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Effect of ageing time on the volatile compounds from cooked horse meat

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ABSTRACT

Volatile compounds from cooked and aged (0, 7, 14, 21 days) Hispano-Bretón horse meat (loin) were analyzed by solid-phase microextraction coupled to gas chromatography–mass spectrometry. A total of 77 volatile compounds were found, from which aldehydes were the predominant family. Most of the identified compounds had their origin in the degradation of lipids, with a negligible contribution of Maillard derived products. Odour impact ratios were calculated and used as indicators of the contribution of each compound to the total aroma and aldehydes were, in general, the major contributors to cooked horse meat aroma. Results revealed that ageing affected 15 of the volatile compounds detected. From them, hexadecanal and 2- and 3-methylbutanal significantly increased during ageing, presumably affecting the cooked meat odour as these have considerable odorant impact. Under the present study conditions, periods longer than 14 days would be necessary for significant changes in the volatile profile of cooked horse meat.

1. Introduction

The characteristic aroma of cooked meat is an important quality attribute for consumer acceptability (Mottram, 1998) which is developed during cooking by the generation of odour active volatile compounds (Calkins & Hodgen, 2007). These compounds result from different complex reactions such as Maillard reaction between amino acids and sugars, lipid (mainly oxidation) and vitamin degradation, and other reactions among intermediate and final compounds derived from these pathways (Mottram, 1998). The formation of these compounds is conditioned by the cooking process and conditions (Domínguez, Gómez, Fonseca, & Lorenzo, 2014a; Kerth, 2016; Morán, Aldai, & Barron, 2021; Wall, Kerth, Miller, & Alvarado, 2019). Indeed, lower cooking temperatures and shorter cooking times are known to favour lipid degradation (thermal oxidation) products, while higher temperatures (especially direct heat sources as grilling) and longer times enhance Maillard reaction compounds (Kerth, 2016; Mottram, 1985). The contribution of volatile compounds to cooked meat aroma depends on their concentration and odour threshold (Zellner, Dugo, Dugo, & Mondello, 2008), and in this regard, Maillard reaction products (pyrazines, pyrroles, oxazoles, thiophenes and other heterocyclic compounds) are in general described as potent odorous compounds (Mottram, 1998). In addition, other factors including animal species (Calkins & Hodgen, 2007; Gasser & Grosch, 1988), breed (Insausti, Beriain, Gorraiz, & Purroy, 2002), sex (Gorraiz, Beriain, Chasco, & Insausti, 2002) muscle (Van Ba, Park, Dashmaa, & Hwang, 2014) and ageing time (Koutsidis et al., 2008) have been reported to influence the volatile profile of cooked meat.

Among all the main precursors of volatiles in cooked meat (amino acids, sugars, lipids and vitamins), a special attention has been placed on lipids (content and composition) (Estevez, Morcuende, Ventanas, & Cava, 2003; Mottram, 1998), the most important contributors to the characteristic aroma of different animal species (Calkins & Hodgen, 2007; Mottram, 1998). Relationships between meat lipids and generated volatile compounds have been found in cooked meat from different species (Elmore, Mottram, Enser, & Wood, 1999; Rivas-Cañedo et al., 2013).

Overall, extensive research has been conducted in the identification of volatile compounds in meat and to elucidate their contribution to cooked meat aroma (Gasser & Grosch, 1988; Van Ba, Hwang, Jeong, & Touseef, 2012). However, despite the recent increase in its consumption (Belaunzaran et al., 2015), very few works have studied the volatile profile of cooked horse meat (Domínguez et al., 2014a; Domínguez, Gómez, Fonseca, & Lorenzo, 2014b; Maggiolino et al., 2019; Tateo et al., 2020).

Meat ageing, practice especially applied for meat tenderness improvement (Koohmaraie, 1994) can also affect meat aroma due to

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changes in the concentration of precursors and to the development of odorous compounds in raw aged meat by enzymatic reactions (Koutsidis et al., 2008), bacterial action (Casaburi, Piombino, Nychas, & Villani, 2015) and lipid oxidation (Resconi et al., 2012; Rivas-Cañedo et al., 2013). Volatile composition of aged meat (different periods) has been widely explored in cooked meat of the most consumed species such as beef (Gorraiz et al., 2002; Van Ba et al., 2014; Watanabe et al., 2015), lamb (Rivas-Cañedo et al., 2013) and pork (Estevez et al., 2003). In addition, sensory studies addressing the implication of ageing in the odour of cooked meat reported that long ageing periods can result in unpleasant aromas (Spanier, Flores, McMillin, & Bidner, 1997). These results have been attributed to decreased levels of volatile compounds with pleasant odour notes (Van Ba et al., 2014) or to an excess concentration of hexanal, one of the major volatile compound in cooked meat (Melton, 1983).

In this sense, to our knowledge, only two studies have reported the ageing time effect on the volatile compounds of cooked meat from Italian Heavy Draught Horses (several muscles aged up to 14 days; Maggiolino et al., 2019; Tateo et al., 2020). To this extent, considering the crucial role of meat aroma in the acceptance and preference of the consumer (Beldarrain et al., 2020), the aim of the present work was to study the changes in the volatile profile of cooked horse meat previously vacuum aged for 0, 7, 14 and 21 days.

2. Materials and methods

2.1. Animal handling, experimental design and sampling

Ten Hispano-Bretón horses (five females and five males) were reared in a commercial farm under grazing conditions while suckling their mothers from birth (May–June 2017) until weaning (6–8 months of age). Then, they continued grazing until 11–13 months of age, when they were moved to a commercial feedlot and finished for 100–120 days (d) on a high-grain diet and straw *ad libitum*. Concentrate was composed by barley, soybean hulls, molasses, palm oil and salts (13.3% protein, 2.70% fat, 7.60% fibre).

Horses were slaughtered in a commercial abattoir, following the specifications in the European legislation (Council Regulation, 2009), at 15–17 months of age. The average carcass weight was of 246 ± 14.0 kg (250 ± 15.0 kg for females and 242 ± 12.5 kg for males). All carcasses were classified as U (conformation) and 2 (fat cover) according to the Community scale for the classification of carcasses of adult bovine animals (Council Regulation, 1981) as there is no specific classification system for horses at EU level.

Two horses (female and male) were slaughtered per week during five consecutive weeks. After 48 h (h) *post mortem* at 4 °C (day 0), both right and left rib sections were removed from carcasses and transported to the laboratory under refrigerated conditions ($n = 20$). The loin, *Longissimus thoracis et lumborum* (LTL) muscle ($n = 20$), was excised, trimmed of visible adipose and connective tissues and cut into 1.5 cm thick steaks.

