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Bio-Preservation Challenge for Shelf-Life and Safety Improvement of Minced Beef

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Abstract: Bio preservation systems are of increasing interest for food industry and consumers. Bacteriocinogenic lactic acid bacteria and/or their isolated bacteriocins are considered safe additives (GRAS), useful to control the frequent development of pathogens and spoilage microorganisms in foods. The role of lactic acid bacteria (*L. acidophilus*) as a trail (I) compared to a probiotic bacteriocin (nisin) as a trail (II) individually and in combination (combined tail, trail III) as bio-preservative agents on shelf-life and safety of *L. monocytogenes* injected in minced beef samples was studied during cold storage (4°C). The results showed that pH, total volatile basic nitrogen (TVB-N) and thiobarbituric acid values were markedly decreased in all treated samples as compared to untreated (control) sample. On the other hand, there was a good antibacterial activity of the investigated trails on total aerobic (TAC), Enterobacteriaceae (TEC) and total coliform (TCC) counts but the activity decreased through out the storage time. Consequently, results indicated that probiotic bacteria and bacteriocin had a positive effect on the shelf-life of treated samples compared to untreated ones by the manner of trail (I), combined trail and lastly trail (II). The samples treated with nisin (trail I) showed the best results as long shelf-life (6 days) compared to those of other trails. It could also be observed that the potential of probiotics to inhibit growth of common foodborne *L. monocytogenes* was different as combined trail (18.2, 29.1,40, 47.3 and 61.8%), trail (I) (12.7, 23.6, 32.7, 43.6, 4.8 and 50.9%), trail (II) (14.5, 20, 25.5 and 38.2%) and control samples (zero%) where, combined trail samples that were treated with both nisin and *L. acidophilus* culture was the superior one. In conclusion, no single probiotic agent could cover all the requirements for food preservation and safety. It is often practical, however, to use a combination of bio preservatives and preservative factors to achieve this goal.

Key words: Probiotic · Lactic acid bacteria · Nisin · Shelf-life · Safety-meat

INTRODUCTION

Meat is a nutritious protein-rich food which is highly perishable and has a short shelf-life unless preservation methods are used [1]. However, it gets easily contaminated by pathogenic microorganisms present in animal prior to slaughter. It is therefore important to make meat safe for consumers in terms of stability, transportation and storage. Shelf-life and maintenance of meat quality are influenced by a number of interrelated factors including holding temperature, which can result in determinable changes in the quality attributes of meat. Spoilage by microbial growth is the most important factor in relation to keeping quality of meat [2]. Moreover, spoilage of meat has remained a serious challenge in developing countries, including Egypt for decades, this has been due to poor storage systems in such countries where necessary facilities that could help to promote preservation are unavailable. Wherever, in most developing countries fresh meat forms a significant proportion of meat intake [1]. It is either eaten cooked or processed into other forms to avoid associated spoilage [3]. To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovation technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and / or their bacteriocins. Until now, approaches to seek improved food safety have relied on the search for more efficient chemical preservatives or on the application of more drastic physical treatments (high temperatures). Nevertheless, these types of solutions

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have many drawbacks, the proven toxicity of many of the commonest chemical preservatives (nitrites), the alteration of the organoleptic and nutritional properties of foods and especially recent consumer trends in purchasing and consumption, with demands for safe but minimally processed products without additives [4].

Bio-preservation has gained increasing attention as natural means for controlling the shelf-life and safety of food products. The application of bio protective cultures to ensure the hygienic quality is a promising tool although, it should be considered only as an additional measure to good manufacturing, processing, storage and distribution pracortices [5]. LAB have been shown a major potential for use in bio-preservation because of safety for human consumption (GRAS status) and the prevalent microflora during storage in many foods [6]. LAB can produce a wide range of antimicrobial metabolites such as organic acids, diacetyl, acetion, hydrogen peroxide and bacteriocin. These antimicrobial activities can contribute in the microbiological safety by controlling the growth of other microorganisms and inhibition of pathogenic bacteria such as *L. monocytogenes* [7]. The nutritious and therapeutic benefits of probiotic microorganisms have been most extensively investigated in dairy products (yogurt and cheese) [8, 9]. Probiotics have been also incorporated in edible spreads [10], meat [11], ras cheese [12] beef burger [13] and sausages, fish, cereals (bread and beer), fruits (wine) and vegetables [3]. LAB have been exploited for thousands of years for the production of fermented foods due to their ability to produce desirable changes in the taste, flavor and texture as well as inhibit pathogenic and spoilage microorganisms. LAB are a group of Gram-positive bacteria widely distributed in nature [14] and include the genera lactococcus, streptococcus, lactobacillus, pediococcus, leuconostoc, enterococcus, carnobacterium, Aerococcus, oenococcus,tetragenococcus, vagococcus and weisella [15].

