



# Quality Improvement of Broiler Chicken Breasts by Nisin and Lactic Acid

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## ABSTRACT

The present study was conducted to evaluate the effect of nisin, lactic acid and their combination on quality parameters and shelf life of broiler chicken breasts during chilling storage at  $3 \pm 1$  °C. For achieving this goal, broiler chicken breasts were collected and divided into control and treated groups by dipping in each of 1% lactic acid, 2% lactic acid, nisin 50µg, nisin 100µg, nisin 50µg followed by 1% lactic acid, and nisin 100µg followed by 1% lactic acid. The samples were packaged and stored at  $3 \pm 1$  °C. Control and treated groups were examined periodically at day zero and every three days until spoilage with chemical and microbiological methods. The results revealed that nisin, lactic acid and their combination improved the quality and extended the shelf life of broiler chicken breasts for 3 to 12 days during chilling storage. The use of nisin followed by lactic acid would be promising to provide a new hurdle technology for poultry meat decontamination.

**Keywords:** Broiler chicken breasts, Quality improvement, Lactic acid, Nisin, Chemical examination, Microbiological examination

## INTRODUCTION

Poultry meat is easy to prepare at home and widely used in restaurants and fast food establishments. There is no primary religious restriction on the consumption of poultry meat (Mulder, 1999). Poultry meat is an excellent substrate for the growth of a wide variety of microorganisms including pathogens and spoilage microorganisms. Other properties influence the growth of microorganisms such as water activity and pH value, as well as the skin, which harbors many microorganisms while serving as a physical barrier to microorganisms (ICMSF, 1998). However, poultry meat is highly perishable with a relatively short shelf life even when it is kept under refrigeration (Mantilla et al., 2012). Thus, the application of decontamination treatments during processing could be highly useful. Several decontaminants have been reported to be effective in destroying disease-causing pathogens, decontaminating, preserving and extending shelf-life of poultry carcasses and cuts. These treatments can be classified as physical, chemical, and biological which include chlorine, organic acids, tri-sodium phosphate, bacteriocin, hydrogen peroxide, ozone, water, ultrahigh pressure, irradiation, pulsed-field electricity, ultrasonic energy and UV (Bolder, 1997 and Capita et al., 1999 a, 1999 b).

Lactic acid is an organic acid with proven effectiveness as a decontaminant with different kinds of food. It can delay the proliferation of spoilage microorganisms, prevent the generation of undesirable chemicals, improve the levels of sensory attributes, and extend the shelf life of chicken during refrigerated storage (Smaoui et al., 2012). Lactic acid is used as a decontaminant in different concentrations and treatment of chicken breast samples with 3% lactic acid gave the highest initial reduction in aerobic mesophilic and psychophilic bacteria (Cosansu et al., 2011).

Nisin is a bacteriocin successfully used as an antibacterial agent in various food products. It is generally recognized as safe for use as a biopreservative in food systems (Jay, 2000; FDA, 2008). It is a 3,500-Da polypeptide produced by *Lactococcus lactis subsp lactis* that inhibits growth of Gram positive organisms. Nisin's spectrum of inhibitory activity could be extended to include gram negative bacteria when combined with agents such as disodium ethylenediamine tetraacetate (EDTA), lactate, citrate, irradiation and vacuum packaging (Cutler and Siusa, 1995; Cosby et al. 1999; Long and Phillips, 2003 and Zahran, 2015). Therefore, the aim of this work was to evaluate the effect of lactic acid and nisin applied

singly or in combination to improve the quality and extend the shelf life of broiler chicken breasts during refrigerated storage.

## MATERIALS AND METHODS

### Preparation of broiler chicken breasts

Fresh broiler chicken breasts (each breast was about 600 g) were purchased from a retail poultry shop at Beni-Suef City, Egypt. Each broiler chicken breast was divided into four parts of average weight 150 g, then wrapped in sterile polyethylene bags and directly transferred in sterile ice box as soon as possible to the laboratory of food hygiene department, faculty of veterinary medicine, Beni-Suef university, Egypt.

### Preparation of treatment solutions:

**Lactic acid:** lactic acid 1, 2% (v/v) solutions were prepared using pure lactic acid liquid (2010/1, ADWIC, Egypt) and distilled water.

**Nisin:** nisin 50, 100µg/ml (w/v) solutions were prepared using nisin (Aplin and Barrelet Ltd. Trobridge, U K) and distilled water.

### Samples treatment

Broiler chicken breasts were divided into seven groups as follow: Group one was used as a control (untreated); Group two was dipped in lactic acid 1% (v/v) solution for 10 minutes; Group three was dipped in lactic acid 2 % (v/v) solution for 10 minutes; Group four was dipped into nisin 50µg/ml (w/v) solution for 30 minutes; Group five was dipped into nisin 100µg/ml (w/v) solution for 30 minutes; Group six was dipped into nisin 50µg/ml (w/v) solution for 30 minutes followed by lactic acid 1% (v/v) solution for 10 minutes and Group seven was dipped into nisin 100µg/ml (w/v) solution for 30 minutes followed by lactic acid 1% (v/v) solution for 10 minutes.

