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Characterization of volatile compounds of cooked wild Iberian red deer meat extracted with solid phase microextraction and analysed by capillary gas chromatography - mass spectrometry

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ABSTRACT

Wild Iberian red deer meat demand and interest are increasing and, therefore, an in-depth characterization of meat quality is needed to meet consumer demands. The objective of the present work was to assess, for the first time, the volatile profile of cooked wild sport-hunted Iberian red deer meat. Twenty-three loin samples from male red deer (*Cervus elaphus hispanicus*) were cooked and the volatile profile was analysed using solid phase microextraction, followed by capillary gas chromatography-mass spectrometry. Fifty-five volatile compounds were found. The major ones in number and relative abundance were aldehydes (84%), followed by alcohols (11%), hydrocarbons (2.4%), ketones (1.7%), furans (0.34%) and sulphur compounds (0.18%). Hexadecanal was the major compound and other long-chain compounds such as (*E*)-2-tetradecen-1-ol or 2-pentadecanone were also reported in considerable abundance. Several compounds related to grass-based diets were identified (2,3-octanedione, hexadecane or 1-pentadecanol). Odour impact ratio of volatile compounds was calculated and dimethyl trisulphide, (*E,E*)-2,4-decadienal, decanal and dodecanal were the most odorant compounds affecting the flavour of the cooked deer meat.

1. Introduction

Deer (Order Artiodactyla, Family Cervidae, Genera *Cervus*) are native to all continents except Australia and Antarctica (CABI, 2020). In Europe, the most common species is *Cervus elaphus* (red deer) and among the listed sub-species in the Iberian Peninsula the most common is *Cervus elaphus hispanicus* (TSN: 898521) (Wilson & Reeder, 2005). Thousands of red deer stags are hunted every year in Spain from October to February. In this type of game hunting, dogs are released within a shrub area to move the deer outward to the sites where hunters are placed (Torres-Porras, Carranza, & Pérez-González, 2009). The Spanish legislation permits the commercialization of game meat (Ministerio de la Presidencia, 1994) and Spain is the second major producer worldwide of deer venison, most of it coming from hunting (Lorenzo, Munekata, Barba, & Toldrá, 2019). In 2018, the number of harvested deer in Spain accounted for more than 144,134 animals representing around 11,531 tonnes of deer meat with an economic value close to 30 M€ (Ministerio para la Transición Ecológica y el Reto Demográfico, 2020), although this value is probably overestimated (Lorenzo, Munekata, et al., 2019). Moreover, the European Federation of Deer Farmers Association estimated the presence of around 10,000 deer farms in the European Union. Deer venison consumption and prices are quite variable among European countries, and prices continue increasing due to the growing demand of Asian countries (Lorenzo, Munekata, et al., 2019).

Despite wild red deer venison can be an economic opportunity for the meat industry, there is very few research on its quality properties probably due to the poor control of *ante mortem* factors, as well as slaughter conditions, which can influence on the quality of the final product (Serrano et al., 2020a,b). Deer venison is known for its low muscle lipid content although values can be quite variable according to several studies (0.4–10.9 g/100 g meat; Bureš, Bartoň, Kotrba, & Hakl, 2015; Hutchison, Mulley, Wiklund, Flesch, & Sims, 2014; Maggiolino et al., 2019). Several other studies performed in Iberian red deer meat indicated even lower intramuscular fat contents (0.05–1.0 g/100 g meat;

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Maggiolino et al., 2019; Quaresma et al., 2012; Soriano et al., 2020). In this regard, very low values appear to be questionable since the content of phospholipids in muscle is relatively independent of the total fat content and is known to vary between 0.2% and 1% of muscle weight (De Smet, Raes, & Demeyer, 2004). The content of muscle triacylglycerols, on the other hand, is strongly related to the total fat content and varies from 0.2% to more than 5%, indicating that some of the reported variations in fat content may be related to differences in extraction methods. The use of appropriate extraction methods, which also extract phospholipids, are highly recommended to obtain more accurate and reliable total muscle fat content values (Aldai et al., 2012). Continuing with the fat content of products, consumers increasing concern about healthiness and sustainability of foods could make wild deer meat an interesting niche market (Milczarek et al., 2021).

In terms of sensory attributes, together with tenderness, flavour is one of the main attributes contributing to the sensory quality of meat and derived products (Neethling, Hoffman, & Muller, 2016). Flavour is determined after a cooking procedure, and the cooking technique, temperature and time may affect the generation of volatiles (Domínguez, Gómez, Fonseca, & Lorenzo, 2014). A wide variety of volatile compounds is generated during meat cooking, which will contribute to the meat odour (Resconi, Escudero, & Campo, 2013). It is well established that, in general, meat from grass-fed cattle is linked to livery, gamey, grass/hay like, and bloody/metallic terms (O'Quinn (2012). In addition, in few sensory studies performed in red deer meat the same pattern has been reported: higher odour intensity values compared to beef (Bureš et al., 2015).

