



## Aroma evolution in aged horse meat: dry vs. vacuum aging

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### ABSTRACT

This study investigates the impact of aging method (dry vs. vacuum) and duration (0, 3, and 6 weeks) on the volatile compound profile of horse loin meat. Twenty-seven Italian Heavy Draft Horse foals were reared under uniform conditions and slaughtered at 17 months. Loin samples were aged under controlled dry or vacuum conditions and analyzed for volatile organic compounds using headspace solid-phase microextraction and gas chromatography–mass spectrometry. Results revealed that, compared with vacuum aging, dry aging led to a significantly higher accumulation of aldehydes, ketones, alcohols, and sulfur compounds, especially after three weeks, reflecting increased lipid oxidation and microbial activity. Notably, hexanal and nonanal levels surged in dry-aged meat, enhancing flavor complexity but also indicating oxidative degradation. Conversely, vacuum aging limited oxidation, preserving a fresher profile with fewer oxidative markers, but saw a moderate increase in certain furans and hydrocarbons, possibly due to anaerobic microbial metabolism. Overall, three weeks of dry aging emerged as the optimal balance, enhancing desirable aroma compounds without excessive oxidation. In conclusion, these findings contribute to the understanding of flavor development in horse meat and suggest that aging strategies can be tailored to optimize sensory quality and product stability.

## 1. Introduction

Although less commonly consumed than beef or pork, horse meat remains integral to the culinary heritage of several Western European countries, including Italy, France, Belgium, and Spain (Stanisławczyk *et al.*, 2023). Its growing popularity is linked to its nutritional value—rich in essential amino acids, B vitamins, iron, and omega-3 fatty acids (De Palo *et al.*, 2016; Lorenzo *et al.*,

2017; Maggiolino *et al.*, 2019; Marino *et al.*, 2022). Beyond its nutritional properties, sensory characteristics, such as flavor and aroma, play a crucial role in consumer acceptance and are closely linked to the presence of volatile organic compounds (VOC). Aging, whether by vacuum or dry methods, is commonly used to enhance these sensory traits, with each technique influencing VOC formation differently depending on time and environmental conditions (Terjung *et al.*, 2021; Xu *et al.*, 2021). Due

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to its biochemical composition, horse meat is particularly susceptible to oxidative reactions during aging, resulting in the production of VOCs, such as aldehydes, ketones, alcohols, and furans (Forte et al., 2024). These compounds shape the aroma profile and can have both positive and negative effects on meat quality (Beldarrain et al., 2020; Xu et al., 2021). Despite this, research on VOC evolution in aged horse meat is limited, and comparative studies between vacuum and dry aging are lacking. This study aims to fill this gap by assessing the impact of aging method and duration on the volatile profile of horse loin.

## 2. Materials and methods

### 2.1 Animal management and muscle sampling

The trial involved 27 male Italian Heavy Draft Horse foals, all born and raised on the same farm until 17 months of age and fed a uniform diet (35% oat hay, 65% commercial feed: rolled corn 33%, soybean meal 21%, wheat bran 17%, rolled barley 13.5%, rolled oats 13.5%, vitamin/mineral supplements 2%). All animals were transported and slaughtered at an EU-approved abattoir, following Maggiolino et al. (2019), in 9 sessions (3 foals/session). From each carcass, both left and right loins (between 13<sup>th</sup> and 18<sup>th</sup> thoracic vertebra) were collected, totaling 54 loins. Left loins were dry aged (D), right loins vacuum aged (V), with 27 loins per method. Each loin was divided into three sections, randomly assigned to aging times of 0, 3, or 6 weeks; cranial-caudal and caudal sections were randomly distributed. Dry aging occurred in chambers at 2°C, 82% relative humidity (RH), and 0.4 m/s air velocity. Vacuum aging used Besser Vacuum® film (65 µm thick, 63 g/m<sup>2</sup>, O<sub>2</sub> transmission ≤ 65 cm<sup>3</sup>/m<sup>2</sup>/day/bar at 23°C, 0% RH; H<sub>2</sub>O vapor transmission ≤ 3.5 g/m<sup>2</sup>/day at 23°C, 85% RH), with storage also at 2 °C.

