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Control of biogenic amines in fermented sausages: role of starter cultures

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Biogenic amines show biological activity and exert undesirable physiological effects when absorbed at high concentrations. Biogenic amines are mainly formed by microbial decarboxylation of amino acids and thus are usually present in a wide range of foods, fermented sausages being one of the major biogenic amine sources. The use of selected starter cultures is one of the best technological measures to control aminogenesis during meat fermentation. Although with variable effectiveness, several works show the ability of some starters to render biogenic amine-free sausages. In this paper, the effect of different starter culture is reviewed and the factors determining their performance discussed.

Keywords: starter cultures, biogenic amines, amino acid decarboxylase, fermented sausages, amino oxidase, autochthonous

INTRODUCTION

Biologically active amines are nitrogenous low-molecular-weight substances with biological functions in animals, plants, and microorganisms. The biologically active amines known as biogenic amines include tyramine, histamine, phenylethylamine, tryptamine, putrescine, and cadaverine. They are mainly derived from the bacterial decarboxylation of precursor amino acids and can be found in nearly all types of foods in a wide and variable range of concentrations (Halász et al., 1994; Vidal-Carou et al., 2007).

Histamine, tyramine, and to lesser extent phenylethylamine are the main dietary biogenic amines associated with certain health disruptions, mainly involving vasoactive and psychoactive reactions: histaminic intoxication, food intolerance due to enteral histaminosis, food-induced migraines, and interaction between tyramine and MAOI drugs (Mariné-Font et al., 1995; Spano et al., 2010; Linares et al., 2011). Additionally, high contents of biogenic amines in foods have traditionally been used as an index of undesired microbial activity as a result of hygienically defective manufacturing or storage practices (Mariné-Font et al., 1995; Suzzi and Gardini, 2003; Ruiz-Capillas and Jiménez-Colmenero, 2004). However, the manufacture of fermented foods involve the activity of a variety of microorganisms, not only associated with the desired technological fermentative properties but also undesired contaminants. Both types of microbial activities entail noticeable risk of biogenic amine production. In this respect, the control of biogenic amine accumulation in fermented products is one of the present challenges of the food industry (Vidal-Carou et al., 2007). In this review, the influence of starter cultures as a technological measure to control aminogenesis during meat fermentation is reviewed and the factors determining their performance discussed.

BIOGENIC AMINE CONTENTS IN FERMENTED SAUSAGES

Fermented meat sausages, together with other fermented foods or beverages, constitute one of the food products that can accumulate higher biogenic amine contents (Suzzi and Gardini, 2003; Spano et al., 2010; EFSA Panel on Biological Hazards (BIOHAZ), 2011). **Table 1** summarizes the occurrence of biogenic amines in retail fermented meat products of different countries. Tyramine is usually the most frequent and abundant biogenic amine found in fermented sausages. In terms of average values, it has been reported that fermented sausages show the highest tyramine content among fermented products (EFSA Panel on Biological Hazards (BIOHAZ), 2011). In fermented sausages, tyramine is produced by fermentative microbial population, mainly lactic acid bacteria (LAB, including lactobacilli and enterococci) and more rarely coagulase negative staphylococci (CNS; Straub et al., 1994; Masson et al., 1996; Montel et al., 1999; Bover-Cid et al., 2001a; Aymerich et al., 2006; Martín et al., 2006; Latorre-Moratalla et al., 2010a; Talon and Leroy, 2011). Latorre-Moratalla et al. (2010a) reported that 48% of LAB and 13% of staphylococci isolated from spontaneously fermented sausage are able to decarboxylate one or more amino acids.

The occurrence of putrescine and cadaverine is also quite common but more variable than tyramine. Although the contents of diamines in fermented sausages are relatively low, in a few cases their levels are extremely high, exceeding those of tyramine. The production of diamines is usually attributed to contaminant Gram-negative bacteria, such as enterobacteria and pseudomonas (Bover-Cid et al., 2001a; Durlu-Özkaya et al., 2001; Suzzi and Gardini, 2003). However, a number of publications show several LAB and CNS strains with a powerful capability of producing putrescine and/or cadaverine (Straub et al., 1995; Bover-Cid et al., 2001a; Martín et al., 2006; Latorre-Moratalla et al., 2010a).