From each loin, four consecutive steaks, starting from the 2nd rib, were obtained. Steaks were vacuum packaged and randomly assigned to an ageing time of 0, 7, 14 and 21 d. Ageing was performed under refrigeration conditions (4 ± 1 °C), without illumination. When each ageing day was reached, steaks were frozen (−80 °C) until volatile compound analysis was performed.

The aforementioned procedure was considered as a full-randomized block design with slaughter day as a blocking factor and ageing time as split plot factor where ageing levels were randomly allocated to different individual steaks obtained from each loin. The experimental unit (loin) was considered as a plot and the steaks were the subplots (sampling units) in which ageing time (factor) was assessed. Animal sex, carcass side and carcass weight were distorting variation sources controlled by the experimental design in order to minimize the residual variation.

2.2. Volatile compound analysis

2.2.1. Meat cooking procedure

Cooking was carried out according to AMSA (2015) recommendations. Before cooking, horse steaks were thawed (4 °C) overnight and kept at room temperature for 2 h covered with an oxygen permeable polyvinylchloride film (580 mL/m^2/h of permeability). Eight steaks from two loins coming from different animals (4 ageing times from female and 4 ageing times from male) were cooked at the same time in four plane double clamp electric grills (Dalyko MB-30, Sogo, Spain). Same grill was used for the steaks belonging to the same ageing time (0, 7, 14 and 21 d), grills were set at 200 °C and meat cooked for 9–13 min until an internal temperature, monitored individually with a multichannel (Lutron electronic, Pennsylvania, USA), of 71 ± 1 °C was reached. Cooked steaks were allowed to cool at room temperature, minced, vacuum packed and frozen (−80 °C) until volatile analysis.

2.2.2. Solid-phase microextraction-gas chromatography–mass spectrometry (SPME-GC–MS) analysis

Minced meat samples were thawed for 2 h at room temperature. Then, approximately 10 g of cooked meat, to which anhydrous sodium sulphate (Sigma Aldrich) was added in a ratio of 4:1 (by weight), were homogenized in a blender. 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, 99.8% purity, Sigma Aldrich) were added as the internal standard (IS). Vials were sealed with PTFE septa and steel magnetic cap (Agilent Technologies).

Volatile compounds were extracted by SPME on a 30/50 μm DVB/Carboxen/PDMS fibre (Supelco) using a PAL RSI 85 autosampler (CTC Analytics AG, Zwingen, Switzerland). Extraction was done over 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 during 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 GC equipment coupled to a 5975E MS detector (Agilent Technologies). Volatile compounds were separated on a Supelcowax-10 capillary column (60 m, 250 μm i.d., and 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 10 °C/min until 240 °C, and finally held at 240 °C for 15 min. Helium (99.999% purity, Air liquid, Madrid, Spain) was used as the carrier gas (constant pressure of 30 psi) and volatiles were transferred to MS detector throughout a transfer line at 280 °C. Chromatographic data were registered with the MSD ChemStation Data Analysis software (version 5.52, Agilent Technologies). MS 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.

Volatile compounds in the cooked horse meat samples were quantified using the chromatographic peak area. The limit of detection (LOD) was set as two times the average baseline noise calculated in three different chromatographic regions from the analysis of 10 blanks (empty vial). Peak areas (arbitrary units) of volatile compounds were used to calculate the abundance relative to the IS area according to the following equation:

$$\text{Relative abundance} = \frac{\text{peak area}}{\text{IS area}} \cdot \frac{2.5\ \text{g}}{\text{mixture weight (g)}} \cdot 100$$

Tentative identifications of volatile compounds were performed by comparing the mass spectra of the peaks with those of NIST 2.0 (National Institute of Standards and Technology, Gaithersburg, USA) library, using a matching factor > 700. Mixtures of C7-C24 alkanes (Sigma Aldrich) were used for the calculation of the experimental linear retention indices (LRI) for cooked horse meat and commercial standard peaks. Experimental LRI values were also compared to values obtained in the literature under similar chromatographic conditions.

Additionally, when available, several high purity commercial standard compounds were used for positive identification of cooked meat volatiles: 1-penten-3-ol, 1-pentanol, 1-octen-3-ol, 1-heptanol, 6-methyl-2-heptanone, 1-octanol, (*E*)-2-octen-1-ol, 1-tetradecanol, 1-tridecanol, acetaldehyde, hexanal, 1-hexanol, (*E*)-2-hexenal, heptanal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, 2-butyl-2-octenal, toluene, benzaldehyde and 1-hexadecene were purchased from Sigma Aldrich (Madrid, Spain), and 1-propanal, 2-methylbutanal, 3-methylbutanal, octanal and 2-heptanone from Honeywell-Fluka Research Chemical-Fisher Scientific (Madrid, Spain).

2.2.3. Odour impact ratio calculation

In order to obtain an indicator of the odour intensity of each volatile compound detected by SPME-GC–MS in cooked horse meat samples, odour impact ratio (OIR) was calculated as previously described (Abilleira, Schilichtherle-Cerny, Virto, de Renobales, & Barron, 2010) with minor modifications. Available odour threshold (OT) values measured in water were obtained from Van Gemert (2011) unless otherwise indicated in the table (Table 1), and subsequently used to calculate OIR for each of the volatile compound:

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

The mean relative abundance of each volatile compound detected in cooked horse meat samples (all 4 ageing times) was used for OIR calculations, not quantitative values. Moreover, it is noteworthy to point out that the present meat protein content might cause a matrix effect and alter the OT values of volatile compounds, compared to literature values obtained in water. However, OIR values allowed the comparison of odour impacts of the different volatile compounds detected in cooked horse meat samples.

2.3. Data treatment and statistical analysis

Peak areas of individual volatile compounds present in both replicates (> LOD) and in over 70% of the samples from each ageing time were used to calculate the mean relative abundance in cooked horse meat samples. The general linear model of analysis of variance (GLM-ANOVA) (IBM-SPSS statistics software (version 25.0, IBM, New York, USA) was used to investigate the ageing effect on the volatile composition of cooked horse meat. The GLM included ageing time and the controlled distorting variation factors (animal sex and carcass side) as fixed effects, and carcass weight as covariate. Slaughter day was also included as a random effect in the model because this blocking factor was a simultaneous distorting factor of uncontrolled variation coming from at least individual animal, feeding, transport or slaughter conditions. Moreover, GLM included binary interactions among all factors. Fisher's Least Significance Difference test of estimated marginal means was used for pairwise comparisons among ageing time levels.

