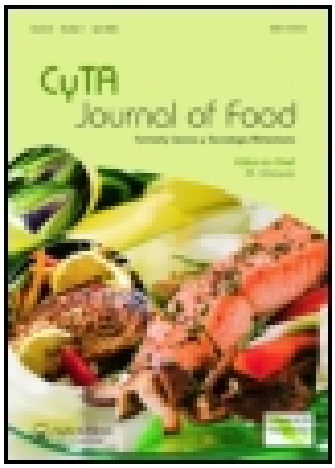

CyTA - Journal of Food
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tcyt20

Reduction of biogenic amine concentration in fermented sausage by selected starter cultures

Chong Xie[a], Hu-Hu Wang[a], Xiao-Kai Nie[a], Lin Chen[a], Shao-Lin Deng[a] & Xing-Lian Xu[a]
[a] Key Laboratory of Meat Processing and Quality Control, Ministry of Education, Key Laboratory of Animal Products Processing, Ministry of Agriculture, Synergetic Innovation Center of Food Safety and Nutrition, College of Food Science and Technology, Nanjing Agricultural University, Nanjing, 210095, PR China
Published online: 23 Feb 2015.

CyTA – Journal of Food, 2015
http://dx.doi.org/10.1080/19476337.2015.1005027

Taylor & Francis
Taylor & Francis Group

Reduction of biogenic amine concentration in fermented sausage by selected starter cultures

Reducción de las proporciones de aminas biógenas en salchichas fermentadas mediante cultivos iniciadores seleccionados

Chong Xie, Hu-Hu Wang, Xiao-Kai Nie, Lin Chen, Shao-Lin Deng and Xing-Lian Xu*

Key Laboratory of Meat Processing and Quality Control, Ministry of Education, Key Laboratory of Animal Products Processing, Ministry of Agriculture, Synergetic Innovation Center of Food Safety and Nutrition, College of Food Science and Technology, Nanjing Agricultural University, Nanjing, 210095, PR China

(*Received 12 September 2014; final version received 5 January 2015*)

We determined the effects of selected starter cultures for the production and ripening of fermented sausages on their ability to reduce biogenic amines accumulation. Starter cultures used were: Group L: *Lactobacillus plantarum*; Group X: *Staphylococcus xylosus*; Group M: *Lactobacillus plantarum* together with *Staphylococcus xylosus*; and Group C: control group without added starter cultures. At the end of ripening period, those inoculated with *S. xylosus* had only a slight effect on reduction of tyramine (21%), histamine (25%) and cadaverine (22%) compared with the control group. Addition of *S. xylosus* plus *L. plantarum* effectively reduced tryptamine, phenylethylamine, putrescine, cadaverine, histamine and tyramine by nearly 100, 100, 86, 63, 82 and 43%, respectively. There were no statistically significant ($P > 0.05$) differences between batches M and L. Results indicated that *L. plantarum* used in this work had a strong effect on inhibiting the production of biogenic amines.

Keywords: biogenic amines; *Lactobacillus plantarum*; amine oxidases; starter cultures; fermented sausage

Se determinaron los efectos de los cultivos iniciadores seleccionados para la producción y maduración de salchichas fermentadas en su habilidad para reducir la acumulación de aminas biógenas. Los cultivos iniciadores que se utilizaron fueron: Grupo L: *Lactobacillus plantarum*; Grupo X: *Staphylococcus xylosus*; Grupo M: *Lactobacillus plantarum* juntamente con *Staphylococcus xylosus* y Grupo C: grupo control sin cultivos iniciadores. Al final del periodo de maduración, aquellos que inocularon con *S. xylosus* únicamente tuvieron un ligero efecto secundario de reducción de tiramina (21%), histamina (25%), cadaverina (22%) en comparación con el grupo control. La adición de *S. xylosus* más *L. plantarum* redujo efectivamente la triptamina, feniletilamina, putrescina, cadaverina, histamina, tiramina cerca de 100%, 100%, 86%, 63%, 82% y 43%, respectivamente. No se produjeron diferencias estadísticas significativas ($P > 0{,}05$) entre los lotes M y L. Los resultados indicaron que el *L. plantarum* utilizado en este estudio tuvo un fuerte efecto en la inhibición de la producción de aminas biógenas.

Palabras clave: aminas biógenas; *Lactobacillus plantarum*; diamino oxidasa; cultivos iniciadores; salchicha fermentada

1. Introduction

Most fermented meat sausage products are sold for consumption through either delicatessen sections of supermarkets or specific delicatessen stores. They are composed of raw minced meat which is mixed with lard, salt, sugars, spices and other ingredients. After stuffing into casings the mixture is fermented and ripened under controlled temperature and humidity. During fermentation, as a result of the acidification produced by the growth of lactic acid bacteria (LAB), certain microbial, physico-chemical and organoleptic changes occurred which gradually gave the product an appealing colour, taste, flavour and texture (Eerola, Sagues & Hirvi, 1998). However, relatively high levels of biogenic amines (BAs) have been found in fermented sausage products in many countries (Latorre-Moratalla et al., 2008; Lu et al., 2010; Riebroy, Benjakul, Visessanguan, Kijrongrojana, & Tanaka, 2004). Biogenic amines are nitrogenous basic compounds which have undesirable physiological effects on human health, and may be responsible for hypotension or hypertension, allergic reactions, nausea, palpitations, intracerebral haemorrhage and death in very severe cases (Shalaby, 1996). In fermented sausage products the major BAs are tryptamine (TRY), phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR) spermine (SPM) and spermidine (SPD), and these are mainly products of microbial decarboxylation of amino acids (Santos, 1996). The most frequent foodborne intoxication caused by BAs involves histamine and tyramine (Anastasio et al., 2010).

