

Effect of different levels of nisin on the microbial quality of chicken cutlets

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Abstract

The present study was conducted to evaluate the efficacy of nisin on the microbial quality of chicken cutlets. Three levels of nisin viz. 400 I.U/g, 600 I.U/g and 800 I.U/g were incorporated in the formulation to study the effect of nisin on storage quality of chicken cutlets based on various microbiological counts. The chicken cutlets were aerobically packaged in low density polyethylene pouches along with control and evaluated for microbiological quality for a period of 15 days under refrigerated conditions ($4\pm 1^{\circ}\text{C}$). The products were analyzed for various microbiological parameters viz. Total plate count, Psychrophilic count, Coliform count and Yeast and mould count. All nisin treated preparations recorded lower total plate count, psychrophilic counts and yeast and mould count as compared to control. The microbiological counts of all nisin treated products were within acceptable limits up to 15 days of refrigerated storage ($4\pm 1^{\circ}\text{C}$) in LDPE bags.

Keywords: Chicken cutlets; Nisin; Refrigerated storage; Microbiological count.

Introduction

Chicken cutlets are convenient ready-to-eat meat products that are famous throughout the world and are commonly consumed in breakfast or supper. Meat cutlets are flat croquettes of minced meat and other ingredients like flours, pulses, shredded potato, condiments, and spices and are often coated with rusk crumbs. Though a highly desirable, nutritious and protein-rich meat product, chicken cutlets at the same time are also highly perishable because it provides the nutrients needed to support the growth types of microorganisms. Until now, approaches to seek improved food safety have relied on chemical preservatives, antibiotics or on more drastic physical treatments (e.g. high temperatures or refrigeration). Nevertheless, these methods have many drawbacks. Since food safety has become an increasingly important international concern, the application of antimicrobial peptides from lactic acid bacteria (LAB) that target food pathogens without toxic or other adverse effects has received great attention. The antimicrobial proteins or peptides produced by bacteria are termed bacteriocins. They are ribosomally synthesized and kill closely related bacteria (Klaenhammer, 1993). The use of bacteriocins, such as nisin, in food systems has been extensively documented (Nettles and Barefoot, 1993). Numerous reports have also addressed the addition of bacteriocins to intact or processed meat products as a means of inhibiting pathogenic or spoilage bacteria (Chung *et al.*, 1989; Vignolo *et al.*, 1996). As an antimicrobial, the bacteriocin, nisin, has numerous advantages over other available antimicrobial compounds. Currently, it is a generally recognized as safe (GRAS) substance and approved for use in pasteurized processed cheese spreads or pasteurized liquid whole egg. Nisin is stable under refrigerated conditions, demonstrates heat stability, and is degraded by gut enzymes. The spectrum of activity of this compound not only includes Gram-positive pathogens such as *Clostridium botulinum*, *Staphylococcus aureus*, *Listeria monocytogenes* or *Bacillus cereus*, but also meat spoilage organisms (*Brochothrix thermosphacta*, *Lactobacillus spp.*). In these laboratories, it has previously been demonstrated that nisin can effectively suppress the meat spoilage isolate, *B. thermosphacta*, when the bacteriocin is applied directly to the meat surface (Cutter and Siragusa, 1994a), immobilized in a calcium alginate gel (Cutter and Siragusa, 1996b, 1997), or combined with vacuum packaging and refrigerated storage (Cutter and Siragusa, 1996a). Main antimicrobial activity of nisin is to form pores in the cytoplasmic membrane, which leads to a loss of small intracellular molecules and ions and a collapse of the proton motive force. To exert its antimicrobial activity, nisin seems to require a specific receptor (Hasper *et al.*, 2006) or a sufficient trans-negative electrical membrane potential (Abee *et al.*, 1995). Considering the extensive documental evidence of use of nisin as a food preservative and its general safety the present study was conducted with an objective of determining the effect of nisin on microbial quality of chicken cutlets during their storage in refrigeration conditions.

