THE EFFECT OF NISIN AND BOVINE LACTOFERRIN ON THE MICROBIOLOGICAL QUALITY OF TURKISH-STYLE MEATBALL (TEKIRDAĞ KÖFTE)

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Accepted for Publication August 14, 2007

ABSTRACT

Tekirdağ köfte, a Turkish-style meatball, is one of the most popular ground meat products in Turkey. The aim of this study was to evaluate the effect of different nisin and lactoferrin (Lf) concentrations and their combinations on the microbiological quality of Turkish-style meatball (Tekirdağ köfte). For this purpose, the meatball dough was divided into six equal groups, and each of the groups was treated separately with different nisin and/or Lf concentrations (0, 100 and 200 μg/g). Analyses were performed on each meatball group at 0, 1, 3, 5, 7, 10 and 12 days for microbiological parameters (total mesophilic aerobic bacteria, lactic acid bacteria, coliforms, Escherichia coli, *total staphylococcae,* Staphylococcus aureus, *total psychrophilic bacteria,* Pseudomonas *spp., sulfite-reducing anaerobic bacteria, and yeast and mold) and physicochemical analyses (pH, water activity and moisture). Treatment with Lf alone and its combination with nisin significantly reduced ($P < 0.05$) the total aerobic bacteria, coliform,* E. coli, *total psychrophilic bacteria,* Pseudomonas *spp., and yeast and mold counts in meatballs. The largest reduction in these counts occurred on the meatballs treated with a mixture of Lf (200 μg/g) and nisin (100 μg/g). This combination prolonged the refrigerated shelf life of naturally contaminated Turkish-style meatballs to 10 days compared to 3 days for nontreated control.*

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Journal of Food Safety **28** (2008) 355–375.

PRACTICAL APPLICATIONS

Meatballs are prepared from ground meat in Turkey. Studies have shown that total bacteria, coliform, and yeast and mold counts were usually high in meatball samples in Turkey, and these products were also usually contaminated with pathogenic bacteria. Therefore, this product mostly poses a risk to consumer health and has a short shelf life (about 3–4 days). In order to control the growth of spoilage microorganisms, the use of natural antimicrobial preservatives has been preferred in the food industry. For this purpose, nisin and lactoferrin were preferred in this study. Although there are many reports on the use of nisin to inhibit pathogenic bacteria, especially *Listeria monocytogenes* and *Staphylococcus aureus* in food, research on the antimicrobial activity of lactoferrin in food systems is limited.

According to the results of our study, treatment with lactoferrin alone and its combination with nisin significantly reduced spoilage bacteria counts and extended the refrigerated shelf life of Turkish-style meatballs. Therefore, the use of these natural compounds in meatballs may be useful for consumer health, and may also be a practical application for the producer because of the short shelf life of this product.

INTRODUCTION

Tekirdağ köfte, a Turkish-style meatball, is one of the most popular ground meat products in Turkey. It is produced from ground meat (most manufacturers prefer veal), toasted bread crumbs, salt, onion, garlic and various spices (Yilmaz *et al.* 2002).

Meat and meat products are usually marketed in small butcher shops as steaks and/or in the ground form in Turkey, and most people prefer to consume meat and meat products in the ground form (Yilmaz *et al.* 2005). Many meat products, such as meatballs, kebabs and patties prepared from ground meat, are consumed in Turkey (Ulu 2006). The safety of these products principally depends on the quality of the ground meat and other ingredients, especially spices. It has been reported by a number of researchers that total bacteria, coliform, and yeast and mold counts were usually high in ground meat and meatball samples in Turkey, and these products were also usually contaminated with pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. (Sancak *et al.* 1993; Yilmaz *et al.* 2002; Gokmen and Alisarli 2003; Kuplulu *et al.* 2003; Baskaya *et al.* 2004).

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 because of consumers' demands (Dufour *et al.* 2003). Nisin and lactoferrin (Lf) are two natural compounds that have received

considerable attention in recent years (Murdock and Matthews 2002). In the literature, there are many reports on the use of nisin to inhibit spoilage and pathogenic bacteria in food (Chung *et al.* 1989; Pawar *et al.* 2000; De Martinez *et al.* 2002). Nisin is a 34-residue long peptide bacteriocin produced by strains of *Lactococcus lactis* ssp. *lactis*, and it exerts bactericidal effects against gram-positive bacteria, especially against strains of *Listeria monocytogenes* in laboratory media or in model food system. Primarily, the antimicrobial activity of nisin is based on pore formation in the cytoplasmic membrane of target organisms. Nisin is in the status of generally recognized as safe, and at present, it is the only bacteriocin permitted for use in foods in many countries. This compound is commercially available and has successfully been applied as a biopreservative in dairy and meat products (Reunanen and Saris 2004; Samelis *et al.* 2005).

Lf, an iron-binding glycoprotein (approximately 80 kDa) of the transferrin family, is an antimicrobial component of milk and other external secretions such as tears and saliva (Hoek *et al.* 1997; Murdock and Matthews 2002; Farnaud and Evans 2003). Within the last few years, considerable interest has arisen regarding the possible use of bovine Lf for the surface decontamination of beef carcasses, and subsequently its possible use as a natural food preservative (Al-Nabulsi and Holley 2006). Lf exerts an antimicrobial effect against a wide range of gram-negative and gram-positive bacteria, fungi, and parasites (Shimazaki 2000; Masschalck *et al.* 2001). Its actions can be classified into several different modes of action: by sequestering free iron, thereby restricting the growth of gram-positive and gram-negative bacteria; by interfering with bacterial membrane function; and by binding bacterial lipopolysaccharide, thereby impairing bacterial cell wall/membrane function (IUCCI *et al.* 2007). Studies have indicated that Lf has the potential to be used as a natural antimicrobial preservative in the food industry (Payne *et al.* 1994; Salamah and Al-Obaidi 1995; Dionysius and Milne 1997). However, research on the antimicrobial activity of Lf in food systems is limited.

This study was planned to evaluate the effect of different nisin and Lf concentrations and their combinations on the microbiological quality of Turkish-style meatballs (tekirdağ köfte).

