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Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage

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ABSTRACT

The generation of aroma compounds through the curing process of dry fermented sausages was studied. The most important aroma compounds were determined using their odour-activity values (OAVs). The compound quantification in the headspace (HS) was carried out by solid phase microextraction (SPME) and the total concentration in the sausage by multiple headspace SPME (multiple HS-SPME) using gas chromatography and mass spectrometry. The main compounds that contributed to the aroma of dry fermented sausages were those with the highest oil OAVs such as 3-methyl butanal, 2-methyl butanal, octanal, diacetyl and ethyl 2-methyl butanoate that were important from the beginning of the process. Other compounds were important contributors as they were generated at the end of process, including propanal, pentanal, hexanal, ethyl 3-methyl butanoate, 1-octen-3-ol, 3-methyl butanoic acid, 2-methyl propanoic acid, ethyl hexanoate and nonanal. However, the aroma perceived in the HS was due to compounds with the highest air OAVs such as 3-methyl butanoic acid, ethyl 2-methyl butanoate, nonanal and octanal. In many cases, the percentage of the aroma compound released to the HS was around 10–20% of the total concentration in the sausage.

1. Introduction

Aroma is a very important characteristic for the overall quality of dry fermented sausages. It is mainly developed by bacterial metabolism and lipid oxidation reactions that occur in the sausage matrix during the manufacture process. The addition of spices also influences the content of volatile compounds, as well as several factors of the fabrication process, such as curing agents and fermentation stage (Marco, Navarro, & Flores, 2008).

The typical aroma of dry fermented sausage is not due to a single compound, but to the mixture of volatile compounds in the appropriate amounts (Marco, Navarro, & Flores, 2007). Hundreds of volatile compounds have been identified in fermented sausages. However, many of those volatiles do not contribute to the aroma because of their high sensory threshold values (Tjener & Stahnke, 2007). In previous work, Marco et al. (2007), using gas chromatography–olfactometry (CG–O) and detection frequency method, reported 55 compounds as odour-active constituents in dry fermented sausages.

Aroma perception in meat products depends on the interactions of volatile compounds with other meat components (fat and protein) (Grosch, 2001) that affect its gas phase concentration and also on the interaction among volatile compounds (Adhikari, Hein, Elmore, Heymann, & Willott, 2006). A criterion for the selection of the most important aroma compounds in a food is their odour-activity value (OAV), which can be easily calculated if the concentration and the odour threshold of the aroma compounds are known (De Roos, 2007). However, Grosch (2001) indicated that the estimation of the odour threshold values of volatiles should be done in a medium that predominates in the food, e. g. water, oil. In the case of dry fermented sausages, the high proportion of fat (around 20%) produces a high effect on threshold calculations.

The composition of the odorants in the air above the food can be obtained by headspace (HS) analysis; however, it is necessary to estimate the proportion of volatile compounds in the whole product to predict their impact on the aroma. In this sense, the quantitative analysis of volatile compounds in a complex matrix was solved by Kolb (1982) with a procedure called multiple headspace extraction (MHE). The method is based on a stepwise gas extraction at equal time intervals, allowing the total area for the compound to be calculated and eliminating the influence of the matrix. Multiple headspace solid phase microextraction (multiple HS-SPME) has the same aim as MHE. The amount of the analyte extracted by the fibre is proportional to the initial amount, and it is assumed that the analyte concentration will decay with the number of extractions. This method has already been applied to study the content of volatile compounds in dry fermented sausages (Flores & Hernández, 2007; Marco et al., 2007).

In fermented sausages, many studies have been focused on protein and lipid degradation during processing (Lücke, 1985). However, the release of volatile compounds from the matrix could be

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also affected by the drying process because the content of fat, protein and salt continually rise during ripening as a result of moisture release.

However, nothing is known about the generation of aroma compounds through the different ripening stages and when those compounds are essential for the aroma. Therefore, the objective of this study was to determine which compounds and at which stage of the curing process are essential for the aroma, and also to determine if the use of different curing agents can affect their generation throughout the process.

2. Materials and methods

2.1. Reagents and standards

The chemical compounds used for the identification and to prepare the standard dilutions were all obtained from Fluka Chemie AG (Buchs, Switzerland) except diacetyl (97%) and 2-ethyl furan (97%), which were obtained from Aldrich (St. Louis, MO).

2.2. Dry fermented sausages

Two batches of fermented sausages, one containing sodium nitrite and another containing potassium nitrate were manufactured and submitted to a slow fermentation process. Dry fermented sausages were made with lean pork (80%) and pork back fat (20%). The following additives were added in gram per kilogram quantities to the meat mixture: sodium chloride (27), lactose (20), dextrin (20), sodium caseinate (20), glucose (7), sodium ascorbate (0.5), and either sodium nitrite (0.15) or potassium nitrate (0.3). The meat was ground through a 10 mm diameter mincing plate, vacuum minced with the remaining ingredients and inoculated with a commercial starter culture (0.1) SP-318 (Danisco, Cultor, Madrid, España) containing *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus*, and *Staphylococcus carnosus*. The mixture was stuffed into collagen casings (Fibran, S.A., Girona, España, 75–80 mm diameter), with an approximate final sausage weight of 500 g. The sausages were kept in a chamber at 3–5 °C for 24 h, and then dried during 10 d at 9 °C, 10 d at 11 °C and 21 d at 9 °C and with 85–75% relative humidity. The total drying time was 42 d. At the end of the process, the sausages were vacuum packed and stored at 4 °C for 50 d, giving a total processing time of 92 d.

The process was followed by performing microbial, moisture and pH analyses in the two batches (Marco et al., 2008). In addition, at day 0, 200 g of the minced meat mixture were collected and at day 11, 21, 42 and 92, 3 sausages from each batch were randomly chosen. Each sausage was sliced, vacuum packed and stored at −20 °C for chemical (TBARS, nitrite and nitrate) and volatile compound analyses. Results are expressed as the mean of the three replicates per kg of dry matter (dm) at each sampling time. Two sensory analyses were carried out at different times of processing, at the end of drying (42 d) and after vacuum storage (92 d).

