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SPME-GC-MS versus Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) Analyses for the Study of Volatile Compound Generation and Oxidation Status during Dry Fermented Sausage Processing

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ABSTRACT: The use of selected ion flow tube mass spectrometry (SIFT-MS) and gas chromatography−mass spectrometry together with solid phase microextraction (GC-MS-SPME) has been compared in the analysis of volatile compounds during dry fermented sausage processing. Thus, the headspace (HS) of samples of dry fermented sausages with different fat contents was analyzed during their manufacture using both techniques, and significant and positive correlations were found between SIFT-MS and SPME-GC-MS measurements for the compounds pentanal, hexanal, 2-heptenal, octanal, 2-nonenal, 2-butanone, 2-pentanone, ethanol, acetic acid, and hexanoic acid. The oxidative status of fermented sausages during processing was also evaluated, and a significant correlation was obtained between the HS concentration of lipid autoxidation volatile compounds measured by SIFT-MS and SPME-GC-MS and the level of thiobarbituric acid reactive substances (TBARS) in the sausage. The hexanal measured by SIFT-MS resulted in a higher correlation coefficient ($r = 0.936$) than that obtained using SPME-GC-MS ($r = 0.927$). SIFT-MS is shown to be a fast, real time analytical technique for monitoring changes in the profile of volatile compounds in dry fermented sausages during processing and a useful tool to evaluate the oxidative status of meat products.

KEYWORDS: SIFT-MS, dry fermented sausages, oxidation, volatile organic compounds

INTRODUCTION

The typical aroma of dry-fermented sausages is due to a mixture of volatile compounds generated by bacterial metabolism and lipid oxidation during processing. However, among the hundreds of volatile compounds identified, only a limited number are the odorants responsible for the dry-cured aroma.[1−3] Gas chromatography−mass spectrometry (GC-MS) is the most widely used technique for the identification and quantification of flavor compounds. This technique requires preconcentration of the volatiles in the gas (headspace, HS) sample using methods such as vacuum distillation or solid phase microextraction (SPME). Using the latter method, the volatile compounds are released from the adsorbent thermally and injected into the column to separate them; however, the chromatographic separation takes time. Although GC-MS is a highly sensitive and reliable technique, the demands of the food industry for rapid analysis indicate that GC-MS is not so convenient, and faster analytical techniques are required. This has led to the development of direct mass spectrometric techniques without chromatographic separation in which the mixture of the emitted volatile compounds from a food matrix is sampled directly into a mass spectrometer and the compounds are immediately detected and quantified. Direct analyses of volatile compounds in air have been demonstrated using different ionization techniques, including atmospheric pressure chemical ionization (APCI[4]), proton transfer reaction mass spectrometry (PTR-MS[5]), and selected ion flow tube mass spectrometry (SIFT-MS; [6,7]).

SIFT-MS is a direct mass spectrometric technique based on the chemical ionization of a gas (analyte) sample (to the exclusion of the major air gases N_2, O_2, H_2O, and CO_2) using specific, selected precursor (reagent) positive ions. Using SIFT-MS, real time quantification of volatile compounds in humid air can be achieved without external calibration. This is because the absolute concentrations are calculated from the ratios of the count rates of the product analyte-derived ions to those of the precursor while taking into account known values of the reaction rate coefficients, reaction time, and the influence of diffusion and mass discrimination.[8] Even so, SIFT-MS analyses have been validated using standard mixtures,[9] and the technique has been widely applied in biology and medicine.[7,10,11] With respect to flavor analysis, Španěl and Smith[6] described the kinetics of the reactions of the precursor ions used in SIFT-MS with some food flavor compounds and studied the real-time release of volatile compounds from freshly cut onion, crushed garlic, and ripe banana. Recently, several papers have demonstrated the value of SIFT-MS for flavor research; the applications continue to broaden as this paper goes to press. Xu and Barringer used SIFT-MS to study headspace tomato volatiles and their release during chewing.[12,13] Davis et al.[14] and Davis and McEwan[15] monitored the major volatile compounds emitted by olive oil to establish differences in their quality. SIFT-MS has also been employed to quantify volatile basic nitrogen compounds released from cod fillets[16] and the

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release of alkylpyrazines and other volatiles by cocoa liquors[17] and aldehydes from malt.[18] Most recently, we have successfully exploited SIFT-MS to quantify the volatile aroma compounds released by dry fermented sausages.[19]

SIFT-MS has also been described as a tool for the evaluation of other quality parameters in food products apart from flavor. For instance, Noseda et al.[16] compared SIFT-MS with the traditional methods for the quantifiation of amines (steam distillation of an alkalized sample) and proposed SIFT-MS as a fast nondestructive technique for the evaluation of raw fish freshness. Davis and McEwan[15] have positively correlated SIFT-MS measurements with oxidative status as determined by the peroxide value (PV) and developed a fast reliable method for PV prediction by SIFT-MS in olive oil. To date, the results of only one study have been reported in which both SIFT-MS and GC-MS have been used in tandem, this study involving the differentiation of malt varieties.[18]

Therefore, the aim of the present work was to evaluate the potential of SIFT-MS to monitor the generation of volatile compounds in fermented sausages during ripening by comparing conventional SPME-GC-MS analyses with real-time SIFT-MS analyses. Additionally, the capability of SIFT-MS to determine the oxidative status of dry fermented sausages during processing was investigated. The results reveal the usefulness of SIFT-MS in quality control applications.

