PE6.08 Microbiological profile of PDO Soprèssa Vicentina 290.00

Stefania Balzan (1) stefania.balzan@unipd.it, Francesca Busin(1), Leonardo Alberghini (1), Valerio Giaccone (1) Enrico Novelli (1)

(1)Dept. of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padua, 35020 Legnaro, Italy

Abstract— Soprèssa Vicentina (SV) is a Protected Designation of Origin (PDO) fermented sausage produced throughout the territory of the province of Vicenza (North-Est. Italy). The SV is made up with all the primal meat cuts of the carcass. The present work aimed to evaluation of the hygienic traits and the evolution of the microflora of SV. Samples at different ripening stages (0, 6, 15, 25, 60 and 90 days) were analyzed for pathogens and spoilage microorganisms. Salmonella spp. was never detected while L. monocytogenes was found until 30 days of ripening. pH at the end of processing was between 5.7 and 6.3 while aw decreased to finally values of 0.87-0.91.

S. Balzan is with the Dept. of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padua, 35020 Legnaro, Italy (corresponding author to provide phone: 39-049-8272966; fax: 39-049-8272604; e-mail: stefania.balzan@unipd.it).

F. Busin is with the Dept. of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padua, 35020 Legnaro, Italy (e-mail: francesca.busin@unipd.it).

L. Alberghini is with the Dept. of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padua, 35020 Legnaro, Italy (e-mail: leonardo.alberghini@unipd.it).

V. Giaccone is with the Dept. of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padua, 35020 Legnaro, Italy (e-mail: valerio.giaccone@unipd.it).

E. Novelli is with the Dept. of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padua, 35020 Legnaro, Italy (e-mail: enrico.novelli@unipd.it).

Index Terms—fermented raw salami, microbiological profile, ripening.

I. INTRODUCTION

Soprèssa Vicentina (SV) is a Protected Designation of Origin fermented sausage [1] product throughout the territory of Vicenza (North-Est. Italy). The annual certificated production is about 150.000 kg of product by 9 local plants. SV is manufactured using all the primal lean meat cuts (ham, shoulder, loin, neck,) belly and backfat from Large White, Landrace and Duroc breeds (above 130 kg carcass weight). The length of SV can vary from 25-30 to 40-50 cm, while the diameter varies from 7-8 to 10-12 cm. The size is depending by the horse or bovine casing employed. The weight of the ripened product range from 800 g to over 7 kg. The recipe other than lean pork and backfat, include salt and other ingredients like species, sugar and nitrate/nitrite. The length of ripening depends by the weight of product: from 60 days (1-1.5 kg at stuffing) to 120 days (3.5 – 8 kg at stuffing) as minimum time. The seasoning gives an external whitish color, which is grayish-brown underneath due to the mould that covers it. Upon cutting the meat appears reddish, tending to rose coloured, with a characteristic irregular white marbling due to the component of fat surrounding the muscular part. As well as other seasoned fermented raw salami, the microflora of SV resulted from the complex interaction among the addition of the species and salt and the progressive reduction of water activity, the presence of microbiota from raw materials and productive environments, etc. The present study investigated the evolutions of microflora and microbiolgical safety of Soprèssa Vicentina PDO during the production processes.

II. MATERIALS AND METHODS

Products from three traditional producers located in the province of Vicenza were studied. They were small size enterprises. No substantial differences in the formulation of the sausages and in the conditions used for fermentation and maturation were registered among plants.

Primal cuts were kept at 0 – 3 °C and debonned 24h after slaughter. Fresh lean pork meat (70% w/w) and pork fat (30% w/w) were chopped to 7 mm particle size and mixed with salt (27 g/kg), pepper (1/4 chopped, 3 g/kg), raw garlic paste (1 g/kg), species mixture (rosemary, chinnamon and cloves, 0.5 g/kg) sugar (1.5 g/kg), nitrate/nitrite. Starter cultures were used. The sausage mixture was amalgamated just before smearing. The sausage mixture was stuffed into natural casings for sausage sizes of approximately 1000-1500 g. The ripening

parameters were as follow: to remove the excess of water from casing the raw sausage stood for about 12h at 20 – 24 °C than during the next 5 days the temperature dropped from 24 to 12 °C.

