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# Effect of the pig rearing system on the final volatile profile of Iberian dry-cured ham as detected by PTR-ToF-MS

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ABSTRACT

The volatile compound profile of dry-cured Iberian ham lean and subcutaneous fat from pigs fattened outdoors on acorn and pasture (*Montanera*) or on high-oleic concentrated feed (*Campo*) was investigated by proton transfer reaction time-of-flight mass spectrometry. In addition to the usual proton transfer ionization the novel switchable reagent ions system was implemented which allows the use of different precursor ions ($H_3O^+$, $NO^+$ and $O_2^+$). The analysis of the lean and subcutaneous fat volatile compounds allowed a good sample discrimination according to the diet. Differences were evident for several classes of compounds: in particular, *Montanera* hams showed higher concentrations of aldehydes and ketones and lower concentrations of sulfur-containing compounds compared to *Campo* hams. The use of $NO^+$ as precursor ion confirmed the results obtained with $H_3O^+$ in terms of classification capability and provides additional analytical insights.


## 1. Introduction

Iberian dry-cured ham is a Spanish high-valued product, highly appreciated by consumers due to its unique sensory characteristics that depend on ripening conditions (Ruiz, Ventanas, Cava, Andrés, & García, 1999) and raw meat characteristics — i.e. animals' age, pig genotype and type of feed during the fattening period (Carrapiso, Bonilla, & García, 2003; Pérez-Palacios et al., 2010). Currently different commercial types of Iberian dry-cured hams are available according to the fattening of the pigs (R.D., 1469/2007). The most valuable dry-cured hams are produced from pigs fattened outdoors, with feeding based on acorn and pasture (*Montanera*), whereas the less expensive come from pigs fattened indoors on a concentrated feed. A new Iberian dry-cured ham type (*Campo*) produced from free range fattened Iberian pigs reared on a concentrated feed has been recently added to the Iberian ham commercial grading (R.D., 1469/2007), and several studies about these hams have been performed (Daza, Rey, Ruiz, & López-Bote, 2005; Ventanas, Ventanas, Tovar, García, & y Estévez, 2007).

Different methods have been proposed for the commercial classification of Iberian dry-cured ham, which analyse the subcutaneous fat (SCF) fatty acid profile (Ruiz et al., 1998) to the n-alkanes (Tejeda, Antequera, Martín, Ventanas, & Garcia, 2001), n-alkenes and branched hydrocarbons of the intramuscular fat (IMF) (Petrón, Tejeda, Muriel, Ventanas, & Antequera, 2005, 2006), the volatile hydrocarbon profile (Narváez-Rivas, Vicario, Alcalde, & León-Camacho, 2010) and the fatty acid profile of the IMF (Pérez-Palacios, Ruiz, Tejeda, & Antequera, 2009). Even if some of these methods, especially the IMF fatty acid profile analysis, allow good discrimination according to the fattening diet, all of them are complex and time-consuming techniques.

Proton transfer reaction mass spectrometry (PTR-MS) is an established method for the rapid, non-destructive detection of volatile organic compounds (VOCs) released from several food matrices or emitted during dynamic processes such as food processing or consumption (Aprea, Biasioli, Carlin, et al., 2007; Aprea, Biasioli, Gasperi, Märk, & van Ruth, 2006; Aprea, Biasioli, Gasperi, et al., 2007; Araghipour et al., 2008; Biasioli et al., 2003, 2006; Gasperi et al., 2009). The technique's fundamentals and its applications to food, environmental or medical purposes have been extensively reviewed (Biasioli, Gasperi, Yeretzian, & Märk, 2011; Biasioli, Yeretzian, Märk, Dewulf, & Van Langenhove, 2011; Blake, Monks, & Ellis, 2009). Very briefly, PTR-MS is based on the reaction of protonated water with VOCs molecules with a proton affinity higher than water's and the successive detection by a quadrupole mass spectrometer. Recently a PTR-MS coupled to a high resolution time-of-flight (ToF) mass analyser was commercialised (Jordan et al., 2009), which partially overcomes the limitations of the slower and low resolution quadrupole version, offering higher mass resolution ($m/\Delta m$ up to 5000), high sensitivity (pptv) and a higher time resolution (0.1 s) (Jordan

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et al., 2009). PTR-ToF-MS has been successfully applied to the study of the volatile compounds of milk during fermentation (Soukoulis et al., 2010) and cheese (Fabris et al., 2010), and its capacity to discriminate dry-cured hams according to geographical origin and ripening processing has been also demonstrated (Sánchez del Pulgar et al., 2011).

Jordan et al. (2009) proposed a Switchable Reagent Ions (SRI) system which allows the use of different reagent ions in PTR-MS. This system introduces in PTR-MS apparatuses, without loss of sensitivity, a possibility that characterises competing techniques for VOCs detection such as the selected ion flow technique (SIFT-MS) (Spanel & Smith, 1999). Based on SRI, Jordan et al. (2009) demonstrated that the use of $NO^+$ and $O_2^+$ precursor ions in the PTR-MS apparatuses improves the analytical performance, particularly for the separation of isobaric compounds (i.e. aldehydes, 2-ketones and alpha-diketones) and for the detection of compounds with proton affinities lower than that of water (e.g. alkanes, alkenes). In general, the usefulness of $NO^+$ and $O_2^+$ as precursor ions for the analysis of volatile compounds in meat products has been demonstrated by SIFT-MS (Olivares et al., 2010, 2011).

For these reasons, it is proposed to use $NO^+$ and $O_2^+$ as reagent ions for the analysis of the volatile compound profile of dry-cured Iberian ham, to detect separately aldehydes and ketones ($NO^+$) and linear and branched alkanes ($O_2^+$), which could be useful to investigate the effect of rearing system and diet (Narváez-Rivas et al., 2010).

Therefore, the aims of this study were:

i) to identify the possible effect of different rearing systems on the final volatile compounds of dry-cured Iberian hams by a rapid direct injection mass spectrometric technique.
ii) to set up discrimination methods for the classification of the different products and to obtain qualitative and quantitative analytical information on the volatile compounds useful for that discrimination.
iii) to evaluate, for the first time in food science, whether the use of different precursor ions ($H_3O^+$, $NO^+$ and $O_2^+$) in PTR-MS apparatuses allows a better separation and/or provides better analytical information.

## 2. Material and methods

### 2.1. Ham samples

Twenty five Iberian dry-cured hams were obtained from twenty Iberian x Duroc 75% pigs (pure Iberian female × Iberian × Duroc male). Ten of these pigs were fattened outdoors on acorn and grass (*Montanera*), while the other fifteen pigs were fattened outdoors on a high-oleic concentrated feed (*Campo*), according to the regulations for Iberian pork products (R.D., 1469/2007). All the hams were simultaneously processed in the same way and ripened for 720 days according to García et al. (1996) and the regulations for Iberian pork products (R.D., 1469/2007) and Dehesa de Extremadura hams (Orden de 2 de Julio de, 1990).

### 2.2. Sample preparation

The analysis was carried out following the procedure described in Sánchez del Pulgar et al. (2011). From each ham a piece of the *Biceps femoris* muscle containing its corresponding subcutaneous fat (SCF) was taken, vacuum packaged and kept at 2 °C. Just before the analysis of the lean, the superficial layer and the visible fat of each piece of muscle was removed, and 3 cubes of 1 $cm^3$, approximately 1.2 g lean (3 replicates) were prepared. The cubes were introduced into 40 ml Pyrex glass vials for analytical/chromatography use (Supelco, Bellefonte, USA), capped by PTFE/Silicone septa (Supelco, Bellefonte, USA). In the case of the subcutaneous fat, the surface of each piece was also removed and 3 fat cubes of 1 $cm^3$ (approximately 0.9 g) were prepared from each sample.

The same procedure was followed for the analysis with $H_3O^+$, $NO^+$ and $O_2^+$ as reagent ions preparing three different sample series of lean.

### 2.3. Proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS)

In order to standardise the measurement, the prepared vials were equilibrated at 37 °C for 30 min in a water bath prior to analysis. Next all the samples were analysed by direct injection of the head space mixture into the PTR-ToF-MS drift tube via a heated (110 °C) peek inlet for 30 s, taking 30 average spectra (Fabris et al., 2010). The PTR-ToF-MS analysis, using $H_3O^+$ as reagent ion was performed on the ten dry-cured *Montanera* hams and ten *Campo* hams. Due to limitation in sample availability, when using $NO^+$ and $O_2^+$ as reagent ion, the same hams measured with $H_3O^+$ were analysed except for five *Campo* hams, which were replaced with a different five *Campo* hams.

