grubbs test ($\alpha = 0.05$) was run to detect outliers.

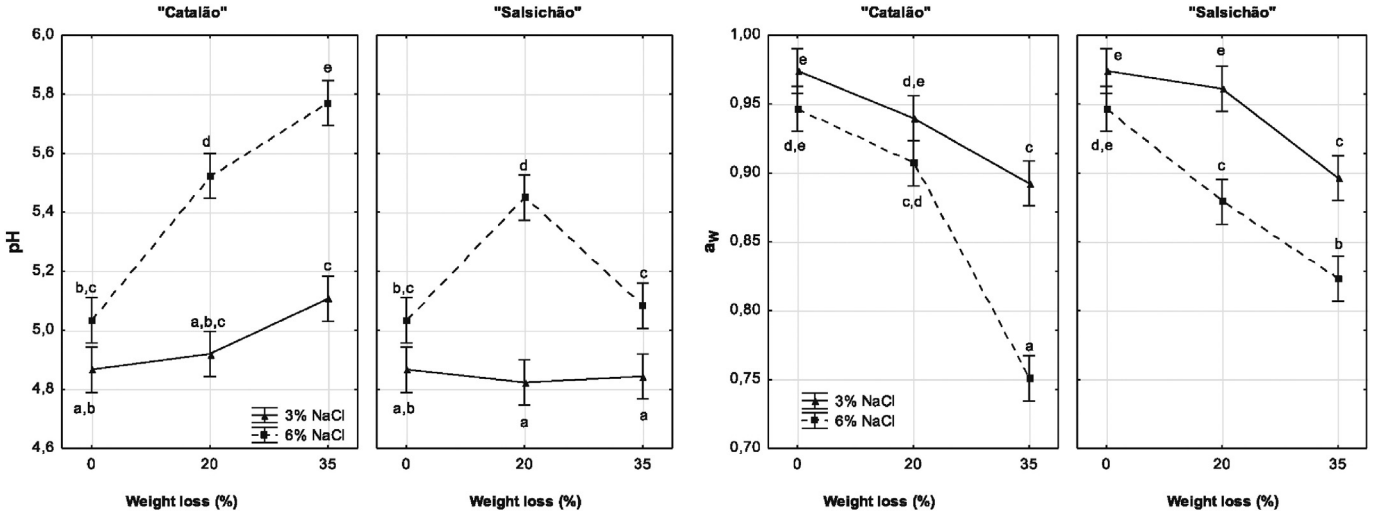

Fig. 1. pH and a_W values throughout the ripening process according to salt content and product type. Vertical bars denote 0.95 confidence intervals and different letters represent significantly different arithmetic means (HSD test, $P = 0.05$).

3. Results

3.1. Physicochemical parameters

Results regarding salt final content showed slight variations, ranging from 3.09 up to 4.19% and from 7.01 to 7.15% in products with 3 and 6% salt, respectively. Despite of the observed fluctuations, they remained at controlled levels, thus not affecting the obtained results.

pH changed considerably with salt throughout ripening, with lower values being found in low-salt products as shown in Fig. 1 ($P < 0.05$). Irrespective of salt content, pH consistently increased in "Catalão", especially in 6% NaCl sausages, where a sharped increment (about 0.5 units) was found at the beginning of the ripening process (between 0% and 20% weight loss). When sausages reached 35% weight loss, the final pH was 5.11 and 5.77 for products containing 3 and 6% salt, respectively. Despite the increment observed in "Salsichão" at 20% weight loss, the pH of the final product significantly decreased to 5.08 ($P < 0.05$). On the other hand, low-salt "Salsichão" did not differ throughout ripening with a final pH of 4.84.

After the initial two days maturation period, the regular-salt meat batter had an a_W of 0.95, which was already slightly lower than that of the low-salt meat batter (0.97). This difference became more pronounced throughout the ripening process both for "Catalão" and "Salsichão". Low-salt "Catalão" had a final a_W of 0.89, whereas regular-salt had 0.75; for "Salsichão", a_W values of 0.90 (low-salt) and 0.82 (regular-salt) were obtained.

3.2. Microbiological analyses

Foodborne pathogenic bacteria like *Salmonella* spp. and *Campylobacter* spp., as well as moulds, were absent from all tested samples. *E. coli* was detected in a few samples, but counts were below 1 log CFU/g.

ANOVA for microbiological results denotes a significant salt effect for mesophilic bacteria, LAB, coagulase-negative staphylococci and yeasts (Table 1) ($P < 0.05$).