Samples (n=90) at different stages of production were collected: sausage mixture (0 days), end drying (6 days), maturation at 15 and 25 days, minimum ripening time (60 days) and long ripening time (90 days). At each time of sampling two samples were analyzed. The appropriate decimal serial dilution were plated onto appropriated selected media: Plate Count Agar for Total Mesophilyc Bacteria (TMB), MRS agar for Lactic acid bacteria (LAB), M17 for Micrococcus spp., Baird Parker medium for Staphylococcus aureus, XLT4 agar for Salmonella spp. (presence/absence), Palcam Listeria selective agar for Listeria monocytogenes (presence/absence), McC Agar for total and fecal coliforms, Pseudomonas spp. on Cetrimide-Fucidin-Cephaloridine agar, SPS agar for anaerobic sporoformers. Plates were incubated at 30 °C for 48 h for TMB; at 37 °C for Micrococcus spp., LAB, Staphylococcus aureus, Anaerobic sporoformers, coliforms (44 °C for fecal ones), Salmonella spp. and Listeria monocytogenes; at 25 °C for 48 h for Pseudomonas spp. Phenotypic identifications were made on the basis of biochemical traits using the API 50 CH, API Listeria and the API Staph - systems. The residual sample was used for chemical analysis.

Water activity (aw) was measured with Aqua Lab CX2 instrument (Decagon Devices, Pullman, WA, USA) and pH was relieved using the INLAB 427 electrode (Mettler Toledo, Urdof, Switzerland) and portamess 910 pH-meter (Knick, Germany). The determination of pH was carried out by sample homogenisation with distilled water (1/10 weight/volume). For pH and aw measurements two replicates were taken for each sample. Data were tested by variance analysis (ANOVA) using SPSS 13 statistical software (Chicago, IL).

III. RESULTS AND DISCUSSION

Ripening of fermented sausage is a complex process characterized by deep changes on meat components resulting in the production of characteristics taste and aroma [2]. Microbiota in fermented raw sausage was composed of useful microorganisms (LAB, yeast/mould, coagulase negative cocci) important for fermentation and flavour development, but also spoilage microorganisms (Pseudomonas, enterobacteria) and enterococci were present. This microbiota could arise from the raw material, from the environment and from the starter culture. The type of microbiota depends also from the recipe adopted and the fermentation and ripening processes [3].

In our study TMB was about 106 cfu/g in the raw mixture that increased during ripening until 25 days (109 cfu/g) than a certain decrease was observed ($P<0.001$). Spoilage microrganisms (Table 1), like Pseudomonas spp., tendentially decreased during ripening but in some cases Pseudomonas remained constant till the end or it was not detect. This agrees with other Authors [4]. Regarding the hygienic status of the SV, total coliforms (generally >103 cfu/g) showed a light decreasing trend during ripening whereas fecal coliforms remained constant becoming an important population influencing the final organoleptic characteristics of the product. Since they are able to produce ammonia and other amines, they possibly contributed to the final flavour of the product [4]. About 30% of samples showed a load of >104 cfu g-1 whereas the carcass contaminations were < 102 cfu g-1. Concerning Aeromonas spp. it is well known that it remained constant or disappeared [5].

Yeasts and moulds were also counted. Yeasts increased during drying than stability in the population count was observed during ripening. On the contrary moulds were not regularly found. [2] reported moulds value <50 cfu/g at 60 days of ripening of some Italian salami. Anaerobic sporoformers were never detected. Lactobacillus spp. (Fig.1) was the predominant microbiota from the fermentation step to the end of ripening ($P<0.001$) with a slight descending trend on the later stages of processing. Micrococcus spp. reached mean value of 105 cfu g-1 and they remained stable through seasoning. Another study reported an increased of Micrococcaceae loads during ripening (Comi) or confirmed our results [6]. Considering the pathogenic bacteria, Salmonella spp. was never detected. S. aureus (Fig.1) was found from the beginning of ripening until the end of processing. At the end of ripening the level was not considered hazardous (lower than 104 cfu g-1) but unsatisfactory (higher than 102 cfu g-1) [7]. Listeria spp. (presence/absence) were detected in 52% of samples. L. monocytogenes was found in 4.3% of the samples while L. innocua was detected in the 25% of the samples.