Table 1 | Biogenic amine contents (mg/kg fresh matter) in fermented sausages of the retail market from several countries.

	Reference	Product	*n*	Tyramine	Histamine	Phenylethylamine	Tryptamine	Cadaverine	Putrescine
Spanish sausage	Vidal-Carou et al. (1990)	Chorizo	11	176 ± 149[a] (2–509)[b]	76 ± 80 (2–249)	–[c]	–	–	–
	Salchichón	19	133 ± 62 (35–270)	18 ± 27 (1–103)	–	–	–	–	
	Salami	5	6 ± 3 (3–12)	66 ± 39 (2–102)	–	–	–	–	
	Sobrasada	3	8 ± 6 (3–14)	55 ± 36 (14–78)	–	–	–	–	
	Hernández-Jover et al. (1997a)	Chorizo	20	282 ± 129 (30–627)	18 ± 27 (0–314)	1 ± 3 (0–52)	16 ± 20 (0–88)	20 ± 16 (0–658)	60 ± 141 (3–416)
	Salchichón	22	281 ± 109 (53–513)	7 ± 14 (0–151)	7 ± 6 (0–35)	9 ± 11 (0–65)	12 ± 23 (0–342)	103 ± 76 (6–400)	
	Fuet	11	191 ± 73 (32–743)	2 ± 40 (0–358)	2 ± 4 (0–34)	9 ± 8 (0–68)	19 ± 18 (5–51)	72 ± 41 (2–222)	
	Sobrasada	7	332 ± 131 (58–501)	9 ± 17 (3–143)	2 ± 6 (0–39)	12 ± 23 (0–65)	13 ± 14 (3–42)	65 ± 50 (2–501)	
	Bover-Cid et al. (1999a)	Secallona	15	92 ± 72 (1–218)	1 ± 2 (0–5)	4 ± 8 (0–29)	5 ± 11 (0–39)	43 ± 48 (1–115)	80 ± 152 (1–513)
	Fuet delgado	23	119 ± 64 (22–272)	12 ± 34 (0–158)	8 ± 13 (0–47)	8 ± 11 (0–36)	28 ± 42 (2–156)	49 ± 43 (1–169)	
	Salchichón	19	141 ± 124 (3–490)	14 ± 20 (0–59)	12 ± 28 (0–126)	15 ± 33 (0–142)	18 ± 30 (0–127)	99 ± 96 (0–325)	
	Ruiz-Capillas and Jiménez-Colmenero (2004)	Chorizo	3	129 ± 100 (19–214)	6 ± 9 (1–16)	nd	nd	103 ± 113 (9–229)	92 ± 92 (0.8–185)
French sausage	Montel et al. (1999)	Saucisson (industrial)	5	220 (172–268)	71 (16–151)	4 (0–8)	4 (0–9)	103 (31–192)	279 (195–410)
	Saucisson (traditional)	3	164 (84–217)	15 (15–16)	1 (0–4)	nd	71 (39–110)	223 (61–317)	
Italian sausage	Parente et al. (2001)	Soppressata	9	178 (0–557)	22 (0–101)	3 (0–20)	–	61 (0–271)	99 (0–416)
	Salsiccia	10	77 (0–339)	nd	nd	–	7 (0–39)	20 (0–78)	
	Coïsson et al. (2004)	Salamini Italiani	10	205 ± 105 (60–372)	46 ± 54 (8–165)	14 ± 20 (nd–53)	20 ± 25 (nd–69)	–	–
Finnish sausage	Eerola et al. (1998)	Finnish sausage	11	88 (4–200)	54 (0–180)	13 (2–248)	14 (0–43)	50 (0–270)	79 (0–230)
	Russian sausage	4	110 (6–240)	89 (0–200)	11 (1–33)	22 (0–43)	10 (3–18)	93 (3–310)	
	Danish sausage	8	54 (5–110)	9 (1–56)	2 (0–4)	27 (0–91)	180 (0–790)	130 (0–450)	
	Meatwurst	12	72 (5–320)	21 (0–170)	3 (0–5)	18 (0–54)	6 (0–16)	77 (2–580)	
	Lubeck	9	73 (9–150)	6 (0–40)	4 (0–7)	10 (0–20)	3 (0–8)	49 (0–220)	
	Salami	13	93 (3–200)	3 (0–9)	5 (0–8)	20 (0–51)	14 (0–71)	54 (0–210)	
	Pepperoni	11	94 (5–190)	21 (0–200)	6 (0–48)	18 (0–42)	82 (0–390)	61 (0–230)	