With the exception of enterococci and coagulase-negative staphylococci, all other microbial groups showed higher counts in the low-salt meat batter. In the case of mesophilic bacteria and LAB, this relationship remained generally unchanged throughout ripening, for both product types. In the early processing stages (0% weight loss), mesophilic counts were 7.86 to 6.70 log CFU/g (for 3 and 6% salt, respectively), which have dropped in the final product ranging from 4.92 to 6.21 log CFU/g. The initial counts for LAB were 7.62 and 4.91 log CFU/g (3 and 6% salt, respectively) remaining in similar levels in the end-product. Regular-salt "Salsichão" was the only product for which LAB counts were higher in the end-product (7.44 log CFU/g) than in the meat batter (4.91 log CFU/g) ($P < 0.05$). Coagulase-negative staphylococci and yeasts evolved in fairly the same way during ripening of regular-salt dry-cured sausages, where significant lower counts were found for both microbial groups at the end of ripening ($P < 0.05$). Regarding regular-salt formulations, no significant differences were identified between coagulase-negative staphylococci at the beginning and at the end of ripening ($P > 0.05$). However, low-salt sausages generally showed significantly lower counts for coagulase-negative staphylococci and yeasts ($P < 0.05$). For these samples, coagulase-negative staphylococci final counts ranged from 0.00 to 2.29 log CFU/g ("Catalão" and "Salsichão", respectively), while yeasts varied from 3.11 to 3.25 log CFU/g (respectively for "Salsichão" and "Catalão"). Following the general trend, enterobacteria substantially decreased throughout the ripening process, irrespective of salt content or product type, where 6.83 and 6.40 log CFU/g were observed in low-salt and regular-salt meat batters, respectively ($P < 0.05$). In end-products, counts ranged between 0.43 and 3.96 log CFU/g, with regular-salt "Salsichão" showing the highest values. Concerning enterococci, no significant differences were observed during ripening, as function of salt content or product type, with final counts ranging between 1.24 and 2.96 log CFU/g. Low-salt "Salsichão"

Table 1
Factorial ANOVA for microbial counts (expressed in log CFU/g) regarding product type, salt content and weight loss.

Counts (log CFU/g)	"Catalão"						"Salsichão"					
	3% NaCl			6% NaCl			3% NaCl			6% NaCl		
	0%	20%	35%	0%	20%	35%	0%	20%	35%	0%	20%	35%
Mesophilic bacteria	$7.86^{b} \pm 0.06$	$8.19^{b} \pm 0.18$	$6.03^{a,b} \pm 0.08$	$6.70^{a,b} \pm 0.09$	$4.42^{a} \pm 3.83$	$4.92^{a,b} \pm 0.31$	$7.86^{b} \pm 0.06$	$7.02^{a,b} \pm 0.18$	$5.47^{a,b} \pm 0.89$	$6.70^{a,b} \pm 0.09$	$6.50^{a,b} \pm 0.14$	$6.21^{a,b} \pm 0.38$
Lactic acid bacteria (LAB)	$7.62^{d} \pm 0.95$	$8.20^{d,e} \pm 0.40$	$7.50^{c,d} \pm 0.07$	$4.91^{a} \pm 0.04$	$6.25^{b,c} \pm 0.44$	$5.23^{a,b} \pm 0.28$	$7.62^{d} \pm 0.95$	$9.37^{e} \pm 0.09$	$7.82^{d} \pm 0.09$	$4.91^{a} \pm 0.04$	$8.26^{d,e} \pm 0.12$	$7.44^{c,d} \pm 0.16$
Enterobacteria	$6.83^{d} \pm 0.08$	$4.00^{b,c} \pm 0.03$	$1.74^{a} \pm 0.02$	$6.40^{d} \pm 0.02$	$5.00^{c,d} \pm 0.06$	$0.43^{a} \pm 0.75$	$6.83^{d} \pm 0.08$	$3.96^{b,c} \pm 0.00$	$3.46^{a,b} \pm 0.71$	$6.40^{d} \pm 0.02$	$3.96^{b,c} \pm 0.00$	$3.96^{b,c} \pm 0.00$
Enterococci	$1.24^{a} \pm 1.13$	$5.56^{d} \pm 0.08$	$1.93^{a,b} \pm 1.08$	$2.68^{a,b,c} \pm 0.07$	$4.42^{c,d} \pm 0.51$	$2.22^{a,b,c} \pm 0.05$	$1.24^{a} \pm 1.13$	$5.14^{d} \pm 0.03$	$4.07^{b,c,d} \pm 1.83$	$2.68^{a,b,c} \pm 0.07$	$1.96^{a,b} \pm 0.00$	$2.96^{a,b,c,d} \pm 0.00$
Coagulase-negative staphylococci	$3.58^{b,c} \pm 0.26$	$2.54^{c} \pm 2.34$	$0.00^{a} \pm 0.00$	$3.65^{c} \pm 0.08$	$5.65^{d} \pm 0.13$	$4.21^{c} \pm 0.15$	$3.58^{b,c} \pm 0.26$	$3.12^{b,c} \pm 0.26$	$2.29^{b} \pm .15$	$3.65^{c} \pm 0.08$	$6.14^{d} \pm 0.03$	$2.96^{b,c} \pm 0.00$
Yeasts	$5.09^{c,d,e} \pm 0.04$	$4.79^{b,c,d} \pm 0.27$	$3.25^{a,b} \pm 0.27$	$4.88^{b,c,d} \pm 0.06$	$6.07^{d,e} \pm 0.14$	$4.29^{b,c} \pm 0.22$	$5.09^{c,d,e} \pm 0.04$	$5.15^{c,d,e} \pm 0.08$	$3.11^{a} \pm 0.21$	$4.88^{b,c,d} \pm 0.06$	$6.72^{e} \pm 0.25$	$4.45^{b,c,d} \pm 0.39$

