Effect of fat content on aroma generation during processing of dry fermented sausages

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Abstract

Dry fermented sausages with different fat contents were produced (10%, 20% and 30%). The effect of fat content and ripening time on sensory characteristics, lipolysis, lipid oxidation and volatile compounds generation was studied. Also, the key aroma components were identified using gas chromatography (GC) and olfactometry. High fat sausages showed the highest lipolysis and lipid oxidation, determined by free fatty acids content and thiobarbituric acid reactive substances (TBARS), respectively. A total of 95 volatile compounds were identified using SPME, GC and mass spectrometry (MS). Fat reduction decreased the generation of lipid derived volatile compounds during processing while those generated from bacterial metabolism increased although only at the first stages of processing. The consumers preference in aroma and overall quality of high and medium fat sausages was related to the aroma compounds hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol who contributed with green, medicinal, tallowy, fruity and mushroom notes.

Keywords: fermented sausages, low fat, lipolysis, aroma compounds.

1. Introduction

The high fat content of dry fermented sausages (40-50 %) is essential for sensory properties, such as hardness, juiciness and flavour, and also is responsible for technological functions (Wirth, 1988). However, from a health point of view, an excessive fat intake is not recommended. For this reason, several authors have focused in the reduction and partial substitution of fat in dry fermented sausages (Mendoza, García, Casas & Selgas, 2001; Muguerza, Fista, Ansorena, Astiasarán & Bloukas, 2002; Muguerza, Ansorena, Bloukas & Astiasarán, 2003; Liaros, Katsanidis & Bloukas, 2009; Olivares, Navarro, Salvador & Flores, 2010).

Low fat sausages become hard due to high weight losses and have unacceptable appearance because of wrinkled surfaces and case hardening (Muguerza et al., 2002). Nevertheless, Liaros et al. (2009) proposed the use of vacuum packaging during ripening as an effective strategy to produce low fat fermented sausages without negative effect on the external appearance. However, high fat sausages still have the highest acceptability scores (Mendoza et al., 2001; Olivares et al., 2010) due to other sensory characteristics such as flavour.

Flavour formation in dry fermented sausages is mainly related to lipolysis (Gandemer, 2002) through the generation of free fatty acids (FFA) that are further subjected to lipid oxidation reactions producing a large variety of volatile compounds (Zanardi, Ghidini, Battaglia & Chizzolini, 2004). Although the role of fat as precursor of aroma compounds is known, there is little information about the effect of fat content on dry fermented sausages flavour. Fat is important for dry fermented sausage flavour not only for the generation of precursors of flavour compounds but also it acts as a solvent for aroma compounds (Leland, 1997).

However, the reduction of fat in fermented sausages has given controversial results in relation to flavour. While Mendoza et al. (2001) and Olivares et al. (2010) obtained higher aroma scores in high fat dry fermented sausages compared to low fat ones, other authors reported no differences in flavour (Muguerza et al., 2002; Liaros et al., 2009). Muguerza et al., (2003) reported an increase of the oxidation process and total volatile compounds in fat reduced sausages that was attributed to the higher intramuscular fat content of reduced fat products. Also, Chevance, Farmer, Desmond, Novelli, Troy & Chizzolini (2000) indicated that fat reduction in salami increased the release of odour compounds. Nevertheless, it has never been determined how fat content through the generation of volatile compounds affects consumer's acceptance.

Therefore, the aim of this work was to study the effect of fat content on lipid changes and headspace volatile compounds during the processing of dry fermented sausages. Moreover, the ripened sausages were evaluated by consumers in order to determine which aroma compounds are responsible for consumers' acceptability.

2. Materials and Methods

2.1 Dry fermented sausages

Three batches of dry fermented sausages with different pork back fat contents (10 %, 20 % or 30 %) were manufactured; low fat (LF), medium fat (MF) and high fat (HF) respectively. The manufacture process was done as described by Olivares et al. (2010). Briefly, the lean pork and the pork back fat were minced and vacuum mixed with additives (sodium chloride, lactose, dextrin, sodium caseinate, glucose, sodium ascorbate, sodium nitrite and potassium nitrate) and commercial starter culture (*Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus* and *S. carnosus*). The meat mixture was stuffed into collagen casings (75-80 mm diameter)

previously dipped in a solution of natamycin and potassium sorbate (Floracid N3, Ceylan, Spain) in order to prevent overgrowth of undesirable surface moulds.

From each batch (LF, MF and HF), 200 g of the meat mixture were collected at day 0. Also, at 9 and 18 d days of ripening and at two different final ripening times (42 and 63 d), four sausages from each batch were randomly removed from the storage chamber, sliced, vacuum packaged and frozen at -80 ºC to await analysis.

2.2 Lipolysis and lipid oxidation analyses.

The lipolysis was studied by the analysis of the free fatty acid (FFA) content. Total lipids were extracted from 5 g of minced sausage according to Folch, Lees & Stanley (1957) and the free fatty acids were separated from the lipid fraction using an ion exchange resin, as described by Needs, Ford, Owen, Tuckley & Anderson (1983). Heneicosanoic acid (C21:0) was used as the internal standard. FFAs were converted into fatty acid methyl esters (FAME) using boron fluoride-methanol (Sigma-Aldrich, Chemical Co., Milwaukee, WI) as the methylating reagent. Analysis of the FAME was carried out in a Fisons 8160 gas chromatograph (GC) equipped with a flame ionisation detector and a split injector (split ratio used 2:1). The capillary column was a CP-SIL 88 (Agilent, Las Rozas, Spain; 100 m, 0.25 mm i.d., 0.2 μm film thickness). The oven temperature program began at 140 ºC for 10 min, ramped to 190 ºC at 4 ºC/min, held at 190 ºC for 10 min, ramped to 220 ºC at 2 ºC/min, held at 220 for 5 min, ramped to 230 at 2 ºC/min, and finally, held at 230 ºC for 20 min. Helium was used as carrier gas at a linear velocity of 17.7 cm/s. Detector and injector temperatures were 240 and 220 ºC respectively. The individual FAME were identified by comparing their retention times with those of standard fatty acid methyl esters. For quantification, the response factors of the standard FAME with respect to the internal standard were used. FFA content was

expressed as mg per 100 g in dry matter (dm). Moisture content was determined according to the official method for analysis of meat products BOE (1979) by dehydration at 100 ºC until constant weight. The results were expressed as the mean of four replicates at each batch and sampling time

The lipid oxidation in the sausages was analysed by thiobarbituric acid reactive substances (TBARS) method as described by Bruna, Ordóñez, Fernández, Herranz and de la Hoz (2001), using tricloroacetic acid instead of perchloric acid as solvent. The results were expressed as mg malonaldehyde (MDA) per kg dm.

2.3 Analysis of headspace volatile compounds by SPME GC-MS.

The analysis of volatile compounds present in the headspace (HS) of the sausages was done as described Marco, Navarro & Flores (2004). The extraction was done using a solid phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA) with a 85 μm carboxen/polydimethylsiloxane StableFlex fibre (CAR/PDMS SF). 3 g of minced sausage was weighted into a 10 mL headspace vial, and 0.75 mg of BHT were added. The vial was left for 1 h in a thermoblock (J.P., Selecta, Barcelona, Spain) at 37 ºC for equilibration. The CAR/PDMS fibre was then exposed to the headspace for 3 h while maintaining the sample at 37 ºC. The identification and quantification of volatile compounds were performed in a gas chromatograph HP 7890A equipped with a HP 5975C mass selective detector (Hewlett Packard, Palo Alto, CA). The compounds adsorbed by the fibre were desorbed in the injection port of the GC for 15 min at 220 ºC with the purge in splitless mode. Then, the compounds were separated using a DB-624 capillary column J & W Scientific (Agilent Technologies, USA) (30 m, 0.25 mm i.d., film thickness 1.4 μm). The GC oven temperature program began at 38 °C, held for 13 min, ramped to 110 °C at 3 °C/min, then to 150 °C at 4 °C/min1 and to 210 °C at 10

°C/min, and, finally, held at 210 °C for 5 min. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range 29–400 amu (scan mode). The compounds were identified by comparison with mass spectra from the library database (Nist' 05), kovats retention index (Kovats, 1965) and by comparison with authentic standards. The standards used for the identification were all obtained from Fluka Chemie AG (Buchs, Switzerland) except 2-methyl furan, 2-nonenal, diacetyl, methyl ethyl sulphide, 3-methyl thiophene, 3-methyl-2-buten-1-ol, 2-methylpyrazine, 2,6-dimethylpyrazine, dimethyl tripsulphide, 3-methylthio-propanol, benzeneacetaldehyde, methyl acetate, methyl 2-hydroxy-propanoate, methyl 3-hexenoate, methyl heptanoate, ethyl 2,4-hexadienoate, 2,4-hexadienoic acid and 4-methyl-phenol which were obtained from Aldrich (St. Louis, MO). Quantification was based on the total extracted area (TIC) or the area of a target ion when different compounds coeluted. The results were expressed in dry matter as abundance units (AU) 10^{-6} per g dm and comprised the mean of three replicates at each batch and sampling time.

2.4 Gas chromatography-olfactometry

The volatile compounds were adsorbed by the SPME fibre as described above but using 4 g of sausage from LF and HF batches at 63 d of ripening. Then, the fibre was desorbed in the gas chromatograph (Agilent 6890, USA) injection port for 15 min at 240 ºC in splitless mode, the split valve was opened after 1 min. The compounds were separated using a DB-624 capillary column (60 m, 0.32 mm i.d., film thickness 1.8 µm). The capillary column was split (2:1) into deactivated and uncoated capillary tubing connected with the sniffing port (ODP3, Gerstel, Mülheim an der Ruhr, Germany) and flame ionization detector (FID), respectively. The sniffing port ODP3 was equipped with a humidified air make up and a computer voice recorder integrated in the

154 Chemstation software (Agilent, USA). Helium was used as the carrier gas with a linear
155 velocity of 35.14 cm/s. The oven temperature program began at 38 ºC for 13 min,
156 ramped to 100 ºC at 3 ºC/min and maintained at 100 ºC during 10 min, then ramped to
157 150 ºC at 3 ºC/min, ramped to 210 ºC at 5 ºC/min, and finally held at 210 ºC for 20 min,
158 the total run time was 82.3 min. Detector temperature was set at 240 ºC.

159 The detection frequency method was used to estimate the aromatic impact of
160 each volatile compound (Pollien, Ott, Montigon, Baumgartner, Muñoz-Box &
161 Chaintreau, 1997). Four trained assessors evaluated the odors from the GC-effluent.
162 Each assessor evaluated two high fat and two low fat sausages (63 d of ripening),
163 therefore a total of 16 assessments were carried out. The final detection frequency value
164 (DF) for each compound was obtained by summation of the 16 sniffings. The detection
165 of an odor by less than three assessors was considered to be noise, therefore the
166 minimum DF value was 4 and the maximum was 16. For each assessment, evaluation
167 of the odor took place over two different time intervals (0-35 and 35-70 min) in order to
168 avoid olfactory fatigue of the assessors. Aroma compounds were identified by three
169 different ways; comparison with mass spectra, comparison with the Kovats retention
170 indices of authentic standards injected in the GC-MS and GC-O; and by coincidence of
171 the assessors' descriptors with those in the Fenaroli's handbook of flavour ingredients
172 (Burdock, 2002).

