



**CHANGES IN COUNTS OF MICROORGANISMS AND BIOGENIC AMINES
PRODUCTION DURING THE MANUFACTURE OF FERMENTED SAUSAGES
POLIČAN**

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ABSTRACT

Poličan is classic raw fermented sausage with low acidity. Dry fermented sausages Poličan were used for the analysis and drawn once a week during production from ripening chambers of meat-packing plants. Those sausages ripened for 35 days under controlled temperature and humidity conditions. The aim of this article is to evaluate microorganisms accompanying ripening of fermented sausages Poličan and characterize relationships between activity of microorganisms and content of biogenic amines. Lactic acid bacteria are the most important group of microorganisms. Their counts are relatively high already at the beginning of ripening - about 10^6 CFU.g⁻¹. During the first week of ripening, their numbers increased to 10^7 CFU.g⁻¹ and has not changed much further. Due to the minimal counts of coliform bacteria and micromycetes at the end of ripening, both series of these products can be considered as good and safe. In freshly made sausages, the amount of biogenic amines was

low. Spermidine, spermine and tyramine were quantitatively the most important. During ripening, the content of biogenic amines was increasing. Significant difference in biogenic amines between I. and II. series was found only for tyramine which was detected in difference exceeding 100 mg.kg^{-1} at the end of ripening. Sausages from II. series with high levels of tyramine may present a risk for sensitive individuals. Due to low levels of biogenic amines, other samples may be regarded as unproblematic.

Keywords: microorganisms, biogenic amines, decarboxylase activity, fermented sausages

INTRODUCTION

Poličan is classic raw fermented sausage with low acidity (pH 5.3 - 6.2, typically 5.8 - 6.2). Shelf-life is determined mainly by reduced water activity (a_w 0.90 - 0.65). These products are characterized by relatively long production time - 3 weeks to several months due to significant loss of moisture, **Pipek (1998)** reported more than 20 %. Low microbial contamination is especially important in the initial period of maturation, then microflora providing cultural maturation eliminates undesirable microorganisms. The decisive stage of production is fermentation - maturation. It is complex of mostly microbial processes which have influence on each other and determine the shelf-life, texture, color, flavor and aroma of the final products (**Pipek, 1998**).

The most important group of microorganisms is formed by lactic acid bacteria, especially in rapidly ripening sausages. From relatively small number at the beginning, their counts rapidly increase and thus they compete with other microflora. Important group are also micrococci, aerobic microorganisms primarily growing in the surface layer of sausages and surviving in environments with lower a_w . They are catalase positive, can break down hydrogen peroxide and reduce nitrates to nitrites. Both of mentioned groups are components of starter cultures that are added into filling while production. The starter cultures are most often formed by lactobacilli *Lactobacillus plantarum*, *L. brevis*, *L. fermentis*, *L. sakei*, *L. curvatus*. Furthermore *Staphylococcus xylosus*, *Staphylococcus carnosus* with, *Pediococcus pentosaceus*, *P. cerevisiae*, or some species of the genus *Micrococcus*, yeast of genus *Debaryomyces* are used. Lactic acid bacteria are added to filling in a amount of 10^6 - 10^7 CFU.g⁻¹.

Raw fermented products are susceptible to microbial spoilage. There is the greatest risk in the first hours and days of ripening because the raw content has still relatively high pH and a_w . (Görner et Valík, 2004).