Taken into account the increasing consumer demand of game meat, particularly deer meat, and the scarcity of studies related to its quality, the main objective of the present work was to characterize the volatile profile of cooked wild (sport-hunted) Iberian red deer meat. In addition, the odorant impact of volatile compounds was estimated.

2. Material and methods

2.1. Chemicals

Sodium sulphate (American Chemical Society reagent, ≥99.0%), methyl isobutyl ketone (≥99.5%), hexane (American Chemical Society reagent, ≥97%), 3-methyl-1-butanol (≥98.5%), 1-penten-3-ol (99%), 1-pentanol (≥99.0%), 1-hexanol (≥99.5%); 1-octen-3-ol (98%), 1-heptanol (98%), 1-octanol (≥99%), (*E*)-2-octen-1-ol (97%), 1-tetradecanol (97%), 1-tridecanol (97%), acetaldehyde (≥99.5%), hexanal (98%), (*E*)-2-hexenal (≥95%), heptanal (≥95%), (*E*)-2-octenal (≥95%), (*E*)-2-nonenal (≥95%), (*E*)-2-decenal (≥95%), 2-butyl-2-octenal (≥95%), 2-butanone (≥99.0%), 2-hexanone (98%), toluene anhydrous (99.8%), benzaldehyde (≥99%), 1-hexadecene (≥98.5%), hexadecane (≥99%), heptadecane (≥99%), 2-ethyl furan (≥99%) and saturated n-alkanes standard certified reference material (49452-u, C7–C40, 1000 μg/mL each component in hexane) were purchased from Sigma Aldrich (Madrid, Spain); 1-propanal (97%), 2-methyl butanal (97%), 3-methyl butanal (97%), octanal (98%) and 2-heptanone (≥99%) were supplied from Honeywell-Fluka Research Chemical (Fisher Scientific, Madrid, Spain).

2.2. Sampling

This study was carried out in accordance with the Spanish legislation as recorded in the National Hunting Code (Boletín Oficial del Estado, 2022) and the Spanish regulation of management and safety of wild meat for human consumption (Ministerio de la Presidencia, 1994).

Twenty-three male Iberian wild red deer (*Cervus elaphus hispanicus*) hunted in Spain between August 2017 and March 2018 were selected, discarding those with wounds and bruises in the muscle of interest. In concordance with the National legislation, animals were exsanguinated, eviscerated, and decapitated at the atlanto-occipital junction in the countryside and subsequently transported under refrigerated conditions to the processing industry (Cárnicas Dibe, Cáceres, Spain) where carcasses were subjected to veterinary examination after hide removal, washed with cold water and maintained in a chill room at 0–2 °C for 4 days. The average carcass weight was of 54 kg, ranging from 29 to 72 kg. Average ultimate pH was of 5.61 ± 0.12.

From each carcass, the *Longissimus thoracis et lumborum* (LTL) (from thoracic vertebra T8 to lumbar vertebra L6) were collected, vacuum packed, and transported to the laboratory in refrigerated conditions (2 ± 2 °C). After removing the subcutaneous fat from a representative LTL portion (1–3 steaks), the meat was mince and homogenized. Then, a subsample of around 100 g was vacuum packed and frozen at −80 °C.

2.3. Sample preparation

On the day of analysis, samples were thawed by introducing them in a circulating water bath at 20 °C. By doing this, the initial temperature of samples was homogenized. Then, vacuum packed samples (100 ± 0.1 g) were cooked to an internal temperature of 80 ± 1 °C by immersing them in another water bath set at 80 °C for 50 min approx. (Selecta Unitronic 320 OR, Barcelona, Spain). Sample internal temperatures were monitored using a 4-channel thermocouple (Lutron electronic, PA, USA). More details about sample preparation were given in Moran, Aldai, and Barron (2021).

After cooking, approximately 10 g of cooked meat and anhydrous sodium sulphate in a 4:1 ratio (meat/salt by weight) were homogenized in a blender (Eloane 445W, Taurus, Barcelona, Spain). From this mixture, 2.5 ± 0.001 g were weighted on a 10 mL amber vial, and 20 μL of a deionized water solution of methyl isobutyl ketone (1 g/L) were added as internal standard. Vials were sealed with PTFE septa and steel magnetic cap (Agilent Technologies, Madrid, Spain) and vortexed for 15 s.