### Packaging and storage

Samples of control and treated groups were aerobically packed as triplicates inside fiber dishes and stored at 3± 1°C. Samples were examined chemically and microbiologically at day zero and periodically every three days until spoilage (0, 3rd, 6th, 9th, 12th, and 18th).

### Examination techniques

#### Deterioration criteria

**Determination of Thiobarbituric Acid-Reactive Substances Value (TBA-RS):** The technique of Taraldgis et al. (1960) with additional modification of Pikul et al. (1983) was applied by blending ten grams of broiler chicken breast sample with 50 mL of distilled water in food blender for 2 min. The mixture was

transferred to Kjeldahl flask by washing with additional 47.5 mL of distilled water. Then, the pH was adjusted to 1.5 by addition of 2.5 mL of 4 N HCl solution. Antioxidant (butylated hydroxytoluene (BHT) and anti pumping stones were added. Apparatus and heat flasks were assembled at the highest heat obtainable on the Kjeldahl distillation apparatus. Five mL of the mixed distillate were pipetted into 50 mL glass stoppered tube and 5 mL of TBA reagent were added. The tubes were stoppered and their contents were mixed, and immersed in a boiling water bath for 35 minutes. A blank solution was prepared by distilled water and TBA reagent and treated the same as the samples. After heating, the tubes were cooled under tap water for 10 min. A portion was transferred to a cuvette and the optical density of sample (D) was determined against the blank at a wavelength of 538 nm of the spectrophotometer.

Calculation:

TBA value =  $D \times 7.8$  (mg malondialdehyde (MDA) / kg of flesh).

### Determination of Total Volatile Basic Nitrogen Value (TVB-N):

The technique recommended by Food and Agriculture Organization (1986) was applied. Briefly, 10 gm of minced broiler chicken breast sample were homogenized with 100 mL of distilled water in a food blender for two minutes. Sample was washed into distillation flask with a further 200 mL of water, then two grams magnesium oxide and two drops of antifoaming agent were added. The mixture was boiled for 10 minutes and distilled for exactly 25 minutes, using the same rate of heating, into 25 ml of 2% boric acid solution with few drops of screened methyl red indicator in a 500 ml flask. The heating was stopped and the condenser washed down with distilled water. Then, the contents of the flask and the blank solution (25 mL of 2% boric acid) were titrated with 0.1 N H<sub>2</sub>SO<sub>4</sub> (titer).

Calculation: Total volatile base (mg N/100g flesh) = 14 (titer–blank).

### Bacteriological examination

**Preparation of samples:** Preparation of broiler chicken breasts samples was carried out according to International Commission on Microbiological Specifications for Food, ICMSF (1986). Ten grams of broiler chicken breast (skin and muscle) were homogenized with 90 ml of 0.1% sterile peptone water at 2000 rpm for 2.5 minutes using a sterile homogenizer (MPW 302, Universal Laboratory Aid, made in Poland). Ten fold serial dilutions up to 10<sup>-6</sup> were done.

**Determination of aerobic mesophilic and psychrophilic counts:** The applied technique

recommended by Association of Official Analytic Chemists, AOAC (1990) was used by standard plate count agar (6G2307, Biolife, Italy) tempered at 45 °C, then inoculated plates were incubated in inverted position at 35 °C for 48 hours and at 7 °C for 10 days for mesophiles and psychrophiles respectively.

**Coliforms, fecal coliforms and E.coli (Most Probable Number):** The three tubes method (MPN) recommended by AOAC (1990) for coliform, fecal coliform, and *E.coli* was applied.

#### **Staphylococcus aureus count**

The applied technique recommended by American public Health Association, APHA (1992) was used by Baired-Parker medium (BP Biolife, Italy) then the inoculated plates were incubated at 35 °C for 48 hours for *S. aureus* count.

#### **Statistical analysis**

Data were subjected to analysis of variances (one way-ANOVA) according to Knapp and Miller (1992) using (SPSS Statistics 20.0) software program. Differences among the mean values of the various treatments were determined by the Least Significant Difference (LSD) test, and the significance was defined at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

#### **Deterioration criteria**

##### **Thiobarbituric Acid-Reactive Substances**

**(TBA-RS):** TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content. MDA formed through hydro peroxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez et al., 1997).

It was cleared that there is no significant differences between all groups ( $P < 0.05$ ) at day zero of storage except nisin (50µg/ml) treated group which significantly higher than lactic acid 2% treated group Figure 1.

A significant increase with storage time ( $P < 0.05$ ) in TBA-RS values was observed in all groups. This was in agreement with Alasnier et al. (2000); Ali et al. (2007) and Rahman et al. (2012). Significant differences ( $P < 0.05$ ) were appeared between control and groups treated with nisin 50µg/ml + lactic acid 1%, and nisin 100µg/ml + lactic acid 1% at 3rd day of storage.

TBA-RS values of control group were the highest allover other groups. On the other hand, nisin + lactic acid treated one had the lowest TBA-RS values, this difference was significant ( $P < 0.05$ ). Lipid oxidation is an important factor of oxidative deterioration of poultry meat. TBA is a measure of malondialdehyde (MA), one

of the degradation products of lipid hydro peroxides formed through oxidation of unsaturated fatty acids. This is in accordance with Nawar (1996); Botsoglou et al. (2002) and Gatellier et al. (2007).

