Dept. of Food Hygiene
Fac. of Vet. Med., Assiut Univ., Egypt
Head of Dept. Prof. Dr. H.Y. Ahmed.

NISIN AS INACTIVATOR TO LISTERIA MONOCYTOGENES IN BROTH AND IN GROUND BEEF

(With 2 Fig.)

By

A. NASSAR and S.A. FARRAG*

(Received at 5/1/1995)

النيسين كقاتل لميكروب الليستريا مونوسيتوجين في الوسط البيئى واللحم المفرى

أحمد نصـــار ، سهام فراج

فى السنوات الأخيره سبب ميكروب الليستريا مونوسيتوجين فى اللحوم خاصة المفرى منها مشكله خطيره ممثله فى انتشار هذا المرض فى كثير من بلاد العالم .

لهذا تم دراسة تأثير تركيزات مختلفه من النيسين التجارى على ميكروب الليستريا مونوسيتوجين فى الوسط البيئى واللحم المفرى فى درجة حرارة التبريد . وكانت التركيزات المستخدمه من النيسين هى ٨٠٠ ، ١٦٠٠ ، ٢٤٠٠ ، ٣٢٠٠ وحده دوليه لكل مللى من الوسط البيئى أو لكل جم من اللحم المفرى .

من الدراسه اتضح تأثير النيسين على الليستريا مونوسيتوجين . كان هذا التأثير يختلف فى الوسط البيئى عنه فى اللحم المفرى بسبب وجود الميكروبات المنافسه فى اللحم المفرى .

وأوصى الباحثين باستخدام تركيز ٢٤٠٠ وحده دوليه لقتل ميكروب الليستريا مونوسيتوجين فى اللحم المفرى فى درجه ٣ م .

---

*: Home Economics Dept., Fac. of Specific Education, Assiut.

SUMMARY

Meatborne listeriosis because of *Listeria monocytogenes* in ground beef in many countries is most serious problem in the last years. The effect of various concentrations of commercially nisin on *Listeria monocytogenes* in tryptose phoshate broth and then in ground beef at refrigerated temperature were investigated. The inoculated numbers of *Listeria monocytogenes* was $9x10^4$ CFU/ml and $5.2x10^3$ CFU/g for tryptose phoshate broth and ground beef respectively. The used concentrations of nisin were 800, 1600 and 2400 RU for this purpose. The effect of nisin on *Listeria monocytogenes* was varied according to its concentration and the substance involved. Its effect on ground beef was differ from that in tryptose phoshate broth due to the competitive flora in ground beef. The use of 2400 RU nisin to inactivate *Listeria monocytogenes* at refrigerator temperature was recommended.

Keywords: Nisin, L.monocytogenes, broth, ground beef.

INTRODUCTION

The last few years, have been intersting time for public health and regulatories officials concerned with foodborne listeriosis. Given the psychrotrophic nature of *Listeria monocytogenes*, the ubiquity of this pathogens with slaughterhouse and meat-packing environments *(RYSER and MARTH, 1991)*, meatborne listeriosis especially ground beef has special attention in recent years in many countries.

Ground beef is one of the most susceptible meat for *Listeria* contamination and spoilage. Surface contamination of the carcasse, time and temperature of aging, sanitary and hygienic standards of meat handling, and the storage condition of the finished product all influence the *Listeria* present in the finished retail product. This type of food is often consumed after a brief heat treatment, which may not be sufficient to kill *L.momocytogenes*, such as grilling.

