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Elucidating the combined effect of sample preparation and solid-phase microextraction conditions on the volatile composition of cooked meat analyzed by capillary gas chromatography coupled with mass spectrometry

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ABSTRACT

Solid-phase microextraction coupled to gas chromatography-mass spectrometry is a common approach to analyze the volatile profile of cooked meat. The present study aims to investigate the combined effect of sample preparation, including meat presentation (minced and steak) and cooking method (stewed and grilled), and extraction temperature (30, 60 and 80 °C) and time (30 and 50 min) on the volatile composition of cooked deer meat. The statistical results indicated that extraction temperature was the most relevant factor affecting the meat volatile profile of cooked meat followed by the extraction time. Higher extraction temperatures improved the detection of heavy volatile compounds, while sample preparation had little influence on the meat volatile profile, probably due to the accurate control of the parameters used for meat presentation and cooking methods. The results of this work can assist in the standardization of analytical procedures for the characterization of volatile compounds in cooked meat.

1. Introduction

Meat aroma is an important trait affecting the sensory quality of cooked meat and consumers' acceptability (Calkins & Hodgen, 2007). For this reason, an accurate analysis of the volatile compounds released from cooked meat is essential to determine their potential impact on meat aroma. The volatile compounds responsible for meat aroma have been extensively studied in cooked samples using diverse extraction methods such as solvent extraction, simultaneous distillation extraction or solvent assisted flavor evaporation. However, solid-phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC–MS) present several advantages compared to other methods; it is simple, cost-efficient, solvent-free and user-friendly (Bueno, Resconi, Campo, Ferreira, & Escudero, 2019; Elmore, Mottram, & Hierro, 2000; Watkins, Rose, Warner, Dunshea, & Pethick, 2012). SPME extraction ability may depend on sample nature and preparation, and extraction conditions (Park, Yoon, Schilling, & Chin, 2009). In addition to matrix effect, another constrain of this technique is the on-fiber absorption competition phenomena among volatile compounds released into headspace (Bueno et al., 2019; Met & Şahin Yeşilçubuk, 2017). In the analysis of meat volatile compounds, most methodological studies using SPME coupled to GC–MS have been focused on the performance of different parameters such as fiber and column type, headspace volume, extraction temperature and time, or different GC temperature programs (Elmore et al., 2000; Kataoka, Lord, & Pawliszyn, 2000; Machiels & Istasse, 2003). Regarding SPME conditions, changes in the extraction temperature and time have been found to have a great influence on the number and abundance of volatile compounds (Wang et al., 2018). However, most studies have followed one-factor-at-a-time analysis approach (Lorenzo, 2014; Machiels & Istasse, 2003; Watanabe, Ueda, Higuchi, & Shiba, 2008), while, when several factors were changed simultaneously, they were limited to mild temperatures (maximum 60 °C) (Ruiz, Cava, Ventanas, & Jensen, 1998; Zhou, Han, Zhuang, Feng, & Xu, 2015) or the interaction effect between the main factors was overlooked (Moon & Li-Chan, 2004) resulting on a biased interpretation of the data. In this regard, Ma, Hamid, Bekhit, Robertson, and Law (2013) applied the response surface methodology to evaluate the interaction between extraction time and temperature although they focused only on the effect of SPME conditions on peak area of some selected odorant compounds, and only mild extraction temperatures were used (20–60 °C).

The volatile profile of cooked meat is complex and can be

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simultaneously affected by its physic-chemical composition, meat storage and the cooking method applied (Kerth, 2016). Maintaining the extraction temperature as low as possible is a general recommendation to avoid lipid oxidation and other structural changes in the food matrix (Panseri, Soncin, Chiesa, & Biondi, 2011). However, this could lead to the non-extraction of heavy volatile compounds present in the food sample, which is particularly relevant when the volatile profile of cooked or heat-treated foods is required (Ma et al., 2013). In cooked meat, the recommended final internal temperature is around 70 °C, and the cooking parameters will depend on the cooking technique and the characteristics of the meat sample (e.g., muscle type, steak width and shape, minced meat, etc.) (AMSA, 1995). Sample preparation for SPME extraction and cooking procedure are performed prior to extraction and both are time-consuming steps that varied widely among meat studies (Kerth, 2016; Lorenzo & Domínguez, 2014). Apart from the cooking method *per se* (grilling, boiling, stewing, frying), there are a number of important parameters that should be taken into account, such as a detailed description of cooking equipment, meat temperature *prior* to cooking, or the final internal and surface temperature of meat. In addition, meat presentation (minced or intact, pieces of different size and thickness) may modify the cooking effect (Kerth, 2016) due to changes in heat transference and, therefore, in the generation of volatile compounds.

Taking into account the need to standardize the volatile analysis conditions in cooked meat, the aim of the present study was to investigate the combined effect of sample preparation, including meat presentation (minced and steak) and cooking method (stewed and grilled setting 70 °C as final internal temperature), and SPME conditions (extraction temperature and time) on the volatile composition of cooked deer meat analyzed by GC–MS. Deer meat was chosen for the present study due to the increasing interest as game meat and its positive nutritional characteristics (Lorenzo, Munekata, Barba, & Toldrá, 2019; Porter, 2020; Vivanco, Morán, Lorenzo, Lavín, & Aldai, 2019).

2. Material and methods

2.1. Meat samples and chemicals

Commercial Iberian red deer (*Cervus elaphus ibericus*) loins (*Longissimus thoracis et lumborum* muscle) were purchased from a specialized commercial retailer (Catedral de la Caza, Toledo, Spain).

Hexane (American Chemical Society reagent, ≥97%), anhydrous sodium sulfate (American Chemical Society reagent, ≥99.0%), 1,3,5-triisopropylbenzene (TIPB; 95%), cyclohexanone (CYAHONE; ≥99.5%), methyl isobutyl ketone (MIBK; ≥99.5%), methyl anteisovalerate (MAIV; ≥99%), 1-penten-3-ol (99%), 1-pentanol (≥99.0%), 1-octen-3-ol (98%), 1-heptanol (98%), 1-octanol (99%), (*E*)-2-octen-1-ol (97%), 1-tetradecanol (97%), 1-pentadecanol (99%), acetaldehyde (≥99.5%), hexanal (98%), heptanal (≥95%), (*E*)-2-octenal (≥95%), (*E*)-2-decenal (≥95%), (*E*)-2-nonenal (≥95%), 2-butyl-2-octenal (≥95%), toluene anhydrous (99.8%), benzeneacetaldehyde (≥90%), 1-hexadecene (≥98.5%), acetone (≥99.9%), 2,3-pentanedione (≥96%), trimethyl pyrazine (≥99%), D-limonene (≥99%), 2-butyl-2-octenal (≥95%) and saturated alkanes standard certified reference material (49452-u, C7-C40, 1000 μg/mL each component in hexane) were supplied by Sigma-Aldrich (Madrid, Spain). Methyl isopropyl ketone (MIPK; ≥98.5%), 1-propanal (97%), 2-methyl butanal (95%), 3-methyl butanal (97%), octanal (98%), 2-butanone (≥99%), 2-heptanone (≥99%) and 3-hydroxy-2-butanone (≥97%) were supplied by Honeywell Research Chemical-Fischer Scientific (Madrid, Spain).

2.2. SPME methodology

SPME procedure was performed using an autosampler (model PAL RSI 85, CTC CombiPAL, Zwingen, Switzerland) equipped with a temperature-controlled air incubator. Volatile compounds were extracted from 2.5 ± 0.001 g of the mixture of ground cooked meat together with the sodium sulfate placed in a 10 mL amber vial (Agilent Technologies, Madrid, Spain) and to which 20 μL of internal standard (IS) solution (0.05 μg/L) were added. Vials were sealed with PTFE septa and steel magnetic cap (18 mm PTFE/SIL, Agilent Technologies) before being placed on the sample tray at room temperature. After 15 min of pre-equilibration time at extraction temperature, volatiles were trapped onto a 1 cm long divinylbenzene/carboxen/polydimethylsiloxane fiber (57298-U, 50/30 μm, Supelco, Madrid, Spain) at studied extraction temperature.

Table 1
Repeatability assay (five runs) for peak abundances (mean and SD; arbitrary area units × 10^7) of the internal standard solutions (0.05 μg/L in hexane) analyzed by SPME-GC–MS using 30 min of extraction time and different extraction temperatures (30, 60 and 80 °C).

Internal standard	LRI		30 °C	60 °C	80 °C
MIPK	931	Mean	327	124	32.1
		SD	12.6	20.3	8.66
		RSD (%)	3.84	16.3	27.0
MIBK	1011	mean	416	151	398
		SD	8.06	15.4	1.06
		RSD (%)	1.94	10.2	2.66
MAIV	1015	mean	4.57	243	43.1
		SD	37.3	33.2	1.04
		RSD (%)	8.15	13.65	2.41
CYHAONE	1293	mean	9.61	5.18	1.68
		SD	1.90	0.63	0.29
		RSD (%)	19.8	12.1	17.0
TIPB	1472	mean	86.8	822	(1)_
		SD	35.1	8.13	
		RSD (%)	40.45	0.99	

MIPK, methyl isopropyl ketone; MIBK, methyl isobutyl ketone; MAIV, methyl anteisovalerate; CYHAONE, cyclohexanone; TIPB, 1,3,5-triisopropylbenzene; LRI, linear retention index; SD, standard deviation; RSD, relative standard deviation.
(1) Thermally degraded compound during SPME procedure.

Five different IS commonly used in the literature were compared (MIPK, MIBK, MAIV, CYHAONE and TIPB) in order to study their performance at different extraction temperatures, and their resolution within the chromatogram of the volatile compounds detected in cooked meat. Hexane solutions (0.05 g/L) of the five IS were analyzed by SPME-GC–MS in quintuplicate using the different extraction temperatures reported in Table 1. Likewise, the hexane solutions of the five IS were added (20 μL) separately to the same cooked meat sample and analyzed by SPME-GC–MS in triplicate using 60 °C for 15 min of equilibration time followed by a further 30 min of extraction.