Measurements were carried out using a commercial PTR-ToF-MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria), in its standard configuration (V mode). The sampling time per channel of ToF acquisition was 0.1 ns, amounting to 350,000 channels for a mass spectrum ranging up to $m/z = 400$. The selected conditions in the drift tube depended on the primary ion and are reported in Table 1.

### 2.4. Spectra analysis

The external calibration automatically done by the acquisition program provided a poor mass accuracy, thus internal calibration of ToF spectra was performed off-line (Cappellin et al., 2010). The procedure allowed achievement of a mass accuracy generally better than 0.001Th for the considered mass range, which was in most cases sufficient for sum formula identification. Data pre-processing on ToF spectra was carried out to remove the baseline and noise reduction was achieved by averaging over the 30 consequent ToF spectra corresponding to the same sample, thereby improving the signal-to-noise ratio by about five times (Cappellin et al., 2011). Peak detection and area extraction were performed as described in Cappellin et al. (2011). Throughout this paper VOC concentrations are expressed in ppbv (parts per billion by volume) and were calculated from peak areas according to the formula described by Lindinger, Hansel, and Jordan (1998). A constant reaction rate coefficient of $2 \cdot 10^{-9}$ $cm^3/s$ was employed in the calculations. In the case of $H_3O^+$ as primary ion, this introduces a systematic error of up to 30% that can be accounted for if the actual rate coefficient is known (Cappellin et al., 2012). For $NO^+$ and $O_2^+$ the same formula was used but the employed constant rate coefficient cannot be safely assumed to be close to the one of the actual reaction and therefore results must be considered as being expressed in normalized counts.

**Table 1**
Drift tube conditions and counts per second (cps) of the primary ion isotopes during the analysis with each primary ion.

| Primary Ion | Drift voltage (V) | Pressure (mB) | Temperature (°C) | E/N (Td) | Mass 21[a] (cps) | Mass 31[b] (cps) | Mass 34[c] (cps) |
|---|---|---|---|---|---|---|---|
| $H_3O^+$ | 600 | 2.25 | 110 | 154 | 1744 | 5 | 86 |
| $NO^+$ | 508 | 2.38 | 110 | 100 | 662 | 3116 | 218 |
| $O_2^+$ | 608 | 2.05 | 110 | 137 | 380 | 247 | 4048 |

[a] Mass corresponding to the M + 2 isotopologue of $H_3O^+$.
[b] Mass corresponding to the M + 1 isotopologue of $NO^+$.
[c] Mass corresponding to the M + 2 isotopologue of $O_2^+$.

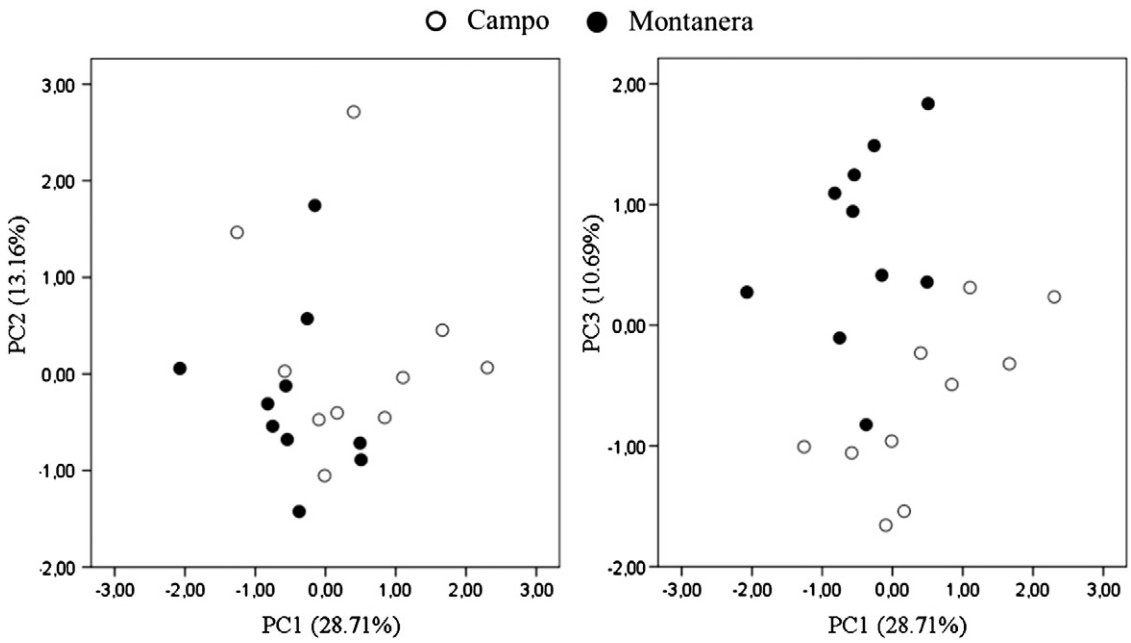


**Fig. 1.** Score plots obtained by the PCA analysis (PC1 vs PC2 and PC1 vs PC3 respectively) of the PTR-ToF-MS $H_3O^+$ fingerprint of the headspace of Iberian dry-cured *Montanera* and *Campo* hams lean.

### 2.5. Statistical analysis

A one-way ANOVA was carried out in order to find the masses significantly different between dry-cured *Campo* and *Montanera* hams ($p<0.05$). Principal component (PCA) and discriminant analysis were also performed. A simplified version of the methods used in Fabris et al. (2010) was used for discriminant analysis on PTR-ToF-MS data, using Penalized Discriminant Analysis (PDA) (Granitto et al., 2007) as classifier. To evaluate the results of the classification method a leave-group-out (LGO) method was used: the process of leaving a group out as test set and using the rest of the data set to fit the models was iterated. The regularization constant of PDA was selected each time at this step by internal cross validation using only the training data sets. The PDA model was then used to individually classify the samples of the independent test batch. Each individual group in this LGO procedure consisted of the 3 replicates of the same ham. The discrimination results were analysed with confusion matrices, in which rows correspond to the true classes and columns to the predicted ones (Witten & Frank, 2005).

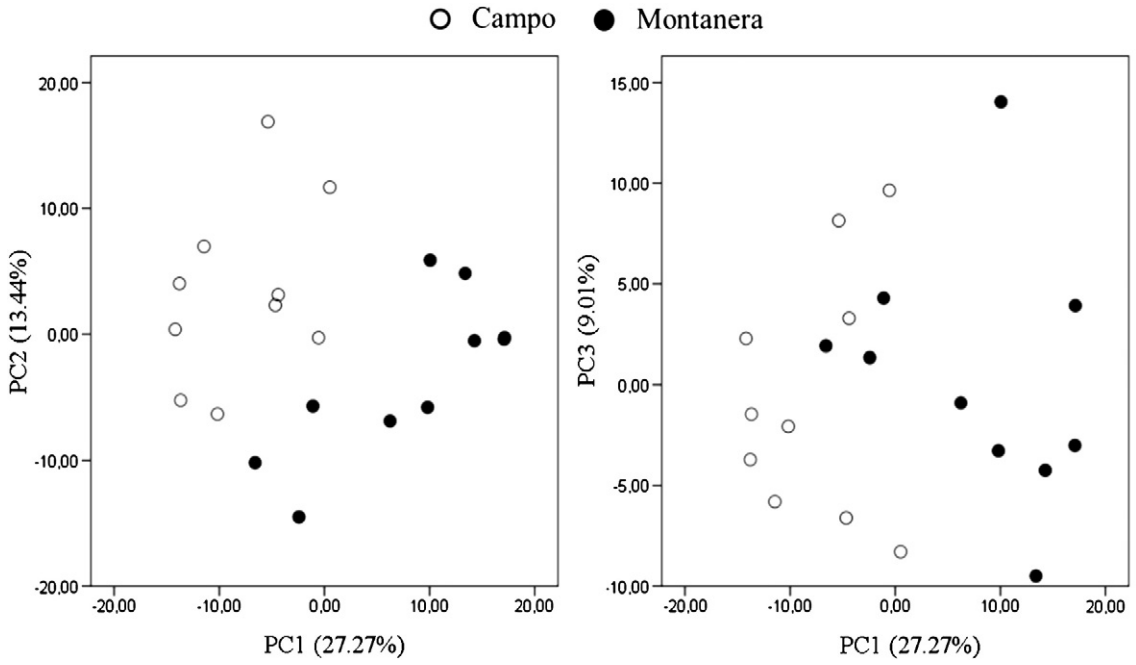


**Fig. 2.** Score plots obtained by the PCA analysis (PC1 vs PC2 and PC1 vs PC3 respectively) of the PTR-ToF-MS $H_3O^+$ fingerprint of the headspace of Iberian dry-cured *Montanera* and *Campo* hams subcutaneous fat (SCF).

