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A new HS-SPME-GC-MS analytical method to identify and quantify compounds responsible for changes in the volatile profile in five types of meat products during aerobic storage at 4 °C

Laura Acquaticci [a], Simone Angeloni [a], Cecilia Baldassarri [a], Gianni Sagratini [a], Sauro Vittori [a], Elisabetta Torregiani [a], Riccardo Petrelli [a], Giovanni Caprioli [a,*]

[a] Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Via Madonna delle Carceri, I-62032 Camerino, MC, Italy

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ABSTRACT

Nowadays, it is important to monitor the freshness of meat during storage to protect consumers' health. Volatile organic compounds (VOCs) are responsible for odour and taste of food, and they give an indication about meat quality and freshness. This study had the aim to seek and select potential new markers of meat spoilage through a semi-quantitative analysis in five types of meat (beef, raw and baked ham, pork sausage and chicken) and then to develop a new quantitative analytical method to detect and quantify potential markers on five types of meat simultaneously. Firstly, a new headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC–MS) method was developed to evaluate the volatile profile of five types of meat, preserved at 4 °C for 5 days. Among the 40 compounds identified, 15 were chosen and selected as potential shelf-life markers on the basis of their presence in most of meat samples or/and for their constant increasing/decreasing trend within the sample. Afterwards, a quantitative HS-SPME-GC–MS analytical method was developed to confirm which VOCs can be considered markers of shelf-life for these meat products, stored at 4 °C for 12 days. Some of the compounds analyzed attracted attention as they can be considered markers of shelf-life for at least 4 types of meat: 1-butanol, 3-methylbutanol, 1-hexanol, 2-nonanone, nonanal, 1-octen-3-ol and linalool. In conclusion, in this study a new quantitative HS-SPME-GC–MS analytical method to quantity 15 VOCs in five types of meat was developed and it was demonstrated that some of the compounds quantified can be considered markers of shelf-life for some of the meat products analyzed.

1. Introduction

Nowadays, it is important to monitor the freshness of meat during storage to protect consumers' health in view of the increase in meat consumption expected in the coming years. In fact, in recent years, the global meat consumption has been increased and it is expected to raise of 14 % by 2030, mainly due to the demographic growth. Moreover, among meat products, poultry meat will be the most involved in this increment, because it's commonly perceived by consumers as healthier when compared to the other types (OECD/FAO, 2021).

Volatile organic compounds (VOCs) are responsible for odour and taste of food, and they give an indication of the meat quality and freshness (Mottram et al., 1998). In fact, changes in the organoleptic characteristics in food products, e.g., odours and colour, are usually related to a certain degree of spoilage and by consequence in an unsuitability of the product for the human consumption (Huis in't Veld et al., 1996; Pellissery et al., 2019). Meat spoilage can be the consequence of the bacterial metabolism, which increases during the storage with the increase of bacterial growth and produces undesirable VOCs (Casaburi et al., 2015). Meat spoilage can be also the consequence of some metabolic changes in meat constituents, such as lipid autoxidation (which leads to the formation of aldehydes, hydrocarbons, alcohols and ketones), microbial esterification (which leads to the formation of esters), amino-acids catabolism (which leads to the formation of aldehydes

* Corresponding author at: Giovanni Caprioli, Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Via Madonna delle Carceri 9/B, I-62032 Camerino, Italy.

E-mail addresses: laura.acquaticci@unicam.it (L. Acquaticci), simone.angeloni@unicam.it (S. Angeloni), cecilia.baldassarri@unicam.it (C. Baldassarri), gianni.sagratini@unicam.it (G. Sagratini), sauro.vittori@unicam.it (S. Vittori), elisabetta.torregiani@unicam.it (E. Torregiani), riccardo.petrelli@unicam.it (R. Petrelli), giovanni.caprioli@unicam.it (G. Caprioli).

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and alcohols such as 2-methylbutanal, 2-methyl-1-butanol) (Andrade et al., 2010; Lorenzo et al., 2012; Meynier et al., 1999; Olesen et al., 2004; Summo et al., 2011). These VOCs can be used as markers of meat spoilage degree to predict the suitability of the product for the human consumption (Tománková et al., 2012; Wojnowski et al., 2018; Chmiel et al., 2020; Mikš-Krajnik et al., 2016; Mikš-Krajnik et al., 2015).

The innovation of this research was firstly to perform a semi-quantitative analysis of the main VOCs occurring in different types of meat (beef, raw ham, baked ham, pork sausage and chicken) with the aim to seek and select potential new markers of meat spoilage and then to the develop a new quantitative analytical method that is able to detect and quantify potential markers on five types of meat simultaneously.

In literature, there are few articles on the volatile profile of raw ham (Sabio et al., 1998; Ruiz et al., 1998; Gianelli et al., 2002; Garcia-Esteban et al., 2004; Pham et al., 2008), beef (Machiels et al., 2003), and some of them are focused on the study of shelf-life of poultry meat (Tománková et al., 2012; Wojnowski et al., 2018; Chmiel et al., 2020; Mikš-Krajnik et al., 2016; Mikš-Krajnik et al., 2015) and beef meat (Bhattacharjee et al., 2011; Argyri et al., 2015; Franke et al., 2019; Bueno et al., 2019; Mansur et al., 2019; Frank et al., 2020). Moreover, to the best of our knowledge, few studies were conducted to perform a quantitative analysis of VOCs in meat (Argyri et al., 2015) and there are no quantitative analyses of VOCs in different types of meat to find possible markers of shelf-life.

Headspace-solid phase microextraction (HS-SPME) technique combined with gas chromatography-mass spectrometry (GC–MS) system were chosen for the determination of VOCs in all meat samples preserved at 4 °C. The semi-quantitative analysis was conducted for five days (at day 0, day 2 and day 5), while the quantitative analysis was carried out until the 12th day (at day 0, day 3, day 6, day 9 and day 12).

2. Materials and methods

2.1. Chemicals

Sodium sulphate (Na_2SO_4) and sodium hydrogen carbonate ($NaHCO_3$) were purchased by J. T. Baker. Sodium chloride (NaCl) was purchased by Chem-lab. Sodium phosphate monobasic (Na_2HPO_4), the alkane mixture (C_7-C_{30}) and pure standards of hexanal (CAS 66-25-1), 1-butanol (CAS 71-36-3), 3-methylbutanol (CAS 123-51-3), 2-pentylfuran (CAS 3777-69-3), 1-pentanol (CAS 71-41-0), acetoin (CAS 513-86-0), octanal (CAS 124-13-0), 1-hexanol (CAS 111-27-3), 2-nonanone (CAS 821-55-6), nonanal (CAS 124-19-6), 1-octen-3-ol (CAS 3391-84-4), 1-heptanol (CAS 111-70-6), linalool (CAS 78-76-6), 1-octanol (CAS 111-87-5), 2-octen-1-ol (CAS 18409-17-1) and 2-methypentanal, used as internal standard (I.S.), (CAS 123-15-9) were purchased by Sigma Aldrich (Milano, Italy). The quantification of the 15 VOCs has been assessed by using authentic analytical standards. The calibration curves were prepared by plotting the standard solution concentrations by the respective Response Factor (RF). RF is the ratio between the peak area of analyte and the peak area of IS.

2.2. Meat samples

Fresh meat samples were bought in a local supermarket and immediately stored during the purchase in the packaging furnished by the store at 4 °C. Five types of meat were chosen: beef, raw ham, baked ham, pork sausage, and chicken. Samples were cut, triturated and weighted in the vial and immediately analyzed. The analyses were performed at days 0, 2 and 5 for the semi-quantitative analysis and at days 0, 2, 6, 9 and 12 for the quantitative analysis.

2.3. Semi-quantitative analysis through HS-SPME-GC–MS

2.3.1. HS-SPME extraction conditions

The extraction was performed using a HS-SPME method and the analysis was conducted on a GC–MS instrument. Briefly, 3 g of sample were put in a 20 mL vial, added with 5 mL of NaCl saturated solution and it was tightly clapped with a PTFE/silicon septum. HS-SPME conditions were optimized: quantity of sample, salt addiction, incubation time, extraction time and temperature, type of fiber. 1 and 3 g of samples were weighted in the vial to choose the optimum quantity for the analysis. After that, the addiction of 5 mL of salt (i.e., Na_2SO_4, NaCl, Na_2HPO_4 and $NaHCO_3$) saturated solution were evaluated to determine the optimum starting conditions for the analysis. Then, samples were incubated at three different temperatures (40 °C, 50 °C and 60 °C) and at three different times (20, 30 and 40 min). Samples were stirred during incubation at 250 rpm with 5 s of on-time and 2 s of off-time. Afterwards, samples were extracted and three different extraction times were evaluated (20, 30 and 40 min). Two different fiber coatings were compared: a Divinylbenzene/Polydimethylsiloxane DVB-PDMS (65 μm) and a Divinylbenzene/Carbon-Wide Range/Polydimethylsiloxane DVB/C-WR/PDMS (80 μm) fibers. The fibers were conditioned for 10 min at 250 °C and then inserted inside the headspace of sample vial with a speed of 20 mm/s and a penetration depth of 35 mm. The extraction was performed and then the fiber was inserted into the injector port at a speed of 100 mm/s and a penetration depth of 40 mm. The desorption occurred at 250 °C for 2 min. After desorption, the fiber was conditioned at 250 °C for 5 min.

2.3.2. GC–MS analysis

GC–MS system was composed of an Agilent 8890 GC coupled to an Agilent 5977B MSD quadrupole detector with an electron ionization (EI) source (Santa Clara, CA, USA). The system was equipped with an autosampler PAL RTC 120 System. The injector temperature was set at 250 °C, and the liner used was recommended for SPME injection, namely, Inlet liner, Ultra Inert, splitless, straight, 0.75 mm id, from Agilent. The gas carrier was helium at flow rate of 1 mL·min^{-1}. A DB-WAX UI capillary column (60 m × 0.25 mm × 0.25 μm) was used. Thermal desorption was carried out at 250 °C in a splitless mode for 2 min. Oven temperature was set at 35 °C held for 3 min, increased up to 70 °C at 3 °C/min, increased up to 210 °C at 5 °C/min, increased up to 250 °C at 15 °C/min held for 10 min. The temperatures of the ionization source and the mass analyzer were set at 230 and 150 °C, respectively. The acquisition was carried out in SCAN mode (35–450 *m/z*).

