Evaluation of vacuum packaging for extending the shelf life of Sardinian fermented sausage

Giuliana Siddi,[1] Francesca Piras,[1] Maria Pina Meloni,[1] Daniele Casti,[2] Carlo Spanu,[1] Carlo Pala,[3] Anna Maria Mocci,[4] Carlo Piga,[5] Riccardo Di Salvo,[5] Enrico De Santis,[1] Christian Scarano[1]

[1]Veterinary Medicine Department, University of Sassari; [2]Local health authority (ASL), Carbonia; [3]Local health authority (ASL), Oristano; [4]AUSL Piacenza; [5]Agris Sardegna, Regional Agency for Agricultural Research, Bonassai, Sassari, Italy

Abstract

Salsiccia sarda or Sardinian fermented sausage is a traditional dry-fermented sausage included in the list of traditional food products of Sardinia (Italy). At the request of some producing plants, the possibility of extending the shelf life of the vacuum-packed product up to 120 days was evaluated. Manufacturing of 90 samples, representing 3 different batches of Sardinian fermented sausage was carried out in two producing plants (A and B). In the packaged product and subsequently every 30 days for four months (T_0, T_{30}, T_{60}, T_{120}), the following analyses were conducted on all samples: physicochemical characteristics, total aerobic mesophilic count, *Enterobacteriaceae* count, detection of *Listeria monocytogenes*, *Salmonella spp.*, mesophilic lactic acid bacteria, and coagulase-positive *Staphylococci*. Moreover, surfaces in contact and surfaces not in contact with food were sampled in both producing plants. Sensory profile analysis was also performed for every analysis time. At the end of the extended shelf life, pH values were equal to 5.90±0.11 (producing plant A) and 5.61±0.29 (producing plant B). Water activity mean values at T_{120} were 0.894±0.02 (producing plant A) and 0.875±0.01 (producing plant B). *L. monocytogenes* was detected in 73.3% (33/45) of the samples from producing plant A, with mean levels of 1.12±0.76 $\log_{10}$ CFU/g. In producing plant B, *L. monocytogenes* was never detected. *Enterobacteriaceae* were detected in 91.1% (41/45) of samples in producing plant A with mean values of 3.15±1.21 $\log_{10}$ CFU/g, and in 35.5% (16/45) samples in producing plant B samples with mean values of 0.72±0.86 $\log_{10}$ CFU/g. *Salmonella* and *Staphylococcus aureus* were never detected. Regarding environmental samples, the sites that were most contaminated by *L. monocytogenes* were the bagging table (contact surface) and processing room floor drains (non-contact surface) with a prevalence of 50% each (8/16 positive samples for both sampling sites). Sensory analysis results showed that at T_{30} the overall sensory quality was at its highest; moreover, the visual-tactile aspect, the olfactory characteristics, the gustatory aspects, and the texture showed significant differences in samples throughout the shelf life, with a decreased intensity at 120 days of storage. Overall, the quality and sensory acceptance of the vacuum-packed Sardinian fermented sausage was not affected until 120 days of shelf-life. However, the possible contamination by *L. monocytogenes* calls attention to the hygienic management of the entire technological process. The environmental sampling was confirmed as a useful verification tool during control.

Introduction

Salsiccia Sarda or Sardinian fermented sausage (SFS) is a traditional Mediterranean-style sausage made from minced pork meat, fermented and dried, with a characteristic horseshoe shape. It represents the main ready-to-eat pork meat product of Sardinia (Italy) and is included in the national list of fermented food products (Italian Republic, 2020). Ingredients include pork meat and fat with the addition of curing ingredients, spices, and authorized additives (nitrates, nitrites, glutamate). Lactic acid bacteria (LAB) and nitrate-reducing coagulase-negative

