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1 **A new cause of spoilage in goose sausages**

2

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17

18 **Abstract**

19 The aim of this work was to determine the microorganisms present and to investigate their

20 metabolites that cause spoilage of many goose sausages produced in Friuli, a northeast region

21 of Italy. The defect was observed by sensorial analysis using the “needle probing” technique;

22 the spoiled sausages were unsafe and not marketable. Despite the addition of starter, the

23 microorganisms, particularly enterococci and Enterobacteriaceae, grew during ripening and

24 produced a large amount of biogenic amines; therefore, these sausages represented a risk to

25 consumers. The production of those compounds was confirmed *in vitro*. Furthermore, a

26 second cause of spoilage was attributed to moulds that grew during ripening; the fungi grew

27 between the meat and casing, producing a large amount of total volatile nitrogen, and
28 consequently an ammonia smell was present either in the ripening area or in the sausages.
29 This is the first description of this type of defect in goose sausages.

30

31 **1. Introduction**

32 In Friuli, a large number of traditional sausages are produced using raw meat of different
33 animals: pork, beef, wild game (deer) and poultry. In particular, goose meat mixed with pork
34 fat is used to produce sausages that are characterized by a slight sour taste and a semi-rigid
35 consistency, which is elastic but not rubbery. These goose sausages are prepared with fresh
36 or frozen goose meat, pork lard, NaCl (2.8% maximum) and additives (nitrates, nitrites,
37 spices). Sugars (mostly sucrose and dextrose) and microbial starters, consisting of
38 coagulase-negative, catalase-positive cocci (CNCPC) and lactic acid bacteria (*Lactobacillus*
39 *sakei*), are also used in both industrial and craft manufacturing. In fact, goose sausages are
40 essentially produced by shops and other small producers (farms, frasche, typical taverns of
41 Friuli) or by artisanal facilities, and consequently the quality is not standardized. Indeed,
42 considering that appropriate drying and ripening chambers or systems with the complete
43 control of relative humidity (R.H.) and temperature do not exist in such small facilities, each
44 lot can have its own history and be completely different from other lots. However,
45 production of an edible product requires evaluation of the choice of raw material, the natural
46 microclimate of the drying/ripening rooms and the aptitude of the producers.

47 Similar to traditional sausages made with pork meat and fat, goose sausage ripening is based
48 on microbial and tissue enzymes (Comi et al., 2005, 2000; Coppola et al., 1998). CNCPC
49 and lactic acid bacteria (LAB) are the main microorganisms responsible for ripening (Talon,
50 2007; Iacumin et al., 2006; Metaxopoulos et al., 2001; Garcia-Verona et al., 2000). Although
51 these bacteria are normally present in salt and both pork and goose meat, they are often
52 intentionally added to fat and meat mixtures for sausages as microbial starters to ensure a

53 consistent aroma and flavor, to improve quality and to reduce the length of the curing period
54 (Iacumin et al., 2006; Comi et al., 2005, 2000; Tjener et al., 2003; Luongo et al., 2001). To
55 meet the increasing needs of new products requested by consumers, goose sausages
56 represent an effort to generate alternatives. Goose and chicken meat and their products are
57 preferred and largely consumed by the public; although chicken meat is often mixed with
58 the meat of other animals, the combination of goose meat with other meat is quite rare
59 (Gulbaz and Kamber, 2008). Recently, the Italian population has rediscovered products
60 based on regional recipes, and goose sausages, which are common in villages throughout
61 Italy, constitute an important resource. Accordingly, these sausages are widely produced and
62 appreciated by consumers, who are weary of eating traditional sausages made with pork
63 meat. The quality of goose sausages is variable and often distinct. However, defects can
64 occur during goose breeding and slaughtering, and sausage manufacturing, making the
65 sausages unfit for consumption. The quality of the raw material, bacterial metabolism, as
66 well as temperature and R.H. values during production and storage can cause these defects.
67 In addition, inadequate ripening may also lead to unpleasant odors or tastes.
68 A small-scale facility produced two lots (a and b) of goose sausages. During their ripening,
69 lot b presented a defect consisting in an ammonia smell, which was confirmed by a sensorial
70 analysis, made by non professional assessors.
71 Therefore, the aim of this work was to study the microorganisms and the metabolites
72 responsible for the defects and spoilages of these goose sausages.

73

74 **2. Material and Methods**

75 2.1 Evaluation and identification of the defect

76 In January, a small-scale facility located in the Friuli area produced two lots of sausages (30
77 each) with two different batches of goose meat (Lots a and b). Five days before the end of

78 ripening, the sausages of Lot b presented an ammonia smell, which was also widespread in
79 the ripening room area. No ammonia smell was perceived in the area of the Lot a sausages.

80

81 2.1.1. *Sensorial analysis*

82 In this facility, the workers are used to tasting each lot before selling in order to value its
83 sensorial quality. Consequently also in this case, all the sausages of both lots were evaluated
84 by the “needle probing” technique, by ten assessors of a non-professional panel (workers at
85 the facility). The technique involves the rapid insertion of a thin horse bone into the
86 sausages, resulting in the perception of odors (Barbuti et al., 2003).

87 Then 5 sausages of Lot b were sliced and tasted by the panelists in order to identify the
88 flavor and to determine the defect.

89

90 2.2. *Sampling*

91 Twenty unspoiled (Lot a) and 20 spoiled goose (Lot b) sausages were analyzed. The samples
92 were collected at the end of the ripening period (45 days). Defects of the spoiled goose
93 sausages were found late during ripening (5 days before the end) due to an ammonia smell
94 that was widespread in the ripening rooms. Both lots of sausages, produced the same day
95 with two different batches of goose meat, had the following composition: goose meat 70%,
96 lard 30%, NaCl 2.8%, KNO₃ 0,02%, dextrose 0.1 %, black pepper 0.002%, nutmeg 0.002%.

97 Before adding each ingredient of the recipe, a starter composed of *Staphylococcus xylosus*
98 and *L. sakei* (1/1 ratio) was added at a final concentration of 6 log CFU/g. A starter of
99 *Penicillium nalgiovense* was spread by aerosol (approximately 3 log/cm²) onto the casings.

100 Natural casing was used.

101 Before analysis, the spoiled and unspoiled products were washed to eliminate moulds on the
102 casings, which were then aseptically removed. Then each sausage was sterile sliced. Four
103 slices of each sausage were used for color determination. The remaining slices were

104 homogenized in stomacher and the homogenate was used for microbial and physico-
105 chemical analysis and for biogenic amines and volatile compounds determination.

