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Effect of Bio Preservation as a Modern Technology on Quality Aspects and Microbial Safety of Minced Beef

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Abstract: The effect of nisin and *Lactobacillus acidophilus* individually and in combination on sensory (color, odor and consistency), chemical (pH, total volatile basic nitrogen "TVB-N" and thiobarbituric acid "TBA") and bacteriological (total colony count "TCC", *Enterobacteriaceae* count and coliform count) quality attributes of fresh minced beef, as well as on the growth and survival of *Staphylococcus aureus*, as a food borne pathogenic bacteria, experimentally inoculated into fresh minced beef, was studied during chilling storage at 4°C. Sensory analysis indicated significant variances in using nisin and the combination trial. The results showed that the applied treatments markedly decreased pH, TVB-N and TBA values as well as TCC, *Enterobacteriaceae* count and coliform count, as compared with the control samples throughout chilling storage, when used separately, but the preservative activity highly increased with the combination trial. In addition, a highly significant differences ($P<0.05$) between the different treatments were noticed. Shelf life of samples treated with nisin, *Lactobacillus acidophilus* and a combination trial, *Lactobacillus acidophilus* and nisin increased and reached over 6, 3 and 5 days of storage at 4°C, respectively, compared to that of control samples which reached 2 days only. Initial count of *S. aureus* in minced meat samples was 8.54 log CFU/g, which decreased following treatment directly with nisin by 1.21 log CFU/g (14.17%), 2.54 log CFU/g (29.74%) and 3.69 log CFU/g (43.21%) in the 1st day, 2nd day and 3rd day, respectively, while after treatment with *Lactobacillus acidophilus* by 1.54 log CFU/g (18.03%) and 4.87 log CFU/g (57.03%) in the 1st day and 2nd day, respectively and finally after treatment with the combination of both by 3.87 log CFU/g (45.32%) in the 1st day. Whereas, the growth of *Staphylococcus aureus* in minced meat samples was completely inhibited after being treated with nisin, *Lactobacillus acidophilus* and combination of both in the 4th day, 3rd day and 2nd day, respectively. The activity of the applied treatments followed the following order: nisin > combination of nisin *and Lactobacillus acidophilus* > *Lactobacillus acidophilus* for shelf life extension, while combination of nisin *and Lactobacillus acidophilus* > *Lactobacillus acidophilus* > nisin, for the growth inhibition of *Staphylococcus aureus*. It could be concluded that the potential of combination of nisin and *Lactobacillus acidophilus* as a probiotic to inhibit the growth of common food spoilage bacteria opens up new perspectives for the bio preservation of meat products.

Key words: Nisin · Bacteriocin · Probiotic · *Lactobacillus acidophilus* · *Staphylococcus aureus*

INTRODUCTION

Meat has long been considered as a highly desirable, nutritious and protein-rich food, but at the same time, unfortunately, it is also highly perishable because it provides the nutrients needed to support the growth of many types of microorganisms. Due to its unique biological and chemical nature, meat undergoes progressive deterioration from the time of slaughter until consumption [1].

Microbial contamination of meat occurs primarily due to raw materials, grinding of meat which will spread exterior contamination essentially throughout the entire muscle mass, post processing handling, cross-contamination and/or equipment, lack of refrigeration facilities, ambient temperatures above 20°C, lack of suitable transportation between the production and marketing areas and improper storage temperature [2].

The microbiological spoilage of foods is due to the biochemical activity of microorganisms as they grow in

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food, causing changes in the food's appearance, odor, texture, or taste. Since food borne pathogens do not typically give an organoleptic indication of their presence, the organoleptic changes caused by spoilage microorganism serve as a warning to the consumer that the food could be unsafe for consumption, thus, protecting millions of people from food borne illness [3]. *Staphylococcus aureus* is generally regarded as a food borne pathogen causing spoilage and intoxication due to the production of heat stable enterotoxins [4].

Food preservation is a continuous fight which aims at either to eliminate or reduce the outgrowth potential of spoilage and pathogenic microorganisms in foods. Until now, approaches to seek improved food safety have relied on chemical preservatives, antibiotics or on the application of more drastic physical treatments (e.g. high temperatures or refrigeration). Nevertheless, these methods have many drawbacks [5]. Currently, there is a strong debate about the safety aspects of chemical preservatives due to impairment/reduction of the nutritional value of food, episodes of adverse food reactions, cardio-vascular disease, many carcinogenic and teratogenic attributes as well as residual toxicity [6]. Processing at high temperatures extensively damages the organoleptic, nutritional and physicochemical properties of food [7]. Refrigerators are either expensive to maintain or means for their maintenance (electricity) are lacking and this method of preservation makes the meat product prone to microbial and other sources of contamination [8]. Nowadays, for these reasons, especially as a consequence of consumer distrust of chemical preservatives, consumers pay a lot of attention to the relation between food and health [9].

Firstly, consumers demand high quality, additive-free, safe, healthy, nutritious, vitamin-rich, minimally processed, fresh tasting, lightly preserved and functional foods with extended shelf life and a natural or "green" image experiencing the trend of Western society of 'green' consumerism [10]. Secondly, legislation has restricted the use as well as the permitted levels of some of the currently approved preservatives in different foods. These consumer and legislative needs have encouraged the food industry to find innovative approaches of food preservation [11].

Recently, a novel scientific approach that has been widely employed in the food industry and gaining more and more attention is the bio preservation technology. By definition, this concept refers to the use of non pathogenic antagonistic microorganisms as protective cultures and / or their antibacterial metabolites (bacteriocins) to inhibit or destroy undesirable microorganisms in foods, improve microbiological safety and extend the shelf life of foods [12].

LAB have great potential for use in bio preservation because of their generally regarded as safe "GRAS" status. LAB are widely used in food industry as starter cultures, co-cultures, or bio protective cultures in wide range of fermented foods since ancient times without any safety risk [13]. In addition to the food applications of LAB, various strains are considered to be protective cultures. Functional protective cultures are microorganisms that possess at least one inherent functional property, which can contribute to improvement in sensory attributes in terms of consumer acceptance through production of flavor and aroma compounds, food safety through competing with food spoilage and pathogenic microflora and improving the nutritional qualities and thereby extension of shelf life of foods [14].

LAB with health-promoting properties, are called probiotic strains. Thus, LAB fermentation is not only of a major economic importance, but it also promotes human health [15]. *Lactobacillus acidophilus* is one of the most common species of LAB which have been used most commonly as probiotics and added as functional components to various food products, primarily because they are the predominant lactobacillus in the intestinal tract of healthy humans, preventive effect against antibiotic-associated diarrhea [16] and their therapeutic activities associated with an intestinal microbial balance [17].

