Effect of freezing method and frozen storage duration on odour-active compounds and sensory perception of lamb

Mónica Bueno[a]; Virginia C. Resconi[b]; M. Mar Campo[b]; Juan Cacho[a]; Vicente Ferreira[a]; Ana Escudero*[a]

[a] *Laboratory for Aroma Analysis and Enology. Aragón Institute of Engineering Research (I3A). Department of Analytical Chemistry, Faculty of Sciences, University of Zaragoza, 50009, Zaragoza, Spain*

[b] *Department of Animal Production and Food Science, University of Zaragoza, 50013, Zaragoza, Spain*

* To whom correspondence should be addressed

Phone: 34976 762503

Fax: 34 976761292

Email: escudero@unizar.es

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ABSTRACT

The effects of different freezing methods (nitrogen freezing tunnel, air blast freezer and home freezer) and frozen storage duration (1 month and 10 months) have been studied by sensory analyses and chemical analyses of odour-active compounds. The sensory analyses showed that fresh meat was significantly ($p < 0.05$) juicier than frozen meat. Meat frozen in the air blast freezer (ABF) and stored during 10 months had the highest lamb flavour intensity, significantly different from meat frozen in a home freezer (stored for 1 and 10 months) or in a nitrogen tunnel and stored for 1 month. The fresh sample was characterized by lower levels of Strecker aldehydes, 2-phenoxyethanol, cresols and to a lesser extent 2-methylbenzaldehyde, and higher levels of furaneol, which was not even detected in frozen samples. These compounds, together with the relative levels of octanoic acid and ethyl hexanoate, could be used as markers to detect whether lamb meat has been frozen. In chemical terms, the ABF samples were the most similar to fresh meat. Remarkably, in most cases the relative chemical aroma composition had no significant differences as a result of freezing. This helps explain the high sensory similarity observed. Only pyrazine levels changed significantly with frozen storage duration. No oxidation aromas or rancidity have been detected in fresh or frozen lamb.

1. Introduction

Lamb meat is widely consumed in Mediterranean countries, Spain being one of the most important producers and consumers of this type of meat (Sanudo, Sanchez, & Alfonso, 1998). While household consumption in Spain is much higher than in restaurants, lamb meat is available in restaurants for most of the year (Albisu & Gracia, 2009). The seasonal supply of lambs is characteristic of the ovine market (Chemineau et al., 1995), with a high supply during spring and a high demand at Christmas time. This results in price fluctuations (Hansen et al., 2004). In order to increase profitability, meat producers freeze meat in an attempt to stabilise its price (Pietrasik & Janz, 2009; Wheeler, Miller, Savell, & Cross, 1990).

Freezing procedures need to ensure not only the nutritional quality, which has already been demonstrated, but also the sensory quality of frozen meat. The latter is closely linked to meat aroma. Consumers consider flavour one of the main sensory properties decisive in their selection, acceptance, and ingestion of a particular food (Fisher & Scott, 1997). Considering the importance of aroma in lamb meat quality, several papers have examined its volatile profile (Elmore, Mottram, Enser, & Wood, 2000; Osorio, Zumalacarregui, Cabeza, Figueira, & Mateo, 2008; Priolo et al., 2004; Young, Lane, Priolo, & Fraser, 2003); but none have studied the effect of freezing on the aroma profile. Meat aroma can only be controlled if the chemical compounds responsible for the most relevant sensory properties of fresh and frozen lamb are quantified (Bueno et al., 2011; Campo et al., 2006; Muela, Sañudo, Campo, Medel, & Beltrán, 2010; Young et al., 2003).

In general, it is thought that frozen meat is of lower quality than fresh meat (Lagerstedt, Enfalt, Johansson, & Lundstrom, 2008). Thus, only 20% of consumers

48 always freeze meat and 19.7% freeze for more than a month. Eighty eight percent of
49 consumers would not buy thawed meat and 63% of consumers do not eat meat frozen-
50 thawed in a restaurant (Muela, Sañudo, Campo, Medel, & Beltrán, 2009). This shows a
51 lack of knowledge of the restoration operation.

52 The quality of frozen meat depends on the specific procedures used to freeze the
53 meat, the conditions of frozen storage (duration, temperature and its fluctuation,
54 exposure to light and/or air), and the thawing rate (Jasper & Placzek, 1980). The
55 freezing rate (related to time-temperature) can affect the quality of meat (Berry &
56 Leddy, 1989; Smith, Spaeth, Carpente, King, & Hoke, 1968; Uttaro & Aalhus, 2007)
57 through the structural changes that occur during freezing. In this work, a nitrogen
58 freezing tunnel was used because of its high freezing rate, an air blast freezer was used
59 for its versatility (in freezing and refrigeration), and a static air chamber was used
60 because of its similarity to a home freezer.