The proportion of sodium sulfate in the mixture was also previously optimized by mixing subsamples of ground cooked meat at 1:1, 2:1 and 4:1 ratios (by weight and in duplicates) with sodium sulfate. The mixtures were analyzed by SPME-GC–MS using 60 °C and 30 min for volatile extraction. In addition, sample stability in the rack was studied by analyzing in duplicate the 4:1 mixture kept for 1, 11, 21 and 33 h on the sample tray at room temperature.

2.3. GC–MS analysis

Volatile compounds were analyzed using a GC instrument (model 7820A, Agilent Technologies) equipped with two split/splitless injectors and coupled to a MS detector (model 5975 series, Agilent Technologies). Volatile compounds trapped onto the fiber were desorbed in the front injection port for 10 min at 240 °C in splitless mode (split valve was opened at 200 mL/min after 25 min of the injection). After thermal desorption, the fiber was directly cleaned in the back injection port for 30 min at 270 °C. Helium (99.999% purity, Air liquid, Madrid, Spain) at a constant pressure of 16 psi was used as carrier gas. Volatile compounds were separated in a Supelcowax-10 (Supelco) fused silica capillary column (60 m length, 0.25 mm i.d.; 0.25 μm film thickness) using the

following temperature program: oven was held at 40 °C for 10 min, then raised at a rate of 5 °C/min until 110 °C, again increased at 10 °C/min until 240 °C, and finally held at 240 °C for 15 min. Volatiles were transferred to the MS detector throughout a transfer line at 280 °C and MS detector operated at 150 °C in full scan mode with total ion current of 70 eV. Chromatographic data were analyzed with MSD ChemStation Data Analysis (vers. 5.52 Agilent Technologies). Tentative identification of volatile compounds was performed by comparing their mass spectra (matching factor > 800) with those of the National Institute of Standards and Technology spectra library (NIST vers. 2.0, Gaithersburg, USA). Mean linear retention index (LRI) value of each chromatographic peak was calculated from the analysis (3 replicates) of cooked meat samples and the saturated alkanes standard mixture (3 replicates × 3 times through the experiment). Additionally, volatile compounds were positively identified by comparison of linear retention indices (LRI) and mass spectra with those of available commercial standards.

2.4. Sample preparation and extraction temperature and time trial

A complete randomized experimental design was used to investigate the combined effect of sample preparation including meat presentation (minced and steak) and cooking method (stewed and grilled at two different cooking temperatures), and extraction temperature (30, 60 and 80 °C) and time (30 and 50 min) on meat volatile composition. Three deer loins from different animals were used (each animal was use as replicate unit). Each loin was split in six steaks and three of them were selected at random and minced. Afterwards, each minced or steak sample for each individual loin was cooked by a different method. Subsequently, the sample preparations (3 loins × 2 presentations × 3 cooking methods = 18) were analyzed by SPME-GC–MS using the different extraction temperatures and times. For each combination of SPME conditions, the vials were randomly ordered on the sample tray with a maximum of nine runs *per* day, and the day of analysis of each vial was randomized. In the automatic sampler, a blank (empty 10 mL amber vial) every 3 sample analyses was performed as a working routine. The full experimental and analytical design is available in supplementary material (Table S1).

Each loin was cut from blade to sirloin end in six steaks of ~2.5 cm thick and the steaks were randomly assigned to one of the aforementioned sample preparations. Those used for steak presentation were trimmed using a commercial round shaper to ensure similar sample shape and weight. For minced presentation, the steaks were trimmed and minced for 20 s at room temperature using a commercial grinder (model HR1393, Philips, Madrid, Spain). Subsequently the same round shaper was used to shape the minced meat to the same shape and thickness as steaks.

Prior to cooking, all samples were individually kept in plastic bags immersed in a water bath at 20 °C for 30 min to ensure the same initial meat temperature. Independently of the cooking method (stewed or grilled), the heating treatment was immediately stopped when the meat reached the intended internal temperature of 70 °C. Temperature in the geometric center of the sample was controlled using a four-channel thermometer (model TM-946, Lutron Electronics, Coopersburg, USA). Stewing was performed in a water bath (model Unitronic 320 OR, Selecta, Abrera, Spain) set at 72 °C. All meat samples were cooked at once where each individual sample was placed in a separate open plastic bag. Plastic bags were hung from a metal bar and fully immersed in water (the sample had no direct contact with water). The same space was kept among bags in order to ensure similar water flow within the bath. Grilling was performed in a horizontal double clamp electric grill (model Dalkyo MB-30, Sogo, Valencia, Spain) set at 197 °C (grilled-high) and at 147 °C (grilled-low). The three different samples *per* presentation were grilled together.

In all samples (stewed and grilled), cooking was stopped by immersion of cooked samples, protected inside a plastic bag, on iced water for one minute. Immediately after cooling, all samples were grounded for 10 s and mixed with sodium sulfate (4:1 meat to salt ratio by weight). Each mixture was divided into six equal parts to be subsequently analysed at different SPME extraction temperatures and times (3 temperatures × 2 times). The mixtures were individually vacuum packed and kept in a freezer at −80 °C until SPME-GC–MS volatile analysis was performed (within 15 days post-cooking). Cooked meat volatiles were analyzed by SPME-GC–MS under the above described conditions (sections 2.2 and 2.3) after thawing for around 2 h, at refrigeration conditions and previous addition to the sample vial of 20 μL of MIBK IS solution (5 μg/L).

2.5. Heavy volatile compound detection trial

A trial was performed to ensure that heavy volatile compounds were only released from cooked deer meat samples when high extraction temperatures were applied. Three batches of stewed subsamples were analyzed in triplicate using different SPME procedures. The first batch was subjected to extraction temperature of 80 °C for 15 min of equilibration time followed by a further 30 min of extraction. The other two batches were preheated at 80 °C of extraction temperature for 15 min of equilibration time followed by a further 30 min of extraction in the autosampler incubator, and then vials were kept in the tray until the sample reached room temperature. Afterwards, the vials were separately subjected to extraction temperature of 30 and 80 °C for 15 min of equilibration time followed by a further 30 min of extraction. The volatile profile was compared among the three batches.

2.6. Data treatment and statistical analysis

Quantification of volatile compounds was based on the chromatographic peak area and the limit of detection (LOD) was calculated from the noise measured in three different zones of the chromatogram obtained from the analysis of ten blanks. LOD was set as twice the average noise for each zone. Volatile compound content of cooked meat samples was expressed as peak area (arbitrary units) relative to the IS area according to the following equation:

$$\text{Volatile relative abundance} = \frac{\text{peak area}}{\text{IS area}} \times \frac{2.5\,\text{g}}{\text{mixture weight (g)}} \times 100$$

Peak areas (>LOD) of individual volatile compounds detected in at least two of the three replicates were used to calculate mean abundances in meat samples of each meat presentation, cooking method, and extraction temperature and time conditions. Relative abundance data of volatile compounds were log-transformed, and normality, homoscedasticity and residual randomness were checked. Data were subjected to Analysis of Variance using a Linear Mixed Model procedure of IBM-SPSS statistical software (vers. 25.0, New York, USA) considering meat presentation and cooking method as fixed whole-plot factors and extraction temperature and time as split-plot factors. Individual animal (loin) as subject (experimental unit) and the interaction between meat presentation and cooking method were included in the mixed model as random effects. The parameters of the model were estimated using the Restricted Maximum Likelihood method and Restricted Scaled Identity Matrix was used for the covariance structure of random effects. Additionally, Least Square Means of dependent variables for the levels of the fixed factor extraction temperature and time were compared using the Least Significance Difference test.

On the other hand, absolute and relative detection frequencies (peak area > LOD) of volatile compounds in the meat samples were estimated using the zero value for a non-detected compound and one for detected volatile compounds. The non-parametric Kruskal-Wallis test was applied to determine the significance of the differences in frequency values among the six resulting SPME procedures for volatile extraction (3 extraction temperatures and 2 times).

Three significant figures were used to express the relative abundance of all volatile compounds. Significance level was declared at $P \leq 0.05$.

Table 2
Relative abundance (mean ± standard deviation) of volatile compounds from deer meat samples with different presentation (minced and steak) and cooking method (stewed, grilled-high and grilled low) analyzed by SPME-GC–MS (arbitrary area units × 10^7).

