Talanta 85 (2011) 386–393

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid characterization of dry cured ham produced following different PDOs by proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS)

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ARTICLE INFO

Article history:
Received 28 October 2010
Received in revised form 13 March 2011
Accepted 28 March 2011
Available online 5 April 2011

Keywords:
PTR-MS
PTR-ToF-MS
Dry cured ham
Volatile compounds
PDO
Flavour compounds

ABSTRACT

In the present study, the recently developed proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS) technique was used for the rapid characterization of dry cured hams produced according to 4 of the most important Protected Designations of Origin (PDOs): an Iberian one (Dehesa de Extremadura) and three Italian ones (Prosciutto di San Daniele, Prosciutto di Parma and Prosciutto Toscano). In total, the headspace composition and respective concentration for nine Spanish and 37 Italian dry cured ham samples were analyzed by direct injection without any pre-treatment or pre-concentration. Firstly, we show that the rapid PTR-ToF-MS fingerprinting in conjunction with chemometrics (Principal Components Analysis) indicates a good separation of the dry cured ham samples according to their production process and that it is possible to set up, using data mining methods, classification models with a high success rate in cross validation. Secondly, we exploited the higher mass resolution of the new PTR-ToF-MS, as compared with standard quadrupole based versions, for the identification of the exact sum formula of the mass spectrometric peaks providing analytical information on the observed differences. The work indicates that PTR-ToF-MS can be used as a rapid method for the identification of differences among dry cured hams produced following the indications of different PDOs and that it provides information on some of the major volatile compounds and their link with the implemented manufacturing practices such as rearing system, salting and curing process, manufacturing practices that seem to strongly affect the final volatile organic profile and thus the perceived quality of dry cured ham.

1. Introduction

Dry-cured ham is a valuable traditional foodstuff with unique quality traits which are influenced mainly by the characteristics of the raw meat (geographical origin, pigs' breed, feeding regime and rearing system) and by the processing conditions (salting, curing and ripening) [1]. Dry cured ham production is often controlled by a protected designation of origin (PDO) in order to achieve products with high quality sensory characteristics and of reproducible quality [2]. In this paper we consider 4 of the most important PDOs for dry cured ham: *Dehesa de Extremadura* produced in a restricted area in Spain and *Prosciutto di Parma*, *Prosciutto di San Daniele* and *Prosciutto Toscano* produced in central and northern regions of Italy. The geographical origin of dry cured ham is a parameter relevant to their quality characteristics as it defines the implemented processing practices, i.e. type of raw materials, use of spices, addition of nitrates, differences in the type and duration of the curing process. Italian PDOs accept hybrid pigs from various crossing breeds such as Large Withe, Landrace and Duroc-Jersey, whereas Spanish Iberian hams are produced only with Iberian pigs or their direct crossbreeds with Duroc-Jersey [3]. Contrary to the Italian ham's salting process, the addition of small amounts of nitrates is permitted during the Spanish ham production [4]. The use of spices like pepper (added at salting or *sugnatura* phases; in the last a mixture of fat, flour and pepper is used to protect the hams) is permitted in the production of Italian hams, whereas it is banned during the production Spanish Iberian dry cured hams. Finally, the duration of curing process of Italian hams is generally shorter (at least 12 months) than that implemented in the production of Spanish Iberian hams that requires more than 18 months.

One of the most important quality attributes of dry cured hams is their unique flavour produced by a complex mixture of volatile organic compounds (VOCs) that is influenced by the characteristics of the raw materials and the implemented processing practices [5].

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0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.03.077

Most VOCs in dry cured ham form during the curing process, and are the result of chemical and biochemical lipid oxidation and of further interaction with proteins, peptides and free amino acids; other VOCs result from Strecker degradation of free amino acids and Maillard reaction with products of the lipid oxidation [6]. The VOCs profile depends also quantitatively and qualitatively on genetic and rearing factors [7,8] that influenced the meat composition as well as on the length of the ripening [9–11]. Therefore the flavour profile as apparent in the VOCs compositions can be used to distinguish differently dry-cured hams in terms of their geographical origin or production process.

Several studies dealing with the volatile compounds profile of different kinds of cured hams have been reported including: Iberian [1,2,10,11] and Serrano Spanish hams [12], Prosciutto di Parma [9,13], Prosciutto di San Daniele [14,15] and Prosciutto Toscano [16] Italian hams. Other researchers investigated the differences in the volatile profile of different kinds of ham are usually based on gas chromatographic (GC) separation preceded by some extraction method as SPME and followed by mass spectrometric identification [17–20]). Other approaches have been proposed to overcome the drawbacks of GC based analysis, namely the time consuming procedure and the need of sample preparation or of a concentration phase [21], a promising possibility being direct injection mass spectrometry and in particular, proton transfer reaction mass spectrometry.

Proton transfer reaction mass spectrometry (PTR-MS) is a novel method that has been successfully applied for the on-line monitoring of VOCs headspace in several model and real food systems as well as the characterization of foods and their production processes [22–28] or origin identification [29,30]. PTR-MS has been described in several review papers [31] and will be not described in detail here. It is based on the protonation of volatiles organic compounds which have a proton affinity higher than that of water and, in its basic version relies on the detection of the product ions by a quadrupole mass spectrometer. To partially overcome the limitations related to the slow and low resolution quadrupole, the coupling of PTR-MS with a time-of-flight (ToF) mass analyzer was recently commercialized [32] offering several advantages including higher mass resolution ($m/\Delta m$ up to 8000) and faster spectra acquisition, see Fabris et al. [27] and Soukoulis et al. [28] for the first applications of PTR-ToF-MS in food science. PTR-ToF-MS is characterized by a high sensitivity with limits down to the low ppt region and a high time resolution (0.1 s) [32].

In this work we studied the volatile compounds profile of dry cured hams produced according to different PDOs, i.e. Italian ham (*Prosciutto di Parma*, *Prosciutto di San Daniele* and *Prosciutto Toscano*) and Spanish Iberian ham (*Dehesa de Extremadura*) aiming (i) at investigating the possibility of using PTR-ToF-MS spectra as fingerprints for their rapid and non invasive classification and (ii) at exploiting the features of PTR-ToF-MS to obtain qualitative and quantitative analytical information on the volatile compounds of the samples considered.

