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Control of biogenic amines in dry sausages inoculated with dairy-originated bacteriocinogenic *Enterococcus faecalis* EF-101

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ABSTRACT

In this study, the dairy-originated bacteriocinogenic *Enterococcus faecalis* EF-101 strain was implemented in traditionally smoked Croatian home-made dry fermented sausages. During ripening, microbiological and physico-chemical changes were observed, and the biogenic amines were monitored. The *Enterococcus faecalis* EF-101 count remained constant during the sausage ripening (10^5 CFU/g). There was no positive correlation of enterococci counts with cadaverine, histamine, tyramine, biogenic amines index, or total biogenic amines content in the sausages with added *E. faecalis.* The histamine and tyramine content correlated moderately with the lactic acid bacteria count in the control sausages. The total biogenic amines content was significantly higher (P<0.05) in the experimental sausages, however only on day 14 of ripening. The bacteriocinogenic strain of *E. faecalis* EF-101 reduced the histamine and cadaverine content, probably by reducing the aminogenic lactic acid bacteria population.

Key words: dry fermented sausage, *Enterococcus faecalis,* biogenic amines

Introduction

The production of traditionally fermented sausages and cured meats occurs worldwide and of course throughout Europe. In the Mediterranean region, sausages are traditionally mold- ripened without smoking, compared to North-European technologies, which usually use smoke (LÜCKE, 2017). Several potential chemical hazards have been recognized in traditional non-starter fermented sausage production, such as: polycyclic aromatic hydrocarbons (smoking process), biogenic

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amines (bacterial contamination) or mycotoxins (mold contamination) (ZDOLEC, 2017, 2018; LORENZO et al., 2007, 2010). One of the main chemical hazards, coupled with microbiological succession during sausage fermentation, would be the development and accumulation of biogenic amines. Sausage batter may be contaminated by persistent environmental microbiota, including an aminogenic population, due to poor hygiene conditions in households. Enterococci are the typical bacteria present for this reason, however, controversially, their presence and activity in sausage fermentation may even be desirable (SPARO et al., 2008). For instance, bacteriocins synthesized by enterococci have been characterized extensively and used in both food and animal production systems to reduce bacterial hazards (LAUKOVÁ, 2012). The antimicrobial activity of enterococci or enterocins in dry sausages has been shown to act mainly toward closely related Gram-positive bacteria, including *Listeria monocytogenes* (SPARO et al., 2008).

Sources of enterocin-producing enterococci are varied, including animal/human feces, raw milk and milk products, meat, fish and fermented food (LAUKOVÁ, 2012; LAUKOVÁ et al., 2016; KUBAŠOVÁ et al., 2017). The knowledge regarding the performance of non-meat originated bacteriocin-producing enterococci in fermented sausages is still very limited (RUBIO et al., 2013; CENCI-GOGA et al., 2016). Thus, the objective of the present work was to evaluate the impact of the bacteriocinogenic *Enterococcus faecalis* EF-101 strain, isolated from raw milk, on the safety and quality properties of traditionally smoked dry fermented sausages. The authors hypothesized that naturally fermented sausages inoculated with *E. faecalis* would pose a hazard, due to the development of biogenic amines.

Materials and methods

The Enterocin-producing culture of Enterococcus faecalis EF-101. Enterococcus faecalis EF-101 was isolated from raw cow milk and identified by MALDI-TOF MS (ZDOLEC et al., 2016; DOBRANIĆ et al., 2016). The inhibitory activity of the strain has been recently reported in foodborne strains of *L. monocytogenes* by enterocin production *in vitro* (CRK and ZDOLEC, 2018).

For the purpose of sausage inoculation, the strain of *Enterococcus faecalis* EF-101 was grown in MRS broth (Merck, Darmstadt, Germany) for 24 h at 37 ºC, followed by enumeration of the cell count in 1 mL of culture. The cells were separated from the culture by centrifugation (14,000 g for 10 min at 4 °C) and washed twice in distilled water. The cells were diluted in 1 mL of sterile distilled water, and the cell number was determined using Compass Enterococcus agar (Biokar, Allonne, France) at 44 ºC for 24 h. For the batter inoculation, a serial dilution was selected to adjust the final number to 1×10^5 cells per gram of sausage mixture.

