Sardinian fermented sausage traditional production process: a preliminary survey in eight establishments

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Abstract

This study aimed to conduct a preliminary investigation in eight Sardinian fermented sausage (SFS) production plants to acquire knowledge about the differences in the applied technological process and their influence on the safety and sensory characteristics of the finished product. Two audits were conducted in each plant to evaluate structural characteristics and process technologies; 72 samples of SFS at the end of seasoning and 48 environmental samples were analyzed. *Listeria monocytogenes*, *Listeria* spp., *Salmonella* spp., and *Yersinia enterocolitica* were investigated, and chemical-physical analyses were also performed. A panel of consumers was subjected to the Check All That Apply test and acceptability test to determine the qualities perceived by consumers and assess the product acceptance rating. A water activity value of >0.941, permissive for the growth of *L. monocytogenes*, was detected in SFS produced by one producing plant; *L. monocytogenes, Salmonella* spp., and *Y. enterocolitica* were detected in 2.8% of SFS samples, and *Listeria* spp. in 20.8% of samples. Environmental samples tested positive for 45.8% of *Listeria* spp. and 16.7% of *L. monocytogenes*. Correct drying and ripening steps, applied for at least 20 days, are critical for the development of hurdles required to guarantee the safety of fermented sausages. The application of proper hygiene and cleaning procedures is required to reduce environmental contamination. Sensory analysis results show how the production processes applied determine the marketing of sensorially different products. The ideal profile suggested by consumers confirms that the attributes that allow for improved liking are "moderate spicing", "moderate spiciness", "seasoned product", and "artisanal character".

Introduction

"Salsiccia Sarda" or Sardinian fermented sausage (SFS) is a traditional ready-to-eat (RTE) pork meat product typical of Sardinia (Italy) and included in the National List of traditional food products (Italian Republic, 2020).

SFS production is widespread in numerous and different production realities, from small and artisanal establishments to larger plants with industrial processing, often heavily influenced by customs and family recipes, with a high degree of variability in the manufacturing process (Siddi *et al.,* 2022). It is a semi-dry sausage made of pork meat and fat fermented, dried, and ripened. SFS-typical microflora, composed of lactic acid bacteria (LAB) and nitrate-reducing coagulase-negative staphylococci, is naturally present in the meat or added by inoculation of starter cultures (Greco *et al.,* 2005). In SFS, pH is the result of the acidification process carried out by LABs. The product's acidity is then decreased by yeasts and mold metabolism of lactic acid during the ripening period. Water activity (a_w) drop is the result of correct drying and control of temperature and relative humidity (Feiner, 2006). Mean pH values between 5.3-5.5 and mean a_w values between 0.90 and 0.92 at the end of ripening indicate correct acidification and ripening processes; SFS's safety is dependent on physicochemical conditions and "hurdles" set during the fermentation and ripening process. A correct production process guarantees proper acidification and

drying (Piras *et al.,* 2019). The aforementioned parameters are crucial in determining compliance with microbiological criteria established by Regulation n. 2073/2005 concerning the permissiveness towards pathogens such as *Listeria monocytogenes* (European Commission, 2005). Inadequate fermentation or inappropriate dry-curing duration and cross-contamination can determine the presence and development of *L. monocytogenes* (Mureddu *et al.,* 2014), rendering their consumption a public health risk (EFSA, 2018). SFS is sold vacuum packaged, with a shelf-life of 120 days (Siddi *et al*., 2023), or without any packaging, and in this case, the shelf life is not higher than 20 days.

Consumers place a higher value on "traditional" products, which have interesting growth potential with strong links to regional traditions. However, very little is known regarding the most desired and appreciated sensory characteristics of SFS by consumers. In this context, the first goal of the research project was to acquire knowledge about differences in the technological process applied by eight SFS production plants, assess the physicochemical characteristics, and detect the presence of *L. monocytogenes*, *Salmonella* spp., and *Yersinia enterocolitica* in the finished products. The second aim of the study was to apply a sensory analysis protocol to determine the influence of technological processes on sensory characteristics and the consumer's choice.

Materials and Methods
Audits in the production plants

Eight SFS production plants (PA, PB, PC, PD, PE, PF, PG, and PH), located in various parts of Sardinia (Italy) and representative of the sector, were selected. Two audits were carried out in each plant to define the production process applied. Production facilities were examined to collect data regarding buildings and equipment, production processes and layouts, ingredients, hygiene management procedures, the system of own-check and Hazard Analysis Critical Control Points plan, and traceability procedures. In the last phase of the audits, a meeting was held with the plant manager to propose possible modifications for the improvement of processes.