The presence or absence of BAs in fermented sausages is a highly complex equilibrium, dependent upon many variables. These include the hygiene of the starting meat ingredients, method of manufacture, qualitative and quantitative composition of the microflora as well as certain physico-chemical parameters (Mercogliano et al., 2013). The selection of appropriate inoculating starter cultures has been widely perceived as one of the most effective strategies to prevent or minimize the presence of BAs (Latorre-Moratalla, Bover-Cid, Veciana-Nogués, & Vidal-Carou, 2012).

LAB are widely used in meat products as starter cultures because of their ability to rapidly reduce pH as well as their contribution to the generation of particular desirable flavours. Effective acidification will inhibit the growth of decarboxylation-positive microorganisms which will then prevent accumulation of BAs (Maijala, Eerola, Aho, & Hirn, 1993). Also, LAB can inhibit the growth of certain pathogenic bacteria during the

*Corresponding author. Email: xlxu@njau.edu.cn

fermentation and ripening periods, thus improving the safety aspects of these products (Kuley, Özogul, Özogul, & Akyol, 2011). Coagulase-negative staphylococci (CNS) and micrococci are often inoculated together with LAB and also contribute to the generation of the typical flavours and textures as a result of their acidic, lipolytic and proteolytic activities (Hammes & Hertel, 1996). During product manufacture, these additional microorganisms generate catalase which prevents sausages developing colour changes and rancidity. In addition, both CNS and micrococci can reduce nitrates to nitrites, thus improving colour formation and stability. Among CNS and micrococci, the use of *S. xylosus* is most frequent (Talon et al., 2008).

A strain of *Lactobacillus plantarum* containing high levels of amine oxidases (AOs) has been isolated from fermented sausages in southern China. The production of AOs in these meat products facilitates the inactivation of BAs through the oxidative deamination of amines, with the production of hydrogen peroxide, aldehydes and ammonia (Cooper, 1997). Under *in vitro* conditions, it has been shown that there was a significant reduction in the concentration of BAs when using strains with AOs as starters (Fadda, Vignolo, & Oliver, 2001; Gardini, Martuscelli, Crudele, Paparella, & Suzzi, 2002; Leuschner & Hammes, 1998; Leuschner, Heidel, & Hammes, 1998; Martuscelli, Crudele, Gardini, & Suzzi, 2000). This has also been demonstrated when some strains were included in sausage formulations (Gardini et al., 2002; Leuschner & Hammes, 1998). However, most researches on starter cultures and AOs have focused on strains of staphylococci and micrococci while these amine-oxidizing microorganisms have shown no distinct effect on BA levels. Latorre-Moratalla et al. (2012) suggested that amine-oxidizing microorganisms have limited effect on amine levels due to low oxygen availability within sausages. Papers published regarding using LAB containing AOs as the starter culture for reduction of BAs number fewer than those written on the use of staphylococci and micrococci as starter cultures.

The aim of this paper was to study the effect of inoculating *L. plantarum* containing AOs as starter cultures in reducing BA accumulation during the fermentation and ripening period of sausages. An amine-negative decarboxylase present in *S. xylosus* was chosen because of its known effects on improving the colour and flavour of the sausages as a result of its lipolytic and proteolytic activities, together with nitrate reductase and catalase activities.

2. Materials and methods

2.1. Starter cultures

The *L. plantarum* strain used in this work was isolated from traditional Chinese sausage obtained from the Meat Processing and Quality Control Center of China. *S. xylosus* was purchased from the China Center of Industrial Culture Collection (CICC).

2.2. Sausage preparation

Sausage manufacture was carried out in the pilot plant of the Meat Processing and Quality Control Center, Nanjing, China. In triplicate (and on different days), four different groups were prepared (5 kg per group) according to the type of starter culture used: control batch without a starter culture (C), *L. plantarum* (L), *S. xylosus* (X) and *L. plantarum* and *S. xylosus* (M). The ingredients used were: lean pork (75%), pork back fat (25%), salt (23 g/kg), glucose (10 g/kg), garlic powder (1.5 g/kg), ginger powder (1 g/kg), black pepper (2 g/kg), white pepper (2 g/kg), ascorbic acid and nitrite (0.15 g/kg). Lean meat was ground to a particle size of 4 mm and fat was chopped to 6–8 mm, then the products were divided into four portions representing different batches. Batch C followed spontaneous fermentation, while batches L, X and M were fermented with each of the respective starter cultures. In batch M, the proportion of the two starters was 1:1. Starter cultures were suspended in 50 mL of cool water (0–4°C) before their addition to batches, to attain a level of 10^5 cfu/g. Each mixture was homogenized and stuffed into collagen casings of external diameter 2.2 cm. All sausages were then placed in a constant temperature and constant humidity box (KBF240, Binder, Germany). All products were ripened under the same temperature/humidity conditions: 30°C/95% day 1, 16°C/85% days 2–7 and 12°C/70% days 8–21.

2.3. Bacterial analysis, measurement of pH and a_w

Microbiological analysis was carried out according to Nie, Zhang, & Lin, 2014): 10 g of sausage was homogenized in 90 mL of sterile water and 10-fold dilutions were prepared. Using the poured plate method, LAB were grown on MRS agar at 3°C for 48 h. Micrococcaceae/Staphylococcaceae were grown on Baird Parker Medium supplemented with egg yolk tellurite and incubated at 37°C for 24 h. Enterobacteriaceae were determined using Violet Red Bile Dextrose Agar (VRBDA) at 30°C for 24 h. After incubation, plates with 30–300 colonies were counted by auto-colony counter (Scan 1200, Interscience, France). Three replicates were carried out for each bacterial analysis. All culture media were purchased from LuQiao Company (Beijing, China).

