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Staphylococcus spp. and *Lactobacillus sakei* Starters with High Level of Inoculation and an Extended Fermentation Step Improve Safety of Fermented Sausages

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Abstract: *Paio do Alentejo* (PA) is one of the most popular dry-fermented sausages in Portugal. The aim of the present work was to evaluate the effect of a high concentration of starter cultures with an extended fermentation step on the safety and quality of PA. Physicochemical parameters, microbiological parameters, biogenic amines, colour, texture profile, and sensory attributes were assessed. Five starters were selected based on our previous works. *Staphylococcus equorum* S2M7, *Staphylococcus xylosus* CECT7057, *Lactobacillus sakei* CV3C2, *Lactobacillus sakei* CECT7056, and a yeast strain (2RB4) were co-inoculated in meat batters at a concentration of 10^8 cfu/g for bacteria and 10^6 cfu/g for yeast strain, and 0.25% dextrose was added. Inoculated starters significantly reduced pH, *Listeria monocytogenes* counts, and total content in biogenic amines. The studied starter cultures did not compromise the sensory characteristics of PA, and thus, their use can be considered to protect these sausages and contribute to their safety.

Keywords: dry-fermented sausages; *Paio do Alentejo*; food safety; starter cultures; biogenic amines; foodborne pathogens; food quality

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1. Introduction

Fermented foods are manufactured through controlled microbial growth and result from the enzymatic conversion of food components. They are strategically present in every European diet due to their importance for human health but also as an intangible cultural heritage for their sustainability, consumer acceptance, and potential for innovation [1].

Natural fermented sausages have a long tradition originating from Mediterranean countries since roman times [2]. Nowadays, dry-fermented sausages are produced in many countries. Traditional Portuguese sausages are unique foods that usually have their origin in geographical areas associated to the respective commercial designation. The exhibited variety in flavour, texture, shape, and calibre results from the diversity of

raw materials, ingredients, and manufacturing processes. According to Dias et al. [3] *Paio do Alentejo* is a popular dry-fermented sausage in Portugal because it is manufactured using pork meat from autochthonous breeds as well as typical nonmeat ingredients in small processing units according to traditional practices specific to each geographical area. This type of sausage has long been spontaneously fermented using empirical methods, but sometimes, the quality of the end products cannot be assured. The microbiota that develops during the fermentation depends on several factors (raw meat, ingredients, and the specific conditions of meat processing and ripening (i.e., temperature relative humidity, and time) and others [4,5]. Chen et al., Rocchetti et al., and Cruxen et al. [6–8] stated that for product safety and quality consistency, starter cultures are commonly used in fermented sausage production. According to Laranjo et al. and Pereira et al. [4,9], the most promising microorganisms selected as starter cultures are those that are isolated from the autochthonous microbiota of products, because they are well adapted to the environmental conditions of food processing.

Some of these microorganisms also play a crucial role as biocontrol agents. Lactic acid bacteria (LAB), namely, *L. sakei*, *L. plantarum*, and *L. curvatus* are considered as the most important contributor in the reduction or elimination of pathogens through the reduction of pH, their antibacterial metabolites, including organic acids, bacteriocins, and hydrogen peroxide [10,11]. Gram-positive catalase-positive cocci (G^+C^+C), such as *S. xylosus*, *S. equorum*, and *Kocuria* spp., are able to reduce lipid oxidation and formation of nitrosomyoglobin, favouring the development of a typical red colour through their nitrate-reducing capacity [4,8,12].Yeasts, such as *Debaromyces* spp., can be inoculated both in meat batters and on the surface of sausages, and moulds of the genus *Penicillium* are used for the superficial inoculation of sausages [13], due to their ability to prevent lipid oxidation and contribute to the gradual dehydration of sausages. Concomitantly, they may also contribute to enhance their sensory properties. The inoculum concentration must take into account the concentrations usually found in non-inoculated sausages. Recently, Oliveira et al. [5] indicated concentrations between 10^5 and 10^9 cfu of bacteria/g of meat bater. However, other authors mention 10^6–10^7 cfu/g for yeasts [14,15] and 10^{11} cfu/g for surface inoculated moulds [16].

Biogenic amines (BA) have been considered hazardous to consumers' health due to their ability to react with nitrites and form potentially carcinogenic nitrosamines [17,18]. The use of autochthonous starter cultures represent one of the main measures to control BA formation in fermented meat products [3,19].

The aim of the present work was to evaluate the effects of different consortia of autochthonous starters, inoculated at high concentrations, and with an extended fermentation step, on the safety and quality of *Paio do Alentejo*, a traditional Portuguese dry-fermented sausage produced in a small manufacturing unit.

2. Materials and Methods

2.1. Dry-Fermented Sausage Manufacturing and Sampling

Paio do Alentejo, a traditional dry-fermented sausage, was manufactured in a local factory using commercial black pig breed (Alentejano pig breed × Duroc pig breed) meat as described previously [3].

Five treatments were considered, using different consortia of starters: (1) control (no starter cultures added), (2) *Staphylococcus equorum* S2M7/*Lactobacillus sakei* CV3C2, (3) *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4, (4) *Staphylococcus xylosus* CECT7057/*Lactobacillus sakei* CECT7056; and (5) *S. xylosus* CECT7057/*L. sakei* CECT7056/yeast 2RB4). Bacteria and yeast strains were co-inoculated at a concentration of 10^8 cfu/g and 10^6 cfu/g, respectively, in meat batters. Food grade dextrose (0.25%) was added to all treatments to boost multiplication of starters, according to previous works [3,13]. All starter strains, *S. equorum* S2M7, *L. sakei* CV3C2, *S. xylosus* CECT7057, and *L. sakei* CECT7056, have been previously studied [3,20,21]. Starter culture composition and concentrations were selected based on previous trials and were inoculated in the meat batter [3]. These inoculated meat batters were subjected to a maturation step of 72 h before stuffing to promote the adaptation of inoculated starters to the meat batter.

Moreover, an extended fermentation step was added after stuffing to maximise fermentation, at room temperature, before smoking.

Three independent manufacturing batches were produced for all five treatments, and triplicate samples were collected throughout the curing process at five different steps: meat batter (immediately before stuffing), fermented sausage (48 h after stuffing and maintained at room temperature in the smokehouse antechamber), and half-cured sausage (12 days after stuffing), 25 days after stuffing and end-product (38–40% weight losses).