staphylococci (CNS) are often naturally present in meat or added by inoculation of starter cultures during the mixing step (Greco *et al.,* 2005); LAB and CNS are responsible for the fermentation of the SFS and the development, during the ripening, of peculiar sensory characteristics such as distinctive aromas and flavors which represent a link between the product and the territory of origin (Flores, 1997). SFS production is widespread throughout the whole island of Sardinia and takes place in both artisanal and industrial producing plants; for this reason, SFS production technology is influenced by numerous local traditions and the product is difficult to standardize (Meloni, 2015). SFS safety is ensured by the presence of multiple factors and specific physicochemical conditions, such as pH values comprised between 5.3-5.5, water activity (a_w) values ≤0.920, sodium chloride, nitrates, and nitrites (Greco *et al.*, 2005; Piras *et al.*, 2019); these values indicate a correct acidification and drying process and act as hurdles that intervene in limiting the microbial growth and in ensuring the safety and shelf stability of the final product (Mangia *et al.*, 2007; Meloni, 2015; Piras *et al.*, 2019). However, during storage, deterioration may lead to unacceptable food quality or safety issues due to oxidative rancidity, an increase in the number of spoilage microorganisms, or the presence of food pathogens (O'Neill *et al.*, 2018). Lipid oxidation phenomena lead to changes in flavor, color, aroma, texture, and safety which give products undesirable characteristics (Hernández- Hernández *et al.*, 2009; Petrón *et al.*, 2013); to inhibit oxidative rancidity occurring during storage, vacuum-packaging is one of the tools used to minimize lipid oxidation and extend the shelf-life of meat products (Parra *et al.*, 2010). Moreover, the environment created in the meat by the vacuum-packaging (low oxygen, presence of NaCl and $NaNO_2$, reduced a_w) inhibits the growth of Gram-negative spoilage bacteria (including *Pseudomonas*, *Acinetobacter,* and *Enterobacter*) (Khorsandi *et al.*, 2019). Regarding safety, contamination with pathogens like *Escherichia coli* O157:H7, *Staphylococcus aureus,* and *Salmonella* is of great concern (Barbuti and Parolari, 2002; Namvar and Warriner, 2006; Malakauskas *et al.*, 2006; Hawken *et al.*, 2013), but the most important hazard in fermented sausages is *Listeria monocytogenes* (*L. monocytogenes*), because of its widespread environmental distribution, frequent post-processing contamination and ability to grow at refrigeration temperatures (EFSA, 2019). Moreover, *L. monocytogenes* can persist in processing environments, which leads to the adaption of certain strains that can also become tolerant to some of the used disinfectants and determine constant contamination of products (Gram *et al.*, 2007; Mureddu *et al.*, 2014). In this framework, the decision around the shelf life duration has to be taken on a product‐by‐product basis, considering the relevant hazards, product characteristics, processing and storage conditions; the intrinsic (*e.g.* pH and a_w), extrinsic (*e.g.* temperature and gas atmosphere) and implicit (*e.g.* interactions with competing background microbiota) factors of each food determine which pathogenic and spoilage microorganisms can grow during storage under reasonably foreseeable conditions until consumption, for these reasons the hazard identification and shelf life studies are product-specific (EFSA, 2020). Very few studies regarding SFS shelf life are available, therefore at the request of producing plants, the possibility of extending the shelf life of vacuum-packed SFS up to four months (120 days) of storage under reasonably foreseeable conditions was evaluated.

Materials and Methods

Samples

The investigation was conducted in collaboration with two SFS industrial-producing plants located in Sardinia (Italy): producing plant A (PA) and producing plant B (PB).

Briefly, the production process involved the selection, chopping, and mincing of pork meat and fat, followed by mixing with curing ingredients, spices, and authorized additives, including nitrates and nitrites (European Commission, 2008). PB used a commercial freeze-dried starter culture consisting of *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Pediococcus pentosaceus,* and *Debaryomyces hansenii*. A starter culture was added during the mixing step. PA did not use starter cultures. After overnight refrigerated storage, the mixture was stuffed in natural bowel (mutton or beef). The next steps were dripping (20-22°C for 24 hours, 70-80% humidity) and drying (2-3 days with progressive decrease of temperature and humidity), in which fermentation took place. Ripening lasted about 20 days in storerooms at 15°C and 70–75% humidity. The production process is summarized in Figure 1.
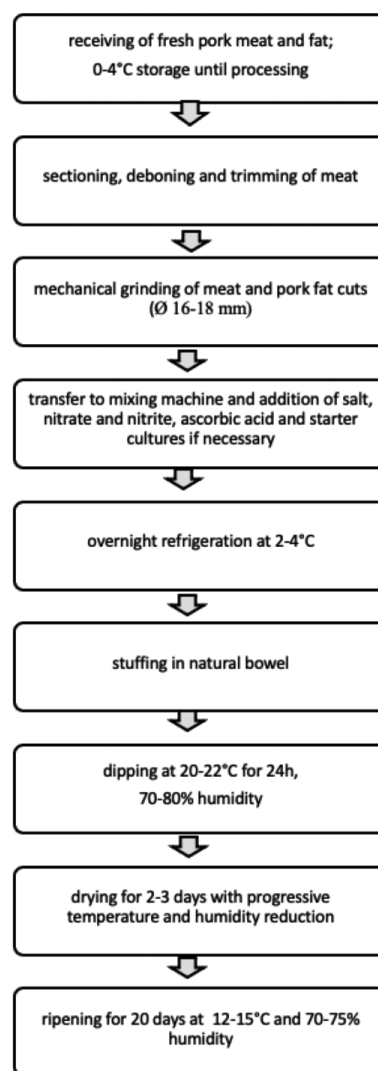

Figure 1. Process flow diagram of Sardinian fermented sausage.

The finished products were cylindrical, with a length of 40-45 cm and a diameter of 3-4 cm, folded with the characteristic horseshoe shape; the weight was between 300 and 600 g. Each SFS was vacuum-packed and regarded as a sample.