2. Materials and methods

2.1. Ham samples

Forty-six ham samples differing in their geographical origin and production process were selected: nine Spanish Iberian dry-cured hams (PDO *Dehesa de Extremadura*) and 37 Italian ones: 12 from PDO *Prosciutto di Parma*, 12 from PDO *Prosciutto di San Daniele* and 13 from PDO *Prosciutto Toscano*. The Italian hams were produced from heavy pigs with at least nine months of age and with 160 kg of minimum live weight, as fixed by the rules of the PDO Consortia [33]. The animals originated from a specific Italian selection obtained from traditional breeds genetically improved by the Italian Breeders Association. In particular the crossed breeds used were Italian Large White and Italian Landrace. The crossings were reared in the same farm, fed with the same diet, based on standard cereals-soybean meal commercial feeds, and slaughtered in the same abattoir, in three lots, within a period of six weeks. The fresh thighs where distributed in three different processing plants, located in Tuscany, Emilia and Friuli regions, in the hill area of the three different PDOs, by sharing the thighs produced in each slaughtering day between the different PDOs. According to the *Dehesa de Extremadura* PDO, Iberian hams were obtained from heavy Iberian pigs (pure Iberian gilt × 50% Iberian-50% Duroc barrow) with 14 months of age and in the range of 130–160 kg of live weight. These pigs were fattened outdoor for 60 days on grazed feedstuffs and a concentrate feeding ("Campo" Iberian hams, according to DOP *Dehesa de Extremdaura*). The hams were processed by applying the usual temperature and relative humidity values of the traditional processing according to their respective PDO's guidance [18,34]. The ripening duration was 399 days for Parma hams, 413 days for San Daniele hams, 396 days for Toscano hams and 720 days for Iberian hams. All pigs used to produce hams according to the Italian PDOs share the same raw material and production period but, since we decided to follow the indication of the PDOs, it was necessary to use, for the Iberian hams, pigs from a different breed and rearing system. Thus, in this study, the differences among the Italian hams originate only from the production process while the differences between Italian and Iberian ham origin from both raw meat and production process.

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

From each ham, a piece of the muscle *biceps femoris* was taken and kept under vacuum at 2 °C. In the moment of the analysis, the external layer of each piece of ham was removed, and 3 meat cubes of 1 cm^3 (3 replicates) were prepared. Each cube was introduced into a 40 ml vial (Supelco, Bellefonte, USA), capped by a PTFE/Silicone septum (Supelco, Bellefonte, USA). To standardise the measurement, all samples were equilibrated at 37 °C for 30 min in a water bath prior to analysis. They were then measured 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, allowing to take 30 average spectra [27].

Measurements were carried out following the procedure described in previous works for other food samples [27,28] 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 is 0.1 ns, amounting to 350,000 channels for a mass spectrum ranging up to m/z 400, with the following conditions in the drift tube: drift voltage 600 V, temperature 110 °C and pressure 2.25 mbar.

2.3. 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 [35]. Data pre-processing on ToF spectra was carried out in order 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. Peak identification and area extraction then followed the procedure described in details by Cappellin et al. [36]. Throughout this paper we report experimental m/z values up to the third decimal, the expected exact m/z values up to the fourth, VOCs concentration is expressed in ppbv (part per billion by volume) and has been calculated from peak areas

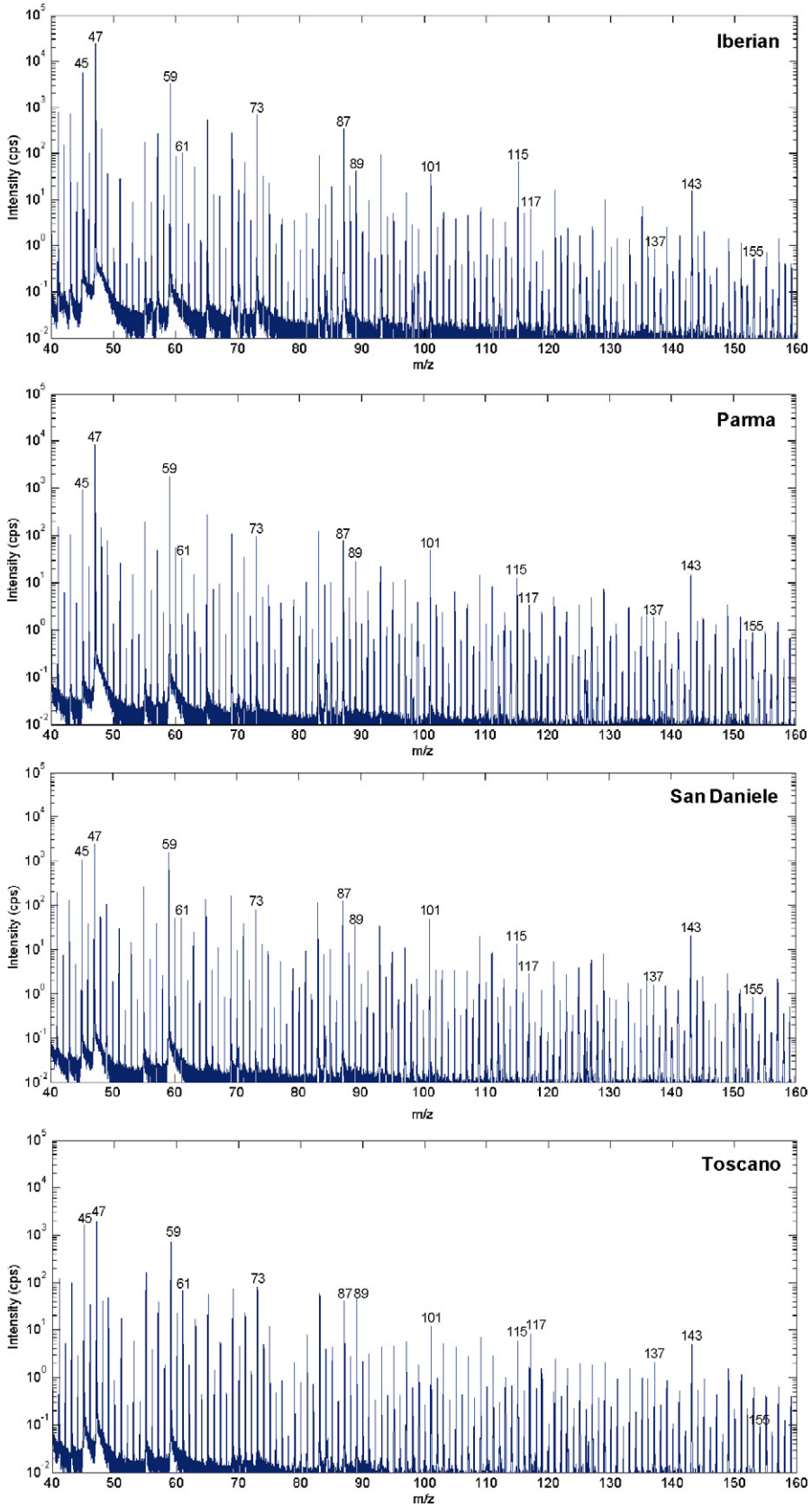

Fig. 1. Low mass region of the average PTR-ToF-MS spectra of the four kinds of ham considered in this work.

according to the formula described by Lindinger et al. [37] using a constant value for the reaction rate coefficient ($k_R = 2 \times 10^{-9}$ cm^3/s). This introduces a systematic error for the absolute concentration for each compound that is in most cases below 30% and can be accounted for if the actual rate constant is available [38,39].

