

*Virginia C Resconi* (1) *virginiareconi@hotmail.com*, *A Escudero* (2), *JA Beltrán* 1, *C Sañudo* 1 *V Ferreira* 2  
*MM Campo* 1

(1) *Department of Animal Production and Food Science, University of Zaragoza, Spain*

(2) *Department of Analytical Chemistry, University of Zaragoza, Spain*

**Abstract— High oxygen modified atmosphere packaging (MAP) is used to extend the colour stability of meat. However, it can promote rancidity. An experimental design with beef stored in modified atmosphere with increasing levels of oxygen (50, 65 or 80 %), at different storage conditions (4 and 8 days) was performed. A dynamic headspace - solid phase extraction (DHS-SPE) coupled with GC-O analysis was conducted to identify the aroma compounds responsible of aroma perception, comparing meat under vacuum conditions with meat packaged in high oxygen atmosphere during 8 days. Identified volatiles were mainly carbonyl compounds: ketones and aldehydes principally coming from lipid oxidation reactions. No significant effect of the different concentration of O<sub>2</sub> was observed in sensory attributes although rancid flavour and TBARS values increased with storage time.**

V. C. Resconi, Department of Animal Production and Food Science, University of Zaragoza, Miguel Servet 177, 50013 Spain (phone: +34-976-761000; fax: +34-976-761590; e-mail: *resconi@unizar.es*). A. Escudero, Department of Analytical Chemistry, University of Zaragoza, 50009 Spain (e-mail: *escudero@unizar.es*). J.A. Beltrán, Department of Animal Production and Food Science Technology Department, University of Zaragoza, 50013 Spain. (e-mail: *jbeltran@unizar.es*). C. Sañudo, Animal Production and Food Science Technology Department, Veterinary Faculty of Zaragoza, Miguel Servet 177, E-50013, Spain. (e-mail: *csanudo@unizar.es*). V. Ferreira, Department of Analytical Chemistry, University of Zaragoza, 50009 Spain (e-mail: *vferre@unizar.es*). M. M. Campo, Department of Animal Production and Food Science, University of Zaragoza, 50013 Spain (e-mail: *marimar@unizar.es*).

**Index Terms—GC-O, high oxygen packs, lipid oxidation, meat aroma**

## I. INTRODUCTION

HIGH oxygen modified atmosphere packaging (MAP) is a very important tool to extend the period where the colour of meat is acceptable. The gas combination typically used in beef varies from 75 to 80 % of O<sub>2</sub> and 20 to 25 % of CO<sub>2</sub> [1], but increasing package oxygen content to levels higher than 55 % may not provide additional benefits to

colour deterioration [2]. In contrast a high oxygen level can promote rancidity, which could develop off-flavours. The aim of this study was to assess the aroma of beef steaks stored under different levels of O<sub>2</sub> in MAP, with the use of GC-O, sensory and chemical analysis.

## II. MATERIALS AND METHODS

A. Samples and treatments Beef loins (*Longissimus dorsi*) were purchased in a local abattoir from 2 males of Spanish Frisian, of 12 months old and raised in the same farm. After ageing for 7 days vacuum packaged at 4 °C, 2-cm thick steaks were obtained, vacuum packaged and frozen at -18°C. Prior to each analysis, samples were thaw at 4°C for 24 hours and placed individually in polystyrene trays. They were flushed with a food grade gas mixture with increasing levels of O<sub>2</sub>: 50, 65 and 80 %, a constant level of CO<sub>2</sub> (20%) and the make up gas Ar and sealed with an impermeable top-web. These 3 types of MAP were displayed under retail display conditions with 16 hours of light every day, at 4 °C for 4 or 8 days. B. Gas

Chromatography-Olfactometry 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 a mixture of 10 g of minced grill-cooked steaks (similarly to the sensory analysis) and 40 ml of Milli-Q water. The mixture was continuously stirred with a magnetic stir bar (450 rpm) and kept at a constant temperature of 37 °C by immersion in a water bath. A controlled stream of nitrogen (100 ml/min) was passed through the sample during 3 hours. The volatile fraction retained in the trap was eluted with 1.6 mL of dichloromethane with 1 % in methanol. The extract was frozen (at -30 °C) and decanted to eliminate any water content. The most contrasting treatments were chosen: vacuum and 80 O<sub>2</sub> displayed for 8 days. There were made 3 extractions per treatment that were jointed and concentrated under a stream of nitrogen to a final volume of 300 µL. Sniffings were

