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Microbial ecology of a small-scale facility producing traditional dry sausage

I. Chevallier [a,*], S. Ammor [a], A. Laguet [a], S. Labayle [a], V. Castanet [a], E. Dufour [a], R. Talon [b]

[a] *Unité de Recherche Typicité des Produits Alimentaires, ENITA-CF, site de Marmilhat, 63370 Lempdes, France*
[b] *Unité de Recherche sur la Viande, INRA-Theix, 63122 Saint-Genès Champanelle, France*

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Abstract

The microbial ecology of a small-scale facility producing traditional dry sausage (without addition of starter cultures) was investigated at 17 control points (12 processing surfaces and equipments samples, 5 raw materials and products samples) and at different periods: winter and spring.

Technological flora (lactic acid bacteria, staphylococci/*Kocuria* and yeast/moulds) populations were low in processing surfaces and equipments (below 10^2 c.f.u/cm^2), but reached a level of about 10^6–10^7 c.f.u/g for lactic acid bacteria and staphylococci/*Kocuria* and about 10^4 c.f.u/g for yeasts/moulds in the final product. Counts of enterococci were low in most of processing surfaces and equipments and increased in the batter and the sausage-1 week; but declined after 8 weeks of ripening.

Differences in temperatures of the processing facility recorded in winter and in spring influenced the hygienic quality of raw materials. *Pseudomonas* and Coliforms populations enumerated from raw materials in spring were about 3 $\log_{10}$ c.f.u/g superior compared to enumeration results recorded in winter. While *Pseudomonas* population declined remarkably in the final product, Coliforms population remained higher. *Staphylococcus aureus* was undetected in spring; however in winter, its population was about 2.5 $\log_{10}$ c.f.u/g in the final product after 9 weeks ripening. One isolate originating from the stuffing machine was identified as *Listeria monocytogenes*. Finally, 9 presumptive *Salmonella* were identified as belonging to *Hafnia alvei*, *Providencia alcalifaciens*, *Proteus vulgaris*, *P. penneri* and *Morganella morganii* species.

These results emphasize the necessity of improving the general hygienic conditions and also technological process of sausage production in this processing facility.

Keywords: Microbial ecology; Small-scale facility; Traditional dry sausage; Hygiene

1. Introduction

Traditional dry sausage production has increased overall Europe since the 1980's (François, 1995). The development is due to several factors such as the decrease of employment in many industrial sectors and the consumer's request for natural and authentical products (Lagrange & Lelièvre, 1995). France with an annual production of approximately 100,000 tons, is the fourth producer of dry sausage in Europe (Fisher & Palmer, 1995). The Rhone-Alpes and Auvergne regions ensure more than 70% of this production, which is largely traditional.

Traditional producers must be in agreement with EC Directive 93/43 (Council of the European Communities, 1993) and others Directives referring to meat, which imposes limits on practice, ensuring that safe products are

* Corresponding author. Tel.: +33 4 7398 1393; fax: +33 4 7398 1390.
E-mail address: chevallier@enitac.fr (I. Chevallier).

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delivered. However, in spite of this directive, the hygiene management in small-scale facilities is still very variable (Gauer, Legoff, Leriche, & Chevallier, 1998). Few sporadic studies on traditional meat products have shown that hygienic shortcomings can lead to 25% of product loss with high economic consequences and may undermine consumer confidence for traditional products (Chevallier & Leriche, 1998).

Traditional dry sausage fermentation relies on natural contamination of raw materials by environmental flora. This contamination occurs during slaughtering and increases during manufacturing. Each processing facility has a specific house flora, composed of useful microorganisms for the fermentation and flavour of sausage, as well as of spoilage and pathogenic flora. Thus, the characterization of this house flora is crucial because safety (pathogenic flora), acceptability (spoilage flora) and sensorial quality (technological flora) of the product depend totally on it.

