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Effect of starter culture and storage temperature on the content of biogenic amines in dry fermented sausage poličan

T. Komprda[a,*], J. Neznalovà[a], S. Standara[b], S. Bover-Cid[c]

[a]*Department of Food Technology, Mendel University of Agriculture and Forestry Brno, Zemědělská 1, CZ-61300 Brno, Czech Republic*
[b]*Czech Agricultural and Food Inspection Brno, Květná 15, CZ-60300 Brno, Czech Republic*
[c]*Department of Nutrition and Food Science-CeRTA, University of Barcelona, Av. Joan XXIII s/n, E-08028 Barcelona, Spain*

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Abstract

Water activity, pH, microbial counts (total counts/TCM/, coliforms, *Enterobacter*, *Proteus*, *Pseudomonas*, *Escherichia* and *Lactobacillus* spp., respectively), and seven biogenic amines (BA) were determined in dry fermented sausage 'poličan' produced using starter culture A (*Lactobacillus sakei*, *Staphylococcus carnosus*, *S. xylosus*) or B (*L. sakei*, *S. carnosus*, *Pediococcus pentosaceus*), ripened 42 days, and subsequently stored at 8 or 22°C 60 days. Counts of lactobacilli were higher and TCM lower in the A-sausage when ripening was finished. Tyramine (quantitatively most important BA) content was not different ($P > 0.05$) in A- (90 mg/kg of dry matter, DM) or B-sausage (91 mg/kg DM) at the end of ripening. The effect of the storage temperature on BA content was not significant ($P > 0.05$) in the case of either tyramine or any other tested BA. The increase of total BA content during ripening was not different ($P > 0.05$) between A- and B-sausages (final value 190 and 222 mg/kg DM, respectively). However, sum of BA was significantly higher ($P < 0.05$) in B-sausage as compared with A-sausage at the end of either refrigerated storage (304 and 236 mg/kg DM) or room temperature storage (468 and 206 mg/kg DM, respectively). It is concluded that legislative limits should be established for tyramine and total BA content in dry fermented meat products.

Keywords: Biogenic amines; Fermented sausage; Storage; Starter culture; Tyramine

1. Introduction

Biogenic amines are nitrogenous low-molecular organic bases synthesised within microbial, vegetable and animal metabolism. They are formed mainly by decarboxylation of amino acids. Despite their important physiological functions (they promote growth, metabolic activity and immunological system of gut, are active in the nervous system and in the control of blood pressure, some are free-radical scavengers), BA may have toxicological effects in humans when consumed in food in high amounts: dilatation of peripheral blood vessels, resulting in hypotension and headache, or contraction of intestinal smooth muscle, causing abdominal cramps, diarrhoea and vomiting (Silla-Santos, 1996).

The toxicological level of a particular amine depends among other things on the ability of detoxification system of the intestinal tract and on the presence of other amines, and is therefore very difficult to establish. A limit 100 mg histamine/kg food was suggested. An ingestion of 100 mg of histamine or 10–100 mg of tyramine can cause poisoning (Eerola, Roig-Sagues, & Hirvi, 1998).

Availability of substrate (amino acids, fermentable carbohydrates) for microorganisms with a decarboxylase activity, presence of these microorganisms, and storage of foods in conditions enabling their growth are the main factors affecting the formation of BA in foods (Silla-Santos, 1996). Fermented foods, including fermented meat products are, apart from scombroid fish which most commonly cause BA (histamine) intoxication in humans, most hazardous in this context. Tyramine is usually the most common BA in fermented meat products (Eerola et al., 1998; Treviño, Beil, & Steinhart, 1997).

* Corresponding author. Tel.: +42-5-4513-3261; fax: +42-5-4521-2044.

E-mail address: komprda@mendelu.cz (T. Komprda).

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It is possible to restrain the formation of BA in fermented meat products by addition of starter cultures which inhibit the growth of proteolytic or decarboxylating microorganisms (Ayhan, Kolsarici, & Ozkan, 1999; Roig-Sagués & Eerola, 1997; Bover-Cid, Hugas, Izquerdo-Pulido, & Vidal-Carou, 2000a).

The objectives of the present study were as follows. (1) To compare two starter cultures, most frequently used in the production of the typical Czech dry fermented sausage poličan, from the viewpoint of the formation rate of biogenic amines during the ripening of the sausage. (2) To assess whether the starter cultures influence the production of biogenic amines in a different way also during storage of the sausage up to the expiration date. (3) To evaluate the production of biogenic amines during storage (under the influence of different starter cultures) at two different temperatures: refrigeration temperature and room temperature, which is used by many consumers in households.

2. Material and methods

2.1. Material

Experiments were carried out with a typical Czech dry fermented meat product, poličan. The basic raw materials were lean beef meat, lean pork and pork fat used in equal parts, nitrite salt mixture (2.5%), sugars (1%), spices and bacterial starter culture. Chopped and blended ingredients were stuffed into the cutisine casing: the diameter of the sausage was 40 mm.

