ISSN 1330-9862
(FTB-1676)

original scientific paper

Investigation of Microbial Association of Traditionally Fermented Sausages

Lidija Kozačinski[1], Eleftherios Drosinos[2], Faruk Čaklovica[3], Luca Cocolin[4], Judith Gasparik-Reichardt[5] and Slavica Veskovič[6]*

[1]University of Zagreb, Faculty of Veterinary Medicine, Department of Hygiene and Technology of Foodstuffs of Animal Origin, Heinzelova 55, HR-10000 Zagreb, Croatia

[2]Agricultural University of Athens, Iera Odos 75, GR-11855 Athens, Greece

[3]University of Sarajevo, Veterinary Faculty, Zmaja od Bosne 90, BA-71000 Sarajevo, Bosnia and Herzegovina

[4]University of Udine, Dipartimento di Scienze degli Alimenti, via Marangoni 97, IT-33100 Udine, Italy

[5]Hungarian Meat Research Institute, Gubacsi út 6/b, HU-1097 Budapest, Hungary

[6]Institute of Meat Hygiene and Technology, Kacanskoga 13, RS-11000 Belgrade, Serbia

Received: April 3, 2006
Accepted: March 19, 2007

Summary

The investigation included fermented sausages produced in the countries of west and south-east Europe, Greece, Bosnia and Herzegovina, Croatia, Hungary, Italy and Serbia. The sausages were produced in local meat industries in a traditional way without the use of starter cultures. Samples were collected from three production batches on day 0 and again after 2, 4, 7, 14 and 28 days. Microbiological analyses included the principal ingredients (meat, fat tissue), casings and additives (sugar, mixture of spices, salt), and the finished products. From all three production batches of fermented sausages from each individual country, 150 strains of lactic acid bacteria and 150 strains of coagulase-negative cocci were isolated. Biochemical characteristics of the isolated microorganisms were determined by API system (bioMérieux), *i.e.* by API 50 CHL and API® Staph. Identification of all strains was made using the computer program APILAB Plus. From the hygienic standpoint, it is highly important that *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* were not found in the finished product. During all stages of investigation, lactobacilli and staphylococci prevailed.

Key words: autochthonous fermented sausages, microflora

Introduction

Fermented sausages have a long tradition of production in certain regions of the world. In the production of fermented sausages, meat processors generally use starter cultures. One of their roles is to stabilise meat fermentation and to ensure its safety. Although their use is recommended and they have advantages over traditional microflora, the final product can have poor aroma and taste. Lactic acid bacteria (LAB) represent a significant part of the naturally fermented sausage microflora. The presence of *Lactobacillus plantarum, Lactobacillus curvatus, Lactobacillus sakei* as well as *Pediococcus* spp. and *Leuconostoc* spp. in fermented sausages has often been reported (*1–3*). From traditional sausages coagulase-negative cocci (CNC) are mainly represented by *Staphylococcus* spp., but *Kocuria* spp. can also be isolated (*4-8*).

For two years, research has been conducted under the EU sponsorship (FP5 'Safety of traditional fermented

*Corresponding author; Phone: ++385 1 2390 190; Fax: ++385 1 2390 192; E-mail: klidija@vef.hr

sausages: Research on protective cultures and bacteriocins') in the countries of west and south-east Europe (Greece, Bosnia and Herzegovina, Croatia, Hungary, Italy, and Serbia). The first phase of research included the isolation of pathogenic bacteria and the identification of LAB and CNC from the natural microflora of dry sausages during various stages of maturation.

Material and Methods

The dry sausages were produced in local meat industries in a traditional way without the use of starter cultures. Their composition is presented in Table 1. Maturation parameters differed and were characteristic of the mode of production of traditional sausages in each member country of the research team (*9*). The sausages were considered ready for consumption on the 28th day. Samples were collected from three production batches on day 0 and after 2, 4, 7, 14 and 28 days of ripening. Every sampling day, three sausages were transported to the laboratory and subjected to microbiological analysis that included the raw material (meat, fat tissue), casings and additives (sugar, mixture of spices, salt), and finished products. Physicochemical parameters of sausages were also monitored (pH, moisture, salt) according to AOAC (*10*). Changes in physicochemical characteristics during fermentation and ripening of sausages are presented in Table 2. Through all fermentation processes, the values of pH decreased during the first 7 days, and then they increased to 4.7–5.7, the value of the final product. This is explained by considering the classical trend of microbial growth in fermented sausages, where the number of LAB increases at the very beginning of the fermentation.

Table 1. Sausage composition

Country												
Greece – traditional sausage		Bosnia and Herzegovina – sudzuk		Croatia – traditional sausage		Hungary – traditional sausage		Italy – Friuli sausage		Serbia – Sremska sausage	
w(ingredients)/%		*w*(ingredients)/%		*w*(ingredients)/%		*w*(ingredients)/%		*m*(ingredients)/kg		*m*(ingredients)/kg	
Pork meat	35	Beef meat	96	Pork meat	60	Pork meat	63.5	Pork meat	60	Pork meat	70
Beef meat	35	Mixture of salt and sodium nitrite	2.5	Beef meat	10	Pork back fat	32.5	Pork back fat	40	Pork back fat	30
Pork back fat	30	Black ground pepper	0.2	Pork back fat	24	Salt	2.3	Salt (NaCl)	2.5	Salt with 0.6 % $NaNO_2$	2.6
Salt	2.5	Garlic	1.0	Salt with 0.5 % $NaNO_2$	2.5	Sugars	0.1	Sugars	1.5	Fresh garlic	0.8
Sugars	1.5	Sugars	0.3	Sugars	0.5	Mixed spices (black pepper, red pepper, clove)	1.5	Sodium nitrite	0.02	Paprika	0.6
Skim milk powder	2.5			Spices (ground black pepper, minced red pepper, garlic)	3.0	Garlic	0.4	Black pepper	0.07	Dextrose	0.5
Mixed spices (black pepper, red pepper, clove)	0.3					Sodium nitrite	0.01				
Garlic	0.1										
White wine	0.2										
Sodium nitrate	0.02										
Sodium nitrite	0.02										
Sodium ascorbate	0.06										

Table 2. Changes in physicochemical characteristics during fermentation and ripening of sausages

