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Combined Use of Starter Cultures and Preservatives To Control Production of Biogenic Amines and Improve Sensorial Profile in Low-Acid Salami

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The combined effect of starter culture, nitrites, and nitrates has been studied in low-acidity salamis, typical products of northern Italy. Nine batches have been prepared, combining three different inoculations of starter cultures (control, *Lactobacillus plantarum*, and *Lactobacillus plantarum* together with *Kocuria varians*) with three different preservatives (control, sodium nitrate, and sodium nitrite). All of the batches showed a good fermentation process with a proper pH decrease, which was quicker in batches inoculated with *L. plantarum*. The use of starter cultures and in particular the use of nitrites allowed the control of the proliferation of Enterobacteriaceae and enterococci. The accumulation of biogenic amines, especially putrescine, cadaverine, tryptamine, and tyramine, in salami ready for consumption (60 days of ripening) was strongly affected by the presence of Enterobacteriaceae and enterococci. Results obtained showed that the combined use of adequate preservatives and starter cultures allows the production of safer products with improved sensorial profile.

KEYWORDS: Salami; biogenic amines; nitrate; nitrite; starter cultures; sensorial profile

INTRODUCTION

The production of salami in Italy is achieving noticeable importance, due to the interest of customers in typical products, guaranteed through Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) acknowledgment (Reg. CE 510/06). Salami consumption has been criticized by nutritionists describing salami as a food rich in fat and salt as well as potentially toxic compounds such as biogenic amines.

Biogenic amines (BA) are organic bases with aliphatic (putrescine and cadaverine), aromatic (tyramine and phenylethylamine), or heterocyclic (histamine and tryptamine) structure. They can be found in several foods and are mainly produced through microbial decarboxylation of amino acids (*1*). Although BA in low concentrations are essential for many physiologic functions, in high concentration they can have deleterious effects (*2*). For instance, histamine and tyramine are particularly involved in the "histaminic intoxication", normally known as poisoning from scombroid fish (*3*) and in the "cheese reaction" (*2*). Excessive oral consumption of BA causes headaches, hypo- and hypertension, nausea, cardiac palpitation, renal intoxication, serious cases of cerebral hemorrhage, and even death (*4*). BA are normal constituents of food products such as cheese, wine, beer, sauerkraut, fish products, and fermented meat as the result of enzymatic degradations or fermentative processes (*1*). Accumulation of BA in food requires the availability of reaction precursors (amino acids) and the presence of microorganisms having amino acid-decarboxylase as well as adequate conditions that allow microbial growth and activity (*5*).

Recently, several authors have discussed the problem of the presence of BA in fermented salami and have set up analytical methods for their determination and quantification. In fermented salami, BA originate from the presence of amino acids, accumulated during the ripening phase as a result of proteolytic activity, as well as several microbial groups with decarboxylasic activity: *Pseudomonas*, Enterobacteriaceae, *Enterococcus*, and *Lactobacillus* (*6, 7*).

Many authors (*8*−*10*) suggest the use of selected starter cultures to control the level of BA in salami, whereas other authors have indicated that sugar can also be used (*11, 12*). Gonzalez-Fernandez et al. (*12*) suggest the simultaneous use of both methods.

The use of nitrite alongside the use of selected starter cultures, which limits the growth of Enterobacteriaceae, has been suggested in traditional Spanish (*9*) and Turkish (*13*) sausages as a method to control the amount of biogenic amines.

Typical Italian salami, such as Felino, are characterized by features that can promote the accumulation of BA: high pH if compared to other northern European salami products, low sugar levels, and prolonged ripening (*7*).

On the basis of these considerations, this work evaluates the role of adding starter culture and preservatives to control the accumulation of BA during the production of Felino style salami

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Table 1. Experimental Design: Letters Indicate the Batches Obtained

	preservative		
starter culture	$NaNO_3$ (250 mg/kg)	$NaNO_2$ (150 mg/kg)	none
Lactobacillus plantarum VLT 73 (10^6 cfu/g) + *Kocuria varians* MIAL 12 (10^5 cfu/g)	A	B	C
Lactobacillus plantarum VLT 73 (10^6 cfu/g)	D	E	F
none	G	H	I

with low sugar content. For this purpose, a starter culture of lactic acid bacteria (LAB) has been selected and used for the production of salami with the addition of nitrites or nitrates.

MATERIALS AND METHODS

Selection of Lactic Acid Bacteria. Sixty-five strains of *Lactobacillus* from the Dipartimento di Scienze degli Alimenti Collection (University of Bologna) were tested for their fermentative strength according to the method of Buckenhuskes (*14*) and for the absence of decarboxylasic activity in MRS broth, modified according to the method of Bover-Cid and Holzapfel (*15*). Biogenic amines in the broth were analyzed as reported below.