2.4. Statistical analysis

A first statistical analysis was carried out by applying Principal Component Analysis (PCA), a multivariate technique which is often used to graphically assess the data under evaluation [40]. PCA only provides unsupervised information. A second analysis includes the use of several supervised classification methods, which investigates the separability of the classes. Following the work of Granitto et al. [42] and its recent implementation for PTR-ToF-MS data [27], we applied Random Forest (RF) [41], Penalized Discriminant Analysis (PDA) [43] and Discriminant Partial Least Squares (dPLS) [44].

To evaluate the results of the classification methods we use a leave-group-out (LGO) method: we iterated the process of leaving a group out as test set and using the rest of the data set to fit the models. The free parameters of each classifier (the number of dimensions considered in dPLS and the regularization constant in PDA) were selected at this step by internal cross validation using only the training data sets. After that, those models were 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, in order to evaluate really independent test sets (the good reproducibility of PTR-MS evaluations results in a high correlation among replicates of the same sample, which bias the result of the discriminant analysis if not taken into account). We analyzed the classification results using confusion matrices, in which rows correspond to the true classes and columns to the predicted ones [44].

RF is also used to analyze the data set in a graphical way, complementing PCA. RF graphical outputs are Multidimensional Scaling projections [45] of the data set that utilise a particular measure of distance among samples based on the internal designation of classes in the RF ensemble. Granitto et al. [42] discussed the use of this tool in the analysis of food data, showing that RF visualizations can be very informative for discrimination tasks, as they use information about the real classes (opposite to PCA) and also can be less biased than other supervised visualizations as LDA or PLS.

As a final step in this analysis, one-way ANOVA followed by Tukey's post hoc comparison was performed on the identified volatile compounds concentration data in order to identify some relevant compounds for the discrimination of the different dry cured hams.

3. Results and discussion

3.1. Preliminary data analysis

VOCs concentration in their headspace using PTR-ToF-MS of a total of 138 dry cured ham samples was measured i.e. 3 replicates for each of the 46 different hams. According to the procedure described by Cappellin et al. [36] the spectra have been aligned and the baseline has been removed. The average mass spectra obtained for the dry cured hams (in the range of m/z 40–200) classified according to their geographical origin and ripening process are displayed in Fig. 1. Peak extraction allows the detection of more than 700 peaks in the range of m/z 20–200, derived from the protonation or fragmentation of various VOCs, with an estimated headspace concentration higher than 1 ppbv. It is interesting to note that the spectral profile of the dry cured ham is dominated in terms of intensity at low molecular weight masses. Iberian hams are characterized by the highest signal intensities whereas Toscano show the lowest ones (Fig. 1). The observation is in accordance with the available literature data and with the maturation conditions (temperature and ageing duration) that favor proteolytic and lipolytic breakdown in the case of Iberian hams [1,17] as will be further discussed in Section 3.3.

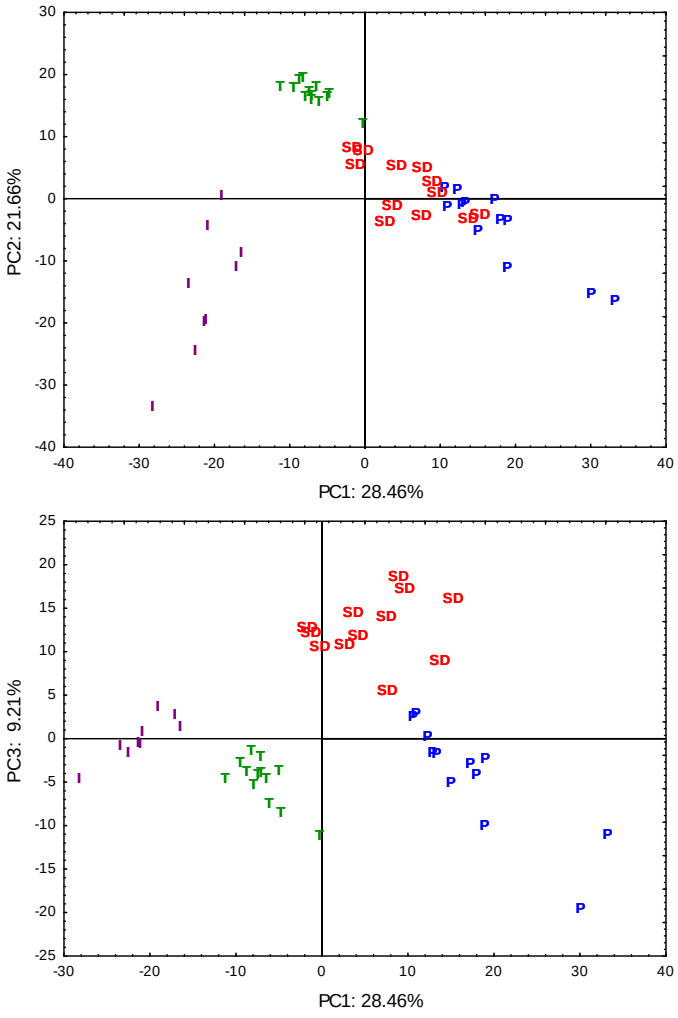

Fig. 2. Score plots obtained by the PCA analysis of the PTR-ToF-MS fingerprint of the headspace of Iberian (I), Parma (P), San Daniele (SD) and Toscana (T) ham samples.

3.2. Classification of dry cured hams

In order to derive useful information from the spectral data, we investigated the capability of the PTR-ToF-MS technique to discriminate the samples under study. For classifying the dry cured ham samples we used the data set comprising the spectral fingerprints from m/z 20 to 200 for the 138 analyzed samples. Thus the data matrix has 138 rows, one for each sample, and 1338 columns for the intensity of the identified peaks.

Principal components analysis (PCA) was performed as an exploratory non-supervised data analysis and the results are displayed in Fig. 2. The three first principal components explain 59% of the total variance and indicate a good discrimination of the samples. The PC1–PC2 plot allows the total discrimination of the Iberian (I) from the Italian dry cured hams as well as the Toscano (T) from Parma (P) and San Daniele (SD) hams while the PC1–PC3 score plot also indicates the possibility to distinguish between Parma and San Daniele hams.