Measurement of pH values was performed according to Chinese GB/T 9695.5–2008 (meat and meat products: measurement of pH) on 10 g of sample homogenized in 100 mL of 1 N KCL, using a pH meter (320 T-81, Thermo). The values of a_w were measured using a water activity meter (Labmaster-aw, Novasina AG) after sectioning. Three independent values were obtained for each sample.

2.4. Biogenic amine determination

Extraction and pretreatment of BAs in samples and the preparation of standard solutions was carried out using the method described by Lu et al. (2010). Subsequent quantification was carried using high performance liquid chromatography techniques (Waters Alliance 2695). Separation was carried out on a C18 column (Agilent ZORBAX SB-C18, 4.6*250 mm^2, 5 μm) at 30°C and peaks were detected at 254 nm. A gradient elution programme was used with a mixture of ultra-pure water as solvent A and with acetonitrile as solvent B. The gradient elution procedure was 35% A + 65% B at 0 min, 30% A + 70% B at 5 min, 0% A + 100% B at 20 min, 0% A + 100% B at 24 min, 35% A + 65% B at 25 min and 35% A + 65% B at 30 min. Standard amines were purchased from Sigma (USA).

2.5. Statistical analyses

All experiments were repeated three times and tests performed in triplicate. Statistical analysis was performed using the software package SPSS 20.0 for Windows. LSD testing and Analysis of the Variance (ANOVA) were employed to determine any significant differences among samples.

3. Results and discussion

3.1. Bacterial growth and physico-chemical parameters

Table 1 shows microbial growth during the ripening period. Initial LAB in batches L and M were significantly higher than in batches X and C ($P < 0.05$), due to the inoculation of the starter strains. Because the *S. xylosus* starter culture used in this work can partly grow on MRS agar, the count for LAB appeared higher than for batch C. On the day 1, as a consequence of the high temperature and nutritional circumstances, LAB in all groups showed a four log unit growth. Between days 1 and 21, LAB in all groups was slightly reduced during the first three days but then grew slowly to day 21. Our results for final LAB counts are similar to those obtained by Ciuciu Simion, Vizireanu, Alexe, Franco, and Carballo (2014) for both inoculated batches and control. However, LAB in batches L and M remained significantly higher ($P < 0.05$) than in batches X and C at day 21 and there was no significant difference ($P > 0.05$) between batches L and M.

According to Gücükoğlu and Küplülü (2010), *Micrococcus/Staphylococcus* spp. represent the second most important group in the natural flora of meat following LAB. The level of Micrococcaceae/Staphylococcaceae in groups C and L was 3.41 and 3.67 log CFU/g, respectively. In batches X and M, due to the high inoculum level, Micrococcaceae/Staphylococcaceae were present at an initial concentration of about 4.89 log CFU/g and 4.10 log CFU/g, respectively. Micrococcaceae/Staphylococcaceae levels increased by over four log units in all batches during the first two weeks and later decreased. Over the whole process, increase in Micrococcaceae/Staphylococcaceae population in all batches showed a marked dependence on pH. In batches L, M, X and C, growth over the whole process was 3.26, 3.76, 4.31 and 5.31 log units, respectively.

As observed in other studies, Enterobacteriaceae counts in all batches were characterized by high initial values that decreased significantly during ripening, independent of the presence of the starter culture used (Gardini et al., 2002; Tosukhowong et al., 2011). At the end of ripening, the Enterobacteriaceae counts in batches X and C were slightly higher than the initial values; however, the corresponding counts in batches L and M had significantly decreased. This difference can likely be attributed to the fact that LAB can suppress the growth of wild Enterobacteriaceae (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000; González-Fernández, Santos, Jaime, & Rovira, 2003; Hu, Xia, & Ge, 2008).

Values of pH and a_w during the production process are shown in Table 2. As found in other studies, pH values in all batches decreased rapidly initially followed by a characteristic increase. Meanwhile, inoculation of the starter cultures resulted in more marked acidification than that found in the control group (González-Fernández et al., 2003; Lu et al., 2010). Groups L and M, especially had pH values significantly lower ($P < 0.05$) than the other two groups over the whole ripening process and there was no significant differences ($P > 0.05$) between groups L and M between days 1 and 14. Meanwhile, pH values of batches X and C showed no significant difference ($P > 0.05$) at end of ripening.

The values of a_w decreased gradually during the manufacturing process, reaching final values lower than 0.85 in all batches. Lu et al. (2010) reported that there were no differences between control and modified samples while in this study, similar to results described by González-Fernández et al. (2003), the final value of a_w in sausages with *L. plantarum* as starter culture was significantly lower ($P < 0.05$) than the control group.

3.2. Biogenic amines

The concentration of BAs in all four batches is presented in Table 3. The initial samples contained low amounts of BAs and only CAD, TYR and SPM were detected. The concentration

Table 1. Changes in bacterial count (log CFU/g) during the manufacture of sausages prepared using different starters cultures (mean ± standard deviation of the values from three replicates).

Tabla 1. Cambios en el recuento de bacterias (log CFU/g) durante la manufactura de salchichas que fueron preparadas utilizando diferentes cultivos iniciadores (promedio ± desviación estándar de los valores de tres réplicas).