Microbiological parameters and contents in biogenic amines were determined at all curing steps, except at 25 days after stuffing. Samples were analysed at this additional step only to evaluate the reducing effect of starters on safety (*L. monocytogenes* and *Salmonella* spp.) and hygiene *(Enterobacteriaceae*) indicators. Additionally, pH and aW were determined at all curing steps.

Evaluations of colour, texture profile analysis, and sensory analysis were only performed in end-products.

Samples were immediately processed for microbiological, sensory, and most physicochemical analyses but stored at −20 °C for later analysis of biogenic amines.

2.2. Physicochemical Analyses

2.2.1. Determination of pH and aW

For determination of pH and aW, five replicates per sample were used. Samples were prepared, and measurements were made as described in [13]. pH measurements followed ISO 2917 [22].

2.2.2. Colour

Determination of colour CIELab chromatic coordinates (L*, a*, and b* were measured and C* and H° calculated) made with a Konica Minolta CR-400 colorimeter (Konica Minolta Inc., Tokyo, Japan) as described in [13]. Five replicates per sample were used.

Using CIEL*a*b* coordinates, the total colour difference (ΔE) between controls and inoculated treatments was calculated, ΔE = [(L*0 − L*1)2 + (a*0 − a*1)2 + (b*0 − b*1)2]0.5, where ΔE is the total colour difference; L*0, a*0, b*0 are the means of colour parameters determined for the control sausages; and L*1, a*1, b*1 are the means of colour parameters determined for the inoculated sausages.

Regarding the interpretation of results, the scale proposed by Mokrzycki and Tatol [23] was used: the observer does not notice the difference when $0 < \Delta E < 1$; only an experienced observer may notice the difference when $1 < \Delta E < 2$; an unexperienced observer also notices the difference when $2 < \Delta E < 3.5$; a clear difference in colour is noticed when $3.5 < \Delta E < 5$, and an observer notices two different colours when $5 < \Delta E$.

2.2.3. Texture Profile Analysis (TPA)

Texture profile analysis (TPA) was performed using a Stable Micro System TA-Hdi (Stable Micro Systems, Godalming, England) following the procedures described in [24,25]. Five replicates per sample were used. Hardness (N), adhesiveness ($N \cdot s^{-1}$), springiness, cohesiveness, resilience, and chewiness (N) were calculated from the obtained force-time curves.

2.3. Microbiological Analyses

Microbiological parameters were performed following international standards and established procedures: mesophiles (ISO 4833-1 [26]), psychrotrophic microorganisms (ISO 17410 [27]), lactic acid bacteria (ISO 15214 [28]) staphylococci [29], yeasts and moulds (ISO 21527-2 [30]), enterobacteria (ISO 21528-2 [31]), and listeria monocytogenes (ISO 11290-2 [32]). The eventual presence of Salmonella spp. was detected using a VIDAS enzyme-linked fluorescent immunoassay (bioMérieux, Marcy-l'Étoile, France), and positive samples were confirmed following ISO 6579-1 [33]. All microbiological analyses were performed in triplicate, and the results were expressed as log colony-forming units (cfu)/g.

2.4. Biogenic Amines Profile

The content of biogenic amines was determined as previously described [29,34], using eight grams of homogenized sample extracted with 0.4 M perchloric acid. 1,7-Diaminoheptane was used as internal standard, and biogenic amines were derivatized with dansyl chloride under alkaline conditions and injected into an HPLC system (Thermo Scientific Dionex, Ultimate 3000, Waltham, MA, USA) with an RP-18 reverse phase column (5 µm of 4.0 × 125 mm and 100 Å) (Merck, Kenilworth, NJ, USA).

All samples were extracted in duplicate, and each replicate was twofold derivatized, and injected in duplicate. Tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine were quantified and are expressed in mg/kg of fresh weight. The content of vasoactive amines (tryptamine, β-phenylethylamine, histamine, and tyramine) was calculated. Chromatographic data were analysed with Chromeleon software version 6.8 (Thermo Scientific Dionex, Waltham, MA, USA).

2.5. Sensory Analysis

Sensory analyses were performed with a panel of ten assessors following the established protocols (ISO 8586-1 [35] ISO 8589-1 [36]). The experts were asked to evaluate sausages using a quantitative descriptive analysis with a scale ranging from 0 to 100 corresponding to "no perception" or "maximum perception". The evaluated attributes were colour intensity, off-colours, marbled, aroma intensity, off-aromas, hardness, fibrousness, succulence, flavour intensity, off-flavours, salt perception, and overall appreciation. For hardness and salt perception, an optimum value of 50 was considered.

Regarding the control treatment, only the sausages with Listeria monocytogenes counts below the detection limit were offered to panellists.

2.6. Statistical Analysis

Data were analysed using STATISTICA v.12.0 software from Statsoft (StatSoft Inc., 1984–2014, Tulsa, OK, USA). Outliers were detected using the Grubbs test ($\alpha = 0.05$).

Factorial or one-way ANOVAs were performed, and significantly different means were compared with Tukey's HSD test ($p < 0.05$).

3. Results

3.1. pH and aW of Fermented Sausages

pH values significantly decreased throughout the first curing steps, with almost insignificant changes after the half-cured sausage stage (Table 1). Within each curing step, inoculated sausages showed significantly lower pH values, when compared to the control sausages, which contributes to their enhanced safety.

Regarding aW values, significant differences were observed along the cure, with significantly lower mean values for end-products. End-product sausages inoculated with *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4 showed significantly lower mean values (0.851 ± 0.004) than control sausages.

3.2. Microbiota of Fermented Sausages

The results for all microbiological parameters are summarised in Table 2.

Significant differences were observed between control and inoculated meat batters for mesophiles (control roughly log 7, while inoculated around log 8), psychrotrophic microorganisms (control roughly log 7, while inoculated around log 8), LAB (inoculated meat batters about 3 logs higher), and staphylococci (inoculated meat batters about 2 logs higher), which could be due to the addition of starters. However, no significant differences were observed between inoculated and control sausages regarding yeasts counts.

Enterobacteria counts were significantly lower in the last two curing steps, probably due to the inoculation with LAB and the consequent pH decrease.

L. monocytogenes was present along the cure. However, inoculated end-product sausages showed a significant reduction in *L. monocytogenes* counts, almost always be-

low the detection limit (100 cfu/g), evidencing the bactericidal effect of starters over this pathogenic bacterium.

