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Role of commercial starter cultures on microbiological, physicochemical characteristics, volatile compounds and sensory properties of dry-cured foal sausage

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ABSTRACT

Objective: To assess the effect of three commercial starter cultures on microbial counts, physicochemical changes, volatile profile and sensory characteristics of dry-cured foal sausage.
Methods: Microbial counts (lactic acid bacteria, Enterobacteriaceae, total viable counts and yeast), proximate parameters (moisture, fat and protein), colour analysis, texture analysis (texture profile analysis test), volatile compounds (solid-phase microextraction-gas chromatography-mass spectrometer technique) and sensory analysis were evaluated in the dry-cured foal sausages using the standard food analysis techniques.
Results: The results revealed that the use of starter cultures increased the number of lactic acid bacteria and total viable counts, while completely reduced Enterobacteriaceae count. Started sausages presented the lowest value of pH, while CX and FL batches had the highest protein amount. In contrast, the use of starter cultures did not affect the other physicochemical parameters. According to volatile profile, there were no differences between batches in total volatile compounds, however, control batch presented the highest amount of aldehydes, derived from lipid oxidation. The sensory analysis showed low differences. Control batch presented higher flavour intensity and lower acid taste score and black pepper odour than inoculated batches.
Conclusions: As a general conclusion, the use of starter cultures contributed to improve the hygienic quality with low impact in physicochemical and sensory properties.

1. Introduction

The acceptance of horsemeat as a food for humans has changed due to changes in attitude from aversion to qualified approval of this meat[1]. From the nutritional point of view, horsemeat is excellent, since it is low in fat, rich in iron and it has a favourable dietetic fatty acid profile with a high content of unsaturated fatty acids and vitamin B[1]. "Salchichón" is a Spanish fermented dry-cured sausage. It has been reported that this kind of fermented dry sausage could contain, during processing and in the final product, some of the pathogenic bacteria which is often associated with meat products[2]. For this reason, to guarantee the safety of consumers and also the quality, maintaining the typical "salchichón" characteristics as to colour and flavour, it is very important to use starter cultures. The final product is the result of a complex microbiological activity, which consists of a lactic fermentation and several biochemical reactions characterizing more or less prolonged ripening period[3].

Poteolysis and lipolysis reactions are responsible for the most important biochemical changes occuring during the ripening of dry fermented sausages[4]. Both reactions are catalysed by either endogenous enzymes present in the meat tissues or by those of microbial origin from added starter cultures. All volatile compounds generated during the process of a dry fermented sausage are of great importance to the aromatic character of the final product.

Given what has been said above, the aim of this work was to study the effect of different commercial starter cultures on microbial counts, chemical composition, colour and textural parameters, production of volatile compounds and on sensory characteritics of foal "salchichón".

2. Materials and methods

2.1. Sausage production and sampling procedures

Four different batches of foal sausage were manufactured

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according to traditional techniques, one of them without starter cultures and the other three batches with addition of different commercial starter cultures (Cargill and Sacco) in a proportion defined by the manufacturer in each case. The batches were named as follows: (i) CNT batch, control without starter culture; (ii) CX batch, with CXP (Cargill) [*Staphylococcus carnosus* + *Staphylococcus xylosus* (*S. xylosus*) + *Pediococcus pentosaceus*]; (iii) FL batch, with flavor start P406 (Cargill) [*Debaryomyces hansenii* (*D. hansenii*) + *S. xylosus*]; (iv) TH batch, with lyocarni THM-17 (Sacco) (*Pediococcus pentosaceus* + *S. xylosus*). Sausage manufacture was done at two different times. The four batches mentioned before were manufactured with the same ingredients, formulation and technology in May and June 2014.

Foal sausage formulation includes foal lean meat (85%), pork back fat (15%), NaCl (25.0 g/kg), lactose (10.7 g/kg), dextrin (9.3 g/kg), sodium caseinate (20 g/kg), glucose (7 g/kg), black pepper (1.5 g/kg), white pepper (1.0 g/kg), sodium ascorbate (0.5 g/kg), sodium nitrite (0.15 g/kg) and potassium nitrate (0.15 g/kg). The foal lean meat and the pork back fat were ground through 12- and 8-mm diameter mincing plates, respectively, and in a vacuum (Industrial Fuerpla, Mod. AO-85, Spain) mixed together with the other ingredients for 3 min. The mix was maintained at 4 ℃ for 24 h and then stuffed into natural casings with a diameter of 60 mm and a length of 40 cm. The sausages were fermented for 2 days at 20 ℃ and 80%–85% of relative humidity and then transferred into a drying-ripening chamber where they were kept for 51 more days at 12 ℃ and 75%–80% relative humidity. Samples were taken at the end of the ripening for subsequent analysis.