2.4. Volatile compound analysis

Volatile compounds were extracted by solid-phase microextraction on a 30/50 μm DVB/Carboxen/PDMS fibre (Supelco, Bellefonte, PA, USA) using a PAL RSI 85 autosampler (CTC Analytics AG, Zwingen, Switzerland). Volatiles were extracted from cooked deer meat for 50 min at 80 °C after 15 min of pre-equilibration time at extraction temperature. Volatiles trapped onto the fibre were desorbed in the injection port of the gas chromatograph for 25 min at 240 °C in splitless mode (split valve was opened at 200 mL/min after 30 min of the injection). Analyses were performed using a 7820A gas chromatograph coupled to a 5975E mass spectrometer detector (Agilent Technologies). Volatile compounds were separated in a Supelcowax-10 fused silica capillary column (60 m, 250 μm i.d., 0.25 μm film thickness; Supelco) using the following temperature gradient: oven temperature was held at 40 °C for 10 min, then increased at a rate of 5 °C/min until 110 °C; increased again at a rate of 10 °C/min until 240 °C and finally held at 240 °C for 15 min. Helium (99.999% purity, Air liquid, Madrid, Spain) was the carrier gas at a constant pressure of 30 psi, and volatiles were transferred to the mass spectrometer detector throughout the transfer line set at 280 °C. Chromatographic data were registered with the MSD ChemStation Data Analysis software (version 5.52, Agilent Technologies). Mass spectrometer detector operated at 150 °C in full scan mode (1.4 scan/s; m/z

range 26–350) using 70 eV as total ion current. Two replicates were done for each cooked meat sample.

2.5. Odour impact ratio calculation

In order to obtain an indicator of the odour intensity of each volatile compound detected in cooked deer meat samples, odour impact ratio (OIR) was calculated as previously described in Abilleira et al. (2010). Available odour threshold (OT) values measured in water were collected from databases and other scientific literature (references in Table 1 footnote), and used for OIR calculation:

$$\mathrm{OIR} = \frac{\text{mean relative abundance}}{\mathrm{OT}(\mu g/kg)}$$

As mean relative abundance values were used in the calculation, OIR values were not quantitative. On the other hand, it is noteworthy to point out that the protein content present in meat might cause a matrix effect and alter the odour threshold values of compounds, compared with the literature values (in water). However, OIR values allowed the comparison of the odour impact of the volatile compounds in cooked deer meat.

Table 1
Mean relative abundance (arbitrary area units x 10^7) of individual volatile compounds from cooked deer meat samples analysed by solid-phase microextraction coupled to a gas chromatography-mass spectrometer.

Chemical family	LRI	Volatile compound	Relative abundance	SD
Aldehydes				
	917	3-Methyl butanal[b]	13.4	0.19
	1075	Hexanal[b]	157	4
	1186	Heptanal[b]	19.0	0.43
	1294	Octanal[b]	66.7	3.8
	1430	(*E*)-2-Octenal[a]	10.0	0.21
	1509	Decanal[a]	95.1	2.2
	1545	Benzaldehyde[b]	539	5
	1726	Dodecanal[a]	55.8	1.0
	1749	3-Ethyl benzaldehyde[a]	66.7	1.1
	1776	2-Undecenal[a]	50.7	0.7
	1836	Tridecanal[a]	116	3
	1845	(*E*,*E*)-2,4-Decadienal[a]	27.7	0.6
	1945	Tetradecanal[a]	419	9
	2055	Pentadecanal[a]	703	17
	2064	4-Pentyl benzaldehyde[a]	49.3	1.5
	2164	Hexadecanal[a]	688×10^1	108
	2193	(*Z*)-9-Hexadecenal[a]	73.6	1.5
	2371	Octadecanal[a]	292	4
Alcohols				
	1209	3-Methyl-1-butanol[b]	17.8	0.7
	1253	1-Pentanol[b]	9.37	0.36
	1354	1-Hexanol[b]	15.6	0.4
	1584	1-Octanol[b]	8.99	0.19
	1617	(*E*)-2-Octen-1-ol[b]	26.9	0.5
	2002	2-Dodecen-1-ol[a]	78.5	1.8
	2076	1-Tridecanol[b]	35.1	1.2
	2109	(*E*)–2-Tetradecen-1-ol[a]	378	8
	2178	1-Tetradecanol[a]	66.3	1.7
	2222	Cyclododecanol[b]	205	4
	2273	1-Pentadecanol[b]	134	2
	2235	(*Z*)-14-Methyl-8-hexadecen-1-ol[a]	263	7
Ketones				
	816	Acetone[a]	18.7	0.3
	903	2-Butanone[b]	4.98	0.10
	1057	2-Hexanone[b]	15.0	0.2
	1065	2,3-Pentanedione[b]	11.4	0.2
	1184	2-Heptanone[b]	10.8	0.4
	1331	2,3-Octanedione[a]	15.9	0.2
	1344	6-Methyl-5-hepten-2-one	28.2	0.6
	1575	2-Undecanone[a]	10.8	0.2
	1874	(*E*)-6,10-Dimethyl-5,9-undecadien-2-one[a]	30.3	0.7
	2041	2-Pentadecanone[a]	77.5	6.1
Hydrocarbons (non-aromatic)				
	834	3-Methyl heptane[a]	6.84	0.11
	959	2,2,4,6,6-Pentamethyl heptane[a]	94.3	1.2
	1034	3,5-Dimethyl-2-hexene[a]	6.71	0.11
	1364	3-Nonane[a]	9.15	0.24
	1601	Hexadecane[b]	30.1	0.8
	1649	1-Hexadecene[b]	18.8	0.3
	1699	Heptadecane[b]	31.4	0.5
	1973	Cyclodecane[a]	19.3	0.5
	2283	Cyclohexadecane[a]	59.8	1.1
Furans				
	980	2-Ethyl furan[b]	23.7	0.3
	1233	2-Pentyl furan[b]	15.8	0.5
Sulphur compounds				
	—[c]	Methanethiol[a]	7.10	0.11
	1395	Dimethyl trisulphide[a]	13.2	0.2

