THE EFFECT OF NISIN ON LISTERIA MONOCYTOGENES IN CHICKEN BURGERS

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ABSTRACT

In this study, the effect of nisin on the growth and survival of L. monocytogenes has been investigated in chicken burger samples. Chicken burger samples inoculated with L. monocytogenes (ATCC 7644) (L.m.) at the levels of 4 log cfu/g and 6 log cfu/g and were added nisin at the amounts of 25 µg/g, 50 µg/g and 100 µg/g to form eight experimental groups (C-1: 4 log cfu/g L.m., A-1: 4 log cfu/g L.m. + 25 ppm nisin, A-2: 4 log cfu/g L.m. + 50 ppm nisin, A-3: 4 log cfu/g L.m. + 100 ppm nisin, C-2: 6 log cfu/g L.m., B-1: 6 log cfu/g L.m. + 25 ppm nisin, B-2: 6 log cfu/g L.m. + 50 ppm nisin, B-3: 6 log cfu/g L.m. + 100 ppm nisin). No nisin was added into the control group. They were stored at 4°C for 7 days. At the present study, although the amounts of nisin used did not totally inhibit L. monocytogenes, the numbers of L. monocytogenes were reduced average 1-2 log cfu/g in the experimental chicken burgers. Therefore, it might be necessary to explore other different amounts of nisin to ensure possibilities of using nisin in meat and meat products.

Key words: Chicken, Chicken Burgers, Listeria monocytogenes, Nisin.

INTRODUCTION

Poultry meat is a very popular product in Western countries (Bruhn, 1994). Poultry products are consumed widely because of their relatively low cost, low fat content, and short preparation time. Even though poultry meat is considered relatively healthy and economical, it is also a common source of pathogenic bacteria, because contamination can occur during slaughter and production, and postprocessing contamination (Kampelmacher, 1987).

Listeria monocytogenes is one of the most common food-borne pathogens (Farber and Peterkin, 1991; Swaminathan, 2001) in raw poultry meat and poultry products all over the world (Kaczmarski and Jones, 1989; Schuchat et al., 1991). The epidemiological data collected from chicken meat products in Turkey revealed that, such as frozen broiler carcasses, minced chicken meat, chicken meatballs, and chicken burgers, were contaminated with Listeria spp. at the percentages of 17%, 20%, 20-35%, 38%, 76%, and 94% as reported by Çiftçioglu (1992), Çolak et al. (2008), Sireli et al. (2002), Akkaya et al. (2005), Özmen and Kılıç (2006), and Erol and Sireli (1999), respectively.

Food-borne outbreaks due to Listeria monocytogenes present considerable threats to public health because of the outbreaks' high mortality rates and their wide distribution across raw products. Moreover, the bacteria can grow at low temperatures and can establish colonies in various food products and food-processing environments (Muriana, 1996). Therefore, the decontamination of carcasses and inhibition of Listeria spp. in poultry and other meat products have been the focus of many studies over a long period of time. Recently, food legislations regarding L. monocytogenes became tight, requiring the total absence of L. monocytogenes in every 25-g or 25-ml food sample. To meet this requirement, researchers have explored various methods and antibacterial agents.

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Nisin, a bacteriocin, produced from Lactococcus lactis subsp. lactis (Delves et al., 1996). It has been used as an antibacterial food additive since 1960s (Delves et al., 1996), and it is still the only bacteriocin used as a preventive food additive in many countries, including China, Brazil, USA, and Europe (Hurst, 1981; Papagianni, 2003). Nisin is considered as a safe substance and has been approved for use in food (Frazer et al., 1962; FDA, 1988). Nisin intake as higher amounts is not cause any toxic effects (ADI level of Nisin is 0.13 mg/kg) (Hagiwara et al., 2010). Nisin exhibited antibacterial effect against L. monocytogenes and other gram-positive organisms. (Singh et al., 2001; Mahadeo and Tatini, 1994; Ming et al., 1997). The efficacy of bacteriocins is concentration dependent, endogenous enzymes and other meat and non meat additives (Horbaek et al., 2006). Therefore, it is imperative to evaluate its effectiveness in-vitro conditions.