2.2. Microbiological analysis

Microbiological analysis was carried out following the procedure described by Lorenzo *et al.*[5]. After incubation, plates with 30–300 colonies were counted. The microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

2.3. Chemical composition and pH values

Moisture[6], fat[7] and protein[8] were determined according to standards recommended by International Organization for Standardization. The pH of samples was measured using a digital pH meter (model 710 A+, Thermo Orion, Cambridgeshire, UK) equipped with a penetration probe.

2.4. Colour analysis

Colour parameters were measured using a portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) with pulsed xenon arc lamp filtered to illuminant D65 lighting conditions, 0° viewing angle geometry and 8-mm aperture size, to estimate meat colour in the CIE L*a*b* space: lightness, redness, yellowness. The colour was measured in three different points of each sample.

2.5. Texture analysis

Texture profile analysis was determined on "salchichón" slices of 1 cm × 1 cm × 2 cm (height, width, length) using a texture analyzer (TA.XTplus, stable micro systems, Godalming, UK). Textural parameters were measured by a 60% compression with a compression probe of 19.85 cm^2 of surface contact. Force-time curves were recorded at a crosshead speed of 3.33 mm/s. Hardness, springiness, cohesiveness and chewiness values were obtained using the software TEE32 Exponent 4.0.12 (stable micro systems, Godalming, UK).

2.6. Volatile compound profile

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME). A SPME device (Supelco, Bellefonte, USA) containing a fused silica fibre (10 mm in length) coated with a 50/30 layer of divinylbenzene/ carboxen/ polydimethylsiloxane was used. Headspace SPME extraction (from 1 g of sample) and chromatography were carried out under the conditions described by Gómez and Lorenzo[9]. The results were expressed as AU (area units) × 10^6/g of dry matter.

2.7. Sensory analysis

Sensory analysis was conducted with ten panellists selected from the Meat Technology Centre of Galicia. The panellists were trained for 2 weeks according to the attributes and scale recommended by International Organization for Standardization[10]. Thirteen sensory traits of dry-cured foal sausages, grouped as appearance (fat distribution and colour intensity), odour (odour intensity, black pepper odour and mould odour), taste (acid taste and saltiness), texture (hardness, juiciness and pastosity) and flavour (flavour intensity, cured flavour and rancid flavour), were assessed.

The casings were removed and the sausages were cut into slices approximately 4 mm thick and served at room temperature on white plastic dishes. The samples were individually labelled with three-digit random numbers. The intensity of each attribute was expressed on an unstructured scale from 0 (sensation not perceived) to 9 (the maximum sensation). The samples were evaluated by panellists in two sessions (four samples per session). During sensory evaluation, the panellists were situated in separate cubicles illuminated with red light. Water was used to clean the palates and remove residual flavours at the beginning of the session and in between samples.

2.8. Statistical analysis

A total of 80 sausages (ten sausages for each batch × four batches × two replicates) were analyzed for different parameters. The effect of different commercial starter cultures on microbial counts, free amino acids, biogenic amines and free fatty acids content was examined using a mixed-model ANOVA, where these parameters were set as dependent variables, commercial starter cultures as fixed effect, and replicate as random effect. The pairwise differences between least-square means were evaluated by Duncan's method. Differences were considered significant if $P < 0.05$. The values were given in terms of mean values and SEM. All statistical analysis was performed using international business machine SPSS statistics 19 software[11].

3. Results

3.1. Microbial counts

The effect of starter cultures on the microbial counts was shown in

Figure 1. The highest value in the lactic acid bacteria (LAB) counts was reached with FL commercial starter culture (8.21 log CFU/g). It is interesting to show that LAB counts at the end of ripening, after 53 days, were found significantly ($P < 0.001$) higher in inoculated batches than in control.

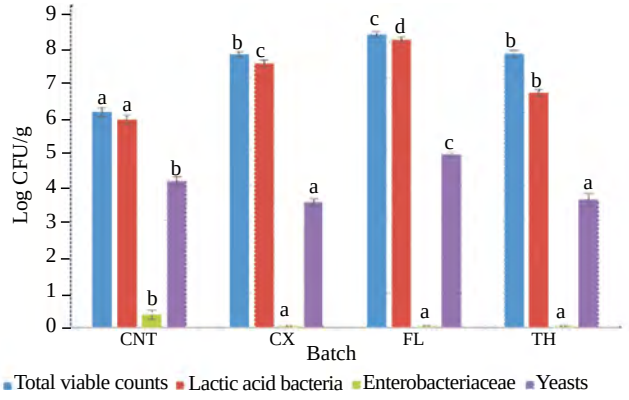

Figure 1. Effect of commercial starter cultures on microbial counts of dry-cured foal sausage (mean ± SD of twenty replicates).
[a–d]: Values in the same row (corresponding to the same parameter) were not followed by a common letter differ significantly ($P < 0.05$).

Regarding Enterobacteriaceae count, all batches inoculated showed zero count at the end of ripening, unlike control batch. After ripening of any kind of sausage, the number of Enterobacteriaceae decreased if a starter culture was added during its manufacture.