2.3. Chemical analyses (TBARS, nitrite and nitrate)

Thiobarbituric reactive substances (TBARS) were determined according to Bruna, Ordoñez, Fernández, Herranz, and de la Hoz (2001), using trichloroacetic acid instead of perchloric acid as solvent.

The nitrite and nitrate content was determined using an enzymatic kit (Cat No. 10905658, 2r-Biopharm, Roche, Darmstadt, Germany) according to the procedure described by Arneth and Herold (1988) and as described Marco et al. (2008).

2.4. Sensory analysis

Sausages from both batches were submitted to sensory analysis at the end of the drying (42 d) and after vacuum storage (92 d). The casing was removed and the sausage was cut into slices of approximately 5 mm thickness. Before serving, the slices were equilibrated at room temperature for 30 min. Samples were assessed by a consumer panel of 50 members. A preference test (ISO-5495) was carried out to determine panellist's preference in terms of colour, aroma, taste and overall quality. Sensory evaluations were recorded by computer software using Compusense® *five* releases 4.6 (Compusense Inc., Guelph, ON, Canada).

2.5. Extraction of volatile compounds

Extraction of HS volatile compounds was done using a solid phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA) with a 85 μm carboxen/polydimethylsiloxane StableFlex fibre (CAR/PDMS SF).

2.6. Gas chromatography–mass spectrometry (GC–MS)

The identification and quantitation of volatile compounds was done in a gas chromatograph HP 5890 series II equipped with an HP 5972 mass-selective detector (Hewlett Packard, Palo Alto, CA). The compounds adsorbed by the fibre were desorbed in the injection port of the GC–MS for 15 min at 220 °C with the purge valve off (splitless mode). The compounds were separated on a DB-624 capillary column J & W Scientific (Agilent Technologies, USA) and analysed as described Flores and Hernández (2007). The compounds were identified by comparison with mass spectra from the library database (NIST 98), Kovats retention index (Kovats, 1965), and by comparison with authentic standards. The quantification of the volatile compounds was done in selected ion monitoring (SIM) mode using the area of a specific ion for each compound (Table 1). The analysis was focused on those compounds previously identified as aroma active compounds in fermented sausages using olfactometry (Marco et al., 2007) and the selection of the volatile compounds was done as described Flores and Hernández (2007) by using the detector in SCAN mode. The aroma properties of the compounds, odour description, air and oil thresholds and hydrophobicity values (Log K_{ow}) are indicated in Table 1.

2.7. Quantification of odour-active compounds of dry fermented sausages by multiple HS-SPME

The analysis was done as previously optimised by Flores and Hernández (2007). For the extraction procedure, a sausage slice was diced, 0.75 mg of butylated hydroxytoluene (BHT) were added, and minced with liquid nitrogen. One gram of the finely pulverised sausage was homogenised with 15 ml of double-distilled water. Then, 1 ml of the homogenised sample was added to a 10 ml HS vial, containing 0.5 g of NaCl and equilibrated at 30 °C for 1 h. The sample was extracted for four consecutive times by exposing the SPME fibre for 90 min at 30 °C each extraction. This procedure was done per triplicate on each batch and sampling time. After each extraction, the fibre was desorbed in the GC injection port of the GC–MS, using the conditions described previously.

The concentration of analyte decayed exponentially through the successive extractions and the total peak area (A_t) corresponding to an exhaustive extraction was calculated from Eq. (1):

$$A_t = \frac{A_1}{1 - e^k} \quad (1)$$

where A_1 is the peak area in the first extraction and k is the slope obtained when representing the natural logarithm of the peak area

Table 1
Volatile compounds analysed by multiple HS-SPME.

Chemical group /compound	KI[a]	Ion (m/z)	Stock solution (ng/ml)	Log K_{ow}[b]	Threshold range (ng/g)[c]		Descriptor
					Air	Oils	
Aldehydes							
Propanal	522	58	24.24	0.33		9.4[2]	Pungent[d]
Butanal	621	72	21.21	0.82	0.84–8800[1]	150[1]	Sweet, snacks[e]
3-Methyl-butanal	687	58	21.21	1.23	2–4[1]	13–13000[1]	Rancid, dry cured ham[e]
2-Methyl-butanal	698	58	21.21	1.23		2.21[2]	Cacao, coffee[d]
Pentanal	733	58	27.27	1.31	120–17500[1]	240[3]	Fresh cut grass, rancid[e]
Hexanal	839	58	27.27	1.80	20–330[1]	32–200[1]	Fresh cut grass, rancid[e]
2-Hexenal	905	41	21.21	1.58	50–1800[1]	850[1]	Salty meat, dry cured ham[e]
Heptanal	941	70	12.12	2.29	60–260[1]	250[1]	Citrus, soap, rancid cured ham[e]
2-Heptenal	1011	41	21.21	2.07	34–2800[1]	1500[1]	Rancid, dirty[e]
Octanal	1039	41	21.21	2.78	5–20[1]	56[2]	Geranium, herbal, floral[e]
Nonanal	1148	98	21.21	3.27	5–230[1]	1000[1]	Plastic, soap[e]
Ketones							
2-Butanone	630	72	15.15	0.26			Apricot[d]
Diacetyl (2,3-butanedione)	625	43	27.27	−1.34	5–20[1]	4.5–10[1]	Cheese, snacks[e]
2-Ethyl-furan	719	81	27.27	2.40		8000[1]	Roasted, garlic[e]
2-Pentanone	728	43	24.24	0.75	6700–30000[1]		Roasted, sweet[e]
2,3-Pentanedione	739	57	27.27	−0.85	10–60[1]		Butter, cheese[e]
2-Heptanone	934	43	18.18	1.73	45–330[1]	1500–392000[1]	Medicinal, fruity[e]
2-Nonanone	1140	58	21.21	2.71	75–5500[1]		Roasted, burnt[e]
Esters							
Ethyl acetate	634	43	30.30	0.86	340–623000[1]	10,000–1,00,000[1]	Fruity, toffees[e]
Ethyl butanoate	831	43	21.21	1.85	2.7–200[1]	28[1]	Strawberry[e]
Ethyl 2-methyl-butanoate	877	57	21.21	2.26	0.06–10[1]	0.26[1]	Strawberry[e]
Ethyl 3-methyl-butanoate	881	88	27.27	2.26		0.62[2]	Fruity[d]
Ethyl pentanoate	928	85	27.27	2.34	0.3–330[1]		Strawberry[e]
Ethyl hexanoate	1023	88	24.24	2.83	3–90[1]	40[1]	Sweet, fruity, cherry[e]
Ethyl octanoate	1156	88	24.24	3.81	5–92[4]		Fruity[d]
Alcohols							
3-Methyl-1-butanol	795	43	24.24	1.26			Whiskey, malt[d]
1-Octen-3-ol	1025	57	27.27	2.60	12–110[1]	34–900[1]	Mushroom[e]
Acids							
2-Methyl-propanoic acid	868	43	30.30	1.00	5–240[1]	755[1]	Fatty, savoury snacks[e]
3-Methyl-butanoic acid	950	60	18.18	1.49	0.22–14[1]	22–66[1]	Cheese, feet, dirty socks[e]
Hydrocarbons							
Limonene	1036	68	24.24	4.83	4–229[4]		Citrus, orange[e]