Preliminary work was conducted to determine the concentration of inoculum needed to yield 4 to 6 log cfu/g on the chicken burger samples. Therefore this study investigates the effect of nisin on the growth and survival of L. monocytogenes at the levels of 4 log cfu/g and 6 log cfu/g in chicken burger samples.

**MATERIAL AND METHODS**

**L. monocytogenes strain and preparation of inoculations:** In this study was used referances strain of L. monocytogenes (ATCC 7644). Experimental inoculations of the L. monocytogenes refers strain were prepared according to the methods of Nolte (1982) and Alibarly (1997), and chicken burger meat batter mixture parts were inoculated at the levels of 4 log cfu/g and 6 log cfu/g.

**Preparation of nisin solution:** Nisin (Sigma, N5764) solution was prepared in 0.02 N HCl by following the method of Schillinger et al. (2001).

**Preparation of chicken burger samples:** Poultry minced meat, fat and additives were supplied form local poultry company in Afyonkarahisar, Turkey. Chicken burgers (80.00% lean meat, 10.00% fat, 7.00 % bread crumbs and 3.00 % additives) were made in the Veterinary Faculty laboratory plant. Spices used were ground black pepper (0.4%), red pepper (0.3%) and cumin (0.3%); salt (2%) in the formulation. The mix was kneaded for 10 min by hand and the chicken burger dough was divided into eight equal batches (2 kg each).

**RESULTS AND DISCUSSION**

This study investigated whether nisin inhibits the development and survival of L. monocytogenes at the levels of 4 log cfu/g (Group A) and 6 log cfu/g (Group B). Various doses of nisin (25 ppm, 50 ppm, and 100 ppm for groups 1, 2, and 3, respectively) were introduced to all the treated samples of chicken burger samples. Chicken burger samples were inoculated with L. monocytogenes (L.m.) at the levels of 4 log cfu/g and 6 log cfu/g, and then nisin was added to the samples in the amounts of 25 µg/g, 50 µg/g, and 100 µg/g to form eight experimental groups (C-1: 4 log cfu/g L.m.; A-1: 4 log cfu/g L.m. + 25 ppm nisin; A-2: 4 log cfu/g L.m. + 50 ppm nisin; A-3: 4 log cfu/g L.m. + 100 ppm nisin; C-1: 6 log cfu/g L.m.; B-1: 6 log cfu/g L.m. + 25 ppm nisin; B-2: 6 log cfu/g L.m. + 50 ppm nisin; and B-3: 6 log cfu/g L.m. + 100 ppm nisin). No nisin was added to the C-1 or C-2 control group. All samples were stored at 4°C for 7 days.

**Microbiological analysis:** The chicken burgers were initially examined for the presence of Listeria monocytogenes before inoculation. Subsequently, samples were taken on days 1, 3, and 7, and were analysed in terms of L. monocytogenes, Standart Plate Count (SPC), and lactobacilli counts. For the analysis, each 10-g sample of chicken burger was homogenised with 90 ml of sterile peptone serum physiological saline (0.85% NaCl + 0.1% peptone) for 2 min. using a stomacher (Interscience, UK). After diluting the samples at a scale of 1:10, decimal solutions were prepared up to 10⁻⁷. The prepared dilutions were enumerated on a Plate Count Agar (PCA, Oxoid, CM325) for SPC at 30°C for 72 h, MRS Agar (Oxoid, CM 361) for Lactobacillus at 30°C for 48 h (Pichhardt, 1993), and a MOX (Modified Oxford Agar) for L. monocytogenes at 37°C for 48 h (Porto-Fett et al., 2008).

**Chemical analysis:** The pH values of the sampled chicken burgers were measured by means of pH metre (InoLab pH 720 model, Germany), based on method as prescribed to ISO 2917 (2002), and their moisture was determined according to ISO 1442 (1997). All measurements were performed in duplicate.