Two batches 100 kg each were produced using bacterial starter culture containing *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus* (culture A), and *L. sakei*, *S. carnosus* and *Pediococcus pentosaceus* (culture B), respectively.

Both products (designated A or B) were placed on the same smoking trolley to assure the same conditions during ripening. Ripening of the sausages was carried out in following steps regarding temperature/humidity conditions: 22°C/92% until the 4th day, 18°C/85% until the 12th day and 13°C/70% until the 42nd day, and included smoking by the cold smoke.

The samples (one sausage from the batch A and B each time) were taken zero, 7th, 14th, 28th, 35th and 42nd day of ripening. Microbiological analysis and determination of water activity and pH were performed immediately after the refrigerated transportation from the producer to the laboratory. An aliquot was stored at −18°C till the analysis of BA.

Six more sausages from the batch A and B, respectively were taken 42nd day of ripening for the purpose of storage (42nd day of ripening was considered to be 0 day of storage). Conditions of the storage simulated the different possible handling the product in shop or household. Samples were, therefore, stored either in a refrigeration chamber with the regulated temperature 8°C or at the room temperature (22°C). Aliquots were taken 10th, 20th, 30th, 40th, 50th and 60th day of the storage and after taking were maintained at −18°C until the BA analysis.

2.2. Microbiological determinations

Ten grams of the material was aseptically removed from the inside of the sausage and homogenised in the Colworth stomacher with 50 ml of 0.85% NaCl solution. Serial decimal dilutions were prepared. Following microorganisms were determined after 72 h cultivation at 37°C: total counts of aerobic and facultative anaerobic mesophilic microorganisms on Plate Count Agar (Hi Media, India), coliforms on Endo Agar (Hi Media, India), *Enterobacter* spp. on Endo agar (Hi Media, India), and *Proteus* spp. on blood agar (Oxoid, England). *Pseudomonas* spp. were enumerated on pseudomonas agar (Oxoid, England) at 22°C after 72 h, *Escherichia* on Chrom agar (Merck, Germany) at 44°C after 48 h and *Lactobacillus* on tomato agar (Oxoid, England) at 30°C after 96 h.

2.3. Water activity and pH determination

Water activity was determined using Thermoconstanter TH-200 apparatus (Defensor AG-Novasina, Switzerland). Concentration of H^+ ions was measured in water extract on PHM 210 apparatus (Radiometer Analytical, France).

2.4. Biogenic amines analysis

Twenty grams of softly grated sample was homogenised in blender with 40 ml of 5% trichloroacetic acid 5 min three times. Substantial portions of lipids were removed after sample cooling to 3°C. Sample was then centrifuged 8 min at 9000 rpm. Supernatants were filtered through microfilter 0.45-μm. Amines in the filtrate were separated on ion-exchange column of the amino acid analyser Mikrotechna 339 T (Mikrotechna, Czech Republic) and determined after derivatization with ninhydrin using spectrophotometric detection at 520 nm and integrator HP 3392 B (Hewlet-Packard). Column size was 5.5×0.37 cm, column temperature 60°C, ionex Ostion LG ANB (Spolek PCHHV, Czech Republic). Flow rate of ninhydrin was 12 ml/h, that of buffers 14 ml/h. Elution buffers pH 6.0 (sodium citrate dihydrate 21 g/l, citric acid monohydrate 1.05 g/l, NaCl 5.0 g/l, KBr 41.65 g/l, isopropanol 300 ml/1; buffer A) and pH 5.45 (citric acid monohydrate 4.0 g/l, KCl 171.5 g/l, NaOH 11.2 g/l; buffer B), and dosing buffer pH 2.2 (citric acid monohydrate 14.0 g/l, NaCl 11.5 g/l, thiodiglycol 5 ml/l; buffer C) were used. Buffer A was used

within 0–68 min, buffer from B 68–96 min. Samples in buffer C were dosed in 21st min. Ninhydrine agent was composed of ethylenglycol monoethylether (750 ml/l), ninhydrine (20 g/l), $SnCl_2.2\ H_2O$ (0.4 g/l) and acetate buffer (250 ml/l). Biogenic amine mix standard solution in the concentration of 0.05 ml of each BA/ml of the dosing buffer was prepared using standard solutions of histamine dihydrochloride (Merck, Germany), tyramine hydrochloride (Calbiochem, USA), cadaverine dihydrochloride (Calbiochem, USA), tryptamine hydrochloride (Fluka, Switzerland), agmatine sulphate (Aldrich, USA), spermidine trihydrochloride (Fluka, Switzerland), spermine tetrahydrochloride (Fluka, Switzerland) and putrescine dihydrochloride (Sigma, USA).

The precision of the method, calculated as variation coefficient (percent) after six times repeated analysis was 9.4 for histamine, 4.5 for tyramine, 6.6 for putrescine, 2.1 for cadaverine, 5.5 for tryptamine, 4.7 for agmatine, 2.4 for spermine and 9.0 for spermidine. Recovery rates were in the range 107.2 (cadaverine) to 92.7% (agmatine). Detection limit for all amines was 1.0 mg/kg with the exception of tryptamine (3.0 mg/kg).

