Synergistic Effect of some Chemical Preservatives on Nisin Inhibition of Shiga Toxin Producing Escherichia coli O111:H4 in Minced Beef

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**Abstract**

A total of 96 frozen beef samples were minced and inoculated with *E. coli* O111:H4 previously isolated from minced meat samples by an intensity 1×10⁶ g. The samples were divided into 4 groups (24 each) to study the effect of nisin and other chemical preservatives on survival of such pathogen after 12, 24 and 48 hours of inoculation. The obtained results indicated that the addition of nisin alone whatever its concentration was not effective for complete elimination of *E. coli* O111:H4 inoculated into minced beef samples. However, a complete elimination of such organisms was not achieved either by adding nisin alone or combined with 0.2% sodium acetate or potassium sorbate. On the other hand, an evidence of complete elimination of *E. coli* was successfully revealed by adding nisin (10 ppm) combined with sodium lactate (0.2%) for 24 hours. Hence, the applied concentration of nisin and their durations of treatment had a significant effect (P<0.05) on the viability of *E. coli*. Accordingly, a combination of nisin and sodium lactate was the best effective and economic formula to eliminate such serious pathogen for production of safe meat products.

**Introduction**

In Egypt, meat products are gaining popularity because they represent quick easily prepared meat meals and solve the problem of the shortage in fresh meat of high price which is not within the reach of large numbers of families with limited income. Contamination of meat products with *E. coli* during further processing need to establish appropriate control measures to get rid of such contaminants.

Pathogenic *E. coli* have been broadly classified into two major categories; the diarrheagenic *E. coli* and the extra intestinal pathogenic *E. coli*. Among the diarrheagenic *E. coli*, there are currently six categories including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusively adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC). (Xiaodong., 2010).

Shiga toxin–producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VT) or enterohaemorrhagic *E. coli* (EHEC), have been known as a group of highly pathogenic *E. coli* strains producing one or more Shiga toxins (Monaghan et al., 2011). The term verocytotoxin producing *E. coli* was derived form observation of strains producing a toxin with a profound and irreversible cytopathic effect on Vero cells "African green monkey kidney" (Konowalchuk et al., 1977). STEC represent a hazardous public health problem worldwide causing various human gastrointestinal tract diseases, including watery or bloody diarrhea and might develop a life-threatening diseases, such as haemorrhagic colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP) and Haemolytic Uraemic Syndrome (HUS). The later is characterized by thrombocytopenia, microangiopathic haemolytic anaemia and acute renal failure (Pennington, 2010).

As Shiga toxin–producing *E. coli* (STEC) are considered public health hazards , various approaches for minimization and controlling of STEC in meat product were tried . Natural preservatives as nisin alone or combined with other chemicals to be effective against *E. coli* were used for biocontrol of *E. coli*. (Catherine
Nisin “bacteriocin” is an antimicrobial peptide produced by some strains of *Lactococcus lactis* (Bender and Bender, 1995) and is used in meat technology as a chemical preservative where it has a powerful inhibitory effect against Gram-positive bacteria, but probably has not the same effect on Gram-negative ones as *E.coli* (Delves & Gasson, 1994 and Thomas et al., 1998). However, the effect of nisin when combined with various chelators such as citrate, lactate and phosphate is questionable against Gram-negative pathogens particularly *E.coli* (Catherine & Gregory, 1995 and Eleiwa-Nesereen, 2003). Therefore, the main purpose of the present study is to modify the action of nisin through its combination with other agents to be effective against *E.coli* especially *E.coli* O111:H4.

**Material and methods**

**Preparation of minced meat:** A total of 96 frozen beef samples (50 g each) were separately minced and packaged and then divided into 4 groups (24 each). Each group was subdivided into 3 classes to study the effect of nicin, either alone or combined with other chemical preservatives, on the viability of *E.coli* O111:H4 in tested samples.

**Inoculation of minced meat samples with *E.coli* O111:H4.**

All tested minced beef samples proved to be free from *E.coli* by using conventional isolation of *E.coli* were evenly inoculated with *E.coli* O111:H4 strain suspended in peptone water by an intensity 1×10⁶ organisms per each gram. The Population of investigated pathogen was evaluated in peptone water by McFarland's nephelometer standards according to Finegold et al.(1978) , and confirmed in inoculated meat samples by enumeration technique on plates of eosin methylene blue(EMB) medium. The growing colonies, having green metallic shine, were enumerated and the initial population as well as the survivors of organism per each gram of tested meat samples was then calculated.

