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Using lactobacillus probiotic in extending the shelf life of minced meat

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Abstract

The practical application of certain probiotic strains (*Lactobacillus casei, Lactobacillus delbrueckii, and Lactobacillus acidophilus*) were investigated to evaluate their antibacterial effects on chilled minced meat. The study focused on Aerobic plate count (APC), coliform count, and experimentally inoculated *Staph-aureus* count during refrigerated storage at 4°C.The meat was divided into two equal groups, each consisting of 1000 grams: the first group was subdivided into four groups (250g for each); groups (A), (B), and (C) were inoculated with *Lactobacillus casei, Lactobacillus delbrueckii, and Lactobacillus acidophilus,* respectively, while the fourth group served as the control. The second group was experimentally inoculated with *staphylococcus aureus* separately in fresh minced meat which firstly were exposed to gamma irradiation at a dose of 5 kGY to ensure complete sterilization of the samples, then the samples were inoculated with the same previous *lactobacillus* strains. The results indicated that the different probiotics had a significant inhibitory effect, with high reduction percentages of APC, coliform count and staphylococci *count* over ten days of refrigerated storage at 4˚C. The duration of shelf life of the treated samples prolonged to ten days, compared to only six days for the control samples. The reduction percentages of *APC* after ten days in samples treated with *L. casei, L. delbrueckii and L. acidophilus* were 99.7%, 99.91%, and 99.86%, respectively. For coliform count, the reduction percentages were 99.68%, 99.92%, and 99.80%, respectively. For experimentally inoculated *Staph aureus*, the reduction percentages were 100%, 100%, and 100% for the same probiotic treated groups, respectively. These results suggest that *Lactobacillus delbrueckii* exhibited the most potent antimicrobial effects among the groups.

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Introduction

Meat is a highly nutritious food, rich in proteins, fats, essential vitamins, and minerals, while being low in carbohydrates and adequate water activity. However, these properties produce an excellent media supporting the proliferation of both microbial contaminants and pathogens, leading to health and economic burdens **(Komba *et al*., 2012).** Unfortunately, the meat products are prone to contamination during preparation, processing, and serving by a variety of microorganisms from numerous sources. The manufacturing process, the quality of the non-meat materials utilized, and the degree of contamination throughout the processing chain, packaging, and storage all affect these bacteria. One of the most prevalent food-borne infections in the world is staphylococcal food-borne disease (SFD), which result from consumption of contaminated food with *Staphylococcus aureus* enterotoxins **(Kadariya *et al*., 2014).** Food preservation efforts aim to minimize the levels of contaminating bacteria in food. In recent years, the demand for high-quality, safe, less processed, fresh, vitamin-rich, functional, additive-free and nutritional foods has increased interest in natural preservatives **(Sarika *et al*., 2010).** As a result, researchers are exploring novel techniques to reduce the microbial burden of raw meat using different antimicrobial compounds to prevent food-borne infections. Recently, probiotic foods have gained special attention. The most common probiotic microorganisms used and marketed in food worldwide belong to the genera Lactobacillus, due to its importance for consumer health. This microorganism is normally part of the gastrointestinal tract's microflora in humans and is frequently incorporated as natural bio-preservatives in food products **(Carlos *et al*., 2015; Zhang *et al*., 2023).** Studies on probiotics have been significantly increased in the last few years. Fermented dairy products, vegetables, juices, and animal products are the main types of probiotic foods. **(Martins *et al*., 2013).** The use of *Lactobacillus acidophilus* probiotics has been significantly increased due to their impact on lowering the acidity while being stored. **(Munekata *et al*., 2022).** The concept of probiotics emerged from observations in the early 19th century by Russian immunologist Elie Metchnikoff, who hypothesized that the long and healthy lives of Bulgarian peasants were due to their consumption of fermented milks containing beneficial Lactobacillus, which positively influenced colonic health **(Dixon, 2002).** Probiotics are defined as microorganisms that provide health benefits to the host when administered in appropriate amounts. Most probiotics fall into the group of lactic acid-producing bacteria. Probiotics can prevent gastrointestinal infections, enhance host immunity, improve intestinal tract health, relieve diarrhea, reduce symptoms of lactose intolerance, decrease the prevalence of allergies in susceptible individuals, and reduce the risk of certain cancers **(Parvez *et al*., 2006).** Using probiotics as bio-preservatives in chilled minced beef meat is a promising trend due to their health benefits and antibacterial characteristics **(Ibrahim-Samar *et al*., 2019).** The antibacterial activity of lactic acid bacteria (LAB) against spoilage and pathogenic microbes can be driven by several mechanisms. These include outcompeting harmful organisms for vital nutrients and adhesion sites on mucosal surfaces, and producing a range of antimicrobial substances. Among these are organic acids such as lactic, formic, acetic, and propionic acids that lower the food's pH and suppress pathogenic growth. LAB also generate bacteriocins, which are the key to natural food preservation, as well as hydrogen peroxide, a potent oxidizing agent that disrupts bacterial enzymes by targeting sulfhydryl groups. Additional inhibitory compounds include carbon dioxide, antifungal agents like fatty acids and phenyl lactic acid, lysozymes, and various enzymes such as proteases, amylases, and lipases. **(Biswas *et al*., 2018).** Thus, the current investigation aimed to evaluate the antibacterial impact of Lactobacillus probiotics on APC, coliform bacteria, and Staphylococcus bacteria in fresh minced meat.