The prevalence of *Listeria* spp. especially *L.monocytogenes* in ground beef were studied by many investigators in the world. Surveys in Germany by many scientists; *ERDLE (1988)* found that 8% of ground beef were positive for *L.monocytogenes;* while *OZARI and STOLLE (1990)* found that 15.2%. In France, 8% of the examined ground beef was positive for *L.monocytogenes (LE*

GUILLOUX et al., 1980). Working in Asutria, revealed that 36% of the examined ground beef samples were contaminated with this pathogen *(BREUER and PRÄNDLE, 1988).* Working in Denmark, *SKOVGAARD and MORGEN, 1988)* found that incidence of *L.monocytogenes* in Danish ground beef was 28%. In New Zealand; 92% of minced beef harboured this bacterium *(LOWRY and TIONG, 1988).* Study Swizerland by *BREER and SCHOPFER, 1989* reported the presence of *L.monocytogenes* in 33% of the examined ground beef. In canada, *FARBER et al., 1988* found that *L.monocytogenes* in 80% of the retail ground beef. *McCLAIN and LEE, 1988* reported that 48.7% of ground beef in USA was positive for this bacterium. In Japan, the incidence of *L.monocytogenes* in ground beef was 37.9% *(KOKUBO et al. 1990).* In croatia, *ZIVROVIC et al. 1992* found that 14.67% of the examined ground beef was positive for *L.monoctogenes.*

Listeria monocytogenes is listed among psychrotrophic spoliage bacteria, in this cooncern *HEFNAWY et al. 1993* found that the storage temperature of ground beef at 4°C for 6 days had no effect on *L.monocytogenes.*

Nisin is antimicrobial polypeptide substance or bacteriocin produced by certain strain of *Streptococcus* lactis *(EL-GENDY et al. 1989).* Its antibacterial activity against Gram-positive especially spore-forming bacteria was referred by *DELVES-BROUGHTON, 1990.* The use of nisin in food preservation was established as safe as recognized by joint FAO/WHO Committee on food additive since, 1968. The use of nisin in milk industry was recommended by *SHEHATA et al., 1982),* in meat and meat products *(DELVES-BROUGHTON, 1987a,b).*

From these available data, ground beef constitute a public health hazard concerning meatborne listeriosis, and nisin may be used as a safe guard toward this aim. Therefore, this work was planned to investigate the effect of various nisin concentrations on *L.monocytogenes* in broth and in ground beef at refrigerator temperatures.

MATERIALS AND METHODS

1. Nisin:

Nisin N-5764 was obtained from Sigma chemical Co. One gram of nisin contained 10^6 nisin RU (100 RU=40 ppm). The stock solution was prepared by disolving 1 g of nisin in 100 ml sterile distilled water as previously described by *(BENKERROUM and SANDINE, 1988).* Sufficient volume of nisin solution was added to tryptose broth or to ground beef to achieve 800, 1600, 2400 RU of nisin per ml or per gram for broth and ground beef respectively.

2. Listeria monocytogenes culture:

L,monocytogenes Scott A (clinical isolate, Serotype 4 B) was obtained from R.M. Twedt, Food and Drug Administration; Cincinnati, OH, USA. The culture was activated by three successive transfers. The first transfer was from typtose agar slant (Difco) followed by incubation at 35 °C for 47 h; the second transfer was from tryptose agar slant to tryptose phoshate broth (Difco), and third was from tryptose phoshate broth to tryptose phoshate broth. Broth culture was incubated at 35°C for 24 h. Sufficient volume of the working culture was dispensed in tryptose phoshate broth or ground beef to achieve population ca 9 X 10^4 CFU / ml of broth or 5.2 X 10^3 CFU/g of ground beef.

3. Effect of nisin on L.monocytogenes in tryptose phoshate broth at 5°C:

Tryptose phoshate broth was prepared in 25 ml quantity, in sterile flasks, each of 9X10^4 CFU of *L.monocytogenes* per ml and classified into 5 categories:

Control: contain no nisin; A: contain 800 RU nisin; B: contain 1600 RU nisin; C: contain 2400 RU nisin; D: contain 3200 RU nisin. Examinations were done after 1, 2, 3 and 4 d at 5°C storage for duplicate samples.

