Effect of nitrate reduction on the development of oxidized aroma in dry fermented sausages during storage

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Abstract

The effect of nitrate reduction on the development of fermented sausage aroma and its stability during vacuum storage has been studied. Different sausage formulations were manufactured with different nitrate contents as a source of nitrite as preservative. The oxidation of sausages was evaluated by analyzing TBARS compounds and extraction of the volatile compounds using solid phase microextraction (SPME) and gas chromatography mass spectrometry. Aroma compounds related to oxidation processes were identified by olfactometry technique. The study revealed the relation of nitrate reduction and fat content on aroma compounds related to oxidation process and their effect on sausage aroma during vacuum storage

Introduction

Aroma characteristics of fermented sausage depend on processing factors such as raw material, meat ingredients, preservatives, technological parameters and presence of starter cultures. Despite the role of nitrites and nitrates in meat product safety and technological properties, there is a trend to reduce its use [1]. However, the effect of nitrite on flavor formation in meat products is essential to develop cured aroma. Thomas et al., [2] indicated that cured cooked ham aroma is due to the balance of sulfur compounds and oxidation compounds produced during cooking and in the absence of nitrite, the aroma is disturbed due to the excessive formation of oxidation compounds that mask the sulfur meaty notes. In dry fermented sausage aroma, nitrite plays a fundamental role in developing the typical dry cured aroma [3] although it is not known the effect of nitrite reduction on aroma generation and stability during shelf life. Therefore, our aim is to determine the effect of reduced nitrate concentrations used as preservatives on the development of sausage aroma in dry fermented sausages after storage under vacuum at ambient temperature.

Experimental

Dry fermented sausages preparation

Dry fermented sausages were manufactured using lean pork (50%), pork fat (50%) and the following additives added in g/kg to the sausage formulation: lactose (20); dextrose (20); sodium chloride (20.25); glucose (7); potassium chloride (6.75); sodium ascorbate (0.5); starter culture (0.1) and sodium nitrate (0.25 for control sausage (C) or reduced in 15% (RN15) and 25% (RN25). The starter culture TRADI-302 (Danisco, Cultor, Madrid, Spain) was added containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus* and *Staphylococcus carnosus*. The sausages were submitted to a slow fermentation process as described by Corral et al., [4]. At the end of ripening, sausages were vacuum packed and stored at room temperature to study its shelf life at 0, 36, 70 and 100 days.

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-10, CC BY-NC-ND 4.0

Physicochemical analysis

The lipid content was determined by organic extraction with Cl_2CH_2:CH_3OH (2:1) [5]. Lipid oxidation was evaluated using the thiobarbituric acid reactive substances test (TBARS) [6] and expressed as μg of malonaldehyde per gram of dry mater (μg MDA/g dm).

Volatile compound analysis

The analysis of volatile compounds was carried out by solid phase micro extraction (SPME) with an 85 μm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber (Supelco, Bellefonte, PA). 5 g of sausage sample (with 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. 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) was used [7]. The compounds were identified by comparison with mass spectra from the library database (Nist'05), with linear retention indices [8] and with authentic standards.

Aroma compound analysis

A gas chromatograph (Agilent 6890, USA) equipped with a FID detector and sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) was used to analyze aroma compounds [6]. Each assessment was carried out with 5 g of sample using the detection frequency method [9]. Four trained panelists evaluated the odors from the GC-effluent. 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 flavor ingredients [10].

Statistical analysis

Analysis of variance (ANOVA) using the statistic software XLSTAT 2011, version 5.0 (Addinsoft, Barcelona, Spain) was performed at each storage time among sausage formulations. Correlation tests (Pearson) among variables were also studied.

Results and discussion

Fat content in sausages was analyzed as it is responsible for the generation of lipid oxidation compounds during sausage fermentation [3]. Although all sausages were manufactured with the same lean and fat content, slightly differences among formulations were obtained due to pork back fat variability. Control sausages had a fat content of 33-38%, while nitrate reduced sausages RN15 and RN25 contained between 29-33% and 29-31%, respectively.

The lipid oxidation level (TBARS values) during sausage vacuum storage is shown in Figure 1A. It showed a slight increase during the first month of storage and a decrease during the following months [11]. This behavior may be due to the high reactivity of malonaldehyde with sugars, aminoacids and nitrite [12]. In addition, the absence of oxygen in vacuum storage prevent the sausages for an increase in oxidation. However, differences among formulations were observed ($p<0.001$): the lipid oxidation was the highest in the control formulation. This fact can be due to the highest fat content of this control sausage as a positive ($p<0.05$) relation between lipid oxidation (TBARS values)

and fat content was obtained among sausages analyzed at the end of the ripening process (Figure 2A) [13].

Regarding sausage aroma, GC-O analysis revealed 23 odour active zones (Table 1). The main odorants were Ethyl butanoate, Hexanal, Ethyl 2-hydroxypropanoate, 1-hexanol, 2-acetyl-1-pyrroline, 3-(Methylthio)propanal, 1-Octen-3-ol and 1 unknown compound. Among them, only three aroma compounds were derived from lipid oxidation reactions: Hexanal (Figure 1B), Heptanal (Figure 1C) and 2-Pentylfuran (Figure 1D) which contributed to fresh cut grass, green-unpleasant and garlic-grass odour notes. The concentration of the three volatile compounds showed a general slight increase during vacuum storage of sausages. However, few differences were observed at each storage time among formulations. Hexanal was the most abundant compound and was positively related to fat sausage content at the end of the ripening (Figure 2B).