Materials and Methods

Preparation of Probiotic strains:

Lactobacillus acidophilus, Lactobacillus delbrueckii, and Lactobacillus casei, with an adjusted concentration of (10^7 CFU/g), were obtained ready-to-use from Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

Collection of samples:
A 2000 g sample of chilled minced beef meat was collected from a local market in El-Mansoura city, Dakahlia governorate, Egypt, and subsequently was packed in separate plastic bags and transferred directly to the laboratory in a completely aseptic conditions within an insulated icebox. The sample was divided into two portions one for the experimental inoculation and the 2nd part divided into four portions (250 g each): the first portion was left as the control group. Group A, representing the second portion, was treated with *Lactobacillus casei* inoculum (10^7 CFU/g). The third portion, designated as Group B, was inoculated with *Lactobacillus delbrueckii* (10^7 CFU/g), while the fourth portion (Group C) was inoculated with *Lactobacillus acidophilus* (10^7 CFU/g). All inoculated groups and the control group were appropriately sealed in polyethylene bags, labeled, and refrigerated at 4°C for storage. Sensory analysis (color, odor, and texture) for overall acceptability was conducted daily during the storage period. Bacteriological examinations, including aerobic plate count and total coliform count were conducted daily, starting from zero day (1 hour after mixing the probiotic strain with the meat sample).

Sensory evaluation of the treated groups:
Overall acceptability of all samples was assessed using a nine-point standardized numerical scale, where ten corresponded to components characteristic of the highest quality. The panel consisted of nine staff members familiar with meat characteristics, and the evaluation was conducted during storage according to **Pearson and Tauber. (1984).**

Preparation of the samples (ISO 6887-2, 2017):
Accurately, from each treatment group, 25 grams of sample were collected and blended with 225 ml of 0.1% buffered peptone water at 2000 rpm for 1–2 minutes to produce a 1:10 dilution homogenate. This homogenate was then transferred into another sterile test tube, and 1 ml was subsequently pipetted into another sterile tube containing 9 ml of 0.1% peptone water. From this, ten-fold serial dilutions were prepared up to a dilution factor of 10^6.

Bacteriological examination:

Aerobic plate count (ISO 4833-1, 2013):
One milliliter from each serial dilution was poured into two separate sterile Petri dishes using the pour plate technique. Each dish was then filled with approximately 15 ml of sterile, melted, and tempered plate count agar maintained at 45°C. After thoroughly mixing, the plates both the inoculated and the control were left to solidify at ambient temperature. Subsequently, they were incubated in an inverted position at 30 ± 1°C for 72 hours. Total aerobic bacterial counts (CFU/g) were determined from plates containing 15 - 300 colonies, with each count documented individually.

Coliform count (ISO 4832, 2006):
One ml from each of the previously prepared serial dilutions was evenly spread on sterile Violet Red Bile (VRBL) agar using a bended glass rod and incubated at 37±1ºC for 24hours. Suspected colonies, which showed purplish - red colonies surrounded by a red zone of precipitated bile acid, were enumerated to obtain coliforms count/g.

Preparation of inoculated minced meat samples
Part of the collected chilled minced meat sample (1000 g) was transported separately immediately to the laboratory in a separate insulated icebox. Upon arrival, it was divided into four equal portions of 250 grams each. Every portion was sealed in a polyethylene package and subjected to sterilization through gamma irradiation at a dose of 5 kGy, using cobalt-60 as the radiation source. The irradiation process was conducted at the National Center for Radiation Research and Technology (NCRRT) in Nasr City, Cairo, Egypt. **(Nassif *et al.,* 2015).** The 3 portions were inoculated with *Staph. aureus* to reach final concentration in minced beef 10^4cfu/g. The first portion remained untreated (control Group), the second portion (Group A) was inoculated with *Lactobacillus casei* to reach final conc. in samples 10^7cfu/g, the third portion (Group B) was inoculated with *Lactobacillus delbrucekii* to reach final conc. in samples 10^7cfu/g and the fourth group (group c) was inoculated with *Lactobacillus acidophillus* to reach final conc. 10^7cfu/g. Analysis of samples was conducted at zero

time (2 hr. after addition of probiotics) then they were refrigerated stored at 4°C. The samples were periodically examined at 2nd,4th,6th,8th, 10th days. Each experiment was conducted in triplicate across three distinct days to ensure consistency and validity of the results.