**Table 2**
Confusion matrices for the penalized discriminant analysis with the lean and subcutaneous fat analysis data obtained by using $H_3O^+$ as reagent ion. Numbers in bold indicate correct classified replicates.

| Lean | | | | Subcutaneous fat | | | |
|---|---|---|---|---|---|---|---|
| | Campo | Montanera | % | | Campo | Montanera | % |
| Campo | **27** | 3 | 95 | Campo | **28** | 2 | 95 |
| Montanera | 0 | **30** | | Montanera | 1 | **29** | |

%: Percentage of correct classified replicates in both dataset.

## 3. Results and discussion

### 3.1. PTR-ToF-MS $H_3O^+$ headspace analysis of lean and SCF samples

The rapid analysis (30 s) of the headspace of the *Campo* and *Montanera* dry cured hams by PTR-ToF-MS using $H_3O^+$ as reagent ion resulted in more than 600 mass peaks. Multivariate analysis of PTR-ToF-MS spectra is a useful tool to exploit a rapid VOC fingerprint to classify dry-cured hams according to different criteria. Principal components analysis (PCA) was performed as an exploratory non-supervised data analysis, and the results are displayed in Figs. 1 and 2. In this analysis the average spectra of the three replicates of each sample were used. In Fig. 1, which corresponds to the analysis of the lean data, the three first principal components (PC) explain 53% of the total variance. Although the PC1–PC2 plot does not show a good discrimination of the samples, the PC1–PC3 plot indicates the possibility of a very good discrimination according to the rearing system of the pigs. In Fig. 2, which corresponds to the analysis of the SCF data, the two first PC explain 41% of the total variance and allow a better discrimination of the ham samples according to the fattening diet of the pigs.

A PDA (Penalized Discriminant Analysis) classifier was used to better assess the possibility of distinguishing ham samples according to the rearing system. Table 2 displays the confusion matrices of the PDA for the $H_3O^+$ lean and SFC analysis: a very good classification was achieved on both datasets, with only 3 replicates being misclassified in each case. In both cases two of the three errors correspond to the same sample. These results confirm the good separability suggested by the PCA analysis.

Tables 3 and 4 show the concentrations of different tentatively identified mass peaks from the spectral data acquired using $H_3O^+$ as primary ion from the analysis of the lean and the subcutaneous fat (SCF) samples respectively. Among the more than 600 peaks identified, only those corresponding to concentrations higher than 1ppbv and significantly different ($p<0.05$) between ham types are listed. For lean (Table 3) and fat headspace (Table 4), aldehydes and ketones with less than 9 carbon atoms were more concentrated in *Montanera* dry-cured Iberian hams than in *Campo* ones. The opposite holds for aldehydes and ketones with 9 and 10 carbon atoms.

The peak observed at m/z = 87.081, corresponding to protonated linear and branched aldehydes and ketones with 5 carbon atoms ($C_5H_{11}O^+$), showed a higher concentration than the other peaks corresponding to aldehydes and ketones, both in lean and SCF, probably due to the presence of 3-methylbutanal, and in less proportion to 2-methylbutanal, pentanal an 2-pentanone. In fact, 3-methylbutanal is the most abundant branched aldehyde found in dry-cured ham, followed by 2-methylbutanal (Andrade, Córdoba, Sánchez, Casado, & Rodríguez, 2009; Andrés, Cava, & Ruiz, 2002; Carrapiso, Jurado, & García, 2003; Dirinck, Van Opstaele, & Vandendriessche, 1992; García et al., 1991; Jurado, Garcia, Timón, & Carrapiso, 2007; Martín, Córdoba, Aranda, Córdoba, & Asensio, 2006; Ruiz, García, Muriel, Andrés, & Ventanas, 2002; Sabio, Vidal-Aragón, Bernalte, & Gata, 1998). 2 and 3-methylbutanal are originates mainly from Strecker reactions of the aminoacids and Maillard reactions (Berdagué, Denoyer, Le Quere, & Semon, 1991; Flores, Grimm, Toldrá, & Spanier, 1997; García et al., 1991; Ruiz et al., 2002; Toldrá, Aristoy, & Flores, 2000; Ventanas et al., 1992). Nevertheless, in the SCF the major contributor to this peak is probably pentanal, followed by 3 and 2-methylbutanal (Timón, Ventanas, Carrapiso, Jurado, & García, 2001). For these isobaric compounds the concentration in dry-cured *Montanera* hams lean was double than in *Campo* hams, while in the SCF the concentration was six times higher in *Montanera* hams. Saturated aldehydes and ketones with 6 carbon atoms (measured at m/z = 101.098) were also two-fold higher in *Montanera* than in *Campo* ham lean, and four times in the SCF of *Montanera* than in the SCF of *Campo* hams. In this case the major contributor to the abundance is probably hexanal, the most concentrated aldehyde in dry-cured ham lean (Andrade et al., 2009; Andrés et al., 2002; Carrapiso, Jurado, et al., 2003; Dirinck et al., 1992; García et al.,

**Table 3**
Peaks from the PTR-ToF-MS $H_3O^+$ lean analysis significantly different between both ham batches ($p<0.05$) and with intensity higher than 1ppbv (mean ± standard error).

| Measured mass (m/z) | Sum Formula | Theoretical mass (m/z) | Tentative identification | Campo (ppbv) | Montanera (ppbv) |
|---|---|---|---|---|---|
| 33.033 | $CH_5O^+$ | 33.0335 | Methanol | 765 ± 25 | 692 ± 17 |
| 55.055 | $C_4H_7^+$ | 55.0542 | C4 aldehydes fragment | 221 ± 19 | 361 ± 43 |
| 83.087 | $C_6H_{11}^+$ | 83.0855 | C6 aldehydes fragment | 105 ± 13 | 211 ± 34 |
| 87.081 | $C_5H_{11}O^+$ | 87.0804 | C5 aldehydes and ketones | 499 ± 62 | 1057 ± 108 |
| 91.058 | $C_4H_{11}S^+$ | 91.0576 | Butanethiol/1-(methylthio)-propane | 5.3 ± 0.5 | 3.9 ± 0.3 |
| 95.059 | $C_5H_7N_2^+$ | 95.0604 | Methylpyrazine | 5.3 ± 0.3 | 4.3 ± 0.2 |
| 97.067 | $C_6H_9O^+$ | 97.0648 | Ethylfurane | 4.0 ± 0.4 | 5.2 ± 0.3 |
| 98.101 | $C_6H_{12}N^+$ | 98.0964 | Hexanenitrile | 4.4 ± 0.3 | 5.4 ± 0.3 |
| 99.082 | $C_6H_{11}O^+$ | 99.0804 | C6 unsaturated aldehydes and ketones | 3.1 ± 0.1 | 3.9 ± 0.3 |
| 99.118 | $C_7H_{15}^+$ | 99.1168 | 1-Heptene/Methyl-cyclohexane | 2.7 ± 0.2 | 2.1 ± 0.1 |
| 101.098 | $C_6H_{13}O^+$ | 101.0961 | C6 aldehydes and ketones | 44 ± 5 | 92 ± 10 |
| 105.072 | $C_5H_{13}S^+$ | 105.0732 | Pentanethiol | 5.7 ± 0.6 | 3.3 ± 0.3 |
| 111.083 | $C_7H_{11}O^+$ | 111.0804 | 2,4-Heptadienal | 2.4 ± 0.1 | 2.8 ± 0.1 |
| 119.089 | $C_6H_{15}S^+$ | 119.0889 | 1-(Methylthio)-pentane/Hexanthiol | 1.1 ± 0.1 | 0.8 ± 0.1 |
| 125.099 | $C_8H_{13}O^+$ | 125.0961 | Octadienal/octadienone | 2.2 ± 0.1 | 2.6 ± 0.2 |
| 133.106 | $C_7H_{17}S^+$ | 133.1045 | Heptanethiol | 2.3 ± 0.3 | 1.4 ± 0.2 |
| 139.115 | $C_9H_{15}O^+$ | 139.1117 | Nonadienal/pentyl-furane | 3.2 ± 0.5 | 6.3 ± 0.8 |
| 141.130 | $C_9H_{17}O^+$ | 141.1274 | C9 unsaturated aldehydes and ketones | 2.1 ± 0.2 | 1.3 ± 0.1 |
| 143.115 | $C_8H_{15}O^+$ | 107.1066 | 2,3-octanedione/2,4-octadienal | 1.9 ± 0.1 | 1.4 ± 0.1 |
| 143.146 | $C_9H_{19}O^+$ | 143.143 | C9 aldehydes and ketones | 21 ± 1 | 11.7 ± 0.9 |
| 149.138 | $C_{11}H_{17}^+$ | 149.1325 | Pentylbenzene | 1.0 ± 0.1 | 0.66 ± 0.05 |
| 157.163 | $C_{10}H_{21}O^+$ | 157.1587 | C10 aldehydes and ketones | 1.7 ± 0.1 | 1.3 ± 0.1 |
| 177.167 | $C_{13}H_{21}^+$ | 177.1678 | Heptylbenzene | 1.2 ± 0.1 | 0.8 ± 0.1 |
| 201.188 | $C_{12}H_{15}O_2^+$ | 201.184 | Decanoic acid | 1.2 ± 0.1 | 0.60 ± 0.05 |