Strains without decaboxylasic activity were tested to analyze fermentative activity in meat (*16*) in order to choose the strain with highest acidogenic power and the best influence on sensorial characteristics. For this purpose, overnight cultures of each strain grown at 30 °C in MRS broth (Oxoid, Basingstoke, U.K.) were centrifuged (17000*g* for 15 min, Centrifuge 5415 R, Eppendorf, Hamburg, Germany), washed twice in 0.9% (w/v) NaCl, and resuspended in the same solution. Each strain was then inoculated in minced pork loin (10^7 cfu/g) and incubated at 25 °C in sterile bags under vacuum. After 48 h of incubation, pH and the absence of unpleasant smell were evaluated as described below.

Formulation of Starter Culture and Sausages Manufacture. The LAB strain selected as described above was inoculated in MRS broth (Oxoid) and incubated at 30 °C for 24 h under anaerobic conditions. After centrifugation and rinsing steps using 0.9% (w/v) NaCl, each strain was frozen (−18 °C) in the same solution until its use in the manufacture of salami.

Kocuria varians MIAL 12, previously selected for its technological properties (*17*), was maintained on a slant of Mannitol Salt Agar (MSA, Oxoid) at 4 °C until use. This strain was inoculated in the same liquid medium at 30 °C for 48 h and treated as reported for the LAB strain.

Before use, microorganism was revitalized for 16 h at 30 °C in MRS broth (Oxoid) for the LAB and MSA broth (Oxoid) without phenol red for the *Kocuria* strain.

Sausages were produced by using 73% lean pork, 27% pork fat, NaCl (23 g/kg), and glucose (2 g/kg), without spices. After chopping and mixing, the mixture was divided into nine batches; starter cultures and preservatives were added to each batch according to the distribution shown in **Table 1**. Batches A−C were inoculated with a starter culture composed of *L. plantarum* VLT73 and *K. varians* MIAL12 prepared as reported above, whereas batches D−F were inoculated with *L. plantarum* VLT73 alone. Batches G−I were not inoculated with starter cultures to serve as controls. Moreover, sodium nitrate (250 mg/kg) was also added to batches A, D, and G, whereas sodium nitrite (150 mg/kg) was added in batches B and E. Mixtures were stuffed into natural casings.

Twelve sausages of approximately 1000 g were produced for each batch. Sausages were placed in a drying chamber at 23 °C and 90% relative humidity (RH) for 48 h. Thereafter, sausages were held in the ripening chamber at 13 °C and 80−70% RH for a further 58 days.

Microbiological and Physicochemical Analyses. Microbiological analyses were performed at time zero (meat mixture prior to stuffing) and after 3, 10, 24, 45, and 60 days of ripening. For this purpose, 20 g of sausage (without casing) was removed under aseptic conditions and homogenized for 2 min with 180 mL of 0.9% (w/v) NaCl using a Stomacher (Laboratory Blender Seward, London, U.K.). The solution was then used to prepare decimal dilutions.

Enterobacteriaceae were counted on VRBGA (Oxoid) incubated for 24 h at 37 °C and Enterococci on Slanetz and Bartley medium (Oxoid) after 48 h at 45 °C. LAB were cultured on MRS agar (Oxoid) at 30 °C for 96 h under anaerobic conditions. Gram positive coagulase negative cocci were cultured on MSA (Oxoid) at 30° for 72 h. Three replicates were carried out for each microbial count.

A pH-meter (Orion) equipped with a penetration probe electrode (Orion) was used to measure acidity directly in sausages.