Assessment of microbial growth (ISO 6888-1, 2021):

One milliliter from each serial dilution was evenly distributed onto Baird Parker agar plates using a sterile bent glass spreader. The inoculated plates were then incubated at 37°C for 24 - 48 hours. After incubation, the characteristic shiny black colonies were counted, and the total number of Staphylococcus organisms per gram of sample was calculated and recorded. Typical colonies are black or grey, shining and convex encircled by a clear zone with opalescent ring in contact with the colonies while, the atypical colonies appear shining black with or without a narrow white edge; the clear zone and the opalescent ring are absent.

Statistical Analysis:

The obtained results were statistically evaluated by applying One Way ANOVA, Ver. 20. The Handbook Of Statistical Analysis Using SPSS, according to **Petrie and Watson (2013).**

Results

Table (1). Overall acceptability of the examined chilled minced beef samples treated with different probiotics during refrigeration storage at 4°C.

Groups	Control	*L.casei*	*L. delbrueckii*	*L. acidophilus*
Zero day	9	9	9	9
2nd day	8^a	9^b	9^b	9^b
4th day	7^a	9^b	9^b	9^b
6th day	6^a	8^b	9^b	8^b
8th day	3^a	7^b	8^b	8^b
10th day	1^a	6^b	7^b	6^b

Score system for sensory evaluation **(Pearson and Tauber. (1984).)**

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

Table (2). The effect of different probiotics on APC (log^{10}cfu/g) in the examined chilled minced meat samples

Groups	Control group	Group A	Group B	Group C
Zero day	5.63±4.95 "a"	5.57±4.8 "b"	5.18±4.4 "c"	5.42±4.5 "b"
2nd day	5.86±4.7 "a"	5.39±4.2 "b"	4.89±3.9 "c"	5.12±4.4 "b"
4th day	5.99±4.5 "a"	4.98±3.6 "b"	4.75±3.6 "c"	4.89±3.6 "b"
6th day	6.51±5.7 "a"	4.73±3.0 "b"	4.39±3.5 "b"	4.59±3.6 "b"
8th day	6.80±5.7 "a"	4.64±3.0 "b"	4.09±3.0 "b"	4.38±3.3 "b"
10th day	6.96±5.3 "a"	4.36±3.0 "b"	3.91±2.9 "b"	4.09±3.0 "b

*Group A: The samples treated with *L. casei*
* Group B: The samples treated with *L. dellubrueckii*
* Group C: The samples treated with *L. acidophillus*
*Letters within the same row represent significance of differences (P<0.05) .

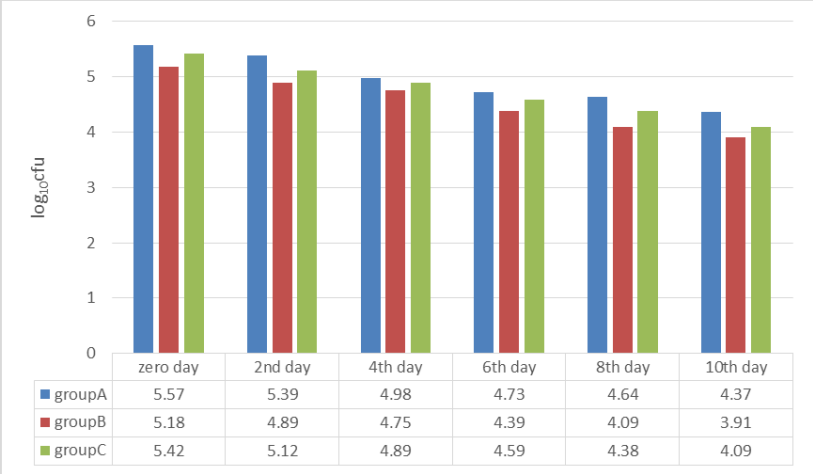

Fig (1). The effect of different used probiotics on APC (log^{10}cfu/g) in the examined chilled minced meat samples

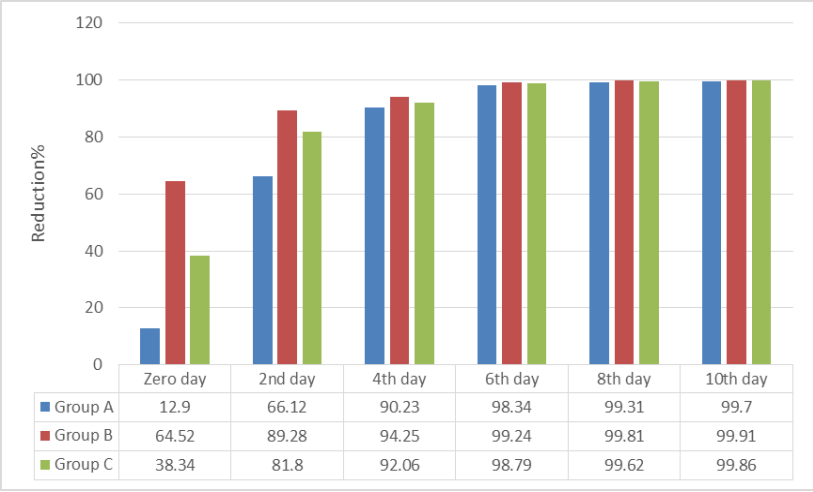

Fig (2). Reduction % of APC (log_{10}cfu/g) in the examined chilled minced meat samples treated with different used probiotics

Table (3). The effect of different used probiotics on coliforms count (log^{10}cfu/g) in the examined chilled minced meat samples.