[a] Kovats index calculated for DB-624 capillary column (J & W Scientific: 30 m, 0.25 mm i.d., 1.4 μm film thickness) installed on a gas chromatograph equipped with a mass-selective detector.
[b] Log of octane/water partition coefficient.
[c] Threshold values obtained from the following sources; (1) Van Gemert and Nettenbreijer (2004); (2) Reiners and Grosch (1998); (3) Meijboom (1964); (4) Burdock (2002).
[d] Odour description according to Burdock (2002).
[e] Odour description defined by Marco et al. (2007) using gas chromatography–olfactometry (GC–O).

versus the number of extractions minus one. The initial mass of compound in the sample can be calculated with the value of A_t by using a calibration curve obtained using standard solutions (Tena & Carrillo, 2007).

The quantification of each volatile compound was done by the external standard method. Stock standard solutions of pure compounds were prepared in methanol (Table 1). The stock solution was diluted 1/5, 1/10, 1/20, 1/200, 1/500, and 1/800 in methanol, and all the dilutions were analysed by multiple HS-SPME using similar conditions as for the sausage. Each dilution was analysed in triplicate and data was acquired in SIM mode. Eq. (1) was used to calculate the total area of standard present in the vial. Then, a linear calibration was obtained by representing the total area against the standard concentration added in the vial. Limits of detection (LOD) and quantification (LOQ) were calculated from the first extraction of a blank plus three and ten times the standard deviation of four blank replicates, respectively.

2.8. Quantification of odour-active compounds in the headspace (HS) of dry fermented sausages

At the end of the drying process, 3 g of minced sausage made with nitrite was weighed into a 10 ml HS vial sealed with a PTFE faced silicone septum and 0.75 mg of BHT were added. The vial was left in a thermoblock for 30 min at 30 °C. The released compounds were extracted by exposing the SPME fibre to the HS while the sample was maintained at 30 °C for different times (0.5, 1, 2 and 3 h). Each experiment was done in triplicate. The compounds adsorbed by the fibre were identified and quantified by GC–MS using SIM mode as described previously.

2.9. Calculation of odour-activity values (OAV)

The odour-activity values (OAVs) (De Roos, 2007) in air or oil of each compound present in the HS or in the matrix were calculated. For this purpose, the concentration of the compound in the HS or in the sausage (total concentration) was divided by the detection threshold in air or oil respectively, obtained from van Gemert and Nettenbreijer (2004), Burdock (2002), Reiners and Grosch (1998) and Meijboom (1964). The threshold value selected for OAV calculation was the minimum of the range (Table 1) because it indicates the minimum concentration necessary to detect the compound.

2.10. Statistical analysis

The effect the addition of curing agents (nitrite or nitrate) and ripening time on the volatile content and chemical parameters

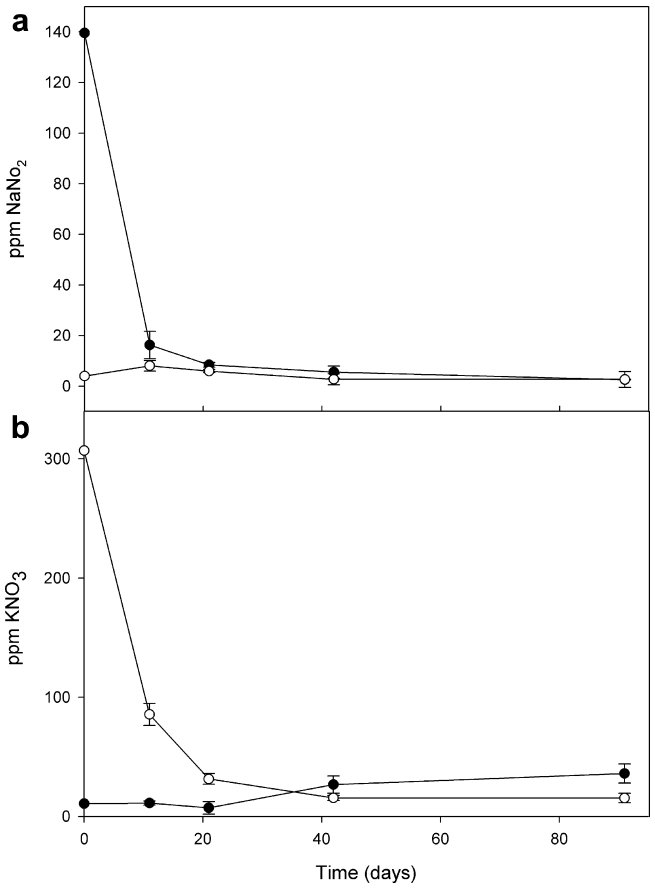

Fig. 1. Concentration (ppm in dm) of nitrite (a) and nitrate (b) during the ripening process of dry fermented sausages manufactured with added nitrite (●) or nitrate (○). Symbols represent the mean and standard error of the mean.

were tested by two-factor analysis of variance (ANOVA) using the statistic software Statgraphics plus (v. 5.1). Significant effects were compared using Fisher's least significant difference (LSD) test. The effect of nitrite and nitrate on the sensory preference was evaluated by ANOVA.

