Biogenic amine producing capability of bacterial populations isolated during processing of different types of dry fermented sausages

Biogenic amine producing capability of bacterial populations isolated during processing of different types of dry fermented sausages

S. Guerrini[1], S. Mangani[1], O. Franci[2], M. Vincenzini[1]

[1] Dipartimento di Biotecnologie Agrarie. Università di Firenze, Italy
[2] Dipartimento di Scienze Zootecniche. Università di Firenze, Italy

Corresponding author: Simona Guerrini. Dipartimento di Biotecnologie Agrarie. Università di Firenze. P.le delle Cascine 24, 50144 Firenze, Italy - Tel. +39 055 3288309 - Fax: +39 055 3288272 - Email: simona.guerrini@unifi.it

ABSTRACT: In order to assess the distribution of the biogenic amine (BA) producing capability within the bacterial populations occurring during production of dry fermented sausages, four different types of sausage processing, three with the use of starter cultures and one without, were investigated. All the main bacterial populations involved in the sausage processing showed a diffuse and strain dependent capability to produce BAs. However, quantitative determination of individual BAs produced by the bacterial isolates suggests an important role of enterococci in the accumulation of tyramine, the most abundant biogenic amine found in all investigated sausages.

Key words: Biogenic amines, Dry fermented sausages.

INTRODUCTION – Biogenic amines (BAs) are organic bases that can be found in several fermented foods (ten Brink *et al*., 1990). BA accumulation in foods requires the availability of amino acids, the presence of microorganisms with aminoacid decarboxylases and favourable conditions to their growth and to their decarboxylating activity (ten Brink *et al*., 1990). The consumption of BA containing foods is of health concern because BAs are known as vaso-active and/or psycho-active molecules. Information about the distribution of the BA producing capability within the bacterial groups occurring in sausage processing is often contradictory, even if high amounts of tyramine and putrescine are generally considered as due to lactic acid bacteria, whereas high amounts of cadaverine and histamine are generally ascribed to a low microbiological quality of raw materials. To get more information on this topic has been the main objective of this work.

MATERIAL AND METHODS – Four different types of sausage processing were studied: *Napoli* (0.7 kg weight and 3 cm diameter), *Toscano* and *Ungherese* (3 kg weight and 10 cm diameter), produced at industrial level using meat of improved pigs and starter cultures, and an artisan sausage (0.5 kg weight and 6 cm diameter) produced using meat of *Cinta Senese* cross pigs without inoculating starter cultures. Sausage samples were taken immediately after stuffing, after eight days and at the end of ripening. Three aliquots of each sample were homogenized in a stomacher and analysed in triplicate for their content in both bacterial populations and biogenic amines. Bacterial counts were performed according to Bover-Cid *et al*. (2001). Bacterial isolates were obtained by removing a significant number of representative colonies from plates of each sausage sample. Qualitative test for BA production by bacterial isolates was carried out according to Bover-Cid and Holzapfel (1999); quantitative determination of individual amines produced by the bacterial isolates grown on TSB medium was carried out by RP-HPLC analysis (Guerrini *et al.*, 2002). Experimental data were statistically treated by analysis of variance (ANOVA) and Tukey's test.

RESULTS AND CONCLUSIONS – Immediately after stuffing, the differences among the sausage samples, especially in terms of bacterial counts, appeared mainly as the consequence of the use of starter cultures, rather than of the sausage type (Table 1). After the first week of ripening, at the end of the drying phase, no significant change in bacterial counts was observed, with the exception of lactobacilli, which increased to more than 10^8 CFU/g, independ-

Table 1. Main bacterial populations (CFU/g + SD) and biogenic amine concentrations (mg/kg + SD) occurring immediately after stuffing.