Partial least squares for discrimination analysis (PLS-DA) (Unscrambler X software version 10.3, CAMO ASA, Oslo, Norway) was applied to the volatile composition of cooked horse meat samples to discriminate among ageing times. Ageing day was used as categorical *Y*-variable and weighed volatile relative abundances (used as the inverse of standard deviation) as *X*-variable. Full (leave-one-out) cross-validation with an uncertainty test was applied to extract the optimal number of model factors. Variable importance in projection scores were used to estimate the *X*-variables contribution to PLS-DA model and uncertainty limits were used to estimate the significance of the weighted coefficients that correlated *X*-variables to categorical *Y*-variables (Martens & Martens, 2000).

Statistical significance was declared at $P \leq 0.05$ and numerical values of volatile relative abundances were log transformed to assure data normality. Three significant figures were used to express the data.

3. Results and discussion

3.1. Volatile composition of cooked horse meat

A total of 77 individual volatile compounds were found (peak area > LOD) in the headspace of cooked horse meat samples with aldehydes constituting most of compounds (30 compounds), followed by alcohols (13), non-aromatic hydrocarbons (11), ketones (8), benzenoic compounds (4), furans (2) and a sulphur compound (Table 1). In addition, 8 volatile compounds were detected but their chemical nature could not be established as their MS signal was not clear enough, and they have been labelled as unknowns.

From the compounds identified, the predominant chemical family in terms of mean relative abundance were the aldehydes (around 75% of the total abundance) as observed in cooked beef (Wall et al., 2019), pork (Estevez et al., 2003) and in horse meat (Domínguez et al., 2014a). Among them, hexanal was the dominating aldehyde followed by nonanal (15% and 8% of the total abundance, respectively). The next major chemical family was that of alcohols (10%), and the remaining families together accounted for around 13% of the total volatile abundance.

It is noteworthy that some of the chemical families reported in relatively high concentrations in cooked horse meat and other species meats were absent in the present study, namely short-chain fatty acids (C4-C10) (horse meat, Tateo et al., 2020), esters (horse meat, Domínguez et al., 2014a) or nitrogen and sulphur compounds (beef, Wall et al., 2019) (only dimethyl trisulphide was detected). Short-chain fatty acid results are controversial, as several studies have not reported them in the volatile profile of cooked beef (Gorraiz et al., 2002). The reason of the high variability usually found in the literature has been attributed to several reasons such as the complexity in the formation of these compounds and their possible further degradation into other aroma compounds as observed in different types of meat (Casaburi et al., 2015), their dissociation equilibrium and pH of the matrix analyzed, or different sensitivities on extraction methods as observed in beef (Song et al., 2011). Certainly, the extraction conditions used in the present study do not allow the detection of > C3 carboxylic acids and, consequently, they are not reported.

The absence of esters and nitrogen and sulphur compounds (except for dimethyl trisulphide) is likely related to the cooking process of the meat. In fact, widely differing cooking methods applied in studies performed in meat from different species (considering sample preparation, type of heat treatment, temperature and cooking time) may lead to diverse concentrations of these compounds; thus, comparisons need to be carefully done (deer meat, Morán et al., 2021; pork, Mottram, 1985). In the present study, steaks were grilled at 200 °C for 9–13 min until an internal temperature of 71 °C was reached (medium degree of doneness according to beef steak colour guide; AMSA, 1995). It has been reported that only when the meat is grilled under severe conditions the Maillard reaction derived nitrogen and sulphur compounds are major components of cooked meat (Mottram, 1998). In this line, Mottram (1985) proved that when beef steaks were grilled at the same temperature but for a longer time (15 min *per* side, medium degree of doneness *versus* 30 min *per* side, well-done degree of doneness), the concentration of nitrogen and sulphur heterocyclic compounds increased and pyrazines dominated the volatile profile. Moreover, Wall et al. (2019) proved that the concentration of these compounds also increased significantly when grilling temperature was increased (232 °C *versus* 177 °C) even if all beef steaks were cooked until a medium degree of doneness (internal temperature of 71 °C). Kerth (2016) reported the influence of steak thickness on the type of volatile compound generated in cooked beef and concluded that the volatile profile of thinner steaks (~ 1.27 cm) was dominated by volatiles originating from lipid degradation, while thicker steaks (~ 2.81 cm) presented higher Maillard reaction derived products under the same cooking conditions. In the present study, a combination of the aforementioned factors related to the cooking process could have been partially responsible for the low contribution of Maillard reaction

Table 1
Mean relative abundance and estimated mean odour impact ratio (OIR), odour threshold (OT) (Van Gemert, 2011) and odour description of volatile compounds of cooked horse meat analyzed by solid-phase microextraction coupled to gas chromatography–mass spectrometry. Mean relative abundance values were calculated from volatile compounds detected in aged (0, 7, 14 and 21 days) and cooked horse meat samples ($n = 80$).

