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Biogenic amines in dry fermented sausages: a review

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Abstract

Biogenic amines are compounds commonly present in living organisms in which they are responsible for many essential functions. They can be naturally present in many foods such as fruits and vegetables, meat, fish, chocolate and milk, but they can also be produced in high amounts by microorganisms through the activity of amino acid decarboxylases. Excessive consumption of these amines can be of health concern because their not equilibrate assumption in human organism can generate different degrees of diseases determined by their action on nervous, gastric and intestinal systems and blood pressure. High microbial counts, which characterise fermented foods, often unavoidably lead to considerable accumulation of biogenic amines, especially tyramine, 2-phenylethylamine, tryptamine, cadaverine, putrescine and histamine. However, great fluctuations of amine content are reported in the same type of product. These differences depend on many variables: the quali-quantitative composition of microbial microflora, the chemico-physical variables, the hygienic procedure adopted during production, and the availability of precursors. Dry fermented sausages are worldwide diffused fermented meat products that can be a source of biogenic amines. Even in the absence of specific rules and regulations regarding the presence of these compounds in sausages and other fermented products, an increasing attention is given to biogenic amines, especially in relation to the higher number of consumers with enhanced sensitivity to biogenic amines determined by the inhibition of the action of amino oxidases, the enzymes involved in the detoxification of these substances.

The aim of this paper is to give an overview on the presence of these compounds in dry fermented sausages and to discuss the most important factors influencing their accumulation. These include process and implicit factors as well as the role of starter and nonstarter microflora growing in the different steps of sausage production. Moreover, the role of microorganisms with amino oxidase activity as starter cultures to control or reduce the accumulation of biogenic amines during ripening and storage of sausages is discussed.

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1. Introduction

Biogenic amines (BA) are organic bases with aliphatic, aromatic or heterocyclic structures that can be found in several foods, in which they are mainly produced by microbial decarboxylation of amino acids, with the exception of physiological polyamines (Silla Santos, 1996). BA accumulation in foods requires the availability of precursors (i.e. amino acids), the presence of microorganisms with amino acid decarboxylases, and favourable conditions for their growth and decarboxylating activity (ten Brink et al.,

1990). Amino acid decarboxylation can have an important energetic role in nutritionally poor environments. In fact, bacterial decarboxylation systems can generate a translocation of charge across the cytoplasmatic membrane, influencing the membrane potential (Konings et al., 1997).

In general, histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine and spermidine are the most important BA in foods (Shalaby, 1996). BA can be found as a consequence of microbial activity in foods such as wine, fermented meat and fish products, cheeses and fermented vegetables (Silla Santos, 1996; Shalaby, 1996). High amounts of amines can be found in fermented foods derived from raw materials with high protein content, such as salami and dry sausages.

Amino acid decarboxylases are enzymes present in many microorganisms of food concern. They have been found in species of the genera *Bacillus* (Rodriguez-Jerez et al., 1994a), *Pseudomonas* (Tiecco et al., 1986), *Photobacterium* (Morii et al., 1988; Jørgensen et al., 2000), as well as in genera of the family *Enterobacteriaceae*, such as *Citrobacter, Klebsiella, Escherichia, Proteus, Salmonella* and *Shigella* (Edwards et al., 1987; Butturini et al., 1995; Roig-Sagués et al., 1996; Marino et al., 2000) and *Micrococcaceae*, such as *Staphylococcus, Micrococcus* and *Kocuria* (Rodriguez-Jerez et al., 1994b; Martuscelli et al., 2000). Furthermore, many lactic acid bacteria (LAB) belonging to the genera *Lactobacillus, Enterococcus, Carnobacterium, Pediococcus, Lactococcus* and *Leuconostoc* are able to decarboxylate amino acids (Maijala et al., 1993; Edwards and Sandine, 1981; de Llano et al., 1998; Bover-Cid and Holzapfel, 1999; Lonvaud-Funel, 2001).

Several authors reviewed the occurrence of BA in foods (Silla Santos, 1996; Shalaby, 1996; Askar and Treptow, 1986; Halász et al., 1994; Rice et al., 1976) as well as their biological activities (Bardócz, 1995). In addition, the presence of these compounds has been reviewed in specific foods, such as cheese (Stratton et al., 1991) and wine (Lonvaud-Funel, 2001). Dry fermented sausages can potentially support the accumulation of BA. In fact, the high amounts of proteins present in these products and the proteolytic activity during ripening provide the precursors for decarboxylase activity of starter cultures and wild microflora. The aim of this work is to give an overview of the presence of BA in fermented sausages, focusing the attention on the factors affecting their accumulation, which have been deeply studied in the last decade.

2. Biogenic amine content in fermented sausages

The only amines present at significant levels in fresh meat used for fermented sausage production are spermidine and spermine, and, to a lesser extent, putrescine (Hernandez-Jover et al., 1997). High concentrations of putrescine and the presence of other amines have been attributed to microbial growth and depend on meat freshness.

Nevertheless, a great variability characterises the BA content in fermented meat products. Sausages with comparable microbiological profiles may differ in their BA content, indicating that the production of such compounds depends on a complex interaction of factors (Table 1).