Concerning the permissible limit of TBA value in poultry meat (0.9 mg MDA/kg) recommended by Egyptian organization for standardization, EOS (2005), only control and treated groups with each of nisin 50µg/ml+lactic acid 1% and lactic acid exceeded such limit in the last occasion of examination.

#### **Total volatile basic nitrogen**

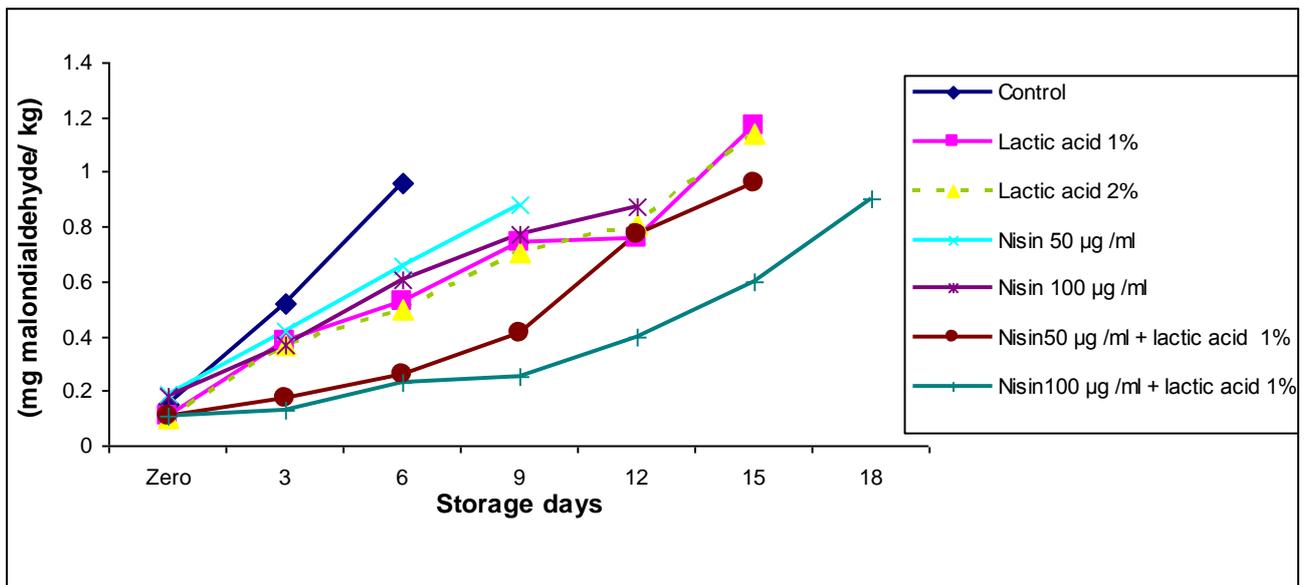
The TVB-N content in chicken, as an important reference index, has been being used to evaluate chicken's freshness (Fatih and Yeşim, 2000 and Castro et al., 2006). TVB-N compounds in chicken contain mainly ammonia, trimethylamine (TMA) and dimethylamine (DMA) and the levels of TVB-N compounds increase with spoilage by either bacterial or enzymatic degradation.

TVB-N values of control and treated groups were illustrated in Figure 2. There were no significant differences between all groups ( $P < 0.05$ ) at day zero. Nearly similar results were detected by Rukchon et al. (2011) and Smaoui et al. (2011 and 2012). Higher values were reported by Balamatsia et al. (2007).

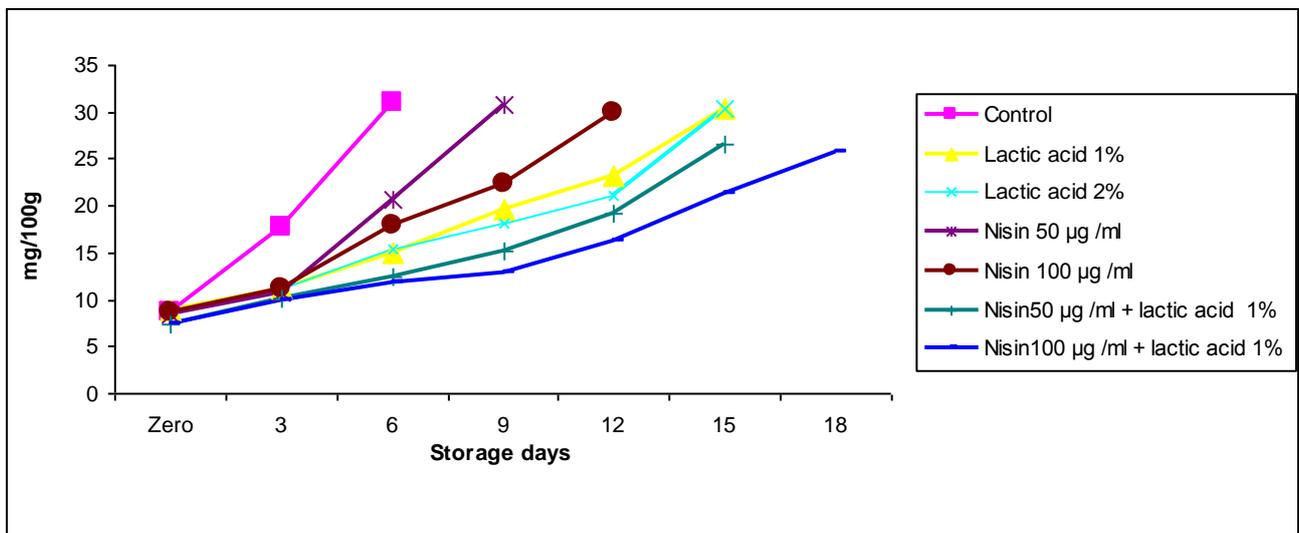
Control group was significantly higher than all treated groups at 3rd and 6th days of storage. Broiler chicken breasts treated with nisin + lactic acid were lower than all groups during all days of storage, this indicates that treatment of broiler chicken breasts with nisin + lactic acid has strong effect on microbial and enzymatic activities.

