“Salt reduction in slow fermented sausages affects the generation of aroma active compounds”

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Abstract

Slow fermented sausages with different salt content were manufactured: control (2.7 % NaCl, S), 16 % salt reduced (2.26 % NaCl, RS) and 16 % replaced by KCl (2.26 % NaCl and 0.43 % KCl, RSK). The effect of salt reduction on microbiology and chemical parameters, sensory characteristics, texture and volatile compounds was studied. The aroma compounds were identified by GC-MS and olfactometry analyses. Small salt reduction (16%) (RS) affected sausage quality producing a reduction in the acceptance of aroma, taste, juiciness and overall quality. The substitution by KCl (RSK) produced the same acceptability by consumers as for high salt (S) treatment except for the aroma that was not improved by KCl addition. The aroma was affected due to the reduction in sulfur and acids and the increase of aldehyde compounds. Aroma compounds that characterized the high salt treatment (S) were dimethyl trisulfide, 3-methyl thiophene, 2,3-butanedione, 2-nonanone and acetic acid.

Keywords: fermented sausages, salt reduction, volatile compounds, aroma, flavour.

1. Introduction

The relation between high salt intake and incidence/prevalence of hypertension has led the European Union (EU) to implement salt reduction initiatives in the EU framework (European Commission, 2008). EU proposed salt reduction of 16 % in 4 years, decreasing 4 % per year in order to allow consumers to adapt to the slightly decreasing salty taste. In some products, salt reduction means lower salty taste, but others products, such as dry curing and processed meats, can lead to safety and technological problem.

Salt is an essential ingredient in dry fermented sausages; it is involved in myofibrillar protein solubilization, improve texture; decrease water activity (a_w) controlling the growth of pathogens microorganism and finally, it controls the biochemical and enzymatic reactions during ripening, affecting the final flavour (Ruusunen & Puolanne, 2005). The reduction of salt in fermented meat products has been studied through different strategies such as the use of KCl alone (Gou, Guerrero, Gelabert, & Arnau, 1996) or together with other chloride salts ($CaCl_2$, $MgCl_2$) (Gimeno, Astiasarán & Bello, 1998; Zanardi, Ghidini, Conter & Ianieri, 2010) and also different flavour enhancers have been used (lactate, amino acids and yeast extracts) (Gou et al., 1996; Guàrdia, Guerrero, Gelabert, Gou & Arnau, 2008; Campagnol, dos Santos, Wagner, Terra & Pollonio, 2011). However, KCl provides metallic or bitter tastes when it is used at concentrations equal or higher than 40% (Gou et al., 1996, Gelabert, Gou, Guerrero & Arnau, 2003). Furthermore, sausage texture is affected depending on the type of salts used in the substitution. When KCl is used alone it produced an increase in sausage hardness (Gou et al., 1996; Guardia et al., 2008) while the use of KCl in combination with other divalent salts resulted in a decrease in hardness (Gimeno, Astiasarán & Bello, 1999).

Moreover, other sensory characteristics are affected by salt substitution such as a decrease in aroma and taste when KCl is used at high percentages (>40%) (Guardia et al., 2008, Campagnol et al., 2011). Nevertheless, the effect of salt substitution on aroma has been poorly studied only Campagnol et al. (2011) studied the volatile compounds generated in fermented sausages when NaCl was substitute by KCl and yeast extracts. These authors reported few differences in aroma among sausages when NaCl was reduced in a 25% but after 50% substitution, the decrease in aroma and taste was evident. In addition, other studies performed on sausage models indicated that salt modifications affected volatile compounds but it depended on ripening time and the type of starter culture used (Olesen, Meyer & Stahnke, 2004; Tjener, Stahnke, Andersen & Martinussen, 2004). In contrast, Ravyts, Steen, Goemaere, Paelinck, De Vuyst & Leroy (2010) indicated that modifications of salt concentrations in sausages produced a very limited impact on the growth and composition of the microbiota without detecting an effect on volatile composition. However, all these previous studies did not evaluate the effect of salt reduction on aroma active compounds as they mainly focused on several volatile compounds.

Furthermore, these studies were done in fermented sausages but there are no reports about the reduction of NaCl content in slow fermented sausages (i.e. Chorizo de Cantimpalos, Cacciatore salami, Hungarian type salami, and others). These slow fermented sausages are typically produced in Southern European countries by using low temperatures during ripening (Flores, 1997) and the rate of acidification is low allowing the activity of acid-sensitive bacteria (*micrococcaceae* and *staphylococci*). The flavour of these sausages is mostly formed by endogenous or bacterial enzymatic activities and the oxidation of the lipid fraction (Ravyst et al., 2010). Nothing is known about the effect of salt reduction on volatile aroma compounds in slow fermented sausages. Therefore, it is necessary to evaluate if salt reduction may affect flavour quality because the acceptability of slow fermented sausages is largely dependent upon its flavour (Flores, 1997). NaCl plays an important role in flavour development, since it provides the salty taste, enhances savory and meaty flavours and improves the release of volatile aroma compound from the food matrix (Ruusunen & Puolanne, 2005). However, the adaptation to less salty taste by consumers is important as it can be a way to reduce salt content in meat products. For all these reasons, the aim of this work was to study the effect of a 16 % salt reduction in the production of aroma active compounds in slow fermented sausages and to determine the effects produced by KCl used during salt reduction.

2. Materials and methods

2.1 Dry fermented sausages preparation

Three treatments of dry fermented sausages were manufactured with different salt contents; control treatment (S) with 2.7 % NaCl, low salt treatment (RS) with 2.26 % NaCl and a third treatment (RSK) with 2.26 % NaCl and 0.43 % KCl.

Sausages was prepared with lean pork (75 %) and pork back fat (25 %) and the following additives (g/Kg): lactose (30); dextrin (10); sodium caseinate (20); glucose (7); sodium ascorbate (0.5); sodium nitrite (0.15); potassium nitrate (0.15) and starter culture (0.1) SP318 TEXEL SA-301 (Danisco, Cultor, Madrid, Spain) containing *Lactobacillus sakei, Pediococcus pentosaceus, Staphylococcus xylosus and Staphylococcus carnosus*. The manufacturing process was the same as described (Olivares, Navarro & Flores, 2010). The meat mixture was stuffed into collagen casings of 9.5 cm diameter (FIBRAN, S.A., Girona, Spain) and the sausages were subjected to drying at 10-12 ºC and 70-85 % HR for 57 days. In order to control the ripening process, weight losses and pH were measured during processing (Olivares et al., 2010).

From each treatment, a 200 g portion of the meat mixture (0 days) and three sausages at 9, 29 and 57 days were randomly collected to study the effect of ripening time and formulation. A 150 g portion of the sample was minced and used for moisture, water activity and pH tests. In addition, sausage color was measured and a 10 g portion was taken for microbiological analysis. The remaining minced sample was vacuum packed and frozen at -20

ºC for subsequent analyses (TBARS, lipid, protein and ions content). At 57 days, several slices (1 cm thickness) were wrapped in aluminum foil, vacuum packaged and stored at -80 ºC for volatile and aroma analyses. All results were expressed as the mean of three replicates at each sampling time. Finally, the texture and sensory analysis were carried out at the end of the drying process (57 days).