In the several reports concerning the Spanish dry fermented sausages Chorizo, Fuet, Sobrasada and Salsichon (Hernandez-Jover et al., 1996a,b, 1997; Roig-Sagués et al., 1999; Bover-Cid et al., 2000b), tyramine was generally detected at the higher concentration (exceeding 600 mg kg^{-1} in some sausages with mean values of about 200 mg kg^{-1}), while in some samples, putrescine was produced up to 450 mg kg^{-1}. The diamine cadaverine, despite of the variability of its presence, was detected in relevant amount in some Chorizo and Salsichon sausages (up to 600 mg kg^{-1} but with mean values lower than 20 mg kg^{-1}). 2-phenylethylamine and tryptamine were detected only at few sausages in concentration higher than 50 mg kg^{-1}. Histamine was not detected in all the samples but its presence reached values of health concern (300 mg kg^{-1}) particularly in Chorizo and Fuet. On the other hand, this latter amine was not found in Fuet by Roig-Sagués et al. (1999), and its absence was attributed to the unfavourable conditions for the growth of *Enterobacteriaceae*, which can accumulate high amounts of histamine (Halász et al., 1994). Bover-Cid et al. (2000b) observed significant differences in BA content in dry sausages depending on hygienic quality of the meat used. Sausages produced from frozen meat were characterised only by the presence of tyramine, which reached a maximum level of about 100 mg kg^{-1}. In contrast,

Table 1
Biogenic amine content found in different dry fermented sausages after ripening

Dry sausage	Biogenic amine (mg kg^{-1})								N[a]	Reference
	Histamine	Tyramine	Tryptamine	Cadaverine	Putrescine	2-Phenyl-ethylamne	Spermidine	Spermine		
Saucisson (industrial)	71[b] (16–151)	220 (172–268)	3.9 (0–9)	103 (31–192)	279 (195–410)	4 (0–8)	5.1 (4–6.4)	91 (59–119)	5	Montel et al. (1999)
Saucisson (traditional)	15.3 (15–16)	164.3 (84–217)	0[c]	71.3 (39–110)	223 (61–317)	1.3 (0–4)	4.3 (2–6)	83.7 (82–86)	3	Montel et al. (1999)
Soppressata	21.9 (0–100.9)	178 (0–556.9)	–[d]	60.8 (0–271.4)	98.8 (0–416.1)	3.4 (0–19.9)	40 (0–91.3)	35.5 (0–97.9)	9	Parente et al., 2001
Salsiccia	0	76.7 (0–338.9)	–	6.7 (0–39)	19.7 (0–77.7)	0	18.8 (0–57.2)	2.8 (0–28.1)	10	Parente et al., 2001
Belgian sausages	4.1 (0–19.7)	36.8 (10.2–150.6)	–	2.5 (0–5.6)	15.1 (3.1–40)	0.9 (0–6.1)	–	–	24	Vandekerckhove, 1977
Finnish sausages	54 (0–180)	88 (4–200)	14 (0–43)	50 (0–270)	79 (0–230)	13 (2–48)	4 (2–7)	31 (19–46)	11	Eerola et al., 1998a,b
Russian sausages	89 (0–200)	110 (6–240)	22 (0–43)	10 (3–18)	93 (3–310)	11 (1–33)	5 (2–8)	33 (23–40)	4	Eerola et al., 1998a,b
Danish sausages	9 (1–56)	54 (5–110)	27 (0–91)	180 (0–790)	130 (0–450)	2 (0–4)	7 (3–9)	37 (23–47)	8	Eerola et al., 1998a,b
Meetwurst	21 (0–170)	72 (5–320)	18 (0–54)	6 (0–16)	77 (2–580)	3 (0–5)	6 (3–11)	29 (22–38)	12	Eerola et al., 1998a,b
Thin Fuet[e]	1.8 ± 2.3[f]	121.8 ± 99.4	6.5 ± 14.8	56.3 ± 62	105.2 ± 200.7	5.3 ± 10.2	9.2 ± 7.3	32.8 ± 19.6	15	Bover-Cid et al., 1999a,b
Fuet[e]	15.2 ± 43.5	156.9 ± 85.4	10 ± 13.6	367.2 ± 55.4	64.7 ± 56.5	10.1 ± 16.1	10.3 ± 10.9	30.6 ± 20.5	23	Bover-Cid et al., 1999a,b
Salsichon[e]	19.4 ± 27.6	198.4 ± 183.6	21.3 ± 50.4	26.4 ± 46.5	138.5 ± 143.8	17.4 ± 43.7	5.6 ± 4.1	35.2 ± 18.2	19	Bover-Cid et al., 1999a,b
Poličan[e]	28.5 (25–32)	89 (86–92)	5 (5–5)	6 (6–6)	54 (54–54)	–	2.5 (2–3)	2 (2–2)	2	Komprda et al., 2001
Chorizo	17.5 (0–314.3)	282.3 (29.2–626.8)	15.9 (0–87.8)	20.1 (0–658.1)	60.4 (2.6–415.6)	1.2 (0–51.5)	–	–	20	Hernandez-Jover et al., 1997
Salsichon	7.3 (0150.9)	280.5 (53.3–513.4)	8.5 (0–65.1)	11.7 (0–342.3)	102.7 (5.5–400.0)	6.7 (0–34.7)	–	–	22	Hernandez-Jover et al., 1997
Fuet	2.2 (0–357.7)	190.7 (31.8–742.6)	8.7 (0–67.8)	18.9 (5.4–51.3)	71.6 (2.2–222.1)	1.9 (0–33.7)	–	–	11	Hernandez-Jover et al., 1997
Sobrasada	9.0 (2.8–143.1)	332.1 (57.6–500.6)	11.5 (0–64.8)	12.6 (3.0–41.6)	65.2 (1.8–500.7)	2.2 (0.2–38.5)	–	–	7	Hernandez-Jover et al., 1997
Egyptian sausages	5.3 (7.5–41)	14 (9.5–53)	13 (2.5–34)	19 (5.6–39)	39 (12–100)	9.7 (1.5–81)	2.3 (5.3–12)	1.8 (1.5–5.3)	50	Shalaby, 1993