61 Although frozen foods are microbiologically stable, they are prone to
62 deterioration during storage due to chemical reactions (Akkose & Aktas, 2008), since
63 enzymatic activity slows down but does not cease (Devine, Graham, Lovatt, &
64 Chrystall, 1995; Jiménez & Carballo, 2000). The main deterioration in frozen meat is
65 due to the processes of lipid oxidation and protein degradation (Zhang, Farouk, Young,
66 Wieliczko, & Podmore, 2005), muscle tissues being especially susceptible. These
67 processes can determine the end point of the display life of frozen products (Jiménez &
68 Carballo, 2000). Storing frozen meat during one month is a common practice in
69 households, but to cover the period of sexual inactivity of small ruminants or even
70 export, long periods are necessary (10 months, for example).

71 This study assesses the effects of freezing methods and frozen storage durations
72 on sensory perception and odour active compounds. Both thawed and fresh meat are
73 evaluated.

74

2. Materials and methods

2.1 Samples, freezing and frozen storage

This study used 70 Rasa Aragonesa male lambs (approximately seventy days old) with a cold carcass mean weight of 11.5 ± 0.1 kg. The animals were fed in the same facilities under intensive husbandry conditions with no grazing (Pastores Grupo Cooperativo), with natural suckling during the first 40 days and fodder with concentrated ad libitum, mainly barley and maize, until slaughtering in a EU-licensed abattoir following standard protocols. The slaughter date was 1 or 10 months (frozen storage duration, FSD) before meat analysis. For each FSD, 30 carcasses were selected randomly among the commercial lambs slaughtered on the corresponding slaughter date. The slaughtering protocol has been described by Muela et al. (2010) in a previous work. As can be seen in Fig. 1, at 4 days post-slaughter, the left side of the carcasses, minus the neck, shoulder, flank and leg, were divided into two parts (one from the 5^{th} thoracic to the 13^{th} thoracic, T5-T13, and the other from the 1^{st} lumbar to the 6^{th} lumbar vertebrae, L1-L6) which were randomly allocated to one of three freezing methods (FM) (10 carcasses for each FM): air blast freezer (dynamic air chamber) (−30ºC, 90% relative humidity, 1–2 m s^{-1}, 3 days), nitrogen freezing tunnel (−40 °C, 96% relative humidity, 0.3 m min^{-1}, 15 min), and home freezer (static air chamber) (−18 ºC). To prevent freezer burn and water losses, each part of the carcass (T5-T13, L1-L6) was over-wrapped in a retractile oxygen-permeable plastic film (permeability 10 g m^{-2} water, 200 cc m^{-2}/24 h O_2, and 650 cc m^{-2}/24 h CO_2) matching similar conservation procedures at home. After being frozen using one of the FMs, the samples were stored at −18 °C, allocated for each FM for 10 months or 1 month. Before conducting the instrumental measurements and the sensorial analysis of the meat, the samples were thawed in a refrigerator (2–4 °C) for 24 h inside their plastic over-wrap. After thawing,

the *Longissimus thoracis* and *lumborum* muscles were excised with the subcutaneous fat, vacuum packed and kept at 2-4 ºC 24 h before being analysed. T5-T13 samples were used for the carbonyl compound quantification and L1-L6 samples were used for the sensorial analysis, the thiobarbituric acid-reactive substances method (TBARS) and other volatile quantifications. The remaining 10 carcasses were not frozen and served as a control group. These animals were subjected to the same slaughter, chilling, splitting and wrapping procedures as described above, but instead of freezing, the fresh meat samples were aged in a refrigerator (0–4 °C) for 6 days. This is equivalent to the total time of the ageing of the thawed meat (96 h previous to freezing + 24 h during thawing + 24 h after excision).

2.2 Reagents, standards and materials

2.2.1 Solvents

Dichloromethane, methanol, hexane and diethyl ether (gas chromatography quality) were purchased from Merk (Darmstadt, Germany). Ethanol was supplied by Panreac (Barcelona, Spain). Water was purified in a Milli-Q system from Millipore (Bedford, Germany).