All papers on the microbial ecology of traditional dry sausage considered only the product and did not investigate the global microbial ecology of the processing facility. Therefore, the aim of this study was to survey the typical house flora of a small-scale facility producing traditional dry sausage without addition of starter cultures.

2. Materials and methods

2.1. Samples collection

Samples used in this study were collected from a meat small-scale facility located in a mountain farm (altitude: 1080 m; Haute-Loire region, France). Dry sausage was manufactured with pork lean (2/3), pork fat (1/3), NaCl (2.5%) and spices, mainly pepper, but without inoculation of starter cultures to the batter. The batter was stuffed into natural casings, fermented for 1 week at 12 °C and dried 8 weeks in a natural dryer.

Samples were collected at 17 control points determined at the basis of previous work dealing with the application of HACCP approach in this processing facility (Chevallier, Talon, Laguet, Labayle, & Labadie, 2001). Twelve samples were collected from processing surfaces and equipments (tables A and B, wall, block, cold room, bowls, knifes, dryer wall, dryer bars, mincing, mixing and stuffing machines) (Fig. 1). Sampling was performed after the cleaning and disinfection procedures and before the manufacturing process to have a better representation of the residual flora. Sampling was carried out under aseptic conditions. A delimited area of 169 cm^2 was swabbed by sterile rags (13×13 cm^2) containing 15 ml of neutralizing solution (10% v/v) (ATL®, Laboratories Humeau, La Chapelle-sur-Erdre, France) to remove the inhibiting action

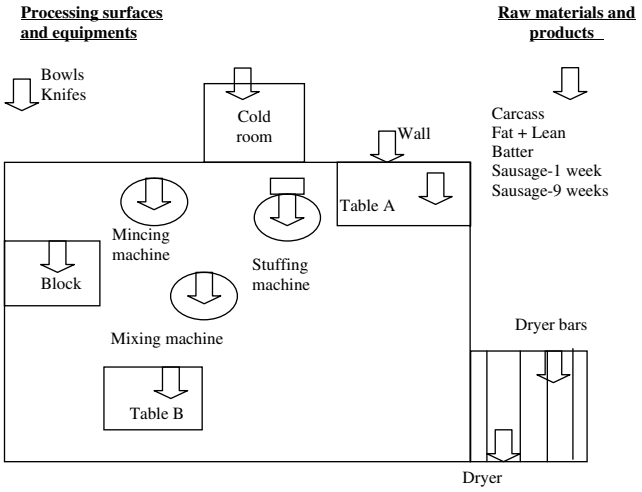

Fig. 1. Scheme of the control points position in the studied small-scale facility (CP1: table A, CP2: table B, CP3: wall, CP4: block, CP5: cold room, CP6: bowls, CP7: knifes, CP8: dryer wall, CP9: dryer bars, CP10: mincing machine, CP11: mixing machine, CP12: stuffing machine, CP13: carcass, CP14: batter, CP15: fat + lean, CP16: sausage-1 week, CP17: sausage-9 weeks).

of the residual disinfectant. Rags were then placed in sterile stomacher bags (AES Laboratories, Combourg, France) containing 100 ml of saline tryptone (Biokar Diagnostics, Beauvais, France) and kept at 4 °C before analysis.

Five samples of 65 g were collected from raw materials (carcass, fat and lean) and products (batter, sausage-1 week, dry sausage-9 weeks). They were kept at −20 °C before analysis. Each sample was placed in sterile stomacher bag as fallow: 10 g in 90 ml of peptoned buffer (Biokar Diagnostics), 25 g in 225 ml Fraser 1/2 broth (Biokar Diagnostics), 25 g in 225 ml Selenite–cystine broth (Biokar Diagnostics) and 5 g in 25 ml of distilled water. The first was used for microbial flora enumeration, the second and the third ones for the detection of *Listeria monocytogenes* and *Salmonella*, respectively. The last one was used for measuring the pH of the product. Sampling was performed in four periods over one year: two repetitions in winter (February and March) and two repetitions in spring (May and June). A total of 68 samples were collected.

