



Article

Co-Inoculation with *Staphylococcus equorum* and *Lactobacillus sakei* Reduces Vasoactive Biogenic Amines in Traditional Dry-Cured Sausages

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Abstract: Dry-cured sausages are traditional in Mediterranean countries, and Paio do Alentejo (PA) is one of the most popular in South Portugal. The aim of the present work was to evaluate the effect of combined starters on the safety and quality of PA preserving its sensory quality. Physicochemical parameters, namely pH and water activity (aw), microbiological parameters, biogenic amines, color, texture, and sensory attributes were assessed. Three starter cultures were used, namely *Staphylococcus equorum* S2M7 and *Lactobacillus sakei* CV3C2, both separate and combined with the 2RB4 yeast strain at a concentration of 10⁶ cfu/g. Dextrose 0.25% was added to the meat batter. Starters had a significant effect on the reduction of aw values (0.845 to 0.823). The treatment with *L. sakei* as well as the co-inoculation of *L. sakei* with *S. equorum* effectively reduced the *L. monocytogenes* counts to undetectable levels. Sausages co-inoculated with *S. equorum* S2M7/*L. sakei* CV3C2 showed a significant reduction in the content of vasoactive amines, namely tryptamine (26.21 to 15.70) and β-phenylethylamine (4.80 to 3.69). Regarding texture, control PA showed higher hardness values, and the starters promoted the cohesiveness of the batter while reducing chewiness. The studied starters did not compromise the sensory characteristics of PA.

Keywords: dry-cured sausages; starter cultures; staphylococci; lactic acid bacteria; food safety; biogenic amines; *Listeria monocytogenes*; food quality

1. Introduction

Dry-cured sausages are traditional food products that are greatly diverse in terms of raw materials, organoleptic characteristics, and manufacturing methods. Paio do Alentejo is a popular dry-cured 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 sensory characteristics of the final products vary. In Portugal, the use of starters has not been a common practice in micro and small processing units [1]. However, manufacturing units are becoming more interested in the use of starter cultures in the production of fermented sausages, due to their potential improvement in safety and in standardizing the desirable technological properties [2–4]. These starter cultures should be autochthonous, i.e., isolated from the native microbiota of these products, so that they will be better adapted to the specific environmental conditions [5].

In fermented meat products, bacteria including lactic acid bacteria (LAB) and Gram-positive catalase-positive cocci (G⁺C⁺C), such as coagulase-negative staphylococci (CNS), but also yeasts and molds influence the technological properties of the product and its quality and safety [3,6]. Therefore, the most frequently used starters in the meat processing industry belong to the four cited microbial groups.

LAB ferment sugars thus boosting the production of lactic acid. The consequent reduction in pH reduces the growth rate of undesirable microorganisms [7,8]. However, and given the fact that LAB are among the most competitive microorganisms throughout the manufacturing process, they are considered to be biopreservatives and bioprotectors. In fact, together with intrinsic food factors, such as pH, temperature and *a_w*, they can impair the growth of pathogenic and spoilage microorganisms, making food products safer even without the use of conservation techniques, such as modified atmospheres, high pressure treatments, and chemical or other preservatives [9].

CNS are able to reduce nitrate and degrade hydrogen peroxide, with advantages at the quality and color stability level, and metabolize nitrogenous and lipid compounds, improving flavor [10,11]. According to Cocconcilli and Fontana [12], CNS have the ability to release enzymes, lipases, and proteases capable of forming low molecular weight compounds, such as peptides, amino acids, aldehydes, amines, and fatty acids that influence texture and the development of aroma compounds.

Yeasts and molds are used less frequently as starter cultures. The application of molds and yeasts as surface starter cultures, normally by immersion or spraying, can improve specific sensory and external characteristics [13]. Surface starters form a protective layer, which favors color formation and hinders the occurrence of premature autooxidation phenomena of fats due to the activity of catalase [14].

Biogenic amines (BA) are nitrogenous compounds of low molecular weight formed from amino acids by decarboxylation or from aldehydes and ketones by amination and transamination [15]. The most prevalent biogenic amines in meat and meat products are tyramine, putrescine, cadaverine, and histamine [16,17]. Formation of BA depends on the availability of specific amino acids, the presence of bacteria with decarboxylase activity, and on the establishment of conditions favorable to bacterial growth and enzymatic activity [4]. It should be noted that BA are thermostable, as further steps do not eliminate them [18] and could contribute to the formation of nitrosamines with the nitrite derivatives [19,20]. Despite some studies that have reported the inefficiency of starters in reducing the content of BA [21,22], recent works have shown that autochthonous starter cultures may control the accumulation of BA in fermented meat products [1,23,24].

The aim of the present study was to evaluate the effects of different autochthonous starter cultures used both in separate and in mixed cultures on the safety and quality of Paio do Alentejo, a traditional Portuguese sausage manufactured on a small scale in a local manufacturing unit in the Alentejo region. Moreover, starters were used to help in the

control of an existing problem with *Listeria monocytogenes* in the manufacturing unit, together with other corrective and preventive measures.

2. Materials and Methods

2.1. Dry-Cured Sausage Manufacturing and Sampling

Paio do Alentejo, a traditional dry-cured sausage, was manufactured in a local factory using commercial black pig breed (Alentejano pig breed × Duroc pig breed) meat.

Pork meat trimmings (70% lean meat/30% fat) were mechanically cut into cubes of approximately 25 mm and mixed with white wine (8.0% v/v), salt (2.5% w/w), red pepper (*Capsicum annuum* L.) paste (2.5% w/w), garlic (*Allium sativum* L.) paste (0.8% w/w), polyphosphates (0.06% w/w), nitrates (0.007% w/w), nitrites (0.003% w/w), ascorbic acid (0.03% w/w), and sodium ascorbate (0.02% w/w). A total of 150 kg of meat batter was prepared and then divided into five portions of 30 kg each. Five treatments were considered: 1—control (no starter cultures added); 2—*Staphylococcus equorum* S2M7; 3—*Lactobacillus sakei* CV3C2; 4—*S. equorum* S2M7/*L. sakei* CV3C2; and 5—*S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4.

Staphylococcus starter strains were selected for their performance in the phenotypic characterization tests, namely nitrate reductase, lipolytic and proteolytic activities, as well as absence of resistance to antimicrobials and decarboxylase activity [25]. *Lactobacillus* starter strains were selected for their bacteriocinogenic profile and the absence of both resistance to antimicrobials and decarboxylase activity [25,26].

Starter culture composition and concentrations were selected based on previous trials [27] and were inoculated in the meat batter. All cultures were inoculated to achieve a final concentration of each starter strain of 10^6 cfu/g of meat batter.

Three independent manufacturing batches of each treatment were prepared. Food grade dextrose (0.25%) was added to all treatments.

Seasoned and inoculated meat batter was stored under controlled conditions at 5 °C and 90% relative humidity (RH) for 72 h and then stuffed into desalted pork natural casings 50 to 55 mm in diameter. Sausages were smoked for 24 h at 18.0 to 24.0 °C and 28.0–72.0% RH in a traditional smokehouse. After smoking, drying was carried out in a controlled storeroom at 8.0–12.0 °C and at an RH between 60–80% for approximately 30 days 38–40% initial weight loss was reached.