(*continued on next page*)

Table 1 (*continued*)

Chemical family	LRI	Volatile compound	Relative abundance	SD
Other compounds				
	1036	Toluene[b]	7.42	0.13
	1050	Unknown	6.50	0.14
	2019	(*E*)-Pinane[a]	41.1	0.8

LRI: Linear retention index; SD, standard deviation.
[a] Tentative identification.
[b] Positive identification.
[c] LRI not calculated because the retention time of the compound was **lower** than that of the shorter-chain alkane.

2.6. Data treatment and statistical analysis

In order to calculate the limit of detection (LOD) of the method, the average noise (arbitrary units) of 10 blanks (empty vial) was determined in three different zones of the chromatogram. LOD was set at two times the average noise in each zone. Peak areas (arbitrary units) of volatile compounds were used to calculate the relative abundance, relative to the area of internal standard, according to the following equation:

$$Relative\ abundance = \frac{peak\ area}{internal\ standard\ area}\ x\ \frac{2.5\ g}{mixture\ weight\ (g)}\ x\ 100$$

Tentative identifications of volatile compounds were performed by comparing the mass spectra of peaks with those of NIST 2.0 (National Institute of Standards and Technology, Gaithersburg, MD, USA) library, using matching factor >700. Mixtures of C_7–C_{24} n-alkanes were used for the calculation of the experimental linear retention indices (LRI) for sample and standards peaks. Experimental LRI values were confirmed by comparing to published data obtained under similar chromatographic conditions. Additionally, high purity commercial standard compounds were analysed for positive identification of cooked deer meat volatiles.

Peak areas of individual volatile compounds present (>LOD) in both replicates of each sample and in more than 70% of cooked deer meat samples were used to calculate the mean relative abundance.

IBM-SPSS Statistics Software version 25.0 (IBM, NY, USA) was used to perform descriptive statistics. Three significant figures were used to express the data.

3. Results and discussion

The relative abundance of individual volatiles detected (>LOD) in the cooked deer meat samples are reported in Table 1. Since there are not previous scientific articles describing the volatile profile of cooked meat from Iberian red deer, the present work will discuss these results in comparison with the few studies describing the volatile composition of other game meats and with those given the volatile profile of domesticated ruminants subjected to a grazing system.

Table 1 shows 55 individual volatile compounds identified. Similarly, around 40 volatile compounds were detected in roe deer and fallow deer meat samples (Ivanović, Pisinov, Pavlović, & Pavlović, 2020). According to the number of different individual compounds, the main chemical families of the cooked deer meat volatile profile were aldehydes (18) and alcohols (12), followed by non-aromatic hydrocarbons (9), ketones (10), furans (2) and sulphur compounds (2). Regarding relative abundances, aldehydes (84%) were the major chemical family followed by alcohols (11%) in line with previous studies in cooked veal (Wei, Wan, Luo, & Zhang, 2014) or lamb (Almela et al., 2010), being hexanal usually the most abundant volatile compound in cooked meat (Shahidi & Pegg, 1994). However, Ivanović et al. (2020) did not find hexanal in roe or fallow cooked deer meats extracted at 40 °C.

In the present study, extraction temperature was set at 80 °C, as it was previously reported that extraction temperatures over 60 °C allow the extraction of compounds with low volatility (Moran et al., 2021). High relative abundances (>100 arbitrary units) were observed for tridecanal, tetradecanal, pentadecanal and hexadecanal. Among saturated aldehydes, hexadecanal was by far the most abundant individual aldehyde in cooked deer meat samples (Table 1). Previous studies reported that hexadecanal and octadecanal were also major aldehydes in the volatile profile of cooked bull meat (Dannenberger et al., 2006). Hexadecanal has been related to oily odour notes (Table 2), but odour thresholds for most of the long-chain aldehydes have not been reported in the literature.

