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Effect of Chlorine and Natamycin on the hygienic status of cattle meat

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Abstract

A total of 4200 g of fresh meat sample was divided into two equal parts (2100 g) each contain 7 equal groups (300 g each), the first part was used for study the effect of chlorine and natamycin on the hygienic status of meat,1st group was untreated used to determine the initial Aerobic Plate Count (APC) and mold count. Natamycin was used at a concentration of 50 and 100 ppm in the treatment of the 2st and 3nd groups, while chlorine was used with the same concentrations for the treatment of the 4rd and 5th groups. In addition, the 6th group was treated with a mixture of chlorine and natamycin at concentration of 50 ppm for each. Meanwhile, the 7th group was treated with a combination of chlorine and natamycin with 100 ppm for each, respectively. The second part was used for study the effect of chlorine and natamycinon the shelf life of marinated meat. The experiment was performed in triplicate. The obtained results revealed that mixture of chlorine and natamycin (100 ppm of each) was the best treatment which had a significance effect in reducing APC by 1.28 log_{10}cfu/g (from log 4.91±0.48 to log 3.63±0.54) with 26.07% reduction rate and by 1.48 log_{10}cfu/g (from 4.54±0.11 to 3.06±0.10) with 32.16% reduction rate for mold count. The obtained results also declare that chlorine alone (50 and 100 ppm) has the lowest effect on mold count while, the same concentrations of natamycin had the lowest effect on APC. Fresh meat with traditional marinades remained sound till the 4th day of refrigeration storage, while those marinades with 50 and 100 ppm of chlorine and natamycin mix remained sound till the 8th day of refrigeration storage.

***Keywords**: Natamycin, Chlorine, APC, Mold count, Reduction rate, Hygienic status of meat, Shelf-life.*

Introduction

Naturally occurring antimicrobial compounds could be applied as food preservatives to protect food safety and quality and subsequently, extend the shelf-life of foods. These, compounds are naturally produced by various sources, including plants, animals and microorganisms. Many naturally occurring compounds, such as natamycin, nisin and plant essential oils, have been reported to be effective in their potential role as antimicrobial agents against spoilage and pathogenic microorganisms, with special reference to natamycin which considered a very stable powder with efficacy against several molds and its toxin production (**Juneja *et al.*, 2012)**. Natamycin is a natural antifungal agent with a wide range of antimicrobial spectrum against yeasts and molds andis widely used in the food industry for the prevention or decrease the risk resulted from mold contamination in meats (**Jay *et al.*, 2005**and **Welscher *et al.*, 2008**). Natamycin is considered effective antifungal agent without changing organoleptic characteristics of the food products such as cheese and meats (**Food Standards Australia New Zealand, 2004**and **Dzigbordi *et al.*, 2013).** The European Food Safety Authority (EFSA) has approved the use of natamycin (E 235) as a food additive as a natural mold inhibitor for treatment of meat surface (**EFSA, 2009**).

Unlike many other preservation options, the application of Natamycin in foods does not produce changes in taste, color or odor. This makes it suitable as a food preservative in a wide range of products. Natamycin remains stable at a wide pH range (3-9). As most foods have a pH value between pH 4 and pH 7, this makes Natamycin a very versatile food preservative. Moreover, natamycin was re-evaluated by the Joint FAO/WHO Expert Committee on Food Additives (**JECFA, 2002 and 2007**) and confirmed as safe for human consumption proportionally with its intended use and use levels with respect to cheese and meats proposed in the draft of Codex Alimentarius Commission-General Standard for Food Additives **(CAC-GSFA, 2018)**. **JECFA (2002)** also reconfirmed the existing Acceptable Daily Intake (ADI) of 0.3 mg/kg body weight for natamycin, the use of natamycin as foods additive, would provide improved protection against microbial spoilage, benefiting both consumers and manufacturers by reducing product losses, extending shelf-life and protecting public health and safety. An indirect benefit could be reduced the use of other antimicrobial preservatives.

Food safety is ensured by controlling the presence and growth of pathogenic organisms in and on foods. Numerous cleaning compounds and processes have been developed to remove and/or destroy bacteria, from the surface of fresh animal carcasses. Antimicrobial compounds are used to eliminate or limit the growth of pathogens in foods. Spoilage microorganisms and molds cause a sever deterioration of food components resulted in acceleration of food spoilage (**Doyle, 2005).**

Today, food losses are a major concern worldwide especially with an ever-growing world population and the fact that approximately one-third of all food produced for human consumption is either lost or wasted, each year estimated 1.3 billion tons of food are lost or wasted in which meat and dairy products constituted 20%. The natural antifungal agents are a key factor, it can only be considered in good practices and within the HACCP context as one of the hurdle technologies to prevent fungal spoilage (**Gustavsson *et al.,* 2011 and FAO "Food and Agriculture Organization of the United Nations", 2017).**