Volatile compound	IM	LRI	Relative abundance	OT[a]	OIR	SEM	Odour description	Ref.
Aldehydes								
Acetaldehyde	P	704	7.07	25	0.283	0.009	Fruity	1
Propanal	P	796	5.78	145	0.0399	0.0025	Nut like	2
2- Methylbutanal	P	911	2.88	4.40	0.655	0.115	Cinnamon, toast	3
3- Methylbutanal	P	915	7.04	1.20	5.87	1.18	Chocolate, caramel, green, nutty	4
Pentanal	T	981	46.5	12.0	3.88	0.09	Almond, malt, pungent, acrid	5
Hexanal	P	1078	501	1.00	500	32	Grassy, tea, vegetable, lemony, sour, beefy	5
2-Methyl-2-butenal	T	1085	6.72	458	0.0147	$8.02{\cdot}10^{-4}$	Coffee like	6
Heptanal	P	1183	95.2	2.80	34.0	0.9	Fruity, nutty	4
(*E*)-2-Hexenal	P	1223	5.20	110	0.0473	0.0045	Eucalyptus, fruit/flower, potato, toast	3
Octanal	P	1282	134	0.700	191	6	Soap/orange	3
(*E*)-2-Heptenal	T	1333	31.8	13	2.44	0.08	Fishy	3
2-Methyl-2-heptenal	T	1363	5.60					–
Nonanal	T	1398	278	2.80	99.2	0.6	Grassy, tea, vegetable, lemony, sour, beefy	7
(*E*)-2-Octenal	P	1441	44.2	3.00	14.7	0.017	Green, nut, fat	5
Decanal	T	1505	13.3	0.150	88.6	0.12	Powerful, waxy, aldehydic, orange, citrus peel	5
(*E,E*)-2,4-Heptadienal	T	1509	8.65	15.4	0.562	0.036	Roast meat, fried potatoe	8
(*E*)-2-Nonenal	P	1550	69.5	0.0800	868	21	Earthy, fermented, burnt	5
(*E*)-2-Decenal	P	1662	171	0.350	487	11	Tallow, orange	5
2-Butyl-2-octenal	P	1683	36.8	20.0	1.84	0.22		–
Dodecanal	T	1722	53.6	55	0.974	0.026	Onion, green, yeast,vomit	9
(*E,E*)-2,4-Nonadienal	T	1726	43.1	0.100	430	1	Meaty, burnt, chocolate	4
(*E*)-2-Undecenal	T	1774	217	1.40	155	2		–
(*E,Z*)-2,4-Decadienal	T	1786	11.1	0.070	158	7	Fried onion, lemon	10
Tridecanal	T	1831	108	70	1.54	0.05	Nutty	11
(*E,E*)-2,4-Decadienal	T	1839	60.1	0.0270	2226	58	Fatty, fried potatoe	12
Tetradecanal	T	1940	219	53	4.14	0.07	Roasted, fried meat	13
(*E,E*)-2,4-Undecadienal	T	1955	14.1	0.01	1410	0.4		–
Pentadecanal	T	2049	222	1000	0.222	0.004	Hot timber	9
Hexadecanal	T	2156	96.9	0.910 (14)	106	17	Sweet	9
cis-11-Hexadecenal	T	2189	17.3				Waxy	15
Ketones								
2-Heptanone	P	1181	8.74	140	0.0624	0.0061	Rancid, flower, vinegar, soap/orange	3
6-Methyl-2-heptanone	P	1242	9.71	8.10	1.20	0.09	Cloves, menthol, eugenol	5
3-Octanone	T	1259	4.17	28	0.149	0.024	Herbal	6
5-Methyl-3-hepten-2-one	T	1342	35.8					–
(*E*)-3-Octen-2-one	T	1415	7.18	250	0.0287	0.0008	Nut, crushed bug, earthy, spicy, herbal, sweet, mushroom, hay	5
(*E,E*)-3,5-Octadien-2-one	T	1586	13.5	125	0.108	0.008	Fruity, green, grassy	15
6,10-Dimethyl-(*E,E*)-5,9-undecadien-2-one	T	1870	12.0	60	0.200	0.011	Fresh, green, fruity, waxy, rose, woody, magnolia tropical	15
2- Pentadecanone	T	2038	8.65				Fresh, jasmin, celery, fatty, oily, waxy, burnt	–
Alcohols								
1-Penten-3-ol	P	1055	0.674	400	$1.69{\cdot}10^{-3}$	$6.50{\cdot}10^{-5}$	Flower, burnt, meaty	3
1-Pentanol	P	1251	36.5	$4.00{\cdot}10^{3}$	$9.12{\cdot}10^{-3}$	$4.78{\cdot}10^{-4}$	Mild odour, fusel oil, fruit, balsamic	5
1-Hexanol	P	1352	14.8	5.60	2.64	0.11	Woody, cut grass, chemical-winey, fatty, fruity, weak metallic	5
1-Octen-3-ol	P	1446	83.3	1.50	55.5	1.8	Fishy, fatty, mushroom, grassy	16
Heptanol	P	1453	42.6	5.40	7.88	0.11	Fragant, woody, oily, green, fatty, winey, sap, herb	5
2-Ethyl-hexan-1-ol	T	1488	2.79	$2.54{\cdot}10^{4}$	$1.09{\cdot}10^{-4}$	$4.74{\cdot}10^{-6}$	Resin, flower, green	5
1-Octanol	P	1555	76.8	190	0.404	0.010	Fatty, waxy, citrus, oily, walnut, moss, chemical, metal, burnt	5
3,5-Octadien-2-ol	T	1581	6.47					–
(*E*)-2-Octen-1-ol	P	1613	24.7	20.0	1.24	0.05	Green, citrus	5
9-Decen-2-ol	T	1781	8.04					–
1-Dodecanol	T	1966	18.8	158	0.119	0.005	Earthy, soapy, waxy, fatty, honey, coconut	15
1-Tridecanol	P	2083	10.8				Musty	15
1-Tetradecanol	P	2174	27.4				Fruity, waxy, coconut	15
Non-aromatic hydrocarbons								
2,2,4,6,6-Pentamethyl-heptane	T	958	5.12					–
Butyl-cyclopentane	T	1038	2.24					–
Tridecane	P	1297	14.5	$2.14{\cdot}10^{3}$(17)	$6.75{\cdot}10^{-3}$	$2.13{\cdot}10^{-4}$	Alkane	18
3-Ethyl-2methyl-1,3-hexadiene	T	1430	23.0					–
Pentadecane	P	1498	23.7	$1.30{\cdot}10^{7}$*	$1.82{\cdot}10^{-6}$	$3.10{\cdot}10^{-8}$	Waxy	15
1- Pentadecene	T	1525	8.14	$3.60{\cdot}10^{3}$	$2.26{\cdot}10^{-3}$	$1.08{\cdot}10^{-4}$		
5,5-Dimethyl-1,3-heptadiene	T	1591	7.58	$1.30{\cdot}10^{7}$*	$5.87{\cdot}10^{-7}$	$5.98{\cdot}10^{-8}$		–
Hexadecane	P	1598	12.1	500*	0.0242	0.011	Mild waxy	15
1-Hexadecene	P	1622	8.60	$3.20{\cdot}10^{3}$*	$2.69{\cdot}10^{-3}$	$1.45{\cdot}10^{-4}$		–
1-Heptadecene	T	1647	11.3	$8.00{\cdot}10^{3}$	$1.42{\cdot}10^{-3}$	$4.65{\cdot}10^{-5}$		–

(*continued on next page*)

Table 1 (*continued*)

Volatile compound	IM	LRI	Relative abundance	OT[a]	OIR	SEM	Odour description	Ref.
1,15-Hexadecadiene	T	2270						–
Benzenoic compounds								
Toluene	P	1035	3.78	24.0	0.158	0.0032	Chemical solvent aroma	19
Benzaldehyde	P	1545	75.9	350	0.217	$4.92{\cdot}10^{3}$	Almond oil, bitter almond, burning aromatic taste	5
3-Ethyl-benzaldehyde	T	1743	109					–
4-Pentyl-benzaldehyde	T	2057	23.3					–
Furans								
2-Ethyl-furan	T	950	3.14	8000	$3.92{\cdot}10^{-4}$	$5.59{\cdot}10^{-5}$	Sweet corn	20
2-Pentyl-furan	T	1231	50.2	5.80	8.65	0.19	Green, vean, butter	5
Miscelaneous								
Dimethyl trisulphide	T	1394	17.4	0.100	173	53	Sulfury, burnt,onion	4
Unknown[m/z: 97, 55, 41, 71, 84]		1471	80.5					
Unknown[m/z: 67, 95, 41, 81, 12]		1630	7.70					
Unknown[m/z: 43, 84, 71, 57, 128]		1696	16.5					
Unknwon[m/z: 121, 91, 77, 150, 65]		1889	70.5					
Unknwon[m/z: 95, 81, 43, 55, 67]		1918	24.9					
Unknwon[m/z: 43, 41 57, 83, 69]		1997	13.2					
Unknown[m/z: 55, 43, 69, 83, 97]		2070	27.3					
Unknown[m/z: 45, 55, 67, 73, 41]		2295	22.5					