**Statistical analysis:**

Statistical evaluation including analysis of variance (ANOVA) was used. Rosner, (2002).

**Results**

Table (1): Biocontrol effect of different nisin concentrations on *E.coli* O111:H4 inoculated into minced beef samples by an intensity of 1×10⁶/g.

![Graph showing the effect of different nisin concentrations on *E.coli* O111:H4 inoculated into minced beef samples](graph.png)
Table (2): Biocontrol effect of different nisin concentrations combined with 0.2% Sodium lactate on E.coli O111:H4 inoculated into minced beef samples by an intensity of 1×10^6/g.

Table (3): Biocontrol effect of different nisin concentrations combined with 0.2% Potassium sorbate on E.coli O111:H4 inoculated into minced beef samples by an intensity of 1×10^6/g.

Table (4): Biocontrol effect of different Nisin concentrations and durations of treatment. Accoring to Table (1), data indicated that all E.coli were relatively resistant to the bactericidal effect of Nisin.

In general, Nisin has a wide spectrum of activity against Gram-positive bacteria including spore-formers but its action is not significant against Gram-negative bacteria (Thomas et al., 1998). In this respect, Gram-positive bacteria are characterized by presence of high contents of anionic lipids in their cell membranes which can be easily penetrated by nisin (Masschalck et al., 2003). Thus, disruption of the bacterial cell membrane results in the passage of Nisin to the cytoplasmic membrane as the site of nisin action (Carneiro et al., 1998). On the other hand, the resistance of E.coli as Gram-negative bacteria against nisin depends on the type of lipopolysaccharide of the cell membrane. Consequently, addition of an agent to change the nature of the outer membrane of E.coli is very necessary to render the organism sensitive to nisin (Ganzle et al., 1999).

Table (2) reveals that the combination of nisin (10 ppm) and sodium acetate (0.2%) has a slight effect on the viable count of E.coli inoculated into minced beef samples (1×10^6/g) to become of mean values of 1.4×10^3 ± 7.9×10^3, 5.0×10^3 ± 2.5×10^3 and 2.1×10^3 ± 8.6×10^2 organisms per gram after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Furthermore, nisin at concentrations of 30 ppm mixed 0.2% sodium acetate decreased the initial intensity of inoculated E.coli (1×10^6/g) to average of 2.9×10^3 ± 1.3×10^4, 1.3×10^3 ± 9.3×10^2 and 4.4×10^2 ± 2.9×10^2 organisms/gram after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Although the concentrations of nisin and durations of treatment had significant effect (p <0.05) on viability of E.coli, yet the complete elimination of this organism was not attained by nisin and sodium acetate mixture. Similar findings were obtained by Stevens et al. (1997), who failed to eliminate E.coli organisms by nisin and sodium acetate formula.

Data in table (3) declare that the combination of nisin (10 ppm) and potassium sorbate (0.2%) has a slight effect on the viable count of E.coli

