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INHIBITORY EFFECT OF NISIN ON STAPHYLOCOCCUS AUREUS AND LISTERIA MONOCYTOGENES IN MINCED BEEF

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SUMMARY

Twenty minced beef samples collected from Cairo and Giza markets were examined for aerobic count, psychrotrophic count and incidence of *Staphylococcus aureus* and *Listeria monocytogenes* were determined to throw the light on their microbiological status. The inhibitory activity of nisin (500 IU /gm and 750 IU /gm) on Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) was also determined. Results showed that the mean log values of aerobes and psychrotrophic counts were 7.48 and 7.52/ gm respectively while incidence of *Staphylococcus aureus* and *Listeria monocytogenes* were 35% and 10% respectively. Application of nisin (500 IU /gm and 750 IU /gm) reduce *Staphylococcus aureus* count by 2.1, 2.6 and *Listeria monocytogenes* by 3.1, 3.6 log/gm respectively. Dramatic decrease was noticed within the first two days of refrigerating storage for both *Staphylococcus aureus* and *Listeria monocytogenes*.

INTRODUCTION

In recent years, environmental pollution and safety of processed foods have become a major issue of concern. Therefore, red meat processors are actively looking for reasonable interventions that minimize the risk of introducing undesirable microorganisms and bacterial pathogens from contaminated raw carcasses into processed meats (Surve et al., 1991).

Chemical preservatives have been used as additional barriers to limit the number of microorganisms capable to grow in foods, but customer preferences have led researchers to develop the use of natural inhibitors from plant, animal, and microbial sources (Shahidi et al., 1999).

In order to increase the food safety, new approaches such as using bacteriocinogenic lactic acid bacteria cultures and/or their bacteriocins to control pathogenic and spoilage microorganisms

have been developed (Rozbeh et al., 1993; Zhang and Mustapha, 1999).

Listeria monocytogenes and *Staphylococcus aureus* are unacceptable in foodstuffs, because of their pathogenicity. Existing methods of preservation may not be sufficient to preclude foodborne Listeriosis (Crandall and Montville, 1998). Therefore, the development of complementary methods to inhibit the growth of both pathogenic bacteria using bacteriocins can suppress effectively the growth of Gram-positive bacteria (Delves-Broughton et al., 1996). It is not suggested that bacteriocins be used as the primary barrier to control large numbers of undesirable microorganisms, but rather, to eliminate those few that may survive other processing steps.

Nisin, the most extensively studied bacteriocin, produced by Lactococcus lactis subsp. lactis. It is a 34-residue- long Lantibiotic that contains the unusual amino acid residues dehydrobutyrine, dehydroalanine, lanthionine and B-methylelanthionine. It has antimicrobial activity against a broad spectrum of Gram-positive bacteria such as *L. monocytogenes and Staph. aureus* (Schillinger et al., 1998). It has been shown to be a strong inhibitor of *L.monocytogenes* growth (Ennahar et al.,2000 and Mota-Meira et al., 2000). The mechanism of action of nisin involves binding to the peptidoglycan layer, causing destabilization of the membrane by the formation of pores which allow leakage of ions and dissipation of the proton motive force (Ruhr and Sahl, 1985).

Nisin has been shown to be non toxic and recognized as safe by the American Food and Drug Administration in 1969. It has widely been used in the food industries as a safe and natural preservative (Delves-Broughton et al., 1996).

Thus the objective of present investigation was designed to examine the market minced meat for microbial quality and to evaluate the effectiveness of nisin on growth of *Staphylococcus aureus* and *Listeria monocytogenes.*

MATERIAL AND METHODS

Twenty random samples of minced beef were collected from different markets in Cairo and Giza Governorates. Samples were examined bacteriologically for total aerobic count, psychrotrophic count, incidence of *Staphylococcus aureus* and *Listeria monocytogenes.*

1-Laboratory experimental work

1.1. Preparation of bacterial inoculums

Staphylococcus aureus strains and *Listeria monocytogenes* previously isolated from examined ground beef samples were maintained on tripticase soy agar at 4°C. Strains were grown separately in tryptose broth at 37°C for 18-24 hs. (Cells propagated to provide approximates concentration of 10^7 cfu/ml).

1.2. Experimental design

Meat samples which proved bacteriologically free from *Staphylococcus aureus* and *Listeria monocytogenes* 6 Kg were ground and divided into two portions (each weighing 3 Kg).

To the first portion 3 ml of washed *Staphylococcus aureus* cells 10^8 cfu/ml suspension were added and 3 ml of washed *Listeria monocytogenes* cell 10^8 cfu/ml suspension were added to the second portion. Each portion was mixed well in sterile stainless steel container then subdivided into 3 subsamples (each weighting 1 Kg), to the first subsample 1 gm of nisin was added to obtain a final concentration of 500 IU /gm and to the second sample 1.5 gm of nisin was added to make a final concentration of 750 IU /gm, while the third one was left untreated. After mixing, each treated and untreated sample was divided into 7 portions and held at 4°C for seven days and examined daily to determine the counts of both inoculated microorganisms.