Volatile compounds were identified through the comparison of their mass spectra with those of NIST library (US National Institute of Standards and Technology) in combination with the calculation of their experimental linear retention indexes, which have been compared to those reported in literature. Compounds abundances were deter-mined using the relative percentage of the area (%) of each peak that was calculated by dividing the area of each component by the total area of all separated components. Data results were managed using MSD ChemStation Software (Agilent, Version G1701DA D.01.00, Santa Clara, CA, USA). Samples were analysed in triplicate. Acceptable relative standard deviation (% RSD) were set up below 20 %.

2.4. Quantitative analysis through HS-SPME-GC–MS

2.4.1. Sample preparation and HS-SPME extraction conditions

The extraction was performed using a HS-SPME method and the analysis was conducted on a GC–MS instrument. Briefly, 5 g of samples were put in a 50 mL centrifuge tube and 15 mL of distilled water was added. Then, samples were homogenized with an Ultra Turrax S 18 N-10G homogenizer (IKA-Werke Gmbh & Co., Germany). After that, 1500 μL of water added with 25 % of NaCl and 40 μL of I.S. 50 mg/mL were added to 500 μL of supernatant in a 20 mL vial which was tightly clapped with a PTFE/silicon septum. The incubation of the sample was performed at 40 °C for 40 min under agitation (250 rpm, 5 s of on-time and 2 s of off-time). The grey fiber (DVB/C-WR/PDMS) from Supelco (Bellefonte, PA, USA) was selected for this work. The fiber was conditioned for 10 min at 250 °C and then, it was inserted inside the headspace of

sample vial with a speed of 20 mm/s and a penetration depth of 40 mm. The desorption occurred at 250 °C for 1 min. After desorption, the fibre was conditioning at 250 °C for 10 min.

2.4.2. GC–MS analysis

The GC–MS was the same used for the semi-quantitative analysis. The separation of target molecules was established on DB-WAX capillary column (60 m, 250 μm i.d., 0.25 μm film thickness) with the following ramp of temperature: 35℃ at 3 °C/min, increased up to 70℃ at 3℃/min, increased up to 210℃ at 5℃/min, increased up to 250℃ at 15℃/min, maintained for 10 min. The run time was about 66 min. The transfer line was set at 250 °C and the temperature of the ionization source and the mass analyser were set at 230 and 150 °C, respectively. The ion species selection and optimization for all the volatile compounds were carried out by injecting a standard solution (10 μg/mL) in SCAN mode (35–450 m/z) and the three most abundant ions were selected for each analyte. The acquisitions were carried out in 'Selected Ion Monitoring' (SIM) mode and detection was divided into time windows to enhance the sensitivity. The most abundant ions were used for quantitation, while the others to confirm the presence of the analytes. The GC–MS parameters including the retention time (Rt) and time windows are reported in **Table 1S (Supplementary material).** Data results were managed by MSD ChemStation Software (Agilent, Version G1701DA D.01.00). Volatile compounds were quantified using calibration curves. Samples were analysed in triplicate. Acceptable relative standard deviation (% RSD) were set up below 20 %.

2.5. Statistical analysis

The results were reported as the mean value ± standard deviation. Significant differences between data were calculated by unidirectional analysis of variance (ANOVA) followed by Tukey's post-hoc comparison test, with a significance level of $p < 0.05$.

Data on selected volatile compounds were examined by principal component analysis (PCA) using Statistica v.7.1 (Stat Soft Italia, Vigonza, Italy). A covariance data matrix including 15 variables and 25 samples (type of meat and different timepoints) was created and analysed to visualize correlation groups at different days of analysis for each type of meat analysed.

3. Results

3.1. HS-SPME-GC–MS semi-quantitative analytical method optimization

HS-SPME technique is one of the most common analytical approaches for the VOCs analysis because it is simple, cheap, solvent-free, easy to handle and very sensitive. Moreover, this technique best represents what happens inside the packaging due to the use of low extraction temperatures and the lack of preparation steps.

Chicken samples were used to optimize parameters before the analysis. 1 g and 3 g of sample were weighted into a 20 mL vial and analyzed to evaluate the best results. 1 g of chicken resulted to give less than 50 % of peaks when compared to 3 g. Moreover, the total area also resulted to be higher for 3 g with respect to 1 g. Amount higher than 3 g led to a fibre saturation with important loss of time for its cleaning. Statistical analysis performed through the ANOVA system revealed that values of total peak area were statistically significant. Then, 5 mL of satured solutions of sodium sulfate (Na_2SO_4), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4) and sodium bicarbonate ($NaHCO_3$) were added to 3 g of chicken. The addiction of sodium sulphate gave the highest total peak area, but sodium chloride was chosen as the best salt to be addicted because this resulted to give the highest number of peaks, even if with no statistically significant differences. Two fibers were tested: a divinylbenzene-polydimethylsiloxane fiber (DVB/PDMS) and a divinylbenzene/carbon-wide range/polydimethylsiloxane fiber (DVB/C-WR/PDMS). Results showed there were no statistically significant differences between the two fibers, anyway the DVB/C-WR/PDMS fiber was chosen because of its triple coating which generally makes it a broad spectrum one. The temperature was also optimized and chosen among 60 °C, 50 °C and 40 °C. The highest value of peak number was reached using a temperature of 40 °C with the highest corresponding total peak area value, which is statistically different when compared to the others. Three incubation times were evaluated: 20, 30 and 40 min. The highest values of number of peaks and total peak area were reached with an incubation time of 40 min with statistically significant differences. Finally, the extraction time was optimized, testing samples with 20, 30 and 40 min of extraction. The highest values of number of peaks and total peak area were reached with an extraction time of 20 min suggesting the equilibrium realization. **Table 2S** summarizes all the parameters monitored and optimized.

These results indicated that 3 g of meat sample, added with 5 mL of NaCl saturated solution, incubated for 40 min at 40 °C and extracted for 20 min with a DVB/C-WR/PDMS fiber were adequate conditions for this type of analysis.

3.2. Semi-quantitative analysis of VOCs in five types of meat through HS-SPME-GC–MS

Firstly, a semi-quantitative analysis was performed to select the main compounds that can be considered potential markers of meat spoilage and to subsequently include in the quantitative analytical method. The analysis was performed on five types of meat at day 0, 2 and 5. Table 1 summarizes the abundance of VOCs in meat samples.

In beef 23 compounds were identified, among which there are esters, hydrocarbons, alcohols, ketones, aldehydes, furans, ethers and organic acids. Ethanol, 1-butanol, 3-methylbutanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-octanol, 2-octen-1-ol and phenylethyl alcohol were the alcohols identified in beef. Peak areas of ethanol, 3-methylbutanol and phenylethyl alcohol increased from day 0 to day 5 and this was in agreement with their production. In fact, alcohols are the products of lipid oxidation (Pham et al., 2008), which is an increasing process with the degradation. In particular, linear alcohols are the products of the oxidative decomposition of fatty acids (Sánchez-Peña et al., 2005; Narváez-Rivas et al., 2012). The branched alcohols, instead, can be the product of the reduction of branched aldehydes, of the catabolism of aminoacids by Streker degradation (Pérez-Palacios et al., 2010; Domínguez et al., 2016b) or of the microbial activity that leads to an increased formation of branched aldehydes (Narváez-Rivas et al., 2012). For what concern the compounds identified, 1-hexanol comes from the reduction of hexanal (Montanari et al., 2018) and 1-octen-3-ol is the product of the autoxidation of polyunsaturated fatty acids, like linoleic acid (Pham et al., 2008). The aroma given by the presence of alcohols is due to their low odour threshold values (Lorenzo et al., 2014) and it is mainly characterized by herbaceous, woody and fatty notes (García and Timón, 2001; Lorenzo et al., 2013) and sweet, fruity or onion and mushroom like odours (Bosse et al., 2017). In particular, 1-octen-3-ol gives mushroom aroma (Purriños et al., 2012; Lorenzo et al., 2015; Petričević et al., 2018) and earth, dust, fatty, sharp and rancid odours (García-González et al., 2008; Théron et al., 2010). Moreover, the peak area of 1-hexanol had an overall increase, even if its increase was not constant. Among the ketones, 3-methyl-2-butanone, acetoin and 2-nonanone have been identified. The peak areas of acetoin and 2-nonanone increased from day 0 to day 5. This behaviour was in agreement with their production which involved microorganism or oxidation processes (Bosse et al., 2017; Petričević et al., 2018; Pastorelli et al., 2003). Among the aldehydes, hexanal, heptanal, octanal, nonanal have been identified and their peak areas decreased from day 0 to day 5. Aldehydes, in general, are the products of lipid oxidation or amino acid degradation and this means that their concentration should increase with the degradation of food. Aldehydes can be the result of lipid oxidation of fatty acids (linear aldehydes); in particular octanal and nonanal come from oleic acid autoxidation (Montanari et al., 2018), hexanal comes from linoleic,

Table 1
. Peak areas of VOCs in five types of meat analyzed on t0, t2 and t5 (n.d. means "not detected").