106

107 2.3. Microbiological analysis

108 Ten g of the meat homogenate was serially diluted with saline-peptone water (8 g/l NaCl, 1
109 g/l bacteriological peptone; Oxoid, Italy, distilled water 1000 ml) in stomacher bags. An
110 aliquot of 1 or 0.1 ml of each serial dilution was plated onto agar for counts of different
111 groups of microorganisms: the Total Viable Count (TVC) was evaluated on Plate Count
112 Agar (PCA, Oxoid, Italy) incubated at 30 °C for 48-72 h; LAB were grown on De Man
113 Rogosa Sharpe agar (MRS, Oxoid, Italy) incubated at 42 °C for 48 h; yeasts and moulds
114 were grown on Malt Agar (MA, Oxoid, Italy) incubated at 25 °C for 72-96 h and
115 distinguished by macroscopical and microscopical examination (Samson et al., 2004);
116 *Escherichia coli* was grown on Violet Red Bile Lactose Agar (VRBLA, Oxoid, Italy)
117 incubated at 44 °C for 24 h; Enterobacteriaceae were grown on Violet Red Bile Glucose
118 Agar (VRBGA, Oxoid, Italy) incubated at 37 °C for 24 h; coagulase-positive, catalase-
119 positive cocci (CPCPC) were grown on Baird-Parker agar medium (BP, Oxoid, Italy)
120 supplemented with egg yolk tellurite emulsion (Oxoid, Italy) incubated at 35 °C for 24-48 h
121 and confirmed by a coagulase test; coagulase-negative, catalase-positive cocci (CNCPC)
122 were grown on Mannitol Salt Agar (MSA, Oxoid, Italy) incubated at 30 °C for 48 h;
123 enterococci were grown on Kanamycin Aesculin Azide Agar (KAA, Oxoid, Italy) incubated
124 at 37 °C for 48 h; sulfite-reducing Clostridia were quantified on Differential
125 Reinforced Clostridia Medium (DRCM, VWR, USA) incubated at 37 °C for 24-48 h in an
126 anaerobic jar with an anaerobic kit (gas pack anaerobic system, BBL, Becton Dickinson,
127 USA). *Salmonella* spp. were evaluated by the ISO (6579-1 2002 Cor.1:2004 Microbiology
128 of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.)
129 method and *Listeria monocytogenes* by another ISO (11290-1,2:1996 Adm.1:2004.

130 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of
131 *Listeria monocytogenes*) method.

132

133 2.3.1. Isolation and identification of enterococci

134 One hundred colonies were randomly isolated from KAA agar plates of the spoiled sausages
135 containing between 30 and 50 colonies and purified on PCA (Oxoid, Italy), which was
136 incubated at 37 °C for 48 h. After purification, the colonies were subjected to Gram staining
137 and to a catalase test. Gram-positive streptococci and catalase-negative colonies were
138 identified by API 20 Strep, according to the manufacturer's method (BioMerieux, France).

139

140 2.3.2. Isolation and identification of moulds

141 One hundred mould colonies grown on MA were isolated from the spoiled goose sausages,
142 purified and transferred onto three different agar media: Czapek Dox Agar (Oxoid, Italy), MA
143 and Salt-Malt Agar (5% malt extract, 5% NaCl, distilled water 1,000 ml, pH 6.2; Oxoid,
144 Italy). The moulds were identified by morphological characters by macroscopical and
145 microscopical examination (hyphas, spores and reproduction, colour of colony and type of
146 mycelium) according to Samson et al. (2004). The identification was confirmed by PCR-
147 DGGE and sequencing according to method reported in Iacumin et al. (2009) briefly: The
148 DNA of each colony was amplified by Nested PCR (2 step amplification). Each amplicon was
149 run in acrilamide gel (DGGE), then it was excised by gel cutting tips and subjected to a re-
150 amplification with the same primers without GC clamp. The product was cloned into pGEM-
151 T easy vector (Promega, Milan, Italy), following the instructions of the manufacturer. The
152 insert of the appropriate clone was sequenced by a commercial facility (Eurofins MWG
153 GmbH, Martinsried, Germany). Sequence comparisons were performed using the Blast
154 program (Altschul et al., 1997).

155

156

157 *2.4. In vitro reproduction of the defect by moulds*

158 One hundred g of the unspoiled meat homogenates of goose sausages was boiled in water
159 (200 ml) for 1 h. After boiling, the mixture was filtered through cotton wool and sterilized at
160 115 °C for 15 minutes. The sterilized mixture was adjusted to 300 ml with distilled sterile
161 water and distributed among 10 Petri plates (30 ml each). A loop of each isolated mould
162 species was inoculated in the plates (one strain per plate), which were incubated for 7 days
163 at 25 °C. Three replicates of each strain were performed. At the end of the incubation period,
164 each mixture was filtered and analyzed for the presence of TVB-N (Total Volatile Basic
165 Nitrogen), biogenic amines and acetic acid.

166

167 *2.5. Total Volatile Basic Nitrogen (TVB-N), pH, acetic acid and color determination*

168 TVB-N was evaluated by the Pearson (1976) method; briefly: “The TVB-N is released by
169 boiling the sample directly with magnesium oxide, which also prevents volatile acids from
170 distilling over into the boric acid. The distillate is titrated with standard acid”. The pH of the
171 product was measured directly by inserting a pH meter probe (Radiometer, Denmark) into
172 the sample. The water activity (A_w) was determined using a Hygromer AWVC (Rotronic,
173 Italia). Acetic acid was detected using an Acetic acid kit (R-Biopharm, Italy) according to
174 the manufacturer’s instructions. The color was measured using a Minolta Chromameter CR-
175 200 (Singapore) and the CIE Lab system. After calibration with standard white tiles, the
176 chromameter was positioned perpendicular to the patty surface, and 10 different positions
177 were evaluated for each sample immediately after slicing. The evaluated parameters were
178 L^* , a^* and b^* . L^* describes the white intensity or brightness, with values ranging from 0
179 (black) to 100 (white). The a^* value describes the redness ($a^* > 0$), and b^* describes the
180 yellowness ($b^* > 0$). The final value was expressed as the respective average of ten
181 measurements.

