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THE INFLUENCE OF YOGURT MARINADE IN COMBINATION WITH ESSENTIAL OILS ON THE MICROBIOLOGICAL QUALITY OF BEEF

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ABSTRACT

This study investigated the influence of yogurt marinade enriched with coriander and basil essential oils (EOs) on the microbiological quality of beef during refrigerated storage (4 ± 1 °C). Specific objectives included evaluating the effects of air exposure (vacuum-packed vs aerobic storage), marinade addition (with or without), and EOs addition (with or without) on selected culturable bacteria. The selected groups of bacteria monitored were coliform bacteria (CB), lactic acid bacteria (LAB), *Pseudomonas* spp., and total viability count (TVC). The quantitative abundance of selected microbial groups was evaluated by dilution plating method and the identification of microorganisms was assessed using MALDI-TOF mass spectrometry. The results demonstrated that the specific combination of yogurt marinade, vacuum packaging and EOs, significantly reduce bacterial counts and diversity in beef samples. These combinations showed significantly lower TVCs on the 20th day of storage ($p \leq 0.05$) compared to control and other treatments. The TVCs likely decreased not only due to the antimicrobial properties of the added EOs but also as a result of the beneficial LAB present in the yogurt marinade. Overall, the storage condition played a crucial role, with vacuum-packed samples exhibiting lower microbial counts compared to aerobically stored samples, regardless of the marinade or EO treatment. The Lactobacillaceae family, with *Latilactobacillus sakei* as a representative, was the most abundant bacterial group. *L. sakei* was consistently present across all samples at every time point, indicating its dominance in the microbial community of the studied beef samples. *Ralstonia pickettii* from the Burkholderiaceae family was the second most prevalent species. The study provides valuable insights into the microbial quality of marinated beef products aligning with general consumer preferences for natural food additives that can prolong the shelf life of foods.

Keywords: marinade, essential oil, beef, vacuum packaging, microbiological quality

INTRODUCTION

Maintaining the microbiological safety of beef meat products is a critical concern for both the food industry and consumers. One of the serious concerns is the threat of foodborne illnesses, which can lead to severe health risks. Another concern arises from the spoilage of meat since meat is a highly perishable commodity. Both concerns may lead to substantial economic losses. Preservation techniques play a crucial role in extending the shelf life and maintaining the safety of perishable foods. Although traditional preservation methods of meat exist, exploring natural alternatives over synthetic additives has gained increasing attention among consumers (**Hussain & Dawson, 2013; Singh *et al.*, 2020; Sofos, 2014**). Essential oils (EOs) derived from plants are becoming more popular as natural alternatives due to their natural origin, and antimicrobial and antioxidant properties. These features make them promising food additives that can prolong the shelf life of various foods, including meat and beef (**Nychas & Tassou, 2014**). The EOs of coriander and basil have been reported to contain bioactive compounds (such as linalool, cineole, and eugenol), which exhibit broad-spectrum of well-known antimicrobial activity (**Al-Khayri *et al.*, 2023; Elgndi *et al.*, 2017; Kačániová *et al.*, 2020; Nadeem *et al.*, 2022; Šojić *et al.*, 2023**). Unfortunately, one of the drawbacks of using plant EOs as a shelf-life prolonging additive is their sensory influence on treated foods, particularly at higher concentrations (**Pateiro *et al.*, 2018**). However, coriander and basil are widely used in various cuisines, with flavours and aromas that are generally well-accepted and preferred as a meat flavouring. Using these EOs in meat marinades could enhance the sensory appeal of meat products without introducing unfamiliar or undesirable flavours. Research conducted on various EOs indicates that using low concentrations of a carefully chosen EO in food does not have a negative impact on taste or smell and may even enhance the sensory experience for consumers (**Leite *et al.*, 2022; Mohamed & Mansour, 2012; Olaniran *et al.*, 2024; Ruiz-Hernández *et al.*, 2023**). Yogurt-based marinades, invented for their flavour and structure-enhancing properties, have also emerged as a promising approach for improving the microbiological quality of meat (**Ehsanur Rahman *et al.*, 2023**). Yogurt's potential to reduce unwanted microbial growth stems from its inherent acidity and the presence of beneficial bacteria, primarily lactic acid bacteria (LAB) (**Latoch *et al.*, 2023**). The pH level of yogurt made from cow's milk typically falls within the range of approximately 4.2 ± 0.3 (**Miocinovic *et al.*, 2016; Peters *et al.*, 2023; Vargas *et al.*, 2008**). During storage, the pH of yogurt can even slightly decrease (**Shah *et al.*, 1995**). The acidic conditions prevent the growth of many harmful bacteria. In addition to the acidic nature of yogurt, the beneficial bacteria present in yogurt can further contribute to its preservative effect through competitive inhibition with spoilage microbiota and pathogens. The production of antimicrobial bacteriocins by these beneficial bacteria should also be considered as a contributing factor. When proper hygiene practices and storage conditions are followed, yogurt bacteria can therefore actively help to prevent the growth of harmful microorganisms (**Bhattacharya *et al.*, 2022; Da Costa *et al.*, 2019; Lahiri *et al.*, 2022**). There are few studies and reviews assessing the combined effect of EOs and biocontrol agents such as LAB and/or bacteriocins (**Abdollahzadeh *et al.*, 2014; Bukvicki *et al.*, 2023; Osaili *et al.*, 2021; Oussama *et al.*, 2023; Sarmast *et al.*, 2023**). However, the potential overall yogurt marinade impact on the microbiological quality of meat has not been extensively studied, particularly in combination with EOs. Therefore, this study aimed to investigate the influence of yogurt marinades enriched with basil and coriander EOs on the microbiological quality of beef during refrigerated storage and their synergistic effects with air exposure (vacuum-packed vs. packed aerobically). This study could contribute to the knowledge about treated safe meat products with longer shelf life aligning with consumer preferences for natural food additives.