3. Results

3.1. Dry fermented sausages

The growth of lactic acid bacteria (LAB) and Staphylococci was monitored during the manufacture process (data not shown). The growth was within the range expected for slow fermented sausages (Marco, Navarro, & Flores, 2006). Moisture content and pH decreased during the process without significant differences between batches. Moisture content decreased from 62% to 40% and the pH reached the lowest value 4.8 after 21 d of processing.

Nitrite content was higher in the samples with added nitrite than nitrate at day 0 (Fig. 1a). Then, the concentration decreased rapidly and only residual concentrations were found in both batches. The nitrate concentration decreased from 300 to 15 ppm from 0 d to 42 d, while a low concentration of nitrate was detected in nitrite added samples (Fig. 1b).

TBARS value increased throughout the drying process in both batches (Fig. 2), and it was significantly higher in the nitrate added batch. However, after vacuum storage the nitrate sausage showed a reduction in the TBARS value that resulted in the absence of differences between batches.

Results of the sensory analyses performed at 42 and 92 d are shown in Table 2. The preference tests did not show significant differences ($p < 0.05$) in aroma, taste and overall quality between batches except for a preference in the colour of nitrite samples at 42 d. However, the panel preferred the nitrate batch in aroma at 92 d although with a low significance value ($p < 0.12$).

3.2. Determination of volatile compounds in fermented sausages by multiple HS-SPME

A total of 42 odour-active compounds were identified in fermented sausages. Of them, 30 presented exponential area decay when multiple HS-SPME was applied. The volatile compounds listed by their chemical classes were: 11 aldehydes, 7 ketones, 7 esters, 2 alcohols, 2 acids and 1 hydrocarbon (Table 3).

The total area for each volatile compound in the dry fermented sausage analysed by multiple HS-SPME was calculated using Eq. (1). In order to calculate the concentration of the volatile compounds corresponding to the total area, the external standard calibrations were obtained. Table 3 shows the measurement ranges of

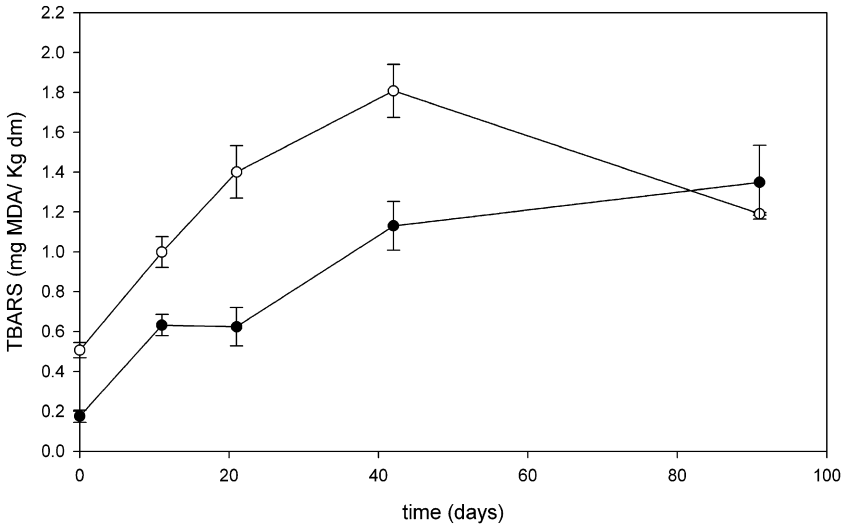

Fig. 2. Levels of TBARS (mg of manoladehyde (MDA) per kg of dm) during the ripening process of dry fermented sausages manufactured with added nitrite (●) or nitrate (○). Symbols represent the mean and standard error of the mean.

Table 2
Sensory analysis (preference test) of the dry fermented sausages at the end of ripening (42 d) and storage (92 d).

	42 d Preferred sample			92d Preferred sample		
	N_2[a]	N_3[b]	*P*	N_2	N_3	*P*
Colour	35	15	0.007	27	23	0.672
Aroma	21	29	0.322	19	31	0.119
Taste	23	27	0.672	22	28	0.480
Overall quality	24	26	0.888	24	26	0.888

P: significant value between both samples, tendency of the preference of one sample over the other.
[a] N_2: samples with added nitrite.
[b] N_3: samples with added nitrate.

the volatile compounds, correlation coefficients and slopes of the standard compounds. The quantification of the 30 odour compounds during the ripening process and vacuum packaged is shown in Table 4. As can be observed, all the concentrations of the compounds were above LOQs and LODs.

Many compounds were absent in the mince meat mixture, although a few volatile compounds were detected such as 3-methyl butanal, diacetyl and others (Table 4). Throughout the drying process and vacuum packed storage, there was a significant increase in the concentration of almost all the compounds, aldehydes, ketones, esters and 3-methyl butanoic acid, except for 2-heptenal, 2-butanone, 2,3-pentanedione, ethyl pentanoate, 2-methyl propanoic acid, 3-methyl-1-butanol, 1-octen-3-ol and limonene (Table 4).

The addition of nitrate or nitrite affected the concentration of 10 volatile compounds (Table 4). Several volatile compounds were more concentrated in the nitrate added sausages such as those originated from amino acid degradation (3-methyl butanal, 2-methyl butanal and 3-methyl butanoic acid), staphylococci esterase activity (ethyl 2-methyl butanoate, ethyl 3-methyl butanoate and ethyl hexanoate) and lipid autooxidation products (propanal, hexanal and 2-heptenal). However, 2-pentanone, derived from lipid β-oxidation, was in high concentration in the nitrite added sausage.

With regards to the detection thresholds of the 30 odour-active compounds quantified (Table 1), all of them were above their air detection threshold, except for 2-pentanone and 2-heptanone. However, only 14 were found in a concentration higher than their oil thresholds values, although not all oil thresholds were available in the literature.