On the other hand, batch inoculated with FL starter culture showed the highest values of LAB and total viable count (8.21 and 8.06 log CFU/g, respectively). Moreover, inoculated batches showed to achieve significantly ($P < 0.001$) higher levels of total viable count and LAB at the end of ripening than control. In addition, at the end of ripening, batch inoculated with FL commercial starter culture had higher ($P < 0.001$) values of yeast than the other batches (4.86 *vs.* 4.10, 3.51 and 3.59 log CFU/g for control, CX and TH batches, respectively). Batches inoculated with CX and TH commercial starter cultures showed the lowest values, and they were not significant differences between them. These two batches presented counts significantly ($P < 0.001$) lower than control, which showed intermediate values (4.10 log CFU/g) regarding inoculated.

3.2. Chemical composition, pH, colour properties and texture profile analysis

The effect of starter cultures on pH, moisture, fat and protein values was shown in Table 1. The highest pH value at the end of ripening was reached in control batch (5.83), without starter culture, and the lower value in batch inoculated with FL commercial starter culture (5.63). Control batch showed a pH value significantly ($P < 0.001$) higher than those inoculated by CX and FL starter cultures, but not by TH. There were no significant differences in moisture (between 30.23% and 31.81%) and fat (between 15.69% and 17.20%) values in any batch. Nevertheless, significant ($P < 0.01$) differences between batches have been found in protein content, where batches inoculated with CX (37.17%) and FL (36.94%) starter cultures reached the highest values, and batch inoculated with TH (35.67%) starter culture and control (35.42%) had the lowest values.

Table 1
Effect of commercial starter cultures on chemical composition and colour parameters of dry-cured foal sausage (mean of twenty replicates).

	Batch				SEM	Significance
	CNT	CX	FL	TH		
pH	5.83[c]	5.72[ab]	5.63[a]	5.76[bc]	0.017	***
Moisture (%)	31.81	30.51	31.66	30.23	0.286	ns
Protein (%)	35.42[a]	37.17[b]	36.94[b]	35.67[a]	0.222	**
Fat (%)	15.71	15.69	15.89	17.20	0.230	ns
Lightness	30.42	29.33	30.10	29.76	0.271	ns
Redness	8.39	8.48	8.85	8.76	0.118	ns
Yellowness	4.73	4.19	4.33	4.41	0.123	ns

[a–c]: Values in the same row (corresponding to the same parameter) were not followed by a common letter differ significantly ($P < 0.05$); **: ($P < 0.01$); ***: ($P < 0.001$); ns: Not significant.

No significant difference was found in colour parameters values between the different batches (Table 1). The lightness values were in all batches about 30, redness values between 8.39 and 8.85, and yellowness values between 4.19 and 4.73.

The effect of starter cultures on textural parameters was shown in Table 2. There were not significant differences between batches in any of the textural parameters studied. Although, it can be observed a higher value in hardness, gumminess and chewiness in batch inoculated with FL starter culture. Moreover, control batch showed a lower value with respect to inoculated batches in the same parameters, but the difference was not so marked as in case of batch inoculated with FL starter culture regarding the rest of batches.

Table 2
Effect of commercial starter cultures on textural parameters of dry-cured foal sausage (mean of twenty replicates).

Texture profile analysis test	Batch				SEM	Significance
	CNT	CX	FL	TH		
Hardness (N)	326.15	346.16	378.44	351.26	7.289	ns
Springiness (mm)	0.55	0.55	0.57	0.56	0.003	ns
Cohesiveness	0.39	0.38	0.38	0.38	0.003	ns
Gumminess (N)	127.66	131.84	143.47	136.58	3.540	ns
Chewiness (N•mm)	70.72	72.17	81.73	76.63	2.087	ns

ns: Not significant.

3.3. Volatile compound analysis

Sixty-five volatile compounds were identified at the end of the manufacturing process of the dry-fermented "salchichón" by the SPME-gas chromatography-mass spectrometer technique. Nevertheless, aroma perception in meat products depended not only on the concentration and odour thresholds of volatile compounds, but also on their interactions with other food components and among volatile compounds. The compounds were grouped by chemical and their linear retention index (Table 3), comprising six acids, six alcohols, fourteen aldehydes, thirty-five hydrocarbons and four ketones. Statistical analysis showed that total volatile compounds content was no affected by starter culture incorporation (2118.99, 2210.14, 2212.60 and 2012.95 AU × 10^6/g dry matter for CNT, CX, FL and TH batches, respectively).

Regarding hydrocarbons, they were the most abundant chemical group at the end of the ripening process in dry-fermented "salchichón". They represent between 62.3% and 69.7% of total volatile compounds in all batches. This fact was related with the high amounts of volatile compounds from the spices representing 58% of total volatile compounds in control samples and between 64% and 68% in started sausages.

Table 3
Effect of commercial starter cultures on volatile compounds (AU × 10^6/g dry matter) of dry-cured foal sausage (mean of twenty replicates).