	Napoli[1]	Toscano[2]	Ungherese[2]	Artisan sausage
Lactobacillus spp.	(5.1 + 0.2) 106 ^{A}	(6.6 + 0.1) 105 ^{A}	(6.9 + 0.2) 105 ^{A}	(1.4 + 0.1) 103 ^{B}
Micrococcaceae	(2.10 + 0.04) 106 ^{A}	(9.6 + 0.3) 105 ^{B}	(9.0 + 0.4) 105 ^{B}	(2.5 + 0.5) 104 ^{C}
Enterococcus spp.	(3.00 + 0.04) 102	(3.0 + 1,4) 102	(4.0 + 2.8) 102	(1.5 + 0.7) 102
Enterobacteriaceae	(7.0 + 0.3) 103 ^{A}	(1.1 + 0.1) 104 ^{AC}	(1.4 + 0.1) 103 ^{B}	(1.5 + 0.1) 104 ^{C}
Phenylethylamine	nd	nd	nd	2.7 + 3.8
Putrescine	nd	nd	nd	2.2 + 2.3
Cadaverine	nd	nd	nd	2.3 + 3.2
Histaminen.d.	nd	nd	nd	
Tyraminen.d.	nd	nd	1.4 + 0.2	
Spermidine	6.4 + 0.5 ^{A}	3.3 + 0.5 ^{B}	3.0 + 0.3^{B}	8.2 + 3.7 ^{A}
Spermine28 + 4 A	32 + 4 ^{A}	29 + 3 ^{A}	64 + 14 ^{B}	

[1] Starter culture (Salum 50): Lactobacillus sakei, Lb. curvatus, Staphylococcus xilosus;

[2] Starter culture (Lyflore 2M): Lb sakei, S. xilosus, S. carnosus;

nd = not detectable under the analytical conditions used; A, B: = P<0.05.

ently of the use of starters (Table 2). At this processing stage, total BA concentration was found at noticeable values, ranging from 160 to more than 270 mg/kg, with tyramine and putrescine as the most abundant amines. Since the commercial starters used in this investigation proved to be unable to produce BAs, these were produced by the wild microflora. In this connection, it is to underline that the artisan sausage samples produced without bacterial starters did not present the highest BA concentration. At the end of ripening, in spite of its different duration depending on the sausage type, all samples still bore high concentrations of viable cells of both lactobacilli and microstaphylococci and contained even increased amounts of BAs (Table 3). The high level of tyramine (more than 200 mg/kg in all samples) is of particular concern because it is often reported that concentrations higher than 100 mg/kg are to be considered as unsafe. When bacterial isolates taken from plates of all samples at each sampling point were assayed for their BA producing capability, both qualitatively and quantitatively, the data reported in Table 4 were obtained (none of the isolates produced cadaverine, spermidine and/or spermine).

Table 2. Main bacterial populations (CFU/g + SD) and biogenic amine concentrations (mg/kg+ SD) occurring eight days after stuffing.

	Napoli	Toscano	Ungherese	Artisan sausage
Lactobacillus spp.	(7.1 + 0.2) 108 ^{A}	(2.8 + 0.2) 108 ^{B}	(3.9 + 0.1) 108 ^{C}	(1.1 + 0.1) 108 ^{D}
Micrococcaceae	(4.3 + 0.1) 105 ^{A}	(1.5 + 0.1) 105 ^{B}	(7.6 + 0.6) 104 ^{C}	(4.8 + 0.9) 104 ^{D}
Enterococcus spp.	(9.0 + 2.8) 102 ^{A}	(1.2 + 0.1) 104 ^{B}	(1.1 + 0.1) 103 ^{A}	(2.1 + 0.1) 104 ^{C}
Enterobacteriaceae	(5.5 + 2.4) 103 ^{A}	(7.2 + 0.1)103 ^{A}	(2.1 + 0.1) 102 ^{A}	(5.9 + 0.1) 105 ^{B}
Phenylethylamine	11 + 5 ^{A}	1.3 + 0.4 ^{B}	3.0 + 1.2 ^{B}	1.2 + 0.1 ^{B}
Putrescine	68 + 13 ^{A}	28 + 16 ^{B}	54 + 3.0 ^{B}	35 + 2 ^{B}
Cadaverine	23 + 4 ^{A}	2.4 + 0.7 ^{B}	3.2 + 0.6 ^{B}	42 + 8 ^{C}
Histamine	nd	nd	4 + 1	nd
Tyramine	143 + 33 ^{A}	77 + 17 ^{B}	101 + 3 ^{A}	68 + 1 ^{B}
Spermidine	5.4 + 0.7	3.7 + 0.4	3.7 + 0.1	7.8 + 0.7
Spermine	22 + 3 ^{A}	34 + 3 ^{B}	35 + 0.4 ^{B}	7.5 + 1.4 ^{C}

n.d. = not detectable under the analytical conditions used; $^{A, B, C, D}$: = P<0.05.

