

## Effects of Disodium Dihydrogen Pyrophosphate and Sodium Tripolyphosphate on the Microbiological Quality and *Enterobacteriaceae* Species During Fermentation Period of Turkish Fermented Dry Sausage (Sucuk)

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**Abstract:** The effects of disodium dihydrogen pyrophosphate and sodium tripolyphosphate were investigated during ripening period of sausage. For this purpose, fermented dry sausages (sucuk) containing 0.3% disodium dihydrogen pyrophosphate (Group A), 0.3% sodium tripolyphosphate (Group B) and control group were manufactured. It was not determined any important effects of disodium dihydrogen pyrophosphate and sodium tripolyphosphate application on the pH value, Enterobacteriaceae, coagulase positive staphylococci and lactobacilli. On the other hand, the total aerobic mesophilic counts increased rapidly in Group A and B and found important ( $p < 0.05$ ) at the second day of fermentation. There was important effect of disodium dihydrogen pyrophosphate application ( $p < 0.05$ ) on the Micrococcus/Staphylococcus at the first day of fermentation. *Enterobacter sakazakii* and *Enterobacter cloacae* were found dominant species in each group and 48 of the 96 isolates was identified as *E. sakazakii*. Isolation rates of *E. sakazakii* in control group, Group A and B were determined as 39.39, 48.73 and 61.76%, respectively. In conclusion, it could be said that any important effects of disodium dihydrogen pyrophosphate and sodium tripolyphosphate on the microbiological quality of sucuk have not been determined.

**Key words:** Sucuk, sausage, disodium dihydrogen pyrophosphate, sodium tripolyphosphate, microbiological quality, *Enterobacteriaceae* sp.

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### INTRODUCTION

Turkish fermented dry sausage which is called sucuk is produced by either natural microflora or the addition of a starter culture (Ockerman and Gokalp, 1987; Soyer *et al.*, 2005). Sausage dough is filled into either natural or artificial casings and then fermented and dried. The safety of fermented sausage is based on different factors including added salt, antimicrobial metabolites such as bacteriocins, lactic acid produced by Lactic Acid Bacteria (LAB), additives, i.e., nitrate and/or nitrite, low pH and water activity ( $a_w$ ) which develop during fermentation and drying (Acton and Keller, 1974; Tekinsen and Dogruer, 2000). However, from a food processing point of view, major risk enhancing factors in fermented foods are the use of contaminated raw materials, lack of pasteurization and poorly controlled natural fermentations. Also sub-optimal fermentation starters, inadequate storage and maturation conditions as well as consumption without prior cooking may reduce the safety of fermented foods (Nout, 1994).

Sausage dough could be contaminated by different microorganisms during production. Food pathogens such as Salmonella, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* 0157:H7 surviving fermentation-drying period are a major concern for the safety of sausage. The Enterobacteriaceae population indicates the hygienic quality of the product. The health interest of Enterobacteriaceae is based on the possibility that various members of the Enterobacteriaceae family such as Salmonella, Yersinia, Shigella and Escherichia could be enteropathogenic due to their capacity to produce enterotoxins that cause important gastrointestinal alterations (Tornadijo *et al.*, 2001).

To obtain high quality characteristics in fermented sausages, several studies have been done. For this purpose starter cultures and some chemical additives especially nitrate and nitrite have been used (Gurakan *et al.*, 1995; Bozkurt and Erkmén, 2002; Oz *et al.*, 2006). Use of mixed lactic cultures of *Lactobacillus plantarum*, *L. casei* and *Lactococcus lactis* caused substantial reduction in coliforms, *E. coli*, *S. aureus* and