MATERIALS AND METHODS

Reagents and Standards. The chemical compounds used for volatile identification were all obtained from Fluka Chemie AG (Buchs, Switzerland) except 2-octenal and 2,3-butanedione, which were obtained from Aldrich (St. Louis, MO).

Dry Fermented Sausages. Three batches of dry fermented sausages with different pork back fat percentage were selected for analysis: 10%, low fat (LF); 20%, medium fat (MF); and 30%, high fat (HF). The processing conditions of the sausages are described in Olivares et al.[20] From each batch, three sausages (LF, MF, and HF) were chosen at 0, 9, 18, 42, and 63 days of processing, after which they were sliced, vacuum packaged, and frozen at −80 °C to await analysis. The lipid oxidation in the sausages was determined using the thiobarbituric acid reactive substances (TBARS) method, as described by Bruna et al.,[21] using trichloroacetic acid instead of perchloric acid as solvent. The results are expressed as milligrams of malonaldehyde (MDA) per kilogram. The lipid oxidation determinations were replicated three times and the results expressed as the mean of the three values.

SPME-GC-MS Analyses. The analysis of volatile compounds present in the HS of the sausages was carried out as described by Marco et al.[22] The compounds shown in Table 1 were quantified on the basis of their aroma properties described previously for Dry Fermented Sausages.[1−3] For each measurement, 3 g of minced sausage was weighed into a 10 mL headspace vial, and 0.75 mg of antioxidant (butylated hydroxytoluene, BHT) was added. The vial was left for 1 h in a thermoblock (J.P., Selecta, Barcelona, Spain) at 37 °C to equilibrate. The volatile compounds were extracted by SPME using an 85 μm carboxen/polydimethylsiloxane StableFlex fiber (CAR/PDMS SF, Supelco, Bellefonte, PA) that was exposed to the headspace for 3 h while the sample was maintained at 37 °C. The fiber was then placed in the injection port of a gas chromatograph (HP 7890A) equipped with a HP 5975C mass selective detector (Hewlett-Packard, Palo Alto, CA). The compounds adsorbed onto the fiber were desorbed in the injection port of the GC with the purge in the splitless mode. The released compounds were separated using a DB-624 capillary column (J&W Scientific, Agilent Technologies, USA) and identified by comparison with the mass spectra constructed from the (NIST 05) library database and by the Kovats linear retention index[23] using authentic standards. The volatile compounds were analyzed by the SCAN mode, and the total ion current (TIC) across the m/z range of 29−400 was acquired. Quantification was based on total extracted area (TIC). The headspace of the sausages was analyzed in triplicate for each fat batch and each processing time. The measuring order of the samples was randomized.

Table 1. Molecular Weight, Precursor Ion, and Mass-to-Charge Ratio (m/z) of the Characteristic Product Ions of the Aroma Compounds Analyzed by SIFT-MS

compound	reliability[a]	M_w[b]	precursor ion[c]	product ion (m/z)[d]
aldehydes				
propanal	a	58	NO^+	57
butanal	a	72	NO^+	71
pentanal	a	86	NO^+	85
hexanal	a	100	NO^+	99
heptanal	a	114	NO^+	113
2-heptenal	a	112	NO^+	111 + 142
octanal	a	128	NO^+	127
2-octenal	a	126	NO^+	125 + 156
nonanal	a	142	NO^+	141
2-nonenal	a	140	NO^+	139 + 170
2,4-decadienal	a	152	NO^+	151
ketones				
2-butanone	a	72	NO^+	102
2,3-butanedione	a	86	NO^+	86
2-pentanone	a	86	NO^+	116
2-heptanone	a	114	NO^+	144
2-octanone	a	128	NO^+	158
2-nonanone	a	142	NO^+	172
esters				
ethyl acetate	a	88	NO^+	118
alcohols				
ethanol	a	46	H_3O^+	47 + 65 + 83
1-propanol	a	60	H_3O^+	43
acids				
acetic acid	a	60	NO^+	90 + 108
hexanoic acid	a	116	H_3O^+	117 + 135
sulfur compounds				
dimethyl disulfide	a	94	NO^+	94
methanethiol	a	48	H_3O^+	49 + 67