LRI	Compound	Meat presentation				Cooking method						Significance		
		Minced		Steak		Stewed		Grilled-high		Grilled-low		MP	CM	MP*CM
Alcohols														
1156	1-Penten-3-ol[2]	1.06	±1.20	0.765	±0.407	1.27	±1.45	0.741	±0.321	0.735	±0.372	ns	ns	ns
1244	1-Pentanol[2]	3.39	±1.81	3.57	±2.29	4.11	±2.61	2.99	±1.61	3.37	±1.72	ns	ns	ns
1342	1-Hexanol[2]	1.49	±0.67	1.33	±0.67	1.60	±0.78	1.25	±0.51	1.34	±0.65	ns	ns	ns
1433	1-Octen-3-ol[2]	16.1	±10.0	13.6	±9.6	17.3	±11.0	13.1	±8.7	14.2	±9.5	ns	ns	ns
1440	1-Heptanol[2]	1.21	±0.48	1.38	±0.66	1.30	±0.56	1.39	±0.67	1.18	±0.49	ns	ns	ns
1473	2-Ethyl-1-hexanol[1]	0.870	±0.358	0.910	±0.390	0.871	±0.362	1.00	±0.39	0.768	±0.320	ns	*	ns
1571	1-Octanol[1]	0.868	±0.419	0.974	±0.385	0.586	±0.081	0.962	±0.368	0.984	±0.547	ns	ns	ne
1593	(E)-2-Octen-1-ol[2]	3.30	±2.79	4.23	±3.56	3.72	±3.51	3.84	±3.17	3.73	±2.95	ns	ns	ns
1997	(E)-2-Dodecen-1-ol[1]	2.39	±1.66	4.65	±6.05	3.01	±4.32	4.20	±5.87	3.10	±2.24	ns	ns	ns
2075	1-Tridecanol[2]	2.17	±1.04	2.68	±1.40	2.56	±1.14	2.32	±1.45	2.32	±1.11	ns	ns	ns
2109	(E)-2-Tetradecen-1-ol[1]	10.3	±11.4	23.7	±46.6	14.3	±32.3	27.4	±51.9	12.5	±16.7	ns	ns	ns
2181	1-Tetradecanol[1]	3.03	±1.73	3.43	±1.95	3.36	±1.69	3.36	±2.12	2.99	±1.78	ns	ns	ns
2217	1-Pentadecanol[1]	2.04	±1.10	1.94	±0.82	1.89	±0.92	2.06	±0.79	1.98	±1.09	ns	ns	ns
Aldehydes														
707	Acetaldehyde[1]	0.901	±0.284	0.883	±0.310	0.778	±0.295	1.00	±0.31	0.869	±0.238	ns	***	ne
797	Propanal[2]	0.464	±0.210	0.354	±0.113	0.443	±0.147	0.456	±0.227	0.349	±0.152	*	ns	ne
913	2-Methyl butanal[2]	0.289	±0.009	0.367	±0.128	ND		0.388	±0.118	0.261	±0.087	ns	ns	ne
917	3-Methyl butanal[2]	0.429	±0.121	0.446	±0.187	0.355	±0.080	0.535	±0.177	0.361	±0.111	ns	***	ns
978	Pentanal[2]	4.39	±2.49	3.42	±1.90	3.78	±2.08	4.06	±2.42	3.88	±2.31	ns	ns	ns
1081	Hexanal[2]	87.2	±59.6	71.3	±57.1	84.7	±63.6	76.5	±59.5	76.4	±53.6	ns	ns	ns
1181	Heptanal[2]	3.65	±2.16	3.34	±2.09	3.90	±2.37	3.22	±1.94	3.37	±2.03	ns	ns	ns
1284	Octanal[2]	3.89	±1.80	5.06	±4.13	4.17	±3.40	4.50	±2.33	4.51	±3.63	ns	ns	ns
1388	Nonanal[2]	19.9	±14.7	18.0	±15.0	19.8	±14.8	18.9	±15.8	18.1	±14.1	ns	ns	ns
1429	(E)-2-Octena[2]	2.14	±1.56	2.02	±1.44	2.09	±1.51	2.14	±1.50	2.04	±1.53	ns	ns	ns
1491	Decanal[2]	1.60	±0.66	1.54	±0.66	1.78	±0.72	1.46	±0.55	1.46	±0.68	ns	ns	ns
1539	(E)-2-Nonenal[1]	3.60	±2.17	3.28	±2.12	3.73	±2.28	3.16	±1.98	3.42	±2.16	ns	ns	ns
1647	(E)-2-Decenal[2]	2.07	±0.99	2.62	±1.24	2.42	±1.34	2.07	±0.96	2.36	±1.00	ns	ns	ns
1668	2-Butyl-2-octenal[1]	2.46	±1.38	2.62	±1.55	2.68	±1.34	2.59	±1.74	2.29	±1.26	ns	ns	ns
1708	Dodecanal[2]	2.87	±1.37	3.07	±1.76	2.84	±1.40	2.99	±1.80	3.05	±1.54	ns	ns	ns
1760	2-Undecenal[1]	2.53	±1.50	2.42	±1.74	2.63	±1.95	2.37	±1.38	2.38	±1.48	ns	ns	ns
1820	Tridecanal[2]	8.00	±5.00	9.29	±7.70	7.50	±5.14	9.52	±7.81	8.98	±6.36	ns	ns	ns
1830	(E,E)-2,4-Decadienal[1]	2.04	±1.32	2.23	±0.62	2.16	±0.93	1.71	±0.83	2.40	±1.41	ns	ns	ns
1939	Tetradecanal[2]	11.5	±12.2	11.2	±15.5	11.4	±12.5	12.0	±16.0	10.7	±13.4	ns	ns	ns
2051	Pentadecanal[2]	11.7	±11.0	13.6	±15.4	11.4	±11.2	14.0	±15.5	12.6	±13.6	ns	ns	ns
2161	Hexadecanal[2]	12.0	±13.2	18.4	±19.9	12.9	±14.0	19.3	±21.7	13.3	±14.3	ns	ns	ns
2374	Octadecanal[1]	2.01	±0.71	2.19	±0.93	1.88	±0.47	2.44	±1.00	1.96	±0.89	ns	ns	ns
Aromatic hydrocarbons														
1037	Toluene[2]	0.579	±0.445	0.837	±0.692	0.603	±0.467	0.630	±0.354	0.948	±0.905	ns	ns	ns
1532	Benzaldehyde[2]	13.7	±12.1	14.2	±12.2	12.7	±11.7	14.7	±12.2	14.6	±12.8	ns	*	*
1656	Benzeneacetaldehyde[1]	1.98	±0.70	1.87	±0.62	1.70	±0.59	1.98	±0.72	2.14	±0.58	ns	ns	ns
1731	3-Ethyl benzaldehyde[1]	4.46	±3.59	4.55	±3.30	4.74	±3.57	4.53	±3.48	4.26	±3.38	ns	ns	ns
2062	4-Pentyl benzaldehyde[1]	2.07	±0.78	2.17	±0.79	2.15	±0.76	2.34	±0.90	1.91	±0.68	ns	ns	ns
Furans														
951	2-Ethyl furan[2]	0.467	±0.251	0.671	±0.296	0.720	±0.381	0.643	±0.194	0.375	±0.208	ne	ne	ne
1226	2-Pentyl furan[2]	3.31	±2.70	3.78	±3.05	3.54	±2.79	3.87	±2.97	3.20	±2.88	ns	ns	ns
Aliphatic hydrocarbons														
>701	Pentane[2]	0.369	±0.249	0.556	±0.414	0.697	±0.547	0.548	±0.254	0.276	±0.172	ns	ns	ne
701	Heptane[2]	0.554	±0.393	0.797	±0.534	0.628	±0.441	0.720	±0.508	0.668	±0.509	ns	ns	ne
737	1-Heptene[2]	0.733	±0.360	0.469	±0.013	0.469	±0.013	0.589	±0.426	0.948	±0.020	ne	ne	ne
801	Octane[2]	0.915	±0.629	0.857	±0.695	0.893	±0.738	0.936	±0.632	0.842	±0.614	ns	ns	ns
958	2,2,4,6,6-Pentamethyl heptane[2]	0.854	±0.854	1.59	±2.25	0.625	±0.324	1.81	±2.45	0.825	±0.592	ns	ns	ns
1288	Tridecane[2]	5.09	±3.21	5.29	±3.60	4.01	±3.85	5.44	±3.27	ND		ns	ns	ne
1418	3-Methyl-2-ethyl-1,3-hexadiene[1]	1.01	±0.47	0.987	±0.491	1.03	±0.49	1.00	±0.44	0.962	±0.513	ns	ns	ns
1484	Pentadecane[1]	1.80	±1.12	1.93	±1.34	1.55	±0.89	2.07	±1.50	1.91	±1.16	ns	ns	ns
1577	Hexadecane[1]	0.512	±0.161	0.492	±0.200	0.508	±0.158	0.550	±0.196	0.443	±0.140	ns	ns	ns
1629	1-Hexadecene[1]	1.30	±0.56	1.62	±0.85	1.30	±0.64	1.50	±0.89	1.52	±0.53	ns	ns	ns
Ketones														
816	Acetone[2]	0.645	±0.324	0.717	±0.451	0.597	±0.362	0.758	±0.410	0.677	±0.387	ns	*	ns
903	2-Butanone[2]	0.600	±0.326	0.454	±0.285	ND		0.546	±0.348	0.463	±0.213	ns	ns	ns
1060	2,3-Pentanedione[2]	0.810	±0.303	0.715	±0.447	0.750	±0.419	0.889	±0.448	0.640	±0.281	ns	ns	ns
1179	2-Heptanone[2]	1.53	±1.18	1.25	±0.85	1.45	±0.97	1.43	±1.14	1.29	±1.03	ns	ns	ns
1236	6-Methyl-2-heptanone[1]	0.904	±0.290	0.850	±0.345	0.724	±0.181	1.03	±0.36	0.829	±0.309	ns	ns	ns
1286	3-Hydroxy-2-butanone[2]	22.9	±8.5	24.1	±10.5	23.8	±8.8	23.4	±9.6	23.3	±10.3	ns	ns	ns
1319	2,3-Octanedione + 2,5-Octanedione[2]	26.9	±13.3	19.6	±14.2	24.1	±14.7	21.7	±14.4	23.6	±13.7	ns	ns	ns
1333	5-Methyl-3-hepten-2 one[1]	4.12	±2.54	3.65	±2.48	3.86	±2.18	4.00	±2.90	3.77	±2.43	ns	ns	ns

(*continued on next page*)

Table 2 (*continued*)

LRI	Compound	Meat presentation				Cooking method						Significance		
		Minced		Steak		Stewed		Grilled-high		Grilled-low		MP	CM	MP*CM
1384	2-Nonanone[2]	1.46	±1.60	0.950	±0.551	0.730	±0.521	1.34	±1.27	1.65	±1.50	ns	ns	ns
1857	6,10-Dimethyl-(E)-5,9-undecadien-2-one[1]	0.987	±0.398	1.07	±0.43	1.05	±0.36	1.09	±0.47	0.940	±0.416	ns	ns	ns
2014	2-Pentadecanone[1]	1.25	±0.49	1.22	±0.51	1.00	±0.29	1.20	±0.44	1.43	±0.63	ns	ns	ns
Nitrogen and sulfur compounds														
729	Dimethyl sulfide[1]	0.519	±0.406	ND		ND		0.298	±0.104	0.740	±0.537	ne	ne	ne
1398	Trimethyl pyrazine[2]	1.11	±0.38	1.71	±0.73	ND		1.69	±0.70	1.23	±0.80	ns	ns	ne
Terpenes														
1191	*cis*-Linalool oxide[1]	0.440	±0.211	0.378	±0.198	0.485	±0.286	0.328	±0.128	0.437	±0.208	ns	ns	ns
1465	D-Limonene[2]	0.416	±0.147	0.440	±0.182	0.425	±0.179	0.475	±0.133	0.390	±0.146	ns	ns	ns

LRI, linear retention index; MP, meat presentation; CM, cooking method;[1]Tentative identification by comparison with mass spectra from NIST (probability match > 800); [2]Positive identification by comparison of LRI and mass spectra of authentic standards; ND, not detected; ns, not significant ($P > 0.05$); ne, not estimated by the linear mixed model due to lack of data at several levels of factors; * $P \leq 0.05$; *** $P \leq 0.001$.