Salmonella spp. was absent in all end-product sausages.

3.3. Biogenic Amines Profile of Fermented Sausages

Biogenic amines profile for *Paio do Alentejo* fermented sausages along the cure is shown in Table 3. An increase in the content of biogenic amines along the cure was generally observed for all amines, but β-phenylethylamine and tyramine exhibited a more heterogenous behaviour.

Table 1. Effect of starter cultures on pH and aW of fermented sausages.

Parameters	Treatment	Curing Steps				
		Meat Batter	**Fermented Sausage**	**Half-Cured Sausage**	**25 Days after Stuffing**	**End-Product**
pH	1	$5.87^{A,a} \pm 0.06$	$5.73^{B,a} \pm 0.10$	$5.05^{CD,a} \pm 0.09$	$5.01^{D,a} \pm 0.03$	$5.06^{CD,a} \pm 0.09$
	2	$5.64^{A,b} \pm 0.24$	$5.35^{B,b} \pm 0.11$	$4.97^{C,b} \pm 0.04$	$4.95^{C,bc} \pm 0.02$	$5.02^{C,bc} \pm 0.04$
	3	$5.60^{A,b} \pm 0.28$	$5.30^{B,b} \pm 0.11$	$5.00^{C,b} \pm 0.06$	$4.97^{C,b} \pm 0.02$	$5.02^{C,bc} \pm 0.06$
	4	$5.68^{A,b} \pm 0.27$	$5.33^{B,b} \pm 0.06$	$5.00^{C,b} \pm 0.03$	$4.95^{C,c} \pm 0.03$	$5.00^{C,c} \pm 0.03$
	5	$5.68^{A,b} \pm 0.03$	$5.35^{B,b} \pm 0.04$	$5.00^{C,b} \pm 0.04$	$4.96^{C,bc} \pm 0.05$	$5.03^{C,b} \pm 0.04$
aW	1	$0.963^{A,a} \pm 0.010$	$0.957^{B,ab} \pm 0.012$	$0.932^{C,a} \pm 0.006$	$0.903^{D} \pm 0.007$	$0.856^{E,a} \pm 0.007$
	2	$0.959^{A,ab} \pm 0.012$	$0.955^{A,abc} \pm 0.009$	$0.934^{B,a} \pm 0.005$	$0.905^{C} \pm 0.004$	$0.852^{D,ab} \pm 0.005$
	3	$0.961^{A,ab} \pm 0.007$	$0.952^{B,c} \pm 0.008$	$0.933^{C,a} \pm 0.007$	$0.902^{D} \pm 0.004$	$0.851^{E,b} \pm 0.004$
	4	$0.956^{A,b} \pm 0.010$	$0.953^{A,bc} \pm 0.005$	$0.927^{B,b} \pm 0.003$	$0.904^{C} \pm 0.008$	$0.852^{D,ab} \pm 0.005$
	5	$0.962^{A,a} \pm 0.007$	$0.959^{A,a} \pm 0.011$	$0.931^{B,bc} \pm 0.009$	$0.906^{C} \pm 0.005$	$0.855^{D,a} \pm 0.007$

Data are expressed as means ± SD. (1) Control; (2) *S. equorum* S2M7/*L. sakei* CV3C2; (3) *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4; (4) *S. xylosus* CECT7057/*L. sakei* CECT7056; (5) *S. xylosus* CECT7057/*L. sakei* CECT7056/yeast 2RB4. For the same treatment and in the same row, distinct capital letters ($^{A–E}$) represent significantly different means ($p < 0.05$). For each curing step and in the same column, distinct lowercase letters ($^{a–c}$) represent significantly different means ($p < 0.05$).

Tyramine showed the lowest concentrations throughout the curing process with values below 2.20 mg/kg for all treatments at all sampling steps. On the other hand, tryptamine showed values of 196.65 ± 116.26 mg/kg for *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4 inoculated end-product sausages and of 269.77 ± 131.28 mg/kg for control sausages.

Regarding natural polyamines, spermine contents were much higher than those of spermidine.

Concerning vasoactive amines (tryptamine, β-phenylethylamine, histamine, and tyramine), although mean values were higher for control sausages (279.11 ± 131.94 mg/kg), no significant differences were observed between control and inoculated end-products.

Furthermore, the content in vasoactive amines in end-products was higher than 200 mg/kg, mainly due to the contribution of tryptamine. However, the total content in biogenic amines is lower than 1000 mg/kg. Moreover, in end-products, inoculation showed a significant effect on the content of biogenic amines, with significantly lower values in inoculated sausages (between 452.58 ± 55.11 mg/kg and 533.96 ± 63.65 mg/kg) when compared to control sausages (731.95 ± 206.23 mg/kg), with the sausages inoculated with *S. xylosus* CECT7057 10^8/*L. sakei* CECT7056 10^8 showing the lowest mean values.

3.4. Colour

Table 4 summarizes the effect of starters on the colour of fermented sausages.

Table 2. Effect of starter cultures on microbiological parameters of fermented sausages.