**Table 4**
Peaks from the PTR-ToF-MS $H_3O^+$ subcutaneous fat (SCF) analysis significantly different between both ham batches ($p<0.05$) and with intensity higher than 1ppbv (mean ± standard).

| Measured mass (m/z) | Sum Formula | Theoretical mass (m/z) | Tentative identification | Campo (ppbv) | Montanera (ppbv) |
|---|---|---|---|---|---|
| 33.034 | $CH_5O^+$ | 33.0335 | Methanol | 310 ± 18 | 218 ± 14 |
| 41.039 | $C_3H_5^+$ | 41.0386 | Alkyl fragment | 422 ± 27 | 701 ± 38 |
| 43.018 | $C_2H_3O^+$ | 43.0178 | Alkyl fragment | 130 ± 12 | 169 ± 8 |
| 43.054 | $C_3H_7^+$ | 43.0542 | Alkyl fragment | 383 ± 25 | 590 ± 43 |
| 55.055 | $C_4H_7^+$ | 55.0542 | C4 aldehydes fragment | 143 ± 20 | 373 ± 41 |
| 57.034 | $C_3H_5O^+$ | 57.0335 | C3 aldehydes and ketones | 66 ± 5 | 99 ± 4 |
| 69.036 | $C_4H_5O^+$ | 69.0335 | Furan | 7.8 ± 0.6 | 10.7 ± 0.7 |
| 69.070 | $C_5H_9^+$ | 69.0698 | C5 aldehydes fragment | 113 ± 9 | 260 ± 23 |
| 71.086 | $C_5H_{11}^+$ | 71.0855 | Alkyl fragment | 36 ± 4 | 62 ± 7 |
| 73.065 | $C_4H_9O^+$ | 73.0648 | C4 aldehydes and ketones | 180 ± 13 | 399 ± 36 |
| 79.055 | $C_6H_7^+$ | 79.0542 | Benzene | 5.1 ± 0.4 | 6.5 ± 0.4 |
| 80.053 | $C_5H_6N^+$ | 80.0495 | Pyridine | 1.00 ± 0.05 | 1.23 ± 0.04 |
| 81.071 | $C_6H_9^+$ | 81.0699 | Alkyl fragment | 5.8 ± 0.3 | 7.5 ± 0.4 |
| 83.051 | $C_5H_7O^+$ | 83.0491 | Methyl-furan | 9.4 ± 0.5 | 12.7 ± 0.6 |
| 83.086 | $C_6H_{11}^+$ | 83.0855 | C6 aldehydes fragment | 72 ± 12 | 186 ± 23 |
| 85.066 | $C_5H_9O^+$ | 85.0648 | C5 unsaturated aldehydes and ketones | 5.6 ± 0.7 | 8.9 ± 0.7 |
| 87.080 | $C_5H_{11}O^+$ | 87.0804 | C5 aldehydes and ketones | 225 ± 32 | 1540 ± 285 |
| 97.065 | $C_6H_9O^+$ | 96.0648 | Ethylfurane | 22 ± 2 | 40 ± 3 |
| 98.100 | $C_6H_{12}N^+$ | 98.0964 | Hexanenitrile | 4.0 ± 0.3 | 7.1 ± 0.7 |
| 99.081 | $C_6H_{11}O^+$ | 99.0804 | C6 unsaturated aldehydes and ketones | 2.0 ± 0.1 | 3.7 ± 0.3 |
| 101.060 | $C_5H_9O_2^+$ | 101.0597 | 2.3-pentanedione | 9 ± 1 | 15 ± 2 |
| 101.096 | $C_6H_{13}O^+$ | 101.0961 | C6 aldehydes and ketones | 30 ± 4 | 130 ± 19 |
| 107.050 | $C_7H_7O^+$ | 107.0499 | Benzaldehyde | 1.5 ± 0.2 | 2.2 ± 0.1 |
| 111.080 | $C_7H_{11}O^+$ | 111.0804 | 2.4-Heptadienal | 3.0 ± 0.3 | 4.7 ± 0.2 |
| 111.117 | $C_8H_{15}^+$ | 111.1168 | C8 aldehydes fragment | 4.1 ± 0.6 | 6.5 ± 0.9 |
| 113.060 | $C_6H_9O_2^+$ | 113.0597 | 2.5-Dimethyl-3(2 H)-Furanone | 0.89 ± 0.03 | 1.10 ± 0.04 |
| 113.096 | $C_7H_{13}O^+$ | 113.0961 | C7 unsaturated aldehydes and ketones | 2.9 ± 0.2 | 3.9 ± 0.1 |
| 115.074 | $C_6H_{11}O_2^+$ | 115.0754 | Hexanedione/5-ethyldihydro-2(3 H)-furanone | 2.3 ± 0.1 | 3.4 ± 0.2 |
| 115.112 | $C_7H_{15}O^+$ | 115.1117 | C7 aldehydes and ketones | 54 ± 6 | 134 ± 17 |
| 123.082 | $C_8H_{11}O^+$ | 123.0804 | Dimethyl-phenol | 1.3 ± 0.1 | 1.8 ± 0.2 |
| 125.096 | $C_8H_{13}O^+$ | 125.0961 | Octadienal/octadienone | 4.7 ± 0.7 | 7.0 ± 0.8 |
| 127.112 | $C_8H_{15}O^+$ | 127.1117 | 1-Octen-3-one | 1.4 ± 0.1 | 2.3 ± 0.2 |
| 129.127 | $C_8H_{17}O^+$ | 129.1274 | C8 aldehydes and ketones | 18 ± 2 | 27 ± 3 |
| 136.022 | $C_7H_6NS^+$ | 136.0215 | Benzothiazole | 1.75 ± 0.04 | 1.59 ± 0.04 |
| 143.142 | $C_9H_{19}O^+$ | 143.143 | C9 aldehydes and ketones | 7 ± 1 | 4.0 ± 0.4 |

1991; Jurado et al., 2007; Martín et al., 2006; Ruiz et al., 2002; Sabio et al., 1998) and fat (Timón et al., 2001). An hexanal fragment caused by the loss of a water molecule was also tentatively identified at m/z = 3.087, and its concentration in the lean was double in the dry-cured *Montanera* hams than in *Campo* hams, while in the SCF its concentration was almost triple in *Montanera* hams. The origin of pentanal and hexanal is fatty acid oxidation, mainly the oxidation of linoleic acid (Larick, Turner, Schoenherr, Coffey, & Pilkington, 1992; Ruiz et al., 2002), which seems to be more concentrated in dry-cured hams from pigs fattened on acorn and grass than in pigs fattened on a high-oleic concentrated feed (Pérez-Palacios et al., 2010). Also saturated aldehydes and ketones with 7 and 8 carbon atoms (measured at m/z = 115.112 and 129.127, Table 4) were more concentrated in the SCF of *Montanera* hams than in *Campo* ones. In both cases the linear aldehydes (heptanal and octanal respectively) are probably the major contributors to the peak concentration, but in the case of the peak at m/z = 115.112 (Table 4), 2-heptanone, which was also found at a high concentration in the SCF of dry-cured Iberian ham (Timón et al., 2001), can play a role. These compounds are produced by the oxidation of unsaturated fatty acids, like oleic acid (Ruiz et al., 2002b), the most concentrated fatty acid in pork, and also more concentrated in hams from pigs fattened on acorn and grass than in hams from pigs fattened on concentrated feed (Carrapiso, Jurado, Martin, & García, 2007; Carrapiso, Jurado, Timón, & García, 2002; Ventanas et al., 2007), even when the concentrated feed is enriched with oleic acid (Pérez-Palacios et al., 2010). Peaks corresponding to unsaturated aldehydes and ketones with less than 9 carbon atoms were also found in higher concentration in *Montanera* than in *Campo* hams, both in lean (Table 3) and SCF (Table 4), probably due to the higher concentration in these hams of the unsaturated fatty acids that are their precursors (Pérez-Palacios et al., 2010), but the concentration in both kinds of ham was much lower than that of

