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Effects of sodium lactate on the presence of *Staphylococcus aureus* and enterotoxins in çiğ köfte (raw meatball)

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Abstract: Çiğ köfte is a traditional raw meat product consumed almost everywhere in Turkey. It has a poor hygienic quality and can contain several pathogenic bacteria as no heating or cooking process is applied during any stage of manufacturing. The effects of sodium lactate on the presence of *Staphylococcus aureus* were investigated during the display-life of çiğ köfte and the formation of enterotoxins was also investigated. For this purpose, çiğ köfte samples were manufactured with different concentrations of sodium lactate (1%, 2%, 3%, and 4% NaL) and examined either in ambient (20 ± 2 °C) or refrigerator (4 ± 2 °C) storage. As a result, the shelf-life of çiğ köfte was improved with NaL addition; the growth of *S. aureus* was delayed dependent on NaL concentration used, and enterotoxin generations were retarded up to 12 h in cold storage.

Key words: Çiğ köfte, sodium lactate, *Staphylococcus aureus*, enterotoxin, shelf-life

1. Introduction

Çiğ köfte (raw meatball) is a popular meat product consumed traditionally almost everywhere in Turkey (1). It is generally manufactured from raw (lean) minced beef and/or lamb's meat, boiled wheat (bulgur), salt, tomato paste, fresh onion, parsley, garlic, cumin, special paprika (isot), black pepper, allspice, and various other spices (2,3). These ingredients are added at different rates according to the consumer's preferences (4), but no heating or cooking process is applied during any stage of manufacturing. It is hand-kneaded thoroughly after mixing and shaped into small portions by squeezing in the hand. Çiğ köfte should be consumed in a few hours after production since it is eaten totally raw (5,6).-

The hygienic quality of çiğ köfte is dependent on various factors, such as personal hygiene, production method, spices, qualities of all ingredients, and the raw, ground meat used. It has been reported by numerous researchers in Turkey that ground meat and spices were contaminated by several pathogenic bacteria (7,8). Recent studies reported that çiğ köfte had a very poor hygienic quality and was contaminated with *S. aureus*, coliforms, *E. coli*, *E. coli* O157:H7, *Salmonella* spp., and fecal streptococci (3,5,9,10).

S. aureus is considered the third most important cause of disease in the world amongst the reported food-borne illnesses. The presence of *S. aureus* in foods constitutes a significant risk of contamination by food handlers and it can be also used as an indicator of cross-contamination (11). Staphylococcal food poisoning is a persistent cause of gastroenteritis worldwide, especially in developed countries (12). The US Food and Drug Administration established that effective doses of staphylococcal enterotoxin can be achieved when populations of *S. aureus* are greater than 10^5 organisms per gram of contaminated food (13).

In recent years, the use of antimicrobial ingredients is one of the widely used methods to maintain microbiological safety and prolong the shelf-life of food products. Lactates, salts of L(+)-lactic acid, show a preservative effect by decreasing the water activity (a_w) of the products to which they are added, and by exhibiting a specific inhibitory effect (14,15). In addition to their antimicrobial effects, lactates are also shown to improve sensory characteristics of the products such as color, texture, and flavor, and they are shown to exert an antioxidant effect (15).

Because heat treatment is not suitable for attaining the desired raw characteristics of çiğ köfte, treatment with sodium lactate (NaL) appears to be a potential technique for improving the shelf-life of the product by inactivating pathogenic and spoilage bacteria. Therefore, this study was performed to investigate the effects of sodium lactate

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on the microbiological quality and enterotoxin presence of çiğ köfte during its display-life both in ambient and refrigerator storage.

2. Materials and methods

2.1. Preparation of çiğ köfte

The lean ground beef and other ingredients were purchased from local supermarkets in Avcılar, İstanbul. The preparation of çiğ köfte was carried out in our laboratory according to the Şanlıurfa-style production as recommended by Ardic and Durmaz (9). The ingredients used to produce çiğ köfte are lean ground beef (18%), bulgur (40%), onion (2%), spring onion (10%), traditional paprika (isot) (3.5%), black pepper (0.2%), salt (0.3%), tomato paste (3%), parsley (3%), and cold water (20%).