173

174 ***2.5 Sensory analysis***

175 At the end of the process (42 and 63 d), dry fermented sausages were tested by a
176 panel of 75 consumers. The analysis was carried out in a sensory laboratory equipped
177 with individual booths (ISO 8589, 1988). The casing was removed and the sausages
178 were cut in slices of approximately 4 mm thickness and served at room temperature on

white plastic dishes. Water and unsalted toasts were provided to consumers to cleanse the palate between samples. Consumers tasted, in two different sessions, three samples (HF, MF and LF) of two different ripening times (42 and 63 d) identified with random, three-digit codes, following a balanced complete block experimental design. For each sample, consumers scored the aroma and overall quality using a 9-box hedonic scale. Data acquisition was performed using Compusense five release 5.0 software (Compusense Inc., Guelph, Ont., Canada).

2.6 Statistical analysis

Two-way analysis of variance (ANOVA) (ripening time, fat content and interaction of ripening time and fat content) was performed on lipid, volatile compounds and sensory parameters to evaluate differences among samples. Differences between particular sample means were analysed according to Fisher's least significant difference (LSD) test. A correlation procedure was performed to evaluate any relationship among lipolysis, lipid oxidation and volatile compounds. Furthermore, principal component analysis (PCA) was used to find the relationships among sausages with different fat content and ripening time (LF, MF and HF at 42 and 63 d of processing) and the parameters related to lipid changes (FFA and TBARS), aroma-active volatile compounds and sensory analysis (aroma and overall quality). Statistical analysis was performed using the statistical software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain).

3 Results

3.1 Lipolysis and lipid oxidation.

The fat content of the sausages was different among batches and at 63 d of processing was 22.0 ± 0.9 in LF, 24.1 ± 1.8 in MF and 28.4 ± 1.2 in HF. Also the chemical composition of the three batches during ripening was reported in Olivares et al. (2010). The levels of FFA in LF, MF and HF sausages during processing were shown in table 1. At day 0, no differences in total FFA concentration were detected among batches and the total FFA concentration ranged from 162 to 220 mg / 100 g dm, which represented 0.38-0.58 % of the total lipid content. The total FFA levels increased during processing as a result of lipolysis reactions ($p < 0.01$). At the end of the processing, total FFA concentration was significantly higher ($p < 0.01$) in high fat sausages (1257 mg / 100 g dm) than in low and medium fat sausages (977 and 1081 mg / 100 g dm, respectively) although total FFA content represented a similar percentage (2.9 %) of the total lipid content in the three batches. Therefore, total FFA levels were proportional to the amount of pork back fat used in the manufacture of the sausages. These results are in agreement with Molly Demeyer, Civera, & Verplaetse (1996) and Marco, Navarro & Flores (2006) who reported that the major proportion of FFA comes from triglycerides present in the subcutaneous fat.

At day 0, FFA in all batches maintained the relationship MUFA>SFA>PUFA as previously observed Navarro, Nadal, Nieto & Flores (2001) in fresh meat paste. During ripening, all the main FFA increased ($p < 0.01$), however PUFA showed a greater release than SFA in all batches resulting in a free MUFA>PUFA>SFA profile previously reported in dry fermented sausages (Molly et al., 1996; Navarro et al., 2001; Zanardi et al, 2004; Marco et al., 2006). With respect to fat content, differences among batches were detected at 42 and 63 d, since the release of all FFAs was significantly higher ($p < 0.01$) in the HF sausage than in MF and LF sausages. In a previous work, Soyer and Ertas (2007) pointed out that high fat fermented sausages showed greater

lipolysis than reduced fat ones, as we also observed. In addition, Molly et al. (1996) and Marco et al. (2006) indicated that there was a very high specific fatty acid (FA) release from the polar fraction compared to triglycerides (TG) when the release is expressed as percentage of the initial amount of FA, however, the majority of FFA were derived from the TG fraction that is the most abundant lipid fraction in sausages. Also, Molly et al. (1996) pointed out the specificity of lipases for the position 3 of the triglycerides molecules where unsaturated FA are predominantly placed.

The level of TBARS in the sausages was measured throughout the processing as an index of lipid oxidation (Figure 1). TBARS increased during fermentation and drying in all batches ($p < 0.001$) from approximately 0.3 to 1.3-1.7 mg MDA /kg dm. However, no differences among batches were detected until the end of the process, when HF sausages showed higher TBARS values than LF sausages ($p < 0.05$). The highest lipid oxidation values detected in high fat sausages was also reported by Soyer and Ertas, (2007) and Liaros et al., (2009) in contrast Muguerza et al. (2003) reported higher TBARS values in low fat sausages that they attributed to the higher intramuscular fat content of reduced-fat products.

In summary, the highest amount of pork back fat in HF sausages produced an increase in both lipolysis and lipid oxidation reactions. These reactions are related to flavour formation in dry sausages (Gandemer, 2002) by the generation of flavour precursors, free fatty acids. However, until now it has not been elucidated how fat can act not only as a source but also as a solvent of flavour compounds in dry sausages and so, how both facts affects consumer's acceptance. Therefore, it is essential to study the volatile compounds present in the headspace of sausages to determine the reasons for consumer's acceptance of hig fat sausages.

3.2 Generation of volatile compound during processing

The proportion of volatile compounds analyzed in this study depends on the stationary phase of the SPME fibre employed. The HS abundance and the volatile compounds profile can not be compared with other works in which other SPME fibres or other extraction techniques have been used. However, our extraction technique allows to determine the effect of the studied factors (fat content and ripening time) on the HS volatile compounds of sausages.

The analysis of the volatile compounds present in the HS of sausages gives an indication of the chemical and metabolic processes that occur during manufacture. In this sense, the volatile compounds listed in table 2 were grouped according to their possible origin (Ordóñez, Hierro, Bruna & de la Hoz, 1999): lipid autooxidation, baterial metabolism (lipid β-oxidation, carbohydrate fermentation, amino acid degradation, and Staphylococci esterase activity), and unknown origin (derived from meat or food contaminants). However, several of the compounds can have more than one origin.

A total of 95 volatile compounds were extracted by SPME and identified by GC-MS in the HS of the sausages and 90 of them were confirmed using authentic standards (table 2). The mixture comprised 20 aldehydes, 17 esters, 15 hydrocarbons, 14 alcohols 11 ketones, 7 sulphur compounds, 6 acids, 3 furans and 2 pyrazines. All the compounds have been previously identified in dry fermented sausages except for ethyl 2,4-hexadienoate.

Lipid autooxidation was responsible for the generation of 36 volatile compounds comprising aldehydes, hydrocarbons, alcohols, acids and ketones (table 2). Ripening time affected ($p < 0.05$) the extracted area of all the compounds, except for nonane, 2-heptenal, 1-heptanol and 2,4-decadienal. Generally, the HS abundance of volatile

compounds increased until 42 d and then it maintained constant (figure 2a). At the end of the process (63 d), this group represented 20-25 % of the total extracted area. A similar evolution was seen in dry fermented sausages using the same extraction technique (Marco et al., 2006). Hexanal was the most abundant compound throughout processing, followed by hexanoic acid, octanoic acid, 4-hexen-1-ol, heptanal, pentanal, nonanal and decane. In relation to fat content, it affected the HS concentration of 21 out of 36 volatile compounds ($p < 0.05$) and generally, these 21 compounds showed the highest extracted areas in the high fat sausage. In addition, a positive relationship was found between FFA and the total extracted area of volatile compounds derived from lipid autooxidation ($p < 0.0001$, $r = 0.926$). For instance, the compounds 2-hexenal, 2-heptenal and 2,4-decadienal were significantly higher in HF sausages than in low fat ones at 42 and 63 d. These compounds are generated from the degradation of linolenic (C18:3) and linoleic (C18:2) acids which also showed the greatest concentration in high fat sausages at 42 and 63 d. Also, a significant correlation was detected between the TBARS values and the total extracted area of volatile compounds derived from lipid autooxidation ($p < 0.0001$, $r = 0.928$). As indicated above, these results are in contrast to Muguerza et al. (2003) who reported higher oxidation values (TBARS) and an increase of lipid oxidation products, such as aldehydes, in low fat ripened sausages than in high fat ones. Nevertheless, in our work a significant relationship among lipolysis, lipid oxidation and fat content was found, and the greatest lipolysis and oxidation were observed in HF sausages.

The HS abundance of volatile compounds produced by β-oxidation of lipids was affected by processing time ($p < 0.01$) except for 2-octanone. The HS abundance increased drastically until 18 d and then it remained constant (figure 2b). Fat content affected the HS abundance of 2,3-pentanedione, 2-heptanone, 1-octen-3-ol, 2-nonanone

303 and 2-undecanone ($p < 0.05$). At 18 d, LF sausages showed significantly higher
304 extracted area than high fat ones for 2-heptanone and 2-nonanone. Also, at the end of
305 the process (42 and 63 d) LF sausages showed the greatest abundance of 2-nonanone
306 and 2-undecanone, while HF sausages showed the highest abundance of 2,3-
307 pentanedione and 1-octen-3-ol. The highest content of fat in HF sausages, and therefore
308 of free fatty acids, was the reason to find a high proportion of lipid β-oxidation
309 degradation products such as 1-octen-3-ol. Also Bovolenta et al. (2008) detected mould
310 flavour which they attributed to 1-octen-3-ol in high fat sausages. However, other
311 compounds derived from lipid β-oxidation showed the highest abundance in LF
312 sausages. These results probably mean that lipid β-oxidation depends not only in the
313 amount of substrate but in the environmental conditions for bacterial growth which
314 were more favourable in LF sausages that had the highest water content (Olivares et al.,
315 2010).

316 The area of volatile compounds coming from carbohydrate fermentation was
317 affected by both processing time and fat reduction ($p < 0.01$) (table 2), except for 3-
318 hydroxy-2-butanone that was not affected by fat content. The extracted area increased
319 drastically after 9 d until 18 d, especially in LF sausages, and then it decreased until the
320 end of the process (figure 2c) when this group comprised 30-40 % of the total extracted
321 area. The most abundant compound was acetic acid, followed by butanoic acid,
322 acetaldehyde and ethanol. These compounds showed greater abundance in LF sausages
323 except for ethanol that had the largest abundance in HF sausages. Carbohydrate
324 fermentation by microorganisms takes place during the first days of processing and
325 produces the pH decline and the generation of volatile compounds. Olivares et al.
326 (2010) reported that fat reduction produced a faster pH decrease in low fat sausages at
327 the beginning of the process, although no differences were observed in further stages. In

328 our study, volatile compounds derived from carbohydrate fermentation showed greater
329 abundance in LF sausages at the beginning of the manufacture that is in agreement with
330 the higher lean and water content in LF sausages producing a faster carbohydrate
331 fermentation process.

332 The evolution of volatile compounds derived from amino acid degradation was
333 shown in fig 2d. Ripening time ($p < 0.01$) significantly affected the extracted area
334 except for dimethyl trisulphide and 3-methylthio-propanol that were not detected at the
335 earliest stages. The abundance increased until day 18 and thereafter it was maintained
336 during the ripening process. Moreover, fat content affected the HS abundance of this
337 group ($p < 0.05$) except for 2-methylpyrazine, 2,6-dimethylpyrazine, dimethyl
338 trisulphide, benzeneacetaldehyde and phenyl ethyl alcohol. During sausage ripening,
339 proteins of the lean tissue are subjected to hydrolysis producing free amino acids which
340 are transformed into different volatile compounds (Toldrá, Sanz and Flores, 2001). A
341 sharp increase was observed at 18 d mainly due to the generation of 3-methylthiophene,
342 3-methyl-2-buten-1-ol and benzaldehyde. However, the differences among batches for
343 almost all compounds of this group were more marked at the longest ripening time (63
344 d) when LF sausages, which contained the largest proportion of lean meat, showed
345 higher abundance than MF and HF sausages (table 2).