Parameters	Treatment	Curing Steps				
		Meat Batter	Fermented Sausage	Half-Cured Sausage	25 Days after Stuffing	End-Product
mesophiles	1	$6.58^{B,b} \pm 0.38$	$7.53^{A,b} \pm 0.70$	$7.76^{A,b} \pm 0.63$	-	$7.83^{A} \pm 0.30$
	2	$7.66^{B,a} \pm 0.30$	$8.07^{A,a} \pm 0.13$	$8.04^{A,a} \pm 0.16$	-	$8.06^{A} \pm 0.27$
	3	$7.54^{B,a} \pm 0.37$	$8.07^{A,a} \pm 0.09$	$8.05^{A,a} \pm 0.11$	-	$7.96^{A} \pm 0.34$
	4	$7.94^{a} \pm 0.21$	$7.97^{ab} \pm 0.08$	$8.19^{ab} \pm 0.22$	-	8.27 ± 0.59
	5	$7.97^{a} \pm 0.49$	$8.09^{a} \pm 0.12$	$8.25^{a} \pm 0.25$	-	8.08 ± 0.38
psychrotrophic microorganisms	1	$6.66^{B,b} \pm 0.41$	$7.47^{A} \pm 0,66$	$7.53^{A} \pm 0.86$	-	$7.78^{A} \pm 0.35$
	2	$7.58^{a} \pm 0.32$	$7.53 \pm 0,97$	7.93 ± 0.21	-	8.13 ± 0.54
	3	$7.61^{a} \pm 0.42$	$7.40 \pm 0,99$	7.85 ± 0.17	-	8.03 ± 0.51
	4	$7.93^{a} \pm 0.21$	$7.23 \pm 1,07$	7.65 ± 0.99	-	8.13 ± 0.69
	5	$7.92^{a} \pm 0.49$	$7.48 \pm 1,14$	8.04 ± 0.25	-	8.14 ± 0.54
LAB	1	$4.75^{C,b} \pm 0.36$	$7.37^{B} \pm 0.69$	$8.14^{A} \pm 0.33$	-	$7.89^{AB} \pm 0.59$
	2	$7.84^{a} \pm 0.41$	8.09 ± 0.25	8.08 ± 0.16	-	8.06 ± 0.60
	3	$7.72^{B,a} \pm 0.49$	$8.19^{A} \pm 0.20$	$8.11^{AB} \pm 0.11$	-	$8.12^{AB} \pm 0.50$
	4	$8.17^{a} \pm 0.42$	8.00 ± 0.11	8.20 ± 0.26	-	8.25 ± 0.79
	5	$8.13^{a} \pm 0.72$	8.20 ± 0.18	8.17 ± 0.16	-	8.14 ± 0.51
staphylococci	1	$4.55^{C,b} \pm 0.43$	$5.41^{AB} \pm 0.66$	$5.46^{A} \pm 0.60$	-	$4.66^{BC} \pm 0.69$
	2	$5.88^{A,a} \pm 0.35$	$5.71^{A} \pm 0.51$	$5.45^{A} \pm 0.53$	-	$4.75^{B} \pm 0.52$
	3	$5.73^{A,a} \pm 0.45$	$5.59^{AB} \pm 0.32$	$5.11^{B} \pm 0.16$	-	$4.33^{C} \pm 0.63$
	4	$6.26^{A,a} \pm 0.41$	$6.06^{A} \pm 0.50$	$5.19^{B} \pm 0.75$	-	$4.67^{B} \pm 0.86$
	5	$5.80^{A,a} \pm 0.52$	$5.76^{A} \pm 0.45$	$4.88^{A} \pm 0.70$	-	$4.69^{A} \pm 0.85$
enterobacteria	1	$5.28^{B,b} \pm 0.50$	$6.48^{A,a} \pm 0.79$	$5.59^{AB} \pm 0.89$	$4.20^{C} \pm 0.51$	$3.49^{C,a} \pm 0.72$
	2	$5.66^{A,ab} \pm 0.43$	$5.77^{A,ab} \pm 0.44$	$5.02^{B} \pm 0.39$	$3.35^{C} \pm 0.81$	$2.71^{C,ab} \pm 0.33$
	3	$5.54^{A,ab} \pm 0.64$	$5.70^{A,ab} \pm 0.35$	$5.00^{A} \pm 0.48$	$3.15^{B} \pm 0.80$	$3.00^{B,a} \pm 0.48$
	4	$5.92^{A,ab} \pm 1.00$	$5.46^{A,b} \pm 0.68$	$4.80^{A} \pm 0.91$	$2.78^{B} \pm 1.11$	$2.08^{B,b} \pm 0.94$
	5	$6.39^{A,a} \pm 0.94$	$6.13^{A,ab} \pm 0.17$	$5.56^{A} \pm 1.18$	$3.00^{B} \pm 0.96$	$2.85^{B,ab} \pm 0.31$
yeasts	1	$4.58^{A} \pm 0.33$	$4.02^{B} \pm 0.35$	$4.71^{A} \pm 0.65$	-	$4.75^{A} \pm 0.23$
	2	$4.40^{A} \pm 0.37$	$3.84^{B} \pm 0.14$	$4.81^{A} \pm 0.33$	-	$4.65^{A} \pm 0.43$
	3	$4.50^{AB} \pm 0.34$	$3.77^{C} \pm 0.24$	$4.47^{B} \pm 0.31$	-	$4.88^{A} \pm 0.38$
	4	$4.29^{AB} \pm 0.63$	$3.92^{B} \pm 0.25$	$4.53^{AB} \pm 0.76$	-	$4.67^{A} \pm 0.54$
	5	$4.54^{AB} \pm 0.57$	$4.08^{B} \pm 0.50$	$4.85^{A} \pm 0.50$	-	$4.71^{AB} \pm 0.43$
moulds	1	0.44 ± 0.88	0.74 ± 0.93	0.94 ± 1.50	-	<DL
	2	0.50 ± 0.84	1.28 ± 1.80	<DL	-	<DL
	3	0.84 ± 1.31	0.11 ± 0.33	0.22 ± 0.67	-	<DL
	4	0.45 ± 0.90	0.11 ± 0.33	0.22 ± 0.67	-	<DL
	5	0.57 ± 1.17	0.37 ± 0.56	<DL	-	<DL
L. monocytogenes	1	$1.33^{B} \pm 1.59$	$2.80^{AB} \pm 3.62$	$3.41^{A,a} \pm 0.43$	$2.86^{AB} \pm 1.60$	$2.44^{AB,a} \pm 1.43$
	2	$1.55^{AB} \pm 1.86$	$2.09^{AB} \pm 1.61$	$2.19^{A,ab} \pm 1.60$	$1.03^{AB} \pm 1.60$	$<DL^{B,b}$
	3	2.03 ± 1.54	1.67 ± 1.60	$2.29^{ab} \pm 1.33$	1.90 ± 1.49	$0.90^{b} \pm 1.35$
	4	2.24 ± 1.72	1.55 ± 1.66	$1.34^{b} \pm 1.60$	1.52 ± 1.68	$<DL^{b}$
	5	1.64 ± 1.95	1.63 ± 1.98	$1.92^{ab} \pm 1.85$	1.09 ± 1.73	$<DL^{b}$
Salmonella spp.	1	Present in 6/9 samples	Present in 4/9 samples	Present in 6/9 samples	Present in 5/9 samples	ND
	2	Present in 6/9 samples	Present in 5/9 samples	Present in 4/9 samples	Present in 4/9 samples	ND
	3	Present in 6/9 samples	Present in 6/9 samples	Present in 4/9 samples	Present in 4/9 samples	ND
	4	Present in 7/9 samples	Present in 5/9 samples	Present in 3/9 samples	Present in 3/9 samples	ND
	5	Present in 6/9 samples	Present in 4/9 samples	Present in 5/9 samples	Present in 3/9 samples	ND

Data are expressed as means ± SD. <DL: below the detection limit of the corresponding analytical method (10 cfu/g for moulds and 100 cfu/g for *L. monocytogenes*). ND—Not detected (absence in 25 g). Results are expressed in log cfu/g. (1) Control; (2) *S. equorum* S2M7/*L. sakei* CV3C2; (3) *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4; (4) *S. xylosus* CECT7057/*L. sakei* CECT7056; 5-*S. xylosus* CECT7057/*L. sakei* CECT7056/yeast 2RB4. For the same treatment and in the same row, distinct capital letters ($^{A–C}$) represent significantly different means ($p < 0.05$). For each curing step and in the same column, distinct lowercase letters (^{a,b}) represent significantly different means ($p < 0.05$).