There was a significant increase ( $P < 0.05$ ) in TVB-N values with storage time in all groups. A clear relationship was found between the microbiological quality of broiler chicken breasts and level of TVB-N formation, this is in agreement with Rukchon et al. (2011). The TVBN is related to protein breakdown (Egan et al., 1981) and the observed increases may be attributed to the formation of ammonia or other basic compounds due to microbial activity (Banwart, 1981).

TVB-N values of all treated groups exceeded the limit recommended by EOS (2005) at last days of storage. Regarding the permissible limits of TVB-N (40mg/100g) suggested by Balamatsia et al. (2007), it was cleared that none of the samples exceeded such limit even at the last day of storage life time of each group. In this respect, Patsias et al. (2008) reported that the initial TVB-N value of 12 mg/100g sharply increased in the chilled chicken fillets stored in air resulting in high TVB-N values (49 g/100g) after 9 day of storage. Further more, Rukchon et al. (2011) stated that TVB-N was markedly detected in fresh chicken and its level increased with storage time.



**Figure 1.** Effect of treatments on Thiobarbituric Acid-Reactive substance values of packed broiler chicken breast samples during chilled storage at  $3\pm 1^{\circ}\text{C}$  (mg malondialdehyde/ kg).



**Figure 2.** Effect of treatments on total volatile basic nitrogen values of packed broiler chicken breast samples during chilled storage at  $3\pm 1^{\circ}\text{C}$  (mg /100g flesh).

### Bacteriological examination

**Aerobic mesophilic count:** From Figure 3, it was cleared that the highest reduction in log CFU/g mesophilic bacteria were recorded in nisin (100µg/ml) + lactic acid (1%) treated group while the lowest reduction was determined in nisin 50µg/ml treated one. Nearly similar results were obtained by Tosun and Tamer's (2000); Sinhamahapatra et al. (2004) and Anang et al. (2010), low results were observed by Morshedy and Sallam (2009). High results were recorded by Ismail et al. (2001). Initially 2% lactic acid was not found significantly more effective in reducing colony counts than 1% lactic acid, this in accordance with Marcel et al. (1988).

The microbial counts in the control and treated groups gradually increased during storage time, but

those for the treated groups were significantly lower than the control; this is in accordance with that reported by Ismail et al. (2001) and Gu et al. (2011).

Improper cleaning and disinfection of machines in poultry abattoirs may lead to contamination of poultry meat during processing. Bean and Griffin (1990) reported that quantifying the total mesophiles is an excellent indicator of contamination which has taken place during processing and is a useful tool to assess microbiological safety and sanitation conditions during processing.

The results indicated that nisin alone was less effective on aerobic mesophilic count. However it was cleared that dipping of broiler chicken breast in nisin solution followed by lactic acid had a synergistic effect on the aerobic mesophilic count. Thus, it can be clearly

