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Abstract

We studied the influence of the IMF content (high –HI- vs. low –LI-) and the fatty acid saturation profile on cooked cured pork ham volatiles. LI hams had higher PUFA and lower MUFA contents than HI hams. Using SPME we identified 29 compounds novel to cooked cured pork ham profiles, mostly lipid derivatives. Group differences were related to the PUFA/MUFA contents but not to the IMF content. Differences were also identified in amino acid breakdown derivatives with potential aroma implications. The SBSE method, a novelty in pork meat science, revealed differences which included board taint-related volatiles,

terpenes and 36 novel compounds; 14 of these compounds were only found by the SBSE

method.

Keywords: *cooked cured pork ham; volatiles; intramuscular fat; SPME; SBSE*

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13 **1. Introduction**

14 Cooked cured pork ham (hereinafter referred to as cooked ham) is one of the most

15 highly consumed, ready-to-eat meat products worldwide. The aroma of cooked ham

16 seems to be one of the main drivers of consumer choice (Morrissey, Sheehy, Galvin,

17 Kerry, & Buckley, 1998). The development of cooked ham aroma arises mainly during

18 the cooking process. For example, fatty acid oxidation results in the release of

19 aldehydes, alcohols, ketones and medium chain fatty acids which, in turn, can react

20 with Maillard reaction and Thiamine degradation compounds (Thomas, Mercier,

21 Tournayre, Martin, & Berdagué, 2013). Intramuscular fat (IMF) appears to be the most

22 important source for volatiles (Mottram, Edwards, & Macfie, 1982). High levels of

23 polyunsaturated fatty acids (PUFA) in IMF, which are extremely sensitive to oxidative

24 deterioration, have been related to the rapid propagation of rancid notes (Carrapiso &

25 García, 2004). In addition, health concerns such as cholesterol related cardiovascular

26 pathologies have contributed to increase the public attention towards dietary fats. As

27 a consequence, monounsaturated fatty acids (MUFA) have become regarded as

28 healthier than polyunsaturated (PUFA) or saturated fats (Rebollo, et al., 1998).

29   Solid phase microextraction (SPME) has been demonstrated to be a useful method

30 to analyze the effect of lipid oxidation on pork aroma (Barba, Santa-María, Herraiz, &

31 Calvo, 2012). Complementary to SPME, solid stir bar sorptive extraction (SBSE) allows

32 improved accuracy in the extraction of volatile and semi-volatile compounds in

33 aqueous systems (Guerrero, Marín, Mejías, & Barroso, 2006). However, the use of

34 SBSE in meat studies has been very limited and no research has been reported in

35 cooked ham.

36   This study aims to compare the volatile compounds present in cooked ham from

37 two commercial ham samples with differing amounts of IMF (LI compared to HI) and

38 differing ratios of MUFA to PUFA, using two non-invasive micro extraction techniques:

39 SPME and SBSE, both being coupled to a GC-MS setup.

40 **2. Material and methods**

41 *2.1. Sample selection and preparation.*

42   Five pork ham samples from two genetic backgrounds, a commercial crossbreed

43 Large White x Landrace (LwL), widely used by the pig industry, and a crossbreed

44   Iberian x Duroc (IbD), were selected based on different IMF contents and composition.

45   The LwL ham had relatively low IMF (LI) contents and low levels of the MUFA oleic acid

46   (C18:1, n-9) together with high levels of PUFA, in particular linoleic acid (C18:2)

47   (Pugliese & Sirtori, 2012). In contrast, IbD ham had a characteristically high

48   intramuscular fat (HI) content and was rich in oleic and low in linoleic acids (Pugliese &

49   Sirtori, 2012). All hams were from left-side gilt carcasses. Post-mortem pH was checked

50   to meet quality standards (pH in the *Semimembranosus* muscle at 45 min *post mortem*

51   was above 6.0 and at 24 h (pH_{24}) was lower than 6.2. Ten whole-leg green hams were

52   deboned and trimmed of subcutaneous intermuscular fat, connective tissue and rind.

53   Brine was injected into the meat to increase their weight by 21% reaching 0.3%

54   pentasodium tripolyphosphate, 0.05% sodium ascorbate, 1.8% NaCl and 0.01% sodium

55   nitrite after injection (Sárraga, Guàrdia, Díaz, Guerrero, García-Regueiro, Arnau; 2007).

56   Hams were then individually placed in a vacuum tumbler at 4 °C at a pressure of 200

57   mbar. The tumbling schedule was set for the ham to rotate a total of 2000 times at 14

58   rpm. Then, the hams were packed in bags (CN330, Sealed Air, Italy), matured at 2 °C±1

59   °C for 8 days, moulded in stainless steel moulds, placed in a steam oven and cooked to

60   an internal temperature of 68 °C using an external temperature of 70 °C. The cooked

61   hams were then refrigerated at 2 °C±1 °C for 48 h and vacuum-packaged individually in

62   PA/PE sealed bags and stored at 0 °C±1 °C until required for analysis (3 days later).

63   Before analysis, each cooked ham was entirely ground and mixed and then two

64   homogeneous subsamples were taken.