Recently, food spoilage considered a major issue for the food industry, leading to food waste, substantial economic losses for manufacturers and consumers, fungal contamination can be encountered at various stages of the food chain. In order to avoid microbial spoilage and thus extend product shelf life, different treatments including fungicides and chemical preservatives are used. In parallel, public authorities encourage the food industry to limit the use of these chemical compounds and develop natural methods for food preservation. This is accompanied by a strong societal demand for 'clean label' food products, as consumers are looking for more natural, less severely processed and safer products (**Salas *et al.,* 2017).**

Chlorine was one of the first chemical treatments used to decontaminate carcass in the beef industry. Its antibacterial activity is mainly due to its strong oxidative effect on bacterial cell wall. Reasonably good reductions in microbial counts have been reported using water chlorinated at 200 to 500 ppm, while chlorine is not effective at 10 ppm (**FSA, 2006**).

The aim of the current study was planned to investigate the antibacterial effect of chlorine (50 and 100 ppm), natamycin (50 and 100 ppm) as well as a combination of chlorine and natamycin (50 and 100 ppm of each) on APC and mold count of contaminated meat surface and their effect on the shelf-life of marinated fresh meat.

Materials and Methods

Sample collection

A total of 4200 g of fresh meat sample was purchased from Cairo market and aseptically transferred in thermal insulator container without delay to the laboratory, divided into two equal parts (2100 g) each contain 7 equal groups (300 g each), the first part used for study the effect of chlorine and natamycin on the hygienic status of meat, the second part was used for study the effect of chlorine and natamycin on shelf life of marinated meat.

1. Study the effect of chlorine and natamycin on the hygienic status of meat

1.1. The 1[st] group was untreated group and used to carry out the following examinations.

1.1.1 Preparation of food homogenate according to ISO 6887/1 (2017)

225 mL. sterile 0.1% peptone water with 25 g of tested sample was stomached for 30-60 se-

conds, 1 mL was transfer into tube containing 9 ml. of sterile diluent, mixed thoroughly to obtain 1:100, the operation was repeated to obtain the appropriate dilutions.

1.1. 2. Enumeration of total aerobic plate counts (APHA, 2001)

0.1 mL of each dilution was inoculated onto the center of a dried plate count agar plates, distributed using glass spreader, the plates were left for 15 minutes, inverted and incubated at 35°C for 48±2hours and the results were recorded as cfu/g sample.

1.1. 3. Enumeration of mold according to ISO 21527/1 (2008)

0.1 mL. of each dilution was transferred to DG18 dichloran rose Bengal agar plates, distributed by sterile glass spreader, plates were incubated at 25°C±1°C for 5 to 7 days, results were recorded as cfu/g sample.

1.2. The 2nd and 3rd groups were treated with 50 and 100 ppm chlorine for 5 minutes and carry out APC and mold count

1.3. The 4th and 5th groups were treated with 50 and 100 ppm natamycin (E235) for 5 minutes and carry out APC and mold count

1.4. The 6th and 7th groups were treated with a mixture of chlorine and natamycin (50 and 100 ppm) of each for 5 minutes and carry out APC and mold count.

2. Study the effect of chlorine and natamycin on shelf life of marinated meat.

The seven groups were marinated using traditional marinades and natamycin chlorine mixture were used and stored on refrigerator at 4°C, APC and mold count were done daily.

2.1. The 1st group was marinated group without adding natamycine or chlorine and carry out APC and mold count

2.2. The 2nd and 3rd groups were marinated and were added 50 and 100 ppm chlorine and were carried out APC and mold count

2.3. The 4th and 5th groups were marinated and were added 50 and 100 ppm natamycin (E235) and were carried out APC and mold count

2.4. The 6th and 7th groups were marinated and were added a mixture of chlorine and natamycin (50 and 100 ppm) for each and were carried out APC and mold count.

2.5. Statistical analysis

Statistical analysis of the data was done using of Statistical Packaging for the Social Science (SPSS) Ver. 20. Statistical comparisons were made by using one-way analysis of variance (ANOVA). The results were considered significantly different with $P<0.05$.

Results and Discussions

Table (1) and Fig. (1) illustrated the effect of different treatments using chlorine and natamycin either separately or combined on APC, the obtained results revealed a significance difference (P<0.05) between mean (4.91 ± 0.48 $\log_{10}$cfu/g) of untreated group(1) and group (3) treated with 100 ppm chlorine which recorded 3.89±0.36 $\log_{10}$cfu/g such difference also was significance with samples of group 7 (3.63 $\log_{10}$cfu/g) which were treated with a mixture of both chlorine and natamycin (100ppm). From the obtained results it can be concluded that natamycin had a little inhibitory effect on APC unless it is mixed up with chlorine, that's explain the synergistic action of both chlorine and natamycin. This also confirmed by the results obtained in table and fig. (2) which showed that the highest reduction proportion of APC $\log_{10}$cfu/g was in the 7th group (1.28) with 26.07% reduction rate followed by chlorine 100 ppm (1.02) with reduction rate of 20.77%, while natamycin and chlorine mixture (50ppm) resulted in 0.87 log reduction (17.72%).

