Effect of Some Preservatives on Bacterial Load of Some Poultry Meat Products

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A B S T R A C T

A total of 45 random samples of processed poultry meat products represented by burger, luncheon and frankfurter (15 of each) were collected from different supermarkets in Qalyubiya governorate for evaluation of their bacteriological quality. The mean values of total aerobic count, total coliform and total anaerobic count were estimated as $1.8 \times 10^7 \pm 1.3 \times 10^7$, $4.2 \times 10^3 \pm 2.6 \times 10^3$, and $2.1 \times 10^4 \pm 1.4 \times 10^4$, respectively for burger, $7.4 \times 10^6 \pm 3.1 \times 10^6$, $3 \times 10^2 \pm 0.9 \times 10^2$, and $8.5 \times 10^2 \pm 4.6 \times 10^2$, respectively for luncheon and $5.8 \times 10^7 \pm 2.5 \times 10^7$, $5.3 \times 10^2 \pm 5 \times 10$, and $4.8 \times 10^2 \pm 4.3 \times 10^2$ CFU/g for frankfurter, respectively. In addition, experimental trial was pointed toward the ability to control the outgrowth of Clostridium perfringens in minced poultry meat using sodium nitrite, nisin and potassium sorbate singly and in combinations in different concentrations. Moreover, sodium nitrite with nisin had synergistic inhibitory effect on the outgrowth of C. perfringens. Meanwhile, sodium nitrite combined with nisin and potassium sorbate have a greater inhibitory effect with higher concentrations.

KEY WORDS: Preservatives, bacterial load, poultry product

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1. I N T R O D U C T I O N

In processing plants, contamination of poultry meat products can occur through processing, packaging and storage until the product is sufficiently cooked and consumed. Heavy bacterial loads enter the processing operations with the living birds and these bacteria can be disseminated throughout the plant during processing. Improperly cooked and post-processing contaminated poultry products lead to public health hazards (Zhang et al., 2001).

Traditional methods of preservation may not be adequate in controlling some food borne pathogens in a meat environment, and the time has come to use a new generation of preservatives. Nisin is used as a natural additive to inhibit spores outgrowth or reduce their heat resistance. Nisin also can be combined with nitrite, although the combined application may allow for less nitrite to exert an identical degree of inhibition of clostridia compared to nitrite alone. The antimicrobial potential of nisin is considerably influenced by physical, chemical and microbial environments (Smid and Gorris, 1999).

Sodium nitrite (50 ppm) has a role in meat products through stabilizing the pink coloration of meat and enhances the meat product flavor (Eleiwa-Nesreen, 2003).

Food preservation techniques can cause a variety of stresses that interfere with bacterial homeostasis to prevent growth or to kill bacteria. However, as a result of the stress response, some bacteria can survive and grow after the application of stress (Jones and Inouye, 1994). Although C.

perfringens is sensitive to cold temperatures (De Joung et al., 2004), it responds to cold shock by synthesizing five cold-shock proteins that increase its cold tolerance (Villarreal et al., 2002).
Concerning to the reality of that cold temperatures are commonly used for food preservation (Beals, 2004), the ability of C. perfringens to adapt to low temperatures could be a safety concern in the food industry (Buchanan et al., 2004).
The aim of this study was carried out to investigate the bacterial profile of some poultry meat products (luncheon, burger and frankfurter), with testing the inhibitory effect of several concentrations of nisin, sodium nitrite and potassium sorbate on C. perfringens growth on freezing temperature (-18 OC).

2. MATERIALS AND METHODS

2.1. Bacteriological examination:

Forty five random samples of chicken burger, chicken luncheon and chicken frankfurter (15 of each) which were collected from different supermarkets in Qalyubiya governorate for bacteriological evaluation of total aerobic count, total coliform count and total anaerobic count.

2.1.1. Preparation of sample:

It was carried out according to APHA (1992)
2.1.2. Total aerobic count according to ICMSF (1996).
2.1.3. Total coliform count according to ICMSF (1996).
2.1.4. Total anaerobic count according to Stanly et al. (1992) and Mossel et al. (1995).