Parameter/day	0	2	4	7	14	28
Greece						
pH						
Batch 1	6.38±0.04	5.49±0.02	4.84±0.03	4.74±0.04	4.75±0.02	4.78±0.01
Batch 2	6.18±0.01	5.42±0.04	4.89±0.06	4.84±0.02	4.82±0.01	4.83±0.00
Batch 3	6.19±0.00	6.19±0.00	5.38±0.02	5.15±0.00	4.97±0.04	5.10±0.11
Moisture/%						
Batch 1	54.58	53.30	51.14	48.88	37.91	28.22
Batch 2	52.60	52.05	49.53	46.27	41.89	34.70
Batch 3	52.24	50.74	50.61	49.09	38.19	29.80
a_w						
Batch 1	0.86	0.86	0.85	0.85	0.83	0.77
Batch 2	0.84	0.84	0.84	0.83	0.81	0.80
Batch 3	0.87	0.87	0.87	0.84	0.85	0.78
NaCl/%						
Batch 1	2.215	2.508	2.584	3.022	3.927	4.224
Batch 2	2.405	2.507	3.614	3.649	3.756	3.887
Batch 3	2.540	2.978	2.988	3.227	3.972	4.035
Bosnia and Herzegovina						
pH						
Batch 1	6.30±0.02	5.50±0.02	4.98±0.02	4.70±0.01	4.75±0.06	4.90±0.02
Batch 2	6.05±0.01	5.95±0.04	5.10±0.01	4.85±0.02	4.80±0.02	4.82±0.05
Batch 3	6.10±0.01	5.30±0.01	5.00±0.03	4.88±0.02	4.90±0.03	4.85±0.07
Moisture/%						
Batch 1	57.69±0.49	49.72±1.22	43.97±2.87	41.51±1.68	38.49±1.12	31.73±0.67
Batch 2	56.78±0.37	46.73±2.88	43.97±2.14	41.50±0.53	36.63±0.62	32.59±1.02
Batch 3	57.36±0.17	49.20±3.15	42.82±1.56	42.94±1.04	39.01±1.19	35.75±0.96
a_w						
Batch 1	0.95±0.01	0.94±0.02	0.92±0.03	0.91±0.01	0.90±0.02	0.90±0.02
Batch 2	0.96±0.00	0.94±0.00	0.92±0.01	0.91±0.00	0.90±0.01	0.90±0.01
Batch 3	0.96±0.01	0.93±0.03	0.93±0.01	0.92±0.01	0.90±0.03	0.89±0.01
NaCl/%						
Batch 1	2.422±0.17	3.411±0.17	3.511±0.16	3.321±0.12	4.082±0.22	4.232±0.39
Batch 2	2.261±0.08	3.263±0.09	3.303±0.16	3.323±0.07	3.631±0.28	4.510±0.37
Batch 3	2.391±0.19	3.372±0.19	3.274±0.12	3.694±0.23	3.694±0.19	4.212±0.36
Croatia						
pH						
Batch 1	6.00±0.01	5.95±0.03	5.67±0.01	5.12±0.06	5.30±0.05	5.27±0.01
Batch 2	6.44±0.02	6.25±0.05	6.12±0.01	5.23±0.02	5.30±0.01	5.35±0.05
Batch 3	6.00±0.00	5.80±0.01	5.72±0.04	5.27±0.02	5.08±0.03	5.52±0.04
Moisture/%						
Batch 1	58.8	59.6	48.4	32.4	22.4	15.2
Batch 2	51.6	50.8	42.0	32.8	23.6	12.8
Batch 3	59.6	58.8	56.0	50.4	22.4	13.2
a_w						
Batch 1	0.97	0.96	0.96	0.95	0.94	0.91
Batch 2	0.97	0.97	0.97	0.96	0.94	0.94
Batch 3	0.97	0.96	0.96	0.96	0.96	0.96
NaCl/%						
Batch 1	1.56	1.64	2.32	2.32	2.32	2.36
Batch 2	1.56	1.59	1.16	2.02	2.32	2.40
Batch 3	1.40	1.38	1.60	1.82	2.02	2.12

Table 2. – continued

Parameter/day	0	2	4	7	14	28
Hungary						
pH						
Batch 1	5.99±0.04	5.78±0.02	5.75±0.03	5.68±0.04	5.41±0.02	5.57±0.01
Batch 2	5.84±0.01	5.86±0.04	5.81±0.06	5.60±0.02	5.25±0.01	5.35±0.00
Batch 3	5.83±0.00	5.89±0.00	5.81±0.02	5.65±0.00	5.78±0.04	5.67±0.11
Moisture/%						
Batch 1	51.22	50.34	44.15	39.64	36.29	23.48
Batch 2	57.24	52.89	45.17	41.55	33.20	22.34
Batch 3	55.79	47.45	41.17	46.83	28.78	19.44
a_w						
Batch 1	NT	NT	NT	NT	NT	NT
Batch 2	NT	NT	NT	NT	NT	NT
Batch 3	NT	NT	NT	NT	NT	NT
NaCl/%						
Batch 1	2.70	2.72	3.93	3.41	3.60	4.99
Batch 2	2.42	2.89	3.48	3.50	4.34	4.70
Batch 3	2.38	2.97	2.91	3.37	3.87	4.44
Italy						
pH						
Batch 1	5.72±0.03	5.42±0.03	5.28±0.03	5.23±0.15	5.63±0.15	5.73±0.06
Batch 2	5.75±0.05	5.62±0.03	5.53±0.06	5.40±0.00	5.33±0.06	5.62±0.03
Batch 3	5.73±0.06	5.57±0.06	5.40±0.00	5.40±0.00	5.53±0.06	5.62±0.10
Moisture/%						
Batch 1	54.67±0.91	47.43±1.22	47.17±1.04	48.33±1.04	46.00±1.00	40.83±1.04
Batch 2	55.77±1.08	53.83±2.88	53.67±2.31	51.67±1.15	50.33±0.58	46.33±0.58
Batch 3	56.80±0.82	53.37±3.15	50.40±0.61	48.43±0.12	46.50±0.50	43.50±0.50
a_w						
Batch 1	0.97±0.01	0.94±0.01	0.92±0.01	0.91±0.01	0.92±0.01	0.91±0.01
Batch 2	0.97±0.01	0.96±0.01	0.95±0.01	0.94±0.01	0.94±0.01	0.92±0.01
Batch 3	0.97±0.00	0.95±0.01	0.93±0.00	0.93±0.01	0.92±0.01	0.92±0.00
NaCl/%						
Batch 1	2.63±0.06	2.83±0.06	2.93±0.06	3.20±0.10	3.23±0.12	3.37±0.06
Batch 2	2.45±0.05	2.46±0.05	2.53±0.23	2.80±0.17	2.97±0.06	3.37±0.12
Batch 3	2.47±0.16	2.70±0.17	2.70±0.05	2.85±0.05	3.13±0.15	3.28±0.03
Serbia						
pH						
Batch 1	5.34±0.03	5.31±0.02	5.15±0.04	5.11±0.03	5.12±0.07	5.16±0.03
Batch 2	5.72±0.03	5.44±0.04	5.27±0.01	5.23±0.04	4.92±0.06	5.30±0.05
Batch 3	5.34±0.02	5.28±0.04	5.36±0.01	5.11±0.03	5.15±0.01	5.35±0.09
Moisture/%						
Batch 1	54.13±0.91	51.56±0.33	47.67±0.32	45.96±0.37	43.41±1.37	36.70±0.55
Batch 2	52.51±0.64	49.49±0.51	43.88±0.99	38.56±0.31	38.69±0.55	23.50±2.03
Batch 3	50.60±1.21	50.60±0.19	40.03±1.82	35.74±0.72	30.58±0.81	36.70±1.28
a_w						
Batch 1	0.92±0.005	0.93±0.003	0.90±0.004	0.91±0.006	0.89±0.000	0.88±0.003
Batch 2	0.92±0.010	0.92±0.008	0.89±0.017	0.91±0.002	0.87±0.005	0.85±0.001
Batch 3	0.91±0.000	0.92±0.003	0.92±0.002	0.89±0.005	0.86±0.003	0.81±0.001
NaCl/%						
Batch 1	2.46±0.15	2.67±0.04	2.86±0.09	3.02±0.04	3.17±0.15	3.48±0.10
Batch 2	2.43±0.11	2.76±0.06	2.83±0.05	3.13±0.03	2.91±0.02	3.62±0.11
Batch 3	2.36±0.13	2.63±0.18	2.64±0.15	3.11±0.11	3.37±0.11	4.09±0.35

NT=not tested

LAB produce acids and determine a decrease in the pH, followed, in the phases of maturation, by the activity of micro- and staphylococci that are able to neutralise the produced acid. The a_w decreased gradually during ripening, reaching values of 0.80-0.92, but with different moisture percentage depending on the composition of sausages. Sodium chloride was added to reach a value of 1.5 to 2.5 %, which further increased because of dehydration process during ripening. Both nitrates and nitrites were characterised by a decrease in the concentration during ripening and the final values were about 10 and 8 ppm, respectively.