[a] Number of samples examined.
[b] Mean value (minimum – maximum).
[c] Not detectable.
[d] Not determined.
[e] Values calculated on dry matter.
[f] Mean value ± S.D.

temperature-abused raw materials were characterised by higher counts (more than three log cycles) of enterococci and *Enterobacteriaceae* with a consequent faster and higher accumulation of tyramine (at about 250 mg kg^{-1}), cadaverine (340 mg kg^{-1}) and putrescine (80 mg kg^{-1}).

The data reported by many authors (Maijala et al., 1995a; Eerola et al., 1998a; Komprda et al., 2001) confirmed the key role played by the raw material quality. However, other variables (such as pH, a_w, redox potential, NaCl, etc.) can have an important effect on the production of BA in sausages. The increasing BA content observed during the last production phases and storage has been attributed to amine-positive aciduric LAB or to the residual activity of *Enterobacteriaceae* decarboxylases (Bover-Cid et al., 2000b). Enterococci were probably the microbial group responsible for tyrosine decarboxylase activity in Fuet during the first days of production, while some LAB contribute to the accumulation of this BA in the latter stages of ripening (Roig-Sagués et al., 1999).

Tyramine and putrescine were the most abundant BA also in different types of dry fermented sausages produced in Finland (Eerola et al., 1998b). High amounts of cadaverine were found in Danish and Pepperoni sausages, while spermine and spermidine were found at low levels in all the samples analysed, as already observed in Spanish sausages (Hernandez-Jover et al., 1997). Histamine was prevalently detected in Domestic and Russian sausages in which, however, its concentration was usually lower than 100 mg kg^{-1}. The sum of vasoactive BA (tyramine, histamine, tryptamine, 2-phenylethylamine) exceeded 200 mg kg^{-1} in 26% of samples and this value was proposed as a possible indicator of hygienic conditions and good manufacturing practices in sausage production (Eerola et al., 1998a).

High amounts of BA were present in Turkish sausage Sucuks (aenöz et al., 2000) in which tryptamine was found in 75% of samples while tyramine and histamine presence in some sample was noteworthy (up to 1100 and 350 mg kg^{-1}, respectively). In another study relative to the Turkish sausage Soudjoucks, histamine was never detected while the most important amines were putrescine (more than 400 mg kg^{-1}), followed by tyramine (250 mg kg^{-1}) (Ayhan et al., 1999).

Putrescine, cadaverine, tyramine and histamine were detected in French sausages, both artisanal and industrial. Histamine was found at concentration higher than 100 mg kg^{-1} only in industrial product, while the higher levels of putrescine and tyramine (400 and 270 mg kg^{-1}, respectively) were detected in the sausages with the highest microbial counts of pseudomonads, Gram positive cocci and yeasts (Montel et al., 1999).

In Salsiccia and Soppressata, dry fermented sausages produced in Southern Italy, tyramine was the most important BA reaching levels higher than 500 mg kg^{-1}, followed by putrescine and cadaverine, while values of histamine higher than 50 mg kg^{-1} were detected only in some samples of Soppressata (Parente et al., 2001). 2-phenylethylamine was found in these sausages at low levels. This amine can be present in considerable amounts in cheeses (Shalaby, 1996), but it is sporadically found in sausages (Vandekerckhove, 1977; Koehler and Eitenmiller, 1978; Pechanek et al., 1983). 2-Phenylethylamine generally occurs when a high quantity of tyramine is present and its formation can be related to the non specific activity of tyrosine decarboxylase (Joosten, 1988).

Parente et al. (2001) found that in industrial products, BA accumulation was not lower than in artisanal ones, as already observed by Montel et al. (1999). The presence of commercial starter cultures did not always inhibited BA production, as reported by Rice and Koehler (1976) and Bauer et al. (1994), in contrast with the results of other authors (Eitenmiller et al., 1978; Santos-Buelga et al., 1986; Maijala et al., 1995a)

3. Biogenic amine production by microorganisms isolated from sausages

Microorganisms have a different ability in synthesising decarboxylases. The production of BA in meat has been attributed to the action of several microorganisms: pseudomonads, *Enterobacteriaceae*, enterococci and lactobacilli (Halász et al., 1994; Silla Santos, 1996).

Many procedures have been proposed to evaluate the decarboxylase activity of microorganisms isolated from foods (Joosten and Northold, 1989; Choudhury et al., 1990; Maijala, 1993; Bover-Cid and Holzapfel, 1999). Within the same species, the presence, the

activity and the specificity of decarboxylases is strain-specific (Bover-Cid and Holzapfel, 1999). Rapid screening methods can have some limitations in terms of sensitivity in detecting BA production leading to contradictory results. The presence of false-positive and false-negative strains is not negligible. For these reasons, BA production has to be confirmed by analytical quantitative methods (Maijala, 1993). Moreover, the negative (or positive) responses in screening media do not necessarily imply a similar behaviour in food products (Bover-Cid and Holzapfel, 1999).