Odour-activity values (OAV) in oil of the compounds throughout the process were calculated and shown in Table 5. The oil OAV was 1 or higher when the oil threshold was achieved and the compound would contribute to the aroma of the sample. Since the beginning of the curing process, the compounds 3-methyl butanal, 2-methyl butanal, octanal, diacetyl and ethyl 2-methyl butanoate showed oil OAVs above 1. All of them, with the exception of ethyl 2-methyl butanoate, also increased during the drying process. At the end of the fermentation stage (11 d), other compounds showed oil OAVs higher than 1 such as propanal, pentanal, hexanal, ethyl 3-methyl butanoate and 1-octen-3-ol. Other compound that contributed to the aroma (oil OAVs higher than 1) in the middle of the process (21 d) were 3-methyl butanoic acid while

Table 3
Linearity, correlation coefficients, LODs and LOQs of the standard compounds analysed by multiple HS-SPME.

Chemical group/compound	Measurement range (ng)	Correlation coefficient (r^2)	*m* (A_t/ng × 10^{-3})*	LOD (ng)	LOQ (ng)
Aldehydes					
Propanal	6.06–121.21	0.999	16.65	0.26	0.57
Butanal	0.13–10.61	0.993	21.36	0.22	0.58
3-Methyl-butanal	5.30–21.21	0.967	26.76	0.02	0.05
2-Methyl-butanal	0.13–5.3	1.000	21.14	0.04	0.08
Pentanal	0.68–136.36	0.999	16.39	0.16	0.39
Hexanal	0.17–136.36	0.991	16.78	9.49	27.64
2-Hexenal	0.21–106.06	0.977	0.80	5.64	11.91
Heptanal	0.30–60.61	0.991	30.71	0.21	0.56
2-Heptenal	0.13–21.21	0.992	3.81	0.14	0.35
Octanal	0.53–106.06	0.990	3.15	1.79	4.81
Nonanal	0.53–106.06	0.990	1.42	4.01	11.01
Ketones					
2-Butanone	3.79–15.15	0.924	74.24	0.47	1.18
Diacetyl (2,3-butanedione)	0.68–136.36	0.990	242.26	0.06	0.14
2-Ethyl-furan	0.17–0.68	0.992	421.35	0.00	0.00
2-Pentanone	0.15–0.61	0.999	284.42	0.00	0.00
2,3-Pentanedione	0.68–13.64	0.996	9.39	0.13	0.32
2-Heptanone	0.11–18.18	0.995	55.33	0.05	0.11
2-Nonanone	0.13–21.21	0.977	9.85	0.17	0.45
Esters					
Ethyl acetate	0.19–30.30	0.883	118.19	0.53	1.19
Ethyl butanoate	0.13–0.53	0.999	181.78	0.01	0.02
Ethyl 2-methyl-butanoate	0.53–106.06	0.997	43.53	0.00	0.00
Ethyl 3-methyl-butanoate	0.17–13.64	0.991	23.92	0.00	0.01
Ethyl pentanoate	0.17–6.82	0.999	12.76	0.07	0.15
Ethyl hexanoate	0.15–24.24	0.997	10.13	0.01	0.03
Ethyl octanoate	0.15–12.12	0.990	7.56	0.08	0.24
Alcohols					
3-Methyl-1-butanol	0.61–121.21	1.000	0.80	0.21	0.46
1-Octen-3-ol	0.27–27.27	0.962	2.97	0.07	0.16
Acids					
2-Methyl-propanoic acid	7.58–151.52	0.996	51.29	0.00	0.01
3-Methyl-butanoic acid	4.55–90.91	0.990	172.49	0.00	0.01
Hydrocarbons					
Limonene	0.24–6.06	1.000	5.96	2.32	6.23

* *m*: slope of the calibration curve, A_t is total area of compound calculated using Eq. (1) obtained in the multiple HS-SPME analysis.

Table 4
Volatile compound concentration (ng/g dm) obtained by multiple HS-SPME in dry fermented sausages during drying and storage.

Chemical group/compound	0 d	11 d		21 d		42 d		91 d		SEM	S[g]	B	S × B
		N_2[e]	N_3[f]	N_2	N_3	N_2	N_3	N_2	N_3				
Aldehydes													
Propanal		463.7a	546.8a	404.9b	994.7b	792.0b	906.3b			5.4	**	**	*
Butanal	6.4a	14.8a	14.1a	20.4a	46.0a	32.1a	43.9a	108.0b	77.6b	16.6	**	ns	ns
3-Methyl-butanal	120.1a	122.7a	220.8a	148.8ab	215.7ab	150.0bc	343.4bc	186.9c	379.8c	33.8	**	**	*
2-Methyl-butanal	11.3a	12.5ab	35.6ab	30.6bc	42.6bc	39.7 cd	77.0 cd	41.1d	60.2d	6.8	**	**	ns
Pentanal		238.5a	291.8a	240.8a	500.2a	208.0ab	394.4ab	738.6b	462.0b	96.1	*	ns	ns
Hexanal		898.7a	984.5a	813.0bc	2799.7bc	803.6b	2307.4b	1911.0c	2620.3c	246.0	**	**	*
2-Hexenal				43.4a	60.8a	179.0a	300.2a	794.3b	580.5b	117.0	**	ns	ns
Heptanal	126.7a	117.8a	128.5a	138.7a	142.8a	93.5a	167.3a	197.9b	179.0b	16.1	*	ns	ns
2-Heptenal		41.4	53.1	60.8	156.4	55.7	291.0	92.7	256.1	51.9	ns	*	ns
Octanal	375.3a	300.2a	359.4a	776.6a	658.9a	316.8a	1120.4a	2329.9b	1044.0b	277.0	**	ns	*
Nonanal	266.5ab	275.9a	302.7a	579.0b	722.3b	263.5ab	833.0ab	1332.5c	701.3c	135.6	**	ns	**
Ketones													
2-Butanone	175.0	85.1	195.9	142.8	119.4	152.3	222.2	167.4	269.0	48.3	ns	ns	ns
Diacetyl (2,3-butanedione)	750.5b	730.2c	1584.7c	772.3b	544.5b	279.5a	373.6a	345.3a	386.0a	72.0	**	**	**
2-Ethyl furan		6.5d	6.8d	5.5c	5.7c	4.5a	4.7a	5.0b	5.0b	0.2	**	ns	ns
2-Pentanone	0.4a	4.1ab	2.7ab	5.0ab	2.2ab	4.2b	2.4b	8.1c	5.0c	1.0	**	*	ns
2,3-Pentanedione						30.1	36.2	25.8	29.1	3.9	ns	ns	ns
2-Heptanone	2.5a	101.4b	58.3b	86.1b	52.1b	109.8c	166.7c	132.3c	185.0c	17.7	**	ns	*
2-Nonanone		103.8a	8.5a	91.0a	20.1a	161.2b	248.5b	291.5b	198.5b	32.8	**	ns	ns
Esters													
Ethyl acetate						42.5a	17.8a	411.3b	257.0b	36.8	**	ns	ns
Ethyl butanoate		6.6b	7.1b	4.6a	4.8a	6.6b	7.8b	7.2b	7.8b	0.8	*	ns	ns
Ethyl 2-methyl-butanoate	90.5d	81.7d	95.7d	68.8c	73.1c	58.7a	66.4a	63.7b	69.2b	1.2	**	**	**
Ethyl 3-methyl-butanoate		22.6ab	86.5ab	25.3a	43.9a	44.1b	93.1b	86.2c	130.5c	12.1	**	**	ns
Ethyl pentanoate				0.8	1.4	0.1	2.6	2.2	8.6	2.1	ns	ns	ns
Ethyl hexanoate		18.9a	29.3a	19.1a	34.1a	29.6b	93.2b	101.5c	126.0c	12.1	**	**	ns
Ethyl octanoate							55.5	66.9	92.2				
Alcohols													
3-Methyl-1-butanol						241.67	93.8	500.2	948.0	361.6	ns	ns	ns
1-Octen-3-ol		69.4	70.6	62.2	62.7	63.1	62.7	61.4	67.4	3.0	ns	ns	ns
Acids													
2-Methyl-propanoic acid				368.8	493.6	839.7	672.2	803.4	580.5	108.8	ns	ns	ns
3-Methyl-butanoic acid				752.7a	996.9a	1194.6b	1830.9b	1138.6b	2009.6b	204.1	*	*	ns
Hydrocarbons													
Limonene	32.6	63.2	55.8	53.9	70.1	35.6	101.6	56.9	84.9	16.9	ns	ns	ns