During maturation of fermented salami, the number of microorganisms increases. Therefore proteolysis and acidification is significant and amount of free amino acids, which can be decarboxylated by microbial decarboxylases, is increasing. Thus conditions for the formation of biogenic amines are created. The only biogenic amines occurring in significant amount in fresh meat intended for the production of fermented sausages are spermidin, spermine and a less putrescine (Hernández-Jover et al., 1997). Kalač et Křížek (2005) states that already less treated material brings increased levels of putrescine and cadaverine into the product. High concentration of putrescine and presence of other amines is a sign of microbial growth and depends on the freshness of meat (Suzzi et Gardini, 2003). Depending on the technology and used starter cultures, the final content of biogenic amines may vary considerably. The data published by many authors (Maijala et al. 1995; Eerola et al. 1998a; Komprda et al., 2001) confirms that a key role in the formation of biogenic amines is played by the quality of fresh material as well as other important characteristic like pH, a_w , redox potential, NaCl, and others. Velišek (1999) states that the increase of biogenic amines is evident mainly in the early stages of fermentation and depend on the present microorganisms. Bover-Cid et al. (2001a) reported that rapid decrease of pH caused by decarboxylase negative starter cultures prevents formation of biogenic amines. Increase of the content of biogenic amines, observed during the final stages of production and storage, is attributed to lactic acid bacteria which are able to create them or to the residual activity of *Enterobacteriaceae* decarboxylases (Bover-Cid et al. 2000b). At the beginning of fermentation, mainly cadaverine and histamine occurs, at the end, there is mostly putrescine and tyramine (Tiecco, 1986). Langer (1980) and Shalay (1993) reported that in addition to those biogenic amines, tryptamine, cadaverine, fenylethylamine, spermine and spermidine may occur there as well. In products with the addition of glucono delta-lactone, higher levels of tyramine, histamine and putrescine were found (Maijala et al. 1993; Maijala et Eerola, 1993; Teodorovic et al., 1994). A comprehensive overview of the biogenic amines content in fermented salami manufactured in Spain and other European countries is provided in work of Suzzi et Gardini (2003). According to Eerola et al. (1998b), tyramine and putrescine are the most representative biogenic amines in fermented salami manufactured in Finland. Putrescine, cadaverine, tyramine and histamine were detected in French salami. Histamine was detected

at concentration higher than 100 mg.kg⁻¹ only in industrially produced salami. In rare cases, the products may contain 100 - 1000 mg.kg⁻¹ of histamine (Velišek, 1999).

The aim of this article is to evaluate microorganisms accompanying ripening of fermented sausages Poličan and characterise relationships between activity of microorganisms and content of biogenic amines.

MATERIAL AND METHODS

Material

Dry fermented sausages Poličan weighing 500 g were used for the analysis and drawn once a week during production (bruises and ripening - 35 days) from ripening chambers of meat-packing plants. Minced meat (pork and beef), pork fat, salt, sugar, spices mixture Rokomplett (Rovita) for fermented sausage Poličan and starter culture Biobak Sal (Wiberg), with *Pediococcus pentosaceus*, *Staphylococcus carnosus* and *Staphylococcus xylosus* were used for the production of sausage. Meat and fat were stored at -5 ° C for 48 h before processing. The finished filling was stuffed to the cutisin gut with 55 mm in diameter. Those sausages ripened for 35 days under controlled temperature and humidity conditions.

Microbiological analysis

Sausages for microbiological analysis were taken during once a week in two replicates (I and II). Samples for analysis were taken from the surface and central part of the sausage bar. Sample weighting 20 g together with 180 ml of sterile distilled water was homogenized in homogenizer Stomacher-type. The resulting suspension was prepared to decimal dilutions. 1 ml of the dilution was inoculated on a Petri dish and bathed in the appropriate nutrient medium. These groups of microorganisms were determined: the total aerobic counts of microorganisms (TAC) on PCA agar (Biokar Diagnostics, France) at 30 °C for 72 h, total counts of anaerobic microorganisms (TANC) on PCA agar (Biokar Diagnostics, France) at 37 °C for 72 h in Anaerobic jar with Anaerocult A (Merck, Germany), lactic acid bacteria (LAB) on MRS agar (Biokar Diagnostics, France) at 37 °C for 72 h, coliform bacteria (COLI) on VRBL (Biokar Diagnostics, France) at 37 °C for 24 h, yeast and moulds (YM) on agar with glucose, yeast extract and chloramphenicol (Biokar Diagnostics, France) at 25 °C for 120 h.

In the sample of spices mixture, the same method was used for analyzing the following groups of microorganisms: the total counts of microorganisms, anaerobic microorganisms, coliform bacteria, yeasts and moulds.

After cultivation on Petri dishes, accrued colonies were counted and viable counts were expressed as CFU.g⁻¹.