2.2.2 SPE cartridge materials

LiChrolut EN® resins (styrene/divinylbenzene copolymer) and 1 mL internal volume polypropylene cartridges were supplied by Merk. Glass wool was purchased from Panreac. Semiautomated solid phase extraction was carried out with a VAC ELUT 20 station system from Varian (Wallnut Creek, CA, USA).

2.2.3. Chemical Standards

Sodium sulfate anhydrous 99% and sodium hydrogencarbonate 99.7% were supplied by Panreac; (E,E)-2,4-decadienal and 3-methylbutanoic acid 98% by Lancaster

(Eastgate, UK); sulfuric acid (95–97%, synthesis grade) by Scharlau (Barcelona, Spain); 2,6-dimethylpyrazine ⩾98% by SAFC (Steinheim, Germany); m-cresol 99%, 2-phenoxyethanol 98%, 2-isopropyl-3-methoxypyrazine 97%, 2-methylbenzaldehyde 97%, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) 98%, 2-ethyl-3(5 or 6)-dimethylpyrazine 95%, 2-methylpropanal (isobutyraldehyde) 99%, 2-methylbutanal 95%, 3-methylbutanal (isovaleraldehyde) 97%, pentanal (valeraldehyde) 97%, hexanal 98%, octanal 99%, nonanal 95%, decanal (95%), (E)-2-hexenal 98%, (E)-2-octenal 94%, (E)-2-nonenal 97%, (E)-2-decenal >92%, (E,E)-2,4-heptadienal 90%, (E,E)-2,4-nonadienal ⩾85%, 2-heptanone 98%, 2-octanone 98%, 2-nonanone 99% and 2,3,6-trichloroanisole 99% used as internal standard for carbonyl compounds by Aldrich (Madrid, Spain). Heptanal ⩾95%, (E)-2-heptenal 98%, 1-octen-3-one >99%, p-cresol ⩾99%, octanoic acid 98%, butanoic acid 99.5%, ethyl hexanoate 99%, 2-octanol 99.5% used as internal standard for other volatile compounds, 5-chloro-2-pentanone ⩾97% used as surrogate for carbonyl compounds and O-(2,3,4,5,6-pentafluorobencyl)hydroxylamine hydrochoride (PFBHA) 99% used as derivatization reagent for carbonyl compounds were supplied by Fluka (Madrid, Spain). The 5 mg mL^{-1} solution of PFBHA was prepared daily.

2.3 Instrumental measurements

2.3.1 pH

To determine the instrumental characteristics of the raw meat before freezing, pH analysis was performed prior to chilling at 24 hours after slaughtering. A portable CRISON 507 pH-meter equipped with a penetrating electrode was used to measure the pH of the left *Longissimus thoracis* and *lumborum* muscles. The average values found were 5.61 ± 0.03.

148 *2.3.2. Level of oxidation (TBARS)*

149 To assess lipid oxidation, meat samples were obtained from the 1st lumbar
150 vertebra. The assay was performed using the thiobarbituric acid-reactive substances
151 method (TBARS) (Pfalzgraf, Frigg, & Steinhart, 1995). For this purpose a UNICAM
152 5625 UV/VIS spectrometer was used.

153 *2.4 Loin grilling*

154 Samples were kept at ambient temperature (20 ºC) during 1 h before analysis to
155 achieve an internal muscle temperature of 15ºC prior to cooking. The muscles (T5-T13
156 muscle mean weight = 154 ± 5 g and L1-L6 muscle mean weight = 202 ± 7 g) were
157 wrapped in aluminium foil and grilled (with the fat side up) on an industrial double-
158 plate grill (GRS-5 SAMMIC) at 200 ºC until the internal temperature was 70 ºC
159 (Resconi, Campo, Furnols, Montossi, & Sanudo, 2009), which was monitored by a
160 JENWAY 2000 internal thermocouple. Once grilled, the subcutaneous fat and external
161 connective tissue were removed.

162 *2.5 Sensory analysis*

163 A trained nine-member tasting panel (ISO 8586-1, 1993) evaluated the meat
164 samples using a quantitative descriptive analysis. A total of 63 samples, 9 replicates per
165 treatment, were tasted, obtaining a total of 81 judgements per treatment. Prior to sensory
166 analysis, the cooked muscle (L1-L6) was cut into 9 portions which were wrapped in
167 aluminium foil and identified with a single random three-digit code. The samples were
168 kept warm in a heater at 50 ºC until they were served to the tasting panel (<10 min after
169 being cooked). To minimize the possibility of an effect of the order of presentation and
170 carryover effects, the samples were presented in a sequence that followed a balanced
171 design (Macfie, Bratchell, Greenhoff, & Vallis, 1989). The sessions took place in a

controlled sensory analysis laboratory (ISO 8589, 1988) equipped with individual booths and red lights. To cleanse their palate between samples, panellists were given bottle water and breadsticks.