[a] Reliability of identification: a, mass spectrum and retention time identical with an authentic standard. [b] Molecular weight. [c] Precursor ion used for quantification. [d] Product ion generated after ionization as described in Olivares et al.[19]

SIFT-MS Analyses. The optimization of SIFT-MS for quantification of the volatile compounds in the headspace of sausages was achieved as described in Olivares et al.[19] For each measurement, 5 g of crushed sausage was weighed into a 15 mL headspace vial, together with 0.75 mg of BHT used as antioxidant. The emitted volatiles were allowed to develop in the HS of the sealed vial (initially purged with laboratory air) at 37 °C for 1 h. A SIFT-MS *Profile 3* instrument manufactured by Instrument Science Limited (Crewe, U.K.) was used to measure the volatile compounds. The air/volatile compounds of the sealed vial were sampled directly by piercing the septum with a stainless steel needle connected directly to the SIFT-MS sampling line. The sample entered the helium carrier gas via a heated (70 °C) capillary tube at a measured rate of 0.45 Torr L/s. A second syringe needle pierced the septum to maintain the pressure in the vial at atmospheric pressure by introducing laboratory air at a rate that balances the small loss rate due to the sampling into

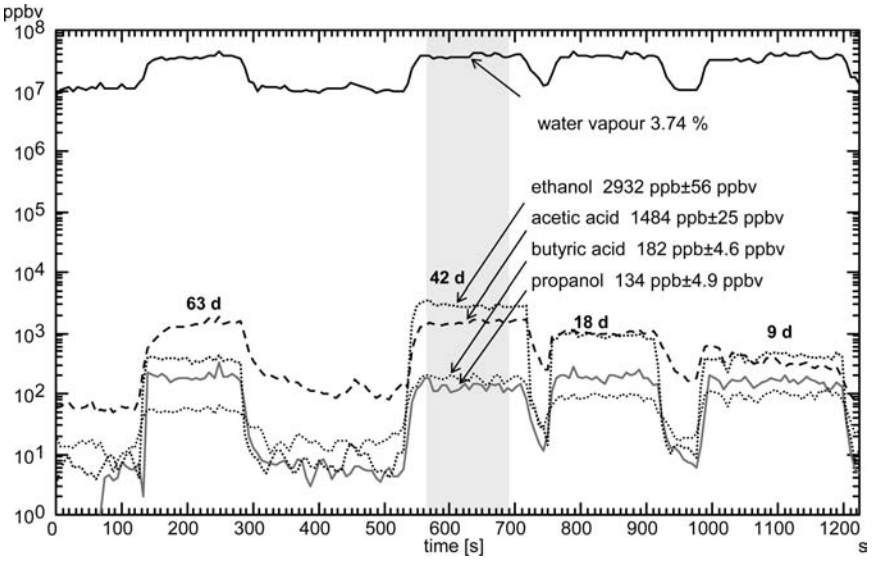

Figure 1. Real time monitoring of the HS of sausages by SIFT-MS. Four high-fat sausages (HF) at different ripening times, 9, 18, 42, and 63 days (indicated as d in the figure), were analyzed. The levels of several compounds indicated in parts per billion by volume (ppbv) are given together with their estimated uncertainties for the 42 day sample, the vertical shading indicating the integration interval.

the SIFT-MS instrument. Background (laboratory air) concentrations of all the volatile compounds included in the analysis were routinely recorded before and after the analysis of each sample. H_3O^+, NO^+, and O_2^+ were used as precursor ions. Flow tube temperature was 26 °C, flow tube pressure was 1.0 Torr, flow tube diameter was 1 cm, and reaction length was 4 cm. For accurate quantification, the multiple ion monitoring (MIM) mode was used to target specific volatile compounds.[11] In this mode, the analytical mass spectrometer is rapidly switched between selected m/z values of both the precursor ions and the characteristic product ions. Precursor ion count rates were in the range from 10,000 to 1,000,000 counts/s. The known rate coefficients for the analytical reactions were then used to quantify the absolute HS concentrations of the compounds using the standard SIFT-MS data analysis software and the general method of quantification.[24] Ionic diffusion and mass discrimination were corrected by the SIFT-MS software according to procedure described in Smith et al.[8] The absolute quantification was continuously verified by analyses of absolute humidity.[25] In Table 1 is shown the volatile compounds quantified together with the precursor ion and product ions used for each compound. Data for each precursor ion were collected and integrated for a period of 200 s, and the mean values over this sampling time were recorded. The results were then expressed in parts per billion by volume of the headspace (ppbv; nL of volatile compound/L of air). The headspace of the sausages was analyzed in duplicate for each fat batch and each processing time. The measuring order of the samples was randomized.