Groups	Control group	Group A	Group B	Group C
zero day	4.68±3.8 "a"	4.58±3.9 "b"	4.28±3.5 "c"	4.49±3.6 "b"
2nd day	4.91±3.3 "a"	4.42±3.8 "b"	3.90±2.8 "c"	4.16±3.2 "c"
4th day	4.98±3.7 "a"	4.09±3.0 "b"	3.74±2.6 "c"	3.91±2.9 "b"
6th day	5.28±4.6 "a"	3.92±2.5 "b"	3.54±2.3 "b"	3.77±2.9 "b"
8th day	5.64±4.9 "a"	3.80±2.7 "b"	3.24±2.7 "b"	3.53±2.8 "b"
10th day	5.91±4.06 "a"	3.42±2.7 "b"	2.81±1.8 "b"	3.21± 2.7 "b"

*Group A: The samples treated with *L. casei*
* Group B: The samples treated with *L. dellubrueckii*
* Group C: The samples treated with *L.acidophillus*
*Letters within the same row represent significance of differences (P<0.05)

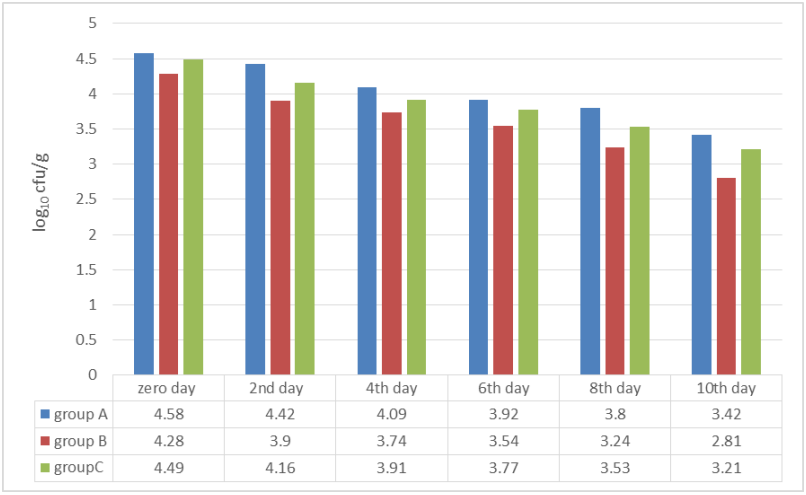

Fig (3). The effect of different used probiotics on coliforms count (log^{10} cfu/g) in the examined chilled minced meat samples

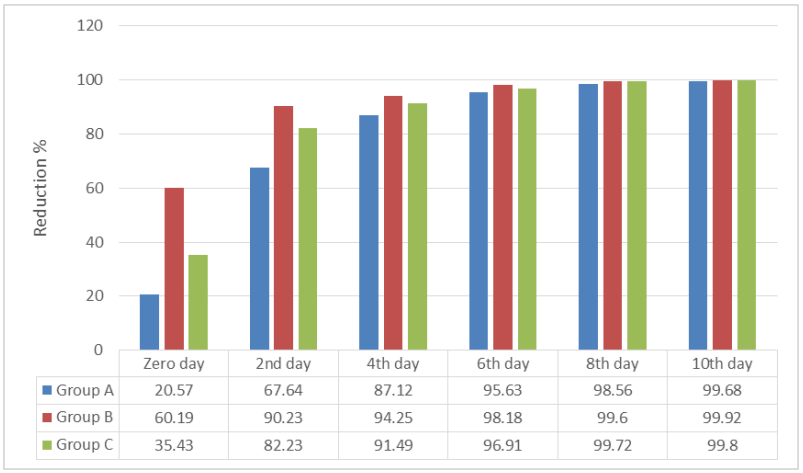

Fig (4). Reduction % of coliforms count (log_{10}cfu/g) in the examined chilled minced meat samples treated with different used probiotics

Table (4). The effect of different used probiotics on *staph aureus* count (log^{10} cfu/g) experimentally inoculated in the examined chilled minced meat samples

Groups	Control	Group A	Group B	Group C
Zero day	4.25±0.24	4.25±0.24	4.25±0.24	4.25±0.24
2nd day	4.51±0.26 ^{a}	3.81±0.12^{b}	3.51±0.16^{b}	3.90±0.12^{b}
4th day	4.92 ±0.05 ^{a}	2.61±0.21^{b}	2.45±0.2^{b}	2.70±0.3^{b}
6th day	5.31±0.25^{a}	1.73±0.12^{b}	1.47±0.24^{b}	1.75±0.2^{b}
8th day	5.88±0.09^{a}	1.42±0.11^{b}	1.25±0.11^{b}	1.61±0.11^{b}
10th day	6.25±0.05^{a}	<1^{b}	<1^{b}	<1^{b}

*Group A: The samples treated with *L. casei*
* Group B: The samples treated with *L. dellubrueckii*
* Group C: The samples treated with *L. acidophillus*
*Initial load of inoculated Staph. aureus 4 log^{10} cfu/g.
* <1 log_{10}cfu/g was calculated by zero when applying statistical analysis.
*Letters within the same row represent significance of differences (P<0.05) .

