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**Abstract—** With the purpose to study the effect of finishing diets on lipid-derived aroma compounds, 13 aldehydes and 4 ketones from grilled lamb were assessed by DHS-SPE (dynamic headspace - solid phase extraction), derivatized with PFBHA (O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride) directly on the trap and analysed by GC-MS. The compounds were also related to sensory data with a Principal Component Analysis (PCA). The four treatments applied differed in the combination of pasture and concentrates offered to animals. The diet based only in pasture had markedly lower quantity in most of the unsaturated aldehydes and ketones studied. Lamb flavour was associated with 1-octen-3-one and 2-heptanone, these compounds were higher in the diet mainly based on concentrates.

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**Index Terms—**aldehydes, finishing diet, ketones, meat volatiles.

## I. INTRODUCTION

LIPID oxidation is one of the primary mechanisms of quality deterioration in meat products, which can cause adverse changes in flavour, mainly due to the carbonyl compounds produced [1]. Post-mortem vacuum ageing increase the release of the fatty acids from triglycerides and phospholipids [2] and reduce the antioxidant protection [3]; which could affect lipid oxidation after cooking. Nevertheless, during cooking, since reactions occur quickly and

the volatile profile is changed, lipid oxidation rather may contribute to desirable meat flavour [4].

Because of their intramuscular lipid composition, pasture-fed produced meat is more susceptible to lipid oxidation than concentrate-fed ones [5] and the higher presence of certain aroma oxidation products could be also affected by their distinctive fatty acid profile [6]. Other differences in the antioxidant/oxidative balance have been found between finishing diets [7]. These changes could explain the differences in sensory quality found for heavy lambs after 20 days of aging finished on pasture, concentrate and combinations of both feeding sources [8]. The aim of this study is to assess aromatic carbonyl compounds released to the headspace from grilled cooked lamb produced on different diet systems; and their relationship with the sensory flavour attributes previously reported [8].

## II. MATERIALS AND METHODS

### A. Animals and treatments

Castrated male Uruguayan Corriedale lambs were finished into 4 different treatments, with different proportions of pasture (P) (mainly *Lotus corniculatus* cv. INIA Draco) and concentrate-maize based (C), where: T<sub>1</sub>, P (6% of live weight, LW); T<sub>2</sub>, P (6% LW) plus C (0.6% LW); T<sub>3</sub>, P (6% LW) plus C (1.2 % LW); and T<sub>4</sub>, C and Lucerne hay (*ad libitum*). Animals were slaughtered at around 40 kg LW and 10-12 months old. After 36 h, a 5-cm portion of *L. lumbarum* (with subcutaneous fat) was vacuum packaged, aged at 2-4 °C for 20 days and frozen at -20 °C.

### B. Sensory analysis

The sensory tests were carried out in a controlled sensory analysis laboratory. Prior to sensory analysis, meat was thawed at 4°C over 24 h. The whole loin was cooked on a pre-heated double hot-plate grill at 200°C until the internal temperature reached 70°C. Then, it was cut into 8

portions (free of connective tissue), which were wrapped in aluminium foil and identified with a single random 3-digit code. A nine-member trained taste panel was used to evaluate the samples using a quantitative descriptive analysis in a complete balanced block design. The descriptors (Lamb, Rancid, Metallic, Acid and Fat Flavour) used were quantified using an unstructured line scale of 100 points (0=no flavour to 100=very intense flavour).

### C. Carbonyl quantification

The loin and subcutaneous fat was thaw for 24h at 4°C and grilled until 70 °C of internal temperature. Muscle volatiles were collected into a SPE cartridge packed with 200 mg Lichrolut EN® resins (Merck, Darmstadt), conditioned with dichloromethane and methanol. The trap was placed on the top of a bubbler flask containing 10 g of minced lamb and 40 mL of Milli-Q water. The mixture was stirred with a magnetic stir bar (450 rpm) while 100 mL/min of N<sub>2</sub> pass through during 3 h, into a water bath at 37 °C.

Derivatization was carried out in the SPE cartridge. The reagents used were purified by passing them through a cleaning cartridge. The interferences were eluted with 10 mL of 1% of NaHCO<sub>3</sub> in water and 4 mL of H<sub>2</sub>O. The oximes were formed adding 4 mL of PFBHA (5 mg / mL, Fluka, Madrid, Spain) and letting the trap imbibed for 15 min at room temperature. Excess of reagent was removed percolating 10 mL of H<sub>2</sub>SO<sub>4</sub> (0.05 M in H<sub>2</sub>O) and water. The derivatives were eluted with 2 mL of CH<sub>2</sub>Cl<sub>2</sub>, using 54 mg/L of 2,3,4,5,6-pentafluorobenzophenone (Aldrich, Madrid, Spain) as an internal standard.