Species	Batch	Storage time (days)					
		0	1	3	7	14	21
LAB	C	3.71 ± 0.07 ^{a1}	8.51 ± 0.17 ^{a2}	8.39 ± 0.14 ^{a2}	8.92 ± 0.89 ^{a23}	9.23 ± 0.09 ^{a34}	9.86 ± 0.17 ^{a4}
	L	5.11 ± 0.09 ^{d1}	9.14 ± 0.19 ^{c3}	8.87 ± 0.21 ^{b2}	9.2 ± 0.07 ^{b3}	10.7 ± 0.14 ^{c4}	10.6 ± 0.14 ^{c4}
	X	4.40 ± 0.23 ^{b1}	8.90 ± 0.11 ^{b3}	8.55 ± 0.06 ^{a2}	9.03 ± 0.18 ^{a3}	10.17 ± 0.1 ^{b4}	10.38 ± 0.1 ^{b4}
	M	5.07 ± 0.05 ^{c1}	9.46 ± 0.05 ^{d2}	9.11 ± 0.10 ^{c2}	9.11 ± 0.10 ^{b2}	10.4 ± 0.03 ^{c3}	10.5 ± 0.04 ^{c3}
M/S	C	3.41 ± 0.25 ^{a1}	5.77 ± 0.11 ^{b2}	7.86 ± 0.05 ^{b3}	8.04 ± 0.10 ^{c3}	9.41 ± 0.02 ^{b4}	8.72 ± 0.10 ^{c5}
	L	3.67 ± 0.03 ^{a1}	5.01 ± 0.08 ^{a2}	7.17 ± 0.16 ^{a4}	6.64 ± 0.12 ^{a3}	8.23 ± 0.02 ^{a5}	6.93 ± 0.43 ^{a43}
	X	4.89 ± 0.09 ^{c1}	5.92 ± 0.04 ^{c2}	8.14 ± 0.11 ^{c3}	7.99 ± 0.09 ^{c3}	9.49 ± 0.05 ^{b4}	9.20 ± 0.11 ^{d4}
	M	4.10 ± 0.02 ^{b1}	5.56 ± 0.15 ^{b2}	7.70 ± 0.09 ^{b3}	7.38 ± 0.07 ^{b3}	8.25 ± 0.15 ^{a4}	7.86 ± 0.17 ^{b3}
Ent	C	5.20 ± 0.17 ^{a1}	5.88 ± 0.07 ^{a2}	6.65 ± 0.21 ^{b3}	6.95 ± 0.16 ^{b34}	6.34 ± 0.13 ^{b3}	5.92 ± 0.21^{b2}
	L	5.21 ± 0.12 ^{a2}	6.07 ± 0.21 ^{b3}	6.39 ± 0.22 ^{a3}	6.14 ± 0.05 ^{a3}	4.92 ± 0.13 ^{a2}	3.32 ± 0.1 ^{a1}
	X	5.23 ± 0.14 ^{a1}	6.02 ± 0.13 ^{b2}	7.07 ± 0.09 ^{c3}	7.25 ± 0.08 ^{c3}	6.04 ± 0.13 ^{b2}	5.70 ± 0.11 ^{b12}
	M	5.17 ± 0.16 ^{a2}	6.32 ± 0.10 ^{c3}	6.55 ± 0.10 ^{b3}	6.85 ± 0.09 ^{b3}	5.11 ± 0.12 ^{a2}	3.21 ± 0.05 ^{a1}

Notes: LAB: lactic acid bacteria; M/S: *Micrococcus/Staphylococcus*; Ent: Enterobacteriaceae; batch C: not inoculated with starter cultures; batch L: inoculated with *Lactobacillus plantarum*; batch X: inoculated with *Staphylococcus xylosus*; batch M: inoculated with *L. plantarum* and *S. xylosus*.
Values (a–d) in the same column with different letters are significantly different ($P < 0.05$). Values (1–5) in the same row with different numbers are significantly different ($P < 0.05$).

Notas: LAB: Bacteria acido-láctica, M/S: *Micrococcus/Staphylococcus*, Ent: *Enterobacteriaceae*. Lote C: sin inocular con cultivos iniciadores; Lote L: inoculado con *Lactobacillus plantarum*; Lote X: inoculado con *Staphylococcus xylosus*; Lote M: inoculado con *Lactobacillus plantarum* y *Staphylococcus xylosus*.
Los valores (a–d) en la misma columna con diferentes letras son significativamente distintos ($P < 0,05$). Los valores (1–5) en la misma fila con diferentes números son significativamente distintos ($P < 0,05$).
LAB: Bacteria acido-láctica, M/S: *Micrococcus/Staphylococcus*, Ent: *Enterobacteriaceae*.

Table 2. Changes in pH and water activity (a_w) during the ripening of sausages prepared using different starters (mean ± standard deviation of the values from three replicates).

Tabla 2. Cambios en el valor pH y en la actividad del agua (a_w) durante la maduración de las salchichas que fueron preparadas utilizando diferentes iniciadores (promedio ± desviación estándar de los valores de tres réplicas).

Fermentation time (days)	Batch			
pH	C	L	X	M
0	6.32 ± 0.01 ^{a6}	6.30 ± 0.01 ^{a5}	6.29 ± 0.02 ^{a5}	6.30 ± 0.02 ^{a5}
1	4.80 ± 0.03 ^{c2}	4.15 ± 0.04 ^{a1}	4.43 ± 0.02 ^{b1}	4.18 ± 0.01 ^{a1}
3	4.44 ± 0.05 ^{c1}	4.20 ± 0.02 ^{a1}	4.34 ± 0.02 ^{b1}	4.22 ± 0.03 ^{a1}
7	5.18 ± 0.02 ^{c3}	4.31 ± 0.02 ^{a2}	4.65 ± 0.04 ^{b2}	4.32 ± 0.03 ^{a2}
14	5.65 ± 0.04 ^{d4}	5.15 ± 0.03 ^{a3}	5.47 ± 0.02 ^{c3}	5.23 ± 0.01 ^{b3}
21	5.76 ± 0.05 ^{c5}	5.24 ± 0.04 ^{a4}	5.72 ± 0.04 ^{c4}	5.34 ± 0.04 ^{b4}
a_w				
0	0.963 ± 0.001 ^{a5}	0.962 ± 0.002 ^{a5}	0.961 ± 0.001 ^{a5}	0.963 ± 0.001 ^{a5}
1	0.962 ± 0.002 ^{a5}	0.961 ± 0.001 ^{a5}	0.960 ± 0.001 ^{a5}	0.962 ± 0.001 ^{a5}
3	0.949 ± 0.001 ^{a4}	0.946 ± 0.001 ^{a4}	0.949 ± 0.001 ^{a4}	0.950 ± 0.002 ^{a4}
7	0.939 ± 0.003 ^{b3}	0.930 ± 0.002 ^{a3}	0.940 ± 0.003 ^{b3}	0.932 ± 0.002 ^{a3}
14	0.877 ± 0.003 ^{b2}	0.855 ± 0.005 ^{a2}	0.874 ± 0.004 ^{b2}	0.854 ± 0.003 ^{a2}
21	0.845 ± 0.004 ^{b1}	0.813 ± 0.004 ^{a1}	0.838 ± 0.006 ^{b1}	0.816 ± 0.004 ^{a1}