*Salmonella* sp. (Sakhare and Narasimha Rao, 2003). In fermented sausage, it was evident from the early stages of the processing, the use of nitrite in sausage processing was found to inhibit the undesirable flora and to reduce hygienic risk (Sanz *et al.*, 1997a) but sausage without additives had the highest level of mould and yeast counts (Bozkurt and Erkmén, 2007). On the other hand, different nitrite doses (100, 150 and 200 ppm NaNO<sub>2</sub>) and starter culture (*L. plantarum* + *Staphylococcus carnosus*) on *E. coli* O157:H7 were found insignificant (Oz *et al.*, 2006). Even though the reduction of nitrate to nitrite is important because of the inhibitory effect of nitrite on the population of Enterobacteriaceae, nitrite could also influence the development of lactic acid bacteria (Castano *et al.*, 2002) and also, N-nitrosamines formed by chemical reaction between nitrite and amines during fermentation and storage are considered important health problem (Byun *et al.*, 2004; Rywotycki, 2007).

Multiple forms of polyphosphates are widely used throughout the meat, poultry and seafood industries to improve binding, water-holding capacity and yield and to retard oxidative rancidity of meat products (Knipe *et al.*, 1985; Lin and Chuang, 2001; Kamal *et al.*, 2005). Although, they are not considered primary antimicrobial agent, there are several studies that have reported antimicrobial effect of phosphates in meat (Sebranek, 2009). Kim and Marshlall (1999) have reported that Aerobic Plate Count (APC) of chicken legs dipped 10 min in either 5% mono potassium phosphate, sodium pyrophosphate and Trisodium Phosphate (TSP) were significantly lower than control group. Rinsing with 10% TSP solution reduced the numbers of *Salmonella typhimurium* and *E. coli* O157:H7 (Kim and Slavik, 1994) and also trisodium phosphate was found effective against *Listeria monocytogenes* in chicken meat, especially after several days of refrigerated storage (Capita *et al.*, 2001). There are few researches determining antimicrobial effect of phosphates in meat products. In a study, the effects of 0.5% sodium acid pyrophosphate, sodium tripolyphosphate, tetrasodium pyrophosphate and sodium polyphosphate glassy on aerobic mesophilic and psychrotrophic bacterial growth and on survival of inoculated *S. aureus* were investigated in uncooked bratwurst (Molins *et al.*, 2006). In another study, addition of starter culture and high concentration of additives (nitrite, nitrate,  $\alpha$ -tocopherol, potassium sorbate, potassium pyrophosphate and di-potassium hydrogenphosphate) were determined on the quality of Turkish style sausage (Bozkurt and Erkmén, 2007).

The objective of this study was to evaluate the effects of disodium dihydrogen pyrophosphate and sodium tripolyphosphate on the microbiological quality and Enterobacteriaceae species of Turkish dry fermented sausage during fermentation.

## MATERIALS AND METHODS

**Materials:** The beef meat and sheep tail fat were obtained from a local butcher shop and other ingredients were purchased from local markets. Sodium nitrate (NaNO<sub>3</sub>), sodium nitrite (NaNO<sub>2</sub>), disodium dihydrogen pyrophosphate (Na<sub>2</sub>H<sub>2</sub>P<sub>2</sub>O<sub>7</sub>) and sodium tripolyphosphate (Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>) were obtained from Merck (Darmstadt, Germany).

**Sausage production and sampling:** Three batches of sucuk were produced using a standard sausage formulation according to Tekinsen and Dogruer (2000). Two trials were performed at a week interval. Sucuk dough contained 80% lean meat and 20% fat and the following ingredients were added based on the weight of meat and fat: 2% NaCl, 1.8% cumin, 1.8% garlic, 1% hot red pepper, 0.75% sweet red pepper, 0.4% black pepper, 0.35% allspice, 0.3% sugar (saccharose), 0.03% ascorbic acid, 0.03% NaNO<sub>3</sub> and 0.015% NaNO<sub>2</sub>.

Afterwards the mixing, the dough was placed overnight in the refrigerator at 4°C and then was minced through a plate with 3 mm hole diameter using medium scale grinder (Model No:32, OMT Machine Ltd. Konya/Turkey). Homogenous mixture added with ascorbic acid was separated to three groups. One group was used as control. In other two groups, 0.3% disodium dihydrogen pyrophosphate (Group A) and 0.3% sodium tripolyphosphate (Group B) which were dissolved in 25 mL of distilled water were added, respectively. Afterwards, sausage dough was stuffed into 38-40 mm diameter natural casing produced from cow intestine using a sausage filling machine (Patron Model, MADO, NORMECH, UK).