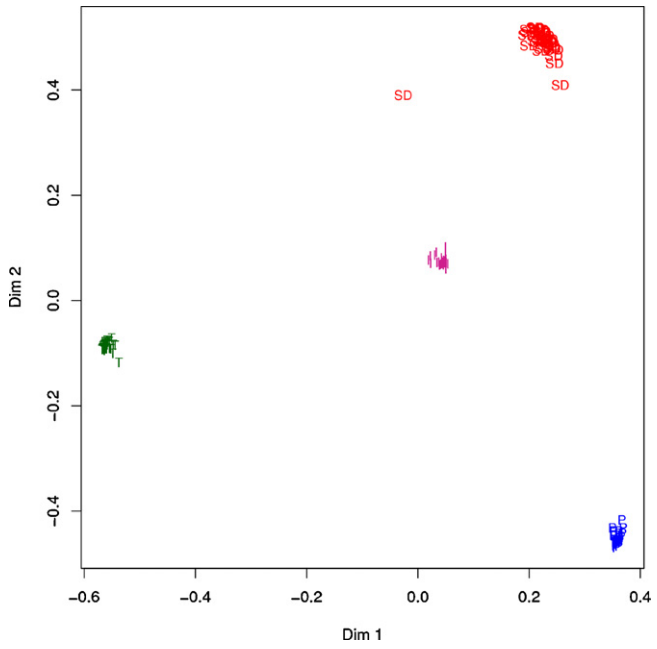

Fig. 3. Graphical output of the Random Forest analysis of the PTR-ToF-MS fingerprint of the head space of Iberian (I), Parma (P), San Daniele (SD) and Toscana (T) ham samples.

The supervised graphical analysis with Random Forest, shown in Fig. 3, produced similar results. The samples belonging to the same PDO ham form compact clouds that indicate a clear separation among different classes. Only one replicate from the San Daniele ham is separated from the others and is wrongly classified. The graphical result is confirmed by the confusion tables for the three different classifiers, shown in Table 1, where only one replicate (of the three related to one sample) is misclassified by RF and dPLS, and none by PDA. Considering the different origins of the samples, a separate study on the Italian ones, which are produced from the same raw material, would, in principle, be necessary. However, it is clear from the discussed data that all classes are well separated: Italian from Iberian but also different Italian PDOs from each other.

3.3. *Rapid detection of volatile compounds using PTR-ToF-MS*

On the basis of literature data, some of the peaks which either are characterized by high intensities or are significantly different ($p < 0.05$) among the different hams have been identified, see Table 2. In order to have more reliable results, we considered only literature data on the volatile compounds profile of Italian and Spanish Iberian dry cured hams [1,2,9,11,14,47] as well as available fragmentation patterns of pure standards [48–50]. Forty-three masses were tentatively identified including important compounds such as methanol (m/z 33.034), acetaldehyde (m/z 45.033), ethanol (m/z 47.048), 2-propanone (m/z 59.048), 2,3-butanedione (m/z 87.045), 2,3-pentanedione (m/z 101.061), terpenes (m/z 137.134) and terpineol (m/z 155.142).

PTR-ToF-MS does not allow for the separation of isobaric ketones and aldehydes using H_3O^+ as primary ion (Table 2), thus, the abundant intensities observed at m/z 59.048, 73.065 and 87.081 were attributed to the complementary contribution of both 2-propanone/propanal, 2-butanone/butanal and 2-pentanone/pentanal respectively. Similarly, the present technique cannot distinguish between branched and linear aldehydes and 2-ketones as in the case of 2-methyl butanal from pentanal/2-pentanone. However, PTR-ToF-MS allows to distinguish other isobaric carbonyl compounds such as 2,3-butanedione from 2-pentanone/pentanal and 2,3-pentanedione from 2-hexanone/hexanal. Aldehydes and 2-ketones are among the most important volatile compounds in the case of dry cured hams [9,14,16]. Linear aldehydes, such as hexanal, heptanal, octanal and nonanal, occur mainly from the oxidation of unsaturated fatty acids, like oleic, linoleic, linolenic and arachidonic [51], while branched aldehydes are originating mainly by Strecker-degradation of aminoacids [1], and also from the oxidation of methyl branched fatty acids, naturally present in low quantities in animal tissues [52]. The Iberian dry cured hams are characterized by the highest concentrations in 2-ketones and aldehydes, whereas the Toscana showed for carbonyl compounds the lowest concentrations with an exception in the case of acetaldehyde (Table 2).

Alcohols are considered as the second most important volatile compound in dry cured hams. Saturated linear (methanol, ethanol, 1-pentanol, 1-hexanol, and 1-heptanol) and branched alcohols (2 methylbutan-1-ol and 3-methybutan-1-ol) as well as unsaturated alcohols (1-octen-3-ol) have been reported as the most abundant alcohols present in Italian (Parma, Toscana and San Daniele) and Iberian dry cured hams [1,14,53]. In principle, they are produced by oxidation of the corresponding aldehydes [1], but they can also be generated by microbial activity [54]. Protonated methanol and ethanol are identified at m/z 33.033 and 47.049 respectively. Generally, alcohols undergo significant fragmentation under the present drift tube conditions: besides the protonated molecule, alkyl fragments are generated by the splitting off of water [48]. The signals at masses 41.038, 43.054, 57.074 and 71.085 have been reported as possible alkyl fragments originating from linear and branched saturated alcohols [48]. Moreover, a significant signal found at m/z 69.070 probably is originating from the fragmentation of 1-octen-3-ol which has been reported as the most abundant unsaturated alcohol both in Iberian and Italian dry cured hams [1,9,14,20,48,52]. The Iberian hams were characterized by the highest concentrations for both protonated alcohols (CH_5O^+ and $C_2H_7O^+$) and alkyl fragments potentially related to alcohols ($C_3H_7^+$, $C_4H_9^+$, $C_5H_9^+$ and $C_5H_{11}^+$). This observation finds support in the fact that in the case of Iberian hams the maturation is intense enough to promote the microbial oxidation of their precursor aldehydes [10]. In the case of Italian dry cured hams, the Toscano samples had the lowest concentrations for the previously specified masses. A comparison of the intensities of all peaks considered can be found in Table 2.

A significant number of masses corresponding to fragments from protonated fatty acids and esters fragments were identified during the headspace analysis of the dry cured hams using PTR-ToF-MS. Moreover, alkyl fragments originating from the fragmentation of fatty acids were also observed e.g. m/z 43.018 is attributed to $C_2H_3O^+$ which is generated by splitting off a water

Table 1
Confusion matrices for the three classification methods used in this works: Random Forest (RF), Penalized Discriminant Analysis (PDA) and Discriminant Partial Least Squares (dPLS) From left to right, the columns correspond to Iberian (I), Parma (P), San Daniele (SD) and Toscana (T) hams. In Bold non-zero values.