carried out in a CP 3800 Varian (California, USA) GC equipped with a flame ionization detector (FID) and a sniffing port (ODO-1 from SGE International) connected by a flow splitter to the column exit. 50  $\mu$ L was injected in a solvent split mode with a PTV injector. During the injection, the injector was kept to low temperature (40°C) and the split valve opened (split ratio=30) to promote solvent evaporation. After most of the solvent was removed (0.25 minutes), the split valve was closed. The injector was then heated with a ramp of 200°C/min until reaching 250°C. Following 3 minutes the split valve was again open (split ratio=20). The column used was a DB-WAX from J&W (Folsom, CA), 30 m x 0.32 mm and 0.5  $\mu$ m film thickness. The carrier gas was He at 1 mL/min. The temperature program was the following: 40°C for 4 min, then raised at 6.7°C/min up to 200°C, maintaining the temperature during 5 minutes, and a ramp of 25°C/min up to 220°C, temperature kept 1 minute. FID detector was kept at 300 °C. A panel of five expert judges carried out the sniffings. Panellists were asked to rate the intensity of the eluted odour using 7-point category scale (0 = not detected; 1 = weak, hardly recognizable odour; 2 = clear but not intense odour, 3 = intense odour), half values being allowed. Then was calculated the modified frequency (MF, in %), with the formula proposed by Dravnieks [3]:  $MF (\%) = \frac{F (\%)}{I (\%)}$ , where F (%) is the detection frequency expressed as percentage and I (%) is the average intensity expressed as percentage of the maximum intensity. The identification of the odorants was carried out by comparison of their odours and chromatographic retention index with those of the pure reference compounds. C. Sensory analysis and lipid oxidation Samples were thawed at 4°C over 24 h. A 9-member trained panel used a quantitative descriptive analysis in a complete unbalanced block design. Steaks were cooked on a pre-heated double hot-plate grill at 200°C until 70°C of internal temperature. Panelists scored each attribute in a 10-cm non-structured line (0: no flavour to 10: very intense). Lipid oxidation was measured with TBARS (Thiobarbituric acid reactive substances) methodology [4], in mg malonaldehyde (MDA) kg<sup>-1</sup> muscle. D. Statistical analysis Multivariant GLM (SPSS, 14.0) was applied, with MAP, display and their interaction and session as fixed effects).

### III. RESULTS AND DISCUSSION

Huge differences were found between the GC-O scores from vacuum and 80 O<sub>2</sub> groups (Table 1). Twenty six out of 35 aromas differed in at least 20 units of MF between both treatments, but among them, only four were higher in samples vacuum packaged. The most discriminant compounds were ketones (2,3-butanedione, 2-octanone, 2,3-pentanedione, 2-heptanone, 4-methyl-2-pentanone), aldehydes (pentanal, hexanal, 2-methyl-butanal) and 2-furfurylthiol + 1-octen-3-ol, being at higher concentrations in the high oxygen atmosphere; whereas 2-methylpropil-acetate was more intense in the steak vacuum packaged. Among all of them, 2-heptanone was described as rancid, vinegar, which could explain the sensory results.

The majority of these volatiles are lipid oxidation products. According to Insausti et al. [5] four of the mentioned compounds can be present in raw meat and their concentration would increase through storage time in steaks packaged with high oxygen atmosphere. Although the vacuum stored beef had shown to have less aroma values of lipid oxidation products than the high oxygen packed group, some of these compounds could be impact odours in this group, since compounds such as hexanal, 1-octen-3-one, (E)-2-heptenal, octanal and 3-nonen-2-one are among the 10 compounds with higher MF. In this case, since TBARS values were very low (0.20 mg MDA/kg), these compounds are supposed to arise mainly from the cooking procedure. Many volatile lipid oxidation products increased in cooked compared with raw beef, although the greatest increases were found when the cooked meat was further stored [6].

Although the higher influence of lipid oxidation aroma compounds has been associated to undesirable flavours [7], a recent study has shown that even with high rancid notes, grilled steaks stored in packs containing 50 % of oxygen were well accepted. The authors explained this result to the familiarity of oxidised flavours [8].

The GC-O analysis also showed that pyrazines were important odorants for both treatments. Kerler and Grosh [9] had shown that meat patties oxidised did not change their concentration in pyrazines, while desirable odours (furanones) were reduced. In the present study, the lower intensity of beefy flavour in meat displayed for 8 days in comparison with vacuum packaged meat was not supported by the meaty aroma compounds detected in GC-O analysis, such as 2-furfurylthiol or 2-methyl-3-furanthiol, which were increased in meat displayed in MAP. This effect

was perhaps due to the masking of other aromas, such as rancid, which was perceived three times more intense in meat displayed in MAP than in vacuum packaged meat, coincidentally with previous findings [10]. TBARS value in high oxygen packaging displayed for 8 days reached 2.28 mg MDA/kg muscle. This value would be over the limit of acceptability due to the development of off-flavours [10]. Although the present GC-O results are mainly due to differences in oxidation between both treatments, other differences caused by MAP and storage time, such as microbiology [11] may play a role in the results.