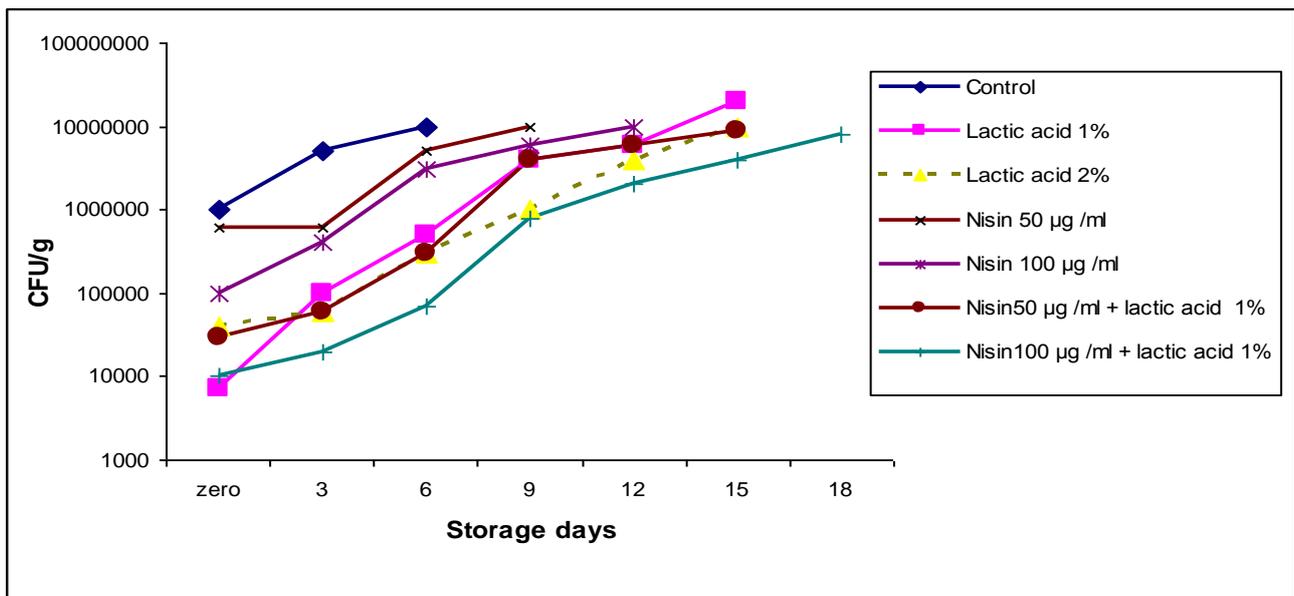
expressed that nisin should be applied as combined with lactic acid application, rather than its alone application. This greater inhibition of nisin + lactic acid 1% may be due to the lactic acid's increasing effect of nisin's penetration into Gram-negative bacteria, by decomposing the cell wall prior to nisin application (Helander, 2000). Regarding to the acceptability limit recommended by ICMSF (1986) for total viable count in processed chickens ( $7 \log_{10}$  CFU/g flesh), it could be observed that all groups exceeded such limit at last day of the chilling storage of each group.

### Aerobic psychrophilic count

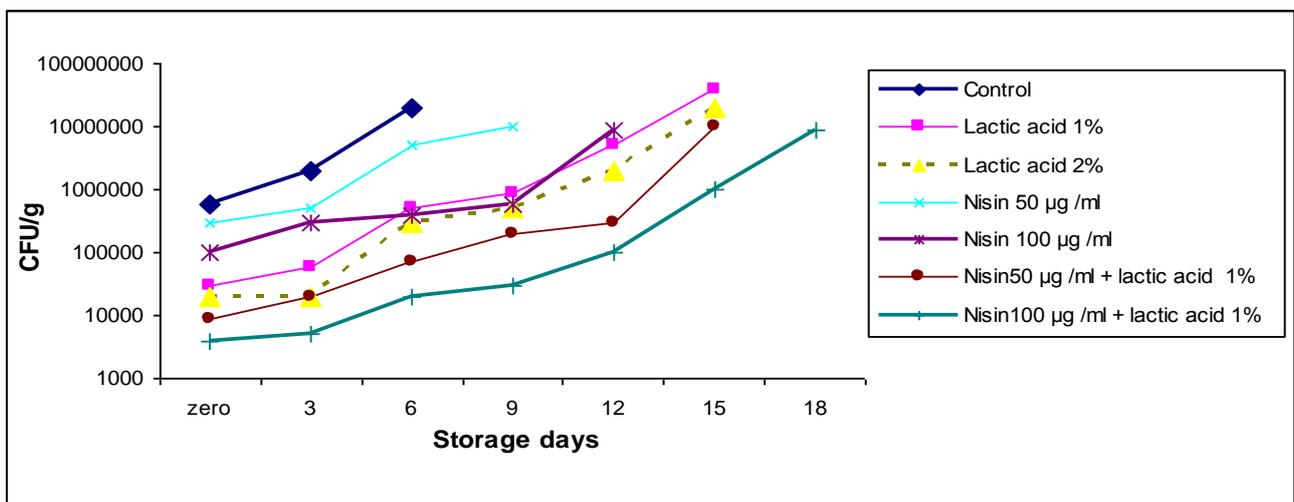
The illustrated results as shown in Figure 4 revealed that treated groups with nisin (100µg/ml) + lactic acid (1%) had the highest reduction in log CFU/g at day zero of storage. Nearly similar results were obtained by Ellerbroek et al. (1996), while lower results

were observed by Morshedy and Sallam (2009) and Hecer and Guldaz (2011). The psychrophilic counts in control group was significantly ( $p < 0.05$ ) higher than other treated groups at day zero of chilled storage. A gradual increase in aerobic psychrophilic count was observed during chilled storage which was significantly lower than that of control group. This is in agreement with Vatansever et al. (2008) and Hecer and Guldaz (2011).

Nisin (50µg/ml) + lactic acid (1%) and nisin (100µg/ml) + lactic acid (1%) treated groups were lower than other groups at each occasion of examination. This suggested a synergistic effect occurred between nisin and lactic acid. The antimicrobial effect of lactic acid is the result of a decrease in pH and a specific antimicrobial effect of non-dissociated molecule (Debevere, 1987 and Smulders, 1987).