Experimental design

Overall, 90 samples of SFS were produced (45 samples from PA and 45 samples from PB), representing 3 different production batches. For each batch, 3 SFSs were sampled after stuffing, in order to assess the initial values of pH and a_w. At the end of the production process, SFS samples were vacuum-packed at the producing plants, then collected and stored at refrigeration temperature ($4°C±1°C$) for 120 days. Refrigerated storage under controlled conditions had the purpose of reproducing the storage conditions that occur during the commercial life of the product in the refrigerated counters of large-scale retailers. The shelf-life study was conducted by analyzing SFS samples at different times: 24 hours after packaging (T_0), after 30 days (T_{30}), after 60 days (T_{60}), after 90 days (T_{90}), and after 120 days (T_{120}) of refrigerated storage in vacuum-packaging.

Physicochemical parameters on the products

On each sample, pH and a_W were determined using pH-meter GLP 22 (Crison Instruments SA, Barcelona, Spain) and Aqualab CX3 (Decagon, Pullman, Washington, USA). Physicochemical parameters were evaluated during processing and storage: immediately after the stuffing phase, at the end of ripening (after 20 days), and at every time point (T_0, T_{30}, T_{60}, T_{90} and T_{120}) during the shelf-life study.

Microbiological profile of the products

At each time point, microbiological analyses were conducted according to international standard methods. Serial decimal dilutions were prepared in buffered peptone water (BPW, Biolife) solution according to ISO (2017c) and used to inoculate the appropriate culture media. Analyses included the following: total aerobic mesophilic count (ISO, 2013), *Enterobacteriaceae* (ISO, 2017d), *Salmonella spp.* (ISO, 2020), mesophilic LAB (ISO, 1998), and coagulase-positive and coagulase-negative *Staphylococci* (CPS and CNS; ISO, 2021). On each sample *Listeria spp.* and *L. monocytogenes* detection and enumeration were conducted according to ISO standards (2017a, 2017b); all the strains showing typical growth characteristics referable to *L. monocytogenes* were identified by specific polymerase chain reaction according to the protocol by Ryu *et al.* (2013) modified by Mazza *et al.* (2015).

Microbiological profile of the environment

Surfaces in direct contact with food and surfaces not in contact with food were selected for environmental sampling in both processing plants to assess the hygienic and sanitary level of the production environment. For each producing plant, three samplings in the time frame of three months were carried out; samples were taken during processing, for a total of 76 environmental samples (38 samples from each producing plant). The selected surfaces in contact with food were: the Teflon tables for the bagging and binding of the product, the meat grinder, the meat mixer, and the stuffer. The surfaces not in contact with the food were represented by the floor and the drainage channel of the processing room, and the floor and walls of the storage cell. Following the approach proposed by the International Commission on Microbiological Specifications for Foods (ICMSF, 2002), the sampling sites were grouped into 3 different zones based on the concept of risk: zone 1 (highest risk) consisted of exposed product contact surfaces, which included direct contact surfaces (work tables, meat grinder, meat mixer, and stuffer); zone 2 (medium risk) included indirect or secondary contact surfaces that were physically close to exposed product but not in contact with it (surfaces adjacent to the machinery or worktables); zone 3 included surfaces away from exposed product but still in the exposed product area (floors, drains, walls, and undersides of equipment) (ICMSF, 2002; Malley *et al.*, 2015). The sampling of the surfaces was carried out using a commercial kit (3M, St. Paul, Minnesota, USA) containing a sterile sponge pre-moistened with 10 mL of BPW, sterile gloves, and a sterile bag. Environmental samples were transferred to the laboratory at refrigerated temperature. Qualitative detection of *L. monocytogenes* (ISO, 2017a) was carried out on each sample.

Sensory profile analysis

Sensory profile analysis (ISO, 2016) was performed on SFS samples to identify the product sensory characteristics and to measure their perceived intensity during shelf life.

The analyses were carried out for every analysis time (T_0, T_{30}, T_{60}, T_{90} and T_{120}) in triplicate and for 3 different production batches of SFSs. Each sample consisted of three slices of sausage, each of 3 mm thickness, cut perpendicularly to the length and served with the bowel. The judging panel consisted of 8 expert judges (ISO, 2005), 4 females and 4 males, aged between 25 and 50 years, with specific experience in sensory profile development.

After acclimatization in a thermostatic oven at a temperature of 16°C, the sausage samples were marked with a random three-digit number and presented to the judges in a tray of odorless material with a cracker and a glass of water, inside the tasting booths (ISO, 1989). The samples were presented to the judges in a randomized and balanced block design (Macfie *et al.*, 1989). The judges identified and quantified the intensity of 22 attributes in a structured 9-point scale (1=no or very low intensity and 9=high intensity), 5 of which belonged to the visual-tactile characteristics (color of meat, uniformity of color, color of fat, cohesion of the slice and peelability), 4 belonging to the olfactory characteristics (spicy smell, seasoned smell, raw meat smell, and oxidized smell), 4 to the gustatory characteristics (salty, sour, spicy and bitter), 4 to the aromatic characteristics (seasoned aroma, spicy aroma, oxidized aroma and off-flavor) and 5 to structure characteristics (tenderness, cohesion, chewability, solubility, and greasiness); in addition, 2 general attributes were evaluated (overall quality and typicality).