#### IV. CONCLUSION

The olfactometry results had shown that the main aroma volatiles were mainly carbonyl compounds which differed in aroma hierarchy between vacuum and high oxygen MAP, which probably explained the higher rancidity of the last group.

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**Table 1.** Odorants found in grilled cooked beef packed in vacuum or high O<sub>2</sub> atmosphere: Linear Retention Index (LRI) in DB-WAX column, Olfactory Description, Chemical Identity, and Modified Frequency Percentage.

| LRI  | Compound  | Aroma description                       | Vaccum | 80 O <sub>2</sub> |
|------|---|---|--------|-------------------|
| 915  | <i>2-methyl-butanal</i>                           | cinnamon, toast                         | 0      | 47                |
| 960  | <i>2,3-butanedione</i>                            | butter, strawberry/cream                | 14     | 91                |
| 973  | unknown   | burnt                                   | 31     | 16                |
| 978  | pentanal  | herb                                    | 0      | 63                |
| 1009 | <i>2-methylpropyl-acetate</i>                     | strawberry/cream, candy                 | 63     | 14                |
| 1019 | <i>4-methyl-2-pentanone</i>                       | strawberry, plastic                     | 28     | 66                |
| 1070 | <i>2,3-pentanedione</i>                           | butter, cheese, strawberry/cream        | 0      | 59                |
| 1088 | hexanal   | herb                                    | 86     | 91                |
| 1106 | <i>hexanal tail</i>                               | flower                                  | 0      | 49                |
| 1120 | unknown   | forest, tobacco, meat, garlic, sweat    | 51     | 67                |
| 1121 | unknown   | spice, acid, fat, dust                  | 31     | 0                 |
| 1155 | 1-penten-3-ol                                     | flower, burnt, meaty                    | 31     | 28                |
| 1167 | unknown   | potato, raw meat, rusty                 | 28     | 61                |
| 1187 | <i>2-heptanone</i>                                | rancid, flower, vinegar, soap/orange    | 16     | 63                |
| 1219 | (E)-2-hexenal                                     | eucalyptus, fruit/flower, potato, toast | 23     | 51                |
| 1239 | (E)-2-heptenal                                    | fishy                                   | 61     | 89                |
| 1287 | octanal   | soap/orange                             | 53     | 78                |
| 1299 | 1-octen-3-one                                     | mushroom, metallic                      | 82     | 57                |
| 1321 | <i>2-octanone</i>                                 | pine, soap, mushroom, food, cinnamon    | 16     | 86                |
| 1341 | 2-methyl-3-furanthiol                             | barbecue, meat, toast                   | 40     | 72                |
| 1381 | dimethyltrisulphide +<br>2,4,5-trimethyl thiazole | forest, glue, flower                    | 40     | 66                |
| 1398 | nonanal + 2-nonanone                              | soap/orange, rancid, herb               | 38     | 49                |
| 1435 | <i>2-furfurylthiol + 1-octen-3-ol</i>             | toast, barbecue, tobacco, flower, green | 14     | 71                |
| 1442 | methional   | lamb, pop-corn, dairy                   | 77     | 63                |
| 1455 | 2,3-diethyl-5-methylpyrazine                      | grilled meat                            | 67     | 88                |
| 1480 | 2-ethyl-3,(5/6)-dimethylpyrazine                  | wet earth, vinegar                      | 0      | 35                |
| 1516 | 3-nonen-2-one                                     | mushroom, chlorine, meat, fatty         | 42     | 51                |
| 1556 | (E)-2-nonenal                                     | new shoes, dust                         | 16     | 49                |
| 1628 | 2-acetylpyrazine                                  | burnt                                   | 49     | 65                |
| 1734 | (E,Z)-2,4-decadienal                              | rancid oil, green, sausage              | 16     | 33                |
| 1776 | 2-acetyl-2-thiazoline                             | barbecue, toast/meat, chicken broth     | 28     | 61                |
| 1870 | (E)-2-dodecenal                                   | rancid oil, fried meat                  | 28     | 51                |
| 1950 | unknown   | animal                                  | 31     | 0                 |
| 2097 | unknown   | manure                                  | 32     | 65                |
| 2119 | unknown   | burnt, urine, stable                    | 0      | 35                |

**In *cursive* the compounds with higher differences in MF between treatments.**