3. Results and discussion

3.1. IS selection, sample to sodium sulfate ratio in the mixture and sample stability

The use of IS is a common practice for an accurate quantification of volatile compounds by SPME-GC techniques avoiding analytical errors and systematic bias (Bueno et al., 2019). The five IS tested were selected from those commonly used in the literature for volatile analysis in animal food products (Abilleira et al., 2011; Bueno et al., 2019; Karabagias, 2018). Abundance and repeatability values for IS solutions were different depending on the chemical nature of the compounds and extraction temperature used (Table 1). With the exception of TIPB which was thermally degraded at 80 °C, the IS abundance of others was modified with increasing extraction temperature and the relative standard deviation (RSD) ranged among 2 to 27%. In this regard, MIBK and MAIV showed the lowest mean RSD values (4.9 and 8.1%, respectively). Vials containing a mixture of ground cooked meat and sodium sulfate, and IS solution (except TIPB) were analyzed at the intermediate extraction temperature of 60 °C. The results showed overlapping between CYHAONE and MIPK with some volatile compounds present in the cooked deer meat. In particular, CYHAONE co-eluted with tridecane while MIPK was identified as a potential compound of cooked deer meat volatile profile. On the other hand, MIBK and MAIV were the most suitable IS and provided very similar LRI values. Finally, MIBK was selected because of its higher average repeatability (Table 1). To our knowledge, the specific effect exerted by extraction temperature on IS abundance has hardly been studied although higher RSD values have been reported when extraction temperatures increased from 40 to 60 °C (Ruiz et al., 1998). In essence, the IS selection for semi-quantitative methods based on the relative abundance of peak area under SPME techniques must be carefully done. This selection will be valid only for the comparison of volatile profiles among samples extracted at the same extraction temperature, since the correction of the peak areas relative to the IS can generate over or underestimation of volatile compounds.

Salt addition to the sample has been highlighted as a good procedure to improve volatile extraction efficiency due to salting-out effect, which increases retention of water-soluble components (Kataoka et al., 2000). Previous studies added anhydrous sodium sulfate to meat sample at 1:1 ratio (by weight) (Gkarane et al., 2018; Rivas-Cañedo, Juez-Ojeda, Nuñez, & Fernández-García, 2011). In the present study, and as others reported (Moon & Li-Chan, 2004), no major differences were found in the volatile profile extracted by SPME using different meat to salt ratios (1:1 to 4:1, by weight). However, the best results in terms of better definition of smallest peaks and higher volatile compound extraction yields were observed when low salt amounts were added to the sample (4:1 meat to salt ratio; data not shown). The observed higher efficiency was probably related to a lower sample dilution in the vial (Lee, Diono, Kim, & Min, 2003).

Sample stability was also studied in order to determine the maximum time that a sample could stay on the sample tray at room temperature without suffering significant changes in its volatile profile. In this regard, the results of the SPME-GC–MS analyses showed hardly any differences in the volatile profile of the same sample kept for 1 to 33 h on the sample tray. Overall, peak frequency was stable over time and only a significant ($P \leq 0.05$) increase of 3-ethyl benzaldehyde (LRI 1731) abundance over time was observed, particularly after 21 h (data not shown). This compound has been associated to the amino acid degradation (Calzada, del Olmo, Picon, Gaya, & Nuñez, 2014) and when volatile compounds were grouped in chemical families no significant abundance differences ($P > 0.05$) were found over time (supplementary material, Table S2). The high stability over time of the volatile profile of the samples (4:1 cooked meat to salt ratio) can be explained by the low water content of cooked meat compared to raw meat, and by the effect of other factors such as structure and nature of protein matrix, mineral content, as well as the degree of cooking of meat. In addition, the fact that immediately after cooking, the meat samples were homogeneously mixed with salt and frozen at −80 °C for a maximum of 15 days until volatile analysis could help the sample stability on the tray at room temperature.

3.2. Effect of sample preparation and SPME temperature and time

3.2.1. General volatile profile of cooked deer meat

A maximum of 68 compounds were identified in cooked deer meat samples regardless of sample preparation and SPME temperature and time conditions. A list of identified compounds is provided in Table 2, where aldehydes, ketones and alcohols were the major chemical families of cooked deer meat and all together represented over 85% of the total content of volatile compounds. Hexanal, 3-hydroxy-2-butanone, 2,3 + 2,5-octanedione and 1-octen-3-ol were also very abundant volatiles, regardless of the analytical conditions used. Hexanal has been reported as major compound in cooked meat from other species (Mottram, 1998), and 3-hydroxy-2-butanone and 1-octen-3-ol have also been found in other species (cooked meat) (Almela et al., 2010). Likewise, most volatiles reported as key-aroma compounds in other cooked meats such as 2- and 3-methyl butanal, 2,3-pentanedione, nonanal, decanal, dimethyl sulfide, heptanal, octanal, 2-octenal, and 2-nonenal (Cerny & Grosch, 1992; Guth & Grosch, 1994; Specht & Baltes, 1994) were also detected in the present study (cooked deer meat; Table 2). Moreover, cooked deer meat also showed a considerable abundance of long chain aldehydes and alcohols, which according to other studies on cooked meat, could be derived from lipid oxidation (Kerth, 2016).

3.2.2. Effect of sample preparation

The effect of sample preparation including meat presentation

Table 3
Relative abundance (mean ± standard deviation) of volatile compounds from cooked deer meat samples extracted using different extraction temperature (30, 60 and 80 °C) and time (30 and 50 min) and analyzed by GC–MS (arbitrary units × 10^7).