346 Esterase activity of Staphylococci produced 15 ester compounds, 12 methyl and
347 3 ethyl esters (table 2). Ripening time affected the HS abundance of all esters except for
348 methyl hexanoate. During processing, it was detected an increase in the HS abundance
349 which reached a maximum at 18 d, and then, a slow decrease (figure 2e). The most
350 abundant esters extracted by SPME were methyl acetate, methyl butanoate, methyl 2-
351 hydroxy-propanoate, methyl hexanoate and methyl octanoate (table 2). On the other
352 hand, fat content significantly affected ($p < 0.05$) the extracted area of all esters except

353 for methyl acetate, methyl propanoate, methyl 2-hydroxy-propanoate and methyl
354 decanoate. Generally, LF and MF sausages showed greater abundance of methyl esters
355 in the first stages although few differences were detected among batches at the end of
356 the process. In contrast, ethyl esters were extracted in higher amounts in the HF
357 sausages at days 42 and 63. Esters have very low odour detection thresholds
358 contributing to the aroma with fruity notes (Stahnke, 1994). Ethyl esters showed lower
359 abundance than methyl esters, however, ethyl esters have lower air thresholds than
360 methyl esters (Burdock, 2002) which result in a higher aroma impact, especially in HF
361 sausages where the highest abundance was detected.

362 With respect to those volatiles of unknown origin (figure 2f) it was observed a
363 significant effect of ripening time ($p < 0.01$) in all cases expect for tetradecane since the
364 extracted areas increased until 18 d and then decreased. Moreover, fat content also
365 affected the extracted area of this group ($p < 0.05$) that generally was the highest in LF
366 sausages during the first stages of processing, with the exception of p-xylene, methyl
367 2,4-hexadienoate and tetradecane that were unaffected. It was remarkable the detection
368 of 2,4-hexadienoic acid (sorbic acid) and also its ethyl and methyl esters that came from
369 the mixture of sorbic acid and natamycin applied to the sausage casing to prevent
370 desirable surface molds (Holley, 1981) as previously described. The compounds, 2,4-
371 hexadienoic acid and methyl 2,4-hexadienoate, have already been reported in other dry
372 fermented sausages (Mateo and Zumalacárregui, 1996; Muguerza et al., 2003).

373 Generally, the volatile compounds coming from bacterial metabolism showed
374 similar generation trends (figure 2). Volatile compounds derived from lipid β-oxidation
375 (fig 2b), carbohydrate fermentation (fig 2c) and amino acid degradation (fig 2d) showed
376 a higher abundance in LF sausages in the first stages of processing (9 and 18 d) than HF
377 sausages. This behaviour is related to the faster pH decrease and higher lean and

moisture content detected in LF sausages (Olivares et al., 2010). The moisture content would favour the fermentation stage producing a faster pH decline, however after 18 d no differences were observed among batches in the HS abundance of volatile compounds coming from bacterial metabolism.

With respect to the role of fat in fermented sausages, it is well known that it acts as a solvent for flavour compounds and thus delays their release, particularly for lipophilic compounds (Leland, 1997). When fat is reduced, different flavour profiles may result. According to this, it was reported that the release of spice- and smoke-derived volatile compounds such as terpenes and phenols was higher in low fat frankfurter and salami (Chevance and Farmer, 1998; Chevance et al., 2000). The sausages used in our study were not smoked and did not contain spices in order to avoid interferences in the volatile compounds analysis. However, dry fermented sausages are meat products subjected to a ripening process where numerous metabolic and chemical processes occur. Therefore, in the case of dry fermented sausages with different fat contents, fat by itself affects flavour not only due to its role as a solvent but also as a flavour precursor.

In conclusion, the effect of fat reduction on volatile generation during processing was seen in two facts. First, lower generation of volatile compounds derived from lipid oxidation reactions during the whole ripening process and second, a higher generation of volatile compounds derived from lipid β-oxidation, carbohydrate fermentation and amino acid degradation during the fermentation stage, although at the end of the process fat reduction did not affect the volatile abundance of volatile compounds produced by bacterial metabolism.

3.3 Volatile compound analysis by GC-O

In order to determinate how fat reduction affects the aroma of sausages, GC-O analyses were applied in the sausages and 30 different aroma-active zones were detected (table 3). The aroma compounds detected are present at a concentration higher than its threshold value contributing to the aroma of sausages. The use of detection frequency (DF) value gives information about the contribution of each compound to the aroma. In this sense, those compounds showing the maximum DF value (16) were always detected by the panellist being important for the sausage aroma, such as acetic acid (vinegar), 1-octen-3-ol (mushroom) and 2-nonenal (medicinal). Also, ten of the 23 aroma compounds identified presented DF values higher than 12. Those compounds were hexanal (fresh cut grass), pentyl furan (meath broth, savory), 2-octanone (floral, geranium), butanoic acid (cheese), methional (cooked potato), methyl 3-methyl butanoate (fruity, strawberry, sweet) and 4-methyl-phenol (stable). Also, two unknown compounds showed DF equal to 15 and were described as onion, garlic-like (unknown 3) and roasted nuts (unknown 5). All the aroma compounds identified have already been detected as odour active compounds in dry fermented sausages (Schmidt and Berger, 1998; Meynier, Novelli, Chizzolini, Zanardi & Gandemer, 1999; Marco, Navarro & Flores 2007; Söllner and Schierberle, 2009), except for methyl ethyl sulphide, methyl 2-hydroxy-propionate, methyl 3-methyl-butanoate, 2-octanone and methyl octanoate.

3.4 Sensory analysis

The consumer sensory analyses were carried out at 42 and 63 d and results are shown in table 4. There were not differences in aroma and overall quality due to ripening time. Fat content did not affect aroma and overall quality at 42 d, although significant differences were detected at 63 d as MF and HF sausages showed the highest acceptance in aroma and overall quality. The highest acceptance of high fat sausages

has been also reported by Mendoza et al. (2001) although Liaros et al. (2009) did not appreciate differences.

3.5 Principal component analysis (PCA)

In order to establish which aroma compounds are responsible for the highest acceptance of MF and HF sausages, a principal component analysis (PCA) was performed using the following parameters; lipolysis (FFA), lipid oxidation (TBARS), aroma compounds abundance and sensory attributes (aroma and overall quality) at the two ripening times (42 and 63 d). Only the aroma active volatile compounds reported in table 3 were selected for the analysis. Results from PCA applied to mean scores of the parameters are summarized in figure 3. The PCA showed that about 68.9% of the variability was explained by two first principal components. Principal component 1 (PC 1) was the most important variable in terms of differences among samples as it accounted for 44.2% of the total variability. PC1 was positively related with TBARS value, free fatty acids (SFA, MUFA and PUFA), lipid content, the aroma compounds; hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol and the sensory parameters; aroma and overall quality. In addition, PC 1 was inversely related to protein content and the aroma compounds methanethiol, methional, 2-methyl-propanal, 2-octanone and octanoic acid. High fat sausages at both ripening times (HF-42 and HF-63) had the greatest component 1 value therefore, they were related to the consumers acceptance. In contrast, LF sausages at both ripening times (LF-42 and LF-63) were inversely correlated to aroma and overall quality while MF (MF-42 and MF-63) sausages were in an intermediate position. These differences were also observed in the sensory analysis (table 4). On the other hand, principal component 2 (24.73%) was positively related to the aroma compounds; 3-methyl-butanal, methyl 3-methyl-

butanoate, octanal, benzeneacetaldehyde and methyl 2-hydroxy-propanoate and inversely to 4-methyl-phenol, methyl octanoate, acetic acid, methyl ethyl sulphide and 2-pentyl-furan. Sausages with 63 d of ripening were on the positive PC 2 axis while those with 42 were in the negative PC 2 axis.

In conclusion, PC1 differentiated the sausages based on fat content while PC2 on ripening time. Fat content was related to the aroma compounds hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol which were more abundant in the HS sausages followed by MF sausages and they contributed to the aroma with green, medicinal, tallowy, fruity and mushroom notes. On the other hand, ripening time was related to the aroma compounds 3-methyl-butanal, methyl 3-methyl-butanoate, octanal, benzeneacetaldehyde and methyl 2-hydroxy-propanoate which were more abundant in the sausages with longer ripening times (63 d) and they contributed to the aroma with green, strawberry, citrus, roses and green-fresh notes.

4. Conclusions

In summary, fat reduction in dry fermented sausages decreased lipolysis, lipid oxidation and lipid derived volatile compounds during processing while the volatile compounds generated from bacterial metabolism increased although only at the first stages of processing. The sensory analysis revealed that consumers prefer the aroma and overall quality of high and medium fat sausages at longer ripening times (63 d). The aroma compounds associated with the highest consumers' acceptability of high fat sausages were hexanal, 2-nonenal, 2,4-nonadienal, ethyl butanoate and 1-octen-3-ol who contributed to the aroma with green, medicinal, tallowy, fruity and mushroom notes. The results obtained in this work indicate that consumer acceptance is strongly linked to high fat content and ripening time, although fat content can be reduced until

20 % as medium fat sausages (MF) were not differentiate by consumers from HF sausages.

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589

590 ***Figure legends***

591

592 **Figure 1**. Levels of TBARS (mg MDA/kg dm) during processing of dry fermented
593 sausages manufactured with different pork back fat contents; low fat (LF, □), medium
594 fat (MF, ○) and high fat (HF, Δ). Symbols represent the mean and standard error of the
595 mean.

596

597 **Figure 2**. Levels of total volatile compounds extracted from the HS of dry fermented
598 sausages grouped according to their origin. Volatile compounds coming from: a) lipid
599 autooxidation; b) lipid β-oxidation; c) carbohydrate fermentation; d) amino acid
600 degradation; e) staphylococci esterase activity; d) unknown origin or contaminants. Low
601 fat sausages (LF, □) medium fat sausages (MF, ○) and high fat sausages (HF, Δ).
602 Symbols represent the mean and standard error of the mean.

603

604 **Figure 3**. Loadings of the first two principal components (PC1-PC2) of the selected
605 variables for dry fermented sausages with different fat contents (low fat LF; medium fat
606 MF; high fat HF) at two different ripening times (42 and 63 d). The selected variables
607 were the aroma active compounds, TBARS values (TBARS), protein content (prot dm),
608 lipid content (lip dm), saturated free fatty acids (SFA), monounsaturated free fatty acids
609 (MUFA), polyunsaturated free fatty acids (PUFA), aroma and overall quality scored by
610 consumers.

611

Table

Table 1. Free fatty acid (FFA) concentrations (mg/100 g dm) of dry fermented sausages with different fat contents.