Table (1). Effect of Natamycin and Chlorine with different concentrations alone or combined on APC (log_{10}cfu/g) in fresh and treated meat samples.

Treatment used	APC (log_{10} cfu/g)			
	Trial (1)	Trial (2)	Trial (3)	Mean±SD
Untreated samples(group 1)	5.26	4.36	5.11	4.91"AB"±0.48
Chlorine				
50 ppm (group 2)	4.70	3.67	4.30	4.22±0.52
100 ppm (group 3)	4.18	3.48	4.00	3.89"a"±0.36
Natamycin				
50 ppm (group 4)	4.78	3.95	4.81	4.51±0.48
100 ppm (group 5)	4.70	3.58	4.70	4.33±0.49
Natamycin and chlorine mix				
50 ppm (group 6)	4.30	3.53	4.30	4.04±0.44
100 ppm (group 7)	3.93	3.00	3.95	3.63"b"±0.54

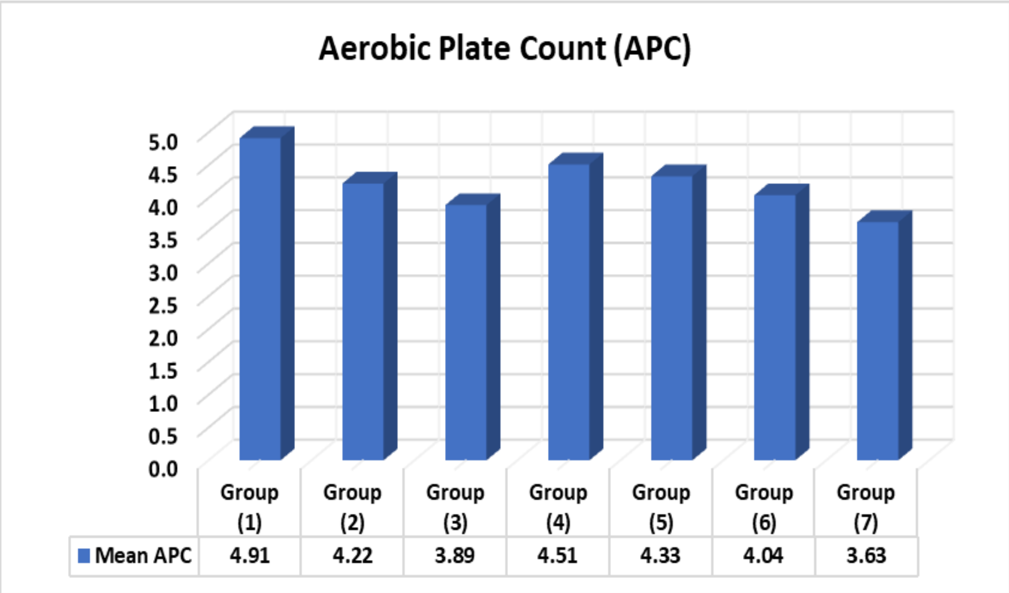

Fig. (1): Effect of Natamycin and Chlorine on APC in treated meat samples.

Table (2). Reduction % of APC (log_{10}cfu/g) in accordance to the different concentrations of natamycin and chlorine treatments through three trials.

Treatment used	APC reduction (count and incidence)							
	Trial (1)		Trial (2)		Trial (3)		Mean reduction	
	Reduction ratio							
	Log	%	Log	%	Log	%	Log	%
Chlorine								
50 ppm (G2)	0.56	10.65	0.69	15.83	0.81	15.85	0.69	14.05
100 ppm (G3)	1.08	20.53	0.88	20.18	1.11	21.72	1.02	20.77
Natamycin								
50 ppm (G4)	0.48	9.13	0.41	9.40	0.30	5.87	0.40	9.98
100 ppm (G5)	0.56	10.65	0.51	11.70	0.41	8.02	0.49	8.02
Natamycin and chlorine mix								
50 ppm (G6)	0.96	18.25	0.83	19.04	0.81	15.85	0.87	17.72
100 ppm (G7)	1.33	25.29	1.36	31.19	1.16	22.70	1.28	26.07

NB: Mean log reduction and incidence compared with untreated samples

The results in the present study were nearly similar to **Kotula *et al* (1974)** and **Richardson (2003)** found that chlorinated water with 200 ppm resulted in 1.5 log_{10}cfu/g reduction of APC on beef carcasses, the author added that treated carcasses with solution of up to 250 ppm chlorine have been variable, with some very poor reductions being reported. Furthermore**, Ku *et al.* (2008)** recorded 0.93 reduction rate of APC in chicken legs treated with 100 ppm chlorine dioxide. The obtained results also more or less agreed with **Kim *et al.* (2005)** who reported that chlorine dioxide (50 ppm) proved to be most significantly in reducing APC of saury type fish by 0.94 log_{10}cfu/g (from 6.43 log_{10}cfu/gto 5.47 log_{10}cfu/g).

