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Effect of nisin and its combination with sodium chloride on the survival of *Listeria monocytogenes* added to raw buffalo meat mince

D.D. Pawar, S.V.S. Malik, K.N. Bhilegaonkar, S.B. Barbuddhe *

Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar 243 122, India

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Abstract

Antilisterial activity of nisin (Nisaplin), alone at concentrations of 400 and 800 IU/g and in combination with 2% sodium chloride was incorporated in raw buffalo meat mince. Samples of the raw meat mince were inoculated with 10^3 colony forming units (cfu)/g of *L. monocytogenes* and stored at 4°C for 16 days and at 37°C for 36 h. Initial estimates of pH, extract release volume, mesophilic and psychrophilic counts were found to be 5.74, 48 ml, 3.5×10^5 and 1.0×10^5 cfu/g of meat, respectively. The growth of *L. monocytogenes* in the treated groups was significantly ($P<0.05$) inhibited compared to the control group. The degree of inhibition increased with increasing concentration of nisin and decreasing storage temperature. Addition of 2% sodium chloride in combination with nisin increased the efficacy of nisin at both storage temperatures. The pH in the treated groups remained significantly lower ($P<0.01$) than in the control groups at both 4 and 37°C.

Keywords: Spoilage; Extract release volume; Mesophilic; Psychrophilic; Salt

1. Introduction

Listeria monocytogenes has been recognized as a foodborne zoonosis (Pearson & Marth 1990). The organism has been isolated from a variety of foods including raw meats (Breer & Schopfer, 1988), raw milk (Bhilegaonkar, Kulshreshtha, Kapoor, Kumar, Agarwal & Singh, 1997), and vegetables (Arumugaswamy, Rahamat ali, Abd. Hamid, 1994).

Listeria monocytogenes can survive and grow in a wide range of environments: including refrigeration temperatures, high levels of salts and relatively low pH. It is among the most heat resistant of vegetative bacterial cells (Lovett, Wesley, Vandermaaton, Bradshaw, Francis, Crawford et al., 1990). Addition of organic acids (Surve, Sherikar, Bhilegaonkar & Karkare, 1991), bacteriocins, like nisin (Benkerroum & Sandine, 1988) and pediocin (Murray & Richard, 1997), lactic acid bacteria (Harris, Daeschel, Shires & Klaehammer, 1989) and polyphosphates (Sofos, 1986) have been tried to retard the growth of spoilage as well as pathogenic organisms in foods.

Nisin is a broad spectrum bacteriocin produced by strains of *Lactococcus lactis,* and is active against Gram-positive bacteria, including *L. monocytogenes* (Delves-Broughton, 1990) and its effectiveness and that of other bacteriocins has been studied extensively (Chung, Dickson & Crouse, 1989; Taylor & Somers, 1985). Addition of common salt to growth media containing nisin has been reported to antagonise its sporicidal action by interfering with nisin adsorption by the spores of *Bacillus licheniformis* (Bell & Delacy, 1985). The purpose of this study was to look at the efficacy of two different concentrations of nisin both alone and in combination with 2% sodium chloride on the survival of pathogenic *L. monocytogenes* in raw buffalo meat mince stored at 4 and 37°C.

2. Materials and methods

2.1. Bacteria

A pathogenic strain of *L. monocytogenes* MTCC 1143 (NCTC 11994) was obtained from The Institute of Microbial Technology, Chandigarh, India. The growth of the standard strain in bacterial suspensions was done by the McFarland Nephelometric technique (Paik &

* Corresponding author at present address; ICAR Research Complex for GOA, Ela, Old Goa 403 402, India. Tel.: +91-832-286-181; fax: +91-832-286-249.

E-mail address: icar@goa.goa.nic.in (S.B. Barbuddhe).

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Suggs, 1974). Briefly, The nephelometer standard tubes, from one to ten were prepared using specific amounts of barium chloride and sulphuric acid. The standard strain of *L. monocytogenes* was subcultured on brain heart infusion (BHI) agar slants and incubated at 37°C for 24 h. The bacterial growth on one BHI agar slant was harvested in 5 ml of normal saline solution (NSS; 0.85% sodium chloride) and washed by agitating it with a sterile pasteur pipette. The bacterial suspension was pipetted into another test tube. The density of the bacterial suspension was adjusted by adding either sterile NSS or bacterial suspension so as to correspond to the density of nephelometer tube number one, which is reported to be equivalent to approximately 3×10^8 million bacterial cells per millilitre. The growth quantification of bacterial suspension was carried out by serial dilution and spread plating methods (International Commission for Microbiological Specifications fro Foods [ICMSF], 1978) and the bacterial suspension was accordingly diluted to give counts of approximately 10^3 cfu per 0.1 ml of suspension.