4. Effect of nisin on L.monocytogenes in ground beef at 3°C:

650 g of raw fresh lean beef meat was purchased from retail market in Assiut City. The meat was grounded under sterile conditions. This ground beef was examined for *L.monocytogenes* and proved free before inoculation. The ground beef was divided into parts in sterile flasks, each of 25 g. The samples were inoculated with 5.2X10^3 CFU *L.monocytogenes* per gram. The samples were classified into 4 categories:

Control: not contain nisin; A: contain 800 RU nisin; B: contain 1600 RU nisin; C: contain 2400 RU nisin. Examinations were done duplicate after 1, 2 and 3 d at 3 °C storage.

5. Enumeration of L.monocytogenes:

One ml of well mixed inoculated tryptose phoshate broth was taken under sterile condition and decimal dilutions were made in sterile 0.1% peptone water. The various dilutions were surface plated on tryptose agar *(RYSER and MARTH, 1991)* in duplicate sets. The plates were incubated at 35 °C for 48 h. For ground beef, the all 25 g of ground beef was mixed with 225 ml of sterile peptone water and blended in a waring blender (8000 rpm) for 3 min. Serial dilutions were made and plated onto Mcbride Listeria lithium chloride agar (MLLA); (35.5 g phenyl ethanol agar (Difco), 10 g glycine, 4 g lithium chloride, 1000 ml distilled water) according to *OINI and GILBERT (1988).*

RESULTS

Are Presented in Fig. 1-2.

DISCUSSION

1. Effect of nisin on the growth of *L. monocytogenes* in broth.

Growth of *L. monocytogenes* in tryptose phoshate broth in absence and presence of nisin is show in Figure 1. *L. monocytogenes* grew well in broth held at 5 °C for 4 days. The initial number of the bacterium was $9X10^4$ CFU/ml, this number increased to $2X10^7$ after one day and reached the maximun numbers of pathogen after 4 days of storage at 5 °C. The concentration of 800 RU/ml in tryptose phosphate broth decreased the population of pathogen from $9X10^4$ CFU/ml to $1X10^4$ CFU/ml after 3 days of storage at 5 °C. After 4 days, the pathogen was completely inactivated (no survivors) were detected on tryptose agar medium. The higher concentration of nisin tested had the same effect on *L. monocytogenes* in tryptose broth kept at 5 °C. This finding established that 800 RU had completly inhibitory effect on the bacterium after 4 days of storage at 5 °C. Our results are consistent with those of *BENKERROUM and SANDINE, 1988*, who studied inhibitory action of nisin against *L. monocytogenes*. Most importantly, nisin conserves its effectiveness at a low temperature for a long time *(GIBBS and HURST, 1964)*.

2. Effect of nisin on the growth of *L. monocytogenes* in ground beef.

Growth of *L. monocytogenes* in ground beef without and with nisin addition is shown in Figure 2. Ground beef inoculated with *L. monocytogenes* $5.2X10^3$ CFU/g and without nisin addition (control) were stored at controlled temperature of 3 °C, and examined after 1, 2 and 3 d storage in duplicate state. The inoculum number of the pathogen increased to $1.8X10^4$ after one day and changed to $1.5X10^4$ CFU/g after 2 and 3 d respectively. This result proved thepsychrotrophic nature of this bacterium and comply with *HEFNAWY et al. 1993)*. The concentration of 800 RU in ground beef at 3 °C declined the count of the pathogen from $5.2X10^3$ to $8.5X10^2$ after one day,. After 2 and 3 days was $2X10^2$ CFU/g. This finding indicate that 800 RU had slightly inhibitory effect on the target pathogen. *MOHAMED et al. 1984* showed that only 32 RU/ml of nisin are necessary to inhibit *L. monocytogenes* 4379 at pH 7.4 and 37 °C. They also revealed that the sensitivity of this strain decreases when the temperature of the incubation decreases. 265 RU/ml nisin are

required to inhibit completely *L.monocytogenes* 4379 growth at 22 °C and pH 7.4. The use of 1600 RU of nisin concentration in ground beef decreased the count of inoculated *L.monocytogenes* to $1X10^2$ after one day and remain constant after 2 and 3 days. concentration of 2400 RU of nisin had a clear inhibitory effect on this bacterium, it decreased to $1X10^2$ after one day and a complete inhibitory effect (no growth) after 2 and 3 d.