Notes: Batch C: not inoculated with starter cultures; batch L: inoculated with *Lactobacillus plantarum*; batch X: inoculated with *Staphylococcus xylosus*; batch M: inoculated with *L. plantarum* and *S. xylosus.*
Values (a–d) in the same row with different letters are significantly different ($P < 0.05$). Values (1–6) in the same column with different number are significantly different ($P < 0.05$).

Notas: Lote C: no inoculado con cultivos iniciadores; Lote L: inoculado con *Lactobacillus plantarum*; Lote X: inoculado con *Staphylococcus xylosus*; Lote M: inoculado con *Lactobacillus plantarum* y *Staphylococcus xylosus.*
Los valores (a–d) en la misma fila con diferentes letras son significativamente distintos ($P < 0,05$). Los valores (1–6) en la misma columna con diferente número son significativamente distintos ($P < 0,05$).

ranged from 9.88 to 12.17 mg/kg for CAD, 19.21 to 24.28 mg/kg for TYR and 100.56 to 108.87 mg/kg for SPM.

We found that PUT, CAD and TYR were the most abundant BAs in batch C, which is similar to results previously reported (González-Fernández et al., 2003; Komprda et al., 2004). In the initial samples, there was no significant difference ($P > 0.05$) in regard to the three amines among batches. At day 1 of fermentation, batch C had the lowest numbers of Enterobacteriaceae but the concentrations of TYR, PUT and CAD were significantly higher than in other batches. Enterobacteriaceae have been identified as being high producers of BAs, particularly for PUT and CAD (Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2001; Durlu-Özkaya, Ayhan, & Vural, 2001; Hortensia Silla, 1998). This paradoxical finding may have resulted from the differences in both pH values and AO content. Between days 1 and 21, the three amines in groups L and M remained significantly lower ($P < 0.05$) than in the other two groups.

From a toxicological aspect, histamine is the most potent biogenic amine (Shalaby, 1996) with an ability to cause poisoning at high concentrations (Naila et al., 2012). In this work, histamine was detected in batches X and C on day 3 and had the highest growth rate from days 3 to 14. Histamine concentration in batches L and M first appeared between days 7 and 14, respectively. In the final product, the concentration of histamine in groups C, L, X and M was 177.4, 28.93, 133.3 and 32.59 mg/kg, respectively. These significant differences may have resulted from the suppression by LAB of the growth of Enterobacteriaceae, which have been described as being able to produce large quantities of histamine (Pircher, Bauer, & Paulsen, 2007).

The aromatic amines tryptamine and phenylethylamine are thought to be generated by non-starter LAB but not by Enterobacteriaceae species (Bover-Cid et al., 2001). The *L. plantarum* strain used in this work has no tryptophan or phenylalanine decarboxylase activities, and it represents the majority of gross LAB strains used in the manufacture of these sausages. That is why there was no TRY or PHE detected in batches L and M during the entire process. In contrast, the TRY content of batches X and batch C was 32.76 and 31.01 mg/kg, respectively, and PHE content in the final product of batches X and C was 2.00 and 1.24 mg/kg, respectively.

SPM is the major biogenic amine present in fresh meat (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1996). Sugita, Takao, Toyama, and Shirahata (2007) demonstrated that frequent ingestion of aliphatic polyamines, such as SPM and SPD, may increase the permeability of the intestinal mucosa to food allergens and thereby facilitate the induction of food allergies. Some studies have indicated that these two amines are transformed from other amines such as PUT but are not formed by microbial decarboxylation (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 1999). Between days 1 and 7, accumulation of SPM in all groups showed a slight increase. Between days 14 and 21, SPM in group C showed a large and sudden decrease. Also, the level of SPM in batches L and M showed a major increase. No SPD was detected in any group.

At the end of fermentation, the total amount of all seven BAs in the control group was 2021 mg/kg, a concentration with the potential to be a safety issue. In regard to batch X, the value of final total BAs was 1788 mg/kg. Though *S. xylosus* inoculated in this batch had no amino acid decarboxylase, increased proteolysis due to its presence may have increased the availability of amino acids and led to greater biogenic amine formation (Ciuciu Simion et al., 2014). The final content of BAs in batches L and M was less than 1000 mg/kg and there was no statistical difference in regard to amine production between them except for TYR and SPM. Previous studies have indicated that the AOs of this *L. plantarum* strain were unable to degrade spermine and

Table 3. Changes in BA concentration (mg/kg dry matter) during the manufacture of sausages prepared using different starters (mean ± standard deviation of the values from three replicates).

Tabla 3. Cambios en la concentración de BAs (mg/kg materia seca) durante la manufactura de las salchichas que fueron preparadas utilizando diferentes iniciadores (promedio ± desviación estándar de los valores de tres réplicas).

