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(Research Article)

Improving the bio-stability of *Bifidobacterium lactis* in vacuum-packed beef

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Abstract

This review evaluates recent findings on the application of a UV-inactivated Bifidobacterium lactis strain to improve the biostability of vacuum-packed beef stored at Zero±1 °C. The comparison between control, active and UV-inactive (B. *lactis*), samples across 124 of storage days reveals that the UV-inactivated strain offers significant improvements in microbial control, chemical stability, and sensory preservation. This approach introduces a sustainable bio-preservative technique for cold stored meat

Keywords: *Bifidobacterium Lactis (B. Lactis*); Active and UV-Partial Inactive *B. Lactis; Escherichia coli*

1. Introduction

Beyond pork and poultry, beef stands as the most widely consumed meat globally. Its primary constituents include water, protein, carbohydrates, and lipids, while vitamins, enzymes, pigments, and flavor compounds form its subsidiary components. The interaction of these constituents grants upon beef its distinct structure, texture, flavor, color, and nutritional value. Beef and its derivatives are excellent sources of high-quality proteins, whose amino acid profiles often complement the deficiencies found in other staple foods (Awoyinka et al., 2024).

Food spoilage, and foodborne pathogens have always been among the main problems in food industry worldwide, due to the increasing trend of meat consumption, industrial manufacturers, have made great efforts to produce healthy products with good quality (Kheyri et al., 2014).

Meat spoilage is primarily driven by microbial growth, lipid oxidation, and protein degradation, all of which compromise food quality and sensory attributes. Vacuum packaging is widely used to extend meat shelf life, but it cannot prevent spoilage entirely especially during long-term storage unless the packaging and storage conditions are consistent with good manufacturing and hygienic practices (GMP and GHP). Traditional approaches such as synthetic preservatives, irradiation, and modified atmosphere packaging (MAP) have been implemented, but consumer concerns regarding chemical additives have intensified the demand for natural and safe preservation methods (Rasheed et al., 2025).

Bio-preservation using probiotics has emerged as a viable alternative. Among the commonly studied probiotics, Bifidobacterium lactis is notable for its strong antimicrobial activity, production of lactic and acetic acids, and its health-promoting effects in the human. In combating pathogenic microorganisms, lactic acid bacteria create a competitive environment, compete for nutrients, modulate target cell immunity, and produce antimicrobial compounds such as acetic acid, propionic acid, exopolysaccharides (Putri et al., 2024).

Escherichia coli has a superior ability to persist at very low pH levels stems from its evolutionary adaptation to the harsh acidity of the mammalian stomach. It has developed an extensive and redundant array of acid resistance systems,

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particularly the highly effective amino acid decarboxylase pathways, which actively consume protons and expel basic products. Staph. aureus, while possessing mechanisms to cope with acidic environments (like urease and cation/proton antiporters), it does not exhibit the same level of extreme acid resistance, likely because its primary niches do not typically expose it to such severe and prolonged acidic challenges (Richard and Foster 2023).

Several studies demonstrate the efficacy of Bifidobacterium lactis in meat preservation, where it contributes to inhibiting spoilage flora and pathogenic bacteria through bacteriocin production and pH reduction. Moreover, B. lactis exhibits antioxidant properties, such as radical scavenging and prevention of lipid oxidation, which are vital for maintaining the color, flavor, and shelf life of meat products. However, the application of native strains often falls short under harsh storage conditions due to limited stress tolerance (Rodríguez-Marca et al., 2025).

To address this, researchers have turned to microbial inactivation techniques. UV-induced partial inactivation is a simple and effective method that generates partial inactivity of B. lactis with enhanced functional properties like acid resistance, oxidative stability, and competitive colonization potential. This partial inactivation can significantly improve strain viability and performance during storage and under meat matrix stress (Marcos-Fernández et al., 2023).

Induced mutagenesis is widely used for selection of microorganisms producing biologically active substances and further improving of their activities. (Alireza Goodarzi, 2016).

The present study explores the use of a UV-partial inactivated B. lactis strain in vacuum-packed beef stored at Zero±1 °C for 124 days. By comparing its performance to both untreated control and active B. lactis, the study aims to evaluate the effectiveness of this bio-preservative approach in delaying spoilage based on microbiological, chemical, and sensory parameters.

