Application of Bacteriocin as Bio-preservative in Foods

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Abstract: Food Preservatives aim either to destroy or inhibit the growth of harmful microorganisms in food by making an environment unsuitable for them. Traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their bacteriocins. The use of LAB and/or their bacteriocins, either alone or in combination with mild physicochemical treatments and low concentrations of traditional and natural chemical preservatives, may be an efficient way of extending shelf life and food safety through the inhibition of spoilage and pathogenic bacteria without altering the nutritional quality of raw materials and food products. In this study, both of chemical preservative as sodium nitrite and natural preservative as Bacteriocin and Nisin were used at different concentrations to examine the effect of each one alone and in combined with each other against different food pathogens. Minced meat samples were inoculated with the prepared cultures of different pathogen as *E. coli* O111:K58, *E. coli* O124:K72, *E. coli* O128:K67, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus cereus* at dose of 1×10^6/ CFU per gram. It was found that both of Sodium nitrite at 125 ppm and Nisin at 10 ppm has inhibitory effect on the examined pathogens as their count was minimized but still viable, while Nisin at 30 ppm prevent the growth of gram – positive pathogens. When the two preservatives (Sodium nitrite at 125 ppm and Nisin at 10 ppm) were used in combined with each other in presence of Lactate (0.01%) no growth observed for both gram – positive or gram – negative pathogens.

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1. Introduction

Since the earliest times humankind has searched for ways to make the food supply safer and to make food last longer. Without the use of some preservation technique, the natural microorganisms that are present everywhere in the environment will grow and multiply in foods.

Preservation aims either to destroy or inhibit the growth of harmful microorganisms in food by making an environment unsuitable for them.

Preservation techniques that limit the availability of water, such as drying, salting, and smoking, and those that use heat, such as canning and pasteurization, dramatically alter the nature of the food itself. These processes degrade the color, flavor, texture, and nutrients in food. Today's consumers want their food to appear fresh and natural, as close to just-picked or just-slaughtered as possible. They don't want preservatives and other chemicals added to their foods, and at the same time they want convenience (Thorne, 1986).

As consumers have become chemical- and preservative-phobic, food preservation using natural antimicrobials has evolved. This concept involves a more natural and milder alternative to making food safer. By their very nature of being milder, natural Antimicrobials by themselves are not sufficient to control pathogens. However, when used in combination with other food preservation methods they can improve the safety of foods without the use of traditional chemical preservatives such as sorbate or benzoate, which consumers no longer consider natural and healthy(Rahman, 1999).

Sodium nitrite is a water soluble, inorganic salt. Nitrite and nitrate are readily interconverted in the body by means of oxidation-reduction reactions. Hence the pharmacokinetics and metabolism of nitrite cannot appropriately be considered in complete isolation from nitrate.

Nitrite and, by extension, nitrate, can also serve as precursors for the in vivo formation of carcinogenic N-nitroso compounds. Both nitrite and nitrate occur naturally in many foods, particularly vegetables. Both compounds also have food additive uses in the production of cured meat products. Both nitrite and nitrate can be found as contaminants of drinking water (JECFA, 1988).

Nitrite is used as a preservative in cured meat products, to prevent the growth of the botulism

toxin-producing organism, *Clostridium botulinum* (Sen and Baddoo, 1997).

Additionally, formation of nitroso-compounds of myoglobin and hemoglobin enhance the red color of cured meats. USDA regulations allow addition of sodium nitrite to cured meats at a maximum concentration of 156 ppm (Cassens, 1997).

Residual nitrite levels in retail cured meat products are considerably lower, with current assays showing a mean of approximately 10 ppm.

The consumption of more food that has been formulated with chemical preservatives has also increased consumer concern and created a demand for more natural and minimally processed food.

As a result, there has been a great interest in naturally produced antimicrobial agents (Cleveland *et al,* 2001).

To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their bacteriocins.

The use of LAB and/or their bacteriocins, either alone or in combination with mild physicochemical treatments and low concentrations of traditional and natural chemical preservatives, may be an efficient way of extending shelf life and food safety through the inhibition of spoilage and pathogenic bacteria without altering the nutritional quality of raw materials and food products (Montville *et al.,* 1997 and Ross *et al.,* 2002).