3.1. Enterobacteriaceae

Enterobacteriaceae isolated from sausages are generally considered as microorganisms with a high decarboxylase activity, particularly in relation to the production of cadaverine and putrescine. Studies carried out in vitro indicated that Citrobacter freundii and Proteus vulgaris were weaker decarboxylating species, whereas Enterobacter cloacae and Serratia species were high putrescine and cadaverine producers (Bover-Cid et al., 2001a). In contrast, Durlu-Özkaya et al. (2001) reported that strains of C. freundii and Enterobacter aerogenes can form in vitro high amounts of putrescine and cadaverine, respectively. Many Enterobacteriaceae can also produce considerable levels of histamine (Halász et al., 1994) and particularly E. cloacae, E. aerogenes and Klebsiella oxytoca (Roig-Sagués et al., 1996), as well as Escherichia coli (Silla Santos, 1998) and Morganella (Proteus) morganii (Bover-Cid et al., 2001a).

Although these microorganisms are usually present in low numbers in the final product, an not correct storage of raw materials, as well as an uncontrolled fermentation, can induce a proliferation of Enterobacteriaceae, which can release their decarboxylases in the early steps of sausage production. The enzyme released, and not the microbial cells, is responsible for the BA accumulation and its action can continue also in the absence of viable cells (Bover-Cid et al., 2001b).

3.2. Lactic acid bacteria

Food-fermenting LAB are generally considered to be not toxinogenic or pathogenic. Some species of LAB, however, can produce BA. For this reason, the decarboxylating activities of LAB isolated from dry fermented sausages have been widely studied. Straub et al. (1995) reported that lactococci, pediococci, streptococci (Streptococcus thermophilus) and leuconostocs, including Leuconostoc oenos (now Oenococcus oeni), did not possess amino acid decarboxylase activity. In contrast, some strains of Lactococcus and Leuconostoc have been described as tyramine producers (Choudhury et al., 1990; de Llano et al., 1998). Strains of lactobacilli belonging to the species L. buchneri, L. alimentarius, L. plantarum, L. curvatus, L. farciminis, L. bavaricus, L. homohiochii, L. reuteri and L. sakei were amine-positive and tyramine is quantitatively the most important BA produced (Masson et al., 1996; Montel et al., 1999; Bover-Cid et al., 2001a; Pereira et al., 2001).

Many authors (Maijala, 1993; Silla Santos, 1998; Montel et al., 1999; Bover-Cid et al., 2001a) did not observe histidine decarboxylase activity in lactobacilli isolated from sausage. In contrast, this enzyme was present in many Lactobacillus spp. and in L. sakei isolated from fish (Dapkevicius et al., 2000), Lactobacillus spp. from sausages (Maijala, 1993, 1994), L. bulgaricus (Chander et al., 1989; Bover-Cid and Holzapfel, 1999), and in a strain of L. acidophilus (Bover-Cid and Holzapfel, 1999). The ability to produce histamine has been found in some leuconostocs (Dapkevicius et al., 2000) and in O. oeni (Lonvaud-Funel, 2001).

As far as enterococci are concerned, many strains were able to produce tyramine (Bover-Cid et al., 2001a; Masson et al., 1996; Gardini et al., 2001). As observed in cheeses (Joosten, 1988), these microorganisms were also able to accumulate 2-phenylethylamine in foods (Montel et al., 1999), but they are not able to produce relevant amounts of the diamines putrescine and cadaverine (Bover-Cid and Holzapfel, 1999; Bover-Cid et al., 2001a).

The accumulation of BA by Carnobacterium was studied by Masson et al. (1996) who observed a considerable tyramine production by C. divergens, C. piscicola and C. gallinarum. If inoculated in a meat–fat mixture, a strain of C. divergens confirmed this aptitude (Masson et al., 1999).

3.3. Micrococcaceae

Few information concerning BA production by Micrococcaceae is available. Histidine decarboxylase activity was observed in some species belonging to the

genera *Micrococcus* and *Staphylococcus* (Tiecco et al., 1986). Histamine production was observed also in the 76% of *Staphylococcus xylosus* strains isolated from Spanish sausages (Silla Santos, 1998) and in some strains of *Kocuria* spp. (Straub et al., 1995). *Staphylococcus carnosus* and *Staphylococcus piscifermentans* can have a high amino acid decarboxylase activity (Straub et al., 1995; Montel et al., 1999), and can produce 2-phenylethylamine, histamine, putrescine and cadaverine. However, Masson et al. (1996) observed that coagulase-negative staphylococci can be used as safe starter cultures: all 23 strains tested isolated from sausages were characterised by a weak tyramine formation ability. Martuscelli et al. (2000) tested 51 strains of *S. xylosus* from sausages and found that 21 were able to decarboxylate amino acids in vitro, and only 7 produced amount higher than 10 mg kg^{-1} of tyramine, spermine and spermidine. Histamine production was never detected.

3.4. Other microorganisms

There are few reports regarding the contribution of yeasts to BA production in fermented foods. In yeasts belonging to the genera *Debaryomyces* and *Candida* isolated from fermented meat, a histidine decarboxylase activity was found, which was higher than that observed for LAB and staphylococci. In addition, unidentified yeast strains were able to produce high 2-phenylethylamine and tyramine contents (Montel et al., 1999).