2. Material and methods

- Sample Preparation: Fresh beef loin was purchased from a local Cairo market, trimmed and portioned into 200g samples. Each sample was assigned to one of three treatments: (1) G1: control (no probiotic), (2) G2: inoculated with active type Bifidobacterium lactis, and (3) G3: inoculated with UV-partial inactivated Bifidobacterium lactis strain. All probiotic cultures were prepared to a final concentration of 106 CFU/g and evenly distributed on the surface of the meat. Samples were vacuum-sealed in oxygen-impermeable plastic bags.
- UV partial inactivation: The UV-inactivated Bifidobacterium strain was generated; the strain was propagated in De Man-Rogosa-Sharpe (MRS) broth (Difco, Detroit, MI) supplemented with 0.05% (wt/vol) L -cysteine (Sigma, St. Louis, MO) (MRSC). Cultures were incubated at 37°Cin an MG 500 anaerobic chamber (Don Whitley Scientific, West York-shire, United Kingdom) with an atmosphere of 10% (vol/vol) H2, 10% CO_2, and 80% N2. An overnight culture was used to inoculate 50 ml of fresh MRSC (1%, vol/vol). Exponential-phase cultures ($A600_{=}$ 0.4) were washed twice with phosphate-buffered saline (PBS), resuspended in 5 ml PBS, poured onto a petri dish, and then exposed to UV light (UV radiation sterilization desk; JP Selecta, Barcelona, Spain) for 3 min. This exposure produced a viability loss of 15%. Inactivation with a decreased ability to incorporate sodium fluoroacetate were selected in MRSC supplemented with 1.8% (wt/vol) agar and 120 mM sodium fluoroacetate (Margolles and Sánchez, 2012).

2.1. Bacteriological examination

2.1.1. Clostridium perfringens enumeration according to ISO 15213-2:2023

Black characteristic colonies of C. perfringens were enumerated after one milliliter of the initial suspension and subsequent decimal dilutions being incorporated in Tryptone Sulfite Cycloserine (TSC, Biokar Diagnostics) agar and incubated under anaerobic conditions at 37 °C ± 1 °C, for 20 h ± 2 h. GEN box anaer, CO_2 generator sachets (bioMérieux) were used for the culture, in jar, of anaerobic bacteria.

2.1.2. Lactic acid bacteria count (Bifidobacterium lactis) according to APHA (2001)

0.1 ml of tenfold serial dilution was streaked on MRS (Man -Rogosa-Sharpe) agar media. The inoculated plates were incubated anaerobically at 37 °C for 72 hrs. The number of colonies were counted and recoded as log10cfu/g sample.

2.1.3. Enumeration of Escherichia coli according to ISO 16649-2 (2001)

Transfer 1 ml of each dilution into sterile petri dishes then pour 15-18 ml of molten Tryptone Bile X-glucoronide medium (TBX) (previously prepared and cooled at 44 °C – 47 °C in the water bath) to plates then mix and allow to set.

Incubate plates at 44 °C for 24 hr. The count is calculated from the number of typical blue or blue green colonies per plate.

2.1.4. Enumeration of Staphylococcus aureus according to ISO 6888-1, (2021)

The count was performed by plating 1 ml of the initial suspension (10−1 dilution) test sample on Baird Parker agar plate (d=140mm). Incubate at 34 °C to 38 °C for 24 h ± 2 h, then re-incubate for a total of 48 h ± 4 h. Suspected colonies were purified and subjected for further biochemical examination.

Chemical analysis

Hydrogen ion concentration (pH) was measured directly in homogenized samples using a digital pH meter. Total Volatile Nitrogen (TVN) was determined using the Kjeldahl steam distillation method. Lipid oxidation was assessed using the thiobarbituric acid reactive substances (TBARS) assay to determine malondialdehyde (MDA) equivalents

2.1.5. Total volatile basic nitrogen (TVB-N) according to ES (63-9/ 2006)

Accurately 10 g of each sample is added to two gm magnesium oxide + 300 ml distilled water were added. The distillation step generally takes 20 min. about 100 ml of distillate was received in flask containing 25 ml boric acid 2% and two drops of indicator. Flask was boiled tell 100 ml distillate was obtained. Sample was titrated with 0.1 M H_2SO_4 (R1). Steps were repeated using distilled water instead of sample as blank (R2). TVBN expressed as mg/100 gm = (R1-R2) X 14.