2.2 Chemical analysis

pH was measured by introducing a pH meter HI 99163 (Hanna Instruments Inc., Hoonsocket, USA) into a mixture of sausage and distilled water (1:1) (ISO 2917:1999). Water activity was determined using a Fast-lab water activity meter (Gbx, Romans sur Isère Cédex, France) as described Olivares et al., (2010). Color evaluation was made through the CIE L*, a*, b* space. The color of the sausages was measured using a colorimeter CR-400/410 (Konica Minolta Sensing Inc., Japan) with D65 illuminant (Olivares et al., 2010).

Moisture content was determined after dehydration at 100 ºC to a constant weight, according to the official method of analysis of meat products (BOE, 1979). Total lipids were extracted from 5 g of minced sausage according to the method of Folch, Lees & Sloane Stanley (1957), using dichloromethane: methanol (2:1) instead of chloroform: methanol (2:1) as solvent. The extract obtained was evaporated in a rotating vacuum evaporator and weighed to determine the total lipid content. Nitrogen content was determined by the Kjeldahl method and protein was estimated by multiplying the nitrogen content by a factor of 6.25.

Thiobarbituric acid reactive substances (TBARS) were quantified to determine the degree of lipid oxidation, as described Olivares, Navarro & Flores (2011) using trichloroacetic acid as solvent instead of perchloric acid. The results were expressed as mg malonaldehyde (MDA)/ kg in dry matter.

Cations (sodium, potassium) were analyzed by ion chromatography as described Armenteros (2009). Chloride anion in sample solutions was determined by using Metrohm 761 Compact IC with Metrohm 833 IC Liquid Handling Suppressor unit to improve chromatographic signal. Guard column A-Supp 4/5 (5.0 x 4.0 mm) and analytical column Supp 5-250 (4.0 × 250 mm) were used to analyze chloride anion. The mobile phase consisted of 1 mM $NaHCO_3$ and 3.2 mM Na_2CO_3 with 30 ml/l acetone. The concentration of each ion was determined from respective calibration curves, using a set of standard solutions of Na^+, K^+ and Cl^- (Fluka, Switzerland, Sigma, St. Louis, MO). The results (means of three determinations) were expressed as mg/100 g of sample in dry matter.

2.3 Microbiological analysis

Minced sausage sample was aseptically homogenized with peptone water (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 min and decimal dilutions were prepared. Lactic acid bacteria population was determined by the overlay technique to promote anaerobic

156 growth using MRS agar (Scharlau Chemie SA, Barcelona, Spain). *Staphylococci* counts were
157 obtained on Mannitol salt agar (Scharlau Chemie SA, Barcelona, Spain). Both mediums were
158 incubated at 30 ºC for 3 days.
159
160 **2.4 Analysis of volatile compounds**
161 **2.4.1 Gas chromatography-mass spectrometry (GC-MS)**
162 An Agilent HP 7890 series II GC (Hewlett- Packard, Palo Alto, CA) with an HP 5975C
163 mass selective detector (Hewlett-Packard) equipped with Gerstel MPS2 multipurpose sampler
164 (Gerstel, Germany) was used in all experiments. Extraction of headspace volatile compounds
165 was performed using a solid-phase microextraction (SPME) with an 85 µm Carboxen/
166 Polydimethylsiloxane (CAR/PDMS) fibre for automatic holder (Supelco, Bellefonte, PA). Before
167 the analysis, the fibre was preconditioned as indicated by the manufacturer.
168 For each experiment, 5 g of dry fermented sausages was minced and weighted into a
169 20 ml headspace vial sealed with a PTFE faced silicone septum and 0.75 mg of BHT was
170 added. The vial was maintained at 37 ºC during 30 min to equilibrate its headspace. Then, the
171 SPME fibre was exposed to the headspace while maintaining the sample at 37 ºC during 3 h.
172 Before each injection, the fiber was baked at 250 ºC for 15 min. The compounds adsorbed by
173 the fibre were desorbed in the injection port of the GC-MS for 5 min at 240 ºC with purge valve
174 off (splitless mode). The analysis of volatile compounds in the GC-MS was done as described
175 Olivares et al. (2011). The compounds were identified by comparison with mass spectra from
176 the library database (Nist'05), kovats retention index (Kovats, 1965) and by comparison with
177 authentic standards. The quantification of volatile compounds was done in SCAN mode using
178 either total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale.
179 **2.4.2 Gas-chromatrography-olfactometry**
180 A gas chromatograph (Agilent 6890, USA) equipped with a FID detector and sniffing
181 port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) was used to analyze aroma compounds
182 as described Olivares et al. (2011) using SPME technique. The detection frequency method
183 was used to estimate the aromatic impact of each volatile (Pollien, Ott, Montigon, Baumgartner,
184 Muñoz-Box & Chaintreau, 1957). Each assessment was carried out according to Olivares et al.
185 (2011). Four trained panellists evaluated the odours from the GC-effluent. Each assessor
186 evaluated 3 sausages per treatment (57 d of ripening), therefore a total of 12 assessments
187 were carried out. The final detection frequency value (DF) for each compound was obtained by
188 summation of the 12 sniffings. The detection of an odor by less than three assessors was
189 considered to be noise.
190 Compounds were identified using the following techniques: comparison with mass
191 spectra, comparison with kovats retention indices of authentic standards injected in the GC-MS
192 and GC-O, and by coincidence of the assessors's descriptors with those in the Fenaroli's
193 handbook of flavour ingredients (Burdock, 2002).

2.5 Texture profile analysis

Texture profile analysis (TPA) was performed using TA-XT.plus Texture Analyzer with Texture Exponent software (version 2.0.7.0. Stable Microsystems, Godalming, UK). Four dry fermented sausage slices (diameter 3.5 cm and thick 1.5 cm) of three sausages per treatment were compressed twice to 50 % of their original height as described Olivares et al. (2010). TPA curves were obtained and the main parameters of texture were calculated: hardness, springiness, cohesiveness and as secondary parameter chewiness.

2.6 Sensory analysis

Testing was carried out in a sensory laboratory equipped with individual booths (ISO 8589, 1988). A panel of 85 untrained consumers was used. The casing was removed and the sausages were cut into slices of 4 mm thickness. Samples from each treatment (S, RS, RSK) were labeled with random, three-digit codes and presented on a plate at room temperature with water and bread without salt to cleanse the palate between samples. An acceptability test was carried out using 9-box hedonic scale (1extremely dislike – 9 extremely like). The attributes evaluated were: appearance, flavour, taste, hardness, juiciness and overall quality. One slice of each treatment was placed inside a camera with D65 illuminant to evaluate the appearance. Data acquisition was performed using Compusense® five release 5.0 (Compusense Inc., Guelph, Ontario, Canada).