Two sausages per treatment and per batch were analyzed throughout the curing process at three different steps: meat batter (immediately before stuffing), half-cured sausage (10 days after stuffing), and end-product (38–40% weight loss).

pH, a_w , microbiological parameters, and contents of biogenic amines were determined at all curing steps. Color, texture profile and sensory analyses were performed only for end-products. Samples were immediately processed for physicochemical, microbiological, and sensory analyses and stored at −20 °C until analysis of the content of biogenic amines.

2.2. Physicochemical Analyses

2.2.1. Determination of pH and a_w

For the determination of pH and a_w , samples were prepared and measurements were made as described previously [1], following ISO 2917 [28], for pH measurements. Five replicates per sample were used for both determinations.

2.2.2. Color

Color CIELab chromatic coordinates were measured as described previously [1]. Five replicates per sample were examined.

2.2.3. Texture Profile Analysis (TPA)

Texture profile analysis (TPA) was performed at room temperature (20 ± 1 °C) using a Stable Micro System TA-Hdi (Stable Micro Systems, Godalming, United Kingdom), as described previously [29,30]. Samples were prepared according to the procedures described by Dias et al. [1]. Five replicates per sample were analyzed.

2.3. Microbiological Analyses

Several microbiological parameters were analyzed following international standards and established procedures: mesophiles ISO 4833-1 [31]; psychrotrophic microorganisms ISO 17410 [32]; lactic acid bacteria ISO 15214 [33]; staphylococci [34]; yeasts and molds ISO 21527-2 [35]; enterobacteria ISO 21528-2 [36]; and *Listeria monocytogenes* ISO 11290-2 [37]. *Salmonella* spp. detection was performed with VIDAS (bioMérieux, Marcy-l'Étoile, France) and confirmed according to ISO 6579-1 [38] as described previously [34]. All microbiological analyses were performed in triplicate, and the results are expressed as log colony-forming units (cfu)/g, except for *L. monocytogenes* counts, which are reported as cfu/g.

2.4. Biogenic Amine Profiles

The content of biogenic amines was assessed as described previously [34,39]. Briefly, eight grams of each previously homogenized sample were extracted with 0.4 M perchloric acid aqueous solution and filtered. 1,7-Diaminoheptane was used as internal standard. Biogenic amines were then derivatized with dansyl chloride under alkaline conditions. The extract was diluted in acetonitrile; filtered through an Acrodisc 25 mm GHP, GF 0.45 μ m membrane (Gelman Sciences, Inc., Port Washington, NY, USA); and injected into an HPLC system (Thermo Scientific Dionex, Ultimate 3000, Waltham, MA, USA). Chromatographic conditions were as follows: A RP-18 reverse phase column (5 μ m of 4.0×125 mm and 100 Å) was used (Merck, Kenilworth, NJ, USA), coupled to an Alliance Separation Module 2695 (Waters, Milford, MA, USA), along with a gradient elution program that combines aqueous ammonium acetate solution and acetonitrile (Panreac, Barcelona, Spain), and detection was conducted at 254 nm using a Dual k UV/Vis Detector 2487 (Waters, Milford, MA, USA).

All samples were extracted in duplicate; 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 was calculated, summing tryptamine, β -phenylethylamine, histamine, and tyramine [15]. The total content of biogenic amines was the sum of each individual amine. Chromatographic data were analyzed with Chromeleon software version 6.8 (Thermo Scientific Dionex, Waltham, MA, USA).

2.5. Sensory Analysis

Panelists were selected and trained according to ISO 8586-1[40] in a sensory evaluation room prepared in accordance with ISO 8589-1 [41].

Thirty minutes prior to each session, sausages were sliced (3 mm thick) and slices randomly distributed in white dishes, each identified with a random three-digit number. Crackers and mineral water were supplied to the panelists as palate cleansers.

The sensory evaluation attributes studied were color intensity, off-colors, marbled appearance, aroma intensity, and off-aromas. The panelists were asked to evaluate these attributes using a quantitative descriptive analysis with a scale ranging from 0 to 100 corresponding to “no perception” or “maximum perception”. Due to the presence of *Salmonella* spp. in some samples, only a visual and olfactory sensory analysis was performed. Each of the 10 panelists evaluated six samples per session.

2.6. Statistical Analysis

Data were analyzed 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 a_w

Table 1 summarizes the results for pH and a_w of sausages subjected to the different treatments throughout the curing process.

For pH, significantly different mean pH values were observed between meat batter and the other two curing steps (half-cured sausages and end-products). Regarding an evaluation by curing step, the sausages inoculated with *S. equorum* S2M7/*L. sakei* CV3C2 showed an initial mean value (5.29 ± 0.51) significantly lower than that of sausages with *L. sakei* CV3C2 (5.48 ± 0.31). Regarding end-products, sausages inoculated with *L. sakei* CV3C2 had the lowest mean pH value (4.94 ± 0.07), and the only pH mean value lower than that of the control (4.97 ± 0.14).

As for a_w , significant differences were observed between curing steps, with significantly lower mean values for the end-products. Concerning meat batter, inoculated sausages generally showed lower a_w values. Regarding half-cured sausages, control sausages still had a significantly higher mean a_w (0.948 ± 0.007). Except for the end-products inoculated with *L. sakei* CV3C2 (0.852 ± 0.002), all other sausages presented a significant reduction in the a_w mean values when compared to the control (0.845 ± 0.024), thus contributing to their safety.

Table 1. Effect of starter cultures on pH and a_w of sausages.

Parameters	Treatment	Curing Steps		
		Meat Batter	Half-Cured Sausage	End-Product
pH	1	5.48 ^{A,ab} ± 0.25	5.05 ^{B,c} ± 0.08	4.97 ^{B,bc} ± 0.14
	2	5.46 ^{A,ab} ± 0.28	5.20 ^{B,a} ± 0.09	5.05 ^{B,ab} ± 0.14
	3	5.48 ^{A,a} ± 0.31	5.13 ^{B,b} ± 0.09	4.94 ^{B,c} ± 0.07
	4	5.29 ^{A,b} ± 0.51	5.06 ^{B,c} ± 0.09	5.10 ^{AB,a} ± 0.01
	5	5.42 ^{A,ab} ± 0.32	5.19 ^{B,a} ± 0.09	5.10 ^{B,a} ± 0.10
a_w	1	0.967 ^{A,a} ± 0.008	0.948 ^{B,a} ± 0.007	0.845 ^{C,a} ± 0.024
	2	0.962 ^{A,bc} ± 0.006	0.937 ^{B,bc} ± 0.009	0.826 ^{C,b} ± 0.031
	3	0.960 ^{A,b} ± 0.008	0.941 ^{B,b} ± 0.004	0.852 ^{C,a} ± 0.002
	4	0.960 ^{A,b} ± 0.007	0.941 ^{B,b} ± 0.004	0.823 ^{C,b} ± 0.030
	5	0.963 ^{A,ab} ± 0.002	0.934 ^{B,c} ± 0.004	0.824 ^{C,b} ± 0.014

Data are expressed as means $\pm$ SD. 1—Control; 2—*S. equorum* S2M7; 3—*L. sakei* CV3C2; 4—*S. equorum* S2M7/*L. sakei* CV3C2; 5—*S. equorum* S2M7/*L. sakei* CV3C2/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–c}) represent significantly different means ($p < 0.05$).

3.2. Characterization of the Microbiota of Sausages

Table 2 shows no differences between control and inoculated sausages for the same curing step. However, in end-products, inoculated sausages tended to have higher counts of mesophiles, psychrotrophic microorganisms, and LAB. Regarding staphylococci, the sausages inoculated with *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4 showed the highest number.