Volatile compounds			Batch				SEM	Significance
	LRI	R	CNT	CX	FL	TH		
Acetic acid	665	m, lri	9.59^{a}	26.68^{c}	39.49^{d}	22.07^{ab}	1.419	***
Butanoic acid	898	m, lri	14.53^{a}	16.61^{bc}	18.17^{c}	14.97^{ab}	0.340	***
Butanoic acid, 3-methyl	971	m, lri	1.25^{a}	1.57^{ab}	2.03^{c}	1.85^{bc}	0.071	**
Hexanoic acid	1 136	m, lri	25.02	26.76	23.79	22.58	0.554	ns
Octanoic acid	1 335	m	3.59^{a}	4.79^{b}	5.00^{b}	4.47^{ab}	0.184	*
Nonanoic acid	1 426	m	0.93	1.19	0.95	0.74	0.063	ns
Total acids			54.26^{a}	77.25^{c}	86.55^{d}	64.93^{b}	1.838	***
1-Butanol, 3-methyl	718	m, lri	0.72^{b}	0.48^{a}	0.50^{a}	0.37^{a}	0.040	*
1-Pentanol	719	m, lri	27.88^{c}	20.78^{b}	16.82^{a}	17.18^{a}	0.761	***
1-Hexanol	956	m, lri	10.34^{c}	5.89^{a}	8.32^{b}	5.13^{a}	0.434	***
1-Octen-3-ol	1 091	m, lri	46.66	45.25	44.00	40.29	1.376	ns
3,5-Octadien-2-ol	1 170	m, lri	4.16^{b}	3.11^{a}	3.47^{ab}	3.47^{ab}	0.130	*
Benzyl alcohol	1 196	m, lri	28.83^{b}	26.69^{ab}	26.88^{ab}	24.89^{a}	0.453	*
Total alcohols			116.76^{c}	102.86^{b}	100.09^{ab}	90.85^{a}	2.373	***
Pentanal	638	m, lri, s	32.25^{b}	18.88^{a}	15.82^{a}	15.08^{a}	1.315	***
Hexanal	830	m, lri, s	532.19^{b}	377.52^{a}	346.05^{a}	349.82^{a}	11.176	***
2-Hexenal	934	m, lri	3.11	3.22	3.14	2.70	0.076	ns
Heptanal	983	m, lri, s	17.99^{b}	13.54^{a}	12.94^{a}	14.39^{a}	0.338	***
2-Heptenal	1 073	m, lri	25.80^{b}	22.63^{a}	21.10^{a}	22.89^{ab}	0.550	*
Benzaldehyde	1 084	m, lri	27.94	32.34	31.97	32.49	0.747	ns
2,4-Heptadienal	1 148	m	8.13^{a}	7.39^{a}	10.25^{b}	8.30^{a}	0.301	**
Benzeneacetaldehyde	1 188	m, lri	2.77^{a}	2.51^{a}	2.56^{a}	9.84^{b}	0.339	***
2-Nonenal	1 303	m, lri	4.02^{c}	3.00^{ab}	3.77^{bc}	2.89^{a}	0.164	*
Benzaldehyde, 3-ethyl	1 314	m, lri	2.02^{ab}	2.42^{b}	1.73^{a}	1.60^{a}	0.077	**
Benzaldehyde, 4-ethyl	1 314	m, lri	1.90	1.86	1.83	2.11	0.075	ns
2,4-Nonadienal	1 366	m, lri	2.48	2.58	2.56	2.23	0.056	ns
2,4-Decadienal	1 463	m, lri	1.74^{c}	1.45^{ab}	1.20^{a}	1.56^{bc}	0.051	***
Piperonal	1 501	m, lri	0.49^{a}	0.73^{b}	0.57^{ab}	0.49^{a}	0.032	*
Total aldehydes			667.57^{b}	487.47^{a}	454.44^{a}	465.04^{a}	13.165	***
Heptane	700	m, lri, s	4.49^{c}	3.92^{bc}	1.42^{a}	3.25^{b}	0.201	***
Toluene	744	m	1.82	2.03	1.58	1.85	0.067	ns
Octane	800	m, lri, s	37.83^{c}	28.45^{b}	18.88^{a}	29.65^{b}	0.983	***
Heptane, 3-ethyl	885	m	2.60	2.03	2.83	2.4	0.113	ns
Undecane, 6-methyl	885	m	0.00^{a}	1.52^{b}	2.07^{c}	1.93^{c}	0.183	***
p-Xylene	915	m, lri	3.55	3.75	3.92	3.52	0.102	ns
α-Thujene	976	m, lri	6.94	8.45	8.54	7.55	0.256	ns
1S-α-Pinene	987	m, lri	90.44	100.80	91.57	89.53	3.291	ns
1R-α-Pinene	987	m, lri	86.32	94.11	101.17	89.67	2.861	ns
Pentane, 3-ethyl-2-methyl	996	m	4.34	3.46	4.07	3.55	0.200	ns
3-Ethyl-3-methylheptane	1 000	m	2.46	2.49	2.48	2.61	0.095	ns
Decane	1 000	m, lri, s	3.75^{a}	2.97^{a}	3.71^{a}	4.61^{b}	0.148	**
Camphene	1 012	m	3.87	4.04	4.33	4.24	0.155	ns
Nonane, 3-methyl	1 025	m, lri	4.50	4.15	4.90	4.09	0.138	ns
β-Thujene	1 048	m	22.93^{a}	45.16^{c}	32.40^{b}	31.36^{b}	2.018	***
β-Pinene	1 050	m, lri	162.82^{a}	192.24^{ab}	198.34^{b}	170.17^{ab}	5.324	*
L-β-Pinene	1 050	m, lri	149.61	157.57	191.06	157.26	7.807	ns
Heptane, 2,2,4,6,6-pentamethyl	1 055	m, lri	17.24^{b}	11.24^{a}	12.82^{a}	11.73^{a}	0.519	***
β-Phellandrene	1 063	m, lri	44.97^{b}	45.80^{b}	36.03^{b}	22.96^{a}	2.770	**
α-Phellandrene	1 084	m, lri	8.35^{a}	44.04^{d}	35.06^{c}	26.68^{b}	2.387	***
2-Carene	1 088	m	303.43^{a}	352.96^{ab}	389.57^{b}	328.62^{ab}	10.966	*
D-Limonene	1 113	m, lri	236.35^{a}	290.57^{b}	287.74^{b}	245.13^{ab}	8.510	*
o-Cymene	1 118	m, lri	63.39^{a}	67.04^{a}	79.80^{b}	65.74^{a}	1.775	**
1,3-Hexadiene, 3-ethyl-2-methyl	1 158	m	7.63^{b}	6.43^{a}	6.18^{a}	5.93^{a}	0.188	*
4-Carene	1 179	m	4.08^{a}	6.86^{b}	4.46^{a}	4.64^{a}	0.305	**
Dodecane	1 200	m, lri, s	2.62	2.60	2.66	2.71	0.102	ns
5-Undecene, 9-methyl	1 203	m, lri	12.43	9.88	11.09	13.28	0.550	ns
Undecane, 3-methyl	1 254	m	3.13	2.11	2.74	2.42	0.138	ns
Undecane, 3-methylene	1 273	m	1.69	1.86	1.79	2.12	0.092	ns
Tridecane	1 300	m, lri, s	1.44	1.22	1.13	1.24	0.065	ns
Copaene	1 450	m, lri	3.46	3.90	4.50	4.65	0.186	ns
α-Cubebene	1 479	m	3.40	3.78	4.29	5.71	0.300	ns