Cutter and Siragusa (1995a) concluded that sprays of 50, 100, 250, 500, and 900 ppm chlorine were only slightly effective (<1-log reduction) in reducing *E. coli* attached to the surface of beef carcass. A relatively novel use of sanitizers including chlorine and chlorine derivatives for the inactivation either by reducing or mitigating microorganisms including bacteria and fungi on raw, unprocessed food products, including meat and poultry carcasses (**Davidson and Harrison, 2002).**

Higher reduction rate of **APC** using chlorine dioxide was recorded by **Cutter-Catherine and Dorsa-Warren, 1995** (2.07 log10cfu/cm2)

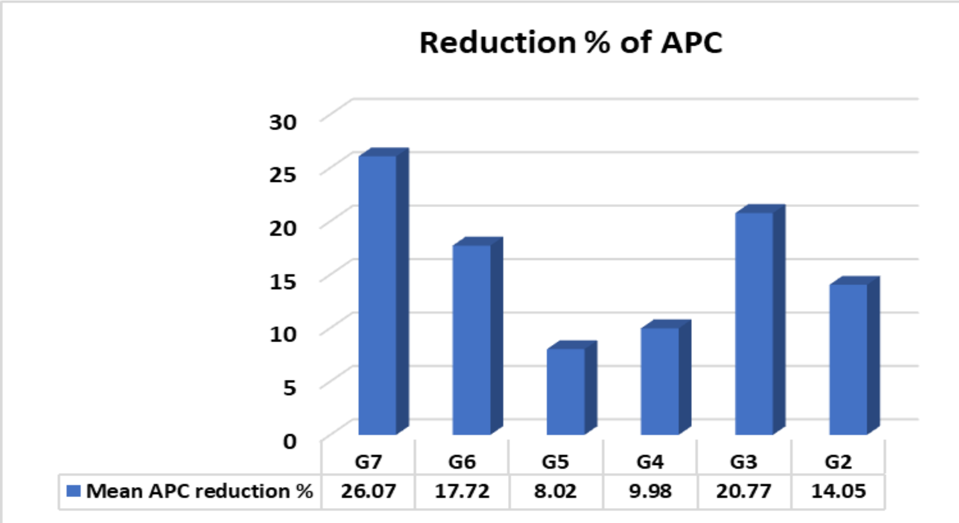

Fig. (2): Mean reduction % 0f APC of different treatments compared to untreated samples.

In this respect, FDA (2000) mentioned that antimicrobial compounds serve as the primary microbial controls specially when using a combination of microbial inhibitors, such combination serve in control of several microorganisms. Moreover, natamycin is effective against nearly all molds and yeasts but which has little effect on bacteria. This substantiates the obtained results which indicated presence of synergistic action when using a mix up of chlorine as antibacterial with natamycin as antifungal agents. While, such combination may be sometimes called "hurdle technology" as the combination of other antimicrobials work in antagonistic manner (**Leistner, 2000).** Raw meat remains a major source of foodborne pathogens, and a variety of techniques have been devised to reduce bacterial contaminants on animal carcasses and on pieces of raw meat including chemical decontamination with chlorine, organic acids and trisodium phosphate; or using natural antimicrobials such as natamycin and bacteriocins and combination treatments **(Dincer and Baysal, 2004 and Koohmaraie *et al.*, 2005).** Decontamination processes reduce microbial populations on the meat surface and subsequently assist in prolong its shelf-life (**Hegerding *et al.*, 2005**).Natamycin binds irreversibly to the cell membrane of fungi because of its high affinity for ergosterol. This causes membrane hyperpermeability leading to rapid leakage of essential ions and peptides and ultimately cell lysis. As bacterial membrane does not contain sterol, natamycin has little or no effect against bacteria (**Adams and Moss, 2008).** This illustrated the slight decrease in bacterial count with the use of natamycin alone.