Sausage production and sampling. Two series of traditional dry fermented sausages were produced during the winter season in the study household. The raw materials were purchased from an export meat factory, and were thus expected to have a low level of microbial contamination. The pork neck meat and fat were chopped, and then mixed manually with 2% of NaCl and commercial spice mixture (Derma, Varaždin, Croatia). The batter was divided into two equal parts and the second was inoculated with a freshly prepared culture of *E. faecalis* EF-101 (10^5 CFU/g). The sausage mixtures were stuffed into natural swine casings, desalted in warm water (φ 38-42 mm, Derma, Varaždin). After stuffing, the sausages were hung in smoking cabins to drain and equilibrate to the outdoor temperature for one day. On the second day, the sausages were smoked by slowly combusting beech (*Fagus*) and hornbeam wood (*Carpinus*). The smoke was applied for several hours every second day and repeated five times. Smoke was generated in a firebox separated from the smoking area, and transferred to the hanging sausages by metal pipe (φ 100 mm). After smoking, the sausages were ripened and dried in an attic for 40 days after stuffing. The sausages were sampled on the 0th, 7th, 14th, 30th and 40th days of production.

Microbiological analyses. For the microbiological analyses, 25 g of sample was diluted in 225 mL of saline peptone water and homogenized for 2 min at 200 rpm (Stomacher Circular 400, Seward, United Kingdom). The initial dilutions were serially

diluted and then, as appropriate, 1 mL or 0.1 mL of the relevant dilutions were used for enumeration of the microbiota, as follows: total viable count was checked on Plate Count agar (BioMérieux, Marcy l'Etoile, France) incubated at 30 ºC for 72 h, lactic acid bacteria onto MRS agar (Merck, Darmstadt, Germany) at 30 ºC for 48 h, enterococci onto Compass Enterococcus agar (Biokar, Allonne, France) at 44 ºC for 24 h, yeast and molds onto Yeast Glucose Chloramphenicol agar (Merck) at 25 ºC for 5 days, coagulase positive staphylococci onto Baird-Parker agar (Merck) at 37 ºC for 48 h, *Listeria monocytogenes* onto ALOA agar (Oxoid, Basingstoke, United Kingdom) at 37 ºC for 48 h and *Yersinia enterocolitica* onto CIN agar (BioMérieux) at 30 ºC for 24 h. Each sample was analyzed twice and, on the basis of the results obtained, the mean values were calculated.

Physico-chemical analyses. The quantity of protein, fat, and water was determined using a NIR spectrophotometer (Bruker Tango FT-NIR). The NIR spectrum encompasses a wide range of overlapping spectral absorption bands which correspond to more complex molecular vibrations and frequencies (overtones and combinations of basic vibrations). The NIR region covers electromagnetic radiation over the wavelengths from 750-2500 nm. In that range molecules that contain C-H, N-H and O-H bonds (fats, proteins, carbohydrates, organic acids, alcohol and water) are absorbed. Homogenized samples were placed into glass dishes and subsequently measured by the NIR spectrophotometer. Each sample was measured twice and, on the basis of the results obtained, the mean values were calculated. The NaCl quantity was obtained using the Mohr method (KAMENÍK et al., 2017), the pH value was determined using a digital pH meter in the extract (pH 510 Eutech Instruments, Landsmeer, Netherlands), and the water activity (a_w) was measured by means of a HigroPalm AW1 device (Rotronic, Bassersdorf, Switzerland). Each sample was measured twice, and on the basis of the results obtained, the mean values were calculated.