IM, identification method; LRI, linear retention index; SEM, standard error of the mean; P, positive identification; T, tentative identification; [a]OT expressed as μg/kg of water; *OT expressed as μg/kg of oil; Ref, reference.
References: (1) Sollner and Schieberle (2009); (2) Frankel (1993); (3) Resconi et al. (2012); (4) Machiels et al. (2004); (5) Calkins and Hodgen (2007); (6) Giri, Osako, and Ohsima (2010); (7) Moon, Cliff, and Li-Chan (2006); (8) Frank et al. (2017); (9) Gkarane et al. (2018); (10) Resconi et al. (2010); (11) Sutherland and Ames (1995); (12) Gasser and Grosch (1988); (13) Xie, Sun, Zheng, and Wang (2008); (14) Xie, He, Lv, Zhang, and Li (2016); (15) The Good Scents Company Information System (2021); (16) Tao, Wu, Zhou, Gu, and Wu (2014); (17) Kataoka, Lord, and Pawliszyn (2000); (18) Van Ba et al. (2012); (19) Moio, Dekimpe, Etievant, and Addeo (1993); (20) Evans, Moser, and List (1971)

derived compounds to cooked horse meat volatile profile.

Mechanisms of lipid oxidation in foodstuff are still an area of controversy and remain as a subject of active research. Indeed, several researchers have highlighted that lipid oxidation should be considered as multiple interrelated pathways rather than a single radical chain based on hydrogen abstraction (Schaich, 2013). Moreover, the interrelation between lipid and protein oxidation has been proposed to affect the odour of cooked meat from different species (Estevez, 2011), and the complexity of the issue becomes evident.

In the present study, most volatiles detected in cooked horse meat samples derived from the thermal degradation of lipids (Table 1). In this regard, high proportions of polyunsaturated FA (PUFA) are known to increase the susceptibility of meat to undergo lipid oxidation and the subsequent formation of volatile compounds during cooking. This has already been proved in other species in which cooked meat from animals with differing PUFA/saturated FA (SFA) ratios were studied (beef, Insausti et al., 2002; pork, Estevez et al., 2003; lamb, Gkarane et al., 2018). Moreover, high PUFA concentrations seem to form free radicals that promote the oxidation of other FA and inhibit the formation of Maillard reaction derived products, as confirmed in model system experiments (Elmore, Campo, Enser, & Mottram, 2002).

The unsaturated FA content of raw meat is relevant to understand the volatile profile of cooked horse meat, as the unsaturation degree of FA determines the overall concentration of volatiles coming from lipid thermo-oxidation (lamb, Rivas-Cañedo et al., 2013). More concretely, heptanal, octanal, nonanal, decanal, (*E*)-2-decenal and (*E*)-2-undecenal which are relevant contributors of cooked horse meat aroma have been reported as major oxidation products derived from oleic acid (Van Ba et al., 2012), which was the predominant FA in raw horse meat (31%; Beldarrain et al., 2021). 1-Octanol, the second most abundant alcohol, and 1-heptanol also originate from oleic acid (Schaich, 2013). Although this monounsaturated FA is less susceptible to lipid oxidation than PUFA, Elmore et al. (1999) hypothesized that a promoting effect of heating on the degradation of PUFA increased the amount of free radicals capable of attacking other less susceptible FA in beef.

The most abundant alcohol in the cooked horse meat samples, 1-octen-3-ol (Table 1), has been reported to be an oxidation product derived from linoleic acid (Rivas-Cañedo et al., 2013), and this is also true for 1- pentanol, 1-hexanol and some aldehydes such as pentanal, hexanal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal and (*E,E*) and (*E, Z*)-2,4-decadienal (Elmore et al., 2002; Van Ba et al., 2012). On the other hand, (*E,E*)-2,4-heptadienal and benzaldehyde have been reported to arise from the oxidation of linolenic acid (Van Ba et al., 2012; Van Ba et al., 2014).

It becomes evident that most relevant volatile compounds detected in the present study have been reported to be thermal oxidation/degradation products of oleic, linoleic and linolenic acids. But, some compounds (mean relative abundance <8) formed *via* Strecker amino acid degradation namely acetaldehyde and 2- and 3-methylbutanal were also identified. Regarding dimethyl trisulphide, it has been suggested that this volatile is formed from dimethyl disulphide, which is a consequence of the degradation of methionine or methanethiol (Baines & Mlotkiewicz, 1983). In contrast, Golovjna and Rothe (1980) attributed the origin of dimethyl trisulphide in beef to the reaction between hydrogen sulphide and ethanol. Other compounds such as methyl alcohols and ketones can be originated from the thermal degradation of branched-chain amino acids, although their presence in the volatile profile of cooked horse meat samples was not relevant, and benzaldehyde has also been suggested to derive from Strecker amino acid degradation according to studies performed in beef (Watanabe et al., 2015).

3.2. OIR values and aroma of cooked horse meat

In order to estimate the contribution of each volatile compound to the aroma of cooked horse meat, OIR values were calculated and odour descriptors reported by other authors or gathered in specialized flavour data bases were considered (Table 1). Overall, aldehydes showed the highest OIR values in cooked horse meat samples and they were, in consequence, the most odour-active compounds. Low detection thresholds have been reported for aldehydes, thus they contribute to cooked beef aroma even when present in low concentration (Elmore et al., 1999). In addition, aldehydes have been reported as the main compounds responsible for species-specific odours in cooked meat due to

aldehydes are essentially derived from lipid oxidation, and the diverse FA composition of different species constitutes a big source of variation in generated volatile aldehydes (Calkins & Hodgen, 2007).

