- 1 NITRATE REDUCTION IN THE FERMENTATION PROCESS OF SALT
- 2 REDUCED DRY SAUSAGES: IMPACT ON MICROBIAL AND
- 3 PHYSICOCHEMICAL PARAMETERS AND AROMA PROFILE.
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11 Abstract

12 Slow fermented sausages with reduced ingoing amounts of sodium nitrate were
13 manufactured: control (250 ppm), 15 % (212.5 ppm) and 25 % (187.5 ppm) reduction.
14 The effect of nitrate reduction on microbiology and chemical parameters, volatile
15 compounds and aroma production was studied. Parameters like, pH, a_w and color
16 decreased during ripening, without being affected by nitrate reduction. Lipid oxidation
17 increased during ripening and it was higher in control sausages due to fat content.
18 Residual nitrite was below the detection limit during the whole process and residual
19 nitrate decreased during ripening, with higher reduction in RN25 sausages. Lactic acid
20 bacteria, total mesophilic bacteria and yeasts and molds increased during ripening but
21 Gram positive cocci decreased. Microbial counts from nitrate reduced sausages at the
22 end of the manufacturing process were not statistically different from the control
23 sausages with nitrate. Regarding volatile compounds formation, compounds derived
24 from amino acid degradation were increased by nitrate reduction. Aroma compounds
25 derived from amino acid degradation and responsible for strong odours, dimethyl
26 disulphide (toasted, garlic) and methional (cooked potato) and, to a lesser extent,
27 compounds derived from esterase activity producing fruity odours (ethyl acetate, ethyl
28 butanoate, ethyl-2-hydroxypropanoate, ethyl-2-methylbutanoate and ethyl-3-
29 methylbutanoate) and several compounds from carbohydrate fermentation acetic acid
30 (vinegar odour) and 2-butanone (fruity) were related to the high nitrate reduction (25%).
31 Despite nitrate reduction up to 25% produced minor effect on microbial growth, their
32 metabolism is regulated by nitrate content and therefore by nitrite generation affecting
33 the production of key aroma compounds that alter the sausage aroma profile.

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35 Keywords: nitrate reduction; fermented sausage; flavor; aroma; microbial safety.

1. Introduction

Nitrite has several important functions in the manufacturing of meat products. Among them, colour improvement through its reduction to nitric oxide and development of cured flavour, are the most important for consumer acceptability (Sindelar et al., 2011). Moreover, nitrite is able to control the oxidative stability by preventing lipid oxidation (Berardo et al., 2016) and plays an important role inhibiting the growth and toxin production of *Clostridium botulinum* (Rahman, 2007). On the other hand, generation of nitrosamines in meat products by reactions between nitrosating agents, derived from nitrite, and secondary amines, derived from protein and lipid degradation, has been of safety concern due to their carcinogenic potential (De Mey et al 2015). Generally, intensive heat treatments are necessary to obtain carcinogenic nitrosamines and in case of fermented sausages, the mild acidic conditions and low water activities prevents their formation (De Mey et al., 2015). The search for alternatives to nitrite have focused on the use of spices and fruits (Gassara et al., 2015), organic acids, antimicrobial peptides, etc (Alahakoon et al., 2015). However, none of them fulfils all technological needs (colour, flavour, microbiological safety and antioxidant activity) and the actual trend is directed to reduce the ingoing amount of nitrite and nitrate in meat products (EFSA, 2010, FCEC 2016).

Previous studies have demonstrated that the reduction of nitrate and nitrite in dry fermented sausages produces changes in quality (Hospital et al., 2015) and microbial safety (Hospital et al., 2012, 2014, 2016). Dry sausages with reduced amounts of nitrate and nitrite contained the highest amounts of volatile compounds derived from carbohydrate fermentation and amino acid degradation (Hospital et al., 2015) which could be attributed to increased counts of Gram-positive catalase-positive cocci and Enterobacteriaceae at the end of the ripening. Furthermore, the presence of nitrite is

essential to control *Salmonella typhimurium* growth in fermented sausages; although, reduced concentrations of the compound are sufficient to provide equal protection (Hospital et al., 2014). Regarding *Listeria*, nitrite reduction increased the growth of inoculated *L. innocua* in the final sausage. Production of *C. botulinum* toxin has not been detected in nitrate and nitrite reduced fermented sausages, although this could depend on existing hurdles and specific ripening conditions (Hospital et al., 2016).

The effect of reduced ingoing amounts of nitrate and nitrite in fermented meat products seems to depend on the presence of both agents (nitrite and nitrate) and specific ripening conditions. Nevertheless, the specific provision concerning nitrites and nitrates (Regulation (EC) nº 1333/2008) indicates the possibility of the exclusively use of nitrates (250 ppm) without added nitrite in traditional slow ripened sausages such as “salchichón” and “chorizo” with maturation period of at least 30 days. Presently, nothing is known about the effect of nitrate reduction on these traditionally cured meat products where nitrate is used exclusively as curing agent.

Slow-fermented sausages are typical Mediterranean products subjected to a first ripening step at low temperatures and a second step at higher temperatures in order to develop special sensory characteristics (Toldrá & Flores, 2014). These sausages have a mild pH decline, although starters are added in the process to improve the microbial safety of the product. The long process allows the exclusively use of nitrate as a nitrite reservoir by the activity of acid sensitive bacteria from the *Staphylococcaceae* family (Sánchez Mainar & Leroy, 2015). Nitrate slow fermented sausages have a preferred flavour than those made with nitrite and provide better organoleptic characteristics (Marco et al., 2008). In fact, the use of only nitrate instead of nitrite in dry fermented sausages produces the inhibition of lipid oxidation and affects the generation of volatile

compounds derived from microbial metabolism, but as Marco et al. (2006) indicated it depends greatly on the fermentation process applied.

The longer ripening times employed in traditional dry sausages and the use exclusively of nitrates without added nitrite can produce different effects on the quality and safety of reduced nitrate sausages. Factors such as final pH, temperature and a_w are closely interrelated with microbial growth. Moreover, the actual trends to reduce sodium in meat products may also affect microbial growth and sausage safety (Aaslyng et al., 2014; Laranjo et al., 2016). In view of these actual trends, the aim of this study was to evaluate the reduction of nitrate used exclusively without added nitrite on the overall quality of traditional dry sausages with reduced sodium content.

2. Materials and methods

2.1 Dry fermented sausage manufacture

Three different formulations (6 kg/formulation) of dry fermented sausages were manufactured: sodium nitrate at 250 ppm (C), 15% reduction (RN15) and 25 % reduction (RN25). Three replicates of each formulation **were carried out. Pork's lean** and belly fat were purchased from a local producer (Cárnicas La Cope, Torrente, Spain).