Hence, the last two decades have seen intensive investigation on LAB and their antimicrobial products to discover new bacteriocinogenic LAB strains that can be used in food preservation.

Biopreservation, as commented above, can be defined as the extension of shelf life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds (Deegan *et al*, 2006).

LAB bacteriocins: The antimicrobial ribosomally synthesized peptides produced by bacteria, including members of the LAB, are called bacteriocins. Such peptides are produced by many, if not all, bacterial species and kill closely related microorganisms (Jack *et al.,* 1995).

Due to their nature, they are inactivated by proteases in the gastrointestinal tract. Most of the LAB bacteriocins identified so far are thermostable cationic molecules that have up to 60 amino acid residues and hydrophobic patches. Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding, forming pores and killing the cells after causing lethal damage and autolysin activation to digest the cellular wall (Muñoz *et al*, 2007).

The LAB bacteriocins have many attractive characteristics that make them suitable candidates for use as food preservatives, such as:

• Protein nature, inactivation by proteolytic enzymes of gastrointestinal tract.

• Non-toxic to laboratory animals tested and generally non-immunogenic.

• Inactive against eukaryotic cells.

•Generally thermo resistant (can maintain antimicrobial activity after pasteurization and sterilization).

• Broad bactericidal activity affecting most of the Gram-positive bacteria and some, damaged, Gram-negative bacteria including various pathogens such as *L. monocytogenes, Bacillus cereus, S. aureus, and Salmonella.*

• Genetic determinants generally located in plasmid, which facilitates genetic manipulation to increase the variety of natural peptide analogues with desirable characteristics.

For these reasons, the use of bacteriocins has, in recent years, attracted considerable interest for use as biopreservatives in food, which has led to the discovery of an ever-increasing potential of these peptides.

Nisin is effective against food-borne pathogens such as *L. monocytogenes* and many other Gram-positive spoilage microorganisms (Thomas and Delves, 2001).

Bacterioncins are used as a tool to control the growth of undesirable microbial growth including spoilage and pathogenic bacteria and to keep the food more acceptable to consumers (Deegan *et al*., 2006).

Bacteriocins are classified into three or four groups (Klaenhanner, 1993; Nes *et al*., 1996). Lantibiotics are belonging to class I which divided to class Ia and IIa.

Class Ia include nisin that consist of cationic and hydrophobic small peptides containing lanthionine and B-methyl lanthionine that form pores in target membranes (Altena *et al*., 2000).

Nisin is listed in Spain as E-234, and may also be cited as nisin preservative or natural preservative. In addition to the work on nisin, several authors have outlined issues involved in the approval of new bacteriocins for food use (Fields, 1996). Both of bacteriocins as natural metabolites of *Lactobacillus acidophilus*, Nisin A (AGCH- 9470 Bucks, E234) and sodium nitrite were used to examine the viability of the common six food pathogens in addition to Enterobacteriacae, coliform and staphylococci control .

The aim of this work: studying the effect of sodium nitrite, Bacteriocin and Nisin either alone, or in combined with each other on the growth of different food pathogens.

2. Material and Methods

2.1. Preparation of Bacterial cultures (Duffy *et al.*, 2000)

Reference strains of Enterohaemorrhagic *E. coli* O111:K58, *E. coli* O124:K72, *E. coli* O128:K67, *Salmonella typhimurium, Staphylococcus aureus and Bacillus cereus* were obtained from Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt.

All strains were kept on tryptone soya agar slants at 4°C. Before being used, the strains were grown twice in 1% peptone water at 37°C for 24 hours. Therefore, the inoculums were determined by dilutions and subsequent enumeration on their specific media. The level of inoculants of each pathogen was 1×10^6/ CFU per gram of the used sample (minced meat).

2.2. Preparation of minced meat samples (Hassan, 1999)

Block samples of minced meat were wrapped in polyethylene pockets. Each block sample was divided into 3 portions to apply any test three times. The collected block samples were classified into different groups to study the effect of certain treatments on the viability of tested pathogens.

Before being inoculated, the minced meat samples were examined for naturally occurring entire pathogens by using of their selective agar media. Accordingly, the minced meat samples were then inoculated separately with the prepared cultures of each pathogen at dose of 1×10^6/ CFU per gram.