65   *2.2. Moisture content and Intramuscular fat (IMF) content.*

66   Moisture was determined according to the AOAC method (AOAC, 1984). The

67   intramuscular fat content (expressed on wet base) was determined according to the

68   AOAC (2006) method using the Foss Soxcap 2047 for the hydrolysis and the Soxtec

69   Extraction 2055 system for the extraction with hexane (Foss analytical, Denmark).

70   *2.3. Fatty acid profile.*

71   Fat was extracted using the chloroform–methanol procedure of Folch, Lees, and

72   Stanley (1957). After evaporation of the extract, fatty acids were converted to fatty

73   acid methyl esters (FAME) following the method ISO 5509-1978 (E) by using 14% BF3 in

74   methanol, and analyzed by gas chromatography (GC Agilent 6890). Individual fatty

75   acids were identified by retention time with reference to a standard solution (FA

76   methyl ester mixture 189-19; Sigma-Aldrich, Madrid, Spain). The separation of FAMES

77   was carried out with a capillary column coated with polyethyleneglycol-TPA modified

78   (SUPELCO SP-2380) (60 m long, 0.25 mm internal diameter and 0.20 μm film

79   thickness). The injector and detector temperature were 280 °C and the oven was

80   programmed from 120 °C to 220 °C by using a linear gradient of 4 °C /min. The carrier

81   gas was helium (He) at a split ratio of 1:50 and 0.5 μl of sample solution was injected.

82   Results were expressed as g of fatty acid per 100 g of sample.

83   *2.4. Crude protein content.*

84   Total nitrogen content (TN, % w/w) was measured by the Kjeldahl method (ISO 937,

85   1978) and the protein content was estimated by multiplying the TN by 6.25.

86   *2.5. SPME extraction.*

87   Two-gram subsamples from the previously homogenized samples described in

88   section "Sample selection and preparation" were placed into a 20 mL glass vial and

89   capped with a Silicone-PTFE septum. The vial was transferred into a CTC SPME

90   AutoSampler (CTC Analytics AG, Zwingen, Switzerland). The SPME fiber used was a

91   30/50 µm Carboxen/PDB/DVB fibers (Supelco, Bellefonte, PA). The fibers were

92   exposed to the headspace of the vial for 30 min at 40 °C and the volatiles were

93   desorbed in the injection port of the chromatograph for 10 min at 250 °C in split-less

94   mode. An empty vial was used as a blank sampler to clean the column and the fiber

95   before each sample run.

96   *2.6. SBSE extraction.*

97   Post-cooking exudate samples of 5 mL were collected immediately after unsealing

98   and stored in 50 ml vials. SBSE extraction and injection parameters were performed

99   according to Ibáñez and Solá (2006). Volatile compound extraction of each sample was

100   performed for 90 min at 1400 rpm using 10 mm x 0.5 mm PDMS phase thickness stir

101   bars (Twister®, GERSTEL GmbH & Co.KG, Mülheiman der Ruhr, Germany). Afterwards,

102   the stir bars were rinsed with distilled water, dried with a clean tissue and transferred

103   to desorption tubes in the MPS2 Gerstel Autosampler System. The Agilent 6890 GC

104   (Agilent Technologies, Palo Alto, California, USA) was equipped with a Multipurpose

105   Automatic Sampler (MPS2), a thermal desorption unit (TDU) and a cooled injection

106   system (CIS4) by Gerstel (Gerstel, Mülheiman der Ruhr, Germany). For the injection of

107   the volatiles absorbed on the stir bars into the GC, the TDU was held at 30 °C for 1 min,

108   raised to 250 °C at a rate of 90 °C/min and then held at this temperature for 10 min.

109   The split 1:15 was used for TDU injection during thermal desorption. The desorption

110   flow was kept to 50 mL/min at 103 kPa, while the injection port was maintained at -

111   110 °C (liquid N2 cooled). After total desorption of volatiles, the CIS4 was ramped at a

112   rate of 12 °C/s from -110 °C to 250 °C and held at this temperature for 3 min.

113   *2.7. Gas Chromatography and Mass Spectrometry (GC-MS) analysis.*

114   All analyses were performed with an Agilent 6890 gas chromatograph coupled to a

115   5973N mass selective detector from Agilent (Agilent, Palo Alto, USA). The separation of

116   volatiles was performed using a Supelcowax 10 (30m x 0.25 mm x 0.25 μm) capillary

117   column and helium was used as the carrier gas. The oven program includes an initial

118   temperature of 60 °C, and a program rate of 4 °C/min up to 230 °C and was held at this

119   temperature for 15 min. The mass spectrometer (MS) transfer line temperature was

120   held at 250 °C. Electronic impact at 70eV was used to obtain the mass spectra. The MS

121   scanned from 35 m/z to 300 m/z keeping the ion source temperature at 230 °C and the

122   quadrupole temperature at 150 °C. Volatile compounds were identified comparing

123   their mass spectra with those of authentic standards and with references from several

124   commercial libraries databases NIST 08 library (NIST 08, version 2.0, Gaithersburg,

125   USA) and Wiley (Willey & Sons Inc., Germany). The volatile standards used were from

126   Sigma Aldrich (St Louis, USA). Each compound was further confirmed by comparing its

127   linear retention index with those obtained from the standards and/or from literature

128   sources. Identification of volatiles was done using AMDIS deconvolution software (NIST

129   08, Gaithersburg, USA) to clean chromatographic peaks from interferences in the

130   studied matrices. If the standards were unavailable, the identification of some volatile

131   compounds was performed by comparing their mass spectra with NIST and Wiley

132   database resulting in tentatively identified compounds. Results from the GC-MS

133   analysis were expressed in area units (AU x 10^{-6}). Each value in Table 2, Table 3 and

134   Table 4 is the mean of five measurements.

135   *2.8. Data analysis.*

136   The effect of the IMF profile (LI and HI) on the proportion of identified volatiles was

137   determined by the analysis of variance (GLM) procedure (SAS, 2007). The Tukey test

138   was used for mean comparisons. Statistical significance was set at $p<0.05$.