Discussion

Tabulated results in Table (1) indicate that the addition of nisin to minced beef samples experimentally inoculated with 1×10^6/g E.coli O111:H4 by concentrations of 10 ppm and 30 ppm could decrease their population from 1×10^6/g to 2.2×10^3±9.9×10^3 and 5.6×10^3±2.3×10^3/g mean values after 12 hours, while the same concentrations of nisin could reduce the initial intensity of such pathogen to averages of 7.5×10^3 ± 2.8×10^3 and 2.6×10^3±1.7×10^3/g after 24 hours, whereas more reduction was revealed when the initial count declined to mean values of 5.2×10^3 ± 2.3×10^3 and 1.4×10^3 ± 7.7×10^2/g after 48 hours, respectively. Such variations in population were significant (P<0.05) as a result of nisin concentrations and durations of treatment. Accordingly, the addition of nisin alone whatever its concentration was not effective for complete destruction of E.coli O111:H4 inoculated into minced beef samples. The current results agree, to some extent, with those recorded by Demel et al. (1996), Hassan (1999) and Gill &Holly (2004) who reported...
inoculated into minced beef samples (1×10^6/g) to become of mean values of 1.3×10^4 ± 95×10^3, 6.1×10^2 ± 2.5×10^2 and 5.4×10^2 ± 2.0×10^2 /g after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Furthermore, nisin at concentrations of 30 ppm mixed 0.2% potassium sorbate decreased the initial intensity of inoculated E. coli (1×10^6/g) to average of 2.3×10^3 ± 9.7×10^2 , 3.2×10^2 ± 2.4×10^2 and 6.6×10 ± 2.4×10 organisms per gram after 12, 24 and 48 hours from the incorporation of such formula in tested samples, respectively. Although the concentrations of nisin and durations of treatment had significant effect (p <0.05) on viability of E. coli, yet the complete elimination of this organism was also not attained by nisin and potassium sorbate formula. Similar findings were obtained by Hassan (1999) and Eleiwa- Nesereen (2003) who failed to eliminate E. coli organisms by nisin and potassium sorbate formula.

Actually, potassium sorbate inhibits certain amino acids uptake by E. coli interfering with its growth and the action of potassium sorbate is greatly dependant on PH of the food article where the low PH (1- 4) had an apparent synergistic inhibition action caused by sorbate on the amino acid uptake by E. coli (Eklund, 2003). Therefore, the minor role of potassium sorbate with nisin for elimination E. coli in the present study may be attributed to the fact that the PH of fresh minced meat is above 5.5 at which the action of potassium sorbate is significantly affected. (Eleiwa- Nesereen., 2003) Table (4) declaired that nisin (10 ppm and 30 ppm) combined with sodium lactate (0.2 %) could decrease the initial count of E. coli in tested minced beef samples from 1×10^6/g to mean values of 4.0×10^3 ± 2.0×10^2and 15×10 ± 7.5×10 /g after 12 hours from adding this preservative formula in the tested samples, respectively. While, the incorporation of nisin either 10 or 30 ppm with 0. 2% Sodium lactate lead to a complete elimination of E. coli after 24 and 48 hours of this treatment.

It is postulated that Sodium lactate destabilize the cell membrane of E. coli and other Gram-negative bacteria by chelating Mg++ and /Ca++ that affecting its permeability to be sensitive to the action of nisin (Catherine & Gregory, 1995 and Tipayanate et al., 1999).

It is concluded that addition of nisin in combination with sodium lactate is effective for elimination of E. coli. Consequently, from the economic point of view, the later formula is wonderful for the meat processors to produce a meat product of good keeping quality by adding the correct preservative against the target organism instead of application of many other preservative of higher cost and lower effect.

Finally, the present results allow to conclude that the best formula to destroy E. coli especially E. coli O111H4 in minced beef is the application of nisin by concentration of 10 ppm and 30 ppm coupled with sodium lactate (0.2%) for at least 24 hours to obtain a maximum margin of human safety against such serious pathogen.

Reference


Xiaodong, X. (2010): Pathogenic E.coli in retail meats. Dissertation submitted to the Faculty of the Graduate School of the University of Maryland, College Park, in partial fulfillment of the requirements for the degree of Doctor of Philosophy 2010.
تأثير بعض المواد الحافظة مع نيسين على ميكروب الأشيزشيا كولاي المفرز لسموم الشيجا توكسين

وخصوصاً في اللحم المفروم H4 O111

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نظراً للأهمية الصحية للميكروب الولوني المفرز لтокسين شيجا ودوره في حدوث الإسهال في الإنسان والحيوان فقد أجريت هذه الدراسة على 96 عينة من اللحوم المفروم بعد حفظها بالميكروب الأشيزشيا كولاي H4 O111

أحد تأثير نيسين وبعض المواد الحافظة على نمو الميكروب من تحضيرات 12 و24 و48 ساعة. واشارت النتائج استخدام نيسين بعدهو كافيا لقتل الميكروب سواء بتركيز صغير اوكثير. تناولت الدراسة تأثير كل من nisin , sodium acetate , potassium sorbate and sodium lactate و مدة تخزين اللحوم ومنتجاتها على كثافة نمو ميكروب الأشيزشيا كولاي H4 O111.