2.3. Addition of sodium nitrites (Catherine and Gregory, 1995)

Addition of sodium nitrite to the inoculated minced meat samples to study their effects on the growth and viability of different pathogens was adopted as follow:

* Addition of sodium nitrite at dose of 125 ppm
** Addition of sodium nitrite (125 ppm) + Nisin (10 ppm)
*** Addition of sodium nitrite (125 ppm) + Nisin (10 ppm) + sodium lactate (0.01%)

The treated samples were left in refrigerator at 4°C for 2 weeks and then examined for determination the survival of such pathogens by culturing on their selective media.

2.4. Addition of Nisin (Rhee *et al.*, 2002)

Nisin at doses of 10 and 30 ppm as well as 10 ppm mixed with sodium lactate (0.01%) were added separately to the samples of inoculated minced meat (3 for each dose). The treated samples were left in refrigerator at 4°C for 24 hours and the enumeration of such pathogens was applied by culturing on their selective media. Also, the effect of addition of Nisin on the growth of different bacterial groups (Total bacterial, Enterobacteriaceae, Coliforms and Staphylococci counts) was studied.

2.5. Addition of bacteriocin (*L. acidophilus*) (Narrashima, 1995)

The inoculated minced meat samples with certain pathogens were treated with bacteriocin concentration of 160 I/U per gram sample. Thus, the detection and enumeration of the tested pathogens and other bacterial groups were carried out at zero time (Control), 1 and 2 weeks of cold storage at 4°C. In general, bacteriocin was obtained from lactic acid culture "*L. acidophilus*" (Ezal My 087, Texel, 86220 Dange Saint, Romaine, France).

3. Results and Discussion

3.1. Effect of sodium nitrite on the growth of different bacterial pathogens

Table (1) showed the results observed for the effect of sodium nitrite on the growth of the six pathogens as following:

E. coli O111:K58 showed sensitivity to sodium nitrite as its count is the least one observed between the three *E. coli* strains with average 5×10^3 cfu/g, while *E.coli* O124:K72 showed viability with the count at average 9.6×10^3 cfu/g, at the same time *E. coli* O128:K67 is the strain that showed viability with the highest count at average 3×10^4 cfu/g.

Table 1. Effect of sodium nitrite on the growth of different bacterial pathogens

Tested pathogens	Nitrite (125 ppm)
E. coli O111:K58	5.0×10^3
E. coli O124:K72	9.6×10^3
E. coli O128:K67	3.0×10^4
S. typhimurium	2.7×10^3
Staph. aureus	6.0×10^2
Bacillus cereus	2.0×10^2

The results observed for *S. Typhi* refers to that it is more sensitive to sodium nitrite than *E. coli* at the count observed for the three samples was 9.7×10^2 cfu/g, 1.4×10^3 cfu/g and 5.6×10^3 cfu/g respectively with average 2.7×10^4 cfu/g.

Both of *Staph. aureus* and *B. cereus* as G+ve bacteria showed high sensitivity to sodium nitrite higher than other G-ve strains as the latter one which is *B. cereus* showed the lowest count observed for the three samples 1.0×10^2 cfu/g, 1.0×10^2 cfu/g and 4.0×10^2 cfu/g respectively with count average 2.0×10^2 cfu/g that is marginally satisfactory according to food hygiene criteria for hygiene stuff No.1441/2007.

3.2. Effect of Bacteriocin (*Lactobacillus acidophilus*) on the growth of different bacterial pathogens

Table (2) revealed that at zero time "control" the count observed was 5×10^6 cfu/g .After incubation for 1 week at 4°C, G +ve bacteria showed high sensitivity to bacteriocin as no growth observed for both of *Staph. aureus and Bacillus cereus*, on the other hand G–ve bacteria (*E. coli, S. typhimurium*) showed less sensitivity to bacteriocin as still have viable count which is less than control count, However, *E. coli* O124:K72 is similar to G +ve bacteria as no growth observed for it. After two weeks, no growth observed for all six pathogens.