Class of compounds	No	*Beef*			*Raw Ham*			*Baked Ham*			*Sausage*			*Chicken*		
		Peak Area			**Peak Area**			**Peak Area**			**Peak Area**			**Peak Area**		
		T0	**T2**	**T5**	**T0**	**T2**	**T5**	**T0**	**T2**	**T5**	**T0**	**T2**	**T5**	**T0**	**T2**	**T5**
Esters	**1**	n.d.	1.1*10⁴ ± 11.47	6.4*10³ ± 8.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.1*10⁴ ± 11.47	6.4*10³ ± 8.41	n.d.	n.d.	n.d.
	2	n.d.	n.d.	7.1*10³ ± 9.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.1*10³ ± 9.45	n.d.	n.d.	n.d.
	3	n.d.	n.d.	n.d.	1.1*10⁴ ± 1.87	1.3*10⁴ ± 2.56	2.6*10⁴ ± 3.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.9*10⁴ ± 15.95	1.3*10⁴ ± 12.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	5	n.d.	n.d.	n.d.	3.4*10⁴ ± 39.84	4.2*10⁴ ± 41.29	3.3*10⁴ ± 37.51	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hydrocarbons	**6**	3.9*10⁴ ± 557.14	3.5*10⁴ ± 512.84	3.6*10⁴ ± 520.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.9*10⁴ ± 557.14	3.5*10⁴ ± 512.84	3.6*10⁴ ± 520.10	n.d.	n.d.	n.d.
	7	8.1*10⁴ ± 619.35	7.4*10⁴ ± 594.76	7.7*10⁴ ± 557.79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.1*10⁴ ± 619.35	7.4*10⁴ ± 594.76	7.7*10⁴ ± 557.79	n.d.	n.d.	n.d.
	8	2.6*10⁴ ± 284.44	2.2*10⁴ ± 266.11	1.8*10⁴ ± 216.55	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.6*10⁴ ± 284.44	2.2*10⁴ ± 266.11	1.8*10⁴ ± 216.55	n.d.	n.d.	n.d.
	9	1.2*10⁵ ± 34.74	9.9*10⁴ ± 12.55	8.9*10⁴ ± 28.79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.2*10⁵ ± 34.74	9.9*10⁴ ± 12.55	8.9*10⁴ ± 28.79	n.d.	n.d.	n.d.
	10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.4*10⁴ ± 124.61	4.0*10⁴ ± 138.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	11	6.8*10⁵ ± 446.21	6.6*10⁵ ± 428.11	6.9*10⁵ ± 657.52	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.8*10⁵ ± 446.21	6.6*10⁵ ± 428.11	6.9*10⁵ ± 657.52	n.d.	n.d.	n.d.
	12	3.6*10⁴ ± 351.54	3.4*10⁴ ± 317.24	3.5*10⁴ ± 345.31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.6*10⁴ ± 351.54	3.4*10⁴ ± 317.24	3.5*10⁴ ± 345.31	n.d.	n.d.	n.d.
Alcohols	**13**	4.7*10⁴ ± 194.75	5.5*10⁵ ± 395.14	5.1*10⁶ ± 373.52	1.1*10⁵ ± 325.48	1.3*10⁵ ± 360.12	5.3*10⁶ ± 451.95	4.5*10⁴ ± 125.75	1.9*10⁵ ± 341.84	4.9*10⁶ ± 516.95	4.7*10⁴ ± 194.75	5.5*10⁵ ± 395.14	5.1*10⁶ ± 373.52	6.3*10⁴ ± 254.61	1.2*10⁵ ± 349.86	5.3*10⁶ ± 579.16
	14	n.d.	n.d.	1.5*10⁴ ± 111.74	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5*10⁴ ± 111.74	n.d.	n.d.	n.d.
	15	n.d.	n.d.	n.d.	n.d.	1.1*10⁴ ± 8.73	3.0*10⁴ ± 18.73	n.d.	3.5*10⁴ ± 15.94	1.7*10⁴ ± 13.46	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	16	6.7*10³ ± 11.79	3.5*10⁴ ± 35.45	8.1*10⁴ ± 67.11	n.d.	n.d.	n.d.	n.d.	1.1*10⁴ ± 9.68	3.6*10⁵ ± 21.75	6.7*10³ ± 11.79	3.5*10⁴ ± 35.45	8.1*10⁴ ± 67.11	1.3*10⁴ ± 9.68	4.3*10⁴ ± 12.57	1.8*10⁵ ± 12.62
	17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.8*10⁴ ± 39.52	1.4*10⁴ ± 32.85	1.2*10⁴ ± 31.43
	18	1.6*10⁴ ± 134.11	4.2*10⁴ ± 193.44	2.9*10⁴ ± 169.84	6.8*10³ ± 178.86	6.8*10³ ± 170.50	7.0*10³ ± 192.30	n.d.	1.7*10⁴ ± 124.97	7.9*10³ ± 113.51	1.6*10⁴ ± 134.11	4.2*10⁴ ± 193.44	2.9*10⁴ ± 169.84	3.4*10⁴ ± 174.85	7.2*10⁴ ± 310.49	1.2*10⁵ ± 432.41
	19	2.8*10⁵ ± 323.88	2.8*10⁵ ± 365.40	1.5*10⁵ ± 299.75	n.d.	n.d.	n.d.	4.6*10⁴ ± 57.84	6.9*10⁴ ± 68.71	3.7*10⁴ ± 51.12	2.8*10⁵ ± 323.88	2.8*10⁵ ± 365.40	1.5*10⁵ ± 299.75	3.6*10⁴ ± 45.84	3.5*10⁴ ± 43.91	3.6*10⁴ ± 47.12
	20	n.d.	n.d.	8.1*10³ ± 24.16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.1*10³ ± 24.16	n.d.	n.d.	1.3*10⁴ ± 21.31
	21	n.d.	n.d.	n.d.	1.6*10⁴ ± 762.35	2.0*10⁴ ± 793.80	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.7*10⁵ ± 758.48	1.5*10⁵ ± 715.14	1.2*10⁵ ± 729.46
	22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.1*10⁴ ± 23.75	2.0*10⁴ ± 21.68

(*continued on next page*)

Table 1 (*continued*)

Class of compounds	No	*Beef*			*Raw Ham*			*Baked Ham*			*Sausage*			*Chicken*		
		Peak Area			**Peak Area**			**Peak Area**			**Peak Area**			**Peak Area**		
		T0	**T2**	**T5**	**T0**	**T2**	**T5**	**T0**	**T2**	**T5**	**T0**	**T2**	**T5**	**T0**	**T2**	**T5**
	23	3.6*10⁴ ± 147.81	2.6*10⁴ ± 135.75	2.3*10⁴ ± 131.55	n.d.	n.d.	n.d.	2.4*10⁴ ± 27.88	3.3*10⁴ ± 32.75	2.5*10⁴ ± 29.51	3.6*10⁴ ± 147.81	2.6*10⁴ ± 135.75	2.3*10⁴ ± 131.55	n.d.	n.d.	n.d.
	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.1*10⁴ ± 29.52	3.1*10⁴ ± 36.75
	25	2.4*10⁴ ± 77.54	1.8*10⁴ ± 75.11	1.4*10⁴ ± 65.95	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.4*10⁴ ± 77.54	1.8*10⁴ ± 75.11	1.4*10⁴ ± 65.95	n.d.	n.d.	n.d.
	26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.2*10⁴ ± 39.08	3.3*10⁴ ± 41.64	1.0*10⁴ ± 21.67
	27	1.2*10⁵ ± 84.56	9.8*10⁴ ± 75.65	7.1*10⁴ ± 71.99	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.2*10⁵ ± 84.56	9.8*10⁴ ± 75.65	7.1*10⁴ ± 71.99	n.d.	n.d.	n.d.
Aldehydes	**28**	n.d.	n.d.	n.d.	n.d.	2.0*10⁴ ± 127.59	2.3*10⁴ ± 138.75	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	29	2.5*10⁴ ± 284.11	2.3*10⁴ ± 271.65	n.d.	2.2*10⁴ ± 397.50	6.3*10⁴ ± 425.20	2.2*10⁴ ± 390.30	n.d.	n.d.	n.d.	2.5*10⁴ ± 284.11	2.3*10⁴ ± 271.65	n.d.	1.8*10⁵ ± 364.50	8.3*10⁴ ± 284.51	n.d.
	30	n.d.	n.d.	n.d.	8.5*10³ ± 52.86	1.5*10⁴ ± 71.20	9.3*10³ ± 61.24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31	2.3*10⁴ ± 469.54	1.6*10⁴ ± 448.45	n.d.	3.4*10⁴ ± 515.27	4.6*10⁴ ± 575.90	3.8*10⁴ ± 528.84	5.8*10⁴ ± 486.14	4.3*10⁴ ± 437.95	n.d.	2.3*10⁴ ± 469.54	1.6*10⁴ ± 448.45	n.d.	7.4*10⁴ ± 538.46	4.2*10⁴ ± 476.64	n.d.
	32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.7*10⁴ ± 127.11	3.3*10⁴ ± 139.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	33	n.d.	n.d.	n.d.	2.1*10⁴ ± 95.47	2.0*10⁴ ± 93.75	1.7*10⁴ ± 85.49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ketones	**34**	1.6*10⁴ ± 133.61	1.4*10⁵ ± 125.84	5.0*10⁴ ± 176.55	n.d.	n.d.	n.d.	n.d.	5.2*10⁴ ± 154.52	5.1*10⁵ ± 148.69	1.6*10⁴ ± 133.61	1.4*10⁵ ± 125.84	5.0*10⁴ ± 176.55	4.5*10⁴ ± 187.22	2.8*10⁵ ± 296.47	4.4*10⁵ ± 379.51
	35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5*10⁴ ± 56.15	1.2*10⁴ ± 62.75	2.8*10⁴ ± 84.91
	36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.3*10⁴ ± 68.81
	37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5*10⁵ ± 12.75	1.6*10⁵ ± 15.41	1.2*10⁵ ± 31.75	n.d.	n.d.	n.d.	n.d.	n.d.	1.0*10⁴ ± 10.76
Ethers	**38**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.1*10⁵ ± 7.12	1.1*10⁵ ± 5.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Organic acids	**39**	1.1*10⁵ ± 50.11	9.5*10⁴ ± 43.84	1.6*10⁵ ± 65.49	n.d.	n.d.	n.d.	3.2*10⁴ ± 54.69	3.0*10⁴ ± 52.15	7.1*10⁴ ± 75.42	1.1*10⁵ ± 50.11	9.5*10⁴ ± 43.84	1.6*10⁵ ± 65.49	n.d.	1.8*10⁴ ± 61.24	8.0*10⁴ ± 70.49
Furans	**40**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.9*10⁴ ± 79.40	2.2*10⁴ ± 82.75	6.4*10³ ± 61.42
Total identified (%)		93.33	70.24	92.46	70.93	90.48	96.12	84.98	86.17	93.16	75.4	72.14	94.29	90.11	83.07	96.18

1. Ethyl acetate (R.I. lit.: 884; R.I. calc.: 883); **2.** Ethyl esanoate (R.I. lit.: 1239; R.I. calc.: 1231); **3.** Ethyl octanoate (R.I. lit.: 1429; R.I. calc.: 1429); **4.** Isorbornyl acetate (R.I. lit.: 1582; R.I. calc.: 1588); **5.** Ethyl decanoate (R.I. lit.: 1637; R.I. calc.: 1638); **6.** β-pinene (R.I. lit.: 1108; R.I. calc.: 1106); **7.** 3-carene (R.I. lit.: 1146; R.I. calc.: 1148); **8.** α-phellandrene (R.I. lit.: 1177; R.I. calc.: 1164); **9.** D-limonene (R.I. lit.: 1198; R.I. calc.: 1195); **10.** Dodecane (R.I. lit.: 1200; R.I. calc.: 1197); **11.** Caryophyllene (R.I. lit.: 1610; R.I. calc.: 1610); **12.** Humulene (R.I. lit.: 1682; R.I. calc.: 1686); **13.** Ethanol (R.I. lit.: 933; R.I. calc.: 933); **14.** 2-methylpropanol (R.I. lit.: 1110;