For each manufacture batch, lean (50 % lean pork meat) and fat (50% pork belly) were ground through a 10 mm diameter mincing plate and mixed with the following ingredients: 20 g/kg lactose, 20 g/kg dextrin, 7 g/kg glucose, 20.25 g/kg sodium chloride (NaCl), 6.75 g/kg potassium chloride (KCl), 0.5 g/kg sodium ascorbate, and sodium nitrate at 250 ppm (C), 212.5 ppm (RN15) or 187.5 ppm (RN25) depending on the batch. Also, a commercial starter culture (0.125 g/kg) TRADI-302

(Danisco, Cultor, Madrid, Spain) containing *Lactobacillus sakei*, *Staphylococcus xylosus* and *Staphylococcus carnosus* was added.

Each formulation was kept at 3–5°C for 24 h and then stuffed into 95 mm diameter collagen casings (Fibran, S.A., Girona, Spain), being the final weight of each sausage approximately 500 g. Sausages were dried for 62 days at 10°C and 75–90% relative humidity (RH). In order to control the ripening process, temperature and RH of the ripening chambers were continuously recorded. Two sausages from each batch were weighed almost every day to control weight losses. Also, one sausage from each batch was used to control the pH by introducing a pH meter HI 99163 (Hanna Instruments Inc., Hoonsocket, USA) into the centre of the sausage as described by Olivares et al. (2010).

A total of 9 batches were produced and approximately 10 sausages were obtained from each batch. Before stuffing, approximately 200 g of the minced meat mixture were collected for further analysis. At the end of the process, two sausages from each batch were randomly chosen for analysis. A slice of approximately 25 g was taken for microbial analyses. Several slices were taken, wrapped in aluminium foil, vacuum packed and storage at –80°C for volatile analysis. Sausage colour was measured and approximately 100 g of sausage were minced and used for moisture, water activity (a_w) and pH analysis. The remaining minced sausage was vacuum packed and frozen at –20°C for subsequent physicochemical analyses (TBARS, lipid, protein, residual nitrite and nitrate).

2.2 Physicochemical analysis.

2.2.1 pH, a_w , colour and moisture

pH was measured with a pH meter HI 99163 (Hanna Instruments Inc.) using minced sausage and distilled water (1:1, p/v) (ISO 2917:1999). Water activity was measured with a Fast-lab water activity meter (Gbx, Romans sur Isère Cédex, France) and colour was analysed with a colorimeter (CR-400/410, Konica Minolta Sensing Inc., Japan) with D65 illuminant (Olivares et al., 2010). Moisture was determined by drying at 100°C until constant weight (BOE, 1979).

2.2.2 Lipid and protein content

Lipid content was determined by organic extraction with $\text{Cl}_2\text{CH}_2:\text{CH}_3\text{OH}$ (2:1) (Folch, Lees & Stanley, 1957) as described Olivares et al. (2010). Nitrogen content was determined by the Kjeldahl method and protein estimated by multiplying the nitrogen content by 6.25.

2.2.3 Oxidation and nitrifying agents

Lipid oxidation was evaluated using the thiobarbituric acid reactive substances test (TBARS) as described Corral et al., (2013). Values were expressed as μg of malonaldehyde per gram of dry matter (μg MDA/g dm). Nitrate and nitrite contents were extracted with hot water (Mohamed et al., 2008) and determined using an enzymatic kit (Boehringer) (Arneth & Herold, 1998).

2.3 Microbiological analysis

Microbial counts were done on 25 g of dry fermented sausage. Samples were finely sliced, blended with 225 ml of buffered peptone water (Pronadisa, Spain) and homogenized in a Pulsifier (Microgen Biotech, Spain). Homogenates were used to prepare decimal dilutions which were spread on appropriate media plates. Microbial counts were determined on the following media: bacterial starter containing lactic acid bacteria (LAB) on MRS Agar (Scharlau, Spain) at 30 °C for 3 days and Gram positive

cocci on Mannitol Salt Agar (MSA) (CN-M) (Scharlau, Spain), at 30°C for 3 to 5 days and Baird Parker Agar (BP) (CN-BP) (Pronadisa, Spain) at 37°C for 48 hours. Coagulase test was carried out in Gram positive cocci isolates from BP (EN ISO 6888–1) using lyophilised rabbit plasma (Scharlau, Spain). Mesophilic bacteria (TMB) were determined on Plate Count Agar (Pronadisa, Spain) at 30 °C for 3 days, yeasts and moulds on Rose Bengal Agar with chloranphenicol (Scharlau, Spain) at 30°C for 5 to 7 days. Enterobacteriaceae were counted on Violet Red Bile Agar with Glucose (VRBG) (Pronadisa, Spain) at 37°C for 24 hours in anaerobiosis. Sulphite reducing clostridia were determined from 1 ml homogenate sample inoculated in freshly prepared Lactose Sulfite Broth supplemented with sodium metabisulfite and ferric ammonium citrate (Pronadisa, Spain) dispensed into tubes with Durham gas collecting tubes and incubated in anaerobiosis (bioMerieux, Spain) at 46°C for 48 hours. Twenty-five ml of homogenated sample were used for enrichment of *Yersinia enterocolitica* in Sorbitol Peptone Broth and Bile Salts (PBS) (Pronadisa, Spain) at 25°C for 5 days and subsequently plated on Yersinia Selective Agar (YSA) (Pronadisa, Spain) at 30°C for 48 hours. Twenty-five ml of homogenated sample were used for enrichment of *Listeria* spp. in ½ Fraser and Fraser Broth supplemented with ferric ammonium citrate (Pronadisa, Spain) at 30°C for 24 and 48 hours, respectively. Dilutions of the *Listeria* enriched Fraser medium were inoculated onto Listeria Chromogenic Agar (Pronadisa, Spain) and incubated at 37°C for 24 hours.

The remaining homogenate was incubated at 37°C during 16–20 hours for Salmonella pre-enrichment. One millilitre of the incubated homogenate was used for enrichment of *Salmonella* spp in Rappaport Soy Broth (Pronadisa, Spain) at 41.5°C for 24 hours and Muller Kauffmann Broth Base w/Brilliant Green and Novobiocin supplemented with iodine and potassium iodide solution (MKTTN) (Pronadisa, Spain)

at 37°C for 24 hours. Enriched cultures were plated on Xylose Lysine Desoxycholate Agar (XLD) (Pronadisa, Spain) and incubated at 37°C for 24 hours.