2.2. Meat

The fresh raw buffalo meat, purchased from retail outlets in Bareilly city, Uttar Pradesh, India was finely minced in sanitized meat mincers (Electrolux, Model 320064,Sweden) using 8 and 6 mm sieves and samples of the minced meat (150 g) were transferred to sterilized polyethylene sachets. Physico-chemical parameters viz., extract release volume (ERV) (Shelef, 1974) and pH (Association of Official Anaylitical Chemists [AOAC], 1975) and the microbiological parameters total viable count (TVC) for mesophiles and psychrophiles (ICMSF, 1978) and a presumptive *L. monocytogenes* count, were determined. To estimate mesophiles, psychrophiles and presumptive *L. monocytogenes* counts, 25 g meat mince was homogenized with 225 ml 0.85% salt solution. For mesophilic and psychrophilic counts, the homogenate was serially diluted in 10-fold dilutions in 0.85% salt solution, inoculated onto plate count agar and incubated at 37°C for 24–48 h and at 7°C for 10–14 days, respectively. For *L. monocytogenes* detection, the homogenate was first inoculated in University of Vermont broths I and II for enrichment and then streaked onto Dominguez-Rodriguez isolation agar (DRIA) plates (Dominguez-Rodriguez, Fernandez, Garayazabal & Ferri, 1984). The inoculated plates were incubated at 37°C for 48 h.

2.3. Nisin and its treatment

Nisin under the trade name Nisaplin (Batch No.72), was provided by Aplin and Barret Limited (Trowbridge, Wiltshire, England). The activity was indicated as 3.7×10^7 IU/g.

The working solution was prepared as described by Benkerroum and Sandine (1988). The commercial nisin was solubilized in distilled water acidified to pH 2.0 with hydrochloric acid so as give 10000 IU/ml of solution. The solution was sterilized using a 0.22 μm filter and stored at −20°C.

Each of the meat samples, contained in polyethylene sachets, was treated separately with the three combinations of nisin and sodium chloride:

Group A: 400 IU nisin per gram of meat.

Group B: 800 IU nisin per gram of meat.

Group C: 400 IU nisin per gram of meat + 2% sodium chloride

Group D: 800 IU nisin per gram of meat + 2% sodium chloride

Control: no treatment.

2.4. Inoculation of the samples

The contents of the polyethylene sachets were inoculated with the calibrated bacterial suspension at approximately 10^3 bacterial cells per gram of meat. Sachets were sealed and the contents were mixed in a stomacher (Model BA 6021, Seward Laboratory, London) for 3 min and then incubated at 4 and 37°C for 16 days and 36 h, respectively, for each group.

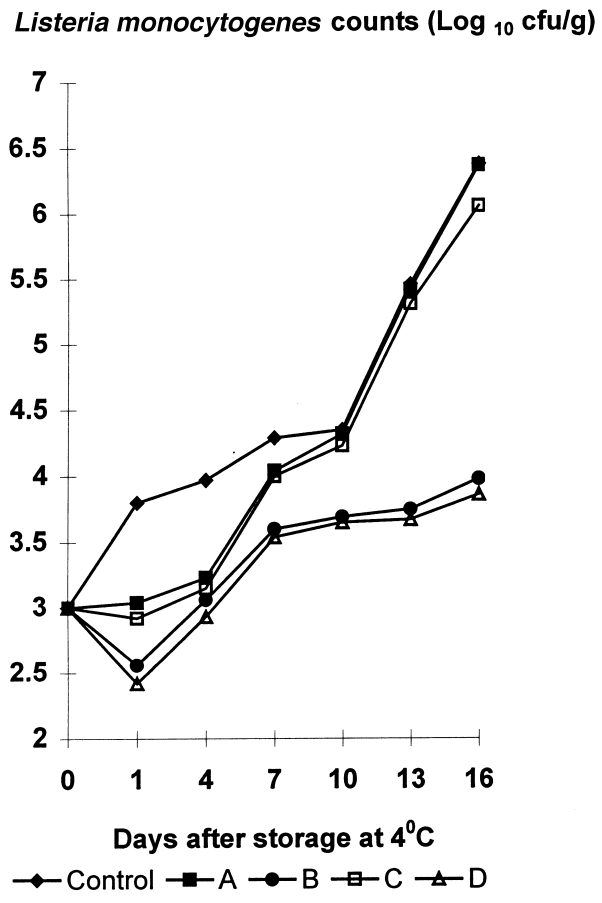

Fig. 1. *Listeria monocytogenes* counts (cfu $\log_{10}$/g) in control and treated raw buffalo meat mince stored at 4°C.