	0 days			9 days			18 days			42 days			63 days						
FFA[A]	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF	SEM[B]	Ps[C]	Pb	Psxb
C14:0	2.6 ^{h}	1.9 ^{h}	2.8 ^{h}	6.4 ^{g}	7.5 ^{efg}	8.9 ^{def}	6.6 ^{g}	9.3 ^{cdef}	11.1 ^{bc}	7.2 ^{fg}	9.4 ^{cde}	12.5 ^{ab}	9.8 ^{cd}	11.4 ^{bc}	13.9 ^{a}	0.82	**	**	*
C16:0	48.7 ^{d}	34.7 ^{d}	44.2 ^{d}	94.5 ^{c}	101.0 ^{c}	111.4 ^{c}	101.5 ^{c}	107.1 ^{c}	112.3 ^{c}	102.2 ^{c}	115.4 ^{bc}	142.4 ^{ab}	141.7 ^{ab}	150.9 ^{a}	167.1 ^{a}	11.1	**	**	ns
C18:0	22.7 ^{g}	16.8 ^{g}	22.8 ^{g}	41.6 ^{f}	43.3 ^{f}	47.9 ^{ef}	57.2 ^{de}	51.5 ^{def}	53.2 ^{def}	53.7 ^{def}	62.6 ^{cd}	77.3 ^{ab}	73.4 ^{bc}	77.7 ^{ab}	87.6 ^{a}	4.7	**	**	ns
SFA	74.1 ^{f}	53.4 ^{f}	69.8 ^{f}	142.6 ^{e}	151.9 ^{de}	168.2 ^{de}	165.3 ^{de}	167.9 ^{de}	176.6 ^{de}	163.2 ^{de}	187.4 ^{cd}	232.2 ^{ab}	225.0 ^{bc}	240.0 ^{ab}	268.6 ^{a}	16.5	**	**	ns
C16:1	6.8 ^{h}	4.9 ^{h}	6.4 ^{h}	17.9 ^{g}	19.4 ^{g}	21.2 ^{fg}	21.0 ^{fg}	25.7 ^{def}	27.7 ^{de}	22.5 ^{efg}	28.0 ^{d}	36.6 ^{ab}	30.9 ^{cd}	35.1 ^{bc}	41.4 ^{a}	2.0	**	**	*
C18:1	89.3 ^{j}	65.8 ^{j}	87.3 ^{j}	205.4 ^{i}	229.1 ^{hi}	255.1 ^{ghi}	293.9 ^{fgh}	304.6 ^{fg}	324.6 ^{ef}	306.1 ^{fg}	377.8 ^{de}	502.7 ^{ab}	423.3 ^{cd}	479.5 ^{bc}	565.1 ^{a}	25.3	**	**	ns
C20:1 n9	1.6 ^{hi}	1.0 ^{i}	1.5 ^{hi}	3.2 ^{gh}	3.8 ^{h}	4.2 ^{fg}	6.7 ^{de}	5.6 ^{ef}	6.1 ^{e}	8.0 ^{d}	9.8 ^{c}	14.0 ^{b}	11.1 ^{c}	14.6 ^{b}	17.6 ^{a}	0.5	**	**	**
MUFA	97.7 ^{j}	71.7 ^{j}	95.1 ^{j}	226.5 ^{i}	252.4 ^{hi}	280.5 ^{ghi}	321.6 ^{fgh}	335.9 ^{fg}	358.4 ^{ef}	336.7 ^{fg}	415.6 ^{de}	553.3 ^{ab}	465.4 ^{cd}	526.0 ^{bc}	624.1 ^{a}	27.6	**	**	ns
C18:2 n6	36.7 ^{g}	27.3 ^{g}	40.2 ^{g}	99.4 ^{f}	100.9 ^{f}	110.8 ^{f}	148.4 ^{e}	157.8 ^{de}	165.2 ^{de}	156.9 ^{e}	186.3 ^{d}	250.3 ^{b}	216.3 ^{c}	237.9 ^{bc}	280.1 ^{a}	10.5	**	**	**
C18:3 n3	1.6 ^{g}	1.2 ^{g}	1.9 ^{g}	4.9 ^{f}	5.1 ^{f}	6.1 ^{f}	8.6 ^{e}	8.8 ^{e}	10.2 ^{de}	9.3 ^{e}	11.2 ^{d}	16.1 ^{b}	12.9 ^{c}	14.5 ^{bc}	18.0 ^{a}	0.7	**	**	**
C20:2 n6	1.0 ^{g}	0.7 ^{g}	1.0 ^{g}	2.6 ^{f}	2.8 ^{f}	3.2 ^{f}	5.3 ^{de}	5.0 ^{e}	5.1 ^{e}	6.3 ^{d}	8.0 ^{c}	11.4 ^{b}	9.0 ^{c}	10.5 ^{b}	12.9 ^{a}	0.4	**	**	**
C20:3 n6	0.8 ^{g}	0.8 ^{g}	1.0 ^{g}	2.1 ^{f}	1.9 ^{f}	2.0 ^{f}	3.4 ^{de}	3.0 ^{e}	2.9 ^{e}	4.0 ^{cd}	4.4 ^{c}	5.5 ^{b}	5.5 ^{b}	5.8 ^{ab}	6.2 ^{a}	0.2	**	**	*
C20:4 n6	6.0 ^{h}	4.4 ^{h}	6.3 ^{h}	12.8 ^{g}	12.4 ^{g}	12.1 ^{g}	20.7 ^{de}	18.2 ^{ef}	16.2 ^{f}	21.5 ^{de}	23.0 ^{cd}	25.5 ^{bc}	28.6 ^{ab}	30.4 ^{a}	30.6 ^{a}	1.1	**	ns	ns
C22:4 n6	0.7 ^{g}	0.6 ^{g}	0.8 ^{g}	1.5 ^{f}	1.5 ^{f}	1.4 ^{f}	2.4 ^{e}	2.2 ^{e}	1.7 ^{f}	2.9 ^{d}	3.4 ^{c}	3.6 ^{c}	3.8 ^{bc}	4.1 ^{ab}	4.4 ^{a}	0.1	**	ns	ns
C20:5 n3	0.2 ^{h}	0.1 ^{h}	0.2 ^{gh}	0.5 ^{fg}	0.5 ^{fg}	0.4 ^{fg}	0.6 ^{def}	0.7 ^{cde}	0.5 ^{ef}	0.8 ^{bc}	0.5 ^{def}	0.7 ^{cd}	0.9 ^{abc}	1.0 ^{ab}	1.1 ^{a}	0.1	**	ns	ns
C22:5 n3	1.5 ^{i}	1.3 ^{i}	2.0 ^{hi}	3.3 ^{fg}	2.7 ^{gh}	3.4 ^{fg}	6.9 ^{d}	4.7 ^{e}	4.0 ^{ef}	6.5 ^{d}	7.0 ^{d}	8.1 ^{c}	8.5 ^{bc}	9.2 ^{ab}	9.5 ^{a}	0.3	**	ns	**
C22:6 n3	0.4 ^{fg}	0.3 ^{g}	0.5 ^{fg}	0.7 ^{efg}	0.5 ^{fg}	0.8 ^{def}	1.4 ^{abc}	1.1 ^{cd}	0.9 ^{de}	1.3 ^{bc}	1.2 ^{cd}	1.4 ^{abc}	1.7 ^{a}	1.6 ^{ab}	1.7 ^{ab}	0.1	**	ns	ns
PUFA	48.9 ^{g}	36.7 ^{g}	53.8 ^{g}	127.9 ^{f}	128.2 ^{f}	140.3 ^{f}	197.8 ^{e}	201.4 ^{e}	206.7 ^{e}	205.3 ^{e}	245.0 ^{d}	322.5 ^{b}	287.2 ^{c}	315.0 ^{bc}	364.3 ^{a}	13.2	**	**	*
Total FFA	220.7 ^{h}	161.9 ^{h}	218.7 ^{h}	497.0 ^{g}	532.5 ^{g}	589.0 ^{fg}	684.7 ^{ef}	705.2 ^{def}	741.8 ^{de}	705.1 ^{def}	848.0 ^{cd}	1108.1 ^{b}	977.6 ^{bc}	1081.0 ^{b}	1257.0 ^{a}	56.3	**	**	ns
Fat (g/100g)																			

[A] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[a-j]: Identical letters in each parameter indicate the absence of significant differences at $p>0.05$ (Fisher's test).

[B] SEM: Standard error of the mean.

[C] P_s: P value of ripening time effect; P_b: P value of fat content effect; P_{sxb}: P value of interaction between fat content and ripening time effects. **: P< 0.01, *: P<0.05, ns: P>0.05

Table 2. Volatile compounds quantified as AU x 10-6 per g dry matter in the headspace of dry fermented sausages during processing.