**Table 5**
Odour-impact compounds tentatively identified from the PTR-ToF-MS $H_3O^+$ analysis of the dry-cured Iberian ham lean headspace and their mean concentration (ppbv) in *Campo* and *Montanera* hams.

| Measured mass (m/z) | Sum formula | Theoretical mass (m/z) | Tentative identification | Mean concentration (ppbv) |
|---|---|---|---|---|
| 49.011 | $CH_5S^+$ | 49.0106 | Methanethiol | 44.1 |
| 75.045 | $C_3H_7O_2^+$ | 75.0440 | Propanoic acid | 21.3 |
| 87.047 | $C_4H_7O_2^+$ | 87.0441 | 2,3-butanedione | 26.1 |
| 89.061 | $C_4H_9O_2^+$ | 89.0597 | Butanoic acid | 45.5 |
| 103.078 | $C_5H_{11}O_2^+$ | 103.0754 | 3-methylbutanoic acid | 5.6 |
| 105.039 | $C_4H_9OS^+$ | 105.0369 | 3-(methylthio)-propanal | 1.0 |
| 109.077 | $C_6H_9N_2^+$ | 109.0760 | 2,6-diemthylpyrazine | 8.9 |
| 121.067 | $C_8H_9O^+$ | 121.0648 | Benzeneacetaldehyde | 20.6 |
| 129.057 | $C_6H_9O_3^+$ | 129.0546 | 2,5-dimethyl-4-hydroxy-−3(2 H)-furanone | 0.6 |
| 131.110 | $C_7H_{15}O_2^+$ | 131.1067 | 2-methylbutanoic acid, ethyl ester + 3-methylbutanoic acid, ethyl ester | 1.7 |

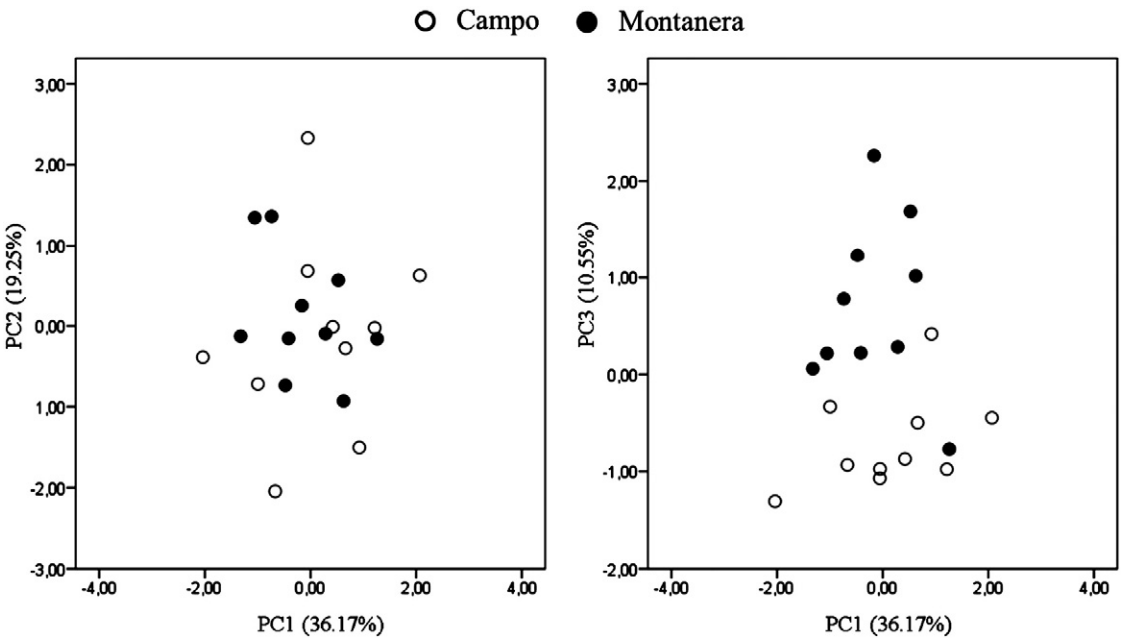


**Fig. 3.** Score plots obtained by the PCA analysis (PC1 vs PC2 and PC1 vs PC3 respectively) of the PTR-ToF-MS $NO^+$ fingerprint of the headspace of Iberian dry-cured *Montanera* and *Campo* hams lean.

the saturated aldehydes and ketones, which agree with previous results in dry-cured Iberian ham (Andrés et al., 2002; Martín et al., 2006; Ruiz et al., 2002).

Aldehydes and ketones with 3 and 4 carbon atoms were also tentatively identified at m/z = 59.051 and 73.065 respectively. The peak at m/z = 59.051, with propanone as probable major contributor (Ruiz et al., 2002), was saturated and the concentration was estimated on the basis of its isotopologue at m/z = 60.053. It showed no significant differences between ham batches. In the case of the aldehydes and ketones with 4 carbon atoms (m/z = 73.065) the major contributors to the peak signal were probably butanal and methylpropanal, found at higher concentrations than their corresponding ketones in previous studies (Andrés et al., 2002; Carrapiso, Jurado, et al., 2003; Ruiz et al., 2002). This peak was significantly different between both ham batches in the fat headspace (Table 4) but not in the lean headspace (Table 3), while the concentration of the butanal fragment, tentatively identified at m/z = 55.055, was double in both lean and SCF of dry-cured *Montanera* hams than in *Campo* hams (Tables 3 and 4).

On the contrary, peaks corresponding to aldehydes and ketones with 9 carbon atoms in lean and SCF (measured m/z = 143.146 and 143.142 respectively) and to aldehydes and ketones with 10 carbon atoms in lean (measured m/z = 157.163) were more concentrated in dry-cured *Campo* hams.

According to Sánchez del Pulgar et al. (2011), many peaks corresponding to alkyl fragments were found in the head-space of dry-cured Iberian ham, some of them being significantly different between *Montanera* and *Campo* hams. Peaks at m/z = 41.038, 43.054 and 71.085 were more concentrated in *Montanera* hams, and are related to alkyl fragments probably from the split off of water from

**Table 6**
Peaks from the PTR-ToF-MS $NO^+$ lean analysis significantly different between both ham batches ($p<0.05$) and with intensity higher than 1 normalized count (mean ± standard error.

| Measured mass (m/z) | Sum Formula | Theoretical mass (m/z) | Tentative identification | Campo (nc)[a] | Montanera (nc) |
|---|---|---|---|---|---|
| 33.033 | $CH_4OH^+$ | 33.0335 | Methanol | 1331 ± 50 | 1142 ± 52 |
| 57.033 | $C_3H_5O^+$ | 57.0335 | C3 aldehydes | 98 ± 10 | 128 ± 9 |
| 58.042 | $C_3H_6O^+$ | 58.0413 | C3 ketones | 241 ± 19 | 385 ± 30 |
| 72.057 | $C_4H_8O^+$ | 72.057 | C4 aldehydes | 67 ± 5 | 81 ± 4 |
| 76.004 | $CH_2NO_3^+$ | 76.0029 | Cluster formic acid | 4.1 ± 0.2 | 4.6 ± 0.2 |
| 80.062 | $C_6H_8^+$ | 80.0621 | Cyclohexadiene | 3.5 ± 0.2 | 4.6 ± 0.3 |
| 82.076 | $C_6H_{10}^+$ | 82.0777 | Cyclohexene/methyl-cyclopentene | 8.2 ± 0.8 | 12.6 ± 1.2 |
| 86.072 | $C_5H_{10}O^+$ | 86.0726 | C5 ketones | 89 ± 11 | 197 ± 23 |
| 87.080 | $C_5H_{10}OH^+$ | 87.0804 | C5 aldehydes and ketones | 1380 ± 176 | 2440 ± 248 |
| 99.080 | $C_6H_{11}O^+$ | 99.0804 | C6 aldehydes | 68 ± 8 | 116 ± 13 |
| 99.114 | $C_6H_{13}N^+$ | 99.1043 | Methyl-piperidine | 4.9 ± 0.3 | 6.2 ± 0.3 |
| 100.082 | $C_6H_{12}O^+$ | 100.0883 | C6 ketones | 15 ± 2 | 25 ± 2 |
| 101.096 | $C_6H_{12}OH^+$ | 101.0961 | C6 aldehydes and ketones | 82 ± 12 | 145 ± 15 |
| 102.056 | $C_4H_8NO_2^+$ | 102.055 | cluster C4 ketones | 22 ± 1 | 32 ± 2 |
| 112.084 | $C_7H_{12}O^+$ | 112.0883 | C7 unsaturated aldehydes | 8.2 ± 0.8 | 11.4 ± 0.9 |
| 114.060 | $C_5H_8NO_2^+$ | 114.055 | Cluster C5 unsaturated ketones | 4.1 ± 0.3 | 5.6 ± 0.4 |
| 116.070 | $C_5H_{10}NO_2$ | 116.0706 | cluster C5 ketones | 56 ± 9 | 171 ± 23 |
| 130.085 | $C_6H_{12}NO_2^+$ | 130.0863 | cluster C6 ketone | 8.3 ± 1.1 | 16.2 ± 1.8 |
| 144.063 | $C_6H_{10}NO_3^+$ | 144.0655 | Cluster hexanedione/ethyldihydro-2(3H)-furanone | 3.7 ± 0.3 | 5.9 ± 0.6 |

[a] Normalized counts.