Table 2. Effect of Bacteriocin *(L. acidophilus)* on the growth of different bacterial pathogens

Tested pathogens	Control	Count after 1 week	Count after 2 weeks
E.coli O111:K58	5.0×10^6	1.0×10^2	No growth
E.coli O124:K72	5.0×10^6	No growth	No growth
E.coli O128:K67	5.0×10^6	2.0×10^2	No growth
S. typhimurium	5.0×10^6	1.0×10^2	No growth
Staph. aureus	5.0×10^6	No growth	No growth
Bacillus cereus	5.0×10^6	No growth	No growth

3.3. Effect of Bacteriocin (*Lactobacillus acidophilus*) on the growth of different bacterial groups

Table (3) showed that Bacteriocin has inhibitory effect against Enterobacteriaceae count as after one week of incubation at 4°c the count of the first sample minimized to 4.8×10^3 cfu/g while the second sample showed high sensitivity to bacteriocin as the count minimized to 6.2×10^3 cfu/g and the third sample 1.3×10^3 cfu/g.

After the second week, the inhibitory effect of Bacteriocin increased against Enterobacteriaceae count for the three samples as 7.5×10^2 cfu/g, 1.1×10^3 cfu/g and 3.0×10^2 cfu/g, respectively.

Additionally, the same inhibitory impact for bacteriocin was observed against Coliform count as after one week the first sample showed high sensitivity as the count minimized to 1.6×10^3 cfu/g while the second sample 2.9×10^3 cfu/g and the count of the third sample minimized to 5.0×10^2 cfu/g.

After the second week, all the samples showed higher sensitivity as the count minimized to 3.4×10^2 cfu/g, 5.9×10^2 cfu/g and 8.0×10 cfu/g for the three samples respectively.

Staphylococci count was affected by bacteriocin as high sensitivity observed that after the first week the count minimized to 2.0×10^2 cfu/g for the first sample which is satisfactory according to the standards, however no growth observed for the second and the third sample. After the second week no growth observed for the first sample.

Table 3. Effect of Bacteriocin (*L. acidophilus*) on the growth of different bacterial groups

Bacterial counts	Control	Count after 1 week	Count after 2 weeks
Enterobacteriaceae	2.7×10^4	4.1×10^3	7.1×10^2
Coliform	9.9×10^3	1.5×10^3	3.3×10^2
Staphylococci	2.9×10^3	2.0×10^2	No growth

3.4. Effect of Nisin on the growth of different bacterial pathogens

Table (4) showed the effect of Nisin on the growth of the common six pathogens which inoculated 1×10^6 cfu/g in minced meat. Nisin was used at different concentrations 10 ppm, 30 ppm and 10 ppm+Lactate (0.01%).

At Nisin concentration, 10 ppm *Staph. aureus* showed high sensitivity to Nisin, as the count average value was 8.0×10^2 cfu/g, while *E. coli* O111:K58 showed sensitivity to Nisin 10 ppm which is similar to *Staph. aureus*, as the count average value was 8.3×10^2 cfu/g.

Additionally, Nisin 10 ppm has inhibitory effect on *E. coli* O124:K72 as the count average value was 1.5×10^3 cfu/g, at the same time the count average value observed for *Bacillus cereus*, *Staph. aureus* and *E. coli* O128:K67 were 1.7×10^3, 2.6×10^3 and 3.7×10^3 cfu/g, respectively.

However, when Nisin was added at 30 ppm no growth was observed for G +ve bacteria which were *Staph. aureus* and *Bacillus cereus*.

On the other hand, it is observed that there was viability for the other G –ve bacteria as *E. coli* O111:K58 is the strain that showed high sensitivity to Nisin 30 ppm as the count average value was 2.5×10^2 cfu/g. Both of *E. coli* O124:K72 and *E. coli* O128:K67 showed sensitivity the count average value observed was 5.0×10^2 and 9.0×10^2 cfu/g respectively. The count average value observed for *S. typhi* was 5.6×10^2 cfu/g.

Table 4. Effect of Nisin on the growth of different bacterial pathogens

Tested pathogens	Nisin (10 ppm)	Nisin (30 ppm)	Nisin (10 ppm) + Lactate (0.01%)
E. coli O111:K58	8.3×10^2	2.5×10^2	No growth
E. coli O124:K72	1.5×10^3	5.0×10^2	No growth
E. coli O128:K67	3.7×10^3	9.0×10^2	No growth
S. typhimurium	2.6×10^3	5.6×10^2	No growth
Staph. aureus	8.0×10^2	No growth	No growth
Bacillus cereus	1.7×10^3	No growth	No growth

As Nisin has a broad spectrum and active against G +ve bacteria, G –ve bacteria are only

affected when their outer membranes are weakened or disrupted by treatment with EDTA or osmotic shock. So when Nisin was added in combination with surfactant, chelator and adjurants, Nisin was added at 10 ppm in combination with Lactate 0.01%, so all G –ve bacteria were sensitive so no growth observed.