Statistical analysis

Differences among pH, a_w, and average microbiological group counts ($\log_{10}$ CFU/g) between samples at the end of ripening (after 20 days) and at every time point (T_0, T_{30}, T_{60}, T_{90}, and T_{120}) during the shelf-life study were compared using the analysis of variance (ANOVA) model with post-hoc Tukey HSD test for comparing multiple treatments. Statistical analyses were performed with Statgraphics Centurion XIX software (Stat Point Technologies, Warrenton, VA, USA). Sensory panel result statistical analysis was conducted using a three-way ANOVA model (assessor, samples, and replicate), and a two-way ANOVA with interactions was used to evaluate the reliability of the panel's judgments for each descriptor (Montouto-Graña *et al.*, 2002; Pagliarini 2002).

A two-way ANOVA (factor: assessor and samples) was used to define the sensory profile of the SFS. If ANOVA detected significant variations, the least significant difference test was applied to detect significant differences among the sausage samples. Data analysis was carried out with Statgraphics Plus 5. Sensory data acquisition was carried out using a special computer application (Smart Sensory box v2.2.39).

Results

Physicochemical parameters on the product

As for PA, pH showed initial mean values [$\bar{x}\pm$standard analysis (SD)] of 5.76±0.21 at the stuffing phase, followed by a decrease to mean values of 5.51±0.17 after 20 days of ripening. In PB, pH showed average values of 5.78±0.34 after the stuffing phase, and at the end of maturation (20 days), pH levels stabilized on mean values of 5.59±0.19. During the shelf life, pH values at T_{120} reached values of 5.90±0.11 and 5.61±0.29 respectively in PA and PB samples.

In PA samples, a_W had average initial values (at the stuffing phase) of 0.962±0.01; then the values showed a gradual reduction during the ripening period, reaching mean values of 0.905±0.01 after 20 days of ripening. In PB, a_W showed mean values of 0.967±0.00 at the stuffing phase, then the values decreased reaching mean values equal to 0.879±0.03 after 20 days of ripening. At the end of the shelf life (T_{120}), values were 0.894±0.02 and 0.875±0.01 respectively in PA and PB. Table 1 reports pH, a_w and physicochemical values in SFS samples.

Microbiological profile of the products

The results showed differences in the microbiological profile of the SFS samples produced by the two producing plants. For PA, the total aerobic mesophilic count showed mean values ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD) that ranged between 7.40±0.43 at T_0 and 7.33±0.62 at T_{120}, reaching the highest levels at T_{60} (7.41±0.74). As for LABs enumeration ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD), values were 7.42±1.15 at T_0 and reached the highest level of 8.38±0.61 at T_{120}.

CNS counts ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD) showed values equal to 6.01±0.71 at T_0 and 4.74±0.81 (T_{120}); the highest CNS levels were detected at T_{30} with values equal to 6.27±0.71. The count of *Enterobacteriaceae* ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD) showed values between 3.80±0.48 (T_0) and 3.04±1.42 (T_{120}), reaching the highest mean levels at T_{60} (3.62±1.02).

L. monocytogenes was detected in all batches produced by PA with values ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD) ranging between 1.74±0.39 at T_0 and 0.29±0.68 at T_{120}. 100% of the strains that showed typical growth characteristics referable to *L. monocytogenes* were confirmed through PCR.

The microbiological profile of SFS samples manufactured in PB was influenced by the use of the starter culture. The total aerobic mesophilic count ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD) showed values between 7.85±0.60 at T_0 and 8.15±0.70 at T_{120}, the highest mean levels were detected at T_{30} with values equal to 8.03±0.38. As regards LABs count ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD), the minimum value was detected at T_0 (7.86±0.73) and the highest at T_{120} (8.60±0.65). CNS counts ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD) showed values equal to 7.66±0.39 at T_0 and to 7.46±0.43 at T_{120} and reached the highest levels of 7.98±0.52 at T_{30}. *Enterobacteriaceae* mean values ($\log_{10}$ CFU/gram; $\bar{x}\pm$SD) were equal to 0.56±0.87 at T_0 and were not detectable at T_{120}, however, at T_{60} mean levels of 1.11±0.94 were detected. None of the samples showed higher values than the method sensitivity limit for the detection of *Staphylococcus aureus* and *Salmonella spp*. Results regarding CMT, LAB, CNS, *Enterobacteriaceae,* and *L. monocytogenes* counts in PA and PB samples are reported in Table 2.