N[A]	Compound/origin	KI[B]	R[C]	0 d			9 d			18 d			42 d			63 d			Ps[D]	Pb	Psxb
				LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF			
	Lipid autooxidation																				
3	Pentane	500	a				0.41 ^{f}	0.49 ^{ef}		0.50 ^{ef}	0.82 ^{de}		1.03 ^{cd}	2.34 ^{b}	2.55 ^{ab}	0.97 ^{cd}	1.37 ^{c}	3.41 ^{a}	**	**	**
5	Propanal	523	a				0.06 ^{d}	0.04 ^{d}	0.78 ^{c}	0.07 ^{d}	0.39 ^{d}	0.87 ^{c}	1.03 ^{bc}	1.80 ^{a}	1.93 ^{a}	1.23 ^{b}	1.73 ^{a}	2.02 ^{a}	**	**	ns
8	Hexane	600	a	0.19 ^{fghi}	0.16 ^{ghi}	0.11 ^{i}	0.14 ^{hi}	0.90 ^{a}	0.66 ^{bc}	0.50 ^{cd}	0.73 ^{ab}	0.52 ^{cd}	0.39 ^{def}	0.42 ^{de}	0.50 ^{cd}	0.29 ^{efgh}	0.37 ^{defg}	0.42 ^{de}	**	**	**
9	1-Propanol	611	a							0.04 ^{d}	0.07 ^{c}	0.11 ^{b}	0.02 ^{e}	0.07 ^{c}	0.08 ^{c}	0.02 ^{e}	0.01 ^{e}	0.14 ^{a}	*	**	**
10	2-Methyl-furan	615	a				1.35 ^{abc}	1.26 ^{abcd}	0.80 ^{ef}	1.51 ^{ab}	1.56 ^{a}	1.35 ^{abc}	0.96 ^{cdef}	1.12 ^{bcde}	0.77 ^{ef}	0.90 ^{def}	0.72 ^{f}	0.84 ^{ef}	*	ns	ns
11	Butanal	621	a				0.14 ^{a}	0.18 ^{cd}	0.16 ^{d}	0.16 ^{d}	0.11 ^{d}	0.16 ^{d}	0.31 ^{b}	0.32 ^{b}	0.46 ^{a}	0.27 ^{bc}	0.28 ^{bc}	0.35 ^{b}	**	*	ns
19	Heptane (71)	700	a	0.09 ^{b}	0.08 ^{b}		0.70 ^{b}	0.69 ^{b}	1.65 ^{a}	0.42 ^{b}	0.54 ^{b}	0.44 ^{b}	0.25 ^{b}	0.50 ^{b}	0.33 ^{b}	0.24 ^{b}	0.44 ^{b}	0.42 ^{b}	*	ns	ns
22	Pentanal	738	a				5.92 ^{c}	1.44 ^{e}	2.89 ^{cde}	5.09 ^{cd}	2.80 ^{de}	9.29 ^{b}	11.94 ^{ab}	11.54 ^{ab}	13.43 ^{a}	9.93 ^{b}	10.58 ^{ab}	11.33 ^{ab}	**	**	*
30	Octane	800	a	2.88 ^{e}	1.62 ^{e}	1.73 ^{e}	8.34 ^{cd}	11.03 ^{abc}	3.58 ^{e}	8.56 ^{cd}	9.36 ^{bcd}	9.56 ^{bcd}	7.94 ^{d}	11.60 ^{ab}	12.69 ^{a}	9.23 ^{bcd}	11.07 ^{abc}	11.08 ^{abc}	**	*	**
32	1-Pentanol	826	a	1.42 ^{efg}	0.93 ^{fg}	0.96 ^{g}	2.60 ^{acb}	2.43 ^{bcd}	2.99 ^{ab}	1.61 ^{def}	1.38 ^{efg}	2.81 ^{ab}	2.76 ^{abc}	1.89 ^{cde}	3.36 ^{a}	2.34 ^{bcd}	2.44 ^{abcd}	2.69 ^{abc}	**	**	ns
35	Hexanal	840	a	4.03 ^{e}	3.83 ^{e}	2.97 ^{e}	15.48 ^{e}	13.20 ^{e}	54.14 ^{cde}	36.06 ^{de}	19.46 ^{e}	70.26 ^{cd}	161.72 ^{ab}	84.50 ^{c}	187.19 ^{a}	139.02 ^{b}	142.94 ^{ab}	169.75 ^{ab}	**	**	ns
41	Nonane	900	a				0.35 ^{de}	0.56 ^{abc}	0.65 ^{ab}	0.26 ^{e}	0.48 ^{bcd}	0.66 ^{ab}	0.45 ^{cd}	0.64 ^{ab}	0.67 ^{a}	0.49 ^{bcd}	0.63 ^{abc}	0.63 ^{ab}	ns	**	ns
42	2-Hexenal (Z)	905	a				0.00			0.13 ^{d}	0.47 ^{bc}	0.39 ^{bc}	0.43 ^{bc}	0.31 ^{c}	0.89 ^{a}	0.42 ^{bc}	0.51 ^{b}	0.99 ^{a}	**	**	**
43	2-Butyl-furan	909	b				0.19 ^{bcd}	0.23 ^{bc}	0.18 ^{cd}	0.16 ^{cd}	0.14 ^{d}	0.18 ^{cd}	0.25 ^{ab}	0.13 ^{d}	0.20 ^{bcd}	0.26 ^{ab}	0.20 ^{bcd}	0.31 ^{a}	**	ns	*
45	1-Hexanol	923	a	0.39 ^{g}	0.38 ^{g}	0.33 ^{g}	5.61 ^{ef}	2.29 ^{g}	1.41 ^{g}	8.78 ^{de}	3.04 ^{fg}	10.35 ^{bcd}	12.32 ^{ab}	14.88 ^{a}	11.29 ^{b}	7.65 ^{cde}	5.91 ^{def}	10.20 ^{bc}	**	ns	**
46	4-Hexen-1-ol	927	b				3.21 ^{f}	1.16 ^{f}	2.36 ^{f}	7.46 ^{ef}	7.56 ^{ef}	49.62 ^{a}	11.20 ^{cde}	17.51 ^{c}	26.78 ^{b}	10.06 ^{de}	14.22 ^{cd}	31.73 ^{b}	**	**	**
48	Heptanal	940	a	1.33 ^{cd}	0.84 ^{d}	0.78 ^{d}	3.91 ^{cd}	2.54 ^{cd}	1.72 ^{cd}	4.67 ^{c}	2.48 ^{cd}	3.84 ^{cd}	13.78 ^{a}	14.71 ^{a}	9.90 ^{b}	12.00 ^{ab}	12.16 ^{ab}	13.37 ^{ab}	**	ns	ns
53	Decane	1000	a	3.27 ^{c}	3.76 ^{c}	6.37 ^{c}	11.82 ^{b}	14.62 ^{ab}	11.12 ^{b}	11.22 ^{b}	13.00 ^{ab}	17.11 ^{a}	11.24 ^{b}	12.50 ^{b}	14.21 ^{ab}	12.24 ^{b}	13.18 ^{b}	12.00 ^{ab}	**	ns	ns
55	2-Pentyl-furan (81)[E]	1009	a	0.31 ^{def}	0.15 ^{ef}	0.13 ^{f}	0.39 ^{de}	0.35 ^{def}	0.25 ^{def}	0.44 ^{cd}	0.29 ^{def}	0.38 ^{de}	0.89 ^{b}	0.86 ^{b}	1.22 ^{a}	0.75 ^{b}	0.71 ^{bc}	0.91 ^{b}	**	ns	ns
56	2-Heptenal (Z) (41)	1011	a							0.26 ^{ab}	0.20 ^{bc}	0.09 ^{e}	0.12 ^{de}	0.21 ^{bc}	0.32 ^{a}	0.13 ^{de}	0.18 ^{cd}	0.29 ^{a}	ns	**	**
58	1-Heptanol	1024	a				2.03 ^{ab}	1.83 ^{ab}	1.05 ^{b}	2.06 ^{ab}	2.20 ^{ab}	2.33 ^{ab}	1.64 ^{b}	1.59 ^{b}	1.57 ^{ab}	1.34 ^{b}	1.11 ^{a}	1.32 ^{b}	ns	ns	ns
63	Octanal	1048	a	0.09 ^{b}	0.08 ^{b}	0.03 ^{b}	0.14 ^{b}	0.12 ^{b}	0.10 ^{b}	0.22 ^{b}	1.80 ^{a}	0.15 ^{b}	0.59 ^{b}	0.45 ^{b}	0.44 ^{b}	0.51 ^{b}	0.45 ^{a}	0.56 ^{b}	*	*	ns
67	Hexanoic acid	1078	a	2.36 ^{f}	1.90 ^{f}	2.09 ^{f}	13.28 ^{d}	10.67 ^{d}	6.55 ^{e}	22.07 ^{ab}	20.52 ^{abc}	21.56 ^{abc}	20.69 ^{abc}	22.88 ^{a}	20.02 ^{abc}	21.81 ^{ab}	19.46 ^{bc}	18.69 ^{c}	**	**	ns
68	2-Ethyl-1-hexanol	1083	a				4.18 ^{a}	4.18 ^{a}	3.10 ^{bc}	4.03 ^{ab}	3.16 ^{bc}	3.19 ^{abc}	2.79 ^{cd}	3.10 ^{bc}	2.39 ^{cde}	2.35 ^{cde}	1.97 ^{de}	1.78 ^{e}	**	ns	ns
69	Undecane	1100	a	0.36 ^{d}	0.43 ^{d}	0.48 ^{d}	1.07 ^{bc}	1.19 ^{b}	0.84 ^{c}	1.28 ^{ab}	1.08 ^{bc}	1.54 ^{a}	1.31 ^{ab}	1.18 ^{b}	1.36 ^{ab}	1.12 ^{bc}	1.06 ^{bc}	1.22 ^{b}	**	ns	ns
72	2-Octenal (E)	1116	a	0.22 ^{ef}	0.19 ^{f}	0.11 ^{f}	0.60 ^{de}	0.42 ^{ef}	0.36 ^{ef}	0.58 ^{de}	0.44 ^{ef}	0.93 ^{d}	1.37 ^{bc}	0.48 ^{ef}	1.81 ^{b}	1.31 ^{c}	1.53 ^{bc}	2.24 ^{a}	**	**	**
73	1-Octanol	1124	a	0.44 ^{g}	0.50 ^{g}	0.29 ^{g}	1.52 ^{de}	1.47 ^{de}	1.06 ^{f}	1.57 ^{de}	1.08 ^{fg}	1.52 ^{de}	1.82 ^{cd}	1.42 ^{ef}	2.39 ^{b}	1.92 ^{cd}	1.78 ^{ef}	2.97 ^{a}	**	**	**
76	Nonanal	1149	a	3.48 ^{def}	3.30 ^{def}	1.88 ^{f}	5.15 ^{d}	4.21 ^{de}	2.54 ^{ef}	7.71 ^{c}	3.81 ^{def}	4.36 ^{de}	13.50 ^{a}	7.62 ^{c}	8.46 ^{c}	11.10 ^{b}	12.11 ^{ab}	11.73 ^{ab}	**	**	**
81	Dodecano	1200	a	0.43 ^{c}	0.53 ^{c}	0.57 ^{c}	1.15 ^{ab}	1.04 ^{ab}	1.01 ^{b}	1.25 ^{ab}	1.02 ^{ab}	1.10 ^{ab}	1.04 ^{ab}	1.17 ^{ab}	1.37 ^{a}	1.14 ^{ab}	1.05 ^{ab}	1.08 ^{ab}	**	ns	ns
82	2-Nonenal (Z)	1222	a	0.61 ^{de}	0.50 ^{e}	0.36 ^{e}	0.65 ^{cde}	0.65 ^{cde}	0.42 ^{e}	0.60 ^{de}	0.45 ^{e}	0.63 ^{de}	1.03 ^{ab}	0.87 ^{bcd}	1.19 ^{a}	0.93 ^{abc}	0.87 ^{bcd}	1.12 ^{ab}	**	ns	ns
85	Octanoic acid	1267	a	1.89 ^{g}	1.58 ^{g}	1.25 ^{g}	18.25 ^{e}	14.36 ^{e}	10.00 ^{f}	31.68 ^{abc}	25.75 ^{d}	26.03 ^{d}	34.40 ^{a}	28.33 ^{bcd}	27.42 ^{cd}	32.53 ^{ab}	28.33 ^{bcd}	26.07 ^{d}	**	**	ns
86	2,4-Nonadienal (Z, Z)	1287	b				0.00			0.12 ^{c}		0.09 ^{c}	0.18 ^{b}	0.21 ^{ab}	0.24 ^{ab}	0.21 ^{ab}	0.18 ^{b}	0.25 ^{a}	**	ns	ns
87	Tridecane	1300	a	0.18 ^{cd}	0.18 ^{cd}	0.14 ^{d}	0.26 ^{ab}	0.23 ^{abc}	0.19 ^{cd}	0.23 ^{abc}	0.20 ^{bc}	0.26 ^{a}	0.20 ^{c}	0.19 ^{cd}	0.23 ^{abc}	0.21 ^{ab}	0.22 ^{abc}	0.21 ^{abc}	**	ns	ns
98	2-Decenal (Z)	1327	a	0.25 ^{c}	0.27 ^{c}	0.16 ^{c}	0.34 ^{c}	0.26 ^{c}	0.26 ^{c}	0.31 ^{c}	0.21 ^{c}	0.24 ^{c}	0.62 ^{b}	0.60 ^{b}	0.64 ^{b}	0.66 ^{abc}	0.59 ^{b}	0.96 ^{a}	**	ns	*
92	2,4-Decadienal (Z, Z)	1392	a										0.20 ^{bc}	0.19 ^{bc}	0.29 ^{a}	0.18 ^{c}	0.19 ^{bc}	0.25 ^{ab}	ns	**	ns
94	Decanoic acid	1451	a	0.22 ^{f}	0.20 ^{f}	0.19 ^{f}	1.03 ^{e}	0.83 ^{e}	0.86 ^{e}	1.87 ^{cd}	1.56 ^{d}	1.91 ^{cd}	2.86 ^{a}	2.15 ^{bc}	2.74 ^{a}	2.80 ^{a}	2.45 ^{ab}	2.51 ^{ab}	**	*	ns
	Lipid b-oxidation																				
21	2-Pentanone	733	a	0.50 ^{d}	0.41 ^{d}	0.53 ^{d}	4.04 ^{a}	3.26 ^{b}	2.95 ^{bc}	2.89 ^{bc}	3.00 ^{bc}	2.72 ^{c}	0.51 ^{d}	0.75 ^{d}	0.60 ^{d}	0.57 ^{d}	0.70 ^{d}	0.54 ^{d}	**	ns	*
23	2,3-Pentanedione	743	a				0.20 ^{cd}	0.40 ^{bc}	0.30 ^{bcd}	0.17 ^{d}	0.26 ^{bcd}	0.37 ^{bcd}	0.33 ^{bcd}	0.30 ^{bcd}	0.96 ^{a}	0.41 ^{bcd}	0.49 ^{b}	0.87 ^{a}	**	**	**
47	2-Heptanone	934	a				7.32 ^{bc}	4.30 ^{e}	2.22 ^{f}	9.45 ^{a}	8.17 ^{ab}	7.58 ^{b}	5.68 ^{de}	6.10 ^{cd}	5.20 ^{de}	6.01 ^{cd}	5.40 ^{de}	5.16 ^{de}	**	**	**
59	2,3-Octanedione	1029	b				0.88 ^{de}		0.58 ^{e}	1.09 ^{cde}	1.34 ^{bcd}	1.40 ^{bcd}	1.42 ^{bcd}	0.82 ^{de}	1.55 ^{bc}	1.91 ^{ab}	2.41 ^{a}	2.39 ^{a}	**	ns	ns
60	1-Octen-3-ol	1031	a	0.50 ^{de}	0.38 ^{e}	0.45 ^{e}	1.78 ^{de}	1.32 ^{de}	0.95 ^{de}	1.65 ^{de}	0.96 ^{de}	2.03 ^{d}	4.15 ^{d}	6.01 ^{ab}	6.46 ^{a}	3.63 ^{c}	4.53 ^{bc}	6.05 ^{ab}	**	*	ns
61	2-Octanone	1039	a				0.34 ^{ab}	0.29 ^{ab}	0.17 ^{c}	0.38 ^{a}	0.28 ^{ab}	0.28 ^{ab}	0.33 ^{ab}	0.28 ^{ab}	0.26 ^{bc}	0.34 ^{ab}	0.28 ^{ab}	0.30 ^{ab}	ns	ns	ns
74	2-Nonanone	1141	a				3.79 ^{cd}	1.87 ^{e}	1.12 ^{e}	5.96 ^{a}	4.60 ^{bc}	4.28 ^{bcd}	4.94 ^{b}	4.12 ^{bcd}	3.65 ^{d}	4.65 ^{bc}	3.74 ^{cd}	3.90 ^{cd}	**	**	ns
89	2-Undecanone	1349	a							0.39 ^{bcde}	1.25 ^{a}	0.43 ^{bcd}	0.54 ^{b}	0.42 ^{bcd}	0.28 ^{de}	0.49 ^{b}	0.45 ^{bcd}	0.34 ^{cde}	**	**	**