Among linear saturated aldehydes, C6-C10 aldehydes and hexadecanal showed the highest OIR values (> 34). C6-C10 aldehydes are in general related to grassy and fruity odours, and of them, hexanal should be highlighted with the highest OIR value (500) (Table 1). Grassy, tea, vegetable, lemony, sour and beefy are odour notes that have been attributed to hexanal in cooked beef, so this aldehyde is considered to positively contribute to beef aroma (Calkins & Hodgen, 2007). In contrast, Kerler and Grosch (1997) reported that C6-C10 linear saturated aldehydes contribute to unpleasant odour notes in poultry and Melton (1983) concluded that an excessive concentration of hexanal might produce off-flavours in beef. Hexadecanal presented a high OIR value (106) and has been related to sweet odour notes. This fact, together with other taste precursors, may influence in the sweet perception of cooked horse meat, as recently reported by Beldarrain et al. (2020).

The contribution of unsaturated aldehydes to cooked horse meat aroma should not be underestimated, especially that of C9-C11 2-alkenals and 2,4-alkedienals, which have been described as aroma contributors in other cooked meats (Calkins & Hodgen, 2007). Even though seldom detected or reported in very low concentrations in cooked horse meat (Domínguez et al., 2014a; Domínguez et al., 2014b; Maggiolino et al., 2019; Tateo et al., 2020), (*E*)-2-nonenal, −decenal and -undecenal showed high OIR values (> 155) in the present study, and so did (*E,E*)-2,4-nonadienal, −decadienal, (*E*)-2-octenal and (*E,Z*)-2,4-decadienal (OIR > 14). These unsaturated aldehydes have been described to generate fatty/burnt odour notes in beef (Machiels, Istasse, & Van Ruth, 2004).

OIR values calculated for alcohols in studied cooked horse meat samples were in general low (≤ 1), with the exception of two linear saturated alcohols (1-hexanol and 1-heptanol) and 1-octen-3-ol. These three compounds have already been described as active odorants in other cooked meats (Calkins & Hodgen, 2007) and the 1-octen-3-ol presented a relatively high OIR value in the present cooked horse meat samples (55.5) which has been agreed to contribute with a mushroom-like odour in beef (Van Ba et al., 2012). Some studies did not report this compound in grilled horse meat (Domínguez et al., 2014a; Domínguez et al., 2014b), whereas others, under similar meat cooking and volatile extraction conditions, did (Maggiolino et al., 2019; Tateo et al., 2020). The rest of the alcohols found in the cooked horse meat samples (Table 1) are generally related to pleasant odours, but as previously stated, they did not seem to significantly contribute to cooked horse meat aroma.

Detected ketones showed low OIR values (≤ 1), poorly contributing to cooked horse meat aroma (Table 1). 2,3-Butanedione, a typical odour active volatile compound reported to contribute to cooked beef aroma with buttery odour notes (Gorraiz et al., 2002; Machiels et al., 2004; Van Ba et al., 2014) was not detected in the present study. Again, this diketone was not detected by Domínguez et al. (2014a and 2014b) in cooked horse meat, although Maggiolino et al. (2019) and Tateo et al. (2020) did detect.

Benzaldehyde has been related to unpleasant odours in cooked meat from different species (Calkins & Hodgen, 2007) and some studies have suggested that a high abundance of benzaldehyde might cause off-flavour in lamb (Elmore et al., 2002), however, this compound showed a low OIR value (0.217) in cooked horse meat. OT values of 3-ethyl- and 4-pentyl-benzaldehyde were not available in the scientific literature although these volatiles have been reported in cooked meat and meat products (Maggiolino et al., 2019).

From the two detected furans, 2-pentylfuran showed a relevant OIR value (8.65) (Table 1). This compound has been described as characteristic of grilled meat aroma in several meats (Shibamoto, 1980) and has been previously reported in cooked pork (Estevez et al., 2003), lamb (Rivas-Cañedo et al., 2013), beef (Calkins & Hodgen, 2007) and horse meat (Maggiolino et al., 2019; Tateo et al., 2020).

On the other hand, dimethyl trisulphide, generally related to unpleasant odours in beef (Machiels et al., 2004), has been reported as an important contributor of cooked beef aroma (Van Ba et al., 2014). This sulphur compound showed the highest OIR value (173) after that of alkenals and alkedienals, due to its moderate abundance in the cooked horse meat samples but low OT (Table 1). Thus, it appears feasible that this compound contributes significantly to the cooked horse meat aroma.

Non-aromatic hydrocarbons, which show high odour detection thresholds, poorly contribute to the aroma of cooked meats (Ho & Chen, 1994), with OIR values below 1 (Table 1).

3.3. Effect of ageing time on the volatile profile of cooked horse meat

Changes in volatile composition of cooked meat caused by the ageing process may indicate chemical, enzymatic or microbial degradation of volatile precursors such as peptides, amino acids, sugars and lipids of raw meat happened during cooling storage of meat from different species (Casaburi et al., 2015; Estevez et al., 2003; Insausti et al., 2002; Koutsidis et al., 2008). Lipid oxidation can occur in raw meat during storage, before lipid thermal oxidation related to the cooking process (Jelen & Wąsowicz, 2012), altering the volatile profile of the aged and cooked meat. These events also depend on the type of packaging used during the ageing process. In the present study, steaks were aged in an anoxic environment (vacuum), thereby limiting oxidative processes as observed by Spanier et al. (1997) in beef.

From the 77 individual volatile compounds detected in the cooked horse meat samples, the relative abundance of 15 changed significantly with ageing time ($P \leq 0.05$; Table 2). The relative abundance of some volatile compounds, including three aldehydes (hexadecanal and 2- and 3-methylbutanal), benzaldehyde and 2-pentadecanone increased with ageing time. Interestingly, both 2- and 3-methylbutanal were not detected in the cooked unaged meat samples but their relative abundance increased from 7 to 14 days of ageing. These branched aldehydes were previously found in higher amounts in cooked beef aged for 28 d than for 7 d (Van Ba et al., 2014), and the reason may be an increase in the content of free leucine and isoleucine due to proteolysis during beef ageing process (Koutsidis et al., 2008). Relative abundance of benzaldehyde also increased during ageing as observed in previous horse meat studies (Maggiolino et al., 2019; Tateo et al., 2020). As aforementioned, hexadecanal has been related to sweet odour notes, and in consequence, its higher relative abundance in the aged and cooked meat samples could be translated in a sweeter aroma in comparison to the unaged and cooked meats. This increase is likely related to oxidative degradation of lipids although autoxidation did not seem to be relevant during the ageing process as other volatile compounds normally derived from lipid oxidation did not change, or even, decreased their relative abundance with ageing time (Table 2) as observed by others in vacuum aged beef (Watanabe et al., 2015). In this sense, some other volatile compounds related to lipid oxidation such as hexanal, 2-methyl-2-heptenal, 2-butyl-2-octenal, hexanal, dodecanol, 5-methyl-3-hepten-2-one, (*E,E*)-3,5-octadien-2-one, and 1-pentadecene decreased significantly over time in the cooked horse meat samples ($P \leq 0.05$). The reported decrease in abundance of these compounds is in contrast with the published literature about vacuum aged and cooked meat, where an increase in lipid oxidation derived compounds have been described (Maggiolino et al., 2019). In agreement to our observations, Rivas-Cañedo et al. (2013) reported a decrease in the content of some oxidation compounds (aldehydes and ketones) of cooked meat over ageing time in n-3 enriched grazed lambs which was attributed to the presence of antioxidants from pasture plants (Resconi et al., 2010). Furthermore, reactions or interactions among products of proteolysis and lipid oxidation yielding non-volatile compounds could also explain the decrease in the abundance of lipid oxidation derived volatile compounds as observed in pork and lamb (Estevez et al., 2003; Rivas-Cañedo et al., 2013). In this regard,

