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Effect of lemon juice on the survival of *Salmonella* Enteritidis and *Escherichia coli* in cig kofte (raw meatball)

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Abstract

Purpose – The purpose of this study is to investigate the effect of lemon juice on the survival of *Salmonella* Enteritidis and *Escherichia coli* in cig kofte (raw meatball).

Design/methodology/approach – Cig kofte samples were inoculated one by one with both bacteria at high inoculum levels and were treated with different doses of fresh lemon juice (2, 5, 10 and 15 ml) for 10 seconds, 30 seconds, and 1, 5, 15, 30 and 60 minutes.

Findings – Treatments of lemon juice for different exposure times caused reduction ranging between 0.1 and 1.7 log CFU/g for *Salmonella* Enteritidis and 0.1 and 2.1 log CFU/g for *E.coli*. Results showed that lemon juice caused slight decrease in *Salmonella* Enteritidis and *E.coli* as an immediate inhibitor, but this effect increased with concentration and time.

Originality/value – This is a research study to provide information on the effectiveness of lemon juice which is squeezed generally before eating cig kofte, on the presence of the surface flora to strengthen the hygienic quality of the product. Inactivation effect of lemon juice on *Salmonella* Enteritidis and *E.coli* may give a practical and easy way of providing food safety for cig kofte.

Keywords Cig kofte, Lemon juice, *Salmonella* Enteritidis, *Escherichia coli*, Food safety, Bacteria, Hygiene

Paper type Research paper

1. Introduction

Cig kofte (raw meatball) is a domestic meat product consumed traditionally almost everywhere in Turkey, especially Southeast and East Anatolia regions of Turkey (Ocal, 1997). It is generally produced from raw (lean) minced beef and/or lambs meat, bulgur (boiled wheat), salt, tomato paste, fresh onion, parsley, garlic, cumin, special paprica (isot), black pepper, all spice and various spices (Cetin *et al.*, 2008; Durmaz *et al.*, 2007). These ingredients are added at different rates according to consumer's preferences (Sagun *et al.*, 2003), but no heating or cooking process is applied during any stage of manufacture and it is eaten totally raw after mixing all. It is hand kneaded thoroughly and shaped into small portions by squeezing in the hand. Before serving cig kofte, it is generally recommended to wrap each piece with a leaf of lettuce and is mostly consumed with fresh lemon juice. Cig kofte should be consumed in a few hours after production (Daglioglu *et al.*, 2005).

The hygienic quality of cig kofte is depended on personal hygiene, production method, spices, qualities of all ingredients and raw ground meat used. It has been reported by numerous researches in Turkey that ground meat and spices were

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contaminated by several pathogenic bacteria (Bingol *et al.*, 2007; Gokmen and Alisarli, 2003; Hampikyan *et al.*, 2009; Sagun, Sancak, Durmaz and Ekici, 1997). Recent studies reported that cig kofte had a very poor hygienic quality and were contaminated with *S.aureus*, coliforms, *E.coli, E.coli* 0157:H7, *Salmonella* spp. and faecal streptococci (Ardic and Durmaz, 2008; Cetin *et al.*, 2008; Daglioglu *et al.*, 2005; Sagun, Sancak, Durmaz and Akkaya, 1997; Uzunlu and Yildirim, 2003).

Recently weak organic acids such as citric acid, acetic acid and lactic acid are frequently used in food technology. Citric acid is preferred for the tenderness of meat (Burke and Monahan, 2003), decontamination of home-made mayonnaise (Xiong *et al.*, 1999) and the decontamination of vegetables such as carrot, rocket and spring onion (Sengun and Karapinar, 2004, 2005).

Since heat treatment is inappropriate for the raw characteristics of the product, treatment of lemon juice appears to be a potential technique to preserve cig kofte through inactivating pathogenic and spoilage bacteria without adversely affecting the characteristic freshness of products.

This study was performed to introduce the effectiveness of lemon juice in the elimination of *Salmonella* Enteritidis and *E.coli* in cig kofte.

2. Materials and methods

Preparation of cig kofte

The lean ground beef and other ingredients were purchased from local supermarkets in Avcilar-Istanbul. The preparation of cig kofte was carried out in our laboratory according to the Sanliurfa style production as recommended by Ardic and Durmaz (2008). The ingredients used to produce cig kofte are lean ground beef (18 per cent), bulgur (40 per cent), onion (2 per cent), spring onion (10 per cent), traditional paprika (isot) (3.5 per cent), black pepper (0.2 per cent), salt (0.3 per cent), tomato paste (3 per cent), parsley (3 per cent), and cold water (20 per cent).