The sensory profile and specific training were developed in an additional session using 7 samples from each of the seven treatments (three different FM x two different FSD + not frozen meat). The test used a quantitative descriptive method within an incomplete and balanced design (Cochran & Cox, 1978) that included 21 plates containing three samples each. There were five testing sessions (over three days in three consecutive weeks) with four plates per session (five in the first session). The analysis was based on 11 sensory descriptors (Table 1) chosen by the panellists during the training session. The panel evaluated the meat samples on a 10 cm semi-structured and continuous scale in which intensity ranged from very low (0) to very high (10), and overall likeness ranged from very bad (0) to very good (10).

2.6 Extraction of volatile compounds

The cartridges hand-making, conditioning, drying and grill extraction procedures have been described in a previous work (Bueno et al., 2011). Meat volatiles were collected during grilling until the muscle internal temperature of two loins (requirement to have enough sensitivity) was 70 ºC.

2.7 Carbonyl compounds quantification

2.7.1 Derivatization

Derivatization was carried out directly in the cartridge after the extraction procedure using a variation of the method described in the literature (Zapata, Mateo-Vivaracho, Cacho, & Ferreira, 2010). In our case, the cartridges contained 100 mg of LiChrolut EN® resins so that only half of the reagents, cleaning solvents and

derivatizing agent aqueous solution were necessary. Before the derivatization process, 200 μL of 5-chloro-2-pentanone 2.5 mg L^{-1} standard solution in 25% ethanol (v/v) in Milli-Q water (surrogate), prepared daily, were loaded onto the cartridge. Prior to the elution process, glass wool was removed from the cartridges. Derivatized analytes were finally eluted with 1 mL of 10% diethyl ether (v/v) in hexane and 30 μL of 2,3,6-trichloroanisole 20 mg L^{-1} were added as internal standard. The extracts were dried with sodium sulfate anhydrous and analyzed by GC-NCI-MS. Two blank samples must be prepared per session by applying the whole procedure to a cartridge.

2.7.2 GC-NCI-MS

Two microlitres of each extract were injected in a Shimadzu QP-2010 gas chromatograph with a quadrupole mass spectrometric detection system. The standard split/splitless injector was operated in splitless mode. The temperature of the injector was kept at 250ºC, and a pressure pulse of 467 KPa was applied during 1.5 min (the column flow during this period of time was 2.69 mL min^{-1}). The carrier gas was He at a constant linear velocity of 35 cm s^{-1} (≈ 0.62 mL min^{-1} flow rate). The column was a DB-5 capillary column from J&W (Folsom, CA, USA), 20 m x 0.18 mm i.d., with 0.18 μm film thickness, preceded by a silica precolumn from Supelco (Bellefonte, PA, USA), 3 m x 0.25 mm i.d.. The chromatographic oven was held at 45ºC for 2 min, then raised to 200ºC at 10ºC min^{-1} and finally to 280ºC at 25ºC min^{-1}; remaining at that temperature for 3 min. The ion source was operated in NCI mode using methane at 3 bars as reagent gas. The temperature of the ion source was 220ºC and the transfer line was kept at 250ºC. The mass analyzer was operated in single ion monitoring (SIM) mode. The selected ions for each analyte are shown in Table 2. The derivatization process was monitored using a surrogate (5-chloro-2-pentanone). For the quantification of the internal standard (2,3,6-trichloroanisole) and the surrogate, mass 35 was used.

221 The solvent cut window was 9.4 min. Quantitative data were obtained by interpolation
222 of ionic peak areas relative to the internal standard in the calibration graphs built by the
223 analysis of water containing known amounts of the analytes. The quantity (ng) is
224 referred to the litres of headspace trapped during the grilling of two lamb loins.