No significant differences were observed for enterobacteria between treatments; however, their mean values were significantly lower in end-products, probably associated with the increase in LAB and the consequently lower pH and the lower a_w values.

L. monocytogenes was present in all curing steps. When *L. sakei* CV3C2 was inoculated alone or combined with *S. equorum* S2M7, the elimination of *L. monocytogenes* was more effective, to levels below the legal limit of 100 cfu/g, according to regulation 2073/2005 [42].

Salmonella spp. were present throughout the curing process but absent in end-products, with the exception of sausages inoculated with *S. equorum* S2M7.

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

Parameters	Treatment	Curing Steps		
		Meat Batter	Half-Cured Sausage	End-Product
mesophiles	1	7.00 ^B ± 0.77	8.46 ^A ± 0.67	7.38 ^B ± 0.60
	2	7.23 ^B ± 0.96	7.70 ^A ± 0.65	7.65 ^{AB} ± 0.54
	3	7.16 ± 0.70	7.77 ± 0.29	8.39 ± 0.97
	4	7.72 ± 1.20	8.61 ± 0.82	8.03 ± 1.06
	5	7.35 ± 0.76	8.12 ± 1.07	8.48 ± 1.19
psychrotrophic microorganisms	1	6.60 ± 1.17	7.01 ± 1.22	5.66 ± 0.29
	2	6.81 ^A ± 0.99	6.50 ^{AB} ± 0.37	5.69 ^B ± 0.26
	3	6.74 ± 0.75	6.51 ± 0.58	6.20 ± 0.18
	4	7.32 ± 1.50	7.45 ± 1.29	5.89 ± 0.44
	5	7.18 ± 1.09	7.01 ± 1.22	6.48 ± 0.52
LAB	1	6.64 ^B ± 0.57	7.95 ^A ± 0.30	8.06 ^A ± 0.77
	2	6.58 ^B ± 0.39	7.59 ^A ± 0.28	7.96 ^A ± 0.67
	3	6.32 ^B ± 0.27	7.70 ^A ± 0.45	8.49 ^A ± 1.15
	4	6.80 ^B ± 0.60	7.93 ^{AB} ± 0.14	8.15 ^A ± 1.09
	5	7.01 ^B ± 0.26	8.18 ^{AB} ± 1.11	8.56 ^A ± 1.10
staphylococci	1	9.14 ± 0.66	10.17 ± 1.77	8.68 ± 1.03
	2	8.97 ± 1.42	9.26 ± 0.98	8.34 ± 0.49
	3	7.57 ± 1.48	9.14 ± 0.79	8.49 ± 0.71
	4	10.88 ± 3.96	10.47 ± 1.47	8.38 ± 2.14
	5	8.40 ± 1.74	9.66 ± 1.10	10.31 ± 1.29
enterobacteria	1	5.99 ^A ± 0.49	6.35 ^A ± 1.13	2.75 ^B ± 0.36
	2	6.55 ^A ± 1.16	5.54 ^A ± 0.24	2.69 ^B ± 0.50
	3	6.73 ^A ± 0.67	5.63 ^A ± 0.40	2.24 ^B ± 0.39
	4	7.02 ^A ± 1.34	6.45 ^A ± 1.04	2.48 ^B ± 0.40
	5	6.54 ^A ± 0.42	6.62 ^A ± 1.30	2.51 ^B ± 0.55
yeasts	1	3.88 ± 0.48	4.33 ± 1.09	4.61 ± 0.35
	2	3.78 ^B ± 0.23	3.15 ^C ± 0.18	4.70 ^A ± 0.47
	3	3.90 ± 0.90	3.82 ± 0.85	4.85 ± 0.42
	4	5.73 ± 2.44	4.56 ± 0.69	4.96 ± 0.74
	5	4.04 ± 0.37	4.47 ± 0.77	4.74 ± 0.69
molds	1	0.17 ± 0.41	0.67 ± 1.21	0.58 ± 1.20
	2	0.50 ± 0.84	0.25 ± 0.60	<DL
	3	<DL	<DL	0.33 ± 0.82
	4	<DL	<DL	<DL
	5	<DL	<DL	<DL
<i>L. monocytogenes</i>	1	2.22 ± 2.38	1.52 ± 1.91	2.06 ± 2.38
	2	2.00 ± 2.10	1.82 ± 1.91	2.17 ± 2.37

	3	2.12 ± 2.17	2.47 ± 2.73	<DL
	4	2.14 ± 2.22	2.26 ± 2.06	<DL
	5	2.50 ± 2.55	1.92 ± 1.87	2.17 ± 2.43
<i>Salmonella</i> spp.	1	present in 6/6 samples	present in 6/6 samples	ND
	2	present in 5/6 samples	present in 6/6 samples	present in 1/6 samples
	3	present in 1/6 samples	present in 2/6 samples	ND
	4	present in 5/6 samples	present in 4/6 samples	ND
	5	present in 3/6 samples	present in 3/6 samples	ND

Data are expressed as means ± SD. < DL: below the detection limit of the corresponding analytical method (10 cfu/g for molds 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; 3—*L. sakei* CV3C2; 4—*S. equorum* S2M7/*L. sakei* CV3C2; 5—*S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4. For the same treatment and in the same row, distinct capital letters (^{A–C}) represent significantly different means ($p < 0.05$).

3.3. Biogenic Amines

Table 3 generally shows that the content of biogenic amines decreased throughout the curing process. Moreover, the content of biogenic amines of inoculated sausages was lower than that of control sausages throughout the entire process.

Natural polyamines, namely spermidine and spermine, did not show large variations in their mean values during the curing process. Cadaverine, putrescine, and tyramine were the most abundantly detected biogenic amines in end-products, in descending order.

The contents in histamine and tyramine reduced over time and were lower in end-products (13 and 114 mg/kg, respectively).

Sausages inoculated with *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4 had the highest mean values (148.12 ± 20.75 mg/kg), while the co-inoculation of *S. equorum* S2M7/*L. sakei* CV3C2 significantly reduced the content of vasoactive biogenic amines. Moreover, the total content of biogenic amines globally decreased during ripening, with higher contents in control end-product sausages (973.01 ± 140.14 mg/kg) and sausages inoculated with *S. equorum* S2M7/*L. sakei* CV3C2 (792.72 ± 175.93 mg/kg) showing significantly lower contents. Concerning end-products, all treatments showed a mean content of total biogenic amines below 1000 mg/kg.

Table 3. Effect of starter cultures on the content of biogenic amines (mg/kg fresh weight) of sausages.

