# INCREASE OF ODOROUS COMPOUNDS IN RAW BEEF DURING RETAIL DISPLAY ACCORDING DEGREE OF LIPID OXIDATION

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**Abstract – This study aims to visualize the changes in the aroma of beef over time when stored in retail display under high oxygen conditions. A solid phase microextraction (SPME) coupled with gas chromatography and mass spectrometry (GC-MS) analysis was conducted for monitoring targeted volatile compounds that increased over time in raw knuckle samples. Results were analysed according to different levels of thiobarbituric acid reacting substances (TBARS) values after 9 days of display. The resulting groups were identified as low oxidation group and high oxidation group when reaching less than 1 or more than 2 mg MDA/kg muscle, respectively. Differences in rancid odour and the amount of seven compounds were found (P≤0.05). In both groups, hexanoic acid and 1-hexanol were the most abundant and sensitive to storage time, showing an exponential increase. Therefore, they are proposed as shelf-life markers.**

**Key Words – meat aroma, modified atmosphere packaging, SPME-GCMS.**

## I. INTRODUCTION

The volatile compounds responsible of beef aroma have been extensively studied using cooked samples, but less is published about raw meat [1,2]. However, when opening the package at home, the odour must be agreeable for the consumer, and short or extended periods of acceptability would happen depending on numerous factors, such as the antioxidant status of the meat. Furthermore, some compounds already present or developed in the raw meat remain after cooking, and could affect the flavour perception [1,3,4].

The aim of this study was to evaluate the effect of display time in rancid odour development and their potential volatile compounds involved in knuckle beef steaks vacuum-aged during 15 days, according to the extent of lipid oxidation after 9 days of display in modified atmosphere packaging (MAP).

## II. MATERIALS AND METHODS

### *Samples and display*

The knuckle (including the muscles *vastus lateralis* and *rectus femoris* of the *cuadriceps femoris*) from 48 crossbred, 12 months bulls, raised with concentrates and straw, were aged for 15 d in vacuum conditions. Then, two-cm thick steaks were placed individually in polyethylene and polyamide laminate trays flushed with $O_2$ (80 %) and $CO_2$ (20%). Samples were displayed with cool white fluorescent illumination, 1200 lux, 16 h daily on, at 4 ± 2 ºC for 0, 5, 7 or 9 d.

### *Lipid oxidation and TBARS groups*

Thiobarbituric Acid Reacting Substances (TBARS) values were expressed as mg malonaldehyde (MDA) per kg of meat [5]. Based on the results obtained at day 9 of display, samples were grouped according to the extent of lipid oxidation in low oxidative, when less than 1 mg MDA/kg was reached on day 9 of display, and high oxidative, when TBARS exceeded 2. Samples from animals reaching values from 1-2 mg MDA/kg were excluded.

*Odour assessment*
Four g of minced meat were placed in a snap clap glass vial (20 mL) and six panellists rated the intensity of rancid odour, from 0 (no detected) to 10 (very intense). The evaluation was performed at each day of display in a room at 20 ºC. Panellists sniffed the same sample on intervals of at least 15 min to allow the aroma compounds reach the balance in the headspace.

*SPME-GCMS analysis*
Four g (± 0.001) of minced meat was collected in the bottom of 20 mL SPME vials, exposed to $N_2$ flow for 20 sec and sealed with HS crimp PTFE/S septa (Agilent Technologies, USA). Samples were kept at 4 ± 2 ºC until analysis. The vial was then equilibrated at 37 ºC for 10 min in the automated sample preparation unit. After that, the polydimethylsiloxane/divinilbenzene (PDMS/DVB) 65 µm film thickness fibre (Supelco-Spain) was exposed to the headspace for 40 min at 37 ºC. After desorption, the fibre was cleaned for 10 min at 250 ºC in the back out unit.

The instrument was a CP-3800 chromatograph coupled to a Saturn 2200 ion trap mass spectrometric detection system (Varian, Sunnyvale, CA, USA). The fibre was desorbed directly in the injection port of the GC-MS in splitless mode for 5 min at 250 ºC and at 30 psi of pulse pressure (column flow during this period was 2 mL/min). Helium was the carrier gas at a flow rate of 1 mL/min. A DB-WAXETR capillary column (J&W Scientific, Folsom, CA, USA) of 60 m × 0.25 mm I.D., a film thickness of 0.25 µm, and preceded by a 3 m × 0.25 mm uncoated (deactivate, intermediate polarity) precolumn from Supelco-Spain was used. The oven temperature was initially at 40 ºC during 10 min, then raised by 4 ºC/min to 140 ºC, followed by a rate of 10 ºC/min to reach 220 ºC and held for 5 min. The MS parameters were a transfer line temperature of 230 ºC and a trap temperature of 170 ºC with emission current of 80 µA. The global run time was recorded in full scan mode (33–300 m/z mass range). The chromatographic data were analysed by Varian Saturn GC–MS Workstation Version 5.52 software.