Material and methods

Raw materials

Broilers were purchased from local market of Chomu, Jaipur. The birds were slaughtered using halal method. The body fat was trimmed and deboning of dressed chicken was done manually removing all tendons and separable connective tissue. The lean meat was packed in polythene bags and frozen at -18 ± 2 °C until use. Refined cottonseed oil of brand name 'Ginni' containing energy (900 Kcal/100 gram), saturated fatty acids (24g %) and cholesterol 0% was purchased from local market used. Condiments used were fresh onion, garlic and ginger in a ratio of 3:2:1 and ground in a mixer to the consistency of fine paste. The spice mix formula used for preparation of the chicken patties was standardized in the laboratory and contained aniseed (*Pimpinella anisum*) 12%, coriander (*Coriandrum sativum*) 20%, cumin seed (*Cuminum cyminum*) 15%, black pepper (*Piper nigrum*) 10%, red chilli (*Capsicum frutescens*) 8%, green cardamom (*Elettaria cardamomum*) 6%, white pepper (*Piper nigrum*) 5%, Black cardamom (*Amomum subulatum*) 5%, cinnamon (*Cinnamomum zeylanicum*) 6%, degi mirch (*Capsicum annum*) 5%, bay leaves (*Laurus nobilis*) 2%, cloves (*Syzygium aromaticum*) 2%, mace (*Myristica fragrans*) 2% and nutmeg (*Myristica fragrans*) 2%. Nisin was obtained from Hi Media. The minimum potency of nisin used was 900 I.U/mg and containing NaCl ≥ 50.0 %.

Method of preparation of chicken cutlets

Several preliminary trials were conducted to optimize the basic formulation and processing conditions for the preparation of chicken cutlets. The standardized formulation contained lean meat 75%, added water 2%, shredded potato 6%, condiment mixture 9%, gram flour 3%, whole egg liquid 1%, spice mixture 2%, common salt 1.75%, sugar 0.25% and sodium nitrite 150ppm.

Lean meat from round part of chicken was cut into smaller chunks and minced in a Sirman mincer (MOD-TC 32 R10 U.P. INOX, Marsango, Italy) with 6mm plate twice. The common salt, sugar, sodium nitrite and added water in the form of crushed ice was added to weighed meat according to above formulation and was kept at refrigeration temperature ($4\pm 1^{\circ}\text{C}$) for 15-20 minutes. The mixture was shallow fat fried in 2.5 percent w/w refined oil for 8 minutes. The condiment and spice mixture was fried separately till golden brown colour. The fried meat, condiment and spice mixture, gram flour, shredded potato and whole egg liquid were mixed in a domestic mixer. The batter so formed was used in the preparation of raw cutlets by using moulds. The raw cutlets were kept at refrigeration temperature for 15-20 minutes and dipped in whole egg liquid and then rolled in rusk powder till uniform coating was formed on the surface and were deep fat fried in refined oil till golden brown colour. The internal core temperature was measured with the help of a thermometer ($80\pm 2^{\circ}\text{C}$) and the excess fat was removed from the fried cutlets by using tissue paper.

Analytical procedures***Microbiological profile***

Total plate count, psychrophilic count, coliform count and yeast and mold count were determined by methods of APHA (1984).

Analysis

The results were analyzed statistically for analysis of variance and least significant difference tests as per Snedecor and Cochran (1997). In significant effects, least significant differences were calculated at appropriate level of significance for a pair wise comparison of treatment means.

Results and discussion***Microbiological characteristics***

The mean values of various microbiological characteristics of chicken cutlets incorporated with 400 I.U/g, 600 I.U/g and 800 I.U/g nisin along with control during refrigerated storage ($4\pm 1^{\circ}\text{C}$) are presented in Table-1.

Total plate count (log cfu/g)

Total plate count followed a linear increasing trend from day 0 to 21 in treated products and control. Total plate count of treated chicken cutlets was lower than control during entire period of storage. Total plate count of all the treated products and control had a comparable count on day 0. However, at day 5 and 10 TPC count of 600 I.U/g nisin and 800 I.U/g nisin treated products were significantly ($P<0.05$) lower as compared to control. At day 15, the counts of all the treated products were significantly ($P<0.05$) lower as compared to control. The reduction of total plate count in nisin treated preparations was very much as per expectations, since nisin is a broad spectrum bacteriocin with bactericidal activity, even in low concentrations, towards a wide range of Gram-positive bacteria, including *Staphylococcus aureus* and *Listeria monocytogenes* (Parada *et al.*, 2007). Similar effects on total colony count (TCC) due to incorporation of nisin have been reported (Reham, 2012). Behnam *et al.* (2013) also reported similar results for the influence of nisin treatment on total viable count (TVC) in vacuum packaged rainbow trout.