LRI	Compound	30 °C				60 °C				80 °C				Significance		
		30 min		50 min		30 min		50 min		30 min		50 min		T	time	T*time
Alcohols																
1156	1-Penten-3-ol	0.818	± 0.491	0.732	± 0.254	1.30	± 0.44	0.734	± 0.273	0.622	± 0.226	1.14	± 1.93	***	*	*
1244	1-Pentanol	4.99	± 2.85	4.08	± 1.97	3.21	± 1.85	3.30	± 1.74	2.64	± 1.30	2.67	± 1.45	***	ns	ns
1342	1-Hexanol	1.27	± 0.60	1.87	± 0.88	1.42	± 0.63	1.46	± 0.72	1.11	± 0.44	1.23	± 0.36	***	**	ns
1433	1-Octen-3-ol	8.76	± 6.49	14.0	± 10.0	19.6	± 12.0	20.9	± 11.4	12.8	± 6.3	13.2	± 6.6	***	***	*
1440	1-Heptanol	0.791	± 0.387	0.717	± 0.227	1.58	± 0.47	1.83	± 0.60	1.32	± 0.32	1.45	± 0.42	***	ns	ns
1473	2-Ethyl-1-hexanol	0.542	± 0.185	0.767	± 0.184	0.987	± 0.347	1.11	± 0.43	0.77	± 0.19	1.14	± 0.67	***	*	ns
1571	1-Octanol	ND		ND		0.674	± 0.170	1.38	± 0.27	0.75	± 0.35	ND		ns	*	ne
1593	(E)-2-Octen-1-ol	0.295	± 0.143	1.49	± 0.75	2.78	± 1.81	4.88	± 3.90	5.51	± 3.71	4.82	± 2.72	***	***	***
1997	(E)-2-Dodecen-1-ol	ND		ND		1.07	± 0.36	1.53	± 0.60	4.05	± 4.16	5.56	± 6.22	***	*	ns
2075	1-Tridecanol	ND		ND		ND		1.01	± 0.34	2.28	± 0.84	2.96	± 1.34	***	ns	ne
2109	(E)-2-Tetradecen-1-ol	ND		ND		2.56	± 1.21	3.28	± 2.30	15.7	± 21.1	29.4	± 52.2	***	*	ns
2181	1-Tetradecanol	ND		ND		ND		0.770	± 0.352	2.82	± 1.03	3.97	± 2.09	***	ns	ne
2217	1-Pentadecanol	ND		ND		ND		ND		1.19	± 0.39	2.61	± 0.69	ne	***	ne
Aldehydes																
707	Acetaldehyde	0.638	± 0.160	0.598	± 0.090	0.694	± 0.200	0.791	± 0.200	0.991	± 0.193	1.20	± 0.28	***	ns	ns
797	Propanal	0.420	± 0.113	0.439	± 0.184	0.465	± 0.287	0.350	± 0.080	ND		ND		ns	ns	ns
913	2-Methyl butanal	0.479	± 0.148	ND		0.298	± 0.136	0.367	± 0.100	0.336	± 0.097	ND		ns	ns	ne
917	3-Methyl butanal	0.366	± 0.194	0.356	± 0.000	0.414	± 0.160	0.463	± 0.137	0.476	± 0.186	0.460	± 0.146	***	ns	ns
978	Pentanal	5.72	± 3.16	4.15	± 2.41	3.49	± 1.78	4.45	± 2.16	2.51	± 0.93	3.14	± 0.92	***	ns	***
1081	Hexanal	116	± 80	98.5	± 61.2	88.8	± 56.1	82.1	± 49.5	45.9	± 27.6	44.2	± 26.3	***	ns	ns
1181	Heptanal	3.23	±2.31	3.91	± 2.57	4.22	± 2.13	4.08	± 2.33	2.51	± 1.16	3.01	± 1.62	***	ns	ns
1284	Octanal	ND		ND		ND		6.61	± 1.39	4.17	± 2.94	4.21	± 3.35	ns	ns	ne
1388	Nonanal	5.43	± 3.61	9.79	± 6.68	22.9	± 16.3	29.7	± 18.2	21.4	± 10.2	24.5	± 12.4	***	***	ns
1429	(E)-2-Octenal	0.366	± 0.085	0.668	± 0.205	2.53	± 1.16	2.74	± 1.58	2.49	± 1.34	2.76	± 1.38	***	**	*
1491	Decanal	ND		ND		1.75	± 0.83	1.60	± 0.85	1.38	± 0.40	1.49	± 0.19	***	ns	ns
1539	(E)-2-Nonenal	0.888	± 0.399	1.20	± 0.59	3.64	± 1.59	4.75	± 1.72	4.54	± 1.32	5.35	± 1.42	***	***	ns
1647	(E)-2-Decenal	ND		ND		1.29	± 0.27	1.79	± 0.62	2.13	± 0.49	3.53	± 1.22	***	***	ns
1668	2-Butyl-2-octenal	ND		ND		1.61	± 0.81	2.47	± 1.18	2.67	± 1.29	3.35	± 1.85	***	*	ns
1708	Dodecanal	ND		ND		1.68	± 0.36	2.72	± 1.05	2.98	± 1.40	4.26	± 1.84	***	ne	ns
1760	2-Undecenal	ND		ND		1.40	± 0.23	1.66	± 0.72	2.51	± 1.08	4.20	± 2.00	***	***	ns
1820	Tridecanal	ND		ND		3.87	± 1.44	6.98	± 4.11	9.41	± 5.55	13.0	± 8.2	***	*	ns
1830	(E,E)-2,4-Decadienal	ND		ND		1.03	± 0.14	4.03	± 3.23	2.16	± 0.60	2.28	± 0.87	ns	**	**
1939	Tetradecanal	0.786	± 0.279	ND		3.27	± 2.19	6.78	± 5.44	15.6	± 12.4	23.8	± 19.7	***	*	ns
2051	Pentadecanal	ND		ND		3.34	± 1.59	5.93	± 3.78	14.6	± 10.6	23.7	± 17.7	***	***	ns
2161	Hexadecanal	ND		ND		1.91	± 0.71	3.54	± 1.52	21.1	± 10.0	34.3	± 19.0	***	***	ns
2374	Octadecanal	ND		ND		ND		ND		1.72	± 0.62	2.50	± 0.86	ne	***	ne
Aromatic hydrocarbons																
1037	Toluene	0.697	±0.314	0.509	± 0.166	1.33	± 0.95	0.375	± 0.128	0.354	± 0.076	0.446	± 0.101	ns	**	*
1532	Benzaldehyde	1.41	±0.68	3.31	± 3.48	3.65	± 1.54	15.1	± 6.1	22.4	± 3.2	31.3	± 6.4	***	***	***
1656	Benzeneacetaldehyde	ND		ND		1.14	± 0.27	1.39	± 0.26	1.95	± 0.56	2.29	± 0.64	***	**	ns
1731	3-Ethyl benzaldehyde	ND		ND		2.31	± 0.73	3.05	± 1.72	4.98	± 2.99	6.88	± 4.57	***	***	ns
2062	4-Pentyl benzaldehyde	ND		ND		ND		1.19	± 0.43	2.10	± 0.58	2.29	± 0.89	ns	ns	ne
Furans																
951	2-Ethyl furan	0.811	±0.262	0.706	± 0.084	0.397	± 0.048	0.371	± 0.065	0.205	± 0.052	ND		***	ns	ne
1226	2-Pentyl furan	2.64	±1.97	6.07	± 4.41	3.21	± 2.57	4.68	± 3.03	2.32	± 1.64	3.09	± 2.32	ns	ns	ns
Aliphatic hydrocarbons																
>701	Pentane	0.508	±0.369	ND		0.129	± 0.026	ND		0.484	± 0.311	ND		ne	ne	ne
701	Heptane	0.667	±0.432	0.531	± 0.152	0.806	± 0.626	0.096	±0.090	ND		ND		ns	ns	ns
737	1-Heptene	0.658	±0.321	ND		ND		ND		ND		ND				

(*continued on next page*)

Table 3 (*continued*)

LRI	Compound	30 °C				60 °C				80 °C				Significance		
		30 min		50 min		30 min		50 min		30 min		50 min		T	time	T*time
801	Octane	1.39	±0.87	1.13	± 0.53	0.518	± 0.142	0.460	± 0.13	0.431	± 0.042	ND		***	ns	ns
958	2,2,4,6,6-Pentamethyl heptane	0.973	±0.760	3.79	± 3.37	0.623	± 0.422	0.809	± 0.416	0.493	± 0.193	ND		***	***	***
1288	Tridecane	ND		ND		ND		ND		5.01	± 3.69	5.32	± 3.13	ne	ns	ne
1418	3-Methyl-2-ethyl-1,3-hexadiene	0.421	±0.148	0.534	± 0.104	1.31	± 0.45	1.29	± 0.50	1.03	± 0.30	0.95	± 0.24	***	ns	ns
1484	Pentadecane	ND		ND		0.855	± 0.393	1.28	± 0.90	2.51	± 0.93	3.02	± 1.35	***	**	ns
1577	Hexadecane	0.437	±0.192	0.494	± 0.114	0.618	± 0.156	0.682	± 0.135	0.382	± 0.089	ND		***	ns	ns
1629	1-Hexadecene	ND		ND		ND		1.28	± 0.36	1.14	± 0.51	2.10	± 0.79	*	**	ne
Ketones																
816	Acetone	1.38	±0.44	0.459	± 0.140	0.57	± 0.10	0.62	± 0.15	0.48	± 0.12	0.51	± 0.09	***	***	***
903	2-Butanone	0.656	±0.333	ND		0.32	± 0.10	ND		0.346	± 0.020	ND		*	ne	ne
1060	2,3-Pentanedione	0.825	±0.379	1.04	± 0.50	0.565	± 0.209	0.535	± 0.141	ND		ND		***	ns	ns
1179	2-Heptanone	1.15	±0.87	1.90	± 1.07	1.27	± 0.69	1.80	± 1.57	0.98	± 0.56	1.33	± 0.96	*	**	ns
1236	6-Methyl-2-heptanone	ND		ND		ND		0.928	± 0.244	0.668	± 0.136	0.970	± 0.364	ns	ns	ne
1286	3-Hydroxy-2-butanone	36.8	±4.9	31.1	± 3.2	22.8	± 3.0	22.0	± 5.0	12.3	± 4.8	14.8	± 4.5	***	ns	*
1319	2,3-Octanedione + 2,5-Octanedione	16.7	±7.2	35.5	± 15.0	28.4	± 15.8	31.2	± 14.9	16.3	± 5.2	11.4	± 3.7	***	ns	***
1333	5-Methyl-3-hepten-2 one	2.05	±1.15	3.36	± 2.02	3.42	± 2.04	4.55	± 2.49	3.67	± 1.87	5.67	± 3.43	***	***	ns
1384	2-Nonanone	ND		ND		0.326	± 0.117	1.34	± 1.95	1.33	± 1.02	1.39	± 0.83	*	ns	ns
1857	6,10-Dimethyl-(E)-5,9-undecadien-2-one	ND		ND		ND		0.818	± 0.309	0.840	± 0.355	1.32	± 0.33	ns	**	ne
2014	2-Pentadecanone	ND		ND		ND		ND		1.31	± 0.54	1.16	± 0.46	ne	ns	ne
Nitrogen and sulfur compounds																
729	Dimethyl sulfide	0.298	±0.104	0.740	± 0.537	ND		ND		ND		ND		ne	ne	ne
1398	Trimethyl pyrazine	ND		ND		1.26	± 0.50	2.35	± 0.20	1.67	± 1.11	1.74	± 0.75	ns	ns	ns
Terpenes																
1191	D-Limonene	ND		ND		0.343	± 0.269	0.347	± 0.165	0.382	± 0.068	0.477	± 0.169	ns	ns	ne
1465	cis-Linalool oxide	0.215	±0.039	ND		0.444	± 0.148	0.524	± 0.117	0.397	± 0.104	ND		***	ns	ne

T, temperature; ND, not detected; ns, not significant ($P > 0.05$); ne, not estimated by the linear mixed model due to lack of data at several levels of factors; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 4
Relative abundance (mean ± standard deviation) of volatile chemical families from cooked deer meat samples extracted using different extraction temperature (30, 60 and 80 °C) and time (30 and 50 min) and analyzed by GC–MS (arbitrary area units × 10^7).