2.2. Experimental part:

2.2.1. Preservatives used:

• Nisin (nisaplin) was obtained from Danisco cultor (Denmark).
• Sodium nitrite was obtained from Merck (Darmstadt, Germany).
• Potassium sorbate was obtained from Wegochem Mexicana (Mexico).
The preservatives were used as recommended by Hassan (1999), who used nisin at concentrations (20, 40, and 60 ppm); sodium nitrite (50 and 125 ppm) and potassium sorbate (0.1%, 0.2%, and 0.3%).

2.2.2. Strain used:

Clostridium perfringens (C. perfringens) type (A) was obtained from Anaerobic Unit, Microbiology Department, Animal Health Research Institute, Dokki, Giza governorate. Cultures were maintained in thioglycolate broth at 4oC and propagated to provide approximately 107 CFU/g as recommended by Eleiwa-Nesreen (2009).

2.2.3. Experimental application:

Fresh poultry meat samples (2800 g) were minced and irradiated; the irradiation process was carried out at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt, then divided into 14 groups (200 g of each) for addition of different food additives experimentally to estimate their effect on inoculated C. perfringens as follows:
The method used was applied according to Eleiwa-Nesreen (2003)
• 1st : C. perfringens strain + 0.4 ml nisin (20 ppm).
• 2nd : C. perfringens strain + 0.8 ml nisin (40 ppm).
• 3rd : C. perfringens strain + 1.2 ml nisin (60 ppm).
• 4th : C. perfringens strain + 2 ml sodium nitrite (50 ppm).
• 5th : C. perfringens strain + 2.5 ml sodium nitrite (125 ppm).
• 6th: C. perfringens strain + 0.2g potassium sorbate (0.1%).
• 7th : C. perfringens strain + 0.4g potassium sorbate (0.2%).

• 8th : C. perfringens strain + 0.6g potassium sorbate (0.3%).
• 9th : C. perfringens strain + 2 ml sodium nitrite + 0.8 ml nisin.
• 10th : C. perfringens strain + 0.4 ml potassium sorbate + 2 ml sodium nitrite.
• 11th : C. perfringens strain + 0.8 ml nisin + 0.4 ml potassium sorbate + 2 ml sodium nitrite.
• 12th : C. perfringens strain + 2.5 ml sodium nitrite + 1.2 ml nisin + 0.6 ml potassium sorbate.
• 13th :control (+ve) inoculated by C. perfringens strain only without any preservatives.
• 14th: control (-ve) neither inoculated by C. perfringens nor any chemical preservatives.

The samples after inoculation were kept at -18oC till be used, 25 g. of minced meat samples were homogenate with 225 ml of buffered peptone water (0.1%) then 1 ml from the homogenate was transferred into a tube containing 9 ml peptone water (0.1%), then tenfold serial dilutions were obtained till 10-7.

2.2.4. Enumeration of Clostridium perfringens in minced poultry meat samples was carried out according to ICMSF (1978).
The samples were investigated bacteriologically every 3hours along 48hours. This experiment was repeated for 3 trials.