Parameters	Treatment	Curing Steps		
		Meat Batter	Half-Cured Sausage	End-Product
tryptamine	1	50.42 ^{A,ab} ± 5.78	38.46 ^{B,ab} ± 5.80	26.21 ^{C,ab} ± 5.59
	2	35.66 ^{A,c} ± 11.95	26.79 ^{B,c} ± 4.32	14.73 ^{C,c} ± 4.61
	3	43.44 ^{A,bc} ± 5.42	31.50 ^{B,bc} ± 5.40	19.28 ^{C,bc} ± 5.20
	4	40.32 ^{A,bc} ± 6.28	25.88 ^{B,c} ± 9.38	15.70 ^{C,c} ± 6.08
	5	59.61 ^{A,a} ± 16.64	47.60 ^{AB,a} ± 16.97	35.60 ^{B,a} ± 16.43
β-phenylethylamine	1	20.22 ^A ± 0.84	12.75 ^{B,a} ± 0.87	4.80 ^{C,a} ± 0.83
	2	17.63 ^A ± 5.50	11.75 ^{B,ab} ± 0.31	3.85 ^{C,b} ± 0.28
	3	19.36 ^A ± 0.72	11.90 ^{B,ab} ± 0.72	3.98 ^{C,b} ± 0.70
	4	19.08 ^A ± 0.56	10.77 ^{B,b} ± 3.09	3.69 ^{C,b} ± 0.51
	5	20.47 ^A ± 0.47	13.00 ^{B,a} ± 0.46	5.16 ^{C,a} ± 0.57
putrescine	1	466.47 ^{A,a} ± 51.42	401.79 ^B ± 51.69	329.11 ^C ± 50.82
	2	366.30 ^b ± 145.51	327.98 ± 91.38	255.70 ± 90.69
	3	407.36 ^{A,ab} ± 50.99	342.86 ^B ± 51.05	270.25 ^C ± 50.34
	4	422.68 ^{A,ab} ± 79.72	324.99 ^B ± 116.88	278.92 ^B ± 65.91
	5	417.69 ^{A,ab} ± 67.86	352.77 ^A ± 67.19	283.86 ^B ± 77.31
cadaverine	1	570.34 ^A ± 100.89	517.32 ^{AB,a} ± 101.11	439.42 ^C ± 98.35

	2	488.14 ± 157.62	483.83 ^{ab} ± 46.10	407.69 ± 47.15
	3	533.10 ^A ± 72.79	480.35 ^{A,ab} ± 73.51	403.29 ^B ± 71.73
	4	492.71 ^A ± 90.66	393.79 ^{AB,b} ± 128.61	353.27 ^B ± 49.91
	5	485.12 ^A ± 51.29	431.95 ^{B,ab} ± 51.66	360.81 ^C ± 89.05
histamine	1	32.81 ^{A,ab} ± 9.10	25.72 ^{A,ab} ± 9.03	10.58 ^{B,ab} ± 8.01
	2	29.54 ^{A,ab} ± 11.34	24.81 ^{A,ab} ± 6.72	10.13 ^{B,ab} ± 5.03
	3	30.99 ^{A,ab} ± 7.51	23.93 ^{A,ab} ± 7.46	8.20 ^{B,ab} ± 6.92
	4	26.50 ^{A,b} ± 2.24	18.06 ^{B,b} ± 5.57	3.17 ^{C,b} ± 2.20
	5	36.21 ^{A,a} ± 4.01	29.13 ^{B,a} ± 4.01	12.96 ^{C,a} ± 3.92
tyramine	1	162.13 ^A ± 33.62	139.50 ^{AB} ± 33.49	113.99 ^B ± 32.99
	2	141.65 ± 60.07	134.18 ± 41.76	108.80 ± 40.96
	3	137.87 ^A ± 17.37	115.31 ^B ± 17.25	89.72 ^C ± 16.95
	4	136.85 ^A ± 21.41	104.22 ^B ± 35.39	88.44 ^B ± 21.49
	5	142.85 ^A ± 19.95	120.22 ^B ± 20.06	94.40 ^C ± 19.52
spermidine	1	12.48 ^A ± 1.34	12.01 ^{AB} ± 1.33	11.02 ^B ± 1.38
	2	11.16 ± 3.71	11.79 ± 1.36	10.86 ± 1.34
	3	12.19 ^A ± 1.14	11.72 ^{AB} ± 1.12	10.78 ^C ± 1.12
	4	12.24 ± 0.73	10.83 ± 3.11	11.37 ± 0.94
	5	12.75 ^A ± 0.90	12.28 ^{AB} ± 0.90	10.82 ^b ± 0.78
spermine	1	46.81 ± 11.12	42.89 ± 11.06	37.88 ± 10.92
	2	40.71 ± 16.30	40.96 ± 10.39	35.97 ± 10.21
	3	43.28 ± 9.46	39.37 ± 9.39	34.40 ± 9.31
	4	44.26 ^A ± 6.05	36.77 ^B ± 11.58	38.16 ^{AB} ± 5.95
	5	47.17 ^A ± 6.16	43.24 ^{AB} ± 6.16	35.29 ^B ± 6.24
vasoactive amines	1	265.58 ^A ± 36.90	216.44 ^{B,a} ± 36.64	155.58 ^{C,a} ± 37.29
	2	224.48 ^A ± 80.05	197.53 ^{A,ab} ± 39.78	137.51 ^{B,ab} ± 39.95
	3	231.66 ^A ± 21.72	182.64 ^{B,ab} ± 21.41	121.18 ^{C,ab} ± 21.95
	4	222.75 ^A ± 22.37	158.93 ^{B,b} ± 48.96	111.00 ^{C,b} ± 23.66
	5	259.15 ^A ± 20.21	209.95 ^{B,a} ± 20.03	148.12 ^{C,a} ± 20.75
total amines	1	1361.68 ^A ± 141.42	1190.45 ^{B,a} ± 141.92	973.01 ^{C,a} ± 140.14
	2	1130.79 ^A ± 381.98	1062.09 ^{AB,ab} ± 146.00	847.73 ^{C,ab} ± 149.84
	3	1227.58 ^A ± 102.13	1056.95 ^{B,ab} ± 103.01	839.90 ^{C,ab} ± 101.51
	4	1194.64 ^A ± 177.13	925.30 ^{A,b} ± 295.33	792.72 ^{B,b} ± 175.93
	5	1221.88 ^A ± 95.62	1050.20 ^{B,ab} ± 94.67	838.90 ^{C,ab} ± 94.05

Data are expressed as means ± SD. 1—Control; 2—*S. equorum* S2M7; 3—*L. sakei* CV3C2; 4—*S. equorum* S2M7/*L. sakei* CV3C2; 5—*S. equorum* S2M7/*L. sakei* CV3C2/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–c}) represent significantly different means ($p < 0.05$).

3.4. Color

Table 4 summarizes the color data for each treatment. Regarding L*, significant differences were observed between treatments with the sausages co-inoculated with *S. equorum*/*L. sakei* CV3C2/yeast 2RB4 being the darkest. No significantly different results were obtained for all other color parameters.

Table 4. Effect of starter cultures on the color parameters of end-product sausages.

Treatment	Color Parameters				
	L * (Lightness)	a * (Redness/Greenness)	b * (Yellowness/Blueness)	C * (Chroma)	H° (Hue Angle)
1	42.32 ^a ± 4.63	18.58 ± 2.86	15.64 ± 5.00	24.44 ± 6.74	39.16 ± 6.74
2	43.41 ^a ± 5.03	19.43 ± 3.66	15.72 ± 5.26	25.14 ± 5.81	38.13 ± 5.91
3	41.32 ^{ab} ± 4.26	19.15 ± 3.80	15.87 ± 5.53	25.00 ± 6.17	38.69 ± 6.14
4	42.00 ^a ± 4.66	19.13 ± 2.97	16.26 ± 4.40	25.21 ± 4.79	39.80 ± 5.31
5	38.14 ^b ± 5.24	18.37 ± 2.61	15.02 ± 4.79	23.90 ± 4.62	38.55 ± 6.42

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

3.5. Texture Profile Analysis (TPA)

The results for the texture profile analysis (TPA) are shown in Table 5. Hardness values tended to be higher in control sausages. Regarding cohesiveness and resilience, no statistical differences were observed between treatments. Nevertheless, sausages inoculated with *L. sakei* CV3C2 showed the highest values, which might indicate more cohesive meat batter. For chewiness, higher values were obtained in the control treatment, which indicates that the inoculated sausages were easier to chew.