182

183 *2.6. Biogenic amines in vitro and in spoiled and unspoiled sausages*

184 All the identified strains were tested for biogenic amine production on agar media,
185 according to the Bover-Cid and Holzapfel (1999) method. Ten out 20 of spoiled and
186 unspoiled meat homogenates were randomly sampled in order to detect the biogenic amines
187 using the method proposed by Eerola et al., (1993) briefly: “Amines were separated using
188 HPLC (HPLC Jasco 2089 quaternary pump, AS 2057 autosampler; Jasco, Ishikawa-cho,
189 Japan). The separation was carried out by gradient elution with 0.1 mol L⁻¹ ammonium
190 acetate/acetonitrile on a reverse-phase column (Spherisorb ODS-2; 5 µm, 125 × 4 mm;
191 Waters Corporation, Milford, MA, USA) at a flow rate of 1 mL min⁻¹ using UV/VIS 2075
192 detector operating at 254 nm for biogenic amine (Jasco, Ishikawa-cho, Japan).

193

194

195 *2.7. Volatile compound determination*

196 Ten out 20 of spoiled and 10 out 20 of unspoiled meat homogenates were randomly
197 collected and analyzed for the presence of volatile compounds using SPME-GC-MS and a
198 Finnigan Trace DSQ (Thermo Scientific Corporation, USA) with a Rtx-Wax capillary
199 column (length 30 m x 0.25 mm id.; film thickness 0.25 µm; Restek Corporation, USA),
200 according to the method reported by Chiesa et al. (2006). The volatile compounds were then
201 identified by comparing the spectra obtained experimentally with spectra available in the
202 Commercial Wiley library and an in-lab library. The results represent the average of all 10
203 samples.

204

205 2.8. *Statistical analysis*

206 The values of the various parameters were compared using a one-way analysis of variance.
207 Averages were compared with Tukey's honest significant test using the StatGraphics
208 software package from Statistical Graphics (Rockville, Maryland).

209

210 **3.0 Results**

211 The ten assessors of the non-professional panel (workers at the facility) confirmed the
212 ammonia smell in lot b by "needle probing" technique. Moreover, after tasting lot b, some of
213 the panel members suffered from headaches (3 of 10), facial flushing (7 of 10) and bright
214 red rash (7 of 10), symptoms that are typical in consumers who eat foods rich in biogenic
215 amines. Consequently the sausages were also analysed for biogenic amine presence. Indeed
216 all the panelists perceived also a vinegar odor.

217 The microbial and physico-chemical characteristics of the unspoiled and spoiled goose
218 sausages are shown in Tables 1 and 2, respectively. The PCA and MRS counts are typical of
219 sausages, and there was no significant difference ($p > 0.05$) between the spoiled and
220 unspoiled sausages. In particular, the PCA counts of both the sausages were approximately 6
221 log CFU/g, and the MRS counts were approximately 8.6 log CFU/g. Yeasts and CNCPC
222 concentrations also did not differ significantly ($p > 0.05$) between the spoiled and unspoiled
223 sausages. The yeast counts were less than 3 log CFU/g, and the CNCPC concentration was
224 approximately 6 log CFU/g; these concentrations are also typical of traditional Italian
225 sausages made with pork meat. The moulds, the enterococci and the Enterobacteriaceae
226 concentrations differed significantly between the spoiled and unspoiled samples ($p < 0.05$).
227 The values of enterococci and moulds in the spoiled sausages were 3 logs higher than in the
228 unspoiled samples. The high level of moulds in the spoiled sausages was not due to
229 contamination during sampling because the casings of both groups were first brushed and
230 washed. In the spoiled goose sausages, the moulds probably grew in the space between the

231 meat and casing. Indeed in some part of the spoiled sausages, between the casing and the
232 meat, a white mycelium was observed, consequently this can prove the higher level of
233 moulds in spoiled sausages. In the spoiled goose sausages, Enterobacteriaceae reached log
234 4.0 ± 0.4 CFU/g and were log 2.0 ± 0.1 CFU/g in the unspoiled samples. CCPPC, sulfite-
235 reducing Clostridia and *E. coli* were present at concentrations below the detection limit of
236 the method (LOD < 10 CFU/g). *Listeria monocytogenes* was present at less than 100 CFU/g,
237 and *Salmonella* was absent in a 25-g sample, according to REG. EEC 2073/05 (15/11/2005,
238 L 338/1). The physico-chemical parameters demonstrated significant differences for the
239 levels of TVB-N and histamine ($p < 0.05$). Indeed, the spoiled products had a high
240 concentration of histamine (415.25 ± 115.01 mg/kg), a level higher than the limit proposed
241 for fish and fish products (REG. EEC 2073/05) and considered unsafe for consumers. In
242 contrast, the values of histamine in the unspoiled sausages were low, approximately 80 times
243 less than in the spoiled sausages. The cadaverine concentration of the spoiled sausages
244 reached 339.3 ± 31.40 mg/kg, 10 times more than the concentration found in the unspoiled
245 sausages. Putrescine, spermine and spermidine concentrations were below the limit of
246 detection (< 1 mg/kg). Moreover, the TVB-N value of the spoiled sausages was
247 approximately twice that of the unspoiled sausages. The pH and A_w values of both sausage
248 lots were similar at a level of 6.3 and 0.92, respectively. The brightness of the spoiled
249 sausages, as expressed by the evaluation of L^* , was not significantly different ($p > 0.05$)
250 from that of the unspoiled sausages (Table 2). Moreover, parameters a^* and b^* showed no
251 changing, and the observed differences were not significant ($p > 0.05$). It is clear that natural
252 oxidative phenomena induced by microorganisms are involved with spoilage, and it is well
253 known that heterofermenting LAB release small amounts of hydrogen peroxide and
254 hydrogen sulfide, which produce discoloration and greening (Comi and Iacumin, 2012).
255 However, it was not observed any greening or discoloration in the spoiled samples.

256 Table 3 shows the ability to produce biogenic amines of 100 enterococci isolates issued
257 from spoiled sausages *in vitro*. Isolates were identified as *Enterococcus faecium* (N=70) and
258 *Enterococcus faecalis* (N=30), two species which are typical sugar fermenting but able to
259 decarboxylate amino acids and produce amines. As observed *in vitro*, all the isolated strains
260 produced histamine, and 60 out of 70 *E. faecium* and 25 out 30 *E. faecalis* strains produced
261 cadaverine. Conversely, only 10 out of 70 (both species combined) produced tyramine; 6 out
262 of 70 *E. faecium* and 4 out of 30 *E. faecalis* were able to produce putrescine, spermine and
263 spermidine. These data justify the low concentration of these biogenic amines found in the
264 sausages (below the detection limit).