The lean ground beef was mixed with paprika (isot), black pepper, onion, and salt. Half of this mixture was then kneaded with bulgur by hand and cold water was gradually added during the kneading process. When the mixture became softer, the rest of the mixture was added and stirred together and tomato paste was added. Afterwards, finely chopped parsley and spring onion were added. Çiğ köfte was then contaminated with *S. aureus* strains, mixed gently, and stored at room temperature (20 ± 2 °C) for 10 min to allow the attachment of bacteria. The mixture was then treated with sodium lactate (Merck ME.106522, Darmstadt, Germany; 1%, 2%, 3%, and 4% NaL) and mixed well before being formed by hand manipulation and divided into portions of 25 g. Five groups of çiğ köfte samples were placed on Styrofoam trays (200 × 275 × 45 mm), packaged with oxygen-permeable polyvinyl chloride overwrap film (oxygen transmission rate of 6.500 cm^3/m^2 per 24 h at 0% relative humidity), and stored at room (20 ± 2 ° C) and refrigerator (4 ± 2 °C) temperature under 1.614 lx of GE "Natural" fluorescent light (model no. F40N; 24-h continuous lighting) simulating retail conditions in a supermarket until further analysis

The experimental çiğ köfte samples were manufactured at room temperature in triplicate for each group in different dates, and samples taken from each trial were subjected to microbiological and physicochemical analyses immediately.

2.2. Culture and preparation of inoculum

Test strain *S. aureus* ATCC 25923 (Microbiologics 0360P, Saint Cloud, MN, USA) from the laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, İstanbul University (Avcılar, İstanbul, Turkey) was maintained on brain-heart infusion broth (Oxoid CM225, Basingstoke, UK). In preparation for use, this strain was grown in 50 mL of brain heart infusion broth at 37 °C for 16 h, pelleted by 5 min of centrifugation at 3000 × *g*, washed, and then resuspended in sterile peptone water (Oxoid CM0009) to obtain a population of 10^8 CFU/mL cells in the early stationary growth phase. Before use, the optical density of the suspension was measured using a spectrophotometer (Shimadzu UV-1202 UV-VIS, Japan) at 620 nm and the suspensions were diluted in sterile peptone water to the bacterial density required. This population was also verified by subsequent plating on standard plate count agar (Oxoid CM0463) incubated at 35 °C for 24 h.

2.3. Microbiological analyses

Portions of çiğ köfte samples (25 g) were transferred to a sterile stomacher bag with 225 mL of 0.1% peptone water (Oxoid CM0009) and homogenized for 2 min in a stomacher (Lab Blender 400, Model BA 6021, Steward Lab., London, UK). Serial decimal dilutions were prepared using the same diluents up to 10^{-6}. A 0.1- or 1-mL inoculum of appropriate dilutions was spread on plate count agar (Oxoid, CM0463). Plates were incubated at 35 °C for 48 h for determination of total aerobic plate counts (TAPC) (16).

Lactic acid bacteria (LAB) counts were determined by plating with overlay on de Man, Rogosa, Sharpe agar (Oxoid, CM0361) and incubated at 35 °C for 48 h (17); *Pseudomonas* spp. were enumerated on *Pseudomonas* agar with a cetrimide, fucidin, cephaloridine supplement (Oxoid, CM0559 and SR0103); and spread plates were incubated at 25–30 °C for 48 h (16). Numbers of *S. aureus* were defined on Baird–Parker agar (Oxoid CM0275) supplemented with egg yolk–tellurite emulsion (Oxoid SR0054). Spread plates were incubated at 35 °C for 24 h. Colonies with typical *S. aureus* morphology were subjected to Gram staining, examined microscopically, tested for catalase and coagulase reaction, and confirmed with DNase Agar (Oxoid CM0321) incubated at 35 °C for 18–24 h (16). All microbiological tests were carried out in duplicate, and the results were expressed as log CFU/g.

Çiğ köfte samples were also analyzed before inoculation to identify the present microflora. At this time, the initial bacterial population was determined to be approximately 4.5 log CFU/g and no *S. aureus* were detected.