Other studies have reported that aldehydes with 6–10 carbons were major compounds related to odour properties due to their low OT values and high concentration in cooked meat (Mottram, 1998). However, the effect of aldehydes in cooked meat odour should be carefully studied since their abundance in raw meat may vary during storage, related to increased lipid oxidation. Although aldehydes have, in general, been related with characteristic odour notes of cooked meat (Calkins & Hodgen, 2007), an increase in their abundance during storage of raw meat may lead to the formation of lipid-derived off-flavours associated with rancid odour notes (Domínguez et al., 2019). In this situations (increased storing times), long-chain aldehydes can decrease in content due to the degradation processes of long-chain carbonyl compounds, while short-chain aldehydes, such as hexanal, can significantly increase (Siegmund & Pfannhauser, 1999).

In our case, as reported in Table 2, (*E,E*)-2,4-decadienal and hexanal are the compounds with higher OIR values among aldehydes. Both compounds have been described as degradation products from linoleic acid (Priolo et al., 2004; Wood et al., 2008), which has been reported as the major polyunsaturated fatty acid in Iberian deer meat (Lorenzo, Munekata, et al., 2019). At the same time, (*E,E*)-2,4-decadienal and (*E*)-2-octenal are usually degraded to hexanal during the oxidative process. These both unsaturated aldehydes have been related with fatty odour notes in cooked meat (Table 2) (Machiels & Istasse, 2003a). In beef samples, these compounds have been found in considerable abundance and related to liver-like descriptors (Yancey et al., 2006), while in game meat liver-like and metallic odour notes have been also reported (Neethling et al., 2016).

Three aromatic aldehydes were also detected in cooked deer meat samples, from which benzaldehyde and 3-ethyl benzaldehyde have been previously described in cooked meat (Shahidi, Rubin, D'Souza, Teranishi, & Buttery, 1986). In general, they present high OT values and, therefore, they seem to have low influence in cooked meat aroma (Table 2). The origin of these compounds has been related with thermal oxidation of linoleic acid, and/or Strecker degradation of phenylalanine (Mottram & Edwards, 1983). The presence of 4-pentyl benzaldehyde is not very common in the literature of cooked meat, which could be related to its low volatility (LRI 2064, Table 1).

Alcohols found in cooked deer meat are mainly secondary products from aldehydes and more than likely derived from lipid oxidation (Bueno, Resconi, Campo, Ferreira, & Escudero, 2019). As observed for aldehydes, in cooked deer meat alcohols were important volatile compounds both in number and in relative abundance (Table 1). Despite it has been reported that alcohols contribution to cooked meat odour is less important compared to aldehydes due to their higher OT values, their impact on aroma can become stronger with longer carbon chains (Shahidi et al., 1986) and higher degree of unsaturation (Evans, Moser, & List, 1971). Special mention should be made to the branched alcohol

Table 2
Ranges of literature odour threshold (OT) and calculated odour impact ratio (OIR) values for volatile compounds found in cooked deer meat samples. Odour type and descriptors compiled according to the information from specialized literature.