Chemical families	30 °C				60 °C				80 °C				Significance		
	30 min		50 min		30 min		50 min		30 min		50 min		T	time	T*time
Alcohols	17.5	±10.9	23.7	±14.2	35.2	±14.9	42.2	±14.5	51.6	±18.7	70.1	±51.9	***	ns	ns
Saturated	7.59	±3.94	7.44	±3.13	7.86	±2.81	10.9	±3.12	12.9	±4.59	16.0	±6.12	ns	ns	ns
Unsaturated	9.87	±7.04	16.3	±11.3	27.3	±12.7	31.3	±11.8	38.7	±20.5	54.1	±53.2	***	*	ns
Branched	0.542	±0.37	0.767	±0.410	0.987	±0.409	1.11	±0.426	0.771	±0.414	1.14	±0.402	***	*	ns
Linear	16.9	±10.7	22.9	±14.1	34.2	±15.1	41.1	±14.6	50.8	±18.8	69.0	±51.9	***	***	ns
≤8 carbons	17.5	±10.9	23.7	±14.2	31.6	±15.5	35.6	±15.5	25.5	±9.30	25.6	±11.1	***	*	ns
>8 carbons	ND		ND		3.63	±1.52	6.59	±3.63	26.1	±23.5	44.5	±56.7	***	**	ns
Aldehydes	134	±89	120	±73	149	±90	174	±95	162	±82	205	±112	***	ns	ns
Saturated	133	±89	118	±73	137	±85	156	±88	145	±75	184	±103	***	ns	ns
Unsaturated	1.25	±0.59	1.87	±0.91	11.5	±5.3	17.4	±7.0	16.5	±6.7	21.5	±9.9	***	***	ns
Branched	0.845	±0.390	0.356	±0.120	2.32	±0.98	3.30	±1.43	3.48	±1.38	3.81	±2.01	***	ns	ns
Linear	133	±90	119	±73	146	±89	171	±94	158	±81	201	±110	***	ns	ns
≤8 carbons	127	±89	109	±73	103	±79	104	±75	62.1	±41.9	62.3	±44.7	**	ns	ns
>8 carbons	7.10	±3.8	11.0	±7.3	46.1	±27.5	69.5	±38.8	99.5	±52.4	143	±83	***	***	ns
Aromatic hydrocarbons	2.11	±1.04	3.82	±3.58	8.42	±2.9	21.1	±7.7	31.7	±6.7	43.2	±4.9	***	***	***
Furans	3.45	±2.36	6.78	±4.58	3.61	±2.73	5.91	±3.39	2.53	±1.66	11.4	±2.3	ns	ns	ns
Aliphatic hydrocarbons	5.06	±2.00	6.48	±2.99	4.86	±1.46	5.91	±2.04	11.5	±4.8	11.4	±5.3	ns	ns	ns
Saturated	1.61	±0.69	1.02	±0.41	2.41	±0.89	2.06	±0.90	8.38	±3.88	8.35	±4.21	***	ns	ns
Unsaturated	3.45	±0.84	5.46	±3.09	2.45	±0.87	3.85	±1.24	3.09	±1.19	3.06	±1.52	ns	ns	ns
Branched	1.39	±0.76	4.33	±3.09	1.93	±0.87	2.10	±0.94	1.52	±0.63	0.952	±0.510	ns	ns	ns
Linear	3.66	±1.72	2.15	±0.86	2.93	±0.93	3.80	±1.42	9.95	±4.36	10.5	±5.1	***	ns	**
≤8 carbons	4.62	±2.07	5.99	±3.00	3.39	±1.10	2.66	±0.99	2.43	±0.78	0.952	±0.510	***	ns	ns
>8 carbons	0.437	±0.262	0.494	±0.247	1.47	±0.594	3.24	±1.35	9.04	±4.23	10.5	±5.1	***	ns	ns
Ketones	59.6	±8.4	73.3	±22.2	57.7	±19.3	63.8	±21.5	38.3	±12.7	38.6	±9.2	***	ns	ns
Saturated	57.5	±7.5	69.9	±20.5	54.2	±17.4	58.5	±19.2	33.8	±11.4	31.6	±6.7	***	ns	ns
Unsaturated	2.05	±1.4	3.36	±2.11	3.42	±2.04	5.36	±2.61	4.51	±2.05	6.99	±3.64	***	***	ns
Branched	38.8	±4.32	34.5	±2.9	26.2	±2.1	28.3	±3.9	17.5	±6.4	22.8	±4.5	***	ns	**
Linear	20.8	±10.9	38.9	±22.5	31.5	±19.4	35.5	±20.8	20.8	±9.7	15.8	±7.5	ns	ns	ns
≤8 carbons	59.6	±8.4	73.3	±22.2	57.3	±19.3	61.7	±20.0	34.8	±11.9	34.7	±8.4	***	ns	ns
>8 carbons	ND		ND		0.326	±0.161	2.15	±1.49	3.48	±1.45	3.87	±1.17	***	ns	ns
Nitrogen and sulfur compounds	0.298	±0.100	0.740	±0.272	1.26	±0.72	2.35	±0.90	1.67	±0.73	1.74	±0.71	ns	ns	ns
Terpenes	0.215	±0.106	ND		0.787	±0.407	0.871	±0.323	0.779	±0.279	0.477	±0.260	**	ns	ns
Σ Volatiles	222	±111	234	±114	260	±126	315	±139	300	±96.0	374	±127	***	*	ns

T, temperature; ns, not significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

(minced and steak; 2.5 cm thickness) and cooking method (stewed and grilled-high and -low, setting 70 °C as final internal temperature) on the volatile profile of cooked deer meat samples analyzed by SPME-GC–MS is provided in Table 2. Statistical results indicated that both meat presentation and cooking method, together with the interaction term, were not significant ($P > 0.05$) effects on the abundance of most volatile compounds. Regarding meat presentation, the abundance of propanal was significantly ($P \leq 0.05$) higher in minced than in intact samples. This could be related to the shorter time needed to reach the target temperature in minced compared to steak sample, independently of the cooking method (e.g., stewed 15.4 *versus* 17.5 min, or grilled-high 1.2 *versus* 2.2 min, respectively) as previously reported in other study (Gardner, 2017). Gardner's study also indicated that meat mincing increased the volatile extraction yield although, in our case, the total abundance of individual volatile compounds and chemical families of minced meat did not differ ($P > 0.05$) from that of steak samples (supplementary material, Table S3). This apparent contradiction could be related to differences in fat content; patties having 1.5% more fat content than steaks (Gardner, 2017) while the fat content of minced and intact samples was the same in the present study.

Despite cooking method has been highlighted as an important factor influencing meat volatile profile (Lorenzo & Domínguez, 2014), the abundance of most volatile compounds did not significantly ($P > 0.05$) change among cooking methods (stewed, grilled-high and -low). In contrast to other studies on cooked meat reported in the literature, it should be noted that in the present study cooked meat internal temperature (70 °C), portion dimensions and sampling protocol were similar for all cooking methods studied. This could explain that the volatile profile of differently cooked meat samples was very similar, with small differences in several compounds such as trimethyl pyrazine, dimethyl sulfide, benzaldehyde or 2- and 3-methyl butanal. These compounds were either not detected in stewed meat, or were present in lower concentration than in grilled meat, particularly at high grill temperature (Table 2). Despite the small differences found among cooking methods, these differences could have an effect on the cooked meat aroma since pyrazines, sulfur compounds, aromatic and branched-chain aldehydes have low odour threshold values. In addition, as expected, not significant differences ($P > 0.05$) were found among cooking methods in the abundance of chemical families, except for nitrogen and sulfur compounds that were only detected in grilled samples (supplementary material, Table S3).

Relative frequencies of detection for individual compounds were also examined among different cooking methods (supplementary material, Table S4) and results indicated statistical differences in some compounds among cooking methods. For instance, (E)-2-octen-1-ol and (E)-2-decenal showed higher relative frequency of detection in stewed compared to grilled meat samples. On the contrary, acetaldehyde, 2- and 3-methyl butanal, showed higher ($P \leq 0.05$) relative frequency of detection in grilled-high than in stewed samples, as previously reported (Roldán, Ruiz, del Pulgar, Pérez-Palacios, & Antequera, 2015). As mentioned before, trimethyl pyrazine and dimethyl sulfide were detected exclusively in grilled meat samples most likely due to a further progression of the Maillard reaction as the surface was in direct contact with the hot plate (Kerth, 2016; Takakura et al., 2014). Summarizing, in contrast to previous studies on cooked meat, the results of the present work indicate very few differences among cooking methods (stewed and grilled) likely because sample preparation and cooking procedure were accurately controlled (i.e., the same final internal temperature). These

Table 5
Relative detection frequencies (%) of volatile compounds from cooked deer meat samples extracted using different extraction temperatures (30, 60 and 80 °C) and times (30 and 50 min) and analyzed by GC–MS.