265 The moulds isolated belonged to 3 different species: *Penicillium nalgiovense* (85 isolates),
266 which predominated, being inoculated as a starter; *P. chrysogenum* (8 isolates) and *P.*
267 *viridicatum* (7 isolates) were also present. The compounds found *in vivo* (TVB-N, acetic
268 acid) were produced by all the mould strains *in vitro* (Table 4), though no biogenic amines
269 were produced by the moulds *in vitro* (Table 4). Either TVB-N or acetic acid contributed to
270 the off-odor and off-flavor of the spoiled goose sausages, as perceived by the panelists.

271 The volatile compounds and their concentrations are shown in Table 5, which also shows the
272 retention times, individual compounds and means of concentrations expressed in µg/kg
273 product from ten analytical runs. The data demonstrate that the same substances were
274 present in both sausage groups tested. The concentrations of these substances were
275 determined by their amount relative to the internal standard (ethylpropionate).

276 For a better interpretation of the results obtained from the headspace, the 37 observed
277 substances were divided into 6 classes: aldehydes (2), ketones (6), esters (1), hydrocarbons
278 (7), alcohols (7), carboxylic acids (10), and others (4). Some classes and compounds are
279 typical of well-ripened dry sausages. The concentrations of only 13 out of 37 compounds
280 exhibited a significant difference between the spoiled and unspoiled goose sausages ($p <$
281 0.05). In fact, the amounts of 2-butanone, 2-pentanone, formic acid pentylester, 2,4-

282 dimethylexane, 3-ethylexane, ethylbenzene, 2-butanol, 1-propanol, 2-pentanol, acetic acid,
283 2-methylpentanoic, butanoic acid and 3-methylpentanoic acid in the spoiled sausages were
284 significantly different from the unspoiled ones. In addition, the differences in the
285 concentrations of volatile organic compounds between the spoiled and unspoiled samples
286 only partially influenced the off-odor. In fact, it appears that only the concentrations of
287 TVB-N and acetic acid were important to the production of the off-odor and off-flavor of the
288 spoiled product.

289

290 **4.0. Discussion**

291 The spoilage of the goose sausages examined was due to the large growth of enterococci and
292 moulds. As shown, the values of moulds and enterococci in the spoiled sausages were 3 logs
293 higher than the values of these microbial groups in the unspoiled samples. The compounds
294 responsible for the spoilage mainly included biogenic amines and TVB-N. The former are
295 common in fermented meats and other foods (Roig-Sagués et al., 1999; Gardini et al., 2008),
296 and the most important are histamine, putrescine, cadaverine, tyramine, tryptamine,
297 spermine, and spermidine. These compounds originate from the decarboxylation of amino
298 acids, and consequently foods rich in proteins are a potential risk (Hernández-Jover et al.,
299 1997). The effects observed in some of the panelists during the tasting and the sensorial
300 analysis are justified by the presence of histamine and cadaverine, over 300 mg/kg. Biogenic
301 amines are often present in Spanish and Italian sausages. (Hernandez-Jover et al., 1996,
302 1997; Roig-Sagués et al., 1999; Bover-Cid et al., 2000). However, their data do not agree
303 with ours because in the present work, only cadaverine, and histamine were found, whereas
304 a large amount of tyramine (600 mg/kg), putrescine (up to 450 mg) were found in those
305 earlier studies. Tyramine production, as well as histamine, depends on lactobacilli and
306 enterococci (Suzzi and Gardini 2003; Buňková et al., 2009; Pircher et al., 2007; Ladero et
307 al., 2012; Marcobal et al., 2012; Gardini et al., 2008), but in this work, only a few of the

308 isolated enterococci were able to produce tyramine. Tyramine was not found consequently it
309 must be excluded that lactobacilli could have contributed to the production of biogenic
310 amines, considering that only lactobacilli possess the tyrosine decarboxylase enzyme (Suzzi
311 and Gardini, 2003; Buňková et al., 2009; Pircher et al., 2007).

312 LAB, CNCPC and CCPPC lack histidine decarboxylation capability (Landete et al., 2007,
313 2008), whereas LAB and staphylococci cannot produce cadaverine and putrescine (Pircher
314 et al., 2007). Consequently, it appears that only Enterobacteriaceae and enterococci could be
315 responsible for the production of biogenic amines, particularly histamine, in fermented foods
316 (Suzzi and Gardini, 2003; Gardini et al., 2008), considering that Enterobacteriaceae
317 decarboxylase activity can continue after the cell autolysis (Rossi et al., 2011; Kanki et al.,
318 2007). In the tested spoiled goose sausages, the Enterobacteriaceae could have had a limited
319 activity, being present at level of 4 log CFU/g. Conversely, the number of enterococci in the
320 spoiled goose sausages was higher, up to 6 log CFU/g; therefore, it is possible to speculate
321 their main role in biogenic amine production. Usually in traditional Italian sausages
322 Enterococci grow during the first days of fermentation, but their concentration never
323 exceeds 3.2 log CFU/g at the end of ripening (Comi et al. 2000, 2005; Comi and Iacumin,
324 2013). It is possible to speculate that in the spoiled samples starters cultures could not
325 inhibit the growth of enterococci and enterobacteriaceae, resulting in defects. Also
326 contaminated raw meat, kept at 8 °C, could be a cause of the production of biogenic amines
327 by Enterobacteriaceae and enterococci (Bover-Cid et al., 2000). The spoiled goose sausages
328 in the present study were produced with different batches of goose meat compared to the
329 unspoiled sausages, and this could further explain the spoilage. The raw meat used could not
330 be analysed, however, considering that both the lots of sausages were produced with the
331 same recipe and technology, it is possible to speculate that the batch of meat used for the
332 spoiled sausages could have been dubious and more contaminated than the batch of meat
333 used for the unspoiled sausages. The lack of tyramine, spermine and spermidine do not

334 represent a novelty. Indeed, the presence or absence of the different types of biogenic
335 amines appears to depend on the microorganisms that grow in the product, and this has been
336 confirmed in many studies by various authors (Montel et al., 1999; Parente et al., 2001;
337 Gardini et al., 2008). In the spoiled goose sausages, the presence of only two types of
338 biogenic amines was confirmed by the *in vitro* test, in which a large number of
339 *Enterococcus* strains were able to produce histamine and cadaverine, but only a few
340 tyramine, spermidine, spermine and putrescine .