Some Authors [7] reported that L. monocytogenes is absent in the final product but is frequently detect in raw materials, in the early stages of fermentation and in food processing environments. In our work Listeria spp. was sometimes detected on pig carcasses (data not reported) and in the final product whereas L. monocytogenes was never found in the raw materials but was detected in the raw mixture until 30 days of ripening than disappeared. It's worth to note that L. innocua is able to survive in acid environment with pH values between 4.0 and 4.4 [8].

The presence of competitive microbiota and the decline of water activity could have affected the L. monocytogenes growth [7]. Microbial growth during the salami fermentation significantly affected the pH (Fig. 2), an important physico-chemical parameter of raw fermented product [2]. All the samples were characterized by a pH comprised from 4.9 to 5.3 at the end of drying (6 days) while the pH at the end of processing was between 5.7 and 6.3 ($P<0.001$).

The sausage weight loss and the salt addition caused the aw decreasing ($P<0.001$) to finally values of 0.87-0.91 (Fig. 2). [2] found a similar trend for pH in three typical raw fermented salami of Northern Italy of 60 days ripening. [6] and [9] reported that during the ripening the pH go back to similar values of those of the fresh meat mixture due to the liberation of peptides, amino acids and ammonia by proteolytic reactions attributed to micro/staphylococci activity. Meat products dried at low temperatures could limit the fermentation processes thus the pH does not decrease more of 0.2-0.4 units [9].

IV. CONCLUSION

L. monocytogenes did not represent a risk as it was never detected after 30 days of ripening. Salmonella spp. was never found. S. aureus load was not satisfactory and the presence of toxins other than positive coagulase strains has to be investigated. The presence of total and fecal coliforms up to the end of processing suggests to improve, a) the Good Hygienic Practices, b) some technological steps that are pivotal for the reduction of spoilage bacteria (i.e., fast activity water reduction at the beginning of drying, water loss from meat before chopping).

ACKNOWLEDGEMENT

Study financial supported by Regione Veneto (D.g.r.2196/2005- A.R.I.S.C.O.). The Authors also wish to thanks the farmers and producers for the technical assistance.

REFERENCES

[1] European Regulation n.2081/92. Disciplinare di produzione della denominazione di origine protetta (d.o.p.) 'Soprèssa vicentina'.

[2] Cocolin, L., Dolci, P., Rantsiou, K., Urso, R., Cantoni, C., & Comi, G. (2009). Lactic acid bacteria ecology of three traditional fermented sausages produced in the North of Italy as determined by molecular methods. Meat Science, 82, 125-132.

[3] Talor, R., Leroy, S., & Lebert, I. (2007). Microbial ecosystems of traditional fermented meat products: The importance of indigenous starters. Meat Science, 77, 55-62.

[4] Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., & Cocolin, L. (2005). Characterisation of naturally fermented sausages produced in the North East of Italy. Meat Science, 69, 381-392.

[5] Tiecco, G. (2000). Microbiologia degli alimenti di origine animale. Bologna, Edagricole.

[6] Spaziani, M., Dal Torre, M., & Stecchini, M.L. (2009). Changes of physicochemical, microbiological, and textural properties during ripening of Italian low-acid sausages. Proteolysis, sensory and volatile profiles. Meat Science, 81, 77-85.

[7] Ferreira, V., Barbosa, J., Silva, J., Gibbs, P., Hogg, T., & Teixeira, P. (2009). Microbiological profile of Salpicão de Vinhais and Chouriça de Vinhais from raw materials to final products: Traditional dry sausages produced in the North of Portugal. Innovative Food Science and Emerging Technologies, 10, 279-283.