**Figure 3.** Effect of treatments on mesophilic counts of packed broiler chicken breast samples during chilled storage at  $3 \pm 1^\circ\text{C}$ .



**Figure 4.** Effect of treatments on psychrophilic counts of packed broiler chicken breast samples during chilled storage at  $3 \pm 1^\circ\text{C}$ .

**Coliforms, fecal coliforms and *E. coli* (MPN)**

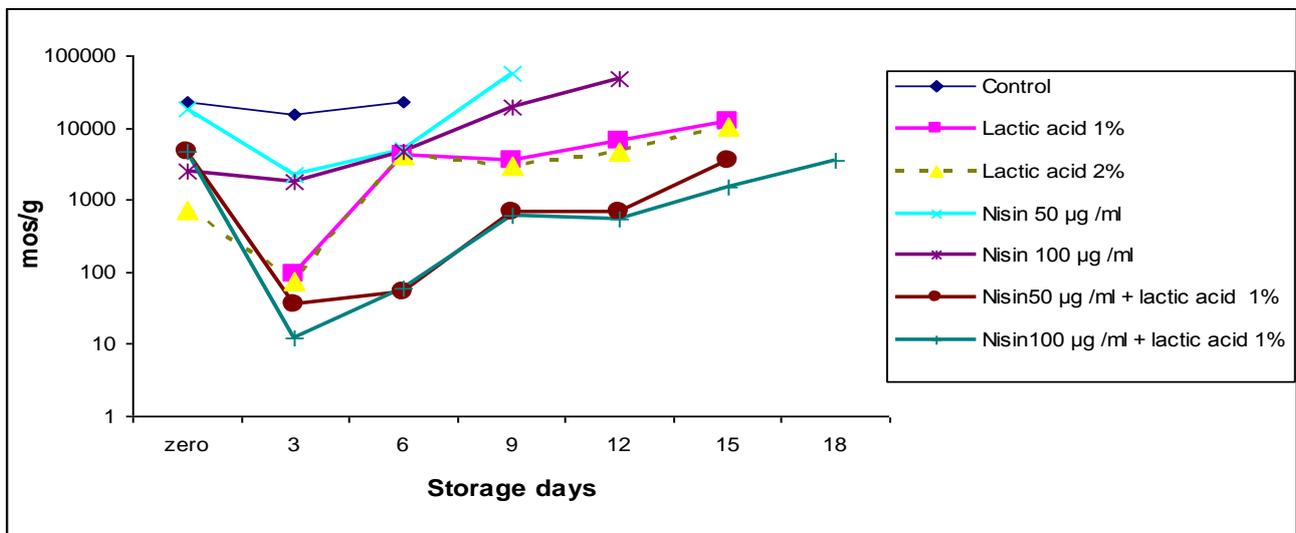
There was a significant difference ( $p < 0.05$ ) in coliforms (Most probable number) between control and treated groups except nisin (50µg/ml), and nisin (100µg/ml) treated one at day zero of chilled storage. Lactic acid (1 and 2%) treated groups had a higher significant reduction in coliforms (MPN) than nisin (100µg/ml) treated group Figure 5.

Nearly similar results were obtained by Tosun and Tamer (2000); Sinhamahapatra et al. (2004) and Gulmez et al. (2006) however higher values were recorded by Vatansever et al. (2008) While low values were recorded by Sinhamahapatra et al. (2004) and Killinger et al. (2010).

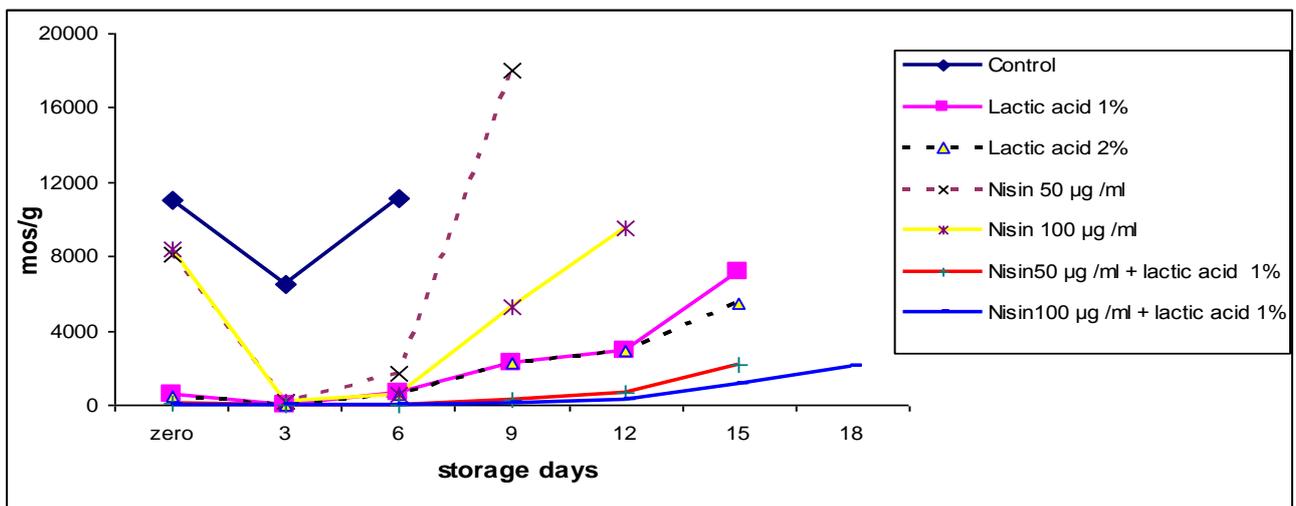
There was a gradual increase in coliforms (MPN) with chilled storage duration; this is in accordance with Vatansever et al. (2008). On contrary Gulmez et al. (2006) recorded that coliforms and fecal coliforms counts of lactic acid treated wings decreased during