MATERIALS AND METHODS

Nisin and Lactoferrin

Pure nisin from *Lactococcus lactis* (ssp. *lactis*) was obtained from Sigma (N5764) Darmstadt, Germany. A 2.5-g nisin was solubilized in 200 mL 0.02 M HCl with heating (60–70C) to aid solubilization. Bovine Lf (17% iron saturated) was obtained from Sigma (L4765). A 3.2-g quantity of Lf was

dissolved in 100 mL 0.05 M phosphate buffer (pH 7.5). The solutions were sterilized by filtration through 0.22-μm membrane filters (Millex, Millipore, Maidstone, UK). For each of three production groups, the nisin and Lf solutions were prepared daily.

Preparation of Meatball (Tekirdağ Köfte) Samples

Well-matured and minced (about 0.2–0.3 cm) veal was purchased from a local market and was used in experimental meatball production. The samples were produced according to the following traditional recipe. The ground veal (84%), which contains 10% fat, was mixed with ground black pepper (0.1%), cumin (0.4%), red pepper (2.0%), onion rind (3.0%), garlic clove rind (0.5%), salt (2.0%) and toasted bread (made of wheat flour) crumbs (8.0%). The mix was kneaded for 30 min by hand (with nonsterile glove) to obtain a homogeneous dough. The whole meatball dough was divided into six equal groups, and each of the groups was treated separately with different Lf and/or nisin concentrations as follows:

Group I: 100 μg Lf/g of meatball dough
Group II: 100 μg nisin/g of meatball dough
Group III: 100 μg Lf + 100 μg nisin/g of meatball dough
Group IV: 200 μg Lf + 100 μg nisin/g of meatball dough
Group V: 100 μg Lf + 200 μg nisin/g of meatball dough
Control: no treatment.

All groups were kneaded for an additional 15 min to obtain a homogeneous mixture, and were then shaped by hand into 5-cm-diameter meatballs with a weight of 40–50 g. Fifteen meatballs were placed on styrofoam trays (200 × 275 × 45 mm), wrapped with polyethylene film and stored at 4C until further analysis. The experimental meatball samples were manufactured at room temperature in triplicate for each group in different dates.

Analyses

Analyses were performed on each meatball group at 0, 1, 3, 5, 7, 10 and 12 days for microbiological parameters (total mesophilic aerobic bacteria, lactic acid bacteria [LAB], coliforms, *E. coli*, total staphylococcae, *S. aureus*, total psychrophilic bacteria, *Pseudomonas* spp., sulfite-reducing anaerobic bacteria, and yeast and mold) and physicochemical analyses (pH, water activity and moisture). All analyses were performed in duplicate.

Microbiological Analyses

Ten grams of meatball sample from each group was transferred to a sterile bag with 90 mL sterile Peptone water (Oxoid, CM0009), and was homogenized for 90 s using a stomacher (Lab Blender 400, Model BA 6021, Steward

Lab., London, U.K.). Serial decimal dilutions were prepared using the same diluent. A 0.1- or 1-mL inoculum of appropriate dilutions was spread on the following media: aerobic plate counts on plate count agar (PCA, Oxoid, CM0325, Hampshire, UK); pour plates incubated at 35C for 48 h; total psychrophilic bacteria (PCA, Oxoid, CM0325); spread plates incubated at 7C for 10 days; coliforms in violet red bile agar (VRBA, Oxoid, CM0107); pour plates, with overlay added before incubation, incubated at 35C for 24 h; *E. coli* in tryptone bile X-glucuronide agar (TBX, Oxoid, CM0945); pour plates incubated at 44C for 24 h; LAB counts in de Man, Rogosa, Sharpe agar (MRS, Oxoid, CM0361); pour plates, with overlay, incubated at 35C for 48 h; *Pseudomonas* spp. on *Pseudomonas* agar with Cetrimid, Fucidin, Cephaloridin supplement (Pseudomonas CFC, Oxoid, CM0559 and SR0103); and spread plates incubated at 25–30C for 48 h.

Sulfite-reducing anaerobic bacteria were analyzed in sulfite polymyxin sulfadiazine agar (SPS, Merck, 1.10235, Darmstadt, Germany) by means of roll tube method and incubated at 35C for 24 h. For yeast and molds, yeast extract glucose chloramphenicol agar (YGC, Merck 1.16000) was used and spread plates were incubated at 25C for 3–5 days. Total staphylococcae and *S. aureus* were defined on Baird–Parker agar (BPA, Oxoid CM0275) supplemented with egg yolk–tellurite emulsion (E-Y-T Emulsion, Oxoid SR0054). Spread plates were incubated at 35C for 24 h. Colonies with typical *S. aureus* morphology were subjected to Gram staining, examined microscopically, tested for catalase reaction and confirmed with DNase agar (DNase, Oxoid CM0321) incubated at 35C for 18–24 h.

Isolation of *Salmonella* spp. was carried out in four steps. Two hundred twenty-five milliliters of buffered peptone water (BPW, Oxoid, CM0509) was added to 25-g meatball sample of each group, and incubated at 35–37C for 16–20 h for preenrichment step; 0.1 and 1 mL of the homogenate was transferred to Rappaport Vassiliadis medium (RV, Oxoid, CM0669) and tetrathionate broth (TT, Oxoid, CM0671) for selective enrichment with an incubation period of 42 and 43C for 24 h, respectively. After incubation, a loopful from each tube was streaked on bismuth sulfite agar (BS, Oxoid, CM0201), xylose lysine desoxycholate agar (XLD, Oxoid, CM0469) and hectoen enteric agar (HE, Oxoid, CM0419), and was incubated for 20–24 h at 35C. Typical colonies were checked and selected for growing on nutrient agar (NA, Oxoid, CM0003) at 35C for 18–24 h, and were then identified by triple sugar iron agar (TSI, Oxoid, CM0277), lysine iron agar (LIA, Oxoid, CM0381) fermentation tests, urease test (urea broth, Oxoid, CM0071), Voges Proskeuer, indole, O-, Vi- and H-antigen tests (Murex Salmonella polyvalent agglutinating sera; Biotech Ltd., Kent, UK).

For *L. monocytogenes* detection, 225 mL *Listeria* enrichment broth (LEB, Oxoid, CM862) without a selective supplement was added to 25-g

erately, 8 = like very much, and 9 = like extremely) was used. Water and bread were served for cleaning the mouth between samples.