LRI	Compound	30 °C		60 °C		80 °C		Significance
		30 min	50 min	30 min	50 min	30 min	50 min	
Alcohols								
1156	1-Penten-3-ol	89	67	67	56	44	72	ns
1244	1-Pentanol	100	100	94	100	100	100	ns
1342	1-Hexanol	100	94	83	89	89	67	ns
1433	1-Octen-3-ol	100	100	100	100	100	100	ns
1440	1-Heptanol	100	89	100	100	100	89	ns
1473	2-Ethyl-1-hexanol	61^{a}	61^{a}	94^{a}	100^{a}	61^{a}	11^{b}	***
1571	1-Octanol	0^{b}	0^{b}	22^{ab}	22^{ab}	50^{a}	0^{b}	***
1593	(E)-2-Octen-1-ol	33^{b}	78^{a}	94^{a}	94^{a}	100^{a}	100^{a}	***
1997	(E)-2-Dodecen-1-ol	0^{b}	0^{b}	50^{b}	78^{ab}	100^{a}	100^{a}	***
2075	1-Tridecanol	0^{b}	0^{b}	0^{b}	22^{b}	67^{a}	67^{a}	***
2109	(E)-2-Tetradecen-1-ol	0^{c}	0^{c}	22^{cb}	50^{b}	100^{a}	100^{a}	***
2181	1-Tetradecanol	0^{b}	0^{b}	0^{b}	11^{b}	67^{a}	72^{a}	***
2217	1-Pentadecanol	0^{b}	0^{b}	0^{b}	0^{b}	67^{a}	83^{a}	***
Aldehydes								
707	Acetaldehyde	39^{b}	11^{b}	89^{a}	100^{a}	100^{a}	100^{a}	***
797	Propanal	67^{a}	22^{abc}	61^{ab}	67^{ab}	0^{c}	0^{c}	***
913	2-Methyl butanal	17^{ab}	0^{b}	22^{ab}	17^{ab}	39^{a}	0^{b}	**
917	3-Methyl butanal	50^{ab}	11^{b}	67^{a}	56^{ab}	94^{a}	72^{a}	***
978	Pentanal	100	100	100	100	100	100	ns
1081	Hexanal	100	100	100	100	100	100	ns
1181	Heptanal	100	94	100	100	100	94	ns
1284	Octanal	0^{b}	0^{b}	0^{b}	11^{b}	61^{a}	56^{a}	***
1388	Nonanal	100	100	100	100	100	100	ns
1429	(E)-2-Octenal	67^{ab}	56^{b}	72^{ab}	89^{ab}	100^{a}	94^{a}	**
1491	Decanal	0^{b}	0^{b}	94^{a}	78^{a}	78^{a}	67^{a}	***
1539	(E)-2-Nonenal	94	94	100	100	100	100	ns
1647	(E)-2-Decenal	0^{b}	0^{b}	39^{ab}	56^{a}	67^{a}	67^{a}	***
1668	2-Butyl-2-octenal	0^{b}	0^{b}	89^{a}	83^{a}	100^{a}	89^{a}	***
1708	Dodecanal	0^{b}	0^{b}	61^{a}	67^{a}	89^{a}	72^{a}	***
1760	2-Undecenal	0^{b}	0^{b}	61^{a}	89^{a}	72^{a}	78^{a}	***
1820	Tridecanal	0^{b}	0^{b}	61^{a}	67^{a}	72^{a}	78^{a}	***
1830	(E,E)-2,4-Decadienal	0^{b}	0^{b}	33^{ab}	11^{b}	56^{a}	67^{a}	***
1939	Tetradecanal	33^{b}	0^{b}	100^{a}	94^{a}	100^{a}	94^{a}	***
2051	Pentadecanal	0^{b}	0^{b}	72^{a}	83^{a}	100^{a}	94^{a}	***
2161	Hexadecanal	0^{b}	0^{b}	100^{a}	100^{a}	100^{a}	100^{a}	***
2374	Octadecanal	0^{b}	0^{b}	0^{b}	0^{b}	78^{a}	78^{a}	***
Aromatic hydrocarbons								
1037	Toluene	94^{a}	50^{ab}	72^{a}	44^{ab}	22^{b}	56^{ab}	***
1532	Benzaldehyde	94^{a}	100^{a}	39^{b}	100^{a}	94^{a}	100^{a}	***
1656	Benzeneacetaldehyde	0^{b}	0^{b}	22^{b}	39^{b}	100^{a}	94^{a}	***
1731	3-Ethyl benzaldehyde	0^{b}	0^{b}	61^{a}	94^{a}	100^{a}	94^{a}	***
2062	4-Pentyl benzaldehyde	0^{b}	0^{b}	0^{b}	11^{b}	67^{a}	67^{a}	***
Furans								
951	2-Ethyl furan	33	11	17	22	11	0	ns
1226	2-Pentyl furan	56^{b}	56^{b}	89^{ab}	83^{ab}	94^{ab}	100^{a}	***
Aliphatic hydrocarbons								
700	Pentane	83^{a}	0^{b}	11^{b}	0^{b}	22^{b}	0^{b}	***
701	Heptane	94^{a}	22^{bc}	50^{ab}	6^{bc}	0^{c}	0^{c}	***
737	1-Heptene	39^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	***
801	Octane	100^{a}	94^{a}	83^{a}	89^{a}	11^{b}	0^{b}	***
958	2,2,4,6,6-Pentamethyl heptane	83^{a}	56^{ab}	94^{a}	72^{ab}	28^{bc}	0^{c}	***
1288	Tridecane	0^{b}	0^{b}	0^{b}	0^{b}	28^{ab}	33^{a}	***
1418	3-Methyl-2-ethyl-1,3-hexadiene	56^{ab}	22^{b}	67^{b}	72^{a}	72^{a}	44^{ab}	*
1484	Pentadecane	0^{b}	0^{b}	61^{a}	78^{a}	78^{a}	50^{a}	***
1577	Hexadecane	67^{a}	72^{a}	50^{a}	28^{ab}	39^{ab}	0^{b}	***
1629	1-Hexadecene	0^{c}	0^{c}	0^{c}	22^{cb}	78^{a}	44^{ab}	***
Ketones								
816	Acetone	100^{a}	72^{b}	100^{a}	100^{a}	100^{a}	100^{a}	***
903	2-Butanone	44^{a}	0^{b}	22^{ab}	0^{b}	11^{b}	0^{b}	***
1060	2,3-Pentanedione	94^{a}	72^{a}	78^{a}	56^{a}	0^{b}	0^{b}	***
1179	2-Heptanone	100	78	94	100	94	100	ns
1236	6-Methyl-2-heptanone	0^{b}	0^{b}	0^{b}	22^{ab}	33^{ab}	61^{a}	***
1286	3-Hydroxy-2-butanone	100	100	100	100	94	94	ns
1319	2,3-Octanedione + 2,5-Octanedione	72	67	72	72	72	72	ns
1333	5-Methyl-3-hepten-2 one	72^{b}	94^{ab}	100^{a}	100^{a}	100^{a}	100^{a}	***
1384	2-Nonanone	0^{b}	0^{b}	28^{ab}	33^{ab}	72^{a}	39^{ab}	***
1857	6,10-Dimethyl-(E)-5,9-undecadien-2-one	0^{b}	0^{b}	0^{b}	11^{b}	100^{a}	72^{a}	***
2014	2-Pentadecanone	0^{b}	0^{b}	0^{b}	0^{b}	56^{a}	61^{a}	***

(*continued on next page*)

Table 5 (*continued*)

LRI	Compound	30 °C		60 °C		80 °C		Significance
		30 min	50 min	30 min	50 min	30 min	50 min	
Nitrogen and sulfur compounds								
729	Dimethyl sulfide	11	11	0	0	0	0	ns
1398	Trimethyl pyrazine	0^{b}	0^{b}	44^{a}	17^{ab}	17^{ab}	17^{ab}	**
Terpenes								
1191	D-Limonene	0^{b}	0^{b}	33^{ab}	11^{ab}	11^{ab}	39^{a}	**
1465	*cis*-Linalool oxide	33^{ab}	0^{b}	56^{a}	67^{a}	33^{ab}	0^{b}	***

ns, not significant ($P > 0.05$); * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
[a-c] Different letter superscripts indicate significant differences ($P \leq 0.05$) among SPME methods corresponding to the eight combinations of extraction temperature and time.

results highlight the need to standardise sample preparation conditions and cooking methods in order to be able to make reliable comparisons of the volatile profile of different types of cooked meat.

3.2.3. *Effect of extraction temperature and time*

The effect of extraction temperature and time on the relative abundance of individual volatile compounds of cooked deer meat is provided in Table 3. Extraction temperature and time significantly affected ($P \leq 0.05$) the abundance of many volatile compounds, with the number of compounds affected by temperature being greater than those affected by time. In general, the relative abundance of volatiles increased with extraction temperature and time, and a combined effect of both factors was noticed for a few compounds (below 20% of the total number of volatiles).

Regarding the effect of extraction time on the abundance of individual compounds, less than half of identified compounds were significantly ($P \leq 0.05$) affected and, in general, longer extraction times (50 min) increased the relative abundance of these compounds. However, this effect was in many cases simultaneously dependent on the extraction temperature used (Table 3). When examining chemical family groupings, the abundance of unsaturated, branched and linear alcohols, long chain (>8 carbons) unsaturated aldehydes, aromatic hydrocarbons and unsaturated ketones were significantly ($P \leq 0.05$) increased at 50 min, as well as the total volatile content (Table 4).

The effect of extraction time on the relative detection frequency of individual compounds was also dependent on extraction temperature although most volatile compounds did not change ($P > 0.05$) with extraction time within each of the extraction temperature assayed (Table 5). In addition, the effect was different depending on the compound and extraction temperature. In this regard, 2-ethyl-1-hexanol, 1-octanol and 2-methyl butanal showed a significant ($P \leq 0.05$) decrease in their relative detection frequency when long extraction time (50 min) was combined with high extraction temperature (80 °C), and lower values were also recorded at 50 min and 30 °C for other compounds such as 2-butanone, pentane, heptane and 1-heptene. On the contrary, the longest extraction time (50 min) significantly ($P \leq 0.05$) increased the relative detection frequency of (E)-2-octen-1-ol at 30 °C. Regarding chemical families, the effect of extraction time on the frequency of detection (absolute mean values) confirmed the results observed for individual volatiles. In this regard, lower ($P \leq 0.05$) absolute detection frequencies were found for branched- and short chain alcohols and saturated aldehydes at 50 min and 80 °C, and of saturated, unsaturated and linear aliphatic hydrocarbons at 50 min and 30 °C.

Extraction time has been highlighted as a relevant factor affecting the total peak area and the number of peaks detected in meat samples (Wang et al., 2018). In general, greater abundance of volatile compounds analyzed by SPME-GC has been reported at longer extraction times (from 20 to 60 min; Moon & Li-Chan, 2004). However, in the literature (Wang et al., 2018), the effect of extraction time have been discussed separately from that of extraction temperature and the abovementioned results indicate that both the relative abundance and frequency of detection of volatiles may be affected differently depending on their chemical nature and SPME conditions used. Some studies reported a detrimental effect in both the abundance and the number of volatile compounds when long times at high extraction temperatures (15 to 60 min at 25 to 70 °C) were used in liquid and water-rich matrices due to the increased competition phenomena between water vapor pressure and volatile compounds to ward active sites in the fiber solid phase (Lee et al., 2003). This detrimental effect in the present study could also be related to the greater abundance of heavy volatile compounds released from the matrix during long extraction times.