MATERIAL AND METHODS

Sample preparation

The cold-stored ground beef used for analysis was obtained from a local hypermarket (Nitra, Slovakia). The meat was processed immediately on the day of purchase after transport to the laboratory. Beef samples were analysed in 5 g portions. All analyses were performed on raw uncooked meat samples subjected to various treatments under strict antiseptic conditions. A total of 8 following groups of samples were analysed: (1) untreated control samples stored under aerobic conditions (C); (2) untreated samples in vacuum-sealed packaging (CV); (3) marinade treated samples stored under aerobic conditions (MM); (4) marinade treated samples stored in vacuum-sealed packaging (MV); (5) marinade treated samples stored under aerobic conditions with basil EO (MB); (6) marinade treated samples stored in vacuum-sealed packaging with basil EO (MBV); (7) marinade treated samples stored under aerobic conditions with coriander EO (MC); and (8) marinade treated samples stored in vacuum-sealed packaging with coriander EO (MCV). Vacuum packaging was performed using a vacuum packaging machine (Concept, Choceň, Czech Republic). The essential oils (EOs) of the basil (*Ocimum basilicum* L.) and coriander (*Coriandrum sativum* L.) plants were purchased from Hanus - Herbal Preparations, Ltd., Slovakia. Both plant EOs were used at 1% (w/v) concentration. Yogurt marinade was prepared using (g/100g meat): full fat yogurt (20.0 g); olive oil (3.5 g); crushed tomatoes (5.0 g; tomatoes were washed with water and disinfected with 70 % ethanol before use); vinegar (3.5 g); crushed onion (15.0 g); salt (2.0 g) and seasoning - black pepper (0.8 g); paprika powder (1.5 g). All samples were cold stored at 4 ± 1 °C for a certain period.

Microbiological analysis

All microbiological analyses were done in triplicates. The Total Viable Count (TVC), Coliform Bacteria (CB), Lactic Acid Bacteria (LAB), and *Pseudomonas* spp. counts were monitored. Identification of cultivated isolates was performed using mass spectrometry MALDI TOF-MS Biotyper (Bruker Daltonics, Germany). Microbial testing was conducted on the 1st (Day 1); 10th (Day 10); and 20th (Day 20) day of storage. On the day of purchase (Day 0) were analysed just untreated control (C) samples.