Data are given as mean ± standard deviation (n = 6). In the same line, different letters represent significant different arithmetic means ($P < 0.05$).

Table 2
Factorial ANOVA for biogenic amines content (expressed on a dry matter basis in mg/kg) regarding product type, salt content and weight loss.

Biogenic amines	"Catalão"						"Salsichão"					
	3% NaCl			6% NaCl			3% NaCl			6% NaCl		
	0	20	35	0	20	35	0	20	35	0	20	35
Tryptamine	$110.22^{c,d,e} \pm 6.99$	$95.73^{c,d} \pm 13.09$	$111.75^{c,d,e} \pm .03$	$90.69^{c} \pm 7.39$	$108.35^{c,d,e} \pm 8.25$	$117.22^{d,e} \pm 29.04$	$110.22^{c,d,e} \pm 6.99$	$3.01^{a} \pm 4.70$	$44.37^{b} \pm 17.34$	$90.69^{c} \pm 7.39$	$124.73^{e} \pm 12.49$	$119.87^{d,e} \pm 7.05$
Phenylethylamine	ND	$34.27^{b} \pm 17.29$	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Putrescine	$17.10^{d} \pm 2.52$	$11.05^{c,d} \pm 1.71$	$8.31^{b,c} \pm 1.54$	$14.80^{d} \pm 4.20$	$7.75^{b,c} \pm 4.13$	$4.43^{a,b} \pm 2.44$	$17.10^{d} \pm 2.52$	$8.08^{b,c} \pm 2.35$	$24.57^{e} \pm 6.31$	$14.80^{d} \pm 4.20$	$6.95^{b,c} \pm 0.65$	ND
Cadaverine	$305.19^{e} \pm 18.77$	$121.80^{b,c} \pm 48.41$	$178.37^{d} \pm 15.32$	$162.47^{c,d} \pm 14.82$	$87.25^{b} \pm 13.39$	$103.36^{b} \pm 10.81$	$305.19^{e} \pm 18.77$	$5.70^{a} \pm 3.56$	$577.87^{f} \pm 44.43$	$162.47^{c,d} \pm 14.82$	$84.12^{b} \pm 25.65$	$83.34^{b} \pm 41.62$
Histamine	ND	$6.12^{b} \pm 0.55$	ND	ND	ND	$5.86^{b} \pm 1.06$	ND	$12.50^{c,d} \pm 5.66$	$15.02^{d} \pm 6.53$	ND	$8.32^{b,c} \pm 0.71$	$9.04^{b,c} \pm 0.59$
Tiramine	$160.28^{c} \pm 10.19$	$196.81^{e} \pm 25.71$	$214.33^{e} \pm 5.19$	$14.38^{a} \pm 2.55$	$59.91^{b} \pm 8.35$	$43.31^{b} \pm 12.47$	$160.28^{c} \pm 10.19$	$13.09^{a} \pm 3.48$	$173.91^{d} \pm 13.27$	$14.38^{a} \pm 2.55$	$102.43^{c} \pm 9.11$	$122.62^{c} \pm 9.39$
Spermidine	$33.60^{b,c} \pm 3.93$	$27.53^{a,b,c} \pm 11.34$	$25.76^{a,b,c} \pm 11.22$	$89.10^{d} \pm 18.89$	$45.78^{c} \pm 18.44$	$53.22^{c} \pm 36.33$	$33.60^{b,c} \pm 3.93$	ND	$42.28^{c} \pm 13.23$	$89.10^{d} \pm 18.89$	ND	$6.39^{a,b} \pm 1.35$
Spermine	$55.96^{b} \pm 32.95$	$46.70^{b} \pm 29.01$	$54.30^{b} \pm 25.29$	$51.96^{b} \pm 6.74$	$57.68^{b} \pm 2.71$	$50.62^{b} \pm 3.50$	$55.96^{b} \pm 32.95$	ND	$37.81^{b} \pm 11.07$	$51.96^{b} \pm 6.74$	$55.38^{b} \pm 4.43$	$48.90^{b} \pm 2.43$
Total[1]	$682.35^{d} \pm 44.14$	$540.01^{c} \pm 103.43$	$592.81^{c,d} \pm 47.79$	$423.41^{c,d} \pm 35.32$	$366.72^{b} \pm 22.78$	$378.01^{b} \pm 59.01$	$682.35^{d} \pm 44.14$	$42.39^{a} \pm 6.62$	$915.84^{e} \pm 73.62$	$423.41^{b} \pm 35.32$	$381.93^{b} \pm 28.20$	$390.14^{b} \pm 125.06$
Vasoactive[2]	$270.50^{d} \pm 15.52$	$332.93^{e} \pm 46.70$	$326.09^{e} \pm 9.51$	$105.08^{a} \pm 8.86$	$168.26^{c} \pm 9.46$	$166.39^{c} \pm 22.80$	$270.50^{d} \pm 15.52$	$28.61^{a} \pm 6.62$	$233.30^{d} \pm 23.33$	$105.08^{a} \pm 8.86$	$235.47^{d} \pm 16.13$	$251.52^{d} \pm 51.86$