2.2. Microbiological analysis

Samples were stomached in Stomacher bags with a Lab Blender 400 Stomacher (Seward, UK) for 6 min. Serial dilutions were then poor plated in duplicate on the appropriate selective medium and incubated under the appropriate conditions (Table 1). Except Rambach agar which was purchased from Merck (Darmstadt, Germany), all other media were purchased from Biokar Diagnostics. The results were expressed in c.f.u/g for meat samples and in c.f.u/cm^2 for processing environmental samples.

Table 1
Normalized methods used for the enumeration of technological, spoilage and pathogenic flora in the environment and meat samples

Target flora	Guidance norm	Selective agar medium	Incubation conditions		
			Time (d)	T °C	An[a]/Ae[b]
Lactic acid bacteria	NF V 04-503[f]	MRS + A[c] + B[d]	2	30	An
Staphylococci/*Kocuria*		Chapman + A + B	2	37	Ae
Yeast and moulds	NF ISO 13681[f]	PDA + C[e]	5	25	Ae
Enterococci		KEA	2	37	Ae
Pseudomonas spp.	NF V04-504[f]	CFC	2	25	Ae
Coliforms	NF ISO 4832[f]	VRBL	1	30	Ae
Listeria spp.	NF ISO 11290-2[f]	Palcam	2	37	Ae
Salmonella spp.	NF ISO 6579[f]	Rambach	2	37	Ae
Staphylococcus aureus	NF V08-014[f]	Baird Parker	2	37	Ae

[a] An: anaerobic.
[b] Ae: aerobic.
[c] A: nalidixic acid (40 mg/l) (Sigma-Aldrich Chemie, Germany) to inhibit Gram-negative bacteria.
[d] B: delvocid (200 mg/l) (Gist-Brocades, Netherlands) to inhibit yeast and moulds.
[e] C: oxytetracycline to inhibit the growth of bacteria.
[f] AFNOR (1998).

Colonies isolated from Palcam agar were checked for their belonging to *L. monocytogenes*. After preliminary tests (Gram-staining, oxydase and catalase activities), identification with the API *Listeria* gallery (BioMérieux, Marcy-l'étoile, France) was performed according to the manufacturer's instructions. Hemolytic activity was researched on Columbia agar (Biokar Diagnostics) added with a sheep blood (Capita, Alonso-Calleja, García-Fernández, & Moreno, 2001). Finally, a simple color test based on a DL-alanine-beta-naphthylamide reaction which differentiates *L. monocytogenes* from other *Listeria* species (Clark & McLauchlin, 1997) was carried out. *L. ivanovii subsp. ivanovii* (CIP 7842T), *L. innocua* (CIP 20595) and *L. monocytogenes* (LO28) were used as reference strains.

Colonies isolated from Rambach agar were checked for their belonging to *Salmonella*. After preliminary tests (Gram-staining, oxydase and catalase activities), identification with the API 20E gallery (BioMérieux) was performed according to the manufacturer's instructions.

2.3. Evaluation of process control

The internal product temperature and pH were recorded using a temperature probe and a pH meter CG 840 (Schott, Mainz, Germany). The ambient temperature and relative humidity were measured with a digital thermometer and hygrometer (ATL). pH of surfaces were recorded using pH paper (Lyphan, Germany). Characteristics of surfaces were noted.

2.4. Statistical analysis

A principal component analysis (PCA) was applied on data of bacterial enumerations. The computer software used was XLSTAT (Addinsoft, Paris, France).

3. Results

3.1. Microbial ecosystem of the small-scale facility

Table 2 summarizes microbial counts of technological, spoilage and pathogenic flora in the processing surfaces and equipments samples ($\log_{10}$ c.f.u/ml) and in raw materials and products samples ($\log_{10}$ c.f.u/g).