Statistical Analysis. The effect of ripening time on the HS volatile compounds concentration obtained by both techniques was assessed using analysis of variance (ANOVA). Pearson correlation analysis was performed to correlate the results obtained by SIFT-MS and SPME-GC-MS analyses. In addition, Pearson correlation analysis was performed between the analysis of volatile compounds (SIFT-MS and GC-MS) and the oxidative status of the sausages (TBARS values). The statistical software XLSTAT 2009.4.03 (Addinsoft, Barcelona, Spain) package was used for these analyses.

RESULTS

Comparison of SIFT-MS and SPME-GC-MS throughout Sausage Processing. Quantification of volatile compounds (Table 1) was achieved by GC-MS for the three batches of sausages throughout the processing period (0, 9, 18, 42, and 63 days). Each SPME-GC-MS analysis required a total time of 5 h (1 h of equilibration, 3 h of CAR/PDMS fiber extraction, 1 h of GC-MS run). A single fiber was used, so only two analyses per day were possible. However, the number of analyses per day can be improved if an automatic device is used. The volatile compounds were selected on the basis of their aroma properties described for Dry Fermented Sausages by previous GC−olfactometric data.[1−3,26−29] The mixture comprised 11 aldehydes, 6 ketones, 2 alcohols, 2 acids, 2 organosulfur compounds, and 1 ester (Table 1).

The volatile compounds were also quantified during ripening using SIFT-MS to determine the potential of this real time analytical method. The sample preparation conditions were exactly the same as those used in SPME-GC-MS analyses (crushing the sample, antioxidant addition, ratio sample/HS volume in the vial, temperature, and equilibration time). For each sample a little more than 1 h was needed (1 h of equilibration, 200 s for SIFT-MS data acquisition with each precursor ion species). Even though preparation of the sample was identical for both techniques, SIFT-MS analysis required a much shorter time because it does not involve SPME and GC separation, even though an automatic device is used. Several samples were crushed and, after equilibration, were measured consecutively. For instance, Figure 1 shows SIFT-MS real-time monitoring data obtained using the MIM mode analysis for the compounds ethanol, acetic acid, butyric acid, propanol, and water vapor in the HS of four high-fat sausages at different ripening times (9, 18, 42, and 63 d). Although Figure 1 represents only one measurement of one sample at each processing time, a change with ripening time of the HS concentration of ethanol, acetic acid, butyric acid, and propanol can be seen, and only 15 min was necessary for SIFT-MS data acquisition, in this case using H_3O^+ precursor ion.

For the quantification of the volatile compounds the parameters described in Table 1 were used. In general, NO^+ was used as the precursor ion, but H_3O^+ was used for ethanol, 1-propanol, hexanoic acid, and methanethiol. This selection was made on the basis of previous work that indicated the m/z values

Table 2. Quantification of Volatile Compounds in Dry Fermented Sausages during Ripening (Values Represent the Mean of the Three Fat Batches Analyzed) by SMPE-GC-MS and SIFT-MS after 0 (Initial), 9, 18, 42, and 63 Days[a]