### 2.2 Volatile compounds analysis

At each aging time, a 5 g portion was taken from every loin sample to analyze VOC. Samples were cooked for 5 minutes on a thermostatically controlled electric griddle (CG660, DeLonghi, Italy) at 130–150°C. Internal temperature was monitored with a copper-constantan thermocouple (Model 5SCTT-T-30-36, Omega Engineering, USA) inserted into the sample center, and the cooked sam-

ples were removed at 70°C, following the method of Forte et al. (2024). Cooked samples were homogenized with a commercial grinder (Moulinex, Swan Holding, UK), and ~1.00 ± 0.05 g of ground meat was placed in 20 mL headspace vials (Agilent Technologies, USA), spiked with 82 ng of 2-octanol (internal standard), and sealed with Teflon-lined septa. VOC were extracted via solid-phase micro-extraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS), as per Maggiolino et al. (2021). Prior to extraction, samples were equilibrated at 35°C for 15 min using a Triplus RSH autosampler (Thermo Fisher Scientific, Italy). Extraction was done for 30 min at the same temperature with a DVB/CAR/PDMS fiber (50/30 µm; Supelco, USA). Volatiles were thermally desorbed into a Trace 1300 GC (Thermo Fisher Scientific, Rodano, Italy) in splitless mode, coupled to an ISQ Series 3.2 SP1 MS (Thermo Fisher Scientific, Rodano, Italy). Separation used a VF-WAX MS capillary column (60 m × 0.25 mm i.d., 0.25 µm; Agilent) with oven program: 35°C (5 min), 1.5°C/min to 45°C, 4°C/min to 160°C, then 20°C/min to 210°C (7 min hold). MS settings: ion source 250°C, 70 eV, 1700 V, scan range 40–300 amu. Compounds were identified via Xcalibur v2.0 (Thermo Fisher Scientific, Rodano, Italy) using NIST spectral database, and semi-quantified with the internal standard method, expressed in µg/kg.

### 2.3 Statistical analysis

The data set was tested for normal distribution (Shapiro-Wilk) and variance homogeneity (Bartlett test). Each loin represented an experimental unit. All data (excluding the sensory profile) were analyzed using two-way ANOVA with SAS (SAS, 2018), according to the following model:

$$y_{ijk} = \mu + \alpha_i + A_j + T_k + (A \times T)_{jk} + \varepsilon_{ijkl}$$

where  $y_{ijk}$  are dependent variables;  $\mu$  is the overall mean;  $\alpha_i$  is the single block random effect (1,...9);  $A$  was the effect of the  $j^{\text{th}}$  aging method adopted (dry or vacuum) ( $j = 1, 2$ ),  $T$  was the effect of the  $k^{\text{th}}$  aging time ( $k = 1, \dots, 9$ ),  $A \times T$  was the effect of the interaction of the  $j^{\text{th}}$  aging method and  $k^{\text{th}}$  aging time (1, ..., 18), and  $\varepsilon_{ijkl}$  was the error term. When not significant, the binary interaction was dropped from the model. A Tukey test was applied to evaluate the differences according to aging time when time effect was significant. All means were expressed as square means and mean standard error. The significance level was set to  $P < 0.05$ .