The obtained results in table and fig. (3) revealed that there was a significant difference ($P<0.05$) of mean mold count (log_{10}cfu/g) between the 7th group which was treated with a mixture of chlorine and natamycin (100 ppm of each) which showed the lowest mold count (3.06 log_{10}cfu/g) and each of 1st untreated group 1 (4.54), group 2which was treated with 50 ppm chlorine (4.43), group 3which was treated with 100 ppm chlorine (4.19) and group 4 (4.08) which was treated with 50 ppm natamycin. Also, there was a significance difference between untreated group (1) and group (5) which was treated with 100 ppm natamycin. While there was no significance difference ($P>0.05$) between each of the other groups. The previous results showed that there was a synergistic action and highly inhibitory effect in reducing mold count when using a combined mixture of chlorine and natamycin. Otherwise, chlorine alone had no inhibitory effect on mold count unless it is combined with natamycin.

Table (3). Effect of Natamycin and Chlorine with different concentrations on mold count of examined fresh meat samples.

Treatment used	Mold count (log_{10} cfu/g)			
	Trial (1)	Trial (2)	Trial (3)	Mean±SD
Untreated samples(G1)	4.49	4.46	4.66	4.54"A"±0.11
Chlorine				
50 ppm (group 2)	4.30	4.40	4.60	4.43"B"±0.15
100 ppm (group 3)	4.15	4.15	4.26	4.19"C"±0.06
Natamycin				
50 ppm (group 4)	4.00	4.00	4.23	4.08"D"±0.13
100 ppm (group 5)	3.56	3.70	3.48	3.58"a"±0.11
Natamycin and chlorine mixture				
50 ppm (group 6)	3.70	3.90	4.05	3.88"a"±0.18
100 ppm (group 7)	3.00	3.18	3.00	3.06"abcd"±0.10

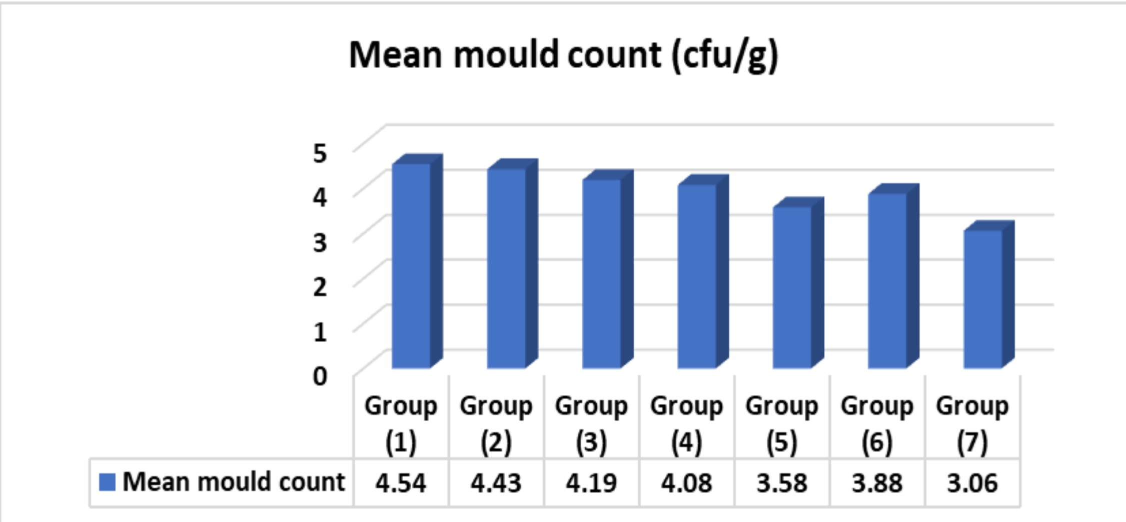

Fig. (3): Effect of natamycin and chlorine on mold count in treated meat samples.

This is explained by the results listed in table and fig. (4) which displayed the highest mean reduction rate of 1.48 log_{10}cfu/g (32.6%) of mold count in the 7th group when used a mixture of chlorine and natamycin (100 ppm of each), followed by natamycin alone (21.15%) as it was evident in the results of group (5) when used at a concentration of 100 ppm with mold reduction count of 0.96 log_{10}cfu/g.

Table (4) Reduction % of molds count (log_{10}cfu/g) in accordance to the different concentrations of natamycin and chlorine treatments through three trials.

Treatment used	Mold reduction (Log count and incidence)							
	Trial (1)		Trial (2)		Trial (3)		Mean reduction	
	Reduction ratio							
	Log	%	Log	%	Log	%	Log	%
Chlorine								
50 ppm (G2)	**0.19**	**4.23**	**0.06**	**1.35**	**0.06**	**1.29**	**0.11**	**2.42**
100 ppm (G3)	**0.34**	**7.57**	**0.31**	**6.95**	**0.40**	**8.58**	**0.35**	**7.71**
Natamycin								
50 ppm (G4)	**0.49**	**10.91**	**0.46**	**10.31**	**0.43**	**9.23**	**0.46**	**10.13**
100 ppm (G5)	**0.93**	**20.71**	**0.76**	**17.04**	**1.18**	**25.32**	**0.96**	**21.15**
Natamycin and chlorine mixture								
50 ppm (G6)	**0.79**	**17.59**	**0.56**	**12.56**	**0.61**	**13.09**	**0.66**	**14.54**
100 ppm (G7)	**1.49**	**33.19**	**1.28**	**28.70**	**1.66**	**35.62**	**1.48**	**32.60**