2.4. Detection of *S. aureus* enterotoxins

RIDASCREEN® SET A, B, C, D, E (Art No.: R4101) is an enzyme immunoassay for the detection of *S. aureus* enterotoxins (SEs) A, B, C, D, and E in fluid and solid foods. According to the test kit manual (R-Biopharm AG, Darmstadt, Germany), 10 g of çiğ köfte sample was minced and homogenized with 15 mL of PBS buffer, then shaken for 15 min. After centrifugation for 10 min at 3500 × *g* and 15 °C, sterile filtration of the resulting supernatant was applied. An aliquot (100 µL per well) of this solution was used in the test.

One hundred microliters of a sample was added to the first 7 wells of the microtiter strip and 100 µL of the positive control to the last well. They were mixed gently

and incubated for 1 h at room temperature (20 ± 2 °C) in the dark. The liquid was dumped out of the wells into a sink in order to remove all remaining liquid from the wells. The wells were then filled with 250 µL per well of washing buffer and the liquid was poured out again. The washing step was repeated 3 more times. The unbound conjugate was removed during washing. Subsequently, 100 µL of enzyme conjugate was added to each well and incubated for 1 h at room temperature (20 ± 2 °C) in the dark after mixing gently. The liquid was dumped out of the wells into a sink and the wells were filled with 250 µL per well of washing buffer. The liquid was poured out again and the wells were emptied for removing all remaining liquid. The washing step was repeated 3 more times again. Afterwards, 50 µL of substrate and 50 µL of chromogen solution was added to each well, mixed gently, and incubated for 30 min at room temperature (20 ± 2 °C) in the dark. Then, 100 µL of the stop solution (1 M H_2SO_4) was added to each well and mixed gently again. The absorbance was measured at 450 nm in an ELISA plate reader (ELX 800, Bio-Tek Inst., Germany).

2.5. Physicochemical analyses

The pH of **çiğ** köfte was determined by blending a 10-g sample with 100 mL of deionized water for 2 min. The pH of the resultant suspension was measured after 10 min at room temperature (20 ± 2 °C) using a Hanna pH meter (Hanna HI-9321, Woonsocket, RI, USA), equipped with a FC220B electrode (Hanna HI-9321) and calibrated with standard buffers of pH 4.0 and 7.0. Three readings were made for each sample and the means were recorded (18).

For determination of water activity (a_w), 20 g of çiğ köfte sample was placed into the cup of a calibrated hygrometer (Lufft, Fellbach, Germany) and measured at room temperature (20 ± 2 °C). Moisture content was determined by drying a homogeneous sample at 103 ± 2 °C until a constant weight was obtained (18).

2.6. Sensory evaluation

Noncontaminated çiğ köfte samples were evaluated by 8 well-experienced panelists, who a usual habit of eating çiğ köfte and had previously participated in training sessions to become familiar with the sensory characteristics of meat and meat products (19). Training sessions were conducted to acquaint the panelists with çiğ köfte samples in order to evaluate the attributes, and these were followed by an open-discussion session to familiarize panelists with the attributes and the scale to be used. The attributes studied were: flavor intensity, spicy taste, salty taste, sweet taste, acidic taste, and overall acceptability. All attributes were scored using a 5-point descriptive scale (1 = dislike extremely, 2 = dislike slightly, 3 = neither like nor dislike, 4 = like slightly, and 5 = like extremely). The sensory panel was carried out in 2 sessions and data were analyzed by analysis of variance (ANOVA) using Fisher's least significant difference test.

2.7. Statistical analysis

All experiments were conducted at 3 independent times in duplicate. In order to determine the effect of sodium lactate in çiğ köfte samples, one-way ANOVA was performed using SPSS 13.00. Moreover, Duncan's multiple range tests were used to evaluate the significance of the differences ($P < 0.05$) between treatment, storage conditions, and exposure times.

3. Results

The microbiological examinations of çiğ köfte samples are presented in Table 1. Treatment with NaL affected the shelf-life of çiğ köfte samples either in ambient or refrigerator storage. In all çiğ köfte samples, addition of NaL delayed the microbial growth depending on the concentration used and differed from control samples during display-life. The inhibition of the microorganisms increased with the increasing concentrations of NaL and cold storage. Significant differences were recorded after 6 h of storage for *Pseudomonas* spp. and 12 h for TAPC and LAB counts ($P < 0.01$; Table 1).

