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Evaluation of an autochthonous starter culture on the production of a traditional dry fermented sausage from Chaco (Argentina) at a small-scale facility

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ABSTRACT

The performance of a mixed starter culture, SAS-1, comprised of the autochthonous strains *Lactobacillus sakei* ACU-2 and *Staphylococcus vitulinus* ACU-10, was evaluated into the production process of a traditional dry sausage. Microbiological, physicochemical and sensory analyses were carried out to accomplish this goal. Results showed an improvement in performance through the introduction of SAS-1; adding mixed starter culture rapidly decreased pH, inhibited the growth of contaminant microorganisms and enhanced the beneficial ones, diminished TBARS, and highlighted color and aroma attributes. However, most influential organoleptic descriptors among consumer acceptance were not affected by the addition of the starter. This starter culture would represent a valuable tool to improve the homogeneity of artisanal manufacture of this traditional food.

1. Introduction

Leading factors determining fermented sausage characteristics and quality are the choice of starter cultures, the ingredients, and the environmental conditions during fermentation and ripening. The selection and interactions of raw materials, microbial strains, technology and ingredients are crucial for the sensory properties, volatile profile, safety, and shelf-life of these products (Talon & Leroy, 2011; Toldrá, 2006).

Artisanal and traditional products have recently grown in popularity, with a return to food consumption with local identity (Leroy, Scholliers, & Amilien, 2015) and its production has been proposed as a development strategy for regional economies of emerging countries (Holzapfel, 2002). Having a great acceptability among local consumers, traditional fermented sausages from Chaco (northeastern Argentina) are manufactured under nonstandard conditions by means of artisan techniques. Their major drawback is the great variability found between batches (Palavecino Prpich, Castro, Cayré, Garro, & Vignolo, 2015a).

According to Ravyts, De Vuyst, and Leroy (2012), the introduction of a native starter culture in the elaboration process of regional dry sausage will constitute a tool for standardizing the final product. The addition of this component could decrease variability in the final product while maintaining typical flavors. Hence, a native starter culture (SAS-1), comprised by the strains *Lactobacillus sakei* ACU-2 and *Staphylococcus vitulinus* ACU-10, was designed through the selection of technologically evaluated autochthonous strains (Palavecino Prpich, Castro, Cayré, Garro, & Vignolo, 2015b). This mixed culture enhanced safety and quality whereas keeping typical sensory attributes of regional dry fermented sausages. Nevertheless, its behavior in different production batches has still not been checked. Consequently, this study was conducted to evaluate the performance of SAS-1 *in situ* during the manufacture of dry fermented sausages at a local small-scale facility (SSF).

2. Materials and methods

2.1. Autochthonous starter culture

Lactobacillus sakei ACU-2 and *S. vitulinus* ACU-10 composed the designed starter culture (Palavecino Prpich et al., 2015a, 2015b). These strains are deposited in the strain repository of Laboratorio de Microbiología de Alimentos (Universidad Nacional del Chaco Austral, Argentina). Lactobacilli are kept in MRS broth (de Man, Rogosa, Sharpe) supplemented with 20% *v*/*v* glycerol, while *Staphylococci* are kept in soy

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triptycase + 0.6% *w/v* yeast extract (TSBYE) added with 20% *v/v* glycerol; both bacterial species are maintained frozen at −80 °C.

Mixed starter culture (SAS-1) was prepared according to Palavecino Prpich et al. (2015b) and was taken to the SSF for direct inoculation of the meat dough.

2.2. Dry fermented sausages manufacture and sampling

Dry fermented sausages were manufactured at a local SSF. The traditional sausage recipe was: pork, beef, and bacon in an average proportion of 60:30:10 thoroughly mixed together with salt (~2%), milk powder (~1%), sucrose (~2%), spices (~1%), and nitrite/nitrate salt (0.07%); spices include chopped garlic, red and black pepper. The meat dough obtained after mixing was used to fill natural casings (sheep pork).Three different production batches −50 kg each; manufactured separately- were inoculated with SAS-1 for evaluation.