Many other Gram negative bacteria (among which pseudomonads) are known to be strong BA producers. Their decarboxylating activity is well known in fish products (Lehane and Olley, 2000; Jørgensen et al., 2000). Nevertheless, the specific environmental conditions characterising production and storage of sausages can be considered prohibitive for their growth and enzymatic activity, unless raw meat has been subjected to temperature abuses or not correct production procedures have been adopted (Paulsen and Bauer, 1997).

4. Proteolytic activity

During dry fermented sausage ripening, proteins change as a consequence of the action of microbial and endogenous proteolytic enzymes (Ordóñez et al., 1999). Proteolysis is favoured by the denaturation of proteins as a consequence of acidity increase, dehydration and action of sodium chloride (DeKetelaere et al., 1974). The nonprotein nitrogen fraction increases during sausage fermentation and drying, and includes the presence of free amino acids, precursors of BA. The production of BA in dry sausages has often been related to the proteolytic activity of microorganisms present in the meat during fermentation and ripening, which yields higher amount of precursors (Maijala and Eerola, 1993; Halász et al., 1994; Paulsen and Bauer, 1997). Straub et al. (1995) found a *L. curvatus* strain able to form tyramine from di- and tripeptide with tyrosine in nonterminal position. Extensive proteolysis, before or during the fermentation process, can be the cause of excessive formation of tyramine (Buncic et al., 1993). On the other hand, no direct correlation has been found between proteolytic activity of *S. xylosus* (used as a starter culture) and BA production (Bover-Cid et al., 1999a).

High temperature, high pH and low salt content can accelerate the amino acid accumulation and, hence, stimulate amine formation (Joosten, 1988), but during fermentation and ripening processes, the factors affecting the activity of the decarboxylating enzyme could be more important than the precursor availability (Edwards and Sandine, 1981; Joosten and van Boeckel, 1988). The same chemico-physical parameters that promoted high proteolysis in *Enterococcus faecalis* did not induce the higher BA formation (Gardini et al., 2001). In addition, the proteolytic activity during sausage ripening can be mainly attributed to the endogenous proteases, while bacteria seem to play a minor role (Hierro et al., 1999; Ordóñez et al., 1999).

Also, moulds have an important role in the manufacturing of some dry fermented sausage, taking part in ripening phenomena through their extracellular proteases and lipases (Toledo et al., 1997; Larsen, 1998). The use of a *Penicillium aurantiogriseum* strain during dry sausage production favoured protease and aminopeptidase activities, with a consequent increase in the concentration of ammonia. In contrast, the amine concentration was not affected by the mould layer of the fermented sausages, showing levels and profiles similar to those of the batches without mould (Bruna et al., 2001).

5. Starter cultures

Lactic acid bacteria are widely used in the meat industry as starter cultures for sausage fermentation. Micrococci and/or coagulase-negative staphylococci, inoculated together with LAB, contribute to development of the typical flavour as a result of their proteolytic and lipolytic activities (Hammes and Hertel, 1996). In addition, they produce catalase that protects from colour changes and rancidity and reduces nitrates to nitrites, improving colour formation and stability.

The addition of pure or mixed selected starter cultures can decrease BA accumulation in sausages. To prevent the formation of BA in sausages, it is necessary that starter organisms do not form BA and have to be competitive in suppressing the growth of wild amine producing microflora, which otherwise might contrast the protective effect of starters (Hammes and Hertel, 1996).

A rapid pH decrease caused by amine negative starter cultures can largely prevent BA accumulation in sausages (Maijala et al., 1993; Bover-Cid et al., 2001a). Moreover, starter LAB able to compete with nonstarter bacteria during the later phase of ripening and throughout storage can further avoid excessive BA production. Selected *L. sakei* strains were able to reduce BA (with the exception of tyramine), also in the presence of an amine-positive *Lactobacillus* strain (Roig-Sagués and Eerola, 1997). Similarly, the presence of a selected starter culture (*L. sakei* CTC494) reduced BA accumulation during ripening, but only if raw meat was characterised by a good quality with *Enterobacteriaceae* count not exceeding 10^3 cfu g^{-1} (Bover-Cid et al., 2001d). The addition of the same strain of *L. sakei*, along with proteolytic *S. carnosus* and *S. xylosus*, decreased BA accumulation in the production of Fuet (Bover-Cid et al., 2000a) and reduced the total BA content of 80–90% with respect to the sausages without starter cultures added. A 50% BA decrease was observed also in sausages fermented by *L. curvatus* CTC371 in association with a proteolytic strain of *S. xylosus*, which increased the free amino acid availability (Bover-Cid et al., 2001b). In contrast, the use of single starter cultures of *Pediococcus cerevisiae* and *L. plantarum* did not decrease tyramine and total BA contents with respect to spontaneous fermentation (Rice and Koehler, 1976; Buncic et al., 1993).