2.3. Statistical analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

Table (1) summarized the results of bacterial counts of the oriental collected samples represented by chicken burger, chicken luncheon and chicken frankfurter.
The mean value of total aerobic counts (CFU/g) were 1.8 x 107± 1.3 x 107 for burger, 7.4 x 106± 3.1 x 106 for luncheon, and 5.8 x 107± 2.5x107 for frankfurter.
Concerning to the mean value of total coliform counts (CFU/g) of examined samples were illustrated in table (2) which were 4.2 x 103 ± 2.6 x 103 for burger, 3 x 102 ± 0.9 x 102 for luncheon, and 5.3 x 102 ± 5 x 10 for frankfurter.
The mean value of total anaerobic bacterial count (CFU/g) in examined samples in table (3) which were 2.1 x 104 ± 1.4 x 104 for burger, 8.5 x 102 ± 4.6 x 102 for luncheon, and 4.8 x 102 ± 4.3 x 10 for frankfurter.
Figure (1) illustrated the inhibitory effect of nisin only in concentrations of 20, 40 and 60 ppm, respectively on experimentally inoculated C. perfringens type (A) along 48 h. C. perfringens counts were decreased to 2.9 x 107 ± 4 x 106, 6.2 x 107 ±1.4 x 106, 5.8 x 107 ± 3.5 x 106 after 3h; 4.2 x 107 ± 1.8 x 106, 7.2 x 107 ± 4.3 x 106, 5.7 x 107 ± 5.2 x 106 after 6h; 3.9 x 107 ± 4.9 x 106, 6.7 x 107 ± 3.3 x 106, 2.8 x 107 ± 8 x 106 after 9h; 2.5 x 107 ± 2.6 x 106, 4.5 x 107 ± 3.1 x 106, 1.9 x 107 ± 6.9 x 106 after 12h; 1.8 x 107 ± 3.3 x 106, 2.8 x 107 ± 1.5 x 106, 8.1 x 106 ± 8 x 105 after 24h; 1.6 x 107 ± 1.7 x 106, 2.1 x 107 ± 5.4 x 106, 7.2 x 106 ± 6.1 x 105 after 48h; respectively.
Figure (2) illustrated the inhibitory effect of sodium nitrite only in concentrations of 50 and 125 ppm, respectively on experimentally inoculated C. perfringens type (A) along 48 h. C. perfringens counts were decreased to 3.8 x 107 ± 7.6 x 106, 6.2 x 107 ± 2.6 x 106 after 3h; 4.9 x 107 ± 6.3 x 106, 8 x 107 ± 4.6 x 106 after 6h; 4.2 x 107 ± 2.3 x 106, 6.9 x 107 ± 1.1 x 106 after 9h; 1.9 x 107 ± 8.5 x 106, 3.5 x 107 ± 2.3 x 106 after 12h; 1.7 x 107 ± 2.5 x 106, 2.3 x 107 ± 9.4 x 106 after 24h; 1.4 x 107 ± 3 x 106, 2 x 107 ± 4.5 x 106 after 48h; respectively.
Figure (3) illustrated the inhibitory effect of potassium sorbate only in concentrations of 0.1%, 0.2% and 0.3%,

respectively on experimentally inoculated C. perfringens type (A) along 48 h. C. perfringens counts were recorded as 6 x 10^7 ± 6.7 x 10^6, 5.1 x 10^7 ± 2.9 x 10^6, 4.7 x 10^7 ± 7.8 x 10^6 after 3h; 4.8 x 10^7 ± 3 x 10^6, 5.3 x 10^7 ± 4.3 x 10^6, 5.9 x 10^7 ± 1.3 x 10^7 after 6h; 5.1 x 10^7 ± 1.2 x 10^7, 6 x 10^7 ± 7.4 x 10^6, 5.1 x 10^7 ± 1.5 x 10^6 after 9h; 5.1 x 10^7 ± 6.8 x 10^6, 4.7 x 10^7 ± 1.8 x 10^7, 7 x 10^7 ± 5.6 x 106 after 12h; 5.9 x 10^7 ± 5 x 10^6, 6.2 x 10^7 ± 1.6 x 10^7, 4.3 x 10^7 ± 5.6 x 10^6 after 24h; 8.4 x 10^7 ± 7.6 x 10^6, 8.8 x 10^7 ± 1.1 x 10^7, 9.3 x 10^7 ± 5.6 x 10^7 after 48h; respectively.

Figure (4) illustrated the inhibitory effect of combined 40 ppm nisin with 50 ppm sodium nitrite in comparison with combined 50 ppm sodium nitrite and 0.2% potassium sorbate. Results revealed that mixture of 40 ppm nisin with 50 ppm nitrite had a greater inhibitory effect on inoculated C. perfringens where the average counts after 48h from inoculation were 4.8 x 10^5 ± 3.8 x 10^4 and 4.8 x 10^6 ± 2 x 10^6, respectively.