From the above reported results, three main conclusions may be drawn: 1) high quantities of BAs can be found also in fermented sausages produced by the use of starter cultures lacking in aminoacid decarboxylating activity, so enlightening the ineffectiveness of starter cultures in avoiding the development and/or the BA producing activity of

Table 3. Main bacterial populations (CFU/g + SD) and biogenic amine concentrations (mg/kg + SD) occurring after ripening.

	Napoli[1]	Toscano[1]	Ungherese[1]	Artisan sausage[1]
Lactobacillus spp.	(1.1 + 0.1) 109 ^{A}	(1.10 + 0.02) 108 ^{BC}	(2.00 + 0.04) 108 ^{B}	(6.2 + 0.1) 106 ^{C}
Micrococcaceae	(3.1 + 0.1) 105 ^{AB}	(1.5 + 0.1) 105 ^{A}	(7.2 + 0.9) 105 ^{C}	(3.8 + 0.2) 105 ^{B}
Enterococcus spp.	(1.4 + 0,4) 103 ^{A}	(3.6 + 1.0) 104 ^{B}	(3.6 + 0.3) 104 ^{B}	nd
Enterobacteriaceae	nd	nd	nd	nd
Phenylethylamine	27 + 2^{A}	5 + 1 ^{B}	35 + 2^{A}	41 + 18 ^{A}
Putrescine	111 + 2 ^{A}	176 + 37 ^{A}	220 + 1 ^{B}	210 + 82 ^{B}
Cadaverine	76 + 4 ^{A}	40 + 3 ^{B}	35 + 4 ^{B}	63 + 4 ^{C}
Histamine	25 + 0.1 ^{A}	40 + 12 ^{A}	148 + 3 ^{B}	223 + 94 ^{B}
Tyramine	238 + 11	215 + 11	265 + 37	292 + 60
Spermidine	6.0 + 0.5	3.1 + 0.3	6.2 + 5.4	6.1 + 0.9
Spermine	27 + 1 ^{A}	36 + 2 ^{B}	19 + 2 ^{C}	43 + 5 ^{D}

[1]Ripening time (days): Napoli, 21; Toscano, 40; Ungherese, 50; Artisan sausage, 130.
nd = *not detectable under the analytical conditions used;* $^{A, B, C, D}$*: = P<0.05.*

Table 4. Distribution of BA capability within the main microbial populations occurring in sausage processing (the number of BA positive isolates is indicated with respect to the total number tested; n.a. = not analysed).

	QLT[1]	QNT[2]			
		Phenylethylamine	Putrescine	Histamine	Tyramine
Lactobacillus spp.	43/180	0/7	3/7	4/7	5/7
Micrococcaceae	UR[3]	5/14	0/14	11/14	4/14
Enterococcus spp.	74/180	7/10	1/10	1/10	10/10
Enterobacteriacea	65/180	na	na	na	na

[1]QLT: qualitative test; [2]QNT: quantitative test; [3]UR: unreliable because of false-positive reaction.

the wild microflora present in raw materials; 2) althuogh BA producing capability is a strain dependent rather than a species specific property, the enterococci may play an important role in determining high tyramine content (see Table 4); 3) it should be highly desirable that starter cultures, especially those constituted by multiple species or strains, besides being unable to produce BAs, possess amine oxidase activity to decontaminate BA containing sausages.

Research supported by MIUR, PRIN 2005 project "Tipizzazione delle popolazioni microbiche per la tracciabilità e la sicurezza di prodotti fermentati tipici ottenuti da suini di razze locali".

REFERENCES – **Bover–Cid,** S., Holzapfel, W. H., 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. Int. J. Food Microbiol. 53: 33-41. **Bover–Cid,** S., Izquierdo-Pulido, M., Vidal-Carou, M.C., 2001. Changes in biogenic amine and polyamine contents in slightly fermented sausages manufactured with and without sugar. Meat Science 57: 215-221. **Guerrini**, S., Mangani, S., Granchi, L., Vincenzini, M., 2002. Biogenic amine production by *Oenococcus oeni*. Curr. Microbiol. 44: 374-378. **ten Brink** B., Damink C., Joosten H. M. L. J., Huis in't Veld J. H. J. 1990. Occurrence and formation of biologically active amines in foods. Int. J. Food Microbiol. 11:73-84.