Dutch sausage	Brink et al. (1990)		14	110 (40–310)	11 (1–63)	14 (5–45)	–	63 (1–150)	52 (1–190)
Egyptian sausage	Shalaby (1993)	Egyptian sausages	50	14 (10–53)	5 (7–41)	10 (2–81)	13 (3–34)	19 (6–39)	39 (12–100)
Thai sausage	Riebroy et al. (2004)	Som-fug	7	87 ± 72 (19–228)	120 ± 82 (55–291)	–	49 ± 25 (19–86)	161 ± 111 (20–328)	127 ± 90 (17–275)
Turkish sausage	Ekici et al. (2004)	Turkish dry sausage	46	–	32 ± 17 (20–87)	–	–	–	–
	Erkmen and Bozkurt (2004)	Sucuk–factory	19	62 ± 69 (1–189)	69 ± 83 (4–255)	9 ± 20 (0–87)	11 ± 14 (0–47)	–	75 ± 123 (0–383)
		Sucuk–butcher	31	77 ± 92 (2–316)	94 ± 151 (2–478)	6 ± 9 (0–32)	25 ± 31 (0–7)	1 ± 2 (0–7)	121 ± 239 (0–919)

[a] *Mean ± standard deviation when available;* [b] *range (minimum–maximum);* [c] *–not reported; nd, not detected.*

Therefore, fermentative activities can also result in a considerable diamine accumulation.

In contrast, histamine is usually more scarcely found in fermented sausages. However, in some particular samples it may reach quite high levels, usually accompanied by high amounts of other biogenic amines. Histamine production seems to be delimited to some strains of a reduced number of isolates of enterobacteria or LAB, which are not commonly found unless specific contaminations occur (Maijala and Eerola, 1993; Roig-Sagués et al., 1996; Silla-Santos, 1998; Bover-Cid et al., 2001a).

Phenylethylamine and tryptamine could be considered minor amines occurring in fermented sausages. Their accumulation seems dependent on the occurrence of high contents of tyramine associated with some LAB or CNS (Vidal-Carou et al., 2007).

Levels of biogenic amines in fermented sausages show a great variation among different types of products, manufacturers and products from the same manufacturer. The influence of the microbiological quality of raw materials, which varies in each production batch, is a key parameter to explain this variability. Additionally, other factors such as ingredients and additives (sugar, curing agents, spices, etc), diameter of sausage and technological ripening conditions (temperature and relative humidity) can also influence the phenomena associated with aminogenesis, including microbial growth, acidification, proteolysis, and activity of decarboxylases (Maijala et al., 1995; Bover-Cid et al., 1999a, 2001b; Parente et al., 2001; González-Fernández et al., 2003; Bozkurt and Erkmen, 2004; Komprda et al., 2004; Latorre-Moratalla et al., 2012).