2.5. Sampling and analysis

For each group, 10 g aliquots were taken from the control and treatment groups on days 1, 4, 7, 10, 13 and 16 from samples stored at 4°C and at 12, 24 and 36 h from those incubated at 37°C. Each aliquot was estimated for *L. monocytogenes* count using direct plating on DRIA and its pH determined.

The data obtained was statistically analyzed using a randomized block design as described by Snedecor and Cochron (1968).

2.6. Results and discussion

The total viable (TVC) mesophilic and psychrophilic counts were 3.5×10^5 and 1.0×10^5 per gram of meat, respectively. No *Listeria* sp. was detected in the raw meat samples before treatment with preservatives. The initial pH and ERV of the meat were 5.74 and 48 ml, respectively. These parameters were acceptable, as normal values have been reported to be 5.8 for pH and 48–53 ml for ERV (Jay, 1986) and less than 10×10^6 organisms per gram for psychrophiles (Nickerson & Sinskey, 1972) and 5.0×10^6 to 1.5×10^7 per gram for mesophiles (Jay, 1986).

Addition of commercial nisin significantly ($P<0.05$) reduced the count of *L. monocytogenes* in groups A and C for up to10 days and for groups B and D for at least 16 days during storage at 4°C (Fig. 1, Table 1). In the control group the count increased from 3 $\log_{10}$ colony

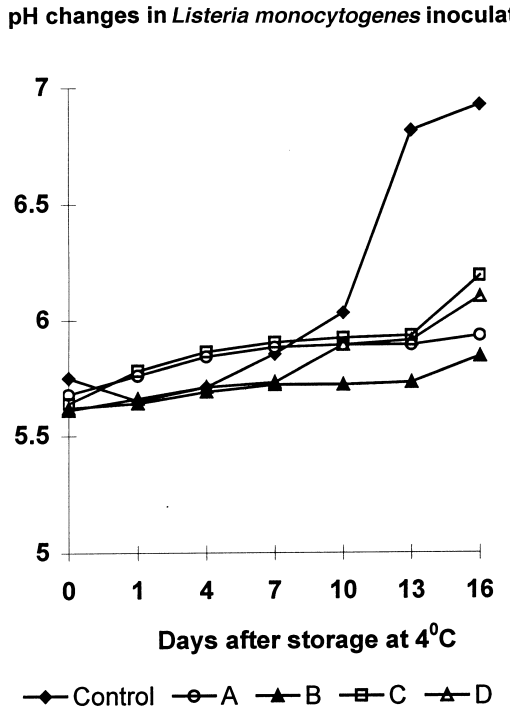

Fig. 2. Changes in pH of *Listeria monocytogenes* inoculated raw buffalo meat containing preservatives at 4°C.

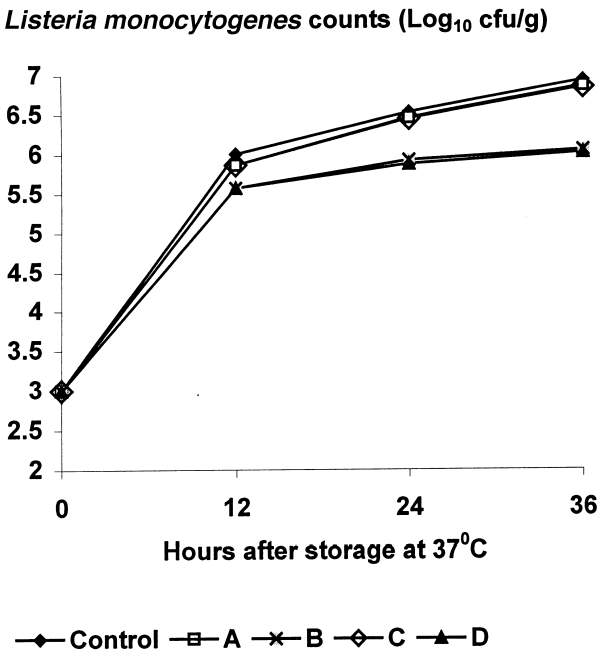

Fig. 3. *Listeria monocytogenes* counts (cfu $\log_{10}$/g) in control and treated raw buffalo meat mince stored at 37°C.