Table 3. Effect of starter cultures in the biogenic amines profile (mg/kg fresh weight) of fermented sausages.

Parameters	Treatment	Curing Steps			
		Meat Batter	Fermented Sausage	Half-Cured Sausage	End-Product
tryptamine	1	18.66 ^{B} ± 12.45	32.41 ^{B} ± 23.55	219.86 ^{A} ± 218.19	269.77 ^{A} ± 131.28
	2	33.49 ^{B} ± 14.35	31.20 ^{B} ± 31.18	192.35 ^{A} ± 189.78	224.96 ^{A} ± 57.06
	3	29.07 ^{B} ± 14.30	43.16 ^{B} ± 39.40	242.02 ^{A} ± 156.04	196.65 ^{A} ± 116.26
	4	44.62 ^{B} ± 23.75	37.49 ^{B} ± 46.19	261.65 ^{A} ± 84.50	221.71 ^{A} ± 47.98
	5	34.47 ^{B} ± 17.26	42.08 ^{B} ± 41.94	230.66 ^{A} ± 236.89	198.98 ^{A} ± 85.30
β-phenylethylamine	1	10.84 ^{A,a} ± 10.45	5.06 ^{B} ± 1.74	9.77 ^{AB,a} ± 2.14	7.02 ^{AB} ± 1.86
	2	3.86 ^{B,b} ± 1.12	5.03 ^{B} ± 1.90	7.49 ^{A,b} ± 2.78	7.02 ^{A} ± 2.63
	3	5.57 ^{B,b} ± 3.37	4.68 ^{B} ± 1.19	8.31 ^{A,b} ± 3.83	6.75 ^{AB} ± 2.17
	4	5.27 ^{BC,b} ± 3.50	4.13 ^{BC} ± 0.62	7.49 ^{A,b} ± 2.83	6.80 ^{AB} ± 1.13
	5	4.46 ^{C,b} ± 0.59	4.32 ^{C} ± 0.49	9.49 ^{A,b} ± 2.79	7.42 ^{B} ± 2.21
putrescine	1	6.65 ^{C} ± 0.62	10.02 ^{C,a} ± 4.50	79.61 ^{B,a} ± 21,44	149.61 ^{A,a} ± 33.01
	2	5.86 ^{C} ± 1.52	7.62 ^{BC,ab} ± 2.04	13.11 ^{B,bc} ± 5.14	29.89 ^{A,bc} ± 13.22
	3	6.34 ^{C} ± 0.33	7.89 ^{BC,ab} ± 1.93	11.83 ^{B,bc} ± 2.48	33.63 ^{A,bc} ± 11.19
	4	6.23 ^{C} ± 0.75	6.61 ^{C,b} ± 0.64	10.76 ^{B,c} ± 1.51	13.13 ^{A,cb} ± 1.72
	5	6.39 ^{B} ± 0.66	8.98 ^{B,ab} ± 3.15	39.13 ^{A,a} ± 22.76	47.35 ^{A,a} ± 35.22
cadaverine	1	9.89 ^{D,ab} ± 1.91	54.39 ^{C,a} ± 7.45	100.45 ^{B,a} ± 9.19	191.75 ^{A,a} ± 98.35
	2	10.68 ^{D,ab} ± 4.30	36.36 ^{C,b} ± 12.30	56.25 ^{B,b} ± 15.94	118.59 ^{A,bc} ± 32.70
	3	11.89 ^{D,a} ± 10.50	37.78 ^{C,b} ± 10.50	82.82 ^{B,b} ± 7.69	102.29 ^{A,bc} ± 34.86
	4	9.27 ^{B,b} ± 2.55	23.51 ^{B,c} ± 8.44	71.78 ^{A,b} ± 14.67	82.00 ^{A,c} ± 18.05
	5	10.36 ^{D,ab} ± 2.14	35.81 ^{C,b} ± 15.13	69.75 ^{B,b} ± 30.27	128.44 ^{A,b} ± 45.48
histamine	1	ND ^{B,b}	ND ^{B,b}	ND ^{B,b}	1.37 ^{A,b} ± 2.10
	2	3.67 ^{B,a} ± 6.35	8.83 ^{B,a} ± 8.82	6.91 ^{B,a} ± 9.56	31.58 ^{A,a} ± 23.97
	3	3.21 ^{B,ab} ± 4.72	10.54 ^{B,a} ± 8.54	5.93 ^{B,a} ± 8.69	29.99 ^{A,a} ± 25.91
	4	ND ^{B,b}	ND ^{B,b}	ND ^{B,b}	5.22 ^{A,b} ± 6.16
	5	ND ^{B,b}	ND ^{B,b}	ND ^{B,b}	3.59 ^{A,b} ± 4.75
tyramine	1	2.17 ^{A} ± 0.73	0.75 ^{C} ± 0.55	1.35 ^{B} ± 0.39	0.95 ^{BC} ± 0.85
	2	1.58 ± 0.74	0.97 ± 0.67	1.25 ± 0.77	1.47 ± 0.85
	3	1.76 ^{A} ± 0.88	0.93 ^{B} ± 0.67	1.18 ^{AB} ± 0.77	1.28 ^{AB} ± 0.81
	4	1.65 ^{A} ± 0.79	0.92 ^{B} ± 0.77	0.95 ^{B} ± 0.63	1.38 ^{AB} ± 0.81
	5	2.09 ^{A} ± 1.03	0.96 ^{B} ± 0.75	0.89 ^{B} ± 0.73	1.26 ^{B} ± 0.77
spermidine	1	12.94 ^{B} ± 4.03	9.19 ^{B} ± 3.98	19.65 ^{A} ± 3.23	19.56^{A} ± 6.67
	2	10.63 ^{B} ± 5.06	10.87 ^{B} ± 3.82	19.24 ^{A} ± 3.39	19.04 ^{A} ± 6.40
	3	13.10 ^{B} ± 3.50	13.75 ^{B} ± 6.41	21.55 ^{A} ± 3.89	16.47 ^{A} ± 7.12
	4	12.71 ^{B} ± 3.96	12.24 ^{B} ± 4.72	19.57 ^{A} ± 5.75	22.17 ^{A} ± 7.53
	5	14.11 ^{B} ± 4.04	11.32 ^{B} ± 6.00	19.63 ^{A} ± 5.53	19.71 ^{A} ± 6.88
spermine	1	47.69 ^{C,b} ± 7.32	62.80 ^{B} ± 7.45	74.65 ^{B} ± 20.45	91.91 ^{A} ± 14.93
	2	51.39 ^{C,ab} ± 18.01	64.72 ^{B} ± 9.68	78.63 ^{B} ± 20.51	101.41^{A} ± 19.54
	3	52.78 ^{B,ab} ± 9.81	61.90 ^{B} ± 7.87	81.02 ^{A} ± 19.24	90.98 ^{A} ± 16.07
	4	53.46 ^{C,ab} ± 10.86	60.57 ^{C} ± 6.17	82.26 ^{B} ± 17.13	100.17 ^{A} ± 20.56
	5	60.80 ^{B,a} ± 14.41	60.99 ^{B} ± 8.36	92.38 ^{A} ± 16.15	103.73 ^{A} ± 18.86
vasoactive amines	1	31.67 ^{B} ± 10.98	38.22 ^{B} ± 23.66	230.97 ^{A} ± 218.17	279.11 ^{A} ± 131.94
	2	42.61 ^{B} ± 15.74	46.02 ^{B} ± 38.75	208.00 ^{A} ± 187.70	265.03 ^{A} ± 59.50
	3	39.61 ^{B} ± 11.44	59.31 ^{B} ± 47.09	257.44 ^{A} ± 154.26	234.66 ^{A} ± 110.36
	4	51.54 ^{B} ± 26.61	42.53 ^{B} ± 47.15	270.10 ^{A} ± 85.59	235.10 ^{A} ± 48.68
	5	41.02 ^{B} ± 17.94	47.37 ^{B} ± 42.71	241.04 ^{A} ± 237.82	211.24 ^{A} ± 84.66
total amines	1	108.83 ^{C,b} ± 14.50	174.62 ^{B,ab} ± 29.93	505.32 ^{B} ± 345.47	731.95 ^{A,a} ± 206.23
	2	121.17 ^{C,ab} ± 35.03	165.60 ^{B,ab} ± 32.54	375.33 ^{B} ± 199.30	533.96 ^{A,b} ± 63.65
	3	123.72 ^{B,ab} ± 19.44	180.63 ^{B,a} ± 35.94	454.66 ^{A} ± 164.28	478.03 ^{A,b} ± 156.31
	4	133.22 ^{B,a} ± 28.69	145.47 ^{B,b} ± 42.12	454.46 ^{A} ± 94.87	452.58 ^{A,b} ± 55.11
	5	132.69 ^{B,ab} ± 26.07	164.46 ^{D,ab} ± 45.58	461.93 ^{A} ± 273.00	510.47 ^{A,b} ± 114.85