In Turkish sausages (Soudjoucks), the natural microflora produced high levels of tyramine and putrescine (more than 250 mg kg^{-1}), whereas the addition of mixed starter cultures (*L. sakei*, *Pediococcus pentosaceus*, *S. xylosus* and *S. carnosus*) avoided the formation of putrescine, but not of tyramine (Ayhan et al., 1999). Mixed starter cultures (*L. sakei*, *S. carnosus* and *S. xylosus*) greatly reduced (about 90%) the presence of putrescine, cadaverine and tyramine in Spanish sausages (Bover-Cid et al., 2000a). Likewise, similar decreases in tyramine, cadaverine, and histamine concentration in sausages using amine-negative mixed (staphylococci plus lactobacilli) starter cultures were observed by Maijala et al. (1995a).

In French sausages, high concentration of histamine were found in industrial product added with starter cultures rather than in artisanal sausages (Montel et al., 1999). The use of starter cultures (*P. pentosaceus* and *S. xylosus*; *L. sakei* and *S. xylosus*) did not reduce BA accumulation also in Italian dry sausages Salsiccia and Soppressata (Parente et al., 2001). A slight reduction of tyramine, cadaverine and putrescine was observed in fermented sausages added with *M. carnosus* plus *L. plantarum* and *M. carnosus* plus *P. pentosaceus* (Hernandez-Jover et al., 1997).

Starter cultures are not always able to control the decarboxylase-positive strains. The discrepancies observed in their efficiency in BA control during sausage fermentation and ripening could depend on the raw meat microbiological quality and the characteristics of natural microflora, in particular amine-positive nonstarter LAB. These microorganisms are often responsible for BA formation in fermented sausages (Maijala and Eerola, 1993; Bauer et al., 1994; Paulsen and Bauer, 1997).

6. Chemico-physical factors influencing biogenic amine production in sausages

6.1. pH

pH is a key factor influencing the amino acid decarboxylase activity. Over 70 years ago, Koessler et al. (1928) suggested that amine formation by bacteria was a physiological mechanism to counteract an acid environment. Bacterial amino acid decarboxylases usually have acid pH optimum (Gale, 1946). A

correlation between BA production and the decrease of pH in sausages caused by lactic fermentation has been evidenced (Eitenmiller et al., 1978; Santos-Buelga et al., 1986). However, amine formation depended on the amount of growth of decarboxylating bacteria rather than on the growth conditions *per se* (Yoshinaga and Frank, 1982). The acidification of MRS broth by adding glucono-δ-lactone decreased the levels of amines and cell counts (Maijala et al., 1993) and its activity was more effective than lactic acid in preventing BA formation in broth (Maijala, 1994). Its addition to dry sausages decreased pH and enterococci and *Enterobacteriaceae* counts without affecting LAB growth; these effects on microbial population resulted in lower histamine and tyramine concentration (Maijala et al., 1993). In fact, a rapid and sharp reduction in pH in sausages is known to reduce the growth of the amine-positive microorganisms, particularly *Enterobacteriaceae* (Maijala et al., 1993; Bover-Cid et al., 2001a). High productions of histamine can be related to inadequate pH decrease in the first days of ripening process (Buncic et al., 1993; Maijala et al., 1993). Also tyramine production by *C. divergens* was lower at pH 4.9 than 5.3, associated with a reduced cell yield. This can explain the low tyramine amount found in Nordic meat generally characterised by lower pH, which limits bacteria growth, and, consequently, tyrosine decarboxylase activity (Masson et al., 1999).

6.2. Sodium chloride

The variation in the quantity of water and in the salt/water ratio during fermentation and storage of fermented sausages has an important role on microbial multiplication. The rate of amine production of a *L. bulgaricus* (now *L. delbrueckii* subsp. *bulgaricus*) strain was considerably reduced when salt concentration in the medium increased from 0% to 6% (Chander et al., 1989). Henry Chin and Koehler (1986) demonstrated that NaCl concentration ranging from 3.5% to 5.5% could inhibit histamine production. This influence can be attributed to reduced cell yields obtained in the presence of high NaCl concentrations and to a progressive disturb of the membrane located microbial decarboxylase enzymes (Sumner et al., 1990). A similar NaCl effect characterised cell yield and BA production in *E. faecalis* EF37 (Gardini et al., 2001).

6.3. Diameter of sausages and redox potential

A different sausage diameter corresponds to different degrees of anaerobiosis, redox potential, salt concentration and a_w values. Bover-Cid et al. (1999b) found a relationship between BA content and the size of dry fermented sausages. The diameter of the sausage affects the environment in which microorganisms grow; for example, salt concentration is usually lower and water activity is higher in sausages with a larger diameter. A larger diameter may be one of the reasons for a higher production of certain amines, such as tyramine and putrescine (Parente et al., 2001). Generally, BA levels in the bigger diameter sausages were higher than in the thinner sausages and in the central part of the sausages than in the edge. Treviño et al. (1997) observed a variable production of BA in three different sections of Cervelat sausage. The edge and the central parts of sausages showed a lower BA concentration (due to the higher drying level and to the higher NaCl concentration, respectively) in comparison with the central area in which the environmental conditions (higher a_w) allowed a more intense microbial activity.