139   **3. Results and discussion**

140   *3.1. Moisture content, Intramuscular fat content, crude protein and fatty acids.*

141   No significant differences regarding moisture content were obtained between LI

142   and HI samples (73.95±0.104% vs 73.49±0.142%, respectively). Consistent with

143   available literature (Arce, et al., 2009; Pugliese & Sirtori, 2012) the LI samples had

144   significantly lower IMF (2.74±0.18%) than HI (4.33±0.31%) and higher crude protein

145   (23.23±0.09%) than HI (20.72±0.04%). In addition, Table 1 shows the fatty acid

146   composition for each sample (g of fatty acid/100 g of sample). HI hams contained

147   2.165±0.066 g oleic acid/100 g, 0.247±0.0624 g linoleic acid/100 g while the IMF from

148   the LI hams had lower contents of oleic acid (1.225±0.056 g/100 g) and higher content

149   of linoleic (0.022±0.006 g/100 g) acids. Consequently, samples of HI compared to the LI

150   had lower levels of PUFA (0.307 g/100 g vs. 0.3836 g/100 g, respectively) and higher

151   levels of MUFA (2.325 g vs. 1.321 g/100 g, respectively).

152   *3.2. Volatile profile of HI and LI cooked ham following SPME extraction.*

153   A list of the HI and the LI cooked ham volatiles identified in our study by GC-MS

154   following SPME extraction is shown in Table 2 (volatiles derived from lipid oxidation)

155   and Table 3 (non-lipid derived volatiles). Overall, our study reveals 100 volatile

156   compounds in cooked ham using SPME. Aldehydes, acids, ketones and alcohols, which

157   are mainly derived from lipid oxidation, were the predominant compounds in both the

158   LI and the HI groups, confirming that lipid composition plays a crucial role in the

159   formation of cooked ham flavour (Elmore, Mottram, & Hierro, 2001). A higher number

160   of lipid-derived volatiles were identified in the LI compared to the HI samples (47

161   compared to 40, respectively) (Table 2).These results are in agreement with those of

162   Machiels and Istasse (2003) in cooked uncured beef and Estévez, Morcuende,

163   Ventanas, and Cava (2003) in cooked pork, who obtained higher number of volatiles

164   from low fat meats when compared to those with higher fat contents. Consequently,

165   the number of lipid-derived volatiles identified does not reflect the IMF content in the

166   two groups. It might be speculated that the higher content of polyunsaturated fatty

167   acids in the LI samples, which are more susceptible to oxidation, may explain the

168   formation of a higher number of lipid-derived volatile compounds (Estévez, et al.,

169   2003).

170   Twenty nine volatile compounds, 11 lactones, 1 alcohol, 5 esters, 1 furan, 10

171   terpenes and one miscellaneous compound were identified for the first time in cooked

172   ham aroma. Lactones are compounds that originate from fatty acid oxidation

173   contributing to the fatty, creamy, fruity and coconut-like nuances associated with

174   cooked meat (Leroy, Vasilopoulos, Van Hemelryck, Falony, & De Vuyst, 2009).

175   The comparison of the HI with the LI hams in our SPME extraction resulted in 22

176   lipid oxidation compounds significantly ($p<0.05$) affected by the IMF profile: 9

177   aldehydes, 6 lactones, 2 acids, 3 alcohols, 1 ketone and 1 furan (Table 2). Aldehydes

178   are the most important lipid-derived volatiles that contribute to the cooked ham

179   aroma due to their low odour threshold. Significant differences ($p<0.01$) between the

180   HI and the LI samples (Table 2) were observed for hexanal, butanal, nonanal, decanal,

181   (E)-2-octenal, (E)-2-decenal, (E)-2-undecenal, (E,Z)-2,4-decadienal and (E,E)-2,4-

182   decadienal levels. The presence of oleic acid-derived aldehydes such as octanal and

183   nonanal has been related to pleasant meaty notes (Muriel, Antequera, Petrón, Andrés,

184   & Ruiz, 2004). In our study, the levels of nonanal ($p<0.01$) were higher in the HI than in

185   the LI.

186   On the other hand, linoleic related compounds such as (E)-2-octenaland and (E)-2-

187   decenal were significantly higher in the LI than in the HI ham while (E,E)-2,4-decadienal

188   showed the opposite trend. The presence of volatile compounds derived in general

189   from PUFA and particularly from linoleic acid, has been related to off-flavours as well

190   as with grass-like and rancid attributes (Estévez, et al., 2003). Hexanal, the most

191   prominent volatile compound related to linoleic acid and a good indicator of lipid

192   oxidation in pork (Ruiz, García, Muriel, Andrés, & Ventanas, 2002) seems to have a

193   significant impact on pig meat aroma. More precisely, high hexanal levels may confer

194   an unpleasant rancid flavour whilst lower levels seem to contribute to a pleasing odour

195   in meat products (Sánchez-Peña, Luna, García-González, & Aparicio, 2005).

196   The IMF profile ($p<0.01$) significantly affected several of the alcohols identified such

197   as 1-pentanol, 1-octen-3-ol and ($p<0.05$) 1-octanol. This compound, which arises from

198   oleic acid oxidation, was higher in the HI hams compared to LI hams and may

199   contribute to cooked ham flavour with green, woody and fatty sensory attributes

200   (Timón, Ventanas, Carrapiso, Jurado, & García, 2001).