Data are expressed as means ± SD. ND–not detected; detection limit for histamine is 0.20 mg/kg. (1) control; (2) *S. equorum* S2M7/*L. sakei* CV3C2; (3) *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4; (4) *S. xylosus* CECT7057/*L. sakei* CECT7056; 5-*S. xylosus* CECT7057/*L. sakei* CECT7056/yeast 2RB4. For the same treatment and in the same row, distinct capital letters ($^{A–D}$) represent significantly different means ($p < 0.05$). For each curing step and in the same column, distinct lowercase letters ($^{a–c}$) represent significantly different means ($p < 0.05$).

Table 4. Effect of starter cultures on the colour parameters of fermented sausages evaluated in end-products.

Treatment	Colour Parameters					
	L* (Lightness)	a* (Redness/Greenness)	b* (Yellowness/Blueness)	C* (Chroma)	H° (Hue Angle)	ΔE
1	37.80 ± 4.09	14.59 ± 2.97	14.18 ± 4.57	20.43 ± 5.11	43.44 ^{a} ± 5.37	—
2	37.10 ± 4.52	13.62 ± 2.12	12.53 ± 3.09	18.61 ± 3.19	42.17 ^{ab} ± 6.19	2.04
3	37.35 ± 4.31	14.50 ± 2.47	13.62 ± 0.43	20.00 ± 3.75	42.79 ^{ab} ± 5.62	0.72
4	36.96 ± 2.66	14.69 ± 2.53	12.41 ± 2.77	19.30 ± 3.40	39.92 ^{b} ± 4.67	1.96
5	36.82 ± 4.08	13.74 ± 3.19	13.46 ± 3.80	19.35 ± 4.49	44.11 ^{a} ± 6.12	1.48

Data are expressed as means ± SD. (1) Control; (2) *S. equorum* S2M7/*L. sakei* CV3C2; (3) *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4; (4) *S. xylosus* CECT7057/*L. sakei* CECT7056; (5) *S. xylosus* CECT7057/*L. sakei* CECT7056/yeast 2RB4. In the same column, different letters represent significantly different means ($p < 0.05$). ΔE—total colour difference between control and inoculated treatments.

Significant differences were only detected for the colour coordinate H°, with sausages inoculated with *S. xylosus* CECT7057 10^8/*L. sakei* CECT7056 10^8 showing more reddish tones.

The total colour difference (**ΔE**) calculated for each inoculated treatment revealed that an unexperienced observer can notice the difference in colour for treatment 2; but only an experienced observer can notice the difference for treatments 4 and 5; and no difference in colour was noticed by the observer regarding treatment 3.

3.5. Texture Profile Analysis (TPA) of Fermented Sausages

The results for the texture profile analysis (TPA) are shown in Table 5.

Table 5. Effect of starter cultures on the TPA parameters of fermented sausages evaluated in end-products.