Bacteriocins are cationic, hydrophobic or amphiphilic, ribosomally synthesized, extracellularly released, primary or modified bioactive peptides or peptide complexes secreted by Gram positive as well as Gram negative bacteria, with a bactericidal or bacteriostatic effect against other closely and non-closely related species. In all cases, the producer cell exhibits specific self immunity to the action of its own bacteriocin [18]. Nisin is the only one internationally accepted as a safe and natural food bio preservative in certain foods for several decades [19]. Nisin is a broad spectrum bacteriocin with bactericidal activity, even in very low concentrations, towards a wide range of Gram-positive bacteria, including *S. aureus* and *L. monocytogenes* [9], decreasing the risk for their transmission through the food chain. Nisin is extremely resistant to heat. It is soluble in dilute acids and stable to boiling in such solutions. It is not toxic, even at levels much higher than those used in foods.

Although a great deal of work has been done on the selection of bacteria exhibiting antimicrobial properties in liquid medium and the number of bacteriocins characterized is increasing every day, very few commercial applications have appeared in meat products. A major hurdle is that these products are not fermented and the selected LAB strain should not change their organoleptic and nutritional qualities. However, there have been very few conclusive attempts to control the spoilage microbiota with protective culture [20].

In Relation to Above, the Main Target of this Work Was to Evaluate the Following:

- The efficacy of nisin and *Lactobacillus acidophilus*, individually and in combination on sensory, chemical and bacteriological quality aspects, as well as, extending the shelf life and improving the microbial safety of fresh minced beef stored at 4°C.
- The efficacy of nisin and *Lactobacillus acidophilus* individually and in combination on improving the microbial safety of fresh minced beef inoculated with food borne pathogenic bacteria as *S. aureus* stored at 4°C.

MATERIALS AND METHODS

Preparation of Bacterial Strains

***Lactobacillus Acidophilus*:** *Lactobacillus acidophilus* was kindly supplied as a reference probiotic strain from Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. The strain was grown on De Man Rogosa Sharpe broth (MRS, Difco Labs. Detroit, MI, USA) for 24 hrs at 30°C. From this culture, dilutions up to 10^8 were plated on MRS agar to reach the recommended cell count of the probiotic in food (10^7 CFU/g) [21].

Staphylococcus Aureus: Staphylococcus aureus, obtained from Microbiology Department, Faculty of Veterinary Medicine, Benha University, Egypt, was cultured in sterile peptone water 0.1% (Merck, Germany) and was then incubated for 24 hrs at 37°C. From this culture, dilutions up to 10^{10} were plated on Baired parker agar to determine the cell concentration. The cell count was adjusted to 10^6 CFU/ml [22]. The effective dose of enterotoxin may be achieved when the population of *S. aureus* reaches a level of > 10^5 CFU/g [23]. *Staphylococcus aureus* was inoculated on decontaminated meat by pouring and swabbing over the meat surfaces [24]. Subsequently, the inoculated meats were kept for 20 min to allow attachment and absorption of bacteria, with the initial count made 2 hrs after inoculation (after 2 hrs = Zero day) [25].

Collection and Preparation of Samples: A grand total of 6 kg of fresh minced beef was collected from different butchers' shops in Tanta, Gharbia governorate, Egypt. The samples were taken and transferred directly to the laboratory using an ice box under complete aseptic conditions without undue delay. All the samples were immediately prepared, inoculated with *S. aureus* (10^6 CFU/g) [22] and then divided into four equal groups (1.5 Kg each). The control group was without any treatment, group I contained nisin, in the form of the commercial product Nisaplin, in an amount of 200 ppm (ig/g) [26]. Group II was inoculated with *Lactobacillus acidophilus* (10^7 CFU/g) [21], while group III contained nisin and *Lactobacillus acidophilus* in combination. Each sample was packed in polyethylene bag, labeled and stored at 4°C. Sensory (color, odor and texture), chemical (pH, TVN-B and TBA) and bacteriological (TCC, *Enterobacteriaceae* count, coliform count and *S. aureus* counts) analyses were conducted initially (after 2 hrs = Zero day) and every day (24 hrs) during storage.

Sensory Examination: It was carried out according to Pearson and Tauber [27].

Chemical Analysis: PH: Standard analytical methods [28] were used to determine pH.

TVB-N: Total volatile basic nitrogen (TVB-N) was determined in a distillatory system (UDK 130A), as described by Antonacopoulos and Vyncke [29].

TBA: Thiobarbituric acid (TBA, mg malonaldehyde/kg) assay was carried out according to the procedure of Schmedes and Holmer [30].

Bacteriological Analysis: Bacterial counts were made every 24 hrs using standard methods [31]. Ten (10) gm of the examined sample were thoroughly homogenized using 90 ml of sterile peptone water 0.1% (Merck, Germany) to obtain a 10^1 dilution from which, other decimal serial dilutions up to 10^7 were prepared. Appropriate media were used for enumeration and identification of microflora. Total colony count (TCC) was determined by plating appropriate dilutions on plate count agar (Difco, USA). Plates were incubated for 48 hrs at 30°C. Total

Enterobacteriaceae and coliform counts were determined using Violet Red Bile Glucose (VRBG) agar and Violet Red Bile (VRB) agar (Biokar, France), respectively. The plates were incubated for 24 hrs at 37°C. *Staphylococcus aureus* count was determined on Baird parker Agar (Merck, Germany). Plates were incubated at 37°C for 48 hrs. *Staphylococcus aureus* showed black and shiny colonies with narrow white margins surrounded by a clear halo zone extending into the opaque medium. After the period of incubation, the number of colonies was enumerated in each Petri dish.

Statistical Analysis: All experiments were conducted in triplicate on separate days and the arithmetic mean was reported as the final result. The means of bacterial population (CFU/ml) were converted to $\log_{10}$ CFU/ml. Statistical analysis was performed using SPSS statistical program for windows (Version 16) (SPSS Inc. Chicago, IL and USA). Standard deviation of mean was used to describe data. Fisher's range test was used to determine differences ($P<0.05$) between tested groups. P value<0.05 was considered as significant.

RESULTS AND DISCUSSION

In spite of the introduction of modern technologies and safety concepts (e.g. HACCP) and the wide range of robust available preservation techniques, the reported number of food borne illnesses and intoxications is in rise and food safety is still an increasingly important public health issue [32].

It is obvious from results obtained in Table (1) that the sensory attributes of different treated minced beef samples during cold storage (4°C) were improved by using groups I, II and III, compared to the control samples during the storage period. Generally, samples of groups I and III, respectively revealed the highest improvement of sensory attributes, while samples of group II demonstrated the lowest one. In addition, the shelf life of samples of groups I, III and II which reached 6, 5 and 3 days, respectively, as compared to that of control samples which reached 2 days only [Fig. 1]. Nearly similar results were obtained by Gelman *et al.* [33].