201 Our study reports six ketones involved in lipid metabolism with different levels in

202 the HI compared to the LI samples. For example, 2-heptanone, a ketone produced

203 during linoleic acid oxidation, which confers blue cheese flavour attributes (St. Angelo,

204 Legendre, & Dupuy, 1980) was higher in the LI than in the HI hams.

205 Another volatile compound derived from linoleic acid oxidation, 2-pentylfuran

206 (Table 2), showed significantly ($p<0.01$) higher levels in the LI, compared the HI

207 samples. It is responsible for roasted nuances on meat aroma and may significantly

208 contribute to the overall aroma of cooked ham (MacLeod, Seyyedain-Ardebili, &

209 Chang, 1981).

210 Volatile compounds derived from other (non-lipid) reactions such as Maillard,

211 amino acid breakdown or Thiamine degradation, are in Table 3. We found that 3-

212 methyl-butanal, a volatile aldehyde derived from amino acid breakdown (Pastorelli, et

213 al., 2003), was significantly ($p<0.01$) higher in the HI, compared to the LI ham (Table 3).

214 The 3-methyl-butanal compound has been related to a fruity, acorn-like, salty and

215 cheesy aroma of high consumer acceptance (Muriel, et al., 2004; Pastorelli, et al.,

216 2003). The combination of a lower concentrations of hexanal together with a high

217 concentration of 3-methyl-butanal seems to significantly contribute to the

218 characteristic flavour of Iberian meat products (Timón, et al., 2001).

219 All the five volatile sulphur and nitrogen compounds from amino acid breakdown

220 identified in our analysis, methanethiol, dimethyl sulfide, dimethyl disulfide, pyridine

221 and methional were significantly ($p<0.01$) higher in the LI than the HI (Table 3).

222 Nuances of hydrogen sulfide, cauliflower, onion, garlic and dirty socks are related to

223 sulphur-containing compounds and their pleasing/unpleasing contribution depends on

10

224 the relative content (Machiels & Istasse, 2003). The balance between these sulphur

225 and nitrogen compounds and the volatiles from lipid oxidation characterize the overall

226 cooked ham aroma. The LI samples presented a higher number of lipid-derived

227 volatiles from linoleic acid oxidation related to off-flavours than the HI samples. These

228 off-flavours may partially mask the 'meaty character' from sulphur compounds and

229 result in a decrease of the cooked ham aroma intensity (Thomas, Mercier, Tournayre,

230 Martin, & Berdagué, 2013). The HI meats showed a rich lipid volatile profile related to

231 oleic acid derivatives, which together with the short branched aldehyde 3-methyl-

232 butanal may confer essential aroma traits such as 'acorn' nuances.

233 *3.3. Volatile profile of HI and LI cooked ham using SBSE extraction.*

234 The SBSE analysis (hereinafter SBSE) resulted in 81 volatile compounds identified

235 between the two IMF profiles, 17 unique to the HI and 15 to the LI hams (Table 4).

236 SBSE allowed identification of peaks with longer retention times and therefore the

237 evaluation of compounds with higher molecular weights and polar compounds, such as

238 medium-chain fatty acids (Horák, Čulík, Jurková, Čejka, & Kellner, 2008). In our study,

239 lauric, miristic and palmitic acid were only identified by SBSE. In addition, it has been

240 demonstrated that SBSE is a useful tool to analyze apolar compounds such as terpenes

241 and derivatives (hereinafter terpenes) in a polar matrix (Jelén, et al., 2012).

242 Consequently, most of the compounds identified by SBSE which were not identified by

243 SPME belonged to that chemical group. Overall 11 terpenes, 4 acids, 2 aldehydes and 2

244 alcohols, 2 nitrogen/sulphur compounds and 2 compounds of other chemical groups

245 were identified by SBSE but not SPME. Moreover, 40 of the compounds identified by

246 SBSE (5 aldehydes, 3 alcohols, 10 acids, 1 ketone, 3 esters, 11 terpenes, 2 lactones, 1

247   nitrogen/sulphur and 3 miscellaneous compounds) differed significantly ($p < 0.05$)

248   between the two IMF profiles (Table 4).

249   By using the SBSE technique, 35 volatile compounds were identified for the first

250   time in cooked ham volatile extraction. Within these compounds, 14 were only

251   identified by SBSE: 1 aldehyde, 1 nitrogen/sulphur compound and 12 terpenes. Thus,

252   to our knowledge 22 of the 26 terpenes found in our study (Table 4) have not been

253   previously reported in cooked ham literature. The terpenes identified in our ham

254   samples may have a high odour threshold and a low impact on the aroma (Timón, et

255   al., 2001). It has been argued that the accumulation of some terpenes in fat, such as

256   dihydromircenol and linalool may reflect diet composition (Muriel, et al., 2004). In our

257   study, both compounds were higher in the HI than in the LI hams (Table 4).

258   Within the group of carboxylic acids, Ruiz, Ventanas, Cava, Andrés, and García

259   (1999) observed an increase in odd-numbered medium chain fatty acids (e.g.

260   pentanoic, heptanoic, nonanoic) in dry-cured Iberian hams compared to other

261   commercial breeds. Our SBSE results are consistent with the previous findings,

262   showing higher ($p < 0.01$) values of odd-chained pentanoic and heptanoic acids in the

263   HI compared to the LI meats.

264   Indole and skatole are related to animal and fecal odours and, together with

265   androstenone, might be responsible for boar taint odour. These compounds have low

266   odour thresholds and result in a deep undesirable impact on the overall flavour,

267   affecting consumer choice. In our study indole and skatole were detected only after

268   SBSE extraction. In particular, the abundance of indole was higher ($p<0.01$) in the LI

269   than in the HI ham while the opposite was true for skatole. Our data agrees with the

12

270 findings by Rius and García-Regueiro (2001) showing that skatole was higher in pig

271 breeds with higher fat content.