Bacteriocins, the antimicrobial substances of LAB have gained tremendous attention as potential bio preservatives. Bacteriocins are ribosomal synthesized, extracellular released bioactive peptides or peptide complexes, having a bactericidal or bacteriostatic activity [16]. They differ from most therapeutic antibiotics in being in proteinaceous agents that can not potentially illict allergic reactions in humans an other medical problems [17].

Attempts have been made to improve safety and to delay spoilage by use of antibacterial sprays or dips [18]. Food application of bacteriocins can provide a good alternative means in protecting food against foodborne pathogens. As products of LAB, they provide natural means of preservation and can be accepted by consumers, in the way nisin became accepted. As the trend of consumption of minimal processed and preserved food is increasing, use of bacteriocins by the food industry could offer solutions and provide alternatives of conventional preservation means [19]. Undoubtedly the most extensively studied bacteriocin is nisin, which has gained widespread applications in the food industry. This FDA-approved bacteriocin is produced by the GARS microorganism *Lactococcus lactis* and used as a food additive at least in 48countries, particularly in processed cheese, dairy products and canned foods. It is extremely resistant to heat, soluble in dilute acids and stable to boiling in such solution. It exhibits antimicrobial activity towards a wide range of Gram-positive vegetative bacteria [20].

In recent years, *L. monocytogenes* an emerging pathogen has caused severe illness from food investigation and therefore, drawn the attention of several investigators to focus their studies on the anti-listerial activity of bacteriocins from lactobacilli [21].

The increasing demand for high quality safe processed food has created a niche for natural food preservatives. The ideal natural food preservatives should fulfill a number of criteria such as acceptable low toxicity, stability to processing and storage, efficacy at low concentration, while most bacteriocins fulfill these criteria to date, nisin is the only one commercially scale as a food preservative dating back to the first half of last century [22].

Microbiological, biochemical and sensory methods have been used to assess freshness and quality during handling and storage with the main attributes of freshness being aroma, texture and appearance response [23]. Biochemical methods based on nucleotide metabolism and total volatile basic nitrogen (TVB-N) have also been commonly used to assess the quality [24]. The development of new meat products which are stable during storage, free of the undesirable odor and taste and retaining all the nutritional advantages, would expand the range of applications of health-giving foods. A promising approach to the creation of such meat products seems to be through the use of LAB. These bacteria have been used for changing the aromatic and textural properties of food and for extending the shelf-life of various products [25].

Therefore, the main target of this work was to investigate the effect of antibacterial metabolites (nisin) and *L. acidophilus* individually and in combination on the quality and safety aspects of minced meat stored at 4 °C.

MATERIALS AND METHODS

Materials: Six kilograms of fresh minced beef were purchased from the local markets in Tanta, Egypt and transported in an ice box as rapidly as possible to the laboratory.

Cultures: *Lactobacillus acidophilus* (*L. acidophilus*) as probiotic strain was kindly supplied from Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. De Man Regosa Sharpe (MRS) broth (Difco, USA) was used for propagation of the LAB. For each culture, MRS broth was inoculated at 1% using a freshly prepared culture of the desired strain of *L. acidophilus* and incubated at 30°C for 24hrs. The cell count was adjusted at the recommended concentration of probiotic in food □107CUF/g [26].