341 Both the spoiled and unspoiled sausages were properly dried as demonstrated by the pH and
342 A_w values, by the LAB and CNCPC concentration, that were not significantly different ($p >$
343 0.05) and these values should be regarded as normal for meat products (Tjener et al., 2003;
344 Comi et al., 2000, 2005; Gounadaki et al., 2008). In particular the pH remained high despite
345 the concentration of acidifying bacteria (MRS counts) and was similar to that usually found
346 in sausages without defects (Coppola et al., 1998; Comi et al., 2000, 2005).

347 The presence of a higher concentration of TVB-N in the spoiled goose sausages ($208.3 \pm$
348 9.5) than in the unspoiled ($p < 0.05$) was demonstrated by the presence of mould mycelium
349 between the meat and casings. All the isolated mould species were able to produce either
350 TVB-N and acetic acid *in vitro*, and this confirms the higher amount of TVB-N present in
351 the spoiled goose sausages and consequently the ammonia smell of the ripening area and of
352 the sausages. The ammonia smell is due to the high TVB-N concentration, which in well-
353 ripened Italian sausages is typically less than 100 mg N/100 g (Cattaneo et al., 2003; Comi
354 and Iacumin, 2013), as found in the unspoiled goose sausages. However, it is also possible
355 that enterococci, Enterobacteria, LAB and CNCPC could have worked together with moulds
356 in TVB-N production, considering that CNCPC and LAB can metabolize amino acids and
357 produce TVB-N, as it has been demonstrated by various authors (Seefeldt and Weimer,
358 2000; Joffraud et al., 2001; Comi and Iacumin, 2013).

359 Moulds could also play an important role on biogenic amines production of enterococci.
360 Their proteolytic activity releases amino acids (Kamenic et al., 2014; Trigueros et al., 1996),
361 which are the main precursors for biogenic amines production.

362 The volatilome of both the goose sausages was almost similar. The analysis was performed
363 on both sausages, and the data demonstrated that organic acids, alcohols, hydrocarbons,
364 ketones and esters are related to intense bacterial activity and to fresh meat. In particular,
365 only 9 types of compounds of the spoiled sausages were present at higher concentrations
366 than those found in the unspoiled sausages. Conversely, many authors have found much
367 more volatile compounds between unspoiled and spoiled pork meat sausages (Meyner et al.,
368 1999; Comi et al., 2000; Luongo et al., 2001; Tjener et al., 2003; Cantoni et al., 2005). In the
369 spoiled goose sausages, there was a strong presence of acetic acid derived from LAB and
370 moulds. The total amount of ketones, alcohols and volatile fatty acids in the spoiled was
371 higher than that in the unspoiled goose sausages. As expected, the concentrations of some
372 individual molecules produced by fermentation or oxidation were increased in the spoiled
373 products. However, differences in the concentrations of volatile organic compounds between
374 the spoiled and unspoiled samples only partially influenced the off-odor, conversely TVB-N
375 and acetic acid concentration did it, as demonstrated by sensorial analysis.

376 A total of 10 carboxylic acids were detected, and these compounds can all originate from the
377 activity of lipolytic enzymes. The concentrations of acetic acid, 3-methylpentanoic, butanoic
378 acid and 3-methylpentanoic acid in the spoiled products significantly differed from the
379 unspoiled samples ($p < 0.05$). Acetic acid can also originate from the metabolism of sugars
380 and lipids by moulds (Motilva et al., 1993; Comi and Iacumin, 2013). Alcohol compounds
381 result from aldehydes reduction, sugar fermentation, oxidative decomposition of lipids and
382 Strecker degradation of amino acids (Ardò, 2006; Smit et al., 2009; Flores et al., 1997). The
383 concentrations of 2-butanol, 2-pentanol and 1-propanol significantly differed between the
384 spoiled and unspoiled goose sausages ($p < 0.05$). Nevertheless, their presence did not cause

385 any pungent and alcoholic characteristics in the spoiled goose sausages. Only 2 aldehydes
386 were detected, and their concentrations did not significantly differ in the spoiled and
387 unspoiled samples ($p > 0.05$). The low number of aldehydes can be explained by their
388 reduction into alcohols or their oxidation into carboxylic acid (Comi and Iacumin, 2013;
389 Flores et al., 1997). Ketones (2-butanone and 2-pentanone) concentrations were significantly
390 different ($p < 0.05$), but they did not lead to unpleasant solvent smells (Flores et al., 1997;
391 Ardò, 2006). Among the hydrocarbons found, 2,4-dimethylexane, 3-ethylhexane and
392 ethylbenzene concentration was significant different between the spoiled and unspoiled
393 products ($p < 0.05$). In the unspoiled products, a great proportion of hydrocarbons were not
394 transformed into aldehydes and ketones, and this was confirmed by the lower concentration
395 of both the compounds with respect to hydrocarbons. Only one ester was detected, and its
396 concentration was significant different in the spoiled and in unspoiled goose sausages ($p <$
397 0.05). However, the lack of esters is unusual because esters are produced by the
398 fermentation of LAB, CNCPC and other bacteria (Stahnke, 1994). Finally no sulfur and
399 pyrazine compounds were detected.

400

401 **Conclusion**

402 The growth of enterococci and Enterobacteriaceae caused the production of high
403 concentrations of histamine and cadaverine. Indeed, both the amines were responsible for
404 the headaches, facial flushing and bright red rashes in some of the panelists. EFSA (2011)
405 has declared that a food is safe if it contains less than 50 mg/kg of histamine, whereas up to
406 400 mg/kg in food is considered absolutely unsafe (Silla Santos, 1996; Ienistea, 1973).
407 Despite significant differences in the levels of many volatile compounds between the spoiled
408 and unspoiled goose sausages, it appeared that the off-odor perceived through the “needle
409 probing” technique and the off-flavor perceived by tasting were mainly due to the high
410 concentration of TVB-N. In addition, the high concentration of acetic acid produced a

411 perception of a light vinegar taste. The *in vitro* test demonstrated that moulds grown between
412 the meat and casing in the spoiled products, produced a high TVB-N concentration.
413 Consequently, moulds were the main organisms responsible of the off-odor of the spoiled
414 sausages and enterococci and Enterobacteriaceae for the production of biogenic amines.
415 Finally, it could be concluded that the control of both microbial groups in the raw meat will
416 permit the production of safe goose sausages.

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419 **References**

420 Ardò, Y., 2006. Flavour formation by amino acid catabolism. *Biotechnol. Adv.* 24, 238-242.

421 Altschul, S.F., Madden, T.L., Shaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J.,

422 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search

423 programs. *Nucleic Acids Res.* 25, 3389–3402.