2.7 Statistical analysis

Effect of reduction/replacement of NaCl and processing time on the variables studied (chemical and microbial) was done by a two-factor analysis of variance (ANOVA) using the statistic software XLSTAT 2009.4.03 (Addinsoft, Barcelona, Spain). Fisher test was used to evaluate differences among treatments. The effect of reduction/replacement of NaCl on texture, sensory parameters and volatile compounds at the end of the process was done by one factor ANOVA analysis. Furthermore, principal component analysis (PCA) was done to evaluate the relationships among sausages and different parameters (pH, TBARS, ions, texture parameters, moisture, lipids and protein content and aroma active compounds).

3. Results

3.1. Chemical and microbiology analyses

At the end of the ripening process, the three treatments showed weight losses of 38.9-39.2 % (data not shown), that are suitable values for this kind of sausage. Salt reduction did not produce differences in weight losses among treatments at the end of process, as also observed other authors (Campagnol et al., 2011).

231 Two essential factors such as a_w and pH, guarantee the stability and safety of the
232 sausage. The pH and a_w values are shown in Fig. 1A. The pH dropped to 4.5 due to the LAB
233 growth in the treatments and then, pH experienced a slight increase due to ammonia formation.
234 No differences in pH were observed among treatments as also was observed in salt reduced
235 small caliber fermented sausages (Gelabert et al., 2003; Gou et al., 1996). Concerning a_w, it
236 decreased throughout the processing to 0.92 value in all treatments. At 9 days, differences
237 were found in a_w as seen by a highest a_w in the RS treatment, nevertheless the final sausages
238 did not show differences ($p>0.05$) as also has been reported by other authors (Campagnol et
239 al., 2011). However, Olesen et al. (2004) also observed a lowest a_w in highly salted sausages
240 as we detected, although they performed a higher salt reduction in their assays (50 %) than the
241 reduction done in our study (16 %).
242 The number of LAB and Staphylococci were within the range of what could be expected
243 in dry fermented sausages and no differences were observed ($p>0.05$) among treatments
244 throughout the process (data not shown). The population of LAB experienced a growth of 3
245 logarithmic cycles during the first 9 days and it was maintained stable until the end of the
246 process. The number of *Staphylococci* suffered a slight decrease of 2 logarithmic cycles during
247 processing.
248 In relation to fat and protein content, an increase in both contents was observed as a
249 result of the reduction in moisture content during ripening. Salt content did not cause significant
250 differences ($p>0.05$) in chemical composition among treatments at the end of the process
251 (Table 1).
252 The color of sausages was also measured along the process, obtaining L*, a* and b*
253 coordinates (data not shown). The trend in the three color coordinates throughout the process
254 was similar to that observed by Olivares et al. (2010). No differences were detected in the final
255 product among treatments as also has been observed in similar fermented sausages
256 (Campagnol et al., 2011).
257 TBARS values increased during the drying process in the three treatments (Fig. 1B) as
258 it has been reported in similar sausages (Olivares et al., 2011). A highest oxidation was
259 observed in the reduced salt treatments at 9 days. TBARS values were significantly higher in
260 the RS treatment than S treatment, but at the end of the process only RSK was significantly
261 higher than S treatment. The effect of NaCl on lipid oxidation is not clear. Several authors have
262 reported a pro-oxidant effect of NaCl in meat and meat products (Kanner, Harel & Jaffe, 1991;
263 Shahidi, Rubin & Wood, 1988) while other authors have not observed this effect in model
264 system (Sárraga & García-Regueiro, 1998). Nevertheless, Zanardi et al. (2010) also observed
265 a highest oxidation in reduced salt sausages and they attributed this highest oxidation to the
266 use of $CaCl_2$ that can favored the lipid oxidation. In our study, the highest oxidation observed in
267 RSK treatment could be due to a slightly highest fat content observed in this treatment (RSK).

As expected, a significant reduction ($p<0.05$) of the Na^+ ion content was achieved in RS and RSK treatments throughout the process (data at the end of the process 57d are shown in Table 1), however, at the end of the process only significant differences were found between S and RSK. The content of K^+ ion was increased ($p<0.05$) in RSK treatment, since this treatment was the one containing KCl (Table 1). Finally, significant differences were detected in Cl^- ion content throughout the process among treatments although at the end of the process there were not significant. The salt reduction detected in the treatments (RS and RSK) could be considered as a healthy benefit, following EU indications.

3.2. Texture profile analysis

Sausages TPA parameters were analyzed at the end of ripening and are shown in Table 2. Salt content did not produced differences in hardness, adhesiveness and springiness. However, reduced/replaced treatments presented lower significant values of cohesiveness and consequently chewiness, since this second parameter is the product of hardness, cohesiveness and springiness. It is well known that salt favors the gel formation in fermented sausages and leads to the desirable texture (Ruusunen, M., & Puolanne, E. 2005). However, we have obtained that in slow fermented sausages the reduction/substitution of low salt percentages can affect the cohesiveness and chewiness although the hardness is not affected. Therefore, it is necessary to determine if these changes can be detected by consumers. Only few studies have detected differences in texture parameters by TPA analyses when the level of salt substitution was 40% or higher but not in lower percentages of substitution as we have performed. In this sense, Gou et al. (1996) did not detect differences in texture parameters when KCl was used as unique salt substitute while only Gimeno et al. (1999) reported a decrease in sausage hardness when KCl was used in combination with other divalent salts.

3.3. Sensory analysis

The results of sensory analysis are shown in Table 3. The sensory panel did not detected significant differences among treatments in appearance and tenderness acceptability, however, S treatment had the highest acceptability in aroma, taste, juiciness and overall quality.

Previous studies detected differences in sensory texture parameters when the level of salt substitution was 40% or higher but not in lower percentages of substitution as we have performed. In this sense, Gou et al. (1996), Gelabert et al. (2003) and Guardia et al. (2008) reported an increase in hardness when KCl was used as unique salt substitute. However, the use of KCl in combination with other divalent salts or lactate resulted in a decrease in sausage hardness (Gimeno et al., 1999; Gelabert et al., 2003). Also, it is important to remark that these previous studies were performed mainly in small diameter fermented sausages and there are no reports in slow fermented sausages. Nevertheless, only the juiciness acceptability was the texture parameter that the consumers detected as lowest in reduced salt treatment (RS). The

lowest juiciness acceptability detected in RS treatment could be due to the lower cohesiveness and chewiness (Table 2) observed in this treatment.

It was remarkable to observe that the addition of KCl removed the differences observed in taste, juiciness and overall quality and the consumers showed the same acceptance between S and RSK treatments in these parameters. Therefore a 16 % substitution by KCl can be carried out although the aroma was the unique parameter that was not improved by KCl addition. Therefore, it is necessary to understand which aroma compounds are affected by the salt reduction and substitution.