2-Bacteriological examination of samples

-Preparing of samples, Total aerobic and Pyschrotrophic count were done according to APHA (1992) while isolation and identification of *Staphylococcus aureus* according to FDA (2001) and isolation and identification of *Listeria monocytogenes* according to FDA (2003).

Suspected colonies were picked up, stained by Gram's stain and examined microscopically to observe the morphological arrangement and staining reaction. Pure cultures of *Staphylococcus aureus* were identified biochemically according to Quinn et al. (1994), while *Listeria monocytogenes* were identified biochemically according to Parker and Collier (1990).

RESULTS AND DISCUSSION

The microbiological quality of raw material is the main factor influencing microbiological quality of the final product, and that contamination during processing has only a secondary role (Cartier, 1993).

Table (1) summarized the results of microbiological counts of the collected minced meat samples. Total aerobic counts ranged between 5.48 and 8.6 with a mean value of 7.48. Lower figures were reported by Samaha et al. (1992) while higher figures were reported by Mouse et al. (1993) and Vorster et al. (1994). Psychrotrophic count ranged between 5.7 and 8.95 with a mean value of 7.52. This high microbial load may be attributed to the differences in manufacture practices, handling and the effectiveness of hygienic measures applied during production. In this concern, Lunden et al. (2002) pointed out that the high microbial load may be due to the complexity of the machinery used in production which can hinder the cleaning process, with bacteria growing in corners and crevices that are difficult to clean.

Table (2) showed that the incidence of coagulase positive *Staphylococcus aureus* was 35 %. Similar result was reported by Ouf (2004). While *Listeria monocytogenes* was isolated from 10% of the samples. Lower result was reported by Hindy (2006). This had been explained by Thevenot et al. (2006) who stated that the inefficiency of cleaning procedures to remove *Listeria monocytogenes* has associated with its ability to adhere to stainless steel and form biofilms.

In the light of these observation, it is clear that traditional methods of preservation may not be adequate in controlling these food borne pathogens in a meat environment, and that the time has come to opt for a new generation of preservatives. In 1988 the US Food and Drug administration (FDA) affirmed nisin as GRAS for use as a direct ingredient in human food. The antimicrobial potential of nisin is considerably influenced by physical, chemical and microbial environments, various factors, such as composition of growth medium, age and size of inoculum, incubation temperature and pH of solutions containing nisin prior to its addition to the medium. (Rogers and Montville 1994).

The results obtained from Figure (1) in this study indicate that the use of nisin (500 IU/g and 750IU/g) increased protection against *Staphylococcus aureus* and resulted in reduction in its count during refrigeration storage by 2.1, 2.6 log 10cfu/g respectively, and the obvious reduction percent was within the first two days of refrigerating storage. Similar finding was reported by Millette et al. (2007) who mentioned that when nisin solution (500 or 1000 IU/g) was mixed with ground beef, 2.2 and 2.81 log 10cfu/g reduction of *Staphylococcus aureus* counts were respectively observed within 14 days of storage. Aideia et al. (2005) stated that reduction of *Staphylococcus aureus* treated with nisin (500 IU) reaches 2.0 log at the end of 4 weeks of storage at -18°C. Higher figures were reported by Scannell et al. (1997) who found that introduction of nisin (500 IU) to the sausage effected a dramatic decrease in *Staphylococcus* population within 24 hours, reducing levels from $4x10^6$ to $3.8x10^3$. The obvious reduction percent was within the first two days of refrigerating storage. The authors further added that reduction was observed over the test period (10 days at 4°C), while not as substantial as the initial decrease. Moreover, Chung et al. (1989) found that when nisin (57μM) was added to exponentially growing cells of *Staphylococcus aureus*, there was an immediate inhibition of growth and optical density decreased, suggesting cell lysis and confirming the high sensitivity of this organism to nisin. Meanwhile, De Martinez et al. (2002) stated that nisin with lactic acid may be useful in controlling microbial contamination. The application of a mixture of nisin with lactic acid was more bactericidal than any of the single antimicrobial treatment alone, and it may be a useful method to inhibit spoilage bacteria and thus extends the

shelf-life of red meat.

Listeria monocytogenes showed reduction by 3.1, 3.6 $\log_{10}$ cfu/g respectively (Fig. 2). Lower figures were reported by Zhang and Mustapha (1999) who stated that treatment with nisin or with nisin combined with EDTA reduced the population of *Listeria monocytogenes* by 2.01 and 0.99 $\log_{10}$ cfu/g as compaired to the control, respectively, under the condition of vacuum package and storage at 4°C for up to 30 days. The results (Fig. 2) showed obvious reduction percent within the first two days of refrigerating storage, these findings agree with the same author, who added that numbers of *Listeria monocytogenes* reduced by nisin treatment on day 30 were not significantly different from that on day.

A possible explanation for the reduction of Gram-positive bacteria by nisin may be that the cell wall was damaged by either osmotic (due to presence of salt or lactate) or cold shock, or a combined effect of the two, allowing the penetration of nisin to the cell membrane (Harris et al., 1992). Davies and Delves-Broughton (1999) added that nisin pass through the cell wall of Gram-positive cells to the cytoplasmic membrane where it interacts with the phospholipids component of cell membrane which allow the efflux of essential cellular components or in severe cases complete lysis of the target cell.