STARTER CULTURES FOR AMINOGENESIS CONTROL IN FERMENTED SAUSAGE

TECHNOLOGICAL ROLE OF STARTER CULTURES IN RELATION TO AMINOGENESIS

The hygienic quality of meat raw materials and ingredients is crucial to minimize the occurrence of microbial contaminants, and it thus constitutes a key point in controlling aminogenesis in fermented meat products (Maijala et al., 1995; Bover-Cid et al., 2000a,b; Naila et al., 2010). However, hygiene is a necessary, though not sufficient condition and additional technological measures focused on the control of aminogenic activity of endogenous microbiota are usually needed. Among the possible technological strategies, the use of starter cultures is one of the most important factors quantitatively affecting the accumulation of biogenic amines during sausage fermentation (Bover-Cid et al., 2000c; Suzzi and Gardini, 2003; Naila et al., 2010; EFSA Panel on Biological Hazards (BIOHAZ), 2011; Talon and Leroy, 2011). Indeed, the mechanism of starter cultures is based on preventing the outgrowth of the potential aminogenic endogenous bacteria together with their own inability to produce biogenic amines (Lonvaud-Funel, 2001; Suzzi and Gardini, 2003).

Strains of LAB and CNS specifically selected as starter cultures have to comply with some technological criteria, among which the adaptation to meat fermentation, the ability to compete with the natural (endogenous) microbiota of raw materials and the lack of amino acid decarboxylase capability are the most relevant for the control of biogenic amine production (Buckenhüskes, 1993; EFSA Panel on Biological Hazards (BIOHAZ), 2011; Talon and Leroy, 2011). Some LAB and CNS species usually

used as meat starters (i.e., *L. curvatus* and *S. carnosus*) have been reported as strong biogenic amine producers, mainly of tyramine (Latorre-Moratalla et al., 2010a; Talon and Leroy, 2011). In contrast, species such as *L. sakei*, *L. plantarum*, and *S. xylosus* are usually described as weak or non-aminogenic microorganisms (Bover-Cid et al., 2001a; Aymerich et al., 2006; Latorre-Moratalla et al., 2010a; Linares et al., 2011). However, though some genera or species are more frequently reported than others to be able to produce specific biogenic amines, the ability to decarboxylate amino acids is a strain-dependent property (Bover-Cid and Holzapfel, 1999; Lonvaud-Funel, 2001; Linares et al., 2011). Therefore, it is necessary to carry out a case-by-case evaluation of the aminogenic activity of the strains to be selected as amine negative starter culture. For this purpose, several procedures have been reported (Marcobal et al., 2006; Landete et al., 2007; EFSA Panel on Biological Hazards (BIOHAZ), 2011). Amino acid decarboxylase potential might be tested by molecular techniques detecting specific genes coding for amino-acid decarboxylase. Nevertheless, the aminogenic potential of a given strain should be confirmed through the study of the phenotypic expression of this activity, both *in vitro* (as a screening procedure) and finally in real fermentation and ripening conditions (EFSA Panel on Biological Hazards (BIOHAZ), 2011).

EFFECTIVENESS OF AMINE-NEGATIVE DECARBOXYLASE STARTER CULTURES IN BIOGENIC AMINE REDUCTION

Several studies have evaluated the use of commercial and experimental starter cultures in order to reduce aminogenesis during the fermentation of sausages. Although a number of studies have demonstrated the beneficial effect of starter cultures in reducing biogenic amine accumulation (Maijala et al., 1995; Hernández-Jover et al., 1997b; Bover-Cid et al., 1999b; González-Fernández et al., 2003; Gençcelep et al., 2007; Gücükoglu and Küplülü, 2010; Lu et al., 2010; Baka et al., 2011), other studies failed to demonstrate the efficiency of starter cultures to reduce the presence of biogenic amines in some fermented meat products (Rice and Koehler, 1976; Buncic et al., 1993; Bauer et al., 1994; Paulsen and Bauer, 1997; Roig-Sagués et al., 1997; Parente et al., 2001; Bozkurt and Erkmen, 2002).