4. Conclusions

273   The comparison of cooked cured ham volatiles from two sets of ham samples

274 selected based on content and profile of IMF showed lower number of volatiles for the

275 HI group than the LI group. However, the LI group appeared to have higher

276 abundances of rancid nuances arising from the oxidation of linoleic acid (PUFA) and

277 components from amino acid breakdown than HI hams. Also, HI samples showed

278 higher terpenes and the components from oleic acid oxidation. Finally, our results

279 show that SBSE extraction is a useful tool to identify volatile compounds of different

280 polarities such as medium chain fatty acids, terpenes and related compounds (such as

281 board taint related indole and skatole) in cooked ham samples.

5. Acknowledgments

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367

368 Table 1. Fatty acid profile (mean ± standard deviation) of the intramuscular fat of
369 cooked cured pork ham samples with low (LI)[a] or high (HI)[b] intramuscular fat content.

Fatty acids (g/100 g sample)	LI[a]	HI[b]	p[c]
C10	-	0.004±0.000	-
C12	-	0.004±0.000	-
C14	0.038±0.003	0.052±0.008	**
C16	0.647±0.026	1.061±0.035	**
C17	0.016±0.004	0.022±0.003	*
C18	0.332±0.029	0.546±0.046	*
C20	0.003±0.000	0.009±0.001	*
∑ SFA	1.036±0.056	1.697±0.100	**
C16:1	0.077±0.012	0.130±0.014	*
C18:1	1.225±0.056	2.165±0.066	*
C20:1	0.019±0.002	0.030±0.001	0.61
∑ MUFA	1.321±0.086	2.325±0.084	**
C18:2	0.318±0.061	0.247±0.062	*
C18:3	0.022±0.006	0.017±0.014	0,05
C20:2	0.014±0.002	0.013±0.002	0.33
C20:3	0.008±0.001	0.009±0.001	0.31
C20:4	0.019±0.003	0.002±0.001	0.29

16

C22:5	0.003±0.001	0.004±0.001	0.29
∑PUFA	0.384±0.082	0.307±0.080	**

370 [a]LI: Low intramuscular fat ham samples from Large White x Landrace pigs. [b]HI: High
371 intramuscular fat ham samples from Iberian x Duroc (IbD) pigs . [c]p: Statistical significance
372 between the two means in the same row. Non-significant differences are given as exact P
373 values whereas significant differences are expressed with asterisks: * when P < 0.05, ** when
374 P < 0.01 and *** when P < 0.001. SFA: saturated fatty acids, MUFA: monounsaturated fatty
375 acids, PUFA: polyunsaturated fatty acids.

376

377 Table 2.Comparative profiles of volatile compounds from lipid oxidation identified in
378 cooked cured pork ham samples with low (LI)[a] or high (HI)[b] intramuscular fats using
379 SPME.

Compounds	LI[a]	HI[b]	p[c]	RT[d]	MI[e]
Aldehydes					
propanal	0.64±0.14	nd[f]	-	2.29	A
butanal	1.29±0.07	0.12±0.00	***	2.67	A
pentanal	nd[f]	0.33±0.05	-	3.45	A
hexanal	0.58±0.24	0.11±0.02	***	4.70	A
heptanal	nd[f]	0.02±0.00	-	6.41	A
(*Z*)-4-heptenal	0.03±0.00	0.03±0.01	0.245	7.45	A
octanal	0.10±0.04	0.12±0.00	0.329	8.75	A
(*E*)-2-heptenal	0.01±0.00	0.01±0.00	0.062	9.84	A
nonanal	0.06±0.01	0.39±0.13	***	11.55	A
(*E*)-2-octenal	0.03±0.01	0.01±0.00	***	12.73	A
decanal	0.02±0.00	0.04±0.02	***	15.54	A
(*E*)-2-nonenal	0.16±0.02	0.02±0.01	0.092	15.78	A
(*E*)-2-decenal	0.19±0.02	0.02±0.01	***	18.85	A
(*E,Z*)-2,4-decadienal	0.17±0.03	0.09±0.02	**	20.95	A
(*E*)-2-undecenal	0.31±0.03	0.18±0.01	***	21.74	A
(*E,E*)-2,4-decadienal	0.02±0.01	0.12±0.01	***	23.55	A
(*E,E*)-2,4-undecadienal	0.04±0.00	0.04±0.01	0.757	23.67	B
Alcohols					
2-methyl-1-propanol	0.03±0.00	0.05±0.01	0.074	4.40	B
1-butanol	0.02±0.00	nd[f]	-	5.41	A
2-methyl-1-pentanol	0.02±0.00	nd[f]	-	5.94	B
1-pentanol	0.04±0.00	0.02±0.00	***	7.82	A
3-methyl-2-buten-1-ol	0.04±0.01	0.04±0.01	0.421	9.23	B
1-hexanol	0.10±0.02	0.03±0.01	0.360	10.52	A
1-octen-3-ol	0.18±0.03	0.08±0.03	***	13.02	A
1-heptanol	0.02±0.01	nd[f]	-	13.32	A
2-ethyl-1-hexanol	0.04±0.02	0.04±0.01	0.425	14.26	A
1-octanol	0.08±0.00	0.17±0.04	*	16.16	A
1-nonanol	0.03±0.00	nd[f]	-	18.72	A
Acids					
hexanoic	0.50±0.14	0.28±0.05	0.125	25.45	A