The lean ground beef was mixed with paprika (isot), black pepper, onion and salt. Then, half of this mixture was 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. Cig kofte was then formed by hand manipulation and divided into portions of 25 g.

Three trials of cig kofte were prepared and samples taken from each trial were transported to the laboratory after inoculation with *Salmonella* Enteritidis and *E.coli*. They were subjected to microbiological and sensorial analyses after treated with lemon juice.

Cultures and preparation of inoculum

E.coli 25922 and *Salmonella* Enteritidis KUEN 349 from the laboratory of Istanbul University, Faculty of Veterinary Medicine, Department of Microbiology, Avcilar, Istanbul, Turkey were maintained in tryptone soy agar (Oxoid CM0131, Basingstoke, UK) slants at 5°C until use. Stock culture of *E.coli* was grown on tryptone soy broth with 0.6 per cent (w/v) yeast extract (Oxoid CM0129, Basingstoke, UK) whereas; *Salmonella* Enteritidis was cultured in tryptone soy broth (Oxoid CM0129, Basingstoke, UK). *E.coli* and *Salmonella* Enteritidis were incubated at 37°C for 18 h

to obtain cells in 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 TSB to the bacterial density required.

Concentrations were then adjusted to 10^8 colonies forming units/millilitre (CFU/ml) using saline peptone water (Oxoid CM0733, Basingstoke, UK). An aliquot of 1 ml of bacterial suspension (*Salmonella* Enteritidis or *E.coli*) at approximately 10^8 CFU/ml was individually added in a sterile jar containing 100 ml of saline peptone water to obtain 10^6 CFU/ml.

Preparation of treatment solutions

Undiluted fresh lemon juice was used as treatment solutions in all experiments. Lemons were purchased from a local supermarket in Avcilar-Istanbul. Lemons were washed with cold water and cut with a sterile knife. A household juicer was used to obtain fresh lemon juice (100 per cent) which was stored in a sterile jar during the experiment. Acidity of test solutions was determined by titration against 0.1 N sodium hydroxide and expressed as citric acid percentage (vol/vol) (Anonymous, 1972), and also the pH value of lemon juice was measured by a pH meter.

Inoculation of cig kofte and treatment with lemon juice

Cig kofte samples were analyzed before inoculation to identify the present microflora. For this purpose, 25 g of cig kofte sample was added in 225 ml of saline peptone water and homogenized in a stomacher (Lab Blender 400, Model BA6021, Steward Lab., London, UK). Then, an aliquot of 0.1 ml of decimal dilution was transferred to plate count agar (Oxoid CM0463, Basingstoke, UK); pour plate incubated at 35°C for 24 h. The initial bacterial population was determined approximately 3.5 log CFU/g. At this time, another 25 g of cig kofte sample was investigated for the presence of *Salmonella* spp. and *E.coli* according to FDA (1995) and ISO 16649-2 (2001) procedures and any *Salmonella* Enteritidis and *E.coli* were detected. After that, a portion of 25 g cig kofte samples were weighed and transferred into sterile petri plates for inoculation with both bacteria.

Each cig kofte sample was plunged separately into a glass of inoculums and stayed in for 10 sec., then was placed in sterile petri plates and stored at room temperature for 10 min to allow the attachment of *Salmonella* Enteritidis and *E.coli* cells. Two, five, ten and fifteen millilitres of treatment solution were poured onto surfaces of cig kofte samples and let them absorb in the petri plates depending on exposure times (10 sec, 30 sec, 1 min, 5 min, 15 min, 30 min and 60 min) at room temperature.

Cig kofte samples were then placed in sterile stomacher bags containing 225 ml of sterile saline peptone water and analyzed depending on bacteria searched.