To determine the number of CFU.g^{-1} present in the samples a dilution plating method was conducted. Serial dilutions were prepared up to 10^{-5}. Surface inoculation was performed using 100 µl of inoculum. The initial dilution (10^{-1}) was prepared by homogenizing the sample (5g) with 45 ml of physiological saline solution (0.9% NaCl). This was followed by a 30-minute homogenization process using an orbital shaker (GFL Orbital Shaker 3005, Germany) at 200 RPM.

Four types of cultivation media were used for the cultivation of microorganisms. Plate Count Agar (PCA, Oxoid, United Kingdom) for the cultivation of Total Viable Counts (TVC); Violet Red Bile Lactose Agar (VRBL, Oxoid, United Kingdom) for the cultivation of Coliform Bacteria (CB); De Man–Rogosa–Sharpe agar (MRS, Oxoid, United Kingdom) for the cultivation of Lactic Acid Bacteria (LAB); and Pseudomonas Agar (PA, Oxoid, United Kingdom) for the cultivation of *Pseudomonas* spp.

The samples were inoculated onto Petri dishes containing the appropriate media and then incubated in a thermostat (PCA at 30 °C for 72 hours; VRBL at 37 °C for 24 hours; MRS at 37 °C for 48 hours with 5% CO_2; and PA for 37 °C for 48 hours). Numbers of plate counts (CFU.g^{-1}) were converted to common logarithm (log10) values. Colonies of cultivated bacteria were suspended in 75% ethanol (Ethyl Alcohol Solvanal 99.8%, Centralchem, Slovakia) and stored at −20 ± 1 °C until the following analyses. Before the next steps of analyses, samples were centrifuged (12 000 RMP, 1 min). The supernatant was discarded, and the pellet was mixed with 30 µl of 70% acetonitrile (Sigma-Aldrich, USA) and 30 µl of formic acid (Honeywell, USA). The mixture was centrifuged (12 000 RMP, 1 minute) and 1 µl of aqueous phase was utilized for mass spectrometry identification.

Isolates identification

The MALDI-TOF MS matrix mixture used for analyses was α-cyano-4-hydroxycinnamic acid (HCCA) (Sigma-Aldrich, USA) diluted in an organic solvent (10 mg/ml). The composition of the organic solvent was as follows: 50% acetonitrile (Sigma-Aldrich, USA), 47.5% ultrapure distilled water, and 25% trifluoroacetic acid (Sigma-Aldrich, USA). A 1 µl of aqueous phase of previously prepared sample solution was transferred to the MALDI-TOF MS metal plate (Bruker Daltonics, Germany) and cowered with 1 µl of matrix mixture. The matrix-coated sample was air-dried and analysed using a MALDI-TOF MS Biotyper instrument (Bruker Daltonics, Germany) with MALDI Biotyper 3.0 software (Bruker Daltonics, Germany).

Statistical analysis

This study utilized fundamental descriptive statistical methods, such as calculating the mean and standard deviation, to analyse the findings. Furthermore, advanced statistical techniques, including the Analysis of Variance (ANOVA), Tukey's Honestly Significant Difference (HSD) test, and Chi-squared test were employed to compare the results and identify any statistically significant differences among the data groups. All statistical analyses were performed using XLSTAT software (developed by Addinsoft, France). Statistical significance was established at a significance level of $p \leq 0.05$.