3.5. Effect of Nisin on the growth of different bacterial groups

Nisin showed great inhibitory effect on the growth of different bacterial groups as Aerobic plate count, Enterobacteriacae, and coliform count as showed in table (5). As Nisin was added at different concentrations; 10 ppm, 30 ppm and 10 ppm in combined with Lactate 0.01 %.

Aerobic plate count is minimized from higher count to the acceptable limit lower than 10^6 cfu/ g when Nisin is added at 10 ppm as for the three samples the count observed was 2.8×10^4, 5.3×10^4 and 7.9×10^3 cfu/ g, respectively.

When Nisin is added at 30 ppm there was a great inhibitory effect observed as APC minimized to lower count 1.2×10^4, 2.7×10^4 and 3.3×10^3 cfu/g respectively.

However, when lactate is added at 0.01% to weak the outer membranes in combined with Nisin 10 ppm the count minimized to 6.5×10^3, 9.1×10^3 and 1.4×10^3 cfu/g respectively.

So, addition of Nisin plays a vital role to minimize APC to be acceptable and conform to the standards.

When Nisin was added at different concentration to examine its effect on Enterobacteriacae count, it was observed that at 10 ppm Nisin lead to minimize the count of the three samples to 9.4×10^3, 1.5×10^4 and 2.8×10^3 cfu/g respectively. By addition of Nisin at 30 ppm the inhibitory effect was increased as the count observed was 8.3×10^3, 1.2×10^4 and 9.9×10^2 cfu/g.

However, when Nisin was added at 10 ppm in combined with lactate 0.01% there was greater inhibitory effect observed as the count minimized to 7.4×10^2 and 1.6×10^3 cfu/g for the first and the second samples while no growth observed for the third sample as the control count was the least one between the three samples.

The same inhibitory effect was observed against coliform count which minimized after addition of Nisin at the same concentrations listed previously.

At 10 ppm there was low inhibitory effect observed as the count for the first sample minimized from 9.8×10^3 to 7.6×10^3 cfu/g and the second sample from 1.7×10^4 to 9.5×10^3 and the third sample from 2.9×10^3 to 1.7×10^3 cfu/g.

When Nisin was added at 30 ppm there was inhibitory effect observed but still low as the count minimized to 5.8×10^3, 8.1×10^3 and 7.4×10^2 cfu/g, respectively.

However, when Nisin is added at 10 ppm in combined with lactate 0.01% no growth was observed for the first and the third sample while the count minimized to 5.4×10^2 cfu/g for the second sample.

Table 5. Effect of Nisin on the growth of different bacterial groups

Bacterial counts	Control	Nisin (10 ppm)	Nisin (30 ppm)	Nisin (10 ppm) + Lactate (0.01%)
Aerobic Plate	2.1×10^6	2.9×10^4	1.4×10^4	5.6×10^3
Enterobacteriaceae	2.7×10^4	9.0×10^3	7.0×10^3	1.1×10^3
Coliform	9.9×10^3	6.2×10^3	4.8×10^3	5.4×10^2

3.6. Effect of Nisin in combined with sodium nitrite on the growth of different pathogens

Nisin was added in combined with sodium nitrite to examine the viability of them against common six pathogens. Nisin was added at 10 ppm in combined with sodium nitrite at 125 ppm then lactate was added at 0.01%.

Table (6) showed the inhibitory effect against the food pathogens which were examined.

When sodium nitrite 125 ppm was added in combined with Nisin 10 ppm there was an inhibitory effect against the food pathogens but G +ve bacteria was more sensitive as no growth observed while G -ve bacteria showed less sensitivity. When lactate 0.01% was added in combined with Nisin 10 ppm and sodium nitrite 125 ppm no growth observed for all the food pathogens (Table 7).

