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Fate of the microbial population and the physico-chemical parameters of “Sanganel” a typical blood sausages of the Friuli, a north-east region of Italy

Lucilla Iacumin [a], Marisa Manzano [a], Simone Stella [b], Giuseppe Comi [a,*]

[a] Department of Agricultural, Food, Animal and Environmental Sciences, University of Udine, Via Sondrio 2/A, 33100, Udine, Italy
[b] Department of Health, Animal Science and Food Safety, University of Milan, via Celoria 10, 20133, Milano, Italy

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ABSTRACT

In Friuli, a Northeastern region of Italy, a blood sausage called Sanganel is produced by farmers, butchers, shops, and factories. This sausage is made with pork meat, boiled blood, lard, spices, and salt. It is stored at 4 ± 2 °C and usually eaten fresh or boiled within 14 days of its manufacture. Little is known about its microbial populations and safety for consumption. The aim of this study is to characterise the microbial populations and the physico-chemical parameters of Sanganel to establish its quality and the safety of consuming it. The microbial population of Sanganel is typical of meat products, and psychrotrophic enterobacteria and lactic acid bacteria (LAB) grow while it is stored. Enterobacteria produce total basic volatile nitrogen (TVB-N) and biogenic amines that, despite the presence of LAB, increase the pH of the sausage to approximately 6.9. Considering the concentrations of Enterobacteriaceae and TVB-N in the sausage, a shelf-life of 14 days is suggested. However, at 30 days the sausage is safe to eat and presents normal odours and flavours. In addition, boiling the sausage for 30 min before consumption eliminates the asporogenous microbial population.

1. Introduction

Blood sausages are popular in many parts of the world. In Italy, they are mainly produced by families, particularly in areas where rural cultures still dominate. Each Italian area has its own ingredients and recipes, which differ by region, and blood sausages are a means of using every edible part of the pig. Recently, blood sausages have been rediscovered by the Italian population, but they continue to be produced by artisans and butchers using the same ingredients, recipes and technology as in the past. In other parts of the world as well, despite being widespread, blood sausages are still produced by butchers and local facilities and distributed and eaten locally (Diez et al., 2008). They are considered an ethnic product, which is one reason for their rediscovery. The European Union has promoted the protection of traditional foods from specific regions, with the aim of improving the traditional food production of rural areas and supporting local livestock production (Santos et al., 2005). In Friuli, a Northeastern region of Italy, a blood sausage called Sanganel (SBS) is produced by farmers, butchers and local factories at the level of local restaurants, taverns, and inns (called Frasche). The SBS is a traditional product, historically eaten in Friuli, frequently with brovada, a typical vegetable dish made with cabbage fermented by pomace, with salad leaves (radicchio), or with cornmeal mush (polenta). SBS is made with bloody pork (bacon rinds and tender muscles from the pig's head, lungs, and kidneys), lard and boiled blood (boiled in water for 45 min). First, 2 kg of boiled blood, mixed with 3 kg of bloody meat and lard, are ground and mixed with salt (3.0%), pepper (0.5%), coriander, cinnamon, and ascorbic acid. The mixture is stuffed into pork bowels and stored at 4 ± 2 °C for 14 days; the SBS must be consumed fresh or boiled for 30 min. Some SBS recipes also include buckwheat flour, stale bread, pine nuts, and dried raisins. SBS processing is performed in local households in areas normally used for the butchering and processing of pigs for family consumption. SBS processing occurs after slaughter, with care taken to cleanse and disinfect the environment and tools used between one operation and the next. No data are available about the microbial and physico-chemical characteristics of SBS, and in particular, no data are available about the microbial populations and physico-chemical parameters of SBS during storage. Because SBS is made with fresh

* Corresponding author.
E-mail address: giuseppe.comi@uniud.it (G. Comi).

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bloody meat, its microbial population likely includes *Pseudomonas* spp, Enterobacteriaceae, and Lactic acid bacteria (LAB). Morcilla de Burgos, a popular cooked blood sausage produced in the region around Burgos, in the North of Spain, and Morcela de Arroz, a popular Portuguese cooked blood sausage from Serra de Monchique (in the South of Portugal) are very similar to SBS. After cooking, Morcilla and Morcela de Arroz are contaminated by the typical aerobic microorganisms causing spoilage, predominantly *Pseudomonas*, which are introduced from handling or during the chilling step (Pereira et al., 2015; Diez et al., 2008, 2009a, b; Santos et al., 2005). Then, after packaging in a Modified Atmosphere Packaging (MAP) or vacuum packaging (VP), these microflora are replaced by homofermentative and heterofermentative LAB (Santos et al., 2005). After being cooked for approximately 1 h at 90–95 °C, air cooled to 8–10 °C, and then stored at 4 °C, the shelf-life of Morcilla is between 14 and 21 days, depending on the amount of initial contamination and the storage conditions (Santos et al., 2005). At the end of its shelf-life, the Morcilla casing is covered by a white, wet slime with a sour odour and taste, caused by LAB. In particular, the bacteria found on spoiled Morcilla packaged in MAP or in VP are primarily heterofermentative LAB of the *Weissella viridescens*, *Leuconostoc mesenteroides* and *Leuconostoc carnosum* species, which cause the packaging to inflate, losing its vacuum seal (Santos et al., 2005).