3.4. Aroma compound analyses

In order to study how salt reduction and substitution affects aroma development in slow fermented sausages, the volatile compounds were extracted by SPME and analyzed by GC-MS and olfactometry analysis (Table 4 and 5, respectively). It is necessary to take into account that the proportion of volatile compounds depends on the extraction method used. In the present study, SPME technique with CAR/PDMS fiber was used. A total of 96 compounds were identified at the end of the process (Table 4) being 20 aldehydes, 11 alkanes, 13 ketones, 1 pyrazynes, 8 sulfur compounds, 8 acids, 17 alcohol, 10 esters, 6 aromatic hydrocarbons and 1 terpene. The volatile compounds present in the sausage treatments classified by chemical groups are shown in Figure 2. The reduction and substitution of salt produced an increase in aldehyde compounds but also a reduction in the abundance of sulphur and acid compounds (Figure 2). One of the chemical groups that was present in highest abundance in the three treatments were the acids, representing 61-72 % of the total extracted area, followed by aldehydes (8-16%) and alcohols (5-9%). Acetic acid was the most abundant compound in headspace (HS) (Table 4). Other abundant compounds were hexanal, 3-methyl-thiophene, octanoic acid, phenol, octane, heptanal, 1-hexanol, hexanoic acid, nonanal, octanal, pentanal, 2-butanone and 3-methyl-2-butenal (Table 4). All the identified compounds have been previously reported in fermented sausages (Marco, Navarro & Flores, 2004, 2006, 2008; Olivares et al., 2011) using the same extraction technique except 3-methyl-2-butenal, 2-hydroxy benzaldehyde and butyl acetate. The identification of compounds was confirmed with authentic standards except benzyl alcohol and methyl 2,4-hexadienoate which were tentatively identified.

In order to study the effect of salt reduction/substitution on the processes involved in the generation of aroma compounds is better to classify the volatile compounds according to their possible origin: lipid autooxidation, bacterial metabolism (lipid β-oxidation, carbohydrate fermentation, amino acid degradation and Staphylococci esterase activity) and unknown or contaminant compounds (Table 4) as indicated Ordoñez, Hierro, Bruna and de la Hoz (1999).

The carbohydrate fermentation volatile compounds were the most abundant compounds, representing 53-58 % of the total extracted area, since acetic acid just represented a 44-52 %. Then, lipid autooxidation volatile compounds represented 16-24 %, amino acid

degradation products 8-10 %, volatile compounds derived from staphylococci esterase activity 0.6-1 % and lipid β-oxidation products 0.6-0.8 %.

Volatile compounds derived from lipid autooxidation have an important role in the odor of dry fermented sausages due to their low olfactory threshold (Marco et al., 2007). Predominantly, the lipid oxidation originates aldehydes among other products such as alkanes, ketones, alcohols, etc. Salt content affected ($p<0.05$) the HS abundance of volatile compounds as observed by a highest abundance in RS and RSK treatments (Table 4). Several compounds have a significant higher abundance in RSK treatment than S and RS treatments such as hexanal, butanal, 2-ethylfuran, 1-pentanol and 2-octenal (Table 4). However, only 2-pentylfuran was more abundant in the HS of RSK treatment than in S treatment while tridecane showed opposite effect. Only two compounds, 1-octanol and 1-heptene, showed more abundance in the HS of RS and RSK than S treatment while hexane and octanoic acid displayed the opposite effect. Finally, octane and heptanoic acid and 1-propanol showed a greater abundance in HS of RS than S and RSK treatments. The higher abundance of compounds derived from lipid oxidation in RS and RSK treatments is in accordance to the TBARS values obtained as they were significantly higher in RS and RSK treatments, probably due to their highest fat content.

On the other hand, salt content did not produced significant differences on volatile compound derived from lipid β-oxidation reactions except for 2-nonanone which was more abundant in the HS of S treatment than in reduced/replaced treatments (RS, RSK). However, several compounds derived from carbohydrate fermentation were affected by salt reduction. The carbohydrate fermentation reactions mainly generate acids, followed by alcohols and ketones. Only ethanol, 2,3-butanediol and 2,3-butanedione showed significant differences among treatments (Table 4). 2,3-butanediol and 2,3-butanedione showed a greater HS abundance ($p<0.05$) in the S treatment while ethanol had the lowest abundance in S treatment. The reduction in 2,3-butanediol abundance was also observed by Olesen et al. (2004), however they detected the opposite effect for 2,3-butanedione. These authors related the 2,3-butanedione concentration to the activity of *Staphylococcus* starter although in the present study we did not detect differences in S*taphylococci* grow among the treatments.

Volatile compounds derived from amino acid degradation depend on free amino acid concentration present in sausages. Branched chain amino acid produces branched aldehydes, alcohols and acids; in addition sulfur amino acids generate sulfur volatile compounds as well as aromatic amino acids produces aromatic compounds. Salt affected the total abundance of this group of compounds as a highest HS abundance ($p < 0.05$) was detected in S than RS treatment. The most abundant compound in HS of sausages within this chemical family was 3-methyl thiophene (Table 4). This compound had a higher abundance in S treatment than RS and RSK treatments, and dimethyl disulfide was also more abundant in S treatment, but it was only significantly different from RSK. Nevertheless, ethyl methyl sulfide showed the lowest abundance in RSK treatment while 3-methyl 2-butenal showed the opposite effect. On the other

hand, several compounds, 3-methyl thiopropanal and benzaldehyde, showed a greater abundance in the HS of reduced treatments (RS, RSK) than in S treatment. However, phenylethyl alcohol and benzeneacetaldehyde were only more abundant in the HS of RS treatment (Table 4).

The compounds derived from the *Staphylococci* activity were also affected by the salt reduction. A higher abundance of total ester compounds was found in the HS of RS treatment, but due to variability among sausages, the differences were not significant. However, several compounds were significantly different among treatments, ethyl acetate, ethyl butanoate, ethyl 2-hydroxy propanoate and ethyl hexanoate were significantly higher in RS treatment than S and RSK treatments (Table 4). These ester compounds provides fruity notes and have been widely detected in slow fermented sausages (Olivares et al., 2011). Talon, Chastagnac, Vergnais, Montel & Berdagué (1998) indicated that the production of esters compounds depended on the presence of the substrates (ethanol and acids) and on the *Staphylococci* esterase activity. In the present study we detected highest ethanol abundance in RS treatment followed by RSK treatment and S treatment that would explain the highest ester production found at the same proportion in the treatments.

About unknown or contaminants compounds, few differences were found among treatments. The presence of sorbic acid and its ethyl and methyl esters came from the potassium sorbate applied to the sausage casing to avoid mold growth as also reported Olivares et al. (2011). Sorbic acid and their esters showed highest abundance in RS treatment while dimethyl sulfone had a highest abundance RSK treatment.