Statistical Analysis. Analysis of variance (ANOVA) of the data was performed using the ANOVA procedure by Duncan's multiple range tests using the SPSS version 9.0 (SPSS Inc., Chicago, IL). Significant differences ($P < 0.05$) between mean values of triplicate samples were determined.

RESULTS AND DISCUSSION

The shelf life of the treated and control meatball samples stored at 4C is given in Table 1. As can be seen from Table 1, in all meatball samples, the total aerobic bacteria counts reached at the end of the shelf life were higher than 7 $\log_{10}$ cfu/g, which is considered a spoilage level for this type of product (Fernandez-Lopez *et al.* 2006). The combination of Lf (200 μg/g) and nisin (100 μg/g) prolonged the refrigerated shelf life of the samples to 10 days, compared to 3 days for the nontreated control.

The average counts of total mesophilic aerobic bacteria, LAB, coliform, *E. coli*, total staphylococcae, *S. aureus*, total psychrophilic bacteria, *Pseudomonas* spp., sulfite-reducing anaerobic bacteria, and yeast and mold were determined as 6.11, 5.68, 4.28, 2.49, 5.54, 3.73, 5.87, 5.23, 2.78 and 5.32 $\log_{10}$ cfu/g, respectively, in the meatball dough. No *L. monocytogenes* and *Salmonella* spp. were detected in the meatball dough.

The effect of nisin and Lf on the total aerobic mesophilic counts in the experimental Turkish meatball (tekirdağ köfte) samples is given in Fig. 1. In the control group, the number of total bacteria increased from the initial level of 6.11 to the level of 9.89 $\log_{10}$ cfu/g in 12 days. During 12 days, the total bacteria counts of the samples were lower in all treatments except in group II (100 μg/g nisin), when compared with the control ($P < 0.05$). No significant difference ($P < 0.05$) was observed in the total plate counts between group II and the control samples. The combination of Lf (200 μg/g) and nisin (100 μg/g) was the most effective treatment ($P < 0.001$), the number of total bacteria being 7.49 $\log_{10}$ cfu/g, while the counts varied from 8.25 to 8.64 $\log_{10}$ cfu/g in other treatments after 12 days.

The changes of LAB counts are shown in Fig. 2. The initial LAB count of the groups was 5.68 $\log_{10}$ cfu/g. The number of LAB increased to about 7–8 $\log_{10}$ cfu/g after 7 days, and remained nearly constant at this level on the 10th day in all groups. The counts on the 12th day varied from 7.70 to 8.65 $\log_{10}$ cfu/g in the groups (Fig. 3). No differences ($P < 0.05$) were determined in the LAB counts between the control and treated groups.

In a study performed by Chun Hui and Chun Chin (2005), the effect of Lf in Chinese sausages was evaluated, and they found that the Lf-treated

TABLE 1.
SHELF LIFE OF THE TREATED AND CONTROL TURKISH-STYLE MEATBALL (TEKIRDAĞ KÖFTE) SAMPLES STORED AT 4C

Group	Shelf life (days)	pH	Mean microorganism counts at the end of the shelf life (cfu/g)					
			Total aerobic bacteria	Total psychrophilic bacteria	*Pseudomonas* spp.	Lactic acid bacteria	Total staphylococcae	Yeast and mold
Control	3	6.38	3.5×10^7	8.1×10^6	1.3×10^6	3.8×10^6	4.4×10^6	8.0×10^6
I	7	6.37	4.1×10^7	1.7×10^5	8.8×10^4	1.8×10^7	5.8×10^6	4.4×10^3
II	5	6.39	4.4×10^7	9.0×10^7	7.9×10^7	3.7×10^6	1.7×10^4	1.8×10^7
III	7	6.34	3.4×10^7	1.2×10^5	7.5×10^4	1.2×10^7	5.8×10^5	4.6×10^3
IV	10	6.32	5.8×10^7	4.7×10^2	3.1×10^2	5.4×10^7	1.2×10^6	7.4×10^2
V	7	6.35	4.2×10^7	6.1×10^4	4.6×10^4	2.6×10^7	6.4×10^2	3.1×10^3

Standard errors ranged from 0.05 to 0.82.
Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g.

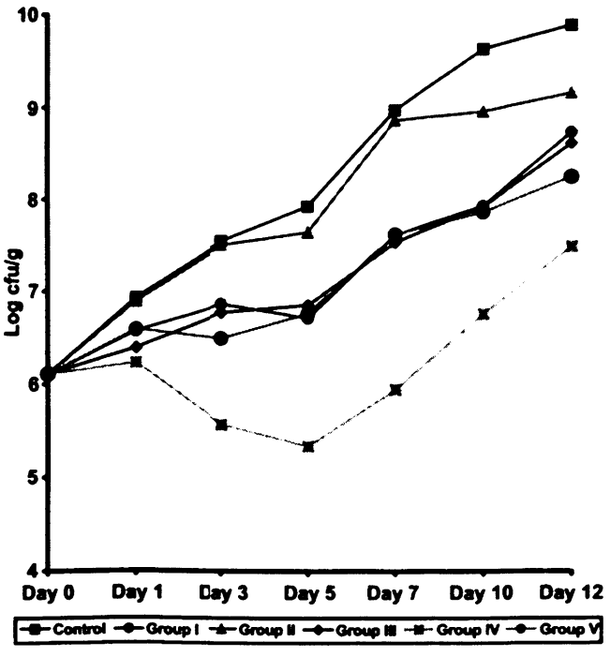

FIG. 1. TOTAL AEROBIC PLATE COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.03 to 0.96.

(40 mg/kg) group had lower total plate and LAB counts than the control at 0 and 15 days, but the differences were not significant at 30, 45 and 60 days. On the other hand, Venkitanarayanan *et al.* (1999) stated that no significant difference was observed in the total plate count between lactoferricin (pepsin digested of Lf)-treated (100 μg/g) and control ground beef samples.

In our country, in a study conducted by Hampikyan and Ugur (2007), the effect of nisin in Turkish fermented sausage (sucuk) was searched. It was found that no significant differences were determined in the total bacteria and LAB counts between the control and nisin-added (5, 10, 25, 50 and 100 μg/g) groups. In a similar study performed by Wang (2000), it was reported that nisin had no effect on the total aerobic mesophilic bacteria and LAB counts in Chinese-style sausages. Similarly, Nykanen *et al.* (2000) stated that different nisin concentrations did not affect the total aerobic mesophilic bacteria counts on cold, smoked rainbow trout.