	Carbohydrate fermentation																			
1	Acetaldehyde	466	a	18.06 ^{abc}	1.05 ^{g}	10.65 ^{f}	25.20 ^{a}	17.55 ^{bcd}	8.29 ^{f}	18.64 ^{bc}	16.37 ^{cd}	23.34 ^{ab}	16.61 ^{cd}	15.76 ^{cde}	13.41 ^{cdef}	14.03 ^{cdef}	13.80 ^{cdef}	8.86 ^{ef}	**	**	**
4	Ethanol	507	a		1.35 ^{e}	1.29 ^{e}	4.95 ^{c}	9.31 ^{b}	12.60 ^{a}	4.41 ^{cd}	9.86 ^{b}	13.21 ^{a}	3.34 ^{cde}	5.83 ^{c}	12.64 ^{a}	2.08 ^{de}	4.31 ^{cd}	9.67 ^{b}	**	**	*
13	2,3-Butanedione	626	a	0.18 ^{cde}	0.22 ^{c}	0.14 ^{c}	4.74 ^{a}	1.24 ^{b}	1.72 ^{b}	0.19 ^{c}	0.10 ^{c}	0.42 ^{c}	0.20 ^{c}	0.08 ^{c}	0.32 ^{c}	0.26 ^{d}	0.02 ^{c}	0.21 ^{c}	**	**	**
14	2-Butanone	630	a	4.53 ^{efg}	3.67 ^{g}	4.21 ^{f}	12.01 ^{ab}	13.14 ^{a}	10.09 ^{bc}	12.15 ^{ab}	10.83 ^{abc}	8.60 ^{cd}	6.35 ^{def}	7.11 ^{de}	4.35 ^{fg}	6.35 ^{def}	4.71 ^{efg}	3.83 ^{fg}	**	**	ns
20	Acetic acid	718	a				276.71 ^{f}	125.35 ^{g}	62.14 ^{g}	651.23 ^{a}	594.19 ^{ab}	505.19 ^{c}	451.95 ^{cd}	535.04 ^{bc}	419.52 ^{de}	415.44 ^{de}	390.26 ^{de}	349.52 ^{ef}	**	**	*
26	3-hydroxy-2-butanone	781	a		5.49 ^{c}	4.34 ^{c}	47.32 ^{a}	45.00 ^{a}	28.60 ^{b}	23.10 ^{b}	24.33 ^{b}	23.54 ^{b}	3.86 ^{c}	6.13 ^{c}	5.42 ^{c}	3.43 ^{c}	1.92 ^{c}	2.17 ^{c}	**	ns	ns
40	Butanoic acid	895	a	6.28 ^{f}	6.90 ^{f}		51.64 ^{b}	43.07 ^{cd}	30.64 ^{e}	69.38 ^{a}	72.73 ^{a}	64.95 ^{a}	36.81 ^{cde}	44.15 ^{bc}	35.97 ^{de}	33.52 ^{e}	33.72 ^{e}	31.44 ^{e}	**	**	*
	Amino acid degradation																			
7	2-Methyl-propanal	593	a				0.77 ^{ab}	0.69 ^{bcd}	0.50 ^{de}	0.95 ^{abcde}	0.76 ^{ab}	0.64 ^{bcd}	0.53 ^{cde}	0.62 ^{bcd}	0.40 ^{e}	0.68 ^{bcd}	0.59 ^{bcde}	0.49 ^{de}	**	**	ns
12	Methyl ethyl sulphide	624	a							0.49 ^{b}	0.23 ^{cd}	1.74 ^{a}	0.36 ^{bc}	0.25 ^{cd}	0.49 ^{b}	0.31 ^{bcd}	0.22 ^{cd}	0.16 ^{d}	**	**	**
17	3-Methyl-butanal	689	a				2.78 ^{c}	3.17 ^{bc}	2.45 ^{cd}	2.50 ^{cd}	2.37 ^{cd}	1.82 ^{d}	2.42 ^{cd}	2.32 ^{cd}	1.76 ^{d}	4.86 ^{a}	3.95 ^{b}	2.92 ^{c}	**	**	ns
18	2-Methyl-butanal (58)	699	a				0.13 ^{bcd}	0.13 ^{bcd}	0.15 ^{abc}	0.11 ^{cde}	0.11 ^{cde}	0.08 ^{e}	0.10 ^{de}	0.13 ^{bcd}	0.08 ^{e}	0.18 ^{a}	0.16 ^{ab}	0.13 ^{bcd}	**	*	ns
25	Dimethyl disulphide	773	a							1.09 ^{a}	1.02 ^{a}	0.54 ^{bcd}	0.31 ^{d}	0.72 ^{b}	0.42 ^{cd}	0.53 ^{bcd}	0.65 ^{bc}	0.54 ^{bcd}	**	**	**
27	Toluene	788	a	1.58 ^{gh}	1.43 ^{h}	1.04 ^{h}	2.92 ^{efg}	2.90 ^{efg}	1.80 ^{fgh}	3.23 ^{e}	3.02 ^{ef}	3.26 ^{e}	4.19 ^{de}	4.84 ^{d}	4.04 ^{de}	13.11 ^{a}	9.90 ^{b}	8.04 ^{c}	**	**	**
29	3-Methyl-thiophene	794	a				11.75 ^{def}	10.72 ^{def}	8.11 ^{ef}	28.35 ^{a}	20.74 ^{b}	15.23 ^{cd}	17.86 ^{bc}	13.40 ^{cde}	11.98 ^{de}	6.58 ^{f}	11.95 ^{de}	11.08 ^{def}	**	**	**
34	3-Methyl-2-buten-1-ol	834	a	0.82 ^{fg}	0.78 ^{g}	0.92 ^{efg}	3.01 ^{c}	1.38 ^{de}	1.59 ^{d}	6.21 ^{a}	5.77 ^{a}	4.27 ^{b}	1.26 ^{defg}	1.35 ^{def}	0.93 ^{efg}	0.90 ^{efg}	0.79 ^{g}	0.71 ^{g}	**	**	**
37	2-Methylpyrazine	860	a				0.10 ^{de}	0.10 ^{e}	0.21 ^{bc}	0.11 ^{de}	0.18 ^{c}	0.23 ^{b}	0.23 ^{b}	0.21 ^{bc}	0.14 ^{d}	0.35 ^{a}	0.24 ^{b}	0.24 ^{b}	**	ns	**
38	Ethylbenzene/2,3-Butanediol	883	a	0.37 ^{e}	0.42 ^{e}	0.42 ^{e}	3.41 ^{a}	2.62 ^{cd}	3.33 ^{ab}	3.49 ^{a}	3.00 ^{abc}	3.43 ^{a}	2.62 ^{cd}	2.69 ^{cd}	2.68 ^{cd}	2.82 ^{bcd}	2.35 ^{d}	2.68 ^{cd}	**	*	ns
49	2,6-Dimethylpyrazine	944	a							0.15 ^{cd}	0.05 ^{e}	0.18 ^{c}	0.14 ^{cd}	0.09 ^{de}	0.12 ^{cde}	0.41 ^{a}	0.39 ^{a}	0.31 ^{b}	**	ns	**
52	3-Methylthio-propanal	966	a	0.37 ^{fg}	0.22 ^{g}	0.31 ^{g}	0.90 ^{de}	0.40 ^{fg}	0.62 ^{efg}	0.78 ^{def}	0.47 ^{efg}	0.90 ^{de}	1.86 ^{a}	1.15 ^{cd}	1.39 ^{bc}	1.74 ^{ab}	1.40 ^{bc}	1.39 ^{bc}	**	**	ns
54	Dimethyl trisulphide	1002	a							0.19 ^{a}	0.06 ^{d}	0.08 ^{cd}	0.09 ^{cd}	0.13 ^{bc}	0.15 ^{ab}	0.14 ^{ab}	0.15 ^{ab}	0.13 ^{bc}	ns	ns	**
57	Benzaldehyde	1018	a	1.31 ^{f}	1.04 ^{f}	0.94 ^{f}	6.58 ^{b}	6.05 ^{b}	3.54 ^{e}	8.27 ^{a}	6.39 ^{b}	6.28 ^{b}	6.41 ^{b}	5.41 ^{bc}	4.49 ^{cde}	6.26 ^{b}	4.81 ^{cd}	3.79 ^{de}	**	**	*
66	3-Methylthio-propanol	1062	a										0.09 ^{c}	0.19 ^{a}	0.10 ^{c}	0.19 ^{a}	0.16 ^{ab}	0.12 ^{bc}	ns	**	**
70	Benzenacetaldehyde	1108	a							0.66 ^{cd}	0.44 ^{d}	0.49 ^{d}	1.37 ^{b}	0.93 ^{c}	1.33 ^{b}	1.83 ^{a}	1.79 ^{a}	1.53 ^{ab}	**	ns	ns
71	Phenol	1111	a		0.14 ^{i}	0.23 ^{i}	2.38 ^{fg}	2.15 ^{g}	1.21 ^{h}	3.61 ^{cd}	2.95 ^{ef}	3.44 ^{de}	4.70 ^{b}	4.54 ^{b}	4.22 ^{bc}	5.38 ^{a}	4.75 ^{ab}	4.39 ^{b}	**	**	ns
79	Phenyl ethyl alcohol	1193	a				0.20 ^{bc}	0.16 ^{cd}	0.13 ^{cd}	0.10 ^{d}	0.13 ^{cd}	0.19 ^{bc}	0.26 ^{ab}	0.33 ^{a}	0.27 ^{ab}	0.19 ^{bcd}	0.16 ^{cd}	0.19 ^{bc}	**	ns	ns
	Esterase activity																			
6	Methyl acetate	551	a				25.19 ^{b}	25.79 ^{b}	13.26 ^{c}	28.73 ^{bc}	40.27 ^{a}	30.08 ^{b}	24.99 ^{b}	31.15 ^{ab}	30.33 ^{b}	22.86 ^{b}	21.98 ^{bc}	28.81 ^{b}	**	ns	*