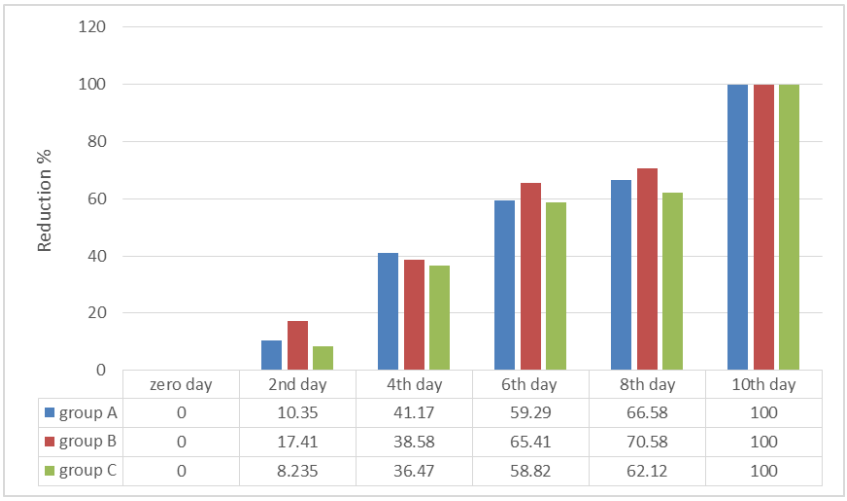

Fig (5). The reduction% of *Staph aureus* count (log^{10} cfu/g) experimentally inoculated in the examined chilled minced meat samples treated with different used probiotics

Discussion

Along centuries of efforts to control foodborne diseases, food safety continues to pose a major challenge even with the numerous advanced technologies. At the same time, consumer demand is shifting toward minimally processed foods with natural preservation methods. One promising approach is biopreservation, which relies on using naturally occurring antimicrobial substances with a long-standing record of safe consumption, thereby reducing the need for synthetic chemical preservatives. **(Muthuvelu *et al.*, 2023).** Enhancing a product's shelf life can have a significant economic impact by reducing the spoilage-related losses and empowering the products to reach new and distant markets. Sensory evaluation ultimately aims to anticipate consumer preferences. It has become an essential tool in fields such as marketing research, product development, and quality assurance. **(Gouin, 2004).** Overall acceptability scores for samples treated with *L. casei* (Group A), *L. delbrueckii* (Group B), and *L. acidophilus* (Group C) during 10

days of refrigerated storage were displayed in Table (1). The score was 9 (excellent) in all examined samples including the Control one. No significance of difference ($p<0.05$) was recorded between all examined groups. At the 2nd day of storage, the Overall acceptability score declined to 8 (very very good) in the Control group while recorded 9(excellent) in the three treated groups (A, B and C). There was no significance of differences ($p<0.05$) in the Control group as compared with the inoculated groups (A, B and C), although the significance of differences ($p<0.05$) was recorded with the treated groups. The Overall acceptance score recorded 7(very good) in the 4th day of storage at 4˚c for the control group while recorded 9 (excellent) for the three treated groups with high significance of differences($p<0.05$) between the Control group and the treated groups (A, B and C). There was no significance of differences ($p<0.05$) between group (A, B and C). On the 6th storage day, a significance of differences($P<0.05$) was recorded between the Control group and groups A, B and C but no significance of differences ($p<0.05$) was recorded between group A, B and C. At the 8th day of storage at 4 ˚c, the Overall acceptance was 6 (good),8(very very good),9(excellent) and 8 (very very good) for the Control, A, B and group C respectively. At the 8th day of storage, the score recorded 3(poor),7(very good),8(very very good) and 8(very very good) for the control group, group A, group B and group C respectively. A significance of differences ($p<0.05$) was recorded between the Control group and the treated groups (A, B, and C) however still, no significance of differences was recorded between the treated groups. On the 10th day of refrigerated storage, the overall acceptance recorded 1(very very poor) for the Control group, 6(good) for group A,7 (very good) for group B and6 (good) for group C. A high significance of difference ($p<0.05$) was recorded between the Control group and the treated groups (A, B and C).