(Continued on next page)

Table 3 *(continued)*

Volatile compounds	Batch						SEM	Significance
	LRI	R	CNT	CX	FL	TH		
Caryophyllene	1531	m	37.40	33.15	34.87	34.63	1.107	ns
α-Caryophyllene	1562	m	1.31	1.17	1.18	1.08	0.056	ns
Eicosane	2000	m, lri, s	0.84	1.01	0.56	0.66	0.090	ns
Total hydrocarbons		1320.91[a]	1517.85[b]	1543.61[b]	1327.64[a]	28.766	**	
Acetoin	724	m, lri	0.00[a]	4.39[b]	6.14[c]	7.16[c]	0.447	***
2-Heptanone	974	m, lri	6.12[c]	5.04[b]	3.92[a]	3.40[a]	0.205	***
3-Octen-2-one	1170	m	4.06	3.13	3.48	3.56	0.122	ns
3,5-Octadien-2-one	1210	m	5.50[a]	8.10[b]	9.18[b]	7.94[b]	0.306	***
Total ketones			15.14[a]	20.33[b]	21.91[b]	22.41[b]	0.639	***
Total compounds			2118.99	2210.14	2212.60	2012.95	33.883	ns

[a–c]: Values in the same row (corresponding to the same parameter) were not followed by a common letter differ significantly ($P < 0.05$); [*]: ($P < 0.05$); [**]: ($P < 0.01$); [***]: ($P < 0.001$); ns: Not significant; AU: area units resulting of counting the total ion chromatogram for each compound; LRI: linear retention index calculated for DB-624 capillary column (J&W scientific: 30 m × 0.25 mm id, 1.4 mm film thickness) installed on a gas chromatograph equipped with a mass selective detector; R: Reliability of identification; lri: volatiles identified by comparing their LRI with those reported in the literature[25-27]; m: mass spectrum agreed with mass database (NIST05); s: mass spectrum and retention time identical with an authentic standard.

Among the pepper terpenes identified, in sausages the most abundant were 2-carene followed by limonene, α and β pinene and o-cymene.

The values of total hydrocarbons showed significant differences ($P < 0.01$) between batches. Sausages from CNT and TH batches (around 1300 AU × 10^6/g dry matter) presented lower amounts of hydrocarbons than sausages from CX and FL (around 1500 AU × 10^6/g dry matter). These differences were due to the CX and FL had higher amounts of terpenes than the other two batches.