### 3. Results

The results of the VOC analysis are presented in Table 1. Aldehydes in D meat significantly increased at 3 w and decreased thereafter at 6 w ( $P < 0.01$ ), while in V meat, they declined at 6 w compared to 0 and 3 w ( $P < 0.01$ ). Moreover, aldehyde levels were significantly higher in D meat than in V meat at both 3 and 6 w ( $P < 0.01$ ). Among alde-

hydes, hexanal increased in both aging methods at 3 w ( $P < 0.01$ ); at 6 w, it markedly decreased in V meat ( $P < 0.01$ ) while remaining stable in D meat ( $P < 0.01$ ). Furthermore, at 6 w, hexanal concentration was significantly higher in D meat compared to V meat ( $P < 0.01$ ). Nonanal increased in D meat at 3 w and remained stable thereafter; at both 3 and 6 w ( $P < 0.01$ ), its concentration was significantly higher in D meat than in V meat ( $P < 0.01$ ). Pentanal in

**Table 1.** Effects of aging method, aging time and their binary interaction on the volatile compound families detected in horse meat.

Family	Aging	Aging week			SEM <sup>1</sup>	P-value		
		0	3	6		A <sup>2</sup>	T <sup>3</sup>	A × T
Aldehydes	D	589.62 <sup>A</sup>	1139.51 <sup>BX</sup>	720.96 <sup>AX</sup>	162.054	< 0.0001	< 0.0001	< 0.0001
	V	586.48 <sup>A</sup>	456.29 <sup>AY</sup>	237.00 <sup>BY</sup>				
Hexanal	D	587.21 <sup>A</sup>	815.64 <sup>B</sup>	459.02 <sup>AX</sup>	92.09	0.0021	< 0.0001	< 0.0001
	V	436.06 <sup>A</sup>	271.20 <sup>B</sup>	65.36 <sup>CY</sup>				
Nonanal	D	58.54 <sup>A</sup>	151.06 <sup>BX</sup>	127.40 <sup>BX</sup>	15.994	< 0.0001	0.0469	0.0101
	V	58.73	52.51 <sup>Y</sup>	44.65 <sup>Y</sup>				
Pentanal	D	30.46 <sup>A</sup>	82.65 <sup>BX</sup>	65.97 <sup>CX</sup>	10.29	< 0.0001	0.0026	0.0011
	V	33.67	20.43 <sup>Y</sup>	3.02 <sup>Y</sup>				
Carboxylic acids	D	248.34 <sup>A</sup>	466.53 <sup>B</sup>	761.37 <sup>CX</sup>	58.376	< 0.0001	< 0.0001	< 0.0001
	V	201.55 <sup>A</sup>	311.77 <sup>B</sup>	287.76 <sup>ABY</sup>				
Hexanoic acid, ethenyl ester	D	72.89	69.66	72.843 <sup>X</sup>	17.775	0.0030	< 0.0001	0.0012
	V	91.92 <sup>A</sup>	97.20 <sup>A</sup>	34.22 <sup>BY</sup>				
Ketones	D	143.71 <sup>A</sup>	411.24 <sup>B</sup>	768.18 <sup>CX</sup>	94.934	0.0007	< 0.0001	< 0.0001
	V	138.70 <sup>A</sup>	251.01 <sup>B</sup>	388.89 <sup>CY</sup>				
2-butanone,3-hydroxy-	D	59.84 <sup>A</sup>	128.87 <sup>B</sup>	206.74 <sup>C</sup>	88.033	0.2321	< 0.0001	0.1186
	V	56.63 <sup>A</sup>	81.98 <sup>B</sup>	298.09 <sup>C</sup>				
Alcohols	D	110.34 <sup>A</sup>	287.64 <sup>B</sup>	315.57 <sup>CX</sup>	95.288	0.0013	< 0.0001	0.0011
	V	86.91 <sup>A</sup>	350.26 <sup>B</sup>	230.26 <sup>CY</sup>				
1-octen-3-ol	D	38.52 <sup>A</sup>	83.30 <sup>B</sup>	89.52 <sup>B</sup>	20.845	0.3531	< 0.001	< 0.001
	V	31.31 <sup>A</sup>	178.85 <sup>B</sup>	81.19 <sup>B</sup>				
1-pentanol	D	20.18 <sup>A</sup>	32.12 <sup>A</sup>	54.79 <sup>BX</sup>	15.0175	0.0036	0.0025	0.0601
	V	10.55	18.98	8.62 <sup>Y</sup>				
Aromatic hydrocarbons	D	15.71	14.05	13.61	2.162	0.0801	0.0043	0.0922
	V	11.24 <sup>A</sup>	19.74 <sup>B</sup>	21.18 <sup>B</sup>				
Furans	D	5.06	5.89 <sup>X</sup>	4.95	1.637	0.0056	0.0028	0.0042
	V	2.04 <sup>A</sup>	18.37 <sup>BY</sup>	6.51 <sup>A</sup>				
Sulfur compounds	D	3.70 <sup>A</sup>	53.67 <sup>BX</sup>	50.15 <sup>BX</sup>	7.351	< 0.0001	0.0043	0.0024
	V	3.77	3.68 <sup>Y</sup>	6.64 <sup>Y</sup>				
Thiols	D	37.92 <sup>A</sup>	14.88 <sup>B</sup>	11.96 <sup>B</sup>	3.794	0.0982	0.0022	0.0019
	V	35.24 <sup>A</sup>	15.22 <sup>B</sup>	13.76 <sup>B</sup>				
Hydrocarbons	D	22.10	27.42 <sup>X</sup>	23.02 <sup>X</sup>	22.747	< 0.0001	0.0102	0.0032
	V	26.98 <sup>A</sup>	86.54 <sup>BY</sup>	73.89 <sup>BY</sup>				