RESULTS

Numbers of Total Viable Counts (TVCs), Coliform Bacteria (CB), Lactic Acid Bacteria (LAB), and *Pseudomonas* spp. are presented in Table 1, Table 2, Table 3, and Table 4. Speaking of TVCs, the treatments vary in their CFU.g^{-1} counts over time, which suggests that different storage conditions and treatments may have an impact on the growth of colonies. The lowest CFU.g^{-1} is observed in MCV samples accounting for 2.777 ± 0.025 log CFU.g^{-1} on Day 1 and the highest 4.92 ± 0.026 log CFU.g^{-1} in C samples on Day 20. Most treatments show an increase in CFU.g^{-1} from Day 1 to Day 20, indicating a growth trend over time. MBV appears to be the most effective treatment, with the lowest mean CFU.g^{-1}. In this sense, MCV is the second most effective. Vacuum-sealed treatments (CV, MV, MBV, MCV) generally perform better than their aerobic counterparts. All marinated treatments (MM, MV, MB, MBV, MC, MCV) show lower CFU.g^{-1} compared to the treatments without marinade addition (C and CV). The untreated control in aerobic conditions (C) has the highest mean CFU.g^{-1}, indicating the highest microbial growth. The growth rate appears to be more pronounced in treatments like C and CV, while overall other treatments show a slower increase. These patterns suggest that some treatments exhibit more significant growth than others. For Days 1, 10, and 20, the ANOVA results show statistically significant differences between the treatment groups ($p \leq 0.05$). These statistically significant differences were observed among the differently treated samples at each time point. The control group (C) consistently shows the highest counts and remains significantly different from other treatments from Day 10 onwards. The differences between treatments become more pronounced over time, with the most diverse groupings on Day 10. By Day 20, some treatments that were significantly different on Day 10 were no longer significantly different. MBV and MCV consistently show lower counts compared to other treatments, especially in the later days. To determine which conditions have the most substantial effect on reducing microbial growth, here's how the data was grouped: (a) impact of air exposure (aerobic conditions: C, MM, MB, MC vs vacuum: CV, MV, MBV, MCV); (b) impact of EOs addition (without EO: C, CV, MM, MV vs with EO: MB, MBV, MC, MCV); (c) impact of marinade addition (without marinade: C, CV vs with marinade: MM, MV, MB, MBV, MC, MCV); (d) combined impact of marinade addition, EOs addition, and vacuum (with marinade, EOs, and vacuum: MCV, MBV vs the rest: C, CV, MM, MV, MB, MC). To determine the most and least effective conditions, we looked at the mean bacterial counts on Day 20, as this represents the endpoint of the study. Lower bacterial counts indicate more effective treatments in controlling bacterial growth. According to this, the most effective treatments are those with the combination of marinade, vacuum packaging and the addition of EOs (MCV, MBV). The least effective treatments are samples exposed to air (C, MM, MB, MC).

CB were identified in only one treatment group, namely the C group, with 2.173 ± 0.015 log CFU.g^{-1} on Day 20. CB were not detected in other treatments at any timepoint (≤ 1 log CFU.g^{-1} when using initial ten-fold serial dilution (10^{-1}) for plate counts).

Speaking of LAB counts, all treatments show an increase in CFU.g^{-1} from Day 1 to Day 20. The lowest log CFU.g^{-1} is observed in MBV samples accounting for 1.993 ± 0.006 on Day 1 and the highest 3.12 ± 0.02 log CFU.g^{-1} in MM samples on Day 20. The treatment with the highest mean CFU.g^{-1} across all days is MB and the lowest is C. The differences between groups are more pronounced on Days 1 and 10 compared to Day 20. According to ANOVA, the p-values are extremely low for all days, indicating statistically significant differences among the treatment groups on each day. As with TVC, to determine the most and least effective conditions, we grouped treatments and looked at the mean bacterial counts on Day 20. According to this, the most effective treatments are those without marinade (C, CV). The least effective treatments are samples exposed to air (C, MM, MB, MC).

Pseudomonas spp. was identified only in the C group, with counts ranging from 1.43 ± 0.02 log CFU.g^{-1} on Day 0 to 1.997 ± 0.006 log CFU.g^{-1} on Day 20. *Pseudomonas* spp. was not detected in other groups (≤ 1 CFU.g^{-1}, when using initial ten-fold serial dilution (10^{-1}) for plate counts).