On the other hand, aldehydes represented between 20.0% and 31.5% of total compounds. Among aldehydes, the most important was hexanal (around 75% of total aldehydes in all batches), following by benzaldehyde and 2-heptenal. In this case, sausages from CNT batch presented the highest values of total aldehydes (667.57 AU × 10^6/g dry matter), while inoculated sausages presented the lowest values (around 460 AU × 10^6/g dry matter in inoculated batches). This was mainly related with the higher amount of hexanal (532.19 *vs.* 377.52, 346.05, 349.82 AU × 10^6/g dry matter in CNT, CX, FL and TH, respectively) and in general the aliphatic aldehydes derive from lipid metabolism (pentanal, heptanal, 2-heptenal and 2-nonenal) in CNT batch than in inoculated sausages. In contrast, the sausages from TH group showed the highest values of benzeneacetaldehyde (9.84 *vs.* around 2.51 AU × 10^6/g dry matter in the other batches).

The third most abundant group were alcohols, representing between 4.5% and 5.5% of total volatile compounds. As occurs in aldehydes, CNT group had higher amounts of alcohols (116.76 AU × 10^6/g dry matter) than in starter sausages (102.86, 100.09 and 90.85 AU × 10^6/g dry matter in CX, FL and TH group, respectively). The main alcohol was 1-octen-3-ol, following by benzyl alcohol, 1-pentanol and 1-hexanol. The highest values of total alcohols in CNT group were due to this batch had the highest amounts of benzyl alcohol, 1-pentanol, 1-hexanol and 1-butanol, 3-methyl.

Regard acids content, they only represent between 2.6% and 3.9% of total volatile compounds. The most abundant acid was hexanoic acid, followed by butanoic and acetic acids, which derived from the carbohydrate fermentation and from lipid oxidation. FL group (started with *D. hansenii*) presented the highest values of acids (86.55 AU × 10^6/g dry matter) and CNT batch the lowest (54.26 AU × 10^6/g dry matter). In addition, all started sausages had higher ($P < 0.001$) content of acids than control batch.

Finally, ketones represented around 1% of total volatile compounds. Inoculated batches showed higher amounts of total ketones (around 21.91 AU × 10^6/g dry matter) than in control batch (15.14 AU × 10^6/g dry matter). Acetoin, found only in started sausages, was a product of the metabolism of LAB. Started sausages also presented higher amounts of 3,5-octadien-2-one than the control sausages. In contrast, the amounts of 2-hetanone, responsible for sweet and fruity notes, were higher ($P < 0.001$) in control batch than in started sausages.

3.4. Sensory analysis

The four types of sausages were subjected to a sensory evaluation carried out by trained panellists, who judged the different characteristics. Figure 2 shows the results of the descriptive sensory analysis of the sausages at the end of the ripening period. Among the 15 descriptors considered in the test, only acid taste, flavour intensity and black pepper odour resulted characterized by significant difference ($P < 0.05$) according to Duncan's test.

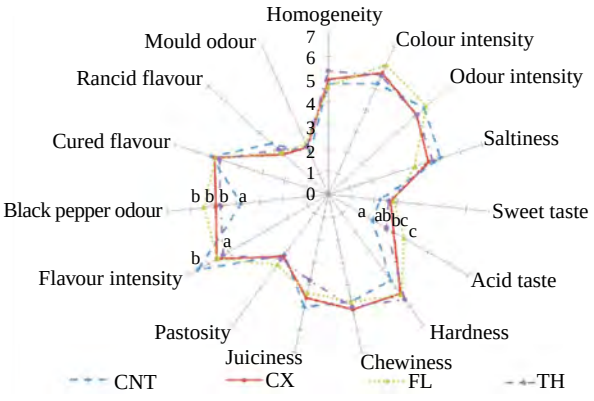

Figure 2. Effect of commercial starter cultures on sensory characteristics of dry-cured foal sausage.
[a–c]: Values in the same row (corresponding to the same parameter) were not followed by a common letter differ significantly ($P < 0.05$).

Acid taste was significantly affected by the addition of commercial starter cultures, with the highest values from FL batch and the lowest values corresponding to control sausages.

On the other hand, control batch showed higher ($P < 0.05$) values of flavour intensity than started sausages. In addition, although rancid flavour did not show significant differences, we could observe slightly higher scores of this descriptor in CNT sausages.

Regarding black pepper odour score, started sausages presented

the highest values. The started sausages also presented higher colour intensity scores, but the results were not significant.

Finally, control batch showed lower hardness scores than inoculated sausages, although this difference was not significant. This score value agrees with the results obtained by texture profile analysis test (Table 2), where control batch showed the lowest hardness.

