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Monitoring volatile compounds during dry-cured ham ripening by solid-phase microextraction coupled to a new direct-extraction device

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Abstract

Key flavour volatile compounds were monitored during ripening of dry-cured ham by solid-phase microextraction (SPME) coupled to a new direct-extraction device (DED). DED allows the insertion of the SPME fibre into the core of solid materials with no damage to the fibre. This enables extraction of volatiles from solid foodstuffs while avoiding sample handling. Major groups of volatile compounds extracted with SPME–DED agreed with available scientific literature about dry-cured ham volatiles. Moreover, volatile compounds previously highlighted as quality markers in dry-cured ham, such as 3-methylbutanal or hexanal, were satisfactorily extracted using SPME–DED. Changes in the profile of volatile compounds throughout the processing followed a typical pattern of volatile compounds formation. Therefore, SPME–DED appears as a new and promising method for monitoring ripening of dry-cured hams with no depreciation of the product, which might substitute traditional subjective methods currently used in the ham processing industry. However, the use of the internal standard method is not possible with this technique. Therefore, results using SPME–DED only point out a trend in the volatile profile. Further attempts relating data obtained using SPME–DED in dry-cured hams with sensory and chemical data from the same samples would be necessary for optimising this method as a quality control method in dry-cured ham industries. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Extraction methods; Solid-phase microextraction; Meat; Food analysis; Volatile organic compounds; Aroma compounds

1. Introduction

A considerable amount of research has been devoted to study volatile compounds from biological material, soil, chemicals, foods and other complex matrixes. Several methods have been developed for volatile compound extraction, such as purge and trap, supercritical fluid extraction, solid-phase extraction or simultaneously distillation/extraction with organic solvents. However, the use of these techniques has several drawbacks. One of the most important problems in volatile compounds analysis is the requirement of a certain amount of sample, which in some circumstances, such as quality control in the industry, is not possible because of depreciation of the product. Furthermore, sample preparation for classical extraction techniques might cause analytical and

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practical problems, such as loss of analytes or artifacts formation [1,2].

Solid-phase microextraction (SPME) is a single-step solvent-free extraction technique for volatile compounds extraction, with a high sensitivity and a reasonable cost [3,4]. To date, SPME device has been used for analysis of volatile compounds in different foodstuff, detection of chemicals in biological and inorganic materials, and so on [5]. Current research has been directed toward remote monitoring, particularly in industrial hygiene application and field environmental [6]. Samples in these cases are liquid or gas, in which SPME fibres can be directly introduced for volatiles extraction. This type of field analysis has many advantages compared to traditional sampling procedures, such as minimization of time between sample collection and analysis, prevention of contamination, losses and/or degradation of the sample during transportation and storage or production of immediate results [7]. However, direct extraction cannot be performed in solid samples, because the SPME fibre is too weak to be pushed into the solid material.

We have recently developed a device that protects the SPME fibre when it is pushed into the core of the solid material [8]. Thus, this direct-extraction device (DED) allows extraction of volatiles from solid samples without manipulation, as it has been previously verified in a couple of meat products [9].

The purpose of this study was to test the recently developed DED coupled to SPME for monitoring those volatile compounds involved in dry-cured Iberian ham aroma during the ripening process.

2. Materials and methods

2.1. Processing of dry-cured Iberian hams

Volatile compounds from five dry-cured Iberian hams were monitored by SPME coupled to DED. Hams were ripened in a controlled humidity and temperature chamber at the Department of Food Science (University of Extremadura) following usual temperature and relative humidity conditions for this product.

2.2. Sampling

Volatile compounds from five dry-cured Iberian hams were sampled in nine different moments of the ripening process (days 115, 149, 163, 177, 191, 219, 252, 308 and 338).

Dry-cured ham volatile compounds extraction was performed using the new DED coupled to a SPME (Supelco, Bellefonte, PA, USA) fibre (10 mm length) coated with carboxen-poly(dimethylsiloxane) (75 μm thickness). This fibre was chosen because of its high sensitivity and wide linear range of response [10]. Prior to analysis the SPME fibre was preconditioned at 280 °C for 45 min in the gas chromatography (GC) injection port.

For carrying out volatile extraction using the new DED, the needle of the SPME holder was placed into the DED and thereafter the DED was inserted into the core of ham by pressing. Once inside the solid material, the fibre was exposed to the small space inside the DED, which has small holes that allow volatiles from the sample to enter into the space in which the fibre is kept. Extraction was carried out for 45 min at the temperature of the chamber. This new technique is thoroughly explained elsewhere [9].