[8] Albano, H., Pinho, C., Leite, D., Barbosa, J., Carneiro, L., Magalhães, R., Hogg, T., and Teixeira, P. (2009). Evaluation of a bacteriocin-producing strain of Pediococcus acidilactici as a biopreservative for ‘‘Alheira”, a fermented meat sausage. Food control, 20, 764-760.

[9] Bover-Cid, S., Izquierdo-Pulido, M., & Vidal-Carou, M. C. (2001). Changes in biogenic amine and polyamine contents in slightly fermented sausages manufactured with and without sugar. Meat Science, 57, 215–221.

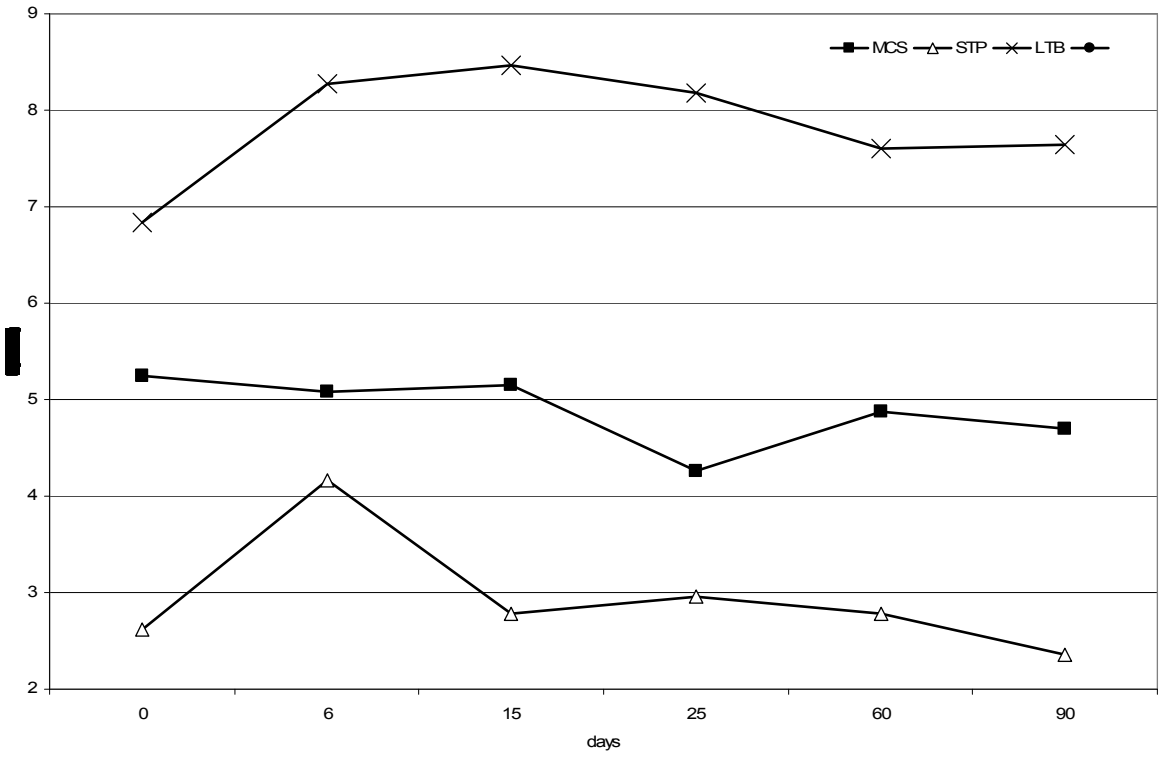

Fig.1. *Micrococcus* spp. (MCS), *S.aureus* (STP) and *Lactobacillus* spp. (LTB) growth dinamycs (means, cfu/g) during ripening.