Listeria monocytogenes (*L. monocytogenes*) was obtained as foodborne pathogen from the Microbiology Department, Faculty of Veterinary Medicine, Benha University, Egypt. The selected strain was grown on Listeria UVM broth for 24hrs at 30 °C. From this culture, dilutions up to 10^3 were plated on Listeria PALCAM agar plate at 30 °C for 24hrs to determine the cell concentration. The amount used to inoculate was approximately around 10^2 to105 CUF/g [27].

Preparation of Nisin: Nisaplin® (2.5% nisin) was standardized to an activity of one million international units per gram (IU/g). The used solution was prepared containing 200ìg/g for inoculation [28].

Preparation of Minced Meat: Minced beef samples were inoculated by the activated selected culture of *L. monocytogenes* (104CUF/g) and divided into four equal portions/groups (500 g each). The initial count was made 2hrs after inoculation, the others were treated with nisin (200 ìg/g) (trial I), *L. acidophilus* (107CUF/g) (trail II) and both of nisin and *L. acidophillus* (combined trail), respectively. Uninoculated meat sample served as control (untreated). Each treatment was packed in a polyethylene bag, stored at 4°C and then examined sensory, physicochemically and microbiologically at predetermined interval (24hrs) through:

Sensory Analyses: It was carried out according to Pearson and Tauber [29]

Physicochemical Analyses: pH value was obtained according to AOAC [30] procedures. Total volatile nitrogen (TVB-N mg/100g) was determined by Conway dish as described by FAO [33] and Thiobarbituric acid (TBA, mg malonaldehyde /Kg) assay, as index for lipid oxidation, was carried out according to the procedure of Vyncke [31]. Microbiological analyses: Twenty five grams of the examined sample were thoroughly homogenized using 225 ml of saline water (NaCl, 0.85% w/v), then serial decimal dilutions up to 107 were prepared. Appropriate media were used for enumeration of microflora. Standard plate count (SPC) was determined by plating appropriate dilution on Plate Count Agar (PCA, Difco, USA). Plates were incubated for 48hrs at 30°C [32]. Total Enterobacteriaceae and coliform counts were assayed in the sample incubated on violet red bile glucose agar (VRBGA, Oxoid) and violet red bile agar (VRBA, Oxoid) for 24hrs at 37°C [33]. *L. monocytogenes* count was determined by homogenizing 25 g of the sample with 225 ml of Listeria UVM as enrichment broth and then 0.1 ml of the homogenate was transferred to 10 ml Listeria UVM2 broth and incubated at 30°C for 24hrs [34]. A loopful of culture was streaked on Listeria PALCAM agar plate media supplemented with Listeria PALCAM supplement, then incubated at 30°C for 24hrs. Bluish grey or black colonies with a black halo and sunken center were enumerated and recorded [35]. The suspected colonies were picked up and streaked into trypticase soy agar plates (Biolife) supplemented with 0.6% yeast extract (Oxoid) (TSAYE) for further identification [36].

Microbiological data were transformed into logarithms to assess the colony forming units (cuf/g). All experiments were conducted in triplicate.

Statistical Analysis: Statistical analysis was performed using SPSS program (Version 16). Standard deviation of mean was used to describe data. Fisher's range test was used to determine differences between tested groups. P value < 0.05 and 0.001 were considered as significant and highly significant, respectively.

RESULTS AND DISCUSSION

The use of LAB as biological preservatives on meat products could confer health benefits to the consumers [37]. Therefore, LAB cultures act as probiotic which are non-pathogenic microorganisms when ingested in certain numbers exerting a positive influence on host physiology and health beyond inherent general nutrition [3].

Organoleptic profile not only determine what we eat, but often allows us to evaluate the quality of food and in some cases, identify unwanted contaminants [38]. Sensory evaluation of minced beef along the time of storage was presented in table (1) and indicated that

Table 1: Sensory evaluation of treated trails of minced beef samples infected with *L. monocytogenes* during cold storage at 4°C

	Time (days)							
Samples	Zero day	1st day	2nd day	3rd day	4th day	5th day	6thday	7th day
Control	Excellent	Very good	Medium	Fair	Very very poor (spoiled)	-	-	-
Trail (I)	Excellent	Very-very good	Very good	Very good	Good	Medium	Fair	Poor (spoiled)
Trail (II)	Excellent	Very good	Good	Medium	Fair	Very poor (spoiled)	-	-
Combined trails (III)	Excellent	Very good	Very good	Good	Medium	Fair	Poor (spoiled)	-