Detection and enumeration of Salmonella *Enteritidis and* E.coli

A pre-detection step was applied to determine if there were any *Salmonella* Enteritidis or *E.coli* in the samples before inoculation. For this purpose, 25 gram of cig kofte was added into 225 ml of buffered peptone water (Oxoid CM0509, Basingstoke, UK) and incubated at 35°C for 24 h. for the detection of *Salmonella*. After the pre-enrichment step, 0.1 ml of aliquot was transferred to 10 ml Rappaport Vassiliadis Broth (Oxoid CM0669, Basingstoke, UK) and another 0.1 ml of aliquot to 10 ml Tetrathionate Broth

(Oxoid CM0671, Basingstoke, UK). RV Broth was incubated for 24 h, at 42°C and TT Broth for 24 h, at 43°C. The test cultures from RV and TT broth separately were streaked onto Bismuth Sulphite Agar (Oxoid CM0201, Basingstoke, UK), Xylose Lysine Desoxycholate Agar (Oxoid CM0469, Basingstoke, UK) and Hectoen Enteric Agar (Oxoid CM0419, Basingstoke, UK), and incubated for 24 h at 35°C (Andrews *et al.*, 1995). Randomly selected typical colonies from the agar plates were confirmed to be *Salmonella* Enteritidis using the following biochemical [triple sugar iron agar (Oxoid CM0277, Basingstoke, UK), lysine iron agar (Oxoid CM0381, Basingstoke, UK), urease test (Oxoid CM0071, Basingstoke, UK) and indole reactions] and serological tests [O-, Vi- and H-antigen tests (Murex Salmonella Polyvalent Agglutinating Sera)] (AOAC, 1990). For the detection of *E.coli*, 25 gram of cig kofte was added in 225 ml of sterile saline peptone water and homogenized in a stomacher (Lab Blender 400, Model BA6021, Steward Lab., London, UK). After that, 1 ml of aliquot was transferred to tryptone bile X-glucuronide agar (Oxoid CM0945, Basingstoke, UK) and incubated at 44°C for 24 h. (ISO 16649-2, 2001). No *Salmonella* Enteritidis and *E.coli* were detected.

To enumerate the level of *Salmonella* Enteritidis and *E.coli* on the surface of inoculated cig kofte samples, 25 gram of cig kofte was added in 225 ml of saline peptone water and homogenized in a stomacher (Labblender 400, Model BA6021, Steward Lab., London, UK). Then, an aliquot of 0.1 ml of decimal dilution was transferred to Bismuth Sulphite Agar, Xylose Lysine Desoxycholate Agar and Hectoen Enteric Agar, and incubated for 24 h at 35°C. The typical colonies were enumerated and the initial *Salmonella* Enteritidis levels were defined as 10^5 CFU/g. For enumeration of *E.coli*, 25 g of cig kofte sample was added in 225 ml of saline peptone water and homogenized well. Then, 1 ml of aliquot was transferred to tryptone bile X-glucuronide agar and incubated at 44°C for 24 h. Typical green colonies were counted and the initial *E.coli* levels were found approximately 10^5 CFU/g. Same practices were done to the suspensions used for *Salmonella* Enteritidis and *E.coli* and it was observed that inoculated cig kofte samples were contained 1 log lower bacteria then the suspensions prepared.

Enumeration of *Salmonella* Enteritidis and *E.coli* in the treated samples was carried out as mentioned.

Determination of pH

The pH value of each cig kofte sample was measured at each exposure time after blended separately with 100 ml of distilled-deionised water (ddH_2O) by a pH meter (Hanna HI 1131, Germany) equipped with a combined electrode (HI 9321 Microprocessor pH meter, Hanna Instruments, Germany) (AOAC, 1990).

Sensory evaluation

Uninfected and treated with lemon juice cig kofte samples were evaluated by a five-member panel, staff of Istanbul University Food Hygiene and Technology Department, who had usually habit to eat cig kofte and had previously participated in training sessions to become familiar with the sensory characteristics of meat and meat products (ISO, 6658:1985; ISO, 8586–1:1993). The panellists were trained in three separate sessions approximately 2 h, in which panellists were served cig kofte samples for evaluation of selected attributes. Training sessions were conducted to acquaint panellists with the products and attributes to be evaluated, and were followed by an

open-discussion session to familiarise panellists with the attributes and the scale to be used. The attributes studied were: flavour intensity, sustained dryness/juiciness, acidic flavour and overall acceptability. All attributes were scored using a five-point descriptive scale (1 = dislike extremely 2 = dislike slightly, 3 = neither like nor dislike, 4 = like slightly, and 5 = like extremely) according to Sørheim *et al.* (1996) and all values were recorded.

The panel members were seated in individual booths in a temperature-controlled and light-controlled room, receiving a set of four samples served in a complete randomised order. Each sample was labelled, at random, with a two-digit code number. Unsalted crackers and water were served to panellists to freshen their mouth between each sub-samples assessment (ISO 8589, 1988). Sensory panel was carried out triplicate in seven sessions followed by the exposure times.