Spoilage characteristics develop in food as microorganisms digest the sugars, complex carbohydrates, proteins and fats of food producing undesirable effects in the food if the spoilage microorganisms grow to significant levels. Typically, the threshold level for observation of food spoilage by odor, taste, or sight is not reached until the spoilage microflora exceeds about 10^7 organisms/g of food [3]. A wide variety of microorganisms including staphylococci produce lipolytic enzymes that hydrolyze lipids, producing readily oxidizable substrates that have a rancid odor. Many spoilage bacteria produce proteolytic enzymes that hydrolyze proteins in foods leading to offensive odor [3]. Most groups of microorganisms can spoil food by growing on the surface. Refrigerated cured meats and cooked products can become slimy or sticky to the touch because of the growth of LAB. This particular spoilage defect is caused simply by the accumulation of very high numbers of microbial cells and not by any specific metabolic activity of the microbes. Similarly, color changes in food can occur because of the surface growth of microorganisms. Examples include the greening of meats, caused by LAB [3].

The pH values of minced beef samples ranged between 5.32±0.34 and 6.74±0.05, 5.60±0.25 and 7.01±0.09 and 5.55±0.25 and 6.94±0.09, for groups I, II and III, respectively, while its value in control sample was 5.82 at zero time of storage as shown in table (2). The values of pH increased in all treatments, especially in control at the end of storage period.

TVB-N content in control recorded 7.37±2.00 mg/100g sample while its content in groups I, II and III ranged from 6.87±2.22 to 19.52±0.58, 7.12±2.05 to 20.5±1.38 and 7.00±2.08 to 21.18±2.81 mg/100g sample, respectively, as shown in Table (2). During storage, control samples recorded a high value of TVB (20.35±0.17 mg/100g sample) in the 2nd day, while its value was low in all treatments.

Table (2) exhibits that TBA values ranged from 0.07± to 0.91±0.04, 0.12±0.09 to 0.93±0.06 and 0.10±0.03 to 0.95±0.08 mg malonaldhyde/kg sample in groups I, II and III, respectively and its value in control samples was 0.13±0.05 at the beginning of storage to 1.00±0.003 mg malonaldhyde/kg sample in the 2nd day of storage.

Similarly, the use of metabolites produced by *Lactobacillus* improved the sensory and biochemical quality criteria (pH, TVN & TBA) of frozen fish fillets [34], fish-based food products [33] and sardine [35].

pH value plays an important role for the microbiological growth affecting the shelf life of the products [17]. Increase in pH value was attributed to formation of volatile basic nitrogen components as affected by biochemical changes under low temperature [34] and to microbial load which may cause protein hydrolysis with the appearances of alkyl groups [36]. The decrease in pH in the inoculated samples was closely related to the production of lactic acid and organic acids by *L. acidophilus*.

Table 1: Sensory evaluation of untreated (control) and treated minced beef samples during cold storage at 4°C

	Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control	9	4	1	Spoiled	-	-	-	-
I	9	8	7	6	5	4	3	Spoiled
II	9	6	4	2	Spoiled	-	-	-
III	9	7	6	5	4	3	Spoiled	-

Score System for Sensory Evaluation [27]:

9: Excellent
8: Very very good
7: Very good
6: Good
5: Medium
4: Fair
3: Poor
2: Very poor
1: Very very poor

Table 2: Chemical evaluation of untreated (control) and treated minced beef samples during cold storage at 4°C

		Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day
pH	Control	5.82±0.09	6.63±0.10^{a}	6.91±0.11^{a}	***	***	***	***
	I		5.32±0.34^{b}	5.89±0.14^{a}	6.19±0.27^{b}	6.26±0.06	6.30±0.06	6.74±0.05
	II		5.60±0.25^{b}	6.30±0.10^{a}	7.01±0.09^{a}	***	***	***
	III		5.55±0.25^{b}	5.97±0.25^{a}	6.32±0.12^{ab}	6.46±0.05	6.94±0.09	***
TVN	Control	7.37±2.00	12.33±1.79^{a}	20.35±0.17^{a}	***	***	***	***
	I		6.87±2.22^{b}	11.53±0.82^{c}	14.17±1.61^{b}	16.49±2.17	17.90±2.22	19.52±0.58
	II		7.12±2.05^{b}	15.33±0.77^{b}	20.5±1.38^{a}	***	***	***
	III		7.00±2.08^{b}	12.23±0.28^{c}	15.16±1.93^{b}	17.50±0.93	21.18±2.81	***
TBA	Control	0.13±0.05	0.77±0.07^{a}	1.00±0.003^{a}	***	***	***	***
	I		0.07±0.02^{b}	0.21±0.05^{b}	0.30±0.02^{c}	0.45±0.14	0.53±0.12	0.91±0.04
	II		0.12±0.09^{b}	0.41±0.19^{b}	0.93±0.06^{a}	***	***	***
	III		0.10±0.03^{b}	0.37±0.12^{b}	0.52±0.09^{b}	0.71±0.09	0.95±0.08	

The values represent Mean ± SD of three experiments. a, b and c are significantly different ($P < 0.05$)
TVN = 20 mg / 100 gm raw minced beef [38]
TBA = 0.9 mg Melanoaldehyde / kg raw minced beef [38]

Table 3: Bacteriological evaluation of untreated (control) and treated minced beef samples during cold storage at 4°C

		Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day
TCC	Control	9.73±0.87	10.67±1.53^{a}	11.67±2.52^{a}	***	***	***	***
	I		7.00±1.00^{b}	8.00±1.00^{b}	9.00±2.00^{a}	9.67±1.53	10.33±0.58	11.73 ± 1.52
	II		9.00±1.00^{ab}	10.00±2.00^{ab}	11.00±3.46^{a}	***	***	***
	III		8.00±1.00^{b}	9.00±1.00^{ab}	9.33±1.15^{a}	10.00±2.00	11.00±1.00	***
EC	Control	7.14±0.63	7.33 ± 0.58^{a}	9.00±1.00^{a}	***	***	***	***
	I		5.00±0.00^{b}	6.67±1.15^{a}	7.00±1.00^{b}	7.67±0.58	8.00±0.00	8.69± 0.81
	II		7.00±1.00^{a}	8.33±3.21^{a}	9.67±1.15^{a}	***	***	***
	III		6.33±0.58^{a}	7.67±0.58^{a}	8.00±1.00^{a}	8.33±1.53	9.33±1.15	***
CC	Control	6.72±0.34	7.00±1.00^{a}	7.33±2.31^{a}	***	***	***	***
	I		4.33±0.58^{b}	5.33±0.58^{a}	5.67±0.58^{a}	6.00±1.00	6.33±0.58	7.32 ± 0.50
	II		6.00±1.00^{ab}	7.00±1.00^{a}	7.33±1.15^{a}	***	***	***
	III		5.67±1.15^{ab}	6.33±1.53^{a}	6.67±0.58^{a}	7.00±1.41	7.33±0.58	
	***							