Generally, all the studies performed on volatile compounds in reduced fermented sausages studied a percentage reduction of 25 % or higher (Olesen et al., 2004; Ravyts et al., 2010; Campagnol et al., 2011). While Campagnol et al. (2011) reported few changes in the profile of volatile compounds when salt was substituted in 25 and 50 % by KCl, other authors such as Olesen et al. (2004) indicated a considerable impact on the volatile profile when NaCl was reduced in a 50 % in fermented sausages. In addition, Olesen et al., (2004) indicated that a half percent salt reduction produced an activation of lactic acid bacteria growth giving a higher pH drop affecting negatively the growth of *Staphylococci*. This effect produced a decrease in the generation of branched derived volatile compounds in low salted sausages. However these authors reported that the differences observed among high and low salted sausages were narrowed as the ripening process continued. In the present study, we only analyzed a reduction of the 16 % percent of the total salt content without observing any effect on the growth of lactic acid bacteria and *Staphylococci*, also no differences in pH were detected along the process among treatments. Therefore, the differences that we have observed in volatile compounds derived from amino acid degradation cannot be attributed to a higher *Staphyloccoci* activity. This fact is also in accordance to Ravyts et al. (2010) who indicated that modifications of salt concentrations had a limited impact on the growth of sausage microbiota and they did not find a

significant effect on volatile production. Probably these authors did not find an effect on volatile compounds production because they only extracted few volatile compounds as they used static headspace gas chromatography analysis. However, it is necessary to remember that the flavour of fermented sausages is affected by recipe and the type of starter culture used (Leroy, Verluyten & De Vuyst, 2006).

In order to reveal the aroma contribution of the volatile compounds present in the slow fermented sausages an olfactometry analysis was performed showing the presence of thirty-five different aroma active zones. Twenty six of them were identified by mass spectra, linear retention indices and odor description, while nine of them could not be identified (Table 5). All of identified compounds have been previously detected as aroma impact compounds in fermented sausages (Chevance, Farmer, Desmond, Novelli, Troy & Chizzolini, 2000; Gianelli, Olivares & Flores, 2011; Marco et al., 2007; Meynier, Novelli, Chizzolini, Zanardi & Gandemer, 1999; Olivares et al., 2011; Schmidt & Berger, 1998a, 1998b; Söllner & Schierberle, 2009; Stahnke, 1995a, 1995b) except 2-hydroxy benzaldehyde (herbal, stable, roasted bread), butyl acetate (spice, rancid, wood, boiled vegetables) and 3-methyl thiophene (cooked potato, green, wood). The detection frequency (DF) method was applied to determine the contribution of the different volatile compounds to the aroma of slow fermented sausages. The highest DF values mean a highest aroma impact. The most potent odorants detected were 2-hexenal (roasted, meat broth), 1-octen-3-ol (mushroom), acetic and butanoic acids (vinegar and cheese odors, respectively), dimethyl trisulfide (onion, cabbage), 2-nonanone (plastic, wood), 3-methyl thiopropanal (cooked potato, savory) and D-limonene (citrus). Four of these aroma compounds (acetic and butanoic acids, 1-octen-3-ol and 3-methyl thiopropanal) were also detected as potent odorants in fermented sausages (Olivares et al., 2011). However other potent aroma compounds, contributing with roasted nuts odors, were detected although they were not identified (unknown compounds with LRI 1179 and 1223). These last unknown aroma compounds were also detected in similar fermented sausages by Olivares et al. (2011) using the same extraction technique.

In order to study which aroma compounds were responsible for the highest acceptability of the salted treatment (S), a principal component analysis was done using the following parameters: chemical composition (fat, protein and moisture content), pH, ions (Na^+, K^+, Cl^-), aroma active volatile compounds (those shown in Table 5) and texture parameters. Figure 3 illustrates the results of the PCA analysis. Two principal components were able to explain the 57.88 % of the total variance observed. PC1 is the most important variable because it accounted for 39.83 % of the variance while PC2 accounted for 18.05 % of the variance. PC1 differentiated the sausages by their salt content. S treatment, with the highest salt content, appeared separately in the positive part of PC1, associated with higher texture parameters, higher Na+ and Cl- ions content and the presence of the volatile compounds such as 2-nonanone, dimethyl trisulfide, 3-methyl thiophene, 2,3 butanedione and acetic acid. However,

PC2 differentiated samples with reduced salt content (RS) to S and RSK treatments showing a negative correlation with RS treatment. Therefore, sausage samples with KCl as substitute (RSK treatments) were differentiated but more similar to the S treatment than the reduced salt treatment (RS).

4. Conclusion

In summary, small salt reduction (16%) affected the quality of slow fermented sausages producing a reduction in the acceptance of aroma, taste, juiciness and overall quality. However, the substitution by KCl removed the differences observed in taste, juiciness and overall quality and the consumers showed the same acceptance for high salt (S) and substituted (RSK) treatments. Therefore a 16 % substitution by KCl can be carried out however, the aroma was the unique parameter that was not improved by KCl addition. The aroma perceived by consumers was affected due to the reduction detected in sulfur and acid compounds and the increase in aldehyde compounds. Moreover, the aroma compounds that characterized the high salt treatment (S) were dimethyl trisulfide, 3-methyl thiophene, 2,3-butanedione, 2-nonanone and acetic acid. In addition, the decrease in chewiness and cohesiveness detected in reduced and substituted treatments (RS and RSK) could affect the perception of the aroma compounds. To improve the aroma of reduced salt slow fermented sausages is necessary to look for other alternatives to KCl addition to improve the aroma perception. Further studies about the use of salt-associated odours which can induce a saltiness enhancement should be performed.

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596

Figure legends

Figure 1. Changes in pH, Aw (A) and TBARS (B) during the ripening of dry fermented sausages: S (control, ○), RS (16 % reduced salt, ∇) and RSK (16% KCl to replace NaCl, □)

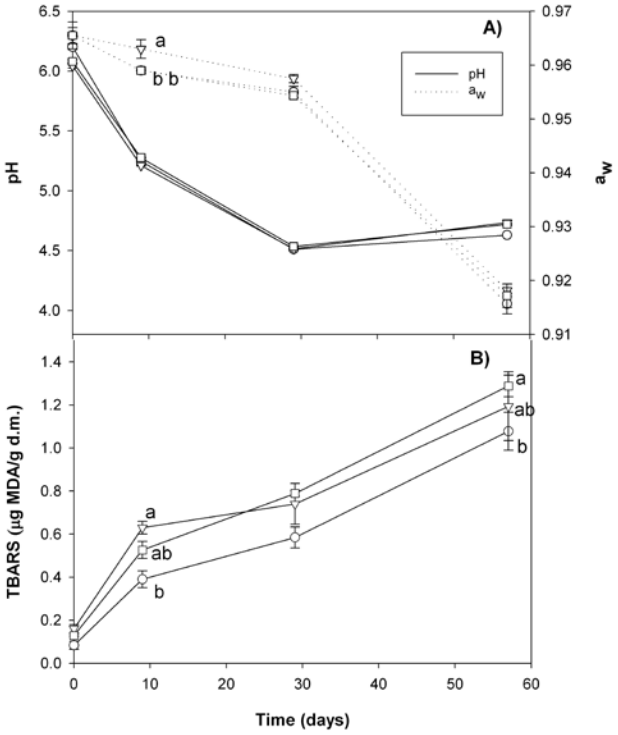

Figure 2. Total volatile compounds abundance expressed as AU x 106 in the headspace of dry fermented sausages with different salt content: S (control), RS (16 % reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process. Different letters in the same chemical group indicate significant differences ($p<0.05$) among treatments.