Figure (5) illustrated the inhibitory effect of combination of three preservatives represented by 40 ppm nisin with 50 ppm sodium nitrite and 0.2% potassium sorbate in comparison with combined 125 ppm sodium nitrite with 60 ppm nisin and 0.3% potassium sorbate where the average counts at the end of experimental period (48h.) from inoculation were 4.3 x 10^5 ± 1.5 x 10^5 and 6.9 x 10^3 ± 1 x 10^3, respectively.

5. DISSCUSION

Results of total aerobic count in examined samples in table (1) are somewhat similar with those reported by Hamada et al. (2008) (1.25 x 10^7, 3.64 x 10^6 and 1.0^6 x 10^8, respectively). This variation is attributed to the curing process of the products which plays a great inhibitory effect on multiplication of microorganisms. The total aerobic bacterial count was also influenced by the bacterial load of raw meat, incorrect temperature of trimming, grinding, curing facilities and incorrect thawing temperature (Ying and Tzer, 1996).

Results of total coliform count illustrated in table (2) did not agree with those reported by Bkheet et al. (2007) (3.9 x 104, 2.3 x 10^4 and 6.4 x 10^3, respectively). While somewhat similar to Hamada et al. (2008) (1.75 x 10^2, 5.46 x 10^3, 1.19 x 10^2, respectively), variations may be attributed to the processing defect and/or post processing contamination from workers, utensils and contact surfaces which indicate inadequate hygiene.

Coliform have an epidemiological interest and importance, as some of which were pathogenic and may cause serious intestinal infection and food poisoning. Coliform count was greatly considered to be suitable indicator for fecal contamination (Mousa et al., 2001).

Table (3) demonstrated results of total anaerobic count in examined samples. This unexpected high anaerobic count in chicken burger may be related to the technical defects in preparation procedures resulted in increasing anaerobic bacterial load. Results are nearly similar to Aiedia (1995) (2.1 x 10^3) and Abo-Zeid-Souzan (1998) (7.4 x 10^3).

Nisin addition at concentrations of 20 and 40 ppm had no inhibitory effect on C. perfringens count, while with increasing nisin concentration to 60 ppm had showed a little inhibitory effect. These results are in harmony with Eleiwa-Nesreen (2009) who examined the effect of nisin in different concentrations on C. perfringens type A throughout a period of storage time (10 days at 4 OC.). Results revealed that 25 ppm of nisin had no effect on C. perfringens, while 50 ppm had little effect, but 100 ppm had the higher effect on such organism, where its count reduced and reached to 3.8 Log 10 CFU/g at the 9th day of storage time. Furthermore, nisin acts in a concentration-dependent fashion both in terms of the amount of nisin applied and the number of vegetative cells or spore to be inhibited or killed. Spores of

a sensitive strain were claimed to be more sensitive to nisin than the vegetative cells Delves-Broughton et al. (1996).

Results illustrated in figure (2) revealed that addition of 50 and 125 ppm sodium nitrite had no inhibitory effect on C. perfringens count. These results are in harmony with Aideia and Yanny (2005) who reported that after addition of 125 ppm sodium nitrite, C.perfringens gradually decreased from 7 log 10 CFU/g to 2.6 log 10 at the end of 7th week of storage and moreover declined to reach <2 log10 after eight weeks interval, and Labbe and Duncan (1970) who mentioned that addition of sodium nitrite up to 20,000 ppm not inhibit germination of heat-resistant C. perfringens.

Results in figure (3) revealed that neither of potassium sorbate concentrations could significantly decline the C. perfringens count. These results were nearly similar to Tompkin et al. (1974) who examined the effect of potassium sorbate (0.1 % wt/wt) on C. perfringens (3.2 x 10^2 CFU/g) and incubated at 27 OC to represent temperature abuse of the product. Results revealed that C. perfringens declined below the detectable levels (<30 CFU/g) in all samples within the first and second day even in presence or absence of potassium sorbate, which re-increased to record <3 x 102 CFU/g at fourth and fifth day of experiment, and from seventh to seventeen days of experiment, count of C. perfringens was over 3 x 103 CFU/g.