424 Barbuti, S., Rastelli, E., Mbe Evane, M.A.E, Grisenti, M.S., Quintavalla, S., Parolari, G.,

425 2003. Alterazione microbica del prosciutto crudo – difetto di vena. *Ind. Conserve* 78, 9-39.

426 Bover-Cid, S., Holzapfel, W. H., 1999. ^{[[SEP]]}Improved screening procedure for biogenic amine

427 production by lactic acid bacteria. *Int. J. Food Microbiol.* 53, 33-41.

428 Bover-Cid, S., Izquierdo-Pulido, M., Vidal-Carou, M. C., 2000. ^{[[SEP]]}Influence of hygienic

429 quality of raw material on biogenic amine production during ripening and storage of dry

430 fermented sausages. *J. Food Protect.* 63, 1544-1550.

431 Buňková, L., Buňka, F., Hlobilová, M., Vaňáková, Z., Nováková, D., Dráb, V., 2009.

432 ^{[[SEP]]}Tyramine production of technological important strains of *Lactobacillus*, *Lactococcus* and

433 *Streptococcus*. *Eur. Food Res. Techn.* 229 (3), 533-538.

434 Cantoni, C., Chiesa, L.M., Cesana, F., Soncin, S., 2005. Composti volatili organici del

435 salame contadino mantovano. *Arch. Vet. It.* 56 (6), 283-302.

436 Cattaneo, P., Stella, L.S., Pensatene, A., Cantoni C., 2003. Alterazioni di salumi preparati
437 con carni suine DFD. Arch. Vet. It. 54 (1/2), 11-20.

438 Chiesa, L., Duchini, M., Iacumin, L., Boscolo, D., Comi, G., Cantoni C. (2006).
439 Caratteristiche chimiche e batteriologiche di salami della Lomellina. Arch. Vet. It. 57 (5),
440 209-224.

441 Comi, G., Procida, G., Aggio, D., Pittia, P., Cantoni, C., Cocolin, L., 2000. Valutazione di
442 uno starter microbico per la produzione di salame tradizionale friulano. Ing. Alim. 4, 9-25.

443 Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., Cocolin, L., 2005.
444 Characterisation of Natural Fermented Sausages Produced in the North East of Italy. Meat
445 Sci. 69, 381-392.

446 Comi, G., Iacumin, L., 2012. Identification and process origin of microorganisms
447 responsible for cavities and volatile off-flavor compounds in artisanal cooked ham. Int. J.
448 Food Sci. Techn. 47, 114-121.

449 Comi, G., Iacumin, I., 2013. Microbial spoilage of traditional dry sausages produced in
450 small-scale facilities in Friuli, a northeastern region of Italy. Acta Alim. 42 (3), 390-399.

451 Coppola, R., Giagnacovo, B., Iorizzo, M., Grazia, L., 1998. Characterization of lactobacilli
452 involved in the ripening of sopressata molisana, a typical southern Italy fermented sausage.
453 Food Microbiol. 15, 347-353.

454 Eerola, S., Hinkkanene, R., Lindfors, E., Hirvi, T., 1993. Liquid chromatographic
455 determination of biogenic amines in dry sausages. J. AOAC Int. 76, 575-578.

456 EFSA, 2011. ^[L]_{SEP} Scientific opinion on risk based control of biogenic amine formation in
457 fermented foods. EFSA J. 9 (10), 2393-2486.

458 Flores, M., Casey Grimm, C., Toldrà, F., Spanier, A.M., 1997. Correlations of sensory and
459 volatile compounds of spanish "serrano" dry cured ham as a function of two processing
460 time. J. Agric. Food Chemistry 45, 2178-2186.

461 García-Varona, M., Santos, E.M., Jaime, I., Rovira, J., 2000. Characterisation of
462 Micrococcaceae isolated from different varieties of chorizo. *Int. J. Food Microbiol.* 54, 189-
463 195.

464 Gardini, F., Bover-Cid, S., Tofalo, R., Belletti, N., Gatto, V., Suzzi, G., 2008. [SEP] Modeling
465 the aminogenic potential of *Enterococcus faecalis* EF37 in dry fermented sausages through
466 chemical and molecular approaches. *Appl. Environ. Microbiol.* 74 (9), 2740-2750.

467 Gounadaki, A.S., Skandamis, P.N., Drosinos, E.H., Nychas, G.J.E., 2008. Microbial ecology
468 of food contact surfaces and products of small-scale facilities producing traditional sausages.
469 *Food Microbiol.* 25, 313-323.

470 Gulbaz, G., Kamber, U., 2008. Experimentally fermented sausage from goose meat and
471 quality attributes. *J. Muscles Foods* 19, 247-260.

472 Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, M. T., Vidal- Carou, M. C.,
473 1996. [SEP] Ion pair liquid chromatographic determination of biogenic amines in meat and meat
474 products. *J. Agricul. Foods Chem.* 44, 2710-2715.

475 Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, M. T., Mariné- Font, A.,
476 Vidal-Carou, M. C., 1997. [SEP] Biogenic amine and polyamine contents in meat and meat
477 products. *J. Agricul. Food Chemistry* 45, 2098-102.

478 Iacumin, L., Comi, G., Cantoni, C., Cocolin, L., 2006. Ecology and dynamics of Coagulase-
479 Negative Cocci isolated from naturally fermented Italian sausages. *Syst. Appl. Microbiol.* 29
480 (6), 480-486.

481 Iacumin, L., Chiesa, L., Boscolo, D., Manzano, M., Cantoni, C., Orlic, S., Comi G., 2009.
482 Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages. *Food*
483 *Microbiol.* 26, 65-70.

484 Ienistea, 1973. [SEP] Significance and detection of histamine in food. *The microbiological*
485 *safety of food*, eds. Hobbs, B. C. and Christian, J. H. B., pp. 327-343.

486 Kameník, J., Saláková, A., Borkovcova I., Pavlík, Z., Lenka Vorlová, V., 2014. The effect
487 of surface mould application to selected properties of dry fermented sausages. J. Microbiol.
488 Biotech. Food Sci. 3, (special issue 3) 22-27.

489 Kanki, M., Yoda, T., Tsukamoto, T., Baba, E., 2007. Histidine decarboxylases and their
490 role in accumulation of histamine in tuna and dried saury. Appl. Environ. Microbiol. 73 (5),
491 1467-1473.

492 Joffraud, J.J., Leroi, F., Roy, C., Bedarguè, J.L., 2001. Characterization of volatile
493 compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon.
494 Int. J. Food Microbiol. 66, 175-184.