225 *2.8 Quantification of other volatiles*

226 For the quantification of other volatiles which are not carbonyls, the compounds
227 were directly eluted after extraction. Glass wool was removed prior to the elution
228 process by cutting the cartridge. The compounds were then eluted with 1 mL of
229 dichloromethane containing 5% methanol (v/v) (Ferreira et al., 2009). 40 μL of 2-
230 octanol 12 mg L^{-1} were added as internal standard. The extracts were dried with sodium
231 sulfate anhydrous and concentrated under nitrogen stream until 200 μL.
232
233 *2.8.1 Multidimensional gas chromatography-olfactometry-mass spectrometry*
234 *(GC-GC-O-MS)*
235 Ten microlitres of each extract were injected into a multidimensional gas
236 chromatograph system supplied by Varian (Walnut Creek, CA, USA). The system
237 consisted of two independent CP 3800 chromatographs interconnected by a
238 thermoregulated transfer line kept at 200ºC equipped with a Deans valve switching
239 system (Valco Instruments, Houston, TX), two olfactory ports, a 1079 PTV
240 (Programmable Temperature Vaporizing) injector, flame ionization detection (FID) and
241 an ion trap mass spectrometric-detector (Varian 240-MS), as described in Bueno et al.
242 (2011).
243 In four chromatographic runs for each extract, selective heart-cuttings were
244 performed to isolate the odorants of interest, detected by GCO in recent studies (Bueno
245 et al., 2011; Resconi et al., 2010). The odorants were transferred to the second oven and

246 monitored by MS detection (Table 2). The columns, oven temperature programs, PTV
247 conditions, delay time and heart-cutting interval were the same as those in Bueno et al.
248 (2011). Compound quantification was determined through two capillary columns and
249 the mass spectra to obtain not only sensitivity but also more selectivity, because direct
250 extracts have a large number of compounds. Heart-cuttings were done to avoid
251 coelutions in the second column. The quantification was carried out with relative ionic
252 peak areas and using response factor so that the mass of the collected analytes was
253 known. The response factor was calculated by the GC-GC-MS analysis of
254 dichloromethane-methanol solutions containing known amounts of the standards.
255 Knowing the volume of fumes extracted during the grilling of two lamb loins, the
256 concentration of the compounds present in the fumes generated is known.

257 *2.9 Statistical analysis and data treatment*

258 Chromatographic data treatment was carried out because of the lack of
259 reproducibility in meat analysis. This is due to the variability between animals, the
260 variability in the shape and weight of the loins and thus the variability in cooking times.
261 For each chromatogram, the analyte in which it was most abundant was assigned a
262 value of 100. Other analytes in the same sample were expressed as percentages in
263 relation to the most abundant. We worked with both the absolute quantity of the
264 analytes (concentration in the generated fumes) and the relative values of the analytes.

265 For the sensory data, the quantitative data of the analytes (absolute and relative),
266 and the TBARS data, a General Linear Model (GLM) was applied with SPSS 15.0
267 software, considering treatment as a fixed effect (3 freezing methods x 2 frozen storage
268 durations + fresh meat). Differences between treatments were assessed using a Duncan
269 test ($p<0.05$). Another GLMs were applied for analyte quantitative data (absolute and
270 relative) and TBARS data considering FM and FSD as fixed effects. Another GLM was

271 applied for analyte quantitative data (absolute and relative) considering frozen and non-
272 frozen as a fixed effect.
273 The data relating to the significant sensory attributes and significant (in any of
274 the GLMs performed) aromatic compounds (ng L^{-1}) were subjected to a Principal
275 Component Analysis (PCA) using The Unscrambler 9.7 statistical software.
276

277 ## 3. Results and discussion

278 *3.1 Level of oxidation (TBARS)*

279 All the samples obtained values from 0.045 to 0.233 μg malonaldehyde g^{-1} meat
280 (average treatment values are shown in Table 3). These oxidation levels were lower than
281 the threshold value for rancid flavour detection of 0.5 μg malonaldehyde g^{-1} meat
282 proposed by Tarladgis, Watts, Younathan, & Dugan (1960) or the even higher threshold
283 of 2 μg g^{-1} for ruminants (Campo et al., 2006) at which consumers detect an off-flavour.
284 Although intensive rearing conditions based on cereals imply less natural antioxidants
285 in the feed than grazing rearing conditions, lipid oxidation was this low after 10 months
286 of storage probably because the loin was kept intact under freezing conditions, which
287 reduces the rate of deterioration.

288 The control samples (non frozen lamb) differ significantly ($p<0.001$) from those
289 of the other 6 treatments (3 FM x 2 FSD). The values from fresh meat were lower than
290 those obtained from frozen meat. Leaving aside this control treatment, the frozen
291 samples were compared and the FM and FSD effects were studied. It was concluded
292 that there were no significant differences resulting from the different FMs but that the
293 FSD did produce such differences ($p<0.05$). No interaction was found between these
294 two effects. This result is slightly different to the findings of Muela et al. (2010) that
295 both FM and FSD had highly significant effects on oxidation ($p<0.001$). As was
296 expected, the longer the storage duration, the higher the amount of malonaldehyde
297 formed. This result is consistent with the finding of Muela et al. (2010). In any case,
298 these TBARS results are proof that all the samples were properly handled since the
299 results are consistent.