RF	I	P	SD	T	PDA	I	P	SD	T	dPLS	I	P	SD	T
I	**27**	0	0	0	I	**27**	0	0	0	I	**27**	0	0	0
P	0	**36**	0	0	P	0	**36**	0	0	P	0	**36**	0	0
SD	0	0	**35**	**1**	SD	0	0	**36**	0	SD	0	0	**35**	**1**
T	0	0	0	**39**	T	0	0	0	**39**	T	0	0	0	**39**

Table 2
A selection of the peaks identified in the PTR-TOF-MS spectrum that are either characterized by high intensities or are significantly different ($p < 0.05$) among the different hams and are discussed in the text. In total more that thousand peaks can be detected. Average concentrations sharing the same apex are not significantly different ($p = 0.05$).

Measured, m/z	Theoretical, m/z	Protonated chemical formula	Tentative identification	Volatile organic compounds headspace concentration (ppbv)				References
				Iberian	Parma	San Daniele	Toscana	
33.033	33.0335	CH_5O^+	Methanol	1028 ± 22c	836 ± 27b	824 ± 25b	709 ± 26a	[1]
41.038	41.0386	$C_3H_5^+$	Alkyl fragment	1761 ± 62c	357 ± 10a	519 ± 32b	264 ± 8a	–
42.034	42.0344	$C_2H_4N^+$	Acetonitrile	353 ± 13b	7.90 ± 0.47a	10.8 ± 0.6a	6.64 ± 0.60a	[3]
43.018	43.0178	$C_2H_3O^+$	Alkyl fragment	343 ± 29b	202 ± 7a	294 ± 42b	192 ± 9a	–
43.054	43.0542	$C_3H_7^+$	Alkyl fragment	1714 ± 66c	246 ± 12ab	352 ± 49b	160 ± 5a	–
45.033	45.0334	$C_2H_5O^+$	Acetaldehyde	11882 ± 910b	2305 ± 182a	2931 ± 427a	3900 ± 177a	[1,6,8,9]
47.048	47.0491	$C_2H_7O^+$	Ethanol	26583 ± 2048c	15989 ± 1608b	6960 ± 715a	4837 ± 290a	[1,6,7,9]
47.012	47.0128	$CH_3O_2^+$	Formic acid/Formates	30.9 ± 1.8c	23.5 ± 0.6b	21.1 ± 0.3b	10.6 ± 0.2a	[1]
49.010	49.0106	CH_5S^+	Methanethiol	72.7 ± 2.6a	191 ± 11b	300 ± 29c	116 ± 12a	[1,6,8,10]
55.054	55.0542	$C_4H_7^+$	Alkyl fragment	442 ± 19c	372 ± 12b	387 ± 27bc	227 ± 13a	–
57.070	57.0699	$C_4H_9^+$	Alkyl fragment	779 ± 84b	131 ± 9a	119 ± 11a	94.1 ± 3.9a	–
59.048	59.0491	$C_3H_7O^+$	2-propanone/propanal	8997 ± 558c	4976 ± 350b	4890 ± 479b	1773 ± 130a	[1,5–7,9]
61.028	61.0284	$C_2H_5O_2^+$	Acetic acid/Acetates	241 ± 11b	92.0 ± 3.9a	136 ± 19a	142 ± 15a	[1,3,5–7,9]
63.027	63.0268	$C_2H_7S^+$	Dimethylsulfide	42.8 ± 2.2d	8.85 ± 0.57a	32.1 ± 1.6c	16.7 ± 0.9b	[1,3,6]
69.070	69.0698	$C_5H_9^+$	Alkyl fragment	826 ± 55d	315 ± 11b	552 ± 31c	212 ± 9a	–
71.085	71.0855	$C_5H_{11}^+$	Alkyl fragment	206 ± 15c	105 ± 5b	128 ± 17b	44.7 ± 1.9a	–
73.065	73.0648	$C_4H_9O^+$	2-butanone/butanal	1901 ± 104b	295 ± 15a	271 ± 10a	230 ± 6a	[1,2,4–9]
75.026	75.0263	$C_3H_7S^+$	2-propane-1-thiol	10.7 ± 1.1a	9.2 ± 0.5a	22.5 ± 4.3a	13.7 ± 0.9a	[6]
75.044	75.0441	$C_3H_7O_2^+$	Propionic acid/Propanates	50.8 ± 3.2b	26.9 ± 0.9a	31.2 ± 1.6a	33 ± 1.2a	[5,6,9]
81.070	81.0699	$C_6H_9^+$	Alkyl fragment	15.7 ± 0.3a	32.3 ± 1.3c	33.3 ± 1.2c	24.4 ± 0.7b	–
83.086	83.0855	$C_6H_{11}^+$	Alkyl fragment	253 ± 18a	415 ± 16b	415 ± 32b	207 ± 14a	[9]
87.045	87.0441	$C_4H_7O_2^+$	2,3-Butanedione	50.5 ± 7.7b	22.6 ± 0.7a	33.1 ± 2.9a	32.1 ± 1.7a	[1,2,5,7,9,10]
87.081	87.0804	$C_5H_{11}O^+$	2-Pentanone/Pentanal	1246 ± 88c	260 ± 13a	465 ± 27b	121 ± 5a	[1–5,7,9,10]
89.060	89.0597	$C_4H_9O_2^+$	Butanoic acid/Butyrates/Acetoin	139 ± 25b	95 ± 8a	136 ± 20b	135 ± 9b	[1–7,9]
95.016	95.0167	$C_2H_7O_2S^+$	Dimethylsulfone	4.82 ± 0.3a	30.8 ± 6.6b	8.62 ± 0.89a	3.86 ± 0.27a	[1]
98.096	98.0964	$C_6H_{12}N^+$	Hexanenitrile	9.40 ± 0.42d	6.41 ± 0.40c	4.61 ± 0.14b	1.66 ± 0.05a	[3]
101.061	101.0597	$C_5H_9O_2^+$	2,3-Pentanedione	24.3 ± 0.83c	18.5 ± 0.2b	23.8 ± 1.2c	13.9 ± 0.2a	[2]
101.096	101.0961	$C_6H_{13}O^+$	2-Hexanone/Hexanal	122 ± 8b	175 ± 6c	200 ± 18c	46.5 ± 3.1a	[1–7,9,10]
103.076	103.0754	$C_5H_{11}O_2^+$	Isobutyric acid Pentanoic acid/Pentanoates	16.9 ± 1.2b	7.09 ± 0.53a	12.4 ± 2.8ab	14.3 ± 1.1b	[1,5–9]
105.038	105.0369	$C_4H_9OS^+$	3-Methylthio-propanal	2.04 ± 0.06c	1.98 ± 0.05bc	1.81 ± 0.08b	1.61 ± 0.05a	[3,8,10]
107.050	107.0499	$C_7H_7O^+$	Benzaldehyde	16.9 ± 0.6c	10.9 ± 0.4a	13.1 ± 0.4b	10.8 ± 0.4a	[4,6–8]
115.113	115.1117	$C_7H_{15}O^+$	2-Heptanone/heptanal	254 ± 13c	48.4 ± 2.0b	57.1 ± 3.9b	22.3 ± 1.4a	[1–7,9]
117.093	117.0910	$C_6H_{13}O_2^+$	Hexanoic/hexanoates	22.3 ± 1.8b	12.4 ± 0.7a	12.7 ± 0.5a	29.6 ± 2.2c	[1–7,9]
121.065	121.0651	$C_8H_9O^+$	Acetophenone	65.5 ± 8.7b	20.5 ± 3.3a	21.6 ± 2.4a	8.90 ± 0.42a	[1]
129.128	129.1274	$C_8H_{17}O^+$	2-Octanone/Octanal	40.2 ± 2.3c	29.1 ± 0.6b	38.0 ± 3.0c	7.74 ± 0.30a	[1–7,9]
131.106	131.1067	$C_7H_{15}O_2^+$	Heptanoic acid/Heptanoates	5.47 ± 0.54b	5.22 ± 0.20b	3.16 ± 0.17a	3.22 ± 0.12a	[4]
137.134	137. 1325	$C_{10}H_{17}^+$	Terpenes	3.10 ± 0.08a	7.01 ± 0.68b	6.54 ± 0.30b	4.49 ± 0.47a	[1,3,6,7,9]
143.110	107.1066	$C_8H_{15}O^+$	2,3-Octanedione/2,4-Octadienal	4.47 ± 0.12a	14.1 ± 0.4c	13.9 ± 0.8c	10.5 ± 0.5b	[1,4]
143.144	143.1430	$C_9H_{19}O^+$	2-Nonanone/Nonanal	67.7 ± 4.5b	63.8 ± 4.7b	94.2 ± 5.1c	19.1 ± 1.5a	[4,7,9]
145.123	145.1223	$C_8H_{17}O_2^+$	Octanoic acid/Octanoates	7.88 ± 0.45b	7.34 ± 0.44b	11.6 ± 0.9c	3.24 ± 0.11a	[2,4–6]
155.142	155.1430	$C_{10}H_{19}O^+$	Terpene alcohol	1.74 ± 0.10b	3.87 ± 0.26c	4.20 ± 0.28c	0.95 ± 0.04a	[1,6,9]
159.139	159.1380	$C_9H_{19}O_2^+$	Nonanoic acid/Nonanoates	1.16 ± 0.08a	2.28 ± 0.13b	1.35 ± 0.13a	0.97 ± 0.08a	[4,6]