Detection of Biogenic Amines. Biogenic amines were determined at time zero and after 60 days of ripening according to the method of Hwang et al. (*18*). Ten grams of salami from each batch were minced and homogenized after the addition of 20 mL of a 5% aqueous solution of trichloroacetic acid using an Ultra-Turrax (Janke & Kunkel, Staufen, Germany). Homogenized samples were then placed in a thermostat at 75 °C for 30 min and subsequently centrifuged (Beckman Centrifuge J2-21, Palo Alto, CA) at 5000*g* for 10 min. The supernatant was filtered on filter paper Whatman no. 40 (Maidstone, U.K.). The extraction procedure was repeated on the pellet, and the acid extracts were combined and made up to a volume of 50 mL using the extraction solvent. One milliliter of 2N NaOH and 20 μL of benzoyl chloride were added to 2 mL of extract (or standard mix). After mixing, the samples were derivatized by incubation in a water bath at 30 °C for 40 min. After this step, 2 mL of a urea-saturated solution was added to samples. Tubes were then mixed for 30 s by vortex and placed again in a water bath at 30 °C for 10 min. To extract the derivatized compounds to the organic phase, samples were treated with 3 mL of diethyl ether and mixed for 1 min by vortex. Phase separation was carried out by centrifugation (3000*g* for 5 min), and the organic phase was dried using a slow flow of nitrogen. The dried extract was solubilized with 2 mL of an *n*-hexane/isopropanol (4:1) solution and filtered using a 0.20 μm filter for the subsequent chromatographic analysis. Analysis was carried out with a Waters HPLC (Milan, Italy), equipped with a Waters 1525 binary pump, a dual wavelength absorbance detector Water 2487 set at 250 nm, and a Symmetry C18 column. Solvent A was HPLC grade water (Carlo Erba reagents), and solvent B was HPLC grade methanol (Carlo Erba reagents). An elution gradient was programmed for solvent B as follows: 50% for 0.5 min, from 50 to 15% in 6.5 min followed by 5 min at 15% then from 15 to 50% methanol in 2 min, followed by 2 min in 50% methanol. A flow rate of 0.8 mL/min was employed, and 20 μL of sample was injected. Breeze 3.30 SPA software (Waters) was used for data acquisition and processing on a personal computer. All of the BA were tentatively identified by comparison of retention time and coelution with the commercial standard compounds (Sigma, St. Louis, MO).

Biogenic Amine Quantification. Calibration curves were performed over the range of 5−500 μg/mL for each amine standard solution using the peak area versus analyte concentration to quantify the BA. The linear range was assessed using seven different concentrations that were injected three times.

Sensory Evaluation. A panel of nine assessors (TINVAL) previously trained in descriptive analysis for meat products according to the method of Chiavari et al. (*19*) was used for the sensory evaluation

Smell and off-flavor of minced pork loin were evaluated to select a strain suitable to produce sausages.

Sausages were evaluated at the end of the ripening, that is, after 60 days, considering appearance, smell, aroma, and texture. Features of the salami were appreciated by observation, light manipulation, and/or taste. The parameters are listed in the order in which they were evaluated during the test. Intensity was marked on an arbitrary scale with points from 1 to 7.

Statistical Analysis. Data regarding sensory analysis and determination of biogenic amines were statistically analyzed using the ANOVA procedure. Pearson correlation was used to find significant relationship between bacterial counts and level of biogenic amines. The analysis of data was conducted by using the statistical package SPSS 13.0 for Windows (SPSS Inc., Chicago, IL).