Table 5. Effect of starter cultures on TPA parameters of end-product sausages.

Treatment	Texture Parameters					
	Hardness (N)	Adhesiveness (N s ⁻¹)	Cohesiveness	Springiness	Resilience	Chewiness (N)
1	63.169 ^a ± 15.151	−3.398 ± 1.741	0.594 ^{ab} ± 0.035	0.881 ± 0.094	0.133 ^{ab} ± 0.014	33.325 ^a ± 10.504
2	49.606 ^c ± 10.171	−2.778 ± 1.529	0.600 ^{ab} ± 0.053	0.913 ± 0.097	0.134 ^{ab} ± 0.029	27.036 ^b ± 6.168
3	58.404 ^{ab} ± 14.308	−2.837 ± 1.852	0.622 ^a ± 0.058	0.901 ± 0.173	0.144 ^a ± 0.022	32.158 ^{ab} ± 8.002
4	52.785 ^{bc} ± 9.826	−3.003 ± 1.827	0.581 ^b ± 0.044	0.889 ± 0.070	0.128 ^b ± 0.025	27.192 ^b ± 5.355
5	51.220 ^{bc} ± 11.199	−2.629 ± 1.553	0.609 ^{ab} ± 0.046	0.966 ± 0.256	0.136 ^{ab} ± 0.016	29.777 ^{ab} ± 8.926

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

3.6. Sensory Analysis

Regarding sensory analysis (Table 6), the panelists did not detect significant differences for any of the evaluated attributes. Nevertheless, *L. sakei* CV3C2 inoculated sausages presented the highest mean color intensity (74 ± 15) and lowest mean value for off colors (0 ± 1). Control sausages had a lower aroma intensity (67 ± 17), and those inoculated with *S. equorum* S2M7/*L. sakei* CV3C2/yeast 2RB4 the highest (74 ± 13).

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

Treatment	Sensory Attributes				
	Color Intensity	Off Colors	Marbled	Aroma Intensity	Off Aromas
1	72 ± 15	1 ± 2	64 ± 16	67 ± 17	3 ± 4
2	73 ± 14	1 ± 2	66 ± 16	71 ± 11	3 ± 4
3	74 ± 15	0 ± 1	67 ± 17	73 ± 17	3 ± 4
4	67 ± 18	1 ± 3	67 ± 16	72 ± 11	3 ± 5
5	69 ± 19	1 ± 3	63 ± 19	74 ± 13	3 ± 6

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

4. Discussion

Paio do Alentejo is a traditionally manufactured dry-cured high-quality sausage with characteristic organoleptic features that however needs to meet all legal standardization and food safety criteria.

Although pH and a_w usually contribute to the stability of sausages [13], in this work, starters did not have a noticeable effect on the pH of sausages. The fact that starters were not able to significantly lower pH, compared to the control, indicates that the lactic microbiota naturally present in the meat batter (to which dextrose was provided) also exhibits a high acidifying ability. In fact, dextrose can be immediately metabolized by all LAB present in the meat batter, autochthonous and starters, as their main source of energy. Our pH values were lower than those of Elias et al. [43] for Paio do Alentejo inoculated with a commercial culture (TEXEL® ELSE BR) of *Lactobacillus* spp., *Micrococcaceae*, and yeast and an experimental starter culture with *L. sakei*/S. *xylosus*, and those of Simion et al. [44] for traditional Romanian sausages (Dacia) inoculated with a mixed culture of *L. sakei* CECT5764 and *S. equorum* SA25. One possible reason for our lower pH values is the use of dextrose (0.25%).

Regarding a_w , inoculated sausages generally showed lower values, therefore contributing to food safety. pH also contributes to the drying process, due to the decrease in the water holding capacity of meat proteins, when pH values reach the isoelectric point (5.0–5.2), with the consequent reduction in a_w [45,46]. Control sausages (0.845 ± 0.024) and sausages inoculated with *L. sakei* CV3C2 (0.852 ± 0.002) had significantly higher a_w values, probably because they had lower pH values when compared to the other treatments. Our a_w values are similar to those of Simion et al. [44] and lower than those of other authors [1,43,47].

Enterobacteria counts were 2.24–2.75 log cfu/g in end-product sausages, which are borderline values for ready-to-eat foods according to the Health Protection Agency guidelines (2–4 log cfu/g) [48]. However, similar results been reported previously for dry-fermented sausages from Portugal and other Mediterranean countries [25,29]. Nevertheless, these values are higher than those reported by other authors for Portuguese and Italian sausages, respectively [1,49], indicating the need to improve hygiene procedures and to use better quality raw materials.

In present study, *L. monocytogenes* was present in most analyzed samples. Other authors reported the presence of *L. monocytogenes* in inoculated and non-inoculated sausages, but this presence was drastically reduced throughout the curing process, in some cases, to values below the detection limit of the method [47,50]. However, Lebert et al. [51] confirmed the presence of *L. monocytogenes* in three of nine ready-to-eat sausages produced in France, with mean values between 1.2 and 2.8 log cfu/g, i.e., values sometimes higher than those obtained in the present study and exceeding the legal limit (100 cfu/g) [42].

Salmonella spp. were absent in end-products, except in those inoculated with *S. equorum* S2M7. Some outbreaks caused by *Salmonella* spp. have been identified in European fermented sausages, such as those reported by Gossner et al. [52] and Kuhn et al. [53] for a French sausage and a Danish salami, respectively. Biogenic amines levels generally decreased throughout the curing process. Although this is not always the case, other authors have reported a similar behavior [54]. On the contrary, Xie et al. [55] verified increases throughout the production process. Laranjo et al. [56] and Simion et al. [44] showed average values that did not follow the same trend for all amines, i.e., some contents increased, others decreased, and others increased until the intermediate stage of curing and decreased again in the finished product. These variations are likely associated with the manufacturing process as well as with the microbiota that has a major influence on the decarboxylation of amino acids, precursors of biogenic amines [13,20].

Dry-fermented sausages can easily accumulate high levels of BA, especially putrescine, cadaverine, and tyramine, the most abundant biogenic amines in the present study

[16,17,57], probably due to the high numbers of enterobacteria, LAB, and staphylococci, the main bacterial groups responsible for the formation of BA [15].

Histamine and tyramine are the most toxic biogenic amines [58,59] and are consequently very relevant for food safety [60]. Nuñez et al. [61] reported that for healthy adults, foods containing more than 500 mg/kg histamine and 1000 mg/kg tyramine are considered toxic or likely to jeopardize consumer health. The concentrations of histamine (3.17 ± 2.20 to 12.96 ± 3.92 mg/kg) and tyramine (88.44 ± 21.49 to 113.99 ± 32.99 mg/kg) obtained in end-products in the present work were much lower than those indicated by [61], and the treatment with *S. equorum* S2M7/*L. sakei* CV3C2 showed the lowest concentrations in all curing steps.

The co-inoculation of *S. equorum* S2M7 with *L. sakei* CV3C2 promoted of 70% reduction in the histamine content when compared to the control sausages in end-products. Authors such as Wang et al. [62] and Casquete et al. [63] also observed pronounced reductions in the content of histamine in sausages inoculated with starter cultures.