Considering technological flora, lactic acid bacteria were not detected in most of processing surfaces and equipments. They were found in only the table B, the block, the dryer bars and wall and in the stuffing machine. However, they were isolated from raw materials and products. Their counts were low in processing surfaces and equipments and in raw materials (0.3–3.9 $\log_{10}$ c.f.u/cm^2) and increased considerably during the fermentation reaching levels of 6.5–7.7 $\log_{10}$ c.f.u/g. Staphylococci/*Kocuria* were enumerated from the 17 samples. Their counts varied from 0.5 to 5.4 in processing surfaces and equipments and in raw materials, related to the season. In the final product, they reached levels varying between 6.2 and 7.3 $\log_{10}$ c.f.u/g. Yeasts/moulds were detected in most of samples. Their counts were comprised between 2 and 4 $\log_{10}$ c.f.u/cm^2 (c.f.u/g).

Counts of enterococci were low in most of processing surfaces and equipments and increased in the batter and in the sausage-1 week; but declined after 8 weeks of fermentation. Moreover their counts were dependent of the season.

Coliforms and *Pseudomonas* were isolated from tables A and B, the block, the stuffing machine, raw materials and products samples. Their counts were especially high in raw materials and products samples. While *Pseudomonas* declined in the sausage-9 weeks compared to the sausage-1 week, Coliforms populations stabilized at the same level in winter and increased slightly in spring reaching a level of about 5.6 $\log_{10}$/g.

Table 2
Microbial counts of technological, spoilage and pathogenic flora in the processing surfaces and equipments samples (log_{10} c.f.u/ml) and in raw materials and products samples (log_{10} c.f.u/cm^2)

Control point	Lactic acid bacteria		Coagulase negative staphylococci		Yeast/moulds		Enterococci		Coliforms		*Pseudomonas*		*Sc. aureus*	
	W	S	W	S	W	S	W	S	W	S	W	S	W	S
Processing surfaces and equipments														
Table A	>0.30	>0.30	1.95	3.91	0.78	2.42	>0.30	1.39	0.86	1.19	1.39	2.06	>0.30	>0.30
Table B	1.02	1.02	2.25	2.52	3.54	3.72	1.77	1.11	2.70	1.79	1.43	1.76	>0.30	>0.30
Wall	>0.30	>0.30	0.54	0.51	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30
Block	2.64	0.54	4.11	4.41	3.01	3.10	0.48	1.11	3.65	3.95	1.95	1.95	0.69	0.87
Cold room	>0.30	>0.30	3.29	3.79	3.83	4.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30
Bowls	>0.30	>0.30	1.15	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	0.30	0.30	>0.30	>0.30
Knifes	>0.30	>0.30	2.58	3.06	0.95	1.29	>0.30	1.98	>0.30	>0.30	>0.30	1.67	>0.30	>0.30
Dryer wall	1.51	1.51	4.27	4.73	1.88	2.44	>0.30	1.43	>0.30	>0.30	0.70	0.70	>0.30	>0.30
Dryer bars	0.30	0.30	4.00	4.67	1.81	4.06	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30
Mincing machine	>0.30	>0.30	1.36	1.83	1.95	1.98	0.65	0.65	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30
Mixing machine	>0.30	>0.30	1.31	1.74	0.81	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30	>0.30
Stuffing machine	3.13	3.08	3.15	2.41	3.28	2.71	3.95	2.08	3.15	2.28	3.67	2.74	0.98	>0.30
Raw materials and products														
Carcass	1.03	1.34	1.87	1.32	>0.30	>0.30	3.67	0.70	2.66	1.44	3.37	1.59	>0.30	>0.30
Fat + lean	>0.30	>0.30	3.33	3.48	2.10	4.79	1.39	2.60	1.61	4.07	3.42	5.51	1.40	>0.30
Batter	1.91	3.94	1.80	5.46	2.20	4.92	2.69	3.82	1.97	5.56	3.59	5.57	3.18	3.48
Sausage-1 week	6.64	7.12	5.30	6.26	2.77	4.08	2.86	3.06	3.67	4.20	2.86	3.00	1.88	2.18
Sausage-9 weeks	6.50	7.74	6.23	7.32	4.23	4.09	1.70	1.30	3.00	4.39	1.70	1.87	2.51	>0.30

W: samples collected in winter (February, March); S: samples collected in spring (May, June).