2.1.6. Thiobarbituric acid (TBA) according to (ES 63-10/2006)

Accurately 10 g sample was homogenized with 97.5 ml distilled water for two min., then washed in distillation flask with 47.5 ml water. 2.5 ml of 4 N HCl was added to adjust pH to 1.5, few drops of antifoam emulsion or 3 to 5 glass beads were added to prevent bumping. Contents well swirled and distilled rapidly until 50 ml distillate is collected. The distillation step generally takes 15 to 20 min. Five ml distillate were pipetted into a screw cap tubes then 5ml of 0.02 M. TBA reagent was added. A reagent blank was prepared (i.e., 5ml of water and 0.02 M TBA), during this, vortex, and heated for 35 min in a boiling water bath, then cooled under running tap water for 10 min, and then the absorbance. The test samples were measured at 538 using a glass cuvette. TBA value mg/kg of sample = Absorbance x 7.8

2.2. Reagents and Chemical

- HCL 4N [One part conc. HCl: two-part D.W (1:1)].
- TBA reagent (0.2883gm/100ml glacial acetic acid 90%).
- Measurement of pH according to ES (63-11/2006)

For pH determination, 50 g sample was blended with 200 ml of distilled water for 2 min. the supernatant was filtered, 50 ml portion of the filtrate was diluted with 50 ml of distilled water. After mixing for 10 min, the pH was measured at room temperature using a digital pH meter (Suntex TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold) through direct immersion of electrode into the mixture.

2.3. Sensory evaluation

It was carried out based on odor, color, tenderness, moisture, dryness and overall acceptability by (10) specialized panelists, the panelists were asked to score independently using 10-point hedonic scale according to Chen et al., (2016).

2.3.1. All samples were evaluated in triplicate and the evaluation was performed according to the following evaluation key

a 9-point hedonic scale (9 = excellent, 1 = unacceptable). Dryness was interpreted as the inverse of moisture perception. Evaluations were performed under controlled lighting and ventilation.

2.3.2. Storage Conditions

All samples were stored at zero±1°C (super chilling conditions) in a commercial cold storage unit. Sampling occurred every 14 days over a total storage period of 124 days.

3. Results and discussion

Table 1 illustrates the relationship between the control vacuum-packed samples (without addition of Bifidobacterium lactis) as 1st group, samples treated with active Bifidobacterium lactis as 2nd group, and samples treated with UV-partially inactivated Bifidobacterium lactis as 3rd group.

Table 1 Statistical Analysis of Bacterial Quality in Vacuum-Packed Chilled Stored Meat

Storage days	**Sample**	**Bacterial counts (log10 cfu/g)**		
	Type	***Bifidobacterium***	***S. aureus***	***E. coli***
1 day	G1	---	2.3^{a}±0.02	1.95^{a} ±0.01
	G2	6.0^{a}±0.02	2.28^{b}±0.02	1.95^{b} ±0.02
	G3	6.0^{b}±0.02	2.3^{c}±0.03	1.94^{c}±0.02
14	G1	---	1.74±0.02	1.93±0.02
	G2	6.2±0.01	1.6±0.04	1.92±0.02
	G3	5.9±0.01	1.48±0.03	1.91±0.01
28	G1	---	1.6^{a}±0.03	1.91±0.01
	G2	6.2±0.02	1.18^{b}±0.09	1.89±0.02
	G3	6.1±0.01	1.01^{c}±0.01	1.9±0.01
42	G1	---	1.0^{a}±0.00	1.89±0.01
	G2	6.4±0.03	<1	1.88±0.02
	G3	6.1±0.01	<1	1.85±0.02
56	G1	---	<1	1.75±0.02
	G2	6.6±0.02	<1	1.7±0.01
	G3	6.4±0.02	<1	1.7±0.03
70	G1	---	<1	1.64±0.03
	G2	6.8±0.04	<1	1.63±0.03
	G3	6.6±0.03	<1	1.64±0.03
84	G1	---	<1	1.60±0.03
	G2	6.9±0.02	<1	1.60±0.01
	G3	6.7±0.01	<1	1.59±0.03
98	G1	---	<1	1.60±0.03
	G2	7.3±0.04	<1	1.54±0.04
	G3	6.9±0.01	<1	1.52±0.04
112	G1	---	<1	1.79±0.05
	G2	7.6±0.02	<1	1.39±0.06
	G3	7.1±0.01	<1	1.31±0.01
124	G1	---	<1	2.6^{a}±0.03
	G2	7.9^{a}±0.01	<1	1.04^{b}±0.08
	G3	7.1^{b}±0.03	<1	0.9^{c}±0.08

Means with different superscripts in the same column (between day one and day 124) for the same group are significantly different (P<0.05)