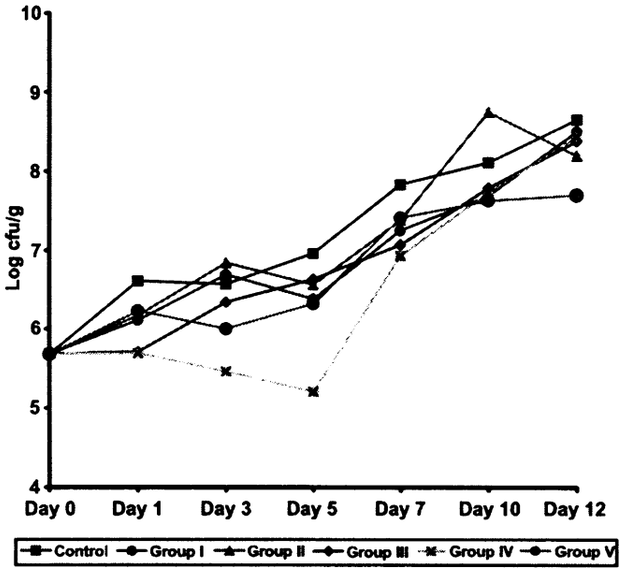

FIG. 2. LACTIC ACID BACTERIA COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.04 to 0.88.

The effect of nisin and Lf on the coliform and *E. coli* counts in the meatball samples is given in Figs. 3 and 4. The initial coliform and *E. coli* counts were 4.28 and 2.49 $\log_{10}$ cfu/g, respectively; and in the control group, the counts increased to 7.63 and 4.89 $\log_{10}$ cfu/g after 12 days. Addition of Lf (groups I, III, IV and V) significantly ($P < 0.05$) reduced the initial coliform and *E. coli* counts when compared with the control and group II. However, the inhibitory effect was more pronounced ($P < 0.001$) in group IV containing 200 μg/g of Lf than in groups I, III and V (100 μg/g Lf). Lf treatments (100 and 200 μg/g) reduced the coliform counts in the samples by about 4.0 and 6.2 log units after 5 days (Fig. 3). As can be seen from Fig. 4, no *E. coli* surviving cells were detected in group IV at day 3. However, the counts on the 12th day in groups I, II, III and V were 1.91, 4.71, 1.66 and 1.83 $\log_{10}$ cfu/g, respectively. These data showed that group IV (200 μg/g Lf–100 μg/g nisin) had a maximum effect on *E. coli* in meatball samples when compared with other treated groups ($P < 0.001$).

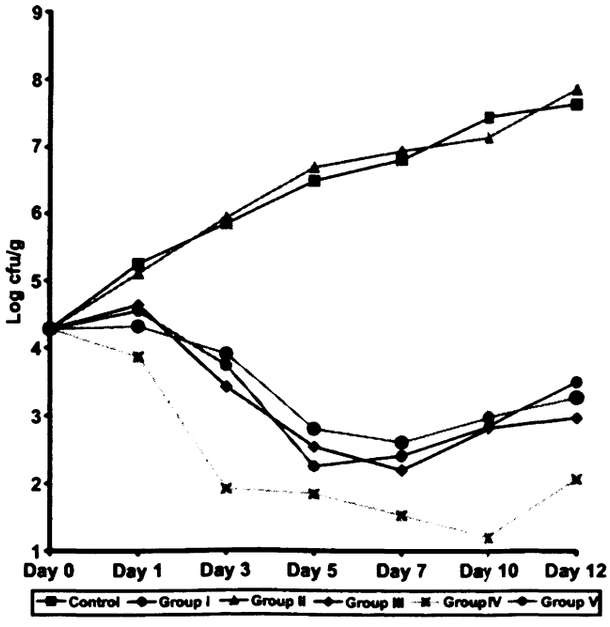

FIG. 3. COLIFORM COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.04 to 0.58.

The inhibition of *E. coli* by Lf recorded in the present study is in agreement with the findings of Batish *et al.* (1988) and Dionysius *et al.* (1993), who reported the *in vitro* antibacterial effect of Lf on strains of *E. coli* using a microassay for bacterial growth. Lf has been shown to interact with lipopolysaccharide (LPS) of the gram-negative bacterial membrane of *E. coli*, with the release of the LPS from the membrane (Hoek *et al.* 1997; Sallman *et al.* 1999; Farnaud and Evans 2003). In a study, Venkitanarayanan *et al.* (1999) reported that lactoferricin B (100 μg/g) reduced the *E. coli* O157:H7 population in ground beef by about 0.8 $\log_{10}$ cfu/g.

In this study, nisin (100 μg/g) alone had no significant effect on the growth of coliform and *E. coli* ($P < 0.05$). Similar results have been reported by other researchers. Lemay *et al.* (2002) found that nisin (0.1%) had no significant effect on the growth of *E. coli* when compared with the control in acidified chicken meat model. Branen and Davidson (2000) also stated that

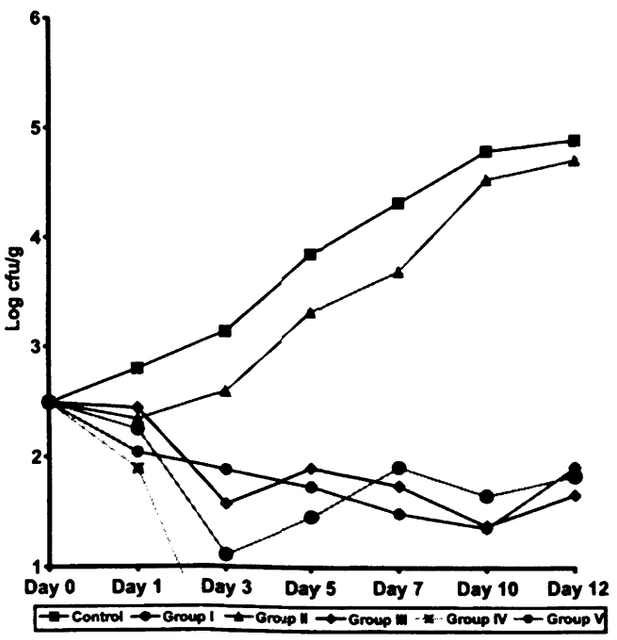

FIG. 4. *ESCHERICHIA COLI* COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.01 to 0.42.

nisin did not inhibit gram-negative bacteria. The poor preservative effect of nisin in meat products has been attributed to possible interference by meat phospholipids, the binding of nisin on meat components and surfaces, high bacterial loads, poor solubility, uneven distribution and possible breakdown by enzymes (Wang 2000; Lemay *et al.* 2002).