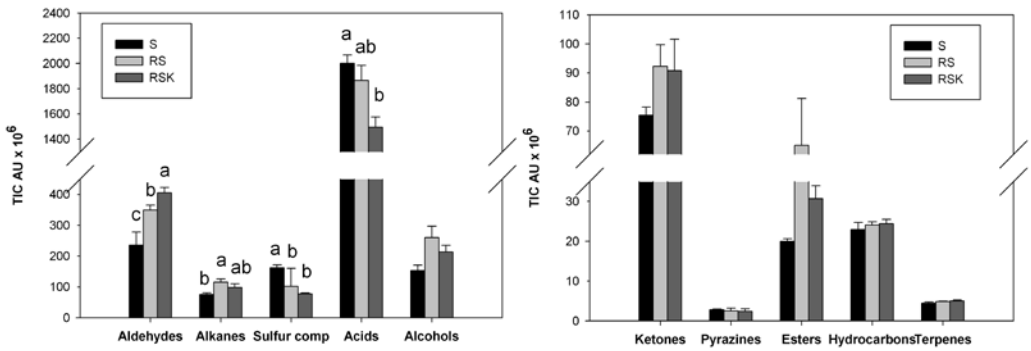

Figure 3. Loadings of the first two principal components (PC1-PC2) of the analyzed parameters (pH, TBARS, ions, texture parameters and aromatic active compounds) of fermented sausages with different salt content: S (control), RS (16 % reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process.

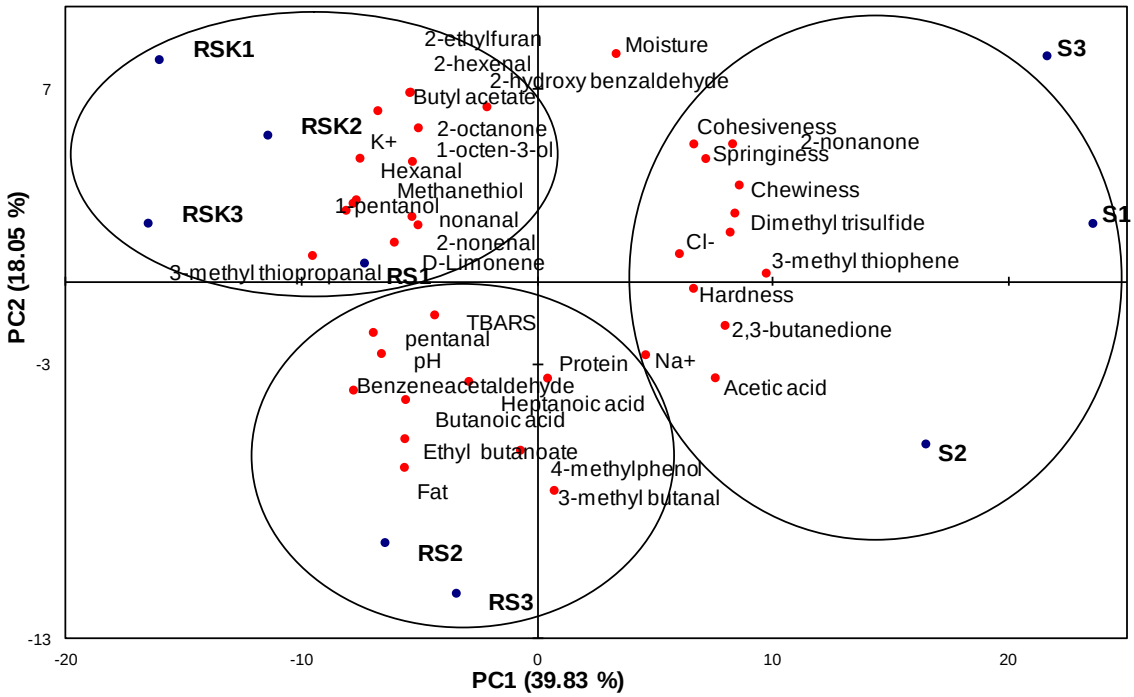

Table 1. Chemical composition and ion contents in dry fermented sausages with different salt content: S (control), RS (16 % reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process (57 days).

		S	RS (red16%)	RSK (red16% +KCl)	P
Moisture	(%)	49.51	48.06	49.23	ns
Fat	(%)	10.75	12.73	12.32	ns
Protein	(%)	35.32ab	37.00	34.88b	ns
Na^+	(g/100 g d.m.)	3435.38 a	3074.55 ab	2748.67 b	***
K^+	(g/100 g d.m.)	952.26 b	996.35 b	1458.14 a	***
Cl^-	(g/100 g d.m.)	3257.52	2946.78	2992.77	ns

P: P value of salt content effect. *** P<0.001, ** P<0.01, * P<0.05, ns: P>0.05. Identical letters in each row indicate the absence of differences at p>0.05 (Fisher test).

Table 2. Texture parameters of dry fermented sausages with different salt content: S (control), RS (16 % reduced salt) and RSK (16% KCl to replace NaCl).

	S	RS (red16%)	RSK (red16% +KCl)	P
Hardness (N)	257.85 (22,60)	245.43 (17.22)	246.23 (14.64)	ns
Adhesiveness (N·s)	-3.34 (0.54)	-3.26 (0.47)	-3.68 (0.58)	ns
Springiness	0.63 (0.02)	0.61 (0.03)	0.61 (0.03)	ns
Cohesiveness	0.64 a (0.02)	0.62 b (0.02)	0.62 b (0.01)	*
Chewiness	103.49 a (9.21)	93.11 b (8.96)	93.24 b (7.94)	**

P_S: P value of salt content effect. *** P<0.001, ** P<0.01, * P<0.05, ns: P>0.05. Identical letters in each row indicate the absence of differences at p>0.05 (Fisher test).The values represent the mean and (standard deviation).

Table 3. Sensory acceptability of dry fermented sausages with different salt content: S (control), RS (16 % reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process.