The results indicated a reduction in both Staphylococcus aureus and E. coli across all three groups. However, the rate of reduction was notably faster for Staphylococcus aureus, which was completely eliminated (from 2.3±0.02 and 2.3±0.03 log10cfu/g at the beginning of the experiment (initial count at the 1st day) for G1 and G3 respectively, and 2.28±0.02 for G2 and decreased continuously to <1 log CFU/g by the 42nd day of storage till the end of the experiment (124 days).At the same time, B. lactis In contrast, E. coli counts in non-treated control vacuum-packed samples (G1) decreased form 1.95±0.01, t0 1.79 log10CFU/g ± 0.05 on day 112, which considered within the acceptable limit. However, by day 124, E. coli count escalated to 2.6 log10CFU/g ± 0.03, exceeding the acceptable limit of (2 log10 CFU/g) as per the 2021 Egyptian Food Safety Authority (EFSA) parameters which are derived from relevant international food safety standards. In contrast, both G2 and G3 treatments maintained acceptable E. coli counts up to day 124 of storage (1.04±0.08 and 0.9±0.08) respectively, with (G3) treated with UV-partially inactivated B. lactis demonstrating a lower E. coli and B. lactis counts compared to G2 which treated with active form of B. lactis.

Kanjee and Houry (2013) assert that E. coli exhibits greater acid tolerance compared to Staphylococcus aureus. The remarkable ability of E. coli to survive extremely low pH conditions suggests its superior capacity to withstand the acidity generated by Bifidobacteria in vacuum-packed meat. Adaptation to acid stress is a critical factor in the transmission of intestinal *Escherichia coli*, which employs a diverse array of physiological, metabolic, and proton-consuming acid resistance mechanisms to survive acid stresses as low as pH 2.0. This aligns with the findings of the present study, where E. coli persisted until the conclusion of the experimental period, while Staphylococcus aureus was completely eliminated. In this regard, Protonated acids are capable of entering microbial cells, subsequently dissociating into protons and their corresponding ions. This dissociation leads to an increase in intracellular acidity, thereby accelerating metabolic disorders within the cells (Trček et al. 2015; Geng et al. 2017).

Furthermore, in response to acid stress, microorganisms have evolved sophisticated mechanisms at both physiological and molecular levels to ensure their survival and adaptation (Fernández-Niño et al. 2015; Hosseini Nezhad et al. 2015; Liu et al. 2015; Ju et al. 2016). Concurrently, a variety of approaches have been employed to elucidate these acid tolerance mechanisms in different microbes across various levels (Sandoval et al. 2011; Zhai et al. 2014; Lee et al. 2015; He et al. 2016; Hu et al. 2017).

E. coli also implements physiological changes, such as modifications to its cell membrane and outer membrane porins, to reduce the influx of protons into the cell. It also utilizes chaperone proteins (e.g., Hsp31, Dps) to protect cytoplasmic and periplasmic proteins and DNA from acid-induced damage (Kanjee and Houry, 2013). Some systems couple proton efflux to energy generation through components of the electron transport chain, and under anaerobic conditions, the formate hydrogen lyase complex can convert cytoplasmic protons to hydrogen gas (Kanjee and Houry, 2013). In contrast, Staphylococcus aureus is a versatile pathogen that colonizes various host niches, some of which are acidic (e.g., skin, vagina, within macrophages) (Zhou, 2019). While S. aureus does possess acid resistance mechanisms, they appear to be less robust and diverse than those found in E. coli. That supports the results in the present study.

Table 2 illustrates that the pH values of G1 (Control), G2 (B. lactis WT), and G3 (B. lactis UV-Mutant) declined from an initial 5.7 across all three groups on Day 1 to 3.1±0.1, 3.3±0.2, and 3.7±0.1 at the end of the experiment (124) days, respectively. A significant difference (P<0.05) was observed between the similar group at day one and day 124. Furthermore, Table 2 clearly indicates that Total Volatile Basic Nitrogen (TVB-N) levels increased during storage from 5.0 mg/100 g at the first day in all groups to 23±0.5, 18.5±0.4, and 15.8 ±0.4mg/100 g at 124 day for G1, G2, and G3, respectively. This signifies that G1 exceeded the permissible limit according to the aforementioned food safety authority at 124 days of storage, though it remained within limits until day 112. Highly significant differences (P<0.01) were observed between the data of each corresponding group separately at the 1st and 124 days. Meanwhile, Thiobarbituric Acid Reactive Substances (TBA) recorded 0.1 mg Malondialdehyde/kg for all groups on Day 1, with these values increasing to 0.82±0.04, 0.64±0.02, and 0.46±0.02 mg Malondialdehyde/kg for G1, G2, and G3at 124 days of storage, respectively. This indicates that TBA levels remained within the permissible limit (0.9 mg Malondialdehyde/kg) for all groups throughout the study. A significant difference (P<0.05) was noted between the beginning and the end of the experiment for the corresponding groups and also between the three groups at 124 days of storage regarding both TVB-N and TBA.