Note: <sup>1</sup>SEM: standard error of the mean; <sup>2</sup>A: aging method; <sup>3</sup>T: aging time.

D meat increased at 3 w ( $P < 0.01$ ), decreased at 6 w ( $P < 0.01$ ), and was higher than in V meat at both time points ( $P < 0.01$ ). Carboxylic acids showed a progressive increase in D meat ( $P < 0.01$ ), whereas in V meat, their levels increased at 3 w and then stabilized ( $P < 0.01$ ). Hexanoic acid and ethenyl ester increased in V meat at 6 w compared to earlier time points ( $P < 0.01$ ), although its concentration was lower than in D meat ( $P < 0.01$ ). Both ketones and alcohols exhibited a progressive increase throughout the aging period in both aging methods ( $P < 0.01$ ). At 6 w, the concentrations of carboxylic acids, ketones, and alcohols were significantly higher in D meat compared to V meat ( $P < 0.01$ ). The 2-butanone,3-hydroxy increased progressively over time in both aging methods ( $P < 0.01$ ). The 1-octen-3-ol increased in both methods at 3 w and remained stable thereafter ( $P < 0.01$ ). In contrast, 1-pentanol increased in D meat at 6 w ( $P < 0.01$ ), reaching higher concentrations compared to V meat ( $P < 0.01$ ). Aromatic hydrocarbons and hydrocarbons increased in V meat at 3 w and subsequently remained stable ( $P < 0.01$ ). Moreover, hydrocarbon levels at both 3 and 6 w were significantly higher in V meat compared to D meat ( $P < 0.01$ ). Furans increased in V meat at 3 w ( $P < 0.01$ ), reaching significantly higher concentrations than in D meat ( $P < 0.01$ ), and then decreased again at 6 w, returning to baseline levels ( $P < 0.01$ ). At both 3 and 6 w, D meat showed significantly higher concentrations of furans compared to V meat ( $P < 0.01$ ). Thiols decreased in both aging methods at 3 w and remained constant thereafter ( $P < 0.01$ ).