R.I. calc.: 1100); **15.** 1-butanol (R.I. lit.: 1150; R.I. calc.: 1149); **16.** 3-methylbutanol (R.I. lit.: 1208; R.I. calc.: 1207); **17.** 1-pentanol (R.I. lit.: 1248; R.I. calc.: 1248); **18.** 1-hexanol (R.I. lit.: 1344; R.I. calc.: 1345); **19.** 2-butoxyethanol (R.I. lit.: 1402; R.I. calc.: 1397); **20.** 1-heptanol (R.I. lit.: 1440; R.I. calc.: 1447); **21.** 1-octen-3-ol (R.I. lit.: 1446; R.I. calc.: 1441); **22.** 2-ethyl-1-hexanol (R.I. lit.: 1484; R.I. calc.: 1482); **23.** Linalool (R.I. lit.: 1540; R.I. calc.: 1540); **24.** 1-octanol (R.I. lit.: 1555; R.I. calc.: 1552); **25.** Terpinen-4-ol (R.I. lit.: 1601; R.I. calc.: 1606); **26.** 2-octen-1-ol (R.I. lit.: 1612; R.I. calc.: 1612); **27.** α-terpineol (R.I. lit.: 1698; R.I. calc.: 1701); **28.** 3-methylbutanal (R.I. lit.: 912; R.I. calc.: 913); **29.** Hexanal (R.I. lit.: 1083; R.I. calc.: 1083); **30.** Octanal (R.I. lit.: 1287; R.I. calc.: 1283); **31.** Nonanal (R.I. lit.: 1389; R.I. calc.: 1387); **32.** Decanal (R.I. lit.: 1496; R.I. calc.: 1495); **33.** Benzaldehyde (R.I. lit.: 1528; R.I. calc.: 1523); **34.** Acetoin (R.I. lit.: 1277; R.I. calc.: 1278); **35.** 3-octanone (R.I. lit.: 1252; R.I. calc.: 1252); **36.** 2-nonanone (R.I. lit.: 1386; R.I. calc.: 1383); **37.** Camphor (R.I. lit.: 1526; R.I. calc.: 1526); **38.** Eucalyptol (R.I. lit.: 1209; R.I. calc.: 1209); **39.** Acetic Acid (R.I. lit.: 1435; R.I. calc.: 1437); **40.** 2-pentylfuran (R.I. lit.: 1228; R.I. calc.: 1228).

linolenic and arachidonic fatty acids oxidation (Montanari et al., 2018; Lorenzo et al., 2014; Lorenzo et al., 2015). Branched aldehydes, instead, are the products of proteolysis and amminoacids degradation (Narváez-Rivas et al., 2012; Lorenzo et al., 2014; Bosse et al., 2017) or of the metabolism of microorganisms, like Staphylococci (Ardö, 2006; Martín et al., 2006). In particular, 3-methylbutanal comes from the oxidative-deamination of leucine (Narváez-Rivas et al., 2012; Purriños et al., 2012; Pastorelli et al., 2003), while benzaldehyde is the product of Strecker degradation of some amino acids as leucine or phenylalanine (Lorenzo et al., 2015). Their presence is related to sweet, floral, grassy and fruity aroma for linear aldehydes (Sánchez-Peña et al., 2005) and to fruity, acorn-like, salty and cheesy aroma for branched aldehydes (Pastorelli et al., 2003; Muriel et al., 2004). In particular, hexanal gives unpleasant rancid aroma at high concentration and pleasant grassy aroma at low concentration (Lorenzo et al., 2014; Petričević et al., 2018; Benet et al., 2015), octanal gives meat-like, green, fresh, grass and fruity aroma (García-González et al., 2008; Carrapiso et al., 2010), nonanal gives sweet and fruity aroma (Nunes et al., 2008), benzaldehyde gives floral, acorn and bitter almond notes (García-González et al., 2008; Martínez-Onandi et al., 2017) and 3-methylbutanal gives fruity, acorn-like, salty and cheesy aroma (Pastorelli et al., 2003; Muriel et al., 2004). Acetic acid, whose peak area increased from day 0 to day 5, is an organic acid, which contribute to give a vinegar note to the meat product and it contributes to the ripened aroma (Domínguez et al., 2016; Marco et al., 2007). Its production could be related to the Maillard reaction (Martín et al., 2006; Andrade et al., 2010).

In raw ham a total of 11 compounds were identified: alcohols, aldehydes and esters. Ethanol, 1-butanol, 1-hexanol and 1-octen-3-ol are the alcohols identified in raw ham. Their peak areas increased from day 0 to day 5, as they are products of lipid oxidation, as described above, suggesting their possible involvement as markers of raw ham shelf-life. Among the aldehydes, 3-methylbutanal, hexanal, octanal, nonanal and benzaldehyde have been identified in raw ham. 3-Methylbutanal peak area increased from day 0 to day 5, that of octanal increased from day 0 to day 2, then it decreased from day 2 to day 5, while peak areas of hexanal, nonanal and benzaldehyde decreased from day 0 to day 5. Aldehydes, in general, are the products of lipid oxidation or amino acid degradation and this means that their concentration should increase with the degradation of food. In this case we found a different behaviour among the aldehydes and this is probably due to the oxidation of some aldehydes to carboxylic acids, of which only those with short-chain can be detected with a HS-SPME-GC–MS method (Stashenko et al., 2006). Ethyl octanoate and ethyl decanoate are the esters identified in raw ham and their peak areas increased from day 0 to day 5, suggesting their possible involvement as food shelf-life markers. This behaviour is in agreement with their production. In fact, esters are mainly produced by the esterification of carboxylic acids and alcohols (Petričević et al., 2018), which can be promoted by the action of some microorganisms such as lactic acid bacteria or Micrococcaceae thanks to their esterase activity (Narváez-Rivas et al., 2012; Purrinos et al., 2011). The aroma given by esters formed by short-chain acids is characterized by fruity notes, while those formed by long-chain acids is characterized by fatty odour (Petričević et al., 2018; Pugliese et al., 2015). Moreover, ethyl esters give to fermented sausages the proper flavour and mask rancid odours (Andrade et al., 2010; Flores et al., 2004).

In baked ham 15 compounds were identified: hydrocarbons, alcohols, ethers and ketones. For what concern alcohols, ethanol, 1-butanol, 3-methylbutanol, 1-hexanol, 2-butoxyethanol and linalool were identified. The peak areas of ethanol, 1-butanol, 3-methylbutanol and linalool increased from day 0 to day 5 and this is in agreement with their production, as described above. Decanal and nonanal are the two aldehydes identified and they had an opposite behaviour. In fact, the peak area of decanal increased from day 0 to day 2, then it decreased, while that of nonanal decreased from day 0 to day 5. This had probably the same explanation given above. Acetic acid is the main organic acid found in cooked ham and this was in agreement with other works that showed the

presence of this VOC in meat samples (Montanari et al., 2018). It contributes to the ripened aroma with its vinegar notes (Marco et al., 2007). Acetoin is a ketone and its peak area increased from day 0 to day 5 suggesting its possible involvement as food shelf-life marker. In fact, its production could be related to the Maillard reaction (Pérez-Santaescolástica et al., 2018) or to the microbial carbohydrate metabolism (Bosse et al., 2017; Petričević et al., 2018; Pastorelli et al., 2003). Acetoin gives a characteristic buttery and sweet odour (Sidira et al., 2015). Camphor is a cyclic ketone and its peak area increased from day 0 to day 5. Its use in food is mainly as a flavouring agent. Eucalyptol levels decreased from day 0 to day 5. It could be derived from spices used in the preparation of the product and its decrease could be associated in a reduction of flavouring characteristics of the product with the time.

In pork sausages 23 compounds were identified, among which there were esters, hydrocarbons, alcohols, nitriles, aldehydes, ketones, ethers and organic acids. 3-Carene, caryophyllene, humulene peak areas increased from day 0 to day 5, while β-pinene peak area decreased from day 0 to day 5. They were all involved in the flavour characteristics of the product. Ethanol, 2-methylpropanol, 3-methylbutanol, 1-hexanol, 1-heptanol, linalool, terpinen-4-ol and α-terpineol were the alcohols identified in pork sausages. The peak areas of ethanol, 3-methylbutanol, 1-hexanol and 1-heptanol increased from day 0 to day 5 and this was in agreement with their production, as described above. Among the aldehydes, hexanal and nonanal have been identified and whose peak areas decreased from day 0 to day 5. This behaviour has been explained before and involved the oxidation of aldehydes to carboxylic acids. Acetoin was the main ketone identified in this meat product and its peak area increased from day 0 to day 5. This behaviour has been explained before and involved microorganism activity. Acetic acid, whose peak area increased from day 0 to day 5, is an organic acid, whose presence and production have been described above.