Sausages were fermented and dried for 7 days in the fermentation room under ambient conditions which are gradually changed (Table 1). Samples of the sausage were taken immediately after stuffing into casing accepted 1st day of fermentation and 2nd, 4th and 7th days of fermentation from each batch.

**Microbiological analysis:** After removing the sausage casing, a 10 g aliquot from each sample was aseptically weighed and diluted in 90 mL of 1/4-strength Ringer solution (Oxoid, BR0052) and homogenised in a Colworth Stomacher Lab-Blender 400 (Seward Medical, London, UK) for at least 2 min. Tenfold dilutions prepared

Table 1: Environmental condition of fermentation room

Environmental condition	Fermentation time (days)			
	1	2	4	7
Temperature	22±1.0	22±1.0	20±1.0	19±1.0
Relative humidity	90.0	87.0	81.0	72.0
Air current (m sec <sup>-1</sup> )	0.8	0.8	0.4	0.1

from the initial 1/10 dilution in 1/4-strength Ringer solution were plated in duplicate on the specific media required for the different microbial groups to be examined (Harrigan, 1998).

Total aerobic mesophilic microorganism were enumerated on standard plate count agar (Oxoid, CM325) after incubation at 30°C for 72 h. Enterobacteriaceae was determined on violet red bile glucose agar (Oxoid, CM0485) after incubation at 37°C for 24 h. Micrococcus/Staphylococcus were counted on Baird Parker agar (Oxoid, CM0961) supplemented with egg yolk tellurite emulsion (Oxoid, SR0054) at 37°C for 48 h and coagulase positive staphylococci were confirmed by a positive coagulase test. For this purpose, up to five typical colony (black or grey colonies) grown on Baird Parker agar were selected and transferred to tubes contained brain heart broth (Merck, 1.10493). The tubes were incubated at 37°C for 24 h. After incubation, coagulase test was applied by using rabbit plasma with EDTA (Merck, 1.13306). Lactobacilli were determined on Rogosa agar (Oxoid, CM 0627) adjusted to pH 5.5 with acetic acid and incubated at 30°C for 5 days.

**Isolation and identification of Enterobacteriaceae strains:**

Up to five colonies were randomly taken from violet red bile glucose agar plates, representing each batch and time of sampling. A total of 108 strains including 36 strains from control group, 36 strains from Group A and 36 strains from group B were purified using alternate subcultures on tryptone soya broth (Merck, 1.0459) and tryptone soya agar (Merck, 1.05458).

Total 96 isolates determined as gram negative, catalase-positive, oxidase-negative short rods were identified with the aid of the API™ 20E System (BioMerieux, Marcy-l’Etoile, France). The following tests were carried out on each isolate: motility; growth and growth characteristics on McConkey agar; methyl red reactions; indole, H<sub>2</sub>S and acetoin production (Voges-Proskauer), NO<sub>3</sub><sup>-</sup> reduction to NO<sub>2</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> reduction to N<sub>2</sub> gas; malonate, gluconate and citrate utilization; gas production from glucose and lactose; fermentation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, L-arabinose; presence of urease, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, gelatinase, α- galactosidase and tryptophane deaminase (Erkmen, 2007).

**Determination of pH:** The pH values of meat and sucuk were measured by pH meter (InoLab pH 720 model; WTW GmbH, Hamburg, Germany).

**Statistical analysis:** A one way analysis of variance was performed on data after a log transformation for bacterial counts, using the SPSS/PC Version 10.00 (SPSS Inc, Chicago, IL, USA). Differences among the groups were identified using Duncan’s Multiple Range test.