Table 2
Mean relative abundance of volatile compounds analyzed by solid-phase microextraction coupled to gas chromatography in aged (0, 7, 14 and 21 days) and cooked horse meat samples ($n = 80$).

Volatile compound	0 d	7 d	14 d	21 d	SEM	*P*-value
Aldehydes						
Acetaldehyde	7.71	6.66	6.80	7.09	0.30	0.791
Propanal	5.60	5.25	6.49	ND	0.26	0.085
2- Methylbutanal	ND	1.88^{b}	3.28^{a}	3.49^{a}	0.17	≤0.001
3- Methylbutanal	ND	4.23^{b}	8.17^{a}	8.73^{a}	0.40	≤0.001
Pentanal	48.4	46.2	48.0	43.5	1.7	0.187
Hexanal	564^{a}	527^{a}	497^{ab}	415^{b}	21	0.005
2-Methyl-2-butenal	7.44	6.12	7.27	6.06	0.38	0.294
Heptanal	98.6	95.4	98.5	88.1	4.0	0.527
(*E*)-2-Hexenal	ND	4.70	5.70	ND	1.91	0.357
Octanal	122	139	143	133	6	0.094
(*E*)-2-Heptenal	34.6	30.2	32.3	29.9	1.5	0.165
2-Methyl-2-heptenal	6.40^{a}	6.12^{a}	6.05^{a}	3.82^{b}	0.39	0.013
Nonanal	277	274	280	280	11	0.866
(*E*)-2-Octenal	46.1	44.0	46.0	40.8	2.0	0.480
Decanal	13.8	13.0	13.2	13.3	0.5	0.765
(*E,E*)-2,4-Heptadienal	8.22	8.04	10.3	8.04	0.43	0.147
(*E*)-2-Nonenal	72.8	67.5	71.9	65.7	2.9	0.364
(*E*)-2-Decenal	177	166	177	162	8	0.179
2-Butyl-2-octenal	45.9^{a}	37.6^{a}	39.1^{a}	24.4^{b}	3.4	0.003
Dodecanal	51.7	57.6	53.5	51.4	2.8	0.357
(*E,E*)-2,4-Nonadienal	43.0	ND	43.2	ND	2.8	0.594
(*E*)-2-Undecenal	225	213	221	210	9	0.549
(*E,Z*)-2,4-Decadienal	12.0	10.0	12.0	10.4	0.5	0.203
Tridecanal	115	108	108	98.9	4.5	0.327
(*E,E*)-2,4-Decadienal	59.8	57.1	64.5	59.0	2.8	0.624
Tetradecanal	224	217	227	210	10	0.730
(E,E)-2,4-Undecadienal	15.0	13.8	14.4	13.0	0.6	0.907
Pentadecanal	230	218	226	214	10	0.715
Hexadecanal	71.2^{c}	74.5^{c}	102^{b}	140^{a}	5.5	≤0.001
cis-11-Hexdecenal	16.6	16.2	18.1	18.2	1.0	0.719
Ketones						
2-Heptanone	ND	9.59	7.88	ND	1.79	0.695
6-Methyl-2-heptanone	11.6	9.69	9.43	8.11	0.51	0.021
3-Octanone	5.78	4.14	3.49	3.27	0.46	0.130
5-Methyl-3-hepten-2-one	45.1^{a}	37.7^{ab}	33.7^{bc}	26.6^{c}	2.1	≤0.001
(*E*)-3-Octen-2-one	6.73	7.33	7.70	6.98	0.35	0.895
(*E,E*)-3,5-Octadien-2-one	12.2^{b}	13.5^{b}	16.4^{a}	11.8^{b}	0.7	0.013
6,10-Dimethyl-(*E,E*)-5,9-undecadien-2-one	12.7	11.3	ND	ND	0.54	0.166
2- Pentadecanone	ND	7.63^{b}	8.92^{ab}	9.41^{a}	0.42	0.001
Alcohols						
1-Penten-3-ol	0.708	0.631	0.730	0.629	0.026	0.239
1-Pentanol	40.5	38.3	35.3	31.7	1.6	0.052
1-Hexanol	15.2^{a}	15.2^{a}	15.6^{a}	13.0^{b}	0.6	0.035
1-Octen-3-ol	88.4	85.4	83.8	75.6	3.2	0.249
1-Heptanol	42.9	42.0	44.0	41.4	1.6	0.766
2-Ethyl-1-hexanol	2.60	2.75	2.66	3.14	0.11	0.191
1-Octanol	77.2	78.6	80.0	71.4	2.9	0.894
3,5-Octadien-2-ol	7.16	ND	6.03	6.23	0.65	0.499
(E)-2-Octen-1-ol	26.9	24.7	24.1	23.1	0.9	0.075
9-Decen-2-ol	9.75	6.32	ND	ND	0.59	0.016
1-Dodecanol	20.5^{a}	19.3^{ab}	18.7^{ab}	16.9^{b}	0.8	0.037
1-Tridecanol	11.9	9.87	10.6	10.6	0.55	0.595
1-Tetradecanol	28.1	27.7	28.7	25.2	1.5	0.345
Non-aromatic hydrocarbons						
2,2,4,6,6-Pentamethyl-heptane	ND	5.1	ND	ND	0.4	
Butyl-cyclopentane	ND	2.2	ND	ND	0.1	
Tridecane	14.6	13.3	15.6	14.3	1.0	0.829
3-Ethyl-2-methyl-1,3-hexadiene	22.2	23.0	23.7	22.9	1.1	0.697
Pentadecane	24.6	22.7	23.5	23.9	1.2	0.946
1- Pentadecene	8.84^{a}	8.60^{a}	8.05^{ab}	7.09^{b}	0.36	0.012
5,5-Dimethyl-1,3-heptadiene	9.14	7.95	7.81	5.43	0.64	0.126
Hexadecane	13.1	11.4	13.0	11.0	0.7	0.187
1-Hexadecene	9.20	7.51	9.52	8.16	0.50	0.468
1-Heptadecene	11.6	10.3	11.5	12.0	0.4	0.099
1,15-Hexadecadiene	23.2	22.0	23.6	23.6	1.0	0.937
Bencenoic compounds						
Toluene	ND	3.54	4.01	3.79	0.14	0.472
Benzaldehyde	67.6^{c}	72.1^{bc}	79.8^{ba}	83.9^{a}	2.5	≤0.001
3-Ethyl-benzaldehyde	115	108	111	102	3	0.387
4-Pentyl-benzaldehyde	25.0	23.4	23.0	21.8	1.0	0.442
Furans						
2-Ethyl-furan	ND	2.69	3.58	ND	0.18	0.022
2-Pentyl-furan	ND	52.3	49.6	48.7	2.5	0.174
Miscelaneous						
Dimethyl trisulphide	32.0	8.90	18.3	10.3	4.73	0.488
Unknown$^{m/z: 97, 55, 41, 71, 84}$	61.5^{b}	87.1^{a}	85.2^{a}	88.2^{a}	3.9	≤0.001
Unknown$^{m/z: 67, 95, 41, 81, 12}$	7.86	7.68	8.00	7.26	0.35	0.736
Unknown$^{m/z: 43, 84, 71, 57, 128}$	16.8	16.2	16.7	16.1	0.8	0.979
Unknwon$^{m/z: 121, 91, 77, 150, 65}$	69.0	67.7	75.6	69.6	3.0	0.560
Unknwon$^{m/z: 95, 81, 43, 55, 67}$	27.0^{a}	25.8^{a}	27.0^{a}	19.6^{b}	1.2	0.032
Unknwon$^{m/z: 43, 41 57, 83, 69}$	15.6	12.7	12.1	12.4	0.7	0.051
Unknown$^{m/z: 55, 43, 69, 83, 97}$	28.6	28.1	27.5	25.2	1.3	0.357
Unknown$^{m/z: 45, 55, 67, 73, 41}$	21.1	21.9	25.4	21.5	1.3	0.172
Total alcohols	372	351	350	319	12	0.218
Total aldehydes	2600	2468	2590	2357	95	0.741
Total benzenoic compounds	207	207	218	211	6	0.646
Total furans	ND	55.0	53.2	48.7	3.0	0.124
Total hydrocarbons	136	134	136	128	6	0.810
Total ketones	94.1^{a}	101^{a}	87.5^{ab}	66.2^{b}	4.1	0.004
Total sulphur compounds	32.0	8.90	18.3	10.3	4.73	0.488
Total volatiles	3442	3325	3453	3141	131	0.702