The effect of extraction temperature on the relative abundance and detection frequency of volatile compounds released from cooked deer meat samples was undoubtedly the strongest. Tables 3 and 5 show, respectively, that relative abundance and frequency of detection of more than 80% of the individual volatile compounds were significantly ($P \leq 0.05$) affected by extraction temperature, as other authors reported for meat samples (Wang et al., 2018). In consequence, the volatile profile of the meat samples was clearly different among extraction temperatures. From a qualitative point of view, the number of compounds detected in the samples analyzed at 80 °C was almost double that of those detected at 30 °C, regardless of extraction time. Likewise, total volatile abundance of cooked meat samples increased by approximately 50% from 30 to 80 °C, as reported by others (Moon & Li-Chan, 2004). This increase was evident for the total content of alcohols, aldehydes and aromatic hydrocarbons, particularly due to the increase of heaviest compounds (>8 carbons) abundance (Table 4). In this regard, all heavy alcohols and aromatic compounds were exclusively detected in cooked deer meat samples analyzed at temperature equal to or higher than 60 °C, and only a few number of long chain aldehydes were detected at 30 °C. In contrast, the lightest (≤8 carbons) alcohols, aldehydes and aromatic compounds either decreased in content or slightly fluctuate as temperature increased. An exception was benzaldehyde, which presented a much higher abundance in meat samples analyzed at 80 °C compared to 30 °C (Table 3).

The most striking feature in the ketones group was the significant decrease ($P \leq 0.05$) of the relative abundance of major ketones such as 3-hydroxy-2-butanone and 2,3- + 2,5-octanedione with high temperatures, and a slight increase of long chain (>8 carbons) ketones which were not detected in meat samples analyzed at 30 °C. Regarding other minor volatiles, in general, the effect of temperature was different depending on their chemical nature. For example, the abundance of some long-chain (>8 carbons) aliphatic hydrocarbons increased with temperature whereas dimethyl sulfide was only detected at 30 °C, and trimethyl pyrazine at temperature equal to or higher than over 60 °C (Table 3).

As reported in the literature (Ma et al., 2013), higher extraction temperatures provide enough energy for volatile compounds to overcome the energy that bind them to the meat matrix, while increasing vapor pressure for mass transfer process. Results indicate that extraction temperatures equal or over 60 °C are required to release compounds with LRI values above 1600, as confirmed by their relative frequency of detection (Table 5). Therefore, the volatile profile of cooked deer meat was particularly enriched in heavy alcohols and aldehydes of which the

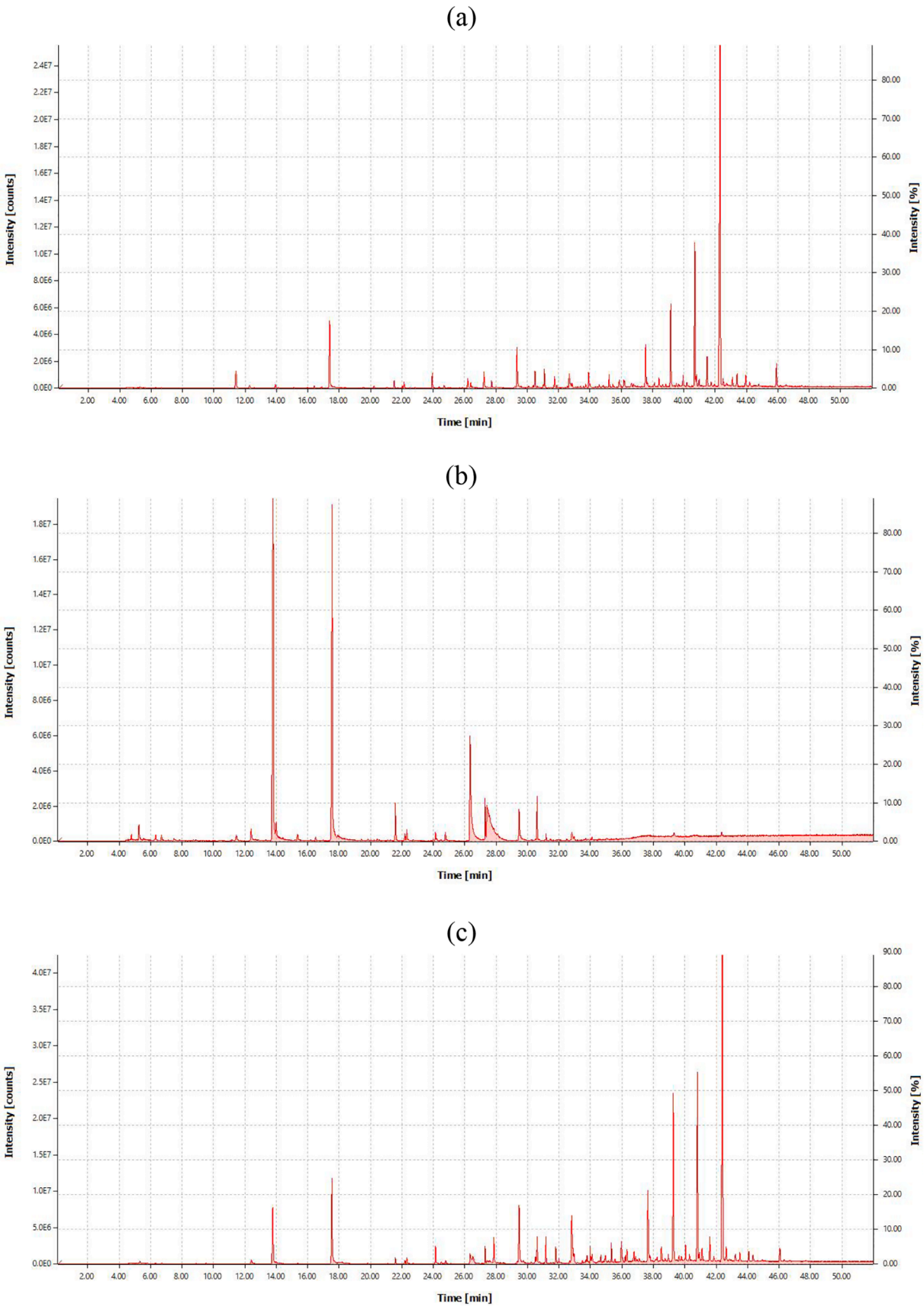

Fig. 1. GC–MS chromatograms of the same stewed deer meat sample analyzed at (a) extraction temperature 80 °C, (b) preheated at 80 °C for 30 min and analyzed at extraction temperature 30 °C, and (c) preheated at 80 °C for 30 min and analyzed at extraction temperature 80 °C. Extraction time was 30 min for all analyses.

relative abundance of (E)-2-tetradecen-1-ol, (E)-2-dodecen-1-ol, tridecanal, tetradecanal, pentadecanal and hexadecanal was remarkably high at 80 °C. In contrast, cooked deer meat samples analyzed at 30 °C were characterized by lighter volatile compounds, in particular by a high relative abundance of hexanal, 3-hidroxy-2-butanone, 2,3- + 2,5-octanedione, 1-octen-3-ol, nonanal and pentanal. However, except for several very minor volatiles, light compounds were also detected at 80 °C (Table 5) while major volatile compounds found at 30 °C were also present in high abundances at 80 °C (Table 3). As other studies discussed (Ma et al., 2013; Moon & Li-Chan, 2004; Wang et al., 2018), extraction temperature and time were determinant of both the release of volatiles from the cooked meat to vial headspace and the competition phenomena among volatiles to be trapped onto the fiber active sites.

3.3. Detection of heavy volatile compounds

As mentioned before, heavy compounds were detected in cooked meat volatile profile analyzed at high extraction temperatures. Regardless of whether such compounds can be generated in the sample after cooking (70 °C internal temperature) and/or during the extraction (60–80 °C), it was necessary to confirm if they were detected or not when samples were analyzed at SPME 30 °C. In this regard, Fig. 1 shows the chromatograms corresponding to the same stewed meat sample analyzed by SPME at 80 °C, and after being preheated for 30 min at 80 °C, cooled and then re-analyzed by SPME at 80 °C and 30 °C. The results showed that the chromatogram of the sample analyzed by SPME at 80 °C showed a very similar volatile profile to the one obtained after double heating at 80 °C. On the contrary, the heaviest volatile compounds of cooked meat were not extracted by SPME at 30 °C despite they were present in the vial due to the previous preheating at 80 °C. Therefore, it is highlighted that high temperatures are required to engage the heavy compounds to the fiber.

4. Conclusions

The volatile profile of cooked deer meat, which has been analysed for the first time in this work, was complex and made up of a large number of compounds of different chemical nature, the most abundant of which were carbonyl compounds and alcohols. The volatile profile of cooked meat was different, both in abundance and frequency of occurrence of compounds, depending on the analytical conditions used. The results showed that extraction temperature, followed by extraction time, were the factors that most significantly affected the occurrence and abundance of volatile compounds in cooked meat. In this regard, higher extraction temperatures (≥60 °C) improved the detection of heavy volatile compounds, particularly alcohols, aldehydes, ketones and hydrocarbons with more than 8 carbons atoms. However, sample preparation before extraction of volatiles had hardly any influence on the volatile profile of meat samples, probably due to the accurate control of parameters used for meat presentation (minced and steak) and cooking methods (stewed, grilled-high and grilled-low).

Ultimately, the results of this work can assist in the standardization of analytical procedures for the characterization of volatile compounds of cooked meat. Likewise, the conditions used in this work for sample preparation and cooking methods may be useful to reduce variability in meat samples due to uncontrolled factors, and allow for adequate and reliable comparisons between different studies.

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CRediT authorship contribution statement

Lara Moran: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Noelia Aldai:** Methodology, Resources, Supervision, Writing - review & editing. **Luis Javier R. Barron:** Conceptualization, Methodology, Formal analysis, Resources, Supervision, Writing - review & editing, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129380.

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