For vasoactive amines, Papavergou et al. [64] suggest 200 mg/kg as an indicator of good manufacturing practices and safe consumption. In the present study we observed a reduction throughout the curing process, and in end-products all sausages showed values below 200 mg/kg. Nevertheless, sausages inoculated with *S. equorum* S2M7/*L. sakei* CV3C2 significantly had the lowest mean value, representing 28.65% fewer vasoactive amines (111.00 ± 23.66 mg/kg), than control sausages (155.58 ± 37.29 mg/kg). This corroborates the previous starter selection, which showed that *S. equorum* S2M7 and *L. sakei* CV3C2 were low producers of biogenic amines [26].

In general, inoculated sausages had lower concentrations of biogenic amines in end-products, except for the treatment with the yeast strain, which seemed to increase the levels of tryptamine and histamine. Higher contents in biogenic amines had been reported previously for sausages inoculated with *Debaryomyces* and *Candida* strains [65].

In the present work, no significant differences were observed between the different treatments regarding most color parameters, as had been reported previously by [62,66–68] contrary to the findings of Ravyts et al. [69] and Talon et al. [70], who reported the positive contribution of starter cultures to sausage color.

The fact that the control sausages were harder could be associated with some proteolytic action of starters that softened the inoculated sausages [71,72].

In general, we may conclude that inoculation with starters did not depreciate the sensory characteristics of the sausages as had been reported previously by others [44] and even seemed to have some positive effect, namely in terms of aroma intensity, which had also been reported by other authors [73,74].

5. Conclusions

The inoculation of Paio do Alentejo with starters did not have a noticeable effect on the pH or improve color. However, significantly lower aw values were obtained for inoculated sausages, except for sausages inoculated with *L. sakei* CV3C2.

The absence of significant differences, particularly for LAB, staphylococci, and yeasts, between inoculated and control sausages could be explained by the fact that starters do not “add” to the established microbiota but rather replace it by competitive exclusion.

The co-inoculation of *S. equorum* S2M7 and *L. sakei* CV3C2 promoted a reduction close to 30% and 20% respectively for vasoactive and total amines.

Regarding texture parameters, control sausages showed higher hardness values, and the use of starter cultures promoted the cohesiveness of meat batter and the reduction of chewiness.

In summary, the co-inoculation of Paio do Alentejo with *S. equorum* and *L. sakei* significantly reduced vasoactive biogenic amines. Moreover, the use of starter cultures did not compromise the quality of traditional dry-cured sausages regarding their sensory acceptability.

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References

1. Dias, I.; Laranjo, M.; Potes, M.E.; Aguilheiro-Santos, A.C.; Ricardo-Rodrigues, S.; Fialho, A.R.; Véstia, J.; Fraqueza, M.J.; Oliveira, M.; Elias, M. Autochthonous starter cultures are able to reduce biogenic amines in a traditional portuguese smoked fermented sausage. *Microorganisms* **2020**, *8*, 16–19.
2. Xiao, Y.; Liu, Y.; Chen, C.; Xie, T.; Li, P. Effect of *Lactobacillus plantarum* and *Staphylococcus xylosus* on flavour development and bacterial communities in Chinese dry fermented sausages. *Food Res. Int.* **2020**, *135*, 109247.
3. Laranjo, M.; Potes, M.E.; Elias, M. Role of starter cultures on the safety of fermented meat products. *Front. Microbiol.* **2019**, *10*, 853.
4. Lorenzo, J.M.; Munekata, P.E.S.; Domínguez, R. Role of autochthonous starter cultures in the reduction of biogenic amines in traditional meat products. *Curr. Opin. Food Sci.* **2017**, *14*, 61–65.
5. Vinicius De Melo Pereira, G.; De Carvalho Neto, D.P.; Junqueira, A.C.D.O.; Karp, S.G.; Letti, L.A.J.; Magalhães Júnior, A.I.; Soccol, C.R. A Review of Selection Criteria for Starter Culture Development in the Food Fermentation Industry. *Food Rev. Int.* **2020**, *36*, 135–167.
6. Dos Santos Cruxen, C.E.; Funck, G.D.; Haubert, L.; da Silva Dannenberg, G.; de Lima Marques, J.; Chaves, F.C.; da Silva, W.P.; Fiorentini, Â.M. Selection of native bacterial starter culture in the production of fermented meat sausages: Application potential, safety aspects, and emerging technologies. *Food Res. Int.* **2019**, *122*, 371–382.
7. Florou-Paneri, P.; Christaki, E.; Bonos, E. Lactic Acid Bacteria as Source of Functional Ingredients. In *Lactic Acid Bacteria-R & D for Food, Health and Livestock Purposes*, 1st ed.; IntechOpen: London, UK, 2013; pp. 589–614.
8. Bassi, D.; Puglisi, E.; Cocconcelli, P.S. Comparing natural and selected starter cultures in meat and cheese fermentations. *Curr. Opin. Food Sci.* **2015**, *2*, 118–122.
9. Kröckel, L. The Role of Lactic Acid Bacteria in Safety and Flavour Development of Meat and Meat Products. In *Lactic Acid Bacteria-R & D for Food, Health and Livestock Purposes*, 1st ed.; IntechOpen: London, UK, 2013; pp. 129–156.
10. Laranjo, M.; Elias, M.; Fraqueza, M.J. The Use of Starter Cultures in Traditional Meat Products. *J. Food Qual.* **2017**, *2017*, 9546026.
11. Flores, M.; Corral, S.; Cano-García, L.; Salvador, A.; Belloch, C. Yeast strains as potential aroma enhancers in dry fermented sausages. *Int. J. Food Microbiol.* **2015**, *212*, 16–24.
12. Cocconcelli, P.S.; Fontana, C. Bacteria. In *Handbook of Fermented Meat and Poultry*, 2nd ed.; Toldra, F., Ed.; Wiley Blackwell: Hoboken, NJ, USA, 2015; pp. 117–128.
13. García-Díez, J.; Saraiva, C. Use of starter cultures in foods from animal origin to improve their safety. *Int. J. Environ. Res. Public Health* **2021**, *18*, 2544.
14. Selgas, M.D.; Garcia, M.L. Starter Cultures: Yeasts. In *Handbook of Fermented Meat and Poultry*, 1st ed.; Toldra, F., Ed.; Wiley Blackwell: Hoboken, NJ, USA, 2007; pp. 159–169.
15. Elias, M.; Fraqueza, M.J.; Laranjo, M. *Biogenic Amines in Food: Presence and Control Measures*; Stadnik, J., Ed.; Nova Science Publishers: New York, NY, USA, 2018; pp. 129–176.
16. Latorre-Moratalla, M.L.; Bover-Cid, S.; Talon, R.; Garriga, M.; Zannardi, E.; Ianieri, A.; Fraqueza, M.J.; Elias, M.; Drosinos, E.H.; Vidal-Carou, M.C. Strategies to reduce biogenic amine accumulation in traditional sausage manufacturing. *LWT Food Sci. Technol.* **2010**, *43*, 20–25.