NB: Mean log reduction and incidence compared with untreated samples

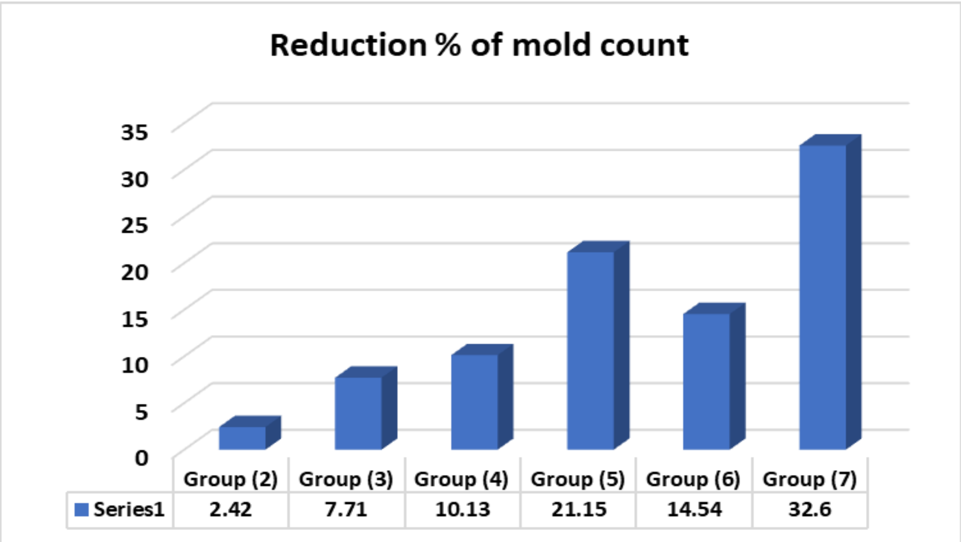

Fig. (4): Mean reduction % 0f mold count of different treatments

The obtained results of using natamycin as an effective antimicrobial agent against molds agreed with that stated by **FSANZ " Food Standards Australian New Zealand, 2004"** this may be attributed to the strong cidal effect of natamycin towards susceptible microorganisms and is particularly effective against fungi, which may produce mycotoxins**.** In addition, extension of use of natural antimicrobials are requested, rather that other chemical preservatives which are generally limited in the range of microorganisms affected and often used at their maximum permissible limit. Even at maximum permitted levels, spoilage problems commonly occur and adverse flavors can result. Natamycin offers many advantages over these preservatives, including a much wider spectrum of activity, no adverse flavor effects, and generally, vastly superior effectiveness and improved product shelf-life. Use of natamycin as antifungal leads to protect the health and safety of consumers and Generally Recognized as Safe (GRAS). Natamycin is widely used in the food industry for the prevention of mold contamination in meats and cheeses (**Chen *et al.*, 2008).** Natamycin has been used for over 30 years as an antimicrobial food preservative as Generally Recognized as Safe (GRAS) and is currently approved in more than 70 countries including USA and United states as antimycotic agent (**CAC, 2017).** Natamycin was also assigned as a natural preservative by the European Union (**Ture *et al.*, 2008).**

The major problem with use of antimicrobial combination is the potential development of acquired resistance. Therefore, the most important issue is to use a combination of appropriate antimicrobials that have different mechanisms with effective concentrations in order to avoid microbial spoilage and thus extend product shelf life **(Davidson and Harrison, 2002)**

Higher reduction rate for mold count was recorded by **Olle-Resa *et al.*, 2014** (0.5 to 1.3 log_{10}cfu/g) by using natamycin concentration of 20 and 50 ppm, respectively. In this respect**, Cerrutti and Alzamora (1996)** stated that growth of yeast was inhibited completely in food containing 2000 ppm natamycin. In addition, **Ku *et al.* (2008)** recorded 1.15 log_{10}cfu/g reduction rate in mold count of chicken legs using chlorine dioxide, **Hassan and El-Lawandy (2006)** reported 1.16 log_{10}cfu/g reduction (33.72%) and 1.14 log_{10}cfu/g reduction (36.77%) in mold count of natamycin treated sausage and frankfurter samples, respectively. In addition**, El-Tawab (2014)** concluded that natamycin (0.1%) had a great inhibitory effect on total mold count as it reduced from 4.69 in control group to 2.96 (1.73 log_{10}cfu/g reduction rate and 36.9% reduction percentage) in treated groups treated with 50 ppm natamycin with reduction in natamycin and potassium sorbate treated groups, Moreover**, Salem-Amany *et al.* (2016)** mentioned that *Aspergillus niger* count was decreased by 2.17 log_{10}cfu/g with reduction rate of 43.4%, 2.21 (44.2%) and 2.24 (44.8%) in minced meat samples treated with 100, 200 and 300 ppm natamycin, respectively. They recommended the use of natamycin (300 ppm), as it is safe.