These findings may be due to that *Lactobacillus spp*. have the ability to produce lactic and organic acids resulted in decrease in pH of the treated minced meat samples and subsequently, stop microbial growth resulted in persistence of product natural color, odor, overall acceptability and subsequently prolong the shelf life of minced meat **(Yassin-Nessrien, 2003).** The results also agreed with **Castellano *et al.* (2010)** who reported that beef treated with the bioprotective culture exhibited a 10-day delay in the onset of tissue degradation compared to the control group.**Cenci-Goga *et al.* (2012)**, **Bahni Dhar (2013**) and **Bomdespacho (2014**) added that using of *L. acidophilus* enhanced the production of a microbiologically safe product, with good sensory and physicochemical properties.

"Lactic acid bacteria (LAB) probiotics serve as natural bio-enhancers, improving the physicochemical characteristics such as sensory attributes, pH levels, total volatile basic nitrogen (TVB-N), and TBA and effectively extending the shelf-life of raw minced beef and other meat products under cold storage **Abdallah *et al* (2024).**

These findings are closely similar to those recorded by **Kalalou *et al*., (2004)** who mentioned that non treated minced meat with lactic acid bacteria was severely deteriorated in its appearance within 3 days of storage at 22°C due to strong elevation of pH (7.2). Also, **Smith *et al.,* (2005)** reported that there were no detectable effects of LAB on the sensory attributes of the refrigerated ground beef stored at 5°C. **Abd El Aziz- Reham (2012) and Salem-Amani (2012)** found that improvement in the sensory attributes and prolonged shelf life of minced meat samples treated with *L. acidophillus* when compared with control non treated samples. **Nassif *et al.,* (2015)** mentioned that treated minced beef with *Lactobacillus acidophilus* were proven to be highly effective in delaying and reducing sensory problems and extend the shelf life.

In addition, **Alireza *et al.,* (2016)** found that addition of *L.acidophilus* prolonged the shelf life of ground beef and keep accepted sensory parameters**.** Also, **Amin-Amira (2018)** concluded that treatment of minced beef samples with probiotics (*Lactobacillus acidophilus)* helped in serving the characteristic minced beef color and odor for longer time than control non treated samples, and prolong the shelf life.

The antimicrobial properties of *lactobacilli* are mainly attributed to their ability to produce organic acids such as lactic, acetic, and propionic

acids as well as hydrogen peroxide, antimicrobial peptides (AMPs), and bacteriocins each with variable degrees of effectiveness **(Cortes-Zavaleta *et al*., 2014., Gemechu, 2015)**.

Lactobacilli strains are capable of producing organic acids via heterofermentative metabolic routes. These acids can disrupt cell membranes, leading to intracellular acidification and protein denaturation. The antimicrobial action of lactic acid is primarily attributed to structural and functional alterations in the bacterial cytoplasmic membrane, which cause the release of intracellular components **(Wang *et al*, 2015).**

Data presented in Table (2) Fig (2,3) demonstrate the effect of different probiotic strains (*L. casei, L. delbrueckii and L. Acidophilus*), on *APC* $\log_{10}$cfu/g in the examined chilled minced meat. At zero day, the Control group, group A, B and C recorded 5.63 ± 4.95, 5.57±4.8, 5.18±4.4 and 5.42±4.5 log $_{10}$cfu/g respectively, representing decreases in the *APC* in the three inoculated groups. This decrease in the counts lead to high significance of differences ($p<0.05$) between the control group and the three inoculated groups.

At the 2nd day of storage there was an increase in *APC* in the Control group (5.86±4.7 l*og* ^{10} cfu/g) while, group A, B and C recorded 5.39±4.2(66.12% reduction%),4.89±3.9 (89.28% reduction %) and 5.12±4.4(81.80% reduction %) respectively leading to a high significance of differences ($p<0.05$) between the Control group and the three inoculated groups A, B and C. There was a high significance of difference($p<0.05$) at the 4th day of storage between the Control group and groups A, B and C as they recorded 5.99±4.5, 4.98±2.6, 4.75±3.6 and 4.89±3.6 l*og* ^{10} cfu/g with reduction% of 90.23, 94.25, 92.06 for the aforementioned groups respectively. At the 6th day of storage at 4˚c, results revealed high significance of differences ($p<0.05$) between the Control group (6.51±5.7 l*og* ^{10} cfu/g), and group A (4.73±3.0 $\log_{10}$cfu/g), group B (4.39±3.5 l*og* ^{10} cfu/g) and group C (4.59±3.6$\log_{10}$cfu/g). At the same day of storage, the reduction % were 98.34, 99.24 and 98.79 for group A, B and C respectively. *APC* continue increasing recorded 6.80±5.7 l*og* ^{10} cfu/g at the 8th day of storage resulted in high significance of differences ($p<0.05$) with group A, B and C. On the 10th storage day, *APC* in the control A, B and C groups were 6.96±5.3, 4.36±3.0, 3.91±2.9 and 4.09±3.0 $\log_{10}$cfu/g respectively, representing high significance of difference($p<0.05$). Moreover, the reduction % was 99.7 (A), 99.91(B) and 99.86 (C).