In each instance, ingredients were thoroughly mixed together and meat dough was divided into two batches; one batch was added with the SAS-1 and the other was kept as a control system. SAS-1 was incorporated once the meat dough had been finished, being completely homogenized before casings were stuffed. Final bacterial concentration of each strain from the starter reached a value of ~10^6 colony forming units per gram (cfu/g). After inoculation and stuffing, the unfermented products were placed in a ripening chamber at the SSF. During the fermentation period (7 days), temperature oscillated between 18 and 22 °C and, along the ripening period (8 days), temperature was kept at 15 °C. Relative humidity (RH) oscillated between 80 and 95%. At 0, 2, 7 and 15 days, two samples were withdrawn from each batch to perform microbiological analyses and to register pH values. Final product consists of an average sausage of 4–5 cm diameter, ~15 cm long, and 150 g weight.

2.3. Microbiological analyses

Ten grams of each sample were aseptically taken and were transferred to a stomacher bag with 90 ml of sterile peptone water to be homogenized. Appropriate dilutions of the homogenate were inoculated into several growth medium to determine viable counts: lactic acid bacteria (LAB) in MRS agar (30 °C, 72 h); *Micrococcaceae* in mannitol salt agar (30 °C, 72 h); yeasts and molds in Chloramphenicol Sabouraud agar (25 °C, 5 days); *Enterobacteriaceae* in Glucose Red Violet Bilis agar (37 °C, 24 h); *Staphylococcus aureus* in Baird Parker agar with egg yolk emulsion and potassium telurite (37 °C, 48 h). Final products were also assayed for: *Escherichia coli* count (MPN/g) following the ICMSF method (1987); sulphite-reducing anaerobes (cfu/g) according to ISO 15213, 2003; absence of *E. coli* O157:H7/NM by the USDA-FSIS method (2010); absence of *Salmonella* spp. by the BAM-FDA method (2007); absence of *Listeria monocytogenenes* according to ISO: 11,290–1/A1 (2004).

2.4. Physicochemical analyses

Moisture, ash and NaCl content were analyzed in the final product according to the AOAC (1995), while pH values were determined at each sampling time with the aim of a pricking probe attached to a pH meter inserted directly into the meat dough (Testo, Germany).

Lipid oxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) test (Sinnhuber & Yu, 1977). TBARS values were expressed as mg malonaldehyde per kilogram of sample (mg MDA/kg).

Free fatty acids (FFA) composition was determined in the fat fraction of dry sausage samples through gas chromatography of their corresponding methyl esters. Detailed methodology can be found in Palavecino Prpich et al. (2015b). FFA concentration was expressed as mg fatty acid per gram of fat (mg FFA/g).

Color measurements were conducted in dry sausages cuts with the aim of an Evolution 600UV–Vis spectrophotometer (Thermo Scientific, equipped with integrator sphere and VISION Lite color Calc. Software, Germany), illuminant D65 and a 2° standard observer, in a room with fluorescent lighting and after standardization of the instrument with respect to the white calibration plate. Two sausages from each treatment were crushed and 5 measurements were performed on each paste.

2.5. Sensory analysis

A quantitative descriptive analysis (QDA) was conducted by an eight-member sensory panel with previous experience in judging fermented sausages. Full ripened dry fermented sausages manufactured with the SAS-1 and those used as control systems were evaluated. Two 30-min-long training sessions were held to define, discuss and clarify each attribute to be evaluated. The overall assessment was performed following Baka, Papavergou, Pragalaki, Bloukas, and Kotzekidou (2011) guidelines. The evaluated attributes were: i) external appearance (descriptors: firmness, cohesiveness and easy peeling capability of the case from the sausage); ii) color (intensity and uniformity); iii) aroma (intensity, cured smell and rancidity); iv) taste (salty, acid and sour); and v) texture (chewing and hardening). The evaluation was conducted in 3 sessions, where each assessor evaluated the samples by duplicate. The answer for every descriptor was determined as the mean value of panelists' answers.