The mean count of *Staphylococcus aureus* in untreated minced meat slightly decreased from 6 ($\log_{10}$cfu/g) from the third day of refrigerated storage to reach 5.89 $\log_{10}$ cfu/g (Fig. 1), this may accentuate stress to the cell in low osmolar conditions as refrigeration may delay the growth or inhibiting survival of bacteria.

Table (1): Statistical analytical results of aerobic and psychrotrophic bacterial count of examined minced beef samples (No=20).

Total aeobic count				Psychrotrophic count			
Min	Max	Mean	Standard error	Min	Max	Mean	Standard error
5.48	8.6	7.48	±7.29	5.7	8.95	7.80	±7.52

Table (2): Incidence of *Staphylococcus aureus* and Listeria monocytogenes in examined minced beef samples stored at 4°C (n= 20).

Types of isolates							
Staphylococcus aureus				*Listeria monocytogenes*			
Suspected colonies		Coagulase +ve		Suspected colonies		*Listeria monocytogenes*	
No	%	No	%	No	%	No	%
10	50	7	35	8	40	2	10

Fig. (1): Effect of Nisin on the growth of *Staphylococcus aureus* in minced beef samples stored at 4°C

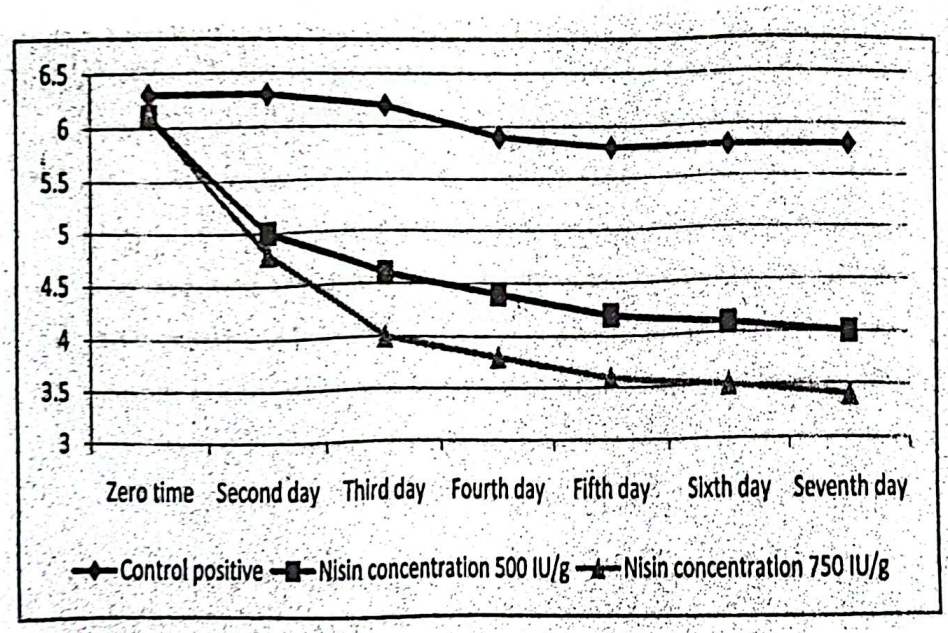
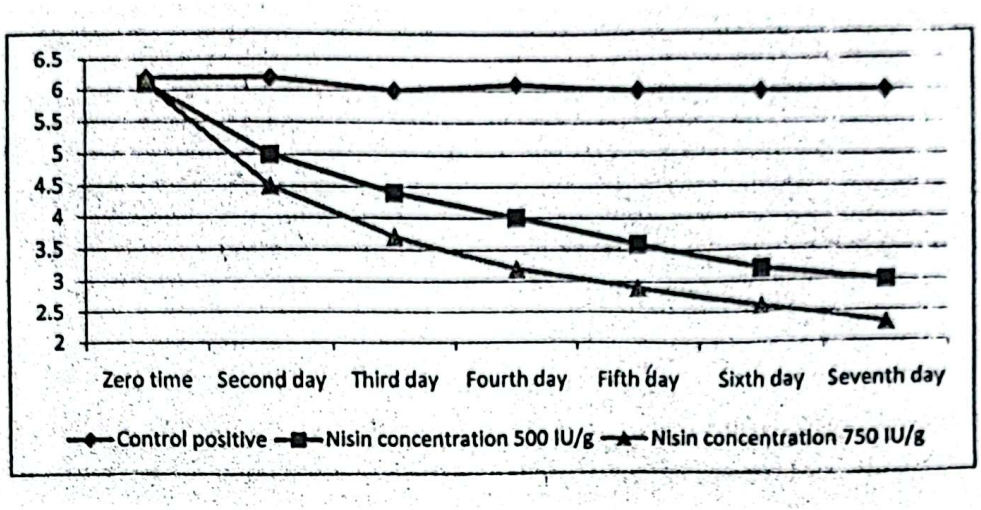


Fig. (2): Effect of Nisin on the growth of *Listeria monocytogenes* in minced beef stored at 4°C.



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