Table-1: Effect of refrigerated storage on microbiological characteristics of aerobically packed chicken cutlets treated with different levels of nisin (Mean±SE)*

TREATMENTS	STORAGE PERIOD (DAYS)			
	0	5	10	15
Total plate count (log cfu/g)				
Control	2.57±0.095 ^{Ac}	3.68±0.081 ^{Ab}	3.89±0.095 ^{Ab}	4.65±0.084 ^{Aa}
Nisin(400 I.U/g)	2.51±0.079 ^{Ac}	3.57±0.064 ^{ABb}	3.67±0.045 ^{ABb}	4.43±0.081 ^{Ba}
Nisin(600 I.U/g)	2.44±0.057 ^{Ad}	3.40±0.076 ^{BCC}	3.61±0.052 ^{Bb}	4.38±0.032 ^{Ba}
Nisin(800 I.U/g)	2.40±0.044 ^{Ac}	3.25±0.051 ^{Cb}	3.34±0.085 ^{Cb}	4.19±0.039 ^{Ca}
Psychrophilic count (log cfu/g)				
Control	Not detected	Not detected	2.48±0.030 ^A	2.55±0.068 ^A
Nisin(400 I.U/g)	Not detected	Not detected	2.06±0.053 ^B	2.22±0.044 ^B
Nisin(600 I.U/g)	Not detected	Not detected	1.76±0.066 ^C	1.85±0.022 ^C
Nisin(800 I.U/g)	Not detected	Not detected	1.68±0.055 ^C	1.73±0.028 ^C
Coliform count (log cfu/g)				
Control	Not detected	Not detected	Not detected	Not detected
Nisin(400 I.U/g)	Not detected	Not detected	Not detected	Not detected
Nisin(600 I.U/g)	Not detected	Not detected	Not detected	Not detected
Nisin(800 I.U/g)	Not detected	Not detected	Not detected	Not detected
Yeast and mould count (log cfu/g)				
Control	Not detected	Not detected	Not detected	1.93± 0.096
Nisin(400 I.U/g)	Not detected	Not detected	Not detected	1.86± 0.082
Nisin(600 I.U/g)	Not detected	Not detected	Not detected	1.83± 0.087
Nisin(800 I.U/g)	Not detected	Not detected	Not detected	1.87±0.099

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05); n=6 for each treatment.

Psychrophilic count (log cfu/g)

Psychrophilic counts were not detected on day 0 and day 5 of storage in both control and nisin treated products. But, it was observed on day 10 and day 15 of storage in all treated groups as well as control. However, the counts of treated products were significantly (P<0.05) lower as compared to control on day 14 and 21 of storage. Kim *et al.* (2006) and Behnam *et al.* (2013) observed a similar trend in psychrophilic count of nisin treated Korean seasoned beef and vacuum packaged rainbow trout respectively.

Coliform count (log cfu/g)

The count was not detected throughout the storage period in both control as well as nisin treated products. It could be due to destruction of these bacteria during cooking at 180 °C much above their death point of 57°C. Further, hygienic practices followed during the preparation and packaging of chicken cutlets could also be one of the reasons for the absence of coliforms. Dawson (1975) and Kumar (2001) also reported zero count of coliform for the product heated to such a high temperature.

Yeast and mould count (log cfu/g)

Yeast and mould count was observed on day 21. No significant (P>0.05) effect of nisin was found in treated products when compared to control. These results are supported by the fact that nisin is generally not active against yeasts and fungi (Hampikyan and Ugur, 2007; Boziaris and Adams, 1999.)

Conclusion

The present study showed that in the preparation of chicken cutlets, the initial microbial count on day 0 was within acceptable limits in both control and treated products and that the incorporation of 400, 600 or 800 IU/g of commercial nisin in chicken cutlets provided significantly lower microbiological counts in its preparations up to 15th day of refrigeration storage (4±1⁰C).

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