Table 2 summarizes the relative reduction of biogenic amines by the use of negative amine producer starter cultures obtained from different experiments. In these studies, different percentages of biogenic amine reduction are observed depending on the bacterial species inoculated, varying from 9% to practically 100%. Moreover, some starters showed an ability to reduce the production of all amines and in other cases only reduce certain amines (Ayhan et al., 1999; Bover-Cid et al., 1999b; Gençcelep et al., 2007; Coloretti et al., 2008; Baka et al., 2011; Casquete et al., 2011).

In general, starters containing LAB species showed a higher effectiveness in biogenic amine reduction than starters including only CNS (Bover-Cid et al., 1999b, 2001b). LAB starter cultures could exert a more efficient replacement of endogenous microbiota with potential aminogenic ability, usually consisting of lactobacilli and enterococci. In particular within starters including LAB species, those containing *L. sakei* or *L. plantarum* are reported to significantly inhibit amine accumulation, though with different intensity depending on the strain and product (Hernández-Jover et al., 1997b; Coloretti et al., 2008; Latorre-Moratalla et al., 2010b; Baka et al., 2011; Tosukhowong et al., 2011). Within CNS, although their proteolytic activity could stimulate the aminogenesis by means of providing the amino acid precursors, Bover-Cid et al. (1999b) showed the potential of proteolytic staphylococci to inhibit biogenic amine production.

Several studies support a greater efficiency in biogenic amine reduction when *L. sakei* was used as the starter in comparison with other species (González-Fernández et al., 2003; Gençcelep et al., 2007; Latorre-Moratalla et al., 2010b; Baka et al., 2011). *L. sakei* are usually well adapted to the ecology of meat fermentation and are competitive between the temperatures of 15 and 25°C, which is the temperature range for sausages manufacture in European Countries (Hugas and Monfort, 1997; Bover-Cid et al., 2001b). In the study carried out by González-Fernández et al. (2003), among all the decarboxylase negative strains tested, *L. sakei* K29 showed the most efficiency in reducing amine production probably because this strain caused a rapid pH drop during sausage fermentation. Bover-Cid et al. (2001b) also described that the amino acid decarboxylase negative strain *L. sakei* CTC494 showed a strong ability to reduce biogenic amine formation in Spanish fermented sausage. However, when this same strain (*L. sakei* CTC494) was combined with *S. carnosus* LHT 2102, *S. xylosus* CTC3037 or *S. xylosus* CTC3050 an even more effective reduction of amine accumulation was achieved compared with the effect of each strain used alone (Bover-Cid et al., 1999b, 2000c). Similarly, Latorre-Moratalla et al. (2010b) described that the rate of reduction was improved when a mixed starter was used. Thus, after the addition of a single strain of *S. equorum,* the contents of cadaverine were reduced by 45% and the single strain of *L. sakei* inoculated in the same product was far more effective, reducing cadaverine by 75%. However, both strains (*L. sakei* and *S. equorum*) used together as a mixed starter reduced cadaverine by 89%. In fact, mixed starters may perform better than single starters being able to control the growth of different bacterial groups (Bover-Cid et al., 2000c; Latorre-Moratalla et al., 2010b; Naila et al., 2010).

Commercial starters usually used in industrial manufacture may not be fully adapted to the meat fermentation environment or more specifically to traditional fermenting conditions. Nowadays, for the fermentation of artisanal sausages the addition of the so-called autochthonous starter cultures consisting of selected strains originating from each specific fermented meat product is recommended (Benito et al., 2007; Talon et al., 2007; Casquete et al., 2011). Thus, an autochthonous starter helps to maintain the typical unique characteristics of artisanal products (Talon et al., 2007). This better adaptation and competitiveness of autochthonous starters compared with commercial ones could explain the greater reduction of biogenic amine contents in artisanal meat fermented products.