Analyses of raw material and intermediate samples were done as follows: (*i*) total viable count (gelysate agar, Oxoid, or 0.8 % bacteriological peptone+agar, incubation: 48 h at 30 °C), (*ii*) LAB (MRS agar overlaid with 5 mL of the same medium, incubation: 48 h at 30 °C), (*iii*) CNC (mannitol salt agar (MSA), incubation: 48 h at 30 °C), (*iv*) enterobacteria – *Escherichia coli* (*E. coli* ID (bioMérieux) overlaid with 5 mL of the same medium, incubation: 48 h at 37 °C), (*v*) faecal enterococcus (kanamycin aesculin azide agar, incubation: 48 h at 37 °C), (*vi*) *Staphylococcus aureus* (Baird Parker agar, incubation: 48 h at 37 °C), (*vii*) yeasts and moulds (malt extract agar+tetracycline 1 μg/mL, incubation: 48 h at 25 °C), (*viii*) *Salmonella* spp. (25 g of sample in 225 mL of buffered peptone water for 24 h at 37 °C and 10 mL in 100 mL of selenite cystine broth and incubation for 48 h at 37 °C. Streaking on Brilliant Green Phenol Red for 24 h at 37 °C) and xylose lysine desoxycholate (XLD) agar for 18–48 h at 37 °C), (*ix*) *Listeria monocytogenes* (25 g of sample in 225 mL of Fraser broth for 24 h at 30 °C and then streaking on Oxford agar and incubation for 48 h at 37 °C, 1 mL of pre-enriched sample in 10 mL of Fraser broth for 48 h at 37 °C and then streaking on Oxford agar and incubation for 48 h at 37 °C. Confirmation with CAMP test and blood agar), (*x*) *Pseudomonas* (cetrimide agar; incubation: 48 h at 25 °C), (*xi*) aerobic sporeformers (pasteurisation of 10^{-1} dilution at 80 °C for 10 min and then pouring of plate with plate count agar, incubation: 48 h at 30 °C), (*xii*) sulphite reducing clostridia (SPS agar, incubation: 72 h at 37 °C and if present, incubation for another 5 days).

A total of 150 (50 per batch) isolates of LAB were collected from MRS agar plate at each sampling day. Gram-positive and catalase-negative strains were further characterised. Biochemical characteristics of the isolated microorganisms were determined by API system API 50 CHL (bioMérieux). Identification of all strains was made using the computer program APILAB Plus. Moreover, a total of 150 (50 per batch) CNC isolates were collected from MSA agar plate at each sampling day. The isolates were rapidly checked for cell morphology by phase contrast microscopy, Gram reaction and catalase production. Gram-positive and catalase-positive cocci were further characterised. The strains were tested with the API® Staph (bioMérieux) and the computer program APILAB Plus performed the identification.

Results and Discussion

In Table 3 the results of total viable count, the count of LAB and CNC of the raw material (meat), and total viable count of spices and natural casings are presented. As it can be seen, the number of LAB in the pork and beef was low in the case of Greek and Croatian sausages (1.93 log CFU/g or they were not isolated), but in others it was 4–5 log CFU/g. In the raw meat of Croatian sausages, CNC were not found and their number was low in the raw pork of Greek, Hungarian and Italian sausages (<100 CFU/g). Total viable count in meat was up to 4 log CFU/g. The high total viable count was observed in mixed spices and garlic, as well as in the natural casings (up to 6.0 log CFU/g in some cases).

The results of the microbiological analyses of fermented sausages made in all countries showed that LAB constituted the major microflora of the sausages (Table 4). The initial population of LAB was in most cases low since no starter cultures were added. Because of the adaptation of LAB to meat environment, they grew fast and became the dominant microflora. The number of CNC differed from country to country and in some cases their count was low at the end of the ripening (Hungary, Croatia, Greece).

In the traditional fermented sausages, the other bacteria were also determined. In Greek sausages *Pseudomonas* had higher initial counts, probably due to the pork back fat used, and their microbial counts exceeded 5 log CFU/g. Enterobacterium *E. coli* population was lower than 3 log CFU/g after stuffing. *Pseudomonas* and enterobacterium *E. coli* were progressively eliminated regardless of their initial population. The low numbers of aerobic sporeformers (<3 log CFU/g) did not increase further; on the contrary, they were reduced during ripening and remained below 2 log CFU/g. The number of yeasts decreased during fermentation. Enterococci increased during early fermentation and remained at a level of 4–5 log CFU/g until the end of the entire process. Pathogenic staphylococci and sulphite-reducing clostridia were not detected. All samples were free of *Salmonella*. The greatest concern associated with sausage safety was the initial presence of *Listeria* spp. in all samples examined, but it should be pointed out that *Listeria* were reduced by the end of fermentation, and were never detected in the final product. The initial presence of *Listeria* spp. was probably due to the used raw material (pork meat and pork back fat) in which *Listeria* were present in all tested samples. This indicates the importance of selecting raw materials of good microbiological quality for dry sausage manufacture.

In Bosnian sudzuk, a relatively high initial count (5 log CFU/g) of aerobic sporeformers originated probably from garlic and black pepper, but their number decreased successively during the fermentation and ripening processes (below 2.5 log CFU/g). The same was observed with the population of enterococci (2 log CFU/g at the end of the ripening), yeast and moulds. *Pseudomonas* spp., *Salmonella* spp., *Listeria* spp. and sulphite-reducing clostridia were not detected in any samples of raw material or sausages examined during the process of production, fermentation and ripening.