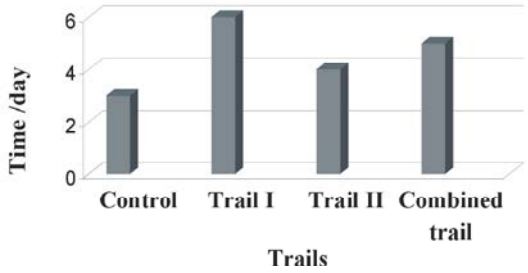

Fig. 1: Shelf life of untreated and treated trails of minced beef samples during cold storage (4°C)

sensory characteristics of minced beef were enhanced by different treatments. The sensory changes were attributed to proteolysis and lipid oxidation in untreated samples (control) that were more obvious in shorter time than those in treatments due to progressive growth of microbial load including *L. monocytogenes*. Properly treatments were proven to be highly effective in delaying and reducing sensory problems in minced beef and extended the shelf-life by the way of trail I, combined trail, trail II and control samples, respectively (Fig. 1). Spoilage characteristics develop in food if the spoilage microorganisms grow to significant levels, typically,the threshold level for observation of spoiled food by odor, taste or sight which is not reached until the spoilage microflora exceeds about 107 organisms/g of food. Therefore, refrigerated meat can become slimy or sticky to the touch because of the growth of LAB; this particular spoilage defect is caused simply by accumulation of high numbers of microbial cells and not by specific metabolic activity of the microbes, while color changes in food can occur because of the surface growth of microorganisms including the greening of meat by LAB [39]. Results are in synchronization with those reported by Leroi [40] who recorded that LAB have no particular negative effect, but in certain cases they are responsible for strong sensory degradation, leading to rejection of the product. On the other hand, Pilet and Leroi [41] reported that LAB do not change the organoleptic characteristics of the products and their use as protective culture could offer an alternative to the use of chemical compounds. Also, they added that the activity of protective culture or bacteriocin is thus directed on increase of sensory shelf-life, or the

Shelf-life means that samples are without any unfavorable changes in color, odor, appearance and no microbial growth observed. A number of interrelated factors influence the shelf-life and keeping quality of meat, specifically holding temperature, atmospheric oxygen, indigenous enzymes, moisture, light and most importantly microorganisms. All of these factors, either alone or in combination can reflect determinable changes in the color, odor, texture and flavor of meat [3]. Moreover, Paulsen and Smulders [42] stated that spoilage is said to be a state of a particular food in which it is offensive to consumer □s senses, usually caused by metabolites of contaminant microorganisms. Wherever, meat spoilage is not always evident and consumers would agree that gross discoloration, strong off-odors and development of slime would constitute the main qualititative criteria for meat rejection. To avoid the associated spoilage, the potential of lactic acid bacteria and bacteriocins as biological preservatives could be exploited in complementing the existing traditional preservation techniques.

Regarding the results recorded in table (2); all groups started with the same initial pH of 5.91± 0.09 and after one day it dropped to 5.61±0.4, 5.4±0.46 and 5.5±0.25 in trail I, II and combined trails samples, respectively ($P < 0.05$). However, at third day of storage, the control (untreated) samples had a higher pH value (6.79±0.37) than the other sets (6.04±0.14, 6.35±0.24 and 5.9±0.25 (Revise with table 2), respectively) ($P< 0.001$). This might be due to the activation effect of microbial load which may cause protein hydrolysis with the appearance of alkyl groups [43] including formation of volatile basic nitrogen components affected by biochemical changes under low temperature [44]. It is necessary to know that production of lactic and organic acids by LAB had an effect on lowering pH values as reported by Kuipers *et al*. [45] and Shah [46]. Therefore, pH plays an important role for

Table 2: Statistical analyses of physicochemical results of treated trails of minced beef samples infected with *L. monocytogenes* during cold storage at 4 °C