In this line, commercial mixed starters combining different LAB and staphylococci species (Hernández-Jover et al., 1997b; Ayhan et al., 1999; Gücükoglu and Küplülü, 2010) showed poorer amine reductions than those provided by mixed autochthonous starter cultures (Talon et al., 2008; Latorre-Moratalla et al., 2010b; Casquete et al., 2011). Talon et al. (2008) and Latorre-Moratalla et al. (2010b) evaluated the effect of autochthonous starter cultures without amino decarboxylase activity on biogenic amine

Table 2 | Different studies on the effect of amine-negative starter cultures on biogenic amine reduction during the manufacture of fermented sausages.

Product	Starter culture	% Of reduction	Reference
Fuet	*Micrococcus carnosus* + *Lactobacillus plantarum* (Texel, France)	25% of TI, 61% of CA, and 25% of PU	Hernández-Jover et al. (1997b)
	M. carnosus + *Pediococcus pentosaceus (Texel, France)*	34% of TI, 50% of CA, and 56% of PU	
Fuet	*Staphylococcus carnosus* LTH 2102	25% of TY, 23% of CA, and 17% of PU	Bover-Cid et al. (1999b)
	S. xylosus CTC3037	69% of TY, 66% of CA, and no effect on PU	
	S. xylosus CTC3050	69% of TY, 17% of CA, and 28% of PU	
Fuet	*L. sakei* CTC494 + *S. carnosus* LTH2102	90% of TY, 87% of CA, and 37% of PU	Bover-Cid et al. (2000c)
	L. sakei CTC494 + *S. xylosus* CTC3037	87% of TY, 87% of CA, and 37% of PU	
	L. sakei CTC494 + *S. xylosus* CTC3050	90% of TY, 87% of CA, and 37% of PU	
Fuet	*L. sakei* CTC494 (high quality raw material)	87% of TY, 38% of HI, 41% of CA, and 67% of PU	Bover-Cid et al. (2001b)
	L. sakei CTC494 (poor-quality raw material)	39% of TY, 29% of HI, 14% of CA ,and 57% of PU	
Fuet	*L. sakei* CTC6626 and *S. xylosus* CTC6013	19% of TY and 46% of PU	Latorre-Moratalla et al. (2010b)
	L. sakei CTC494 and *S. xylosus* CTC6013	45% of TY and 50% of PU	
Salchichón	*P. acidilactici* MS200 + *S. vitulus* RS34*	38% of TY, 74% of HI, and 77% of CA. No effect on PU	Casquete et al. (2011)
		70% of TY, 82% of HI, 64% of CA, and 89% of PU	
	P. acidilactici MS198 + *S. vitulus* RS34*	65% of CA. No effect on HI, TY, and PU	
		58% of HI, 71% of CA, and 72% of PU. No effect on TY	
Chorizo	*L. sakei* K29	98% of TY, 100% of CA and 98% of PU	González-Fernández et al. (2003)
	Pediococcus sp. P22	92% of TY, 67% of CA, and 93% of PU	
	Pediococcus sp. P208 (Rhodia Food, France)	81% of TY, 100% of CA, and 89% of PU	
Chorizo	*L. sakei* CTC6469 + *L. sakei* CTC6626 + *S. xylosus* CTC6013 + *S. xylosus* CTC6169	76% of TY, 97% of CA, and 90% of PU	Garriga et al. (2005)
Xouriço	*L. sakei*	17% of TY and 75% of CA. No effect on PU	Latorre-Moratalla et al. (2010b)
	S. equorum	45% of CA. No effect on TY and PU	
	L. sakei + *S. equorum*	15% of TY and 89% of CA. No effect on PU	
French fermented sausage	*L. sakei* + *S. succinus* + *S. equorum*	87% of TY, 35% of CA, and 38% of PU	Talon et al. (2008)
Salami	*L. plantarum* VLT 73 + *Kokuria varians* MIAL	26% of CA and 27% of PU. No effect on TY and HI	Coloretti et al. (2008)
	L. plantarum VLT 73	47% of CA. No effect on TY, HI, and PU	
Greek fermented sausage	*L. sakei* (+ 0.5% of satureja tymbra extract oil)	62% of TY, 71% of HI, and 100% of PU	Latorre-Moratalla et al. (2010b)
Greek fermented sausage	*L. sakei* 4413	13% of TY and 72% of PU. No effects on CA	Baka et al. (2011)
	L. sakei 8426	25% of CA. No effect on TY and PU	
	L. plantarum 7423	26% of CA. No effect on TY and PU	
	L. curvatus 8427	9% of TY and 29% on PU. No effect on CA	
Finnish fermented sausage	*P. pentosaceus* + *S. carnosus* (Rudolf Müller and Co, Germany)*	41% of TY, 28% of HI, and 86% of CA. No effect on PU	Maijala et al. (1995)
		79% of TY, 62% of HI, and 70% of CA. No effect on PU	
		67% of TY, 77% of HI. No effect on PU and CA	