	S	RS (red16%)	RSK (red16% +KCl)	P
Appearance	6.14 (1.57)	5.86 (1.41)	5.96 (1.48)	ns
Aroma	6.33 a (1.46)	5.93 b (1.37)	5.89 b (1.44)	*
Taste	5.96 a (1.89)	5.34 b (1.80)	5.84 a (1.94)	**
Tenderness	6.13 (1.64)	6.00 (1.69)	6.18 (1.61)	ns
Juiciness	6.22 a (1.63)	5.75 b (1.60)	5.99 ab (1.58)	*
Overall quality	5.92 a (1.72)	5.54 b (1.61)	5.92 a (1.78)	*

P_S: P value of salt content effect. *** P<0.001, ** P<0.01, * P<0.05, ns: P>0.05. Identical letters in each row indicate the absence of differences at p>0.05 (Fisher test). The values represent the mean and (standard deviation).

Table 4. Volatile compounds (expressed as AU x 10^6 extracted by HS-SPME) identified in the headspace of dry fermented sausages with different salt content: S (control), RS (16 % reduced salt) and RSK (16% KCl to replace NaCl) at the end of the ripening process.

Compound	LRI [B]	RI[C]	Sausage Batches S		RS (red16%)		RSK (red16% +KCl)		SEM	P[D]
Lipid autooxidation										
Pentane	500	a	8.25	c	19.98	b	32.45	a	1.66	***
Propanal	524	a	4.78		11.00		9.78		2.62	ns
Isopropyl alcohol	542	a	3.26		6.58		10.41		1.92	ns
Hexane	600	a	12.05	a	5.81	b	5.88	b	1.32	*
1-Propanol (31)[A]	613	a	0.06	b	0.11	a	0.09	ab	0.01	*
2-Methylfuran (82)[A]	615	a	1.23		1.21		0.69		0.15	ns
Butanal	622	a	0.56	b	1.41	b	3.04	a	0.27	**
1-heptene (55)[A]	693	a	0.03	b	0.25	a	0.31	a	0.03	**
Heptane (71)[A]	700	a	1.84		5.55		5.31		1.19	ns
2-Ethylfuran (81)[A]	720	a	1.55	b	1.13	b	3.11	a	0.32	*
Pentanal (44)[A]	737	a	18.82		33.63		33.67		5.19	ns
Octane	800	a	25.95	b	60.93	a	37.00	b	5.21	*
2-octene	810	a	13.15		8.91		6.13		1.66	ns
1-Pentanol	826	a	9.35	b	12.22	b	17.79	a	1.11	**
Hexanal (44)[A]	840	a	72.90	b	92.41	b	137.28	a	10.45	*
Nonane	900	a	3.14		2.56		2.36		0.28	ns
2-Hexenal (Z)	904	a	1.84		1.47		2.56		0.39	ns
2-Butylfuran	908	a	1.71		2.06		1.88		0.25	ns
1-Hexanol	922	a	28.19		45.87		37.14		5.18	ns
Heptanal	940	a	19.06		54.79		55.22		9.72	ns
Decane	1000	a	1.81		1.84		1.85		0.20	ns
2-Penthylfuran	1009	a	3.61	b	8.73	ab	11.78	a	1.51	*
Octanal	1047	a	19.79		31.80		33.30		3.36	ns
Hexanoic acid	1075	a	37.54		42.70		43.92		2.15	ns
2-Ethyl 1-hexanol	1082	a	6.89		7.54		8.00		0.60	ns
Undecane (57)[A]	1100	a	0.16		0.15		0.18		0.02	ns
2-Octenal (Z)	1115	a	1.03	b	1.88	b	4.24	a	0.64	*
1-Octanol	1123	a	1.02	b	2.84	a	2.74	a	0.40	*
Nonanal	1149	a	28.86		37.94		37.95		3.62	ns
Heptanoic acid	1165	a	2.03	b	3.23	a	2.16	b	0.29	*
Dodecane	1200	a	6.32		7.19		5.36		0.62	ns
2-Nonenal (Z)	1221	a	2.12		3.09		2.90		0.39	ns
Decanal	1256	a	1.99		2.36		1.73		0.35	ns
Octanoic acid	1266	a	87.82	a	35.80	b	36.79	b	5.78	***
2,4-Nonadienal (E, E)	1287	a	tr.		tr.		tr.			
Tridecane	1300	a	2.83	a	2.05	ab	1.39	b	0.24	*
Nonanoic acid	1357	a	3.20		2.83		2.85		0.16	ns
Decanoic acid	1449	a	7.98		6.80		6.97		0.90	ns
Total			442.70	b	566.66	a	606.23	a	36.77	*
Bacterial metabolism										
Lipid β oxidation										
2,3-Pentanedione (85)[A]	744	a	0.17		0.14		0.08		0.03	ns
2-Heptanone	933	a	4.64		7.88		7.70		1.10	ns
2-Heptanol	946	a	5.92		4.32		4.01		0.67	ns
1-Octen-3-ol (57)[A]	1030	a	2.13		2.01		3.80		0.56	ns
2-Octanone	1039	a	0.62		0.61		0.90		0.09	ns
2-Nonanone	1140	a	5.60	a	3.01	b	3.78	b	0.29	**
2-Undecanone	1306	a	1.27		1.25		1.11		0.16	ns
Total			20.36		19.22		21.38		2.20	ns
Carbohydrate fermentation										
Acetaldehyde	466	a	9.94		12.44		10.67		0.67	ns
Ethanol	508	a	13.11	c	96.86	a	49.08	b	12.11	**
Acetone	530	a	20.48		27.79		24.48		4.18	ns
2,3-Butanedione (43)[A]	626	a	0.52	a	0.34	b	0.30	b	0.04	*
2-Butanone	631	a	28.96		30.95		28.48		2.41	ns

