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# THE EFFECT OF PRESERVATIVE (NISIN) ON THE SURVIVAL OF LISTERIA MONOCYTOGENES.

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## ABSTRACT

Five samples of beef escalope panee were randomly collected from different localities in Qalyubia governorate in order to clarify the effect of preservative nisin on the survival of *Listeria monocytogenes* (*L. monocytogenes*) and suggest the concentrations of Nisin that could be useful in controlling *L. monocytogenes.* The inhibitory effect of Nisin at concentration of 10, 30 and 50 ppm on the survival of *L. monocytogenes* ($5\times10^6$/g) inoculated into beef escalope pane. It was noticed that the treatment of inoculated beef escalope panee with nisin at concentration of 30 or 50 ppm for 24 hours was useful in controlling *L. monocytogenes.*

**KEY WORDS**: Beef Escalope Pane, Listeria monocytogenes, Nisin.

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## 1. I N T R O D U C T I O N

*Listeria monocytogenes* has become increasingly important as a food-associated pathogen during the past 25 years. Although the number of annual *L. monocytogenes* infections is low, mortality rate is as high as 30% and indicates that it is one of the most important food borne pathogens. Most European Union countries have an annual incidence of human listeriosis, between two and ten reported cases per million and due to its high fatality rate, listeriosis ranks among the most frequent causes of death due to food borne illness. *L. monocytogenes* infections are responsible for the highest hospitalization rates (91%) amongst known food-borne pathogens and have been linked to sporadic episodes and large outbreaks of human illness worldwide. The ability to persist in food-processing environments and multiply under refrigeration temperature makes *L. monocytogenes* as a significant threat to public health. *L. monocytogenes* contamination is one of the leading microbiological causes of food recalls, mainly of meat, poultry, dairy products as well as seafood [6, 7]

Delves-Broughton [2] declared that the suitability of nisin as a food preservative arises from the following characteristics: it is nontoxic, no apparent cross-resistance in bacteria, quickly digested and the producer strains of *Lactococcus lactis* are regarded as safe (food-grade).

Since 1953, nisin has been sold under the trade name of Nisaplin®. Nisin initially forms a complex with Lipid II, a precursor molecule in the formation of bacterial cell walls. The nisin-lipid II complex then inserts itself into the cytoplasmic membrane forming pores and allows the efflux of essential cellular components resulting in inhibition or death of the bacteria. Solomakos et al. [11] stated that nisin is a polypeptide bacteriocin produced

by certain strains of *Lactococcus lactis subsp. Lactis*. It is generally recognized as safe (GRAS) and constitutes the only bacteriocin approved for use as food additives in over 50 countries. Nisin acts mainly against Gram positive bacteria by permeating the cytoplasmic membrane with the formation of transient pores. It is stable under refrigerated conditions, demonstrates heat stability and is degraded in the digestive system.

Several in vitro experiments showed that nisin possesses antibacterial activity against *L. monocytogenes*. The antimicrobial effect of nisin (500 or 1000 IU/g) against *L. monocytogenes* was examined. Addition of nisin to meat samples at 500 IU/g resulted in populations of *L. monocytogenes* strains, not significantly different ($P>0.05$) than those of the control samples during storage at 4 or 10°C, indicating no antimicrobial activity of nisin at this level against the pathogen, while addition of nisin at 1000 IU/g resulted in populations of all examined strains of *L. monocytogenes* significantly lower ($P<0.05$) than the controls at both storage temperatures.

Therefore, this study was planned to clear the effect of preservative Nisin on the survival of *L. monocytogenes*, and determine the concentration of nisin could be applied to control that pathogen.

## 2. MATERIALS AND METHODS

The effect of Nisaplin (2.5% nisin) was studied according to El-Lawendy [4] and Kamat and Nair [8].

*2.1. Preparation of the samples:*

One kg of frozen pre-cooked beef escalope panee was purchased from supermarkets collected at different localities in Qalyubia governorate and was divided into portions, each of 25 gm.

*2.2. Preparation of nisin:*

Nisin (obtained from the Danisco Chemical Co.) was standardized to an activity of one million international units per gram (IU/g). Three solutions were prepared containing 10, 30 and 50 ppm for inoculation.

*2.3. Inoculation of beef escalope panee samples and storage:*

Samples were aseptically inoculated separately with $5\times10^6$/g of isolated *L. monocytogenes* using sterile automatic pipette and treated with 10, 30 and 50 ppm Nisin per kg of beef escalope panee. The inoculated samples were mixed well to be thoroughly distributed of the inoculum and Nisin.

The samples were stored at 4°C to be examined at 0, 12 and 24 hours intervals. Five samples were plated immediately (0 h) to enumerate *L. monocytogenes*.

*2.4. Sampling and analysis:*

Five inoculated treated samples with Nisin were analyzed at 0, 12 and 24 hours of storage at 4°C by blending the entire contents of a package (approximately 25 g) with 225 of sterile 0.1% peptone water. Serial dilutions (1:10) were then made in 0.1% sterile PBS (phosphate buffer saline). Cell count of *L. monocytogenes* was determined by spread plating on PALCAM agar.

Five plates were incubated at 37°C for 48 h and the colonies were enumerated. Mean values were reported as the average number of the five plates.