495 Ladero, V., Fernández, M., Calles-Enríquez, M., Sánchez-Llana, E., Cãnedo, E., Martín, M.
496 C., Alvarez, M. A., 2012. Is the production of the biogenic amines tyramine and putrescine a
497 species-level trait in enterococci? Food Microbiol. 30, 132-138.

498 Landete, J. M., de las Rivas, B., Carrascosa, A. V., Muñoz, R., 2007. Screening of
499 biogenic amine production by coagulase-negative staphylococci isolated during industrial
500 Spanish dry-cured ham processes. Meat Sci. 77 (4), 556-561.

501 Landete, J. M., de las Rivas, B., Marcobal, A., Muñoz, R., 2008. Updated molecular
502 knowledge about histamine biosynthesis by bacteria. Critical Rev. Food Sci. Nutr. 48 (8),
503 697-714.

504 Luongo, D., Giagnacovo, B., Fiume, I., Iorizzo, M., Coppola, R., 2001. Volatile compounds
505 in “Soppressata molisana” style salami fermented by *Lactobacillus sakei*. It. J. Food Sci. 13,
506 19-28.

507 Marcobal, A., De Las Rivas, B., Landete, J. M., Tabera, L., Muñoz, R., 2012. Tyramine and
508 phenylethylamine biosynthesis by food bacteria. Crit. Rev. Food Sci. Nutr. 52, 448-467.

509 Metaxopoulos, J., Samelis, J., Papadelli, M., 2001. Technological and microbiological
510 evaluation of traditional processes as modified for the industrial manufacturing of dry
511 fermented sausage in Greece. It. J. Food Sci. 1, 3-18.

512 Meyneir, A., Novelli, E., Chizzolini, R., Zanardi, E., Gandemer G., 1999. Volatile
513 compounds of commercial Milano salami. *Meat Sci.* 51, 175-183.

514 Montel, M. C., Masson, F., Talon, R., 1999. ^[L]_{SEP}Comparison of biogenic amine content in
515 traditional and industrial French dry sausages. *Sci. des Alim.* 19, 247-254.

516 Motilva, M.J, Toldrà, F., Nieto, P., Flores, J., 1993. Muscle lipolysis phenomena in the
517 processing of dry cured ham. *Food Chem.* 48, 121-125.

518 Parente, E., Martuscelli, M., Gardini, F., Grieco, S., Crudele, M. A., Suzzi, G., 2001.
519 ^[L]_{SEP}Evolution of microbial populations and biogenic amine production in dry sausages
520 ^[L]_{SEP}produced in Southern Italy. *J. Appl. Microbiol.* 90, 882-891.

521 Pearson, D., 1976. *Laboratory Techniques in Food Analysis.* pp. 201-202. London (UK):
522 Butterworths & Co. Publishers Ltd.

523 Pircher, A., Bauer, F., Paulsen, P., 2007. ^[L]_{SEP}Formation of cadaverine, histamine, putrescine
524 and tyramine by bacteria isolated from meat, fermented sausages and cheeses. *Eur. Food*
525 *Res. Techn.* 226 (1,2), 225-231.

526 Roig-Sagués, A. X., Hernández-Herrero, M., López-Sabater, E. I., Rodríguez- Jerez, J. J.,
527 Mora-Ventura, M. T., 1999. ^[L]_{SEP}Microbiological events during the elaboration of “fuet”, a
528 Spanish ripened sausages. Relationships between the development of histidine and tyrosine
529 decarboxylase containing bacteria and pH and water activity. *Eur. Food Res. Techn.* 209,
530 108-112.

531 Rossi, F., Gardini, F., Rizzotti, L., La Gioia, F., Tabanelli, G., Torriani, S., 2011. ^[L]_{SEP}Features
532 of the histidine decarboxylase activity of *Streptococcus thermophilus* PRI60: quantitative
533 analysis of *hdcA* transcription and factors influencing histamine production. *Appl. Environ.*
534 *Microbiol.* 77 (8), 2817-2822.

535 Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., 2004. *Introduction to Food and*
536 *Airborne Fungi*, Seventh edition. CSB, Wageningen.

537 Seefeldt, K.E., Weimer, B.C., 2000. Diversity of sulfur compound production in lactic acid
538 bacteria. *J. Dairy Sci.* 83, 2740-2746.

539 Silla Santos, M.H., 1996. Biogenic amines: their importance in food. *Int. J. Food*
540 *Microbiol.* 29, 213-231.

541 Smit, B.A., Engels, W.J.M., Smit, G., 2009. Branched chain aldehydes: production and
542 breakdown pathways and relevance for flavour in foos. *Appl. Microbiol. Biotechnol.* 81,
543 987-999.

544 Stahnke L.H. (1994). Aroma components from dried sausages fermented with
545 *Staphylococcus xilosus*. *Meat Science*, 38, 39-53.

546 Suzzi, G., Gardini, F., 2003. Biogenic amines in dry fermented sausages: A review. *Int. J.*
547 *Food Microbiol.* 88 (1), 41-54.

548 Talon, R., Leroy, S., Lebert, I. 2007. Microbial ecosystem of traditional fermented meat
549 products: The importance of indigenous starters. *Meat Sci.* 77, 55-62.

550 Tjener, K., Stahnke, L.H., Andersen, L. & Martinussen, J., 2003. A fermented meat model
551 system for studies of microbial aroma formation. *Meat Sci.* 66, 211-218.

552 Trigueros, G., García, M.L., Casas, C., Ordóñez, J.A., Selegas, M.D. 1995. Proteolytic and
553 lipolytic activities of mould strains isolated from Spanish dry fermented sausages. *Zeits.*
554 *Lebensmittel-Unter. Forsch.* 201 (3), 298-302.

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557 **Conflict of interest**

558 None of the authors of this paper has a financial or personal relationship with other people
559 or organisations that could inappropriately influence or bias the content of the paper.

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Table 1: Microbial characteristics of unspoiled and spoiled goose sausages.