2.6. Statistical analyses

Randomized block design — with three blocks (each block corresponding to an independent batch processing) — was applied, using ANOVA to analyze microbiological and chemical results. For the QDA, a three-factor ANOVA for each descriptor was carried out on the trained assessor scores, considering sample, session, assessor and their interaction as sources of variation. The level of significance was set at $P < 0.05$ for all analyses. All the tests were performed with the software Statgraphics Plus 4.0 (Manugistics Inc., USA).

3. Results and discussion

The behavior of the microbiota and the pH variation observed in the three assessed production lots (data not shown) were similar to the results obtained in the first assessment of SAS-1 (Palavecino Prpich et al., 2015b), demonstrating the ability of this culture to reproduce technological behavior. In the inoculated systems, LAB rapidly colonized the meat matrix during the first 48 h, giving a deep decrease of pH values and diminishing the number of *Enterobacteriaceae*. Thereafter, enterobacteria counts in these systems were less than 1 log cfu/g, though they were detected in the control systems. *Micrococcaceae* counts revealed no significant differences between inoculated and control systems in any of the sampled stages. *Staphylococcus aureus* was not detected in any of the samples taken during the productive process.

3.1. Evaluation of the final product

Microbiological assessment of the products from each of the three batches evidenced that these products fulfill the requirements stated by the national legislation (Código Alimentario Argentino "Argentine Food Code", 2015), being safe for consumption.

The results of the determinations performed on the products are shown in Table 1. LAB mean counts were significantly higher in inoculated than in control batches. Molds and yeasts exhibited significantly less counts in inoculated than in control batches. *Enterobacteriaceae* were not detected in inoculated systems, albeit found in control systems, which indicated a good performance of SAS-1 on safety and quality achievement.

Regarding physicochemical parameters inherent to product quality, namely moisture, ash, and NaCl, no significant differences were detected

Table 1
Several parameters analyzed in dry fermented sausages at the end of the ripening period (final product).

Parameters	Inoculated batches		Control batches		*P*-value
	Mean[a]	SD[b]	Mean	SD	
Lactic bacteria (log cfu/g)	8.22	0.19	8.02	0.18	0.0029
Micrococos (log cfu/g)	6.42	0.72	6.54	0.66	0.0513
Molds and yeasts (log cfu/g)	2.63	0.34	3.20	0.44	<0.0001
Moisture %	34.3	2.81	33.6	2.64	0.5532
Ash %	5.76	0.30	5.88	0.53	0.4578
NaCl %	4.47	0.09	4.37	0.25	0.5045
pH	5.00	0.10	5.18	0.12	0.0001
Free amino acids (mM)	4.00	0.81	2.46	0.74	0.0001
TBARS (mg MDA/kg)	1.02	0.41	1.81	0.48	<0.0001
Color L*	42.2	1.91	42.85	1.75	0.2122
Color a*	12.65	1.04	12.00	0.91	0.0216
Color b*	6.38	0.48	6.14	0.60	0.2632
Free fatty acids (mg/g)					
Saturated					
(14:0), myristic	12.19	2.18	12.69	1.95	0.0250
(16:0), palmitic	178.88	29.74	184.36	31.03	0.0428
(17:0), heptadecanoic	3.17	0.62	2.98	0.42	0.1729
(18:0), stearic	92.61	20.01	101.47	19.00	<0.0001
(20:0), behenic	5.41	0.94	5.05	0.68	0.0159
(22:0), decosanoic	8.55	1.16	8.53	1.43	0.8376
Monounsaturated					
(16:1), palmitoleic	15.55	2.48	14.84	2.32	0.0001
(17:1)c, heptadecanoic	2.44	0.47	2.04	0.38	0.0001
(18:1)c, n9 oleic	277.40	53.31	259.98	45.44	0.0023
Polyunsaturated					
α(18:3) n3, heneicosanoic	27.84	5.20	31.11	7.67	0.0188

[a] Mean value of three batches.
[b] Standard deviation.

in inoculated and control batches. Nevertheless, these parameters depend on the process conditions rather than on the microflora.