2.3. Gas chromatography-mass spectrometry

Analyses were performed using a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass-selective detector (HP-5971 A, Hewlett-Packard). Volatiles were separated using a 5% phenyl–methylsilicone (HP-5) bonded-phase fused-silica capillary column (50 m×0.32 mm I.D., film thickness 1.05 mm, Hewlett-Packard), operating at 41.3 kPa of column head pressure, resulting in a flow of 1.45 ml min^{-1} at 40 °C. The SPME fibre was desorbed and maintained in the injection port at 280 °C during the whole chromatographic run. The injection port was in splitless mode. The temperature program was isothermal for 10 min at 40 °C, raised to 200 °C at a rate of 5 °C min^{-1}, and held for 5 min.

The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained by electronic impact at 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of 1 scan s^{-1} over the m/z range of 30 to 500. Compounds were tentatively identified by comparing their

mass spectra with those contained in the US National Institute of Standards and Technology (NIST)/US Environmental Protection Agency (EPA)/US National Institutes of Health (NIH) and Wiley libraries and by comparison of Kováts indexes with those reported in the literature [11,12]. Results from volatiles analysis are provided in total area counts.

3. Results and discussion

A total of 107 volatile compounds were tentatively identified throughout the ripening of dry-cured Iberian ham using SPME–DED (Table 1). These compounds were aliphatic and aromatic hydrocarbons (24 compounds), aliphatic and aromatic aldehydes (22 compounds), aliphatic and aromatic alcohols (15 compounds), aliphatic ketones (13 compounds), organic acids (12 compounds), sulphur compounds (four compounds), nitrogen compounds (four compounds), terpenes (three compounds), lactones (four compounds), esters (two compounds), chloride compounds (two compounds), furans (one compound) and ethers (one compound). These chemical families agree with those previously described in dry-cured hams using different extraction methods [2,13]. Moreover, volatile compounds from the hams of the present study were also extracted in the final ripening stage using purge and trap and major volatile compounds were the same as using SPME–DED (data not shown)

Concerning the general pattern of volatiles extracted by SPME–DED, it was observed an increase in the number and heights of peaks throughout the processing, although some compounds diminished or even disappeared. This trend basically agrees with previous findings in Iberian ham [2] and other types of dry-cured hams [13,14]. Some of the highest peaks in the chromatogram were bleeding (siloxanes) from the stationary phase of the fibre, which has been previously pointed out as one of the main drawbacks of SPME [15,16]. Insufficient conditioning has been suggested as a possible reason explaining bleeding of SPME fibres [15]. However, in some of the samples of the present study conditioning of the fibre was carried out for a longer time than that recommended by the manufacturer and siloxane peaks were also present.

Table 2 shows chromatographic peak area of selected compounds during the processing of dry-cured Iberian hams. These compounds were chosen on the basis of previous results that highlighted either their impact on dry-cured Iberian ham flavour or their significance as ripening markers [2,17]. More concretely, aliphatic saturated aldehydes have been shown as good indicators of lipid oxidation in muscle foods [18], including dry-cured ham [19]. Variations in the amount of these aldehydes during ripening agrees with the general trend previously found in dry-cured Iberian ham [20]: an increase during the drying phase due to the high temperature of this stage, a subsequent decrease during the first part of the cellar phase, probably due to further chemical reactions of aldehydes with other components [21] and a second increase in the amount of these compounds during the cellar phase. This latter increase has been associated to a second rise in the temperature during the cellar phase in previous works [2,20]. However, in the present study there was not such a temperature increase during the cellar phase. The increase in compounds from lipid oxidation was confirmed by other oxidation indicators determined on the hams of the present study, such as thiobarbituric acid reactive substances (TBARs) or amount of hexanal using headspace SPME (data not shown). Therefore, this peak in the oxidation phenomena during the cellar phase should not be due only to the temperature, but to some other reasons such as a reduction in the activity of antioxidative systems or development of an intense lipolysis. Regardless the reasons explaining such enhance in the lipid oxidative phenomena, SPME–DED satisfactorily detected this trend, which highlights the possibilities of this technique for quality control in the dry-cured ham industry.