**RESULTS AND DISCUSSION**

This study was performed to evaluate the effects of disodium dihydrogen pyrophosphate and sodium tripolyphosphate on the microbiological quality and *Enterobacteriaceae* species during the fermentation period of Turkish fermented dry sausage. Microbiological characteristics and pH value of meat used in sausage preparation are shown in Table 2 and changes in microbiological counts and *Enterobacteriaceae* species of sausage samples are shown in Table 3 and 4. The pH values of each group are shown in Fig. 1.

It was not determined any important effects of disodium dihydrogen pyrophosphate and sodium tripolyphosphate application on the pH value, Enterobacteriaceae, coagulase positive staphylococci and lactobacilli. On the other hand, the Total Aerobic Mesophilic Microorganism (TAMM) counts increased rapidly in Group A and B and found important (p<0.05) at the 2nd day of fermentation. There was important effect of disodium dihydrogen pyrophosphate application (p<0.05) on the Micrococcus/Staphylococcus at the 1st day of fermentation.

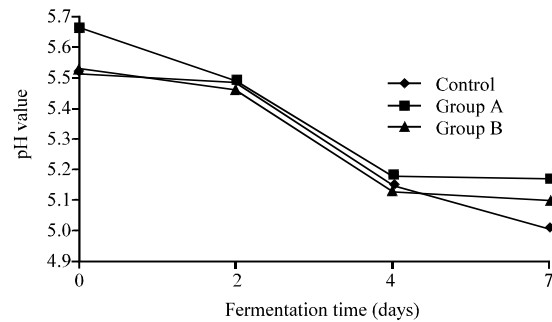


Fig. 1: Changes in pH values during ripening of sausages

Table 2: Microbiological characteristics (log<sub>10</sub> cfu g<sup>-1</sup>) and pH value of meat used in sausage production

Microorganism	Counts
Total aerobic mesophilic	3.63±0.07
Enterobacteriaceae	NG
Micrococcus/Staphylococcus	3.02±0.02
Coagulase positive staphylococci	2.36±0.06
Lactobacilli	NG
pH	5.66

NG: Not Growth

Table 3: Changes in microbiological characteristics of sausage samples during fermentation (log<sub>10</sub> cfu g<sup>-1</sup>)

Microorganism	Groups	Fermentation time (days)				p-value
		1	2	4	7	
Total aerobic mesophilic	Control	5.86±0.15 <sup>b</sup>	7.72±0.08 <sup>bb</sup>	8.41±0.41 <sup>a</sup>	8.15±0.40 <sup>a</sup>	*
	A (DSDHPP)	6.16±0.31 <sup>b</sup>	8.03±0.01 <sup>aa</sup>	8.30±0.09 <sup>a</sup>	8.21±0.03 <sup>a</sup>	**
	B (STPP)	5.83±0.35 <sup>b</sup>	7.84±0.02 <sup>aaB</sup>	8.16±0.13 <sup>a</sup>	7.96±0.04 <sup>a</sup>	**
	p-value	NS	*	NS	NS	
Lactobacilli	Control	<1 <sup>c</sup>	7.27±0.04 <sup>b</sup>	8.00±0.12 <sup>c</sup>	8.02±0.08 <sup>c</sup>	***
	A	1.24±1.24 <sup>c</sup>	7.43±0.04 <sup>b</sup>	8.19±0.04 <sup>a</sup>	7.91±0.20 <sup>b</sup>	**
	B	<1 <sup>c</sup>	7.41±0.07 <sup>b</sup>	8.06±0.10 <sup>a</sup>	7.95±0.13 <sup>a</sup>	***
	p-value	NS	NS	NS	NS	
Micrococcus/Staphylococcus	Control	4.10±0.01 <sup>ba</sup>	6.53±0.07 <sup>a</sup>	6.54±0.77 <sup>a</sup>	6.12±0.42 <sup>a</sup>	*
	A	4.19±0.01 <sup>ba</sup>	6.84±0.05 <sup>a</sup>	6.88±0.41 <sup>a</sup>	6.69±0.38 <sup>a</sup>	**
	B	4.07±0.02 <sup>bb</sup>	6.56±0.07 <sup>a</sup>	6.80±0.46 <sup>a</sup>	6.39±0.09 <sup>a</sup>	**
	p-value	*	NS	NS	NS	
Coagulase positive staphylococci	Control	3.84±0.12 <sup>b</sup>	4.91±0.13 <sup>a</sup>	<1 <sup>c</sup>	<1 <sup>c</sup>	***
	A	3.57±0.13 <sup>b</sup>	5.74±0.26 <sup>a</sup>	<1 <sup>c</sup>	<1 <sup>c</sup>	***
	B	3.37±0.03 <sup>b</sup>	5.48±0.00 <sup>a</sup>	<1 <sup>c</sup>	<1 <sup>c</sup>	***
	p-value	NS	NS	NS	NS	
Enterobacteriaceae	Control	4.31±0.77	3.24±0.76	1.55±1.55	1.50±1.50	NS
	A	4.50±0.30	4.19±1.51	2.09±2.09	1.70±1.70	NS
	B	3.97±0.34	3.84±1.36	2.24±2.24	3.49±0.17	NS
	p-value	NS	NS	NS	NS	