Treatment	Texture Parameters					
	Hardness (N)	Adhesiveness ($N \cdot s^{-1}$)	Cohesiveness	Springiness	Resilience	Chewiness (N)
1	39.025 ± 11.67	−1.685 ^{ab} ± 1.282	0.587 ^{ab} ± 0.083	0.858 ± 0.186	0.135 ^{a} ± 0.032	20.016 ± 9.241
2	44.446 ± 9.724	−2.228 ^{a} ± 1.557	0.599 ^{a} ± 0.047	0.904 ± 0.262	0.133 ^{ab} ± 0.019	23.957 ± 7.857
3	42.188 ± 14.842	−2.037 ^{ab} ± 1.325	0.570 ^{ab} ± 0.047	0.840 ± 0.070	0.124 ^{ab} ± 0.023	20.369 ± 8.244
4	45.221 ± 12.655	−1.641 ^{ab} ± 1.201	0.566 ^{b} ± 0.051	0.930 ± 0.324	0.121 ^{b} ± 0.021	23.885 ± 10.971
5	40.556 ± 10.633	−1.484 ^{b} ± 0.841	0.571 ^{ab} ± 0.046	0.893 ± 0.248	0.126 ^{ab} ± 0.022	19.970 ± 4.916

Data are expressed as means ± SD. (1) Control; (2) *S. equorum* S2M7/*L. sakei* CV3C2; (3) *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4; (4) *S. xylosus* CECT7057/*L. sakei* CECT7056; (5) *S. xylosus* CECT7057/*L. sakei* CECT7056/yeast 2RB4. In the same column, different letters represent significantly different means ($p < 0.05$).

Data analysis showed significant differences for adhesiveness, cohesiveness, and resilience.

Regarding adhesiveness, fermented sausages inoculated with *S. equorum* S2M7 10^8/L. sakei CV3C2 10^8 (-2.228 ± 1.557 $N \cdot s^{-1}$) were significantly more adhesive than those inoculated with *S. xylosus* CECT7057 10^8/*L. sakei* CECT7056 10^8/yeast 2RB4 10^6 (-1.484 ± 0.841 $N \cdot s^{-1}$).

Sausages inoculated with *S. equorum* S2M7 10^8/*L. sakei* CV3C2 10^8 also showed significantly higher cohesiveness values than sausages inoculated with *S. xylosus* CECT7057 10^8/*L. sakei* CECT7056 10^8.

Regarding resilience, control sausages showed significantly higher values than sausages inoculated with *S. xylosus* CECT7057/*L. sakei* CECT7056.

3.6. Sensory Analysis of Fermented Sausages

Regarding the results obtained for the sensory attributes (Table 6), significant differences were only found between inoculation treatments for off colours, off flavours, and overall perception. No off-colours were reported for control sausages. Regarding off-flavours, control sausages showed the lowest mean values (1.21 ± 3.78). Taking all

this into account, control sausages were the most appreciated (overall perception) by the panel, although not significantly different from all inoculated treatments. Generally, the selected yeast strain seems to have a negative effect on the sensory characteristics of fermented sausages.

Table 6. Effect of starter cultures on some sensory attributes of fermented sausages evaluated in end-products.

Treatment	Sensory Attributes						
	Colour Intensity	Off-Colours	Aroma Intensity	Flavour Intensity	Off-Flavours	Salt Perception	Overall Perception
1	74.51 ± 11.16	0.00 ^{b} ± 0.00	70.49 ± 11.25	69.00 ± 10.81	1.21 ^{b} ± 3.78	56.26 ± 9.09	67.72 ^{a} ± 11.14
2	78.69 ± 11.95	0.56 ^{ab} ± 3.06	70.98 ± 12.95	70.07 ± 9.77	2.91 ^{ab} ± 4.77	57.24 ± 8.19	62.98 ^{ab} ± 11.98
3	77.00 ± 11.59	0.47 ^{ab} ± 2.01	73.17 ± 9.66	69.31 ± 15.86	3.39 ^{ab} ± 6.63	57.97 ± 8.12	64.92 ^{ab} ± 13.58
4	77.39 ± 12.02	1.27 ^{ab} ± 4.34	69.12 ± 12.95	65.47 ± 11.00	3.55 ^{ab} ± 5.50	55.84 ± 6.46	59.33 ^{b} ± 15.40
5	77.63 ± 12.56	3.90 ^{a} ± 12.63	68.67 ± 15.96	64.85 ± 15.89	5.10 ^{a} ± 9.41	57.42 ± 11.53	58.73 ^{b} ± 14.23

Data are expressed as means ± SD. (1) Control; (2) *S. equorum* S2M7/*L. sakei* CV3C2; (3) *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4; (4) *S. xylosus* CECT7057/*L. sakei* CECT7056; (5) *S. xylosus* CECT7057/*L. sakei* CECT7056/yeast 2RB4. In the same column, different letters represent significantly different means ($p < 0.05$).

4. Discussion

The extended fermentation step added before smoking had a significant effect on the decrease of pH values and in the product's stabilisation due to the lower aW values.

Regarding microbiological parameters, meat batters showed higher counts of mesophiles, LAB, and staphylococci, as a result of inoculation with starters, provided that these are well adapted to the conditions existent in the meat batters. Similar higher counts of these microbial groups in the initial curing steps have been reported before [37,38]. However, unlike LAB that maintained their numbers almost constant throughout the cure, staphylococci significantly decreased their counts in end-products, probably due to the poor competitiveness of staphylococci against LAB, as reported before [6,13]. Moreover, the early lower aW values due to the additional fermentation step had a pronounced effect on the stabilisation of staphylococci and enterobacteria.

Our results indicate that, although the inoculated meat batters have significantly higher LAB counts, these differences between control and inoculated sausages will gradually fade away throughout the curing process. This could probably be due to the extended fermentation step that allowed both the autochthonous LAB microbiota and the inoculated LAB to grow, resulting in similar LAB numbers for both control and inoculated sausages in end-products.

Considering that enterobacteria are generally considered food hygiene indicators [7], our results show clear evidence of microbiological contamination during processing. Moreover, raw materials have a poor hygienic quality, since high enterobacteria counts are already high in the meat batters (mean values of 5 to 6 log cfu/g). The decline of enterobacteria in inoculated fermented sausages is notorious, due to the decrease in the pH values. Moreover, the extended fermentation step allowed an early control of enterobacteria, due to the dominance of LAB and their metabolites, such as bacteriocins, throughout the curing process. Similar values had been reported in previous studies [3], although these are high when compared to those of other authors [6,7].