SEM, standard error of the mean; ND, not detected; ^{a,b,c} Means with different superscripts indicate statistically significant ($P \leq 0.05$) differences among ageing days.

Goodridge, Beaudry, Pestka, and Smith (2003) reported that hexanal may bind to chicken meat proteins and, in consequence, reduce its volatility.

The PLS-DA methodology was used to assess from a multivariate approach the effect of the ageing time on the volatile profile of the cooked horse meat. The results of PLS-DA confirmed that the volatile profile of the cooked horse meat was affected by ageing time. Fig. 1 shows the scores calculated for the cooked horse meat samples in the two-dimensional plot formed by the factors of the PLS-DA model with the greatest variance explained for volatile relative abundances and ageing times. Unaged samples were clearly separated from the 14 and 21 d aged samples showing, in general, negative scores on factor 1. Likewise, 21 d aged samples were clearly differentiated from the 7 d aged and unaged samples showing positive score values on factor 1. However, horse meat samples aged for 7 and 14 d were very close to each other and both had positive and negative scores on the horizontal axis (factor 2). Therefore, for the vacuum ageing period studied (21 d), a difference of at least two weeks (14 d) in the ageing time resulted in a differentiated volatile profile in the cooked horse meat samples.

4. Conclusions

For the first time, the volatile profile of up to 21 days vacuum aged and cooked horse meat was reported. Aldehydes, which primarily originate from lipid oxidation, were in general the major contributors of

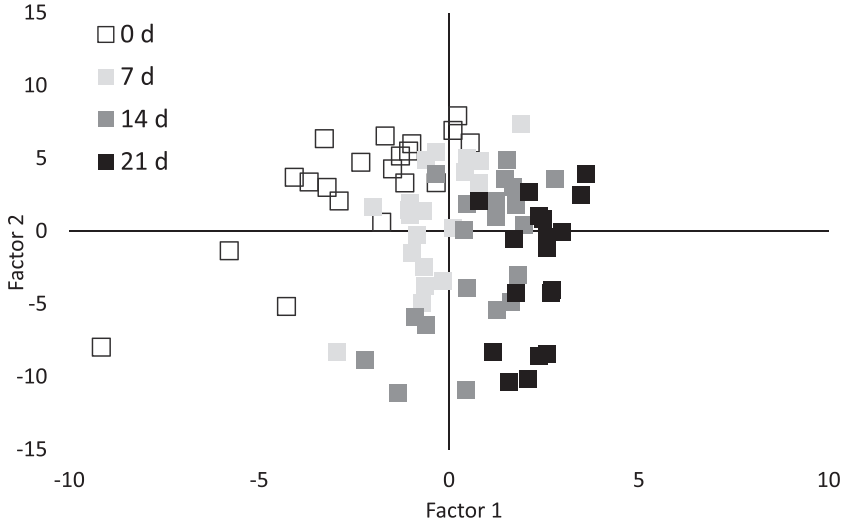

Fig. 1. Partial Least Squares-Discriminant Analysis factor scores depicting cooked horse meat sample distribution according to ageing time (0, 7, 14 and 21 d). *Y*-variable: ageing time; *X*-variable: weighed relative abundance of volatile compounds. Factor 1: explained *X*-variance 18%; explained *Y*-variance 19%. Factor 2: explained *X*-variance 36%; explained *Y*-variance 7%.

cooked horse meat odour in terms of relative abundance and odour impact. In contrast, the contribution of Maillard derived compounds was negligible probably due to cooking conditions utilized.

With ageing time from 0 to 21 days, the abundance of several aldehydes such as hexadecanal, and 2- and 3-methylbutanal increased in the studied cooked horse meat samples, presumably affecting the cooked meat odour as these have considerable odorant impact. Overall, ageing periods longer than 14 days are necessary for significant changes in the volatile profile of cooked horse meat.

The sensory implications that could derive from the odorant impact of volatile compounds should be further studied in order to elucidate their practical repercussion in cooked horse meat quality.

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