Group A: Disodium dihydrogen pyrophosphate; Group B: Sodium tripolyphosphate; NS: p>0.05; \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; <sup>c</sup>Means within a row followed by different superscripts are significantly different (p<0.05); <sup>a, b</sup>Means within a column followed by different superscripts are significantly different (p<0.05)

Table 4: Distribution of *Enterobacteriaceae* species isolated from sausage samples

Application	Species	Days of fermentation (No. of isolates)				Total		
		1	2	4	7	No. of isolates	Percentage	
Control	<i>Enterobacter sakazakii</i>	5	5	2	1	13	39.39	
	<i>Enterobacter cloacae</i>	1	5	2	1	9	27.27	
	<i>Enterobacter amnigenus</i>	-	-	-	1	1	3.03	
	<i>Klebsiella oxytoca</i>	-	1	-	-	1	3.03	
	<i>Serratia ficaria</i>	2	-	-	-	2	6.06	
	<i>Erwinia</i> sp.	2	-	-	-	2	6.06	
	Uninterpretable	2	2	-	1	5	15.15	
	Disodium dihydrogen pyrophosphate	<i>Enterobacter sakazakii</i>	5	2	4	3	14	48.27
		<i>Enterobacter cloacae</i>	1	3	-	-	4	13.73
		<i>Enterobacter aerogenes</i>	-	3	-	-	3	10.34
<i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i>		1	1	-	-	2	6.89	
<i>Pasteurella pneumotropica</i>		1	-	-	-	1	3.44	
<i>Serratia liquefaciens</i>		-	1	-	-	1	3.44	
<i>Pseudomonas aeruginosa</i>		-	1	-	-	1	3.44	
<i>Raoultella ornithinolytica</i>		-	1	-	-	1	3.44	
<i>Kluyvera</i> sp.		1	-	-	-	1	3.44	
Uninterpretable		1	-	-	-	1	3.44	
Sodium tripolyphosphate	<i>Enterobacter sakazakii</i>	12	3	2	4	21	61.76	
	<i>Enterobacter cloacae</i>	1	2	2	-	5	14.70	
	<i>Stenotrophomonas maltophilia</i>	-	1	-	-	1	2.94	
	<i>Serratia liquefaciens</i>	-	2	-	1	3	8.82	
	Uninterpretable	4	-	-	-	4	11.76	
	Total	39	33	12	12	96	-	