Sardinian fermented sausage sampling and analysis

Before the application of the proposed modifications, SFS produced according to each producing plant's regular production process were collected. The finished products were cylindrical, 40-45 cm in length and 3-4 cm in diameter, folded into the typical horseshoe shape, and weighed between 300 and 600 g. Three batches were produced, and two SFS per batch were collected (six SFS for each of the eight producing plants). Before the analyses, the SFS were divided in half: one half was intended for laboratory analyses and the other half for sensory testing. For microbiological analysis, from the two halves of SFS from the same batch, a pool was created; from each pool, three samples were collected (72 samples overall). On each sample, pH (pH-meter GLP 22 Crison Instruments SA, Barcelona, Spain) and a_w (Aqualab CX3, Decagon, Pullman, Washington, USA) were determined.

Listeria spp. and *monocytogenes* (ISO, 2017a, 2017b), *Salmonella* spp. (ISO, 2020) and *Yersinia enterocolitica* (ISO, 2017c) detection was carried out on all SFS samples.

Environmental sampling and analysis

Samplings were carried out after processing on surfaces in contact with meat (SCM) and surfaces not in contact with meat (SNCM). The SCM were the tables for the bagging and binding of the product and the meat mixer. The SNCM were the floor and drains of the processing room, the floor of the storage cells, and the dripping cell. Surface sampling was performed using a kit (3M, St. Paul, Minnesota, USA) containing a sterile sponge pre-moistened with 10 ml of buffered peptone water. A total of 48 environmental samples (six samples from each producing plant) were collected. Microbiological analyses were conducted on the environmental samples as previously described.

Sensory profile analysis

To obtain descriptive information from consumers regarding samples produced by seven producing plants (PA, PB, PC, PD, PE, PF, and PG) the Check All That Apply (CATA) test was conducted (Ares and Jaeger, 2015). PH was excluded because, at the time of the study, the plant had temporarily stopped production. A representative sample of the population (90 consumers) was asked to select sensory attributes/terms in a questionnaire. Afterward, the
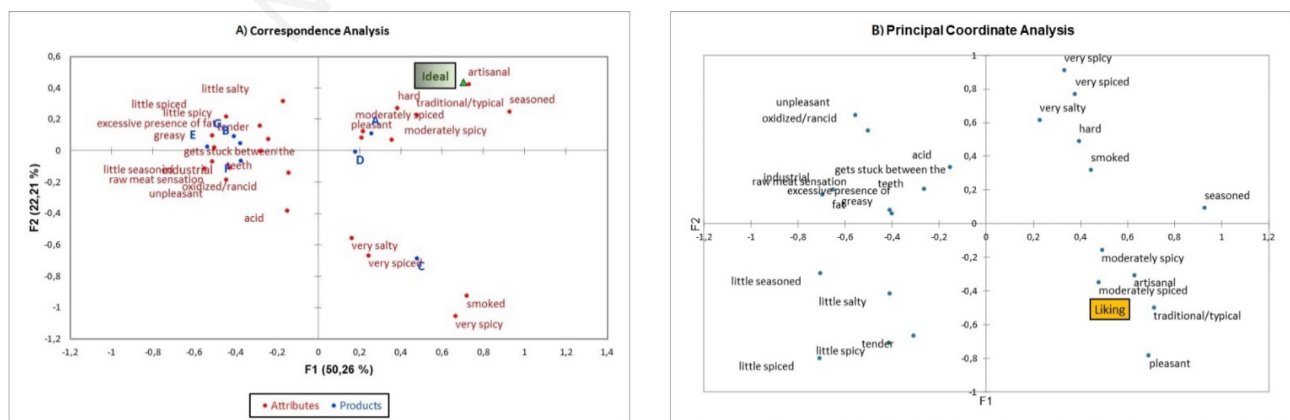

Figure 1. Sensory profile analysis results. **A**) Correspondence analysis between attributes/terms of the Check All That Apply (CATA) test and attributes/terms of an ideal Sardinian sausage; **B**) principal coordinates analysis of hedonic acceptability data (consumer test) and data from the CATA test.

acceptability test (ISO, 2014) was carried out with the aim of understanding which sensory attributes drive SFS preferences. Consumers were asked which sensory characteristics they would like to find in their ideal SFS, by using the same attributes/terms available.