Determination of biogenic amines content

The content of histamine, tryptamine, tyramine, putrescine, phenylethylamine, cadaverine, and spermine and spermidine was determined with high-pressure liquid chromatography with UV detection after derivatization by dansyl chloride. 0.5 ml of internal standard (1,7-diaminoheptan) with concentration of 1 mg.ml⁻¹ was added to the sample (10 g ± 1 mg) held in 85 ml test tube. Then the sample was homogenized by mixer (Moulinex, France) and extracted with 15 ml of 5% trichloroacetic acid disintegrator (Heidolph Diax 900, Germany) for two minutes. The suspension was then centrifuged at 3000 rpm for 10 min at 4 °C in a centrifuge Universal 32R (Hettich, Germany). The supernatant was filtered through paper filter and solid phase was then twice re-extracted the same way. Joined extracts were filled to 50 ml volume with water. The extract was filtered before derivatization through disposable nylon membrane filter (13 mm, 0.45 micron, Chromatography Research Supplies, Addison, USA). Derivatization was performed by dansyl chloride. 1 ml of extract or standard was mixed with 0.5 ml of saturated sodium carbonate and 1 ml derivatization reagent (5 mg of dansyl chloride in 1 ml of acetone) and mixed for 1 minute in mixer (IKA MS2 Minishaker, USA). Derivatization proceeded for 1 hour at 40 °C, protected from light. Derivatives of amines were extracted with diethyl ether (3 x 1 ml). The organic phase was evaporated to dry matter by stream of nitrogen and the residue was dissolved in 0.5 ml acetonitrile. The solution was again filtered through nylon membrane filter 0.45 µm and dispensed to a chromatography column. For the separation of biogenic amines, Liquid Chromatograph HP 1100 (AgilentTechnologies, Waldbronn, Germany; Bold et al., 2004) was used.

RESULTS AND DISCUSSION

Results of microbiological analysis of Poličan sausages are shown in Table 1. The composition of microflora is a decisive factor for maturation and it changes during production, maturation and storage. Changes in microflora depend on pH, a_w (reduction by

pre-drying slows fermentation), temperature and the carbohydrate content. At higher temperatures, the development of undesirable microflora, fast acidification and excessive formation of gas may occur. Too low temperatures slow ripening by undesirable way. In the early stages, proteolytic microorganisms and lactic acid bacteria are competing. Lowering the pH (by breakdown of carbohydrates) decay microflora is suppressed and the lactic acid bacteria are promoted (**Pipek 1998**). In this period, the content of microorganisms is rising from 10^4 to 10^{11} CFU.g⁻¹ (**Sielaff et al., 1986**) due to increasing osmotic pressure. During the maturation, counts and activity of lactic acid bacteria decrease and lipolytic bacteria begin to predominate.

The most important group of determined microorganisms was lactic acid bacteria. Their enzymes allow necessary biochemical transformations during the manufacturing process, therefore they are intentionally added. LAB are added to the filling in the amount of 10^6 to 10^7 CFU.g⁻¹. They multiply rapidly and quickly acidify filling and then usually die. During this period, their numbers can rise up to 10^{11} CFU.g⁻¹. (**Pipek, 1998; Ricke et al. 2001**). Dynamics is shown in Figure 1 in the numbers of lactic acid bacteria. There is a noticeable increase in their numbers between the zero and 7th day of ripening up to the value of the order of magnitude 10^7 CFU.g⁻¹. During further maturation, these numbers (14th-35th day) started to decline but did not fall below 10^7 CFU.g⁻¹. Such values are given also by **Ansorena et al. (2002)** and **Kalhotka (2007)** who found similar values as well.

In filling, the total aerobic counts of microorganisms (TAC) were relatively high. After opening and during ripening, they increased. During 35 days of production, the numbers of this group of microorganisms (Table 1) ranged from 10^7 to 10^8 CFU.g⁻¹. TAC, in a similar range with a predominance of species of lactic acid forming bacteria, were determined also by **Smith et Palumbo (1973)**. Thus the counts of this group logically reflect the number of lactic acid bacteria which are together with other bacteria main component of starter culture. Numbers of both groups of microorganisms, on the 35th day of ripening, were compared to the previous determination. Increasing development of TAC and LAB could be explained by potential development of nonstarter lactic acid bacteria (for example *Lactobacillus plantarum*, *L. casei*, enterococci). Those bacteria might come to filling as a secondary contamination and prevailed over dwindling starter culture bacteria. Therefore, it may be a similar phenomenon that occurs during ripening of cheese, as **Tůma et al. (2004)** and **Kalhotka (2007)** evidenced. Some strains of lactobacilli but particularly enterococci involved in the formation of biogenic amines. **Komprda et al. (2010)** found the end of ripening in dry fermented sausages, LAB in the order of 10^6 CFU.g⁻¹ and relatively low counts enterococci. Counts of aerobic

microorganisms (TAC), LAB and anaerobic microorganisms (TANC) are essentially identical (Fig. 1). This is due to the use of starter cultures that make up the majority of microflora and similar metabolic properties of these bacteria.