The effect of nisin and Lf on the total staphylococcae and *S. aureus* counts in the meatball samples is shown in Figs. 5 and 6. The initial total staphylococcae and *S. aureus* counts were 5.54 and 3.73 $\log_{10}$ cfu/g, respectively. The counts remained nearly constant at day 1 in all groups. After 5 days, the total staphylococcae and *S. aureus* counts in group I and in the control increased from the initial level to the level of 6.14–6.76 and 3.92–4.39 $\log_{10}$ cfu/g, respectively, while in groups II, III, IV and V, the numbers were significantly ($P < 0.05$) reduced by about 1.8–3.5 log units. The largest reduction (3.5 log units) in the total staphylococcae counts occurred in group

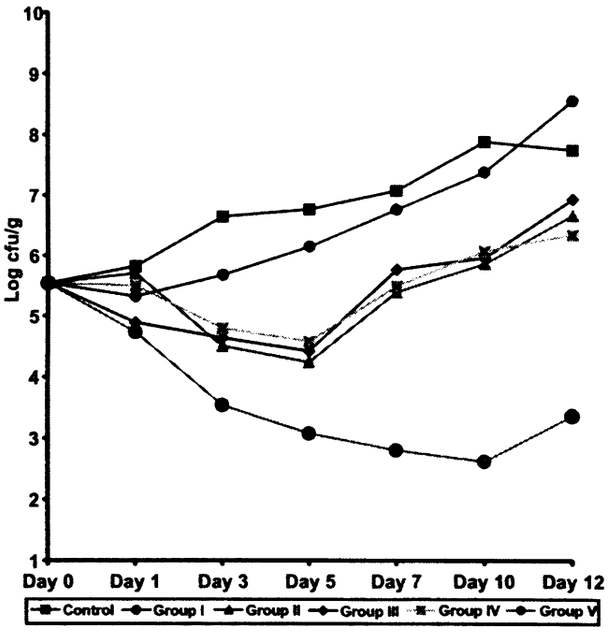

FIG. 5. TOTAL STAPHYLOCOCCAE COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.04 to 0.74.

V containing 200 μg/g nisin–100 μg/g Lf ($P < 0.001$). As can be seen from Fig. 6, the *S. aureus* population decreased steadily during 5 days and was inhibited at day 7 in group V ($P < 0.05$). These data showed that group V (200 μg/g nisin–100 μg/g Lf) had a maximum effect on *S. aureus* in the meatball samples. Lf (group I) alone was ineffective at inhibiting the growth of *S. aureus* and total staphylococcae ($P < 0.05$).

Masschalck *et al.* (2001) reported that *S. aureus* was completely inhibited by nisin (100 IU/mL). Similarly, Chung *et al.* (1989) stated that nisin had an inhibitory effect on gram-positive bacteria, especially *S. aureus* in meat. Nisin causes partial depolarization of *S. aureus* cytoplasmic membranes (Chung and Hancock 2000). Early experiments regarding the mode of action of nisin pointed to the cytoplasmic membrane as the biological target. Nisin disrupts membrane activity via pore formation, membrane insertion and simultaneous depolarization. The increase in membrane permeability disturbs the membrane

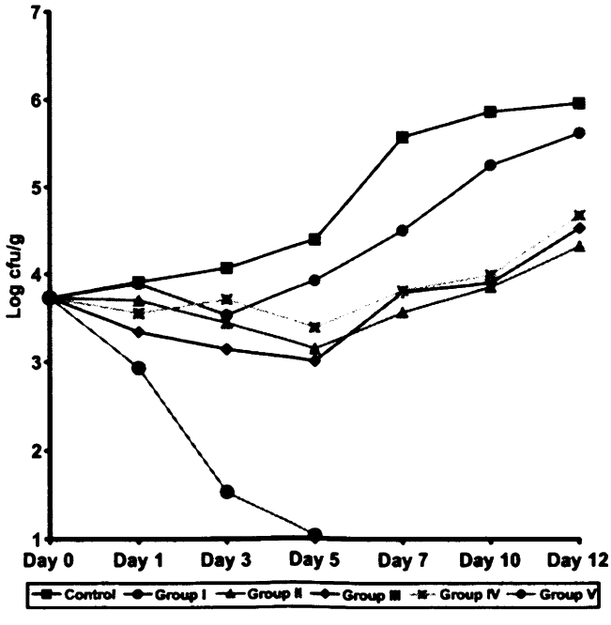

FIG. 6. *STAPHYLOCOCCUS AUREUS* COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.01 to 0.61.

transport, and inhibits the energy production and biosynthesis of proteins or nucleic acids (Bruno *et al.* 1992; Breukink *et al.* 1998; Raju *et al.* 2003).

The changes of psychrophilic bacteria and *Pseudomonas* spp. counts in the meatball samples are given in Figs. 7 and 8. The initial psychrophilic bacteria number was 5.87 log_{10} cfu/g. In the control and in group II, the counts increased steadily at the end of the test period (8.81–8.92 log_{10} cfu/g). At day 3, the counts in the control (6.90 log_{10} cfu/g) and in group II (6.44 log_{10} cfu/g) were higher than the counts in other treated samples (3.93–4.85 log_{10} cfu/g). After 5 days, significant reductions ($P < 0.05$) of 2.29, 2.22, 3.44 and 2.31 log_{10} cfu/g of the bacterial numbers in groups I, III, IV and V were respectively observed (Fig. 7).