Analyses were performed using a CP-3800 Saturn 2200 ion trap GC-MS (Varian, Sunnyvale, USA). The column was a DB-WAXetr (J&W, Folsom, USA, 60 m × 0.25 mm × 0.5µm) and He was used as carrier gas (1 ml / min). The injection (40 µL) was in a solvent split mode at constant speed (5 µL/s) in a programmed temperature vaporization injector. The injector was initially in a split mode (split ratio = 100) at 40°C until 1.37 min. After 1.3 min, the split valve was closed and reopened at the 9<sup>th</sup> min with the same split ratio; and the injector was heated to 300 °C (at 200°C / min.) and held 70.3 min. The oven was held at 40 °C for 10 min, then heated to 220 °C at 10 °C/min, temperature held for 45 min. MS transfer line and chamber ionization were at 200 °C, and the trap

emission current, 70 µA. The global run time was recorded in full scan mode. The ionic peak areas were first normalized to those of the internal standard (*m/z* 272).

### D. Statistical analysis

The mean of the relative areas to the internal standard per animal (from duplicated samples) was obtained. A GLM (SPSS, 14.0) was used, considering treatment as a fixed effect. Means between treatments were compared by the Duncan test. The highest mean per carbonyl compound and treatment (from 5 or 6 animals per treatment) was considered 100 and all data were normalized to this value. A Principal Component Analysis (The results are shown graphically in a biplot.

## III. RESULTS AND DISCUSSION

In order to obtain those lipid-derived carbonyl compounds that could affect the retronasal aroma perception of the cooked meat, an extraction of the volatiles released to headspace was performed in a DHS-SPE system [9]. Directly on the trap with the volatiles retained, a specific isolation of the target compounds were conducted through their derivatization and subsequent quantification by MS to improve the selectivity and sensitivity of these substances [10].

Animal diet system influenced the majority of the unsaturated aldehydes and ketones evaluated, but not the saturated aldehydes. The latest are among the major compounds in the volatile fraction of cooked lamb [11], and they could be related to rancid notes when their concentration is high [1]. However, the higher intensity of rancid flavour of the T1 was not related to saturated aldehydes ([8], Figure 1). Rancid odours have been associated to 3-methylindole rather than hexanal and its analogues [12]. Figure 1 shows that saturated aldehydes were correlated between them, and with acid and metallic flavours.

An increasing level of unsaturated aldehydes with the increase of poli-unsaturated fatty acids (PUFA) in muscle has been found by Elmore *et al.*[11]. In the present study, however, T1 had the highest proportion of PUFA in intramuscular fat [13], while having considerably lower quantity of lipid derived volatiles than the rest of treatments (Table 1). These correspond with the PCA, since T1 is placed separated of the other treatments (Figure 1). These findings could be explained by the high

muscle vitamin E content [13] and probably other antioxidants present in fresh pastures [7].

The diets which include concentrates (T2, T3 and T4) were not statistically different in most of the aldehydes assessed, with the exception of (*E,E*)-2,4-heptadienal. This alkadienal was lower for T4, according to the lower level of its precursor:  $\alpha$ -linolenic acid [13]. Although the treatment based only on pastures (T1) had the highest levels of this fatty acid, lipid oxidation in this group had globally a lower contribution, as mentioned before.

The ketones 1-octen-3-one and 2-heptanone increased with the increase of concentrates in the diet (Table 1) and they were positive related to lamb flavour (Figure 1). Similar tendencies are shown for 2-octanone and (*E,E*)-2,4-decadienal. Lorenz *et al.* [6] also found higher levels of 1-octen-3-one and (*E,E*)-2,4-decadienal in beef for stable animals compared with those kept on pastures, but both compounds were considered key beef aromas in the two groups.

None of the carbonyls quantified were associated to fat flavour (Figure 1), although some of them have been characterized with fatty odours in olfactometries analysis of meat [14]. Other interesting carbonyl compounds have not been detected in the present study, such as 2,3-butanodione. Diketones are low reactive to the derivatization reagent and/or are present at levels over the method detection limits.

It is important to highlight that in this study, the high levels of lipid volatile compounds could be related to desirable flavours developments, since globally European consumers preferred the flavour of those treatments with higher carbonyls [15].

#### IV. CONCLUSION

Grilled cooked lamb from animals finished only on pastures had markedly lower concentration of lipid carbonyl compounds than meats produced with concentrates. The inclusion of concentrates in diets promotes an overall higher contribution of lipid carbonyl compounds in the cooked meat after 20 days of ageing, although this was not associated to undesirable flavours.