Determination of biogenic amines (BA). The eight biogenic amines studied (Cadaverine - CAD, Histamine - HIS, phenyletilamine PHE, Putrescine - PUT, Spermidine SPD, Spermine - SPM, Tryptamine - TRP and Tyramine - TIR) were detected and quantified by high performance liquid chromatography, using a diode array detector (G1315B DAD, Agilent Technologies) at 254 nm, with 550 nm as a reference after precolumn derivatization with dansyl chloride, as described by EEROLA et al. (1993). The extraction of 2.5 g of homogenized sample without casing was performed in 25 mL 0.4 mol/L perchloric acid. A total of 125 µL of an internal standard solution (1.7 heptanediamine, 1000 mg/L) was added to the homogenized sample prior to the extraction with 0.4 M perchloric acid. One milliliter of filtered sausage extract or mixed standard solution of biogenic amines was alkalized by adding 200 µL of NaOH solution (2 mol/L) and 300 µL of a saturated sodium bicarbonate ($NaHCO_3$) solution. Derivatization was obtained by thoroughly mixing with 2 mL of a dansyl chloride solution in acetone (10 mg/mL), followed by incubation (45 min at 40 °C). Residual dansyl chloride was removed by adding 150 µL of ammonia solution (1 mol/L NH_4OH), and the solution was kept in the dark for 45 min. Acetonitrile was added to a total volume of 5 mL followed by filtration using a 0.45 µm syringe filter (Sartorius, Goettingen, Germany).

The HPLC analysis of the BAs was performed using an High Performance Liquid Chromatograph (Agilent 1200 Series HPLC, Santa Clara, CA) equipped with a binary gradient pump (G1312A), and an auto-sampler with a thermostated sample compartment (G1329A), a thermostated column compartment (G1316A) and a DAD detector (G1315D). The separation of the compounds was undertaken in an LiChrospher C18 analytical column (ID 250 mm × 4.0 mm, particle size 5 µm) using a C18 Security Guard Cartridge(ID 4 mm × 3.0 mm) supplied by Phenomenex (Agilent, Santa Clara, CA), which was maintained at 40 °C and processed the injection volume of 20 µL. The mobile phase was a mixture of ACN and 0.1 mol/L ammonium acetate in a gradient mode, flowing at the rate of 1 ml/min (EEROLA et al., 1993). The initial 50% ACN representation was increased to 90% in 19 min. The initial conditions were reached in a minute, and maintained for 9 min before the next run. The

total run time was 29 min. The compounds were quantified using internal calibrations curves plotted for each BA and covering, for eight concentration levels ranging from 0.125-10 mg/L. Limits of quantification (mg/kg wet weight) ranged from 0.30 mg/kg (cadaverine) to 0.40 mg/kg (histamine and putrescine). Each sample of fermented sausages was analyzed in duplicate, and the BAs content values stated herein thereby represent the mean of these two parallel analyses.

Statistical analysis. Statistical analyses were performed using STATISTICA Version 12 (StatSoft. Inc., 2012). Differences in biogenic amines content between the two groups were evaluated by t-test ($P<0.05$). The correlation coefficient R was calculated at the level of significance of $P<0.05$ to evaluate the relationship between the determined microbial counts and biogenic amines.

Results and discussion

Microbiological and physico-chemical parameters. The total viable count in the control sausages was 1 log lower compared to sausages with the *E. faecalis* strain until day 30 (Table 1), while in the final sausages it was equal in both groups. The population of enterococci in the control sausages was low - below the detection limit by this method (2 log CFU/g), suggesting good hygiene standards in meat production. The enterococcal count in the inoculated sausages remained constant during the manufacturing process (5 log CFU/g). The lactic acid bacteria count was 1-2 log higher during the ripening in the sausages inoculated with *E. faecalis* EF-101. A growth of LAB was recorded in the control sausages, while in the experimental group it was constant at the level of 5 log CFU/g. The *L. monocytogenes, Y. enterocolitica, S. aureus* and *Pseudomonas* spp. counts were below the limit of detection (<2 log CFU/g).