Volatile compound	Ranges OT[a] (μg/kg^{1})		Ranges OIR (x10^{7})		Odour type[b]	Odour descriptors[b]	Other odour descriptors
	Min	Max	Min	Max			
3-Methyl butanal	5.00×10^{-7}	1.20×10^{-6}	1.12	2.68	Fruity	Fatty, pungent, nutty.	Pungent, apple-like, malt[c]; chocolate, caramel, green, nutty[d]
Hexanal	4.00×10^{-7}	6.15×10^{-5}	0.256	39.3	Green	Aldehydic, fatty, fruity, green, freshly cut grass and unripe fruit	Fatty-green, grassy, strong green, tallow, fat, unripe fruit[c]
Heptanal	5.80×10^{-7}	2.80×10^{-6}	0.679	3.28	Green	Aldehydic, fatty, oily, powerful, rancid	Oily, fatty, rancid, unpleasant, penetrating fruity odour [c]
Octanal	6.00×10^{-7}	3.40×10^{-6}	1.96	11.1	Aldehydic	Aldehyde, green with citrus orange notes	Harsh, fatty, orange peel, soapy, lemon, green, honey[c]; Fruity[d]
(*E*)-2-Octenal		3.00×10^{-6}	0.334		Fatty	Green, pungent, fresh cucumber, green herbal, banana	Green, nut, fat[c]; earthy[d]
Decanal	8.00×10^{-8}	3.00×10^{-6}	3.17	119	Aldehydic	Sweet, waxy, orange peel, floral	Powerful, waxy, orange, citrus peel[c]; Stewed, burnt, gravy, gas[d]
Benzaldehyde	2.40×10^{-5}	4.50×10^{-4}	0.120	2.25	Fruity	Strong sweet, bitter almond-like, spicy, stinging, cherry	Volatile almond oil, bitter almond, burning[c]; fatty, broth[e]
Dodecanal	1.30×10^{-7}	2.90×10^{-7}	19.2	42.9	Aldehydic	Fatty, sweaty, soapy, fresh clean, waxy, aldehyde, floral	
3-Ethyl benzaldehyde		6.00×10^{-4}	0.011				
2-Undecenal		7.80×10^{-7}	6.50		Fruity	Fresh, fruity, orange peel	Unpleasant, aldehyde-like[f]
Tridecanal		7.00×10^{-5}	0.165		Aldehydic	Powerful waxy, citrus with a hint of grapefruit peel	
(*E*,*E*)-2,4-Decadienal	2.70×10^{-8}	2.00×10^{-7}	13.9	103	Fatty	Oily, cucumber, melon, citrus, pumpkin, nut, meat	Deep fat flavor, chicken flavor citrus, grapefruit [c] rancid, meat[e]
Tetradecanal	5.30×10^{-5}	1.10×10^{-4}	0.381	0.791	Waxy	Fatty, waxy, amber, incense, dry, citrus peel, musk	Roasted, fried meat[g]
Pentadecanal		1.00×10^{-3}	0.070				Geranium, metallic, pungent[h]
4-Pentyl benzaldehyde		9.10×10^{-7}	5.42				
Hexadecanal							Marine, fat[i]
(*Z*)-9-Hexadecenal							Fatty[i]
Octadecanal							Oil[c]
3-Methyl-1-Butanol	3.06×10^{-3}	4.00×10^{-5}	0.001	0.044	Fermented	Fusel, alcoholic, whiskey, fruity, banana	Sweet, malty, rancid, rubber [j]
1-Pentanol	4.00×10^{-3}	1.20×10^{-4}	0.000	0.008	Fermented	Pungent, fermented, bready, yeasty, fusel, winey, solvent	Mild odour, fusel oil, fruit, balsamic[c]
1-Hexanol	1.60×10^{-3}	5.60×10^{-6}	0.001	0.279	Herbal	Ethereal, fusel, oily, fruity alcoholic, sweet green	Woody, cut grass, chemical-winey, fatty, fruity, weak metallic[c]
1-Octanol	1.90×10^{-4}	1.26×10^{-4}	0.007	0.005	Waxy	Waxy, green orange, aldehydic rose, mushroom	Penetrating aromatic odour, fatty, waxy, citrus, oily, walnut, moss, chemical, metal[c] burnt[k]
(*E*)-2-Octen-1-ol		2.00×10^{-5}		0.135	Green	Green citrus vegetable fatty	Green citrus[c]
2-Dodecen-1-ol					Fatty	Fatty	
1-Tridecanol							Must[l]
(*E*)-2-Tetradecen-1-ol							
1-Tetradecanol					Waxy	Fruity, waxy, orris, coconut	
Cyclododecanol							
1-Pentadecanol							
(*Z*)-14-Methyl-8-hexadecen-1-ol							
Acetone		8.32×10^{-4}	0.002		Solvent	Solvent, ethereal, apple, pear	Chemical[d]
2-Butanone		3.54×10^{-2}	0.000		Ethereal	Acetone, ethereal, fruity, camphor	
2-Hexanone	5.60×10^{-4}	4.00×10^{-5}	0.003	0.037	Fruity	Fruity, fungal, meaty, buttery	
2,3-Pentanedione	2.00×10^{-5}	3.00×10^{-5}	0.038	0.057	Buttery	Pungent, sweet, buttery, creamy, caramel, nutty, cheesy	Caramel, buttery, fruity[d]
2-Heptanone		1.40×10^{-4}	0.008		Cheesy	Fruity spicy sweet herbal coconut woody	Fruity, spicy, cinnamon, penetrating fruity odour in liquid[c]
2,3-Octanedione		1.20×10^{-5}	0.132		Dill	Dill asparagus cilantro herbal aldehydic earthy fatty cortex	
6-Methyl-5-hepten-2-one	5.00×10^{-5}	8.50×10^{-4}	0.003	0.056	Citrus	Citrus, green, musty, lemongrass, apple, creamy, cheesy, banana	

(*continued on next page*)

Table 2 (*continued*)