Already feedstock may be significantly contaminated with undesirable microorganisms (Comi et al; 2005; Chevalier et al., 2006). Coliform bacteria (COLI) are a group of indicator microorganisms. Their increased numbers point defects in hygiene and sanitation and possible presence of pathogens. In Poličan sausages, coliform bacteria counts were are very low. The highest values, several tens of the order of magnitude CFU.g⁻¹, were reached in freshly stuffed filling. During ripening their numbers dropped to minimum or were not detected at all (Table 1). Lebert et al. (2007) reported counts of *Enterobacteriaceae* in the final products of the order of 10² CFU.g⁻¹ (mean value). Bover-Cid et al. (2001c) report that in meat of good quality, the numbers of *Enterobacteriaceae* should not exceed 10³ CFU.g⁻¹. Our findings of low numbers therefore indicate of good quality of feedstock and effective suppression of coliforms by lactic acid bacteria during ripening.

The occurrence of micromycetes in the analyzed samples of sausages was not high. The highest numbers were found in freshly stuffed filling, around 10⁴ CFU.g⁻¹ (Table 1).

During ripening, there was a reduction in their numbers to the minimum amount. Higher numbers of micromycetes (yeast) may indicate later defects. In the first series of analysis, yeast prevailed over fiber micromycetes (moulds). In the second series, the ratio reversed. This could be due to lower level of hygiene, since the second series were made of last sausages produced in the meat dressing plants.

The microbiological analysis was also performed by determining TAC, TANC, coliform bacteria and the counts of micromycetes (moulds and yeasts) in the spice mixture Rokomplett – sausages (Rovita) which was used for the production of analyzed sausages. The results of the analysis are shown in Figure 2.

TAC and TANC were relatively high, reaching values of 4.5 x 10⁵ CFU.g⁻¹, respectively 5,7 x 10⁴ CFU.g⁻¹. Counts of coliform bacteria were low - 25 CFU.g⁻¹, the presence of *Escherichia coli* was not confirmed. In the investigated compounds, micromycetes (moulds and yeasts) also occurred in small amounts and did not exceeded the order of magnitude 10² CFU.g⁻¹. The results showed that the seasoning mix used for making sausage may be an important vehicle of contaminating microorganisms and thus may represent, as stated Görner et Valík (2004), considerable hygienic and technological risk.

Table 1 Changes in counts of microorganisms in samples of sausages expressed in CFU.g⁻¹ during ripening

		day					
		0.	7.	14.	21.	28.	35.
TAC	I	1.10 x 10 ⁷	1.28 x 10 ⁷	1.22 x 10 ⁸	4.25 x 10 ⁷	2.41 x 10 ⁷	3.66 x 10 ⁸
	II	3.21 x 10 ⁷	7.89 x 10 ⁷	8.82 x 10 ⁷	5.31 x 10 ⁷	6.34 x 10 ⁷	4.85 x 10 ⁷
	mean	2.15 x 10⁷	9.51 x 10⁷	1.05 x 10⁸	4.78 x 10⁷	4.37 x 10⁷	2.07 x 10⁸
LAB	I	5.07 x 10 ⁶	7.91 x 10 ⁷	8.24 x 10 ⁷	8.17 x 10 ⁶	7.95 x 10 ⁶	1,52 x 10 ⁷
	II	2.75 x 10 ⁶	7.25 x 10 ⁷	2.94 x 10 ⁷	3.83 x 10 ⁷	1.12 x 10 ⁷	3.39 x 10 ⁷
	mean	3.91 x 10⁶	7.47 x 10⁷	5.59 x 10⁷	2.32 x 10⁷	9.58 x 10⁶	2.46 x 10⁷
TANC	I	7.76 x 10 ⁶	9.34 x 10 ⁷	6.61 x 10 ⁷	3.46 x 10 ⁷	2.63 x 10 ⁷	4.67 x 10 ⁷
	II	5.25 x 10 ⁶	6.80 x 10 ⁷	7.24 x 10 ⁷	4.09 x 10 ⁷	2.99 x 10 ⁷	5.08 x 10 ⁷
	mean	6.51 x 10⁶	7.64 x 10⁷	6.93 x 10⁷	3.77 x 10⁷	2.81 x 10⁷	4.88 x 10⁷
COLI	I	44	ND	3	ND	1	ND
	II	58	ND	ND	ND	ND	ND
	mean	51	ND	1	ND	1	ND
YM	I	2.17 x 10 ³	43	28	6	ND	ND
	II	4.07 x 10 ⁴	123	63	138	321	1
	mean	2.14 x 10⁴	96	45	72	160	1

Legend: TAC – Total Counts of Microorganisms, LAB – Lactic Acid Bacteria, TANC – Total Anaerobic Counts, COLI - Coliform bacteria, YM – Yeasts and Moulds, I, II – series, ND – not detected

