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Abstract

The aim of this work was to determine the microorganisms present and to investigate their metabolites that cause spoilage of many goose sausages produced in Friuli, a northeast region of Italy. The defect was observed by sensorial analysis using the “needle probing” technique; the spoiled sausages were unsafe and not marketable. Despite the addition of starter, the microorganisms, particularly enterococci and Enterobacteriaceae, grew during ripening and produced a large amount of biogenic amines; therefore, these sausages represented a risk to consumers. The production of those compounds was confirmed *in vitro*. Furthermore, a second cause of spoilage was attributed to moulds that grew during ripening; the fungi grew

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

1. Introduction

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

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

consistent aroma and flavor, to improve quality and to reduce the length of the curing period (Iacumin et al., 2006; Comi et al., 2005, 2000; Tjener et al., 2003; Luongo et al., 2001). To meet the increasing needs of new products requested by consumers, goose sausages represent an effort to generate alternatives. Goose and chicken meat and their products are preferred and largely consumed by the public; although chicken meat is often mixed with the meat of other animals, the combination of goose meat with other meat is quite rare (Gulbaz and Kamber, 2008). Recently, the Italian population has rediscovered products based on regional recipes, and goose sausages, which are common in villages throughout Italy, constitute an important resource. Accordingly, these sausages are widely produced and appreciated by consumers, who are weary of eating traditional sausages made with pork meat. The quality of goose sausages is variable and often distinct. However, defects can occur during goose breeding and slaughtering, and sausage manufacturing, making the sausages unfit for consumption. The quality of the raw material, bacterial metabolism, as well as temperature and R.H. values during production and storage can cause these defects. In addition, inadequate ripening may also lead to unpleasant odors or tastes.

A small-scale facility produced two lots (a and b) of goose sausages. During their ripening, lot b presented a defect consisting in an ammonia smell, which was confirmed by a sensorial analysis, made by non professional assessors.

Therefore, the aim of this work was to study the microorganisms and the metabolites responsible for the defects and spoilages of these goose sausages.

2. Material and Methods

2.1 Evaluation and identification of the defect

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

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

2.1.1. *Sensorial analysis*

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

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

2.2. Sampling

Twenty unspoiled (Lot a) and 20 spoiled goose (Lot b) sausages were analyzed. The samples were collected at the end of the ripening period (45 days). Defects of the spoiled goose sausages were found late during ripening (5 days before the end) due to an ammonia smell that was widespread in the ripening rooms. Both lots of sausages, produced the same day with two different batches of goose meat, had the following composition: goose meat 70%, lard 30%, NaCl 2.8%, KNO_3 0,02%, dextrose 0.1 %, black pepper 0.002%, nutmeg 0.002%. Before adding each ingredient of the recipe, a starter composed of *Staphylococcus xylosus* and *L. sakei* (1/1 ratio) was added at a final concentration of 6 log CFU/g. A starter of *Penicillium nalgiovense* was spread by aerosol (approximately 3 log/cm_2) onto the casings. Natural casing was used.

Before analysis, the spoiled and unspoiled products were washed to eliminate moulds on the casings, which were then aseptically removed. Then each sausage was sterile sliced. Four 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 ºC for 48-72 h; LAB were grown on De Man
113 Rogosa Sharpe agar (MRS, Oxoid, Italy) incubated at 42 ºC for 48 h; yeasts and moulds
114 were grown on Malt Agar (MA, Oxoid, Italy) incubated at 25 ºC 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 ºC for 24 h; Enterobacteriaceae were grown on Violet Red Bile Glucose
118 Agar (VRBGA, Oxoid, Italy) incubated at 37 ºC 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 ºC 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 ºC 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.

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

2.3.1. Isolation and identification of enterococci

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

2.3.2. Isolation and identification of moulds

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

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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 (Aw) 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).

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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

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

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

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

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

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

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

4.0. Discussion

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

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

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

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

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

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

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

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

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

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

Conclusion

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

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

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Conflict of interest

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

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
*Escherichia coli**	< 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_2S+*	< 10	< 10

Legend: PCA: Plate Count Agar; MRS: De Man Rogosa Sharpe; Data in log CFU/g - * CFU/g; $CNCPC_1$: Coagulase Negative Catalase Positive Cocci; $CCPPC_2$: Coagulase Positive Catalase Positive Cocci; Data represent the means ± standard deviations of the total samples; Mean with the same letters within the same lane (following the values) are not significantly differently ($P< 0.05$).

Table 2: Physico-chemical paramaters 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

Legend: Data TVB-N: ^Total Volatile Basic Nitrogen mg N/100 g; Biogenic amines: mg/kg; < L.O.D.: Limit of quantitation (1.7 to 22.5 µg/L); Data represent the means ± standard deviations of the total samples; Mean with the same letters within the same lane (following the values) are not significantly differently ($P < 0.05$).

Table 3: Strains of Enterococci isolated and biogenic amines production

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

Legend: Number of positive strains

Table 4: Identification of the strains isolated from the spoiled goose sausages and their production of TVB-N and acetic acid.

Identification	No. of isolates	TBV-N/acetic acid/biogenic amines production	Source[b]
Penicillium nalgiovense	85	+/+/-	JQ434685.1
Penicillium chrysogenum	8	+/+/-	JQ434684.1
Penicillium viridicatum	7	+/+/-	JQ388751.1

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

652 **Table 5: Volatile compounds of spoiled and unspoiled goose sausages**
653

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 penthylester	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**	

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).