Data are given as mean ± standard deviation (n = 6). In the same line, different letters represent significantly different arithmetic means ($P < 0.05$). ND-not detected.
[1] Total biogenic amount was calculated using the sum of individual amines.
[2] Vasoactive content was calculated based on the sum of tryptamine, phenylethylamine, histamine and tyramine.

was the exception, with significantly higher counts both in half-cured and end-products (5.14 and 4.07 log CFU/g, respectively).

3.3. Biogenic amines profile

The use of different salt contents in dry-cured sausages had a major impact in the amounts of individual biogenic amines, with special emphasis for tyramine and cadaverine, where higher levels were detected in low-salt products ($P < 0.05$). Likewise, total biogenic amines and vasoactive amines (tryptamine, phenylethylamine, histamine and tyramine) also denoted higher contents in low-salt sausages ($P < 0.05$). Higher mean levels of total biogenic amines (682.35 mg/kg) were already detected for low-salt meat batters in contrast to the regular ones (423.41 mg/kg) (Table 2**)**. After the first ripening stage (comprised between 0 to 20% weight losses), lower contents of total biogenic amines were observed for all product types. Such reduction was more pronounced in low-salt "Salsichão" with a general decrease of the eight studied amines ($P < 0.05$). Nevertheless, biogenic amines levels increased again throughout the ripening period, regardless of salt content or product type. Considering the whole drying process, total biogenic amines have globally decreased during ripening, with higher contents being observed in end-product low-salt sausages (592.81 mg/kg and 915.84 mg/kg for "Catalão" and "Salsichão", respectively), while regular-salt products showed 378.01 mg/kg and 390.14 mg/kg, respectively for "Catalão" and "Salsichão". The observed differences were largely due to cadaverine (up to 577.87 mg/kg in low-salt "Salsichão"). The lowest contents of cadaverine were detected in regular-salt sausages, ranging from 103.36 mg/kg ("Catalão") to 93.34 mg/kg ("Salsichão"). A similar effect was also noticed for tyramine, which final mean contents were significantly higher in low-salt products (214.33 mg/kg in "Catalão" and 173.91 mg/kg in "Salsichão") ($P < 0.05$). On the other hand, for regular-salt sausages, tyramine final contents were 43.31 mg/kg and 122.62 mg/kg (in "Catalão" and "Salsichão", respectively). Histamine, which is generally the most problematic amine, remained at low levels. The highest mean content corresponded to 15.02 mg/kg found in low-salt "Salsichão" sausages. Instead, high levels of vasoactive amines were found in "Catalão" end-products (166.39 mg/kg in regular-salt and 326.09 mg/kg in low-salt).

Generally, "Salsichão" has significantly higher values for most biogenic amines than "Catalão" (data not shown). This calibre effect may be explained by a faster dehydration of small calibre sausages, which would then evidence less microbial activity.