The results shown in Table (3) and Fig. (3,4) represent the effect of different probiotics on coliforms count l*og* ^{10} cfu/g. The results recorded at zero day were, 4.68±3.8, 4.58±3.9, 4.28±3.5 and 4.49±3.6 $\log_{10}$cfu/g for control, A, B and C groups respectively. The counts revealed high significance of difference ($p<0.05$) in the treated groups. The reduction % were 20.57, 60.19 and 35.43 for group A, B and C respectively. At the 2nd day of storage, the coliforms count increased in the control group (4.91±33$\log_{10}$cfu/g) in comparing with group A, B and C (4.42±3.8, 3.90±2.8 and 4.16±3.2) respectively, resulting in high significance of differences ($p<0.05$). The reduction % in the 4th day of storage were 87.12% (group A), 94.25% (group B) and 91.49% (group C). High significance of differences ($p<0.05$) was also recorded between the Control group (4.98 ± 3.7l*og* ^{10} cfu/g), group A(4.09 ± 3.0$\log_{10}$cfu/g), group B (3.74 ± 2.6$\log_{10}$cfu/g) and group C (3.91 ± 2.9$\log_{10}$cfu/g). At the 6th day, there was a decrease in the coliforms count in group B (3.54 ± 2.3l*og* ^{10} cfu/g) more than that recorded in group A (3.92 ± 2.5 l*og* ^{10} cfu/g) and C (3.77 ± 2.9 l*og* ^{10} cfu/g) in comparing with the control group (5.28±4.6$\log_{10}$cfu/g) resulting in high significance of difference ($p<0.05$). The reduction % recorded were 95.63% (group A), 98.18% (group B) and 96.91% (group C). At the 8th day of storage the coliforms count was 5.64 ± 4.9, 3.80 ± 2.7, 3.24 ± 2.7 and 3.53 ± 2.8 l*og* ^{10} cfu/g for the control, A, B and C groups. These decreases in the coliforms count result in high significance of differences ($p<0.05$). The reduction% recorded 98.56,99.60 and 99.22 for group A, B and C respectively. Group B recoded significant decrease ($p<0.05$) in the coliforms count (2.81±1.8 l*og* ^{10} cfu/g) with reduction % 99.92% at 10th day of storage at 4˚c, where the counts for group A, and C were 3.42±2.7, and 3.21±2.7 l*og* ^{10} cfu/g; with reduction % 99.68,

and 99.80 respectively. while the control group recorded 5.91 ± 4.06 log_{10}cfu/g.

These findings are in agreement with those reported by **Kalalou *et al*. (2004)** studied the antibacterial effect of *L. delbrueckii* in minced meat and reported that, the initial *APC* of the mixture was around 1.4x 10^6cfu/g, which decreased to 1.2 x 10^4cfu/g after 24 hrs. and 8 x 10^2cfu/g after 7 days of incubation at the ambient temperature (22ºC).

Coliform levels also decreased from 8 × 10^2 cfu/g to 10^2 cfu/g within 24 hours, and dropped to below 1 cfu/g at the 7th day of storage.

The results also agree with that reported by **Salem-Amani (2012)** who investigated the effect of *L. acidophilus* as bio-preservative agents on shelf-life and safety of minced beef samples during cold storage (4°C).

The investigated trials exhibited strong antibacterial effects, significantly reducing both Aerobic Plate Counts (APC) and total coliforms. *The* counts of *total aerobes* (TAC), and *c*oliform (TCC) were 8.5± 0.92 and 5.4 ± 0.28 log cfu/g respectively for control group at zero day. For the Control samples, the previous microbial groups grew and reached high count levels at 3 days of storage (12.1±0.97and 8.6±0.15 log cfu/g, respectively), while TAC and TCC were significantly different (P 0.05 and 0.001), decreased and reached 8.3±1.2, and 5.6+0.57 log cfu/g. While the results disagreed with those reported by **Amin-Reham (2012)** who reported weak activity of *L. acidophilus* against total colony count (TCC), coliform count and *Staphylococcus aureus* in minced meat, with the shelf life of treated samples reaching over 3 days at 4°C rather than 2 days for control samples.