B: batch. S × B: interaction between batches and stage. ns: no significant.
a–d: means with different letters in the same batch indicate significant differences ($p < 0.05$) among processing times.
[e] N_2: samples with added nitrite.
[f] N_3: samples with added nitrate.
[g] S: stage.
* Significant $p < 0.05$.
** Significant $p < 0.01$.

2-methyl propanoic acid and ethyl hexanoate did it at the end of processing (42 d). However, nonanal contributed to the aroma after vacuum storage. In addition, compounds like 2-hexenal, heptanal and butanal showed oil OAVs close to 1.

3.3. Determination of volatile compounds in the HS of fermented sausages

The quantification by HS-SPME showed that the concentration of volatile compounds present in the HS is only a fraction of the total content of the sausage at any extraction time, although, as can be observed in Fig. 3, the content increases with the extraction time. Table 6 shows the concentration found in the HS of a sausage (nitrite added sausage at 42 d of processing) by exposing the CAR/DPMS fibre during 3 h. In many of the compounds, the concentration in the HS was around 10% of the total obtained by multiple HS-SPME. However, there were compounds with a concentration in the HS that represented 20–40% of the total concentration, such as 2-heptenal, ethyl hexanoate, 2-nonanone and nonanal, or even above 70%, as in the case of ethyl acetate and ethyl pentanoate.

The air OAVs were also calculated (Table 6) and the volatile compounds that presented the highest air OAVs values in the HS of sausages were 3-methyl-butanoic acid, ethyl 2-methyl-butanoate, nonanal, octanal, ethyl hexanoate, hexanal and ethyl octanoate (Table 6). In addition, limonene, 2-methyl-propanoic acid, 3-methyl butanal and diacetyl showed OAVs close to 1.

4. Discussion

In this study, sausages made with nitrate or nitrite have been used. The results obtained were similar to those previously reported by Marco et al. (2006). Nitrite decreased very quickly due to its high reactivity (Cassens, Greaser, Ito, & Lee, 1979), while nitrite formation in the nitrate added samples was likely caused by the nitrate reductase activity of staphylococci (Talón, Walter, Chartier, Barriere, & Montel, 1999). The selection of a slow fermentation process was performed to allow the enzyme activity to convert nitrate to nitrite (Lücke, 1985).

In relation to TBARS values, the lower levels reported in the nitrite added batch during the drying process could be due to the potent antioxidant effect of nitrite. On the other hand, the TBARS decrease in the nitrate batch after vacuum storage could be explained, as Talón et al. (1999) observed, by the production and release of catalase in *S. xylosus* in presence of nitrate. The TBARS decrease during vacuum storage was previously observed by

Table 5
Odour-activity values (OAVs) in oil of volatile compounds detected in dry fermented sausages during ripening and storage.