Storage (days)	Batch	TRY	PHE	PUT	CAD	HIS	TYR	SPM
0	C	nd	nd	nd	12.17 ± 3.12 ^{a1}	nd	22.37 ± 3.11 ^{a1}	108.87 ± 2.74 ^{a1}
	L	nd	nd	nd	9.88 ± 1.18 ^{a1}	nd	24.28 ± 1.54 ^{a1}	106.45 ± 3.28 ^{a1}
	X	nd	nd	nd	10.36 ± 1.11 ^{a1}	nd	19.21 ± 2.11 ^{a1}	100.56 ± 6.23 ^{a1}
	M	nd	nd	nd	11.20 ± 0.15 ^{a1}	nd	21.53 ± 0.95 ^{a1}	104.14 ± 2.24 ^{a1}
1	C	nd	nd	24.37 ± 4.10 ^{d1}	164.2 ± 2.13 ^{d2}	nd	131.8 ± 18.3 ^{c2}	119.06 ± 3.52 ^{a1}
	L	nd	nd	4.98 ± 0.28 ^{a1}	84.34 ± 3.55 ^{b2}	nd	56.71 ± 1.96 ^{a2}	117.81 ± 2.02 ^{a1}
	X	nd	nd	16.16 ± 1.1 ^{c1}	105.3 ± 2.77 ^{c2}	nd	85.10 ± 2.97 ^{b2}	113.62 + 3.11 ^{a1}
	M	nd	nd	9.50 ± 0.5^{b1}	71.98 ± 4.26 ^{a2}	nd	51.93 ± 2.82 ^{a2}	119.64 ± 2.91 ^{a1}
3	C	nd	1.10 ± 0.15 ^{1}	89.48 ± 4.6 ^{b2}	220.7 ± 3.54 ^{b3}	9.96 ± 0.68^{1}	177.9 ± 7.51 ^{d2}	118.87 ± 1.71 ^{a1}
	L	nd	nd	19.68 ± 2.02 ^{a2}	131.4 ± 2.25 ^{a3}	nd	35.24 ± 2.56 ^{a2}	126.21 ± 4.21 ^{a1}
	X	19.68 ± 0.9^{1}	0.869 ± 0.15 ^{1}	152.2 ± 5.8 ^{c2}	278.0 ± 0.56 ^{b3}	40.64 ± 0.5^{1}	63.10 ± 9.18 ^{c2}	125.56 ± 3.73 ^{a1}
	M	nd	nd	22.49 ± 0.8 ^{a2}	124.0 ± 3.13 ^{a3}	nd	50.59 ± 2.47 ^{b2}	120.4 ± 4.14 ^{a1}
7	C	23.33 ± 1.8^{1}	1.90 ± 0.11 ^{2}	349.2 ± 7.75 ^{c3}	376.0 ± 8.14 ^{c4}	55.31 ± 17.8 ^{b2}	261.2 ± 2.93 ^{d3}	159.96 ± 6.50 ^{b2}
	L	nd	nd	30.82 ± 1.16 ^{b3}	182.9 ± 8.24 ^{b4}	3.03 ± 0.6 ^{a1}	82.22 ± 4.99 ^{a3}	177.87 ± 3.09 ^{c2}
	X	26.16 ± 1.4^{2}	1.61 ± 0.11 ^{2}	346.3 ± 9.58 ^{c3}	357.9 ± 7.66 ^{c4}	85.49 ± 1.87 ^{c2}	237.7 ± 2.93 ^{c3}	183.62 + 5.18 ^{c2}
	M	nd	nd	15.73 ± 1.06 ^{a3}	91.40 ± 2.32 ^{a2}	nd	33.05 ± 3.22 ^{b12}	129.54 ± 4.98 ^{a1}
14	C	30.14 ± 1.2^{2}	2.12 ± 0.2 ^{2}	556.2 ± 6.06 ^{b4}	654.7 ± 14.4 ^{c5}	155.9 ± 7.19^{b3}	494.4 ± 37.5 ^{d4}	160.02 ± 6.13 ^{b2}
	L	nd	nd	62.22 ± 1.42 ^{a4}	273.5 ± 5.73 ^{a5}	20.71 ± 0.48 ^{a2}	214.4 ± 8.7 ^{a4}	143.35 ± 4.04 ^{a1}
	X	32.55 ± 3.6^{3}	4.28 ± 0.21 ^{3}	561.1 ± 21.4 ^{b4}	480.1 ± 16.9 ^{b5}	131.4 ± 5.11 ^{b3}	395.1 ± 8.97 ^{b4}	155.90 ± 12.8 ^{a3}
	M	nd	nd	55.41 ± 0.91 ^{a4}	253.7 ± 3.01 ^{a4}	14.39 ± 1.66 ^{a1}	213.3 ± 10.8 ^{a3}	144.97 ± 7.70 ^{a2}
21	C	31.01 ± 0.2 ^{2}	1.24 ± 0.11 ^{1}	563.3 ± 4.69^{b4}	644.1 ± 26. 6 ^{c5}	177.4 ± 21.6 ^{c3}	502.8 ± 42.3 ^{d4}	101.2 ± 2.04 ^{a1}
	L	nd	nd	70.03 ± 1.01^{a4}	252.6 ± 6.29 ^{a5}	28.93 ± 0.82^{a2}	268.1 ± 6.05 ^{a4}	266.8 ± 14.5 ^{c3}
	X	32.76 ± 1.7 ^{3}	2.00 ± 0.34 ^{4}	564.5 ± 8.72^{b4}	503.2 ± 4.82 ^{b5}	133.3 ± 1.79 ^{b3}	397.2 ± 2.5 ^{c4}	155.8 ± 3.62 ^{b3}
	M	nd	nd	78.11 ± 4.01^{a5}	232.51 ± 11.1 ^{a4}	32.59 ± 1.07 ^{a2}	288.7 ± 6.13 ^{b3}	328.6 ± 11.5 ^{d3}

Notes: TRY: tryptamine; PHE: phenylethylamine; PUT: putrescine; CAD: cadaverine; HIS: histamine; TYR: tyramine; SPM: spermine; nd: not detected. batch C: not inoculated with starter cultures; batch L: inoculated with *Lactobacillus plantarum*; batch X: inoculated with *Staphylococcus xylosus*; batch M: inoculated with *L. plantarum* and *S. xylosus.*
Values (a–d) in the same column and day with different letters are significantly different ($P < 0.05$). Values (1–5) in the same column and starter with different number are significantly different ($P < 0.05$).