Results demonstrated in figure (4) revealed that mixture of 40 ppm nisin with 50 ppm nitrite had a greater inhibitory effect on inoculated C. perfringens than mixture of 50 ppm sodium nitrite and 0.2% potassium sorbate. These results are in harmony with Aideia and Yanny (2005) who stated that combination both of nisin and sodium nitrite in concentrations of 400 and 125 ppm, respectively revealed greater inhibition on C. perfringens growth since it reach 2 log10 after 3 weeks from inoculation. They concluded that combination of nisin with sodium nitrite can had a synergistic effect leading to greater inhibitory effect on C. perfringens. Moreover, Limón et al. (2011) stated that although using potassium sorbate and/or sodium nitrite at its maximal concentrations, C.perfringens was not inhibited but it increased at 10 OC although presence of preservatives

Results in figure (5) revealed that mixture of 125 ppm sodium nitrite with 60 ppm nisin and 0.3% potassium sorbate had a greater lethality effect on inoculated C. perfringens than mixture of 50 ppm sodium nitrite with 40 ppm nisin and 0.2% potassium sorbate which may be referred to ability of nisin to pass through the cell wall of Gram-positive bacteria to the cytoplasmic membrane where it interacts with the phospholipids component of cell membrane that allows the outgoing of essential cellular components or in severe cases complete lysis of the target cell (Delves and Delves-Broughton, 1999).

These results are in harmony with Eleiwa-Nesreen (2003) and Aideia and Yanny (2005) who stated that combination both of nisin and sodium nitrite in concentrations of 400 and 125 ppm, respectively revealed greater inhibition of C. perfringens growth since it reach 2 log10 after 3 weeks from inoculation. They concluded that combination of nisin with sodium nitrite can lead to greater inhibitory effect on C. perfringens, this reduction may be attributed to that the nisin-nitrite combination which had a synergistic effect that overcome the effect of potassium sorbate.

Results of this study were indicative for contamination and inadequate hygienic conditions in production and processing of chicken meat products.

The best formula could inhibit C. perfringens growth was 125 ppm sodium nitrite with 60 ppm nisin and 0.3% potassium sorbate for the economic and public health importance which decline count of inoculated C. perfringens from 6.3 x 10^7 ± 5.8 x 10^5 CFU/g after 3h from inoculation to 6.9 x 10^3 ± 1 x 10^3 CFU/g

after 48h from inoculation. Moreover, according to EOS (2005), poultry products must be free from C. perfringens viable cells and spores (EOS, 2005).

Table 1. Statistical analytical results of total Aerobic Plate Count/g (APC) of the examined chicken product samples (n=45).

Chicken samples	Count of CFU/g		
	Min	Max	Mean ± SE
Burger	2.0×10^5	2.0×10^8	$1.8 \times 10^7 \pm 1.3 \times 10^7$
Luncheon	1.1×10^3	4.5×10^7	$7.4 \times 10^6 \pm 3.1 \times 10^6$
Frankfurter	2.0×10^2	3.2×10^8	$5.8 \times 10^7 \pm 2.5 \times 10^7$

Table 2. Statistical analytical results of total coliform count (CFU/g) in the examined chicken product samples (n=45).

Chicken Samples	Count of CFU/g.		
	Min	Max	Mean ± SE
Burger	1.6×10	2.6×10^4	$4.2 \times 10^3 \pm 2.6 \times 10^3$
Luncheon	2.5×10^2	1.1×10^3	$3.0 \times 10^2 \pm 0.9 \times 10^2$
Frankfurter	4.5×10^2	6.4×10^2	$5.3 \times 10^2 \pm 5.0 \times 10$

Table 3. Statistical analytical results of total anaerobic count/g in the examined chicken product samples (n=45).