Table 1. Water activity, pH, and physicochemical mean values ($\bar{x}$ ± standard deviation) in Sardinian fermented sausage samples during the shelf life.

Parameters	Producing plants			Analysis time				
		Stuffing	End of curing	T_0	T_{30}	T_{60}	T_{90}	T_{120}
pH	A	5.76±0.21	5.51±0.17	5.55±0.24	5.67±0.30	5.71±0.15	5.94±0.03	5.90±0.11
	B	5.78±0.34	5.59±0.19	5.44±0.08	5.55±0.16	5.72±0.15	5.79±0.06	5.61±0.29
a_w	A	0.962±0.01	0.905±0.00	0.896±0.01	0.904±0.01	0.899±0.01	0.909±0.02	0.894±0.02
	B	0.967±0.00	0.879±0.03	0.889±0.02	0.888±0.01	0.893±0.01	0.880±0.01	0.875±0.01

A, producing plant A samples; B, producing plant B samples; T_0, end of ripening; T_{30}, after 30 days of shelf-life; T_{60}, after 60 days of shelf-life; T_{120}, after 120 days of shelf-life; aw, water activity.

Table 2. *Listeria monocytogenes*, lactic acid bacteria, micrococci, coagulase negative staphylococci, and *Enterobacteriaceae* mean values ($\bar{x}$ ± standard deviation) in Sardinian fermented sausage samples during the shelf-life.

Parameters	Producing plants		Analysis time			
		T0	T30	T60	T90	T120
Listeria monocytogenes $\log_{10}$ CFU/g	A	1.74±0.34	1.58±0.47	1.51±0.18	0.44±0.58	0.29±0.77
	B	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CMT $\log_{10}$ CFU/g	A	7.40±0.43	7.25±0.46	7.46±0.73	7.15±0.77	7.33±0.62
	B	7.85±0.60	7.99±0.38	7.97±0.81	7.76±0.67	8.15±0.70
LAB $\log_{10}$ CFU/g	A	7.42±1.15	7.94±0.42	7.98±0.42	8.33±0.80	8.38±0.61
	B	7.86±0.73	8.48±0.30	8.35±0.42	8.51±0.78	8.60±0.65
Micrococci and CNS $\log_{10}$ CFU/g	A	6.01±0.71	6.27±0.71	5.89±0.80	4.79±0.78	4.74±0.81
	B	7.66±0.39	7.99±0.52	7.51±0.48	7.27±0.56	7.46±0.43
Enterobacteriaceae $\log_{10}$ CFU/g	A	3.80±0.48	3.11±1.02	3.64±0.96	2.16±1.53	3.04±1.42
	B	0.56±0.87	0.44±0.68	1.11±1.00	1.11±0.98	0±0.00

A, producing plant A samples; B, producing plant B samples; T_0, end of ripening; T_{30}, after 30 days of shelf-life; T_{60}, after 60 days of shelf-life; T_{120}, after 120 days of shelf-life; CNS, coagulase negative staphylococci.

Microbiological profile of the producing plant environment

The overall prevalence of *Listeria* in both producing plants' samples was 32.89% (25/76). In PA, *Listeria spp.* prevalence was 50.0% (19/38) and the prevalence of *L. monocytogenes* was 42.1% (16/38). In PB, only *Listeria spp.* was detected, with a prevalence of 15.8% (6/38). Differences in contamination levels were observed in the three different risk zones. In zone 1, *Listeria* was detected with an overall prevalence of 27.6% (21/76), in particular, *Listeria spp.* showed a prevalence of 17.1% (13/76) and *L. monocytogenes* showed a prevalence of 10.5% (8/76). In zone 2, no positivity was found. In zone 3, the prevalence of contamination was 26.3% (20/76) overall, with values of 10.5% (8/76) and 15.8% (12/76) for *Listeria spp.* and *L. monocytogenes* respectively. The sampling sites mostly contaminated by *Listeria spp.* were the drainage channel located in the processing room, with 4/12 (33.4%) positive samples for PA and 2/12 (16.7%) positive samples for PB, and the work-table in which the bagging and binding of the cured meats took place, with a prevalence of 38.5% (5/13) positivity only for the PA. The most contaminated sites by *L. monocytogenes* were the bagging table (contact surface) and processing room floor drains (non-contact surface) with a prevalence of 50% each (8/16 positive samples for both sampling sites).

Sensory profile analysis

Table 3 shows the mean values, SD, and statistical processing of the sensory attributes of the SFS at different storage times. The attributes that showed a significant difference are related to: the visual-tactile aspect (peelability), the olfactory characteristics (spicy smell, spicy aroma, and oxidized aroma), the gustatory aspects (acid and spicy), and the texture (cohesion in the mouth and tenderness). Also, the overall quality was statistically different. The samples reached the highest peelability value at 120 days of storage. However, also samples at T_0, T_{30}, T_{60} and T_{90} showed high peelability values: this is considered a positive characteristic, as the meat mixture does not stick to the bowel.