Table 2 Statistical Analysis of Physico-chemical parameters in Vacuum-Packed Chilled Stored Meat

Day	Sample Type	pH	TVN (mg N/100g)	TBA (mg MDA/kg)
1st Day	G1	5.7^{a}±0.1	5.0^{d}±0.2	0.1^{g}±0.01
	G2	5.7^{b}±0.1	5.0^{e}±0.2	0.1^{h}±0.01
	G3	5.7^{c}±0.1	5.0^{f}±0.2	0.1^{i}±0.01
14	G1	5.4±0.1	7.0±0.3	0.18±0.01
	G2	5.2±0.1	6.5±0.3	0.16±0.01
	G3	5.3±0.1	6.2±0.3	0.14±0.01
28	G1	5.1±0.1	9.0±0.4	0.26±0.01
	G2	4.8±0.1	8.0±0.3	0.22±0.01
	G3	4.9±0.2	7.4±0.3	0.18±0.02
42	G1	4.6±0.2	11.0±0.2	0.34±0.02
	G2	4.4±0.2	9.5±0.3	0.28±0.01
	G3	4.2±0.1	8.6±0.3	0.22±0.01
56	G1	4.1±0.2	13.0±0.3	0.42±0.02
	G2	4.2±0.2	11.0±0.3	0.34±0.01
	G3	4.0±0.3	9.8±0.4	0.26±0.01
70	G1	4.0±0.2	15.0±0.3	0.5±0.01
	G2	4.1±0.2	12.5±0.3	0.4±0.02
	G3	4.0±0.1	11.0±0.4	0.3±0.01
84	G1	3.9±0.1	17.0±0.4	0.58±0.01
	G2	4.0±0.2	14.0±0.4	0.46±0.02
	G3	4.0±0.2	12.2±0.4	0.34±0.02
98	G1	3.6±0.2	19.0±0.5	0.66±0.03
	G2	3.9±0.3	15.5±0.5	0.52±0.01
	G3	3.9±0.2	13.4±0.4	0.38±0.02
112	G1	3.3±0.2	19.7±0.4	0.74±0.02
	G2	3.8±0.1	17.0±0.3	0.58±0.03
	G3	3.9±0.2	14.6±0.4	0.42±0.01
124	G1	3.1^{a}±0.1	23.0^{d}±0.5	0.82^{g}±0.04
	G2	3.3^{b}±0.2	18.5^{e}±0.4	0.64^{h}±0.02
	G3	3.7^{c}±0.1	15.1^{f}±0.4	0.46^{i}±0.02

Means with different superscripts in the same column (between day one and day 124) for the same group are significantly different ($P<0.05$)

Additionally, significant differences were observed among all three groups regarding pH, TVB-N and TBA levels at the first day and 124 days of storage for each corresponding group separately, indicating that G3 performed best, followed by G2, and then G1.

Bacteria, like *Escherichia coli*, face a significant challenge in maintaining their internal stability when external conditions, particularly pH levels, fluctuate. Most bacteria strive to keep their internal pH relatively stable and neutral, even when the outside environment changes drastically. This is a complex process that involves constantly managing

proton gradients (Siegumfeldt et al. 2000). E. coli which considered acid-tolerant bacteria, allow their internal pH to decrease as the environment acidifies, but they always keep it higher than the external pH. This delicate balance is crucial. If the external acid concentration becomes too high, their internal pH can drop sharply, disrupting the vital pH equilibrium. This leads to damage to proteins and DNA, ultimately causing the cells to die (Li et al. 2024). Therefore, maintaining pH homeostasis or a stable internal pH is absolutely essential for microorganisms to survive and thrive in acidic conditions. A remarkable example is E. coli, which can endure for several hours at a pH as low as 2 or 3, even though its optimal growth limit is around pH 4.5. This highlights the reason E. coli was not eliminated, but rather its population began to proliferate during the later stages of the experiment (124 days of storage).

Furthermore, the persistence of E. coli until the end of the experiment aligns with the findings of Kanjee and Houry (2013), who stated that *Escherichia coli* and Staphylococcus aureus, while both common bacteria, differ significantly in their ability to tolerate and survive in acidic environments. E. coli is an enteric bacterium, naturally inhabiting the digestive tracts of mammals, an environment characterized by extreme pH fluctuations, particularly the highly acidic conditions of the stomach (pH 1.5-3.0). Over evolutionary time, E. coli has developed a highly specialized and redundant set of acid resistance (AR) systems to overcome this challenge. Specifically, E. coli possesses at least six distinct acid resistance systems (AR1-AR6), each activated under specific conditions (e.g., glucose-repressed, glutamate, arginine, lysine, ornithine, and serine-dependent).