Statistical analysis

Statistical analysis was performed with Statgraphics-Centurion XIX software (Stat Point Technologies, Warrenton, VA, USA). Differences among pH and a_w between processing plants were compared using the analysis of variance (ANOVA) model with a post-hoc Tukey honestly significant difference test for comparing multiple treatments.

Sensory data analysis was carried out with Statgraphics Plus5. A one-way ANOVA assessed significant differences among the acceptability of the samples. The least significant difference test ($p < 0.05$) was applied to detect significant differences among the samples. Afterward, the acceptability test (ISO, 2014) was carried out with the aim of understanding which sensory attributes drive SFS preferences. At this scope, a hedonic 9-point linear scale was used (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

The data analysis for the CATA test was performed using XLSTAT 2023.1.3 software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France). The Cochran's Q test identified significant differences among SFS from the seven producing plants. Sensory data acquisition was performed using a specific computer application (Smart Sensory box v2.2.39).

Results

Audits in the production plants

During the audits, the SFS production process was successfully defined in all production plants. The eight plants used different production methods (Table 1). The raw materials were pork half carcasses (3/8 producing plants, prevalence of 37.5%) or pork trimmings (5/8, 62.5%). The meat was minced and mixed with fat and other ingredients. The mixture was kept at refrigeration temperature overnight; afterward, it was stuffed in natural pork or calf bowel. The sausages were hung for the dripping phase; this step was variable in the producing plants: 4/8 (50%) plants carried out the dripping for less than 8 hours with temperatures above 24°C (sometimes not defined); 3/8 (37.5%) plants carried out the dripping phase for 12-24 h at 24±2°C; 1/8 (12.5%) dripped SFSs for 3 days at 5°C. The drying phase lasted for less than 3 days at 22±2°C in 4/8 (50%) producing plants and for 5-6 days with a progressive decrease in temperature and humidity in 3/8 (37.5%) plants. The ripening phase time ranged from 10 to 20 days, with a progressive decrease in temperature until 15±2°C and a humidity of 70-75% in 6/8 (75%) plants. In PD (1/8, 12.5%), the drying and ripening steps were carried out for 15 days overall at a non-defined room temperature with no relative humidity control. In PE (1/8, 12.5%), the drying and ripening phases were carried out for 30 days at 2°C without relative humidity control. Overall, in PA, PE, and PH, the ripening phase was >20 days, whereas in PB, PC, PD, PF, and PG, it was <20 days.

Issues in the layout of the production process were detected in 3/8 (37.5%) producing plants, particularly intersections of flows between raw materials and finished products. Another critical issue identified in 2/8 (25%) plants was the application of process parameters that did not allow a correct maturation of the products,

Table 1. Sardinian fermented sausage production processes in the producing plants.

Process phases	Producing plants							
	A	B	C	D	E	F	G	H
Raw ingredients	Heavy pork half carcasses	Finishing pig half carcasses	Heavy pork meat trimmings	Heavy pork meat trimmings	Heavy pork meat trimmings	Heavy pork meat trimmings	Heavy pork meat trimmings	Finishing pig half carcasses
Mixing of curing ingredients and rest	Overnight at 2-4°C	Overnight at 2-4°C	Overnight at 2-4°C	Overnight at 2-4°C	Overnight at 2-4°C	Overnight at 2-4°C	Overnight at 2-4°C	Overnight at 2-4°C
Filling into case and dipping	For 15 h at 24°C	For 12 h at 23°C	For 5 h, nd T°C	For 15 h, nd T°C	For 3 days at 5°C	For 24 h at 25°C	For 8h at 25 °C	For 6 h at 24°C
Drying	For 24 h at 23 °C	For 3 days at 20°C	For 24 h at 24°C	For 5 days, nd T°C	For 15 days at 2°C	For 6 days, ↓ 1°C/day	For 6 days ↓ 1°C/day	For 3 days at 23°C
Ripening	For 18 days, ↓ 1°C/die until 14°C	For 15 days, ↓ 1°C/die until 14°C	For 15 days, ↓ 1°C/die until 17°C	For 10 days, nd T°C	For 15 days at 2°C	For 10 days at 14°C	For 10 days at 15°C	For 20 days, ↓ 1°C/die until 12°C

nd T°C, non-defined temperature; ↓ 1°C/die, gradual reduction of 1°C per day.

e.g., a ripening period shorter than 20 days and a ripening temperature above 15°C. The proposed modifications were generally positively received by the food operators, but in 2/8 (25%) plants, the modifications were not accepted due to the substantial structural changes needed.