The effect of NaL against the counts of *S. aureus* in çiğ köfte samples is shown in the Figure. Significant differences were observed after 3 h of storage ($P < 0.001$) between control and NaL-treated samples. Increasing the level of NaL, with the synergic effect of cold storage, decreased the counts of *S. aureus*. During storage, significant decreases were determined in 2%, 3%, and 4% NaL-treated samples, while a slight increase was found for 1% NaL and control samples. Different concentrations of NaL resulted in 0.5 to 1.5 log CFU/g reductions of *S. aureus* in treated samples. This reduction was also strengthened with refrigerator storage (Figure).

The presence of enterotoxins in çiğ köfte samples is summarized in Table 2. Increasing levels of NaL delayed the toxin formation in çiğ köfte both in ambient and refrigerator storage. However, high temperatures stimulated the toxin production earlier than refrigerator temperature (4 ± 2 °C).

Changes in physicochemical parameters are shown in Table 3. There were significant differences between samples in terms of storage time and condition ($P < 0.001$). pH, a_w, and moisture content varied according to NaL concentrations; the initial level of pH and a_w decreased in NaL treated samples in both storage conditions, while a slight increase was recorded in control groups. Moreover, the change in the moisture content was remarkable in ambient storage with a higher loss of humidity than in refrigerated samples.

Table 1. Mean values and standard errors (SEs) of microbiological parameters in experimental çiğ köfte samples during display life at ambient (20 ± 2 °C) and refrigerator (4 ± 2 °C) temperatures (log CFU/g).