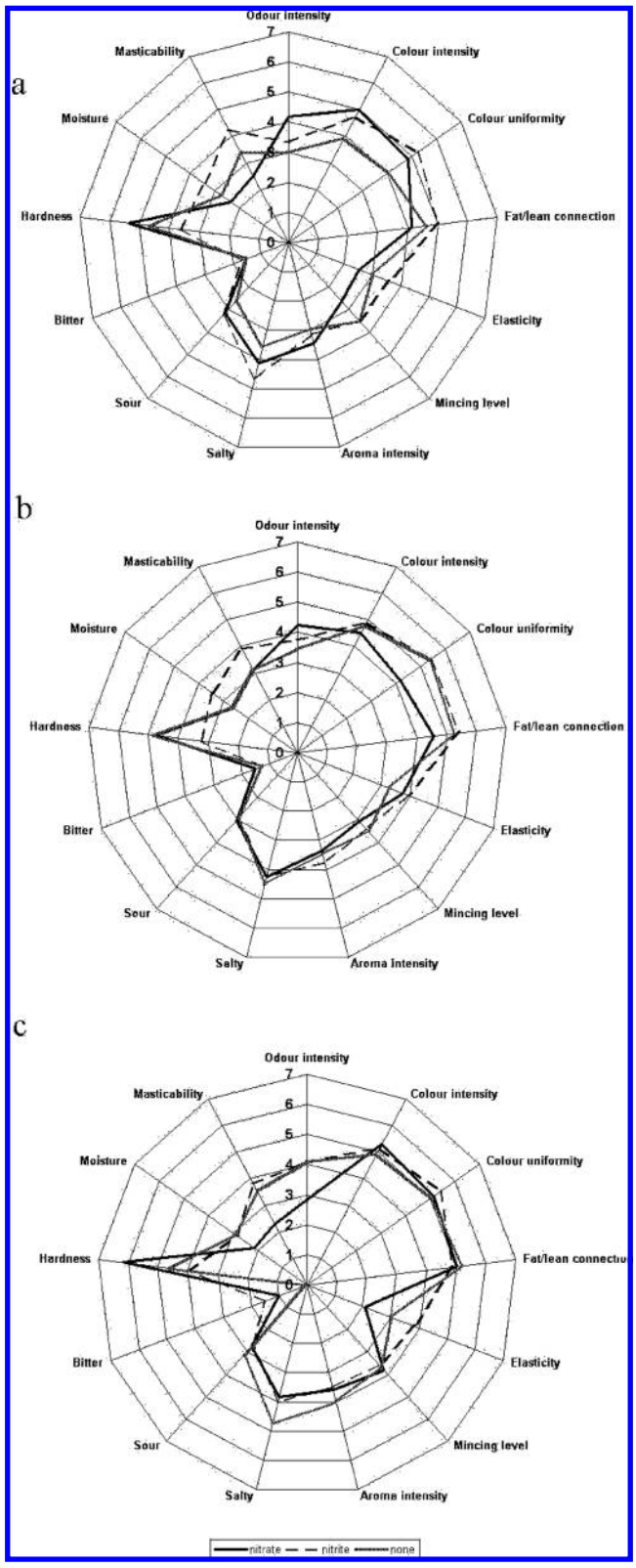

Figure 1. Sensory profile of batches obtained: (**a**) batches with addition of *Lactobacillus plantarum* VLT73 and *Kocuria varians* MIAL 12; (**b**) batches with *L. plantarum* VLT73; (**c**) batches without addition of starter cultures.

RESULTS AND DISCUSSION

***Lactobacillus* Starter Selection.** Of 65 analyzed strains, 25 showed good fermentative strength and high acidogenic power, characteristics that suggested their use for the fermentation process. For eight of these strains the concentrations of BA were found to be instrumentally nondetectable (data not shown). The percentage of positive strains on the total number of strains was in agreement with results reported by other authors (*15*).

Selected strains with nonactive carboxylase and high acidogenic power were tested for their fermentative strength, in a meat model, evaluating the decrease of pH and the sensorial characteristic after 72 h of fermentation under vacuum at 15 °C. Of eight tested strains, *L. plantarum* VLT73 was found to be the most suitable. It did not influence the organoleptic profile of the meat and confirmed a decrease of pH to 5.40 after 24 h of incubation. The next step of this experimentation involved the use of this strain as starter culture to produce different batches of Felino style salami.

Microbial Count. At time zero meat mixtures without inoculum (batches G−I) contained low levels of LAB (<10 cfu/g), whereas in the inoculated batches LAB reached $>10^7$ cfu/g, confirming the effectiveness of the added starter (**Table 2**). In these cases, fermentation was quick and effective, bringing the pH to levels lower than 5.5 after 3 days. In batches G−I (no inoculation), LAB were also present after 3 days with values $>10^7$ cfu/g, but the acidification was slower and less effective. These results confirmed again that short acidification times are mainly due to the use of starter cultures, and therefore it is a necessary step to produce safer higher quality products. Batches in which nitrates and nitrites were added did not show particular differences in microbial count, proving that their use does not affect the development of LAB (*9, 20*).

Gram-positive coagulase negative cocci, counted on MSA, showed a behavior similar to that of lactic acid bacteria. In noninoculated batches (G−I) or in batches inoculated with only *L. plantarum* 32 (D−F), around 10^3 log cfu/g were present, whereas counts in batches inoculated with *K. varians* MIAL12 (A−C) were 1.5 logarithmic cycles higher at least. This difference was particularly appreciated in the first 3 weeks of ripening, whereas in the subsequent steps of ripening counts decreased, reaching the same level for all batches. This result is in agreement with Bover-Cid et al. (*21*).

Enterococci, evaluated in Slanetz and Bartley agar, were particularly influenced by the presence of nitrites and nitrates. At time zero, these microorganisms were present at amounts of <10 cfu/g; after only 3 days, they reached values of $>10^5$ cfu/g in batches without the addition of nitrites, maintaining this level in the other steps of the ripening. The strong inoculation of lactobacilli allowed a partial control of the proliferation of enterococci; however, their level reached values comparable with those of noninoculated batches. In batches B, E, and H (with addition of nitrites), the level of enterococci increased only after the 10th day of ripening, reaching a stable level of about 10^3 cfu/g.

Enterobacteriaceae and enterococci had similar behaviors: in batches B and E (addition of nitrites and inoculation of lactic acid bacteria), their level decreased to 100 cfu/g after 3 days. This level was reached in the other batches only in subsequent steps of the ripening. Other authors (*9, 21*) have also suggested that nitrites and lactic acid bacteria started cultures can be used to control Enterobacteriaceae because these microorganisms are the main factors in the production and accumulation of BA in salami.