Analysis of Sardinian fermented sausage

The detected pH, expressed as mean ± standard deviation (SD), values among SFS samples were 5.72±0.34, with the lowest value of 5.29±0.02 in samples from PF and the highest value of 6.21±0.01 in samples from PH. A significant difference has been observed in pH values between samples with a ripening period longer and shorter than 20 days ($p<0.05$). The values of a_w values (mean±SD) were 0.911±0.01; a value >0.920 was found in samples from PB (0.941±0.04). No significant difference was found in a_w values in SFS samples with a ripening period longer and shorter than 20 days. The physical-chemical characteristics of the SFS samples are summarized in Table 2.

L. monocytogenes was detected in 2/72 (2.8%) SFS samples, produced in PD; *Listeria* spp. in 15/72 (20.8%) samples and in 5/8 (62.5%) producing plants (PA, PB, PC, PF, PG). *Salmonella* was detected in 2/72 (2.8%) samples from PB. *Y. entercolitica* was detected in 2/72 (2.8%) samples from PF. Microbiological results in samples are reported in Table 3.

Environmental samples

As reported in Table 3, *L. monocytogenes* was detected in 8/48 (16.6%) environmental samples and 2/8 producing plants (PA, PF). *Listeria* spp. was detected in 22/48 (45.84%) samples and all producing plants except for PD (7/8; 87.5%). PA and PF showed the highest *L. monocytogenes* prevalence (both 4/6 *L. monocytogenes* positive samples, with a prevalence of 66.7%); PG showed 0/6 *L. monocytogenes* positive samples and the highest *Listeria* spp. prevalence (6/6, 100%). *Salmonella* was never detected in environmental samples. *Y. enterocolitica* was detected in 3/48 (6.25%) samples and in 3/8 (37.5%) different producing plants.

Sensory profile analysis

Table 4 presents the attributes/terms and their statistical analysis. The results indicate that out of 24 attributes/terms, 22 had significantly different citation numbers, confirming that SFS produced by different companies is perceived as different from a sensory and emotional point of view. Table 5 shows the results of the acceptability test. In Figure 1A, the results of the correlation between attributes/terms of the samples evaluated by consumers and the attributes/terms they would have wanted to find in an ideal SFS are shown. The multivariate analysis (correspondence analysis) revealed how the product differs from the ideal one and which attributes might need to be reformulated. The graph illustrates that production plants are separated into two groups along the first factor of variability (F1 50.26%). PB, PE, PF, and PG are on the left, producing SFS with less appreciated attributes like "excessive fat presence", "greasiness", "unpleasant", *etc*. PA, PC, and PD are on the right, producing SFS with attributes like "moderately spiced", "traditional/typical", "seasoned", and "artisanal". The ideal SFS aligns with PA and PD, sharing similar attributes. Along the second factor of variability (F2 22.21%), PC stands out for its "very salty", "spiced", "spicy", and "smoky" attributes. The results of principal

Table 2. Means and standard deviations for the physicochemical characteristics and composition of 72 samples of Sardinian fermented sausage.

Parameters	Producing plants							
	A	B	C	D	E	F	G	H
pH	6.08±0.25[a]	5.90±0.17[a]	5.30±0.08[b]	5.58±0.30[ab]	5.57±0.19[ab]	5.29±0.02[bc]	5.88±0.32[abd]	6.21±0.01[ad]
aw	0.906±0.01[a]	0.941±0.01[b]	0.914±0.01[ac]	0.886±0.01[ac]	0.914±0.02[d]	0.918±0.01[d]	0.911±0.02[abcd]	0.905±0.01[ac]

Means in the same row with different superscript letters denote significant differences ($p \leq 0.05$) depending on the producing plant.

Table 3. Prevalence expressed as positive/total (%) *Listeria monocytogenes*, *Listeria* spp., *Salmonella* and *Yersinia enterocolitic*a in Sardinian fermented sausage and environmental samples.