In conclusions, these results proved the use of nisin not only inhibit the growth of *Listeria* but also kill the bacterium. The antibiotic is therefore able to protect this meat product from *Listeria* with contaminated either from the raw meat or a result of subsequent contamination. Furthermore, nisin seems to delay the growth of psychrotrophs that can exist or contaminate ground beef.

REFERENCES

Benkrrom, N. and Sandine, W. (1988): Inhibitory action of nisin against *Listeria monocytogenes.* J. Dairy Sci., 71, 3237-3245.

Breer, C. and Schopfer, K. (1989): Listerien in Nahrungsmitteln. Schweiz. med W Sch. 119, 306-311.

Breuer, J. and Parändl, O. (1988): Nachweis von Listerien und deren Vorkommen in Hakfleisch und Mettwûrsten in österreich. Arch. Lebensmittelhyg. 39, 28-30.

Delves-Broughton, J. (1987a): Fous on nisin. Food Manufacture 62, 163-164.

Delves-Broughton, J. (1987 b): Nisin in food preservation. North European Dairy J. 53, 1-8.

Delves-Broughton, J. (1990): Nisin and its uses as a food preservative. Food Technology 11. 100-117.

El-Gendy, S.M., Mohran, M.A. and Fahmy, M.A. (1989): Influence of adding nisaplin to Domiati cheese milk and cheese quality and prevention of tins blowing. Assiut J. Agric. Sci., 20, 121-137.

Erdle, E. (1988): Zum vorkommen von Listerien in Käse, Fleisch und Fleischwaren. Vet. Med. Diss. Munich, Germany.

Farber, J.M., Tittiger, F. and Gour, L. (1988): Survellance of raw-fermented (dry-cured) sausages for the presence of *Listeria* spp. Canadian Institute of Food and Technol. J. 21, 430-434.

Gibbs, B.M. and Hurst, A. (1964): Limitations of nisin as a preservative in non dairy foods. 4th Int. Symp. Food. Microbiol., SIK, Cöteborg, Swed.

Hefnawy, Y., Moustafa, S. Marth, E. (1993): Behaviour of *Listeria monocytogenes* in refrigerated and frozen ground

beef and in sausage and broth with and without additives. Lebensm-Wiss u.-Technol., 26, 167-170.

Kokuubo, Y., Takashi, I, Kaneko, S. and Maruyama, T. (1990): Evaluation of enrichment and plating media for isolating *Listeria monocytogenes* from raw meat. Shokohin Eiseigaku Zusshi, 31, 51-56.

Le Guilloux, M.; Dollinger, Cl. and Freyburger, G. (1980): *Listeria monocytogenes* Sa Frequence dans Les Produits de charcuterie. Bull. Soc. Vet. Prat. France, 64, 1, 45-53.

Lory, P.D., and Tiong, I. (1988): The incidence of *Listeria monocytogenes* in meat and meat products: Factors affecting distributions. In Proc. 34 th Int. Congress Meat Sci. Technol., Brisbane, Australia, Part B, pp. 528-530.

McClain, D. and Lee, W.H. (1988): Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J. Ass. of Anal. Chem. 71, 660-664.

Mohamed, G.E., Seaman, A. and Woodbine,M. (1984): Food antibiotic nisin comperative effect on Erysipelothrix and *Listeria.* Proc. 24th Int. Sym. Antibiotic-Agric, March 27-30 (1983) and the Univ. Nottingham. Blackwell Publ. Ltd, London, England.

Ozari, R. and Stolle, A. (1990): Zum vorkommen von *Listeria monocytogenes* in Fleisch und Fleisch-Erzeugnissen einschicsslich Geflugelfleisch des handels. Arch. Lebensmittelhyg. 41, 47-52.