In chicken 19 compounds were identified, among which there were hydrocarbons, alcohols, aldehydes, furans, ketones, ethers and organic acids. Ethanol, 3-methylbutanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-heptanol, 1-octanol, 2-octen-1-ol and 1-hexanol-2-ethyl were the alcohols identified in chicken. Their peak areas increase from day 0 to day 5 and this was in agreement with their production, as described above. Hexanal and nonanal were the aldehydes identified in chicken, whose peak areas decreased from day 0 to day 5 and this behaviour has been explained above. Among the furans, furan, 2-pentyl has been identified in chicken and its peak area increased from day 0 to day 5. 2-pentylfuran likely came from the oxidation of fatty acids, like linolenic and other n-6 acids (Lorenzo et al., 2013; Akköse et al., 2017) and its presence in meat was confirmed by other authors (Lorenzo et al., 2014; Lorenzo et al., 2015; Bermúdez et al., 2015; Domínguez et al., 2016). 2-pentylfuran is responsible for the pleasant aroma (Ramírez et al., 2007) that is characterized by sweet, green, fruity, vegetable aromatic notes and roasted nuances (Lorenzo et al., 2014; García-González et al.,2008). 3-Octanone, acetoin, 2-nonanone and camphor were the ketones identified in chicken. Their peak areas increased form day 0 to day 5. Acetoin production has been described above, while 2-nonanone production was related to the oxidation of free fatty acids (Narváez-Rivas et al., 2012; Ramírez et al., 2007) and this should explain the increment of its concentration over the time. In fact, as the oxidation processes increase over the time, also the concentration of their products increases with the time. Among the organic acids, the acetic acid was found in chicken and its levels increased from day 0 to day 5. This could be related to its production, which is induced by microorganisms though a carbohydrate fermentation process (Andrade et al., 2010). Since microorganism proliferation increases with the time, it is reasonable to explain the increasing concentration of this compound. However, this reaction is mainly involved in products that have a fermented stage during the elaboration process. Instead, in other meat products, the production of acetic acid could be related to the Maillard reaction (Martín et al., 2006; Andrade et al., 2010). Acetic acid has the feature of giving a vinegar note to the meat product and it contributes to the ripened aroma (Domínguez et al., 2016; Marco et al., 2007).

The semi-quantitative analysis allowed to select some compounds that can be considered potential markers of shelf-life for these five types of meat. Compounds were chosen on the basis of their presence in most of meat samples or/and for their constant increasing/decreasing abundance within the sample. Among the 40 compounds identified, 15 were chosen and selected as potential markers of shelf-life: hexanal, 1-butanol, 3-methylbutanol, 2-pentylfuran, 1-pentanol, acetoin, octanal, 1-hexanol, 2-nonanone, nonanal, 1-octen-3-ol, 1-heptanol, linalool, 1-octanol and 2-octen-1-ol. Hexanal was selected because it is present in beef, raw ham, pork sausage and chicken; 1-butanol in raw and baked ham; 3-methylbutanol and acetoin in beef, baked ham, pork sausage and chicken; 2-pentylfuran, 2-nonanone, 1-octanol and 2-octen-1-ol in chicken; octanal in raw ham; 1-hexanol and nonanal in all meat samples; 1-octen-3-ol in raw ham and chicken; 1-heptanol in beef, raw ham and chicken; linalool in beef, baked ham and sausage. The chosen compounds and their presence/trend in samples were summarized in Table 2.

3.3. HS-SPME-GC–MS quantitative analytical method validation

A new analytical method for the quantification of 15 VOCs in five types of meat, stored at 4 °C for 14 days, was developed. HS-SPME is one of the most used techniques for the analysis of VOCs because it's simple, economic, solvent-free, easy to handle and very sensitive. For these reasons, this technique has been chosen to quantify 15 VOCs. The chromatographic separation was characterized by a good resolution for all peaks and Fig. 1 showed a mix standard chromatogram of 15 VOCs quantified in this study.

The new analytical method was validated studying linearity, LOQ and LOD (**Table 3S**). Linearity was studied by injecting five different concentrations of 15 VOCs and plotting the calibration curves with the respective determination coefficients (R^2). All compounds showed a good linearity as R^2 was equal or greater than 0.990. The calculation of LOQs and LODs was conducted by injecting serial dilutions of the standard solutions, taking the signal-to-noise ratio 3:1 for the LOD and 10:1 for the LOQ, respectively.

3.4. Quantitative analysis of VOCs in five types of meat through HS-SPME-GC–MS

3.4.1. Beef

The concentrations of all monitored compounds in beef are summarized in Table 3. Hexanal concentration decreased from t0 (1836 μg/kg) to t9 (47.9 μg/kg) with statistically significant differences for t2, t6 and t9. This result has been observed in other studies (Argyri et al., 2015), where the concentrations of the most important VOCs, that are responsible for the spoilage of beef, are monitored over the time and ranged between 49.3 μg/kg and 1459.4 μg/kg. Octanal and nonanal concentrations had an oscillatory trend from t0 to t12 with no statistically significant differences and their values coincided with those reported by Argyri et al. (26.3–157.4 μg/kg for octanal and 37.0–181.7 μg/kg for nonanal) in almost 7 days of storage at 4 °C (Argyri et al., 2015).

The concentration of aldehydes usually increases with the time as they are the products of oxidation processes; however, in this study the sum of the concentration of aldehydes decreased and this should be due to their conversion to carboxylic acids.

Concerning alcohols, 1-butanol concentration increased from t0 (<LOQ) to t12 (532.5 μg/kg), 3-methylbutanol from t0 (<LOQ) to t12 (1115.5 μg/kg) and 1-pentanol from t0 (120.5 μg/kg) to t2 (240.7 μg/kg) with statistically significant differences. 1-hexanol, 1-octen-3-ol, 1-heptanol, 1-octanol and 2-octen-1-ol concentrations increased from t0 to t6 with statistically significant differences for 1-hexanol and 1-octen-3-ol. Linalool concentration increased from t0 (<LOQ) to t9 (7.7 μg/kg) with statistically significant differences for t6 and t9. The concentrations

Table 2
. Concentration of monitored VOCs in beef (μg/kg). n.d. means "not detected".

		t0	t2	t6	t9	t12
Aldehydes	Hexanal	1836.0 ± 0.33[a]	762.9 ± 0.04[b]	650.0 ± 0.13[bc]	47.9 ± 0.01[c]	250.0 ± 0.04[bc]
	Octanal	14.1 ± 0.00[a]	8.6 ± 0.00[a]	7.2 ± 0.00[a]	6.7 ± 0.00[a]	5.7 ± 0.00[a]
	Nonanal	120.4 ± 0.00[a]	120.8 ± 0.00[a]	133.7 ± 0.02[a]	125.0 ± 0.01[a]	131.0 ± 0.02[a]
Sum		1970.5	892.3	790.9	179.6	386.7
Alcohols	1-butanol	n.d.	33.1 ± 0.01[a]	37.2 ± 0.00[a]	44.0 ± 0.01[a]	532.5 ± 0.08[a]
	3-methylbutanol	n.d.	n.d.	53.0 ± 0.01[a]	938.3 ± 0.08[b]	1115.5 ± 0.22[b]
	1-pentanol	120.5 ± 0.02[ab]	240.7 ± 0.02[b]	143.2 ± 0.00[a]	231.8 ± 0.04[b]	93.9 ± 0.02[a]
	1-hexanol	n.d.	59.5 ± 0.00[a]	485.3 ± 0.05[b]	230.5 ± 0.05[c]	307.5 ± 0.06[c]
	1-octen-3-ol	173.7 ± 0.03[a]	190.0 ± 0.01[a]	285.6 ± 0.01[b]	163.0 ± 0.03[a]	129.3 ± 0.01[a]
	1-heptanol	75.9 ± 0.01[a]	90.0 ± 0.00[a]	101.9 ± 0.01[a]	59.0 ± 0.01[b]	n.d.
	Linalool	n.d.	n.d.	5.1 ± 0.00[b]	7.7 ± 0.00[c]	n.d.
	1-octanol	29.3 ± 0.00[a]	30.0 ± 0.00[a]	39.3 ± 0.01[a]	n.d.	n.d.
	2-octen-1-ol	89.0 ± 0.01[a]	101.0 ± 0.00[a]	109.5 ± 0.00[a]	99.6 ± 0.01[a]	87.9 ± 0.00[a]
Sum		488.4	744.3	1260.1	1773.9	2266.6
Ketones	Acetoin	40.8 ± 0.01[a]	60.0 ± 0.00[ab]	625.0 ± 0.11[bc]	963.5 ± 0.14[c]	2320.8 ± 0.4[d]
	2-nonanone	5.0 ± 0.00[a]	6.0 ± 0.00[a]	11.5 ± 0.00[b]	5.5 ± 0.00[a]	3.7 ± 0.00[a]
Sum		45.8	66.0	636.5	969.0	2324.5
Furans	2-pentylfuran	64277.1 ± 11.67[ab]	70250.0 ± 7.44[a]	81500.0 ± 14.85[b]	20709.8 ± 3.77[c]	17753.5 ± 0.53[c]

[a] Values in the same line that don't share the same letters are statistically significative different ($p < 0.05$).