2.5. Statistical evaluation

Regression analysis was used for testing the significance of the linear or quadratic term of the dependence of BA content on time during ripening or storage of the sausage. The equality test of linear regressions (Rod & Vondráček, 1973) was used for the comparison of the dependencies of BA content on time of ripening or storage, as influenced by the starter culture or by storage temperature. The independent variable (X, time of ripening or storage) and the number of measurements ($n=7$ in the case of ripening and storage, respectively) being the same for any particular pair of compared samples, the decisive criterion (F-value) was calculated according to the simplified formula:

$$F=\frac{n\left(y_1+y_2^2\right)-\frac{n(y_1+y_2)^2}{2}+\frac{s_x\left(b_1^2+b_2^2-2b_1b_2\right)}{2}}{s_{y1}^2+s_{y2}^2}$$

where y_1 and y_2 are the means of compared sums of BA, s_{y1}^2 and s_{y2}^2 their variances, b_1 and b_2 corresponding coefficients of the linear term, and s_x is a standard deviation of the mean of the independent variable.

3. Results and discussion

3.1. Microbiological quality of the sausage during ripening

Proteus, Enterobacter, Escherichia and Pseudomonas spp. were not observed in any of seven samples taken during the ripening of the sausage. Coliform bacteria were found in the first sampling (zero day of ripening) in amounts of 10–30 CFU/g, but their growth was suppressed already at the beginning of ripening. Similarly, the study of Roig-Sagués, Hernandez-Herrero, Lopez-Sabater, Rodriguez-Jerez, and Mora-Ventura (1999) decreased the number of enterobacteria and pseudomonads steadily during the production process of a Spanish ripened sausage fuet and these microorganisms were not present at the end of the ripening period.

Total counts of aerobic and facultative anaerobic mesophilic microorganisms (TCM) and of lactobacilli during ripening within the present experiment are shown in Table 1. Counts of lactobacilli were higher and TCM lower at the end of ripening in the sausage produced using starter bacterial culture A in comparison with culture B. Similar to our results were lactobacilli dominant microorganisms in salami-type sausages in the study of Scheuer and Rödel (2000): pseudomonads decreased rapidly and *Enterobacteriaceae* were practically not detected.

3.2. Water activity and pH

The decrease of water activity was from 0.96 to 0.87 and from 0.95 to 0.89 between zero and 42nd day of ripening in our study when A or B starter culture was used. Weight losses were 28.2 and 27.8%, respectively, in A and B sausages during ripening. During storage weight losses were influenced by the storage conditions: 4.6 and 5.1% at refrigerated and room temperature, respectively, with little difference between A and B sausages.

The course of pH values during ripening and storage of the sausage is presented for both starter cultures and two storage temperatures in Fig. 1. The lowest values were reached 28th day of ripening. Then pH values increased moderately and the increase continued during

Table 1
Microorganisms found in the sausage during ripening with two different starter cultures (A, B)

Time of ripening (days)	Microorganisms (counts$\times 10^7$ per g)			
	Lactobacillus		Total counts[a]	
	A[b]	B[c]	A[b]	B[c]
0	0.20	1.00	0.90	0.60
7	1.00	2.00	3.00	1.00
14	1.00	3.00	0.12	0.21
21	4.00	2.00	0.80	0.70
28	2.00	4.00	0.40	0.38
35	3.00	2.00	0.20	0.50
42	7.00	4.00	0.01	0.60

[a] Mesophilic aerobic and facultative anaerobic microorganisms.
[b] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*.
[c] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*.

storage at both temperatures. This pattern of pH values is rather different when compared with the production of cervelat sausage in the study of Treviño, Beil, and Steinhart (1997), where a slight increase of pH was observed as late as post 72nd day of maturation/storage. In our study, pH started to increase after 28th day of ripening. An increase of pH is related to the breakdown of lactic acid following the depletion of the added sugar (Klettner & List, 1978). However, counts of lactobacilli increased until the end of ripening (42nd day) in our study. In the experiment of Bover-Cid, Izquierdo-Pulido, and Vidal-Carou (1999) increased pH in dry fermented sausage prepared using starter cultures of *Staphylococcus* spp. since 14th day of ripening. This increase is possibly related (similar to our experiment) to proteolytic activity of the starter culture, with the formation of peptides, amino acids and ammonia (Demeyer, Vandekerckhove, & Mormans, 1979).

3.3. BA contents during ripening

The measured values of BA contents during ripening are presented in Table 2. The measurements were performed only once and with one batch of either sausage, A or B. However, it is not unusual in similar experiments, as follows from the paper of Ayhan et al. (1999), as far as the effect of a starter culture on biogenic amines content in Turkish soudjoucks is concerned. Also, Hernández-Jover, Izquierdo-Pulido, and Veciana-Nogués, Mariné-Font, and Vidal-Carou (1997) used only one batch for each of the experimental interventions regarding starter culture for fermentation of fuet sausages.