Every 16 meat samples, an external dipropylene glycol control solution was analysed. This control solution contained a compound of each family and their response factor was calculated. This response factor was subsequently applied to all compounds of the same family: 1-hexanol, 2-pentylfuran, 3-octanone (saturated ketones), 1-octen-3-one (unsaturated ketones), nonanal (saturated aldehydes), (E)-2-nonenal (alkenals) and butanoic acid (acids). Results, in µg of compound from 4 g of raw meat, were divided by 4 and expressed as µg/g.

Compounds were identified by their mass spectra, linear retention indices (LRI) and confirmed by the injection of the pure reference standards when available. Peak integration was conducted using selective mass. Out of 26 quantified, only the seven compounds which more clearly increased with display are shown in the present study. The compounds, LRI and quantitative ions are: 2-heptanone (1192, 99), 2-pentylfuran (1235, 138), 1-hexanol (1354, 69), (E)-2-undecenal (1381, 83), pentanoic acid (1744, 60), hexanoic acid (1851, 60) and heptanoic acid (1975, 60).

*Data analysis*
Multivariant GLM (SPSS, 22.0) was applied with display and TBARS group as fixed effects. Since interactions were significant ($P \leq 0.05$) data was segmented by TBARS group and reanalysed (multivariant GLM with display as fixed effect). A Tukey test was performed to show differences between display days.

## III. RESULTS AND DISCUSSION

A TBARS value of around 2 has been proposed as the limiting threshold for the acceptability of flavour in cooked beef [6]. Therefore, samples from the present study were grouped according to the extent of lipid oxidation in *low oxidative* and *high oxidative*, as explained in the material and methods section.

Seven volatile compounds of raw meat in high oxygen MAP which increase during display and could be related to odour changes are presented. Among them, hexanoic acid and 1-hexanol showed an exponential increase in the low oxidative group (Table 1) with $R^2$ higher than 0.60, but the increase of the rest of compounds was poorer patterned ($R^2 < 0.50$). Rancid odour

increased in the 5th day, but then remained unchanged until day 9.

Table 1. Mean and standard deviation for rancid odour and volatile compounds (µg/g) of raw knuckle steaks in modified atmosphere packaging throughout display from the *low oxidative* group[1] (*n*=16)

| | *0 d* | *5 d* | *7 d* | *9 d* |
|---|---|---|---|---|
| *Rancid odour** | 1.9$^{a}$ | 3.2$^{b}$ | 3.4$^{b}$ | 3.6$^{b}$ |
| (linear, $R^2$ 0.43) | ±0.9 | ±0.7 | ±0.7 | ±0.7 |
| *Pentanoic acid* | 1.3$^{c}$ | 2.3$^{bc}$ | 3.4$^{ab}$ | 4.5$^{a}$ |
| (exp, $R^2$ 0.46) | ±1.0 | ±1.4 | ±1.5 | ±1.7 |
| *Hexanoic acid* | 14.0$^{c}$ | 45.3$^{b}$ | 71.4$^{ab}$ | 89.6$^{a}$ |
| (exp, $R^2$ 0.62) | ±10.3 | ±24.3 | ±40.1 | ±35.3 |
| *Heptanoic acid* | 1.3$^{b}$ | 2.0$^{b}$ | 2.1$^{b}$ | 3.3$^{a}$ |
| (linear, $R^2$ 0.24) | ±0.5 | ±1.2 | ±1.0 | ±1.5 |
| *(E)-2-Undecenal* | 0.1$^{b}$ | 1.5$^{a}$ | 1.1$^{a}$ | 1.2$^{a}$ |
| (linear, $R^2$ 0.22) | ±0.2 | ±1.2 | ±0.7 | ±0.5 |
| *2-Heptanone* | 0.03$^{b}$ | 0.3$^{a}$ | 0.2$^{a}$ | 0.4$^{a}$ |
| (linear, $R^2$ 0.31) | ±0.01 | ±0.2 | ±0.2 | ±0.3 |
| *1-Hexanol* | 2.6$^{b}$ | 24.4$^{b}$ | 99.5$^{a}$ | 149.2$^{a}$ |
| (exp, $R^2$ 0.73) | ±3.9 | ±23.0 | ±102.3 | ±113.9 |
| *2-Pentylfuran* | 0.1$^{b}$ | 1.7$^{ab}$ | 1.9$^{ab}$ | 3.0$^{a}$ |
| (linear, $R^2$ 0.17) | ±0.1 | ±1.6 | ±2.7 | ±3.4 |

[1] < 1 mg MDA/kg on day 9 of display. * 0 (no detected) – 10 (very intense). Means with different superscripts within a row are statistically different, according the Tukey test (P≤0.05). In parenthesis is shown the mode of increase of each volatile compound through display (exp: exponential) and the $R^2$ value.

In the high oxidation group, the intensities of rancid odour were higher compared to the other group (P≤0.01) and increased linearly through the time of display (Table 2). From the 5th day, the mean value was about 5 out of 10 and therefore, might be affecting acceptability. Furthermore, the increase was better patterned (higher $R^2$) in the volatile compounds, and both, the concentration (P≤0.05) and their rates of increase were higher than in the low oxidation group (Table 2). Again, in the high oxidation group, hexanoic acid and 1-hexanol were the most sensitive to storage time.