Chemical group/compound	0 d	11 d		21 d		42 d		91 d	
		N_2[a]	N_3[b]	N_2	N_3	N_2	N_3	N_2	N_3
Aldehydes									
Propanal		49.33	58.18	43.08	105.82	84.26	96.42		
Butanal	0.04	0.10	0.09	0.14	0.31	0.21	0.29	0.72	0.52
3-Methyl-butanal	9.24	9.44	16.99	11.45	16.60	11.54	26.42	14.83	29.22
2-Methyl-butanal	5.14	5.66	16.14	13.86	19.29	17.99	34.85	18.64	27.28
Pentanal		0.99	1.22	1.00	2.08	0.87	1.64	3.08	1.93
Hexanal		28.09	30.77	25.41	87.49	25.11	72.11	59.72	81.89
2-Hexenal				0.05	0.07	0.21	0.35	0.93	0.68
Heptanal	0.51	0.47	0.51	0.55	0.57	0.37	0.67	0.79	0.72
2-Heptenal		0.03	0.04	0.04	0.10	0.04	0.19	0.06	0.17
Octanal	6.70	5.36	6.42	13.87	11.77	5.66	20.01	41.61	18.64
Nonanal	0.27	0.28	0.30	0.58	0.72	0.26	0.83	1.33	0.70
Ketones									
2-Butanone	–*	–	–	–	–	–	–	–	–
Diacetyl (2,3-butanodione)	166.79	162.27	352.17	171.63	121.02	62.12	83.04	76.74	85.79
2-Ethyl furan		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2-Pentanone	–	–	–	–	–	–	–	–	–
2,3-Pentanodione	–	–	–	–	–	–	–	–	–
2-Heptanone	0.002	0.07	0.04	0.06	0.03	0.07	0.11	0.09	0.12
2-Nonanone	–	–	–	–	–	–	–	–	–
Esters									
Ethyl acetate						0.004	0.001	0.04	0.03
Ethyl butanoate		0.24	0.25	0.17	0.17	0.24	0.28	0.26	0.28
Ethyl 2-methyl-butanoate	348.30	314.52	368.44	264.68	281.49	225.85	255.76	245.20	266.17
Ethyl 3-methyl-butanoate		36.53	139.66	40.85	70.81	71.15	150.16	139.07	210.49
Ethyl pentanoate	–	–	–	–	–	–	–	–	–
Ethyl hexanoate		0.47	0.73	0.48	0.85	0.74	2.33	2.54	3.15
Ethyl octanoate	–	–	–	–	–	–	–	–	–
Alcohols									
3-Methyl-1-butanol	–	–	–	–	–	–	–	–	–
1-Octen-3-ol		2.04	2.08	1.83	1.84	1.86	1.85	1.81	1.98
Acids									
2-Methyl-propanoic acid				0.49	0.65	1.11	0.89	1.06	0.77
3-Methyl-butanoic acid				34.21	45.31	54.30	83.23	51.76	91.35
Hydrocarbons									
Limonene	–	–	–	–	–	–	–	–	–

[a] N_2: samples with added nitrite.
[b] N_3: samples with added nitrate.
* OAV not calculated due to absence of the oil threshold in literature.

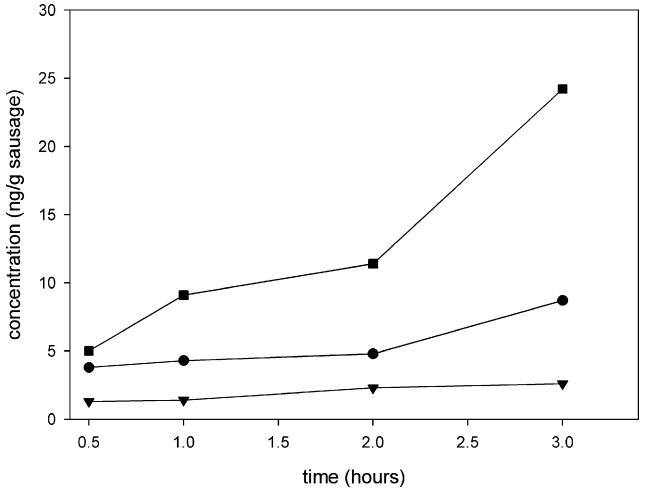

Fig. 3. Concentration (ng/g sausage) of ethyl hexanoate (●), 1-octen-3-ol (▼) and 2-nonanone (■) in the headspace of nitrite added dry fermented sausage (42 d of drying) at different extraction times.

Marco et al. (2006) in nitrite and nitrate dry fermented sausages, although the levels of oxidation were higher in nitrite samples during drying and storage. In contrast, Navarro, Nadal, Nieto, and Flores (2001) reported that TBARS levels were higher in small diameter non-fermented sausages with added nitrate than with nitrite as also occurs in our study.

The effect of the addition of nitrate and nitrite on aroma generation was studied by Marco et al. (2006); however, the total amount of each volatile compound generated at the different stages of processing is not known. In the present study, multiple HS-SPME was applied throughout the drying process and after vacuum storage of dry fermented sausages. The compounds quantified were those previously reported as odour-active compounds in dry fermented sausages using gas chromatography–olfactometry (Marco et al., 2007). The concentrations observed after vacuum packed storage were within the range of concentrations reported by Marco et al. (2007) and Flores and Hernández (2007) using multiple HS-SPME in slow and fast fermented sausages, respectively. The low concentration of volatile compounds in the unfermented mince and the increase of most of them throughout the drying process and vacuum storage were previously observed by several authors using HS techniques, (Croizet, Denoyer, Tran, & Berdagué, 1992; Marco et al., 2006; Olesen, Meyer, & Stahnke, 2004). As Olesen et al. (2004) concluded, volatile compound generation is linked to the fermentation and curing process.

The higher concentration of volatiles derived from amino acid catabolism, staphylococci esterase activity and lipid autooxidation in the nitrate added sausages was already reported by Marco et al. (2006) and Olesen et al. (2004) using HS techniques and this increment could be the reason for the aroma preference of nitrate

Table 6
Odour-activity values in air of volatile compounds detected in nitrite added dry fermented sausage (42 d of drying) and percentage of volatile compounds in the HS respect to the total concentration.

Chemical group/compound	Concentration (ng/g)		%[c]	OAV in air[d]
	HS[a]	Total[b]		
Aldehydes				
Propanal	2.94	488.1	0.6	*
Butanal	0.26	19.8	1.3	0.31
3-Methyl-butanal	1.10	92.5	1.1	0.55
2-Methyl-butanal	0.14	24.5	0.5	*
Pentanal	4.41	128.2	3.4	0.04
Hexanal	33.69	495.3	6.8	1.68
2-Hexenal	2.60	110.3	2.3	0.05
Heptanal	3.80	57.7	6.5	0.06
2-Heptenal	8.31	34.3	24.2	0.24
Octanal	34.28	195.3	17.5	6.86
Nonanal	57.60	162.4	35.4	11.52
Ketones				
2-Butanone	tr	93.9	–	*
Diacetyl (2,3-butanedione)	2.36	172.3	1.3	0.47
2-Ethyl furan	0.08	2.8	3.0	*
2-Pentanone	0.35	2.6	13.2	0.00
2,3-Pentanedione	1.38	19.1	7.2	0.14
2-Heptanone	5.64	67.7	8.3	0.13
2-Nonanone	24.21	99.4	24.3	0.32
Esters				
Ethyl acetate	24.4	26.2	93.1	0.07
Ethyl butanoate	0.35	4.1	8.8	0.13
Ethyl 2-methyl-butanoate	0.79	36.2	2.1	13.17
Ethyl 3-methyl-butanoate	0.53	27.2	1.9	*
Ethyl pentanoate	0.03	0.04	78.8	0.10
Ethyl hexanoate	8.69	18.3	47.6	2.90
Ethyl octanoate	16.44	n.q.	–	3.29
Alcohols				
3-Methyl-1-butanol	23.07	148.9	15.4	*
1-Octen-3-ol	2.63	38.9	6.7	0.22
Acids				
2-Methyl-propanoic acid	3.76	517.5	0.7	0.75
3-Methyl-butanoic acid	3.15	736.2	0.4	14.32
Hydrocarbons				
Limonene	2.67	22.0	12.2	0.67