**Changes in the microbial group counts and pH value during ripening:** there was no differences in pH values among the groups in all fermentation period (Fig. 1). In the illumination of this result, it would be said that polyphosphate application does not seem to have a very significant effect on the pH values during fermentation period. As expected, the pH values of the sucuk samples were significantly decreased (p<0.05) after the 2nd day

and varied from 5.17-5.01 in all groups at the end of the fermentation period. This result is in agreement with that of knowledge that the pH generally attained during fermentation is near 5.1 although, a lower pH is desired for some products (Acton and Keller, 1974). After the stuffing, TAMM counts increased rapidly in Group A and B and found important (p<0.05) at the 2nd day of fermentation. Following the 2nd day, increases in TAMM

counts in all groups were similar (Table 3). On the contrary of this finding, Bozkurt and Erkmen (2007) have reported that some commercial additives changed APC of sausage and lowest APC was determined in samples with potassium pyrophosphate and dipotassium hydrogen phosphate. In the present study, TAMM counts in all groups increased from 5.83-6.16-7.96-8.21 log cfu g<sup>-1</sup> at the end of the fermentation period (Table 3). Similar results were stated by some researchers (Aksu and Kaya, 2004; Gonulalan *et al.*, 2004; Soyer *et al.*, 2005). However, low counts in TAMM are reported by some researchers. Bozkurt and Erkmen (2007) stated that the APC count increased from 5.19-6.09 log cfu g<sup>-1</sup> during the 10 days of ripening period. Sanz *et al.* (1997b) reported that total viable count was 7.6×10<sup>6</sup> at the end of the fermentation period.

No important effects of sodium tripolyphosphate and disodium dihydrogen pyrophosphate application on the lactobacilli were seen during fermentation (Table 3). Similarly, Bozkurt and Erkmen (2007) reported that addition of potassium pyrophosphate, dipotassium hydrogen phosphate and potassium sorbate did not decrease the lactobacilli counts in sausage samples. In the present study lactobacilli counts increased from ca. About <1-7.91-8.02 log cfu g<sup>-1</sup> at the end of the fermentation period (Table 3). This result is in agreement with that of Soyer *et al.* (2005) who reported that initial LAB counts of fermented sausages increased from 4.3 log cfu g<sup>-1</sup> to ca. 8 log cfu g<sup>-1</sup> at the end of the ripening period. However, it is in conflict with those reported by others. Bozkurt and Erkmen (2007) stated that the LAB counts increased from 4.62-5.47 log cfu g<sup>-1</sup> during the 10 days of ripening period. Gonulalan *et al.* (2004) reported that LAB count of fermented sausage without starter culture combinations was 5.75 log cfu g<sup>-1</sup> at the end of the ripening period. The above results conflicted with each other might be explained with the widespread concept that various parameters such as salt, nitrite, temperature, pH and a<sub>w</sub> interact to create the desired affect of dry-curing (Messier *et al.*, 1989) and dry-fermented sausages are produced by using different technological processes in different countries (Garriga *et al.*, 1996).

Disodium dihydrogen pyrophosphate application caused an important increase (p<0.05) on the Micrococcus/Staphylococcus counts at the 1st day of fermentation. This result should be evaluated from a widespread concept that the evolution of the desirable flora (lactic acid bacteria and micrococaceae) as well as the pH is important in order to reduce hygienic risks in the production of fermented sausages (Sanz *et al.*, 1997b).

And also, Micrococcus/Staphylococcus counts increased significantly (p<0.05) in all groups at the 2nd

day of fermentation and there were slightly changes in the other days of fermentation (Table 3). Similar results were determined in fermented sausage during the fermentation period by Aksu and Kaya (2004) and Soyer *et al.* (2005). However, this result is in conflict with Gonulalan *et al.* (2004) who reported that Micrococcus/Staphylococcus count of fermented sausages without starter culture combinations was decreased from 3.34-2.86 log cfu g<sup>-1</sup> during the ripening period. In the present study, coagulase positive staphylococci were not isolated in all groups after the 2nd day of fermentation.

According to the 1st and 2nd days of analysis, there was no effect of disodium dihydrogen pyrophosphate and sodium tripolyphosphate application on coagulase positive staphylococci in the sausage samples. The above results could have been derived from single and/or interaction affect of various parameters such as salt, nitrite temperature, a<sub>w</sub> and pH (Messier *et al.*, 1989). On the contrary to these finding and knowledge, it was reported that after inoculation with 1.1×10<sup>3</sup> *S. aureus*, viable *S. aureus* were still found after 74 days of dry-curing (Messier *et al.*, 1989).