The number of *Pseudomonas* spp. increased from the initial level of 5.23 to the level of 7.59–7.89 log_{10} cfu/g in the control and in group II, while the numbers were reduced by about 2.8–4.0 log units in other treated groups

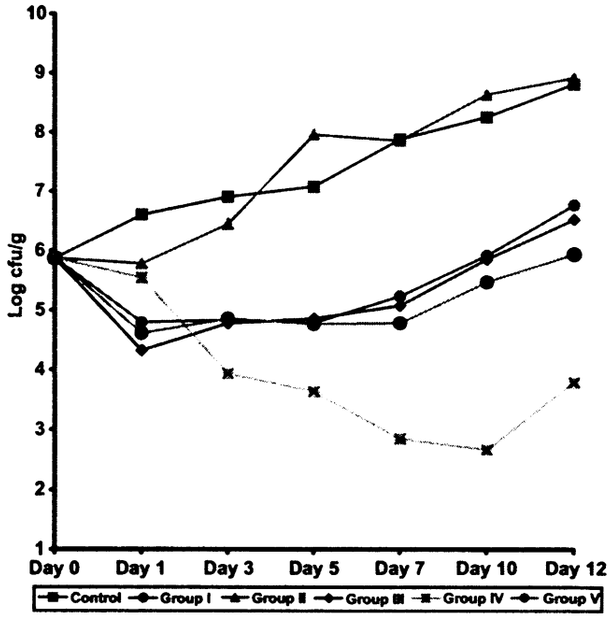

FIG. 7. PSYCHROPHILIC BACTERIA COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.03 to 0.53.

($P < 0.05$). After 5 days, the largest reduction (4.0 log units) in *Pseudomonas* spp. counts was observed in group IV (Fig. 8).

In a study, Masschalck *et al.* (2001) found that Lf (500 μg/L) significantly inhibited *Pseudomonas fluorescens* under high pressure. On the other hand, the authors stated that nisin alone may not be sufficient to prevent meat spoilage because of the presence of gram-negative and other nisin-resistant gram-positive bacteria. Similarly, Chung *et al.* (1989) reported that nisin did not have an inhibitory effect on *Pseudomonas aeruginosa* attached to meat.

No differences ($P < 0.05$) were determined in the anaerobic bacteria counts between the control and treated groups. At day 0, the anaerobic bacteria count of the groups was 2.78 $\log_{10}$ cfu/g. The number of bacteria increased to about 3.0–3.7 $\log_{10}$ cfu/g after 7 days, and remained nearly constant at this level on the 12th day in all groups. Wang (2000) reported that anaerobic counts increased during storage in nisin (100 mg/kg)-treated Chinese-style sausages.

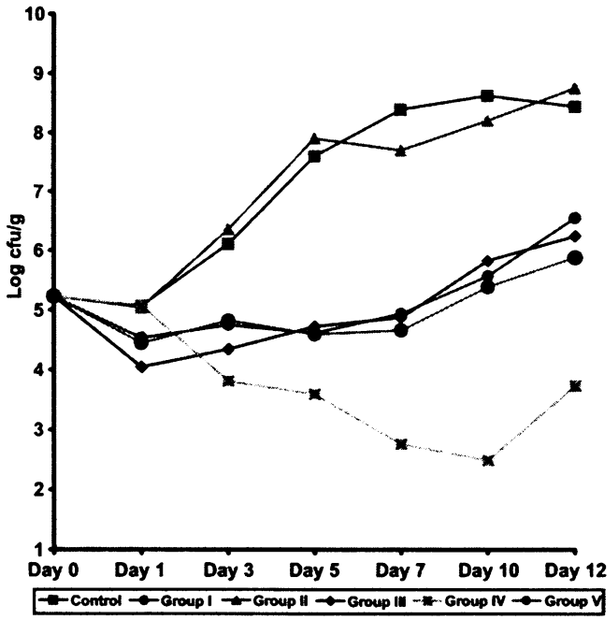

FIG. 8. *PSEUDOMONAS* SPP. COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.06 to 0.71.

The effect of nisin and Lf on the yeast and mold counts in the meatball samples is given in Fig. 9. The initial yeast and mold count was 5.32 $\log_{10}$ cfu/g. At day 3, the counts in the control (6.90 $\log_{10}$ cfu/g) and in group II (6.74 $\log_{10}$ cfu/g) were higher than the counts in other treated meatball samples (3.38–4.85 $\log_{10}$ cfu/g). After 5 days, 3.72, 4.24, 3.94 and 3.89 log units of reductions ($P < 0.05$) were respectively observed in groups I, III, IV and V (Fig. 9). On the other hand, the counts increased to 8.92 and 8.38 $\log_{10}$ cfu/g in the control and in group II at the end of the test period (day 12).

Several studies have shown that Lf has an antifungal activity (Kirkpatrick *et al.* 1971; Bellamy *et al.* 1993; Wakabayashi *et al.* 1996). In a research conducted by Liceaga-Gesualdo *et al.* (2001), the antimicrobial effect of Lf digest on spores of a *Penicillium* sp. was searched, and they found that the compound at concentrations of 60 and 300 μg/mL inhibited spore germination and mycelial growth for up to 9 and 21 days at 30C, respectively.

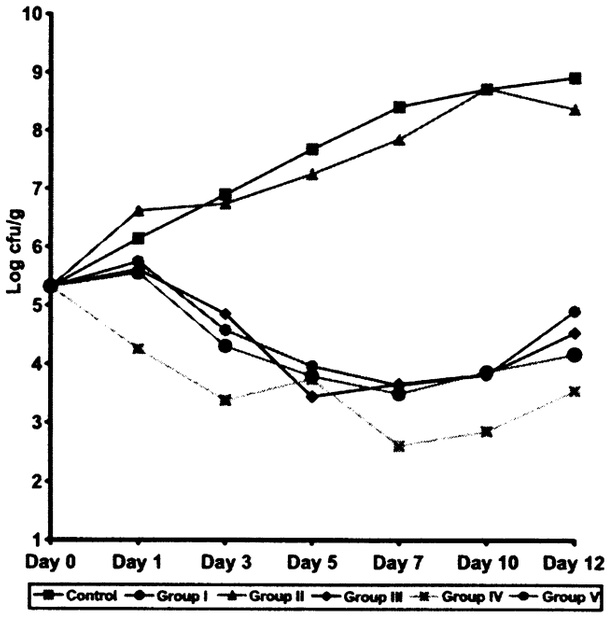

FIG. 9. YEAST AND MOLD COUNTS (LOG_{10} cfu/g) IN CONTROL AND IN TREATED MEATBALL GROUPS STORED AT 4C

Control, no treatment; group I, 100 μg lactoferrin (Lf)/g; group II, 100 μg nisin/g; group III, 100 μg Lf + 100 μg nisin/g; group IV, 200 μg Lf + 100 μg nisin/g; group V, 100 μg Lf + 200 μg nisin/g. The mean values were calculated from three different batches of meatball ($n = 3$). Standard errors ranged from 0.08 to 0.81.