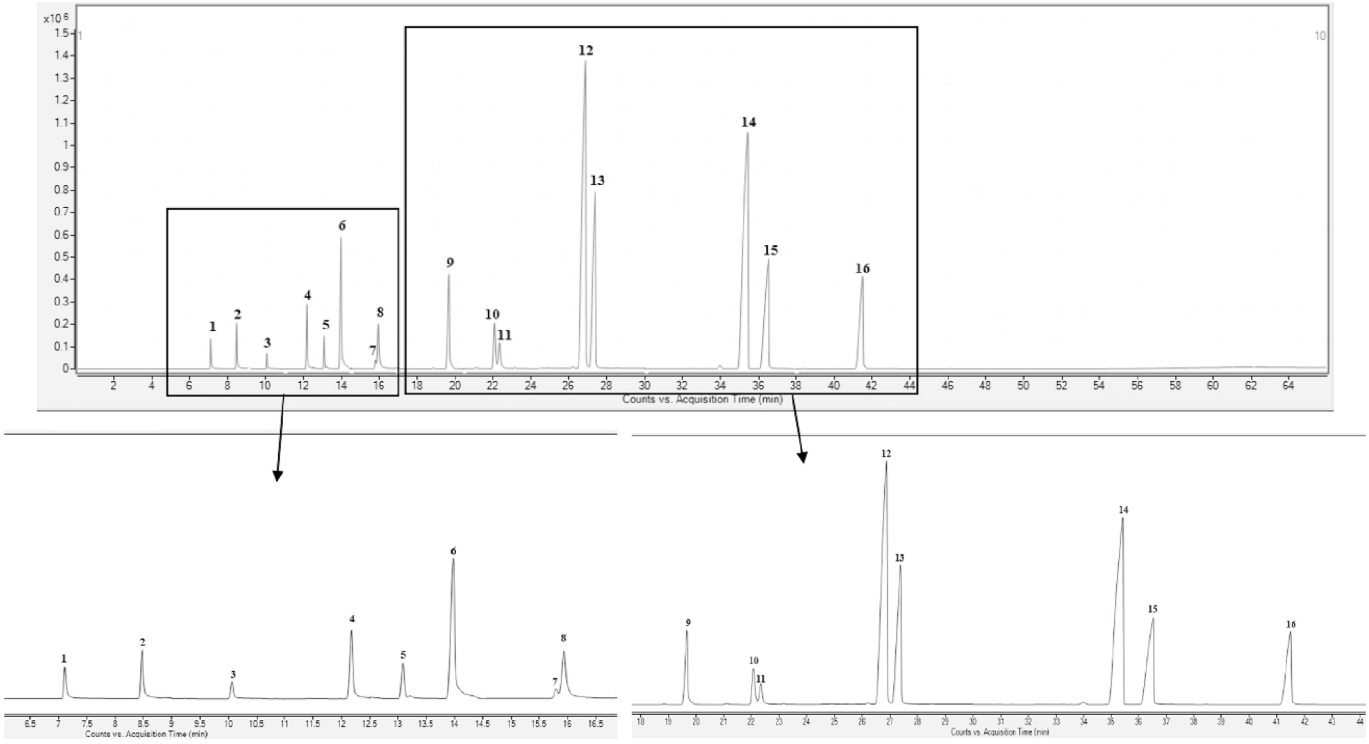

Fig. 1. Chromatogram of all monitored compounds acquired in Selected Ion Monitoring (1: 2-methylpentanal (S.I.); 2. Hexanal; 3. 1-butanol; 4. 3-methylbutanol; 5. 2-pentylfuran; 6. 1-pentanol; 7. Acetoin; 8. Octanal; 9. 1-hexanol; 10. 2-nonanone; 11. Nonanal; 12. 1-octen-3-ol; 13. 1-heptanol; 14. Linalool; 15. 1-octanol; 16. 2-octen-1-ol).

of some alcohols, such as pentanol, hexanol and octanol, are in the same range of those found in the study by Argyri et al. (Argyri et al., 2015). The sum of the concentrations of the monitored alcohols increased with the time (from t0 to t12) and this is in agreement with their formation, as they are formed from the lipid oxidation, which is an increasing process over the time.

Acetoin concentration increased from t0 (40.8 μg/kg) to t12 (2320.8 μg/kg) with statistically significant differences. 2-nonanone concentration increased from t0 (5.0 μg/kg) to t6 (11.5 μg/kg) with a statistically significant difference. The sum of the concentrations of acetoin and 2-

Table 3
Concentration of monitored VOCs in raw ham (μg/kg). n.d. means "not detected".

		t0	t2	t6	t9	t12
Aldehydes	Hexanal	2199.7 ± 0.01[a]	1217.6 ± 0.00[b]	1171.8 ± 0.19[b]	1513.6 ± 0.12[b]	1282.0 ± 0.21[b]
	Octanal	86.8 ± 0.01[a]	70.0 ± 0.00[a]	62.5 ± 0.01[ab]	53.2 ± 0.00[b]	33.5 ± 0.01[c]
	Nonanal	20.5 ± 0.00[a]	24.7 ± 0.00[a]	25.0 ± 0.00[a]	26.3 ± 0.00[a]	27.8 ± 0.01[a]
Sum		2307.0	1312.3	1259.3	1593.1	1343.3
Alcohols	1-butanol	295.0 ± 0.06[a]	43.2 ± 0.00[bc]	44.9 ± 0.00[bc]	61.1 ± 0.01[bc]	88.7 ± 0.00[c]
	3-methylbutanol	n.d.	68.7 ± 0.00[b]	71.2 ± 0.00[b]	76.0 ± 0.02[b]	72.5 ± 0.01[b]
	1-pentanol	197.3 ± 0.02[ad]	112.9 ± 0.00[bc]	90.1 ± 0.01[b]	167.1 ± 0.03[c]	116.1 ± 0.01[bc]
	1-hexanol	n.d.	n.d.	n.d.	n.d.	170.0 ± 0.03[b]
	1-octen-3-ol	102.1 ± 0.02[a]	64.9 ± 0.00[a]	85.0 ± 0.01[a]	75.9 ± 0.02[a]	88.2 ± 0.01[a]
	1-heptanol	157.6 ± 0.02[a]	67.6 ± 0.00[b]	54.9 ± 0.01[b]	73.3 ± 0.01[b]	n.d.
	Linalool	n.d.	0.1 ± 0.00[a]	1.2 ± 0.00[a]	4.5 ± 0.00[b]	6.2 ± 0.00[c]
	1-octanol	83.9 ± 0.01[a]	50.7 ± 0.00[bc]	67.8 ± 0.00[bc]	41.5 ± 0.01[b]	40.0 ± 0.01[b]
	2-octen-1-ol	32.0 ± 0.01[a]	24.3 ± 0.00[a]	39.4 ± 0.00[a]	25.0 ± 0.00[a]	36.0 ± 0.01[a]
Sum		867.9	432.4	454.5	524.4	617.7
Ketones	Acetoin	n.d.	n.d.	n.d.	n.d.	n.d.
	2-nonanone	3.4 ± 0.00[a]	3.4 ± 0.00[a]	3.4 ± 0.00[a]	3.6 ± 0.00[a]	4.8 ± 0.00[a]
Sum		3.4	3.4	3.4	3.6	4.8
Furans	2-pentylfuran	20726.5 ± 1.49[a]	8808.0 ± 0.00[bc]	7185.5 ± 0.93[b]	7000.0 ± 0.16[b]	6523.7 ± 0.81[b]

[a] Values in the same line that don't share the same letters are statistically significative different ($p < 0.05$).

nonanone increased from t0 to t12 and this agrees with their production, as they are formed by the microbial carbohydrate metabolism and the oxidation of free fatty acids, respectively. Also in this case, the range of concentrations agrees with the concentrations found in the study of Argyri et al. (Argyri et al., 2015). 2-Pentylfuran concentration increased from t0 (64277.1 μg/kg) to t6 (81500.0 μg/kg), then, it decreased from t6 (81500.0 μg/kg) to t12 (17753.5 μg/kg) with statistically significant differences.

3.4.2. Raw ham

The concentrations of all monitored compounds in raw ham are summarized in Table 4.

Hexanal and octanal concentrations decreased from t0 to t6 and from t0 to t12 respectively, with statistically significant differences, while nonanal concentration increased from t0 (20.5 μg/kg) to t12 (27.8 μg/kg) with no statistically significant differences. In this meat product, both hexanal and octanal concentrations decreased probably because

Table 4
Concentration of monitored VOCs in baked ham (μg/kg). n.d. means "not detected".

		t0	t2	t6	t9	t12
Aldehydes	Hexanal	0.0 ± 0.00[a]	18.0 ± 0.00[bc]	16.4 ± 0.00[bc]	14.1 ± 0.00[b]	25.0 ± 0.00[c]
	Octanal	6.6 ± 0.00[a]	22.0 ± 0.00[bc]	14.4 ± 0.00[bd]	14.4 ± 0.00[bd]	22.5 ± 0.00[c]
	Nonanal	65.3 ± 0.01[a]	84.5 ± 0.02[ab]	131.7 ± 0.00[bc]	106.1 ± 0.00[ac]	140.6 ± 0.03[c]
Sum		71.9	124.5	162.5	134.6	188.1
Alcohols	1-butanol	53.0 ± 0.01[a]	164.5 ± 0.03[a]	217.6 ± 0.03[a]	250.86 ± 0.01[a]	618.5 ± 0.11[b]
	3-methylbutanol	57.4 ± 0.00[ad]	70.0 ± 0.00[ad]	311.9 ± 0.05[b]	350.0 ± 0.04[b]	552.4 ± 0.00[c]
	1-pentanol	9.4 ± 0.00[a]	19.5 ± 0.00[ab]	32.5 ± 0.00[b]	35.7 ± 0.00[b]	72.2 ± 0.01[c]
	1-hexanol	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
	1-octen-3-ol	0.0 ± 0.00[a]	1.6 ± 0.00[b]	2.5 ± 0.00[b]	1.9 ± 0.00[b]	2.0 ± 0.00[b]
	1-heptanol	0.0 ± 0.00[a]	15.4 ± 0.00[b]	30.5 ± 0.00[c]	26.6 ± 0.00[c]	2.9 ± 0.00[a]
	Linalool	217.0 ± 0.02[a]	195.4 ± 0.01[a]	228.1 ± 0.03[a]	203.2 ± 0.02[a]	171.4 ± 0.02[a]
	1-octanol	7.2 ± 0.00[a]	8.2 ± 0.00[ac]	12.3 ± 0.00[b]	11.0 ± 0.00[bc]	0.0 ± 0.00[d]
	2-octen-1-ol	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
Sum		344.0	474.6	835.4	628.4	1419.4
Ketones	Acetoin	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
	2-nonanone	10.6 ± 0.00[a]	13.5 ± 0.00[a]	22.2 ± 0.00[b]	22.9 ± 0.00[a]	24.0 ± 0.01[b]
Sum		10.6	13.5	22.2	22.9	24.0
Furans	2-pentylfuran	1766.4 ± 0.03[a]	2970.9 ± 0.27[a]	27661.7 ± 3.00[bc]	26770.4 ± 3.57[bc]	3067.9 ± 0.06[a]

[a] Values in the same line that don't share the same letters are statistically significative different ($p < 0.05$).

they are transformed in the corresponding carboxylic acids, while nonanal concentration increased for the oxidative processes. The sum of the concentrations of the monitored aldehydes decreased over the time from t0 to t6 probably because of their transformation in carboxylic acids. Regarding alcohols, 1-butanol and 1-pentanol concentration decreased from t0 to t6 with statistically significant differences and 1-octen-3-ol concentration underwent an overall decrease from t0 (102.1 μg/kg) to t12 (88.2 μg/kg) with no statistically significant differences. The concentration of 1-heptanol and 1-octanol also decreased from t0 to t12 with statistically significant differences. 3-methylbutanol concentration increased from t0 (<LOQ) to t9 (76.0 μg/kg), while 1-hexanol and linalool increased from t0 to t12 with statistically significant differences. 2-octen-1-ol concentration did not vary among the samples. The sum of the concentrations of the monitored alcohols underwent a slight decrease (from t0 to t2) and then a slight increase (from t6 to t14). Acetoin was not present in all samples at all times, while 2-nonanone concentration increased from t0 (3.4 μg/kg) to t12 (4.8 μg/kg) with no statistically significant differences. 2-pentylfuran concentration decreased from t0 (20726.5 μg/kg) to t12 (6523.7 μg/kg) with statistically significant differences.