According to the mass spectrometry results, samples contain 14 distinct bacteria and 9 unique families. The data in Table 6 represents the distribution of reliably identified bacterial species in differently treated samples. All identified species along with their families are graphically depicted in Figure 1. The bacterial community composition at family level is as follows (percentages in the brackets are rounded to two decimal places): Bacillaceae (2.30%), Burkholderiaceae (14.94%), Carnobacteriaceae (6.90%), Hafniaceae (9.20%), Lactobacillaceae (45.98%), Micrococcaceae (1.15%), Pseudomonadaceae (6.90%), Staphylococcaceae (4.60%), Yersiniaceae (8.05%). The most abundant family of bacteria is the Lactobacillaceae family accounting for nearly half of all isolates identified (45.98%). Within this family, only one species of bacterium has been reliably identified, namely *Latilactobacillus sakei*. *Ralstonia pickettii* from the Burkholderiaceae family is the second most abundant (14.94%) species. *Latilactobacillus sakei* is consistently present across all samples at any time point, indicating its dominance in the microbial community. Similarly, *Ralstonia pickettii* also frequently appears in the majority of the samples (except MC, MCV, MB). The dominant presence of these bacteria in samples suggests that they play a significant role in the microbial community of the samples. On the other hand, the least represented families are the Carnobacteriaceae (1.15%) family with *Carnobacterium maltaromaticum* as representative, and Micrococcaceae (1.15%) family with *Micrococcus luteus* as representative. Furthermore, several other species such as *Bacillus subtilis*, *Bacillus mojavensis*, and *Serratia proteamaculans* are also minor components of the bacterial community of samples (1.15% each). Nevertheless, these less abundant species still contribute to the overall microbial ecology of samples. However, the abundance and presence/absence of any identified isolates do not show a clear pattern across the different time points and treatments.

Vacuum packaging seems to slightly reduce the diversity of bacterial species and families present. Samples exposed to air during storage showed the presence of 10 different species. Vacuum treatments showed the presence of 9 different species. Marination seems to increase the diversity of bacterial species and families present. Non-marinated treatments showed the presence of 8 different species. Marinated treatments showed the presence 11 of different species. The presence of EO appears to reduce the diversity of bacterial species and families. Treatments without EO showed the presence of 11 different species. Treatments with EO showed the presence of 7 different species. These findings suggest that vacuum packaging has a slightly reducing effect on bacterial diversity, marination increases bacterial diversity, and the EO addition has the most substantial impact on bacterial diversity in terms of reducing bacterial diversity. Based on the Chi-squared test, we can conclude that there is a statistically significant difference in bacterial presence between samples with and without EO addition ($p \leq 0.05$). This suggests that the addition of EO has a significant effect on the bacterial community structure. The addition of marinade or exposure to air has no significant effect on bacterial diversity based on the Chi-squared test.

Table 1 Total Viable plate Counts (TVC)

Treatment	**Count ± SD (log CFU.g^{-1})**			
	Day 0	**Day 1**	**Day 10**	**Day 20**
C	2.82 ± 0.026	3.02 ± 0.02^{a}	3.963 ± 0.023^{a}	4.92 ± 0.026^{a}
CV	-	$3 \pm 0.01^{a, b}$	3.777 ± 0.021^{b}	$4.1 \pm 0.01^{b, c}$
MM	-	3.023 ± 0.025^{a}	3.72 ± 0.02^{c}	4.11 ± 0.017^{b}
MV	-	$2.96 \pm 0.01^{b, c}$	3.5 ± 0.01^{d}	4.027 ± 0.021^{d}
MC	-	2.913 ± 0.021^{c}	3.593 ± 0.015^{e}	$4.103 \pm 0.006^{b, c}$
MCV	-	2.777 ± 0.025^{d}	3.39 ± 0.01^{f}	3.973 ± 0.021^{e}
MB	-	3.023 ± 0.025^{a}	3.68 ± 0.02^{c}	$4.06 \pm 0.01^{c, d}$
MBV		2.833 ± 0.015^{e}	3.447 ± 0.015^{g}	3.82 ± 0.02^{f}

Note: The plate counts, each carried out in triplicate, are displayed as mean values together with standard deviations (SD). According to Tukey's Honestly Significant Difference (HSD) test, data in the same column categorized by different letters are considered significantly different ($p \leq 0.05$). (C) untreated control samples stored under aerobic conditions; (CV) untreated samples in vacuum-sealed packaging; (MM) marinade treated samples stored under aerobic conditions; (MV) marinade treated samples stored in vacuum-sealed packaging; (MB) marinade treated samples stored under aerobic conditions with 1% basil EO; (MBV) marinade treated samples stored in vacuum-sealed packaging with 1% basil EO; (MC) marinade treated samples stored under aerobic conditions with 1% coriander EO; (MCV) marinade treated samples stored in vacuum-sealed packaging with 1% coriander EO.