		Time (days)						
Parameter	Samples	Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day
pH	Control	5.91± 0.09	6.03±0.02*	6.35±0.14*	6.79±0.37**			
	Trail (I)		5.61±0.4	5.81±0.32	6.04±0.14	6.21±0.1**	6.33+0.21	6.57±0.51
	Trail (II)		5.5±0.46	5.82±0.30	6.35±0.24	6.73±0.7		
	CombinedTrails		5.4±0.25	5.6±0.30	5.9±0.25	6.3±0.40	6.67±0.33	
TVB-N	Control	7.3± 1.8	14.7±1.7**	18.4+2.5**	20.1+2.2*			
	Trail (I)		7.9±1.6	11.5±0.81	14.4±1.6	16.5±1.9*	17.6±1.6*	18.76±2.4
	Trail (II)		9.7±1.8	13.7±1.9	16.7±1.2	19.8±0.55		
	CombinedTrails		9.8±2.2	12.2±3.9	14.3±2.6	17.4±2.4	19.5±4.3	
TBA	Control	0.12± 0.5	0.39±0.13**	0.78±0.03*	1.07±0.15**			
	Trail (I)		0.14±0.08	0.19±0.05	0.28±0.1	0.36+0.13**	0.48±0.16**	0.76±0.24
	Trail (II)		0.25±0.05	0.39±0.11	0.62±0.17	0.92±0.11		
	CombinedTrails		0.11±0.05	0.23±0.10	0.35±0.16	0.59±0.20	0.82±0.10	

-Control: untreated samples.

-Trail (I): treated samples with nisin.

-Trail (II): treated samples with *L. acidophilus* culture.

-Combined Trails (III): treated samples with both nisin and *L. acidophilus* culture.

-*: significant at P< 0.05.

-**: highly significant at P< 0.001.

microbiological growth affecting the shelf-life of the meat products [47]. These results are in contrast with those illustrated by Kuipers *et al.* [45] and Callewaert and De-Vuyst [48].

Total volatile basic nitrogen (TVB-N) measurement is the traditional chemical mean most widely used for evaluation of the degree of meat spoilage. TVB-N content in control samples revealed a highest level (20.1±2.2 mg / 100g), while its content was lower in all treatments and ranged 14.4±1.6, 16.7±1.2 and 14.3±2.6 mg / 100g, respectively at third day of storage (P □ 0.05). In addition during progresse of storage time, the value of TVB-N increased significantly in all treatments (18.76±24, 19.8±0.55 and 19.5±4.3 mg/100g, respectively) to reach the maximum levels at 6th, 4th and 5th day for trail I, trail II and combined trails, respectively (P □ 0.001) (Table 2). This increase in TVB-N might be due to microbial activity under low temperature[44]. On the other hand, Shenouda [49] described the increase in TVB-N is generally caused by autolytic enzymes and desamination and is not related to microbiological activity. Such increase in TVB-N could be explained easily by volatile basis production and non-volatile (histamine) and those compensatory of free fatty acids resulting from lipids deterioration. Moreover, El-Marrakchi *et al.* [50] reported that the TVB-N value is more useful for assessing the degree of deterioration than for evaluating the changes occurring during the first stages. Wherever, EOSQC [51] recorded that 20 mgTVB-N/100g raw samples indicates the spoilage of minced meat. Ndaw *et al*. [25] stated that TBA is a good indicator for the assessment of quality of meat and degree of lipid oxidation. Also, table (2) proved that thiobarbituric acid (TBA) value of initial records was 0.12± 0.5 mg MDA /kg, where values increase significantly with increase the storage period and reach the maximum levels 1.07±0.15, 0.76±0.24, 0.92±0.11 and 0.82±0.10 mg MDA /Kg at third day for control, 6th day for trial I, 4th day for trail II and 5th day for combined trail samples, respectively (P □ 0.001). The increased in TBA values may be due to lipid hydrolysis and secondary products formation under low temperature [52]. Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and poly unsaturated fatty acids. Radical are known to take part in lipid peroxidation, which cause food deterioration, aging organisms and cancer promotion [53]. It has been proposed that a maximum TBA value indicating the good quality of minced meat is 0.9mg MDA /Kg [51] and the rancid flavor is initially detected between TBA values of 0.5 and 2.0 mg MDA/Kg.