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#### REFERENCES

- [1] Gray, J. I.; Gomaa, E. A.; Buckley, D. J. Oxidative quality and shelf life of meats. *Meat Science* **1996**, 43, (Supplement 1), 111-123.
- [2] Hood, R. L.; Allen, E. Influence of sex and postmortem aging on intramuscular and subcutaneous bovine lipids. *Journal of Food Science* **1971**, 36, (5), 786-790.
- [3] Descalzo, A. M.; Rossetti, L.; Sancho, A. M.; García, P. T.; Biolatto, A.; Carduza, F.; Grigioni, G. M. Antioxidant consumption and development of oxidation during ageing of buffalo meat produced in Argentina. *Meat Science* **2008**, 79, (3), 582-588.
- [4] Mottram, D. S. Flavour formation in meat and meat products: a review. *Food Chemistry* **1998**, 62, (4), 415-424.
- [5] Yang, A.; Lanari, M. C.; Brewster, M.; Tume, R. K. Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. *Meat Science* **2002**, 60, (1), 41-50.
- [6] Lorenz, S.; Buettner, A.; Ender, K.; Nürnberg, G.; Papstein, H.-J.; Schieberle, P.; Nürnberg, K. Influence of keeping system on the fatty acid composition in the longissimus muscle of bulls and odorants formed after pressure-cooking. *European Food Research and Technology* **2002**, 214, (2), 112-118.
- [7] Descalzo, A. M.; Insani, E. M.; Biolatto, A.; Sancho, A. M.; García, P. T.; Pensel, N. A.; Josifovich, J. A. Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science* **2005**, 70, (1), 35-44.
- [8] Resconi, V. C.; Campo, M. M.; Furnols, M. F. i.; Montossi, F.; Sañudo, C. Sensory evaluation of castrated lambs finished on different proportions of pasture and concentrate feeding systems. *Meat Science* In Press, Accepted Manuscript.
- [9] Campo, E.; Ferreira, V.; Escudero, A.; Cacho, J. Prediction of the Wine Sensory Properties Related to Grape Variety from Dynamic-Headspace Gas Chromatography - Olfactometry Data. *Journal of Agricultural and Food Chemistry* **2005**, 53, (14), 5682-5690.
- [10] Ferreira, V.; Culleré, L.; Loscos, N.; Cacho, J. Critical aspects of the determination of pentafluorobenzyl derivatives of aldehydes by gas chromatography with electron-capture or mass spectrometric detection: Validation of an optimized strategy for the determination of oxygen-related odor-active aldehydes in wine. *Journal of Chromatography A* **2006**, 1122, (1-2), 255-265.
- [11] Elmore, J. S.; Mottram, D. S.; Enser, M.; Wood, J. D. The effects of diet and breed on the volatile compounds of cooked lamb. *Meat Science* **2000**, 55, (2), 149-159.

[12] Young, O. A.; Berdagué, J. L.; Viallon, C.; Rousset-Akrim, S.; Theriez, M. Fat-borne volatiles and sheepmeat odour. *Meat Science* **1997**, 45, (2), 183-200.

[13] Cañeque, V.; Díaz, M. T.; Álvarez, I.; Sañudo, C.; Oliver, M. A.; Montossi, F.; de la Fuente, J. Fatty acid composition and vitamin E content of lamb fed with different levels of concentrate on a pasture feeding system, *54th International Congress of Meat Science & Technology* **2008**.

[14] d'Acamora Zellner, B.; Dugo, P.; Dugo, G.; Mondello, L. Gas chromatography-olfactometry in food flavour analysis. *Journal of Chromatography A* **2008**, 1186, (1-2), 123-143.

[15] Font i Furnols, M.; Realini, C. E.; Guerrero, L.; Oliver, M. A.; Sañudo, C.; Campo, M. M.; Nute, G. R.; Cañeque, V.; Álvarez, I.; San Julián, R.; Luzardo, S.; Brito, G.; Montossi, F. Acceptability of lamb fed on pasture, concentrate or combinations of both systems by European consumers. *Meat Science* **2009**, 81, (1), 196-202.

**Table 1** Lipid derived carbonyl compounds in grilled cooked lamb from different feeding systems

Carbonyl compound	T1	T2	T3	T4	SD
pentanal	100	64	86	48	42.0
hexanal	100	76	84	70	49.6
heptanal	32	69	100	60	47.1
octanal	100	92	100	72	46.8
nonanal	58	99	100	59	38.3
decanal	100	90	69	57	38.4
(E)-2-hexenal	40	90	100	78	42.3
(E)-2-heptenal	29b	89a	100a	93a	47.4
(E)-2-octenal	20b	85a	100a	92a	51.1
(E)-2-nonenal	11b	70a	100a	61ab	52.2
(E,E)-2,4-heptadienal	4b	100a	95a	23b	66.8
(E,E)-2,4-nonadienal	24	58	100	65	62.6
(E,E)-2,4-decadienal	7b	74ab	86a	100a	63.0
2-heptanone	14c	51bc	76ab	100a	44.3
2-octanone	17b	99a	100a	86a	48.3
2-nonanone	30	91	100	65	51.5
1-octen-3-one	29b	31b	91ab	100a	56.9

For each compound, the treatment where it was most abundant has been assigned a value of 100. The rest of treatments are expressed in relation to this value. Means with different letters within a row, are statistical different ( $p < 0.05$ ).

SD: standard deviation.

**Figure 1** Principal Component Analysis of flavour sensory attributes and lipid carbonyl compounds from cooked lamb from different feeding systems.