3.4.3. Baked ham

The concentrations of all monitored compounds in baked ham are summarized in Table 5**.** Hexanal, octanal and nonanal concentration increased from t0 to t12, from t0 to t2 and from t0 to t6 respectively, with statistically significant differences. In this type of meat, the sum of the concentrations of monitored aldehydes increased from t0 to t12, as they are the products of oxidative process that increases over the time. The concentrations of 1-octen-3-ol and 1-octanol increased from t0 to t6 with a statistically significant difference for 1-octen-3-ol and 1-octanol, while 1-butanol, 3-methylbutanol and 1-pentanol concentrations increased from t0 to t12 with statistically significant differences. 1-heptanol concentration increased from t0 (0.0 μg/kg) to t6 (30.5 μg/kg), with statistically significant differences. Linalool concentration remained quite the same in all samples, while 1-hexanol and 2-octen-1-ol were not present in all samples at all times. The sum of the concentrations of the monitored alcohols increased from t0 to t12 (with a slight decrease on t9) and this agrees with their production. Regarding ketones, acetoin was not present in all samples at all times, while 2-nonanone concentration increased from t0 (10.6 μg/kg) to t12 (24.0 μg/kg) with no statistically significant differences and this is in agreement with their production from oxidation processes. 2-pentylfuran concentration increased from t0 (1766.4 μg/kg) to t6 (27661.7 μg/kg) with statistically significant differences.

3.4.4. Pork sausage

The concentrations of all monitored compounds in pork sausages are summarized in Table 6**.** Hexanal and octanal concentrations increased from t0 to t2 and from t0 to t12, respectively with statistically significant difference. The concentration of nonanal decreased from t0 (9.3 μg/kg) to t12 (6.9 μg/kg) with statistically significant differences. The sum of the concentrations of the monitored aldehydes increased from t0 to t12 and this agrees with their production. 1-Butanol, 3-methylbutanol, 1-octen-3-ol, 1-heptanol and linalool concentrations increased from t0 to t12 with statistically significant differences. 1-Pentanol and 1-octanol concentrations increased from t0 to t6 with statistically significant differences. The concentration of 1-hexanol increased from t0 (3.8 μg/kg) to t2 (28.2 μg/kg), with statistically significant differences, while 2-octen-1-ol was not present in the sample. The sum of the concentrations of all monitored alcohols increased from t0 to t12 and this agrees with their production. Acetoin increased from t0 (5879.0 μg/kg) to t12 (202052.5 μg/kg) with statistically significant differences, while 2-nonanone concentration increased from t0 (0.2 μg/kg) to t2 (0.4 μg/kg) with no statistically significant differences. 2-pentylfuran increased from t0 (1.9 μg/kg) to t2 (27.6 μg/kg) with a statistically significant difference.

3.4.5. Chicken

The concentrations of all monitored compounds in chicken are summarized in Table 7**.**

The concentration of hexanal decreased from t0 to t6 with statistically significant differences, while octanal concentration decreased from t0 (14.6 μg/kg) to t9 (0.00 μg/kg) with a statistically significant difference. The concentration of nonanal increased from t0 (11.6 μg/kg) to t9 (1257.9 μg/kg) with statistically significant differences. The sum of

Table 5
Concentration of monitored VOCs in pork sausage (μg/kg). n.d. means “not detected”.

		t0	t2	t6	t9	t12
Aldehydes	Hexanal	28.9 ± 0.00^{a}	137.7 ± 0.03^{b}	1.8 ± 0.00^{a}	7.5 ± 0.00^{a}	3.4 ± 0.00^{a}
	Octanal	8.4 ± 0.00^{a}	72.7 ± 0.01^{ab}	365.9 ± 0.07^{b}	450.0 ± 0.03^{b}	1095.1 ± 0.20^{c}
	Nonanal	9.3 ± 0.00^{a}	2.1 ± 0.00^{b}	1.9 ± 0.00^{b}	1.7 ± 0.00^{b}	6.9 ± 0.00^{c}
Sum		46.6	212.5	369.6	459.2	1105.4
Alcohols	1-butanol	27.6 ± 0.00^{a}	32.9 ± 0.00^{a}	33.3 ± 0.00^{a}	34.4 ± 0.00^{a}	88.6 ± 0.02^{b}
	3-methylbutanol	95.6 ± 0.01^{a}	324.5 ± 0.04^{b}	982.6 ± 0.04^{c}	1050.0 ± 0.11^{c}	1212.2 ± 0.05^{d}
	1-pentanol	2.8 ± 0.00^{a}	9.7 ± 0.00^{b}	10.4 ± 0.00^{b}	3.4 ± 0.00^{a}	9.5 ± 0.00^{b}
	1-hexanol	3.8 ± 0.00^{ade}	28.2 ± 0.00^{b}	14.1 ± 0.00^{c}	7.3 ± 0.00^{a}	n.d.
	1-octen-3-ol	1.1 ± 0.00^{a}	3.2 ± 0.00^{abc}	4.0 ± 0.00^{bc}	4.3 ± 0.00^{bc}	6.2 ± 0.00^{c}
	1-heptanol	0.6 ± 0.00^{ac}	2.2 ± 0.00^{ab}	2.8 ± 0.00^{b}	3.2 ± 0.00^{b}	3.9 ± 0.00^{b}
	Linalool	0.9 ± 0.00^{a}	1.5 ± 0.00^{a}	3.5 ± 0.00^{b}	3.8 ± 0.00^{b}	7.6 ± 0.00^{c}
	1-octanol	n.d.	n.d.	14.1 ± 0.00^{b}	4.4 ± 0.00^{c}	n.d.
	2-octen-1-ol	0.5 ± 0.00^{a}	n.d.	0.2 ± 0.00^{a}	n.d.	n.d.
Sum		132.9	402.2	1065.0	1110.8	1328.0
Ketones	Acetoin	5879.0 ± 1.05^{a}	27535.0 ± 3.65^{ab}	71483.7 ± 7.90^{b}	73541.0 ± 11.64^{b}	202052.5 ± 37.20^{c}
	2-nonanone	0.2 ± 0.00^{a}	0.4 ± 0.00^{a}	0.1 ± 0.00^{a}	0.1 ± 0.00^{a}	0.0 ± 0.00^{a}
Sum		5879.2	27535.4	71483.8	73541.1	202052.5
Furans	2-pentylfuran	1.9 ± 0.00^{a}	27.6 ± 0.01^{b}	8.7 ± 0.00^{a}	8.2 ± 0.00^{a}	8.3 ± 0.00^{a}

[a] Values in the same line that don’t share the same letters are statistically significative different ($p < 0.05$).

Table 6
Concentration of monitored VOCs in chicken (μg/kg). n.d. means "not detected".

		t0	t2	t6	t9	t12
Aldehydes	Hexanal	707.4 ± 0.09[a]	652.4 ± 0.04[a]	192.4 ± 0.04[c]	1364.6 ± 0.20[d]	1694.0 ± 0.6[d]
	Octanal	62.6 ± 0.01[a]	35.0 ± 0.00[a]	29.5 ± 0.00[a]	986.4 ± 0.00[b]	1025.7 ± 0.02[b]
	Nonanal	11.6 ± 0.00[a]	12.6 ± 0.00[a]	13.2 ± 0.00[a]	1257.9 ± 0.21[b]	161.5 ± 0.03[a]
Sum		781.6	700.0	235.1	2622.5	2881.2
Alcohols	1-butanol	23.3 ± 0.00[a]	59.1 ± 0.01[a]	104.0 ± 0.01[a]	110.0 ± 0.00[a]	1109.8 ± 0.22[b]
	3-methylbutanol	12.4 ± 0.00[a]	21.6 ± 0.00[a]	390.6 ± 0.06[a]	5995.4 ± 1.90[b]	11663.9 ± 2.20[b]
	1-pentanol	44.9 ± 0.00[a]	29.4 ± 0.00[ab]	14.9 ± 0.00[b]	0.0 ± 0.00[b]	169.9 ± 0.02[c]
	1-hexanol	8.5 ± 0.00[a]	9.0 ± 0.00[a]	9.1 ± 0.00[a]	239.8 ± 0.04[b]	259.9 ± 0.05[b]
	1-octen-3-ol	32.5 ± 0.01[a]	19.4 ± 0.00[ac]	5.8 ± 0.00[a]	91.3 ± 0.01[b]	107.7 ± 0.02[b]
	1-heptanol	4.8 ± 0.00[a]	3.9 ± 0.00[b]	0.0 ± 0.00[c]	0.0 ± 0.00[c]	0.0 ± 0.00[c]
	Linalool	0.1 ± 0.00[a]	0.2 ± 0.00[a]	0.3 ± 0.00[a]	0.6 ± 0.00[b]	3.3 ± 0.00[b]
	1-octanol	11.3 ± 0.00[a]	9.7 ± 0.00[b]	0.0 ± 0.00[c]	0.0 ± 0.00[c]	0.0 ± 0.00[c]
	2-octen-1-ol	14.9 ± 0.00[a]	8.3 ± 0.00[b]	2.9 ± 0.00[c]	0.0 ± 0.00[c]	0.0 ± 0.00[c]
Sum		152.7	160.6	527.6	6437.1	13314.5
Ketones	Acetoin	1868.5 ± 0.05[a]	2015.8 ± 0.02[a]	2238.7 ± 0.07[a]	14522.0 ± 28.3[b]	8360.0 ± 5.07[c]
	2-nonanone	0.3 ± 0.00[a]	0.3 ± 0.00[a]	0.4 ± 0.00[a]	2.9 ± 0.00[b]	8.1 ± 0.00[c]
Sum		1868.8	2016.1	2239.1	14524.9	8368.1
Furans	2-pentylfuran	164.9 ± 0.01[a]	78.9 ± 0.01[a]	32.3 ± 0.01[a]	1167.4 ± 0.21[b]	830.7 ± 0.16[b]

[a] Values in the same line that don't share the same letters are statistically significative different ($p < 0.05$).

Table 7
. Trend of 15 VOCs quantified in 5 types of meat.