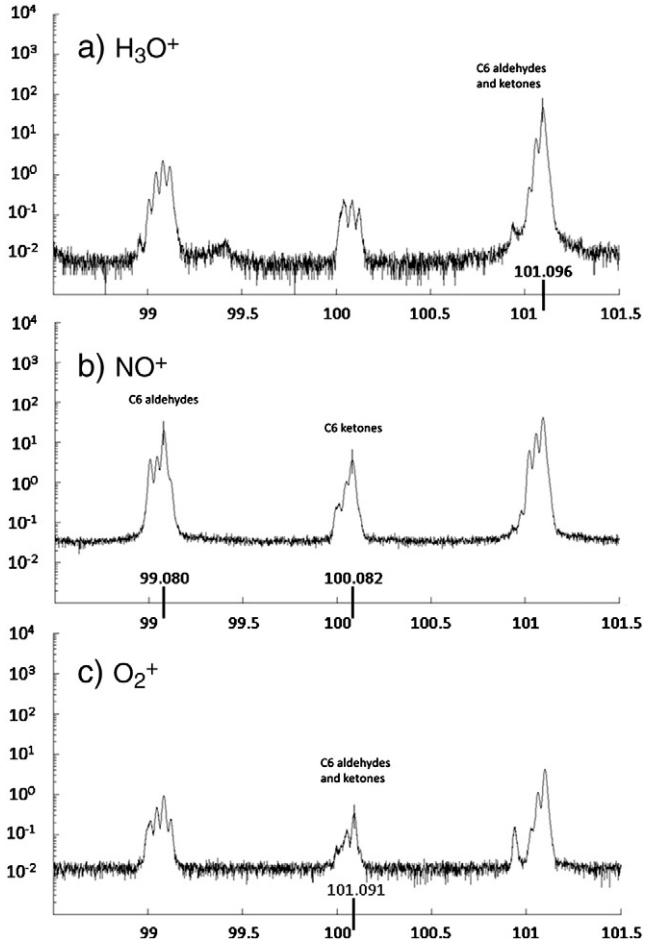


**Fig. 4.** Example of peaks related to aldehydes and ketones measured by using different reagent ions. a: $H_3O^+$. b: $NO^+$. c: $O_2^+$.

linear and branched saturated alcohols (Aprea, Biasioli, Mark, & Gasperi, 2007).

The peak at m/z = 43.018 in the SCF samples was also more concentrated in *Montanera* hams SCF (Table 4), and corresponds to an alkyl fragment from the split off of a water molecule from acetic acid (Aprea, Biasioli, Gasperi, et al., 2007).

Dry-cured Iberian *Campo* ham lean showed higher concentration for the peaks at m/z 91.058, 105.072, 119.089 and 133.106 (Table 3), corresponding to the sum formula of the sulphur-containing compounds $C_4H_{11}S^+$, $C_5H_{13}S^+$, $C_6H_{15}S^+$, and $C_7H_{17}S^+$ (tentatively identified as butanethiol/1-(methylthio)-propane, pentanethiol/1-(methylthio)-butane, hexanethiol/1-(methylthio)-pentane and heptanethiol/1-(methylthio)-hexane respectively), while the SCF of *Campo* hams showed higher amounts of benzothiazol ( m/z = 136.022, $C_7H_6NS^+$) (Table 4). Sulphur-containing compounds arise from Strecker reactions of amino-acids and Maillard reactions (Ruiz et al., 2002) and are potent odorants, major contributors to meat odour (Carrapiso et al., 2002; Ruiz et al., 2002; Sánchez del Pulgar, García, Reina, & Carrapiso, 2012). Moreover, the peak detected at m/z = 95.059 (tentatively identified as methylpyrazine $-C_5H_7N_2^+-$) in lean (Table 3), with the same origin as sulphur compounds and the rest of the nitrogen compounds (Ruiz et al., 2002), was more concentrated in dry-cured *Campo* hams than in *Montanera* ones. Pyrazines are also potent odorants with nutty and roast notes (Ruiz et al., 2002; Sánchez del Pulgar et al., 2012). However, *Montanera* hams SCF showed higher signals of the peak at m/z = 80.053, corresponding to the formula $C_5H_6N^+$ and tentatively identified as pyridine.

Hexanenitrile, already identified in dry-cured ham by PTR-ToF-MS (Sánchez del Pulgar et al., 2011), was more concentrated in *Montanera* hams than in *Campo* ones, both in lean and SCF (Tables 3 and 4). Nitrile compounds have been detected in dry-cured ham (Ruiz et al., 1998), and their most probable formation occurs at the expense of the corresponding aldehydes during lipid oxidation involving nitrite (Mottram, Croft, & Patterson, 1984), so the higher concentration of hexanal found in *Montanera* hams could explain also the higher concentration of hexanenitrile.

The PTR-ToF-MS analysis of the dry-cured Iberian ham headspace allowed the detection of more compounds than those included in Tables 3 and 4, some of them identified as major odorants of dry-cured Iberian ham in previous studies (Carrapiso & García, 2004; Carrapiso et al., 2002; Sánchez del Pulgar et al., 2012), but at concentrations not significantly different between batches. These compounds and their mean concentration in dry-cured *Campo* and *Montanera* hams are listed in Table 5.

### 3.2. Switching reagent ion system

PCA and discriminant analysis (PDA) were also performed on data obtained with $NO^+$ and $O_2^+$ for the lean headspace. For $NO^+$ (Fig. 3), the first three PCs explain 66% of the total variance. Although the PC1–PC2 plot shows a poor discrimination of the samples, the PC1–PC3 plot suggests a good discrimination of the dry-cured Iberian ham samples according to the rearing system of the pigs. On the contrary, spectra obtained using $O_2^+$ as precursor ion do not suggest a simple discrimination of the ham batches.

In the case of the $NO^+$ dataset, PDA classifies correctly 83.3% of the replicates. It is worth mentioning that 5 out of the ten misclassified replicates correspond to five different samples, and the other five correspond to only two samples. In the case of the $O_2^+$ dataset, even if the PCA does not suggest an easy discrimination of the batches, PDA allows the discrimination of 78.3% of the replicates.

As shown in Table 6, the analysis of the samples with $NO^+$ allowed the detection of several peaks significantly different between both hams and corresponding to aldehydes and ketones. In agreement with the results from the ham lean analysis using $H_3O^+$ (Table 3), aldehydes and ketones were more concentrated in dry-cured *Montanera* hams than in dry-cured *Campo* hams. As mentioned in the Introduction, $NO^+$ allows the detection in different peaks of the isobaric aldehydes and ketones due to the ionization reaction that occurs in both cases (Fig. 4). Except for aldehydes and ketones with 3 carbon atoms, each aldehyde was more concentrated than its corresponding ketone. Matching the absence of differences in the concentration of the 5 carbon atom aldehyde fragment in the lean analysis with $H_3O^+$, aldehydes with 5 carbon atoms showed no differences between batches in the $NO^+$ analysis. No difference was found in the concentration of 4 carbon atom aldehydes, although the butanal fragment was more concentrated in *Montanera* hams than in *Campo* hams in the lean analysis with $H_3O^+$. In addition, ketones with 4 and 5 carbon atoms were more concentrated in dry-cured *Montanera* hams than in *Campo* ones. As a first approach to the VOC analysis by PTR-ToF-MS using $NO^+$ as reagent ion in a complex matrix, the data indicate that also $H_3O^+$ has intervened in the ionization of the compounds present in the sample headspace. In fact, Table 6 shows significant differences in the concentration of the peaks corresponding to protonated aldehydes and ketones with 5 and 6 carbon atoms, this was also found in the peaks corresponding to other protonated aldehydes and ketones (data not included). Therefore, the PTR-ToF-MS analysis with $H_3O^+$ allowed the detection of the isobaric aldehydes and ketones in the same peak, and, due to the high energy into the drift tube, also aldehyde fragments caused by the loss of a water molecule (Jordan et al., 2009). Nevertheless, the use of $NO^+$ as reagent ion allowed the detection of aldehydes and ketones in separated peaks, permitting confirmation of the results of the $H_3O^+$ analysis, and even indicating which isobaric compound was responsible for the differences between batches. It was

checked that the sum of the intensity of the peaks corresponding to aldehydes and ketones with 6 carbon atoms in the $H_3O^+$ analysis (ppbv of the hexanal fragment peak and the peak for C6 saturated aldehydes and ketones) and in the $NO^+$ analysis (normalized counts corresponding to the aldehyde peak, the ketone, the cluster ketone·$NO^+$ and the peak of protonated aldehydes and ketones). In both analyses the intensity ratio of the C6 aldehydes and ketones between *Campo* and *Montanera* hams is almost the same.