In the traditional Croatian sausages, yeasts were isolated at the beginning of fermentation, and their number decreased, so that on day 28 there were no yeasts detected. Their higher number at the beginning was pro-

Table 3. Microbial counts* (log (CFU/g)±SD) of raw material and additives

Greece							
Total viable count	Pork	Beef	Pork back fat	Sugar	Mixed spices	Garlic	Casings
Batch 1	3.94±0.24	6.43±0.04	8.39±0.00	<2.00	3.50±0.05	<2.00	
Batch 2	5.51±0.27	5.01±0.34	6.03±0.47	NT	4.06±0.09	NT	
Batch 3	4.53±0.19	6.53±0.31	6.93±0.15	NT	4.02±0.06	NT	
LAB							
Batch 1	1.93±0.23	3.56±0.04	4.53±0.02	NT	NT	NT	NT
Batch 2	2.73±0.21	4.33±0.18	3.96±0.23	NT	NT	NT	NT
Batch 3	2.96±0.36	5.04±0.59	4.07±0.65	NT	NT	NT	NT
CNC							
Batch 1	<2.00	4.28±0.20	4.16±0.32	NT	NT	NT	NT
Batch 2	4.10±0.19	3.40±0.15	5.30±0.26	NT	NT	NT	NT
Batch 3	2.89±0.17	5.28±0.22	5.14±0.41	NT	NT	NT	NT
Bosnia and Herzegovina							
Total viable count		Beef	Mixture of salt and $NaNO_2$	Sugar	Garlic	Black pepper	Casings
Batch 1		5.96±0.20	2.63±0.15	<2.00	5.21±0.14	6.27±0.08	5.64±0.18
Batch 2		5.91±0.37	2.67±0.12	<1.00	5.41±0.13	6.00±0.04	5.68±0.09
Batch 3		6.05±0.30	2.66±0.07	<1.00	5.33±0.20	5.98±0.17	5.82±0.07
LAB							
Batch 1		5.13±0.05	4.53±0.02	NT	NT	NT	NT
Batch 2		5.19±0.11	3.96±0.23	NT	NT	NT	NT
Batch 3		5.72±0.25	4.07±0.65	NT	NT	NT	NT
CNC							
Batch 1		4.12±0.08	4.16±0.32	NT	NT	NT	NT
Batch 2		3.90±0.10	5.30±0.26	NT	NT	NT	NT
Batch 3		4.68±0.22	5.14±0.41	NT	NT	NT	NT
Croatia							
Total viable count	Pork	Beef	Pork back fat	Sugar	Mixed spices	Garlic	Natural casings
Batch 1	5.93±0.12	6.08±0.23	6.40±0.17	5.83±0.11	6.66±0.19	NT	4.40±0.22
Batch 2	5.08±0.42	7.08±0.09	6.63±0.22	5.77±0.24	6.35±0.21	NT	5.28±0.27
Batch 3	5.15±0.21	5.30±0.11	6.11±0.08	5.72±0.43	6.12±0.34	NT	5.18±0.35
LAB							
Batch 1	3.08±0.15	2.40±0.24	/	NT	NT	NT	NT
Batch 2	/	1.30±0.16	/	NT	NT	NT	NT
Batch 3	/	/	/	NT	NT	NT	NT
CNC							
Batch 1	/	/	/	NT	NT	NT	NT
Batch 2	/	/	/	NT	NT	NT	NT
Batch 3	/	/	/	NT	NT	NT	NT

Table 3. – continued

Hungary						
Total viable count	Pork	Pork back fat	Sugar	Mixed spices	Garlic	Casings
Batch 1	5.32±0.15	3.48±0.53	<2.00	5.82±0.84	5.53±0.27	5.67±0.08
Batch 2	5.51±0.27	4.13±0.27	NT	5.88±1.03	4.86±0.16	4.21±0.36
Batch 3	4.53±0.19	3.67±0.25	NT	6.49±0.78	3.98±0.42	4.08±0.39
LAB						
Batch 1	2.72±0.09	3.63±0.24	NT	NT	NT	NT
Batch 2	3.25±0.16	3.26±0.54	NT	NT	NT	NT
Batch 3	2.84±0.26	3.08±0.17	NT	NT	NT	NT
CNC						
Batch 1	2.16±0.17	2.43±0.31	NT	NT	NT	NT
Batch 2	1.84±0.28	1.67±0.16	NT	NT	NT	NT
Batch 3	<1.00	2.01±0.54	NT	NT	NT	NT

Italy			
Total viable count	Meat	Mixed spices	Natural casings
Batch 1	4.33±0.73	4.97±0.03	3.77±0.39
Batch 2	4.84±0.80	6.89±0.39	4.85±0.29
Batch 3	5.80±0.81	4.47±0.09	6.16±0.43
LAB			
Batch 1	4.10±1.57	NT	NT
Batch 2	3.76±0.47	NT	NT
Batch 3	5.77±0.77	NT	NT
CNC			
Batch 1	<100	NT	NT
Batch 2	<100	NT	NT
Batch 3	4.58±0.56	NT	NT

Serbia							
Total viable count	Pork	Sugar	Nitrite salt	Hot paprika	Paprika	Garlic	Casings
Batch 1	4.46±0.45	1	1.78	6.48	0.70	3.00	NT
Batch 2	4.07±0.50	<1	<1	0.77	0.80	0.36	NT
Batch 3	4.53±0.45	<1	<1	0.90	0.90	0.77	NT
LAB							
Batch 1	3.63±0.35	NT	NT	NT	NT	NT	NT
Batch 2	5.86±1.11	NT	NT	NT	NT	NT	NT
Batch 3	2.93±0.33	NT	NT	NT	NT	NT	NT
CNC							
Batch 1	2.50±0.43	NT	NT	NT	NT	NT	NT
Batch 2	4.59±0.97	NT	NT	NT	NT	NT	NT
Batch 3	1.79±0.44	NT	NT	NT	NT	NT	NT

*Each number is the mean of three sausage samples taken from the same batch
NT=not tested
LAB=lactic acid bacteria
CNC=coagulase-negative cocci
SD=standard deviation