A correlation between sensory evaluation and chemical parameters (pH, TVB-N and TBA) were observed in relation to the treatment trails. Therefore, LAB in fresh meat bring about a mild fermentation process without

Table 3: Statistical analyses of microbiological results of treated samples of minced beef samples infected with *L. monocytogenes* during cold storage at 4 °C

Couns Log CUF/g	Samples	Time (days) Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day
TAC	Control	8.5±0.92	9.7±0.7**	11.1±0.47*	12.1±0.97*	-	-	-
	Trail (I)		7.4±0.90	8.1±0.23	8.3±1.2	9.4±1.2*	10.5±1.3*	11.3±1.1
	Trail (II)		9.1+0.24	10.3±0.99	11.1±1.2	12.0±0.34	-	-
	Combined Trails			8.1±0.9	9.5±0.45	9.9±1.3	10.3±1.1	11.9±1.6
TEC	Control	6.1±0.69	7.9±1.1*	9.5.1±0.85*	10.4±0.92**	-	-	-
	Trail (I)		5.1±0.02	6.02±0.05	6.7±0.5	7.4±0.7**	8.1±0.6**	8.4±1.1
	Trail (II)		7.0+0.72	8.1±0.23	9.4±0.26	10.2±1.1	-	-
	Combined Trails			6.4±0.21	7.4±0.60	8.01±.30	8.3±1.6	9.1±0.31
TCC	Control	5.4±0.28	6.8±0.28**	7.7±0.14**	8.6±0.15**	-	-	-
	Trail (I)		4.3+0.29	5..2±0.29	5.6±0.57	6.1±0.5**	6.8±0.26**	7.8±0.60
	Trail (II)		4.4±0.8	6.2±0.4	7.9±0.2	9.1±1.02	-	-
	Combined Trails		5.6±0.6	6.2±0.4	6.8±0.6	7.1±0.3	7.5±0.8	-

Control: untreated samples.-Trail (I): treated samples with nisin.

-Trail (II): treated samples with *L. acidophilus* culture.

-Combined Trail (III): treated samples with both nisin and *L. acidophilus* culture.

-*: significant at P< 0.05.-**: highly significant at P< 0.001.

-TAC: Total aerobic bacterial count.-TEC: Total Enterobactriaceae count.

-TCC: Total coliform count

producing any changes in the sensory characteristics because of low carbohydrate content and the strong buffering capacity of meat. In the same way the growth of LAB in naturally fermented meats after addition of sugar. So, subsequent decrease in pH denaturizes the meat proteins favoring the decrease of water activity which ends up in a microbial stabilization of the transformed product [54]. Similar results were mentioned by Ndaw *et al.* [25] and Ibrahim and Desouky [44].

Results illustrated in table (3) for different microbial groups during storage period revealed that the high initial bacterial counts of total aerobes (TAC), Enterobacteriaceae (TEC) and coliform (TCC) were 8.5± 0.92, 6.1 ± 0.69 and 5.4 ± 0.28, respectively for control group. The relatively high initial counts of samples may be attributed to the grinding process, which increased the problem by introducing the pathogens into the interior of the meat and contributed to the overall keeping quality of the meat product [55]. As shown for the control samples, the previous microbial groups grew and reached high count levels at 3rd day of storage (12.1±0.97, 10.4±0.92 and 8.6±0.15 log CUF/g, respectively). TAC, TEC and TCC were significantly different (P □ 0.05 and 0.001) and decreased in all treatments and reached 8.3±1.2, 6.7±0.5 and 5.6+0.57 log.CUF/g for trail (I), 11.1±1.2, 9.4±0.26 and 7.9±0.2 for trail (II) and 9.9±1.3, 8.01±0.30 and 6.8±0.6 log.CUF/g for combined trail compared to the untreated samples, respectively, then, growth was progressively increased at the end of storage time. Similarly, the activity of protective culture or bacteriocin was thus directed to the increase of sensory shelf-life, or the inhibition of common microbial indicators such as total viable count [40]. In the same time, the reduction in indicator microorganisms such as Enterobacteriaceae and coliforms in the treatments could be due to acidification and / or to some inhibitory compounds formed by LAB [56] and nisin. This reduction might ensure good bio preservation against undesirable and /or hazardous microorganisms [25]. In addition, the combined treatment was much more effective against coliform bacteria [44]. Therefore, preservation of fermented products obviously depends on lactic acid and possibly bacteriocin production. However, other factors might also contribute to the over all keeping fermented products quality [57].