The use of $O_2^+$ as reagent ion in the PTR-ToF-MS analysis of the dry-cured Iberian ham lean showed no peaks significantly different between ham batches, with the exception of the peak at m/z = 82.079, tentatively identified as alkenes with 6 carbon atoms, and the peak at m/z = 86.075, tentatively identified as saturated aldehydes and ketones with 5 carbon atoms, which were more concentrated in dry-cured *Montanera* than in *Campo* hams.

The data demonstrate the feasibility and usefulness of the SRI system in food samples. Nevertheless, it was not possible to obtain for $NO^+$ and $O_2^+$ the same purity of the primary ion beam achieved with $H_3O^+$ (Table 1). The presence of the signals produced by $H_3O^+$ proton transfer during $NO^+$ and $O_2^+$ analysis also makes data analysis more difficult and less conclusive.

## 4. Conclusions

Pig rearing system strongly affects the volatile compound profile of dry-cured Iberian ham.

The rapid PTR-ToF-MS analysis of the headspace of dry-cured Iberian ham, both lean and subcutaneous fat, allows the discrimination of the hams according to the rearing system: *Campo* and *Montanera*.

Many peaks are significantly different between *Montanera* and *Campo* Iberian hams. In particular *Montanera* samples show higher amounts of aldehydes and ketones than *Campo* probably due to the higher amounts of their precursor fatty acids, while dry-cured *Campo* hams have higher concentrations of peaks corresponding to sulphur-compounds.

In this study the switching reagent ion system in PTR-MS instruments was applied for the first time to food products providing the possibility of separating the contribution of isobaric compounds (aldehydes and ketones), however the same purity of the precursor ion beam achieved with $H_3O^+$ was not obtainedand this limits classification efficiency and data interpretation.

The rapid and high sensitive PTR-ToF-MS technique coupled to appropriate chemometric analysis provides both classification models and analytical information. It could be useful for the dry-cured Iberian ham industry. In particular the rapid characterisation of subcutaneous fat seems suited for the implementation of a non-destructive on-line quality control monitoring.

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## References

Andrade, M. J., Córdoba, J. J., Sánchez, B., Casado, E. M., & Rodríguez, M. (2009). Evaluation and selection of yeasts isolated from dry-cured Iberian ham by their volatile compound production. *Food Chemistry*, *113*, 457–463.

Andrés, A. I., Cava, R., & Ruiz, J. (2002). Monitoring volatile compounds during dry-cured ham ripening by solid-phase microextraction coupled to a new direct-extraction device. *Journal of Chromatography*, *963*, 83–88.

Aprea, E., Biasioli, F., Carlin, S., Versini, G., Märk, T. D., & Gasperi, F. (2007). Rapid white truffle headspace analysis by proton transfer reaction mass spectrometry and comparison with solid-phase microextraction coupled with gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*, *2*, 2564–2572.

Aprea, E., Biasioli, F., Gasperi, F., Märk, T. D., & van Ruth, S. (2006). *In vivo* monitoring of strawberry flavour release from model custards: Effect of texture and oral processing. *Flavour and Fragance Journal*, *21*, 53–58.

Aprea, E., Biasioli, F., Gasperi, F., Mott, D., Marini, F., & Märk, T. D. (2007). Assessment of Trentingrana cheese ageing by proton transfer reaction-mass spectrometry and chemometrics. *International Dairy Journal*, *17*, 226–234.

Aprea, E., Biasioli, F., Mark, T. D., & Gasperi, F. (2007). PTR-MS study of esters in water and water/ethanol solutions: Fragmentation patterns and partition coefficients. *International Journal of Mass Spectrometry*, *262*, 114–121.

Araghipour, N., Colineau, J., Koot, A., Akkermans, W., Moreno Rojas, J. M., Beauchamp, J., et al. (2008). Geographical origin classification of olive oils by PTR-MS. *Food Chemistry*, *108*, 374–383.

Berdagué, J. L., Denoyer, J. L., Le Quere, J. L., & Semon, E. (1991). Volatile components of dry-cured ham. *Journal of Agricultural and Food Chemistry*, *39*, 1257–1261.

Biasioli, F., Gasperi, F., Aprea, E., Colato, L., Boscaini, E., & Märk, T. D. (2003). Fingerprinting mass spectrometry by PTR-MS: heat treatment vs. pressure treatment of red orange juice—A case study. *International Journal of Mass Spectrometry*, *223–224*, 343–353.

Biasioli, F., Gasperi, F., Aprea, E., Endrizzi, I., Framondino, V., Marini, F., et al. (2006). Correlation of PTR-MS spectral fingerprints with sensory characterisation of flavour and odour profile of "Trentingrana" cheese. *Food Quality and Preference*, *17*, 63–75.

Biasioli, F., Gasperi, F., Yeretzian, C., & Märk, T. D. (2011). PTR-MS monitoring of VOCs and BVOCs in food science and technology. *TrAC Trends in Analytical Chemistry*, *30*, 968–977.

Biasioli, F., Yeretzian, C., Märk, T. D., Dewulf, J., & Van Langenhove, H. (2011). Direct-injection mass spectrometry adds the time dimension to (B) VOC analysis. *TrAC Trends in Analytical Chemistry*, *30*, 1003–1017.

Blake, R. S., Monks, P. S., & Ellis, A. M. (2009). Proton transfer reaction mass spectrometry. *Chemical Reviews*, *109*, 861–896.

Cappellin, L., Biasioli, F., Fabris, A., Schuhfried, E., Soukoulis, C., & Märk, T. D. (2010). Improved mass accuracy in PTR-TOF-MS: Another step towards better compound identification in PTR-MS. *International Journal of Mass Spectrometry*, *209*, 60–63.

Cappellin, L., Biasioli, F., Granitto, P., Schuhfried, E., Soukoulis, C., Costa, F., et al. (2011). On data analysis in PTR-TOF-MS: From raw spectra to data mining. *Sensors and Actuators B: Chemical*, *155*, 183–190.

Cappellin, L., Karl, T., Probst, M., Ismailova, O., Winkler, P. M., Soukoulis, C., et al. (2012). On quantitative determination of volatile organic compound concentrations using proton transfer reaction time-of-flight mass spectrometry. *Environmental Science & Technology*, *46*, 2283–2290.

Carrapiso, A. I., Bonilla, F., & García, C. (2003). Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. *Meat Science*, *65*, 623–629.

Carrapiso, A. I., & García, C. (2004). Iberian ham headspace: Odorants of intermuscular fat and differences with lean. *Journal of the Science of Food and Agriculture*, *84*, 2047–2051.

Carrapiso, A. I., Jurado, A., & García, C. (2003). Effect of crossbreeding and rearing system on Iberian ham volatile compounds. *Food Science and Technology International*, *9*, 421–426.

Carrapiso, A. I., Jurado, A., Martin, L., & García, C. (2007). The duration of the outdoor rearing period of pigs influences Iberian ham characteristics. *Irish Journal of Agricultural and Food Research*, *46*, 105–115.

Carrapiso, A. I., Jurado, A., Timón, M. L., & García, C. (2002). Odor-active compounds of Iberian hams with different aroma characteristics. *Journal of Agricultural and Food Chemistry*, *50*, 6453–6458.

Daza, A., Rey, A. I., Ruiz, J., & López-Bote, C. J. (2005). Effects of feeding in free-range conditions or in confinement with different MUFA/PUFA ratios and α-tocopheryl acetate, on antioxidants accumulation and oxidative stability in Iberian pigs. *Meat Science*, *69*, 151–163.

Dirinck, P., Van Opstaele, F., & Vandendriessche, F. (1992). Flavour differences between northern and southern European cured hams. *Food Chemistry*, *59*, 511–521.