The initial (day 0) pH was 6.0. The pH levels increased during storage time for all groups, reaching nearly similar values (6.32–6.39) at the end of the shelf life (Table 1). This increase has been observed in other meat products, such as burgers, and it has been attributed to proteolysis (Bond *et al.* 2001; Fernandez-Lopez *et al.* 2006). The pH of the meatball groups was not significantly affected ($P < 0.05$) by the addition of different nisin and Lf concentrations.

The A_w and moisture values observed in the treated samples are in accordance with the results obtained in the nontreated control group (mean A_w and moisture value: 0.945 and 42.90%). Nisin and Lf treatments had no effect on the A_w and moisture values of the meatball samples compared with the control ($P < 0.05$).

Sensory analyses revealed that the addition of nisin and Lf to meatballs did not cause any negative sensory changes in the product. No significant

difference ($P < 0.05$) was found between the control and treated samples for sensory characteristics evaluated. The general score ranged between 7.1 and 8.2 (results not shown).

In conclusion, the results of the present study demonstrate the benefits of using mixtures of Lf and nisin against food spoilage bacteria. Treatment with Lf alone and its combination with nisin significantly reduced the total aerobic bacteria, coliform, *E. coli*, total psychrophilic bacteria, *Pseudomonas* spp., and yeast and mold counts in the meatballs. The largest reduction in these counts occurred on the meatballs treated with a mixture of Lf (200 μg/g) and nisin (100 μg/g). This combination extended the refrigerated shelf life of naturally contaminated Turkish-style meatballs to 10 days.

ACKNOWLEDGMENT

This work was supported by the Research Fund of Istanbul University. Project Number 223/29042004.

REFERENCES

AL-NABULSI, A.A. and HOLLEY, R.A. 2006. Enhancing the antimicrobial effects of bovine lactoferrin against *E. coli* O157:H7 by cation chelation NaCl and temperature. J. Appl. Microbiol. *100*, 244–255.

BASKAYA, R., KARACA, T., SEVINC, I., CAKMAK, O., YILDIZ, A. and YORUK, M. 2004. Istanbul'da satisa sunulan hazir kiymalarin histolojik, mikrobiyolojik ve serolojik kalitesi. Yüzüncü Yil Üniversitesi Veteriner Fakültesi Dergisi *15*(1–2), 41–46.

BATISH, V.K., CHANDER, H., ZUMDEGNI, K.C., BHATIA, K.L. and SINGH, R.S. 1988. Antibacterial activity of lactoferrin against some common food-borne pathogenic organisms. *Aust. J. Dairy Technol. May*, 16–18.

BELLAMY, W., WAKABAYASHI, H., TAKASE, M., KAWASE, K., SHIMAMURA, S. and TOMITA, M. 1993. Killing of Candida albicans by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. Med. Microbiol. Immunol. *182*, 97–105.

BOND, J.M., MARCHELLO, M.J. and SLANGER, W.D. 2001. Physical, chemical and shelf-life characteristics of low-fat ground beef patties formulated with waxy hull-less barley. J. Muscle Foods *12*, 53–69.

BRANEN, J. and DAVIDSON, P.M. 2000. Activity of hydrolyzed lactoferrin against food-borne pathogenic bacteria in growth media: The effect of EDTA. Lett. Appl. Microbiol. *30*, 233–237.

BREUKINK, E., VAN KRAAIJ, C., VAN DALEN, A., DEMEL, R.A., SIEZEN, R.J., DE KRUIJFF, B. and KUIPERS, O.P. 1998. The orientation of nisin in membranes. Biochemistry *37*, 8153–8162.

BRUNO, M.E.C., KAISER, A. and MONTVILLE, T.J. 1992. Depletion of proton motive force by nisin in *L. monocytogenes* cells. Appl. Environ. Microbiol. *58*(7), 2255–2259.

CHUN HUI, C. and CHUN CHIN, K. 2005. Effect of lactoferrin on microbial quality and lipid oxidation of Chinese sausages. Taiwanese J. Agric. Chem. Food Sci. *43*(5), 325–334.

CHUNG, K.H., DICKSON, J.S. and CROUSE, J.D. 1989. Effects of nisin on growth of bacteria attached to meat. Appl. Environ. Microbiol. *55*(6), 1329–1333.

CHUNG, W. and HANCOCK, R.E.W. 2000. Action of lysozyme and nisin mixtures against lactic acid bacteria. Int. J. Food Microbiol. *60*, 25–32.

DE MARTINEZ, Y.B., FERRER, K. and SALAS, E.M. 2002. Combined effects of lactic acid and nisin solution in reducing levels of microbiological contamination in red meat carcasses. J. Food Prot. *65*(11), 1780–1783.

DIONYSIUS, D.A. and MILNE, J.M. 1997. Antibacterial peptides of bovine lactoferrin: Purification and characterization. J. Dairy Sci. *80*, 667–674.

DIONYSIUS, D.A., GRIEVE, P.A. and MILNE, J.M. 1993. Forms of lactoferrin: Their antibacterial effect on enterotoxigenic *Escherichia coli*. J. Dairy Sci. *76*, 2597–2606.

DUFOUR, M., SIMMONDS, R.S. and BREMER, P.J. 2003. Development of a method to quantify in vitro the synergistic activity of natural antimicrobials. Int. J. Food Microbiol. *85*, 249–258.

FARNAUD, S. and EVANS, R.W. 2003. Lactoferrin – a multifunctional protein with antimicrobial properties. Mol. Immunol. *40*, 395–405.

FERNANDEZ-LOPEZ, J., JIMENEZ, S., SAYAS-BARBERA, E., SENDRA, E. and PEREZ-ALVAREZ, J.A. 2006. Quality characteristics of ostrich (*Struthio camelus*) burgers. Meat Sci. *73*, 295–303.

GOKMEN, M. and ALISARLI, M. 2003. Van ilinde tüketime sunulan kiymalarin bazi patojen bakteriler yönünden incelenmesi. Yüzüncü Yil Üniversitesi Veteriner Fakültesi Dergisi *14*(1), 27–34.