	SPME-GC-MS (AU[b] 10^{-6})							SIFT-MS (ppbv)						
compound	0 days	9 days	18 days	42 days	63 days	SEM[c]	p[d]	0 days	9 days	18 days	42 days	63 days	SEM	p
aldehydes														
propanal	0.00 b	0.40 b	0.61 b	2.68 a	3.24 a	0.38	0.000	33.80 c	44.03 bc	61.38 abc	88.61 ab	94.04 a	14.18	0.049
butanal	0.00 c	0.20 b	0.19 b	0.61 a	0.59 a	0.05	<0.0001	6.90 b	9.50 b	20.77 a	17.45 a	20.10 a	1.88	0.001
pentanal	0.00 c	4.25 bc	7.76 b	20.67 a	20.61 a	1.61	<0.0001	6.32 c	10.76 c	11.46 c	27.47 b	38.54 a	2.05	<0.0001
hexanal	4.18 b	36.20 b	56.82 b	243.30 a	293.38 a	29.28	<0.0001	10.32 c	23.30 c	31.38 bc	50.00 ab	57.16 a	6.93	0.004
heptanal	1.13 b	3.37 b	4.87 b	21.40 a	24.24 a	1.23	<0.0001	20.87	17.81	19.04	24.50	24.97	1.96	0.094
2-heptenal	0.00 b	0.00 b	0.24 a	0.36 a	0.39 a	0.07	0.004	31.38	25.27	26.99	34.73	31.94	2.75	0.176
octanal	0.07	0.15	0.95	0.82	1.91	0.50	0.144	3.37 b	3.54 b	3.69 b	5.49 a	4.70 ab	0.47	0.040
2-octenal	0.20 c	0.57 c	0.87 bc	2.06 ab	3.30 a	0.42	0.002	1.55 b	3.50 ab	4.88 a	4.04 a	3.87	0.63	0.039
nonanal	3.31 c	4.91 c	6.99 c	16.50 b	22.60 a	1.56	<0.0001	4.22	4.28	4.78	4.16	4.01	0.46	0.870
2-nonenal	0.56 b	0.71 b	0.74 b	1.73 a	1.88 a	0.12	<0.0001	0.78	2.80 a	3.69 a	3.73 a	3.73 a	0.56	0.016
2,4-decadienal	0.00 b	0.00 b	0.37 a	0.38 a	0.40 a	0.11	0.059	0.25 c	1.09 bc	1.87 ab	2.03 a	1.33 ab	0.28	0.008
ketones														
2-butanone	5.05 c	14.72 a	13.93 a	9.92 b	9.60 b	0.99	0.000	7.37 c	17.71 b	27.51 a	24.19 ab	23.39 b	2.70	0.003
2,3-butanedione	0.21 b	3.18 a	0.32 b	0.34 b	0.31 b	0.58	0.018	9.13	9.67	10.51	11.13	12.31	1.50	0.613
2-pentanone	0.56 c	4.27 a	3.82 a	1.04 b	1.17 b	0.15	<0.0001	3.85 b	8.34 a	9.01 a	7.34 a	8.87 a	1.00	0.024
2-heptanone	0.00 c	5.68 b	11.14 a	9.49 a	10.77 a	0.80	<0.0001	1.37 c	4.63 b	5.11 b	8.28 a	5.28 b	0.93	0.006
2-octanone	0.00 d	0.33 c	0.41 bc	0.49 ab	0.59 a	0.03	<0.0001	1.22	3.96	3.61	5.58	3.77	0.94	0.087
2-nonanone	0.00 c	2.78 b	6.56 a	7.09 a	7.95 a	0.56	<0.0001	0.40	3.69	3.35	5.39	2.86	1.00	0.058
esters														
ethyl acetate	0.00 b	2.94 a	3.63 a	3.34 a	4.38 a	0.58	0.003	27.05 c	62.12 a	59.41 a	45.13 b	31.98 bc	4.49	0.001
alcohols														
ethanol	1.64	11.50	12.38	12.36	10.53	3.92	0.466	69.57	382.11	570.00	647.70	408.64	164.80	0.200
1-propanol	0.00	0.00	0.10	0.10	0.11	0.04	0.238	109.05	102.92	140.63	145.25	180.24	27.46	0.338
acids														
acetic acid	0.00 c	189.48 b	773.71 a	785.62 a	747.03 a	44.47	<0.0001	115.77 d	160.04 d	498.49 c	737.93 b	831.37 a	20.30	<0.0001
hexanoic acid	2.47	12.61	28.47	35.54	38.77	9.30	0.060	11.30	12.67	12.29	14.06	13.23	1.51	0.759
sulfur compounds														
dimethyl disulfide	0.00 b	0.00 b	1.16 a	0.80 a	1.11 a	0.14	0.000	2.70	7.37	11.23	11.37	8.38	2.26	0.113
methanethiol	149.33	181.60	196.66	173.39	192.78	13.98	0.202	5.40 c	11.24 c	22.91 b	39.12 a	42.97 a	2.52	<0.0001

[a] Means with different letters indicate significant differences ($p < 0.05$) among ripening times. [b] Abundance units. [c] Standard error of the mean. [d] p value for the statistical analysis.

of characteristic products ions (Table 1) that did not overlap with those characteristic ions produced by other compounds in the sausage HS.[19] Thus, the MIM mode was used to quantify the volatile compounds, as described under Materials and Methods. All of the analyses were carried out in duplicate for all of the samples and the mean values obtained.

The results of quantification realized by both techniques are shown in Table 2, where the values represent the mean of the three fat batches analyzed. The values of the SPME-GC-MS analysis were expressed in abundance (TIC $\times$ 10^{-6}) as their values depend on the affinity of the compounds to the fiber, and the concentration will not be the real concentration present in the sausage HS. These data indicate significant increases in almost all of the volatile compounds during ripening, except for octanal, 2,4-decadienal, ethanol, 1-propanol, hexanoic acid, and methanethiol. The latter is probably due to the high standard error obtained in the analyses of these compounds due to the different fat batches employed. Nevertheless, SPME-GC-MS can be used to monitor changes during ripening in most of all the emitted volatile compounds. Therefore, the results obtained can be an index of the ripening process and can also relate to sensory properties.[30,31]

On the other hand, the SIFT-MS analyses express the concentrations of the volatile compounds in ppbv in the HS. Many volatile compounds increased significantly during ripening except for heptanal, 2-heptenal, nonanal, 2,3-butanedione, 2-octanone, 2-nonanone, ethanol, 1-propanol, hexanoic acid, and dimethyl disulfide. Previously, it was mentioned that the absence of observable differences can be due to the high standard error that can result when collectively analyzing the three fat batches but, in addition, several of these compounds were at very low concentration in the HS close to the quantification limits of the SIFT-MS (limit of quantification is 10 ppbv).[24] This is the case for nonanal, 2,3-butanedione, 2-octanone, and 2-nonanone, which were present at concentrations of <10 ppbv (Table 2).

