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The fate of two *Listeria monocytogenes* serotypes in "cig kofte" at different storage temperatures

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Abstract

Cig kofte is a traditional Turkish food prepared from minced beef, bulgur, onions, garlic and varieties of spices. It is generally consumed within a few hours. However, leftovers can be kept in refrigerator or in room temperature up to 24 h until they are consumed. In this study, survival and growth of two *Listeria monocytogenes* serotypes were investigated in cig kofte during the storage. For this purpose, the prepared samples were separately contaminated with serotypes 1/2b or 4b of *L. monocytogenes* at the level of 10^4 CFU/g and stored at 4 °C and 21 °C. *L. monocytogenes* colonies were counted at the beginning, 3rd, 6th, 12th and 24th hours of the storage. At 4 °C, *L. monocytogenes* 4b significantly increased ($P < 0.05$) from 4.12 to 5.49 $\log_{10}$CFU/g but *L. monocytogenes* 1/2b remained constant ($P > 0.05$) during the storage period. At 21 °C, both *L. monocytogenes* 1/2b and 4b increased significantly ($P < 0.05$) from 4.56 to 5.16 $\log_{10}$CFU/g and from 4.23 to 5.65 $\log_{10}$CFU/g, respectively. The physicochemical and microbiological characteristics of the cig kofte did not inhibit the growths of *L. monocytogenes* serotypes during the storage. These results indicated that *L. monocytogenes* was able to survive and grow in cig kofte at the both storage temperatures of 4 °C and 21 °C and cig kofte seemed to be a suitable medium for this pathogen.

Keywords: Cig kofte; Survival; *L. monocytogenes* serotypes; Storage temperature

1. Introduction

Cig kofte is a traditional Turkish food prepared from minced beef, bulgur, onions, garlic and various spices particularly in the East and Southeast regions of Anatolia and in Syria. It is made by hand kneading of minced beef with bulgur and spices, including red pepper, cumin, black pepper, cinnamon, parsley, garlic and onion (Goktan & Tuncel, 1988; Ocal, 1997).

The cig kofte is generally consumed within a few hours following preparation. However, they can be kept for up to 24 h in a refrigerator when large amounts of leftovers remain for additional meals (Ocal, 1997). Additionally, it is prepared and sold under unhygienic conditions in restaurants and restaurant-like places. Therefore, the insufficiency of personnel hygiene during manufacture and contamination of the materials used with microorganisms may lead to food borne diseases. Because cig kofte is consumed without heat treatment, it may also cause increased public health problems (Goktan & Tuncel, 1988).

It has been reported that minced beef and spices consumed in Turkey may be highly contaminated with various bacteria (Guven, Gulmez, & Kamber, 1997; Sancak, Boynukara, & Agaoglu, 1993; Sagun, Sancak, Durmaz, & Ekici, 1997a; Tekinsen & Sarigol, 1982; Tekinsen, Yurtyeri, & Mutluer, 1980). Therefore, the quality of minced beef and spices used for preparing the cig kofte is very important for the hygienic quality of this food. Several studies showed that cig kofte is contaminated with *Staphylococcus*

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aureus, coliform bacteria, *Escherichia coli* and enterococci (Arslan, Guven, Saltan, & Patir, 1992; Kuplulu, Sarimehmetoglu, & Oral, 2003; Sagun, Sancak, Durmaz, & Akkaya, 1997b). We previously reported that *S. aureus* could produce enterotoxin in cig kofte stored at room temperature (21–23 °C) for 24 h (Sagun, Alisarli, & Durmaz, 2003). Although Isleyici, Sancak, Sagun, and Ekici (2004) reported that *Listeria monocytogenes* was present in six of 50 samples of the cig kofte, there is no information about the fate of *L. monocytogenes* in the cig kofte.

Food-associated outbreaks of human listeriosis involving many deaths have led to an awakening of interest in controlling it's presence in foodstuffs (Fleming et al., 1985; James et al., 1985; Schlech et al., 1983). Therefore, the purpose of this study was to describe the survival of *L. monocytogenes* in cig kofte manufactured from meat and ingredients contaminated with *L. monocytogenes* during a storage period of 24 h at 4 or 21 °C.

2. Materials and methods

2.1. Inoculum preparation

In this study, two serotypes of *L. monocytogenes*, serotypes 1/2b (RSKK 472) and 4b (RSKK 475), were used. The bacteria were supplied from the Refik Saydam National Type Culture Collection, Ankara, Turkey. The cultures were maintained on tryptone soya agar (TSA, Oxoid, UK) slants at 4 °C with bimonthly transfers and grown in tryptone soya broth (TSB, Oxoid) at 35 °C for 24 h. Population density in peptone water (PW, 0.1%) was assessed spectrophotometrically at 550 nm after centrifugation (2500*g*), washing, and resuspension in sterile distilled water with further dilution to approximately equal concentrations of each serotype, and enumerated on TSA (at 35 °C for 24 h) to verify the number of *L. monocytogenes* in the cell suspension of each serotype. Then the cultures were appropriately diluted in peptone water to obtain an initial level of 10^4 CFU/g in the cig kofte.