**Statistics analysis:** The data obtained from two replications were analysed by ANOVA using the SPSS statistical package program and differences among the means were compared using Duncan’s Multiple Range test.
burger (A-1, A-2, A-3, B-1, B-2, and B-3) except for the controls (C-1 and C-2). In the group C-1, L. monocytogenes increased from 4.07 log cfu/g to 5.60 log cfu/g while the pH of C-1 was in the range of 6.68 to 4.53 (p<0.05) (Table 1). In contrast, it decreased from 4 log cfu/g to 3 log cfu/g at the 1st day in groups A-1, A-2 and A-3 (p<0.05). Then, the level in A-3 decreased to 2 log cfu/g at the 7th day (p<0.05). The L. monocytogenes level in group C-2 did not change while the pH was in the range of 6.77 to 4.54. However, they did decrease from 6 log cfu/g to 5 log cfu/g at the 1st day in groups B-1, B-2, and B-3 (p<0.05). These levels continued to decrease until they reached 4 log cfu/g at the 3rd day in groups B-2 and B-3 (p<0.05). Finally, group B-3 exhibited a further decrease to the level of 3 log cfu/g at the 15th day (p<0.05) (Table 2).

Ruiz et al. (2009) inoculated ready-to-eat diced turkey ham samples with a cocktail of 5 strains of L. monocytogenes, treated the samples with 0.5% of nisin, and then vacuum-packaged and stored them for 63 days at 4 °C ± 1 °C. The nisin reduced the L. monocytogenes by 4.42 log cfu/g when compared to the positive control. On day 0, the L. monocytogenes counts remained less than 2.75 log cfu/g for the turkey ham samples treated with nisin. Although the 0.5% nisin treatment did not completely eliminate L. monocytogenes, the low levels indicate that nisin can reduce significantly the counts of L. monocytogenes in ready-to-eat turkey ham samples under similar conditions. In a recent study, the same team of researchers (Ruiz et al., 2010) treated ready-to-eat diced turkey ham samples with 0.2, 0.3, 0.4, and 0.5% nisin solutions. They concluded that nisin could be used as a post-processing intervention to control L. monocytogenes in ready-to-eat poultry products, because they encountered very similar reduction rates (4 log cfu/g), although they encountered them on different days of storage. The level of L. monocytogenes remained less than 2 log cfu/g through the 63 days of storage. In another study, Soriano et al. (2004) added 0.15% nisin and 1.5% (w/w) lacticin 3147 to minced pork meat. The meat was cooked and inoculated with a L. innocua strain at the counts of 7 and 5 logcfu/g. The batches were stored in a vacuum package for 21 days at 8 °C. Nisin and lactic in 3147 immediately reduced the L. innocua population at the time of inoculation. Nisin showed higher inhibitory activity than did lactic in 3147. During the storage period, a slight growth of L. innocua was observed in the batches that had been inoculated with the larger inoculum, and a bacteriostatic effect was observed against Listeria in the batches inoculated with 5 log cfu/g.

### TABLE 1: Behavior of L. monocytogenes (4 log cfu/g) in chicken burgers*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>pH</th>
<th>Humidity (%)</th>
<th>Listeria monocytogenes</th>
<th>SPC **</th>
<th>Lactobacillus spp.</th>
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<td>C-1</td>
<td>0</td>
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<td>65.22</td>
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<td>4.90</td>
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<td></td>
<td>3</td>
<td>6.68</td>
<td>62.23</td>
<td>4.25</td>
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<tr>
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<td>7</td>
<td>5.92</td>
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<td>5.07</td>
<td>8.38</td>
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<td>4.07</td>
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</tr>
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<td>2.47</td>
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<td>5.07</td>
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</table>