[1] = Gaspardo et al. [14], [2] = Garcia et al. [1], [3] = Ruiz et al. [10], [4] = Pugliese et al. [16], [5] = Bolzoni et al. [9], [6] = Muriel et al. [7], [7] = Luna et al. (2006), [8] = Jurado et al. [11], [9] = Sabio et al. [18], and [10] = Carrapiso et al. [62].

molecule from acetic acid [48]. Although fatty acids and esters are not considered as the most abundant volatile compounds present in dry cured hams, methyl acetate, butanoic acid/butanoates, isobutyric acid/pentanoates and hexanoic acid/hexanoates have been reported by several researchers [1,9,10,14]. Moreover, significant differences in the profile of volatiles fatty acids and esters in dry cured hams are due to production aspects (rearing, salting, curing) and geographical origin and ripening process of raw meat, and were observed. For instance, Gaspardo et al. [14] reported propyl and amyl formates as the most abundant esters present in San Daniele hams whereas butanoates and hexanoates are considered as the most important esters in the case of Iberian and Parma hams [1,9,10]. Fatty acids are the main products formed from the lipolysis by the enzymatic action of muscle lipases and phospholipases on the lipids during the maturation of hams, and it is thought that they act as precursors for the formation of other flavour volatiles including esters [10,47,54,55]. According to the PTR-ToF-MS data, acetic acid/acetates (m/z 61.028) has been found to be the most abundant followed by butyric acid/butanoates (m/z 89.060), formic acid/formates (m/z 47.012) and propionic acid/propanoates (m/z 75.044). The Iberian and San Daniele dry cured hams were determined as the richest in volatile fatty acids and esters (Table 2). Toscana hams, characterized by the lowest concentrations for all the volatiles analyzed, present the highest values for the C4, C5 and C6 acids and the relative esters.

Terpenes are minor volatile compounds usually found in dry cured hams and are originating from the unsaponifiable fraction of the vegetable present in the feed and accumulated in pig fat [56,57]. Limonene, the most common forage related terpene, can be identified at m/z 137.134 [58]. However, the presence of other terpenes such as α-pinene, β-pinene and 2-carene has been also reported by other researchers in dry cured hams [10,16]. The highest terpenes concentration was observed in Parma and San Daniele hams whereas the lowest in Iberian ones. Terpene alcohols (α-terpineol) detected at m/z 155.142 is one of the major volatile compound groups found in black pepper and it was expected to be found in high concentrations in Italian hams given that they were treated with pepper [59]. Our results did not reveal any significant increase of the signal abundance measured at m/z 155.142 for Toscano hams, whereas as San Daniele and Parma hams were characterized by the highest terpineol concentrations.

Some sulfur and nitrogen containing compounds, found in dry-cured hams by other authors using gas-chromatography technique [10,14,15,18,47,54,60,61] were also tentatively identified here. These compounds are not always found in dry-cured ham, probably because their low concentration necessitates the use of specific techniques to detect them [60,61]. Nevertheless, these volatiles are potent odour-active compounds with a low odour threshold, so they have a remarkable effect on the dry-cured ham odour. PTR-ToF-MS has the ability to detect sulfur compounds with very low detection thresholds e.g. dimethylsulfide has been also reported in previous studies [27,28]. Methanethiol (CH_5S^+, m/z 49.011) and dimethylsulfide (m/z 63.027) have been found to be the most abundant sulfur compounds, the first being highest in Italian hams, especially in San Daniele, whereas the second is highest in Iberian ones. Methanethiol is believed to result from the degradation of L-methionine by microbial cultures [62], and it can interact with other molecules to form diverse sulfur compounds during the maturation step [54]. The lower methanethiol concentration in Iberian ham samples (72.7 ± 2.6 ppbv) compared to the mean concentration of Italian ones (200 ± 13 ppbv) might be related to the longer maturation period of the first. The former assumption finds support in the mean data of dimethylsulfide (42.8 ± 2.2 ppbv for Iberian and 27.4 ± 1.6 for Italian) and dimethylsulfone (4.82 ± 0.31 for Iberian and 14.2 ± 2.4 for Italian), though it was not the fact in the case of 2-propane-1-thiol ($C_3H_7S^+$, 75.026) and 3-methylthio-propanal ($C_4H_9OS^+$, 105.038). Thus, the data suggest that differences in the maturation duration alone cannot provide an adequate mechanistic explanation for the observed trends in sulfur compounds, but also synergistic effects from other parameters e.g. raw meat characteristics, salting etc. must be also considered.