heptanoic	0.29±0.07	0.19±0.06	*	27.40	A
octanoic	0.54±0.16	0.65±0.17	0.458	30.72	A
nonanoic	0.61±0.05	0.13±0.03	***	32.50	A
decanoic	0.95±0.03	1.28±0.10	***	35.62	A
Ketones					
3-pentanone	0.62±0.12	nd[f]	-	2.77	B
heptane-2,3-dione	nd[f]	0.02±0.00	-	5.76	A
3-heptanone	0.27±0.05	0.12±0.03	0.051	5.94	A
2-heptanone	0.10±0.02	0.06±0.02	**	6.30	A
2-octanone	0.02±0.00	nd[f]	-	8.65	A
1-octen-3-one	0.02±0.00	nd[f]	-	8.99	B
Furans					
2-pentylfuran	0.12±0.04	0.03±0.01	***	7.15	A
Lactones					
γ-butyrolactone	0.01±0.00	nd[f]	-	18.60	A
γ-hexalactone[g]	0.02±0.00	nd[f]	-	20.47	A
γ-heptalactone[g]	0.01±0.00	nd[f]	-	23.06	A
δ-octalactone[g]	0.01±0.00	0.01±0.00	0.699	27.47	A
γ-octalactone[g]	0.02±0.00	0.01±0.00	*	28.91	A
γ-nonalactone[g]	0.45±0.01	0.34±0.02	**	28.65	B
δ-nonalactone[g]	0.04±0.01	0.02±0.00	***	30.36	A
γ-decalactone[g]	0.02±0.01	0.04±0.01	***	31.62	A
γ-undecalactone[g]	0.21±0.03	0.13±0.01	**	31.81	B
δ-decalactone[g]	0.04±0.01	0.45±0.05	***	32.40	A
δ-undecalactone[g]	nd[f]	0.04±0.01	-	35.38	A
δ-dodecalactone[g]	0.05±0.01	0.67±0.04	***	37.63	B

380   [a]LI: Low intramuscular fat ham samples from Large White x Landrace pigs. [b]HI: High
381   intramuscular fat ham samples from Iberian x Duroc (IbD) pigs . [c]p: Statistical significance of
382   the difference between the two means in the same row due to IMF profile. Non-significant
383   differences are given as exact P values whereas significant differences are expressed with
384   asterisks: * when P < 0.05, ** when P < 0.01 and *** when P < 0.001. [d]RT: retention time. [e]MI,
385   method of identification: A, volatile compound identified by comparison of the retention time
386   and the mass spectrum of volatile standards. B, volatile compound tentatively identified by
387   comparison of the mass spectrum in NIST 98 and Wiley libraries. [f]nd: not detected. [g]Volatile
388   compounds with the superindex have not been reported before in cooked pork ham literature.
389

390   Table 3.Comparative profiles of volatile compounds from non-lipid oxidation identified
391   in cooked cured pork ham samples with low (LI)[a] or high HI[b] intramuscular fats using
392   SPME.

Compounds	LI[a]	HI[b]	p[c]	RT[d]	MI[e]
Aldehydes					
acetaldehyde	3.74±0.12	4.76±0.11	***	2.02	A
2-methyl-butanal	0.33±0.05	0.35±0.08	0.597	2.82	A
3-methyl-butanal	0.21±0.09	2.33±0.26	***	2.97	A
benzaldehyde	0.15±0.05	0.11±0.02	0.215	15.54	A
cinnamaldehyde	0.06±0.01	0.02±0.00	***	28.45	B
Alcohols					
ethanol	0.18±0.06	nd[f]	-	2.81	B