Attributes	Storage condition	Group	Time (h)				
			0	3	6	12	24
			Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
TAPC	Ambient	Control	6.455 ± 0.115	6.644 ± 0.058	6.949 ± 0.023	7.278^{a} ± 0.115	7.462^{a} ± 0.173
		1% NaL	6.376 ± 0.098	6.544 ± 0.115	6.813 ± 0.058	6.851^{bc} ± 0.058	6.919^{b} ± 0.006
		2% NaL	6.346 ± 0.081	6.447 ± 0.115	6.732 ± 0.115	6.778^{cd} ± 0.058	6.820^{bc} ± 0.058
		3% NaL	6.304 ± 0.115	6.415 ± 0.115	6.663 ± 0.173	6.716^{cd} ± 0.058	6.771^{bc} ± 0.058
		4% NaL	6.214 ± 0.058	6.342 ± 0.115	6.519 ± 0.115	6.591^{cd} ± 0.115	6.623^{bc} ± 0.115
	Refrigerator	Control	6.431 ± 0.058	6.533 ± 0.173	6.869 ± 0.058	7.079^{ab} ± 0.040	7.301^{a} ± 0.115
		1% NaL	6.279 ± 0.040	6.462 ± 0.115	6.708 ± 0.115	6.799^{cd} ± 0.058	6.875^{bc} ± 0.058
		2% NaL	6.241 ± 0.023	6.380 ± 0.115	6.690 ± 0.115	6.748^{cd} ± 0.058	6.785^{bc} ± 0.058
		3% NaL	6.214 ± 0.058	6.301 ± 0.115	6.613 ± 0.058	6.681^{cd} ± 0.115	6.699^{bc} ± 0.058
		4% NaL	6.179 ± 0.040	6.255 ± 0.115	6.478 ± 0.173	6.544^{d} ± 0.115	6.579^{c} ± 0.115
	Significance (P-value)		NS	NS	NS	***	***
LAB	Ambient	Control	4.398 ± 0.115	4.978 ± 0.013	5.146 ± 0.058	5.342^{a} ± 0.115	5.491^{a} ± 0.115
		1% NaL	4.380 ± 0.115	4.681 ± 0.115	4.813 ± 0.058	4.924^{b} ± 0.014	5.000^{b} ± 0.058
		2% NaL	4.342 ± 0.115	4.644 ± 0.115	5.778 ± 0.950	4.869^{b} ± 0.058	4.924^{bc} ± 0.058
		3% NaL	4.342 ± 0.173	4.602 ± 0.115	4.699 ± 0.058	4.785^{b} ± 0.115	4.869^{bc} ± 0.058
		4% NaL	4.342 ± 0.115	4.477 ± 0.115	4.544 ± 0.115	4.613^{b} ± 0.115	4.634^{c} ± 0.115
	Refrigerator	Control	4.398 ± 0.115	4.690 ± 0.115	4.826 ± 0.058	4.935^{b} ± 0.017	5.041^{b} ± 0.058
		1% NaL	4.322 ± 0.115	4.557 ± 0.067	4.708 ± 0.115	4.799^{b} ± 0.058	4.875^{bc} ± 0.058
		2% NaL	4.322 ± 0.115	4.532 ± 0.115	4.591 ± 0.115	4.644^{b} ± 0.115	4.672^{c} ± 0.115
		3% NaL	4.322 ± 0.115	4.505 ± 0.115	4.556 ± 0.115	4.613^{b} ± 0.173	4.634^{c} ± 0.173
		4% NaL	4.322 ± 0.115	4.477 ± 0.115	4.519 ± 0.115	4.591^{b} ± 0.115	4.602^{c} ± 0.115
	Significance (P-value)		NS	NS	NS	**	***
Pseudomonas spp.	Ambient	Control	3.798 ± 0.114	3.715 ± 0.113	3.602^{cd} ± 0.002	3.545^{de} ± 0.113	4.301^{a} ± 0.001
		1% NaL	3.833 ± 0.099	3.792 ± 0.058	3.699^{bc} ± 0.033	3.633^{cd} ± 0.015	4.145^{b} ± 0.057
		2% NaL	3.851 ± 0.005	3.763 ± 0.057	3.611^{cd} ± 0.064	3.532^{de} ± 0.058	4.041^{c} ± 0.015
		3% NaL	3.836 ± 0.003	3.699 ± 0.033	3.502^{d} ± 0.001	3.398^{e} ± 0.032	3.924^{d} ± 0.009
		4% NaL	3.836 ± 0.003	3.653 ± 0.058	3.342^{e} ± 0.058	3.175^{f} ± 0.055	3.839^{e} ± 0.006
	Refrigerator	Control	3.806 ± 0.058	3.868 ± 0.057	4.079^{a} ± 0.029	4.146^{a} ± 0.015	4.230^{a} ± 0.002
		1% NaL	3.826 ± 0.058	3.845 ± 0.003	3.806^{b} ± 0.031	3.905^{b} ± 0.058	4.000^{cd} ± 0.001
		2% NaL	3.845 ± 0.058	3.864 ± 0.058	3.785^{b} ± 0.058	3.864^{b} ± 0.059	3.956^{d} ± 0.003
		3% NaL	3.833 ± 0.029	3.851 ± 0.014	3.733^{bc} ± 0.058	3.793^{bc} ± 0.058	3.845^{e} ± 0.001
		4% NaL	3.813 ± 0.058	3.839 ± 0.058	3.699^{bc} ± 0.058	3.741^{bc} ± 0.058	3.773^{e} ± 0.060
	Significance (p value)		NS	NS	***	***	***

a, b, c: Means within a column with different letters are significantly different ($P < 0.05$).
NS: Not significant, **: $P < 0.01$, ***: $P < 0.001$.

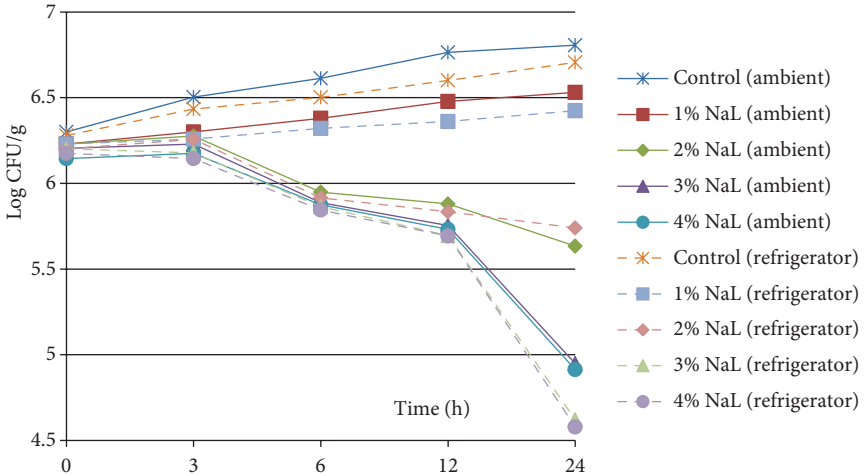

Figure. Presence of *S. aureus* in experimental çiğ köfte samples during display life at ambient (20 ± 2 °C) and refrigerator (4 ± 2 °C) temperatures.