tr: traces.
* odour threshold in air was not reported in literature. n.q.: not quantified.
[a] Concentration obtained after 3 h of CAR/PDMS fibre exposure in the HS of dry fermented sausage.
[b] Concentration obtained by multiple HS-SPME corresponding to the total content of volatile compound in the sausage.
[c] Percentage of volatile compound found in the HS of dry fermented sausages in relation to the total content obtained by multiple HS-SPME.
[d] Odour-activity values calculated using the concentration found in the HS and the air detection thresholds from Van Gemert and Nettenbreijer (2004) and Burdock (2002).

sausages at 92 d. The previous application of multiple HS-SPME by Flores and Hernández (2007) also showed a higher proportion of almost all the volatile compounds in the nitrate fermented sausage. However, Marco et al. (2007) only reported few significant differences between batches.

The application of multiple HS-SPME allowed the selection of the main contributors to the aroma by comparing the total content with the oil thresholds values. In our study, 14 compounds were above their oil thresholds (Table 5). Five compounds showed concentrations above their oil thresholds since the beginning. Some of them, such as 3-methyl butanal, 2-methyl butanal, octanal and diacetyl also increased during the drying process, while ethyl 2-methyl butanoate was at a constant concentration. The other 9 compounds achieved the oil detection threshold throughout the drying process or even during the storage, these were propanal, pentanal, hexanal, 1-octen-3-ol, 3-methyl butanoic acid, 2-methyl propanoic acid, ethyl 3-methyl butanoate, ethyl hexanoate, and nonanal. These results confirmed that ripening time has a considerable effect on the aroma of dry fermented sausages and are partially in accordance with those from Marco et al. (2007) who only detected hexanal, heptanal and 1-octen-3-ol as the main contributors to the aroma (concentrations above their oil thresholds). In addition, Marco et al. (2007) indicated the presence of other compounds (18 compounds) above their air threshold and therefore, they were also considered as odour-active compounds of fermented sausages. On the other hand, Schmidt and Berger (1998) applying olfactometry techniques to Spanish-fermented sausages, reported that, without taking into consideration the contribution of aroma compounds derived from spices, the most potent odorants were 2,3-butanedione, 3-methyl butanoic acid, ethyl propanoate, 2-phenylethanol and acetic acid. Only, two compounds, 2,3-butanedione (diacetyl) and 3-methyl butanoic acid, are in accordance with our results.

Nevertheless, aroma perception in meat products depends not only on the concentration and odour thresholds of volatile compounds, but also on their interactions with other food components and among volatile compounds (Adhikari et al., 2006). In fact, the concentration found in the HS of nitrite added sausages was only a fraction, around 10–20%, of the total content determined by HS-SPME multiple (Table 6). However, other compounds can be in higher proportion (around 70%) such as esters. On the other hand, it was not possible to establish a significant relationship between the fraction released and the hydrophobicity of each compound. For this purpose, the parameter Log K_{ow} (Table 1) was used as it represents the partition coefficient in octanol/water and measures the hydrophobicity of each compound. Only the aldehydes showed a significant relationship ($p < 0.01$) between Log K_{ow} and release. In this case, the higher Log K_{ow} the higher percentage of aldehyde compound found in the HS in relation to the total content. The binding of aroma compounds by muscle proteins could be responsible for this behaviour (Chevance & Farmer, 1998; Pérez-Juan, Flores, & Toldrá, 2007). Other factors that have not been considered in the present study are water and sodium chloride content as they can affect the partition coefficients between matrix and vapour phase (Guichard, 2002).

In relation to air OAVs, the values obtained in the HS are partially in accordance with those calculated considering the total content and the oil OAVs. Reiners and Grosch (1998) indicated that the compounds with OAVs greater than five contribute strongly to the flavor of a sample. In the HS of fermented sausages, 3-methyl butanoic acid, ethyl 2-methyl butanoate, nonanal and octanal showed the highest air OAVs (Table 6). All of them also had high oil OAVs, except nonanal, whose oil OAV value increased after vacuum storage although it was lower than 5 (Table 5). The most important compounds in relation to oil OAVs were those previously mentioned and also propanal, hexanal, diacetyl and ethyl 3-methyl butanoate. However, the air thresholds of propanal and ethyl 3-methyl butanoate were not available, and its air OAVs could not be calculated. With respect to diacetyl and hexanal, their air OAV were close to 1.

In summary, the positive effect of nitrate addition on the aroma of fermented sausages was confirmed. Also, the HS concentration of aroma compounds represents around 10–20% of the total concentration of the compounds in fermented sausages. Therefore, the release of compounds is highly dependent on matrix composition and compound concentration. In this study, the aroma perceived in the HS was mainly due to 3-methyl butanoic acid, ethyl 2-methyl butanoate, nonanal and octanal that showed the highest air OAVs. However, the main compounds that contributed to the aroma of dry fermented sausages during eating were those with the highest oil OAVs, such as 3-methyl butanal, 2-methyl butanal, octanal, diacetyl and ethyl 2-methyl butanoate that were important since the start of the ripening process. Other compounds, such as propanal, pentanal, hexanal, ethyl 3-methyl butanoate, 1-octen-3-ol, 3-methyl butanoic acid, 2-methyl propanoic

acid, ethyl hexanoate and nonanal were also important contributors as they were generated at the end of process. Actually to confirm the results of the aroma analyses, further investigations are been carried out in our laboratory using aroma models in order to reproduce the fermented sausage aroma and the factors that affect their release.

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