Table 4. Microbial growth* (log (CFU/g)±SD) of the naturally fermented sausages

Microorganisms/day	0	2	4	7	14	28
Greece						
Total viable count						
Batch 1	5.65±0.31	7.72±0.02	7.95±0.15	8.77±0.44	8.60±0.04	8.58±0.04
Batch 2	4.33±0.05	7.55±0.03	7.62±0.02	7.51±0.14	7.47±0.01	6.48±0.13
Batch 3	5.88±0.09	7.15±0.18	8.03±0.12	7.90±0.08	7.91±0.26	7.73±0.08
LAB						
Batch 1	5.57±0.03	7.65±0.09	8.02±0.10	8.34±0.07	8.22±0.06	8.31±0.05
Batch 2	3.95±0.12	7.01±0.03	7.53±0.05	7.50±0.04	7.61±0.09	7.43±0.06
Batch 3	3.84±0.21	6.07±0.02	8.44±0.04	8.14±0.07	8.07±0.03	7.61±0.31
CNC						
Batch 1	3.79±0.00	3.57±0.01	2.72±0.12	2.50±0.71	2.00±0.00	1.70±0.00
Batch 2	4.63±0.29	5.39±0.25	3.79±0.29	3.62±0.23	3.48±0.10	2.73±0.40
Batch 3	4.86±0.09	5.18±0.11	5.39±0.05	3.90±0.30	4.05±0.38	4.50±0.13
Bosnia and Herzegovina						
Total viable count						
Batch 1	5.95±0.03	6.89±0.36	7.58±0.24	8.21±0.21	8.48±0.13	8.21±0.20
Batch 2	6.05±0.09	7.20±0.08	7.95±0.37	8.19±0.33	8.33±0.21	8.12±0.08
Batch 3	6.08±0.03	7.10±0.06	8.10±0.13	8.56±0.39	8.50±0.10	8.23±0.12
LAB						
Batch 1	5.65±0.14	6.30±0.25	7.33±0.11	7.55±0.23	8.13±0.20	7.90±0.03
Batch 2	5.74±0.09	6.85±0.10	7.14±0.06	7.25±0.12	7.95±0.08	8.10±0.14
Batch 3	5.81±0.04	6.80±0.13	7.45±0.28	7.33±0.30	8.22±0.31	7.99±0.31
CNC						
Batch 1	5.39±0.21	5.20±0.22	4.33±0.04	3.88±0.40	3.24±0.03	2.95±0.13
Batch 2	5.57±0.06	5.90±0.35	4.88±0.12	4.20±0.15	3.77±0.10	3.10±0.21
Batch 3	5.64±0.11	5.79±0.42	5.21±0.31	4.85±0.22	4.10±0.06	3.68±0.23
Croatia						
Total viable count						
Batch 1	7.11±0.02	6.11±0.11	7.26±0.13	5.83±0.02	6.08±0.50	6.41±0.15
Batch 2	7.28±0.12	7.15±0.21	6.32±0.05	8.08±0.12	7.80±0.47	6.74±0.24
Batch 3	5.51±0.14	7.56±0.13	6.26±0.21	6.18±0.16	6.93±0.21	6.81±0.34
LAB						
Batch 1	4.08±0.02	4.13±0.01	6.34±0.25	7.53±0.03	7.72±0.22	8.08±0.04
Batch 2	/	3.15±0.15	5.32±0.21	7.62±0.12	7.79±0.60	7.72±0.21
Batch 3	/	3.76±0.74	5.79±0.41	6.91±0.14	6.71±0.15	6.92±0.19
CNC						
Batch 1	/	/	/	/	2.64±0.14	/
Batch 2	/	/	/	/	/	3.61±0.02
Batch 3	/	/	/	/	/	/
Hungary						
Total viable count						
Batch 1	5.60±0.14	5.04±0.05	5.61±0.05	6.98±0.39	8.36±0.39	8.53±0.39
Batch 2	5.61±0.19	5.67±0.24	6.87±0.04	7.99±0.01	8.33±0.12	7.78±0.03
Batch 3	5.96±0.07	5.86±0.17	8.05±0.16	8.21±0.16	7.94±0.21	8.32±0.06
LAB						
Batch 1	2.84±0.17	3.12±0.28	5.79±0.28	7.70±0.16	8.12±0.19	8.45±0.21
Batch 2	3.84±0.16	3.97±0.05	6.73±0.04	8.07±0.11	8.61±0.14	8.09±0.12
Batch 3	4.22±0.18	5.49±0.09	7.75±0.16	8.54±0.32	7.54±0.16	8.37±0.14
CNC						
Batch 1	1.84±0.34	1.44±0.16	1.24±0.21	1.13±0.21	1.29±0.16	<1.00
Batch 2	1.37±0.18	2.02±0.43	1.89±0.26	1.76±0.24	1.53±0.46	1.20±0.27
Batch 3	<1.00	1.86±0.16	<1.00	1.59±0.39	1.32±0.29	<1.00

Table 4. – continued

Microorganisms/day	0	2	4	7	14	28
Italy						
Total viable count						
Batch 1	5.01±0.04	4.45±0.28	4.01±0.12	4.90±0.07	7.44±0.34	6.62±0.12
Batch 2	5.09±0.20	5.41±0.22	5.88±0.19	5.33±0.34	6.90±2.06	9.11±1.47
Batch 3	6.22±0.66	7.84±0.25	8.04±0.07	8.30±0.05	8.93±0.65	7.81±0.17
LAB						
Batch 1	4.11±0.19	7.27±0.27	7.86±0.10	8.22±0.15	8.28±0.05	8.39±0.37
Batch 2	4.20±0.17	8.24±0.01	7.81±0.10	8.21±0.03	8.28±0.16	8.45±0.06
Batch 3	5.61±0.19	8.39±0.10	8.15±0.09	8.34±0.07	8.55±0.15	8.47±0.03
CNC						
Batch 1	3.54±006	4.87±0.39	5.77±0.73	5.97±1.13	6.02±0.45	6.08±0.42
Batch 2	3.66±0.10	3.80±0.63	5.67±0.57	4.62±0.69	5.54±0.06	5.01±0.12
Batch 3	4.85±0.14	5.82±1.00	4.80±0.44	5.78±0.18	6.09±0.40	4.57±0.47
Serbia						
Total viable count						
Batch 1	4.69±0.36	4.04±0.15	4.75±0.39	4.85±0.22	4.79±0.28	4.23±0.29
Batch 2	4.97±0.85	6.20±0.56	6.29±0.56	5.46±0.15	6.09±0.12	5.65±0.97
Batch 3	5.97±0.31	5.16±0.48	7.49±0.70	7.10±0.17	6.93±0.89	6.79±0.43
LAB						
Batch 1	3.53±0.50	3.31±0.27	4.00±0.62	4.33±0.37	6.86±0.07	6.79±0.10
Batch 2	4.28±0.49	5.63±0.35	7.70±1.15	8.66±0.16	7.94±2.55	5.07±0.68
Batch 3	4.46±0.28	5.19±0.48	6.98±0.98	7.83±0.43	7.46±0.56	8.13±0.51
CNC						
Batch 1	4.10±0.17	4.10±0.17	4.40±0.62	4.33±0.37	3.73±0.23	3.27±0.55
Batch 2	3.07±0.50	2.67±0.65	5.06±0.39	3.43±0.51	3.18±0.59	2.10±0.17
Batch 3	4.17±0.13	3.33±0.58	4.09±0.83	3.03±0.06	3.27±0.37	4.42±0.39

*Each number is the mean of three sausage samples taken from the same batch
LAB=lactic acid bacteria
CNC=coagulase-negative cocci
SD=standard deviation

bably due to the meat and pork back fat used, which had counts of 3 log CFU/g and higher, as well as to the spices and natural casings. Pathogenic staphylococci detected in the meat (<2 log CFU/g) were absent on day 7 of fermentation. The numbers of aerobic sporeformers were low (<2 log CFU/g in the sugar and in the spices). *Pseudomonas* spp. was found in meat, but it was not detected in the sausages. Enterococci and sulphite-reducing clostridia were not found in the raw material or in the sausages. All samples were free of *Salmonella* and *Listeria* spp.

In the Hungarian sausages, the number of enterococci increased during fermentation and reached relatively high counts at the end of ripening (4–6 log CFU/g). The population of enterobacterium *E. coli* decreased continuously during fermentation and was eliminated at the end of ripening, so was the population of yeasts. *Pseudomonas* spp., *Salmonella* spp., *Listeria* spp., pathogenic staphylococci and sulphite-reducing clostridia were not detected.

As regards the Italian Friuli sausages, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* spp. were always absent from the raw meat. Faecal enterococci and enterobacteria present at the beginning decreased during ripening. The number of yeasts and moulds decreased and in some cases were not detectable. Neither *Pseudomonas* spp. was present after 3 days of fermentation.

In Sremska sausages, enterococci were detected in all three batches. Yeasts were present in all three batches until day 7. Yeasts most probably originated from paprika and minced garlic. All samples were free of pathogenic staphylococci and *Salmonella* spp.

The most important fact is that *L. monocytogenes*, *Salmonella* spp. and *S. aureus* were not found in the finished product. The number of other microorganisms, if present in the raw material or sausage stuffing, decreased during the course of fermentation.