Parameters	Samples SFS	A	B	C	D	E	F	G	H	Total
L. monocytogenes		0/9	0/9	0/9	2/9 (22.2)	0/9	0/9	0/9	0/9	2/72 (2.8)
Listeria spp.		3/9 (33.3)	3/9 (33.3)	3/9 (100)	0/9	0/9	3/9 (33.3)	3/9 (33.3)	0/9	15/72 (20.8)
Salmonella		0/9	2/9 (22.2)	0/9	0/9	0/9	0/9	0/9	0/9	2/72 (2.8)
Y. enterocolitica		0/9	0/9	0/9	0/9	0/9	2/9 (22.2)	0/9	0/9	2/72 (2.8)
Environmental		A	B	C	D	E	F	G	H	
L. monocytogenes	SCM	2/2 (100)	0/2	0/2	0/2	0/2	1/2 (50)	0/2	0/2	8/48 (16.7)
	SNCM	2/4 (50)	0/4	0/4	0/4	0/4	3/4 (75)	0/4	0/4	
Listeria spp.	SCM	2/2 (100)	0/2	1/2 (50)	0/2	1/2 (50)	1/2 (50)	2/2 (100)	0/2	22/48 (45.8)
	SNCM	3/4 (75)	2/4 (50)	2/4 (50)	0/4	0/4	2/4 (50)	4/4 (100)	2/4 (50)	
Salmonella	SCM	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/48
	SNCM	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	
Y. enterocolitica	SCM	0/2	0/2	1/2 (50)	0/2	0/2	0/2	1/2 (50)	0/2	5/48 (10.4)
	SNCM	0/4	0/4	0/4	0/4	0/4	1/4 (25%)	2/4 (50)	0/4	

SCM, surfaces in contact with meat; SNCM, surfaces not in contact with meat.

coordinate analysis correlating hedonic data of acceptability (consumer test) and data related to the CATA test are presented (Figure 1B) and confirm that attributes that improve liking are "moderately spiced", "moderately spicy", "traditional/typical", "seasoned", and "artisanal". Conversely, attributes like "excessive fat presence", "greasy", "raw meat sensation", "acidic", and "industrial" are in direct opposition to the likability index.

Discussion

The audits of the selected producing plants allowed for the description of the production process of the eight facilities, which were different and representative of the regional sector. The main deficiency identified in the plants' production process (37.5% of the plants) was the existence of intersections of flows between raw materials and finished products. In some cases, the process parameters and procedures applied had little to no control over the correct acidification and drying, which are crucial steps to ensuring the product's safety. In this investigation, the ripening of SFS ranged from 16 days (PB) to 33 days (PE). The regulation of temperatures and relative humidity also varied from strict regulation to near absence of control (PD). The length of the ripening stage is often determined by the need to reduce production costs and satisfy market demands, oriented towards not particularly dried sausages; however, final products' consistent quality and safety may suffer (Consigliere et al., 2017).

Table 4. Cochran's Q test for each attribute/term.

Attributes/terms	p	Attributes/terms	p
Excessive presence of fat	0.000	Pleasant	0.034
Little seasoned	0.000	Unpleasant	0.000
Very salty	0.000	Seasoned	0.000
Little spiced	0.000	Moderately spiced	0.111
Gets stuck between teeth	0.000	Very spiced	0.000
Very spicy	0.000	Hard	0.000
Acid	0.037	Greasy	0.000
Oxidized/rancid	0.051	Little spicy	0.000
Moderately spicy	0.000	Smoked	0.000
Tender	0.000	Traditional/typical	0.005
Raw meat sensation	0.000	Little salty	0.000
Artisanal	0.000	Industrial	0.000

Table 5. Acceptability test on Sardinian fermented sausage.

Samples	Mean±SD
A	6.06[a]±1.61
B	6.02[a]±1.66
C	6.17[a]±1.82
D	6.09[a]±1.68
E*	5.11[b]±1.92
F	6.08[a]±1.61
G	6.03[a]±1.68

SD, standard deviation; *significant (p<0.001). Different superscript letters mean significant differences among the samples.

As a result of substantial differences in production procedures, SFS samples had different physicochemical characteristics. Mean pH values ranged from 5.29±0.02 (PF) to 6.21±0.01 (PH), and a significant difference has been observed between samples with a ripening period longer and shorter than 20 days (p<0.05). Mean a_w values ranged from 0.886±0.01 (PD) to 0.941±0.01 (PB), with no significant difference between samples with different ripening periods (p>0.05). According to Regulation n. 2073/2005, the growth of *L. monocytogenes* is not supported in RTE foods, such as fermented sausages, with pH≤4.4 and a_w≤0.92 or pH≤5.0 and a_w≤0.94 (European Commission, 2005). In our study, no sample at the end of ripening showed pH<5.0. On the other hand, SFS samples from PB exhibited a_w values above the >0.920 threshold, and SFS from PF had values close to undesirable (0.918±0.01). These values could lead to food safety issues in the products.