TCC: Total Colony Count EC: Enterobacteriaceae count CC: Coliform Count
The values represent Mean ± SD of three experiments. a, b and c are significantly different ($P < 0.05$)

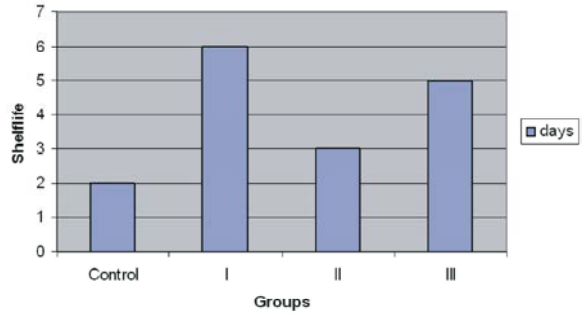

Fig. 1: Shelf life of untreated (control) and treated minced beef samples

TVB-N value was more useful for assessing the degree of meat deterioration than for evaluating the changes occurring during the first storage stages [37]. EOS [38] stated that 20 mg TVN/ 100 gm raw samples indicates the spoilage of minced meat. The increase in TVB-N is generally caused by the breakdown of proteins as a result of microbial activity under low temperature and autolytic and proteolytic enzymes. Such increases in TVB-N can be attributed to by the volatile basis production (NH_3, TMA, DMA and hypoxanthine) and non-volatiles (histamine) and those compensatory of free fatty acids resulting from lipids deterioration [34].

TBA is a good indicator of the quality of meat products. TBA value is a widely used indicator for the assessment of degree of lipid oxidation [39]. Limit of acceptance for TBA is proposed as 0.9 mg Melanoaldehyde / kg raw minced beef [38]. Concerning the increased TBA values for all the stored samples with advancing the chilling storage time; it could be due to lipid hydrolysis, oxidative rancidity and secondary products formation under low temperature [40].

Table (3) clears the effect of treatment in groups I, II and III on TCC for minced beef samples during storage at 4°C. Initial TCC for control samples at zero time was 9.73±0.87 log cfu/g and then sharply increased with advance storage to 11.67±2.52 log cfu/g after 2 days storage. In addition, all treatments had inhibitory activity, especially groups I and III, respectively.

The effect of treatments I, II and III on total *Enterobacteriaceae* counts for minced beef samples was shown in table (3). Initial counts of *Enterobacteriaceae* in control samples were 7.14±0.63 log cfu/g at zero time and then markedly increased with extend storage. *Enterobacteriaceae* was reduced in all treatments especially group I followed by group III.

Table (3) illustrates the effect of groups I, II and III on total coliform counts for minced beef samples during storage at 4°C. Initial coliform count for control minced beef samples at zero time was 6.72±0.34 and then highly increased with advance storage to reach 7.33±2.31 log cfu/g after 2 days of storage. In addition, groups I and III, respectively, throughout storage period had more inhibitory activity on total coliform counts than others. Group II was relatively less active.

The addition of nisin inhibits the microbial growth in fresh veal meat [41], meat products [42] and smoked salmon [43]. Similarly, the use of metabolites produced by *Lactobacillus* improved the microbial aspects (TCC, SAC and CC) and safety of frozen fish fillets [34], fish-based food products [33] and sardine [35].

The relatively high initial counts of control samples may be attributed to the grinding process, which compounds the problem by introducing the pathogens into the interior of the meat and contributes to the increase of total viable counts of meat [44].

The numbers of *Enterobacteriaceae* were higher in control samples than in treated samples. The growth of *Enterobacteriaceae* might have been reduced as a result of antimicrobial activity of the LAB and nisin. The reduction of coliform counts in treated minced beef samples could be due to the acidification by lactic acid and/or to some inhibitory compounds formed by LAB. The reduction of coliforms number might ensure good bio preservation against undesirable and/or hazardous microorganisms [35].

Bacteriocins produced by LAB, especially nisin, have the potential to cover a very broad field of application, including food industry as safe natural food preservatives [19]. Based on target organisms, nisin is used to prevent spoilage by Gram positive endospore formers (particularly in heat processed food) and LAB and to kill or inhibit Gram positive pathogens, as well as, Gram-negative bacteria, but only when used at high concentrations [45]. The direct inoculation of the crude extract of a bacteriocin will eliminate the inconvenience of the organoleptic changes [46].

It is well recognized that the antimicrobial effect of nisin is caused by membrane depolarization and permeability by pore formation in the cytoplasmic membranes of sensitive target species, in a torrid manner after electrostatic interactions with phospholipids negatively charged and binding to lipid II. This step is thought to be the driving force for subsequent events [47]. Nisin dissipates by increasing membrane permeability. Following nisin treatment, whole or intact sensitive cells and membrane vesicles exhibit efflux of ATP, amino acids and cations (mainly potassium and magnesium). Loss of these substances inactivates sulfhydryl groups and depletes proton motive force, which ultimately interferes with cellular biosynthesis of DNA, RNA, protein and polysaccharides, as well as, the production of energy. These events result in collapse of the membrane potential and ultimately cause cellular death and autolysin activation to digest the cellular wall [6].

L. plantarum and L. lactis have showed more bacteriocin producing ability compared to *L. acidophilus* [48]. Bacteriocins are not frequently active against Gram-negative bacteria. The lipopolysaccharide of the outer membrane of this class of bacteria acts as a permeability barrier for the cell. It is responsible for preventing molecules from reaching the cytoplasmic membrane [49].

In some instances, LAB may cause food spoilage since their enzymes may lead to generation of fermentation digests which are responsible for strong sensory degradation, leading to rejection of the products. The carbohydrates fermentation by heterofermentative LAB leads to sour and marinated off-odors and flavors due to organic acid production. Degradation of proteins by this genus may also conducts to bitter taste. Cysteine and arginine can be metabolized into H_2S and NH_3 respectively, responsible for sulphurous and ammonia off-odors [20].