The presence of nitrogen containing compounds is related to the degradation of aminoacids during processing i.e. the decarboxylation of aminoacids under low pH values, and the nitrite–aldehydes interaction as result of the nitrates addition [63]. Several nitrogen compounds have been reported in previous studies including pyrazines, nitrile compounds and amines. The signals at m/z 42.034, and 98.096 were attributed to acetonitrile and hexanenitrile respectively, with Iberian hams showing the highest concentrations. Nitrile compounds have been detected in sausages [64], dry-cured ham [2], dry-cured pork loin [54] and nitrite-cured cooked pork [65]. The latter proposed their formation at the expense of the corresponding aldehydes during lipid oxidation involving nitrite [66]. The formation of nitrile compounds involving nitrite could explain the higher amount of acetonitrile and hexanenitrile in Iberian hams, the only product with added nitrates.

4. Conclusions

On the one side, this study confirms the possibility to implement PTR-ToF-MS as an alternative, rapid and non-invasive method for the characterization and classification of dry cured hams on the basis of their flavour profile. Firstly, PTR-ToF-MS can efficiently classify dry cured ham samples according to the geographical region and production process and, secondly, it provides chemical information (at least sum formula and concentration) on many volatile compounds and a tentative identification, also on the basis of available literature is often possible. Of particular interest is the possibility to identify important nitrogen and sulfur compounds in a very rapid and direct way.

On the other side, the paper presents data on the characterization of the volatile organic compounds profile of dry cured hams demonstrating a strong effect of the production procedures on the final quality. In particular the comparison among different Italian PDOs indicates that quite different product characteristics can be obtained even when starting with the same material and with the same ripening time.

Given this, the availability of rapid, simple, highly sensitive techniques such as PTR-ToF-MS seems to be of interest for supporting food industry, and ham industry in particular, for product development, production, quality control and traceability. The proposed methodology is however not limited to food samples but can be applied to any case where volatile organic compounds play a relevant role.

Acknowledgements

This research was partially supported by the Provincia Autonoma di Trento (APR 2009/2010) and by MIUR-PRIN prot. 2007HCW9HN "Applicable requirements and perceived typicality of dry-cured ham" (E. Piasentier). José Sánchez del Pulgar was beneficiary of a grant from the Spanish Ministeio de Ciencia e Innovación.

References

[1] C. García, J.J. Berdagué, T. Antequera, C. López-Bote, J.C. Córdoba, J. Ventanas, Food Chem. 41 (1991) 23–32.
[2] J. Ruiz, J. Ventanas, J. Cava, M.L. Timón, C. García, Food Res. Int. 31 (1998) 53–58.
[3] A.I. Carrapiso, A. Jurado, C. Garcia, Food Sci. Technol. Int. 9 (2003) 409–419.
[4] I. Astiasarán, D. Ansorena, in: L. Nollet, F. Toldrá (Eds.), Handbook of Processed Meats and Poultry Analysis, CRC Press, Taylor and Francis, Boca Raton, 2009, pp. 69–90.
[5] F. Shahidi, in: F. Shahidi (Ed.), Flavor of Meat and Meat Products, Chapman and Hall Cornwall, UK, 1994, pp. 1–3.