300 *3.2 Sensory analysis*

301 The sensory panel test on the *Longissimus lumborum* muscle showed that most
302 attributes suffered no detrimental effects from freezing. Only 3 of the 12 sensory
303 attributes were significantly affected by treatment. Table 4 illustrates the significant
304 influence of different treatments on the organoleptic quality of lamb.

305 Lamb and fat odour intensities were under the mid 10 cm scale, consistent with
306 the type of lamb studied (light lambs fed with concentrates, Sanudo et al., 1998) and
307 with higher scores than those for the odour intensity of old meat (also consistent with
308 the young age of the lambs, Young, Berdague, Viallon, RoussetAkrim, & Theriez,
309 1997). Furthermore, the lamb odour did not show detrimental freezing effects, in
310 agreement with the findings of Muela, Sañudo, Campo, Medel, & Beltrán (2012). This
311 was also reflected in the lack of significant differences between fresh and thawed meat
312 in odour attributes.

313 In the case of flavour, the lamb intensities differed significantly. Meat frozen in
314 the air blast freezer during 10 months had the highest score, and sensory differences
315 with meat frozen in a home freezer (1 and 10 months) or in a nitrogen tunnel for 1
316 month could be appreciated. The lamb and fat flavour scores were higher than the
317 scores of the other flavour attributes, being at the middle of the 10 cm scale. If the fresh
318 lamb score is removed, there is a correlation between the lamb and fat flavour ($r =$
319 0.91). On the other hand, there were significant differences in the acid flavour between
320 meat frozen in the air blast freezer during 1 month, and in the home freezer and in the
321 nitrogen freezing tunnel during 1 month. In any case, the meat had low acid scores.
322 Acid flavours appear in beef aged more than 7 days, and can be associated with
323 enzymatic degradation processes which occur in meat throughout the ageing process
324 (Spanier, Flores, McMillin, & Bidner, 1997).

The greasiness attribute had low scores but was noticeable enough, maybe due to the cooking methodology with the fat side up (Bueno et al., 2011). The meat was more easily impregnated with the fat. Independently of the treatment, tenderness was perceived on the high part of the scale, either due to the age of the animal (only 70 days) or probably because of the ageing period (6 days). Ageing promotes tenderness through the enzymatic degradation of myofibrils (Penny, 1984) and 6 days are long enough to consider lamb meat as tender. Typically, meat tenderness and juiciness are positively correlated. In the present work, they are correlated ($r = 0.90$) if the fresh meat data is removed, but tenderness was not significantly affected by the treatment probably because all the treatments had the same ageing,period and, as already stated, 6 days is a sufficiently long ageing period. Hopkins et al. (2006) found that all the sensory traits improved as the proportion of intramuscular fat increased. In this study, the lambs had been subject to the same feeding system, had nearly the same carcass mean weight and therefore similar fatness, and were of the same age at slaughter. Consequently, no significant differences would be expected in texture parameters (tenderness, juiciness and untuosity), as was the case reported by Muela (Muela et al., 2012). Ageing prior to freezing was the same and sufficiently long in all the treatments (Vieira, Diaz, Martinez & Garcia-Cachan, 2009). Nevertheless, in our case juiciness was significantly affected by the treatment when differentiating between fresh and frozen meat. Since all the samples were thawed in the same way, it could be said that the significant difference found in juiciness only depends on the freezing methods, probably because changes in juiciness are caused by the loss of water due to cell structure disruptions (Farouk & Swan, 1998).

In conclusion, looking at all the data as a whole, it can be said that freezing did not damage the sensorial quality of the lamb. The hedonic preference of the panel did

not penalise frozen meat, perhaps because these consumers were used to frozen meat at their home, as is the case in other countries (64% in New Zealand and 87% in Australia, Gilbert et al., 2007). A panel is not representative of the population, so its hedonic values must be considered with care. In any case there are studies which state that panel hedonic values can subsequently be correlated with consumer acceptability (Resano, Sanjuan, Cilla, Roncales, & Albisu, 2010).