Notas: TRY: triptamina; PHE: feniletilamina; PUT: putrescina; CAD: cadaverina; HIS: histamina; TYR: tiramina; SPM: espermina; nd: Sin detector; Lote C: no inoculado con cultivos iniciadores; Lote L: inoculado con *Lactobacillus plantarum*; Lote X: inoculado con *Staphylococcus xylosus*; Lote M: inoculado con *Lactobacillus plantarum* y *Staphylococcus xylosus.*
Los valores (a–d) en la misma columna y día con diferentes letras son significativamente distintos ($P < 0,05$). Los valores (1–5) en la misma columna e iniciador con diferente número son significativamente distintos ($P < 0,05$).

would become inactive below 15°C. Thus the degradation of BAs through AOs mainly occurred during the first 7 days. After that, the control of BAs by *L. plantarum* is mainly attributed to a fall in pH and inhibition of amino decarboxylase-positive bacteria.

4. Conclusion

We conclude that the starter culture used in this study, *L. plantarum*, had a beneficial effect on the control of biogenic amines in fermented sausages on account of amino oxidases and its ability to inhibit the growth of Enterobacteriaceae. Even when meat preparations were inoculated with other starter cultures, the inhibition of BAs remained effective. Furthermore, these findings suggest that the presence of amino oxidases is an important factor to consider when selecting starter cultures for use in the manufacture of fermented foods.

Acknowledgements

This work was supported by a Special Fund for Agri-scientific Research in the Public Interest (201303082-2). We are very grateful to Professor Ron Tume from the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Animal, Food and Health Sciences, Australia for his valuable advice and for assistance with language.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Anastasio, A., Draisci, R., Pepe, T., Mercogliano, R., Quadri, F. D., Luppi, G., & Cortesi, M. L. (2010). Development of biogenic amines during the ripening of Italian dry sausages. *Journal of Food Protection*, *73*(1), 114–118.

Bover-Cid, S., Hugas, M., Izquierdo-Pulido, M., & Vidal-Carou, M. C. (2001). Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. *International Journal of Food Microbiology*, *66*, 185–189. doi:10.1016/S0168-1605(00)00526-2

Bover-Cid, S., Izquierdo-Pulido, M., & Vidal-Carou, M. C. (1999). Effect of proteolytic starter cultures of *Staphylococcus* spp. on biogenic amine formation during the ripening of dry fermented sausages. *International Journal of Food Microbiology*, *46*(2), 95–104. doi:10.1016/S0168-1605(98)00170-6

Bover-Cid, S., Izquierdo-Pulido, M., & Vidal-Carou, M. C. (2000). Mixed starter cultures to control biogenic amine production in dry fermented sausages. *Journal of Food Protection*, *63*, 1556–1562.

Ciuciu Simion, A. M., Vizireanu, C., Alexe, P., Franco, I., & Carballo, J. (2014). 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*, *35*(1), 123–131. doi:10.1016/j.foodcont.2013.06.047

Cooper, R. A. (1997). On the amine oxidases of *Klebsiella aerogenes* strain W70. *FEMS Microbiology Letters*, *146*(1), 85–89. doi:10.1111/j.1574-6968.1997.tb10175.x

Durlu-Özkaya, F., Ayhan, K., & Vural, N. (2001). Biogenic amines produced by *Enterobacteriaceae* isolated from meat products. *Meat Science*, *58*(2), 163–166. doi:10.1016/S0309-1740(00)00144-3

Eerola, H. S., Sagues, A. R., & Hirvi, T. K. (1998). Biogenic amines in Finnish dry sausages. *Journal of Food Safety*, *18*(2), 127–138. doi:10.1111/j.1745-4565.1998.tb00208.x

Fadda, S., Vignolo, G., & Oliver, G. (2001). Tyramine degradation and tyramine/histamine production by lactic acid bacteria and *Kocuria* strains. *Biotechnology Letters*, *23*(24), 2015–2019. doi:10.1023/A:1013783030276

Gardini, F., Martuscelli, M., Crudele, M. A., Paparella, A., & Suzzi, G. (2002). Use of *Staphylococcus xylosus* as a starter culture in dried sausages: Effect on the biogenic amine content. *Meat Science*, *61*(3), 275–283. doi:10.1016/S0309-1740(01)00193-0

González-Fernández, C., Santos, E. M., Jaime, I., & Rovira, J. (2003). Influence of starter cultures and sugar concentrations on biogenic amine contents in chorizo dry sausage. *Food Microbiology*, *20*(3), 275–284. doi:10.1016/S0740-0020(02)00157-0

Gücükoğlu, A., & Küplülü, Ö. (2010). The effect of different starter cultures and ripening temperatures on formation of biogenic amine in Turkish fermented sausages. *European Food Research and Technology*, *230*(6), 875–884. doi:10.1007/s00217-010-1220-z

Hammes, W. P., & Hertel, C. (1996). Selection and improvement of lactic acid bacteria used in meat and sausage fermentation. *Le Lait*, *76*(1–2), 159–168. doi:10.1051/lait:19961-214

Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, T., & Vidal-Carou, M. C. (1996). Ion-pair high-performance liquid chromatographic determination of biogenic amines in meat and meat products. *Journal of Agricultural and Food Chemistry*, *44*(9), 2710–2715. doi:10.1021/jf9506803