4. Discussion

4.1. Microbial counts

Inoculated batches had higher value of LAB counts than in control, which agree with the results obtained by other authors in fermented sausages[12-14]. Unlike these cases, Rubio *et al*.[15] observed that after ripening the two inoculated batches reached a number of LAB significantly lower than control and Lorenzo *et al*.[5] reported lower LAB counts in an inoculated group than in control group, although it was not significant.

At the end of ripening LAB were the dominant microorganisms as expected, due to their good adaptation to the meat environment and their faster growth rates during fermentation and sausage ripening[16,17]. FL starter culture turned out to be the starter culture with the highest LAB count at the end of ripening, which showed its aptitude to be used as starter in the manufacture of the foal "salchichón", since LAB play an important role in meat preservation and fermentation processes because they affect both the technological properties and the microbial stability of the final product[18].

On the other hand, the addition of starter cultures completely reduce Enterobacteriaceae count. This result is in agreement with those reported by several authors who also found that control batches had higher counts than inoculated[5,13-15]. However, Ruiz-Moyano *et al*.[19] reported a very similar Enterobacteriaceae count in both control group and groups inoculated by two potential probiotic strains. In our study, the reduction of Enterobacteriaceae count could be due to the rapid decline in pH value during fermentation as well as probably production of bacteriocin by LAB and antagonistic characteristics of *Staphylococcus* strains used[20].

The total viable count was higher in started sausages than in control sausage. These results agree with those obtained in other researches, in which higher counts were also observed in inoculated batches. Nonetheless, this outcome is in disagreement with that reported by Bedia *et al*.[21], who found that the final of total viable count, after drying, of one of the inoculated samples of salami had a significantly lower number than control sample, although, it is noteworthy that the LAB counts were lower in that inoculated sample than in control, which may explain this result.

Finally, the highest values of yeast in batch inoculated with FL commercial starter culture is related with the fact that this starter culture had the yeast *D. hansenii* in its composition.

4.2. Chemical composition, pH, colour properties and texture profile analysis

The highest pH value in control batch agree with the results reported by other authors, who observed that all inoculated batches reached a lower pH value than control[5,13,14,22]. Nevertheless, Simion *et al*.[23] observed a very different result, since in the manufacture of the Romanian sausage Dacia they did not find differences on pH values between batches. Rubio *et al*.[15] also found a different result in the manufacture of Spanish fermented sausages when they found that two inoculated batches were significantly higher than control.

Regarding proximate composition, Santa *et al*.[22] also found significant differences between batches in protein content at the end of ripening of Italian sausages, since they did not report either significant difference in fat content. However, they observed significant differences in moisture values which is not the same with the present study. Lorenzo *et al*.[5], Essid and Hassouna[16] and Simion *et al*.[23] did not find either significant difference in moisture values between batches. Nonetheless, Ruiz-Moyano *et al*.[19] reported significant differences between them in the manufacture of traditional Iberian dry-fermented sausages.

In our study, colour parameters were no affected by starter cultures. This result is in agreement with that reported by Wang *et al*.[24], who did not find any significant difference between batches when they manufactured fermented sausages. The same result was observed by Essid and Hassouna[16], who did not observe either significantly differences among groups, included control, in the manufacture of a Tunisian dry-fermented sausage. They reported that colour values were only affected by the ripening time. However, Lorenzo *et al*.[5] found significantly differences in lightness at the end of the development of a dry-cured foal sausage, reaching values a slightly higher than ours. Bedia *et al*.[21] reported significantly differences in redness and yellowness at the end of drying of salami.

As occurs in colour parameters, the textural parameters were no affected by the addition of starter cultures. In agreement with our results, Lorenzo *et al*.[5] did not observe either significant differences between batches in hardness, springiness, cohesiveness, gumminess and chewiness of a dry-cured foal sausage. In the same way other authors did not report either significant differences in hardness parameter among control batch and inoculated batches[13,16].

4.3. Volatile compound analysis

Most of the 61 volatile compounds identified after 53 days of sausages ripening have been previously described in different types of dry-fermented sausages[4,25,26]. The total volatile compounds content was similar to those found by Lorenzo *et al*.[26], however Andrade *et al*.[25] and Kargozari *et al*.[27] described higher amounts of total volatile compounds. These different results could be explained by the volatile extraction methods used and the characteristics (temperature and time) of the ripening process. In addition, there are many factors that affect SPME fibre performance, such as the choice of stationary phase and the extraction conditions.

Regarding hydrocarbons, volatile compounds from the spices presented the highest amounts. All compounds from spices are classified as terpenes and most of them were previously identified in black and white pepper[28]. Our results were in agreement with those found by Lorenzo *et al*.[26] and Kargozari *et al*.[27], who reported that 32%–76% in their dry-fermented sausages were terpenes, due to the use of spices as an ingredient. Some of the terpenic compounds identified have been described to add menthol, fresh, herbal and lemon notes[29]. In contrast with our results, Andrade *et al*.[25] did not find differences in the volatiles from the spices among batches.