Parameters related to the metabolism of SAS-1 in the batches, i.e. pH, free amino acids, TBARS and color development (mostly redness), showed significant differences between inoculated and control systems. Furthermore, concentration of free amino acids was higher, TBARS was lower, and redness values were higher in inoculated batches. The nitrate-reductase activity of *S. vitulinus* ACU-10 could be accounted for this intensification in redness, which was favored by the rapid reduction of pH. These results confirm the acidogenic, proteolytic, antioxidant and nitrate-reductase activity of SAS-1 already evaluated in previous work (Palavecino Prpich et al., 2015b), and show that these expected features are kept in different production batches.

The lipidic profile of dry fermented sausage manufactured with the addition of SAS-1 and the non-inoculated (control) samples is reported in Table 1. In general, figures were in the same range to the ones reported previously (Palavecino Prpich et al., 2015b). Comparing between inoculated and control batches, there were significant differences in almost all the FFA, with the exception of heptadecanoic (17:0) and decosanoic acids. Moreover, saturated FFA were higher in the control, while the monounsaturated FFA were higher in the inoculated batches. Besides, the only polyunsaturated (PUFA) FFA analyzed in this study, i.e. heneicosanoic acid, was higher in the control batch than the inoculated one. Comparing data from other authors would be ambiguous since FFA were expressed in several units; nevertheless, data from this study are in keeping with Talon et al. (2008) and Casaburi et al. (2008) who found that PUFA were higher in control batches than in inoculated ones.

3.2. Sensory attributes

Dry sausages produced with SAS-1 and their controls were subjected to a quantitative descriptive analysis (QDA); results from the three production batches are shown in Fig. 1. Significant differences were detected in three descriptors from color and aroma attributes. Inoculated systems showed a higher color intensity ($P < 0.0001$) and color uniformity ($P = 0.0115$) and a higher aroma intensity ($P = 0.0179$). The enhancement in the color attributes of inoculated dry sausages can be endorsed to the nitrate-reductase activity of SAS-1. Taste and external appearance attributes showed no differences compared to control systems, confirming that the most influential organoleptic descriptors among consumer acceptance were not affected by the addition of the starter. The addition of autochthonous starter cultures showed a positive sensorial effect on some traditional fermented sausages (Casquete et al., 2011; Baka et al., 2011); nonetheless, flavor defects had also been described (Bedia, Méndez, & Bañón, 2011).

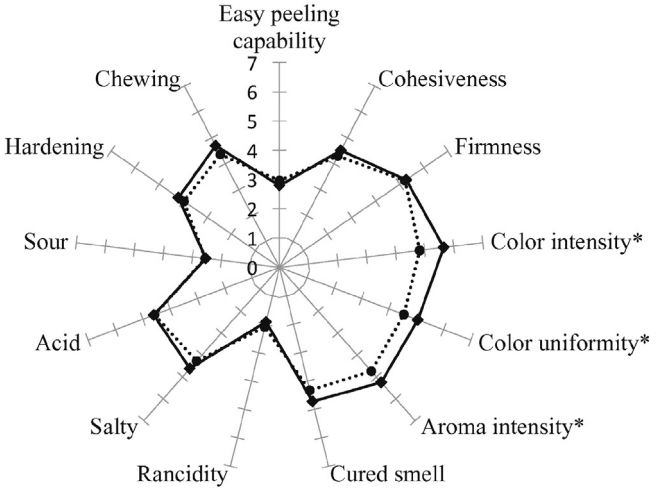

Fig. 1. Spider plot of the sensory profiles by assessor panels for: inoculated (♦-♦), and non-inoculated (●…●) samples. *Significantly different ($P < 0.05$).

4. Conclusions

The *in situ* evaluation of the starter culture SAS-1 during the manufacture of a dry fermented sausage at a local SSF showed a better performance of the inoculated compared to the non-inoculated products. Had not a single modification in the production process been introduced, the starter replied its performance along different batches. Consequently, this autochthonous culture — together with the standardization of raw materials and environmental conditions — would represent a valuable tool to improve the homogeneity of artisanal manufacture of this traditional food.

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