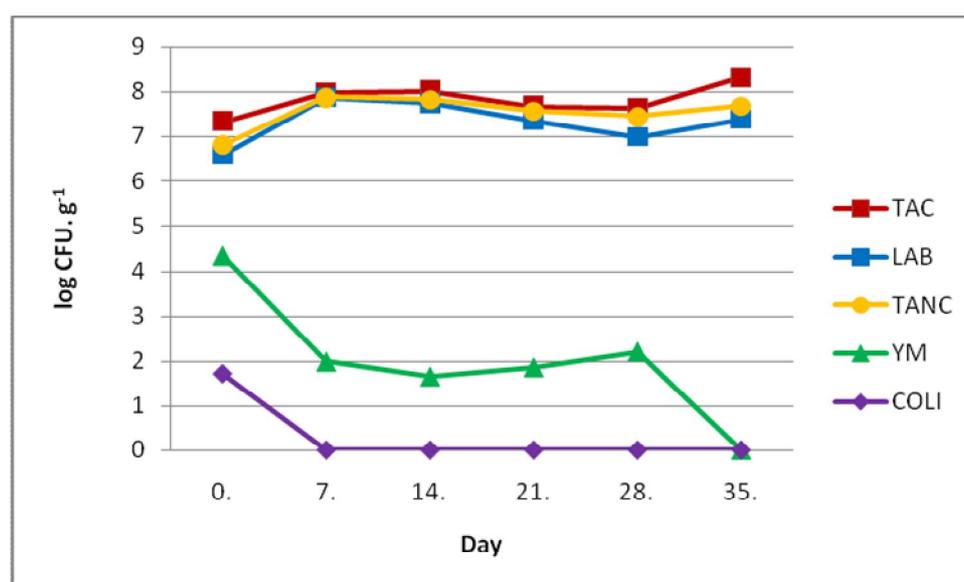


Figure 1 The counts of microorganisms during ripening (mean values)

Legend: TAC – Total Counts of Microorganisms, LAB – Lactic Acid Bacteria, TANC – Total Anaerobic Counts, COLI - Coliform bacteria, YM – Yeasts and Moulds

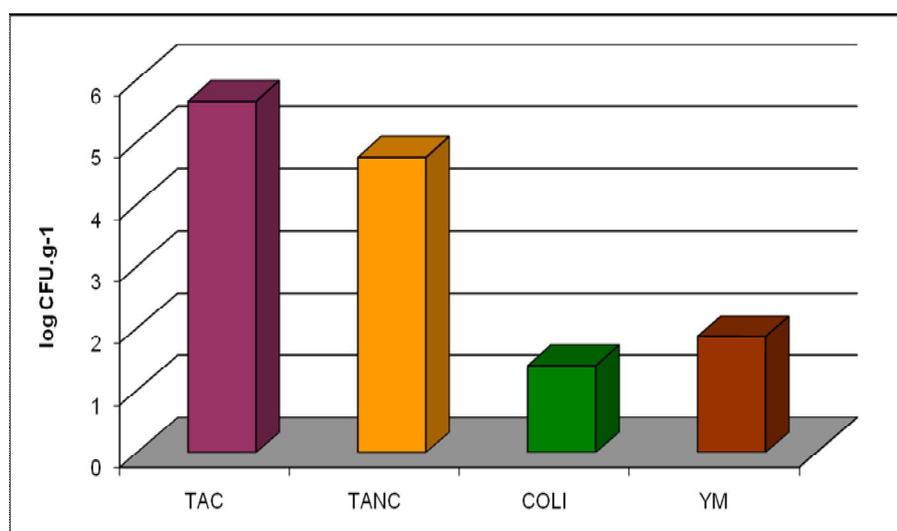


Figure 2 Counts of microorganisms in mixture of spices

Legend: TAC – Total Counts of Microorganisms, TANC – Total Anaerobic Counts, COLI - Coliform bacteria, YM – Yeasts and Moulds