3.4. Fatty acids profile

In general, the FA profile did not change significantly throughout

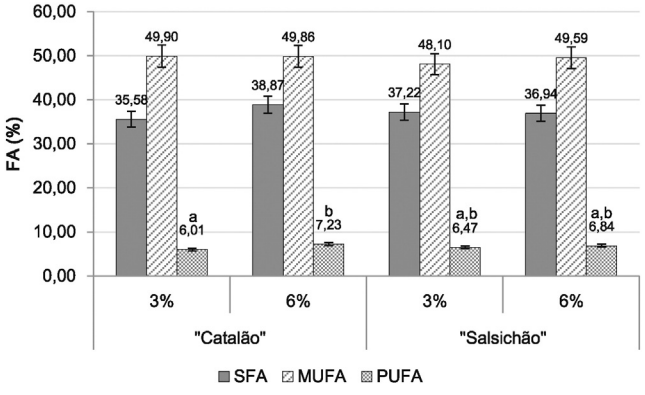

Fig. 2. Fatty Acids groups according to salt content and product type. SFA-Saturated fatty acids; MUFA—Monounsaturated fatty acids; PUFA—Polyunsaturated fatty acids. Vertical bars denote 0.95 confidence intervals and different letters represent significantly different arithmetic means (HSD test, $P = 0.05$).

Table 3
Two-way ANOVA for methyl ester fatty acid-derivatives (expressed in %) regarding product type and salt content.

Fatty acids (FA)	"Catalão"		"Salsichão"	
	3% NaCl	6% NaCl	3% NaCl	6% NaCl
Lauric (C12:0)	$0.058^{a} \pm 0.02$	$0.242^{b} \pm 0.00$	$0.073^{a} \pm 0.03$	$0.078^{a} \pm 0.04$
Myristic (C14:0)	1.13 ± 0.14	1.31 ± 0.00	1.24 ± 0.12	1.19 ± 0.07
Palmitic (C16:0)	22.30 ± 2.60	23.77 ± 0.00	23.37 ± 0.43	23.23 ± 0.43
Palmitoleic (C16:1)	2.40 ± 0.28	2.58 ± 0.00	2.51 ± 0.16	2.47 ± 0.15
Margaric (C17:0)	$0.32^{a,b} \pm 0.04$	$0.439^{b} \pm 0.00$	$0.288^{a} \pm 0.09$	$0.352^{a,b} \pm 0.04$
Margaroleic (C17:1)	0.32 ± 0.04	0.38 ± 0.00	0.27 ± 0.08	0.37 ± 0.04
Stearic (C18:0)	11.77 ± 1.33	13.11 ± 0.00	12.25 ± 0.30	12.09 ± 0.55
Oleic (C18:1)	45.88 ± 5.47	45.61 ± 0.00	43.95 ± 0.94	45.59 ± 1.47
Linoleic (C18:2)	$5.582^{a} \pm 0.73$	$6.663^{b} \pm 0.00$	$6.078^{a,b} \pm 0.28$	$6.332^{a,b} \pm 0.17$
Linolenic (C18:3)	$0.425^{a,b} \pm 0.06$	$0.569^{b} \pm 0.00$	$0.393^{a} \pm 0.05$	$0.511^{a,b} \pm 0.09$
Gadoleic (C20:1)	0.97 ± 0.13	1.05 ± 0.00	1.03 ± 0.10	1.04 ± 0.05
Heneicosanoic (C21:1)	$0.329^{b} \pm 0.09$	$0.240^{a,b} \pm 0.00$	$0.343^{b} \pm 0.07$	$0.128^{a} \pm 0.03$

Data are given as mean ± standard deviation (n = 6). In the same line, different letters represent significant different arithmetic means ($P < 0.05$).

ripening, or according to the amount of salt or product type (data not shown). By decreasing order of importance, oleic (45.26%), palmitic (23.17%), stearic (12.31%) and linoleic (6.16%) were the most abundant FA (Fig. 2). Despite this, as can be seen in Table 3, differences were identified in FA present in smaller amounts, such as lauric, linoleic and heneicosanoic acids. According to our results, heneicosanoic acid denoted an important decrease in "Salsichão" sausages from 0.34 to 0.13%, when higher amounts of salt were used. In opposition, the other two FA increased in "Catalão" with salt content: lauric acid from 0.06 to 0.24% and linoleic acid from 5.58 to 6.66%. The opposite trend was observed for PUFA, which increased with salt content, but only in "Catalão" sausages (Fig. 2) ($P < 0.05$).

3.5. Texture profile analysis

Both salt content and product type had a significant impact in sausages texture profile analysis, leading to noticeable changes on almost parameters assessed ($P < 0.05$) (Table 4). The exception was springiness, where no important differences were observed. Chewiness values are higher in low-salt sausages, with higher values being estimated for "Salsichão", however no significant differences between products were observed for regular-salt sausages. Adhesiveness, on the other hand, is lower in low-salt sausages, irrespective of calibre, while an opposite trend was observed for cohesiveness and hardness, which is in good agreement with the results obtained in sensory analysis.