TVN quantifies volatile amines; like ammonia, trimethylamine, and dimethylamine produced by bacterial and enzymatic breakdown of proteins. A TVN value above 15–20 mg N/100 g is commonly recognized as spoiled for chilled meat. Vacuum or super-chilled meat often retains freshness longer, but once TVN exceeds 20 mg/100 g, sensory quality deteriorates noticeably. According to table (2), TVN of G1 (Control) are steadily increases, nearing 23±0.5 mg/100g by Day 124; surpassing standard freshness limits, implying protein breakdown. Also, G2 (Active B. lactis) and G3 (UV-partially inactivated B. lactis): contained TVN within the permissible limit (18.5±0.4 and 15.1±0.4 respectively, at Day 124, with G3 demonstrating the slowest increase (15.1 mg); indicating superior protein integrity and lower proteolytic activity, this aligns with the findings of Marcelli et al. (2024).

TBA is marker of lipid oxidation it measures malondialdehyde and related aldehydes, reflecting the degree of lipid peroxidation. Typical threshold ranges (≤0.9 mg MDA/kg) are set for acceptability; higher values denote rancidity and off-flavors. G1: TBA increases to 0.82±0.04 mg/kg by Day 124; indicating TBA within the permissible limits, but very close to the maximum acceptable rancidity limit (≤0.9). G2: Peaks at 0.64 mg/kg well within acceptable limits. G3: Shows the lowest TBA (0.46 mg/kg), signaling robust defense against lipid oxidation.

G3's reduced TBA levels as compared with G2 suggest heightened antioxidant activity, possibly due to UV-enhanced expression of antioxidative enzymes or stabilizing metabolites produced by the strain. This aligns with literature on probiotic and UV-mutant antioxidative benefits in meat preservation; Masoumi et al. (2022) and Putri et al. (2024) found that meat treated with probiotics showed lower lipid oxidation products during the storage period, which was in agreement with previous studies. Align with findings showing TVN levels drive freshness declines and sensory rejection in chilled meat (20 mg threshold).

TBA values reflect progressive oxidation control by probiotic inoculation, with G3's low TBA reaffirming UV-partial inactivated antioxidant enhancement. In chemical terms, G3 exhibits superior preservation, maintaining protein integrity and preventing lipid oxidation better than G2, which still outperforms the untreated control. These outcomes underscore the efficacy of the UV-partially inactivated B. lactis strain as a potent natural preservative in extending the shelf-life and improve the chemical criteria of meat under extended cold storage and this is in agreement with Marcelli et al. (2024) and Putri et al. (2024).

G2 and especially G3 effectively suppress proteolysis and microbial decarboxylation pathways, preserving protein quality. The UV-inactivated strain stronger effect could be due to antimicrobial metabolites or competitive exclusion.

Table 3 Sensory attributes of examined groups

Storage day	Sample Type	Odour	Texture	Tenderness	Color	Juiciness
1st Day	G1	9.0^{ab}±0.2	9.0^{ab}±0.2	9.0^{ab}±0.1	9.0^{ab}±0.1	9.0
	G2	9.0^{cd}±0.2	9.0^{cd}±0.2	9.0^{cd}±0.1	9.0^{cd}±0.1	9.0
	G3	9.0^{ef}±0.2	9.0^{ef}±0.2	9.0^{ef}±0.1	9.0^{ef}±0.1	9.0
14	G1	8.7±0.2	8.6±0.3	8.6±0.1	8.6±0.1	8.4
	G2	8.8±0.2	8.7±0.2	8.7±0.1	8.7±0.2	8.6
	G3	8.8±0.2	8.8±0.2	8.8±0.1	8.8±0.1	8.8
28	G1	8.3±0.3	8.2±0.1	8.2±0.2	8.2±0.1	8.0
	G2	8.5±0.2	8.4±0.2	8.4±0.2	8.4±0.2	8.3
	G3	8.6±0.2	8.6±0.2	8.6±0.2	8.6±0.2	8.5
42	G1	8.0±0.2	7.8±0.2	7.8±0.2	7.9±0.3	7.6
	G2	8.2±0.2	8.1±0.1	8.1±0.2	8.2±0.1	7.9
	G3	8.5±0.2	8.3±0.2	8.3±0.2	8.4±0.1	8.3
56	G1	7.6±0.3	7.4±0.2	7.4±0.1	7.5±0.2	7.2
	G2	8.0±0.2	7.8±03	7.8±0.1	7.9±0.1	7.6
	G3	8.3±0.2	8.1±0.2	8.1±0.1	8.2±0.2	8.0
70	G1	7.2±0.4	7.0±0.2	7.0±0.1	7.1±0.1	6.8
	G2	7.8±0.2	7.5±0.2	7.5±0.2	7.6±0.1	7.3
	G3	8.1±0.1	7.9±0.2	7.9±0.1	8.0±0.1	7.7
84	G1	6.9±0.2	6.6±0.1	6.6±0.1	6.7±0.2	6.4
	G2	7.5±0.2	7.2±0.1	7.2±0.2	7.3±0.3	6.9
	G3	7.9±0.2	7.7±0.1	7.7±0.1	7.8±0.1	7.4
98	G1	6.6±0.1	6.2±0.1	6.2±0.3	6.3±0.2	5.9
	G2	7.2±0.2	6.9±0.2	6.9±0.1	7.0±0.2	6.5
	G3	7.7±0.3	7.5±0.1	7.5±0.1	7.6±0.1	7.0
112	G1	6.2±0.2	5.8±0.2	5.8±0.1	6.0±0.1	5.4
	G2	7.0±0.2	6.6±0.1	6.6±0.1	6.8±0.1	6.0
	G3	7.6±0.2	7.2±0.1	7.2±0.1	7.4±0.1	6.6
124	G1	4.8^{ab}±0.2	5.4^{ab}±0.3	5.5^{ab}±0.3	5.6±0.3	5.0
	G2	6.8^{cd}±0.4	6.3^{cd}±0.1	6.25^{cd}±0.2	6.5±0.1	5.8
	G3	7.4^{ef}±0.2	7.0^{ef}±0.1	7.0^{ef}±0.1	7.2±0.1	6.4