Statistical analysis

All experiments were conducted at three independent times in duplicate. In order to determine the effect of lemon juice in cig kofte samples, a one way analysis of variance (ANOVA) was performed using SPSS 10.0 statistical package (SPSS, 1999). Moreover, ANOVA for a four (treatments) × seven (exposure times) factorial design was used to explain whether the interactions between treatment and exposure times were significant ($P < 0.05$). If the effect of lemon juice was found significant, Duncan's multiple range tests was used to evaluate the significance of the difference. Also, the general linear model (GLM) procedures were used to analyse data for sensory characteristics. The model used in the analyses of these characteristics included the fixed effects of lemon juice, exposure times and panellists.

3. Results and discussion

The pH of lemon juice was 2.54 and the level of citric acid was 6.19 per cent. The initial pH of cig kofte was measured 5.02. When it was treated with lemon juice, the average pH of the mixture became to 4.06 and decreased to 3.75 at the end of the experiments (Figure 1).

Table I shows the effects of different doses of lemon juice (2 ml, 5 ml, 10 ml and 15 ml lemon juice) on *Salmonella* Enteritidis and *E.coli* inoculated to cig kofte at different exposure times.

When cig kofte samples were treated with different doses of lemon juice, the reduction on the initial counts of *Salmonella* Enteritidis in each group from 3.93 to 4.50 log CFU/g ranged from 0.1 to 0.7 log CFU/g, 0.1 to 1.4 log CFU/g, 0.1 to 1.7 log CFU/g and 0.2 to 1.7 log CFU/g, respectively.

Although there seemed to be slight reductions in the counts for each inhibitory group, the immediate antimicrobial effect of lemon juice against *Salmonella* Enteritidis at 10 sec, 30 sec and 1 min was observed at statistically significant level ($P < 0.05$). There was a significant difference between 5 and 15 min treatments by using lemon juice ($P < 0.01$), moreover antimicrobial effect at 30 and 60 min was statistically more significant ($P < 0.001$).

While cig kofte samples were treated with different doses of lemon juice, the initial counts of *E.coli* in each group was reduced from 3.84-4.30 log CFU/g to 1.69-3.57 log

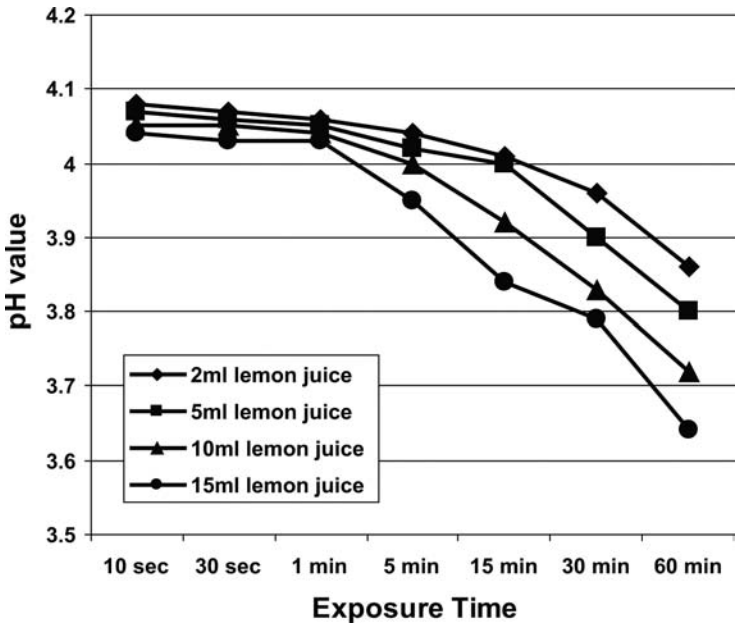

Figure 1.
pH values of cig kofte samples

CFU/g and ranged from 0.2 to 0.8 log CFU/g, 0.2 to 1.1 log CFU/g, 0.1 to 1.7 log CFU/g and 0.1 to 2.1 log CFU/g, respectively.

Even though, slight reductions in the counts of *E.coli* were observed in each group, the statistically significant effects were determined at 10 sec and 60 min of treatment ($P < 0.05$). The statistically difference was more significant at 60 min ($P < 0.01$) while no significance was observed in other exposure times ($P > 0.05$).