The results of biochemical identification of LAB are presented in Table 5. A total of 150 strains were isolated from the MRS agar plates. The isolates having thick, short, straight or curved rod- or coccoid rod-shaped cells were regarded as belonging to the genus *Lactobacillus*. The results of identification of LAB from sausages indicated that lactobacilli were by far the most prevalent microorganisms isolated from MRS agar plates.

The results of identification of LAB from Greek traditional sausages (Table 5) showed that the majority of

the strains isolated from the batches belonged to the species *Lactobacillus plantarum* biotype 1 (43.3 %). The same species was found in sudzuk (40.7 %) and in Croatian traditional sausages (34.0 %). In Hungarian traditional sausages the most frequently isolated strain was *Lactobacillus sakei* (28.7 %), while *Lactococcus lactis* was the main species found in Italian sausages. *Lactobacillus fermentum* was the predominant microorganism (24.0 %) in Sremska sausages.

The results of investigation are very close to the data reported by other authors, who also pointed out that lactic acid bacteria constitute a significant part of the microflora in pork in which *Lactobacillus plantarum, L. brevis and L. viridescens* prevail. *Leuconostoc* spp. and *Pediococcus* spp. can also be isolated from sausages (*11*). Results could be compared with the results of the authors who isolated lactobacilli (90 %), enterococci (4 %), and *Pediococcus* spp. (3 %) from dry, naturally fermented sausages, and also found sporadic isolates of *Weissella viridescens*, *Leuconostoc pseudomesenteroides*, and *Leuconostoc* sp. (*12*). In the Turkish-style dry fermented sausages the dominant strains were *Lactobacillus sakei*, *L. curvatus* and *L. plantarum*, as well as *L. alimentarius* and *L. brevis* (*13*). The presence of *L. plantarum*, *L. curvatus* and *L. sakei* as well as of *Pediococcus* spp. and *Leuconostoc* spp. in fermented sausages has been reported (*1–4,6,14*). Lactobacilli (*L. rhamnosus* LC-705 and *L. plantarum* E-98098) as well as *Pediococcus pentosaceus* E-98098 are considered to

Table 5. Biochemical characterisation of lactic acid bacteria isolated from naturally fermented sausages

No. of isolates at each stage of process (days)*						No. of isolates (%)	Identification (API 50 CHL)
0	2	4	7	14	28		
Greece							
13	5	3	1	19	24	65 (43.3)	*L. plantarum* biotype 1
6	2	1	3	3	1	16 (10.7)	*L. curvatus*
2	3	2	1	4	4	16 (10.7)	*L. pentosus*
0	0	2	0	2	1	5 (3.3)	*L. rhamnosus*
1	2	1	2	0	0	6 (4)	*L. sakei*
1	0	0	1	0	0	2 (1.3)	*L. paracasei* ssp. *paracasei* biotype 1
1	0	0	0	0	0	1 (0.7)	*L. salivarius*
1	3	1	1	2	5	13 (8.7)	*L. brevis* biotype 1
3	1	1	1	2	0	8 (5.3)	*Ln. mesenteroides* ssp. *mesenteroides* biotype 2
1	0	0	0	0	0	1 (0.7)	*Ln. mesenteroides* ssp. *mesenteroides* biotype 1
4	2	0	0	0	0	6 (4)	*Ln. lactis*
5	1	0	1	2	1	10 (6.7)	*Lc. lactis* ssp. *lactis* biotype 1
1	0	0	0	0	0	1 (0.7)	*E. faecium*
Bosnia and Herzegovina							
14	8	4	3	18	14	61 (40.7)	*L. plantarum* biotype 1
3	2	1	1	10	10	27 (18)	*L. pentosus*
9	3	2	1	4	6	25 (16.7)	*L. curvatus*
1	1	0	2	4	5	13 (8.7)	*L. sakei*
8	2	1	0	0	0	11 (7.3)	*L. brevis* biotype 1
6	1	0	0	0	0	7 (4.7)	*P. pentosaceus* biotype 2
2	1	0	2	0	0	5 (3.3)	*Ln. lactis*
0	0	1	0	0	0	1 (0.6)	*L. salivarius*
Croatia							
2	4	5	9	11	20	51 (34.0)	*L. plantarum* biotype 1
0	4	6	5	3	9	27 (18.0)	*L. curvatus*
0	0	1	2	2	5	10 (6.7)	*L. pentosus*
2	1	2	0	1	2	8 (5.3)	*L. plantarum* biotype 2
0	1	0	2	1	2	6 (4.0)	*L. fermentum*
4	3	12	4	4	4	31 (20.7)	*L. brevis* biotype 1
1	0	2	3	2	0	8 (5.3)	*Ln. mesenteroides* ssp. *mesenteroides* biotype 2
2	1	0	0	0	0	3 (2.0)	*Lc. lactis* ssp. *lactis*
0	0	0	0	1	0	1 (0.6)	*Ln. mesenteroides* ssp. *mesenteroides* biotype 1
0	1	0	0	4	0	5 (3.3)	*Pediococcus pentosaceus*

Table 5. – continued

No. of isolates at each stage of process (days)*						No. of isolates (%)	Identification (API 50 CHL)
0	2	4	7	14	28		
Hungary							
4	5	0	3	12	7	43 (28.7)	*L. sakei*
0	1	1	0	0	3	5 (3.4)	*L. plantarum*
0	0	1	1	1	2	5 (3.4)	*L. curvatus*
1	0	1	2	0	0	5 (3.4)	*L. delbrueckii*
0	0	2	1	1	2	5 (3.4)	*L. alimentarius*
0	0	1	1	1	0	4 (2.7)	*L. amylophilus*
0	0	0	0	0	3	3 (2.0)	*L. bavaricus*
0	1	0	0	0	0	1 (0.7)	*L. salivarius*
0	0	0	0	0	0	1 (0.7)	*L. acidophilus*
1	0	0	0	0	0	1 (0.7)	*L. maltoromicus*
0	1	0	0	0	0	1 (0.7)	*L. yamanashiensis*
8	4	1	2	4	4	25 (16.7)	*Lc. mesenteroides mesenteroides*
2	2	3	0	0	0	7 (4.7)	*Lc. mesenteroides dextranicum*
0	1	0	2	0	4	7 (4.7)	*L. sanfrancisco*
2	0	1	0	0	0	3 (2.0)	*W. viridescens*
1	0	1	0	0	0	2 (1.4)	*L. cofosus*
1	0	0	0	0	0	1 (0.7)	*L. halotolerans*
0	0	1	0	0	0	1 (0.7)	*L. fructivorans*
0	1	0	0	0	0	1 (0.7)	*Ln. citreum*
0	0	0	0	0	0	1 (0.7)	*Ln. eonos*
1	5	4	3	4	3	26 (17.4)	Not identified
Italy							
0	0	0	6	3	12	21 (14.0)	*L. fermentum*
1	3	0	2	3	3	12 (8.0)	*L. curvatus*
7	0	0	0	2	0	9 (6.0)	*L. brevis*
3	8	1	0	1	4	17 (11.3)	*L. plantarum*
0	0	0	0	0	2	2 (1.7)	*Lc. lactis*
3	6	3	7	16	4	39 (26.0)	*Lc. lactis* spp. *lactis*
0	1	0	0	0	0	1 (0.7)	*L. cellobiosus*
0	0	0	1	0	0	1 (0.7)	*L. acidophilus*
2	1	0	1	1	1	6 (4.0)	*L. paracasei*
1	0	1	0	0	5	7 (4.7)	*Lc. mesenteroides*
0	1	0	2	0	0	3 (2.0)	*Lc. mesenteroides/dextrinus*
0	0	1	0	2	1	4 (2.7)	*P. pentosaceus*
0	0	0	0	0	1	1 (0.7)	*P. acidilactici*
Serbia							
5	4	6	9	9	3	36 (24)	*L. fermentum*
3	5	3	0	0	0	11 (7.3)	*L. curvatus*
4	5	5	0	0	0	14 (9.3)	*L. brevis*
9	0	0	0	0	0	9 (6)	*L. plantarum*
4	2	4	2	2	0	14 (9.3)	*L. delbrueckii* ssp. *delbrueckii*
2	0	0	0	0	2	4 (2.6)	*L. delbrueckii* ssp. *bulgaricus*
3	3	1	1	2	0	10 (6.6)	*Lc. lactis* ssp. *lactis*
0	0	0	0	4	3	7 (4.6)	*L. cellobiosus*
0	0	1	2	2	2	7 (4.6)	*L. collinoides*
1	0	0	0	0	0	1 (0.6)	*L. acidophilus*
1	0	0	0	0	0	1 (0.6)	*L. paracasei* ssp. *paracasei*
1	1	1	0	1	0	4 (2.6)	*Ln. mesenteroides* ssp. *cremoris*
0	2	6	6	3	2	19 (12.6)	*Ln. mesenteroides* ssp. *mesenteroides*
3	0	3	2	1	1	10 (6.6)	*E. faecalis*
3	0	0	0	0	0	3 (2)	*E. faecium*