Volatile compound	Ranges OT[a] (μg/kg[1])		Ranges OIR (x10[7])		Odour type[b]	Odour descriptors[b]	Other odour descriptors
	Min	Max	Min	Max			
2-Undecanone		5.50×10^{-6}	0.197		Fruity	Waxy fruity creamy fatty orris floral pineapple ketonic	
(*E*)-6,10-Dimethyl-5,9-undecadien-2-one	1.86×10^{-4}	6.00×10^{-5}	0.016	0.050	Floral	Fresh green, fruity, waxy, rose, woody, magnolia, tropical, pear, rose	
2-Pentadecanone					Floral	Fresh, jasmin, celery, fatty, oily, waxy, burnt	
3-Methyl heptane							
2,2,4,6,6-Pentamethyl heptane							
3,5-Dimethyl-2-hexene							
3-Nonene							
Hexadecane		5.00×10^{-4}	0.006				
1-Hexadecene		3.20×10^{-3}	0.001				
Heptadecane							
Cyclodecane							
Ciclohexadecane							
2-Ethyl furan		8.00×10^{-3}	0.000		Chemical	Chemical, beany, bready, malty, sweet, burnt	
2-Pentyl furan		5.80×10^{-6}	0.272		Fruity	Fruity, green, earthy, beany, vegetable, metallic	Green, bean, butter[c]
Methanethiol		2.00×10^{-7}		3.55	Sulphurous	Cabbage, garlic, decomposing	Sulphury, sweaty[d]
Dimethyl trisulphide	7.00×10^{-11}	1.00×10^{-7}	13.2	18920	Alliaceous	Sulphurous, onion,cooked onion, savoury, meaty	Sulphury, burnt, onion[d]
Toluene		5.27×10^{-4}	0.001				
(*E*)-Pinane					Balsamic	Fresh, pine, balsamic, herbal	

[a] Data from water values of otherwise specified (van Gemert, 2011); when available, ranges were selected exclusively from recent literature (>2000).
[b] Data from: http://www.thegoodscentscompany.com/.
[c] (Calkins & Hodgen, 2007).
[d] (Machiels, Istasse, & van Ruth, 2004).
[e] (Flores, 2017).
[f] (Siegmund & Pfannhauser, 1999).
[g] (Xie, Sun, Zheng, & Wang, 2008).
[h] (Varlet, Knockaert, Prost, & Serot, 2006).
[i] (Valim, Rouseff, & Lin, 2003).
[j] (Murnane, Lehocky, & Owens, 2013).
[k] (Zhang, Zhang, Liu, Zhao, & Luo, 2020).
[l] (Mebazaa, Rega, & Camel, 2011).

3-methyl-1-butanol since its origin is usually related to leucine catabolism and this compound has been associated to off-flavour related to meat spoilage (Smit, Smit, & Engels, 2005). In this sense, meat coming from hunting is more prone to suffer certain level of spoilage since the hygienic conditions of hunted animals are not as controlled as the ones that can be reached in a commercial slaughterhouse.

Long-chain alcohols were present in higher relative abundance than short chain homologues, with (*E*)-2-tetradecen-1-ol as the most abundant compound (Table 1). To the best of our knowledge, this compound has not been previously reported in cooked meat in the scientific literature, but its presence has been well described in plant essential oils (Kozhamkulova, Radwan, Zhusupova, Abilov, & Ross, 2011; Liolios, Sotiroudis, & Chinou, 2009; Selim, Aziz, Mashait, & Warrad, 2013). Therefore, it could be hypothesized that this compound could have been transferred to deer meat through the animal's diet. Additionally, (*E*)-2-octen-1-ol showed the highest OIR value in cooked deer meat samples, and the odour descriptors reported in the literature relate to green citrus and fatty aroma (Table 2). Other long-chain alcohols, such as 1-pentadecanol were also found in the cooked deer meat samples. However, long-chain alcohols have been rarely reported in cooked meat, and they are considered as potential biomarkers of meat from grazing animals (Gkarane et al., 2019; Kelman, Bugalho, & Dove, 2003).

Despite the considerably lower abundance of ketones compared to aldehydes and alcohols in the cooked deer meat samples, these carbonyl compounds are considered to exert an important impact on meat aroma (Machiels & Istasse, 2003b). Origin of ketones in meat is variable and methyl ketones are usually associated with the oxidation of free fatty acids. Most ketones detected in cooked deer meat samples were methyl ketones being 2-pentadecanone the most abundant (Table 1). In cooked deer meat samples, maximum OIR values calculated for ketones were, in general, lower than that for aldehydes, indicating a general lower odorant power (Table 2). Among ketones, 2-undecanone and 2,3-octanedione showed the highest OIR values, and the last compound has been previously proposed as a biomarker of meat from grazing animals (Priolo et al., 2004). Several authors hypothesized that this diketone originates from the oxidation of linoleic acid by the action of a lipoxygenase enzyme exclusively present in leafy plants (Keen & Wilson, 1992) but others proposed that 2,3-octanedione can be also formed by thermal oxidation of linoleic acid during meat cooking (Elmore, Campo, Enser, & Mottram, 2002). Sensory attributes for 2,3-octanedione have been related with herbal odour notes (Table 2). Moreover, it should be highlighted the high relative abundance of 6,10-dimethyl-(*E*)-5, 9-undecadien-2-one in cooked deer meat samples, which could be related with pasture diet, being a volatile compound usually found in herbs and fruits. To our knowledge, this is the first time that this compound has been detected in cooked meat, although it has been previously detected in other animal matrices such as eggs (Jin, Gouda, Jin, & Ma, 2019).