The main interest of the present experiment was to evaluate the dynamics of the biogenic amines formation. Therefore, all figs. that refer to the differences between BA content due to the starter culture or storage temperature (Table 5) were calculated using particular regressions based on seven measurements in each case (Table 4). According to the results presented by Bover-Cid et al. 2000a,b), BA content from the same batch might be relatively variable. Therefore, the above differences in the present study do not consider BA contents at the given time interval but the course of BA contents as the dependence on time, and are evaluated using linear regression equality test (Rod & Vondráček, 1973).

As far as the dynamics of BA changes in general is concerned, BA content is usually presented on fresh matter basis in similar experiments (Eerola, Roig-Sagues, Lilleberg, & Aalto, 1997). However, one must be aware of the fact that in some cases the apparent

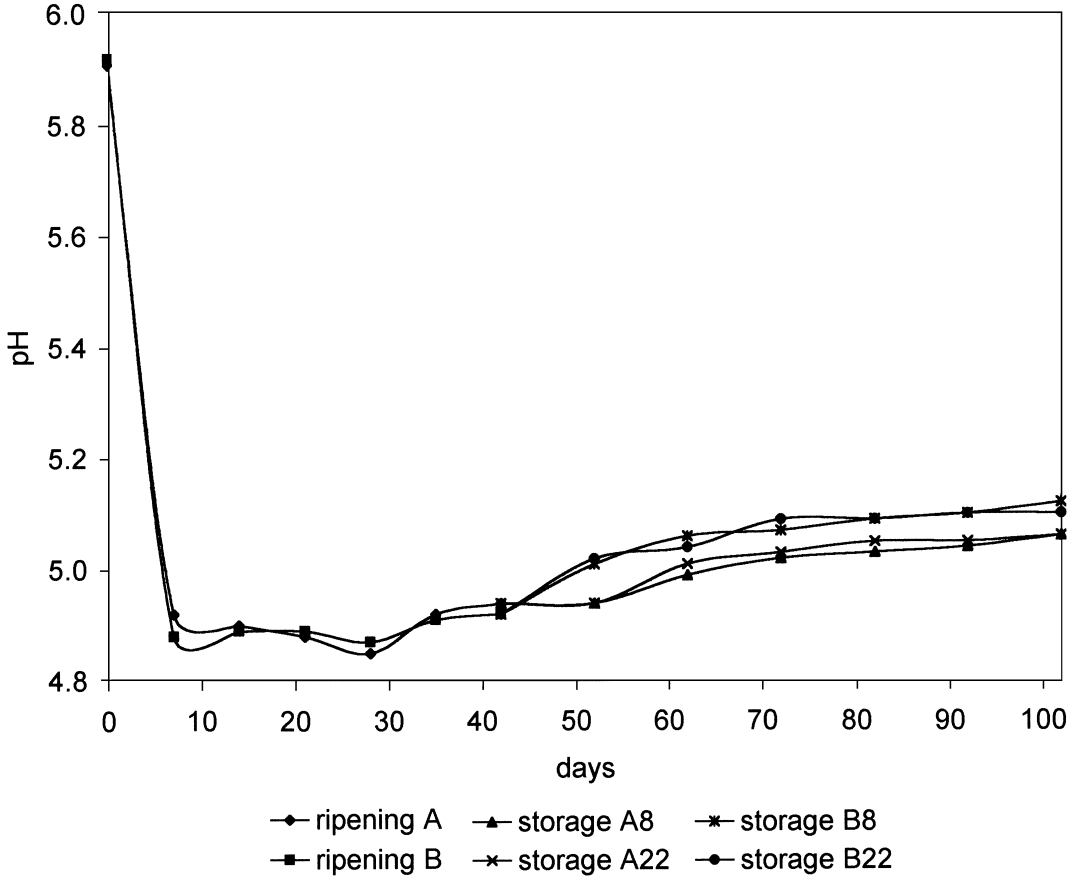

Fig. 1. pH during sausage ripening using starter culture A (*Lactobacillus sakei, Staphylococcus carnosus, Staphylococcus xylosus*) or B (*Lactobacillus sakei, Staphylococcus carnosus, Pediococcus pentosaceus*) and during storage at refrigeration (8°C) or room (22°C) temperature.

formation of BA might be partially explained by the concentration effect due to the loss of water during ripening (Hernández-Jover et al., 1997). Therefore, we expressed the data on a DM basis.