Antifungal agent, is therefore recommended to improve safety of the meat products **Schneider *et al.* (2004)** and **Ariyapitipun *et al.* (2000)** added that sanitizing treatments of meat and beef trimmings was performed by using chlorinated compounds and natamycin.

In contrary, **Reps *et al.* (2002)** and **Fajardo *et al.* (2010)** had another opinion as the use of natamycin as on food surfaces by spraying or dipping would permeate the meat tissue and thus reduce its concentration on the meat surface.

Table and Fig. (5) Clarified the effect of chlorine and natamycin used in different concentrations (50 and 100 ppm) either separate or in a mixture on the APC and mold counts (log_{10}cfu/g) and their reflection on the shelf life of examined marinades meat. Regarding APC, control samples marinades with traditional ingredients without addition of either chlorine or natamycin as well as those samples contained 50 ppm of natamycin were remained sound till the 4th day of storage with APC of 5.78 log_{10}cfu/g, and at the 5th day recorded APC of 5.00 log_{10}cfu/g, for both at the 5th day. While, samples marinades with addition of 100 ppm of chlorine and those with 50 ppm of chlorine and natamycin mix remained sound till the 7th day of storage (5.11 and 5.48 log_{10}cfu/g), respectively. Finally, samples marinades with addition of 100 ppm of chlorine and natamycin mix remained sound till the 8th day of storage (5.63 log10cfu/g).

Table (5). The effect of various concentrations of chlorine and natamycin on the shelf life of fresh meat

Storage days	APC (log_{10}cfu/g)							Mold count (log_{10}cfu/g)						
	Control	Chlorine		Natamycin		Chlorine + Natamycin		Con-trol	Chlorine		Natamycin		Chlorine + Natamycin	
		50	100	50	100	50	100		50	100	50	100	50	100
1st day	4.63	4.00	3.94	4.62	4.52	4.43	3.45	2.70	2.60	2.52	2.30	2.26	2.30	2.15
2nd day	4.65	3.58	3.53	4.63	4.60	4.68	3.48	2.49	2.46	2.34	1.18	1.04	1.18	0.78
3rd day	4.69	3.95	3.88	4.69	4.65	4.79	3.70	2.95	2.93	2.84	1.90	1.59	1.64	1.18
4th day	5.78	4.67	4.59	5.15	4.68	4.92	3.95	3.63	3.61	2.41	2.72	1.94	2.30	1.41
5th day	XX	5.00	4.84	XX	5.00	5.00	4.67	XX	XX	3.23	3.04	2.56	2.61	1.74
6th day	XX	XX	4.91	XX	XX	5.36	4.86	XX	XX	XX	XX	2.84	3.32	1.96
7th day	XX	XX	5.11	XX	XX	5.48	5.34	XX	XX	XX	XX	2.97	3.53	2.49
8th day	XX	XX	XX	XX	XX	XX	5.63	XX	XX	XX	XX	3.23	XX	3.15
9th day	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX

NB: XX means samples spoiled at the corresponding day

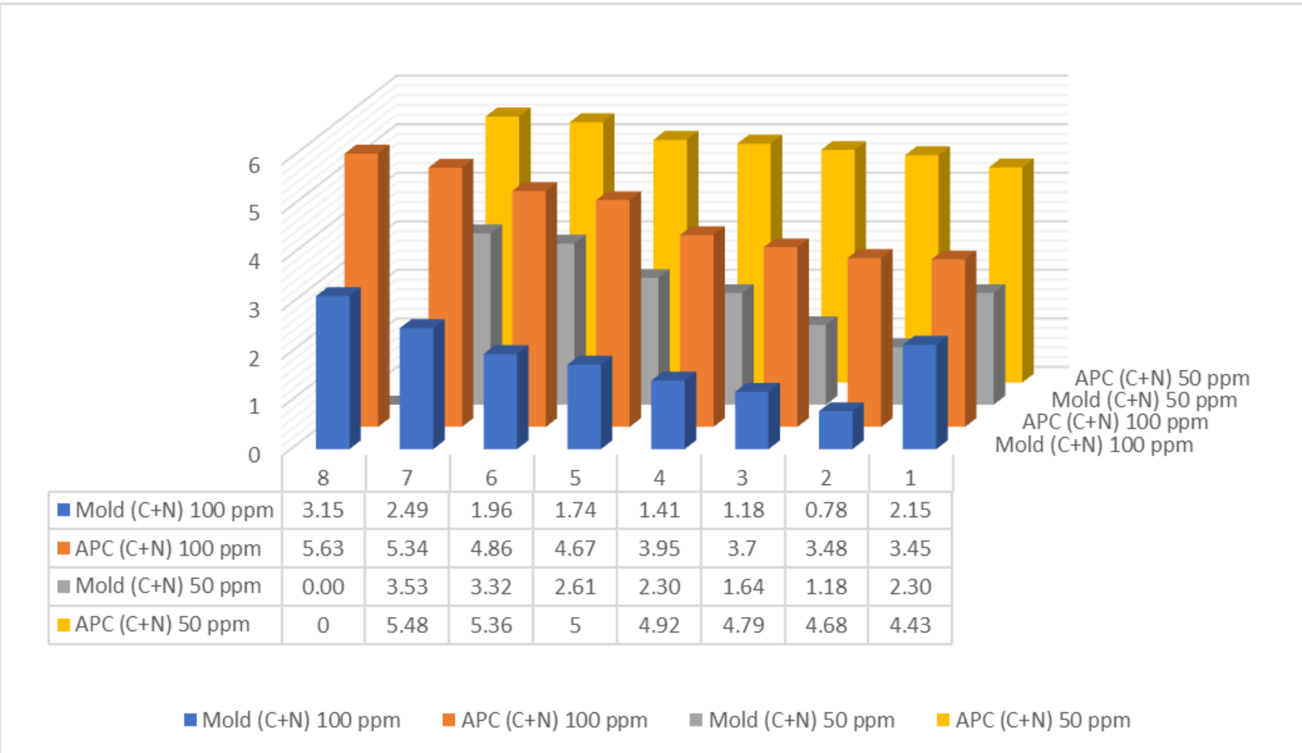

Fig. (5): Effect of chlorine and natamycin mix (50 and 100) ppm on both APC and mold counts

Table and Fig. (5) also revealed that mold count recorded 3.63 and 3.61 log_{10}cfu/g in control samples and those treated with 50 ppm of chlorine at the 4^{th} day of storage respectively, followed by 3.23 and 3.04 for chlorine 100 ppm and natamycin 50 ppm, respectively. At the 5^{th} day Samples treated with natamycin 100 ppm and a mixture of chlorine and natamycin remained sound till the 8^{th} day of storage and recorded 3.23 and 3.15 log_{10}cfu/g, while 50 ppm natamycin and chlorine mix recorded 3.53 log_{10}cfu/g at the 7^{th} day of storage. Overall, the obtained results revealed that chlorine had a strong inhibitory effect on APC while natamycin had the same effect on mold count and both when used together resulted in huge inhibitory effect on both APC and mold count which reflected positively on the shelf life of refrigerated stored meat.

These results agreed with **EFSA (2009)** which reported that natamycin is an antifungal substance produced by *Streptomyces natalensis* that is effective against almost all molds and yeasts; however, it has little or no effect on bacteria. They added that, natamycin has been used in dairy, meats, and other foods for antifungal effects and prolong the product shelf life. Moreover, **Food and Drug Administration, 2016** permitted the use of natamycin as a preservative in livestock foods as it is generally regarded as safe (GRAS). In addition, natamycin and nisin are the only natural preservatives regulated by legislation and permitted for use in over 150 countries in the surface treatment of different kinds of foods **(Adams, 2003; and EFSA, 2009).**

Natamycin is useful in inhibiting the growth of emergent foodborne pathogens and extending the shelf life of the products, no resistant molds and yeast were reported against natamycin, which is considered quite remarkable as compared with other preservatives (**Lule *et al.*, 2016).**

Conclusions and Recommendation

The present work has demonstrated that mixture of natamycin and chlorine with 100 ppm was highly effective against APC and mold counts when used as a spray for treatment of fresh meat surface. Moreover, natamycin alone was highly effective against molds as fungicidal or fungistatic in a concentration 100 ppm followed by 50 ppm for a mixture of natamycin and chlorine. Additionally, chlorine was effective at 100 ppm concentration against APC followed by 50 ppm. From the obtained results, it was recommended that natamycin and chlorine mixture can be potentially used as antifungal and antibacterial agents for controlling bacterial and mold contaminants of fresh meat surface as the data showed synergistic action between the two antimicrobials used in this study.

Due to the lack of published scientific data in the field of using a mixture of chlorine and natamycin and study the influence of both together in reducing the pollution of fresh meat with aerobic bacteria and fungi that causes rapid corruption of meat. Therefore, more researches should be conducted in this field. The documented results in the present study showed that there is a synergistic effect between chlorine and natamycin, which can be used together in a mixture for reduce both APC and mold counts in fresh meat and subsequently prolong the shelf life of the product Compared with each compound used separately

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