3.6. Sensory evaluation

Some of the evaluated sensory parameters were affected by salt content, as highlighted in Table 5. These include aroma intensity, off aromas, hardness, flavour intensity and obviously salt perception. In fact, salt perception, as well as flavour intensity, denoted a significant increase, when larger amounts of sodium chloride were added to the formulation ($P < 0.05$). On the contrary, sausages' hardness followed the opposite trend. Based on a scale of 0 to 100, tasters globally rated the products between 66 and 70, with no significant differences being noticed as a function of the products' salt content. However, it was possible to identify a positive correlation between the overall appreciation and sausages' flavour intensity ($r = 0.71$).

A principal component analysis (PCA) was run for parameters related to sausages texture both from TPA (hardness, adhesiveness, cohesiveness, springiness, resilience and chewiness) and sensory evaluation (hardness, fibrousness, succulence) (Fig. 3). Two principal components were extracted, which explain 91.05% of the observed variance: PC1 accounted for 76.32%, while PC2 for 14.73%. The first component included all assessed parameters, with the exception of fibrousness that was part of the second component. Furthermore, adhesiveness seems to respond to salt content (Fig. 3), although there is no correlation between adhesiveness and NaCl concentration.

4. Discussion

Portuguese traditional dry-cured sausages, recognised by consumers as safe and high quality products, are still being manufactured according to traditional methods. Meat quality combined with the traditional processing technology provides them with unique sensory properties. In this context, salt content reduction alone was carefully investigated, without compromising safety and authenticity of products. For this purpose, two salt concentrations were tested: 6% (regular-salt content) and 3% (low-salt content) in "Catalão" and "Salsichão" sausages, keeping all other processing conditions unchanged. Since they are closely related to safety and dry-cured sausages stability, pH, a_w, microbiota and biogenic amines were analysed throughout the ripening process. Moreover, FA, TPA and sensory evaluation were assessed in end products.

Differences in pH evolution throughout processing were clearly dependent on the amount of salt. Low-salt products showed lower pH values, as a result of higher LAB counts. Irrespective of sausage calibre, low-salt products pH was always below 5.2, a value considered safe for traditional dry-cured sausages (Roseiro et al., 2010). In regular-salt sausages, an increment in pH during processing was observed as a result of a less marked LAB growth (especially in "Catalão") and of the formation of ammonia that usually occurs in these products. Nevertheless, a sharp a_w decrease nsured microbiological stability. Several studies have reported no changes in pH due to salt content (Corral, Salvador,

Table 4
Two-way ANOVA for texture profile analysis (TPA) parameters concerning product type and salt content.

TPA	"Catalão"		"Salsichão"	
Parameter	3% NaCl	6% NaCl	3% NaCl	6% NaCl
Hardness	$42.249^{b} \pm 5.820$	$32.637^{a} \pm 5.376$	$58.610^{c} \pm 8.414$	$35.419^{a} \pm 7.511$
Adhesiveness	$-3.736^{a} \pm 2.447$	$-1.918^{b} \pm 1.534$	$-3.792^{a} \pm 1.555$	$-1.699^{b} \pm 0.895$
Cohesiveness	$0.417^{a,b} \pm 0.038$	$0.391^{a} \pm 0.032$	$0.447^{b} \pm 0.05$	$0.443^{b} \pm 0.04$
Springiness	0.979 ± 0.101	0.914 ± 0.146	1.026 ± 0.278	0.931 ± 0.063
Resilience	$0.096^{a,b} \pm 0.021$	$0.082^{a} \pm 0.016$	$0.116^{b} \pm 0.03$	$0.099^{a,b} \pm 0.016$
Chewiness	$17.29^{b} \pm 3.435$	$11.737^{a} \pm 3.097$	$26.72^{c} \pm 7.809$	$14.525^{a,b} \pm 2.894$

Data are given as mean ± standard deviation (n = 6). In the same row, different letters represent significantly different arithmetic means ($P < 0.05$).

Table 5
Two-way ANOVA for sensory attributes considering product type and salt content.