Lemon juice caused no significant differences between the groups for the sensory attributes of cig kofte samples ($P > 0.05$) except 5, 15 and 30 min of treatments ($P < 0.01$). Lemon juice strengthened the flavour of cig kofte samples without causing any sensory problems and they were gratefully acceptable approximately for 30 min and overall acceptable till the end of the experiment (Table II). Acidic flavour was tasted only in high volume dressed lemon juice samples on the 30 and 60 min of exposure times. However, sustained dryness was tasted only in low volume treated lemon juice sample while slight juiciness was observed in high volume treated samples at the end of the treatment.

There are several reports regarding the use of citric acid or lemon juice as the inhibitors against *Salmonella* for real food systems (Kisla, 2008; Koutsoumanis *et al.*, 1999). Kisla (2008) reported that the treatment of lemon juice and lemon dressing on *Salmonella* typhimurium inoculated to the stuffed mussels at high inoculum level reduced the initial counts of *Salmonella* Typhimurium from 5.94 log CFU/g to the levels ranging from 0.25 to 0.56 log CFU/g and from 0.50 to 0.69 log CFU/g, respectively. She emphasized that there was a significant difference between 5 and 15 min treatments by using lemon juice. These findings were in accordance with the results of the present

Table I. Effects of lemon juice treatment on *Salmonella* Enteritidis and *E.coli* in cig kofte samples (log_{10} CFU/g)

Microorganism	Treatment with lemon juice	Exposure time 10 sec	30 sec	1 min	5 min	15 min	30 min	60 min
Salmonella Enteritidis	2 ml lemon juice	4.50 ± 0.05^{a}	4.46 ± 0.02^{a}	4.36 ± 0.01^{a}	4.30 ± 0.01^{a}	4.25 ± 0.05^{a}	4.04 ± 0.01^{a}	3.81 ± 0.09^{a}
	5 ml lemon juice	4.39 ± 0.07^{a}	4.32 ± 0.02^{a}	4.23 ± 0.02^{a}	3.90 ± 0.03^{b}	3.81 ± 0.02^{b}	3.66 ± 0.02^{b}	3.04 ± 0.01^{b}
	10 ml lemon juice	4.14 ± 0.06^{b}	4.00 ± 0.02^{b}	3.80 ± 0.06^{b}	3.65 ± 0.03^{b}	3.57 ± 0.09^{bc}	3.49 ± 0.01^{b}	2.43 ± 0.03^{c}
	15 ml lemon juice	3.93 ± 0.04^{c}	3.84 ± 0.05^{b}	3.69 ± 0.08^{b}	3.62 ± 0.03^{b}	3.47 ± 0.07^{c}	3.39 ± 0.07^{b}	2.30 ± 0.05^{c}
	P	*	*	*	**	**	***	***
E.coli	2 ml lemon juice	4.30 ± 0.01^{a}	4.14 ± 0.06	4.00 ± 0.03	3.97 ± 0.03	3.84 ± 0.05	3.64 ± 0.03	3.57 ± 0.09^{a}
	5 ml lemon juice	4.17 ± 0.06^{b}	4.07 ± 0.09	3.98 ± 0.02	3.86 ± 0.09	3.72 ± 0.04	3.44 ± 0.07	3.17 ± 0.06^{a}
	10 ml lemon juice	4.00 ± 0.04^{bc}	3.94 ± 0.04	3.85 ± 0.01	3.83 ± 0.08	3.61 ± 0.02	3.23 ± 0.02	2.30 ± 0.01^{b}
	15 ml lemon juice	3.84 ± 0.05^{c}	3.69 ± 0.08	3.65 ± 0.03	3.56 ± 0.08	3.14 ± 0.06	2.77 ± 0.08	1.69 ± 0.08^{b}
	P	*	NS	NS	NS	NS	NS	***

Notes: Mean values within the same column with different superscript small letters are different; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS = not significant

Table II.
Effects of lemon juice treatment on the sensory quality of cig kofte samples