For the olfactory/aromatic aspect, the samples showed different intensities (spice smell and spice aroma) with a slight decrease in intensity at 120 days of storage. During the extension of the shelf life (T_{120}), the characteristics of spiciness and texture (tenderness and cohesion) decreased in intensity. The overall quality was highest at T_{30}. No spoilage or unpleasant taste was observed in any sample.

Discussion

The possibility of extending the shelf life of the vacuum-packed SFS produced in two producing plants in Sardinia was tested. According to results regarding the physicochemical and microbiological profile, no particular issues emerged during the four months following packaging (120 days overall).

As regards pH and a_w, values reported at the end of SFS ripening

Table 3. Means and standard deviations of sensory attributes assessed on Sardinian fermented sausage at different times of storage.

Attributes	SFS samples				
	T_0	T_{30}	T_{60}	T_{90}	T_{120}
Color of meat	3.80±1.37	4.20±1.30	4.21±1.44	4.38±1.74	4.30±1.82
Uniformity of color	5.71±1.51	5.78±1.51	5.52±1.38	5.52±1.64	5.49±1.51[bc]
Color of fat	2.57±1.02	2.59±0.96	2.51±0.84	2.68±1.00	2.56±0.93
Cohesion of the slice	6.89±1.06	7.14±1.15	7.27±1.10	7.17±1.32	7.12±1.05
Peelability**	7.54[a]±1.19	7.77[ab]±1.09	8.08[c]±0.99	8.02[bc]±1.27	7.95[bc]±0.91
Spicy smell**	5.27[c]±1.23	5.13[bc]±1.27	5.05bc±1.22	4.85[ab]±1.44	4.51[a]±1.04
Seasoned smell	4.89±1.74	5.28±1.42	5.25±1.63	4.88±1.87	5.25±1.92
Raw meat smell	2.66±1.77	2.45±1.51	2.67±1.81	2.62±1.83	2.44±1.81
Oxidized smell	1.52±0.79	1.49±0.85	1.48±0.74	1.53±1.02	1.42±0.65
Salty taste	4.50±1.49	4.74±1.66	4.56±1.58	4.50±1.68	4.51±1.54
Acid taste*	2.59[b]±1.25	2.20[b]±1.22	2.29[a]±1.05	2.15[a]±1.02	2.18[a]±0.93
Spicy taste***	4.00[b]±1.38	4.12[b]±1.38	3.48[a]±1.38	3.58[a]±1.46	3.26[a]±1.28
Bitter taste	1.27±0.49	1.45±0.76	1.43±0.61	1.33±0.60	1.49±0.63
Seasoned aroma	4.45±1.63	4.70±1.78	4.62±1.91	4.88±1.54	4.74±1.67
Spicy aroma***	4.91[bc]±1.27	5.03[c]±1.35	4.57[ab]±1.30	4.57[ab]±1.27	4.28[a]±1.01
Oxidized aroma*	1.25[a]±0.51	1.38[ab]±0.69	1.44[b]±0.62	1.43[b]±0.77	1.35[b]±0.58
Off-flavor*	1.43[a]±0.66	1.49[ab]±0.82	1.62[abc]±1.01	1.75[c]±1.00	1.60[bc]±0.92
Tenderness**	5.14[c]±1.10	4.55[a]±1.05	4.83[abc]±0.99	4.85[bc]±1.16	4.49[ab]±1.04
Cohesion*	5.54[b]±1.26	5.19[a]±1.28	5.32[ab]±1.33	5.12[a]±1.29	5.14[a]±1.16
Chewability	7.13±1.06	7.23±1.05	7.02±0.94	6.88±1.30	6.96±1.24
Solubility	5.11±1.15	5.01±1.14	5.08±1.21	5.12±1.14	5.02±1.06
Greasiness	4.61±1.40	4.78±1.21	4.76±1.19	4.62±1.29	5.11±1.19
Typicality	5.43±1.28	5.64±1.43	5.52±1.48	5.35±1.61	5.42±1.36
Overall quality**	5.41[a]±1.14	5.94[b]±1.14	5.60[a]±1.28	5.38[a]±1.42	5.37[a]±1.23

SFS, Sardinian fermented sausage; *significant (P<0.05); **significant (P<0.01); ***significant (P<0.001); T_0, end of ripening; T_{30}, after 30 days of shelf-life; T_{60}, after 60 days of shelf-life; T_{120}, after 120 days of shelf-life. Different letters for each row mean significant differences among the samples.

were consistent with what was found by other authors and typical of the product (Meloni *et al.*, 2009; Piras *et al.*, 2019), with pH values of 5.55±0.24 (x̄±SD) for PA and 5.44±0.08 for PB; a_w values were 0.896±0.13 for PA and 0.889±0.02 for PB. In consideration of the typicality and adequacy of physicochemical values in the SFS samples, these were selected for the application of the extended shelf life. After 120 days of shelf life, a slight increase in pH values was observed in samples (P<0.05); the changes in pH values, in particular, the increase during the prolonged storage could be related to the presence of ammonia in relation to bacterial fermentation of amino acids and the liberation of metabolites (Reddy and Rao, 2000; Sčetar *et al.*, 2013). However, these findings partially disagree with Rubio *et al.* (2006), who observed a reduction of pH in Salchichon packed under vacuum. As regards a_w, values were typical of the product and did not show any relevant modifications (P>0.05): values were 0.896±0.01 at T_0 and 0.894±0.02 at T_{120} for PA, 0.889±0.02 at the end of ripening and 0.875±0.01 at T_{120} for PB.