SBS cannot be spoiled by LAB because it is sold or stored unpackaged at 4 ± 2 °C. The aerophilic atmosphere and partial surface dehydration of the casing experienced during storage delay the growth of LAB and eliminate the production of slime. Considering the absence of data on the microbial and physico-chemical characteristics of SBS, the aim of this study was to determine the microbial populations and physico-chemical parameters of SBS throughout its shelf-life, to determine its quality and utility.

2. Materials and methods

2.1. Sampling and microbiological and physico-chemical analyses

Three different lots of SBS, collected in September and November 2015 and February 2016, were investigated. Each lot included 40 fresh SBS, which were produced by an artisanal laboratory in the Friuli region and stored at 4 ± 2 °C for 30 days. At each time points (0, 7, 14, and 30 days), 10 SBS were analysed to evaluate the microorganisms present, the physico-chemical parameters and to determine the shelf life and safety of the sausages. The casings were aseptically removed from each sausage and the meat was homogenized in a stomacher bag in a laboratory blender (P.B.I., Italia).

2.1.1. Microbial analysis

Twenty-five g of the homogenate was serially diluted in stomacher bags using 225 ml of saline-peptoned water (8 g/l NaCl, 1 g/l bacteriological peptone, Oxoid, Italy, 1000 ml distilled water) and homogenized in a laboratory blender (P.B.I., Italia) for 3 min. One or 0.1 ml of each serial dilution was poured or spread on the following agars to evaluate the microorganisms present. Total Viable Count (TVC) was determined on Plate Count Agar (PCA, Oxoid, Italy) incubated at 30 °C for 48–72 h; LAB on De Man Rogosa Sharpe (MRS) agar (Oxoid, Italy) incubated at 42 °C for 48 h; yeasts and moulds on Malt Agar (MA) (Oxoid, Italy) incubated at 25 °C for 72–96 h; *Escherichia coli* on Violet Red Bile Lactose Agar (VRBLA) (Oxoid, Italy) incubated at 44 °C for 24 h; Enterobacteriaceae on Violet Red Bile Glucose Agar (VRBGA) (Oxoid, Italy) incubated at 37 °C for 24 h; *Pseudomonas* spp. on *Pseudomonas* CFC Agar (Oxoid, Italy) incubated at 25 °C for 48 h; Coagulase positive catalase positive cocci (CPCPC) on Baird-Parker agar medium (BP, Oxoid, Italy), supplemented with egg yolk tellurite emulsion (Oxoid, Italy) incubated at 35 °C for 24–48 h and then confirmed with a coagulase test; Coagulase Negative Catalase Positive Cocci (CNCPC) on Mannitol Salt Agar (MSA, Oxoid, Italy) incubated at 30 °C for 48 h; Enterococci on Kanamicina Aesculina Agar (KA, Oxoid, Italy) incubated at 37 °C for 48 h; and Sulphite-reducing clostridia in Differential Reinforced Clostridia Medium (DRCM, VWR, USA) incubated at 37 °C for 24–48 h in an anaerobic jar with a kit (gas pack anaerobic system, BBL, Becton Dickinson, USA). *Salmonella* spp. was evaluated by the ISO (6579-1 2002 Cor.1:2004 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.) method and *Listeria monocytogenes* was also evaluated by the ISO (11290-1,2:1996 Adm.1:2004 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Listeria monocytogenes*) method. Enterohemorragic *E. coli* were detected by the ISO TS 13136 (EU Commission Regulation No. 209/2013, 11/03/2013; Official Journal European Union, 12/03/2013, L68/19).

2.1.2. Isolation and identification of Pseudomonas and Enterobacteriaceae

At each sampling days of each lot, 25 colonies were randomly isolated from the *Pseudomonas* CFC agar (300 colonies) and twenty-five colonies were isolated from VRBGA agar plates (300 colonies). The colonies were purified in PCA incubated at 30 °C for 48 h. Then, each colony was subjected to gram staining and to an oxidase test. Both oxidase negative and positive strains were identified by an Api system according to the manufacturer's instructions (BioMerieux, France).

2.1.3. Physico-chemical analysis

The total volatile basic nitrogen (TVB-N) was determined by the Pearson method (1976). The pH values were determined directly by inserting a pH-meter probe into the homogenate (Radiometer, Denmark). The water activity (Aw) was determined using a Hygromer AWVC (Rotronic, Italia). The colour was determined using a Minolta Chroma meter CR-200 (Singapore) and a CIE Lab system. After calibration with standard white tiles, the chroma meter was positioned perpendicular to the surface of the sausage, and each sample was evaluated in five different positions immediately after slicing. The evaluated parameters were L^*, a^* and b^*. L^* describes the white intensity or brightness, with values ranging from 0 (black) to 100 (white). The a^* value describes the redness ($a^* > 0$), and b^* describes the yellowness ($b^* > 0$). Moisture (A.O.A.C., 934.01 1995), salt content (Pearson, 1976), ash (A.O.A.C. 920.153 (1995), fat (A.O.A.C. 960.39, 1995), protein (A.O.A.C. 928.08, 1995) were also determined. For all the parameters, the final values were expressed as the respective average of ten measurements for each sampling time of each lot.

2.2. In vitro production of biogenic amines

All of the identified oxidase-negative strains were tested for the production of biogenic amines in various agars according to the method proposed by Bover-Cid and Holzapfel (1999).

2.3. Detection of biogenic amines in SBS

Biogenic amine contents were determined by the method proposed by Gosetti et al. (2007).

2.4. Volatile compounds determination

At 0, 7, 14 and 30 days, ten SBS of each lot were analysed for the presence of volatile compounds, Volatile compounds were