Enterococci are part of the normal microflora of fermented sausages, but vary considerably with regards to the number in the population in different types of products (GARCÍA FONTÁN et al. 2007; MILIČEVIĆ et al., 2014). In our control batter, enterococci were present in very low numbers. The lactic acid bacteria and aerobic mesophilic bacteria showed a trend towards growth, mostly in the initial fermentation phase as reported by others (KOZAČINSKI et al., 2006; ZDOLEC et al., 2008; LORENZO et al., 2012). Enterococci can survive and multiply during fermentation in meat products, especially in products made without the use of competitive starter cultures (HUGAS et al., 2003). After the inoculation of bacteriocinogenic strain *E. faecalis* EF-101 from milk into the sausage batter (10^5 CFU/g), the number of enterococci did not change significantly during ripening. Since the population was stable, we can assume that the strain adapted to the conditions of meat fermentation, although further research and experimental production are required. The sensorial properties of sausages produced with the strain *E. faecalis* EF-101 were reported as acceptable by ČOP (2016). RUBIO et al. (2013) investigated the applicability of three bacteriocinogenic strains of enterococci in low acid fermented sausages, with the aim of reducing the population of *L. monocytogenes* and *S. aureus*. All three strains were shown to successfully transform the natural microflora of enterococci and two strains completely inhibited the growth of *L. monocytogenes*. SPARO et al. (2008) used a dairy culture of *Enterococcus faecalis* CECT7121 in the production of fermented sausages, and found no statistically significant differences between the control and experimental sausages with respect to the production of lactic acid and pH oscillation. Sausages with inoculated *E. faecalis* CECT7121 had a lower count of enterobacteriae, *S. aureus* and other Gram-positive cocci at the end of fermentation (SPARO et al., 2008).

As shown in Table 1, the content of proteins, fat and salt increased with the progression of ripening and drying ($R>0.91$; $P<0.05$), along with a lowering of the moisture and water activity ($R=-0.99$; $P<0.05$). The pH values and their trend were as expected in this kind of product. However, differences between groups were observed in pH values; pH was 0.2-0.3 lower in sausages inoculated with the bacteriocinogenic strain. This finding is in agreement with data reported by LORENZO et al. (2014); lower pH values were noted in sausages produced with the starter strain compared to the control group.

Table 1. Microbiological and physico-chemical progression during the ripening of fermented sausages produced with or without *E. faecalis* EF-101

Microbiological parameters (log CFU·g^{-1})	Day 0		Day 7		Day 14		Day 30		Day 40	
	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*
Total viable count	4.79 ± 0.22	5.79 ± 0.24	5.41 ± 0.16	6.67 ± 0.22	5.36 ± 0.14	6.91 ± 0.15	4.65 ± 0.10	5.24 ± 0.12	5.39 ± 0.22	5.74 ± 0.18
Lactic acid bacteria	3.30 ± 0.20	5.14 ± 0.25	3.50 ± 0.11	5.41 ± 0.16	4.00 ± 0.11	5.67 ± 0.12	3.47 ± 0.16	4.85 ± 0.13	4.92 ± 0.12	5.92 ± 0.15
Enterococci	<2*	5.60 ± 0.15	<2	5.31 ± 0.12	<2	5.44 ± 0.11	<2	4.56 ± 0.16	<2	5.20 ± 0.23
Yeast and moulds	<2	<2	<2	3.83 ± 0.05	<2	4.46 ± 0.03	<2	2.77 ± 0.11	2.69 ± 0.18	2.69 ± 0.22
S. aureus	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Pseudomonas	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
L. monocytogenes	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Y. enterocolitica	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Physico-chemical parameters	Day 0		Day 7		Day 14		Day 30		Day 40	
	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*		Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*
Water activity (a_w)	0.959 ± 0.004	0.955 ± 0.006	0.936 ± 0.004	0.936 ± 0.005	0.908 ± 0.003	0.924 ± 0.005	0.776 ± 0.003	0.780 ± 0.002	0.770 ± 0.002	0.780 ± 0.003
pH	5.69 ± 0.02	5.67 ± 0.03	5.44 ± 0.01	5.35 ± 0.03	5.49 ± 0.03	5.38 ± 0.02	5.67 ± 0.01	5.44 ± 0.01	5.9 ± 0.01	5.6 ± 0.03
NaCl (%)	3.36 ± 0.03	3.13 ± 0.04	4.58 ± 0.03	4.29 ± 0.04	5.8 ± 0.02	5.8 ± 0.02	5.8 ± 0.02	5.8 ± 0.01	5.85 ± 0.02	5.9 ± 0.01
Proteins (%)	16.11 ± 0.25	16.45 ± 0.29	21.20 ± 0.22	21.68 ± 0.02	27.28 ± 0.48	26.30 ± 0.39	36.58 ± 0.48	33.42 ± 0.26	36.46 ± 0.09	39.93 ± 0.05
Fat (%)	18.82 ± 0.28	16.74 ± 0.10	24.93 ± 0.16	20.84 ± 0.18	28.89 ± 0.20	24.61 ± 0.05	35.24 ± 0.02	31.28 ± 0.13	39.27 ± 0.15	36.66 ± 0.01
Moisture (%)	61.04 ± 0.14	63.35 ± 0.12	48.86 ± 0.18	52.46 ± 0.17	37.34 ± 0.18	42.20 ± 0.21	20.86 ± 0.03	27.75 ± 0.21	17.39 ± 0.04	18.37 ± 0.26