Generally, both the GC-MS and SIFT-MS techniques detected differences in volatile compound concentrations and increases with ripening time. However, to determine if both techniques were able to reveal the same differences, a Pearson correlation analysis was done using the data obtained in the three batches during the

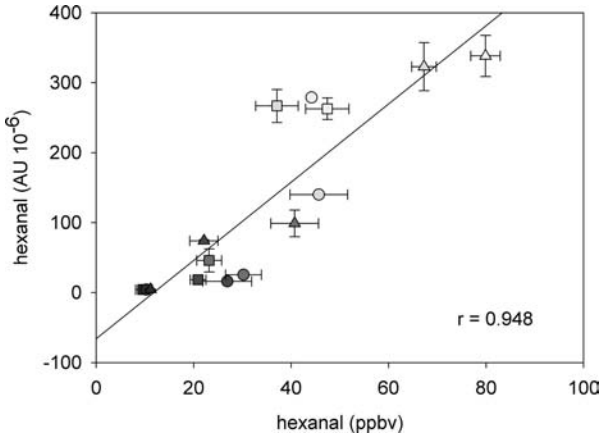

Figure 2. Pearson correlation coefficient (r) of the hexanal levels measured by SIFT-MS (ppbv) and GC-MS (abundance units; AU 10^{-6}) during sausage processing: low-fat (LF, □); medium-fat (MF, ○); high-fat (HF, △) sausages. The plotted values are the mean values for the three sausages, and the bars represent the standard errors for both techniques. Gray intensity in symbols decreases with ripening time.

different ripening times. Figure 2 shows the correlation between the measurement of hexanal by both techniques (GC-MS and SIFT-MS) for the three batches and ripening times. The gray intensity of the symbols decreases with ripening time (Figure 2), so it can be seen that both techniques showed an increase in the hexanal concentration during ripening with a positive and significant correlation coefficient ($r = 0.948$). The same analysis was carried out for all of the volatile compounds analyzed, and the correlation coefficients for each fat batch and the whole set of batches are shown in Table 3. When the three batches were analyzed together, significant and positive correlations were obtained for 13 of the 24 volatile compounds analyzed such as for the linear aldehydes C5−C8 and 2-heptenal, 2-octenal, and 2-nonenal; ketones 2-butanone, 2,3-butanedione, and 2-pentanone;acetic and hexanoic acids; and ethanol. The best correlations ($r > 0.8$) were obtained for hexanal, pentanal, acetic acid, ethanol, and 2-pentanone. However, when the correlation analysis was carried out for the different fat samples of each batch, the LF batch was the one for which a higher number of significant correlations was obtained. Probably the presence of a higher fat content of the MF and HF samples can interfere in the analysis of volatile compounds. Not

Table 3. Pearson Correlation Coefficients of Volatile Compounds Obtained by SIFT-MS and SPME-GC-MS in Dry Fermented Sausages during Processing with Different Pork Back Fat Contents; Low Fat (LF), Medium Fat (MF) and High Fat (HF)

	LF		MF		HF		all batches	
compound	r	p	r	p	r	p	r	p
aldehydes								
propanal	0.920	**0.001**	0.430	0.249	0.751	**0.012**	0.517	0.085
butanal	0.378	0.225	0.045	0.895	0.909	**0.001**	0.337	0.283
pentanal	0.840	**0.001**	0.957	**<0.0001**	0.892	**<0.0001**	0.861	**<0.0001**
hexanal	0.857	**0.002**	0.869	**0.020**	0.960	**<0.0001**	0.948	**0.001**
heptanal	0.804	**0.002**	0.510	0.197	0.239	0.454	0.548	**0.034**
2-heptenal	0.719	**0.044**	0.747	**0.033**	0.871	**0.019**	0.761	**0.029**
octanal	0.892	**<0.0001**	0.701	**0.050**	0.841	**0.001**	0.751	**0.006**
2-octenal	0.846	**0.001**	0.500	0.207	0.597	0.090	0.549	**0.042**
nonanal	0.704	**0.016**	0.148	0.609	0.285	0.425	0.271	0.395
2-nonenal	0.875	**0.001**	0.652	0.030	0.769	**0.006**	0.774	**0.010**
2,4-decadienal	0.298	0.474	0.598	0.117	0.531	0.092	0.316	0.374
ketones								
2-butanone	0.718	**0.019**	0.754	**0.005**	0.858	**0.0004**	0.763	**0.003**
2,3-butanedione	0.495	0.212	0.598	**0.040**	0.583	**0.047**	0.560	**0.049**
2-pentanone	0.848	**0.002**	0.918	**0.0002**	0.671	**0.017**	0.803	**0.001**
2-heptanone	0.205	0.522	0.190	0.553	0.285	0.369	0.254	0.433
2-octanone	0.255	0.448	0.539	0.169	0.032	0.929	0.189	0.557
2-nonanone	0.766	0.016	0.435	0.182	0.084	0.812	0.211	0.678
esters								
ethyl acetate	0.673	**0.047**	0.344	0.406	0.724	0.066	0.474	0.166
alcohols								
ethanol	0.679	**0.031**	0.843	**0.001**	0.796	**0.003**	0.822	0.002
1-propanol	0.423	0.297	0.164	0.729	0.777	**0.014**	0.299	0.435
acids								
acetic acid	0.872	**0.001**	0.934	**<0.0001**	0.786	**0.004**	0.843	**0.001**
hexanoic acid	0.797	**0.003**	0.805	**0.005**	0.752	**0.012**	0.780	**0.002**
sulfur compounds								
dimethyl disulfide	0.871	**0.001**	0.571	0.108	0.527	0.095	0.599	0.081
methanethiol	0.696	**0.050**	0.277	0.470	0.775	**0.041**	0.406	0.133