2.4 Volatile compound analysis.

2.4.1 Gas chromatography-Mass spectrometry

The analysis of headspace (HS) volatile compounds was carried out by solid phase micro extraction (SPME) with an 85 µm Carboxen/Polydimethylsiloxane (CAR/PDMS) fibre (Supelco, Bellefonte, PA) using a gas chromatograph (Agilent HP 7890 series II (Hewlett-Packard, Palo Alto, CA) with a mass detector (HP 5975C (Hewlett-Packard) equipped with an autosampler (Gerstel MPS2 multipurpose sampler (Gerstel, Germany), as described Corral et al. (2015) with minor modifications. Briefly, 5 g of sausage with 0.75 mg BHT to avoid oxidation, was weighed into a headspace vial. The vial was incubated at 37 °C for 30 min. Then, the fibre was exposed into the headspace vial for 120 min while maintaining the sample at 37 °C. The compounds adsorbed by the fibre were desorbed in the injection port of the GC-MS for 5 min at 240 °C in splitless mode. The compounds were identified by comparison with mass spectra from the library database (Nist'05), with linear retention indices (Van Den Dool and Dec. Kratz, 1963) and with authentic standards. The results were expressed as abundance units (AU) 10⁵.

2.4.2 Gas chromatography-Olfactometry/FID

The analysis of aroma compounds was carried out by a gas chromatograph (Agilent 6890, USA) equipped with a FID detector and sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) as described Corral et al. (2015). Each assessment was carried out with 5 g of sample using the detection frequency method (Pollien et al.,

1997). Four trained panellists evaluated the odours from the GC-effluent of the sausages at the end of the maturation (62 days). A total of 12 assessments were carried out. The aroma compounds were identified by comparison with mass spectra, with linear retention indices of authentic standards injected in GC-MS and GC-O and by the coincidence of the assessor's descriptors with those in the Fenaroli's handbook of flavour ingredients (Burdock, 2002).

2.5 Statistical analysis

Data were analysed using the Generalized Linear Model (GLM) procedure of statistical software (XLSTAT 2011, v5.01, Addinsoft, Barcelona, Spain). Data was analysed using the linear mixed model and included nitrate reduction and ripening time as fixed effects, and replicates as random effect. When significant effect of the treatment group was detected ($P < 0.05$), least squares means (LSM) were compared using Tukey test. Principal component analysis (PCA) was done to evaluate the relationships among sausage formulation and different parameters (pH, water activity, TBARS, protein and fat content, nitrate residual, colour, microbiota and aroma compounds).

3. Results and discussion

3.1 Physicochemical analyses

Physicochemical parameters are shown in table 1. Observed differences between batches in fat and protein content were due to random formulation variations which resulted in a higher fat content in control sausages and small differences in weight losses (C 39.78%, RN15 41.97%, RN25 42.58%). Final dry fermented sausages had an average pH value of 4.9, as expected for this type of product (Marco et al., 2008), and

the a_w decreased from 0.977 to 0.893 (average values). These results guarantee the stability and safety of the sausages and, therefore, nitrate reduction did not seem to affect them, as observed in nitrite and nitrate reductions by Hospital et al. (2015). The colour parameters evaluated (L^* , a^* and b^* values) decreased during ripening due to drying with values within the expected range (Olivares et al., 2010). Nitrate reduction did not affect the colour of sausages neither at the beginning nor at the end of process, although a higher L^* value was detected in the control raw sausage than in nitrate reduced ones (RN15 and RN25). Regarding lipid oxidation, it increased with ripening in all sausages, although at the end of process C batch had a significant higher TBARS value than RN15 and RN25. The antioxidant activity of nitrite is well known (Zanardi et al., 2004), therefore, the higher lipid oxidation in C batch could be due to its higher fat content (Olivares et al., 2011).

At the beginning of the process, nitrite was not detected because only nitrate was added and there was not enough time for its reduction. At the same time, residual nitrate content confirmed the different ingoing amounts added to each batch. At the end of the process, the nitrate decline was 33, 39 and 52% respect to the initial amount (C, RN15 and RN25). Nitrite was never detected; this compound was always below the detection limits. Marco et al. (2006, 2008) also observed a decrease of nitrate concentration during ripening of slow fermented sausages with only nitrate added. These authors found a nitrate decline of approximately 30% in sausages with a final pH value of 4.8 (Marco et al., 2006), however, higher nitrate drops were observed in sausages with pH slightly above 5 (Marco et al., 2008). In both cases, residual nitrate amounts were close to 100 ppm dm. In contrast, the residual amounts of the manufactured fermented sausages were above 100 ppm dm but the differences may be due to the fermentation process and its effect on the nitrate reductase enzyme (Cachaldora et al., 2013).

3.2 Microbiological analyses

Microbial counts are shown in the table 2. At the beginning of the process, total mesophilic bacteria (TMB) and lactic acid bacteria (LAB) had mean counts of 6.8 and 5.8 log cfu/g respectively, in accordance with the amount of starter inoculated. Moreover, the increase by 2 log in TMB and 1 log in LAB during ripening is in the range expected for this type of sausage (Fontana et al., 2005). The population of Gram positive cocci that include staphylococcal starters numerated either on MSA (CN-M) or on BP (CN-BP) decreased during the process. This could be due to the pH decrease produced by LAB which is affecting coagulase negative staphylococci (Leroy et al., 2006) as well as NaCl reduction and replacement by KCl as observed by Corral et al. (2014). Coagulase test on CN-BP cocci isolates (about 400 studied) classified all of them as coagulase negative and probably representative of *Staphylococcus* species also included in the bacterial starter. Enterobacteria counts decreased by 2 log from the beginning to the end of the process. Yeasts and moulds increased until 10^3 cfu/g in final sausages. Sulphite reducing clostridia, *Salmonella* spp. and *Yersinia enterocolitica* were not present during the whole process. Listeria counts were also negative in all samples except for two positive colonies grown on LCA media in a replicate of RN25 reduced nitrate sausages at the end of the fermentative process.

In summary, microbial counts in control samples were in the range expected and followed the dynamics observed in slow fermented sausages (Rantsiou et al., 2005; Samelis et al., 1998, Di Luccia et al., 2016, Tabanelli et al., 2016). Hospital et al. (2015) studied the effect of nitrate and nitrite reduction on the microbial growth of dry fermented sausages. In agreement with our results, these authors did not find differences in LAB counts at the beginning of the process, but neither in Gram positive catalase positive cocci. The differences between results in the present study and those of

Hospital et al. (2015) can also be attributed to the initial addition of nitrite together with nitrate and the kind of fermentation (Marco et al., 2008; Sanz et al., 1997). Nitrate reduction appeared to affect mostly growth of yeasts and moulds which counts were significantly higher in nitrate reduced samples.