(Continued)

Table 2 | Continued

Product	Starter culture	% Of reduction	Reference
Turkish Soudjoucks	*L. sakei* + *P. pentosaceous* + *S. carnosus* + *S. xylosus* (Bioback-K, Wiberg, Germany)	100% of PU. No effect on TY	Ayhan et al. (1999)
Turkish Sucuk	*L. sakei* + *S. carnosus* F-SC-111(CHR-HANSEN, Germany)	88% of TY, 54% of CA, and 63% of PU. No effect on HI	Gençcelep et al. (2007)
	P. acidilactici + *L. curvatus* + *S. xylosus* F-LC (CHR-HANSEN, Germany)	86% of TY, 27% of HI, 62% of CA, and 60% of PU	
Turkish fermented sausage	*L. sakei* + *S. xylosus* B-FM (CHR-HANSEN, Germany)	54% of TY and 62% of PU	Gücükoglu and Küplülü (2010)
	L. plantarum + *S. carnosus* TD-66 (CHR-HANSEN, Germany)	52% of TY and 61% of PU	
	L. curvatus + *S. carnosus* + *S. xylosus* RM-10 (CHR-HANSEN, Germany)	55% of TY and 63% of PU	
Xinese fermented sausage	*P. pentosaceus* + *S. xylosus*	66% of TY, 49% of CA, and 30% of PU. No effect on HI	Lu et al. (2010)
	L. farciminis + *S. saprophyticus*	83% of TY, 99% of HI, 99% of CA, and 66% of PU	
Thai fermented sausage	*L. plantarum* BCC9546 (BIOTEC, Thailand)	94% of TY, 75% of CA, and 97% of PU	Tosukhowong et al. (2011)
	L. plantarum BCC9546 + *L. brevis* BCC26756 (BIOTEC, Thailand)	37% of TY, 75% of CA, and 99% of PU	

**Starter has been tested in different technological conditions. It is showed the different% of reduction for each technological condition.*

reduction in different artisanal European fermented sausages. The work demonstrated the importance of case-by-case basis strain selection to obtain a good adaptation to the meat fermentation environment and in turn a good biogenic amine prevention.

It has been suggested that the use of bacterial strains with amine oxidase activity might enhance the reduction of biogenic amine accumulation by metabolizing the amines formed during the fermentation. Amine oxidase activity, metabolizing tyramine and/or histamine under *in vitro* conditions, has been described in microorganisms involved in sausage fermentation, such as specific strains of LAB (*L. sakei* or *L. plantarum*) and CNS (*S. xylosus*; Leuschner and Hammes, 1998; Martuscelli et al., 2000; Fadda et al., 2001; Gardini et al., 2002). However, under real conditions of sausage fermentation, amine-oxidizing microorganisms have shown a limited effect on tyramine and histamine levels, likely due to a low oxygen availability inside the sausage and/or an insufficient number of amine-oxidizing bacteria, e.g., below the minimum 10^7 cfu/g, required for amine degradation (Leuschner and Hammes, 1998; Gardini et al., 2002).

FACTORS THAT INFLUENCE THE PERFORMANCE OF STARTER CULTURES IN REDUCING BIOGENIC AMINE ACCUMULATION

The magnitude of reduction of biogenic amine accumulation achieved by a starter culture depends on the factors that determine the presence of endogenous microbiota as well as the competitiveness and implantation of the added starter culture.