Table 4: The effects of different preservatives on *Staphylococcus aureus* counts (log CFU/g) in artificially inoculated minced beef samples

	Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day
Control	8.54 ± 0.34	9.67 ± 0.58[a]	10.00 ± 1.00[a]	-	-	-	-
I		7.33 ± 0.58[b]	6.00 ± 2.00[b]	4.85 ± 1.38	ND	ND	ND
II		7.00 ± 2.00[b]	3.67 ± 0.58[b]	ND	ND	ND	ND
III		4.67 ± 0.58[c]	ND	ND	ND	ND	ND

ND: Not Detected

The values represent Mean ± SD of three experiments. a, b and c are significantly different ($P < 0.05$).

Table 5: Reduction log and % of *Staphylococcus aureus* artificially inoculated into minced beef samples treated with different preservatives

	1st day		2nd day		3rd day		4th day		5th day		6th day	
	Log	%	Log	%	Log	%	Log	%	Log	%	Log	%
I	1.21	14.17	2.54	29.74	3.69	43.21	8.54	100	8.54	100	8.54	100
II	1.54	18.03	4.87	57.03	8.54	100	8.54	100	8.54	100	8.54	100
III	3.87	45.32	8.54	100	8.54	100	8.54	100	8.54	100	8.54	100

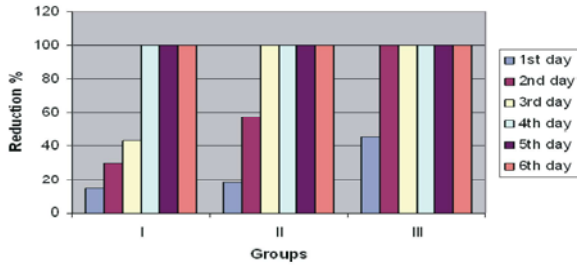

Fig. 2: Reduction % of Staphylococcus aureus artificially inoculated into minced beef samples

Table (4) showed *S. aureus* counts in minced beef samples treated with groups I, II and III. The initial count of *S. aureus* in minced beef samples after inoculation was 8.54±0.34 log CFU/g. *Staphylococcus aureus* counts in minced beef samples treated with groups I, II and III in the 1st and 2nd day of storage were significantly lower ($p < 0.05$). Moreover, the growth of *S. aureus* in minced beef samples was completely inhibited after treatment with groups I, II and III in the 4th, 3rd and 2nd day of storage, respectively. *Staphylococcus aureus* counts in treated minced beef samples declined from 8.54±0.34 to 7.33±0.58 log cfu/g (14.17%), 8.54±0.34 to 7.00±2.00 log cfu/g (18.03%) and 8.54±0.34 to 4.67±0.58 cfu/g (45.32%), when treated with groups I, II and III, respectively, in the 1st day of storage (Table 5 & Fig. 2). The results indicated that *S. aureus* was reduced in all treatments especially group III followed by group II and then group I.

Similarly, the combination of lactic acid cultures and their cell-free bacteriocins has induced a greater inhibitory effect on different spoilage and pathogenic microflora than the use of a single bacteriocin, on fresh meat [50] and many forms of meat products [12, 51, 52].

Even so, several authors reported that certain bacteriocin-producing LAB could be used as bio protective cultures and seemed to be more efficient than bacteriocin to prevent the growth of food borne pathogens such as *S. aureus* in sausage [53], beef burger [54] and many forms of meat products [55-57].

Results are in synchronization with those reported by Hampikyan [26], Cleveland *et al.* [58] and González-Martínez *et al*. [59] who stated that all of the variants of nisin inhibit food borne pathogens including *S. aureus*.

The inhibition of growth of *S. aureus* could be due to the antibacterial metabolites of LAB such as organic acids (which lead to rapid reduction of pH below 5.3), H_2O_2 (where *S. aureus* is 2 to 10 times more sensitive to H_2O_2 than most LAB), bacteriocins (which are more effective against Gram positive bacteria than Gram negative bacteria) and bacteriocin-like substances [60].

The safety of fermented product primarily depends on rapid decrease in pH, which must be achieved as quickly as possible in order to inhibit the growth of spoilage microorganisms in the final product [61].

In mammalian meat, the glycogen content is higher than in fish, leading to a post rigor acidification (pH<5.5) that prevents growth of the pH-sensitive bacteria and allows the implantation of LAB [20].

Bacteriocins (such as Class II acidocin CH5 produced by *L. acidophilus*) have generally a cationic character and easily interact with Gram-positive bacteria that have a high content of anionic lipids in the membrane determining the formation of pores [62].

There are, however, some common mechanisms of action that have been reported for the majority of LAB. LAB exert a strong antagonistic effect against food spoilage and pathogenic microorganisms by competitive

exclusion for essential nutrients or adhesion sites of mucous cells, immune modulation, redox modification, accumulation of D-amino-acids and production of extracellular and diffusible antimicrobial metabolites, such as organic acids (lactic, propionic, formic and acetic acids), diacetyl, hydrogen peroxide, carbon dioxide, antifungal compounds (fatty acids or phenyl lactic acid), lysozymes, enzymes (proteases, amylases and lipases) and bacteriocins, which play an essential role in natural preservation, enhancing the safety and extending the shelf life of food products [63].

Lactic acid, secreted by this probiotic as the primary metabolite of carbohydrate fermentation, lowers pH of the food and directly inhibits the growth of pathogenic bacteria. Furthermore, undissociated lactic acid, on account of its fat solubility, will diffuse into the bacterial cell membranes, thereby lowering the intracellular pH and interfering with metabolic processes such as oxidative phosphorylation [64]. Carbon dioxide interacts with cell membranes by reducing internal and external pH levels and plays a role in creating an anaerobic environment which inhibits enzymatic decarboxylations and the accumulation of CO_2 in the membrane lipid bilayer may cause a dysfunction in permeability. Diacetyl, an aroma and flavor component, is a product of citrate metabolism and acts by interacting with arginine-binding proteins [65]. Hydrogen peroxide is a powerful oxidizing antimicrobial agent which oxidizes sulfhydryl groups and destroys bacterial enzymatic activity destroys bacterial enzymatic activity and form the peroxidation of membrane lipids and cell proteins thus increasing membrane permeability. Hydrogen peroxide may also be a precursor for the production of bactericidal free radicals such as superoxide (O^{-2}) and hydroxyl (OH^-) radicals which can damage DNA [66]. Enzymes hydrolyze food complexes (polysaccharides, proteins, phytates and lipids) into simple non toxic products with desirable textures, aroma that makes them palatable for consumption [67].

The antibacterial effects of probiotic (LAB) against common food borne pathogens depend on the type of product used, species of pathogenic bacteria, interaction between the bacteria, its adaptation to a substrate, the acid production, the microorganism sensitivity, NaCl, redox potential (Eh), water activity (a_w), pH, storage temperature and the cell density of the protective culture [17].