3.3 Volatile compound analysis

The effect of nitrate reduction on the volatile compounds of dry fermented sausages was studied through the analysis of the volatile compounds present in the headspace (HS) of the different sausages analysed by SPME-GC-MS (Table 3). Fifty-three volatile compounds were identified and quantified in the HS of dry fermented sausages using a CAR/PDMS fibre. The volatile compounds identified and quantified in the HS can be compared with previous studies using the same extraction technique and fibre. The volatile compounds present in the HS of sausages were derived from chemical and metabolic reactions during ripening. Table 3 shows the volatile compounds classified by their possible origin: microbial activity: amino acid degradation (14), carbohydrate fermentation (9), esterase activity (6) and β -oxidation (3), and from lipid oxidation reaction (20) and unknown origin (1). All of these compounds have been previously identified in slow fermented sausages (Corral et al., 2013; Marco et al., 2006; Olivares et al., 2011).

Volatile compounds derived from amino acid degradation were significantly affected by nitrate reduction. In RN25 sausages a significant increase was observed for 2-methylpropanal, 2-methylpropanol, 3-methylbutanol, 2-methylbutanol, and 2,6-dimethylpyrazine, which come mainly from branched chain amino acids (Søndergaard & Stahnke, 2002). However, only benzene was significantly reduced in nitrate reduced sausages ($p < 0.01$). Thus, amino acid degradation took place in more abundance when nitrate was reduced. This fact can be related to the growth of LAB, *Staphylococcaceae*,

yeasts and moulds as responsible for the degradation of amino acids (Toldrá, 2008) although only yeasts and moulds were significantly high in RN25 sausages (Table 2). Hospital et al. (2012, 2015) also found an increase in volatile compounds derived from amino acid degradation in reduced nitrite and nitrate sausages. These authors also observed an increase on 3-methylbutanol in reduced nitrite and nitrate sausages. However, it is important to notice that differences among studies are due to the use of different curing agents, since different effect is produced by the use of nitrate versus nitrite in the generation of volatile compounds as already observed by Marco et al., (2006), specially volatiles from high branched chain amino acid degradation (Olesen et al., 2004).

Carbohydrate fermentation was the group with the highest proportion of volatile compounds in all formulations (75–80%) (Corral et al., 2013) being acetic acid, ethanol and 3-hydroxy-2-butanone the most abundant compounds in this group. Nitrate reduction did not affect the total abundance of compounds derived from carbohydrate fermentation. As observed in table 2, starter culture (LAB and Gram positive cocci) were not affected by nitrate reduction, being these microorganisms responsible for the generation of these volatile compounds (Toldrá, 2008). Nevertheless, the effect of nitrate reduction affected the abundance of several compounds especially at the highest reduction (RN25) producing an increase in acetone, 2-butanone and, acetic and butanoic acids. Similar increases in acid compounds was also reported in nitrite and nitrate reduced dry fermented sausages (Hospital et al., 2012, 2015)

Among volatile compounds derived from esterase activity, the most abundant was ethyl acetate as showed by other authors in similar sausages (Corral et al., 2013). The total abundance of compounds derived from esterase activity was higher in RN25 batch, although the increase was not significant. The abundance of compounds ethyl

acetate, ethyl butanoate, ethyl-2-hydroxypropanoate, ethyl-2-methylbutanoate and ethyl-3-methylbutanoate was significantly higher in nitrate reduced than in C sausages. However, Gram positive cocci (CN-M and CN-BP) growth was not significantly different among sausages (Table 2). In contrast, Hospital et al. (2012, 2015) reported a reduction of ethyl acetate in dry fermented sausages containing reduced nitrite and nitrate levels. These differences among studies can be due to the exclusive use of nitrate (Marco et al., 2008) and for the differences in ethanol abundance, which is a substrate in esterase activity (Talon et al., 1998).

Compounds 2,3-pentanedione, 2-heptanone and 1-octen-3-ol were identified as volatiles **derived from β -oxidation** (Corral et al., 2013). These compounds, found in the smallest proportion (<1%), seemed not to be affected by nitrate reduction. Although high fat content has been related to high abundance of these compounds (Olivares et al. 2010), no differences were observed among nitrate reduced sausages nor due to different fat content. Hospital et al. (2012) found significantly higher amounts of 2-heptanone and 1-octen-3-ol in absence of nitrite and nitrate, demonstrating the antioxidant activity of curing agents.

Compounds derived from lipid oxidation reactions have an important role in dry fermented sausages aroma. The highest abundance of volatiles from lipid oxidation was detected in the control batch, although the differences with nitrate reduced sausages were not significant. This is in accordance with the high TBARS values detected in control sausages (Table 1). Among the lipid oxidation compounds, 2-methylfuran, butanal, 1-pentanol, hexanal, heptanal, 2-heptenal and hexanoic acid were produced in significantly increased amounts in control sausages. On the other hand, 1-propanol, 2-hexenal and decane appeared in significantly higher amounts in nitrate reduced sausages. The antioxidant activity of nitrite (Berardo et al., 2016) was difficult to

appreciate in the nitrate reduced fermented sausages because the higher fat content in control sausages produced a higher susceptibility to oxidation. In addition, the nitrate reduced sausages had the lowest residual nitrate content, indicating a higher conversion of nitrate to nitrite and probably a higher antioxidant effect.

3.4 Aroma analysis

Aroma compounds were analysed by gas chromatography-olfactometry (Table 4). Twenty-five aroma active zones were detected. From these, 21 compounds were identified by mass spectra, linear retention indices and by comparison with the odour description in literature. Four odour zones were not identified and were labelled as unknown compounds. The most important aroma compounds with highest DF values were ethyl butanoate (sweet and fruity odour), hexanal (fresh cut grass), ethyl 2-hydroxypropanoate (cheese and fruity odour), 1-hexanol (cheese and rancid odour), 2-acetyl-1-pyrroline (popcorn odour), methional (cooked potato odour), 1-octen-3-ol (mushrooms odour) and 1 unknown aromatic compound (cooked potato, fried corn, toasted and dried fruit odour) as already observed by Corral et al., (2013) and Olivares et al., (2011).