Quantitatively most important BA during ripening in the present study was tyramine, its content increased from 18 to 90 and from 15 to 91 mg/kg of DM in the case of starter culture A and B, respectively (the figures were calculated from corresponding regressions in Table 4 for the first and the last day of ripening). The course of the tyramine increase was not different ($P > 0.05$; Table 5) between the sausages produced with starter culture A or B. Contrary to our findings, Bover-Cid et al. (1999) reported greater differences between batches of dry fermented sausage prepared using different starter cultures (based on *S. carnosus* or different strains of *S. xylosus*) regarding tyramine content. Tyramine showed the dominance also in cervelat sausage after 87 days of the maturity process and storage (59 mg/kg) in the study of Treviño et al. (1997) when the starter culture containing *P. pentosaceus* and *S. carnosus* was used. However, under the influence of the starter culture with *P. pentosaceus, S. carnosus*, *P. acidilactici* and *Micrococcus varians* I, putrescine reached the highest value (484 mg/kg) followed by tyramine (119 mg/kg) in the above mentioned study.

Table 2
Content of biogenic amines in the sausage during ripening when two different starter cultures (A, B) were used; means of two measurements

Biogenic amine (mg/kg of dry matter)	Time of ripening (days)													
	0		7		14		21		28		35		42	
	A[a]	B[b]	A[a]	B[b]	A[a]	B[b]	A[a]	B[b]	A[a]	B[b]	A[a]	B[b]	A[a]	B[b]
Histamine	30	20	16	26	16	16	25	24	27	31	25	31	25	32
Tyramine	24	18	19	23	23	76	72	90	73	91	73	92	86	92
Putrescine	10	8	5	7	15	28	33	48	43	53	54	53	54	54
Cadaverine	8	6	7	5	5	5	8	8	7	7	6	6	6	6
Tryptamine	10	14	7	14	8	12	11	9	7	6	6	6	5	5
Agmatine	6	6	5	7	3	3	3	3	3	5	3	5	3	3
Spermidine	6	6	4	4	2	2	2	3	3	3	2	2	2	3
Spermine	4	4	4	4	2	2	2	3	3	2	2	2	2	2
Σ BA [c]	98	82	67	90	74	144	156	188	166	198	171	197	183	197

[a] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*.
[b] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*.
[c] Sum of all measured biogenic amines.

Table 3
Content of quantitatively most important biogenic amines, including sum of all biogenic amines during storage at 8 or 22°C using different starter culture (A[a],B[b]); mean of two measurements

Time of storage (days)	Starter culture	Biogenic amine (mg/kg of dry matter)							
		Histamine		Tyramine		Putrescine		Σ BA[c]	
		8 (°C)	22 (°C)	8 (°C)	22 (°C)	8 (°C)	22 (°C)	8 (°C)	22 (°C)
0	A	25	25	86	86	54	54	183	183
	B	32	32	92	92	54	54	197	197
10	A	25	23	85	86	50	52	177	186
	B	31	29	157	217	77	94	291	360
20	A	23	23	83	85	64	56	193	190
	B	29	34	154	230	80	107	289	398
30	A	23	25	88	83	65	52	193	182
	B	31	34	159	228	79	105	290	392
40	A	23	22	89	83	64	56	194	181
	B	31	38	154	233	83	104	287	402
50	A	24	22	104	87	92	77	240	205
	B	35	43	152	250	80	103	286	422
60	A	24	24	104	95	96	75	241	216
	B	36	46	154	273	80	104	291	448

[a] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*.
[b] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*.
[c] Sum of all measured biogenic amines.

Our results (Table 2) also did not confirm previous findings regarding the major rate of tyramine formation between the second and third day of ripening, during the sharp decline of pH (Bover-Cid et al., 1999, 2000b). BA production has been (among other things) associated with the protective action of microorganisms against an acidic environment (Eitenmiller, Koehler, & Reagan, 1978). However, the substantial increase of tyramine content in the present experiment (significant regression dependences, $P<0.05$, Table 4) occurred as late as in 2nd week (starter culture B) and 3rd week (starter culture A), respectively, when pH did not decrease any more (Fig. 1), despite the fact that lactobacilli counts further increased (Table 1). Hernández-Jover et al. (1997) found tyramine accumulation from the first day, immediately when the pH dropped, although no significant correlation was found between pH and tyramine content during the whole ripening process.

Putrescine content increased ($P<0.01$, Table 4) from about 10 mg/kg DM to about 60 mg/kg DM concurrently ($P>0.05$, Table 5) under the influence of both starter cultures in our experiment.

Histamine content increased in B-sausage ($P=0.039$) but not in A-sausage ($P=0.668$; Table 4) during ripening. However, the differences in histamine content between A- and B-sausage were not found at the end of ripening ($F=0.28$, $P>0.05$; Table 5). High variability of histamine content in particular sausage in the course of ripening can be concluded from these facts. According to Tschabrun, Sick, Bauer, and Kranner (1990), a decisive increase in histamine content occurs during the first part of ripening. On the other hand, Bover-Cid et al. (1999) reported constant histamine amount (below 0.5 mg/kg of DM) during the ripening of dry fermented sausages using proteolytic starter cultures similar to our experiment (*S. carnosus* or *S. xylosus*).