Characteristics	Treatment with lemon juice	Exposure time 10 sec	30 sec	1 min	5 min	15 min	30 min	60 min
Flavour intensity	2 ml lemon juice	3.40 ± 0.24	3.40 ± 0.24	3.80 ± 0.20	4.05 ± 0.00^{ab}	4.08 ± 0.00^{ab}	3.80 ± 0.20^{b}	3.42 ± 0.24
	5 ml lemon juice	3.42 ± 0.24	3.66 ± 0.24	3.85 ± 0.20	4.08 ± 0.00^{ab}	4.40 ± 0.24^{a}	4.20 ± 0.20^{ab}	3.46 ± 0.24
	10 ml lemon juice	3.46 ± 0.24	3.82 ± 0.20	4.06 ± 0.00	4.40 ± 0.24^{a}	4.60 ± 0.24^{a}	4.65 ± 0.24^{a}	3.24 ± 0.20
	15 ml lemon juice	3.60 ± 0.24	3.80 ± 0.20	4.05 ± 0.00	3.85 ± 0.20^{b}	3.60 ± 0.24^{b}	3.65 ± 0.24^{b}	3.00 ± 0.00
	P	NS	NS	NS	*	*	*	NS
Acidic flavour	2 ml lemon juice	4,25 ± 0.10	4,22 ± 0.10	4,12 ± 0.20	3,82 ± 0.20	3,66 ± 0.20	3,30 ± 0.22^{a}	3,10 ± 0.20^{a}
	5 ml lemon juice	4,22 ± 0.10	4,20 ± 0.10	4,10 ± 0.20	3,80 ± 0.20	3,60 ± 0.20	3,22 ± 0.20^{a}	3,05 ± 0.20^{a}
	10 ml lemon juice	4,08 ± 0.12	4,08 ± 0.12	4,00 ± 0.22	3,62 ± 0.24	3,40 ± 0.22	3,06 ± 0.18^{ab}	2,62 ± 0.24^{b}
	15 ml lemon juice	4,02 ± 0.12	4,00 ± 0.12	3,80 ± 0.26	3,55 ± 0.18	3,28 ± 0.18	2,82 ± 0.14^{b}	2,30 ± 0.26^{c}
	P	NS	NS	NS	NS	NS	*	*
Juiciness	2 ml lemon juice	5.0 ± 0.00	5.00 ± 0.00	4,80 ± 0.10	4.20 ± 0.22	3,80 ± 0.20	3,00 ± 0.18	2,72 ± 0.18
	5 ml lemon juice	5.0 ± 0.00	5.00 ± 0.00	4,80 ± 0.10	4.25 ± 0.24	4,02 ± 0.18	3,20 ± 0.16	3,08 ± 0.14
	10 ml lemon juice	4.8 ± 0.20	4.80 ± 0.20	4.60 ± 0.12	4.22 ± 0.24	3,85 ± 0.22	3,22 ± 0.14	3,00 ± 0.12
	15 ml lemon juice	4.8 ± 0.20	4.80 ± 0.20	4.60 ± 0.12	4.30 ± 0.25	3,85 ± 0.24	3,05 ± 0.10	2,80 ± 0.10
	P	NS	NS	NS	NS	NS	NS	NS
Overall acceptability	2 ml lemon juice	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	4.05 ± 0.02	4.02 ± 0.04	4.00 ± 0.00	4.00 ± 0.00
	5 ml lemon juice	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	4.40 ± 0.24	4.42 ± 0.24	4.25 ± 0.20	4.25 ± 0.20
	10 ml lemon juice	4.85 ± 0.20	4.85 ± 0.20	4.80 ± 0.20	4.22 ± 0.20	4.20 ± 0.20	4.22 ± 0.20	4.22 ± 0.20
	15 ml lemon juice	4.85 ± 0.20	4.85 ± 0.20	4.80 ± 0.20	4.22 ± 0.20	4.20 ± 0.20	4.20 ± 0.20	4.00 ± 0.00
	P	NS	NS	NS	NS	NS	NS	NS

Notes: Mean values within the same column with different superscript small letters are different; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS = not significant

study. The initial counts of *Salmonella* Enteritidis between 3.93-4.50 log CFU/g showed reductions from 0.1 to 0.3 log CFU/g till 1 min. by using lemon juice, whereas when lemon juice was used for 5 min, the reductions were from 0.2 to 0.5 log CFU/g and when it was used for 15 min, the reductions were from 0.3 to 0.6 log CFU/g. The excessive reduction on the amount of *Salmonella* Enteritidis in cig kofte was observed in 30 and 60 min treatments; the reductions were from 0.5 to 0.8 log CFU/g and from 0.7 to 1.7 log CFU/g, respectively.