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only can fat act as a solvent of the volatile compounds, thus decreasing its concentration in the HS, but also it is one of the main precursors of volatile compounds during ripening.[30] Nevertheless, these results indicated that despite the different fat contents present in the sausages, both the GC-MS and SIFT-MS analyses were correlated and were able to detect the same changes with processing time for 13 volatile compounds.

However, several compounds did not show such correlations in any batch; one reason could be because the HS concentrations are too low for accurate SIFT-MS analysis. This was so for 2-heptanone and 2-octanone, the concentrations of which increased significantly during ripening when measured by SPME-GC-MS (Table 2), but their concentrations were close to the quantification limits for SIFT-MS.[24] Another reason could be the fact that SPME-GC-MS concentrates the volatile compounds from the HS, whereas SIFT-MS measures the concentration directly without preconcentration. Similar results were obtained by Pozo-Bayón et al.[32] when comparing GC-MS and PTR-MS. In any case, the positive and significant correlations obtained for many of the compounds analyzed indicate that SIFT-MS is a useful tool for monitoring changes in the concentrations of HS volatile compounds above fermented sausages during processing much more quickly than SPME-GC-MS.

SIFT-MS as a Tool for the Evaluation of Oxidative Status. Lipid oxidation is one of the major causes of deterioration in meat quality, but it is also essential for the development of the characteristic dry cured aroma.[33] In meat products, lipid oxidation is currently evaluated using the peroxide value and TBARS measurements and more recently by volatile compound quantification.[33] TBARS measures secondary lipid oxidation products and has been correlated with consumers' perception of lipid oxidation.[34] However, the TBARS method requires sample preparation and is not convenient for the real-time monitoring of the oxidative status of dry fermented sausages.

To determine if SIFT-MS and SPME-GC-MS can be used to evaluate the oxidative status of the sausages throughout the processing period, a Pearson correlation procedure between the measurements of aldehydes, produced during lipid oxidation reaction in the sausage, and the measurement of TBARS was performed. Figure 3a,c,e,g,i shows the correlation between TBARS and the HS concentration of the aldehydes C3−C7 obtained by SPME-GC-MS, whereas Figure 3b,d,f,h,j includes the correlation between TBARS and SIFT-MS measurement of aldehydes C3−C7. The selection of the aldehydes as markers of the lipid oxidation process in meat has been widely studied.[35] For this study, the linear aldehydes (C3−C7) were chosen as possible markers. The TBARS values increased during processing time (from 0.2 to 1.2 mg of MDA/kg (Figure 3)), in the three fat batches as has been reported in other dry fermented sausages.[30,31,36]

The TBARS data were well correlated with the HS concentration of the linear aldehydes (C3−C7) as detected by both SPME-GC-MS and SIFT-MS techniques (Figure 3). Using the SPME-GC-MS data, high positive correlation coefficients ($r > 0.9$, $p < 0.0001$) were obtained between the TBARS levels and the HS concentration for all of the aldehydes C3−C7 (Figures 3a,c,e,g,i). However, the results obtained by SIFT-MS resulted in good correlation for the aldehydes C3−C6 (Figure 3b,d,f,h) but a poorer correlation for heptanal (Figure 3j). The hexanal measured by SIFT-MS resulted in a higher correlation coefficient ($r = 0.936$) than obtained using SPME-GC-MS ($r = 0.927$). The best correlation coefficients obtained by SIFT-MS were for pentanal and hexanal. Nevertheless, the correlation coefficients for the other