Hortensia Silla, S. M. (1998). Amino acid decarboxylase capability of microorganisms isolated in Spanish fermented meat products. *International Journal of Food Microbiology*, *39*(3), 227–230. doi:10.1016/S0168-1605(97)00129-3

Hu, Y., Xia, W., & Ge, C. (2008). Characterization of fermented silver carp sausages inoculated with mixed starter culture. *LWT-Food Science and Technology*, *41*(4), 730–738. doi:10.1016/j.lwt.2007.04.004

Komprda, T., Smel, D., Pechov, P., Kalhotka, L., Stencl, J., & Klejdus, B. (2004). Effect of starter culture, spice mix and storage time and temperature on biogenic amine content of dry fermented sausages. *Meat Science*, *67*(4), 607–616. doi:10.1016/j.meatsci.2004.01.003

Kuley, E., Özogul, F., Özogul, Y., & Akyol, I. (2011). The function of lactic acid bacteria and brine solutions on biogenic amine formation by foodborne pathogens in trout fillets. *Food Chemistry*, *129*(3), 1211–1216. doi:10.1016/j.foodchem.2011.05.113

Latorre-Moratalla, M. L., Bover-Cid, S., Veciana-Nogués, M. T., & Vidal-Carou, M. C. (2012). Control of biogenic amines in fermented sausages: Role of starter cultures. *Frontiers in Microbiology*, *3*, 1–9. doi:10.3389/fmicb.2012.00169

Latorre-Moratalla, M. L., Veciana-Nogués, T., Bover-Cid, S., Garriga, M., Aymerich, T., Zanardi, E., & Vidal-Carou, M. C. (2008). Biogenic amines in traditional fermented sausages produced in selected European countries. *Food Chemistry*, *107*(2), 912–921. doi:10.1016/j.foodchem.2007.08.046

Leuschner, R. G., Heidel, M., & Hammes, W. P. (1998). Histamine and tyramine degradation by food fermenting microorganisms. *International Journal of Food Microbiology*, *39*(1–2), 1–10. doi:10.1016/S0168-1605(97)00109-8

Leuschner, R. G. K., & Hammes, W. P. (1998). Tyramine degradation by micrococci during ripening of fermented sausage. *Meat Science*, *49*(3), 289–296. doi:10.1016/S0309-1740(97)00124-1

Lu, S., Xu, X., Zhou, G., Zhu, Z., Meng, Y., & Sun, Y. (2010). Effect of starter cultures on microbial ecosystem and biogenic amines in fermented sausage. *Food Control*, *21*(4), 444–449. doi:10.1016/j.foodcont.2009.07.008

Maijala, R. L., Eerola, S. H., Aho, M. A., & Hirn, J. A. (1993). The effect of GDL-induced pH decrease on the formation of biogenic amines in meat. *Journal of Food Protection*, *56*(2), 125–129.

Martuscelli, M., Crudele, M. A., Gardini, F., & Suzzi, G. (2000). Biogenic amine formation and oxidation by Staphylococcus xylosus strains from artisanal fermented sausages. *Letters in Applied Microbiology*, *31*(3), 228–232. doi:10.1046/j.1365-2672.2000.00796.x

Mercogliano, R., De Felice, A., Luisa Cortesi, M., Murru, N., Marrone, R., & Anastasio, A. (2013). Biogenic amines profile in processed bluefin tuna (*Thunnus thynnus*) products. *Cyta - Journal of Food*, *11*(2), 101–107. doi:10.1080/19476337.2012.699103

Naila, A., Flint, S., Fletcher, G. C., Bremer, P. J., Meerdink, G., & Morton, R. H. (2012). Prediction of the amount and rate of histamine degradation by diamine oxidase (DAO). *Food Chemistry*, *135*(4), 2650–2660. doi:10.1016/j.foodchem.2012.07.022

Nie, X., Zhang, Q., & Lin, S. (2014). Biogenic amine accumulation in silver carp sausage inoculated with *Lactobacillus plantarum* plus *Saccharomyces cerevisiae*. *Food Chemistry*, *153*, 432–436. doi:10.1016/j.foodchem.2013.12.093

Pircher, A., Bauer, F., & Paulsen, P. (2007). Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. *European Food Research and Technology*, *226*, 225–231. doi:10.1007/s00217-006-0530-7

Riebroy, S., Benjakul, S., Visessanguan, W., Kijrongrojana, K., & Tanaka, M. (2004). Some characteristics of commercial Som-fug produced in Thailand. *Food Chemistry*, *88*, 527–535. doi:10.1016/j.foodchem.2004.01.067

Santos, M. H. (1996). Biogenic amines: Their importance in foods. *International Journal of Food Microbiology*, *29*, 213–231. doi:10.1016/0168-1605(95)00032-1

Shalaby, A. R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International*, *29*, 675–690. doi:10.1016/S0963-9969(96)00066-X

Sugita, Y., Takao, K., Toyama, Y., & Shirahata, A. (2007). Enhancement of intestinal absorption of macromolecules by spermine in rats. *Amino Acids*, *33*, 253–260. doi:10.1007/s00726-007-0532-1

Talon, R., Leroy, S., Lebert, I., Giammarinaro, P., Chacornac, J. P., Latorre-Moratalla, M., & Lebecque, A. (2008). Safety improvement and preservation of typical sensory qualities of traditional dry fermented sausages using autochthonous starter cultures. *International Journal of Food Microbiology*, *126*(1), 227–234. doi:10.1016/j.ijfoodmicro.2008.05.031

Tosukhowong, A., Visessanguan, W., Pumpuang, L., Tepkasikul, P., Panya, A., & Valyasevi, R. (2011). Biogenic amine formation in Nham, a Thai fermented sausage, and the reduction by commercial starter culture, *Lactobacillus plantarum* BCC 9546. *Food Chemistry*, *129*(3), 846–853. doi:10.1016/j.foodchem.2011.05.033