Chicken Samples	Count of CFU/g.		
	Min.	Max.	Mean ± S.E.
Burger	1.1×10^3	2.1×10^5	$2.1 \times 10^4 \pm 1.4 \times 10^4$
Luncheon	2.6×10^2	6.7×10^3	$8.5 \times 10^2 \pm 4.6 \times 10^2$
Frankfurter	4.6×10^2	5.1×10^2	$4.8 \times 10^2 \pm 4.3 \times 10$

Fig. 1. Effect of 20, 40, 60 ppm nisin after different incubation times.

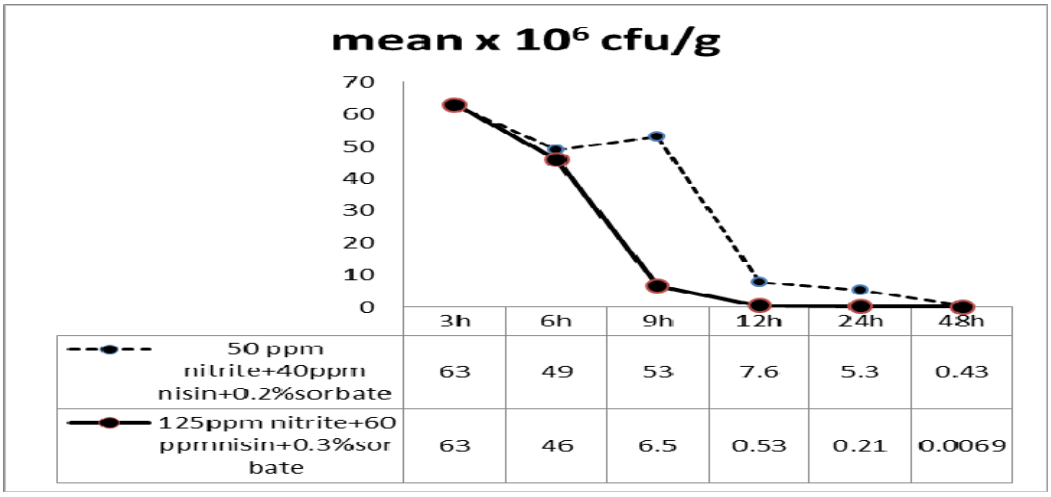


Fig. 2. Effect of 50 ppm, 125 ppm sodium nitrite after different incubation times.

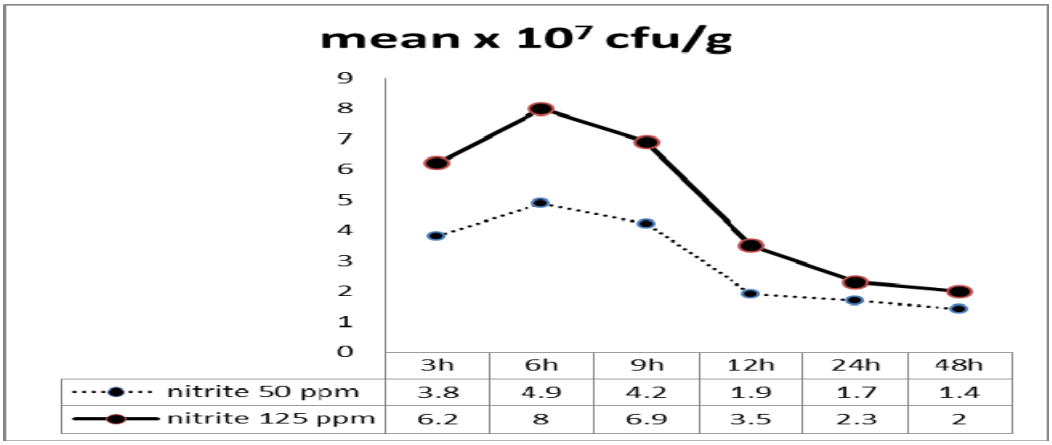


Fig. 3. Effect of 0.1%, 0.2%, 0.3% potassium sorbate after different incubation times.