Table 2 Coliform bacteria (CB) plate counts

Treatment	**Count ± SD (log CFU.g^{-1})**			
	Day 0	**Day 1**	**Day 10**	**Day 20**
C	≤ 1	$\leq 1^{a}$	$\leq 1^{a}$	2.173 ± 0.015^{a}
CV	-	$\leq 1^{a}$	$\leq 1^{a}$	$\leq 1^{b}$
MM	-	$\leq 1^{a}$	$\leq 1^{a}$	$\leq 1^{b}$
MV	-	$\leq 1^{a}$	$\leq 1^{a}$	$\leq 1^{b}$
MC	-	$\leq 1^{a}$	$\leq 1^{a}$	$\leq 1^{b}$
MCV	-	$\leq 1^{a}$	$\leq 1^{a}$	$\leq 1^{b}$
MB	-	$\leq 1^{a}$	$\leq 1^{a}$	$\leq 1^{b}$
MBV		$\leq 1^{a}$	$\leq 1^{a}$	$\leq 1^{b}$

Note: The plate counts, each carried out in triplicate, are displayed as mean values together with standard deviations (SD). According to Tukey's Honestly Significant Difference (HSD) test, data in the same column categorized by different letters are considered significantly different ($p \leq 0.05$). (C) untreated control samples stored under aerobic conditions; (CV) untreated samples in vacuum-sealed packaging; (MM) marinade treated samples stored under aerobic conditions; (MV) marinade treated samples stored in vacuum-sealed packaging; (MB) marinade treated samples stored under aerobic conditions with 1% basil EO; (MBV) marinade treated samples stored in vacuum-sealed packaging with 1% basil EO; (MC) marinade treated samples stored under aerobic conditions with 1% coriander EO; (MCV) marinade treated samples stored in vacuum-sealed packaging with 1% coriander EO.

Table 3 Lactic acid bacteria (LAB) plate counts

Treatment	**Count ± SD (log CFU.g^{-1})**			
	Day 0	**Day 1**	**Day 10**	**Day 20**
C	2.097 ± 0.015	2.15 ± 0.01^{a}	2.147 ± 0.021^{a}	2.93 ± 0.02^{a}
CV	-	2.277 ± 0.015^{b}	2.613 ± 0.015^{b}	$2.953 \pm 0.042^{a, b, d}$
MM	-	2.583 ± 0.021^{c}	2.99 ± 0.01^{c}	3.12 ± 0.02^{c}
MV	-	2.733 ± 0.012^{d}	2.877 ± 0.012^{d}	$3 \pm 0.01^{b, d, e}$
MC	-	2.603 ± 0.015^{c}	2.76 ± 0.01^{e}	$3.02 \pm 0.02^{d, e}$
MCV	-	2.463 ± 0.021^{e}	2.803 ± 0.015^{f}	$2.997 \pm 0.006^{a, b, d, e}$
MB	-	2.703 ± 0.006^{d}	2.98 ± 0.01^{c}	3.023 ± 0.025^{e}
MBV	-	1.993 ± 0.006^{f}	2.713 ± 0.015^{g}	$2.937 \pm 0.032^{a, b}$

Note: The plate counts, each carried out in triplicate, are displayed as mean values together with standard deviations (SD). According to Tukey's Honestly Significant Difference (HSD) test, data in the same column categorized by different letters are considered significantly different ($p \leq 0.05$). (C) untreated control samples stored under aerobic conditions; (CV) untreated samples in vacuum-sealed packaging; (MM) marinade treated samples stored under aerobic conditions; (MV) marinade treated samples stored in vacuum-sealed packaging; (MB) marinade treated samples stored under aerobic conditions with 1% basil EO; (MBV) marinade treated samples stored in vacuum-sealed packaging with 1% basil EO; (MC) marinade treated samples stored under aerobic conditions with 1% coriander EO; (MCV) marinade treated samples stored in vacuum-sealed packaging with 1% coriander EO.