An important number of individual non-aromatic hydrocarbons were found in cooked deer meat (Table 1). Long-chain non-aromatic alkanes such as hexadecane and heptadecane were previously identified as reliable pasture-based diet tracers in meat products (Sivadier, Ratel, & Engel, 2010). Their origin has been related to the decarboxylation and splitting of higher fatty acids (Watanabe & Sato, 1971). The impact of these volatile compounds on meat aroma has been reported as low (Elmore et al., 2002). In this regard, due to the lack of OT data in the scientific literature, OIR values for most hydrocarbons could not be calculated. However, despite their low expected impact on meat odour,

3-methyl heptane has been identified as a grass and gamey odorant compound in cooked lamb (Grabež et al., 2019). In our cooked deer meat samples, the most abundant hydrocarbon was 2,2,4,6,6-pentamethyl heptane (Table 1), which has been previously described as one of the main volatile compounds of raw lamb and beef, being associated to the characteristic fatty meaty flavour (Gorraiz, Beriain, Chasco, & Insausti, 2002; Karabagias, 2018). In contrast, 1-hexadecene and cylohexadecane have been related with rancid odour notes in sheep fat (Young, Berdagué, Viallon, Rousset-Akrim, & Theriez, 1997) and pork salami (Lorenzo, Bedia, & Bañón, 2013).

Very few furans and sulphur compounds were found in the volatile profile of cooked deer meat (Table 1). The origin of these compounds has been linked to Maillard reactions from which usually carbohydrates give off furans (Umano, Hagi, Nakahara, Shyoji, & Shibamoto, 1995) that react with sulphur-containing amino acid cysteine. Thiamine degradation has been also related with the generation of furans and sulphur compounds in cooked meat (Vermeulen, Gijs, & Collin, 2005), and both of them can contribute to roasted odour notes in cooked meat (Brewer, 2006). Dimethyl trisulphide showed a very high OIR value being by far the highest value of all volatiles detected in cooked deer meat due to its very low OT value (Table 2). This sulphur compound is related with Strecker degradation of methionine and the subsequent production of methional. The degradation of the last one can lead to the formation of methanethiol and dimethyl trisulphide compounds (Flores, 2017). Dimethyl trisulphide is, therefore, not only the most potent odorant compound identified in cooked Iberian red deer meat, but also the main responsible for the characteristic cooked meat odour in line with previous studies in other cooked meats (Madruga, Elmore, Dodson, & Mottram, 2009; Rochat, de Saint Laumer, & Chaintreau, 2007; Watkins, Frank, Singh, Young, & Warner, 2013).

Finally, some other volatile compounds that have previously been proposed as pasture-based diet markers were found in cooked deer meat samples. These were benzene compounds such as toluene and benzaldehyde, as well as (*E*)-pinane. Toluene has been commonly related with the degradation of carotenoids, while terpenes are usually transferred directly from diet (Moran, Aldezabal, Aldai, & Barron, 2019; Rios, Fernández-García, Mínguez-Mosquera, & Pérez-Gálvez, 2008).

4. Conclusions

Overall, aldehydes were the major compounds in the volatile profile of cooked Iberian red deer meat, and they could be considered, together with the dimethyl trisulphide, as key odorant compounds of this game meat. In general, the volatile profile of cooked deer meat was, to some extent, similar to that of other grazed ruminants, although the deer meat showed a specific volatile profile in terms of the presence of particular individual compounds and relative abundance ratios. Special mention requires, first, the detection of long-chain compounds as their odour threshold values and sensory descriptors have not yet been reported in the scientific literature, and, second, the high number of compounds found in cooked deer meat that are related with pasture-based diet. To our knowledge, the present work is the first one reporting the volatile profile of cooked Iberian deer meat. Further research about the effect of meat storage conditions (refrigerated and frozen) and the relationship between volatile compounds and sensory characteristics are needed in order to identify relevant sensory descriptors that could relate with the consumer acceptability towards this type of game meat.

CRediT authorship contribution statement

Lara Moran: Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. **Carlos Vivanco:** Methodology, Formal analysis, Investigation, Writing – original draft. **José Manuel Lorenzo:** Conceptualization, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Luis Javier R. Barron:** Methodology, Software, Validation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Noelia Aldai:** Conceptualization, Resources, Writing – review & editing, Visualization, Supervision, Project administration.

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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