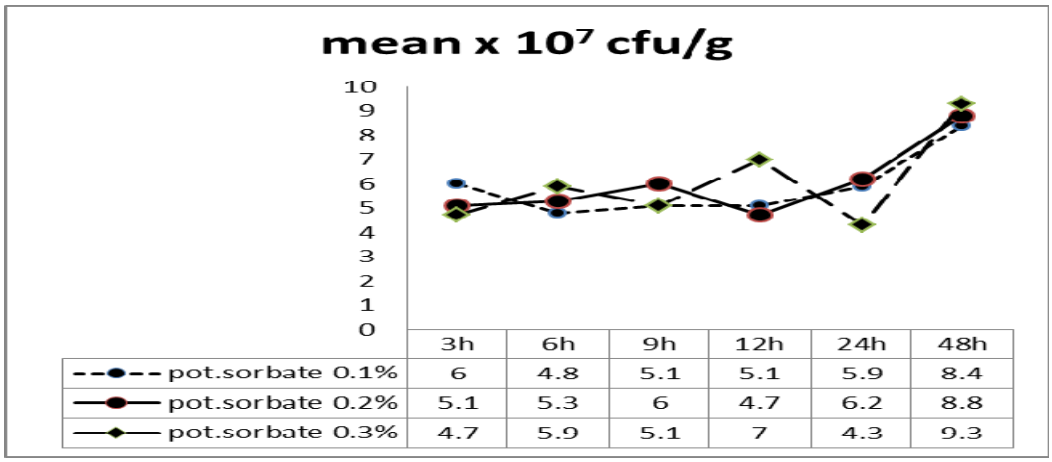
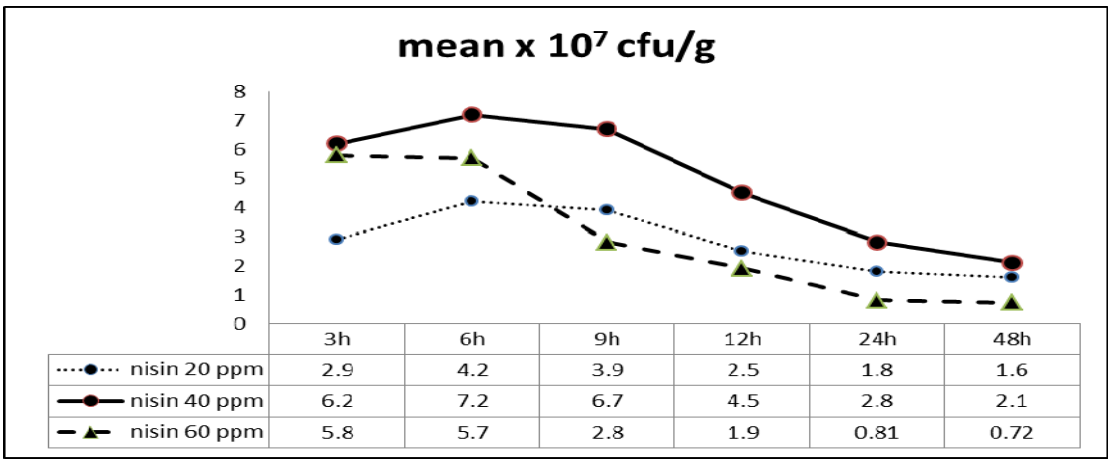
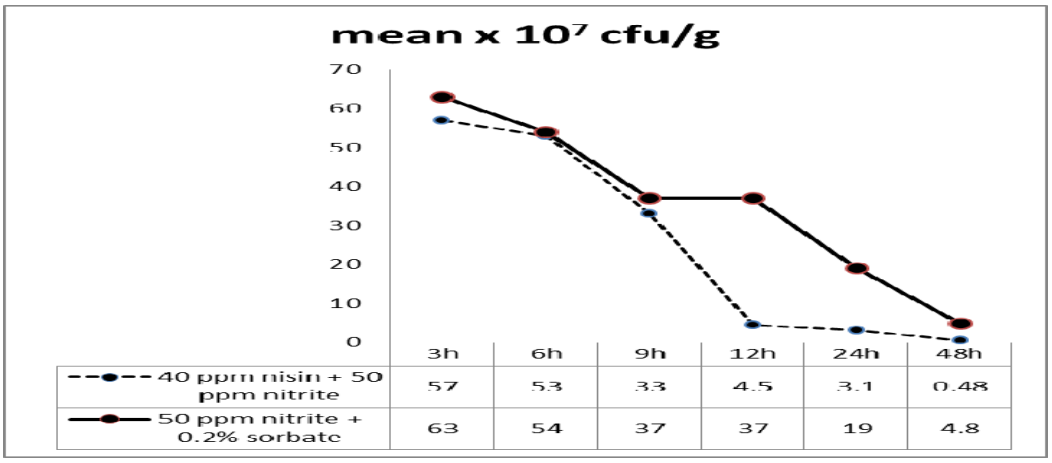