3.3 Relationship between odour-active compounds and sensory perception

Most aroma-active chemical compounds in grilled meat have been quantified in order to gain a more complete understanding of the flavour-related phenomena taking place during the storage of frozen meat. In all, 36 compounds were quantified in the grilled meat samples (Tables 2, 5 and 6). The target aroma chemicals were selected having regard to the scientific literature, taking into account all those that have been found to be odour-active or related to the freezing process. In recent studies (Bueno et al., 2011), ethyl hexanoate and butanoic and 3-methylbutanoic acids were found at high levels in frozen samples. In the same study, pyrazines and octanoic acid were found in samples obtained directly from the grill, and these have also been targeted in this work. In addition, three of the quantified pyrazines have been previously described as important contributors to goat meat (Madruga, Elmore, Dodson, & Mottram, 2009). Other compounds identified for the first time in lamb (Bueno et al., 2011), such as 2-isopropyl-3-methoxipyrazine and 2-methylbenzaldehyde, have also been quantified in this work. Caporaso, Sink, Dimick, Mussinan, & Sanderson (1977) suggested 10 aldehydes, 3 ketones and 1 lactone as the most important compounds in lamb flavour. Six of these carbonyl compounds (hexanal, heptanal, octanal, nonanal, (E)-2-octenal

374 and (E,E)-2,4-decadienal) were found to be sensory active in other studies (Bueno et al.,
375 2011; Resconi et al., 2010) and have also been studied here. Other analytes (strecker
376 derivates, saturated aldehydes, 2-ketones, (E)-2-alkenals, (E,E)-2,4-alkadienals and
377 cresols) have been previously identified in Tenax fractions (Elmore et al., 2005;
378 Sutherland & Ames, 1995; Young et al., 1997) in the headspace aroma of grilled lamb.
379 Finally, some odorants with meaty odours ((E)-2-heptenal, $RI_{DB\text{-}WAX}$ 1473/$RI_{VF\text{-}5}$ 1168
380 barbecue, (E)-2-decenal, (E,E)-2,4-decadienal, 2-acetyl-2-thiazoline) have been
381 identified by GC-O in a previous work (Bueno et al., 2011). Most of these compounds
382 are lipid oxidation products, Strecker reaction products from amino acids or Maillard
383 reaction products.

384 The absolute quantities of the target aroma chemicals, expressed in ng L^{-1},
385 contained in the fumes generated during the grilling of the different meat samples are
386 given in Table 5. Significant differences between the means of the treatments are
387 indicated by different superscripts. The aroma chemical data has been processed in both
388 absolute and relative values (see section 2.9), and the significance of the major factors
389 considered in the study is compiled for both sets of data in Table 6. In addition, a
390 Principal Component Analysis was carried out on discriminant chemical and sensory
391 data, and the projection of the samples and variable loadings on the first two
392 components is plotted in Fig. 2.

393 Regarding the absolute content in aroma compounds, the data in Table 5 and the
394 plot in Fig. 2 reveal that the meat sample frozen in the freezing tunnel after 10 months
395 of storage (sample NTF10m) showed the greatest difference in relation to all the other
396 samples in terms of its absolute aroma composition. This is consistent with the
397 organoleptic data reported by Muela et al. (2012). This sample produced the highest

398 levels of some carbonyl compounds, notably aliphatic aldehydes and 2-ketones, and
399 also of (E)-2-octenal, (E,E)-2,4-heptadienal and (E,E)-2,4-nonadienal.

400 In this paper the lamb oxidation level has been studied in three ways: first with
401 TBARS analyses, secondly with carbonyl compound analyses and thirdly with sensory
402 analysis. It was expected that the results of the first method would not match the results
403 of the second and third methods because the TBARS is carried out on uncooked meat
404 while the carbonyl quantification of the aroma release during cooking and the sensory
405 analysis are carried out on cooked meat. If all the tests had been carried out on
406 uncooked meat, the results might have been the same (Olivares et al., 2011).
407 Nevertheless, the conclusions in our study remain the same: no oxidation aromas or
408 rancidity from lipids have been found in either fresh or frozen lamb.

409

410 The data reveal that the fresh meat sample is characterized by a relatively small
411 number of compositional differences from the other samples, although some of them
412 become significant. In particular, this sample had the smallest levels of Strecker
413 aldehydes (2-methylpropanal, 2-methylbutanal and 3-methylbutanal), p- and m-cresols,
414 and to a lesser extent, 2-methylbenzaldehyde, and the maximum levels of furaneol (see
415 also Table 6, last column). All these compounds, particularly furaneol, can in this sense
416 be considered as general markers for freezing.