#### 4. Discussion

The aging process, both in terms of method and duration, significantly influenced the profile of volatile compounds in horse meat. Across the board, dry-aged samples (D) exhibited higher concentrations of several volatile families compared to vacuum-aged (V) counterparts, particularly after extended storage. This aligns with previous literature data indicating that dry aging, due to its oxygen-rich environment, promotes oxidative reactions and microbial activity that contribute to a more complex volatile profile (Kim et al., 2016). Among the aldehydes, hexanal, nonanal, and pentanal were notably more abundant in dry-aged meat over time, especially at week 3, reflecting an increase in lipid oxidation, particularly of polyunsaturated fatty acids. Hexanal is widely recognized as a primary oxida-

tion product of linoleic acid and a marker of oxidative degradation in meat (Tateo et al., 2020). In contrast, vacuum-aged samples showed lower levels of aldehydes, consistent with the limited oxygen availability that suppresses lipid oxidation (Lee et al., 2021). A significant rise in carboxylic acids, especially in dry-aged meat by week 6, suggests intensified lipolysis and microbial fermentation under aerobic conditions, as also observed by Ahnström et al. (2006). Interestingly, some acid levels in vacuum-aged meat increased at week 3 but plateaued or declined thereafter, which may indicate a temporary microbial or enzymatic activity peak followed by stabilization. Ketone levels, such as 2-butanone-3-hydroxy, followed a similar trend, with dry aging showing a steady increase throughout the aging period, indicative of ongoing oxidation and potential microbial metabolism (Bliznyuk et al., 2024). These compounds contribute sweet, buttery, and creamy notes and are considered important in dry-aged flavor development (Maggiolino et al., 2019). Alcohols, notably 1-octen-3-ol and 1-pentanol, were more prevalent in dry-aged meat and increased with aging, suggesting intensified oxidative degradation of lipids and amino acids (Fu et al., 2022). 1-octen-3-ol, derived from the oxidative cleavage of linoleic acid, contributes to mushroom-like aromas and is often found in oxidized meat (Mottram, 1998). In vacuum-aged meat, a spike in 1-octen-3-ol at week 3 may suggest a transient oxidative episode or microbial contribution (Song et al., 2021; Sun et al., 2024). Aromatic hydrocarbons and furans increased significantly in vacuum-aged meat, particularly at week 3, possibly linked to Maillard reaction intermediates or microbial action under anaerobic conditions (Tateo et al., 2020). Notably, furans are often associated with heat treatment, but low levels may also arise from sugar or amino acid degradation (Liu et al., 2022). In terms of sulfur compounds and thiols, dry-aged meat showed markedly higher levels over time compared with vacuum-aged meat, likely due to the breakdown of sulfur-containing amino acids (e.g., methionine, cysteine) and microbial activity, which are known to generate potent aroma-active molecules (Meinert et al., 2009). These compounds, although present in small quantities, greatly influence the sensory characteristics due to their low odor thresholds (Vilar et al., 2022). Lastly, hydrocarbons increased significantly in vacuum-aged meat, especially at weeks 3 and 6. Although less aromatic, they may act as markers of lipid degradation or microbial fermentation (Fu

*et al.*, 2022). Overall, the volatile profile suggests that dry aging leads to a more pronounced and rapid development of lipid oxidation products and Maillard-derived volatiles, whereas vacuum aging suppresses oxidation but may enhance fermentation and microbial-derived compounds under anaerobic conditions. These findings corroborate previous studies on beef and lamb, and now extend them to horse meat, highlighting the impact of aging strategies on flavor development and potential consumer preference (Kim *et al.*, 2016).

## 5. Conclusion

This study confirms that both aging method and duration significantly influenced the development of volatile compounds in horse meat, affecting

its oxidative stability and flavor profile. Dry aging, particularly for three weeks, led to a higher accumulation of lipid oxidation products such as aldehydes, ketones, and sulfur compounds, resulting in a more complex and intense aroma. In contrast, vacuum aging reduced oxidative reactions and maintained a fresher but less complex volatile profile. A three-week dry aging period proved to be the most effective for enhancing desirable aroma compounds without causing excessive degradation. Vacuum aging, instead, better preserved freshness and could suit products requiring longer shelf-life and milder flavor. The findings highlight how tailoring aging strategies could improve the sensory qualities and marketability of horse meat. Future studies should correlate these chemical patterns with sensory analysis to better understand consumer preferences.

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