## 3. RESULTS AND DISCUSSION

The inhibitory effect of nisin at concentration of 10, 30 and 50 ppm on the survival of *L. monocytogenes* ($5\times10^6$/g) inoculated into beef escalope panee was studied in the present research (Table 1). It was noticed that inoculated beef escalope panee after treatment with Nisin at concentration of 10 ppm showed

significant decrease in the mean *L. monocytogenes* count from $5\times10^6$ to $2.92\pm0.57\times10^5$/g after 12 hours, while the mean count was decreased significantly to $8.30\pm2.11\times10^3$/g after 24 hours. It was noticed that inoculated beef escalope panee after treatment with nisin at concentration of 10ppm showed significant decrease in the mean *L. monocytogenes* count from $5\times10^6$ to $2.92\pm0.57\times10^5$/g after 12 hours, while the mean count was decreased significantly to $8.30\pm2.11\times10^3$/g after 24 hours.

Furthermore, treatment with nisin at concentration of 30ppm in beef escalope panee showed a high significant decrease in the mean *L. monocytogenes* count from $5\times10^6$ to $7.81\pm1.49\times10^4$/g after 12 hours, and the mean count was decreased to 0/g after 24 hours. While, treatment with nisin at concentration 50 ppm in beef escalope panee showed decrease in the mean *L. monocytogenes* count from $5\times10^6$ to $1.64\pm0.35\times10^4$/g after 12 hours and decreased to 0/g after 24 hours. The effectiveness of bacteriocin appears to be depended upon the concentration of the bacteriocin itself and the count of the target pathogen [3].

The investigators found that $4x10^4$ IU of nisin gave an immediate decrease (0.9 log) in *L. monocytogenes* count and the treatments decreased the count of *L. monocytogenes* on meat surface by (1.1 log) in 48 hours at 4°C and minimal or no delayed effects were noticed during storage. Mahadeo and Tatini [9] found that 100 IU/ml was effective in reducing *L. monocytogenes* by 1 to 2 log units.

Results obtained by Nassar and Farrag [10] indicated that the concentration of 800 IU/g nisin in ground beef at 3°C declined the count of such pathogen from 5.2 x $10^3$ to $8.5\times10^2$ after one day storage, while after 3 days was $2\times10^2$ (colony forming units) CFU/g., they added that the use of 1600 IU/g decreased the count of inoculated *L.monocytogenes* to $1x10^2$ after one day storage.

Spray treatments of beef meat with nisin lead to immediate reduction of approximately 2 log10 CFU/$cm^2$ for inoculated *L. innocua* in study conducted by Cutter and Siragusa [1]. They used *L. innocua* as a model pathogen in this study because of its greater resistance to nisin, compared with *L. monocytogenes*.

The effect of 600IU/ml of nisin in broth on the sensitivity of *L. monocytogenes* serotype 1 ($10^8$ CFU/ml) was studied by Ukuku and Shelef [12] and the survivors reached high numbers after 24 hours, but there was no survivors when the inocula was less than $10^4$ CFU/ml.

All Listeria species including *L. monocytogenes* were present at high incidence in the examined samples of poultry products than meat products, which obligate the establishment of good sanitation practice.

Table 1 Influence of different doses of nisin on *Listeria monocytotogenes* inoculated into beef escalope panee.

| Exposure time (h) | Nisin dose | | | | | |
|---|---|---|---|---|---|---|
| | 10 ppm | | 30 ppm | | 50 ppm | |
| | Mean±S.E | Reduction% | Mean±S.E | Reduction% | Mean±S.E | Reduction% |
| 0 | $5\times10^6$ | - | $5\times10^6$ | - | $5\times10^6$ | - |
| 12 | $2.92\pm0.57\times10^5$ | 94.16 | $7.81\pm1.49\times10^4$ | 98.44 | $1.64\pm0.35\times10^4$ | 99.67 |
| 24 | $8.30\pm2.11\times10^3$ | 99.85 | - | 100 | - | 100 |

The antilisterial action of nisin used at two concentrations 800 and 1600 IU/g occurred immediately (day zero) as recorded by El-Lawendy [4]. The same author indicated that nisin treatment at 1600 IU/g in ground beef was useful in controlling *L. monocytogenes* and HACCP systems during manufacturing especially in poultry processing plants.

The antilisterial effect of nisaplin (commercial form of nisin, 0.5% equivalent to 5000 IU/ml of nisin) was evaluated by Georgnaras et al. [5] who recorded a reduced initial levels of inoculated 10 strain composite *L. monocytogenes* cultures originating from different sources in commercially manufactured frankfurters by 2.4 to >3.8 log CFU/cm$^2$.

In conclusion, the treatment of meat products with nisin at concentration of 30 ppm or 50 ppm for 24 hours was useful in controlling *L. monocytogenes*.

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# تأثير بعض إضافات الأغذية( نيسين) علي بقاء ميكروب الليستيريا مونوسيتوجينز

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## الملخص العربى

اجريت هذه الدراسة على عدد 5 عينات عشوائية من منتج الإسكالوب بانيه البقري لبيان تأثير بعض إضافات الأغذية ( نيسين ) علي ميكروب الليستيريا مونوسيتوجينز. بعد التأكد من خلو المنتج من الميكروب تم حقنه بميكروب الليستيريا مونوسيتوجينز بتركيز $5 \times 10^6$ ثم إضافة نيسين في ثلاث تركيزات 10، 30، 50 جم / كجم ودراسة عدد هذا الميكروب في الساعة نفسها ثم بعد 12 ساعة ثم بعد 24 ساعة من حفظها في درجة حرارة 4 درجة مئوية وقد وجد أن الثلاث تركيزات لها تأثير فعال في تقليل أعداد الميكروب.