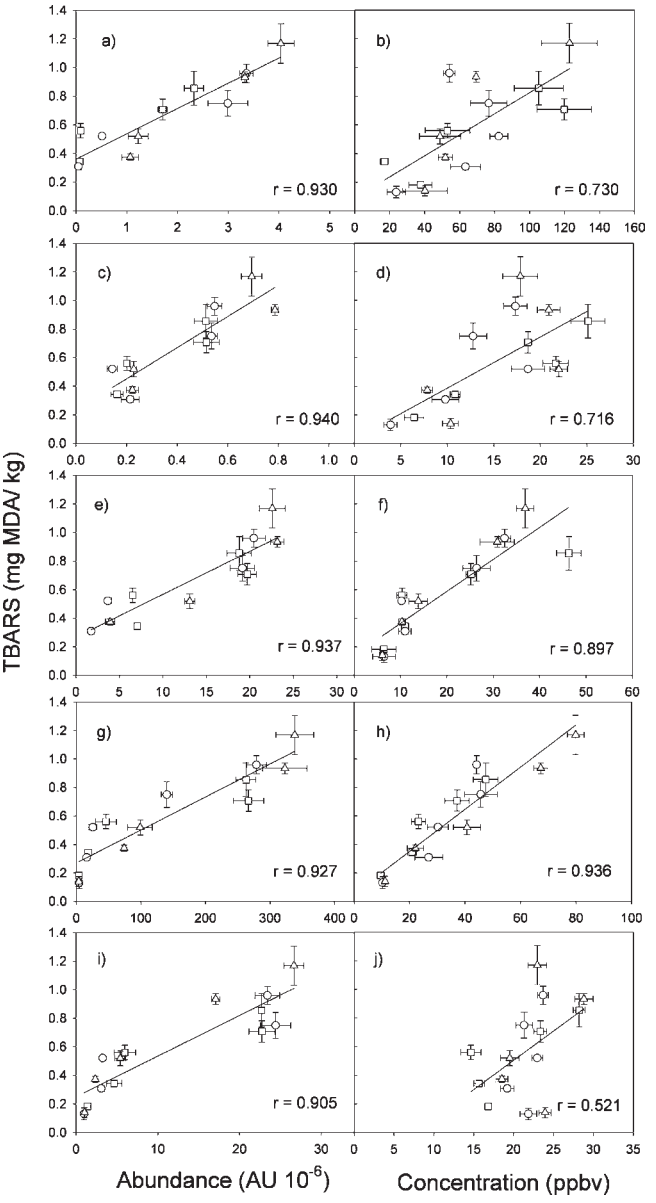

Figure 3. Correlation between lipid oxidation value (TBARS) and volatile compounds monitored by GC-MS and SIFT-MS during the manufacture of dry fermented sausages with different fat contents. Plots in the left column represent abundance obtained by GC-MS (abundance units: AU 10^{-6}) for (a) propanal, (c) butanal, (e) pentanal, (g) hexanal, and (i) heptanal. Plots b, d, f, h, and j in the right column represent concentration obtained by SIFT-MS (ppbv) for the same sequence of compounds. The symbols correspond to low-fat (LF, □), medium-fat (MF, ○), and high-fat (HF, △) sausages. The plotted values are the mean values for the three sausages and the standard errors.

aldehydes using SIFT-MS were lower than those obtained using GC-MS. These results indicate that both the SPME-GC-MS and SIFT-MS analytical techniques can be used to evaluate the oxidation process during dry fermented sausage ripening. Although it would be necessary to analyze a higher number of meat samples to confirm these results. SIFT-MS measurements are obtained more rapidly than both TBARS and SPME-GC-MS measurements. SIFT-MS provided similar information about the hexanal levels, as has been reported as the most abundant volatile compound derived from lipid oxidation in

meat and has often been chosen as an index of lipid oxidation level.[30]

In summary, significant and positive correlations are seen between SIFT-MS and SPME-GC-MS measurements for the volatile compounds pentanal, hexanal, 2-heptenal, octanal, 2-nonenal, 2-butanone, 2-pentanone, ethanol, acetic acid, and hexanoic acid generated during the processing of dry fermented sausages. This study demonstrates that SIFT-MS is a reliable technique for monitoring changes in the volatile compounds, providing information similar to the traditional SPME-GC-MS analyses, but more rapidly and, if desired, in real time. Finally, this study reveals that SIFT-MS can be used to evaluate the oxidative status of meat products by measuring the hexanal content of the sausages much more rapidly than conventional techniques.

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ABBREVIATIONS USED

BHT, butylated hydroxytoluene; CAR/PDMS SF, carboxen/polydimethylxylosane StableFlex; GC, gas chromatograph; HS, headspace; MS, mass spectrometry; MW, molecular weight; SIFT-MS, selected ion flow tube mass spectrometry; SPME, solid phase microextraction; TBARS, thiobarbituric acid reactive substances.

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