To examine the relationship of the instrumental variables with the ingoing amounts of nitrate, a principal component analysis was done using the following parameters; chemical composition, TBARS values, pH, colour parameters, a_w , residual nitrate concentration, aroma active volatile compounds and **microorganism's** groups (Figure 1). Two principal components were able to explain the 52.87% of the total variance. PC2 accounted for 18.19% of the variance and showed the variability among replicates of the same formulation. PC1 accounted for 34.68% of the variance and distinguished sausages by nitrate ingoing amounts placing C sausages on the left quadrant and nitrate reduced sausages, RN15 and RN25, on the centre and right

quadrant, respectively. C batch was related to lipid oxidation and aroma compounds derived from oxidation reactions (hexanal, octanal, heptanal and 2-methylfuran) and carbohydrate fermentation (2,3-butanedione and 3-hydroxy-2-butanone). Other aroma impact compounds, methanethiol and 1-octen-3-ol, were also related to the control sausages. In contrast, nitrate reduced sausages (RN25) were related to aroma compounds derived from amino acid degradation (dimethyl disulphide and methional), esterase activity (ester compounds) and several compounds from carbohydrate fermentation (acetic acid and 2-butanone). Moreover, nitrate reduced sausages (RN25) were related to Enterobacteriaceae, Gram positive cocci (CN-BP) and yeast and moulds growth indicating the presence of other microorganisms which can be responsible for the high volatile compounds derived from microorganism's metabolism. In summary, nitrate reduction affected the aroma profile of fermented sausages due to their effect on microbial metabolism.

4. Conclusion

The reduction of in going amounts of nitrate when used exclusively without nitrite addition in slow fermented sausages produced changes in the production of volatile compounds affecting the aroma profile. Microbial counts dynamics were mostly influenced by fermentation time and only slightly by nitrate reduction in case of yeasts and moulds. Manufacturing with reduced amount of nitrate up to 15% did not have a significant impact on microbial counts. Nitrate reduction resulted in an increase of volatile compounds derived from amino acid degradation and, to a lesser extent, from esterase activity, affecting the production of key aroma compounds that alter the sausage aroma profile. Our results indicate that nitrate reduction does not seem to affect directly microbial growth but affects microbial metabolism. Moreover, microbial metabolism can affect nitrate reduction into nitrite and hence its effect on lipid

oxidation. More studies are necessary to know the extent and significance of nitrate reduction into the manufacturing process of traditional dry sausages.

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References

- Aaslayng, M.D., Vestegaard, C., Koch, A.G., 2014. The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Sci.* 96, 47–55.
- Alahakoon, A.U., Jayasena, D.D., Ramachandra, S., Jo, C., 2015. Alternatives to nitrite in processed meat: up to date. *Trends Food Sci. Tech.* 45, 37–39.
- Arneth, W., Herold, B., 1988. Nitrat/Nitrit-Bestimmung m Wurstwaren nach enzymatischer Reduktion. *Fleischwirtschaft.* 68, 761–764.
- Berardo, A., De Maere, H., Stavropoulou, D.A., Rysman, T., Leroy, F., De Smet, S., 2016. Effect of sodium ascorbate and sodium nitrite on protein and lipid oxidation in dry fermented sausages. *Meat Sci.* 121, 359–364.
- BOE, 1979. Métodos oficiales de análisis de productos cárnicos. *Boletín Oficial del Estado, de 28 de agosto de 1979, Anexo II* (pp. 20233–20240). (Madrid, Spain).

426 Burdok, G.A., 2002. **Fenaroli's** handbook of flavor ingredients, fourth ed. Boca Raton,
427 Florida: CRC Press Inc.

428 Cachaldora, A., Fonseca, S., Franco, I., Carballo, J., 2013. Technological and safety
429 characteristics of Staphylococcaceae isolated from spanish traditional dry-cured
430 sausages. *Food Microbiol.* 33, 61–68.

431 Corral, S., Salvador, A., Flores. M., 2013. Salt reduction in slow fermented sausages
432 affects the generation of aroma active compounds. *Meat Sci.* 93, 776–785.

433 Corral, S., Salvador, A., Belloch, C., Flores, M., 2014. Effect of fat and salt reduction
434 on the sensory quality of slow fermented sausages inoculated with
435 *Debaryomyces hansenii* yeast. *Food Control.* 45, 1–7.

436 Corral, S., Salvador, A., Belloch, C., & Flores, M., 2015. Improvement the aroma of
437 reduced fat and salt fermented sausages by *Debaromyces hansenii* inoculation.
438 *Food Control.* 47, 526–535.

439 De Mey, E., De Maere, H., Paelinck, H., Fraeye, I., 2015. Volatile N-nitrosamines in
440 meat products: Potential precursors, influence of processing, and mitigation
441 strategies. *Crit. Rev. Food Sci. Nutr.* 57, 2909–2923.

442 Di Luccia, A., Tremonte, P., Trani, A., Loizzo, P., La Gatta, B., Succi, M., Sorrentino,
443 E., Coppola, R., 2016. Influence of starter cultures and KCl on some
444 biochemical, microbiological and sensory features of soppressata molisana, an
445 Italian fermented sausage. *Eur. Food Res. Technol.* 242, 855–867.

446 European Food Safety Authority (EFSA), 2010. Statement on nitrites in meat products.
447 *EFSA J.*, 8(5), 1538.

448 Folch, J., Lees, M., & Stanley, G.H.S., 1957. A simple method for the isolation and
 449 purification of total lipides from animal tissues. *J. Biol. Chem.* 226, 497–509.

450 Fontana, C., Cocconcelli, P.S., Vignolo, G., 2005. Monitoring the bacterial population
 451 dynamics during fermentation of artisanal Argentinean sausages. *Int. J. Food*
 452 *Microbiol.* 103, 131–142.

453 Food Chain Evaluation Consortium, 2016. Directorate General for health and food
 454 safety, European Commission. Study on the monitoring of the implementation of
 455 Directive 2006/52/EC as regards the use of nitrites by industry in different
 456 categories of meat products.

457 Gassara, F., Kouassi, A.P., Kaur Brar, S., Belkacemi, K., 2016. Green alternatives to
 458 nitrates and nitrites in meat-based products—a review. *Crit. Rev. Food Sci. Nutr.*
 459 56, 2133–2148.

460 Hospital, X.F., Hierro, E., Fernández, M., 2012. Survival of *Listeria innocua* in dry
 461 fermented sausages and changes in the typical microbiota and volatile profile as
 462 affected by the concentration of nitrate and nitrite. *Int. J. Food Microbiol.* 153,
 463 395–401.