6.4. Temperature

It is well known that temperature has a marked effect on the formation of BA in the fishing industry and in cheese. Several authors report that amine content depends on temperature, and increase with time and storage temperature (Klausen and Lund, 1986; Diaz-Cinco et al., 1992; Halász et al., 1994). *Carnobacterium divergens* produced more tyramine at 25 °C than at 15 °C (Masson et al., 1999). Nevertheless, temperature, influencing the relationships among the activities of the different microorganisms present in sausages, can have opposite effect on BA accumulation. In fact, this variable has different influences on many phenomena related to BA production, such as growth kinetics, cell yields and enzymatic activity. In addition, its effects on the activity of proteolytic and decarboxylating enzymes and the relationships between the microbial populations (Joosten and van Boeckel, 1988; Maijala et al., 1995b) have an important role on the total amount of amines. Higher fermentation temperatures (24 °C) favour starter LAB which outgrew the amine-positive nonstarter microorganisms (Maijala et

al., 1995b). Higher temperature can favour proteolytic and decarboxylating reactions, resulting in increased amine concentration after storage. At 15 °C, microbial decarboxylases might remain active, even if during storage, most microbial populations have reached the stationary growth or death phase (Bover-Cid et al., 2000b). In contrast, at low temperatures, as during a prolonged meat storage at 4 °C before casing, putrescine can be produced due to the action of psychrotrophic pseudomonads (Paulsen and Bauer, 1997). However, lower BA amounts were detected in fermented sausage stored at 4 °C with respect to those stored at 15 °C (Bover-Cid et al., 2000b).

6.5. Additives

The addition of sugars to sausages influences the population dynamics and, consequently, the production of BA because they can enhance the growth of starter cultures. Enterococci developed earlier if sugar (glucose and lactose) was not added and *Enterobacteriaceae* counts were significantly higher (10^5 against 10^3 cfu g^{-1}). Tyramine and cadaverine were the most important BA and their concentration in the samples without sugar is doubled. Moreover, the absence of sugar influenced the susceptibility to formation of BA during storage with marked increase of tyramine, cadaverine, putrescine and tryptamine (Bover-Cid et al., 2000c).

The addition of sodium sulphide to slight fermented sausages at concentrations of 500 and 1000 mg kg^{-1} did not reduce the production of tyramine (which, surprisingly, seems to be stimulated by sulphide) while it inhibited cadaverine accumulation (Bover-Cid et al., 2001c).

The addition of sodium nitrite (150 mg kg^{-1}) in Italian dry sausages was able to consistently reduce putrescine and cadaverine accumulation but the presence of the curing agent caused a three-fold increase of histamine concentration (Cantoni et al., 1994).

6.6. Net effects

The production of biogenic amines is an extremely complex phenomenon, dependant on several variables, such as the growth kinetics of the microorganisms, their proteolytic and decarboxylase activities, which interacts each other. Furthermore, there is not a

univocal rule linking these variables with the different metabolic mechanisms necessary for the formation of biogenic amines. Gardini et al. (2001) evaluated the interactive effects of pH, temperature and NaCl concentration on the growth parameters, proteolytic activity and BA production of *E. faecalis* E37. Using an experimental design, they obtained polynomial equations describing the behaviour of these variables. Table 2 shows the values predicted by the models for the growth extent, the proteolytic activity and BA production after 72 h.

BA accumulation decreased markedly with the increase of NaCl concentration, while proteolytic activity was higher for intermediate concentration of salt, pointing out that there is not necessarily a correlation between these two variables. Moreover, more BA were produced at the higher pH, in correspondence with the higher cell yields. The effects of temperature after 72 h of incubation resulted to be

Table 2

Total biogenic amine production, cell yield and proteolytic activity of *E. faecalis* E37 after a 72 h incubation in synthetic medium in relation to pH, temperature and NaCl concentration

Chemico-physical parameters			*E. faecalis* E37 activities after 72 h		
pH	Temperature (°C)	NaCl (%)[a]	Biogenic amines (mg kg^{-1})	Cell yields (OD)[b]	Proteolytic activity[c]
5.4	20	1	27.81	1.76	–[d]
5.4	20	3	8.52	1.74	75.33
5.4	20	6	3.01	1.71	–
6.0	20	1	35.93	1.95	–
6.0	20	3	13.11	1.94	79.16
6.0	20	6	2.30	1.91	–
5.4	30	1	27.81	1.76	58.11
5.4	30	3	8.52	1.74	138.91
5.4	30	6	3.01	1.71	–
6.0	30	1	35.93	1.95	46.24
6.0	30	3	13.11	1.94	155.76
6.0	30	6	2.30	1.91	–
5.4	40	1	27.81	1.76	5.69
5.4	40	3	8.52	1.74	86.49
5.4	40	6	3.01	1.71	–
6.0	40	1	35.93	1.95	6.84
6.0	40	3	13.11	1.94	116.36
6.0	40	6	2.30	1.91	–

The data are elaborated from the models of Gardini et al. (2001).

[a] (w/v).
[b] Optical density at 590 nm.
[c] Free amino acids expressed as mg of leucine 100 ml^{-1}.
[d] No detectable proteolysis is predicted.

negligible with respect to NaCl concentration and pH and cell yield seems to be the more important parameter associated to BA production.

7. Bacterial amine oxidases

BA are physiologically inactivated by amine oxidases (AO), which are enzymes found in bacteria, fungi, plant and animal cells able to catalyse the oxidative deamination of amines with production of aldehydes, hydrogen peroxide and ammonia (Cooper, 1997). The sequential action (in the presence of an electron acceptor, such as O_2) of an amine oxidase and an aldehyde dehydrogenase leads to the production of an acid and ammonia, which can be used to support microbial growth (Parrot et al., 1987). Monoamine oxidase and diamine oxidase activity has been described in higher organisms as well as in bacteria (Murooka et al., 1976, 1979; Ishizuka et al., 1993). There are relevant differences between microbial AO in terms of substrate specificity and location, as stated by Cooper (1997).