The yeast population was controlled throughout the manufacturing process. Yeasts maintained similar concentrations along the cure, and there were no differences between treatments at any of the sampling times. Since we assured that the starter yeast strain was viable before inoculation, one may speculate that the starter yeast was outcompeted by the autochthonous yeast strains right from the start and did not establish itself during the maturation step (before stuffing).

Moulds showed relatively low numbers throughout the curing process, with no differences between treatments at all curing steps. Moreover, the presence of moulds in non-inoculated sausages is uncommon [39].

L. monocytogenes was present in most analysed samples, which is a persistent problem either in this manufacturing unit or again of the raw materials used [3]. Towards the end of the curing process, numbers were significantly reduced and were below the legal limit of 100 cfu/g according to Reg.2073/2005 [40] for most treatments. Moreover, all inoculated sausages showed a significant reduction in *L. monocytogenes* counts when compared to control sausages, which may indicate an effective bactericidal action of starters against *L. monocytogenes*. Other authors did not detect the presence of *L. monocytogenes* in Italian salami [6].

Salmonella spp. was absent in all end-product sausages.

Concerning biogenic amines, their levels generally increased along the curing process, except for β-phenylethylamine and tyramine, which is in line with the findings of Xi et al. [41] but contradicts the results obtained by Dias et al. [3] and Lu et al. [42]. These different trends might be justified by the fact that β-phenylethylamine and tyramine are mainly produced by enterococci [21], while enterobacteria are the main producers of other amines, such as cadaverine and putrescine.

Tyramine and histamine are considered the most toxic to consumers, with the European Food Safety Authority (EFSA) considering daily levels of up to 50 mg for histamine and of up to 600 mg for tyramine to be safe for healthy individuals [17,43]. In the present study, tyramine contents were lower than 2.20 mg/kg, for all samples, concentrations much lower than those obtained by other authors [44,45]. Regarding histamine, it was present at detectable levels in end-products, although with values that do not threaten the health of consumers, and close to those obtained by other authors [3,45]. Moreover, Kurt and Zorba [46] and Suzzi and Cardini [47] found that the production of β-phenylethylamine is often associated with high tyramine contents, which seems to be corroborated by our results, since both tyramine contents and β-phenylethylamine concentrations were reduced.

Tryptamine was the biogenic amine that showed the highest concentrations. High levels of tryptamine were also detected in Portalegre PGI chorizo [48], in *Catalão* and *Salsichão* [49] and in non-inoculated Serbian sausages [50]. Vidal-Carou et al. [51] refer that high tryptamine concentrations will depend on the existence of high tyramine contents, associated with the decarboxylative activity of certain LAB, but the same authors [51] also highlighted that the occurrence of tryptamine could be associated with diamines (putrescine and/or cadaverine).

The presence of the natural polyamines also followed the pattern usually found in fermented sausages, with the prevalence of spermine. About the proportion between these two amines, there are several examples in the literature where similar results have been reported [3,52].

Vasoactive amines showed values higher than 200 mg/kg in end-products, mainly due to the contribution of tryptamine [50]. Although the content in vasoactive amines is above the recommended, the total content in biogenic amines can be considered quite acceptable (below 1000 mg/kg) and sometimes even lower than the values reported by other authors for similar sausages [3,50,53].

Inoculation with starters has proven to be effective in reducing the content in biogenic amines with significantly lower values in inoculated end-products than in fermented control sausages. This reduced content in biogenic amines is most probably because of the decreased number of enterobacteria, main producers of biogenic amines, whose growth was controlled from the beginning of the manufacturing process, due to the product's stabilisation through fermentation of inoculated LAB that secrete their secondary metabolites, namely bacteriocins against enterobacteria, and the consequent decreased pH and a_W values.

Regarding the colour of fermented sausages, our results showed significant differences in the H° parameter and that the inoculation with *S. xylosus* CECT7057/*L. sakei*

CECT7056 seems to have a positive effect in the setting of the red colour. On the other hand, Bañón et al. [54] obtained similar mean L* and C* values for control and inoculated salami, while the mean H° was lower in control sausages. Differences in H° values have been reported before depending on the type of starters [55,56]. Based on the calculated total colour difference, our results showed that the difference in the colour between control and inoculated sausages was visible to the unexperienced observer regarding treatment 2 (*S. equorum* S2M7/*L. sakei* CV3C2), which has the highest colour intensity value in the sensory analysis.

There are numerous factors that can influence the texture of sausages. However, moisture reduction, pH values, salt concentration, and protein denaturation and proteolysis processes are among the most relevant. The use of starters with proteolytic activity contributes to the breakdown of proteins, with the consequent increase in hardness and adhesiveness, which is not necessarily negatively assessed in the sensory analysis. By comparison with the results obtained by Dias et al. [3], the fermented sausages analysed in the present study were less hard and less adhesive and had lower chewiness values. The hardness values were, in general, lower in 10 N, which strongly influenced chewiness values. One possible explanation for the lower hardness values may be associated to the less extensive proteolysis, which may also contribute to reduce the levels of biogenic amines.

Regarding sensory analysis, the starter consortium *S. equorum* S2M7/*L. sakei* CV3C2 seems to have a generally more positive effect than the co-inoculation with *S. xylosus* CECT7057/*L. sakei* CECT7056. Moreover, the inoculated yeast does not have a marked positive effect in the sensory appreciation of sausages, contrary to what has been reported before by other authors [57,58].

5. Conclusions

The inoculation of *Paio do Alentejo* with starter cultures played a major role on the food safety of these fermented sausages. In fact, the used starter consortia significantly reduced both *Listeria monocytogenes* counts and the total content in biogenic amines.

Furthermore, the use of starters did not compromise the standard quality of traditional fermented sausages regarding their sensory acceptability.

Moreover, the use of an extended fermentation step has contributed to the better establishment of both staphylococci and LAB starter consortia. In fact, this additional fermentation step early in the manufacturing process contributed to the stabilisation of the product due to the lower pH and a_W values, thus controlling the spoilage and pathogenic microbiota.

The present study made an important contribution towards the safety improvement of fermented foods, particularly those that are still manufactured under traditional processes that pose additional challenges to food safety.

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