464 Hospital, X.F., Hierro, E., & Fernández, M., 2014. Effect of reducing nitrate and nitrite
 465 added to dry fermented sausages on the survival of *Salmonella Typhimurium*.
 466 *Food Res. Int.* 62, 410–415.

467 Hospital, X.F., Carballo, J., Fernández, M., Arnau, J., Gratacós, M., Hierro, E., 2015.
 468 Technological implications of reducing nitrate and nitrite levels in dry-fermented
 469 sausages: Typical microbiota, residual nitrate and nitrite and volatile profile.
 470 *Food Control*, 57, 275–581.

471 Hospital, X.F., Hierro, E., Stringer, S., Fernández, M., 2016. A study on the toxigenesis
 472 by *Clostridium botulinum* in nitrate and nitrite reduced dry fermented sausages.
 473 *Int. J. Food Microbiol.* 218, 66–70.

474 ISO 2917, 1999. *Meat and meat products*. Determination of the pH (Reference method).

475 Laranjo, M., Gomes, A., Agulheiro-Santos, A.C., Potes, M.E., Cabrita, M.J., Garcia, R.,
 476 Rocha, J.M., Roseiro, L.C., Fernandes, M.J., Fernandes, M.H., Fraqueza, M.J.,
 477 Elias, M., 2016. **Characterization of “Catalao” and “Salchichao” Portuguese**
 478 traditional sausages with salt reduction. *Meat Sci.* 116, 34–42.

479 Leroy, F., Verluyten, J., De Vuysr, L., 2006. Functional meat starter cultures for
 480 improved sausage fermentation. *Int. J. Food Microbiol.* 106, 270–285.

481 Marco, A., Navarro, J.L., Flores, M., 2006. The influence of nitrite and nitrate on
 482 microbial, chemical and sensory parameters of slow dry fermented sausage.
 483 *Meat Sci.* 73, 660–673.

484 Marco, A., Navarro, J.L., Flores, M., 2008. The sensory quality of dry fermented
 485 sausages as affected by fermentation stage and curing agents. *Eur. Food Res.*
 486 *Technol.* 226, 449–458.

487 Mohamed, A.A., Mubarak, A.T., Fawy, K.F., & El-Shahat, M.F., 2008. Modification of
 488 AOAC Method 973.31 for Determination of Nitrite in Cured Meats. *J. AOAC*
 489 *Int.* 91, 820–827.

490 Olesen, P.T., Meyer, A.S., Stahnke, L.H., 2004. Generation of flavour compounds in
 491 fermented sausages – the influence of curing ingredients, *Staphylococcus* starter
 492 culture and ripening time. *Meat Sci.* 66, 675–687.

493 Olivares, A., Navarro, J.L., Salvador, A., Flores, M., 2010. Sensory acceptability of
 494 slow fermented sausages based on fat content and ripening time. *Meat Sci.* 86,
 495 251–257.

496 Olivares, A., Navarro, J.L., Flores, M., 2011. Effect of fat content on aroma generation
 497 during processing of dry fermented sausages. *Meat Sci.* 87, 264–273.

498 Pollien, P., Ott, A., Montigon, F., Baumgartner, M., Muñoz-Box, R., Chaintreau, A.,
 499 1997. Hyphenated headspace–gas chromatography–sniffing technique: Screening
 500 of impact odorants and quantitative aromagram comparisons. *J. Agr. Food Chem.*
 501 45, 2630–2637.

502 Rahman, M.S., 2007. Nitrite in Food Preservation. In: Shafiur Rahman, M. (Ed.),
 503 *Handbook of Food Preservation* (2nd ed.). Boca Raton, Florida: CRC Press Inc.

504 Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., Cattaneo, P., Comi, G., Cocolin, L.,
 505 2005. Culture-dependent and -independent methods to investigate the microbial
 506 ecology of Italian fermented sausages. *Appl. Environ. Microbiol.* 71, 1977–1986.

507 Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16
 508 December 2008 on food additives. *ANNEX II (Part E)*. (pp. 170 and 182).

509 Samelis, J., Metaxopoulos, J., Vlassi, M., Pappa, A., 1998. Stability and safety of
 510 traditional Greek salami – a microbiological ecology study. *Int. J. Food.*
 511 *Microbiol.* 44, 69–82.

512 Sánchez Mainar, M., Leroy, F., 2015. Process-driven bacterial community dynamics are
 513 key to cured meat colour formation by coagulase-negative staphylococci via
 514 nitrate reductase or nitric oxide synthase activities. *Int. J. Food. Microbiol.* 212,
 515 60–66.

516 Sanz, Y., Vila, R., Toldrá, F., Flores, M., 1997. Effect of nitrate and nitrite curing salts
 517 on microbial changes and sensory quality of rapid ripened sausages. *Int. J. Food.*
 518 *Microbiol.* 37, 225–229.

519 Sindelar, J.J., Milkowski, A.L., 2011. Sodium nitrite in processed meat and poultry
 520 meats: a review of curing and examining the risk/benefit of its use. *American*
 521 *Meat Science Association (AMSA). White Paper Series*, (3).

522 Søndergaard, A.K. Stanhke, L.H., 2002. Growth and aroma production by
 523 *Staphylococcus xylosus*, *S. carnosus* and *S. equorum* –a comparative study in
 524 model systems. *Int. J. Food. Microbiol.* 75, 99–109.

525 Tabanelli, G., Bargossi, E., Gardini, A., Lanciotti, R., Magnani, R., Gardini, F.,
 526 Montanari, C., 2016. Physico-chemical and microbiological characterisation of
 527 Italian fermented sausages in relation to their size. *J. Sci. Food Agr.* 96, 2773–
 528 2781.

529 Talon, R., Chastagnac, C., Vergnais, L., Montel, M.C., Berdagué, J.L., 1998. Production
 530 of esters by staphylococci. *Int. J. Food. Microbiol.* 45, 143–150.

531 Toldrá, F., Flores, M., 2014. Dry and semidry. In: Carrick Devine & Michael Dikeman
 532 (Eds.), *Encyclopedia of Meat Sciences* (pp. 248–255). Oxford: Elsevier, Inc.

533 Toldrá, F., 2008. Biotechnology of flavor generation in fermented meats. In: Fidel
 534 Toldrá (Ed.), *Meat Biotechnology* (pp. 199–216). New York: Springer

535 Van Den Dool, H., Kratz, P.D., 1963. A generalization of the retention index system
 536 including linear temperature programmed gas–liquid partition chromatography.
 537 *J. Chromatogr.* 2, 463–471.