Acetic acid	737	a	1447.65		1295.19		1228.50		59.09	ns
2,3-Butanediol	887	a	1.63	a	0.26	b	0.33	b	0.25	*
Butanoic acid	892	a	72.74		80.23		82.45		4.31	ns
Total			1595.03		1544.06		1424.30		75.20	ns
Amino acid degradation										
2-Methyl propanal	594	a	7.42		7.38		5.59		1.06	Ns
Ethyl methyl sulfide (61)[A]	624	a	0.98	a	0.79	a	0.41	b	0.10	*
Benzene	675	a	0.95		1.00		1.20		0.13	Ns
2-Methyl 1-propanol	682	a	0.36		0.20		0.31		0.08	Ns
3-Methyl butanal (44)[A]	689	a	8.89		9.32		8.36		0.96	Ns
2-Methyl butanal (58)[A]	700	a	2.50		2.30		2.11		0.21	Ns
Dimethyl disulfide	772	a	4.40	a	3.11	ab	2.06	b	0.51	*
Toluene	788	a	12.83		14.11		13.63		0.76	Ns
3-Methyl-3-buten-1-ol (41)[A]	789	a	0.30		0.24		0.29		0.05	Ns
3-Methyl thiophene	794	a	125.16	a	68.25	b	42.68	b	6.88	***
3-Methyl 2-butenal (55)[A]	840	a	14.73	b	17.74	b	28.74	a	2.61	*
Ethyl benzene	883	a	2.46		2.74		2.90		0.20	Ns
2,5-dimethyl pyrazine	944	a	2.81		2.53		2.42		0.52	Ns
3-Methyl thiopropanal	966	a	6.24	b	9.89	a	11.66	a	0.75	**
Dimethyl trisulfide	1002	a	1.80		1.25		1.01		0.20	Ns
Benzaldehyde	1017	a	16.33	b	21.55	a	22.29	a	1.40	*
2-Hydroxy benzaldehyde (122)[A]	1100	a	0.27		0.27		0.28		0.01	Ns
Benzeneacetaldehyde	1107	a	3.69	c	6.82	a	5.65	b	0.30	***
Phenol	1111	a	75.55		74.23		73.78		2.99	ns
Benzyl alcohol	1120	b	1.63		1.66		1.53		0.05	ns
Phenylethyl alcohol (91)[A]	1194	a	0.39	b	2.32	a	0.92	b	0.16	***
Total			289.69	a	247.69	b	227.82	ab	16.78	ns
Staphylococci esterasa activity										
Methyl acetate	551	a	2.70		1.98		1.62		0.27	ns
Ethyl acetate	635	a	6.76	b	26.99	a	13.21	b	2.58	**
Ethyl propanoate (57)[A]	744	a	0.27		0.62		0.47		0.12	ns
Ethyl butanoate (71)[A]	831	a	0.28	c	2.90	a	1.29	b	0.30	**
Butyl acetate	847	a	2.05		2.55		4.69		0.64	ns
Ethyl 2-hydroxy-propanoate	869	a	1.78	b	16.45	a	1.97	b	0.44	***
Ethyl hexanoate (88)[A]	1028	a	0.10	c	1.22	a	0.71	b	0.09	***
Ethyl octanoate	1229	a	2.73		4.42		3.14		0.83	ns
Total			16.68		57.12		27.09		9.78	ns
Unknown or contaminants compound										
Methanethiol	472	a	7.83		9.17		9.84		0.47	ns
Carbon disulfide	537	a	13.78		7.75		8.53		1.59	ns
o-Xylene	916	a	3.24		3.23		3.33		0.23	ns
Styrene	918	a	1.61		1.27		1.36		0.15	ns
2-Butoxyetanol	952	a	2.35		2.74		2.87		0.31	ns
Butyrolactone	1023	a	5.07		7.15		6.49		0.82	ns
p-Cymene	1050	a	1.81		1.72		2.00		0.13	ns
D-Limonene	1045	a	4.48		4.85		5.03		0.20	ns
Dimethyl sulfone	1061	a	1.27	b	1.37	b	1.61	a	0.06	*
Methyl 2,4-hexadienoate (67)[A]	1065	b	0.19	a	0.22	a	0.06	b	0.01	***
Ethyl 2,4-hexadienoate	1144	a	3.13	b	7.72	a	3.57	b	0.75	*
Sorbic acid	1179	a	342.07	a	397.89	a	91.74	b	42.89	**
4-Methyl-phenol (107)[A]	1195	a	0.46		0.58		0.43		0.04	ns
Total			387.29		445.65		136.87		82.35	ns

AU: Abundance units, the result of counting the total ion chromatogram (TIC) for each compound.

[A] Target ion used to quantify the compound when the peak was not completely resolved.

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

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

[D] p value of salt content effect. ***: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05.

Means followed by different letters in the same compound indicate significant differences (p<0.05) among batches.

Table 5. Odor active compounds identified in the HS of dry fermented sausages

Compound	LRI GC-O	LRI standard	Descriptor	DF	Previously reported in dry sausages
Lipid autooxidation					
2-Ethylfuran	725	718	Tallowy, savory, sweet	4	8
Pentanal	734	735	Green, nut, meat broth	7	1,8
1-Pentanol	815	820	Floral, butter, roasted nuts	5	8,10
Hexanal	835	836	Fresh cut grass	8	1,2,5,7-9,11
2-Hexenal (Z)	903	904	Sweet, roasted, meat broth	12	8
Nonanal	1148	1151	Citrus, laurel, carnation	8	1,2,7-10
Heptanoic acid	1163	1162	Herbal, rancid	6	8,10
2-Nonenal (Z)	1219	1222	Herbal, strawberry	4	1,2,8-11
2,4-Nonadienal (E, E)	1290	1288	Herbal, unpleasant, roasted	6	7,9,11
Bacterial metabolism					
Lipid β oxidation					
1-Octen-3-ol	1024	1028	Mushroom	12	5,8,11
2-Octanone	1031	1037	Green, garlic	10	11
2-Nonanone	1137	1142	Plastic, wood, pop-corn, roasted	11	1,3,4,8
Carbohydrate fermentation					
2,3-Butanedione	632	632	Butter	4	1-4,6,8,10,11
Acetic acid	705	700	Vinegar	11	1-3,8-11
Butanoic acid	871	876	Cheese	12	1-4,8,9,11
Amino acid degradation					
3-Methyl butanal	691	691	Green	5	1,2,8,9,11
3-Methyl thiophene	795	796	Cooked potato, green, wood	4	11
3-Methyl thiopropanal	967	969	Cooked potato, savory	11	7-11
Dimethyl trisulfide	1007	1009	Onion, rotten, cabbage	12	6
2-Hydroxy benzaldehyde	1105	1107	Herbal, stable, roasted bread	4	-
Benzeneacetaldehyde	1110	1112	Rancid, musk, jasmine	6	8-11
Staphylococci esterasa activity					
Ethyl butanoate	825	825	Pineapple, strawberry	6	1-5,8-11
Butyl acetate	843	840	Spice, rancid, wood, boiled vegetables	9	-
Unknown or contaminants compound					
Methanethiol	472	471	Rotten, stable	8	8,11
D-Limonene	1046	1048	Citrus	11	3-5,8
4-Methyl-phenol	1194	1190	Plastic, stable, rancid,	7	3,4,9-11
Unknown 1	922		Green, rancid, manure, cheese	6	
Unknown 2	962		Onion, Swiss chard	7	
Unknown 3	1001		Herbal, roasted, damp, vanilla	6	
Unknown 4	1179		Roasted nuts	10	
Unknown 5	1223		Roasted nuts, unpleasant, cardboard	11	

[A] Linear retention indices (LRI) of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column (J&W Scientific 60 m x 0.32 mm i.d. x 1.8 µm film thickness).

[B] DF Detection frequency value

[C] Previously reported in dry fermented sausages by: 1 Stahnke (1994), 2 Stahnke (1995b), 3 Schmidt and Berger(1998a), 4 Schmidt and Berger (1998b), 5 Meynier et al. (1999), 6 Chevance et al. (2000), 7 Blank et al. (2001), 8 Marco et al. (2007), 9 Söllner et al. (2009), 10 Gianelli et al. (2011), 11 Olivares et al. (2011).