Fabris, A., Biasioli, F., Granitto, P. M., Aprea, E., Cappellin, L., Schuhfried, E., et al. (2010). PTR-TOF-MS and data-mining methods for rapid characterization of agro-industrial samples: Influence of milk storage conditions on the volatile compounds profile of Trentingrana cheese. *Journal of Mass Spectrometry*, *45*, 1065–1074.

Flores, M., Grimm, C. C., Toldrá, F., & Spanier, A. M. (1997). Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. *Journal of Agricultural and Food Chemistry*, *45*, 2178–2186.

García, C., Berdagué, J. J., Antequera, T., López-Bote, C., Córdoba, J. C., & Ventanas, J. (1991). Volatile components of dry-cured ham. *Food Chemistry*, *41*, 23–32.

García, C., Ventanas, J., Antequera, T., Ruiz, J., Cava, R., & Álvarez, P. (1996). Measuring sensorial quality of Iberian ham by Rasch model. *Journal of Food Quality*, *19*, 397–412.

Gasperi, F., Aprea, E., Biasioli, F., Carlin, S., Endrizzi, I., Pirretti, G., et al. (2009). Effects of supercritical $CO_2$ and $N_2O$ pasteurisation on the quality of fresh apple juice. *Food Chemistry*, *115*, 129–136.

Granitto, P., Biasioli, F., Aprea, E., Mott, D., Furlanello, C., Märk, T., et al. (2007). Rapid and non-destructive identification of strawberry cultivars by direct PTR-MS headspace analysis and data mining techniques. *Sensors and Actuators B: Chemical*, *121*, 379–385.

Jordan, A., Haidacher, S., Hanel, G., Hartungen, E., Herbig, J., Märk, L., et al. (2009). An online ultra-high sensitivity proton-transfer-reaction mass-spectrometer combined with switchable reagent ion capability (PTR + SRI−MS). *International Journal of Mass Spectrometry*, *286*, 32–38.

Jurado, A., Garcia, C., Timón, M. L., & Carrapiso, A. I. (2007). Effect of ripening time and rearing system on amino acid-related flavour compounds of Iberian ham. *Meat Science*, *75*, 585–594.

Larick, D. K., Turner, B. E., Schoenherr, W. D., Coffey, M. T., & Pilkington, D. H. (1992). Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet. *Journal of Animal Science*, *70*, 1397–1401.

Lindinger, W., Hansel, A., & Jordan, A. (1998). On-line monitoring of volatile organic compounds at pptv levels. *Chemical Society Reviews*, *27*, 347–354.

Martín, A., Córdoba, J. J., Aranda, E., Córdoba, M. G., & Asensio, M. A. (2006). Contribution of a selected fungal population to the volatile compounds on dry-cured ham. *International Journal of Food Microbiology*, *110*, 8–18.

Mottram, D. S., Croft, S. E., & Patterson, R. L. S. (1984). Volatile components of cured and uncured pork: The role of nitrite and the formation of nitrogen compounds. *Journal of the Science of Food and Agriculture*, *35*, 233–239.

Narváez-Rivas, M., Vicario, I. M., Alcalde, M. J., & León-Camacho, M. (2010). Volatile hydrocarbon profile of Iberian dry-cured hams. A possible tool for authentication of hams according to the fattening diet. *Talanta*, *81*, 1224–1228.

Olivares, A., Dryahina, K., Navarro, J. L., Flores, M., Smith, D., & Spanel, P. (2010). Selected ion flow tube-mass spectrometry for absolute quantification of aroma compounds in the headspace of dry fermented sausages. *Analytical Chemistry*, *82*, 5819–5829.

Olivares, A., Dryahina, K., Navarro, J. L., Smith, D., Spanel, P., & Flores, M. (2011). 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. *Journal of Agricultural and Food Chemistry*, *59*, 1931–1938.

Orden de 2 de Julio de 1990 por la que se ratifica el Reglamento de la denominación de origen «Dehesa de Extremadura» y su Consejo Regulador. B.O.E. 158, July 3rd 1990.

Pérez-Palacios, T., Antequera, T., Durán, M. L., Caro, A., Rodríguez, P. G., & Ruiz, J. (2010). MRI-based analysis, lipid composition and sensory traits for studying Iberian dry-cured hams from pigs fed with different diets. *Food Research International*, *43*, 248–254.

Pérez-Palacios, T., Ruiz, J., Tejeda, J. F., & Antequera, T. (2009). Subcutaneous and intramuscular lipid traits as tools for classifying Iberian pigs as a function of their feeding background. *Meat Science*, *81*, 632–640.

Petrón, M. J., Tejeda, M. J., Muriel, E., Ventanas, J., & Antequera, T. (2005). Study of branched hydrocarbon fraction of intramuscular lipids from Iberian dry-cured ham. *Meat Science*, *69*, 129–134.

Petrón, M. J., Tejeda, M. J., Muriel, E., Ventanas, J., & Antequera, T. (2006). Effect of duration of the Montanera diet on the hydrocarbon fraction of intramuscular lipids from Iberian dry-cured ham; characterization by gas chromatography. *Journal of the Science of Food and Agriculture*, *86*, 1040–1045.

Real Decreto 1469/2007, de 2 de noviembre, por el que se aprueba la norma de calidad para la carne, el jamón, la paleta y la caña de lomo ibéricos. B.O.E. n. 264, November 3rd 2007, 45087–45104.

Ruiz, J., Cava, R., Antequera, T., Martín, L., Ventanas, J., & López-Bote, C. J. (1998). Prediction of the feeding background of Iberian pigs using the fatty acid composition of subcutaneous, muscle and hepatic fat. *Meat Science*, *49*, 155–163.

Ruiz, J., García, C., Muriel, E., Andrés, A., & Ventanas, J. (2002). The flavour of Iberian ham. In F. Toldrá (Ed.), *Research advances in the quality of meat and meat products*. Kerala, India: Trivandrum.

Ruiz, J., Ventanas, J., Cava, R., Andrés, A., & García, C. (1999). Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. *Meat Science*, *52*, 19–27.

Sabio, E., Vidal-Aragón, M. C., Bernalte, M. J., & Gata, J. L. (1998). Volatile compounds present in six types of dry-cured ham from south European countries. *Food Chemistry*, *61*, 493–503.

Sánchez del Pulgar, J., García, C., Reina, R., & Carrapiso, A. I. (2012). Study of the volatile compounds and odor-active compounds of dry-cured Iberian ham extracted by SPME. *Food Science and Technology International*, http://dx.doi.org/10.1177/1082013212442199.

Sánchez del Pulgar, J., Soukoulis, C., Biasioli, F., Cappellin, L., García, C., Gasperi, F., et al. (2011). Rapid characterization of dry cured ham produced following different PDOs by proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS). *Talanta*, *85*, 386–396.

Soukoulis, C., Aprea, E., Biasioli, F., Cappellin, L., Schuhfried, E., Märk, T. D., et al. (2010). Proton transfer reaction time-of-flight mass spectrometry monitoring of the evolution of volatile compounds during lactic acid fermentation of milk. *Rapid Communications in Mass Spectrometry*, *24*, 2127–2134.

Spanel, P., & Smith, D. (1999). Selected ion flow tube-mass spectrometry: Detection and real-time monitoring of flavours released by food products. *Rapid Communications in Mass Spectrometry*, *13*, 585–596.

Tejeda, J. F., Antequera, T., Martín, L., Ventanas, J., & Garcia, C. (2001). Study of the branched hydrocarbon fraction of intramuscular lipids from Iberian fresh ham. *Meat Science*, *58*, 175–179.

Timón, M. L., Ventanas, J., Carrapiso, A. I., Jurado, A., & García, C. (2001). Subcutaneous and intermuscular fat characterisation of dry-cured Iberian hams. *Meat Science*, *58*, 85–91.

Toldrá, F., Aristoy, M. C., & Flores, M. (2000). Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Research International*, *33*, 181–185.

Ventanas, J., Córdoba, J. J., Antequera, T., García, C., López-Bote, C., & Asensio, M. A. (1992). Hydrolysis and Maillard reactions during ripening of Iberian cured ham. *Journal of Food Science*, *57*, 813–815.

Ventanas, S., Ventanas, J., Tovar, J., García, C., & y Estévez, M. (2007). Extensive feeding versus oleic acid and tocopherol enriched mixed diets for the production of Iberian dry-cured hams: Effect on chemical composition, oxidative status and sensory traits. *Meat Science*, *77*, 246–256.

Witten, I., & Frank, E. (2005). *Data mining: Practical machine learning tools and techniques* (2nd ed.). Amsterdam, Boston MA: Morgan Kaufman.