17. Singh, V.P.; Pathak, V.; Verma, A.K. Fermented meat products: Organoleptic qualities and biogenic amines—A review. *Am. J. Food Technol.* **2012**, *7*, 278–288.
18. Ruiz-Capillas, C.; Herrero, A.M. Impact of biogenic amines on food quality and safety. *Foods* **2019**, *8*, 62.
19. Fraqueza, M.J.; Laranjo, M.; Elias, M.; Patarata, L. Microbiological hazards associated with salt and nitrite reduction in cured meat products: Control strategies based on antimicrobial effect of natural ingredients and protective microbiota. *Curr. Opin. Food Sci.* **2021**, *38*, 32–39.
20. Barbieri, F.; Montanari, C.; Gardini, F.; Tabanelli, G. Biogenic amine production by lactic acid bacteria: A review. *Foods* **2019**, *8*, 17.
21. Latorre-Moratalla, M.L.; Bover-Cid, S.; Bosch-Fusté, J.; Vidal-Carou, M.C. Influence of technological conditions of sausage fermentation on the aminogenic activity of *L. curvatus* CTC273. *Food Microbiol.* **2012**, *29*, 43–48.
22. Bozkurt, H.; Erkmen, O. Effects of starter cultures and additives on the quality of Turkish style sausage (sucuk). *Meat Sci.* **2002**, *61*, 149–156.
23. Lu, S.; Ji, H.; Wang, Q.; Li, B.; Li, K.; Xu, C.; Jiang, C. The effects of starter cultures and plant extracts on the biogenic amine accumulation in traditional Chinese smoked horsemeat sausages. *Food Control* **2015**, *50*, 869–875.
24. Sun, Q.; Chen, Q.; Li, F.; Zheng, D.; Kong, B. Biogenic amine inhibition and quality protection of Harbin dry sausages by inoculation with *Staphylococcus xylosus* and *Lactobacillus plantarum*. *Food Control* **2016**, *68*, 358–366.
25. Fraqueza, M.J.; Rocha, J.M.; Laranjo, M.; Potes, M.E.; Fialho, A.R.; Fernandes, M.J.; Fernandes, M.H.; Barreto, A.; Semedo-Lemsaddek, T.; Elias, M. What is the Main Processing Factor Influencing *Staphylococcus* Species Diversity in Different Manufacturing Units? *J. Food Sci.* **2019**, *84*, 2932–2943.
26. Alfaia, C.M.; Gouveia, I.M.; Fernandes, M.H.; Fernandes, M.J.; Semedo-Lemsaddek, T.; Barreto, A.S.; Fraqueza, M.J. Assessment of Coagulase-Negative *Staphylococci* and Lactic Acid Bacteria Isolated from Portuguese Dry Fermented Sausages as Potential Starters Based on Their Biogenic Amine Profile. *J. Food Sci.* **2018**, *83*, 2544–2549.
27. Dias, I. *Aplicação de Culturas Microbianas Autóctones na Produção de Enchidos Tradicionais do Alentejo e da Beira Baixa*; Universidade de Évora: Évora, Portugal, 2018.
28. ISO. *Meat and Meat Products—Measurement of pH—Reference Method*; ISO: Geneva, Switzerland, 1999.
29. Fraqueza, M.J.; Laranjo, M.; Alves, S.; Fernandes, M.H.; Cristina, A.; Santos, A.; Fernandes, M.J.; Potes, M.E.; Elias, M. Dry-Cured Meat Products According to the Smoking Regime: Process Optimization to Control Polycyclic Aromatic Hydrocarbons. *Foods* **2020**, *9*, 91.
30. Laranjo, M.; Agulheiro-Santos, A.C.; Potes, M.E.; Cabrita, M.J.; Garcia, R.; Fraqueza, M.J.; Elias, M. Effects of genotype, salt content and calibre on quality of traditional dry-fermented sausages. *Food Control* **2015**, *56*, 119–127.
31. ISO. *Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 1, Colony Count at 30 Degrees C by the Pour Plate Technique*. Geneva, Switzerland: Volume ISO 4833-1, 2013. Available online: <https://www.iso.org/obp/ui/#iso:std:iso:4833:-1:ed-1:v1:en> (accessed on 1 July 2021).
32. ISO. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Psychrotrophic Microorganisms*; ISO: Geneva, Switzerland, 2001.
33. ISO. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria—Colony-Count Technique at 30 Degrees C*; ISO: Geneva, Switzerland, 2013.
34. Laranjo, M.; Gomes, A.; Agulheiro-Santos, A.C.; Potes, M.E.; Cabrita, M.J.; Garcia, R.; Rocha, J.M.; Roseiro, L.C.; Fernandes, M.J.; Fraqueza, M.J.; et al. Impact of salt reduction on biogenic amines, fatty acids, microbiota, texture and sensory profile in traditional blood dry-cured sausages. *Food Chem.* **2017**, *218*, 129–136.
35. ISO. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Part 2, Colony Count Technique in Products with Water Activity Less than or Equal to 0.95*; ISO: Geneva, Switzerland, 2008.
36. ISO. *Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of Enterobacteriaceae—Part 2, Colony-Count Technique*; ISO: Geneva, Switzerland, 2017.
37. ISO. *Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and of *Listeria* spp.—Part 2, Enumeration Method*; ISO: Geneva, Switzerland, 2014.
38. ISO. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection of *Salmonella* spp.*; ISO: Geneva, Switzerland, 2014.
39. Roseiro, C.; Santos, C.; Sol, M.; Silva, L.; Fernandes, I. Prevalence of biogenic amines during ripening of a traditional dry fermented pork sausage and its relation to the amount of sodium chloride added. *Meat Sci.* **2006**, *74*, 557–563.
40. ISO. *Sensory Analysis—General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors*; ISO: Geneva, Switzerland, 2012.
41. ISO. *Sensory Analysis—General Guidance for the Design of Test Rooms*; ISO: Geneva, Switzerland, 2012.
42. EC. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* **2005**, *338*, 1–26.
43. Elias, M.; Potes, M.E.; Roseiro, L.C.; Santos, C.; Gomes, A.; Agulheiro-Santos, A.C. The Effect of Starter Cultures on the Portuguese Traditional Sausage “Paio do Alentejo” in Terms of Its Sensory and Textural Characteristics and Polycyclic Aromatic Hydrocarbons Profile. *J. Food Res.* **2014**, *3*, 45–56.
44. Simion, A.M.; Vizireanu, C.; Alexe, P.; Franco, I.; Carballo, J. Effect of the use of selected starter cultures on some quality, safety and sensorial properties of Dacia sausage, a traditional Romanian dry-sausage variety. *Food Control* **2014**, *35*, 123–131.