An important factor in histamine formation is an adequate decrease in pH (Fig. 1). Eerola et al. (1998) explained high histamine levels in some Finnish dry sausages taken from retail markets (100–200 mg/kg) by an inadequate decrease in pH at the beginning of the ripening process. Also, a poor quality of raw materials can be the reason of a considerable accumulation of histamine during sausage fermentation (Bover-Cid et al., 2000b). The relatively low histamine levels in the

Table 4
Parameters of the regression dependences of the content of quantitatively most important biogenic amines in the sausage (Y; mg/kg DM) on the time (X) of ripening (0–42 days) and storage (0–60 days) using different starter cultures A[a], B[b]

Biogenic amine	Ripening/storage	Starter culture	Parameters of the regression ($Y=a+bX+cX^2$)				
			a	b	c	I^2_{YX} (%)	P
Histamine	Ripening	A	21.9	0.07		4	0.668
		B	19.2	0.31		59	0.039
	Storage 8 °C	A	24.3	0.00		1	0.830
		B	31.9	−0.17	0.0046	92	0.016
	storage 22 °C	A	24.2	−0.01		4	0.675
		B	28.4	0.29		88	0.002
Tyramine	Ripening	A	16.0	1.76		82	0.005
		B	10.2	5.09	−0.0756	92	0.047
	Storage 8 °C	A	80.7	0.43		80	0.007
		B	128.6	0.70		39	0.128
	storage 22 °C	A	87.6	−0.37	0.0082	83	0.027
		B	153.3	2.26		68	0.022
Putrescine	Ripening	A	2.9	1.32		92	0.000
		B	8.5	1.30		85	0.003
	Storage 8 °C	A	46.8	0.80		84	0.004
		B	58.4	1.42	−0.0175	88	0.026
	Storage 22 °C	A	47.9	0.44		68	0.022
		B	60.8	2.59	−0.0320	85	0.038
Σ BA[c]	Ripening	A	71.3	2.83		75	0.011
		B	90.9	3.13		83	0.004
	Storage 8 °C	A	170.3	1.09		78	0.008
		B	247.9	0.94		36	0.156
	Storage 22 °C	A	178.1	0.46		54	0.048
		B	280.7	3.13		68	0.023

[a] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*.
[b] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*.
[c] Sum of all measured biogenic amines.

present study is in accordance with the data of Hernández-Jover et al. (1997) regarding production of the Spanish sausage fuet.

Contents of other detected BA did not change ($P > 0.05$) during ripening and did not differ ($P > 0.05$; results not shown) due to the starter bacterial culture in the present study. Polyamines spermine and spermidine are natural components of fermented sausages, their origin is in raw materials and their levels are not influenced by the starter culture (Bover-Cid et al., 1999; Hernández-Jover et al., 1997).

Total BA content when ripening of the sausage was finished (42nd day; calculated from the particular regressions shown in Table 4) was 190 mg/kg and 222 mg/kg of DM using starter culture A and B, respectively. This difference was not statistically significant ($P > 0.05$) as it is apparent from Table 5.

3.4. BA contents during sausage storage

Tyramine was quantitatively the most important BA also during storage in our experiment (Table 3). The increase of tyramine content during storage was statistically significant in all cases (P in the range 0.005–0.047) except for B-sausage stored at the refrigeration temperature ($P = 0.128$; Table 4). However, the final value (calculated from a corresponding regression in Table 4) was not different ($P > 0.05$; corresponding columns in Table 5) between sausages stored at 8 and 22°C either in A-sausage ($F = 0.9$) or in B-sausage ($F = 4.7$). Ayhan et al. (1999) found tyramine content in Turkish soudjoucks prepared with starter culture (*L. sakei*, *P. pentosaceus*, *S. carnosus* and *S. xylosus*) and without the starter 283 and 253 mg/kg, respectively 30th day of storage, from which the authors concluded that the addition of starter had no effect on tyramine formation. Eerola et al. (1998) found a mean of 82 mg/kg tyramine in 68 samples of Finnish dry sausages taken from retail markets. The more detailed comparison is not possible because the storage time was not reported in the paper of Eerola et al. (1998).

Generally speaking, within the particular sausage (A and B, respectively; corresponding columns in Table 5), we did not prove the differences between content of any tested BA due to the storage temperature. On the other hand, as regards the comparison of the starter cultures in sausages stored at a given temperature (8 and 22°C, respectively; corresponding lines in Table 5), higher ($P < 0.05$) content of tyramine, putrescine and the sum of BA was found in most cases (with the exception of putrescine at 8°C) in the sausage produced with the starter culture B as compared to the starter culture A.