The microbiological profile of the SFSs was characterized by the prevalence of high levels of the mesophilic microflora, mainly composed of LABs and CNS, as typical for this product (Greco *et al.*, 2005; Mangia *et al.*, 2007; Mureddu *et al.*, 2014; Meloni, 2015). The differences observed between the values of the microbiological profile between the SFSs of the two producing plants (Table 2) are attributable to the use of starters in the production technology of PB, in particular concerning total mesophilic bacteria count (P<0.01) and CNS (P<0.01). Similarly, the difference in *Enterobacteriaceae* counts (P<0.01) between the two producing plants is attributable to the use of starters: *Enterobacteriaceae* in PA samples (without the use of starter cultures) were detected in all three production batches, with an overall mean value of 3.15±1.21; counts in PB samples were considerably lower with mean values of 0.724±085. This result is in line with what was stated in previous investigations, in which it was observed that counts of *Enterobacteriaceae* and Coliforms were lower in dry-cured sausages produced with the addition of starter cultures (Cenci-Goga *et al.*, 2012; Casquete *et al.*, 2012; Mangia *et al.*, 2013; Siddi *et al.*, 2022). As regards *L. monocytogenes*, the difference between producing plants' samples was significative (P<0.01): the pathogen was only detected in PA samples and at all analysis times, even though with lower mean values at T_{120}. However, the detected values always complied with the parameters set by EU legislation. In fact, according to Regulation 2073 (European Commission, 2005) microbiological criteria, from the assessment of pH and a_w values, the product is attributable to the category of ready-to-eat foods that do not support the growth of *L. monocytogenes*, therefore the reference values, based on 5 sample rates, must be lower than 100 CFU/gr. The detection of the pathogen in the finished product confirms the presence of strains able to survive during sausage fermentation and maturation, as stated by other Authors (Thévenot *et al.*, 2005; Mureddu *et al.*, 2014; Mataragas *et al.*, 2015). In packed products, the growth of *L. monocytogenes* is scarcely affected by the anaerobic or oxygen-reduced atmosphere (Saraiva *et al.*, 2018) and regarding vacuum packaging, this preservation methodology seems to not affect the growth of *L. monocytogenes* (Nyhan *et al.*, 2018). For this reason, proper technological and hygienic procedures play a decisive role in manufacturing products in line with the Food Safety Criteria set by Regulation 2073 (European Commission, 2005). The fact that *L. monocytogenes* has not been identified in the samples from PB could be due to the use of starter cultures in the producing process: starter culture microorganisms, consisting in this case of *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Pediococcus pentosaceus*, and *Debaryomyces hansenii*, act as competitors for pathogens

potentially present in raw meat (Pedonese *et al.*, 2020; Siddi *et al.*, 2022). Also, *Lactobacillus sakei* has an antimicrobial effect due to its capacity to produce organic acids, hydrogen peroxide, and bacteriocins (Zagorec and Champomier-Vergès, 2017). Hugas *et al.* (1995) demonstrated the ability of *L. sakei* to inhibit the growth of *L. monocytogenes* in a model sausage system and dry fermented sausages. Therefore, in this study, the absence of *L. monocytogenes* in PB samples is most likely due to the microbial components of the starter culture used.

Regarding environmental samples, contamination by *Listeria spp*. and *L. monocytogenes* was observed in both producing plants. The presence at the same time of different *Listeria* species is common in meat processing environments and it is probably due to the fact that *L. monocytogenes* and other *Listeria spp*. (*e.g. L. innocua*) share the same ecological niches (harborage sites where microorganisms can survive and grow) (King *et al.*, 1989). Although with differences in the pathogen prevalence between the two producing plants, the results indicate the presence of contamination in both producing plants and in sites with different risk levels. In zone 1, an overall prevalence of 17.1 and 10.5% for *Listeria spp*. and *L. monocytogenes* respectively was found. In zone 2, no positivity was found. In zone 3, the prevalence of contamination was respectively 10.5 and 15.8% for *Listeria spp*. and *L. monocytogenes*.