15	Ethyl acetate	635	a				2.12 ^{cde}	3.55 ^{a}	1.42 ^{ef}	2.40 ^{bcd}	3.15 ^{ab}	2.63 ^{bc}	1.69 ^{def}	2.16 ^{cde}	2.18 ^{cde}	1.27 ^{bc}	2.72 ^{abc}	2.76 ^{abc}	*	**	**
16	Methyl propanoate	650	a	0.41 ^{ef}	0.22 ^{f}	0.36 ^{ef}	1.47 ^{bc}	1.00 ^{cd}	1.46 ^{bc}	1.52 ^{b}	2.15 ^{a}	1.41 ^{bc}	0.78 ^{de}	1.00 ^{cd}	0.98 ^{cd}	0.68 ^{def}	0.70 ^{def}	0.78 ^{de}	*	ns	ns
24	Methyl butanoate	755	a	16.88 ^{h}	11.91 ^{h}	15.45 ^{h}	87.50 ^{cd}	80.20 ^{cde}	71.33 ^{cde}	115.05 ^{ab}	137.48 ^{a}	92.74 ^{bc}	58.62 ^{efg}	68.43 ^{defg}	47.93 ^{g}	55.22 ^{fg}	55.98 ^{fg}	52.39 ^{fg}	**	*	ns
28	Methyl 2-hydroxy-propanoate	793	a				7.54 ^{ef}	6.72 ^{ef}	3.34 ^{f}	12.87 ^{de}	26.07 ^{ab}	17.26 ^{cd}	13.53 ^{de}	19.98 ^{abcd}	19.16 ^{bcd}	22.24 ^{abc}	21.72 ^{abc}	27.46 ^{a}	**	ns	ns
31	Methyl 3-methyl-butanoate	805	a							0.70 ^{bc}	0.69 ^{bc}	0.55 ^{c}	0.69 ^{bc}	0.60 ^{c}	0.60 ^{c}	0.87 ^{ab}	1.04 ^{a}	0.64 ^{c}	**	*	ns
33	Ethyl butanoate	831	a							0.26 ^{bc}	0.27 ^{bc}	0.52 ^{a}	0.13 ^{d}	0.16 ^{cd}	0.34 ^{b}	0.18 ^{cd}	0.14 ^{d}	0.25 ^{bc}	**	**	ns
36	Methyl pentanoate	855	a	0.83 ^{f}	0.59 ^{f}	0.67 ^{f}	4.02 ^{cd}	3.77 ^{cde}	2.82 ^{de}	5.46 ^{b}	6.65 ^{a}	4.59 ^{b}	3.67 ^{cde}	3.96 ^{cd}	2.73 ^{e}	3.53 ^{cde}	3.41 ^{de}	3.20 ^{de}	**	**	ns
50	Methyl hexanoate	951	a	13.94 ^{f}	9.89 ^{f}	11.45 ^{f}	49.30 ^{cd}	43.53 ^{de}	24.95 ^{ef}	82.77 ^{ab}	87.85 ^{a}	73.61 ^{ab}	85.67 ^{ab}	74.76 ^{ab}	67.15 ^{bc}	81.08 ^{ab}	76.95 ^{ab}	76.61 ^{ab}	**	**	ns
51	Methyl 3-hexenoate (E)	963	a										0.11 ^{b}	0.13 ^{b}	0.14 ^{ab}	0.14 ^{ab}	0.11 ^{b}	0.17 ^{a}	ns	*	ns
64	Methyl heptanoate	1057	a	0.14 ^{e}	0.12 ^{e}	0.11 ^{e}	0.48 ^{bc}	0.43 ^{cd}	0.30 ^{d}	0.65 ^{a}	0.66 ^{a}	0.57 ^{abc}	0.62 ^{ab}	0.46 ^{bc}	0.43 ^{cd}	0.48 ^{bc}	0.44 ^{cd}	0.44 ^{cd}	**	*	ns
77	Methyl octanoate	1156	a	8.47 ^{f}	6.99 ^{f}	7.84 ^{f}	30.29 ^{de}	25.80 ^{e}	21.50 ^{e}	59.45 ^{ab}	52.78 ^{abc}	49.58 ^{abc}	64.75 ^{a}	43.99 ^{bcd}	45.65 ^{bcd}	46.22 ^{bc}	35.93 ^{cde}	41.39 ^{cd}	**	*	ns
83	Ethyl octanoate	1229	a				0.09 ^{f}	0.21 ^{de}		0.20 ^{de}	0.27 ^{d}	0.42 ^{c}	0.18 ^{de}	0.26 ^{d}	0.67 ^{b}	0.13 ^{ef}	0.26 ^{d}	0.87 ^{a}	**	**	**
84	Methyl nonanoate	1260	a	0.15 ^{h}	0.13 ^{h}	0.14 ^{h}	0.41 ^{bcd}	0.41 ^{bcd}	0.29 ^{efg}	0.55 ^{a}	0.48 ^{ab}	0.43 ^{bc}	0.55 ^{a}	0.37 ^{cde}	0.30 ^{efg}	0.31 ^{def}	0.21 ^{fgh}	0.19 ^{gh}	**	**	ns
90	Methyl decanoate	1358	a	0.74 ^{f}	0.76 ^{ef}	0.75 ^{f}	1.09 ^{def}	0.94 ^{ef}	0.67 ^{f}	1.79 ^{ab}	1.64 ^{abc}	1.64 ^{abc}	2.03 ^{a}	1.43 ^{bcd}	1.68 ^{abc}	1.54 ^{bcd}	1.23 ^{cde}	1.41 ^{bcd}	**	ns	ns
	Unknown or contaminants																			
2	Methanethiol	472	a	110.34 ^{cde}	155.31 ^{acb}	116.31 ^{bcde}	164.72 ^{a}	158.32 ^{ab}	113.30 ^{cde}	133.78 ^{abcde}	167.75 ^{a}	141.63 ^{abcd}	117.46 ^{bcde}	99.55 ^{de}	93.48 ^{e}	114.02 ^{cde}	95.72 ^{e}	88.61 ^{e}	**	*	ns
39	*p* -xylene	891	a	1.28 ^{f}	1.34 ^{f}	1.21 ^{f}	10.09 ^{bcd}	8.05 ^{e}	9.02 ^{bcde}	12.14 ^{a}	9.85 ^{bcde}	11.27 ^{ab}	8.34 ^{de}	10.31 ^{abcd}	8.51 ^{cde}	10.54 ^{abc}	7.73 ^{e}	8.99 ^{cde}	**	ns	ns
44	*o* -xylene	917	a	0.31 ^{g}	0.25 ^{gh}	0.35 ^{h}	4.06 ^{a}	3.33 ^{abcde}	2.38 ^{f}	3.89 ^{abc}	3.56 ^{abcd}	3.93 ^{ab}	2.97 ^{cdef}	2.83 ^{def}	3.06 ^{bcdef}	2.99 ^{bcdef}	2.52 ^{ef}	2.88 ^{def}	**	*	ns
62	Limonene	1045	a				1.83 ^{bcde}	1.76 ^{bcde}	3.11 ^{a}	1.95 ^{bcd}	1.59 ^{cdef}	3.02 ^{a}	1.12 ^{f}	1.37 ^{ef}	2.12 ^{b}	1.47 ^{def}	1.11 ^{f}	2.00 ^{bc}	**	**	ns
65+67	Methyl 2,4-hexadienoate (E, E)	1059/1066	b	0.11 ^{e}	0.37 ^{e}	0.19 ^{e}	185.70 ^{b}	180.20 ^{b}	89.80 ^{d}	198.13 ^{b}	252.51 ^{a}	255.62 ^{a}	151.16 ^{bc}	114.94 ^{cd}	123.89 ^{cd}	102.74 ^{cd}	81.75 ^{d}	102.85 ^{cd}	**	ns	**
75	Ethyl 2,4-hexadienoate (E, E)	1144	a				0.22 ^{f}	0.44 ^{ef}	0.33 ^{ef}	0.39 ^{ef}	0.60 ^{de}	1.33 ^{a}	0.60 ^{de}	0.52 ^{def}	0.94 ^{bc}	0.34 ^{ef}	0.73 ^{cd}	1.17 ^{ab}	**	**	**
78	2,4-Hexadienoic acid (E, E)	1180	a				104.73 ^{cd}	58.01 ^{ef}	42.95 ^{f}	146.79 ^{ab}	174.83 ^{a}	129.96 ^{bc}	109.89 ^{bc}	91.66 ^{cde}	87.72 ^{cde}	120.96 ^{bc}	69.84 ^{def}	92.08 ^{cde}	**	*	ns
80	4-Methyl-phenol	1196	a				0.56 ^{a}	0.48 ^{abc}	0.33 ^{cd}	0.54 ^{a}	0.32 ^{d}	0.56 ^{a}	0.63 ^{a}	0.60 ^{a}	0.61 ^{a}	0.63 ^{a}	0.49 ^{ab}	0.34 ^{bcd}	**	**	*
91	Caprolactame	1387	a	0.36 ^{cde}	0.17 ^{g}	0.20 ^{fg}	0.72 ^{a}	0.39 ^{cde}	0.46 ^{cd}	0.63 ^{ab}	0.35 ^{de}	0.50 ^{bc}	0.61 ^{ab}	0.38 ^{cde}	0.39 ^{cde}	0.63 ^{cde}	0.30 ^{ef}	0.62 ^{ab}	**	**	ns
93	Tetradecane	1400	a	0.11 ^{b}	0.18 ^{ab}	0.10 ^{b}	0.13 ^{a}	0.11 ^{b}	0.10 ^{b}	0.15 ^{b}	0.13 ^{b}	0.09 ^{b}	0.12 ^{b}	0.15 ^{b}	0.10 ^{b}	0.14 ^{b}	0.14 ^{b}	0.12 ^{b}	ns	ns	ns