Table 2. Presence of staphylococcal enterotoxin in experimental çiğ köfte samples during display life at ambient (20 ± 2 °C) and refrigerator (4 ± 2 °C) temperatures.

Storage condition	Group	Time (h)				
		0	3	6	12	24
Ambient	Control	-		SEA	SEA, SED	SEA, SED
	1% NaL	-	-	-	SEA	SEA, SED
	2% NaL	-	-	-	-	-
	3% NaL	-	-	-	-	-
	4% NaL	-	-	-	-	-
Refrigerator	Control	-	-	-	SEA	SEA, SED
	1% NaL	-	-	-	-	SEA
	2% NaL	-	-	-	-	-
	3% NaL	-	-	-	-	-
	4% NaL	-	-	-	-	-

SEA: Enterotoxin A, SED: Enterotoxin D.

4. Discussion

In order to eliminate or control the growth of spoilage and pathogenic microorganisms, the use of natural antimicrobial preservatives has been preferred in the food industry as a result of consumer demands (20). Lactates are recommended as food additives while they are naturally present in meat, meat products, and many other fermented foods, and are effective on many spoilage pathogen microorganisms (21).

The enhancement in the shelf-life of meat and meat products has been stated by several researchers (14,22,23). Nevertheless, this study was the first report to evaluate the beneficial effects of sodium lactate on the presence of *S. aureus* in çiğ köfte during its display-life.

The poor hygienic quality of çiğ köfte is hazardous for consumer health due to the presence of pathogenic bacteria contaminated from various sources. In Turkey, the contamination of çiğ köfte with *S. aureus* has been reported by a number of researchers. Cetin et al. (3) found *S. aureus* counts in 45 (44.11%) of analyzed çiğ köfte samples and determined that the mean count was 3.4×10^3 CFU/g. Daglioglu et al. (5) reported that the mean *S.*

Table 3. Mean values and standard errors (SEs) of physicochemical parameters in experimental çiğ köfte samples during display life at ambient (20 ± 2 °C) and refrigerator (4 ± 2 °C) temperatures.