Chi-Zhang *et al.* [68] reported that in the presence of an excess of nisin, *S. aureus* instantaneously developed resistance to the bacteriocin, whereas when nisin was added slowly to the cells, only a temporary tolerance was developed and these cells became nisin-sensitive. The antimicrobial effectiveness of nisin strongly depends on its mode of delivery. Resistance may result probably from alteration of bacterial membrane composition, destruction of the bacteriocin by proteases or altered receptors.

The effectiveness of nisin activity as preservative in food is negatively affected by: (1) its hydrophobic nature, although they are relatively small molecules and so they can easily diffuse into the water phase of food products, (2) its amphiphilic nature, it can be adsorbed to food macromolecules and undergo proteolytic degradation by proteases and other proteolytic enzymes released during cell lysis, especially in non heat-treated foods, the apparent higher proteolytic activity in homogenates can be explained by the proteases being intracellular and released during homogenization of the meat. In whole meat, there will be less contact between the nisin and the proteases. (3) the physicochemical characteristics of food such as nutrients, temperature and pH which affects the growth rate of target microorganisms and changes the degree of ionization (dissociation / association) of the most active chemicals and could change the antimicrobial activity, (4) leaching into the food and cross-reactions with food components such as lipids or proteins, (5) the poor solubility and lack of stability at the pH of raw meat pH (neutral and high pH values), (6) uneven distribution in the food matrix due to strong interaction with phospholipids, (7) sensitivity to food enzymes, (8) inactivation by formation of a nisin glutathione adduct, (9) nisin molecule number implicated in pore formation may be lower to kill cells, (10) development of highly tolerant and/or resistant strains to nisin, (11) inadequate environmental conditions for the biological activity and (12) their inhibition by salt and curing agents [69].

No single antimicrobial agent can cover all the requirements for food preservation [70]. The application of combined preservative factors (nisin and *L. acidophilus*) is significantly greater in controlling the growth of food spoilage and food borne pathogenic bacteria than that of a single bacteriocin used alone [71].

Similarly, lactic acid, secreted by *L. acidophilus*, lowers pH of the food. Nisin has an immediate pH-dependent bactericidal effect, which increases with decreasing pH values [72] resulting in a decreased adsorption of nisin molecules to the producer cells and hence in an increased bioavailability. This response could be attributed to acidic damaging effects on target cells concomitant with the higher stability and solubility of nisin and the increase in net positive charge of nisin [45].

In conclusion, the data obtained in this study suggest that using combination of both nisin and *L. acidophilus* was superior in improving the quality aspects, extending the shelf life and inhibiting the growth of *St. aureus* in minced beef.

Nowadays, bio preservation, by using combination of LAB bacteriocins (nisin) and bacteriocin-producing strains (*L. acidophilus),* can be considered as an integral part of hurdle technology in food industry. Therefore, a synergistic effect clearly plays a role in preventing growth of pathogenic and spoilage microorganisms, extending the inhibitory activity spectrum to such intrinsically resistant bacteria as Gram-negative bacteria through affecting outer-membrane (OM) permeability, improving the sensory, chemical and microbial qualities of food and ultimately, have a significant impact on food safety, shelf life extension and health requirements [15].

Continued research may lead to bacteriocins with increased stability and enhanced features, or extension of the antimicrobial spectrum to Gram-negative bacteria. The genomics will soon become an essential tool for exploring the antimicrobial potency of LAB and may yield characteristics that could be very rewarding from a food safety and an economic point of view, however, they can be counterproductive from the marketing point of view, since they will yield products- bacteriocins made by genetic engineering with all the associated consumer concerns [19].

REFERENCES

1. Kalalou, I., M. Faid and A.T. Ahami, 2004. Extending the shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus delbruekii* subsp. *delbruekii.* Electronic J. Biotechnology, 7: 246-251.
2. Olaoye, O.A. and I.G. Ntuen, 2011. Spoilage and preservation of meat: a general appraisal and potential of lactic acid bacteria as biological preservatives. Review Paper. Int. Res. J. Biotechnol., 2: 33-46.
3. Sperber, W.H., 2009. Introduction to the microbiological spoilage of foods and beverages. In: Sperber, W. H. and Doyle, M. P. (Eds). Compendium of the Microbiological Spoilage 1 of Foods and Beverages, Food Microbiology and Food Safety. DOI 10.1007/978-1-4419-0826-1_1, C_ Springer Science+Business Media, LLC.
4. Pu, S., F. Han and B. Ge, 2009. Isolation and characterization of Methicillin-resistant Staphylococcus aureus strains from Louisiana retail meats. J. Appl. and Environ. Microbiol., 75: 265-267.
5. Rasooli, I., 2007. Food preservation. A biopreservative approach. Food Global Science Books, 1: 111-136.
6. Calo-Mata, P., S. Arlindo, K. Boehme, T. Miguel, A. Pascoal and J. Barros-Velazquez, 2008. Current Applications and Future Trends of Lactic Acid Bacteria and their Bacteriocins for the Biopreservation of Aquatic Food Products. Food Bioprocess Technol., 1: 43-63.
7. Elez-Martinez, P. and O. Martin-Belloso, 2005. Food safety aspects of pulsed electric fields, p. 183-218. In: Emerging technologies for food processing. Ed. Sun, D.W. Academic Press, Boston.
8. Egbunike, G.N. and A.O. Okubanjo, 1999. Effects of processing upon the quality of Nigerian meat products. Livestock Production Science, 59: 155-163.
9. Parada, J.L., C.R. Caron, A.B.P. Medeiros and C.R. Soccol, 2007. Bacteriocins from lactic acid bacteria: purification, properties and use as biopreservatives. Int. J. Brazilian Archives of Biology and Technol., 50: 521-542.
10. Sarika, A.R., A.P. Lipton and M.S. Aishwarya, 2010. Bacteriocin production by a new isolate of *Lactobacillus rhamnosus* GP1 under different culture conditions. Advance J. Food Science and Technology, 2: 291-297.
11. Brul, S. and P. Coote, 1999. Preservative agents in foods - Mode of action and microbial resistance mechanisms. Int. J. Food Microbiol., 50: 1-17.
12. Dortu, C. and P. Thonart, 2009. Bacteriocins from lactic acid bacteria: interest for food products preservation. J. Biotechnol. Agron. Soc. Environ., 13: 143-154.
13. Vignolo, G., P. Castellano, C. Belfiore and S.A. Fadda, 2008. Review of bacteriocinogenic lactic acid bacteria used as bio-protective cultures in fresh meat produced in Argentina. J. Meat Sci., 79: 483-499.
14. El-Nezami, H.N. Polychronaki, S. Salminen and H. Mykkanen, 2002. Binding Rather Than Metabolism May Explain the Interaction of Two Food-Grade Lactobacillus Strains with Zearalenone and Its Derivative {alphaacute}-Zearalenol. J. Appl. Environ. Microbiol., 68: 3545-3549.