Fig. 4. Effect of 50 ppm nitrite+40ppm nisin in comparison with effect of 50ppm sodium nitrite+0.2%potassium sorbate.



	3h	6h	9h	12h	24h	48h
nisin 20 ppm	2.9	4.2	3.9	2.5	1.8	1.6
nisin 40 ppm	6.2	7.2	6.7	4.5	2.8	2.1
nisin 60 ppm	5.8	5.7	2.8	1.9	0.81	0.72

Fig. 5. Effect of 50 ppm nitrite+40ppm nisin+0.2% potassium sorbate in comparison with effect of 125ppm nitrite+60ppmnisin+0.3% potassium sorbate.



	3h	6h	9h	12h	24h	48h
40 ppm nisin + 50 ppm nitrite	57	53	33	4.5	3.1	0.48
50 ppm nitrite + 0.2% sorbate	63	54	37	37	19	4.8

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تأثير بعض المواد الحافظة على العدد البكتيري لبعض منتجات لحوم الدواجن

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الملخص العربى

تم إجراء هذه الدراسة على 45 عينة من مختلف منتجات لحوم الدواجن (البيرجر،اللنشون والفرانكفورتر) بواقع 15 عينة من كل منتج بمحافظة القليوبية، والتي تم تجميعها من مختلف محلات السوبر ماركت بالمحافظة. كان متوسط العدد الكلي للبكتريا الهوائية, $1.8 \times 10^7 \pm 1.3 \times 10^7$, $4.2 \times 10^3 \pm 2.6 \times 10^3$, and $2.1 \times 10^4 \pm 1.4 \times 10^4$ خلية/جرام في كل من البيرجر، اللنشون والفرانكفورتر،على التوالي. بينما كان متوسط العد الكلي للميكروبات المعوية $7.4 \times 10^6 \pm 3.1 \times$ 10^6, $3 \times 10^2 \pm 0.9 \times 10^2$, and $8.5 \times 10^2 \pm 4.6 \times 10^2$ خلية/جرام،على التوالي. بينما كان متوسط العد الكلي للميكروبات اللاهوائية $5.8 \times 10^7 \pm 2.5 \times 10^7$, $5.3 \times 10^2 \pm 5 \times 10$, and $4.8 \times 10^2 \pm 4.3 \times 10^2$، خلية/جرام، على التوالي .كذلك فقد تم إجراء اختبار تجريبي لمعرفة مدى تأثير مركب النيسين، نترات الصوديوم و سوربات البوتاسيوم كل على حدا أو في صورة خليط من المواد محل الدراسة بتركيزات مختلفة على ميكروب الكلوستريديم بيرفرنجس في عينات لحم دجاج مفروم ومعقم بالإشعاع. وجد أن النيسين والنترات لهم تأثير مثبط لنمو الكلوستريديم بيرفرنجس بينما خلط النيسين مع النترات يعطي تأثير يضاعف تأثير كل مركب بمفرده، بينما أظهرت الدراسة أن استخدام خليط من 60 جزء في المليون نيسين و125 جزء في المليون نترات الصوديوم و0,3% سوربات بوتاسيوم كان له التأثير الأفضل لتثبيط نمو الكلوستريديم بيرفرنجس

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(1):94–103, مارس 2014)