45. Grau, R.; Andres, A.; Barat, J. Principles of Dring. In *Handbook of Fermented Meat and Poultry*, 2nd ed.; Toldrá, F., Ed.; Wiley Blackwell: Hoboken, NJ, USA, 2015; pp. 31–38.
46. Ruiz, J.; Pérez-Palacios, T. Ingredients. In *Handbook of Fermented Meat and Poultry*, 2nd ed.; Toldrá, F., Ed.; Wiley: Hoboken, NJ, USA, 2015; pp. 55–65.
47. Casquete, R.; Benito, M.J.; Martín, A.; Ruiz-Moyano, S.; Aranda, E.; Córdoba, M.G. Microbiological quality of salchichón and chorizo, traditional Iberian dry-fermented sausages from two different industries, inoculated with autochthonous starter cultures. *Food Control* **2012**, *24*, 191–198.
48. Health Protection Agency. *Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods*; Health Protection Agency: London, UK, 2009.
49. Montanari, C.; Gatto, V.; Torriani, S.; Barbieri, F.; Bargossi, E.; Lanciotti, R.; Grazia, L.; Magnani, R.; Tabanelli, G.; Gardini, F. Effects of the diameter on physico-chemical, microbiological and volatile profile in dry fermented sausages produced with two different starter cultures. *Food Biosci.* **2018**, *22*, 9–18.
50. Drosinos, E.H.; Mataragas, M.; Xiraphi, N.; Moschonas, G.; Gaitis, F.; Metaxopoulos, J. Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Sci.* **2005**, *69*, 307–317.
51. Lebert, I.; Leroy, S.; Giammarinaro, P.; Lebert, A.; Chacornac, J.P.; Bover-Cid, S.; Vidal-carou, M.C.; Talon, R. Diversity of microorganisms in the environment and dry fermented sausages of small traditional French processing units. *Meat Sci.* **2007**, *76*, 112–122.
52. Gossner, C.M.; Van Cauteren, D.; Le Hello, S.; Weill, F.X.; Terrien, E.; Tessier, S.; Janin, C.; Brisabois, A.; Dusch, V.; Vaillant, V. Nationwide outbreak of *Salmonella enterica* serotype 4,12:I:-infection associated with consumption of dried pork sausage, France, November to December. *Eurosurveillance* **2012**, *17*, 19–22.
53. Kuhn, K.G.; Torpdahl, M.; Frank, C.; Sigsgaard, K.; Ethelberg, S. An outbreak of *Salmonella* Typhimurium traced back to salami, Denmark, April to June 2010. *Eurosurveillance* **2011**, *16*, 5–8.
54. Gücükoğlu, A.; Küplülü, Ö. The effect of different starter cultures and ripening temperatures on formation of biogenic amine in Turkish fermented sausages. *Eur. Food Res. Technol.* **2010**, *230*, 875–884.
55. Xie, C.; Wang, H.H.; Nie, X.K.; Chen, L.; Deng, S.L.; Xu, X.L. Reduction of biogenic amine concentration in fermented sausage by selected starter cultures. *CYTA J. Food* **2015**, *13*, 491–497.
56. Laranjo, M.; Gomes, A.; Agulheiro-Santos, A.C.; Potes, M.E.; Cabrita, M.J.; Garcia, R.; Rocha, J.M.; Roseiro, L.C.; Fernandes, M.J.; Fernandes, M.H.; et al. Characterisation of “Catalão” and “Salsichão” Portuguese traditional sausages with salt reduction. *Meat Sci.* **2016**, *116*, 34–42.
57. Bover-Cid, S.; Latorre-Moratalla, M.L.; Veciana-Nogués, M.T.; Vidal-Carou, M.C. Processing contaminants: Biogenic amines. In *Encyclopedia of Food Safety*; Motarjemi, Y., Moy, G.G., Todd, E.C.D., Eds.; Elsevier Inc.: Burlington, MA, USA, 2014; pp. 381–391.
58. del Rio, B.; Redruello, B.; Linares, D.M.; Ladero, V.; Fernandez, M.; Martin, M.C.; Ruas-Madiedo, P.; Alvarez, M.A. The dietary biogenic amines tyramine and histamine show synergistic toxicity towards intestinal cells in culture. *Food Chem.* **2017**, *218*, 249–255.
59. Gardini, F.; Özogul, Y.; Suzzi, G.; Tabanelli, G.; Özogul, F. Technological factors affecting biogenic amine content in foods: A review. *Front. Microbiol.* **2016**, *7*, 1218.
60. EFSA. Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *EFSA J.* **2011**, *9*, 2393.
61. Nuñez, M.; del Olmo, A.; Calzada, J. Biogenic Amines. In *Encyclopedia of Food and Health*, 1st ed.; Caballero, B., Finglas, P., Toldra F., Ed.; Academic Press: Oxford, UK, 2016; pp. 416–423.
62. Wang, X.; Ren, H.; Wang, W.; Zhang, Y.; Bai, T.; Li, J.; Zhu, W. Effects of Inoculation of Commercial Starter Cultures on the Quality and Histamine Accumulation in Fermented Sausages. *J. Food Sci.* **2015**, *80*, M377–84.
63. Casquete, R.; Benito, M.J.; Martín, A.; Ruiz-Moyano, S.; Córdoba, J.J.; Córdoba, M.G. Role of an autochthonous starter culture and the protease EPg222 on the sensory and safety properties of a traditional Iberian dry-fermented sausage “salchichón”. *Food Microbiol.* **2011**, *28*, 1432–1440.
64. Papavergou, E.J.; Savvaidis, I.N.; Ambrosiadis, I.A. Levels of biogenic amines in retail market fermented meat products. *Food Chem.* **2012**, *135*, 2750–2755.
65. Montel, M.; Masson, F.; Talon, R. Comparison of biogenic amine content in traditional and industrial French dry sausages. *Sci. Aliments.* **1999**, *19*, 247–254.
66. Casquete, R.; Benito, M.J.; Martín, A.; Ruiz-Moyano, S.; Hernández, A.; Córdoba, M.G. Effect of autochthonous starter cultures in the production of “salchichón”, a traditional Iberian dry-fermented sausage, with different ripening processes. *LWT Food Sci. Technol.* **2011**, *44*, 1562–1571.
67. Essid, I.; Hassouna, M. Effect of inoculation of selected *Staphylococcus xylosus* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. *Food Control* **2013**, *32*, 707–714.
68. Dias, I.; Laranjo, M.; Fialho, R.; Potes, M.E.; Véstia, J.; Agulheiro-Santos, A.C.; Fraqueza, M.; Elias, M. Effect of autochthonous starter cultures in the production of Paio, a traditional Portuguese dry-cured sausage. *Archivos de Zootecnia.* **2018**, *66*, 161–165.
69. Ravyts, F.; De Vuyst, L.; Leroy, F. Bacterial diversity and functionalities in food fermentations. *Eng. Life Sci.* **2012**, *12*, 356–367.
70. Talon, R.; Leroy, S.; Lebert, I. MEAT Microbial ecosystems of traditional fermented meat products: The importance of indigenous starters. *Meat Sci.* **2007**, *77*, 55–62.

71. Van Ba, H.; Seo, H.-W.; Kim, J.-H.; Cho, S.-H.; Kim, Y.-S.; Ham, J.-S.; Park, B.-Y.; Kim, H.-W.; Kim, T.-B.; Seong, P.-N. The effects of starter culture types on the technological quality, lipid oxidation and biogenic amines in fermented sausages. *LWT Food Sci. Technol.* **2016**, *74*, 191–198.
72. Kargozari, M.; Moini, S.; Basti, A.A.; Emam-Djomeh, Z.; Gandomi, H.; Martin, I.R.; Ghasemlou, M.; Carbonell-Barrachina, Á.A. Effect of autochthonous starter cultures isolated from Siahmazgi cheese on physicochemical, microbiological and volatile compound profiles and sensorial attributes of sucuk, a Turkish dry-fermented sausage. *Meat Sci.* **2014**, *97*, 104–114.
73. Bañón, S.; Serrano, R.; Bedia, M. Use of Micrococcaceae combined with a low proportion of Lactic Acid Bacteria as a starter culture for salami stuffed in natural casing. *CYTA J. Food* **2014**, *12*, 160–165.
74. Baka, A.M.; Papavergou, E.J.; Pragalaki, T.; Bloukas, J.G.; Kotzekidou, P. Effect of selected autochthonous starter cultures on processing and quality characteristics of Greek fermented sausages. *LWT Food Sci. Technol.* **2011**, *44*, 54–61.