storage days. On the other hand the results indicated that nisin alone was less effective on coliforms (MPN) as their cell wall were less permeable to nisin. However it was cleared that dipping of broiler breast in nisin solution followed by lactic acid had a synergistic effect on the total coliforms.

As shown in Figure 6, nisin + lactic acid treated groups had the highest reduction in fecal coliforms counts at day zero of storage. Nearly similar results were obtained by Gulmez et al. (2006), however higher results were recorded by Vatansever et al. (2008). It was noticed that control group was significantly higher than all treated groups at 3rd day of storage. Fecal coliforms (MPN) decreased then gradually increased from 6th day of storage and through storage days. This is in agreement with Sinhamahapatra et al. (2004). On contrary Gulmez et al. (2006) recorded that coliforms and fecal coliforms counts of lactic acid treated wings decreased during storage days.



**Figure 5.** Effect of treatments on coliforms (MPN) of packed broiler chicken breast samples during chilled storage at  $3\pm 1^\circ\text{C}$ .



**Figure 6.** Effect of treatments on fecal coliforms (Most Probable Number) of packed broiler chicken breast samples during chilled storage at  $3\pm 1^\circ\text{C}$ .

*E. coli* (MPN) in control group was significantly higher than all treated groups at day zero of chilled storage, while the lowest values were recorded in nisin + lactic acid treated groups Figure 7. Nearly similar results were recorded by Hecer and Guldas (2011), higher results were recorded by Tosun and Tamer (2000). At 3<sup>rd</sup> day and 6<sup>th</sup> of storage control group was significantly higher than all treated groups, while at 9<sup>th</sup> day of storage nisin (50µg/ml) treated group was significantly higher than other treated groups. Nisin 50µg/ml + lactic acid 1%, and nisin 100µg/ml + lactic acid 1% treated groups were more effective in reducing *E. coli* than other treated groups, thus suggesting that lactic acid increased the effect of nisin against *E. coli*.

Presence of coliforms, fecal coliforms and *E. coli* in chicken carcasses indicates fecal contamination which may be attributed to the system of manual evisceration and unsatisfactory hygienic measures of handling and processing, this is in agreement with Whyte et al. (2004).

Gram negative bacteria are resistant to nisin because their cell walls are far less permeable than those of Gram positive bacteria. However, any treatment of Gram negative bacteria to make their cell walls permeable to nisin makes them susceptible to nisin. Such treatments include exposure to chelating agents, sub-lethal heat, osmotic shock and freezing (Delves-Broughton, 2005).

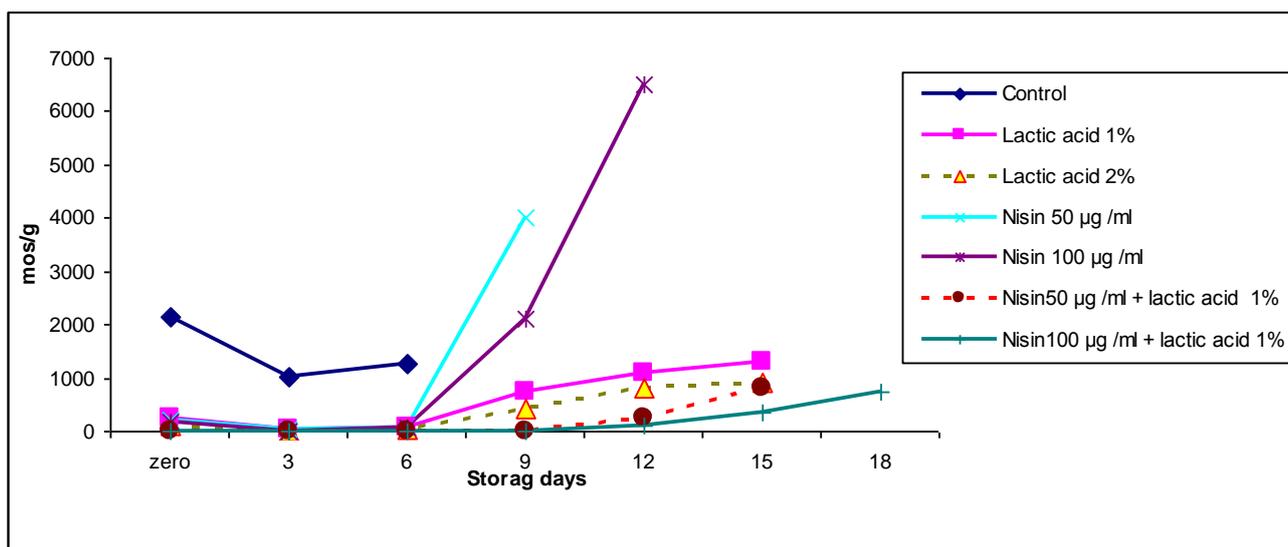


Figure 7. Effect of treatments on *E. coli* (MPN) of packed broiler chicken breast samples during chilled storage at 3±1°C.