It is clearly demonstrated that poor hygienic quality of raw materials and ingredients diminished the protective effect of the amine negative starter culture. Bover-Cid et al. (2001b) reported a significant reduction in the effectiveness of the amino acid decarboxylase negative strain *L. sakei* CTC494 when sausages were made from raw materials of relatively poor hygienic quality, since high bacterial loads (both of Gram-negative and Gram-positive) increase the number of potentially aminogenic microorganisms and also hamper the implantation and competitiveness of amine negative technological microbiota.

Different processing environment (e.g., pilot plant versus traditional processing plant) and the different type of formulation (e.g., *chorizo* or *fuet*) can also significantly influence the performance of the amine negative starter culture. The addition of a mixed starter culture including *L. sakei* (CTC6469 + CTC6626) and *S. xylosus* (CTC6013 + CTC6169) with no amino acid decarboxylase activity was successfully used in a fermentation study carried out in pilot plants by Garriga et al. (2005). The starter prevented the accumulation of tyramine, putrescine, and cadaverine by up to 90% of total amines. However, when the same strains (*L. sakei* CTC6626 + *S. xylosus* CTC6013) were inoculated into products produced in a traditional real processing plant, the starter culture showed a weaker effect, only slightly reducing the contents of tyramine and cadaverine, by 19 and 46%, respectively (Latorre-Moratalla et al., 2010b).

In the literature, several studies have evaluated the effect of factors that influence starter culture on biogenic amine reduction, such as the type and quantity of sugar added (González-Fernández et al., 2003) or the addition of some additives (Bozkurt and Erkmen, 2007; Coloretti et al., 2008). According to the results reported by González-Fernández et al. (2003), when the starter culture, especially *L. sakei* K29, was used with a sugar concentration of

0.5 or 1%, the presence of biogenic amines decreased considerably in comparison with the control and low sugar concentration sausage. Bozkurt and Erkmen (2007) described a higher decrease of tyramine, histamine and putrescine contents when a mixture of antimicrobials (nitrite and nitrate), antioxidants, and flavoring and coloring compounds were added with the starter culture mixture of *Pediococcus acidilactici, L. plantarum*, and *S. carnosus*.

The influence of processing temperatures on the reducing effect of starter cultures has been fully studied (Maijala et al., 1995; Komprda et al., 2001; Gücükoglu and Küplülü, 2010; Casquete et al., 2011). Gücükoglu and Küplülü (2010) reported that higher ripening temperatures (26°C versus 22°C) potentiated the reduction of putrescine by different starter cultures (*L. sakei* + *S. xylosus* B-FM, *L. plantarum* + *S. carnosus* TD66, and *L. curvatus* + *S. carnosus* + *S. xylosus* RM-10) in experimental Turkish fermented sausages. However, the fermentation temperatures did not have any significant effect on tyramine reduction. Similarly, Casquete et al. (2011) described an influence of temperatures on the development and activity of autochthonous starter cultures consisting of *P. acidilactici* MS200 + *S. vitulus* RS34 and *P. acidilactici* MS198 + *S. vitulus* RS34. In this case, higher fermentation temperature (12°C versus 7°C) also obtained the highest reduction of amines, probably due to a better adaptation of the starters to the meat fermentation environment.

In conclusion, the absence of decarboxylase activity should be a criterion for the selection of strains intended for use as starter cultures to obtain fermented sausages free of biogenic amines. Mixed starter cultures consisting of amine negative strains of LAB and CNS species, well adapted to the meat fermentation environment, seem the best alternative. Moreover, the use of autochthonous strains is presented as a promising control measure especially for the manufacture of traditional fermented sausages. High quality raw materials and optimal technological conditions are crucial factors to ensure a proper performance of starter cultures for the reduction of biogenic amine accumulation in fermented sausages.

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