538 Zanardi, E., Ghidini, S., Battaglia, A., Chizzolini, R., 2004. Lipolysis and lipid
539 oxidation in fermented sausages depending on different processing conditions
540 and different antioxidants. *Meat Sci.* 66, 415–423.

541 FIGURA LEGEND

542

543 Figure 1. Loadings of the first two principal components (PC1–PC2) of dry fermented
544 sausages with different nitrate ingoing amounts: C (control batch, 250 ppm), RN15
545 (15% sodium nitrate reduction, 212.5 ppm), RN25 (25% sodium nitrate reduction, 187.5
546 ppm) and instrumental variables (physicochemical, microbiological parameters and
547 aroma compounds). Abbreviations are indicated in tables 1 and 2.

Table 1. Effect of nitrate reduction on physicochemical parameters of dry fermented sausages at initial and end of process.

	0 days			62 days			RMSE ³	P_t^4	P_n	P_{txn}
	C ¹	RN15	RN25	C	RN15	RN25				
pH	5.85 a ²	5.83 a	5.88 a	4.99 b	4.95 b	4.93 b	0.038	***	ns	ns
Aw	0.981 a	0.974 a	0.975 a	0.890 b	0.894 b	0.895 b	0.004	***	ns	*
Moisture (%)	63.12 a	64.18 a	64.88 a	40.33 b	41.03 b	42.61 b	2.091	***	ns	ns
Proteins (% dm)	49.11 d	49.37 cd	50.05 cd	54.58 bc	56.15 b	61.23 a	2.409	***	**	ns
Fat (% dm)	35.97 ab	34.07 abc	31.68 bc	36.30 a	33.15 abc	30.57 c	2.052	ns	***	ns
L*	64.92 a	61.15 a	61.04 a	50.50 b	49.84 b	49.27 b	1.716	***	*	ns
a*	26.05 a	27.14 a	26.47 a	17.66 b	18.21 b	17.88 b	0.994	***	ns	ns
b*	15.73 a	15.69 a	15.32 a	6.59 b	6.76 b	6.26 b	0.538	***	ns	ns
TBARS ⁵	0.56 b	0.46 b	0.41 b	0.93 a	0.69 ab	0.61 b	0.139	***	**	ns
NO ₃ (ppm dm)	445.21 a	378.58 ab	338.50 bc	299.79 c	231.81 d	163.52 e	27.214	***	***	ns

¹C: control batch. RN15: 15% sodium nitrate reduction batch. RN25: 25% sodium nitrate reduction batch. ²Different letters in the same row indicate significant differences at $p < 0.05$ among batches. ³RMSE: Root mean square error. ⁴ P : P value of time (t), nitrate content (n) and time and nitrate content (txn) effect. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: $p > 0.05$. ⁵ TBARS expressed as μg malonaldehyde/g dm.

Table 2. Effect of nitrate reduction on microbial counts (log cfu/g) of dry fermented sausages at initial and end of process.

	Culture medium ⁵	0 days			62 days			RMSE ³	P_t^4	P_n	P_{txn}
		C ¹	RN15	RN25	C	RN15	RN25				
Total mesophilic bacteria (TMB)	PCA	6.84 b ²	6.88 b	6.87 b	8.89 a	8.83 a	8.96 a	0.12	***	ns	ns
<i>Lactobacillus</i> (LAB)	MRS	5.85 b	5.83 b	5.75 b	6.98 a	6.94 a	6.96 a	0.07	***	ns	ns
Yeasts and molds (YM)	RB	–	–	–	2.32 b	3.24 a	3.33 a	0.20	**	**	ns
Enterobacteriaceae (EB)	VRBGA	4.84 a	4.83 a	4.81 a	2.39 b	2.34 b	2.33 b	0.03	***	ns	ns
Gram positive cocci CN (CN-M)	MSA	5.47 a	5.41 a	5.28 a	4.49 b	4.33 b	4.39 b	0.13	***	ns	ns
Gram positive cocci CN (CN-BP)	BP	4.86 b	4.84 ab	4.78 ab	4.45 c	4.56 bc	4.64 abc	0.10	***	ns	ns

¹C: control batch. RN15: 15% sodium nitrate reduction batch. RN25: 25% sodium nitrate reduction batch. ²Different letters in the same row indicate significant differences at $p < 0.05$ among batches. ³RMSE: Root mean square error. ⁴ P : P value of time (t), nitrate content (n) and time and nitrate content (txn) effect. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: $p > 0.05$. ⁵PCA: Plate Count Agar, RB: Rose Bengal Agar, VRBGA: Violet Red Bile Agar with Glucose, MSA: Mannitol Salt Agar, BP: Baird Parker Agar.

Table 3. Effect of nitrate reduction on volatile compounds generated (expressed as AU × 10⁵) in dry fermented sausages at the end of process.

	LRI ¹	RI ²	C ³	RN15	RN25	RMSE ⁴	P _n ⁵
<i>Microbial origin</i>							
Amino acid degradation			400.589 b	392.157 b	534.289 a	72.31	**
2-Methylpropanal	594	a	4.852 ab	4.428 b	7.655 a	1.75	*
Benzene	676	a	2.011 a	1.982 a	1.034 b	0.47	**
2-Methyl-1-propanol	683	a	12.449 ab	9.707 b	17.083 a	4.22	*
3-Methylbutanal	691	a	34.320	27.215	39.208	8.98	ns
Dimethyl disulfide	773	a	2.439	3.904	3.148	1.38	ns
Toluene	788	a	27.531	101.371	70.830	58.49	ns
3-Methyl-1-butanol	795	a	227.773 ab	176.059 b	286.989 a	57.99	*
2-Methyl-1-butanol	797	a	49.137 b	40.051 b	68.571 a	12.58	**
2,6-Dimethylpyrazine	945	a	3.230 b	3.509 b	4.586 a	0.61	**
Methional	968	a	2.186	3.145	5.137	1.24	ns
Benzaldehyde	1020	a	8.538	11.533	11.050	3.45	ns
Benzene acetaldehyde	1110	a	3.253	2.295	3.686	1.14	ns
Phenol	1114	a	17.529	17.635	16.779	1.55	ns
Methanethiol	473	a	12.395	10.746	8.2407	2.62	ns
Carbohydrate fermentation			6845.361	8221.824	7426.739	1448.965	ns
Acetaldehyde	466	a	75.874	72.257	61.998	17.915	ns
Ethanol	507	a	1858.751	2126.592	2326.289	565.348	ns
Acetone	529	a	31.026 b	51.857 a	38.867 ab	8.163	**
2,3-Butanedione	627	a	78.890 a	47.539 b	45.482 b	18.699	*
2-Butanone	631	a	41.494 b	47.258 b	101.245 a	20.633	**
Acetic acid	718	a	2685.579 b	2601.611 b	3031.631 a	233.298	*
3-hydroxy-2-butanone	781	a	1333.706	1021.151	1025.607	448.975	ns
2,3-Butanediol	888	a	702.097	2259.924	1449.120	1172.704	ns