These results are similar to those investigated by Amal and Soher [47], Callewaert and De Vuyst[48], Faid *et al.* [58] and Kantachote and Charenjiratrakul [59].

Recently, there has been significant interest in the development of secondary preservation steps that could reduce *L. monocytogenes* viability and growth in refrigerated ready to eat foods [60]. The effect of probiotic trails on growth of *L. monocytogenes* in minced beef

Table 4: Statistical analyses of *L. monocytogenes* counts in treated trails of minced beef samples infected with *L. monocytogenes* during cold storage at 4 °C

		Time (days)						
	Counts							
Samples	logCUF/g	Zero day	1st day **	2nd day**	3rd day**	4th day**	5th day**	6th day
Control								
(untreated)	*L. monocytogenes*	5.5±0.34	6.4±0.39	7.6±0.50	8.8±0.47	-	-	-
	Reduction %	ND	ND	ND	ND	-	-	-
Trail (I)	*L. monocytogenes*	5.5±0.34	4.8+0.33	4.2±0.51	3.7±0.84	3.1±0.46	2.7±0.19	3.41+1.61
	Reduction %	ND	12.7%	23.6%	32.7%	43.6%	44.8%	50.9%
Trail (II)	*L. monocytogenes*	5.5±0.34	4.7±0.55	4.4+1.6	4.1+1.8	3.4±3.2	-	-
	Reduction %	ND	14.5%	20%	25.5%	38. 2%	-	-
Combined								
Trails (III)	*L. monocytogenes*	5.5±0.34	4.5+0.5	3.9±1.2	3.3+0.18	2.9±0.21	2.1±0.16	-
	Reduction %	ND	18.2%	29.1%	40%	47.3%	61.8%	-

Control: untreated samples.-Trail (I): treated samples with nisin.

-Trail (II): treated samples with *L. acidophilus* culture.

-Combined Trail (III): treated samples with both nisin and *L. acidophilus* culture.

--**: highly significant at P< 0.001

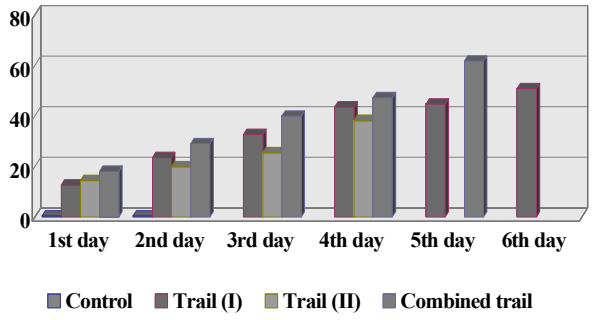

Fig. 2: Reduction% of L.monocytogenes in untreated and treated trails of minced beef samples during cold storage 4°C.