Results are presented as mean ± standard deviation; * below limit of detection by this method (2 log CFU/g)

Table 2. Concentrations of BAs during the ripening of naturally smoked fermented sausages produced with and without *E. faecalis* EF-101

BA (mg/kg fresh matter)	Day 0		Day 7		Day 14		Day 30		Day 40	
	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*	Control	Sausages with *E. faecalis*
Triptamine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	18.61 ± 2.89	<LOQ	16.09 ± 1.28	<LOQ	<LOQ
β-phenyletilamine	4.15 ± 0.37	<LOQ	3.15 ± 0.23	<LOQ	<LOQ	4.76 ± 0.92	3.27 ± 0.6	3.15 ± 1.02	3.48 ± 0.69	<LOQ
Putrescine	<LOQ	<LOQ	2.52 ± 0.37	<LOQ	<LOQ	<LOQ	1.05 ± 0.24	<LOQ	<LOQ	<LOQ
Cadaverine	2.19 ± 0.28	2.37 ± 0.71	63.76 ± 3.13	27.01 ± 1.24	8.22 ± 0.33	30.53 ± 1.86	70.38 ± 1.82	37.32 ± 2.34	41.67 ± 2.10	32.94 ± 2.73
Histamine	<LOQ	<LOQ	6.72 ± 0.25	4.69 ± 0.45	10.71 ± 0.71	9.66 ± 0.53	10.35 ± 0.51	7.66 ± 0.96	10.27 ± 0.99	8.94 ± 1.41
Tyramine	<LOQ	<LOQ	17.73 ± 0.69	21.97 ± 1.25	7.46 ± 0.75	42.73 ± 1.17	16.80 ± 0.26	33.53 ± 1.90	22.03 ± 1.97	31.76 ± 3.13
Spermidine	1.16 ± 0.25	1.08 ± 0.30	1.07 ± 0.28	0.91 ± 0.06	1.05 ± 0.23	2.06 ± 0.19	1.32 ± 0.71	1.64 ± 0.53	1.37 ± 0.40	1.34 ± 0.48
Spermine	21.61 ± 0.57	19.66 ± 2.35	21.87 ± 1.82	16.34 ± 1.01	16.38 ± 0.70	36.34 ± 2.14	21.76 ± 1.09	27.98 ± 0.61	21.29 ± 1.70	23.09 ± 2.18
BA Index*	2.18 ± 0.32	2.37 ± 0.83	90.68 ± 8.92	53.67 ± 0.99	26.34 ± 0.70	82.92 ± 1.52	98.64 ± 2.26	79.54 ± 3.54	74.01 ± 2.88	73.62 ± 2.53
Total content	29.11	23.11	116.82	70.92	43.82a	144.69b	124.93	127.37	100.11	98.07
Student's *t*-test (P value)	0.41		0.25		0.03		0.48		0.48	

BA concentrations are presented as mean ± standard deviation; *∑PUT-CAD-HIS-TYR; Limits of quantification LOQ: triptamine =0.31 mg/kg, β-phenyletilamine=0.35 mg/kg, putrescine= 0.40 mg/kg, cadaverine = 0.30 mg/kg, histamine =0.40 mg/kg, tyramine = 0.33 mg/kg, spermidine = 0.34 mg/kg, spermine = 0.33 mg/kg