Table 5
Differences due to the starter culture and storage temperature in the content of quantitatively most important (including sum of total measured) biogenic amines when ripening and storage, respectively was finished[g]

Biogenicamine	Ripening/storage	Final value[a] of biogenic amine content[b] (mg/kg of dry matter)		
		A[c]	B[d]	F[e]
Histamine	Ripening	24.8	32.2	0.28 NS
	Storage at 8°C	24.3	38.3	34.1 **
	Storage at 22°C	23.6	45.8	15.7 *
	F[e]	0.4 NS	2.7 NS	
Tyramine	Ripening	89.9	90.6	0.45 NS
	Storage at 8°C	106.5	170.6	17.7 *
	Storage at 22°C	94.9	288.9	18.4 *
	F[e]	0.9 NS	4.7 NS	
Putrescine	Ripening	58.3	63.1	0.1 NS
	Storage at 8°C	94.9	80.6	0.4 NS
	Storage at 22°C	74.3	101.0	9.6 *
	F[e]	0.7 NS	3.0 NS	
Σ BA[f]	Ripening	190.2	222.4	0.5 NS
	Storage at 8°C	235.7	304.3	10.1 *
	Storage at 22°C	205.7	468.2	16.9 *
	F[e]	0.5 NS	4.3 NS	

[a] 42nd day of ripening and 60th day of storage, respectively.
[b] Calculated from the particular regression (Table 4); $n = 7$.
[c] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*.
[d] *Lactobacillus sakei*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*.
[e] Linear regressions equality test (Rod & Vondráček, 1973).
[f] Sum of all measured biogenic amines.
[g] *Differences are significant at $P < 0.05$;
**Differences are significant at $P < 0.01$;
NS Not significant ($P > 0.05$).

Tyramine content in the sausage produced using starter culture B was higher in comparison with the starter culture A at the end of both refrigerated (171 vs. 107 mg/kg DM) and room temperature storage (289 vs. 95 mg/kg DM; $P<0.05$, Table 5). According to the potential toxic values of 10–100 mg of tyramine (Eerola et al., 1998), levels of tyramine found in sausages B stored at 22°C (200 mg/kg fresh matter) could provoke symptoms when sensitive individuals ingest from 50 to 500 g of the product.

Putrescine content in the sausages with both starter cultures increased ($P<0.01$ or $P<0.05$; Table 4) both during refrigerated storage and room temperature storage, and was higher ($P<0.05$) in B-sausage as compared to A-sausage when temperature 22°C was applied (Table 5).

As regards the histamine content, significant differences between starter cultures (Table 5) were not relevant, because the dependence of histamine content in A-sausage on the time of storage was not significant at either temperature (Table 4; regression equality test was based on these regressions). Similarly to the period of ripening, histamine content did not change during storage at any temperature when starter culture A was used ($P>0.05$; Table 4). In the case of the starter culture B, histamine content (Y; mg/ kg DM) began to increase from about 40th day of refrigeration storage ($Y=31.9-0.17X+0.0046X^2$) and from the beginning of the storage at the room temperature ($Y=28.4+0.29X$; Table 4).

Generally, better results (lower BA contents) obtained with the starter culture A in comparison with culture B (Table 5) are confirmed in Fig. 2 as regards of the total content of all detected BA. Sum of all BA at the end of the refrigeration storage (60th day; calculated from the particular regressions presented in Table 4) was in the present study 236 mg/kg of DM (starter culture A) and 304 mg/kg of DM (starter culture B), respectively. Similar results were found regarding room temperature storage: 206 and 468 mg/kg of DM in favour of A-sausage (Table 5).

Eerola et al. (1997) suggested two possible explanations for the formation of BA after the ripening of a dry sausage is finished: recontamination by decarboxylase-active microorganisms and/or favourable conditions for the activity of amine-producing bacteria originating from sausage manufacture. These explanations are not