417 The plot in Fig. 2 also reveals a clear separation of freezing methods. The
418 nitrogen freezing tunnel is positively correlated to the first component, the house freezer
419 is negatively correlated with PC1 and positively correlated with PC2, whereas the air
420 blast freezer is negatively correlated with both PCs. This last freezing method is
421 positively correlated to butanoic and 3-methylbutanoic acid, in accordance with Bueno

et al. (2011) who reported high modified frequency percentages in air blast frozen samples. In any case the plot suggests from the aroma chemical composition point of view that the ABF treatment produces samples most similar to fresh meat, and in this sense it is the most efficient. Remarkably, from the chemical point of view the effect of storage time is negligible in HF and ABF treatments, since samples stored for 1 or 10 months have an almost identical chemical composition, suggesting that in these treatments changes can be essentially attributed to the freezing process and not to the storage itself (see also Table 6, columns four and six).

If chemical changes are clear but not very intense in absolute terms, they become almost negligible when it comes to relative terms (Table 6, all the columns headed by "relative values"). When we compare the chemical aroma profile (the relative content of each aroma component in each sample), in most cases differences between samples are purely random and cannot be clearly attributed to any of the studied factors. These results are summarized in Table 6, in which the columns give the significance level reached by the differences between treatments, between frozen treatments without the fresh treatment and between fresh and frozen samples. In this last case, for instance, it can be seen that the relative content of fresh samples does not differ from that of frozen samples except in the levels of furaneol, octanoic acid, ethyl hexanoate and, to a lesser extent, 2-phenoxyethanol. The fumes from fresh meat contained a higher proportion of octanoic acid, ethyl hexanoate (this last result is contrary to what was found recently, Bueno et al., 2011) ($p<0.01$) and, of course, of furaneol which was not even detected in frozen meat ($p<0.001$). On the other hand, 2-phenoxyethanol values are lower in fresh meat than in frozen meat ($p<0.1$).

We cannot yet offer a satisfactory explanation for the absence of furaneol in the frozen meats. It may be related to the release of intermolecular water due to disruptions

447 in the cell walls caused by microcrystals formed during the freezing process (Ballin &
448 Lametsch, 2008). It may be that such water is partly drained off during the thawing
449 process, dragging with it fructose-1,6-diphosphate, the predominant precursor of the
450 odorant furaneol (Schieberle, 1992). It could be that just the change of the water activity
451 has a deep influence on the grilling proces. In any case, the possibility that the absence
452 of this strong odorant in frozen samples can be related to the lower level of juiciness
453 noted in these sets of samples is of considerable interest and deserves further scientific
454 attention.

455 Returning to Table 6, it can be seen that the relative levels of the four pyrazines
456 changed significantly with the duration of the frozen storage, increasing with the
457 duration.

458 Leaving aside furaneol, Table 6 reveals that the differences in relative terms are
459 marginal. This would help explain the small sensory differences noted between the
460 different samples, as previously discussed (Table 4). These low levels of sensory
461 differences can be related to the fact that “odor concepts” and “flavour concepts” in
462 general are more related to the profile of chemicals than to their absolute quantities,
463 which are more closely related to the intensity (Ferreira, 2012a, 2012b). From this point
464 of view, the lack of oxidation aromas or rancidity (Table 4) can be explained by the fact
465 that the sensory levels of rancid-smelling compounds were never too high (Table 5). In
466 cases where they were a little bit higher, the other odorants were also at a higher level so
467 that the profile (relative amount) remained almost unchanged.

468

4. Conclusions

The sensory study has confirmed that frozen meat retains all the sensory characteristics of fresh meat, except juiciness which was significantly smaller in all cases. The different treatments, including storage time, seemed to have a limited effect on the sensory properties. Besides, no oxidation aromas or rancidity have been detected in fresh or frozen lamb. From the chemical point of view, the fresh sample was characterized by smaller levels of Strecker aldehydes, 2-phenoxyethanol and cresols and higher levels of furaneol, which was not even detected in frozen samples, and to a lesser extent, 2-methylbenzaldehyde. These compounds, together with the relative levels of octanoic acid and ethyl hexanoate, could be used as markers to detect when lamb meat has been frozen. In chemical terms, the ABF samples were the most similar to fresh meat. Remarkably, leaving aside furaneol, octanoic acid and ethyl hexanoate, the chemical aroma profiles were very similar between samples, which could help explain the high degree of sensory similarity observed. Only pyrazine profiles changed with the duration of the storage, thus if meat is stored for 10 months under freezing conditions the problem of seasonality in the ovine market can be solved.

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