Pini, P.N. and Gilbert, R.T. (1988): The occurrence in the U.K. of *Listeria* species in raw chickens and soft cheese. Int. J. Food Microbiol. 6, 317-326.

Ryser, T.E. and Marth, E.H. (1991): Listeria, Listeriosis, and Food safety. Marcel Dekker. INC New York.

Shehata, A., Magdoub, E., El Samargy, M.N. and Hassan, A. (1982): Effect of sublethal heat-schok L-alanine, B-alanine and nisin on the destruction of psychrotrophic *Bacillus* species in milk. Egypt. J. Dairy Sci. 21, 197-207.

Skovagaard, N. and Morgen, C.A. (1988): Detection of *Listeria* spp. in faeces from animals, in raw foods of animal origin. Int. J. Food Microbiol. 6, 229-242.

Zivkovic, J., Uhitil, S. and Hadziosomanovic, M. (1992): The occurence of *Listeria* spp. in meat in the Republic of Crotia. 3rd World congress on Foodbrne infections and Intoxications, Berlin, Germany pp. 501-505.

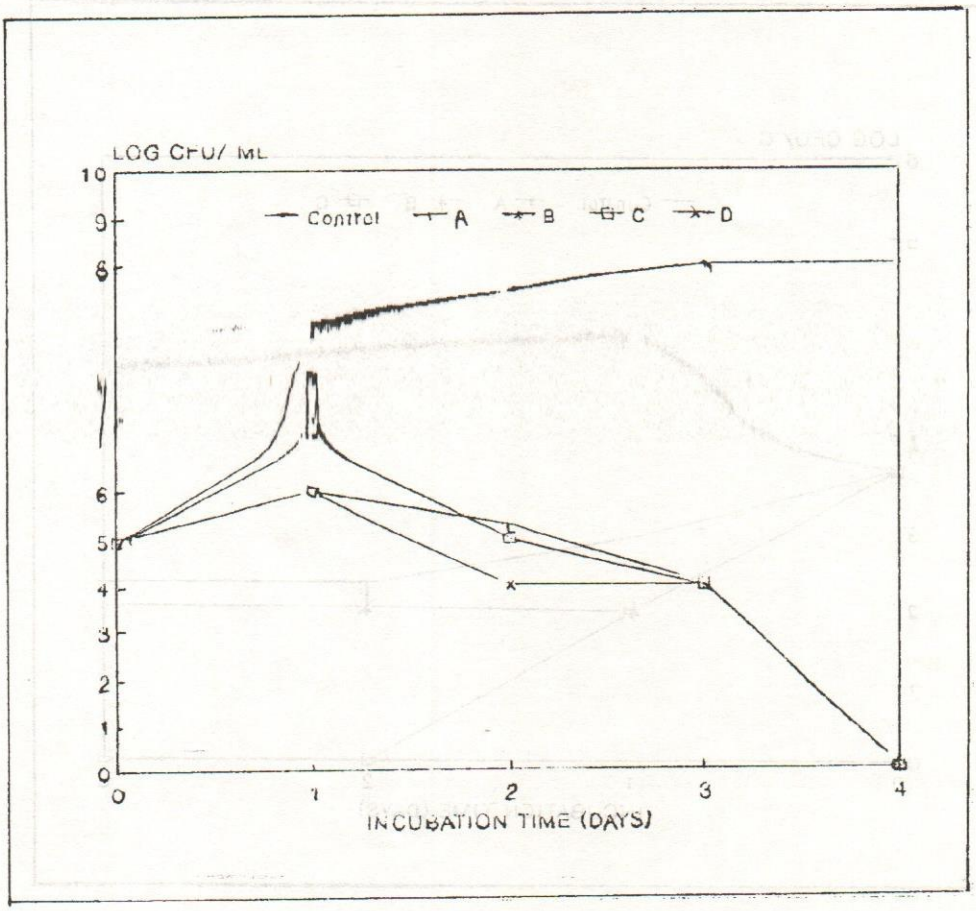

Fig: 1- Effect of nisin on the growth of L. monocytogenes in broth at 5°C.