*All batches (150 isolates)

have the ability to act as the main fermenting organisms in the manufacturing process of dry sausages (*15*). In the fermentation process of dry sausages, their count increased from 6.5–7.0 to 8.0–9.0 log CFU/g. It was also noticed that the presence of these probiotic or protective strains, as major organisms in the sausage after the fermentation and ripening, is in connection with flavour profiles, similar to that produced by the commercial meat starter culture and commercial dry sausage recipe (*15*). A total of 516 strains of lactic acid bacteria were isolated from chorizo made in Castilla-Léon, Spain (*16*). Among the isolated strains, 68.8 % were *Lactobacillus sakei*, 16.5 % were *L. curvatus*, and 6.2 % belonged to the genus *Pediococcus*.

From the naturally fermented sausages, CNC were also isolated (Table 6). *Staphylococcus saprophyticus* was the predominant species in Greek sausages (34.7 %), in Bosnian sudzuk (30.7 %), and in the Sremska sausage (21.2 %). *Staphylococcus xylosus* was the main species in Croatian traditional sausages (29.2 %), in Hungarian fermented sausages (43.0 %) as well as in Friuli sausages (74.0 %).

Other authors also isolated the majority of the strains isolated in our investigation from dry fermented sausages. It was found that among staphylococci, *S. saprophyticus* strains dominated the microflora of traditional Greek dry salami, followed by *S. xylosus* (*6*). Isolated strains were also identified as *S. saprophyticus*, *S. xylosus* and *Staphylococcus carnosus* (*8*). According to the API® Staph system, *Staphylococcus caprae*, *S. capitis*, *S. aureus/intermedius*, *S. sciuri* and *S. hominis* were also identified, in addition to sporadic isolations of *S. auricularis*, *S. warneri*, *S. cohnii* ssp. *cohnii*, *S. cohnii* ssp. *urealyticum* and *S. epidermidis*. Similar isolations from dry fermented sausages were also reported (*8,17,18*). *S. xylosus* was pointed out as the predominant species found in most dry fermented sausages in some investigations (*7,19*). The strains of this species are used as starter cultures, because of their contribution to aroma and taste formation (*20*).

Table 6. Biochemical characterisation of staphylococci isolated from naturally fermented sausages

No. of isolates at each stage of process (days)						No. of isolates (%)	Identification (API® Staph)
0	2	4	7	14	28		
Greece							
3	5	8	11	14	11	52 (34.7)	*S. saprophyticus*
2	2	4	3	8	3	22 (14.7)	*S. xylosus*
0	2	5	4	5	1	17 (11.3)	*S. simulans*
9	5	1	2	0	0	17 (11.3)	*S. haemolyticus*
0	0	0	6	2	4	12 (8)	*S. caprae*
5	3	1	0	0	0	9 (6)	*S. capitis*
2	1	4	1	0	0	8 (5.3)	*S. aureus/intermedius*
2	2	1	0	0	0	5 (3.3)	*S. sciuri*
1	0	0	1	0	1	3 (2)	*S. hominis*
0	0	0	1	0	0	1 (0.7)	*S. auricularis*
0	0	0	1	0	0	1 (0.7)	*S. warneri*
0	1	0	0	0	0	1 (0.7)	*S. cohnii* ssp. *cohnii*
0	0	0	0	0	1	1 (0.7)	*S. cohnii* ssp. *urealyticum*
0	0	0	0	1	0	1 (0.7)	*S. epidermidis*
Bosnia and Herzegovina							
4	6	5	12	8	11	46 (30.7)	*S. saprophyticus*
0	0	4	7	10	12	33 (22)	*S. simulans*
10	6	7	1	0	0	24 (16)	*S. xylosus*
3	3	2	2	3	3	16 (10.6)	*S. epidermidis*
0	0	0	4	6	6	16 (10.6)	*S. caprae*
2	1	4	2	0	0	9 (6)	*S. capitis*
1	2	0	0	1	0	4 (2.7)	*S. aureus/intermedius*
0	0	1	0	0	0	1 (0.7)	*S. auricularis*
0	0	0	0	0	1	1 (0.7)	*S. sciuri*
Croatia							
0	0	0	0	10	11	21 (29.2)	*S. xylosus*
0	0	0	0	8	10	18 (25.0)	*S. capitis*
0	0	0	0	9	9	18 (25.0)	*S. carnosus*
0	0	0	0	7	8	15 (20.8)	*S. saprophyticus*