2.2. Cig kofte production

Equal amounts of fine bulgur and minced beef were combined with blends of spices including cinnamon, cumin, pepper, black pepper, garlic, parsley, onion, tomato paste and salt. The cig kofte was then kneaded by hand and water was added until the desired consistency was reached. Then the cig kofte was divided into two equal portions of 600 g. The first and second groups were separately inoculated with *L. monocytogenes* 1/2b and 4b at the level of 10^4 CFU/g, respectively. Each group was then divided into two subgroups for two different storage conditions. Samples in one subgroup were kept at 21 °C and the others were stored at 4 °C for 24 h. This study was conducted in triplicate on three different occasions using different raw materials in every experiment. All cig kofte samples were analyzed in duplicate after storage for 0, 3, 6, 12 and 24 h.

2.3. Microbiological analysis

All microbiological media were obtained from Oxoid Ltd. (Basingstoke, UK). Ten grams of cig kofte were aseptically obtained from each sample and homogenized in a stomacher (2300/400, Barcelona, Spain) with 90 mL of 0.1% sterile peptone water for 2 min. From this basic dilution (10^{-1}), a series of decimal dilutions were prepared for bacterial analysis. Typical colonies of *L. monocytogenes*, which exhibited a black color, were enumerated by surface plating on Oxford agar containing *Listeria* selective supplement after an incubation period of 48 h at 35 °C. Five selected colonies were confirmed by streaking onto TSA and testing isolated colonies for catalase production and for the following characteristics: tumbling motility at 25 °C, carbohydrate fermentation (maltose, dextrose, mannitol, xylose and rhamnose), nitrate reduction, Methyl Red–Voges Proskauer reactions, umbrella motility in SIM medium at 25 °C, β-hemolysis and Gram staining (Hitchins, 1995).

Total aerobic plate count (APC) was determined on plate count agar after an incubation period at 30 °C for 48 h and lactic acid bacteria (LAB) were enumerated on MRS Agar following an incubation at 30 °C for 5 days under anaerobic conditions (Pichhardt, 1998). Microbial counts are reported as the log number of colony forming units (log CFU/g).

2.4. Chemical analysis

Salt (NaCl) and moisture contents of the cig kofte were determined according to the procedure of the Association of Official Analytical Chemists (AOAC, 2000). The pH-value was measured by inserting the combination electrode of a pH meter (Nel 890, Ankara, Turkey).

2.5. Statistical analysis

The data were analyzed using the SAS statistical package for windows (SAS/STAT Software, 1998). Analysis of variance was applied to determine the existence of significant differences between the values. Significant ($P < 0.05$) differences among means were identified using the Duncan multiple range test.

3. Results and discussion

All of the cig kofte were free (both direct plating and enrichment) of *L. monocytogenes* before they were inoculated with the pathogen.

Growth of *L. monocytogenes* serotypes 1/2b and 4b was compared by monitoring viable cell count in the cig kofte stored at 4 and 21 °C for 24 h. There was no statistically significant difference for survival and growth of *L. monocytogenes* 1/2b between two different storage temperatures up to 6 h of storage. Between 6 h and 24 h, the number of *L. monocytogenes* 1/2b increased significantly ($P < 0.05$) at

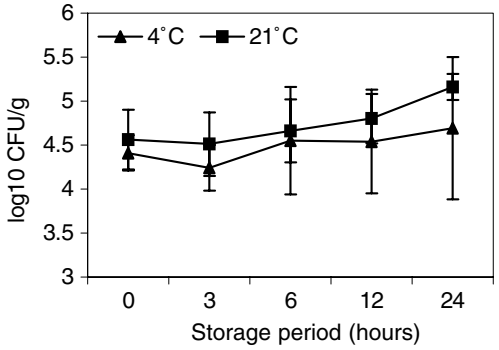

Fig. 1. The fate of *L. monocytogenes* 1/2b in cig kofte during the storage.

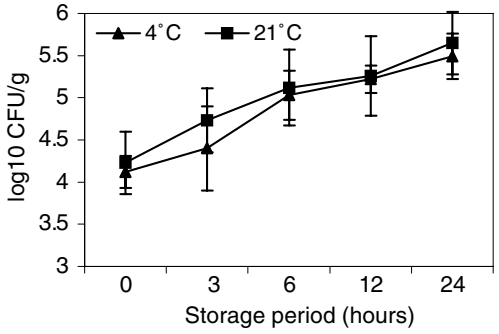

Fig. 2. The fate of *L. monocytogenes* 4b in cig kofte during the storage.