It is interesting to note that spoilage and pathogenic flora was practically undetected from most of processing surfaces and equipments. However, their counts were relatively high in the block and the stuffing machine.

The pathogenic *Staphylococcus aureus* was present in most of raw materials and products samples, especially in winter. Its counts were high in the batter and decreased slightly during the fermentation. In spring, it was undetected; however in winter, its population was about 2.5 $\log_{10}$ c.f.u/g, which is a very high level compared to the French norm (below 25 c.f.u/g).

Sixteen presumptive *Listeria* species were isolated from the stuffing machine, the block, dryer bars, carcass, the batter, the sausage-1 week and the sausage-9 weeks. However, only one originating from the stuffing machine was identified as *L. monocytogenes*. Nine presumptive *Salmonella* species grown on Rambach medium and isolated from the table A, the stuffing machine, the carcass, the batter, the sausage 1 week and the sausage-9 weeks, were identified as belonging to *Hafnia alvei*, *Providencia alcalifaciens*, *Proteus vulgaris*, *P. penneri* and *Morganella morganii* species.

To have a global view, a PCA was performed on $\log_{10}$ c.f.u/g (c.f.u/cm^2) cells counts enumerated for both seasons. Fig. 2 presents the factorial biplot defined by the principal components 1 and 2. The principal component 1 which took into account 58.5% of the total variance separated control points with relative high cell counts from those with relative low cell counts. The principal component 2 that accounted for 15.8% of the total variance separated between technological and spoilage flora. It clearly showed that the table B, the block, the sausage-1 week and the sausage-9 weeks were positively correlated to the technological flora, while the batter, the stuffing machine and the fat and lean were positively correlated to the spoilage and pathogenic flora.

3.2. Evaluation of process control

Table 3 summarizes physical characteristics of the different control point. The seasonal period (winter or spring), influenced greatly the ambient temperature of the processing facility and consequently temperatures recorded in spring were superior of about 3 °C compared to those in spring. pH values of processing equipments and surfaces were lower in spring (5.5 ± 0.2) compared to winter (6.2 ± 0.2).

pH of sausage 1 week and 9 weeks remained high showing that there was no acidification of the product during the fermentation. Sausage-9 weeks sampled in spring had a particularly high final pH (6.5).

Concerning the state of equipment, the block made from wood and tables were characterized by many stripes and organic residues of raw material, which allow bacteria attachment. The stuffing machine was characterized by the presence of fatty layer which constitute a support for spoilage and pathogenic flora development.

The higher temperature in the processing facility in spring was certainly the major factor that explained the different initial contamination or survival of the microorganisms and then their different growth during the ripening (Table 2).