Attributes	Storage condition	Group	Time (h)				
			0	3	6	12	24
			Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
pH	Ambient	Control	$5.120^{a} \pm 0.045$	$5.255^{a} \pm 0.038$	$5.270^{a} \pm 0.058$	$5.265^{a} \pm 0.035$	$5.270^{a} \pm 0.036$
		1% NaL	$5.056^{b} \pm 0.038$	$5.160^{b} \pm 0.058$	$5.155^{b} \pm 0.045$	$5.120^{b} \pm 0.058$	$5.060^{c} \pm 0.058$
		2% NaL	$4.964^{c} \pm 0.056$	$5.102^{d} \pm 0.025$	$5.085^{d} \pm 0.045$	$5.080^{d} \pm 0.058$	$4.955^{d} \pm 0.036$
		3% NaL	$4.862^{d} \pm 0.058$	$4.946^{g} \pm 0.016$	$4.935^{f} \pm 0.045$	$4.910^{e} \pm 0.058$	$4.880^{e} \pm 0.058$
		4% NaL	$4.800^{e} \pm 0.058$	$4.825^{i} \pm 0.058$	$4.740^{g} \pm 0.058$	$4.695^{g} \pm 0.035$	$4.650^{g} \pm 0.058$
	Refrigerator	Control	$5.121^{a} \pm 0.046$	$5.115^{c} \pm 0.034$	$5.125^{c} \pm 0.045$	$5.105^{c} \pm 0.035$	$5.065^{b} \pm 0.036$
		1% NaL	$5.051^{b} \pm 0.046$	$5.040^{e} \pm 0.058$	$5.045^{e} \pm 0.045$	$4.910^{e} \pm 0.058$	$4.750^{f} \pm 0.058$
		2% NaL	$4.965^{c} \pm 0.058$	$5.010^{f} \pm 0.058$	$4.935^{f} \pm 0.045$	$4.765^{f} \pm 0.035$	$4.510^{h} \pm 0.058$
		3% NaL	$4.870^{d} \pm 0.058$	$4.845^{h} \pm 0.038$	$4.790^{h} \pm 0.058$	$4.455^{h} \pm 0.035$	$4.250^{i} \pm 0.058$
		4% NaL	$4.855^{e} \pm 0.056$	$4.695^{j} \pm 0.038$	$4.560^{i} \pm 0.058$	$4.220^{i} \pm 0.058$	$4.145^{j} \pm 0.036$
	Significance (P-value)		***	***	***	***	***
a_w	Ambient	Control	0.961 ± 0.057	$0.973^{a} \pm 0.056$	$0.986^{a} \pm 0.058$	$0.979^{b} \pm 0.058$	$0.972^{b} \pm 0.058$
		1% NaL	0.963 ± 0.056	$0.969^{b} \pm 0.058$	$0.966^{c} \pm 0.058$	$0.961^{c} \pm 0.057$	$0.954^{d} \pm 0.058$
		2% NaL	0.965 ± 0.045	$0.967^{c} \pm 0.058$	$0.964^{d} \pm 0.058$	$0.956^{de} \pm 0.058$	$0.951^{e} \pm 0.057$
		3% NaL	0.967 ± 0.058	$0.966^{cd} \pm 0.058$	$0.963^{d} \pm 0.056$	$0.952^{f} \pm 0.058$	$0.946^{f} \pm 0.058$
		4% NaL	0.968 ± 0.058	$0.965^{de} \pm 0.045$	$0.961^{e} \pm 0.057$	$0.943^{g} \pm 0.056$	$0.936^{g} \pm 0.058$
	Refrigerator	Control	0.961 ± 0.057	$0.970^{b} \pm 0.058$	$0.978^{b} \pm 0.058$	$0.981^{a} \pm 0.057$	$0.976^{a} \pm 0.058$
		1% NaL	0.963 ± 0.056	$0.962^{f} \pm 0.058$	$0.959^{f} \pm 0.058$	$0.958^{d} \pm 0.058$	$0.956^{c} \pm 0.058$
		2% NaL	0.965 ± 0.045	$0.964^{e} \pm 0.058$	$0.961^{e} \pm 0.057$	$0.957^{d} \pm 0.058$	$0.955^{cd} \pm 0.045$
		3% NaL	0.967 ± 0.058	$0.966^{cd} \pm 0.058$	$0.963^{d} \pm 0.056$	$0.955^{e} \pm 0.045$	$0.951^{e} \pm 0.057$
		4% NaL	0.968 ± 0.058	$0.966^{cd} \pm 0.058$	$0.961^{e} \pm 0.057$	$0.951^{f} \pm 0.057$	$0.947^{f} \pm 0.058$
	Significance (P-value)		NS	***	***	***	***
Moisture content (%)	Ambient	Control	38.81 ± 0.058	$46.08^{d} \pm 0.040$	$47.18^{c} \pm 0.040$	$45.26^{c} \pm 0.029$	$43.12^{e} \pm 0.058$
		1% NaL	38.78 ± 0.012	$46.03^{d} \pm 0.012$	$47.16^{c} \pm 0.029$	$45.23^{c} \pm 0.058$	$43.04^{f} \pm 0.012$
		2% NaL	38.75 ± 0.023	$46.01^{d} \pm 0.010$	$47.11^{c} \pm 0.010$	$45.16^{c} \pm 0.029$	$43.01^{f} \pm 0.010$
		3% NaL	38.73 ± 0.012	$45.82^{e} \pm 0.058$	$46.70^{d} \pm 0.058$	$44.33^{d} \pm 0.244$	$42.93^{g} \pm 0.012$
		4% NaL	38.73 ± 0.012	$45.70^{f} \pm 0.058$	$46.56^{e} \pm 0.058$	$44.32^{d} \pm 0.058$	$42.28^{h} \pm 0.058$
	Refrigerator	Control	38.81 ± 0.058	$50.34^{a} \pm 0.017$	$52.32^{a} \pm 0.058$	$52.20^{a} \pm 0.058$	$52.10^{a} \pm 0.003$
		1% NaL	38.79 ± 0.046	$50.24^{ab} \pm 0.017$	$52.10^{b} \pm 0.033$	$52.06^{a} \pm 0.029$	$52.04^{ab} \pm 0.006$
		2% NaL	38.76 ± 0.024	$50.22^{ab} \pm 0.058$	$52.16^{b} \pm 0.029$	$52.02^{a} \pm 0.006$	$52.10^{b} \pm 0.010$
		3% NaL	38.76 ± 0.029	$50.12^{bc} \pm 0.058$	$52.10^{b} \pm 0.029$	$49.97^{b} \pm 0.017$	$49.92^{c} \pm 0.006$
		4% NaL	38.75 ± 0.023	$50.08^{c} \pm 0.012$	$52.04^{b} \pm 0.017$	$49.90^{b} \pm 0.058$	$49.83^{d} \pm 0.012$
	Significance (P-value)		NS	***	***	***	***