Species of many genera such as *Bacillus*, *Staphylococcus*, *Micrococcus*, *Kocuria*, genera of the families *Enterobacteriaceae*, such as *Citrobacter*, *Klebsiella*, *Escherichia*, *Proteus*, *Salmonella* and *Shigella* and many LAB, including *Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Pediococcus*, *Streptococcus*, *Lactococcus* and *Leuconostoc* are able to decarboxylate amino acids. (Silla Santos, 1996; Lebert et al., 2007; Gounadaki et al., 2009; Kameník, 2011). According to Maijala et Eerola (1993) contaminating lactic acid bacteria play an important role in the production of tyramine and histamine. Bacteria *Pseudomonas fluorescens*, *Citrobacter freundii*, *Hafnia alvei* showed a positive reaction to histamine production and *Enterococcus faecalis* to production of tyramine as well (Tiecco et al. 1986; Maijala et al., 1993). High levels of putrescine and cadaverine (400 resp. 270 mg.kg⁻¹) were found in sausages with high numbers of *Pseudomonas*, gram-positive cocci and yeasts. (Montel et al., 1999). Microorganisms used as starter cultures, as well as microorganisms of processed material may participate on the formation of biogenic amines in durable sausages. It should be noted, however, that not all strains of these species are aminepositive. Some strains have a rather wide spectrum and are able to decarboxylase many aminoacids, whereas others have only strictly substrate specific decarboxylases. (Gounadaki et al., 2009)

Within the analysis of samples of sausages Poličan, the determination of biogenic amines was held as well. Spermine, spermidine, putrescine, cadaverine, tyramine, histamine and tryptamine were monitored. The amount of biogenic amines in samples of sausages

during ripening is shown in Table 2. In the freshly made sausage, quantitatively the most important biogenic amines were spermidine, spermine and tyramine. According to **Eerola et al. (1998b)**, tyramine is usually quantitatively the most important biogenic amine in fermented meat products. Spermine and spermidine, as **Hernandez-Jover et al. (1996)** reported, are present in fresh meat which is intended for the production of fermented sausages. High levels of spermine were found by **Bover-Cid et al. (2001d)** in freshly made sausages. Spermidine and spermine content did not change much during ripening, **Komprda et al. (2004)** has also found similar data. During ripening, tyramine, putrescine and spermidine were quantitatively the most important. **Komprda et al. (2002)** quantitatively the most important biogenic amine, during ripening and storage of Poličan, is tyramine. At the end of ripening (on the 35th day), tyramine was detected in excess of 100 mg.kg⁻¹ (average 108.8 mg.kg⁻¹) in two samples analyzed from sausages from the II. series. This is higher amount of tyramine than the one which was detected by **Komprda et al. (2001)** at the end of ripening of sausage Poličan. Their detected levels of tyramine reached 86 respectively 92 mg.kg⁻¹. In salami Hercules and Paprikaš at the end of ripening, value in the range 175 - 218 mg.kg⁻¹ were found (**Komprda et al., 2010**). Higher levels of tyramine as well as cadaverine and putrescine were detected in traditional fermented sausages produced in various European countries (**Latorre-Moratalla et al., 2008**). According to the **Silla-Santos (1996)**, toxicologically significant amount of tyramine is a considered from 100 to 800 mg.kg⁻¹. At higher concentration of putrescine in these samples (average 28.7 mg.kg⁻¹), **Bover-Cid et al. (2001d)** states that toxic effect of tyramine is enhanced. These sausages are considered to be risky for sensitive individuals. **Eerola et al. (1998a)** proposed the sum concentration 200 mg.kg⁻¹ of vasoactive amines (tyramine, histamine, tryptamine and 2-fenylethylamine) as an indicator of hygienic quality of fermented meat products. This value was not obtained in any of the samples, but the sum of biogenic amines in the two previously mentioned samples exceeded 160 mg.kg⁻¹. The content of histamine, tryptamine, cadaverine and spermine was low and did not change during all the time of maturing. Mostly low levels of histamine and tryptamine detected **Latorre-Moratalla et al. (2008)**.