Formation of Biogenic Amines. Spermine (37.3 ± 3.45 mg/kg) and spermidine (12.5 ± 2.23 mg/kg) were the only BA detectable in the meat mixture at time zero; the other BA were present in trace amounts. **Table 3** details the concentration of BA at the end of the ripening (60 days). The most prevalent amines were putrescine and tyramine, particularly in batches

Table 2. pH and Microbial Counts (log cfu/g) during Ripening as Means ± Standard Deviation

batch	starter culture[b]	preservative[b]	days of ripening	pH	lactic acid bacteria	Gram+ coagulase cocci	enterococci	Enterobacteriaceae
A	L + K	$NaNO_3$	0	5.56 ± 0.06	7.07 ± 0.11	4.61 ± 0.13	<1.00	2.23 ± 0.18
			3	5.40 ± 0.06	8.57 ± 0.22	4.42 ± 0.09	5.01 ± 0.13	2.01 ± 0.22
			10	5.22 ± 0.07	8.55 ± 0.22	5.83 ± 0.38	4.52 ± 0.23	2.48 ± 0.15
			24	5.37 ± 0.18	8.42 ± 0.09	3.05 ± 0.08	4.44 ± 0.45	1.71 ± 0.12
			45	5.77 ± 0.22	8.53 ± 0.12	4.70 ± 0.14	3.52 ± 0.55	0.99 ± 0.23
			60	6.06 ± 0.07	7.76 ± 0.10	6.10 ± 0.12	3.66 ± 0.10	<1,00
B	L + K	$NaNO_2$	0	5.51 ± 0.01	7.17 ± 0.09	5.06 ± 0.10	<1.00	2.17 ± 0.05
			3	5.38 ± 0.03	8.38 ± 0.09	4.01 ± 0.13	<1.00	<1.00
			10	5.22 ± 0.02	8.37 ± 0.08	5.75 ± 0.13	<1.00	<1.00
			24	5.32 ± 0.19	8.44 ± 0.16	3.58 ± 0.25	2.45 ± 0.16	<1.00
			45	5.65 ± 0.20	8.35 ± 0.19	5.46 ± 0.14	2.31 ± 0.13	<1.00
			60	5.87 ± 0.08	8.36 ± 0.19	5.27 ± 0.16	2.32 ± 0.17	<1.00
C	L + K	none	0	5.53 ± 0.08	7.20 ± 0.11	4.76 ± 0.23	<1.00	1.98 ± 0.11
			3	5.43 ± 0.04	8.48 ± 0.05	4.08 ± 0.19	5.07 ± 0.22	2.47 ± 0.23
			10	5.21 ± 0.03	8.43 ± 0.10	5.07 ± 0.15	4.08 ± 0.05	<1.00
			24	5.33 ± 0.18	8.51 ± 0.08	3.48 ± 0.16	4.02 ± 0.09	<1.00
			45	5.43 ± 0.18	8.45 ± 0.06	4.54 ± 0.23	4.00 ± 0.18	<1.00
			60	5.92 ± 0.07	8.20 ± 0.20	5.03 ± 0.13	4.66 ± 0.12	<1.00
D	L	$NaNO_3$	0	5.63 ± 0.01	7.16 ± 0.05	3.01 ± 0.22	<1.00	2.37 ± 0.18
			3	5.42 ± 0.09	8.33 ± 0.04	3.68 ± 0.10	4.80 ± 0.15	2.30 ± 0.23
			10	5.32 ± 0.24	8.47 ± 0.05	5.71 ± 0.44	4.80 ± 0.18	2.30 ± 0.30
			24	5.43 ± 0.19	8.48 ± 0.05	3.52 ± 0.39	3.79 ± 0.32	1.49 ± 0.12