a, b, c: Means within a column with different letters are significantly different ($P < 0.05$).
NS: Not significant, ***: $P < 0.001$.

aureus count in çiğ köfte samples collected from İstanbul was 1.5×10^3 CFU/g. Similar results were also reported by Küplülü et al. (24) and Sancak and İşleyici (25).

In a study conducted by Erol et al. (26), the growth magnitude of *S. aureus* was examined in çiğ köfte and it was determined that *S. aureus* did not grow in inoculated samples at a level of 10^3–10^5 CFU/g. Contrary, Sağun et al. (4) indicated that çiğ köfte contaminated with *S. aureus* at a level of 10^5 CFU/g produced enterotoxin while stored at room temperature (21–23 °C) for 24 h and at 30 °C for 12 h. Likewise, in the present study, *S. aureus* was inoculated at a level of 10^8 CFU/g into çiğ köfte samples and we isolated approximately 10^6 CFU/g in the final product, which is enough to produce staphylococcal enterotoxins.

Since *S. aureus* is widely distributed in the environment, raw and processed foods, meat, and meat products (27), this pathogen can spread during different stages of çiğ köfte production. Therefore, *S. aureus* food poisoning occurs from ingestion of staphylococcal enterotoxin produced by certain strains that cause gastroenteritis (28).

It has been previously emphasized that the antimicrobial effect of lactates is greater at lower pH values since under these conditions more undissociated lactate is present (29). Its bactericidal effect is due to the reduction in pH below the growth range and metabolic inhibition by the undissociated molecules that penetrate the bacterial membrane (30).

Lactates do not pose any health risk for consumers and do not affect the sensory characteristics of the products (21). In the present study, sensory analyses revealed that the addition of NaL did not cause any negative sensory changes to the flavor intensity and taste of the product (data not shown). The samples received generally close scores in both storage conditions in respect to panelists' preferences. Taste and flavor evaluation showed differences ($P < 0.05$) among lactate-treated samples, while no difference was observed in flavor intensity of çiğ köfte stored either at ambient or refrigerator temperatures ($P > 0.05$). However, the taste of samples became stronger over time with increasing NaL concentration; the overall acceptability of çiğ köfte was admissible during the whole storage time.

In conclusion, çiğ köfte, which is a one of the ready-to-eat foods sold frequently in Turkey, may be a major cause of food poisoning and food-borne diseases. The growth of *S. aureus* in çiğ köfte prepared from contaminated raw materials under poor manufacturing conditions may cause serious food-safety problems for consumers. In addition, initial conditions of the raw product associated with its manufacturing and storage conditions (pH, a_w, moisture, and temperature) can lead to rapid growth of *S. aureus* and its toxin production. In particular, çiğ köfte sold in the streets and open areas without any safety precautions may heighten the magnitude of health risk. Usage of sodium lactate appears to be a potential technique to preserve hygienic quality of çiğ köfte without adversely affecting the characteristic freshness of the product. As a result, the shelf-life of çiğ köfte can improve with NaL treatment either in ambient or refrigerator storage; the growth of *S. aureus* can be delayed dependent on increasing levels of NaL, and enterotoxin generation can be retarded up to 12 h in cold storage.

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