Another study was performed by Sengun and Karapinar (2004) on the lethal effects of lemon juice, vinegar and the mixture of lemon juice-vinegar (1:1). They stated that the treatment of carrots with lemon juice and vinegar alone for different exposure times caused significant reductions on *Salmonella* Typhimurium ranging between 0.79-3.95 and 1.57-3.58 log CFU/g, respectively, while the number of pathogens was reduced to an undetectable level after 30 min treatment by combined used lemon juice-vinegar.

When rocket leaves were treated with lemon juice, the reduction of *Salmonella* Typhimurium observed ranged from 1.87 to 3.52 log CFU/g and from 1.23 to 4.17 log CFU/g for high and low inoculum levels, respectively in a similar study conducted by Sengun and Karapinar (2005). They determined that, although the population of *Salmonella* Typhimurium on low inoculated rocket samples was reduced to an undetectable level after 60 min treatment with lemon juice, no significant difference was observed between 15, 30 and 60 min treatment times ($P > 0.05$). However, the initial population of *Salmonella* Typhimurium on spring onion samples were reduced to 4.36 and 3.61 log CFU/g after 60 min treatment with lemon juice for high and low inoculum levels, respectively ($P < 0.05$) (Sengun and Karapinar, 2005).

Populations of *Salmonella* Typhimurium in high inoculum carrots were reduced to 2.68 log CFU/g after 15 and 30 min treatments, and a maximum reduction of 3.95 log CFU/g was achieved by dipping 60 min in lemon juice. In low inoculum level, 2.63, 2.57 and 2.64 log CFU/g reductions were achieved after 15, 30 and 60 min treatments, respectively. Although there was no significant difference between 15, 30 and 60 min treatments ($P > 0.05$), the immediate antimicrobial effect of lemon juice against *Salmonella* Typhimurium at 0 min was significant ($P < 0.05$) (Sengun and Karapinar, 2004). Similarly Kisla (2008) found the immediate antimicrobial effect of lemon juice against *Salmonella* Typhimurium at 0 min to be negligible ($P > 0.05$), but the antimicrobial effect was observed starting from 5 min of treatment ($P < 0.05$). However, there was no statistical difference ($P > 0.05$) between the 5 and 15 min treatments. Likewise, in the present study, the immediate antimicrobial effect of lemon juice against *Salmonella* Enteritidis at 10 and 30 sec was negligible, but the antimicrobial effect started from 5 min treatment ($P < 0.05$) and there was no statistical difference ($P > 0.05$) between the 5 and 15 min treatments, but significant differences were determined between 30 and 60 min.

On the other hand, Vijayakumar and Wolf-Hall (2002) established that lemon juice and lime juice showed no significant differences for minimum bactericidal concentrations against all strains of *E.coli*. They had minimum bactericidal effects at a citric acid concentration of approximately 6 per cent.

Giannuzzi and Zaritzky (1993) showed the antimicrobial action of citric acid at 3,500 ppm ($\approx$0.35 per cent) in a model system of refrigerated pre-peeled potatoes. They

stated that the microbial growth rate decreased with increase in citric acid concentration.

Similarly in the present study, the initial counts of *E.coli* were reduced in each group depending on the concentration of lemon juice and exposure time. But statistical differences were observed at 10 sec and 60 min of treatment ($P < 0.05$).

The presence of weak acids at different concentrations in some common household products, such as vinegar, bleach, hydrogen peroxide, lemon juice, lime juice and lemon dressing, led to the hypothesis that these products could be used as sanitizers for in-home use of foods like raw products (Kisla, 2008; Vijayakumar and Wolf-Hall, 2002). These natural products and their mixture can be considered to be potential antimicrobial agents in preventing foodborne outbreaks related to fresh products at household levels (Sengun and Karapinar, 2005). However, treatment of cig kofte with lemon juice for 30 min is not enough to prevent *Salmonella* Enteritidis or *E.coli* outbreaks related to cig kofte. But it is enough to reduce the microbial counts of raw products. Inactivation effect of lemon juice on *Salmonella* Enteritidis and *E.coli* may give a practical and easy way of providing food safety for cig kofte. Consumers generally prefer to eat cig kofte just after squeezing fresh lemon juice and these could be a way to minimize the amount of existing microorganisms. Results of this study showed that lemon juice caused slight decrease in *Salmonella* Enteritidis and *E.coli* as an immediate inhibitor, but this effect increased by concentration and time.

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