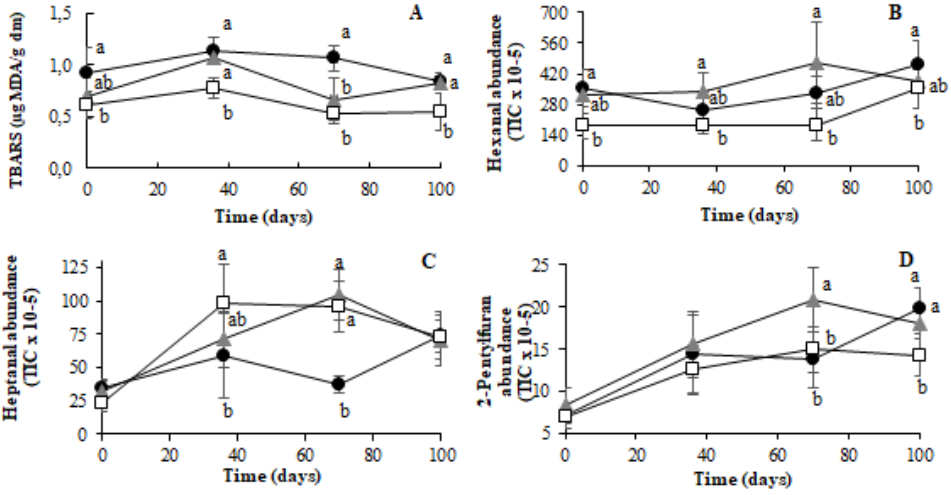

Figure 1: Changes in TBARS (A), Hexanal (B), Heptanal (C) and 2-Pentylfuran (D) during vacuum storage of dry fermented sausages: C (control, ●), RN15 (15% reduced nitrate, ▲) and RN25 (25% reduced nitrate, □).

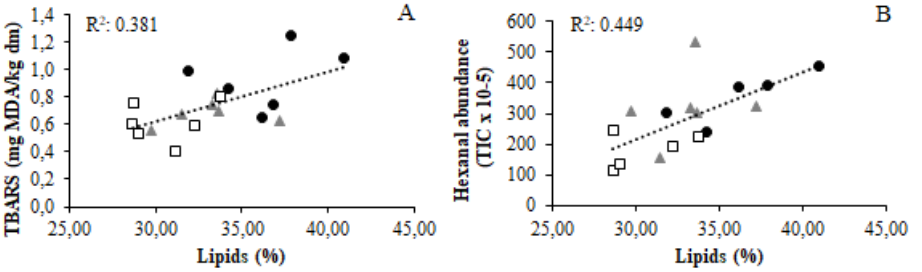

Figure 2: Pearson Correlation between fat content and lipid oxidation (A) or Hexanal (B) in dry fermented sausages at the end of the ripening: C (control, ●), RN15 (15% reduced nitrate, ▲) and RN25 (25% reduced nitrate, □).

Conclusions

Aroma compounds derived from lipid oxidation reactions contribute to the aroma of fermented sausages. The increase in shelf life by vacuum storage produced variation in

Table 1: List of aroma compounds detected in GC-FID/Olfatometry

Compound	*LRI std*[a] *GC-O*	*LRI*[b] *GC-O*	*Descriptor*	*DF*[c]
Methanethiol	471	472	Rotten, unpleasant	8
2-Methylfuran	619	615	Green, garlic, toasted, yeast, malt	4
2,3-Butanedione	632	629	Fruit, cheese, butter, floral, fresh, broth	4
2-Butanone	638	636	Cheese, butter, dairy, strawberries, fruity, sweet, flower	8
Acetic acid	700	699	Vinegar, acid, unpleasant, sweet	8
2,3-Pentanedione	739	740	Sweet, candy, fruit, glue, meat	4
3-hydroxy-2-butanone	777	782	Strawberry, sweet, fruity, apple, orange, acid, fresh, green	9
Ethyl butanoate	825	824	Sweet, apple, banana, orange, fruit, strawberry, floral	10
Hexanal	**836**	**834**	**Fresh cut grass, vegetable, lemon, aromatic herbs, fresh**	**10**
Ethyl 2-hydroxy propanoato	859	865	Cheese, fruit, strawberry, sweet, rancid, acid	11
Ethyl 3-methyl butanoato	876	874	Strawberry, fruit, glue, sweet	9
1-hexanol	919	920	Cheese, oxidized fat, humidity	11
2-Heptanone	931	931	Cheese, rancid, burnt, irritating, garlic, vinegar, strawberry	4
Heptanal	**937**	**938**	**Green, unpleasant, toasted**	**5**
2-acetyl-1-pyrroline	960	960	Toasted, fried corn, bread, citrus, floral	12
3-(methylthio)propanal	969	965	Cooked potato, roast meat	10
2-Pentylfuran	**1011**	**1008**	**Garlic, onion, fried, unpleasant, cured, grass**	**8**
1-Octen-3-ol	1028	1023	Mushrooms, humidity, spicy	11
Unknown	-	1031	Burnt, mushrooms, garlic, unpleasant, humidity, closed, herbs	8
Unknown	-	1037	Green, grass, earth, burnt, spicy, aromatic herbs	6
Unknown	-	1162	Spices, garlic, spicy, fried corn, unpleasant	5
Unknown	-	1178	Cooked potato, fried corn, toasted, dried fruit	10
Ethyl octanoate	1226	1223	Cured sausages, onion, fruit, cooked potato	7

[a]LRI std: Linear retention index of standard compounds in the GC-FID-O. [b]Linear retention index of the compounds eluted from the GC-FID-O. [c]Detection frequency value.

these aroma compounds that are affected not only by the presence of preservatives (curing agents) but also by the matrix composition (fat content).

Acknowledgements

Financial support from AGL 2015-64673-R from MINECO (Spain) and from predoctoral scholarship (ACIF/2019/107) from GVA (Generalitat Valenciana, Spain) to Laura Perea-Sanz are fully acknowledged.

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