There were no differences in Enterobacteriaceae counts in all group during fermentation period (Table 3). This result suggests that polyphosphate application does not seem to have a very significant effect on the Enterobacteriaceae during fermentation period. Even though Enterobacteriaceae was not determined in meat used in the preparation of sausage dough, the Enterobacteriaceae counted in the sausage samples during the fermentation period could have been derived from the working environment, the tools used for the cutting up and mincing of the meat and the handling (Castano *et al.*, 2002).

Enterobacteriaceae count was decreased during ripening period and determined as 1.50-3.49 log cfu g<sup>-1</sup> at the end of the fermentation period. Similarly, Castano *et al.* (2002) reported that the count of Enterobacteriaceae in the sausages manufactured industrially decreased continuously in the 2nd day. The average count of Enterobacteriaceae in fermented sausage samples taken from markets in Turkey was 3.27 log cfu g<sup>-1</sup> (Yaman *et al.*, 1998). Sanz *et al.* (1997a) reported that inhibition exerted by nitrite on the Enterobacteriaceae was evident from the early stage of the processing. On the contrary to the present result, Enterobacteriaceae counts were determined as 3.92, 4.57 and 4.89 log cfu g<sup>-1</sup> at 1st, 3rd and 7th days of ripening (Aksu and Kaya, 2004). Garriga *et al.* (1996) reported that higher content of Enterobacteriaceae throughout the ripening process could be related to the treatment using natural fermentation.

**Enterobacteriaceae species isolated and identified during ripening:**

A total 108 isolates obtained from VRBD agar was subjected to Gram stain, oxidase and catalase tests. Total 96 isolates were determined as gram negative, catalase-positive, oxidase-negative, short rods. According to the identification tests, the distribution of *Enterobacteriaceae* species and the others are shown in Table 4. Total 96 isolates obtained during the fermentation of the sausage samples were identified as *Enterobacter sakazakii* (48), *E. cloacae* (18), *Serratia liquefaciens* (4), *E. aerogenes* (3), *Klebsiella pneumoniae* ssp. *pneumoniae* (2), *Erwinia* sp. (2), *Serratia ficaria* (2), *E. amnigenus* (1), *K. oxytoca* (1), *Pasteurella pneumotropica* (1), *Kluyvera* sp. (1), *Raoultella ornithinolytica* (1), *Stenotrophomonas maltophilia* (1), *Pseudomonas aeruginosa* (1) and ten uninterpretable isolates. According to the results, *E. sakazakii* and *E. cloacae* were found as dominant species in each group. Important pathogens in Enterobacteriaceae such as Salmonella *E. coli*, Yersinia and Shigella have not been detected in this study. Similarly, Warburton *et al.* (1987) reported that neither Salmonella nor Yersinia were found in any of the fermented sausages samples. Tornadijo *et al.* (2001) reported that environmental factors (moisture content, NaCl concentration, water activity, pH and temperature) that limit growth have been described for many organisms, especially the foodborne pathogens. But in the illumination of the present study, it is thought that the results are important because of the fact that 48 of the 96 isolates is *E. sakazakii* considered as a serious foodborne pathogen and also isolation rates of *E. sakazakii* in Group A (48.73%) and Group B (61.76%) are higher than control group (39.39%).

### CONCLUSION

In this study, any important effects of disodium dihydrogen pyrophosphate and sodium tripolyphosphate on the microbiological quality of sausage have not been determined and also higher isolation rate of *E. sakazakii* in Group A (disodium dihydrogen pyrophosphate) and Group B (sodium tripolyphosphate) than control group is to take into consideration. Salt, nitrite, pH,  $a_w$  and lactic acid bacteria have important effect not only on sensory properties of sucuk but also on the control of microbiological quality.

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