3-methyl-1-butanol	nd[f]	0.17±0.07	-	4.31	A
2-methyl-1-butanol	0.02±0.00	nd[f]	-	6.51	B
2-phenyl-ethanol[g]	0.09±0.02	nd[f]	-	25.89	B
Acids					
acetic	0.17±0.02	0.05±0.00	***	13.62	A
butanoic	0.29±0.13	0.45±0.10	0.071	15.43	A
3-methyl-butanoic	0.97±0.12	0.94±0.11	0.637	21.42	A
Ketones					
acetone	0.73±0.13	0.37±0.07	**	2.35	A
2-butanone	0.17±0.07	0.22±0.05	0.215	2.75	A
butane-2,3-dione	0.10±0.03	1.24±0.24	***	3.23	A
pentane-2,3-dione	nd[f]	0.02±0.00	-	4.23	A
3-hidroxybutan-2-one	0.78±0.19	1.20±0.15	**	8.97	A
Esters					
ethyl acetate[g]	0.16±0.05	0.14±0.02	0.464	2.71	B
ethyl butanoate	1.07±0.04	0.59±0.01	***	3.96	A
ethyl-2-methyl butanoate[g]	0.03±0.01	0.04±0.00	0.672	4.14	A
isoamyl acetate	0.13±0.06	0.16±0.01	0.348	5.29	A
2-methylpropyl-3-methyl butanoate	0.03±0.01	nd[f]	-	6.74	B
ethyl caproate	0.02±0.00	0.01±0.00	*	7.47	A
prenyl acetate[g]	nd[f]	0.02±0.00	-	7.75	A
hexyl acetate[g]	0.05±0.01	0.02±0.00	***	8.09	A
isoamyl butanoate	0.04±0.01	0.02±0.01	**	8.16	A
benzyl acetate[g]	nd[f]	0.29±0.03	-	21.06	B
Furans					
tetrahidrofuran[g]	nd[f]	0.01±0.00	-	2.55	B
furfural	0.02±0.01	0.02±0.01	0.371	13.77	A
Terpenes and derivates					
α-pinene[g]	0.10±0.03	0.07±0.01	0.071	3.75	A
β-pinene[g]	0.03±0.01	0.02±0.01	0.383	4.85	A
sabinene[g]	0.02±0.01	0.02±0.01	0.071	4.91	B
myrcene[g]	0.02±0.01	0.01±0.01	*	5.73	B
δ-3-carene[g]	0.03±0.01	nd[f]	-	5.75	B
1,4-cineole[g]	nd[f]	0.26±0.17	-	6.07	B
limonene	0.45±0.05	0.59±0.07	0.127	6.74	A
α-phellandrene[g]	nd[f]	0.07±0.01	-	6.86	B
1,8-cineole	nd[f]	0.12±0.01	-	7.06	A
terpinolene[g]	nd[f]	0.02±0.00	-	8.23	A
p-cymene[g]	0.09±0.01	0.05±0.00	0.052	8.51	A
linalool	0.45±0.02	0.50±0.05	*	15.8	A
linalool acetate[g]	0.04±0.01	nd[f]	-	15.99	B
Nitrogen and sulphur compounds					
methanethiol	0.05±0.01	0.02±0.01	***	1.92	A
dimethyl sulfide	0.04±0.01	0.01±0.00	**	2.12	B
dimethyl disulfide	0.04±0.01	nd[f]	-	4.35	A
pyridine	0.03±0.00	0.02±0.00	**	4.41	A
methional	0.13±0.03	0.05±0.01	***	13.42	A
Miscellaneous					
ethoxyethane[g]	0.01±0.00	nd[f]	-	1.89	B

benzene	0.07±0.01	nd[f]	-	3.05	B
toluene	0.79±0.04	0.43±0.02	*	4.17	A

393 [a]LI: Low intramuscular fat ham samples from Large White x Landrace pigs. [b]HI: High
394 intramuscular fat ham samples from Iberian x Duroc(IbD) pigs. [c]p: Statistical significance of the
395 difference between the two means in the same row due to IMF profile. Non-significant
396 differences are given as exact P values whereas significant differences are expressed with
397 asterisks: * when P < 0.05, ** when P < 0.01 and *** when P < 0.001. [d]RT: retention time. [e]MI,
398 method of identification: method of identification: A, volatile compound identified by
399 comparison of the retention time and the mass spectrum of volatile standards. B, volatile
400 compound tentatively identified by comparison of the mass spectrum in NIST 98 and Wiley
401 libraries. [f]nd: not detected. [g]Volatile compounds with the superindex have not been reported
402 before in cooked pork ham literature.
403

404 Table 4.Comparative profiles of volatile compounds identified in cooked cured pork
405 ham samples with low (LI)[a]or high (HI)[b] intramuscular fats using SBSE.

Compounds	LI[a]	HI[b]	p[c]	RT[d]	MI[e]
Aldehydes					
acetaldehyde	3.14±0.16	2.39±0.15	***	2.02	A
pentanal	nd[f]	7.98±0.52	-	3.45	A
hexanal	2.45±0.32	1.38±0.15	**	4.70	A
octanal	0.64±0.04	0.90±0.19	**	8.75	A
nonanal	1.06±0.04	2.23±0.19	***	11.55	A
benzaldehyde	0.69±0.02	0.31±0.09	***	15.54	A
lauric[g,h]	110.27±5.56	113.31±9.42	0.554	20.44	A
cinnamaldehyde	0.05±0.01	0.07±0.00	0.554	28.45	B
4-anisaldehyde[h]	nd[f]	3.35±0.46	-	29.20	B
Alcohols					
ethanol	0.53±0.03	0.45±0.03	**	2.81	A
2-methyl-1-pentanol	0.85±0.12	nd[f]	-	5.94	B
2-ethyl-1-hexanol	0.88±0.06	nd[f]	-	14.26	A
1-octanol	2.65±0.09	6.39±0.21	***	16.16	A
anethol[h]	0.06±0.01	nd[f]	-	23.98	B
2-phenyl-ethanol[g]	4.06±0.05	10.02±0.12	***	25.89	B
Acids					
acetic	1.02±0.35	3.29±0.36	***	13.62	A
butanoic	1.24±0.02	4.06±0.11	**	15.43	A
3-methyl-butanoic	0.56±0.05	0.65±0.11	0.108	21.42	A
pentanoic[h]	0.94±0.08	2.72±0.27	***	22.10	A
hexanoic	0.79±0.15	1.22±0.11	*	25.45	A
heptanoic	0.45±0.09	1.65±0.26	***	27.40	A
octanoic	1.21±0.19	1.27±0.37	0.784	30.72	A
nonanoic	1.50±0.07	0.75±0.09	***	32.50	A
decanoic	0.85±0.06	1.49±0.22	***	35.62	A
lauric[h]	1.46±0.18	0.96±0.02	***	39.91	A
miristic[h]	2.82±0.13	0.81±0.23	***	43.94	B
palmitic[h]	4.53±0.32	1.98±0.55	***	49.22	A
Ketones					
acetone	nd[f]	9.83±0.25	-	2.46	A
2-octanone	1.74±0.13	nd[f]	-	8.65	A
3-hidroxybutan-2-one	2.44±0.34	8.89±0.54	**	8.97	A