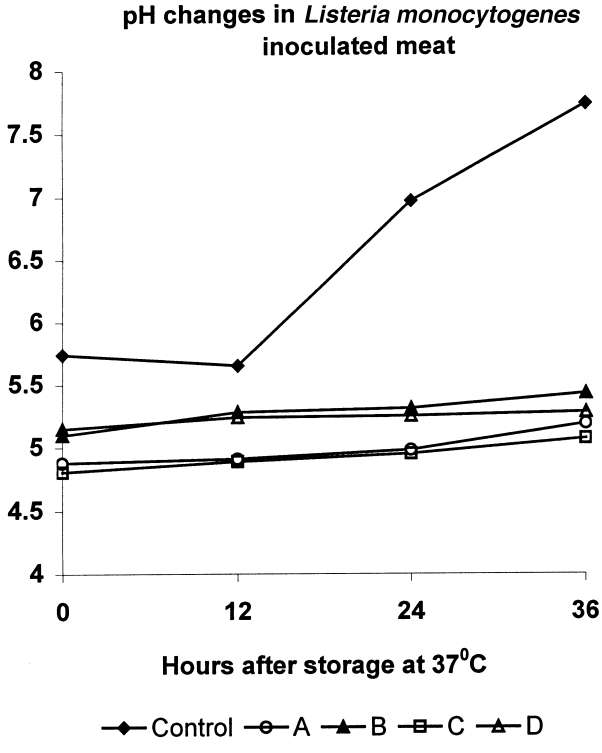

Fig. 4. Changes in pH of *Listeria monocytogenes* inoculated raw buffalo meat containing preservatives at 37°C. A: 400 IU nisin per gram of meat; B: 800 IU nisin per gram of meat; C: 400 IU nisin per gram of meat + 2% sodium chloride; D: 800 IU nisin per gram of meat + 2% sodium chloride; Control: no treatment.

Table 1
Listeria monocytogenes counts (cfu $\log_{10}$/g; mean±S.E.) in control and treated raw buffalo meat mince stored at 4°C[a]

	Days after storage						
	0	1	4	7	10	13	16
Control	3.0	3.8±1.9a	3.9±1.7a	4.2±1.7a	4.3±1.6a	5.4±0.6a	6.3±3.7a
400 IU nisin	3.0	3.0±1.7b	3.2±1.9b	4.0±2.0b	4.3±2.0	5.4±3.2b	6.3±4.2b
800 IU nisin	3.0	2.6±1.5c	3.1±1.8c	3.6±1.1c	3.7±2.0c	3.8±1.9c	3.9±1.9c
400 IU nisin + 2%NaCl	3.0	2.9±1.5b	3.2±1.5b	4.0±2.0b	4.2±2.1b	5.3±3.1b	6.1±4.1b
800 IU nisin + 2% NaCl	3.0	2.4±1.5c	2.9±1.8c	3.5±1.8c	3.5±1.8c	3.7±1.5c	3.9±1.8c

[a] Means bearing dissimilar letters (a,b,c) are significantly ($P < 0.05$) different from each other.

forming units (cfu)/g to 6.38 log cfu/g after 16 days storage. The inhibitory effect was more pronounced in group B, containing 800 IU of nisin than in group A which contained 400 IU. These observations are in agreement with earlier studies that showed that nisin reduces the pathogen count on the surface of meat dipped in a solution containing 10^4 IU/ml (Chung et al., 1989), and in broth containing 10 μg/ml (Harris, Flemming & Klaehammer, 1991).

Addition of commercial nisin in combination with common salt (groups C and D) significantly ($P < 0.05$) reduced the *L. monocytogenes* counts in both the groups when compared to the control. However, the inhibitory effect was more pronounced in group D containing 800 IU of nisin than in group C (400 IU). Addition of common salt to the medium has been reported to increase the listericidal action of nisin (Harris et al., 1991) and there is some suggestion that improved inhibition occurred in this study (Fig. 1).

The pH in the control sample increased from 5.74 to 6.92 during 16 days of storage (Fig. 2, Table 2). A general increase in the pH of the control and all groups was seen throughout storage. The pH was significantly ($P < 0.01$) lower in all the treated groups than the control. Group B had a slightly higher pH than group A throughout storage which indicated that the pH of *L. monocytogenes* inoculated meat increased with increasing concentration of nisin. The pH of groups C and D was higher after the first day of storage but was lower on extended storage than the controls. Addition of 2% common salt with the nisin slightly reduced the pH of the meat compared to incorporation of nisin alone. The sensitivity of *L. monocytogenes* to nisin has been reported to be enhanced at low pH (Ukudu & Shelaf, 1997) either because of increased efficacy of nisin at low pH or as a result of the additive effect of acidity and nisin (Benkerroum & Sandine, 1988).