Diamine oxidases can oxidase several BA, such as putrescine and histamine, and their activity can be affected by substrate inhibition; aminoguanidine, antihistaminic drugs and foodborne inhibitors, such as ethanol, carnosine, thiamine, cadaverine and tyramine, reduce their activity (Lehane and Olley, 2000).

The potential role of microorganisms involved in food fermentations with AO activity has been investigated with the aim to prevent or reduce the accumulation of BA in foods. Leuschner et al. (1998) tested in vitro the potential amine degradation by many bacteria isolated from foods and, in particular, in strains belonging to the genera *Lactobacillus*, *Pediococcus*, *Micrococcus*, as well as to the species *S. carnosus* and *Brevibacterium linens*. They found that this enzymatic activity can be present at very different quantitative levels. Tyramine oxidase activity of several microbial strains was strictly dependent on pH (with an optimum at 7.0), temperature and NaCl, as well as glucose and hydralazine concentration. Moreover, this enzyme was characterised by a higher potential activity under aerobic conditions. Temperature has also an important effect on histamine degradation (Dapkevicius et al., 2000). The highest degradation rate of this amine was observed at 37 °C, but at 22 and 15 °C, degradation

was still considerable. The amine oxidase responsible for this degradation has its optimum temperature at 37 °C and retains about 50% of its maximum activity at 20 °C (Schomburg and Stephan, 1993).

Many *S. xylosus* strains isolated from artisanal fermented sausages in southern Italy showed the ability to degrade BA in vitro (Martuscelli et al., 2000). Among the strains tested, *S. xylosus* S81 completely oxidised histamine, but it degraded, under the adopted conditions, also a part of tyramine. Even if the AO activity in vitro of microorganisms is not quantitatively reproducible in vivo (due to the more severe conditions and, in particular, to the low O_2 tension, pH and salt concentration), reduction of histamine in dry sausages has been observed in the presence of AO-positive staphylococcal starter cultures (Leuschner and Hammes, 1998). In addition, important reduction of the concentration of tyramine and putrescine in the presence of amine oxidase positive *S. xylosus* starter cultures have been observed by Gardini et al. (2002).

In other words, BA presence in foods is the consequence of a complex equilibrium between the composition of the food and the enzymatic activities of the microbial population. Together with the decarboxylating aptitude of the starter cultures, the presence and relative activity of AO should be considered as an important characteristic in the selection of starter cultures used in the production of fermented foods.

8. Conclusions

Tyramine and putrescine are the most common BA found in dry sausages and their presence is often due to the activity of LAB. High amounts of cadaverine and, to a lesser extent, of histamine have been detected in some samples, but the presence of these amines has been related to the low quality of raw materials in which high proliferation of. microorganisms occurs. On the other hand, the same raw material can lead to very different amine levels in final products depending on the presence of decarboxylating microorganisms, either derived from environmental contamination or from starter cultures, and the conditions supporting the growth and activity of amine-producing bacteria. However, the quality of raw materials seems to be only one of the many factors affecting amine formation in dry

sausages. In this perspective, the control of thawing and storage time and temperature of raw material is essential for the reduction of BA accumulation.

Also, chemico-physical productive parameters can select and favour amine-positive microflora and influence the activity of amino acid decarboxylases. While some of these parameters, such as sausage diameter (and, in turn, redox potential), depend on the type of products, other variables, such as temperature and the rate and entity of pH decrease, can be successfully modulated to avoid the growth of undesirable microorganisms. In addition, the activity of other microorganisms, usually present in sausages (for example, nonstarter LAB), can be controlled through a proper selection of starter cultures, which have to remain active and viable throughout all the fermentation, ripening and storage steps with the aim to limit the growth of amine positive wild microflora.

The physiological role of amine formation by microorganisms has not yet been completely elucidated. The capacity to decarboxylate amino acids is generally considered a strain-dependent characteristic rather than a species property. Since during the first stages of sausage fermentation, high contents of nutritional compounds are present, it is difficult that amine production depends only to the necessity of the cell to obtain N or C compounds. The regulatory role of putrescine in the expression of microbial genes, and in particular the *E. coli* oxidative stress response gene *oxyR* and *soxRS*, which are responsible for protecting the cell against hydrogen peroxide and superoxide radicals, respectively, has been recently reported (Tkachenko et al., 2001). These recent studies suggest new interesting hypothesis on the physiological role of amine in microorganisms. Some strains, which possess amino acid decarboxylase activity, could overcome or reduce the effects of temperature, NaCl, and other biological and chemico-physical factors that induce stress responses in the cells, with the production of some BA. Schiller et al. (2000) observed that in *E. coli* periplasmatic concentration of polyamines might fluctuate in response to environmental changes. Polyamines may act as endogenous modulators of outer membrane permeability, possibly as a part of an adaptative response to acidic conditions (Samartzidou and Delcour, 1999).

The choice of starter culture is fundamental to guarantee the quality of final products in relation to their BA content. For this reason, the inability to form BA, to survive during ripening and storage and to possess amine oxidase activity should be relevant criteria to be taken into consideration in the selection of starter cultures for the fermentation of dry sausages.

Acknowledgements

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