Control: Tryptose phosphate broth (TPB) inoculated with L. monocytogenes only.

A: TPB inoculated with *L. monocytogenes* plus 800 RU/ml nisin.

B: TPB inoculated with *L. monocytogenes* plus 1600 RU/ml nisin.

C: TPB inoculated with *L. monocytogenes* plus 2400 RU/ml nisin.

D: TPB inoculated with *L. monocyogenes* plus 3200 RU/ml nisin.

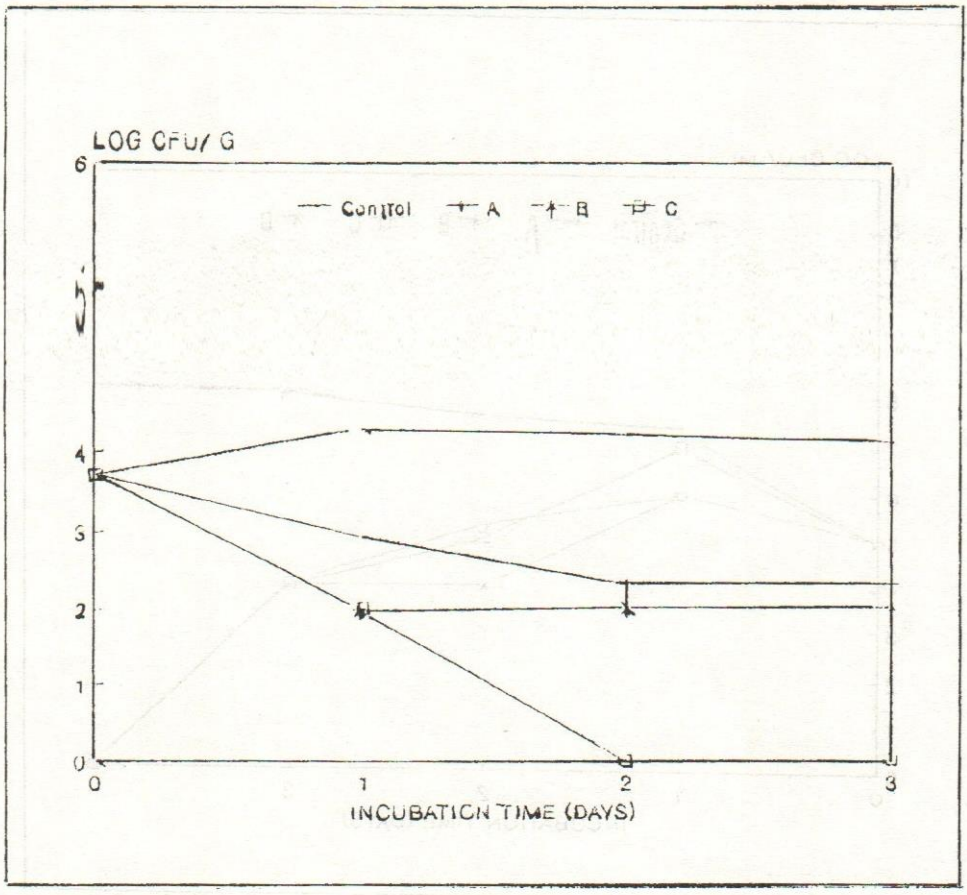

Fig: 2- Effect of nisin on the growth of *L. monocytogenes* in ground beef at 3°C.

Control: Ground beef inculated with *L. monocytogenes only.*

A: Ground beef inoculated with *L. monocytogenes* plus 800 RU/ml nisin.

B: Ground beef inoculated with *L. monocytogenes* plus 1600 RU/ml nisin.

C: Ground beef inoculated with *L. monocytogenes* plus 2400 RU/ml nisin.