			45	6.00 ± 0.18	8.77 ± 0.13	5.52 ± 0.26	3.10 ± 0.12	0.98 ± 0.08
			60	5.98 ± 0.05	8.45 ± 0.31	5.52 ± 0.51	3.75 ± 0.23	<1.00
E	L	$NaNO_2$	0	5.58 ± 0.06	7.12 ± 0.11	3.19 ± 0.18	<1.00	2.45 ± 0.12
			3	5.56 ± 0.06	7.66 ± 0.11	3.72 ± 0.25	<1.00	1.98 ± 0.08
			10	5.32 ± 0.11	9.09 ± 0.42	5.95 ± 0.05	4.39 ± 0.15	1.98 ± 0.09
			24	5.42 ± 0.09	8.04 ± 0.08	3.63 ± 0.21	2.76 ± 0.07	1.46 ± 0.10
			45	5.78 ± 0.17	8.43 ± 0.22	5.38 ± 0.14	2.69 ± 0.27	<1.00
			60	5.63 ± 0.06	7.93 ± 0.22	5.58 ± 0.13	3.50 ± 0.13	<1.00
F	L	none	0	5.49 ± 0.06	7.08 ± 0.03	3.23 ± 0.12	<1.00	2.23 ± 0.27
			3	5.45 ± 0.06	7.85 ± 0.05	4.36 ± 0.18	4.50 ± 0.43	1.95 ± 0.10
			10	5.22 ± 0.05	8.37 ± 0.25	6.03 ± 0.60	4.97 ± 0.12	2.00 ± 0.23
			24	5.24 ± 0.06	8.45 ± 0.22	3.44 ± 0.52	3.73 ± 0.11	0.98 ± 0.09
			45	5.53 ± 0.14	8.88 ± 0.19	5.30 ± 0.18	4.91 ± 0.19	<1.00
			60	5.70 ± 0.07	8.36 ± 0.28	4.76 ± 0.15	4.72 ± 0.17	<1.00
G	N	$NaNO_3$	0	5.61 ± 0.04	nd[c]	3.13 ± 0.29	<1.00	2.37 ± 0.43
			3	5.63 ± 0.04	7.86 ± 0.05	3.96 ± 0.17	5.46 ± 0.02	2.87 ± 0.30
			10	5.29 ± 0.05	8.51 ± 0.40	5.43 ± 0.22	5.71 ± 0.11	<1.00
			24	5.52 ± 0.22	8.52 ± 0.40	4.01 ± 0.19	5.58 ± 0.47	1.23 ± 0.09
			45	5.86 ± 0.16	8.36 ± 0.26	5.39 ± 0.37	5.09 ± 0.38	1.99 ± 0.12
			60	5.90 ± 0.07	7.58 ± 0.22	5.74 ± 0.29	5.08 ± 0.30	<1.00
H	N	$NaNO_2$	0	5.62 ± 0.06	nd	3.21 ± 0.40	<1.00	2.47 ± 0.34
			3	5.62 ± 0.09	7.13 ± 0.09	3.39 ± 0.14	<1.00	2.68 ± 0.45
			10	5.39 ± 0.08	8.12 ± 0.12	3.65 ± 0.20	4.01 ± 0.15	1.01 ± 0.10
			24	5.38 ± 0.10	7.80 ± 0.16	2.98 ± 0.13	3.60 ± 0.15	1.00 ± 0.03
			45	5.77 ± 0.13	7.93 ± 0.09	4.77 ± 0.12	3.25 ± 0.12	<1.00
			60	6.03 ± 0.06	6.65 ± 0.36	5.11 ± 0.30	3.49 ± 0.17	<1.00
I	N	none	0	5.60 ± 0.03	nd	3.23 ± 0.38	<1.00	2.18 ± 0.01
			3	5.63 ± 0.05	7.64 ± 0.18	3.04 ± 0.25	5.41 ± 0.13	2.60 ± 0.12
			10	5.38 ± 0.06	7.79 ± 0.31	3.17 ± 0.26	5.74 ± 0.22	4.45 ± 0.45
			24	5.55 ± 0.21	7.85 ± 0.17	3.41 ± 0.26	5.37 ± 0.32	<1.00
			45	5.74 ± 0.16	8.02 ± 0.07	5.65 ± 0.19	5.06 ± 0.37	2.58 ± 0.23
			60	6.34 ± 0.05	7.50 ± 0.41	5.45 ± 0.75	5.12 ± 0.40	<1.00