Table 6. – continued

No. of isolates at each stage of process (days)						No. of isolates (%)	Identification (API® Staph)
0	2	4	7	14	28		
Hungary							
4	5	8	10	5	4	40 (43.0)	*S. xylosus*
0	2	1	2	5	0	15 (16.0)	*Micrococcus* spp.
5	4	3	1	1	0	24 (16.0)	*S. hominis*
0	2	0	0	1	2	9 (10.0)	*S. lentus*
5	1	0	0	0	0	6 (6.0)	*S. warneri*
0	1	3	0	0	0	4 (4.0)	*S. capitis*
0	1	0	1	0	0	2 (2.0)	*S. epidermidis*
1	0	0	0	0	0	1 (1.0)	*S. haemolyticus*
1	0	0	0	0	0	1 (1.0)	*S. auricularis*
0	0	1	0	0	0	1 (1.0)	*S. saprophyticus*
0	0	0	0	1	0	1 (1.0)	*S. cohnii*
Italy							
2	3	0	0	0	0	5 (3.3)	*S. saprophyticus*
1	0	0	0	0	0	1 (0.7)	*S. saprophyticus/simulans*
3	1	0	0	2	3	10 (6.7)	*S. hominis*
2	2	2	0	0	0	6 (4.0)	*S. hominis/warneri*
3	2	0	0	2	4	11 (7.3)	*S. warneri*
5	11	17	17	27	34	111 (74.0)	*S. xylosus*
0	0	1	1	0	0	2 (1.7)	*S. epidermidis*
0	0	0	0	1	0	1 (0.7)	*S. simulans*
3	2	1	0	2	3	11 (7.3)	*S. warneri*
0	0	0	0	0	3	3 (2.0)	*S. lentus*
Serbia							
8	5	5	1	0	0	19 (21.1)	*S. saprophyticus*
3	2	2	3	0	1	11 (12.2)	*S. auricularis*
4	2	4	5	0	0	15 (16.6)	*S. xylosus* biotype 1
0	0	4	0	0	0	4 (4.4)	*S. xylosus* biotype 2
2	3	4	0	1	1	11 (12.2)	*S. capitis*
0	1	2	1	0	0	4 (4.4)	*S. hominis* biotype 1
4	4	3	2	0	0	13 (14.4)	*S. simulans* biotype 1
2	0	0	0	0	4	6 (6.7)	*S. warneri*
0	0	0	0	1	0	1 (1.1)	*S. cohnii*
0	1	3	2	0	0	6 (6.7)	*S. aureus*

Conclusions

From the hygienic standpoint, it is highly important that *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* were not found in the finished products. The number of other microorganisms, if present in the raw material or sausage stuffing, decreased in the course of fermentation. Enterobacteria, *Escherichia coli* and *Pseudomonas*, when present, were progressively eliminated, regardless of their initial count. In certain cases, a stable level of enterococci was detected in sausages (Greece, Bosnia and Hungary). During all stages of investigation, LAB and CNC prevailed. These microorganisms are responsible for the formation of favourable organoleptic properties during maturation of dry sausages. Among the population of lactobacilli the strains of *L. sakei* and *L. curvatus* prevailed. Most often, the isolated CNC belonged to the genera *Staphylococcus xylosus* and *Staphylococcus saprophyticus.*

API biochemical identification was used for identification of isolates. Sugar fermentation profiles obtained by API 50 CHL ranged from excellent to good identification (ID>90-95 %). Several acceptable, doubtful or low discrimination profiles (ID<80–85 %) were obtained after reading the strips. The identification was performed on the basis of additional tests requested by API 50 CHL. It should be pointed out that API® Staph gave, in general, very good results (ID>90–95 %), in some cases >99 % and only in few cases the strains were not identified.

Acknowledgement

This research was carried out under the umbrella of the SAFETYSAUSAGE project, which is funded by the EC within the framework of the INCO-DEV program (Contract No ICA4-CT-2002-10037).

References

1. F.A. Schved, A. Lalazar, Y. Henis, B.J. Juven, Purification, partial characterisation and plasmid-linkage of pediocin SJ-1 bacteriocins produced by *Pediococcus acidilactici, J. Appl. Microbiol. 74* (1993) 67–77.
2. R.F. Vogel, M. Lohmann, M. Nguyen, A.N. Weller, W.P. Hammes, Molecular characterization of *Lactobacillus curvatus* and *Lact. sake* isolated from sauerkraut and their application in sausage fermentation, *J. Appl. Microbiol. 74* (1993) 295–300.
3. E. Parente, S. Grieco, M.A. Crudele, Phenotypic diversity of lactic acid bacteria isolated from fermented sausages produced in Basilicata (Southern Italy), *J. Appl. Microbiol. 90* (2001) 943–952.
4. T. Aymerich, B. Martin, M. Garriga, M. Hugas, Microbial quality and direct PCR identification of lactic acid bacteria and non-pathogenic staphylococci from artisan low-acid sausages, *Appl. Environ. Microbiol. 69* (2003) 4583–4594.
5. J. Samelis, J. Metaxopoulos, The microbiology of traditional Greek country-style sausage during manufacture followed by storage at 3° and 12°C in air, *Ital. J. Food Sci. 10* (1998) 155–163.
6. J. Samelis, J. Metaxopoulos, M. Vlassi, A. Pappa, Stability and safety of traditional Greek salami – A microbiological ecology study, *Int. J. Food Microbiol. 44* (1998) 69–82.
7. M. Garcia-Varona, E.M. Santos, I. Jaime, J. Rovira, Characterisation of *Micrococcaceae* isolated from different varieties of chorizo, *Int. J. Food Microbiol. 54* (2000) 189–195.
8. E. Papamanoli, P. Kotzekidou, N. Tzanetakis, E. Liopoulou-Tzanetaki, Characterization of *Micrococcaceae* isolated from dry fermented sausages, *Food Microbiol. 19* (2002) 441–449.
9. J. Gasparik-Reichardt, S.Z. Toth, L. Cocolin, G. Comi, E.H. Drosinos, Ž. Cvrtila, L. Kozačinski, A. Smajlović, S. Saičić, B. Borović, Technological, physicochemical and microbiological characteristics of traditionally fermented sausages in Mediterranean and central European countries, *Tehnologija mesa* (*Meat Technology*), *46* (2005) 143–153.
10. Official Methods of Analysis of AOAC, AOAC, Washington, DC, USA (2002).
11. S. Duraković, F. Delaš, L. Duraković: *Moderna mikrobiologija namirnica* (*Modern Microbiology of Foodstuff*), Kugler, Zagreb, Croatia (2002).
12. E. Papamanoli, N. Tzanetakis, E. Litopolou-Tzanetaki, P. Kotzekidou, Characterisation of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties, *Meat Sci. 65* (2003) 859–867.
13. G.C. Gürakan, T.F. Bozogu, N. Weiss, Identification of *Lactobacillus* strains from Turkish-style dry fermented sausages, *Lebensm. Wiss. Technol. 28* (1995) 139–144.
14. J. Samelis, F. Maurogenakis, J. Metaxopoulos, Characterisation of lactic acid bacteria isolated from naturally fermented Greek dry salami, *Int. J. Food Microbiol. 44* (1994) 69–82.
15. S. Erkkila, E. Petaja, S. Eerola, L. Lilleberg, T. Mattila-Sandholm, M.L. Suihko, Flavour profiles of dry sausages fermented by selected novel meat starter cultures, *Meat Sci. 58* (2001) 111–116.
16. E.M. Santos, C. Gonzales-Fernandez, I. Jaime, J. Rovira, Identification and characterization of lactic acid bacteria isolated from traditional chorizo made in Castilla-Léon, *Food Sci. Technol. Int. 3* (1997) 21-29.
17. M.S. Seager, J.G. Banks, C.W. de Blackburn, R.G. Board, A taxonomic study of *Staphylococcus* spp. isolated from fermented sausages, *J. Food Sci. 51* (1986) 295-297.
18. P. Kotzekidou, Identification of staphylococci and micrococci isolated from an intermediate moisture meat product, *J. Food Sci. 57* (1992) 249–251.
19. R. Coppola, M. Iorizzo, R. Saotta, E. Sorrentino, L. Grazia, Characterisation of micrococci and staphylococci isolated from sopressata molisana, a Southern Italy fermented sausages, *Fleischwirtschaft, 72* (1997) 1679–1683.
20. W.P. Hammes, C. Hertel, New developments in meat starter cultures, *Meat Sci. 36* (1998) 155–168.