samples as shown in table (4) revealed that the initial load of *L. monocytogenes* was 5.5±0.34 log.CUF/ g at zero time that was markedly increased in untreated samples with extended cooled storage to reach 8.8±0.47 log CUF/g at 3rd day storage. The pathogen was reduced in all trails with different reduction % especially at trail III (combined trails) (18, 29.1,40, 47.3 and 61.8%), followed by trail I (12.7, 23.6, 32.7, 43.6, 44.8 and 50.9%), then trail II (14.5, 20, 25.5 and 38.2%) (Fig. 2). An important aspect must be taken into consideration in relation to the commercial use of bacteriocins is the tolerance or resistance of certain pathogenic bacteria that are normally sensitive, such as *L. monocytogenes*, since it may compromise the antibacterial efficiency of these compounds [61]. Whenever, ukuku and Shelef [62] stated that there are no survivors when the inocula were less than 10^4 CUF/ml at the same time, Vignolo *et al.* [63] have reported resistant of *L. monocytogenes* to nisin but the combined use of nisin plus one of other bacteriocins would result in more efficient inhibition. Resistance may result probably from alteration of bacterial membrane composition, destruction of bacteriocin by proteases or altered receptors. Moreover, the anti microbial effectiveness of nisin strongly depends on its mode of delivery. In concern, Matinez and De-martiniz [64] described that not all strains of *L. monocytogenes* show the same degree of sensitivity to antilisterial bacteriocins. Bacteriocin-resistant *L. monocytogenes* have been reported to appear at frequencies 10^3 to$10^9$for nisin. The inhibition mechanism varies according to the protective cultures [65]. By contrast, El-kateib *et al.* [66] found that 4x10^4IUof nisin gave an immediate decrease (0.9log) of *L. monocytogenes* count and on meat surface decreased by (1.1log) in 48hrs. Wherever, Mehado and Tatini [67] recorded that 100 IU/ml of nisinwas effective in reducing count by 1 to 2 log units.

Currently, Murry and Richard [68], Aymerich *et al.* [69], Bouttefroy *et al.* [70], Gill and Holly [71], Luke [72], Aasen *et al.* [73] and Chi-Zhang *et al.* [74] records agree with these results.

Finally in conclusion nisin has an immediate pH-dependent bactericidal effect, which increases with decreasing pH values [70] which can be obtained by the effect of *L. acidophilus* growth, so nisin and *L. acidophilus* is more effective together than each alone. This response could be attributed to acidic damaging

effects on target cells concomitant with the higher stability and solubility of nisin [75] and the increase in net positive charge of nisin [76]. *L. lactis* showed more bacteriocin producing ability compared to *L. acidophilus* [77], so it is evident to add nisin to compensate the low producing bacteriocins by *L. acidophilus*.

The application of bacteriocin or bacteriocin producing LAB strains in food has a potential use as part of the hurdle technology. Although, a bacteriocin alone in food is not likely to ensure satisfactory safety, since Gram-negative bacteria do not represent target cells for bacteriocins as they are protected by an outer membrane. Since, bacteriocins have shown synergies with other treatments and could be used to increase their effectiveness and improve food safety [78]. In contrast, the use of a single bacteriocin is not a sufficient safety factor against *L. monocytogenes* and there might be other strategies such as the combination of two bacteriocins [79]. The direct addition of nisin into food results in an immediate reduction of bacterial populations but may prevent the recovery of injured cells or the growth of cells that are not destroyed by direct addition if residues of antimicrobials are rapidly depleted [74]. Other reported that direct addition of nisin could result in more loss of its activity because of instant reaction with other food components such as lipids or proteins [80]. At the same time, protein binding may cause a significant reduction in free bacteriocin in food. This activity loss is caused by proteolytic activity that attributed to proteases in raw meat [68]. The apparent higher proteolytic activity can be explained by the proteases being intracellular and released during homogenization of the meat. Consequently, in whole meat there will be less constant between the bacteriocin and the proteases [73]. Also, the application of nisin to meat is limited due to its low solubility at the meat pH, its strong interaction with phospholipids leading to an uneven distribution of the bacteriocin in the food system [81]. Therefore there are difficulties in using nisin for raw meat applications on accounts of its poor solubility and instability at the pH of raw meat [16]. On the other side, Chelule *et al.* [82] observed in some instance, LAB may cause food spoilage since their enzymes may lead to generation of fermentation digests that have offensive odors or

flavors, making food to be entirely unpalatable.

Therefore, the effect of combined treatments was significantly greater than that of each preservative alone. The use of combination of preservatives provides effective ways to reduce the natural aerobic background microflora and presumably extend the shelf-life of the product [83] and also in controlling the growth of food spoilage and foodborne pathogenic bacteria [84].Then, a synergistic effect clearly plays a role in preventing microbial growth to affect quality and safety of the food.

In conclusion, the utilization of multiple bacteriocins producing strains would provide an additional barrier to ensure that the emergence of resistant populations is even less likely.

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