The overall prevalence of *L. monocytogenes* in SFS (8%) was similar to what was found in previous investigations in Sardinia (Meloni et al., 2014) and lower if compared to other surveys in fermented sausages, which reported a prevalence from 10% to 31.5% (Martin et al., 2004; De Cesare et al., 2007). In our study, the pathogen was detected with the qualitative method in samples from PD, whose production process was characterized by 16-day-long drying and ripening phases without control of temperature or relative humidity. All SFS samples from PB were positive for *Listeria* spp., and 2/3 were also positive for *Salmonella*. The samples from PB had been dried and ripened for 19 days and showed mean pH and a_w values of 5.9±0.17 and 0.941±0.01, which are favorable for both *Listeria* and *Salmonella* survival in the product.

The presence of pathogens in the finished products may be the result of the incorrect application of the hurdle technology in the production of SFS. Correct drying and ripening steps, longer than 20 days at 12-15°C and 70-75% humidity (Greco et al., 2005; Piras et al., 2019), are therefore highly suggested to allow the development of the hurdles required to guarantee the safety of fermented sausages.

In environmental samples, *L. monocytogenes* was detected in 25% (2/8) of producing plants and *Listeria* spp. in 87.5% (7/8) of plants. The presence of different *Listeria* species at the same time is common in meat processing environments and it is probably because *L. monocytogenes* and other *Listeria* spp. (e.g., *L. innocua*) share the same ecological niches (Wagner et al., 2007). Higher levels of contamination (albeit not significantly so) for *L. monocytogenes* and *Listeria* spp. were detected in SNCM (11.9% and 35.7%, respectively). However, the finding of *L. monocytogenes* and *Listeria* spp. from SCM (7.14% and 16.7%) is of particular concern. Floor drains displayed the highest levels of contamination (4.8% for *L. monocytogenes* and 14.3% for *Listeria* spp.). Floor drains are well recognized to serve as a niche for pathogens and a source of cross-contamination (Belias et al., 2022). In these areas, the ongoing humidity and presence of organic substrates promote the growth of *Listeria* and biofilm formation, which offers pathogens resistance to cleaning and disinfection (Thévenot et al., 2005). Effective hygiene practices are necessary as control measures to reduce *L. monocytogenes* presence in processing environments; the strategy should be focused on preventive measures, such as hygienic layouts and effective cleaning and disinfection procedures on manufacturing equipment and the food-processing environment (Saini et al., 2012). *Y. enterocolitica* was also detected in environmental samples collected from SCM and SNCM. In food processing facilities, *Y. enterocolitica* can be used as a marker of the possible presence of *L. monocytogenes* since both can grow at refrigeration temperatures, which may allow colonization in specific

areas of the production plant (Piras *et al*., 2023). Investigating the presence of *Y. enterocolitica* in production plants is, therefore, a useful component of a successful environmental monitoring program.

The results obtained from the CATA test and the acceptability test conducted by consumers showed that sausages produced in different production plants have different sensory characteristics. The results show that SFS have unique and distinctive sensory characteristics, which can be attributed to various factors, including the quality of meat, the type of spices, the effect of different starter cultures, the percentage of fat, the processing method, *etc*. (Fonseca *et al*., 2013; Braghieri *et al*., 2016). The sensory diversity detected highlights the complexity and richness of sausages as a food product. These differences not only enrich the industry but also provide buyers with a range of options to satisfy individual tastes and preferences. The results of sensory analysis provide valuable information for producers to improve and differentiate their products based on consumer expectations.

Conclusions

SFS-producing plants use different production techniques, which are occasionally traditional and hard to standardize. Regarding safety, correct drying and ripening steps lasting longer than 20 days at 12-15°C and 70-75% humidity are highly suggested to allow the development of the hurdles required. Environmental contamination by pathogens, particularly *Listeria* spp., is a widespread problem. The approach should be centered on preventive measures: processing environments, cleaning, and disinfecting procedures remain of paramount importance.

Data obtained will allow producing plants to define correct procedures to guarantee the safety of SFS. Results also highlight how the multidisciplinary approach and the collaboration between the veterinarian inspectors who conducted the audits, the laboratory, and the consumer judges who conducted the sensory analyses allowed for a precise response to the food business operators regarding the safety of the products and consumer preferences.

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