2- and 3-Methylbutanal have been associated with nutty, cheese and salty notes in Parma ham [14], and significantly contribute to the overall flavour of dry-cured Iberian ham [2,17] and other meat products [22]. They have been shown as major contributors to desirable flavour in Iberian ham [17], and therefore a method that successfully permits controlling their formation results very interesting for both industrial and research purposes. The trend found in the present study for these branched aldehydes confirms only in part previous findings in dry-cured Iberian

Table 1
Volatile compounds identified in dry-cured Iberian ham throughout the processing extracted using SPME–DED

Acids	Alcohols	Hydrocarbons
Acetic acid	Ethanol	Pentane
2-Methylpropanoic acid	Propanol	Methylcyclopentane
Butanoic acid	2-Methyl-1-propanol	Hexane
2-Methylbutanoic acid	1-Penten-3-ol	Heptane
3-Methylbutanoic acid	3-Methylbutanol	Toluene
2-Methylhexanoic acid	2-Methylbutanol	1-Octene
Hexanoic acid	1-Pentanol	Ethylbenzene
Heptanoic acid	1-Hexanol	1,4-Dimethylbenzene
Octanoic acid	Cyclohexanol	1,2-Dimethylbenzene
Nonanoic acid	1-Heptanol	Styrene
Decanoic acid	Diethylenglycol	Nonane
Dodecanoic acid	1-Octen-3-ol	Decane
Aldehydes	Benzenemethanol	Branched alkenes
2-Methylpropanal	1-Octanol	Undecane
Butanal	Benzeneethanol	Pentylbenzene
3-Methylbutanal	**Chloro compounds**	Dodecane
2-Methylbutanal	Dichloromethane	Tridecane
Pentanal	Trichloromethane	Tetradecane
Hexanal	**Ketones**	Pentadecane
Heptanal	2-Propanone	Butylated hydroxytoluene
2-Heptenal	2,3-Butanodione	Nonylbenzene
Benzaldehyde	2-Butanone	Hexadecane
Octanal	2-Pentanone	Heptadecane
Benzeneacetaldehyde	3-Hydroxy-2-butanone	
2-Octenal	3-Methyl-2-pentanone	**Sulphur compounds**
Nonanal	2-Hexanone	Dimethylsulphide
2-E-Nonenal	4-Heptanone	Dimethyldisulphide
Decanal	2-Heptanone	Benzothiazole
2,4-Nonadienal	Ciclohexanone	Diphenyl sulphide
2-Decenal	2-Octanone	**Ethers**
2,4-Decadienal (E,Z)-	2-Nonanone	Diethyl ether
2,4-Decadienal(E,E)-	2-Decanone	**Esters**
2-Undecenal	**Lactones**	Acetic acid ethyl ester
Tetradecanal	γ-Butyrolactone	3-Methylbutanoic acid ethyl ester
Octadecenal	δ-Hexalactone	**Nitrogen compounds**
Terpenes	γ-Octalactone	Pyridine
α-Pinene	γ-Nonalactone	2,6-Dimethylpyrazine
3-Carene	**Furans**	Dibutylamine
1-Limonene	2-pentylfuran	Ethylbenzenamine

ham, in which these compounds showed slightly higher amounts than hexanal and nonanal [1], and increased during the cellar phase [2]. However, the cited studies were carried out on long processing hams (more than 450 days processing), whereas in this study a shortened processing was followed. The increase in 2- and 3-methylbutanal occurs subsequent to a rise in the amount of free amino acids due to proteolysis, and to an increase in reactive compounds from lipid oxidation [17]. Generation of Strecker aldehydes at the temperature followed during dry-cured ham ripening takes a considerably long time,

Table 2
Chromatographic peak area (total area counts $\times 10^6$ of five hams) of selected compounds from dry-cured Iberian ham throughout the processing obtained using SPME–DED

Compound	I[a]	Processing day									SEM[b]
		115	149	163	177	191	219	252	308	338	
Hexanal	792	15.6	39.1	21.3	39.6	59.0	52.0	48.5	152.4	36.3	7.9
Heptanal	900	nd	11.6	11.3	20.6	20.5	22.9	24.5	85.1	9.8	5.0
Octanal	1008	5.7	13.4	14.3	27.4	22.9	25.4	42.3	92.6	14.0	5.2
Nonanal	1109	12.5	63.2	24.0	56.2	51.7	28.5	57.6	168.0	22.1	9.0
3-Methylbutanal	649	12.4	7.1	9.5	14.7	4.6	5.2	5.7	4.5	8.4	1.0
2-Methylbutanal	661	17.4	1.4	4.7	14.8	1.9	2.4	3.6	4.6	4.1	1.0
2-Heptanone	888	9.7	2.1	4.8	4.8	4.2	2.9	11.3	8.4	4.5	0.8
2-Octanone	994	nd	1.2	2.3	2.1	1.6	1.8	3.3	4.1	1.3	0.3
2-Pentylfurane	997	nd	3.0	2.0	2.6	5.0	11.3	6.5	14.2	5.9	1.1
γ-Octalactone	1287	2.1	2.2	2.2	7.3	1.8	4.1	6.2	7.3	3.9	0.5

nd, not detected.
Mass spectrum was tentatively identified using NIST and Wiley libraries, the Kováts retention index being in agreement with literature.
[a] I, Kováts retention indices are given for a nonpolar DB-5 capillary column.
[b] SEM, standard error of the mean.