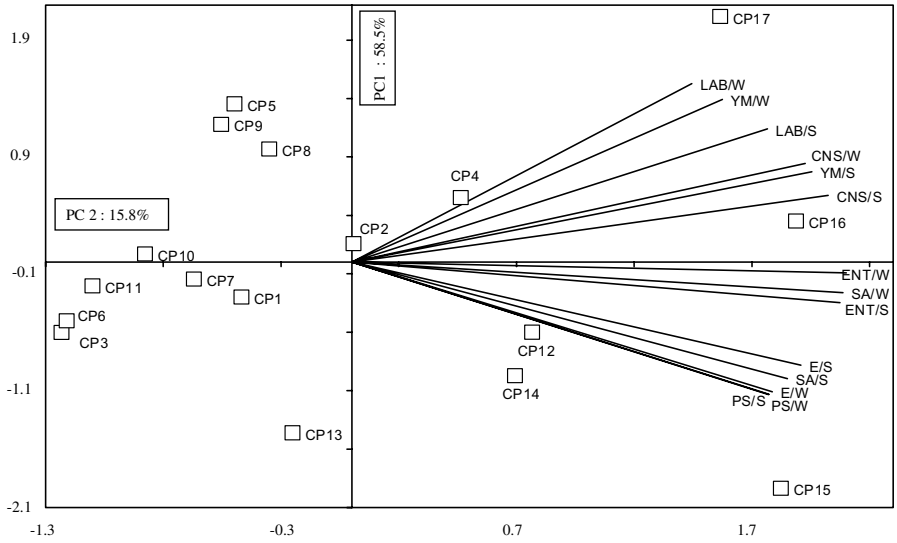

Fig. 2. Factorial biplot defined by the principal components 1 and 2 for viable counts of microorganisms in processing materials and equipments, in raw materials and in products, in winter and in spring. Coding for bacteria: SK: staphylococci/*Kocuria*; LAB: lactic acid bacteria; YM: yeasts/moulds; PS: pseudomonas; E: coliforms; ENT: enterococci. Coding for season: W: winter; S: spring. Coding for control points: TA: table A; TB: table B; W: wall; BL: block; CR: cold room; BW: bowls; K: knifes; DW: dryer wall; DB: dryer bars; M: mincing machine; MM: mixing machine; SM: stuffing machine; CAR: carcass; F + L: fat + lean; BAT: batter; S1W: sausage-1 week; S9W: sausage-9 weeks.

Table 3
Physical characteristics of the different control points

Control point	Ambient temperature (°C)		Ambient relative humidity (% HR)		pH surface or product		Material	State	Apparent moisture
	W	S	W	S	W	S			
Table A	12.4	16.5	70	79	6.3	5.6	Resin	Striped + residues of raw materials	Constantly wet
Table B	12.4	16.5	70	79	6.3	5.6	Resin	Striped + residues of raw materials	Constantly wet
Wall	12.4	16.5	70	79.5	6.3	5.7	Tiling	Smooth	Dry
Block	12.4	16.5	70	79	6.2	5.3	Wood	Damaged + striped + residues of raw materials	Constantly wet
Cold room	−0.4	−0.1	92	71	6.3	5.7	Tiles	Smooth + residues of raw materials	Wet, condensation
Bowls	12.4	16.5	70	79.5	6	5.3	Plastic	Fatty layer	Dry
Knifes	12.4	16.5	70	79.5	6	5.6	Stainless steel	Smooth	Dry
Dryer wall	11.7	11.8	85	82	6	5.5	Pebble-dash	Granulous moulds	Wet, condensation
Dryer bars	11.8	11.8	88	82	6.1	5.7	Iron	Rusted + granulous + moulds	Dry
Mincing machine	13.9	16.5	80	79.5	6.3	5.9	Stainless steel	Smooth + fatty layer	Dry
Mixing machine	6.5	9.5	80	79.5	5.8	6	Stainless steel	Smooth + fatty layer	Dry
Stuffing machine	13.9	16.5	80	79.5	6.3	5.5	Stainless steel	Smooth + fatty layer	Dry
Carcasses	2.3	3.4	60	90.5	6	5.9		Unsoiled	Dry
Fat + lean	2	3.1	77	72	5.7	5.8		Fresh	Wet
Batter	6.5	9.5	87	72	5.8	6		Fresh	Wet
Sausage-1 week	10.3	13	87.5	76.5	6.1	6.2		No moulds	Dry
Sausage-9 weeks	9.5	13.5	86	76.5	6.2	6.5		Moulds	Dry

W: winter (February, March); S: spring (May, June).

4. Discussion

Traditional dry sausage relies on natural contamination originating from raw material and from the processing facility house-flora. This includes useful microorganisms for the fermentation and the flavour of sausage, as well as of spoilage and pathogenic flora. The house-flora is determinate by the environmental characteristics of the processing facility. Among these characteristics, the ambient temperature, the apparent moisture of surfaces and the pH played an important role in the colonization of surfaces by microorganisms.