Butanoic acid	896 a	55.761 b	50.373 b	95.959 a	15.088	**
Esterase activity		718.76	713.97	1093.10	304.53	ns
Ethyl acetate	635 a	571.12 b	704.31 b	1049.16 a	136.75	***
Ethyl butanoate	832 a	47.01	46.30	68.99	14.16	ns
Ethyl 2-hydroxypropanoate	867 a	45.84 b	55.66 ab	67.80 a	13.87	*
Ethyl 2-methylbutanoate	878 a	22.84 b	28.77 ab	40.31 a	9.44	*
Ethyl 3-methylbutanoate	882 a	29.34 b	27.45 b	62.31 a	14.84	*
Ethyl octanoate	123 a	7.51	10.75	9.60	2.60	ns
β -oxidation		43.95	38.70	38.14	10.46	ns
2,3-Pentanedione	745 a	16.64	11.84	19.44	4.51	ns
2-Heptanone	935 a	11.41	11.35	10.40	1.97	ns
1-Octen-3-ol	1033 a	17.49	16.58	12.18	4.20	ns
<i>Chemical origin</i>						
Lipid oxidation		831.43	805.59	560.18	186.39	ns
Pentane	500 a	51.79	55.47	37.52	17.295	ns
Propanal	524 a	8.74	6.40	7.65	2.043	ns
Hexane	600 a	19.80	20.40	15.01	4.808	ns
1-Propanol	612 a	11.88 ab	10.71 b	16.55 a	3.038	*
2-Methylfuran	616 a	1.80 a	1.25 b	1.36 ab	0.317	*
Butanal	622 a	2.11 a	0.48 b	0.93 b	0.703	**
Heptane	700 a	94.23	94.22	78.82	24.149	ns
Pentanal	739 a	55.17	48.45	36.12	12.601	ns
Octane	800 a	126.91	121.26	93.47	22.281	ns
1-Pentanol	827 a	34.12	30.49	21.56	7.228	ns
Hexanal	842 a	355.29 a	322.76 ab	183.14 b	93.305	*
2-Hexenal	907 a	1.55 b	1.78 b	2.96 a	0.520	**
1-Hexanol	924 a	57.22	53.23	46.12	13.174	ns
Heptanal	941 a	34.88 a	31.88 ab	22.97 b	6.441	*

Decane	1000 a	2.59 b	3.19 ab	3.63 a	0.603	*
2-Pentylfuran	1010 a	7.16	8.50	7.08	1.516	ns
(E)-2-Heptenal	1013 a	3.41 a	2.88 ab	1.93 b	0.835	*
Octanal	1049 a	21.69	22.12	16.26	4.872	ns
Hexanoic acid	1079 a	14.34 a	12.27 ab	9.53 b	2.164	*
Nonanal	1151 a	24.45	26.76	21.95	5.178	ns
Unknown compounds						
Carbon disulfide	537 a	57.80 b	73.28 ab	104.65 a	20.871	*

¹LRI: Linear retention index of the compounds eluted from the GC-MS. ²RI: Reliability of identification: a, identification by mass spectrum, coincidence with the LRI of an authentic standard; b, tentative identification by mass spectrum. ³C: control batch, RN15: 15% sodium nitrate reduction batch, RN25: 25% sodium nitrate reduction batch. ⁴RMSE: Root mean square error. ⁵ P_n : P value of nitrate content effect. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: $p > 0.05$, different letters in the same row of each group indicate significant differences at $p < 0.05$ among batches.

Table 4. Odour active compounds identified in dry fermented sausages at the end of process.

	LRI GC- O ¹	LRI std GC-O ²	RI ³	Descriptor	DF ⁴
Methanethiol	472	471	a	Rotten, unpleasant	8
2-Methylfuran	615	619	a	Green, garlic, toasted, yeast, malt	4
2,3-Butanedione	629	632	a	Cheese, butter, floral, fresh	4
2-Butanone	636	638	a	Fruity, sweet, cheese, butter, dairy	8
Acetic acid	699	700	a	Vinegar, acid, unpleasant	8
2,3-Pentanedione	740	739	a	Sweet, candy, fruit, glue	4
Dimethyl disulfide	769	774	a	Toasted, garlic	3
3-Hydroxy-2-butanone	782	777	a	Strawberry, sweet, fruity, green	9
Ethyl butanoate	824	825	a	Sweet, fruity, floral	10
Hexanal	834	836	a	Fresh cut grass, vegetable, fresh	10
Ethyl 2-hydroxypropanoate	865	859	a	Cheese, fruit, strawberry, sweet	11
Ethyl 3-methylbutanoate	874	876	a	Strawberry, fruit, glue, sweet	9
1-Hexanol	920	919	a	Cheese, oxidized fat, humidity	11
2-Heptanone	931	931	a	Rancid, burnt, strawberry, fruit	4
Heptanal	938	937	a	Green, unpleasant, toasted	5
2-Acetyl-1-pyrroline	960	960	a	Toasted, fried corn, bread	12
Methional	965	969	a	Cooked potato, roast meat	10
2-Pentylfuran	1008	1011	a	Garlic, onion, unpleasant, grass	8
1-Octen-3-ol	1023	1028	a	Mushrooms, humidity, spicy	11
Unknown	1031	–	c	Burnt, mushrooms, humidity, herbs	8
Unknown	1037	–	c	Green, grass, earth, burnt, herbs	6
Octanal	1045	1047	a	Orange, sweet	4
Unknown	1162	–	c	Spices, fried corn, unpleasant	5
Unknown	1178	–	c	Fried corn, toasted	10
Ethyl octanoate	1223	1226	a	Cooked vegetable, onion, fruity	7

¹Linear retention index of the compounds eluted from the GC-FID-O. ²Linear retention index of standard compounds in the GC-FID-O. ³Reliability of identification: a, identification by mass spectrum, coincidence with LRI of an authentic standard and by coincidence of the assessors's descriptors with those described in the Fenaroli's handbook of flavor ingredients (Burdock, 2002); b, tentatively identification by mass spectrum; c, unknown compounds. ⁴Detection frequency value.

F1 and F2: 52.87 %