Compounds	*Beef*	*Raw Ham*	*Baked Ham*	*Sausage*	*Chicken*
Hexanal	↓	↓	↑	↑	↓
1-butanol	↑	↓	↑	↑	↑
3-methylbutanol	↑	↑	↑	↑	↑
2-pentylfuran	↑	↓	↓	↑	↑
1-pentanol	↑	↓	↑	↑	↓
Acetoin	↑	n.d.	n.d.	↑	↑
Octanal	↑	↓	↑	↑	↓
1-hexanol	↑	↑	n.d.	↑	↑
2-nonanone	↑	↑	↑	↔	↑
Nonanal	↑	↑	↑	↓	↑
1-octen-3-ol	↑	↓	↑	↑	↑
1-heptanol	↑	↓	↑	↑	↓
Linalool	↑	↑	↔	↑	↑
1-octanol	↑	↓	↑	↑	↓
2-octen-1-ol	↑	↔	n.d.	↔	↓

the concentrations of monitored aldehydes decreased from t0 to t6 and this should be due to their transformation to carboxylic acid. 1-butanol, 3-methylbutanol, 1-hexanol and linalool concentrations increased from t0 to t12 with statistically significant differences. On the contrary, 1-pentanol and 2-octen-1-ol concentrations decreased from t0 to t9 with statistically significant differences and 1-octen-3-ol, 1-heptanol, 1-octanol decreased from t0 to t6 with statistically significant differences for 1-heptanol and 1-octanol. The sum of the concentrations of all monitored alcohols increased from t0 to t12 and this agrees with their formation from the oxidative processes. The concentration of acetoin increased from t0 (1868.5 μg/kg) to t9 (14522.0 μg/kg) with statistically significant differences, while 2-nonanone increased from t0 (0.3 μg/kg) to t12 (8.1 μg/kg) with statistically significant differences. The sum of the concentrations of acetoin and 2-nonanone increased from t0 to t9 and this agrees with their formation. 2-pentylfuran concentration decreased from t0 (164.9 μg/kg) to t9 (32.3 μg/kg) with statistically significant differences.

To summarize, the majority of the compounds monitored can be considered markers of shelf-life for one or more types of meat because their trend is constant over the time (increasing or decreasing), with the exception of acetoin, 1-hexanol, 2-nonanone, linalool and 2-octen-1-ol for some types of meat (not present or with a constant concentration). Anyway, all compounds quantified can be considered markers of shelf-life for at least 2 types of meat and 7 of them (1-butanol, 3-methylbutanol, 1-hexanol, 2-nonanone, nonanal, 1-octen-3-ol and linalool) for at least for 4 types of meat products analyzed. 3-Methylbutanol can be considered a marker of shelf-life for all meat products analyzed.

3.5. Principal component analysis (PCA)

The principal component analysis (PCA) was applied to each type of meat to relate the volatile profile with five different days of analysis. PCA showed clearly that there are differences in the volatile profiles between meat samples and days of analysis (Fig. 2).

In beef (Fig. 2a) the volatile profile is different in each time of analysis, especially between T0, T2 and T6 with respect to T12 and T14. 2-Pentylfuran contributed the most to data variability and was correlated to samples at days 0, 2, and 5. In raw ham (Fig. 2b) the volatile profile was different at each time of analysis, especially between T0 and all other times of analysis. The volatile compound most contributing to data variability was again 2-pentylfuran which was correlated to sample at T0.

In baked ham (Fig. 2c) the volatile profile of sample at T14 was different with respect to samples at T0-T2 and T6-12, while there was not a great difference between samples at T0 and T2 and between T6 and T12. Again 2-pentylfuran influenced mostly data variability and was correlated to samples at T6 and T12.

In pork sausage (Fig. 2d) the volatile profile of samples at T14 and T12 are different with respect to samples at T0-T2 and T6, while there was not a great difference between samples at T0, T2, and T6. The variables most contributing to data variability are 3-methylbutanol, correlated to the sample at T14, and acetoin which is correlated to that at T12.

In chicken (Fig. 2e) the volatile profile of the sample at T14 is different with respect to samples at T0-T2 and T6-12, while there was not a great difference between samples at T0, T2, T6 and T12. The

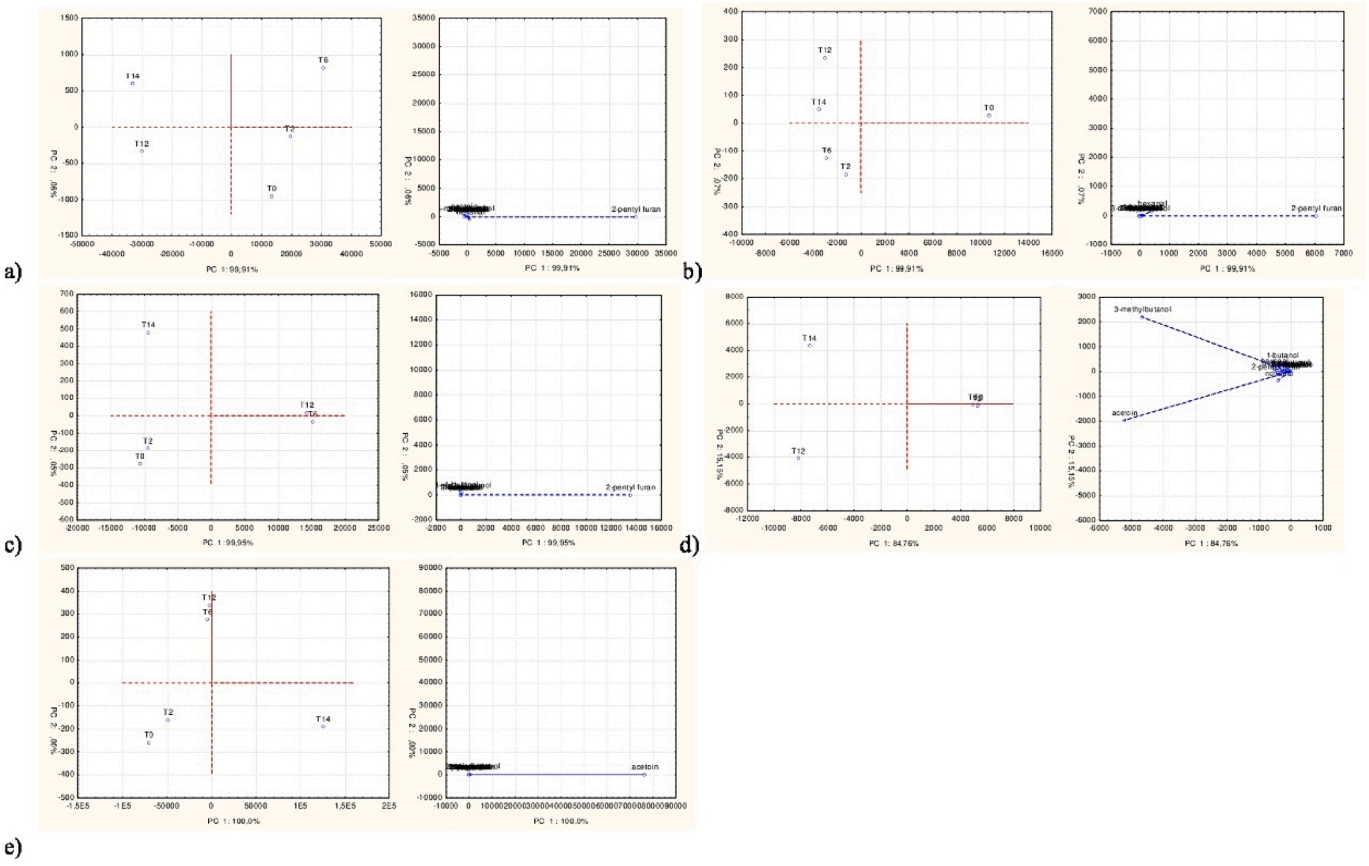

Fig. 2. Principal component analysis (PCA) score and loading plots representing the variance of the volatile profile of five types of meat at different days of analysis.

variable most contributing to data variability is acetoin which is associated correlated to the sample at T14.

4. Conclusions

The semi-quantitative analysis of 5 types of meat allowed to select 15 volatile compounds as potential markers of shelf-life. Then, the 15 selected VOCs were quantified in 5 types of meat over 12 days of storage to assess their reliability as meat shelf-life markers. Table 4S summarizes the trend of the 15 VOCs quantified in 5 types of meat. Results showed that all the compounds monitored can be considered markers of shelf-life for one or more types of meat because their trend is constant over the time (increasing or decreasing), with the exception of acetoin for raw and baked ham (n.d.), 1-hexanol and 2-octen-1-ol for baked ham (n.d.), 2-nonanone and 2-octen-1-ol for sausage (remained constant over the time), linalool for baked ham (remained constant over the time) and 2-octen-1-ol (remained constant over the time). Among the monitored compounds, some can be considered markers of shelf-life for at least 4 types of meat: 1-butanol, 3-methylbutanol, 1-hexanol, 2-nonanone, nonanal, 1-octen-3-ol and linalool. In particular, 1-butanol can be considered a marker of shelf-life for beef, baked ham, pork sausage and chicken; 3-methylbutanol for all types of meat; 1-hexanol for beef, raw ham, pork sausage and chicken; 2-nonanone for beef, raw ham, baked ham and chicken; nonanal for beef, raw and baked hams and chicken; 1-octen-3-ol for beef, baked ham, pork sausage and chicken; linalool for beef, raw ham, pork sausage and chicken. In conclusion, in this study an HS-SPME-GC–MS semi-quantitative analysis allowed to select the most important VOCs (in terms of increasing/decreasing trends) in five types of meat, then a new quantitative HS-SPME-GC–MS analytical method to quantity the 15 VOCs selected in five types of meat preserved for 12 days was developed and it was demonstrated that all compounds quantified can be considered markers of shelf-life for at least 2 types of meat and 7 of them for at least for 4 types of meat products analyzed. This study is innovative because to the best of our knowledge, there are no quantitative analyses of VOCs in different types of meat simultaneously to find possible markers of shelf-life.

CRediT authorship contribution statement

Laura Acquaticci: Writing – original draft, Methodology, Formal analysis. **Simone Angeloni:** Formal analysis, Data curation, Conceptualization. **Cecilia Baldassarri:** Methodology, Investigation. **Gianni Sagratini:** Validation, Supervision. **Sauro Vittori:** Visualization, Validation, Supervision. **Elisabetta Torregiani:** Software, Methodology. **Riccardo Petrelli:** Visualization, Validation, Methodology. **Giovanni Caprioli:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114398.

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