15. Chelule, P.K., M.P. Mokoena and N. Gqaleni, 2010. Advantages of traditional lactic acid bacteria fermentation of food in Africa. Current Research, Technology and Education Topics in Appl. Microbiol. and Microbial Biotechnol., pp: 1160-1167.
16. Beausoleil, M., N.A. Fortier, S. Guinette, A.L. Ecuyer, M. Savoie, K. Weiss, J. Lachaine and M. Franco, 2004. Effect of a Lactobacillus acidophilus and casei-fermented milk in the primary prevention of antibiotic associated diarrhea (lactic trial): a randomized, double-blind, placebocontrolled trial. In the Proceedings of the 69th Annual Scientific Meeting and Postgraduate Course, November 2004. Orlando, FJ: American College of Gastroenterology.
17. Hathout-Amal, S. and E. Aly-Soher, 2010. Role of lactic acid bacteria as a biopreservative agent of Talbina. J. American Sci., 6: 889-898.
18. Saavedra, L., C. Minahk, A.P. Holgado and F. Sesma, 2004. Enhancement of the enterocin CRL35 activity by a synthetic peptide derived from the NH2-terminal sequence. Antimicrob. Agents Chemother. 48: 2778-2781.
19. Papagianni, M. and S. Anastasiadou, 2009. Pediocins: The bacteriocins of Pediococci. Sources, production, properties and applications. Review. Microbial Cell Factories, 8: 1-16.
20. Françoisea, L., 2010. Occurrence and role of lactic acid bacteria in seafood products. J. Food Microbiol., 27(6): 698-709.
21. Reid, G., 2001. Probiotic agents to protect the urogenital tract against infection. Am. J. Clin. Nutrition, 73: 437-443.
22. Kantachote, D. and W. Charernjiratrakul, 2008. Selection of lactic acid bacteria from fermented plant beverages to use as inoculants for improving the quality of the finished product. Pakistan J. Biol. Sci., pp: 1-8.
23. Stewart, C.M., M.B. Cole and D.W. Schaffner, 2003. Managing the risk of staphylococcal food poisoning from cream- filled baked goods to meet a food safety objective. J. Food Prot., 66: 1310-25.
24. Dorsa, W.J., 1997. New and established carcass decontamination procedures commonly used in the beef-processing industry. J. Food Protec., 60: 1146-1151.
25. Dubal, Z.B., A.M. Paturkar, V.S. Waskar, R.J. Zende C. Latha, *et al.* 2004. Effect of food grade organic acids on inoculated *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* in sheep/goat meat stored at refrigeration temperature. J. Meat Sci., 66: 817-821.
26. Hampikyan, H., 2009. Efficacy of nisin against Staphylococcus aureus in experimentally contaminated sucuk, a Turkish-type fermented sausage. J. Food Protec., 72: 1739-1743.
27. Pearson, M.A. and W.F. Tauber, 1984. Processed meat. 2nd ed. AVI Pub. Com. Inc. West port, Connection. pp: 93.
28. AOAC, 2002. Official Methods of Analysis of the Association of Official Analytical Chemists 17th Edn. Vol. 1. The Association of Official Analytical Chemists, Inc. USA.
29. Antonacopoulos, N. and W. Vyncke 1989. Determination of volatile basic nitrogen in fish: a third collaborative study by the West European Fish Technologists Association (WEFTA) Z. Lebensmittel - Untersuchung Und - Forschung, 189: 309-16.
30. Schmedes, A. and G. Holmer, 1989. A new thiobarbituric acid (TBA) method for determination of free malonaldehyde (MDA) and hydroperexides selectivity as a measure of lipid peroxidation. American J. Oil Chem. Soc., 66: 813-7.
31. FDA, 2001. U.S. Food and Drug Administration: Bacteriological Analytical Manual Online.
32. WHO, 2002. Food safety and food borne illness. World Health Organization Fact sheet 237, revised January 2002. Geneva, pp: 1-4.
33. Gelman, A., V. Drabkin and L. Glatman, 2001. Evaluation of lactic acid bacteria, isolated from lightly preserved fish products, as starter cultures for new fish-based food products. Innovative Food Sci. Emerging Technol., 1: 219-26.
34. Ibrahim, S.M. and S.G. Desouky, 2009. Effect of Antimicrobial Metabolites Produced by Lactic Acid Bacteria (Lab) on Quality Aspects of Frozen Tilapia (Oreochromis niloticus) Fillets. World J. Fish and Marine Sciences, 1: 40-45.
35. Ndaw, A.D., M. Faid, A. Bouseta and A. Zinedine, 2008. Effect of Controlled Lactic Acid Bacteria Fermentation on the Microbiological and Chemical Quality of Moroccan Sardines (Sardina pilchardus). Int. J. Agri. Biol., 10: 21-27.
36. Yassin - Nessrien, M.N., 2003. Effect of storage conditions on the quality parameters of differently treated fish. Ph. D. thesis, Fac. Agric. Ain Shams University, Cairo. Egypt.
37. El-Marrakchi, A., M. Bennour, N. Bouchriti, A. Hamama and H. Tagafait, 1990. Sensory, Chemical and Microbiological assessment of Moroccan sardines (Sardina pilchardus) stored in ice. J. Food Protec., 53: 0-5.