Biogenic amines. In general, the highest amount of amines tested was recorded for cadaverine, tyramine and spermine during product ripening (Table 2). The histamine content was lower in sausages with the bacteriocinogenic strain during the whole ripening process. Moreover, the quantity of cadaverine was also lower in sausages with the *E. faecalis* strain, except on day 14. On the other hand, tyramine content was lower in the control sausages on all sampling days. The BA index (the sum of putrescine, histamine, cadaverine, and tyramine) was below 5 mg/kg in both sausage mixtures, indicating the good hygiene standards of the raw material used. The highest differences in the BA index were observed on day 7 (highest in the control) and day 14 (highest in the experimental sausages). The total content of BA was statistically significantly higher ($P<0.05$) in the experimental sausages on day 14, while there was no difference between the sausages during the other production phases ($P>0.05$). There was no positive correlation of enterococci counts and cadaverine, histamine, tyramine, BA index, or total BA content in the sausages with the added *E. faecalis* strain. In the control sausages, the histamine and tyramine content correlated moderately with the LAB count ($R = 0.56$ and 0.54, respectively).

The main biogenic amines developed in fermented meat products are tyramine, cadaverine, putrescine, and histamine (RUIZ-CAPILLAS and JIMÉNEZ-COLMENERO, 2004; DOMÍNGUEZ et al., 2016; PLEADIN and BOGDANOVIĆ, 2017). ANSORENA et al. (2002) reported that the tyramine and putrescine content ranged from 76 to 187 mg/kg and from 33 to 125 mg/kg, respectively. However, in our study, the putrescine content was below the limit of quantification (0.40 mg/kg). Tyramine is another important amine from a toxicology point of view, and its toxicological level ranges from 150-800 mg/kg. Tyramine production has been reported in the majority of enterococci from fermented sausages (BOVER-CID and HOLZAPFEL, 1999), which may be related to our finding of higher tyramine content in sausages with the *E. faecalis* strain. Histamine is the most important biogenic amine from a toxicology point of view (SHALABY, 1996). In our study, a slight accumulation of histamine was observed in both batches (10.27 and 8.94 mg/kg), which is much lower than its upper limits in food (100-400 mg/kg) (ZHANG et al., 2013). In addition, our values were lower than the mean values reported by EFSA (2011) for European sausages (about 25 mg/kg). Besides the microbiological factors (VESKOVIC MORACANIN et al., 2015), the amine content depends on conditions such as pH values, temperature, salt content, and sausage diameter (LATORRE-MORATALLA et al., 2008; LORENZO et al., 2010). The strategies for reduction of biogenic amines in fermented sausages should be primarily based on quality control of the raw material, and the use of competitive starter cultures (LORENZO et al., 2017; BEHERA et al., 2018).

In conclusion, simulation of high-level contamination/inoculation of sausage batter by *Enterococcus faecalis* EF-101 resulted in a higher content of biogenic amines on day 14 ($P<0.05$), but not during the other production phases. In addition, our study showed that the presence of the bacteriocinogenic *E. faecalis* EF-101 strain in sausage fermentation affected the histamine and cadaverine content, probably by reducing the level of aminogenic LAB.

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SAŽETAK

U ovom je istraživanju primijenjena bakteriocinogena kultura *Enterococcus faecalis* EF-101 iz mlijeka u proizvodnji trajnih kobasica u domaćinstvu. Tijekom zrenja praćene su mikrobiološke i fizikalno-kemijske promjene u nadjevu te sastav i količina biogenih amina. Broj *E. faecalis* ostao je stalan tijekom zrenja kobasica (10^5 CFU/g). Nije zabilježena povezanost broja enterokoka i količine kadaverina, histamina, tiramina, indeksa biogenih amina i ukupnih amina. U kontrolnim je kobasicama količina histamina i tiramina umjereno korelirala s brojem bakterija mliječne kiseline. Ukupna količina biogenih amina u pokusnim je kobasicama bila znakovito veća ($P<0,05$) tek 14. dan zrenja. Istraživanje je pokazalo da bakteriocinogena kultura *E. faecalis* EF-101 reducira količinu histamina i kadaverina, vjerojatno sistiranjem aminogene mikroflore.

Ključne riječi: trajne kobasice; *Enterococcus faecalis;* biogeni amini