Hexanoic acid, together with butanoic and nonanoic acids, usually raised in spoiled raw meat stored, imparting fatty, gammy, cheesy and dairy odours [7]. However, using non-inoculated meat results are more controversial. In a study related to the effect of storage in high oxygen packs, acids were not reported [1]. Whereas in another study, butanoic and nonanoic acids decreased in raw pork meat refrigerated in oxygen permeable films during 10 days and hexanoic acid was only found in the cooked and refrigerated meat but not in the raw meat [8]. Differences between studies could be related with different methodology approaches, but also due to the complexity in the development of these compounds. The acids in the meat can derive from several pathways, including enzymatic and chemical reactions and bacterial action, being the source lipids, aminoacids or carbohydrates [9]. At the same time, acids could be further degraded to other aroma compounds [7].

Table 2. Mean and standard deviation for rancid odour and volatile compounds (µg/g) of raw knuckle steaks in modified atmosphere packaging throughout display from the *high oxidative* group[1] (*n*=17)

| | *0 d* | *5 d* | *7 d* | *9 d* |
|---|---|---|---|---|
| *Rancid odour** | 2.4$^{a}$ | 4.2$^{b}$ | 5.3$^{c}$ | 6.3$^{d}$ |
| (linear, $R^2$ 0.77) | ±0.7 | ±0.9 | ±0.7 | ±1.0 |
| *Pentanoic acid* | 1.6$^{c}$ | 4.8$^{bc}$ | 6.7$^{b}$ | 12.2$^{a}$ |
| (exp, $R^2$ 0.68) | ±1.0 | ±5.0 | ±2.1 | ±4.8 |
| *Hexanoic acid* | 19.7$^{b}$ | 125.3$^{a}$ | 141.7$^{a}$ | 177.0$^{a}$ |
| (exp, $R^2$ 0.79) | ±17.2 | ±121.1 | ±35.5 | ±35.9 |
| *Heptanoic acid* | 1.1$^{c}$ | 3.3$^{b}$ | 3.3$^{b}$ | 5.8$^{a}$ |
| (exp, $R^2$ 0.68) | ±0.8 | ±2.3 | ±1.2 | ±2.0 |
| *(E)-2-Undecenal* | 0.8$^{b}$ | 4.7$^{a}$ | 3.8$^{a}$ | 4.3$^{a}$ |
| (linear, $R^2$ 0.54) | ±1.0 | ±5.5 | ±1.4 | ±1.3 |
| *2-Heptanone* | 0.1$^{b}$ | 0.5$^{a}$ | 0.5$^{a}$ | 0.7$^{a}$ |
| (exp, $R^2$ 0.55) | ±0.1 | ±0.5 | ±0.2 | ±0.6 |
| *1-Hexanol* | 63.7$^{c}$ | 438.7$^{bc}$ | 841.1$^{b}$ | 1328.3$^{a}$ |
| (exp, $R^2$ 0.69) | ±107.1 | ±698.5 | ±365.3 | ±551.6 |
| *2-Pentylfuran* | 0.7$^{c}$ | 9.2$^{b}$ | 11.6$^{ab}$ | 18.6$^{a}$ |
| (linear, $R^2$ 0.43) | ±1.2 | ±11.9 | ±5.0 | ±12.9 |

[1] > 2 mg MDA/kg on day 9 of display. * 0 (no detected) – 10 (very intense). Means with different superscripts within a row are statistically different, according the Tukey test (P≤0.05). In parenthesis is shown the mode of increase of each volatile compound through display (exp: exponential) and the $R^2$ value.

Alcohols and alkylfurans are mainly formed when aminoacids and ribose interact with lipid oxidation [10] and therefore, the rise of 1-hexanol and 2-

pentylfuran caused by display time seems reasonable (Tables 1-2). In other studies, 1-hexanol tend to increase with storage time, but the effect is not clear enough [8], probably due to a high variability, as found in the present study as well. The alcohol can also be increased in stored meat due to the growth of natural spoilage bacteria [2], giving pungent, ethereal, fuel oil, fruity, alcoholic and sweet with a green top notes [7]. Although 2-pentylfuran is higher when meat is cooked at high temperatures, it has been also reported in raw meat, but not affected by storage time [8].

The increment of ketones and aldehydes with storage is not surprising, especially in the high oxidation group since they are major lipid oxidation products. 2-Heptanone has been already proposed as a candidate for the aroma differences between cooked beef less or more oxidized [5] and had also shown an increment with storage time in raw meat [1,8], although the increase of 3-hydroxy-2-butanone was more pronounced in pork meat [8]. In the aforementioned study, only (E)-2-decenal out of nine aldehydes compounds detected (including (E)-2-undecenal) in the raw meat increased statistically with refrigeration, although a high variability was generally found. In the study of Insausti *et al*. [1] only saturated aldehydes showed effect of storage in beef stored with high oxygen packs, but alkenals were not reported.

## IV. CONCLUSION

In fresh raw beef packed with high oxygen atmosphere hexanoic acid and 1-hexanol are proposed as potential indicators of the shelf life odour in low and high oxidative samples.

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