The contamination of zone 1 included specifically the work-table where the bagging and binding of cured meats took place; this poses a potential direct risk of food contamination and production of final products with non-compliant values. Zone 1 sites are typically the primary verification sites for the effectiveness of the environmental pathogen control program to prevent product contamination and positivity in zone 1 is a sign of deficiencies in this regard (Tompkin *et al.*, 1999). Contamination in zone 3 interested mostly the drain floors; zone 3 surfaces are exposed during normal operating conditions and are likely to serve as transfer points; therefore, the monitoring of zone 3 sites allows to detect of microorganisms that may be moved from their harborage location to a contact surface or product (Bourdichon *et al.*, 2021). *L. monocytogenes* was detected from samples taken at three different times over three months and this could indicate the presence of persistent strains. The factors that can contribute to the persistence of *L. monocytogenes* in producing environments include the survival attributes of the strain, the existence of niches in the processing environment, and deficiencies in the application of good hygiene practices, cleaning and disinfection (Tompkin *et al.*, 1999; Simmons and Wiedmann, 2018; Spanu and Jordan, 2020). This is with particular reference to PA, where significant structural deficiencies and inadequate management of production flows were found. As regards the sensory analysis test, the acceptability level remained high until 120 days of shelf-life. However, a limit of the present study was the impossibility to evaluate PA samples. The very few modifications in pH and a_w values are helpful during storage time: an excessive decrease in pH values might cause coagulation of the muscle protein that reduces sliceability, firmness, and cohesiveness of the product, while lower a_w values might reduce the acceptability of the product (Daga *et al.*, 2007). Samples maintained pleasant flavors throughout the shelf-life, without any major lack in texture and color; samples reached the highest score of acceptability at analysis time T_{30}. In T_{120} samples, a slight decrease in intensity was observed regarding descriptors of spicy olfactory and gustatory characteristics and texture (tenderness and cohesion). Although the off-flavor attribute (atypical taste often associated with spoilage or processing of the product) was statistically different, its intensity values on all samples was on

average 1.5, which, translated into a verbal scale, means a null or very low intensity. In general, the results showed that the sensory profiles for every analysis time (T_0, T_{30}, T_{60}, T_{90}, and T_{120}) were similar for most of the descriptors, as shown in Figure 2. This result is particularly positive considering that vacuum packaging creates a favorable situation for psychotropic LAB growth, which may lead to souring, slimy exudates, and swelling of the pack due to gas production (Vercammen *et al.*, 2011). The results allow concluding that the quality and sensory acceptance of the vacuum-packed SFS was not affected until 120 days of shelf-life.

Conclusions

The results regarding the physicochemical, microbiological, and sensory characteristics of the product did not highlight any particular issues during the four months following packaging (120 days overall). Given products with specific and typical physicochemical conditions (such as pH<5.5, $a_w \leq 0.920$, sodium chloride, nitrates, and nitrites), the vacuum packaging preserved the quality of the product for at least 120 days. However, based on the results obtained in the samples of PA, the widespread contamination of *L. monocytogenes*, albeit within the limits set by the EU legislation, must emphasize the need for hygienic management of the entire technological process. The obtained data confirmed the usefulness of starter cultures and the importance of the correct management of the technological phases to ensure adequate drying and correct curing of SFSs, create effective hurdles for the product safety, and control the possible contamination by pathogenic microorganisms. Moreover, the need to program environmental sampling plans, as a useful verification tool during control, is confirmed.
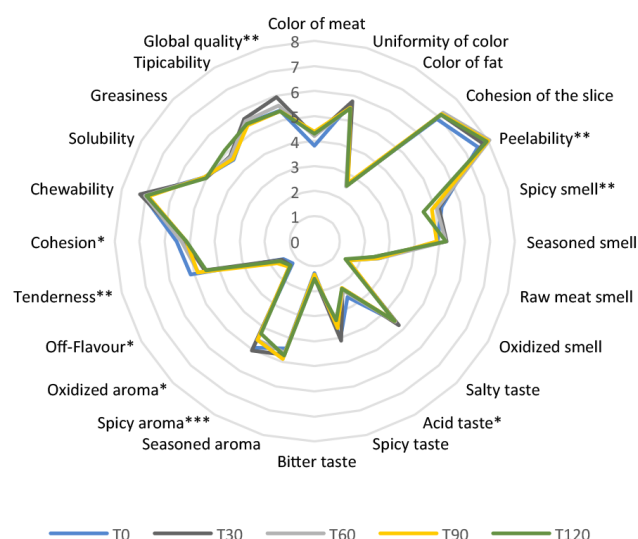

Figure 2. Graphic representation of the sensory profile of Sardinian fermented sausage. Values are the mean of sensory attributes assessed at different times of storage (T_0, T_{30}, T_{60}, T_{90}, and T_{120}). *significant (P<0.05); **significant (P<0.01); ***significant (P<0.001). T_0, end of ripening; T_{30}, after 30 days of shelf-life; T_{60}, after 60 days of shelf-life; $T1_{20}$, after 120 days of shelf-life.

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