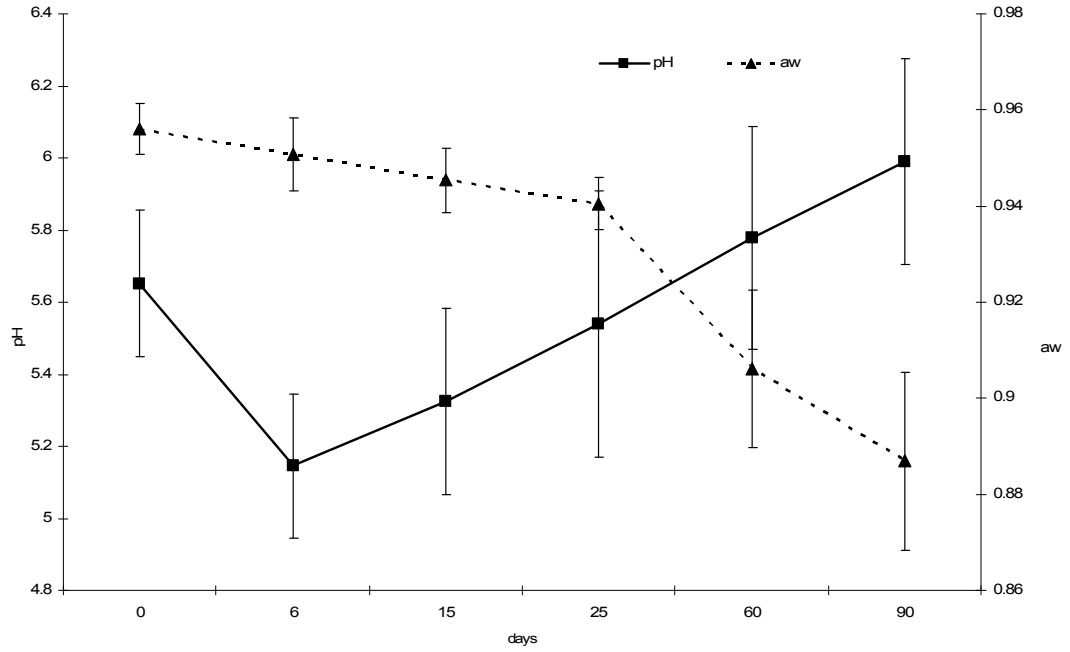

Fig.2. Evolution of pH and water activity (a_w) during ripening (means ± standard deviation).

Table 1. Counts (log_{10} cfu g^{-1}) of the microbial groups during ripening.

	0 d (n= 18)			6 d (n= 18)			15 d (n= 18)			25 d (n= 18)			60 d (n= 18)			90 d (n= 18)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
***Pseudomonas* spp.**	**5.4**	**<1**	**6.6**	**4.4**	**<1**	**5.4**	**3.4**	**<1**	**4.1**	**3.7**	**<1**	**4.5**	**4.3**	**<1**	**5.2**	**2.2**	**<1**	**2.8**
Micrococcus spp.	5.2	<1	5.7	5.1	<1	5.7	5.2	<1	6.0	4.3	<1	4.8	4.9	<1	6.0	4.7	<1	5.8
Aeromonas spp.	2.1	<1	2.7	1.8	<1	2.1	2.0	<1	2.4	2.1	<1	2.7	2.0	1.0	2.3	2.5	<1	3.0
Total coliforms	6.4	<1	7.5	6.2	<1	7.3	4.9	<1	5.7	6.5	<1	7.7	5.4	<1	6.5	4.7	<1	5.2
Fecal coliforms	5.8	1.0	6.9	5.9	<1	6.7	5.5	<1	6.5	6.0	<1	7.1	5.0	<1	5.7	4.7	<1	5.0
Yeasts	5.0	1.7	6.0	6.0	2.0	7.1	4.0	2.0	4.9	3.8	2.0	4.4	4.5	2.5	5.0	4.7	2.0	5.6
Moulds	5.0	<1	6.0	3.4	<1	4.0	3.0	<1	3.4	3.2	<1	3.9	3.6	<1	4.5	2.0	2.0	2.2

d.: days of ripening; Min: minimum; Max: maximum.