Microorganism	Unspoiled	Spoiled
PCA counts	6.0 ± 0.1a	5.7 ± 0.2b
MRS counts	8.6 ± 0.2a	8.6 ± 0.1a
Yeasts	2.0 ± 0.3a	2.3 ± 0.2a
Moulds	2.1 ± 0.1a	5.1 ± 1.5b
Enterococci	3.1 ± 0.3a	6.7 ± 0.2b
<i>Escherichia coli</i> *	< 10	< 10
Enterobacteriaceae	2.1 ± 0.1a	4.0 ± 0.4b
CNCPC ₁	6.1 ± 0.2a	5.8 ± 0.3a
CCPPC* ₂	< 10	< 10
Clostridia H ₂ S+*	< 10	< 10

581 Legend: PCA: Plate Count Agar; MRS: De Man Rogosa Sharpe; Data in
582 log CFU/g - * CFU/g; CNCPC₁: Coagulase Negative Catalase Positive
583 Cocci; CCPPC₂: Coagulase Positive Catalase Positive Cocci; Data represent
584 the means ± standard deviations of the total samples; Mean with the same
585 letters within the same lane (following the values) are not significantly
586 differently (P< 0.05).

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Table 2: Physico-chemical parameters of unspoiled and spoiled goose Sausages.

Parameter	Unspoiled	Spoiled
pH	6.3 ± 0.2a	6.3 ± 0.1a
Aw	0.92 ± 0.01a	0.92 ± 0.01a
TVB-N [^]	80.2 ± 10.1a	208.3 ± 9.5b
Histamine	5.6 ± 1.8a	415.26 ± 115.01b
Putrescine	< L.O.D.	< L.O.D.
Cadaverine	32.1 ± 3.2a	339.3 ± 31.4b
Spermine	< L.O.D.	< L.O.D.
Spermidine	< L.O.D.	< L.O.D.
Tyramine	< L.O.D.	< L.O.D.
L*	38.2 ± 6.0a	36.3 ± 4.1a
a*	16.0 ± 1.2a	17.6 ± 1.6a
b*	1.2 ± 0.4a	1.7 ± 0.9a

598 Legend: Data TVB-N: [^]Total Volatile Basic Nitrogen mg N/100 g;
599 Biogenic amines: mg/kg; < L.O.D.: Limit of quantitation (1.7 to 22.5 µg/L);
600 Data represent the means ± standard deviations of the total samples; Mean
601 with the same letters within the same lane (following the values) are not
602 significantly differently (P< 0.05).
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Table 3: Strains of Enterococci isolated and biogenic amines production

Biogenic amines	<i>E. faecium</i>	<i>E. faecalis</i>
Hystamine	70	30
Putrescine	6	4
Spermine	6	4
Spermidine	6	4
Cadaverine	60	25
Tyramine	10	10
Total isolated	70	30

618 Legend: Number of positive strains
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629 **Table 4: Identification of the strains isolated from the spoiled goose sausages and their**
630 **production of TVB-N and acetic acid.**
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Identification	No. of isolates	TVB-N/acetic acid/biogenic amines production	Source^b
<i>Penicillium nalgiovense</i>	85	+/+/-	JQ434685.1
<i>Penicillium chrysogenum</i>	8	+/+/-	JQ434684.1
<i>Penicillium viridicatum</i>	7	+/+/-	JQ388751.1

Legend: TVB-N, total volatile basic nitrogen; +, positive production; - < LOD; ^bThe accession number of the closest related species found by a BLAST search.

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Table 5: Volatile compounds of spoiled and unspoiled goose sausages

RT	Compounds	Unspoiled (n=10)	s.d.	Spoiled (n=10)	s.d.
Aldehydes					
22,82	dodecanale	0.16	0.02a	0.11	0.03a
24,2	Benzaldehyde	0.01	0.01a	0.02	0.01a
	total	0.19		0.14	
Ketones					
2,35	acetone	0.28	0.13a	0.30	0.03a
3,29	2-Butanone	1.56	0.48a	2.91	0.07b
11,62	2-Pentanone	1.93	0.15a	0.38	0.02b
14,4	2-Heptanone	0.26	0.11a	0.52	0.18a
18,03	2-nonanone	9.20	0.40a	9.88	0.32a
20,21	3-methyl-2-heptanone	0.08	0.01a	0.05	0.02a
	total	13.30		14.04	
Esters					
17,30	Formic acid pentylester	0.09	0.01a	0.17	0.02b
	total	0.09		0.17	
Hydrocarbons					
1,56	2,4-Dimethylexane	50.23	0.33a	42.69	0.02b
2,27	3-Ethylexane	0.67	0.07a	1.32	0.07b
4,72	2,2,4,6-Methylheptane	1.02	0.10a	0.09	0.13a
8,22	Octane	0.06	0.03a	0.09	0.03a
11,41	ethylbenzene	0.11	0.03a	0.02	0.03b
12,48	Benzene	10.09	0.46a	11.40	1.80a
17,64	1-methylbenzene	0.36	0.01a	0.44	0.12a
	total	62.54		56.05	
Alcohols					
6,96	2-Butanol	1.85	0.40a	3.62	0.19b
7,55	2-Pentanol	0.46	0.14a	0.72	0.03b
10,70	2-Methyl-1-propanol	0.19	0.04a	0.24	0.02a
13,31	1-Propanol	0.64	0.03a	0.98	0.09b
15,79	3-Methyl-1-Butanol	0.86	0.01a	0.79	0.22a
20,35	1-Exanol	0.49	0.02a	0.38	0.06a
25,25	2,3-Butanol	0.06	0.01a	0.09	0.02a
	total	4.55		6.82	
Volatile fatty acids					
22.52	Acetic acid	6.11	0.03a	10.40	0.02b
23,41	2-Methylpentanoic	0.11	0.02a	0.06	0.01b
24,57	Propanoic acid	0.24	0.00a	0.29	0.05a
26,32	2-Methylpropionic	0.04	0.01a	0.08	0.03a
26,49	Butanoic acid	0.10	0.02a	0.19	0.04b
27,35	3-Methylpentanoic acid	0.09	0.01a	0.17	0.02b
28,56	Diethylacetic acid	0.05	0.01a	0.07	0.02a
30,65	Hexanoic acid	0.07	0.01a	0.07	0.07a

32,19	2-Ethylheptanoic acid	0.02	0.01a	0.01	0.01a
33,27	Octanoic acid	0.01	0.01a	0.01	0.01a
	total	6.85		11.24	
	Miscellanea				
5,78	Acetonitrile	0.24	0.12a	0.10	0.14a
16,54	Furan	0.06	0.01a	0.10	0.05a
30,91	2-Methoxyphenol	0.05	0.01a	0.07	0.07a
32,73	3-Methylphenol	0.01	0.01a	0.01	0.01a
	total	0.36		0.27	

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Legend: Data (mean of 10 samples) expressed in µg/Kg; Sum of compounds; RT: Retention time. Data represent the means ± standard deviations (S.D.) of the total samples; Mean with the same letters within a row (following the values) are not significantly differently (P< 0.05).