[6] F. Toldrá, Meat Sci. 49 (1998) 101–110.
[7] E. Muriel, T. Antequera, M.J. Petrón, A.I. Andrés, J. Ruiz, Meat Sci. 68 (2004) 391–400.
[8] A.I. Carrapiso, F. Bonilla, C. García, Meat Sci. 65 (2003) 623–629.
[9] L. Bolzoni, G. Barbieri, R. Virgili, Food Chem. 43 (1996) 301–310.
[10] J. Ruiz, J. Ventanas, J. Cava, A. Andrés, C. García, Meat Sci. 52 (1999) 19–27.
[11] A. Jurado, C. Garcia, M.L. Timón, A.I. Carrapiso, Meat Sci. 75 (2007) 585–594.
[12] M. Flores, C.C. Grimm, F. Toldrà, A.M. Spaniel, J. Agric. Food Chem. 45 (1997) 2178–2186.
[13] G. Pastorelli, S. Magni, R. Rossi, E. Paglierini, P. Baldini, P. Dirink, F. Van Opstaele, C. Corino, Meat Sci. 56 (2003) 571–580.
[14] B. Gaspardo, G. Procido, B. Toso, B. Stefanon, Meat Sci. 80 (2008) 204–209.
[15] L. Pizzale, C. Crosera, I. Fenderico, O. Boschelle, L. Conte, in: E. Piasentier (Ed.), Qualità e sicurezza nella filiera del prosciutto (Quality and safety in prosciutto production chain), Nuove Arti Grafiche, Trento, Italy, 2009, pp. 158–167.
[16] C. Pugliese, F. Sirtori, L. Calamai, O. Franci, J. Mass Spectrom. 45 (2010) 1056–1064.
[17] P. Dirinck, F. Van Opstaele, F. Vandendriessche, Food Chem. 59 (1997) 511–521.
[18] E. Sabio, M.C. Vidal-Aragon, M.J. Bernalte, J.L. Gata, Food Chem. 61 (1998) 493–503.
[19] C.S. Sánchez-Peña, G. Luna, D.L. Garcìa-Gonzalez, R. Aparicio, Meat Sci. 69 (2005) 635–645.
[20] G. Luna, R. Aparicio, D.L. Garcìa-Gonzalez, Food Chem. 97 (2005) 621–630.
[21] S. López-Feria, S. Cárdenas, M. Valcárcel, TrAC Trends Anal. Chem. 27 (2008) 794–803.
[22] F. Biasioli, F. Gasperi, E. Aprea, L. Colato, E. Boscaini, T.D. Märk, Int. J. Mass Spectrom. 223–224 (2003) 343–353.
[23] F. Gasperi, E. Aprea, F. Biasioli, S. Carlin, I. Endrizzi, G. Pirretti, S. Spilimbergo, Food Chem. 115 (2009) 129–136.
[24] F. Biasioli, F. Gasperi, E. Aprea, I. Endrizzi, V. Framondino, F. Marini, D. Mott, T.D. Märk, Food Qual. Pref. 17 (2006) 63–75.
[25] E. Aprea, F. Biasioli, F. Gasperi, T.D. Märk, S. van Ruth, Flavour Fragr. J. 21 (2006) 53–58.
[26] E. Aprea, F. Biasioli, F. Gasperi, D. Mott, F. Marini, T.D. Märk, Int. Dairy J. 17 (2007) 226–234.
[27] A. Fabris, F. Biasioli, P.M. Granitto, E. Aprea, L. Cappellin, E. Schuhfried, C. Soukoulis, T.D. Mark, F. Gasperi, I. Endrizzi, J. Mass Spectrom. 45 (2010) 1065–1074.
[28] C. Soukoulis, E. Aprea, F. Biasioli, L. Cappellin, E. Schuhfried, T.D. Märk, F. Gasperi, Rapid Commun. Mass Spectrom. 24 (2010) 2127–2134.
[29] N. Araghipour, J. Colineau, A. Koot, W. Akkermans, J.M. Moreno Rojas, J. Beauchamp, A. Wisthaler, T.D. Märk, G. Downey, C. Guillou, L. Mannina, S. Van Ruth, Food Chem. 108 (2008) 374–383.
[30] E. Aprea, F. Biasioli, S. Carlin, G. Versini, T.D. Märk, F. Gasperi, Rapid Commun. Mass Spectrom. 21 (2007) 2564–2572.
[31] R.S. Blake, P.S. Monks, A.M. Ellis, Chem. Rev. 109 (2009) 861–896.
[32] A. Jordan, S. Haidacher, G. Hanel, E. Hartungen, J. Herbig, L. Märk, R. Schottkowsky, H. Seehauser, P. Sulzer, T.D. Märk, Int. J. Mass Spectrom. 286 (2009) 32–38.
[33] B. Renaville, E. Piasentier, B. Fan, M. Vitale, A. Prandi, M.F. Rothschild, Meat Sci. 85 (2010) 441–445.
[34] J. Flores, F. Toldrà, in: R. Macrae, R. Robinson, M. Sadle, G. Fulleslove (Eds.), Encyclopedia of Food Science, Academic Press, London, 1993, pp. 1977–1982.
[35] L. Cappellin, F. Biasioli, A. Fabris, E. Schuhfried, C. Soukoulis, T.D. Mark, Int. J. Mass Spectrom. 209 (2010) 60–63.
[36] L. Cappellin, F. Biasioli, P. Granitto, E. Schuhfried, C. Soukoulis, F. Costa, T.D. Märk, F. Gasperi, Sens. Actuators B-Chem., in press, doi:10.1016/j.snb.2010.11.044.
[37] W. Lindinger, A. Hansel, A. Jordan, Chem. Soc. Rev. 27 (1998) 347–354.
[38] J. Zhao, R. Zhang, Int. J. Mass Spectrom. 38 (2004) 2177–2185.
[39] L. Cappellin, M. Probst, J. Limtrakul, F. Biasioli, E. Schuhfried, C. Soukoulis, T.D.T.D. Märk, F. Gasperi, Int. J. Mass Spectrom. 295 (2010) 43– 48.
[40] I. Jolliffe, Principal Component Analysis, 2nd ed., Springer, New York, 2002.
[41] L. Breiman, Random forests, Mach. Learn. 45 (2001) 5–32.
[42] P. Granitto, F. Biasioli, E. Aprea, D. Mott, C. Furlanello, T. Märk, F. Gasperi, Sens. Actuators B-Chem. 121 (2007) 379–385.
[43] I. Witten, E. Frank, Data Mining: Practical Machine Learning Tools and Techniques, 2nd ed., Morgan Kaufman, Amsterdam, Boston MA, 2005.
[44] T. Hastie, A. Buja, R. Tibshirani, Ann. Stat. 23 (1995) 73.
[45] S. Wold, M. Sjostrom, L. Eriksson, Chemom. Intell. Lab. Syst. 58 (2001) 109–130.
[47] P.M. Granitto, F. Gasperi, F. Biasioli, E. Trainotti, C. Furlanello, Food Qual. Pref. 18 (2007) 681–687.
[48] A.I. Andrès, R. Cava, J. Ruiz, J. Chromatogr. A 963 (2002) 83–88.
[49] K. Buhr, S. van Ruth, C. Delahunty, Int. J. Mass Spectrom. 221 (2002) 1–8.
[50] E. Aprea, F. Biasioli, T.D. Mark, F. Gasperi, Int. J. Mass Spectrom. 262 (2007) 114–117.
[51] C. Yeretzian, A. Jordan, W. Lindinger, Int. J. Mass Spectrom. 223–224 (2003) 115–119.
[52] E.N. Frankel, W.E. Neff, E. Selke, Lipids 16 (1981) 267–285.
[53] J.L. Berdagué, C. Denoyer, J.L. Le Quere, E. Semon, J. Agric. Food Chem. 39 (1991) 1257–1261.
[54] M.L. Timòn, J. Verdanas, A.I. Carrapiso, A. Jurado, C. Garcia, Meat Sci. 58 (2001) 85–91.
[55] A. Martín, J.J. Córdoba, M.J. Benito, E. Aranda, M.A. Asensio, Int. J. Food Microbiol. 84 (2003) 327–338.
[56] C. Coutron-Gambotti, G. Gandemer, Food Chem. 64 (1999) 95–101.
[57] S. Buscailhon, Influence des caractéristiques de la matière première sur les qualities organoleptiques du Jambon sec, Ph.D. Thesis, Universitè Blaise Pascal, Clermont-Ferrand II, 1992.
[58] S. Buscailhon, J.L. Berdagué, G. Monin, J. Sci. Food Agric. 63 (1993) 69–75.
[59] A. Tani, S. Hayward, C.N. Hewitt, Int. J. Mass Spectrom. 223–224 (2003) 561–578.
[60] L. Jirovetz, G. Buchbahuer, M.B. Ngassoum, M. Geissler, J. Chromatogr. A 976 (2002) 265–275.
[61] A.I. Carrapiso, J. Ventanas, C. Garcia, J. Agric. Food Chem. 50 (2002) 1996–2000.
[62] A.I. Carrapiso, A. Jurado, M.L. Timón, C. García, J. Agric. Food Chem. 50 (2002) 6453–6458.
[63] H.E. Spinnler, C. Berger, C. Lapadatescu, P. Bonnarme, Int. Dairy J. 11 (2001) 245–252.
[64] R. Dainty, H. Blom, in: G. Campbell-Platt, P.E. Cook (Eds.), Flavour Chemistry of Fermented Sausages, Blackie Academic and Professional, London, 1995, pp. 176–193.
[65] L.H. Stanhke, Meat Sci. 41 (1995) 193–209.
[66] D.S. Mottram, S.E. Croft, R.L.S. Patterson, J. Sci. Food Agric. 35 (1984) 233–239.