Table 2 The amount of biogenic amines in samples of Poličan in mg.kg⁻¹

day		TRP	PUT	CAD	HIS	TYR	SPD	SPM	sum BA
0	I	0,6	2,4	3,4	0,2	5,3	22,0	6,7	40,7
	II	1,5	1,3	2,1	0,1	4,7	16,8	3,7	30,3
	II	0,4	1,0	3,1	0,1	2,1	17,6	5,1	29,4
	<i>mean II</i>	<i>1,0</i>	<i>1,2</i>	<i>2,6</i>	<i>0,1</i>	<i>3,4</i>	<i>17,2</i>	<i>4,4</i>	<i>29,9</i>
	mean I+II	0,8	1,6	2,8	0,1	4,0	18,8	5,2	33,3
7	I	0,2	3,2	1,5	0,2	1,8	20,6	6,3	33,8
	II	0,4	4,8	0,8	0,2	37,3	19,1	5,5	68,2
	II	1,2	3,4	0,6	0,4	54,8	17,5	2,6	80,6
	<i>mean II</i>	<i>0,8</i>	<i>4,1</i>	<i>0,7</i>	<i>0,3</i>	<i>46,1</i>	<i>18,3</i>	<i>4,1</i>	<i>74,4</i>
	mean I+II	0,6	3,8	1,0	0,3	31,3	19,0	4,8	60,8
14	I	0,0	8,2	1,9	0,0	5,3	17,6	5,1	38,0
	I	0,0	10,4	0,6	0,1	2,1	20,9	2,7	36,8
	I	0,6	3,9	1,9	0,2	2,2	19,9	6,6	35,2
	I	0,6	6,2	0,8	0,4	1,2	23,2	6,2	38,6
	<i>mean I</i>	<i>0,3</i>	<i>7,2</i>	<i>1,3</i>	<i>0,2</i>	<i>2,7</i>	<i>20,4</i>	<i>5,2</i>	<i>37,3</i>
	II	0,8	14,7	1,8	0,2	64,8	16,8	5,5	104,5
	II	0,6	12,1	1,9	0,7	50,1	15,1	6,2	86,7
	<i>mean II</i>	<i>0,7</i>	<i>13,4</i>	<i>1,9</i>	<i>0,5</i>	<i>57,5</i>	<i>16,0</i>	<i>5,9</i>	<i>95,9</i>
	mean I+II	0,4	9,2	1,5	0,3	20,9	18,9	5,4	56,6
21	I	0,5	11,3	1,5	0,2	5,3	22,3	5,3	46,3
	I	0,0	22,8	2,6	1,3	17,1	18,1	6,5	68,4
	<i>mean I</i>	<i>0,3</i>	<i>17,1</i>	<i>2,1</i>	<i>7,5</i>	<i>11,2</i>	<i>20,2</i>	<i>5,9</i>	<i>64,3</i>
	II	2,5	16,5	1,3	0,6	99,2	19,4	5,7	145,2
	II	1,3	14,7	2,0	0,6	79,9	20,4	4,5	123,4
	<i>mean II</i>	<i>1,9</i>	<i>15,6</i>	<i>1,7</i>	<i>0,6</i>	<i>89,6</i>	<i>19,9</i>	<i>5,1</i>	<i>134,4</i>
	mean I+II	1,1	16,3	1,9	0,7	50,4	20,0	5,5	95,9
28	I	0,1	35,6	2,4	0,1	9,1	23,4	7,8	78,5
	I	0,5	14,6	1,7	1,1	12,1	19,3	6,3	55,5
	<i>mean I</i>	<i>0,3</i>	<i>25,1</i>	<i>2,1</i>	<i>0,6</i>	<i>10,6</i>	<i>21,4</i>	<i>7,1</i>	<i>67,2</i>
	II	1,5	49,4	2,0	1,4	97,1	21,8	5,0	178,3
	II	1,6	13,3	1,8	0,5	88,6	17,2	6,0	128,9
	<i>mean II</i>	<i>1,6</i>	<i>31,4</i>	<i>1,9</i>	<i>1,0</i>	<i>92,9</i>	<i>19,5</i>	<i>5,5</i>	<i>153,8</i>
mean I+II	0,9	28,2	2,0	0,8	51,7	20,4	6,3	110,3	
35	I	0,4	7,4	1,1	0,2	1,2	24,0	7,4	41,8
	I	0,0	5,3	1,6	0,1	1,3	19,6	6,2	34,2
	I	0,2	20,3	1,6	0,1	1,9	17,7	4,8	46,6
	I	0,6	28,1	2,4	0,0	2,4	24,3	6,3	64,0
	<i>mean I</i>	<i>0,3</i>	<i>15,3</i>	<i>1,7</i>	<i>0,1</i>	<i>1,7</i>	<i>21,4</i>	<i>6,2</i>	<i>46,7</i>
	II	1,6	30,2	2,2	2,8	104,5	16,3	3,0	160,5
	II	1,7	27,1	2,1	1,3	113,1	17,6	5,0	167,8
	<i>mean II</i>	<i>1,7</i>	<i>28,7</i>	<i>2,2</i>	<i>2,1</i>	<i>108,8</i>	<i>17,0</i>	<i>4,0</i>	<i>164,5</i>
	mean I+II	0,7	19,7	1,8	0,7	37,4	19,9	5,5	85,7