On the other hand, sausages from CNT batch presented the highest values of total aldehydes. In addition, hexanal is one of the main markers of lipid oxidation in dry-fermented sausages and at high concentrations confers a rancid flavour[30]. These results suggested that the use of starter cultures limited the lipolysis and

lipid autoxidation in comparison with spontaneous fermentation. In contrast, Lorenzo *et al*.[26] found that the use of starter cultures increase the total aldehyde and hexanal content, while Casquete *et al*.[4] did not find differences in hexanal content between started and spontaneous fermented sausages. Among aldehydes interesting differences were also found in the amount of benzeneacetaldehyde, which derive from the deamination of 2-phenylethylamine and impart floral aroma[3]. In this case, the sausages from TH group showed the highest values. The values of the phenylethylamine were also higher in this batch (7.71 *vs*. 4.53, 3.37 and 2.30 mg/kg in CNT, CX and FL, respectively[31]) than in the others. Therefore, the highest values of benzeneacetaldehyde in TH sausages are due to the most intense proteolytic activity of this starter. In accordance with our results, Tabanelli *et al*.[3] also reported a significant and positive correlation between the amounts of phenylethylamine and benzeneacetaldehyde. The aliphatic aldehydes derive from lipid metabolism give grassy, rancid and/or floral notes depending on the concentrations[3].

As occurs in aldehydes, CNT group had the highest amounts of alcohols. Alcohols normally present in fermented products are mainly generated from the reduction of aldehydes[32]. In our case, batch containing higher amounts of aldehydes (CNT) also contain high alcohol quantities. Similar results were reported by Lorenzo *et al*.[26].

The results obtained in the present research regard acids content were agreed with those described by Andrade *et al*.[25] and dos Santos *et al*.[33], who also found higher amounts in inoculated sausages than in control batch. Furthermore, Flores *et al*.[32] also found that the inoculation of *Debaryomyces* spp. increase the content of acids. Consequently, yeast activity may contribute to their production[34]. The acetic acid has a great impact on the flavour and contributes to the ripened aroma, while butanoic acid gives cheese notes[35].

Finally, inoculated batches showed the highest amounts of total ketones. Acetoin, found only in started sausages, is a product of the metabolism of LAB. This compound has a characteristic buttery, sweet odour with a very low threshold value, and therefore with great importance to aroma[36]. Our results agree with those obtained by Andrade *et al*.[25] and Flores *et al*.[32], who reported that inoculated sausages presented higher values of this compound. Moreover, Lorenzo *et al*.[26] reported that started sausages had higher contents of 3,5-octadien-2-one, which give woody, mushroom and fresh aroma.

4.4. Sensory analysis

The highest acid taste score in started batches agree with those reported by Lorenzo *et al*.[26], who also observed that non inoculated foal sausages presented the lowest acid taste. Our result is consistent with the data obtained for pH values, where, as commented above, started sausages presented significantly lower pH values than control sausages. The high rancid flavour in control samples agree with the results reported by Cenci-Goga *et al*.[37], who found higher rancid taste in salami made without addition of starter cultures. Both, flavour intensity and rancid flavour is related with volatile profile. To this regard, Essid and Hassouna[16] found that these attributes are related with the volatile compounds released by microbial and endogenous enzymes throughout the ripening process. As commented above, control sausages had significantly higher amounts of aldehydes and alcohols, who have very low threshold value, and therefore with great importance to aroma, flavour intensity and rancid flavour.

As discussed in volatile section, sausages with addition of started cultures presented higher amounts of terpenes (FL > CX > TH; between 64% and 68% of total volatile compounds) than control batch (58% of total volatile compounds). Therefore, the highest black pepper odour score in started sausages could be related with this fact. Obviously, the higher amount of volatile compounds from spices results in higher score of spices odour.

The starter sausages presented higher ($P > 0.05$) colour intensity than control batch. These findings agree with the results obtained by other authors, who found more intense red colour in inoculated than in control sausages[16,25]. The red colour is related with the nitrate reductase activity and the starters used in this study contain *S. xylosus* with high nitrate reductase activity[16].

The results of the sensory analyses seem to confirm the positive effect of the action of the starter cultures in controlling the traditional process as they limited the lipid oxidation and not lead to great changes in the sensory properties of "salchichón".

According to the results obtained in this study, the use of starter cultures affects the microbial counts, pH values, volatile profile and acid taste, flavour intensity and black pepper odour from sensory analysis. However, they did not affect the physicochemical characteristics. Regarding the results, FL started presented the highest amounts of LAB (which is so important from the point of view of food safety) and acid taste and the lowest pH values. Inoculated batches also had the lowest values of aldehydes which are related with lipid oxidation. This fact demonstrates that the use of the starters reduces lipid degradation. Therefore, the use of starter cultures controls the ripening process with low impact in physicochemical and sensory properties. As a general conclusion, considering all the results FL commercial starter culture could be the most suitable culture to the manufacture foal sausage.

Conflict of interest statement

We declare that we have no conflict of interest.

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