HAMPIKYAN, H. and UGUR, M. 2007. The effect of nisin usage on *L. monocytogenes* in fermented sausages. Meat Sci. *76*, 327–332.

HARRIGAN, W.F. 1998. *Laboratory Methods in Foods Microbiology*, Academic Press Ltd., London, UK.

HOEK, K.S., MILNE, J.M., GRIEVE, P.A., DIONYSIUS, D.A. and SMITH, R. 1997. Antibacterial activity of bovine lactoferrin derived peptides. Antimicrob. Agents Chemother. *41*(1), 54–59.

IUCCI, L., PATRIGNANI, F., VALLICELLI, M., GUERZONI, M.E. and LANCIOTTI, R. 2007. Effects of high pressure homogenization on the activity of lysozyme and lactoferrin against *Listeria monocytogenes*. Food Control *18*, 558–565.

KIRKPATRICK, C.H., GREEN, I., RICH, R.R. and SCHADE, A.L. 1971. Inhibition of growth of Candida albicans by iron-unsaturated lactoferrin: Relation to host-defence mechanisms in chronic mucocutaneous candidiasis. J. Infect. Dis. *124*, 539–544.

KUPLULU, O., SARIMEHMETOGLU, B. and ORAL, N. 2003. The microbiological quality of Çiğ Köfte sold in Ankara. Turkish J. Vet. Anim. Sci. *27*, 325–329.

LEMAY, M.J., CHOQUETTE, J., DELAQUIS, P.J., GARIEPY, C., RODRIGUE, N. and SAUCIER, L. 2002. Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. Int. J. Food Microbiol. *78*, 217–226.

LICEAGA-GESUALDO, A., LI-CHAN, E.C.Y. and SKURA, B.J. 2001. Antimicrobial effect of lactoferrin digest on spores of a *Penicillium* sp. isolated from bottled water. Food Res. Int. *2001*(34), 5001–5006.

MASSCHALCK, B., VAN HOUDT, R. and MICHIELS, C.W. 2001. High pressure increases bactericidal activity and spectrum of lactoferrin, lactoferricin and nisin. Int. J. Food Microbiol. *64*, 325–332.

MURDOCK, C.A. and MATTHEWS, K.R. 2002. Antibacterial activity of pepsin digested lactoferrin on food borne pathogens in buffered broth systems and ultrahigh temperature milk with EDTA. J. Appl. Microbiol. *93*, 850–856.

NYKANEN, A., WECKMAN, K. and LAPVETELAINEN, A. 2000. Synergistic inhibition of *L. monocytogenes* on cold-smoked rainbow trout by nisin and sodium lactate. Int. J. Food Microbiol. *61*, 63–72.

PAWAR, D.D., MALIK, S.V.S., BHILEGAONKAR, K.N. and BARBUDDHE, S.B. 2000. Effect of nisin and its combination with sodium chloride on the survival of *L. monocytogenes* added to raw buffalo meat mince. Meat Sci. *56*, 215–219.

PAYNE, K.D., OLIVER, S.P. and DAVIDSON, P.M. 1994. Comparison of EDTA and apo-lactoferrin with lysozyme on the growth of foodborne pathogenic and spoilage bacteria. J. Food Prot. *57*(1), 62–65.

RAJU, C.V., SHAMASUNDAR, B.A. and UDUPA, K.S. 2003. The use of nisin as a preservative in fish sausage stored at ambient and refrigerated temperatures. Int. J. Food Sci. Technol. *38*, 171–185.

REUNANEN, J. and SARIS, P.E.F. 2004. Bioassay for nisin in sausage: A shelf life study of nisin in cooked sausage. Meat Sci. *66*, 515–518.

SALAMAH, A.A. and AL-OBAIDI, A.S. 1995. Effect of some physical and chemical factors on the bactericidal activity of human lactoferrin and transferring against *Yersinia pseudotuberculosis*. New Microbiol. *18*, 275–282.

SALLMAN, F.R., DESCAMPS, S.B., PATTUS, F., SALMON, V., BRANZA, N., SPIK, G. and LEGRAND, D. 1999. Porins OmpC and PhoE of *E. coli* as specific cell-surface targets of human lactoferrin. J. Biol. Chem. *274*(23), 16107–16114.

SAMELIS, J., BEDIE, G.K., SOFOS, J.N., BELK, K.E., SCANGA, J.A. and SMITH, G.C. 2005. Combinations of nisin with organic acids or salts to control *L. monocytogenes* on sliced pork bologna stored at 4°C in vacuum packages. Lebensm.-Wiss. Technol. *38*, 21–28.

SANCAK, Y.C., BOYNUKARA, B. and AGAOGLU, S. 1993. Van'da tüketime sunulan kiymalarin mikrobiyolojik kalitesi. Yüzüncü Yil Üniversitesi Veteriner Fakültesi Dergisi *4*(1–2), 73–86.

SHIMAZAKI, K. 2000. Lactoferrin: A marvelous protein in milk. Anim. Sci. J. *71*, 329–347.

ULU, H. 2006. Effects of carrageenam and guar gum on the cooking and textural properties of low fat meatballs. Food Chem. *95*, 600–605.

VENKITANARAYANAN, K.S., ZHAO, T. and DOYLE, M.P. 1999. Antibacterial effect of lactoferricin B on *E. coli* O157:H7 in ground beef. J. Food Prot. *62*(7), 747–750.

WAKABAYASHI, H., HIRATANI, T., UCHIDA, K. and YAMAGUCHI, H. 1996. Antifungal spectrum and fungicidal mechanism of an N-terminal peptide of bovine lactoferrin. J. Infect. Chemother. *1*, 185–189.

WANG, F.H. 2000. Effects of three preservatives agents on the shelf life of vacuum packaged Chinese-style sausage stored at 20°C. Meat Sci. *56*, 67–71.

YILMAZ, I., YETIM, H. and OCKERMAN, H.W. 2002. The effect of different cooking procedures on microbiological and chemical quality characteristics of Tekirdağ meatballs. Nahrung *46*(4), 276–278.

YILMAZ, I., ARICI, M. and GUMUS, T. 2005. Changes of microbiological quality in meatballs after heat treatment. Eur. Food Res. Technol. *221*, 281–283.