C-1: Control; A-1: 25 ppm nisin; A-2: 50 ppm nisin; A-3: 100 ppm nisin; * n:6; ** Standart Plate Count; a-e Means in a same column with different letters are significantly different (p<0.05);
Nisin has many advantages over other food preservatives, such as its non-toxicity, immediate digestibility by the enzyme α-chymotrypsin, heat stability at a low pH, and absence of colour and flavour (Pongtharangkul and Demirci, 2004). For decades, nisin has been observed to be effective. For example, Chung et al. (1989) found that 10^5 IU/ml of nisin delayed bacterial growth on meats that they had artificially inoculated with L. monocytogenes. During the meats’ two weeks of storage at 5 °C, the nisin caused 2-4 log cfu/4 cm² reduction in the numbers of L. monocytogenes. Although Reunanen and Saris (2004) concluded that nisin can tolerate the cooking process of sausage and has a reasonably long shelf life in cold-stored cooked sausages, making it a useful food preservative, other researchers have asserted that the use of nisin in meat as a preservative has resulted in little success because of interference by meat components, such as phospholipids and high pH. Under such conditions, nisin becomes far less soluble, indicating the limitation of nisin application (O’Sullivan et al., 2002; Liu and Hansen, 1990; Aasen et al., 2003; Rose et al., 2002, Yonema et al., 2004).

Günes and Çibik (2002) reported that the negöl meatball samples they had contaminated experimentally with L. monocytogenes and then inoculated with 50 μg/g nisin resulted in a 3 log cfu/g reduction in the numbers of L. monocytogenes, whereas 25 μg/g nisin resulted in a 1 log cfu/g decrease in the numbers of L. monocytogenes. The addition of nisin at the levels of 1 and 5 μg/g did not cause any reduction in the numbers of L. monocytogenes. The results of this study were similar to the results reported by Ko et al. (2001) and Pawar et al. (2000). Hampikyan and Ugur (2007) experimentally contaminated samples of Turkish fermented soujouk (fermented sausage) with L. monocytogenes at the 6 log cfu/g level, and tested 5, 10, 25, 50, and 100 μg/g of nisin on various groups. The numbers of L. monocytogenes reduced further as the concentration of nisin increased, which is similar to the results reported by Ko et al. (2001) and Pawar et al. (2000). Hampikyan and Ugur (2007) noted a 1-to-3 log cfu/g increase in the 5-to-25 μg/g nisin groups after 30 days, respectively. They also found that the numbers of L. monocytogenes were higher in the control and in the 5 μg/g nisin groups than they were in the other groups on the storage days of 20 and 25, respectively. However, the groups with 50 and 100 μg/g of nisin experienced significant L. monocytogenes inhibition (P<0.001) on days 20 and 25 during storage, respectively.
commonalities with the results of Hampikyan and Üșür (2007). Likewise, the amounts of nisin used in this study did not totally inhibit L. monocytogenes, yet they did reduce the numbers of L. monocytogenes 1-2 log cfu/g in the experimental chicken burger samples. It has been reported that the inhibitory activity of nisin against L. monocytogenes in minced beef meat was found to depend on the storage temperature, the strain of L. monocytogenes used, and its supplementation level, since 500 IU/g of nisin showed no or a weak inhibition activity, whereas the addition of 1000 IU/g resulted in significantly lower (P< 0.05) populations of all examined strains of L. monocytogenes compared to the controls at both 4 °C and 10 °C (Solomakos et al., 2008). The results from Aasen et al. (2003) also indicate that considerably more nisin was needed than sakacin P to control the growth of L. monocytogenes in almost all instances. In addition, Reunanen and Saris (2004) stated that the amount of nisin in the meat must be rather high, even when used in conjunction with nitrate, to remain in the meat for a substantial period of time.

**CONCLUSION**

The use of nisin has been proven to be very effective in preserving microbiological quality of chicken burger. The nisin adding was reduced L. monocytogenes counts as 1-2 log cfu/g. It is important to note that the L. monocytogenes numbers of chicken burger very important for consumer health. It could be concluded that level of nisin might be good candidates for use in chicken burger in order to extend safety of meat and poultry products. However, another study could also be designed to investigate the effects of different level of nisin on L. monocytogenes counts and the other food borne pathogens.

**REFERENCES**