In fermented meat products, the content of biogenic amines can be quite variable as microflora and its activity. In the analyzed samples of sausages from I. and II. series, significant differences in the numbers of microorganisms were found with the exception of the micromycetes in the II. series. The most of the content of biogenic amines was similar. The exception was detected in tyramine content. This biogenic amine has significantly differed between samples from sausages from I. and II. series already from the 7th day of ripening. When comparing the mean values between I. and II. series on 35th day of ripening (1.7 respectively. 108.8 mg.kg⁻¹), this distinction makes more than 100 mg.kg⁻¹. The amount of this amine significantly influence not only mean values but also the total amount of amines (Fig. 3). When using the same starter culture, high levels of tyramine can be present because of the contaminating microorganisms with high activity of tyrosindecaboxylase. **Ansorena et al. (2002)** reported that enterococci, gram-positive cocci and contaminating lactic acid bacteria may participate in the formation of tyramine. Formation of biogenic amines in this type of product can be eliminated by convenient microbial starter cultures (**Bover-Cid et al. 2000a**).

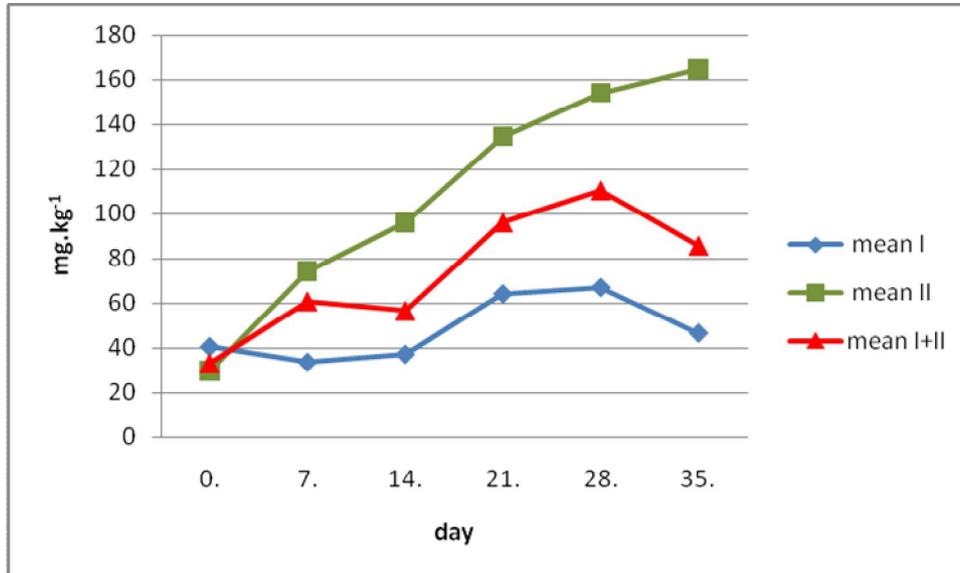


Figure 3 The amount of biogenic amines during ripening (mean values)

CONCLUSION

From the results of analysis, following conclusions can be drawn. Lactic acid bacteria are the most important group of microorganisms. Their counts are relatively high already at the beginning of ripening - about 10^6 CFU.g⁻¹. During the first week of ripening, their numbers increased to 10^7 CFU.g⁻¹ and has not changed much further. Lactic acid bacteria also strongly reflected in the total counts of microorganisms (TAC) and anaerobic microorganisms (TANC). Numbers of coliform bacteria were low and continued to decline during ripening. In the early ripening, relatively high counts of micromycetes were found - 10^3 respectively 10^4 CFU.g⁻¹. Their counts during ripening also significantly decreased. There were significant differences in counts of microorganisms between I. and II. series with the exception of micromycetes counts. High counts of moulds from the II. series, which were detected during ripening, could be caused by hygiene lowered by termination of production. Due to the minimal counts of coliform bacteria and micromycetes at the end of ripening, both series of these products can be considered as good and safe.

In freshly made sausages, the amount of biogenic amines was low. Spermidine, spermine and tyramine were quantitatively the most important. During ripening, the content of biogenic amines was increasing. Significant difference in biogenic amines between I. and II. series was found only for tyramine which was detected in difference exceeding 100 mg.kg⁻¹ at the end of ripening. High levels of tyramine from II. series could be caused, when using the same starter culture, by the presence of contaminating microorganisms with high activity of tyrosindecaboxylase. Sausages from II. series with high levels of tyramine may present a risk for sensitive individuals. Due to low levels of biogenic amines, other samples may be regarded as unproblematic.

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