AU: Abundance units resulting of counting the total ion chromatogram (TIC) for each compound. [A] Number of the Peak in the chromatogram. [B] Kovats index calculated for DB-624 capillary column (J&W Scientific 30m x 0.25 mm i.d. x 1.4 µm film thickness) installed on a gas chromatograph equipped with a mass selective detector. [C] Reliability of identification: a, mass spectrum and retention time identical with an authentic standard; b, tentative identification by mass spectrum. [a-i]: Identical letters in each parameter indicate that there is no significant difference at $p>0.05$ (Fisher's test). [D] P_b: P value of fat content effect; P_s: P value of ripening effect; P_{sxb}: P value of interaction between fat content and ripening effects. **: P< 0.01, *: P<0.05, ns: P>0.05. E Target ion used to quantify the compound when the peak was not completely resolved.

Table 3. Odor-active compounds identified in the HS of dry fermented sausages.

KI*	Compound	GC-O descriptor	DF**	previously reported in dry sausages
472	Methanethiol	Rotten, unpleasant	9	1
589	2-Methyl-propanal	Green grass, fresh	5	5
631	Methyl ethyl sulfide	Rotten onion, unpleasant	4	-
633	2,3-Butanedione	Butter	7	1,4,6
691	3-Methyl butanal	Green, herbal	10	1,2,6
702	Acetic acid	Vinegar	16	1,2,4,6
790	Methyl 2-hydroxy-propionate	Green grass, fresh	4	-
803	Methyl 3-methyl butanoate	Strawberry, sweet	12	-
824	Ethyl butanoate	Fruity, flowery	5	1,2,4,5,6
837	Hexanal	Fresh cut grass, green	14	1,2,3,4,6
873	Butanoic acid	Cheese	15	1,2
904	Unknown 1	Meat broth, snacks, roasted	11	-
926	Unknown 2	Cheese, feet	5	-
939	Heptanal	Unpleasant	7	1,3,6
964	Unknown 3	Onion, garlic	15	-
969	Methional (3-methyl-thiopropanal)	Onion, cooked-potato	12	1,5
1010	2-Pentyl furan	Meat broth, savory, metallic	14	1,5
1023	1-Octen-3-ol	Mushroom	16	1,3
1035	2-Octanone	Floral, geranium	13	-
1046	Octanal	Citrus	11	1
1111	Benzeneacetaldehyde	Roses	8	1,2,5
1148	Unknown 4	Roasted, toasted	7	-
1159	Methyl octanoate	Fruity	7	-
1180	Unknown 5	Roasted nuts, snacks	15	-
1992	4-Methyl phenol	Stable, horse	12	5
1203	Unknown 6	Mustiness, fruity	9	-
1220	2-Nonenal	Medicinal	16	1,2,5,6
1226	Unknown 7	Roasted nuts	8	-
1236	Octanoic acid	Toasted, coffee	5	1
1288	2,4-Nonadienal (E, E)	Tallowy	5	2

* Kovats index calculated for DB-624 capillary column (J&W Scientific 60m x 0.32 mm i.d., film thickness 1.8 µm) installed on a gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port. ** DF Detection frequency value. Previously identified in dry sausages by: 1 Marco et al. (2007), 2 Söllner and Schierberle (2009), 3 Meynier et al. (1999), 4 Schmidt and Berger (1998), 5 Gianelli et al. (2009); 6 Stahnke (1994).

Table 4. Sensory analysis (hedonic test) of dry fermented sausages with different fat contents at 42 and 63 days of ripening.

	42 d			63 d			S	B	S x B
	LF	MF	HF	LF	MF	HF			
aroma	5.88 ^{b}	6.21 ^{ab}	6.20 ^{ab}	5.83 ^{b}	6.15 ^{ab}	6.35 ^{a}	0.942	0.014	0.730
overall quality	6.23 ^{bc}	6.49 ^{ab}	6.68 ^{ab}	5.91 ^{c}	6.60 ^{ab}	6.69 ^{a}	0.605	0.000	0.366

Identical letters in each parameter indicate the absence of significant differences at $p > 0.05$ (Fisher's test). * P_s: P value of ripening time effect; P_b: P value of fat content effect; P_{sxb}: P value of interaction between ripening time and fat content effects.

Figure 1

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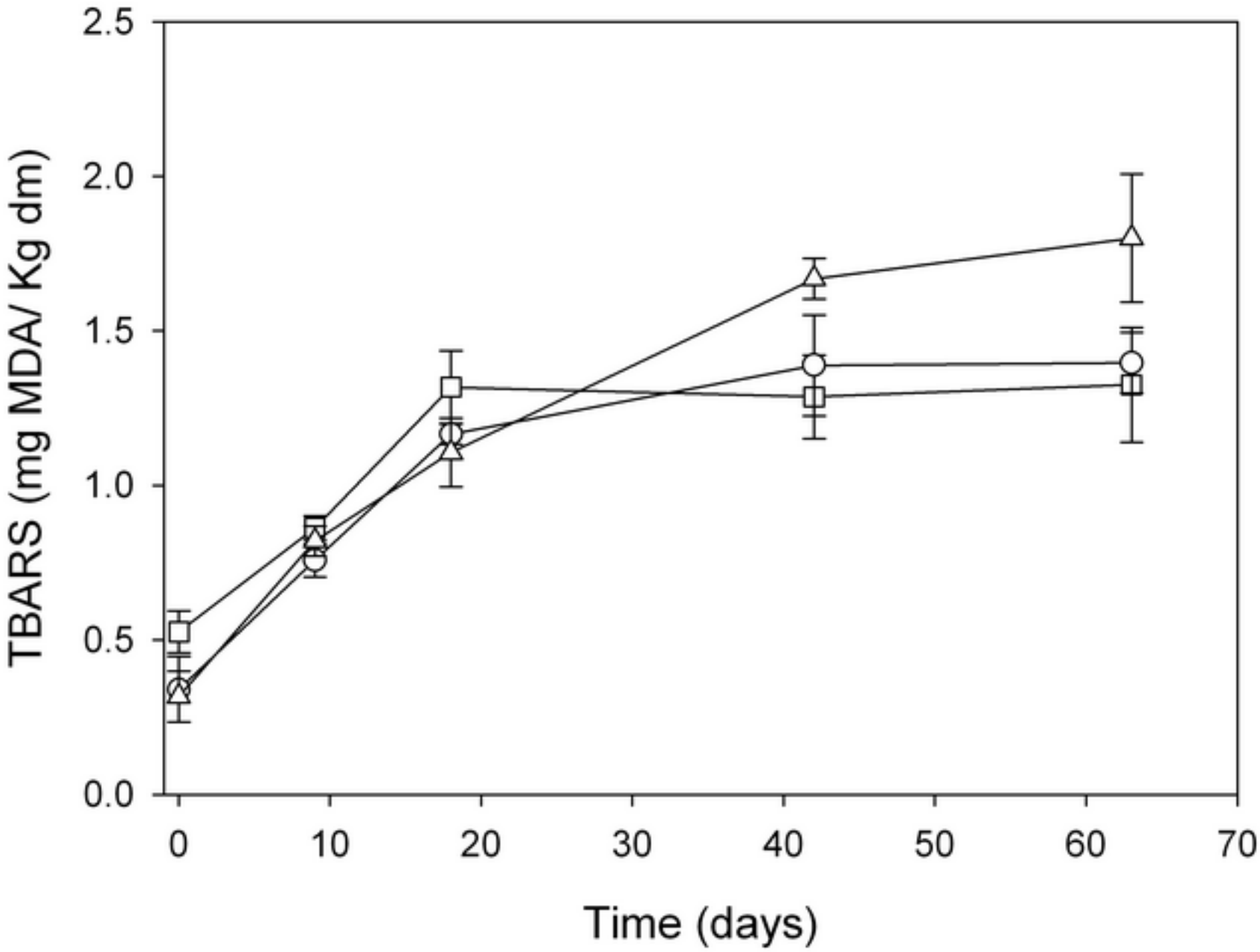

Figure 2

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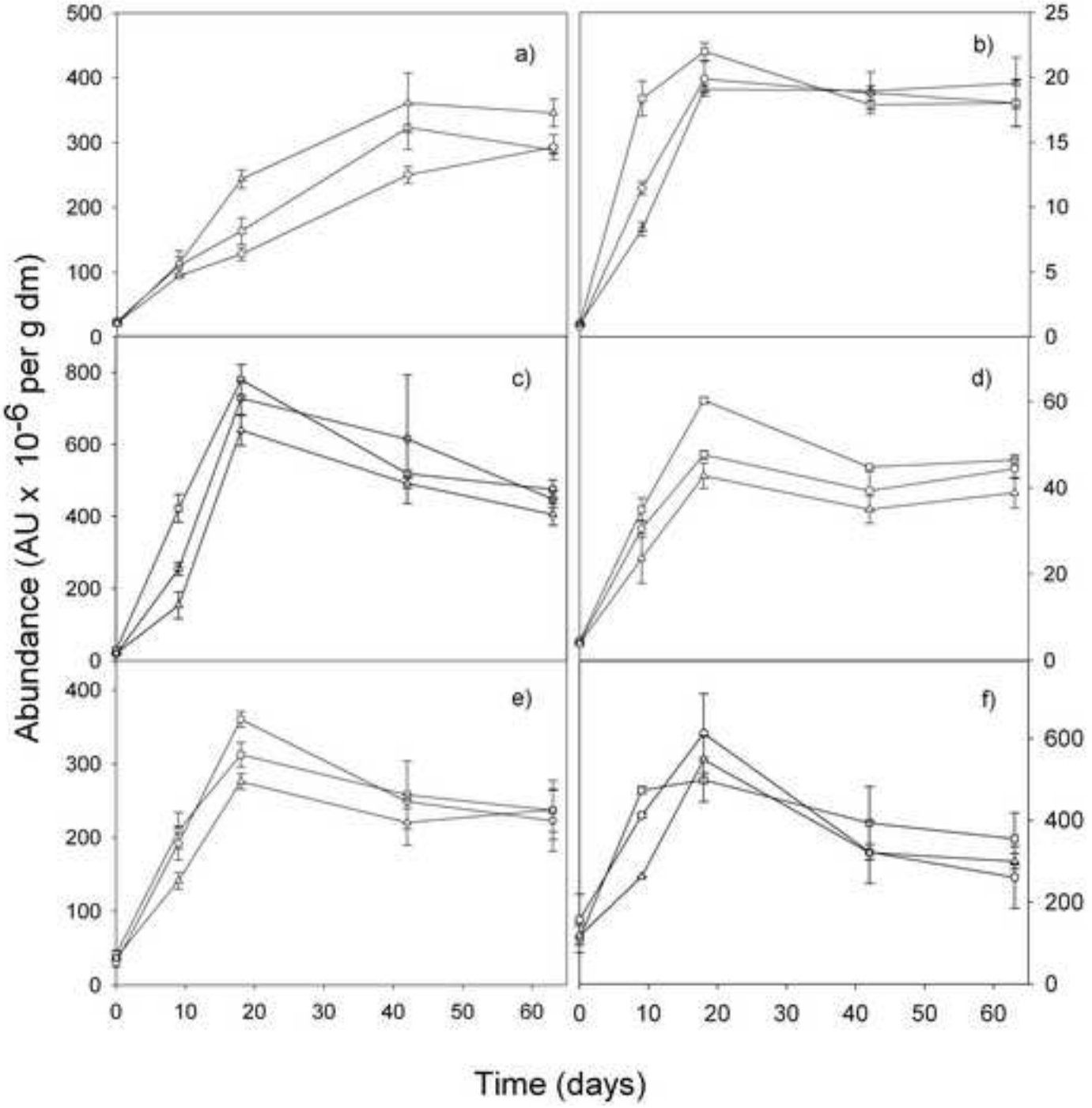

Figure 3

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