Sensory attribute	"Catalão"		"Salsichão"	
	3% NaCl	6% NaCl	3% NaCl	6% NaCl
Colour intensity	68 ± 13	73 ± 10	71 ± 12	74 ± 11
Off colours	0	0	0	0
Marbled	73 ± 11	68 ± 13	64 ± 17	68 ± 13
Aroma intensity	69 ± 14	72 ± 13	68 ± 14	76 ± 11
Off aromas	0	1 ± 3	0	0 ± 1
Hardness	$53^{a,b}$ ± 10	49^{a} ± 8	59^{b} ± 14	$49^{a,b}$ ± 12
Fibrousness	29 ± 28	21 ± 23	16 ± 22	23 ± 24
Succulence	69 ± 11	71 ± 12	64 ± 17	69 ± 11
Flavour intensity	$68^{a,b}$ ± 11	$72^{a,b}$ ± 11	65^{a} ± 12	75^{b} ± 7
Off flavours	0 ± 2	1 ± 3	1 ± 5	1 ± 4
Salt perception	54^{a} ± 8	65^{b} ± 13	53^{a} ± 9	70^{b} ± 15
Overall appreciation	70 ± 11	69 ± 11	66 ± 14	69 ± 9

Data are given as mean ± standard deviation (n = 6). In the same row, different letters represent significantly different arithmetic means ($P < 0.05$).

Belloch, & Flores, 2014; Roseiro et al., 2008). In the meanwhile, the more acidic environment that was established in low-salt meat batters after the two-day maturation period also induced a reduction (especially in "Catalão") on coagulase-negative staphylococci counts. Besides their important effect on products' flavour, organic acids present in wine contribute to reduce the pH of meat batters and to inhibit the undesirable microbiota, and ethanol may also inhibit sensible microorganisms like moulds (Coloretti et al., 2014). According to our results this effect was not detectable, since no moulds were detected.

During the dry-curing process, good environmental conditions (such as pH and a_w) favour growth of both technological and spoilage microbiota, including LAB and enterobacteria, which actively participate in biogenic amines accumulation (González-Tenorio et al., 2013; Li et al., 2014). Furthermore, bacterial proteolytic activity results in the availability of precursor amino acids (Latorre-Moratalla, Bover-Cid, Bosch-Fusté, Veciana-Nogués, & Vidal-Carou, 2014). On the other hand, biogenic amines accumulation is inhibited due to antimicrobial substances used in dry-cured sausages, such as NaCl. Thus, higher content of biogenic amines was found mainly in low-salt products throughout the ripening process. Since LAB, particularly *Enterococcus* spp., are known for their ability to decarboxylate tyrosine (Bargossi et al., 2015), lower LAB counts in regular-salt products, resulted in lower amounts of tyramine (r = 0.49, $P < 0.05$). Similarly, slightly lower

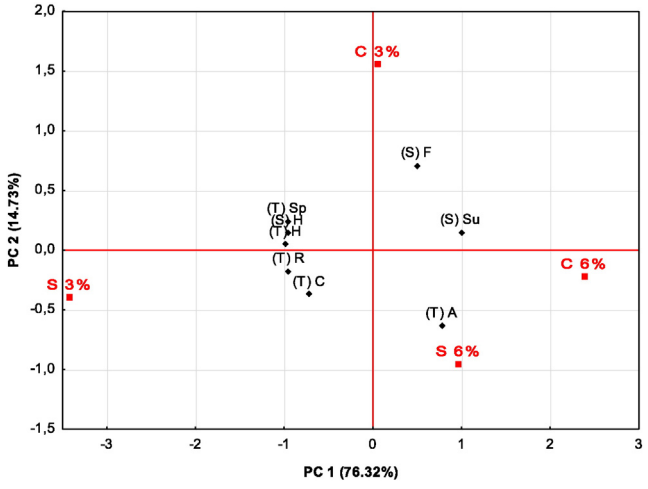

Fig. 3. Principal Component Analysis. Projection of selected variables (textural and sensory data) and samples on the factor-plane, considering two main factors: salt content and calibre. Attributes abbreviations are preceded by S or T depending if they resulted from sensory analysis or texture profile analysis (TPA), respectively. Attributes abbreviations: H (hardness), F (fibrousness), Su (succulence), Ad (adhesiveness), Co (cohesiveness), Sp (springiness), and R (resilience). Products: C 3% (low-salt "Catalão"), C 6% (regular-salt "Catalão"), S 3% (low-salt "Salsichão"), and S 6% (regular-salt "Salsichão").

enterobacteria counts, might have led to lower cadaverine contents. In fact, higher contents in biogenic amines were usually observed at the start of the ripening process.

Total biogenic amines content throughout the curing process was mainly influenced by cadaverine and tyramine (by decreasing order of importance), since they are the most abundant amines in dry-cured sausages. Previous studies had already reported the prevalence of tyramine and cadaverine over other exogenous biogenic amines (Latorre-Moratalla et al., 2008; Lu et al., 2010; Papavergou, Savvaidis, & Ambrosiadis, 2012). Interestingly, relative higher contents of tryptamine, corresponding to nearly 30% of total biogenic amines content, were detected in regular-salt sausages.