38. EOS "Egyptian Organization for Standardization and Quality", 2005. Egyptian Standard, ES.
39. Raharjo, S. and J.N. Sofos, 1993. Methodology for measuring malonaldhyde as a product of lipid peroxidation in muscle tissues. J. Meat Sci. 35: 145- 169.
40. Forrest, J.C., E.D. Aberle, H.B. Hedrick, M.D. Judge and R.A. Merkel, 1975. Principles of Meat Science, WH Freeman, San Francisco, CA, pp: 240-248.
41. Guerra, N.P., C.L. Macias, A.T. Agrasar and L.P. Castro, 2005. Development of a bioactive packaging cellophane using Nisaplin as biopreservative agent. Lett. Appl. Microbiol., 40: 106-1610.
42. Deegan, L.H., P.D. Cotter, C. Hill and P. Ross, 2006. Bacteriocins: biological tools for bio-preservation and shelf-life extension. Int. Dairy J., 16: 1058-1071.
43. Neetoo, H., M. Ye, H. Chen, R.D. Joerger, D.T. Hicks and D.G. Hoover, 2008. Use of nisin-coated plastic films to control Listeria monocytogenes on vacuum-packaged cold-smoked salmon. Int. J. Food Microbiol., 122: 8-15.
44. Mead, P.S. and P.G. Griffin, 1998. Escherichia coli O157:H7. Lancet, Vol. 352, October 10.
45. Jeevaratnam, K., M. Jamuna and A.S. Bawa, 2005 Biological preservation of foods - Bacteriocins of lactic acid bacteria. Ind. J. Biotechnol., 4: 446-454.
46. De Vuyst, L., 1996. Production and applications of bacteriocins from lactic acid bacteria bioactive peptides for future food preservation. Cerevisia, 21: 71-74.
47. Bierbaum, G. and H.G. Sahl, 2009. Lantibiotics: mode of action, biosynthesis and bioengineering. Curr. Pharm. Biotechnol., 10: 2-18.
48. Jagadeeswari, S., P. Vidya, D.J. Mukesh Kumar and M.D. Balakumaran, 2010. Isolation and characterization of bacteriocin producing *Lactobacillus spp.* from traditional fermented foods. Electronic J. Environmental, Agricultural and Food Chemistry, 9: 575-581.
49. Gao, Y., M.J.V. Belkum and M. Stiles, 1999. The outer membrane of Gram negative bacteria inhibits antibacterial activity of Brochocin C. J. Appl. Environ. Microbiol., 65: 4329-4333.
50. Castellano, P. and G. Vignolo, 2006. Inhibition of Listeria innocua and Brochothrix thermosphacta in vacuum-packaged meat by addition of bacteriocinogenic Lactobacillus curvatus CRL705 and its bacteriocins. Lett. Appl. Microbiol., 43: 194-199.
51. Ananou, S., M. Maqueda, M. Martínez-Bueno and E. Valdivia, 2007. Biopreservation, an ecological approach to improve the safety and shelf-life of foods. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, pp: 475-486.
52. Jin, T., 2010. Incorporation of preservatives in polylactic acid films for inactivating Escherichia coli O_{157}:H_7 and extending microbiological shelf life of strawberry puree (dagger). J. Food Protec., 1: 1-10.
53. Lucke, F.K., 2000. Utilization of microbes to process and preserve meat. J. Meat Sci., 56: 105-115.
54. Mohsen, S.M., S. Hathout-Amal and E. El-Sideek-Lamyaa, 2009. Microbiological characteristics of processed beef burger treated with lactic acid bacteria. Egyptian J. Biotechnol., 33: 256-266.
55. Batdorj, B., V. Trinetta, M. Dalgalarrondo, H. Prevost, X. and Dousset, *et al.* 2007. Isolation, taxonomic identification and hydrogen peroxide production by Lactobacillus delbrueckii subsp. Lactis T31, isolated from Mongolian yoghurt: Inhibitory activity on foodborne pathogens. J. Appl. Microbiol., 1(3): 584-593.
56. Olaoye, O.A. and C.E.R. Dodd, 2010. Evaluation of bacteriocinogenic Pediococcus acidilactici as protective culture in the preservation of tsire, a traditional Nigerian stick meat. J. Food Safety, 30: 867-888.
57. Pilet, M. and F. Leroi, 2011. Applications of protective cultures, bacteriocins and bacteriophages in fresh seafood and seafood products. Woodhead Publishing Series in Food Science, Technology and Nutrition No. 201, ISBN-13: 978 1 84569 669 6.
58. Cleveland, J., T.J. Montville, I.F. Nes and M.L. Chikindas, 2001. Bacteriocins: Safe, natural antimicrobials for food preservation. Int. J. Food Microbiol., 71: 1-20.
59. González-Martínez, B.E., M. Gómez-Treviño and Z. Jiménez-Salas, 2003. Bacteriocinas de probióticos. Rev. Salud Pública y Nutrición, pp: 4.
60. Shah, N.P., 2007. Functional cultures and health benefits. Int. Dairy J., 17: 1262-1277.
61. Faid, M., A. Zouiten, A. El-Marrakchi and A. Achkari Begdouri, 1997. Biotransformation of Fish waste into a stable feed ingredient. J. Food Chem., 60: 13-8.
62. Chen, H. and D.G. Hoover, 2003. Bacteriocins and their Food Applications. Comprehensive Review in Food Science and Food Safety, 2: 81-100.

63. Yesillik, S., N. Yildirim, A. Dikici, A. Yidiz and S. Yesillik, 2010. Antibacterial effects of some fermented commercial and homemade dairy products and 0.9% lactic acid against selected food borne pathogens. Asian J. Anim. Vet. Adv., 6: 189-195.
64. Cardirci, B. and Citak, 2005. A comparison of two methods used for measuring antagonistic activity of lactic acid bacteria. Pakistan J. Nutrition, 4: 237-241.
65. De Vuyst, L. and E.J. Vandamme, 1994. Bacteriocins of lactic acid bacteria. London, England: Blackie Academie & Professional.
66. Zalam, Z.E., E. Nemeth, A. Barath and A. Halasz, 2005. Influence of growth medium on hydrogen peroxide and bacteriocin production of Lactobacillus strains. Food Technol. Biotechnol., 43: 21-9-225.
67. Steinkraus, K.H., 1998. Bio-enrichment: production of vitamins in fermented foods. Thomson Science, City.
68. Chi-Zhang, Y., K.L. Yam and M.L. Chikindas, 2004. Effective control of Listeria monocytogenes by combination of nisin formulated and slowly released into a broth system. Int. J. Food Microbiol., 90: 15-22.
69. Jin, T., L.S. Liu, H. Zhang and K. Hicks, 2009. Antimicrobial activity of nisin incorporated in pectin and polylactic acid composite films against Listeria monocytogenes. Int. J . Food Sci. Technol., 44: 322-329.
70. Pérez-Pérez, C., C. Regalado-González, C.A. Rodríguez-Rodríguez, J.R. Barbosa-Rodríguez and F. Villaseñor-Ortega, 2006. Incorporation of antimicrobial agents in food packaging films and coating. Advances in Agricultural and Food Biotechnol., 193-216.
71. Olasupo, N.A., D.J. Fitzgerald, A. Narbad and M.J. Gasson, 2004. Inhibition of Bacillus subtilis and Listeria innocua by nisin in combination with some naturally occurring organic compounds. J. Food Protec., 67: 596-600.
72. Bouttefroy, A., M. Mansour, M. Linder and J. Milliere, 2000. Inhibitory combinations of nisin, sodium chloride, and pH on Listeria monocytogenes ATCC 15313 in broth by an experimental design approach. Int. J. Food Microbiol., 54:109-115.