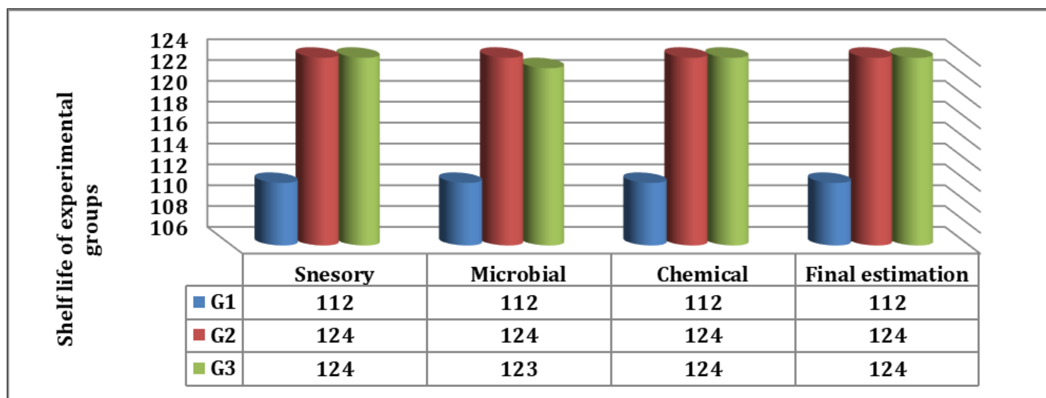
Means with different superscripts in the same column (between day one and day 124) for the same group are significantly different (P<0.05)

Table 3 reveals significant differences in sensory attributes for each group both between the beginning and end of the experiment (124 days), and among the three groups at Day 124. Specifically, for odour, Group 1 initially scored 9 ±0.2 but declined to 4.8±0.2 at the end of the experiment with appearance of highly sour odour, while G2 recorded 9±0.2 at the 1st day of storage and 6.8±0.4, and G3 registered 9±0.2 and 7.4±0.2. Regarding texture, all groups initially scored 9±0.2 at the 1st day, then decreased to 5.4 ±0.3, 6.3±0.1, and 7.0±0.1 for G1, G2, and G3 respectively, by 124 day of storage. Tenderness also started at 9±0.1 for all groups, but by Day 124, it reached 5.5±0.3, 6.25±0.2, and 7.0±0.1 for the respective groups. Similarly, color scored 9±0.1 for all groups initially, but dropped to 5.6±0.3, 6.5±0.1, and 7.2±0.1

for G1, G2, and G3, respectively. Juiciness is a crucial sensory attribute reflecting moisture release during chewing, closely connected to the meat's water-holding capacity (WHC). In this table, G3 (UV-partially inactive B. lactis) consistently shows higher juiciness scores than G2 and G1 at every time point, particularly in later stages (e.g., 7.7 vs. 7.3 and 6.8 at Day 70; 6.4 vs. 5.8 and 5.0 at Day 124). This indicates improved moisture retention and texture, likely due to the strain's capacity to reduce oxidative and microbial degradation factors that otherwise weaken WHC during cooking.