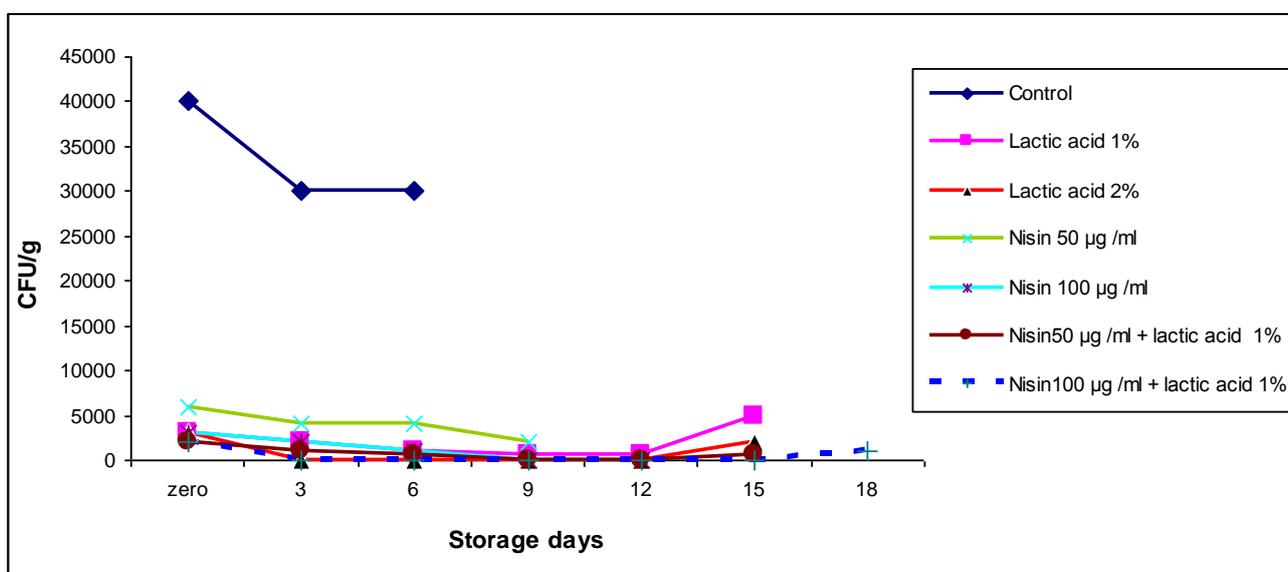


Figure 8. Effect of treatments on *S. aureus* count of packed broiler chicken breast samples during chilled storage at 3±1°C.

### *Staphylococcus aureus* count

The initial *S. aureus* count Figure 8 of control group was significantly higher than all treated groups at

day zero, 3<sup>rd</sup> and 6<sup>th</sup> day of chilled storage (P < 0.05). Lactic acid (1%) treated group was significantly higher than lactic acid (2%), nisin (100µg/ml), nisin (50µg

/ml) + lactic acid (1%), and nisin (100µg/ml) + lactic acid (1%) treated groups at 12th day of storage. Nearly similar reduction effect was recorded by Marcel et al (1988); Antown (2002) and Smaoui et al (2011 and 2012).

This decrease in count during storage days was in accordance with Hwang and Beuchat (1995) and Antown (2002). On contrarily Smaoui et al. (2011 and 2012) found a gradual increase in *S. aureus* counts during chilled storage. In this respect, Grisi and Gorlach-Lira (2005) found that in pure cultures, the growth of *S. aureus* was strongly inhibited by nisin for eight hours. *S. aureus* has often been tested in poultry products to assess microbiological safety, sanitation conditions, and product quality during processing and storage. The presence of *S. aureus* could occur due to inappropriate techniques applied, with regard to personal hygiene, abdomen opening, hand deboning or hand washing (Tompkin, 1983).

Nisin affects several Gram-positive bacteria such as *Staphylococcus spp.* but does not inhibit the majority of Gram-negative bacteria. Nisin initially forms pores in cell membrane and allows the efflux of essential cellular components resulting in inhibition or death of the bacteria (Abee et al. 1994 and Delves-Broughton, 2005).

## CONCLUSION

From the previously mentioned data, it could be concluded that the dipping of broiler chicken breasts into nisin, lactic acid and their combination before refrigeration can retain the quality attributes and extend the shelf life for about 3-12 days more than the control during refrigerated storage. It was cleared that dipping of broiler chicken breasts in nisin solution followed by lactic acid had a synergistic effect on the bacterial load. Thus, it can be clearly expressed that nisin should be applied as combined with lactic acid application, rather than it's alone application.

### Competing interests

The authors have no competing interests to declare.

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