Because lactic acid bacteria can produce massive amounts of hydrogen peroxide and/or other antimicrobial substances at refrigeration temperature that suppress foodborne pathogens and psychrophilic spoilage microorganisms, they are frequently used in food preservation at refrigerator temperatures **(Alireza *et al*., 2016).**

Table (4) revealed the effect of different used probiotics on the growth rate of *staph aureus* in the experimentally inoculated minced meat. There was no significance of differences ($p<0.05$) within all tested groups (Control, A, B and C) at zero day. At the 2nd day of storage at 4˚c, the control group revealed increase in the count recording 4.51±0.26 log^{10} cfu/g. This increase resulting in presence of significance of differences ($p<0.05$) in the three inoculated groups (A, B, and C) however, no significance of difference ($p<0.05$) was recorded between the three groups as they recorded 3.81 ±0.12, 3.51±0.16 and 3.90±0.12 log^{10} cfu/g four group A, B and C respectively. There was high significance of differences ($p<0.05$) at the 4th day of storage between the Control group and groups A, B, and C recording 4.92±0.05, 2.61±0.21, 2.45±0.2 and 2.70±0.3 log^{10} cfu/g respectively, but no significance of differences ($p<0.05$) was recorded between the inoculated groups. On the 6th day, there was an increase in the count of the control group reaching 5.31±0.25 while the counts in group A ,B, and C were 1.73±0.25, 1.47±0.24 and 1.75±0.2 log_{10}cfu/g respectively exhibiting high significance of differences ($p<0.05$) between the Control group and the inoculated groups (A, B, and C) however, no significance of differences ($p<0.05$) was recorded between group A,B and C. At the 8th day of storage, a high significance of differences($p<0.05$) was recorded between the Control group and group A, B, and C and the count recorded 5.88 ±0.09, 1.42±0.11,1.25±0.11 and 1.61±0.11 respectively. At the 10th day, in the experimentally inoculated groups the *staph aureus* growth was suppressed and recorded <1 log^{10} cfu/g with a high significance of differences($p<0.05$) with the Control group (6.25±0.05 log^{10} cfu/g). Fig (5) revealed that the reduction% of the growth rate of *staph aureus* was10.35% at the 2nd day, increased to 29.2% and 59.29% and 66.58 %at the 4th ,6th and 8th days of storage at 4˚c recording 100% at the 10th day for group A. For group B, the reduction %were 17.41, 42.35, 65,41, 70.58 and 100% at the 2nd ,4th,6th,8th and 10th days of the experiment. Group C recorded reduction% of 8.2, 36.47, 58.82, 62.12 and 100% at the 2nd, 4th, 6th, 8th and 10th days of the experiment**.** Comparable findings were reported by **Ibrahim-Hemmat *et al*. (2019),** who observed that the reduction in *Staphylococcus aureus* counts achieved by *Lactobacillus acidophilus* was nearly the same as that achieved by *Bifidobacterium lactis*. Additionally, *S. aureus* growth persisted until the sixth day of storage but was totally inhibited by the eighth day of the experiment. These results are con-

sistent with the antibacterial effects recorded by

Amin-Amira (2018) found that *L. acidophilus* strongly inhibited the growth of *Staphylococcus aureus* and *E. coli*, with growth persisting until the 6th day of refrigeration storage, while the organisms were completely inhibited by the 8th day of the experiment. While **Ali *et al.* (2020)** indicated that using *L. acidophilus* reduced the number of *E. coli* and *S. aureus*. According to **Nassif *et al.* (2015)**, the *Staphylococcus aureus* count declined from 6.48 $\log_{10}$ CFU/g on day zero to 3.52 $\log_{10}$ CFU/g by the 9th day of storage. However, by the 11th day, the samples exhibited complete spoilage. **Bahni and Dhar (2013)** reported a highly significant reduction ($p < 0.01$) in staphylococcal *counts*, decreasing from 2.40 to 1.46 $\log_{10}$ CFU/g over the storage period. This reduction became statistically significant after 14 days of storage in minced fish meat inoculated and pretreated with lactic acid bacteria (*LAB*). **Bomdespacho (2014)** demonstrated that the addition of *Lactobacillus acidophilus* resulted in complete inhibition of coagulase positive staphylococci.

The antimicrobial effects of probiotics *(LAB)* against common foodborne pathogens depend on the product type used, species of pathogenic bacteria, interactions between bacteria, adaptation to a substrate, acid production, microorganism sensitivity, NaCl, redox potential (Eh), water activity (Wa), pH, storage temperature, and cell density of the protective culture **(Hathout-Amal and Aly-Soher, 2010).** Lactic acid, secreted by *LAB*, lowers the food pH and directly suppress the growth of pathogenic microorganisms **(Cardirci and Citak, 2005).**

Due to its ability to flourish in anaerobic environments and its tolerance for salt and nitrite, Staph. aureus poses a greater risk of developing and producing toxins **(Kaban and Kaya, 2006).**

lactic acid bacteria (*LAB*) exhibited broad-spectrum antimicrobial activity against numerous foodborne pathogens (**Zhu *et al.*2000)**, causing a wide range of gastrointestinal illnesses and, in severe cases, may lead to fatal outcomes in humans.

Conclusion

The present study concluded that the different probiotic strains (*L.casei, L. acidophilus and L. delbrueckii*) can be used as natural biological additives improving the sensory characteristics and extending the shelf- life of minced meat throughout refrigeration period through its antibacterial characteristics. The study also revealed that *L. delbrueckii* has the most antimicrobial effect among the probiotic strains.

Conflict of interest: The authors declare that they have no conflict of interest.

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