Among the four products tested, low-salt "Salsichão" showed a quite different evolution pattern. A sharp decline in biogenic amines content was observed from the meat batter to the intermediate ripening step, followed by an important increase afterwards. In our opinion, these results cannot be explained by the existing microbiota. The formation of biogenic amines in sausages is dependent on the interaction of numerous factors (pH, calibre, seasoning, etc), which due to their complexity are not yet fully understood (Bover-Cid, Schoppen, Izquierdo-Pulido, & Vidal-Carou, 1999; Ikonić et al., 2013; Komprda et al., 2004; Lorenzo, Martinez, Franco, & Carballo, 2007; Suzzi & Gardini, 2003). Furthermore, the consumption of biogenic amines as nitrogen source is usually pointed out as the main cause for their decline (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2001; Roseiro et al., 2010). On the other hand, the production of amines is often strain-dependent rather than related to a specific microbial group. Thus, the strong increase detected *a posteriori*, may be due to the growth of strains with higher decarboxylase activity.

The importance of biogenic amines relies on them representing a toxicological hazard, which results in the occurrence of undesirable symptoms when ingested. Despite the lack of legislation applicable to dry-cured sausages, a safety limit of 100 mg histamine/kg of food is usually considered (Eerola, Sagués, & Hirvi, 1998; Silla Santos, 1996). The accumulation of histamine in our end-products never exceeded the mentioned limit. However, vasoactive amines' final contents were generally quite high, resulting mainly from high levels of both tryptamine and tyramine. These results are of particular importance, since putrescine and cadaverine are known for potentiating the toxic effect of histamine and tyramine, even though they do not represent a potential hazard to consumers themselves (Larqué, Sabater-Molina, & Zamora, 2007). Moreover, an important positive correlation was observed between LAB counts and tyramine (r = 0.40; $P < 0.05$), which is in good agreement with previous studies (Curiel et al., 2011; Lu et al., 2010).

Lipolytic activity is often associated with coagulase-negative staphylococci (Martin, Colin, Aranda, Benito, & Cordoba, 2007) and mainly with the activity of endogenous lipases, which preferentially release oleic and linoleic acids (Zanardi, Ghidini, Battaglia, & Chizzolini, 2004). However, lipolytic activity can vary considerably depending on the strain (Ordoñez, Hierro, Bruna, & de la Hoz, 1999). Moreover, according to Zanardi et al. (2004) FA profiles do not depend on the processing conditions, but mostly from the composition of raw material itself, despite the pro-oxidant effect of salt. Our results show an increase of lauric and margaric acids, further denoting a significant positive correlation with salt content (r = 0.99 and r = 0.93, respectively). Likewise, a significant correlation of lauric (r = 0.99), margaric (r = 0.93) and linolenic (r = 0.90) acids with coagulase-negative staphylococci counts. These results suggest the presence of coagulase-negative staphylococci endogenous strains with remarkable lipolytic activity. In contrast, only heneicosanoic acid decreased with salt content. In a general way, in dry-cured sausages, monounsaturated fatty acids (MUFA) are the most abundant, which is positive from the nutritional standpoint, although they are more susceptible to oxidation process. However, a moderate lipid oxidation is desirable, in order to enable the release of aroma and flavour compounds.

Regarding sensory evaluation the salty taste seems to be the outstanding factor, followed by flavour intensity. Clearly, taste attributes

are preponderant over products' overall appreciation. Furthermore, salt content does not necessarily correlate with the lower rates attributed to flavour (Aaslyng et al., 2014). In our study, panellists identified a significant reduction in flavour intensity in low-salt products.

Principal component analysis showed an association between low-salt "Catalão" and sensory attributes, namely fibrousness and succulence, and low-salt "Salsichão" with sausages' textural cohesiveness. There is a positive correlation between regular-salt "Salsichão" and "Catalão", which is highlighted as a result of the sausages' textural adhesiveness. In fact, adhesiveness increased as a result of higher salt contents. Regarding hardness, PCA projection of variables revealed a very close relationship between hardness evaluated by TPA and hardness as sensed by panellists, which means that the tasters accurately evaluated the samples.

5. Conclusion

Regarding salt content and product type, the former factor had the greatest impact on the studied parameters. Low-salt sausages showed higher a_w values and a more intense production of biogenic amines, than regular-salt sausages. Even though, no food safety issues arose regarding microbial contaminations or histamine levels, associated with the salt reduction. Moreover, considering sensory analysis, and despite no clear preference for one of the tested salt contents, a more balanced salt taste was associated to low-salt products. For the abovementioned reasons, a reduction in salt content should be considered in the processing of these dry-cured products, with the obvious health-related advantages.

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