[a] Starter cultures: L, *Lactobacillus plantarum* VLT 73; K, *Kocuria varians* MIAL; N, none. [b] Preservative: $NaNO_3$, sodium nitrate, 250 mg/kg; $NaNO_2$, sodium nitrie 150 mg/kg. [c] nd, not detected.

without the addition of starter culture and preservatives. This is in agreement with previous works (*7, 22*), in particular for traditional Spanish salami (*12, 23, 24*).

As evidenced by the results, the use of nitrite preservatives can control the formation of BA; in particular, the amounts of putrescine, cadaverine, tryptamine, and tyramine showed statistically significant differences ($p < 0.05$) if compared with control samples and salami with nitrate preservatives.

Use of nitrites enabled the control of the production of putrescine in batch B, which also involved the use of a starter culture composed of two strains; however, they were less effective when the *Kocuria* strain was not inoculated ($p < 0.05$).

Table 3. Concentration of Biogenic Amines (Milligrams per Kilogram) at the End of the Ripening Process (60 Days) as Means ± Standard Deviation[a]

		preservative		
amine	starter culture	$NaNO_2$ (150 mg/kg)	$NaNO_3$ (250 mg/kg)	none
putrescine	*Lactobacillus* + *Kocuria*	$11.1^{a1} \pm 2.0$	$193.8^{3} \pm 7.2$	$116.4^{a2} \pm 11.1$
	Lactobacillus	$177.8^{b} \pm 3.8$	220.4 ± 39.4	$222.5^{c} \pm 8.1$
	none	$216.5^{b} \pm 22.3$	183.7 ± 14.2	$159.4^{b} \pm 1.9$
cadaverine	*Lactobacillus* + *Kocuria*	$44.0^{b1} \pm 4.4$	$67.7^{ab2} \pm 1.4$	$59.5^{b12} \pm 6.8$
	Lactobacillus	$10.5^{a1} \pm 1.0$	$55.9^{a3} \pm 0.9$	$42.3^{a2} \pm 0.8$
	none	$16.9^{a1} \pm 1.0$	$80.5^{b2} \pm 5.9$	$80.2^{c2} \pm 0.9$
tryptamine	*Lactobacillus* + *Kocuria*	$2.8^{a1} \pm 0.6$	$14.3^{a2} \pm 0.3$	$17.6^{a2} \pm 2.4$
	Lactobacillus	$7.1^{b1} \pm 0.4$	$26.6^{b2} \pm 0.0$	$28.0^{b2} \pm 1.1$
	none	$12.4^{c1} \pm 0.5$	$32.8^{c2} \pm 0.7$	$34.2^{b2} \pm 2.2$
spermidine	*Lactobacillus* + *Kocuria*	$2.2^{a1} \pm 0.5$	$26.7^{a2} \pm 4.8$	$18.5^{a2} \pm 3.7$
	Lactobacillus	$22.1^{b1} \pm 2.0$	$34.1^{a2} \pm 2.2$	$30.5^{a2} \pm 1.2$
	none	$48.6^{c1} \pm 4.7$	$83.2^{b2} \pm 1.3$	$98.6^{b3} \pm 4.1$
spermine	*Lactobacillus* + *Kocuria*	41.2 ± 7.4	59.6 ± 11.3	49.8 ± 0.6
	Lactobacillus	51.6 ± 2.5	57.6 ± 0.1	52.8 ± 6.1
	none	50.1 ± 0.0	36.6 ± 6.8	38.2 ± 2.7
histamine	*Lactobacillus* + *Kocuria*	54.6 ± 2.1	$60.0^{ab} \pm 5.0$	64.0 ± 3.7
	Lactobacillus	54.6 ± 7.1	$94.4^{b} \pm 10.5$	60.3 ± 12.4
	none	46.0 ± 0.5	$37.4^{a} \pm 5.3$	61.1 ± 26.9
tyramine	*Lactobacillus* + Kocuria	$19.0^{a1} \pm 4.6$	$108.0^{2} \pm 6.0$	$43.8^{1} \pm 23.2$
	Lactobacillus	$95.0^{c2} \pm 1.9$	$86.6^{12} \pm 3.6$	$79.5^{1} \pm 1.5$
	none	$60.9^{b} \pm 7.9$	85.9 ± 17.1	43.4 ± 5.3
total amine	*Lactobacillus* + *Kocuria*	$174.7^{a1} \pm 12.5$	$530.0^{a2} \pm 35.9$	$369.8^{a3} \pm 2.1$
	Lactobacillus	$418.7^{b1} \pm 11.2$	$568.1^{a2} \pm 50.4$	$515.9^{b12} \pm 12.0$
	none	$451.4^{a} \pm 25.5$	$540.2^{b} \pm 51.2$	$515.1^{b} \pm 13.6$

[a] For each amine, any means followed by different characters show statistical differences ($p < 0.05$) according to the post hoc comparisons (Tukey's HSD) of the ANOVA. Letters compare among the different starter cultures, numbers among preservatives.

The production of putrescine, which was the most prevalent biogenic amine in all of the samples, was influenced by the presence of nitrites and by the inoculation of the *Kocuria* strain. Previously, Gardini et al. (*10*) evidenced similar behavior, demonstrating the ability of a *Staphylococcus xylosus* strain to maintain the concentration of putrescine at a level 30 times lower than the control sample. Data related to putrescine are confirmed by the decreased bacterial charge of Enterobacteriaceae (*25*) and fecal enterococci (*7*) in batches that showed the lower content in putrescine.