21 °C, while this serotype remained unchanged at 4 °C (Fig. 1). In contrast to the serotype 1/2b, number of *L. monocytogenes* serotype 4b increased significantly ($P < 0.05$) at both temperatures during storage period (Fig. 2). These observations indicated that at least the growth of *L. monocytogenes* 4b was not reduced at low temperatures. This finding is in accordance with that of Beuchat and Brackett (1990), who also have reported that *L. monocytogenes* 4b can grow on refrigerated samples.

Results of APC and LAB counts and chemical analyses of the cig kofte inoculated with *L. monocytogenes* 1/2 and 4b during storage are shown in Tables 1 and 2, respectively. Although the natural flora significantly increased ($P < 0.05$) to ca. 10^6–10^7 CFU/g during storage, *L. monocytogenes* was able to compete with inherent flora. Shelef (1989) also observed that the flora increased to ca. 10^8 per gram but no effect on growth of *L. monocytogenes* could be observed. Several explanations have been suggested for a lack of competitive effect of the natural microflora against *L. monocytogenes*. Gouet, Labadie, and Serratore (1978) suggested that degradation of muscle by proteolytic bacteria might stimulate the growth of *L. monocytogenes*. Verheul et al. (1995) reported that degradation of muscle could provide stimulatory factors such as large and small peptides and amino acids for the growth of *L.monocytogenes*.

The pH of the cig kofte samples decreased more rapidly during storage at 21 °C than those stored at 4 °C. Probably due to different counts of LAB, which increased from 10^3 to 10^4 at 4 °C and from 10^3 to 10^5 CFU/g at 21 °C. The effectiveness of low pH in controlling *L. monocytogenes* using a broth system was previously demonstrated (Sorrells & Enigl, 1990). However, the low pH of the cig kofte in this study did not prevent the increase of *L. monocytogenes* population. The reason for persistence and growth of *L. monocytogenes* can be explained by low NaCl concentration in cig kofte, which is between 2.01% and 2.75%. Faleiro, Andrew, and Power (2003) demonstrated that exposure to low levels of NaCl protects *L. monocytogenes* against acid shock down to pH 3.5. In another study, McClure, Roberts, and Oguru (1989) also noted that this pathogen

Table 1
Total aerobic plate count (APC), lactic acid bacteria (LAB) counts and chemical characteristics of the cig kofte inoculated with *L. monocytogenes* 1/2b[A]

Characteristics	Temperature	Storage time (h)				
		0	3	6	12	24
APC (log CFU/g)	4 °C	6.42 ± 0.49	6.44 ± 0.45	6.48 ± 0.51	6.58 ± 0.42	6.66 ± 0.45
	21 °C	6.07 ± 0.14^{c}	6.17 ± 0.32^{bc}	6.25 ± 0.26^{bc}	6.50 ± 0.36^{b}	6.95 ± 0.38^{a}
	P	NS	NS	NS	NS	NS
LAB (log CFU/g)	4 °C	3.27 ± 0.19^{c}	3.28 ± 0.15^{c}	3.29 ± 0.13^{c}	3.56 ± 0.13^{b}	4.38 ± 0.22^{a}
	21 °C	3.44 ± 0.25^{c}	3.46 ± 0.21^{c}	3.70 ± 0.27^{c}	4.35 ± 0.24^{b}	5.27 ± 0.21^{a}
	P	NS	NS	**	***	***
pH	4 °C	5.62 ± 0.11^{b}	5.70 ± 0.11^{ab}	5.74 ± 0.09^{a}	5.81 ± 0.04^{a}	5.60 ± 0.10^{b}
	21 °C	5.64 ± 0.11^{b}	5.74 ± 0.04^{ab}	5.83 ± 0.06^{a}	5.82 ± 0.10^{a}	5.26 ± 0.12^{c}
	P	NS	NS	NS	NS	***
Moisture (%)	4 °C	61.00 ± 0.32^{a}	60.61 ± 0.65^{a}	59.84 ± 0.59^{b}	58.28 ± 0.44^{c}	56.94 ± 0.29^{d}
	21 °C	61.24 ± 1.26^{a}	59.70 ± 1.41^{b}	57.14 ± 1.23^{c}	55.68 ± 1.40^{c}	52.94 ± 1.11^{d}
	P	NS	NS	**	**	***
Salt (%)	4 °C	2.01 ± 0.09^{c}	2.02 ± 0.09^{c}	2.06 ± 0.09^{bc}	2.15 ± 0.04^{ab}	2.20 ± 0.06^{a}
	21 °C	2.20 ± 0.11^{d}	2.27 ± 0.07^{cd}	2.39 ± 0.10^{bc}	2.48 ± 0.09^{b}	2.75 ± 0.15^{a}
	P	**	***	***	***	***

[abcd]: Indicate differences ($P < 0.05$) between columns; NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
[A] Mean values ± standard deviation of three trials; *P*: significance between two groups (those stored 4 °C and 21 °C).