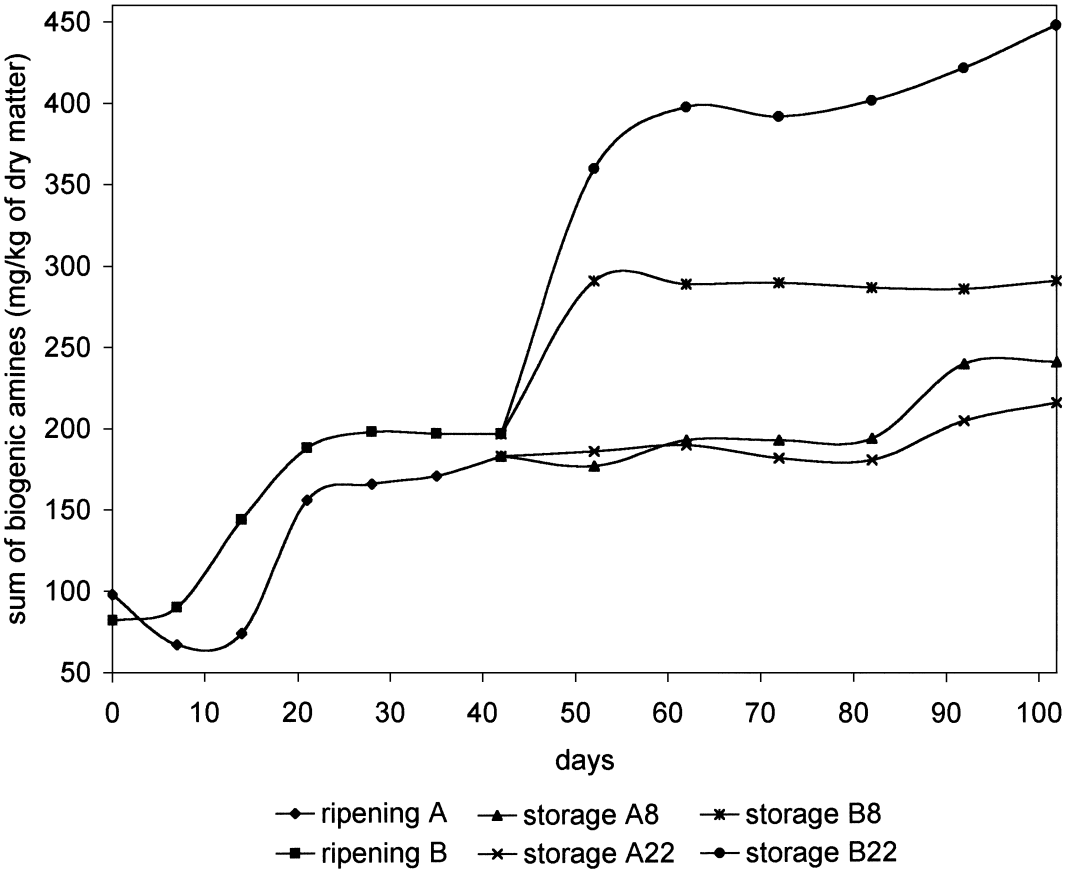

Fig. 2. Sum of all determined biogenic amines in the sausage during ripening and storage using two different starter cultures (A — *Lactobacillus sakei, Staphylococcus carnosus, Staphylococcus xylosus*; B — *Lactobacillus sakei, Staphylococcus carnosus, Pediococcus pentosaceus*) and two storage temperatures (8 and 22°C).

easy to apply in our paper, because the above mentioned authors studied sliced and vacuum packaged sausage. On the other hand, in the present study, the course of the increase of total BA content during storage was significantly different depending on the starter culture A or B respecting both the refrigerated storage ($F = 10.1$, $P < 0.05$) and the room temperature storage ($F = 16.9$, $P < 0.05$; Table 5). It is apparent from Fig. 2, that BA were not produced in B-sausage within 28th to 42nd days of ripening. However, we do not suspect the recontamination of the product (sausages B) because both sausages (A and B) were stored together in the same chamber and, indeed, they were not sliced. Possibly, each of the starter cultures could have influenced the development and activity of the spontaneous microflora in a different way. The different microbial composition (due to both different starter culture and different spontaneous microflora) might have resulted to different BA accumulation during the storage. The production of BA during storage can be related to both surviving microorganisms or to residual activity of decarboxylase enzymes produced by microorganisms in earlier stages (Bover-Cid et al., 2000a,b). Moreover, counts of lactobacilli (most probably due to *L. sakei* development) were substantially higher in A-sausage at the end of ripening (Table 1), presenting a better possibility to inhibit the growth and/or the activity of amine-producing microorganisms as compared to the B-sausage, in which, on the other hand, total counts were higher.

It is recommended that starter cultures for production of BA be tested in model media. Starter culture can influence BA production in dry fermented sausages by two ways: indirectly, by its ability to depress the growth of microorganisms with decarboxylase activity and, directly by its own decarboxylase activity. Regarding this item, Schauer and Rödel (2000), when testing samples of salami-type sausage produced using 15 different mixtures of starter cultures which were all declared as suitable by the producers, found higher tyramine content in eight of these samples as compared with the sausage produced without the starter culture. Decarboxylase activity of the starter cultures was not measured in model media in the present study. These cultures were applied because the producer of the given sausage traditionally uses them. *L. sakei,* which was a component of both starter cultures (A and B) in the present study was reported to have no decarboxylase activity and show good competitiveness in dry sausages (Bover-Cid et al., 2000a; Roig-Sagués & Eerola, 1997). However, it should be mentioned that the capability to decarboxylate amino acids is a strain dependent rather than a species dependent property (Bover-Cid & Holzapfel, 1999). Recently, Bover-Cid and Holzapfel (1999) developed an improved screening method for the detection of amino acid decarboxylase-positive microorganisms.

4. Conclusions

Producers of dry fermented meat products should test starter cultures for decarboxylase activity. Not only genera but also strains within the given genus and species can differ from this viewpoint. Therefore, different amounts of BA can be produced under the influence of two starter cultures, both of which are declared to be negative as far as the decarboxylase activity is concerned.

Consumers should not store dry fermented sausages at room temperature. Hazardous amounts of BA can arise in a product not stored in refrigerated conditions. Legislators are recommended to establish limits for tyramine and total BA content in dry fermented meat products. Tyramine is quantitatively most important BA in fermented sausages, and the presence of other amines can potentiate the negative effect of tyramine on human health.

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