and therefore, it might not have taken place yet due to the short processing followed in the present study. In fact, last sampled pointed out a slight increase of 3-methylbutanal, which could be the beginning of the increase found during the cellar phase in previous studies.

2-Pentylfurane is a non-carbonyl oxidation product from linoleic and other n-6 fatty acids [23], which is frequently found in dry-cured ham [2,24] and other meat products [25]. Furans usually show relatively high odour thresholds and are therefore considered as not very important contributors to the flavour of muscle foods [26]. However, 2-pentylfuran shows a relatively low threshold (about 4 ppb) and a vegetable aromatic note, and therefore, its presence could play an important role in the overall flavour of dry-cured Iberian ham, along with being an indicator of lipid oxidation.

γ-Octalactone shows coconut and creamy aromatic notes [27] and a low odour threshold [28]. It has been shown as a key flavour compound in different foodstuff, specially fruits and wine. It may have different origins, but in the case of dry-cured ham it is most likely originated from lipid oxidation. The presence of γ-lactones in dry-cured hams have been previously described [24], and although its contribution to the flavour of this product has not been established yet, its low threshold and pleasant flavour points out an important significance, as has already been established in other meat products [27]. These two compounds (2-pentylfurane and γ-octalactone) followed a similar trend to that of aliphatic aldehydes. Their successful extraction using SPME–DED could be interesting in the study of dry-cured ham flavour due to the positive aromatic notes they exhibit, and points to the possible use of this technique in other solid material in which lactones are important, such as fruits or the wood of wine barrels.

2-Heptanone and 2-octanone are carbonyl compounds that arise from oxidation or descarboxilation of lipids [29] and both of them have been frequently found in dry-cured ham [2,29]. The amount of these ketones found throughout the ripening of the hams followed a similar trend to that of aldehydes: an increase during the first part of the cellar phase and a decrease thereafter. This confirms their lipid oxidative origin in dry-cured Iberian ham, although in some other products, such as mould-ripened cheeses, a microbial origin has also been suggested [30]. Moreover, the increasing amount of 2-heptanone during the first part of the cellar phase and subsequent decrease thereafter agrees with the trend previously found in other studies on other types of dry-cured hams [13,14].

Therefore, data from the present study confirm the potential use of SPME–DED for extracting volatile compounds in dry-cured Iberian ham without phys-

ically sampling the ham. This feature is very interesting for both research and industrial purposes: in previous studies about dry-cured ham a high number of hams were necessary to study the evolution of volatile compounds, whereas using SPME–DED the number of hams needed is much fewer, and therefore the cost of the study is significantly lower. With regards to industrial purposes, SPME–DED allows the detection of major volatiles formed during ham processing without damaging the aspect of the ham, and therefore, avoiding depreciation of the product and its economical consequences.

Nevertheless, this technique shows a number of possible drawbacks. A major factor influencing volatile compounds extraction in SPME is the temperature, as have been previously stated sampling dry-cured Iberian ham [1]. In the present study the extraction was performed at the temperature in which the hams were kept in each processing stage. In fact, raising the temperature of the hams during the first phases to enhance extraction could be a problem, since microbial spoilage might occur. The same problem could happen in many other foodstuffs. Another possible drawback is the influence of the matrix. Ham is a heterogeneous matrix, showing areas of fat and lean unevenly distributed. Extraction of compounds could be greatly influenced by the proportion of lean and fat in the area where the DED is inserted, since polarities of these components are quite different. Finally, using SPME–DED quantitative results cannot be obtained, since the addition of an internal standard is not possible.

4. Conclusions

SPME–DED appears as a new technique for controlling volatile compound formation during the ripening of dry-cured ham, since the trend followed by major and most significant compounds of the volatile profile of Iberian ham was satisfactorily shown. Nevertheless, evaluation of different SPME fibres and correlation with sensory and chemical parameters should be performed in order to validate this technique for quality control purposes. Its application to other solid materials for aromatic compounds or contaminants extraction is also promising and will be evaluated in next studies.

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