Table 2
Total aerobic plate count (APC), lactic acid bacteria (LAB) counts and chemical characteristics of the cig kofte inoculated with *L. monocytogenes* 4b[A]

Characteristics	Temperature	Storage time (h)				
		0	3	6	12	24
APC (log CFU/g)	4 °C	6.44 ± 0.35^{b}	6.61 ± 0.57^{b}	6.65 ± 0.52^{b}	6.81 ± 0.37^{ab}	7.18 ± 0.17^{a}
	21 °C	6.35 ± 0.47^{c}	6.37 ± 0.45^{c}	6.65 ± 0.24^{bc}	7.05 ± 0.33^{b}	7.48 ± 0.20^{a}
	P	NS	NS	NS	NS	*
LAB (log CFU/g)	4 °C	3.81 ± 0.48^{b}	3.75 ± 0.36^{b}	3.83 ± 0.31^{b}	4.13 ± 0.33^{b}	4.70 ± 0.31^{a}
	21 °C	3.54 ± 0.33^{c}	3.68 ± 0.30^{c}	4.17 ± 0.40^{b}	4.69 ± 0.36^{a}	5.08 ± 0.21^{a}
	P	NS	NS	NS	*	*
pH	4 °C	5.64 ± 0.04^{c}	5.75 ± 0.07^{b}	5.83 ± 0.09^{ab}	5.87 ± 0.08^{a}	5.65 ± 0.06^{c}
	21 °C	5.68 ± 0.12^{b}	5.78 ± 0.09^{ab}	5.84 ± 0.12^{ab}	5.88 ± 0.14^{a}	5.34 ± 0.19^{c}
	P	NS	NS	NS	NS	**
Moisture (%)	4 °C	61.63 ± 0.93^{a}	60.64 ± 1.42^{a}	59.10 ± 1.41^{b}	58.45 ± 1.34^{b}	56.55 ± 0.92^{c}
	21 °C	61.18 ± 0.34^{a}	59.74 ± 0.46^{b}	58.07 ± 0.43^{c}	56.73 ± 0.43^{d}	53.70 ± 0.88^{e}
	P	NS	NS	NS	*	***
Salt (%)	4 °C	2.09 ± 0.06^{c}	2.10 ± 0.05^{c}	2.12 ± 0.04^{bc}	2.16 ± 0.06^{ab}	2.21 ± 0.03^{a}
	21 °C	2.24 ± 0.06^{d}	2.29 ± 0.03^{cd}	2.36 ± 0.02^{bc}	2.44 ± 0.06^{b}	2.57 ± 0.14^{a}
	P	**	***	***	***	***

[abcd]: Indicate differences ($P < 0.05$) between columns; NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
[A] Mean values ± standard deviation of three trials; *P*: significance between two groups (those stored 4 °C and 21 °C).

could survive under salt concentrations as high as 10% NaCl. In our study, the moisture content during storage at 4 and 21 °C decreased from 61% to 56% and 52%, respectively. These moisture contents were higher than 25% which had been referred to as the limit for growth of *L. monocytogenes* (Chen & Shelef, 1992). Therefore, it seems that cig kofte with high moisture and low salt contents provide a good medium for *L. monocytogenes* growth.

In summary, significant changes were observed in environmental conditions including pH, salt and moisture as well as its microbiologic characteristics during storage of the cig kofte. These changes were insufficient to prevent the survival and growth of *L. monocytogenes* . Therefore, the cig kofte supported survival and growth of the two serotypes of *L. monocytogenes* tested and might serve as a vector for the transmission of human listeriosis. Several ingredients used in the manufacture of cig kofte could also serve as a potential source of *L. monocytogenes*. As previously mentioned, the cig kofte does not undergo any thermal treatment to inactive pathogenic microorganisms during or after manufacture. Therefore, cig kofte must be manufactured only from high quality *Listeria*-free ingredients and under the best sanitary conditions, and measurements should be taken to prevent the marketing of the cig kofte on streets. Additionally, it would be advisable to include a bacteriocin against *L. monocytogenes* as suggested for *Salmonella* by Calicioglu, Ilhak, and Dikici (2004).

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