20

2-nonanone	1.29±0.06	nd[f]	-	11.46	A
Esters					
ethyl acetate[g]	8.97±0.09	3.79±0.11	***	2.71	A
ethyl butanoate	0.09±0.01	0.03±0.01	***	3.96	A
isoamyl acetate	4.55±0.10	9.03±0.17	***	5.29	A
hexyl acetate[g]	n.d.[f]	6.29±0.43	-	8.09	A
isoamyl butanoate	nd[f]	1.75±0.16	-	8.16	A
benzyl acetate[g]	nd[f]	2.10±0.25	-	21.06	B
Furans					
2-pentylfuran	4.27±0.12	nd[f]	-	7.15	A
Terpenes and derivates					
α-pinene[g]	0.45±0.04	0.55±0.13	0.065	3.75	A
β-pinene[g]	2.70±0.11	6.85±0.08	***	4.85	A
sabinene[g]	0.32±0.03	0.96±0.32	***	4.92	B
myrcene[g]	0.07±0.02	0.06±0.05	0.232	5.73	B
δ-3-carene[g]	0.06±0.03	0.08±0.04	***	5.75	A
α-terpinene[g,h]	nd[f]	0.23±0.12	-	6.12	A
limonene	26.76±1.37	29.63±1.25	0.868	6.74	A
α–phellandrene[g]	0.13±0.01	0.15±0.01	0.176	6.86	B
1,8-cineole	0.33±0.06	0.19±0.05	0.188	7.06	A
γ-terpinene[g]	0.11±0.02	0.60±0.04	***	7.47	A
terpinolene[g]	0.03±0.01	0.05±0.01	***	8.23	A
p-cymene[g]	1.93±0.23	0.26±0.03	***	8.51	A
linalool oxide[g,h]	nd[f]	0.08±0.01	-	12.66	A
dihydromyrcenol[g,h]	1.54±0.21	5.22±0.09	***	13.58	A
menthone[g,h]	nd[f]	0.05±0.02	-	13.67	A
iso-menthone[g,h]	nd[f]	0.07±0.03	-	14.40	B
linalool	0.08±0.01	0.13±0.03	**	15.80	A
bornyl acetate[g,h]	0.07±0.01	0.27±0.04	***	16.69	A
isoborny lacetate[g,h]	0.09±0.02	nd[f]	-	16.94	A
β-caryophyllene[g,h]	0.16±0.05	0.14±0.03	*	17.20	A
terpenyl acetate[g,h]	0.08±0.02	nd[f]	-	20.29	A
γ-terpineol[g]	nd[f]	0.19±0.04	-	20.45	B
citronelol[g,h]	nd[f]	10.03±0.24	-	22.10	B
nerol[g,h]	nd[f]	0.16±0.02	-	22.80	B
geraniol[g,h]	nd[f]	6.80±0.34	-	24.00	B
thymol	0.12±0.04	0.20±0.02	*	32.40	B
Lactones					
γ-butyrolactone	nd[f]	4.77±0.35	-	18.60	A
δ-octalactone[g]	1.08±0.26	nd[f]	-	27.47	A
γ- octalactone[g]	0.76±0.12	1.53±0.09	***	28.65	B
γ-nonalactone[g]	0.44±0.08	0.69±0.13	***	28.91	A
γ-undecalactone[g]	0.65±0.04	0.66±0.05	0.486	31.81	A
δ-undecalactone[g]	nd[f]	0.54±0.05	-	35.38	A
δ-dodecalactone[g]	0.85±0.09	nd[f]	-	37.63	B
Nitrogen and sulphur compounds					
methanethiol	0.77±0.22	nd[f]	-	1.92	A
dimethyl disulfide	0.02±0.00	nd[f]	-	4.41	A
octanenitrile[g,h]	nd[f]	2.57±0.14	-	9.18	B
3-isothiocyanato-1-propene[h]	0.05±0.01	0.09±0.03	***	10.69	B

Miscellaneous					
ethoxyethane[g]	0.05±0.01	nd[f]	-	1.89	B
benzene	0.02±0.00	0.01±0.01	***	3.05	B
toluene	12.06±0.67	nd[f]	-	4.17	A
indole[h]	0.04±0.01	0.02±0.00	**	37.75	A
skatole[h]	0.01±0.00	0.05±0.01	***	38.94	A

406 [a]LI: Low intramuscular fat ham samples from Large White x Landrace pigs. [b]HI: High
407 intramuscular fat samples from Iberian x Duroc(IbD) pigs. [c]p: Statistical significance of the
408 difference between the two means in the same row due to IMF profile. Non-significant
409 differences are given as exact P values whereas significant differences are expressed with
410 asterisks: * when P < 0.05, ** when P < 0.01 and *** when P < 0.001. [d]RT: retention time. [e]MI,
411 method of identification: A, volatile compound identified by comparison of the retention time
412 and the mass spectrum of volatile standards. B, volatile compound tentatively identified by
413 comparison of the mass spectrum in NIST 98 and Wiley libraries. [f]nd: not detected. [g]Volatile
414 compounds with the superindex have not been reported before in cooked pork ham literature.
415 [h]Identified only by SBSE analysis.

HIGHLIGHTS

Volatiles of pork cooked ham were evaluated comparing high and low IM fat hams.

The majority of volatiles in both breeds were derived from lipid oxidation.

We identified 42 novel volatiles never reported before in cooked cured pork ham.

Low IMF hams had high PUFA and elevated volatiles conferring rancid notes.

Novel SBSE application showed high sensitivity for fatty acids and terpenes.