Most published works that investigate processing equipment flora, focused on the presence of pathogenic flora. In this study, although *Sc. aureus* was practically undetected in processing surfaces and equipments, the final sausage manufactured in winter was contaminated by *Sc. aureus*. The raw materials were the main source of this contamination. This bacterium is endemic in processing and can be widely disseminated in meats (Borch, Nesbakken, & Christensen, 1996). *L. monocytogenes* is ubiquitous and can adhere and forms biofilms on numerous surfaces (Mafu, Roy, Goulet, & Magny, 1990). (Salvat, Toquin, Michel, & Colin, 1995) had reported that more than 68% of environmental samples in a curing plants were positive and 17% remained positive after cleaning. European investigations mentioned between 12% to 16% *Listeria* findings in fermented industrial products (European report, 1999). In this study, the presence of *L. monocytogenes* was only confirmed on the stuffing machine, which was in addition contaminated by many other flora. This result was is in agreement with those of Chasseignaux (1999) who showed that the presence of *L. monocytogenes* in meat processing facilities was associated with strong contamination by other flora. The presence of a fatty layer in the stuffing machine certainly supported the survival of *L. monocytogenes*. All presumptive *Salmonella* isolates were identified as belonging to mainly *Proteus* and *Morganella* species. *Proteus* species are responsible of uroinfections (Burne & Chen, 2000), while *M. morganii* are known to produce a large quantity of histamine which could cause food intoxications (Tham, Karp, & Danielsson-Tham, 1990).

At the beginning of the process, technological flora contaminated the batter at approximately 2 $\log_{10}$ c.f.u/g in winter and between 4 and 5 $\log_{10}$ c.f.u/g in spring. Then, LAB and staphylococci/*Kocuria* grew during the process and reached a final level ranging from 6 $\log_{10}$ c.f.u/g (winter) to 8 $\log_{10}$ c.f.u/g (spring). Their levels in the sausage manufactured in spring were quite similar to those of sausage inoculated with starter cultures (Coppola, Mauriello, Aponte, Moschetti, & Villani, 2000). Nychas and Arkoudelos (1990) showed that levels of staphylococci were similar in traditional and industrial productions.

Low levels of LAB recorded in raw meat were already reported by (Parente, Grieco, & Crudele, 2001) who showed that LAB are usually present in raw meat at maximum 10^2–10^3 c.f.u/g. LAB rapidly dominate during the fermentation due to the anaerobic environment and

the presence of NaCl, nitrate and nitrite (Hammes & Knauf, 1994).

In this study, the final pH of the sausage was not acid (pH 6.2–6.5). There was no acidification because no sugar was added to the batter. Many traditional dry sausages are manufactured without addition of starter cultures but often sugar is added. Thus the pH decreases from 5.8 to 5.2 during the fermentation period and stabilized at this level (Comi, Citterio, Manzano, Cantoni, & Bertoldi, 1992; Coppola et al., 2000) or increases up to 5.8 during ripening period (Viallon, 1996). Thus, *Pseudomonas* population disappears after 7 days of ripening and the level of Coliforms decrease (Solignat & Durand, 1999). In more acidified sausages (pH final about 4.5–5.0), Coliforms and *Pseudomonas* populations decline very quickly and are totally inhibited within 7 days (Lücke, 2000). In non acidified sausages, the safety and the shelf life are ensured by drying which decrease the a_w and thus constitute a limiting factor for growth of undesirable microorganisms. In this study, acidification was absent; drying allowed the decline of *Pseudomonas* but Coliforms remained at high level.

5. Conclusion

In conclusion, the decrease of water activity in traditional sausage is a key for ensuring the shelf life and the safety. However, high level of Coliforms can survive. Addition of sugar seems necessary to decrease the pH during the fermentation. It is necessary to improve hygiene in this processing facility by selecting disinfection procedures adapted to fermentation purposes. Moreover, the selection of appropriate starter cultures and barrier flora from the house flora of the small-scale facility would be a way to improve products safety without affecting their typicality.

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