The effect of combinations of nisin and sodium chloride on *L. monocytogenes* in ground buffalo meat was also studied at 37°C (Fig. 3, Table 3). In the control group the *L. monocytogenes* count increased rapidly from an initial 3 log cfu/g to 6.93 log cfu/g of meat after 36 h. *L. monocytogenes* counts in groups A and B were

Table 2
Changes in pH of *Listeria monocytogenes* inoculated raw buffalo meat containing preservatives at 4°C[a]

	Days after storage						
	0	1	4	7	10	13	16
Control	5.74a	5.65a	5.71a	5.85a	6.03a	6.81a	6.92a
400 IU nisin	5.68b	5.76b	5.84b	5.88b	5.89b	5.89b	5.93b
800 IU nisin	5.64b	5.78b	5.86b	5.90b	5.92b	5.93b	6.19b
400 IU nisin + 2% NaCl	5.62b	5.64b	5.69b	5.72b	5.72b	5.73b	5.84b
800 IU nisin + 2%NaCl	5.61b	5.66b	5.71b	5.73b	5.89b	5.91b	6.10b

[a] Means bearing dissimilar letters (a,b) are significantly ($P < 0.01$) different from each other.

Table 3
Listeria monocytogenes counts (cfu $\log_{10}$/g; mean±S.E.) in control and treated raw buffalo meat mince stored at 37°C[a]

	Hours after storage			
	0	12	24	36
Control	3.0	6.0±2.0a	6.5±4.0a	6.9±4.0a
400 IU nisin	3.0	5.9±4.1b	6.5±4.2b	6.9±4.4b
800 IU nisin	3.0	5.6±3.5c	5.9±3.5c	6.0±3.9c
400 IU nisin + 2% NaCl	3.0	5.9±3.8b	6.4±3.8b	6.8±3.8b
800 IU nisin + 2% NaCl	3.0	5.6±3.8c	5.9±3.8c	6.1±3.5c

[a] Means bearing dissimilar letters (a,b,c) are significantly ($P < 0.05$) different from each other.

Table 4
Changes in pH of *Listeria monocytogenes* inoculated raw buffalo meat containing preservatives at 37°C[a]

	Hours after storage			
	0	12	24	36
Control	5.74a	5.65a	6.97a	7.73a
400 IU nisin	4.88b	4.91b	4.98b	5.19b
800 IU nisin	5.10b	5.28b	5.31b	5.43b
400 IU nisin + 2% NaCl	4.81b	4.89b	4.95b	5.07b
800 IU nisni + 2% NaCl	5.15b	5.24b	5.25b	5.28b

[a] Means bearing dissimilar letters (a, b) are significantly ($P < 0.01$) different from each other.

slightly but significantly ($P < 0.05$) lower than in controls. However, it was more pronounced in the group B. It was evident that the increased inhibition of *L. monocytogenes* is proportional to the concentration of nisin added. The effect of nisin added to UHT skim milk incubated at 30°C on *L. monocytogenes* (approximately 10^4 cfu/ml) was found to be bacteriostatic at 10 IU/ml and slightly bactericidal at 100 IU/ml (Zapico, Medina, Gaya & Nunez, 1998). Davies and Adams (1994) established minimum inhibitory concentration values of nisin from 200 to 400 IU/ml for serotype 4b *L. monocytogenes* in tryptone soya agar at 37°C. Addition of nisin in combination with 2% common salt caused a significant ($P < 0.05$) reduction in both the groups compared to the controls, but as with the samples stored at 4°C the sodium chloride caused little improvement in inhibition.

The pH of the control samples increased from 5.74 at 12 h to 7.73 at 36 h at 37°C (Fig. 4, Table 4). The pH of all treated groups was significantly ($P < 0.01$) lower than the controls. The addition of 2% common salt in combination with nisin reduced slightly the meat pH at 37°C compared to the addition of nisin alone.

The inhibitory effect of nisin was greater at 4°C than at 37°C.

It can be concluded that the initial microbial status of the meat mince was within the normal limits and that the incorporation of 400 or 800 IU/g of commercial nisin per gram of meat alone, or in combination with 2% sodium chloride inhibited pathogenic *L. monocytogenes* at both the storage temperatures.

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