Moreover, the use of nitrites allowed for tryptamine to be limited to levels of <10 mg/kg for the batches with inoculation; in control noninoculated sample this amine was found in a amount of 12.42 mg/kg (*26*). Differences in tryptamine concentration were statistically significant between batches produced with different starters for the inoculation and also if salami were produced with or without nitrates.

Data related to cadaverine showed that the production of this biogenic amine is reduced by the presence of nitrites, but in this case, inoculation with *Kocuria* was not able to decrease its production. Moreover, salami with *Kocuria* showed concentrations of cadaverine that were significantly higher than those inoculated only with the *Lactobacillus* strain. The counts of enterococci, which were not influenced by the presence of *Kocuria* as starter culture, could explain these results.

The concentration of spermidine was also influenced by the use of nitrites, in the same way as the other amines. Concentrations of this amine in salami with the combination of the two microorganisms in the starter culture were always significantly smaller when compared with noninoculated control samples. The use of preservatives and the different combination of the starter culture did not affect the amounts of spermine, which maintained its level in a range between 36 and 59 mg/kg. Other works (*23*) have indicated that spermine and spermidine are already present in meat during mixing. These results demonstrate that is not possible to control the production of these two biogenic amines with technological coadjuvant as preservatives or starter cultures (*13, 27*).

Histamine, found in all of the samples, did not show significant differences between the batches with nitrites and without preservatives. However, batches with nitrates confirmed the results observed by other authors (*10, 28*); the amount of histamine decreased in batches with starter culture. These authors suggested the use of *S. xylosus* to control the production of BA. Moreover, the strict relationship between the accumulation of histamine during ripening (data not shown) and the charge of fecal enterococci must be emphasized, as reported by other authors (*29*). Accumulation of tyramine is linked with the proliferation of lactic acid bacteria, whereas the presence of sugars added to meat allows control of their production (*29*). The concentration of this amine, however, is in agreement with previous results obtained by these authors in salami produced with added sugars. The use of nitrites allowed the control of amines to levels of <200 mg/kg in the batches with the use of *Lactobacillus* and *Kocuria* as starter culture.

Table 4 reports Pearson's correlation coefficients between the counts of Enterobacteriaceae and enterococci and BA amount. It can be seen that a strongly significant correlation ($p < 0.01$) exists between the count of Enterobacteriaceae and the amounts of putrescine, spermine, spermidine, and histamine. A correlation with lower but consistent significance ($p < 0.05$) was observed between the counts of Enterobacteriaceae and

Table 4. Correlation Coefficients between Biogenic Amines and Microbial Counts[a]

	Enterobacteriaceae	enterococci
putrescine	−0.275**	0.376**
cadaverine	−0.099	0.341**
tryptamine	−0.085	0.408**
spermidine	−0.188**	0.247**
spermine	0.185**	0.071
histamine	−0.349**	0.016
tyramine	−0.213*	0.161
total amine	−0.225*	0.322**

[a] Significance: **, $p < 0.01$; *, $p < 0.05$.

tyramine. The total amount of amines showed a less significant relationship with the counts of Enterobacteriaceae, whereas it was more significantly influenced by the counts of enterococci. At the same time a significant relationship between these counts and the concentrations of putrescine, cadaverine, tryptamine, and spermidine was found.

Sensorial Characteristics. A trained panel carried out sensorial analysis on all of the batches previously produced, at the end of ripening. The analysis of batches produced with the same starter (**Figure 1**) showed that the use of nitrites improved the structural characteristics as hardness, elasticity, and masticability. The influence of the starter culture for these salami is limited; in fact, the sensorial profiles of batches B, E, and H were more or less the same.

The starter cultures used in this work notably influenced the sensorial characteristics of salami with added nitrates (A, D, and G) or salami without preservatives (C, F, and I). The role of *K. varians* was appreciable, particularly in terms of color when compared with salami produced using only *L. plantarum* or without starter. *L. plantarum* evidenced its own characteristics only in batches with added nitrates. Use of either culture gave rise to the perception of a slight bitter taste in comparison to those produced without starter culture.

The results obtained clearly show that the use of nitrates and, especially, nitrites, when combined with the use of selected starter culture (as lactic acid bacteria from *Lactobacillus* genus and Gram positive coagulase negative cocci *Kocuria* genus) generates safer salami products with an improved sensorial profile. The disciplined production of PDO salami should take into account this information with strong qualitative and hygienic−sanitary concern.

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