Table 6. Effect of Nisin in combined with sodium nitrite on the growth of different bacterial pathogens

Tested pathogens	Nitrite (125 ppm) + Nisin (10 ppm)	Nitrite (125 ppm) + Nisin (10 ppm) + Lactate (0.01%)
E.coli O111:K58	2.0×10^2	No growth
E.coli O124:K72	3.5×10^2	No growth
E.coli O128:K67	4.3×10^2	No growth
S. typhimurium	4.5×10^2	No growth
Staph. aureus	No growth	No growth
Bacillus cereus	No growth	No growth

Sen and Baddoo, 1997 reported that Nitrite is used as a preservative in cured meat products, to prevent the growth of the botulism toxin-producing organism, *Clostridium botulinum*. Cassens, 1997, mentioned that formation of nitroso-compounds of myoglobin and hemoglobin enhances the red color of cured meats. USDA regulations allow addition of

sodium nitrite to cured meats at a maximum concentration of 156 ppm.

Table 7. Comparison of the results of Sodium Nitrite & Nisin

Tested pathogens	Nitrite (125 ppm)	Nisin (10 ppm)	Nitrite (125 ppm) + Nisin (10 ppm)	Nitrite (125 ppm) + Nisin (10 ppm) + Lactate (0.01%)
E. coli O111:K58	5.0×10^3	8.3×10^2	2.0×10^2	No growth
E. coli O124:K72	9.6×10^3	1.5×10^3	3.5×10^2	No growth
E. coli O128:K67	3.0×10^4	3.7×10^3	4.3×10^2	No growth
S. typhimurium	2.7×10^3	2.6×10^3	4.5×10^2	No growth
Staph. Aureus	6.0×10^2	8.0×10^2	No growth	No growth
Bacillus cereus	2.0×10^2	1.7×10^3	No growth	No growth

When Nisin was added as food preservative at 30 ppm no growth observed for G +ve bacteria which were *Staph. aureus* and *Bacillus cereus.* On the other hand, it is observed that there was viability for the other G –ve bacteria as *E. coli* O111:K58*, E. coli* O124: K 72 and *E. coli* O128:K67.

Nisin was added at 10 ppm in combination with Lactate 0.01%, so all G –ve bacteria were sensitive so no growth observed .Though some researchers concluded that Nisin is not effective in meat applications due to high pH (Rayman *et al*., 1983) inability to uniformly distribute Nisin, and interference by meat components such as phospholipids (De Vuyst and Vandamme, 1994). Others find contradictory results (Chung *et al.,* 1989).

A presentation at the Workshop on the Bacteriocins of Lactic Acid Bacteria showed that Nisin is inactivated by glutathione in a reaction catalyzed by glutathione S-transferase (Cleveland *et al* ., 2001).

Glutathione is found in raw meat, and the reaction greatly diminishes the activity of Nisin. Other work shows that Nisin can be used in meat under certain conditions.

Mohanasrinivasan *et al.* (2012) reported Nisin was active against a broad spectrum of G +ve bacteria, G –ve bacteria are only affected when their outer membranes are weakened or disrupted by treatment with EDTA or osmotic shock. So Nisin was added in combination with surfactant, chelator and adjurants.

Other studies (Ariyapiti-pun *et al.,* 2000) have used Nisin in combination with lactic acid to show an increased effect when the preservatives are used together to inhibit gram negative organisms.

When sodium nitrite 125 ppm was added in combined with Nisin 10 ppm there was an inhibitory effect against the food pathogens but G + ve bacteria was more sensitive as no growth observed while G – ve bacteria showed less sensitivity as *E. coli* O111:K58, two samples of *S. typhi*. showed viability in the presence of Nisin in combined with sodium nitrite, when lactate 0.01% was added in combined with Nisin 10 ppm and sodium nitrite 125 ppm no growth observed for all the food pathogens.

Rayman *et al.* (1983) reported that Nisin or its combination with lower levels of nitrate can prevent the growth of Clostridia.

4. Conclusion

- This paper studied Application of Bacteriocin and Nisin as Bio-preservatives in Foods.
- Sodium nitrite, Bacteriocin and Nisin were used as food preservatives at different concentrations.
- Gram positive bacteria showed higher sensitivity against the listed preservatives than gram negative bacteria.
- The addition of sodium nitrite in combined with Nisin and lactate prevent the growth of the tested pathogens, which allow using those preservatives in combined with each other at large scale in meat products.

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