All sensory metrics (odour, texture, tenderness, color, juiciness) decline gradually over time, with G3 maintaining the highest values, followed by G2, and then G1. The cohesive pattern across attributes in G3 suggests an integrated protective effect of the UV-partially inactivated B. lactis, possibly by reducing muscle protein degradation and oxidative changes. Higher juiciness in G3 indicates stronger WHC maintenance, likely due to reduced protein denaturation and oxidation effects commonly observed with probiotic interventions.

The alignment between juiciness and other sensory traits underscores broader quality retention, facilitated by the combined actions of probiotic metabolism and UV-inactivation enhanced strain resilience. Our results agree with Hu et al., 2022 who stated that, lactic acid bacteria are incorporated into meat product formulations and, shortly after beginning their metabolic activity, they notably alter key sensory properties such as flavor, aroma, and texture. Additionally, they enhance product safety by generating antimicrobial peptides and other inhibitory compounds. Acidification significantly influences the sensory characteristics of the product by contributing a tangy flavor. The associated pH reduction facilitates meat protein coagulation and enhances color development reactions, as noted by Martin and Dea Lindner (2022). While flavor development is mainly attributed to lactic acid production, other compounds generated through hetero fermentation; such as acetic acid, ethanol, carbon dioxide, and pyruvic acid; may also contribute to the overall product profile (Zdolec et al., 2022).



	Snesory	Microbial	Chemical	Final estimation
G1	112	112	112	112
G2	124	124	124	124
G3	124	123	124	124

Figure 1 Estimated shelf life through the deteriorative criteria and bacterial proliferation

Control G1 exhibits Shelf-life limited to 112 days across all criteria (sensory, microbial, chemical). This suggests that, under zero±1 °C storage without probiotic treatment, deterioration occurs uniformly after day 112 and before 124 days of storage. Reflects typical spoilage progression: sensory quality decline, microbial proliferation beyond acceptable limits, and chemical oxidation coincide.

Active form of Bifidobacterium lactis(G2) exhibits full stability up to day 124 in all measured aspects. Indicates that active type B. lactis significantly enhances shelf-life compared to control. This enhancement likely stems from its moderate antimicrobial and antioxidant activity, delaying spoilage.

Ultraviolet-partially-inactivated B. lactis (G3) showed great stability in sensory and chemical indicators through day 124. Microbial shelf-life slightly limited to the end of the experiment (124 storage days), likely due to total bacterial count nearing but not exceeding the spoilage threshold. Despite this minor lag, the final shelf life remains 124 days, meaning that deterioration wasn't triggered by accelerated microbial growth.

Both probiotic treatments offer substantial shelf-life extension compared to control, highlighting their bio preservative effectiveness. G2 and G3 outperform G1, but interestingly G2 (active strain) showed no microbial constraint; potentially due to the parameters used in the model (e.g., microbial growth rate assumptions). G3's slight microbial limit at day 124 reflects realism; even the UV-partially inactivated strain mutant enhanced stress tolerance may be slightly offset by slower microbial control ramp-up. Nonetheless, overall shelf life remains determined by sensory and chemical stability,

not microbial thresholds they ensure greater safety and stability of some compounds during shelf life. Our results agree with Shao et al., 2024 who stated that probiotics ensure greater safety and stability of some compounds during shelf life.

Bacteriocins are antimicrobial peptides produced by the ribosomal machinery of lactic acid bacteria and other types of microorganisms (Choi et al., 2023). They are generally described as having inhibitory activities versus other closely related bacterial species. Bacteriocins were extensively explored regarding their applications as natural preservatives in food to extend shelf life and enhance the safety stability of food products. Consequently, bacteriocins can provide an additional layer of protection against microbial contamination. This can help extend the shelf life of packaged foods (Abouloifa et al., 2024).

Lactic acid producing bacteria and their metabolites, not only bacteriocins as bio preservatives, but antioxidant properties of beneficial strains in meat and meat products for extending their shelf life (Carneiro et al., 2024).

4. Conclusion

The current study's results indicate that G1 (control) samples remained acceptable until 112 days of cold storage in vacuum-packed meat at zero ±1 OC. However, by day 124 of storage, these samples became unfit for consumption based on bacteriological (E. coli count: 2.6 ± 0.03), biochemical (TVB-N: 23 ± 0.5 mg/100g), and sensory (4.8 ± 0.2) parameters, exhibiting a very strong sour odor. In contrast, both G2 and G3 remained acceptable across all parameters until the end of the experimental period (124 days). Notably, G3 samples, treated with UV-inactivated Bifidobacterium lactis, showed significantly improved results ($P<0.05$) compared to G2 samples (treated with active Bifidobacterium lactis) across all sensory, chemical, and bacteriological standard parameters.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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