Table 4 *Pseudomonas* spp. plate counts

Treatment	**Count ± SD (log CFU.g^{-1})**			
	Day 0	**Day 1**	**Day 10**	**Day 20**
C	1.43 ± 0.02	1.753 ± 0.035^{a}	1.9 ± 0.01^{a}	1.997 ± 0.006^{a}
CV	-	$\leq 1^{b}$	$\leq 1^{b}$	$\leq 1^{b}$
MM	-	$\leq 1^{b}$	$\leq 1^{b}$	$\leq 1^{b}$
MV	-	$\leq 1^{b}$	$\leq 1^{b}$	$\leq 1^{b}$
MC	-	$\leq 1^{b}$	$\leq 1^{b}$	$\leq 1^{b}$
MCV	-	$\leq 1^{b}$	$\leq 1^{b}$	$\leq 1^{b}$
MB	-	$\leq 1^{b}$	$\leq 1^{b}$	$\leq 1^{b}$
MBV		$\leq 1^{b}$	$\leq 1^{b}$	$\leq 1^{b}$

Note: The plate counts, each carried out in triplicate, are displayed as mean values together with standard deviations (SD). According to Tukey's Honestly Significant Difference (HSD) test, data in the same column categorized by different letters are considered significantly different ($p \leq 0.05$). (C) untreated control samples stored under aerobic conditions; (CV) untreated samples in vacuum-sealed packaging; (MM) marinade treated samples stored under aerobic conditions; (MV) marinade treated samples stored in vacuum-sealed packaging; (MB) marinade treated samples stored under aerobic conditions with 1% basil EO; (MBV) marinade treated samples stored in vacuum-sealed packaging with 1% basil EO; (MC) marinade treated samples stored under aerobic conditions with 1% coriander EO; (MCV) marinade treated samples stored in vacuum-sealed packaging with 1% coriander EO.

Table 5 Families and species of isolated bacteria

Family	Species
Bacillaceae	*Bacillus mojavensis, Bacillus subtilis*
Burkholderiaceae	*Ralstonia pickettii*
Carnobacteriaceae	*Carnobacterium divergens, Carnobacterium maltaromaticum*
Hafniaceae	*Hafnia alvei*
Lactobacillaceae	*Latilactobacillus sakei*
Micrococcaceae	*Micrococcus luteus*
Pseudomonadaceae	*Pseudomonas fragi, Pseudomonas lundensis*
Staphylococcaceae	*Staphylococcus epidermidis, Staphylococcus haemolyticus*
Yersiniaceae	*Serratia liquefaciens, Serratia proteamaculans*

Table 6 Bacteria isolated from meat subjected to various treatments

Treatment	Species
C	*Hafnia alvei*, *Latilactobacillus sakei*, *Pseudomonas fragi*, *Pseudomonas lundensis*, *Ralstonia pickettii*, *Serratia liquefaciens*, *Serratia proteamaculans*
CV	*Hafnia alvei*, *Latilactobacillus sakei*, *Serratia liquefaciens*, *Ralstonia pickettii*, *Staphylococcus haemolyticus*
MM	*Hafnia alvei, Latilactobacillus sakei*, *Micrococcus luteus*, *Pseudomonas fragi*, *Ralstonia pickettii*
MV	*Carnobacterium divergens*, *Carnobacterium maltaromaticum*, *Latilactobacillus sakei*, *Ralstonia pickettii*
MC	*Carnobacterium divergens*, *Latilactobacillus sakei*, *Serratia liquefaciens*
MCV	*Bacillus mojavensis*, *Bacillus subtilis, Latilactobacillus sakei*, *Ralstonia pickettii*
MB	*Latilactobacillus sakei, Staphylococcus epidermidis*
MBV	*Carnobacterium divergens*, *Latilactobacillus sakei*

Note: (C) untreated control samples stored under aerobic conditions; (CV) untreated samples in vacuum-sealed packaging; (MM) marinade treated samples stored under aerobic conditions; (MV) marinade treated samples stored in vacuum-sealed packaging; (MB) marinade treated samples stored under aerobic conditions with 1% basil EO; (MBV) marinade treated samples stored in vacuum-sealed packaging with 1% basil EO; (MC) marinade treated samples stored under aerobic conditions with 1% coriander EO; (MCV) marinade treated samples stored in vacuum-sealed packaging with 1% coriander EO.

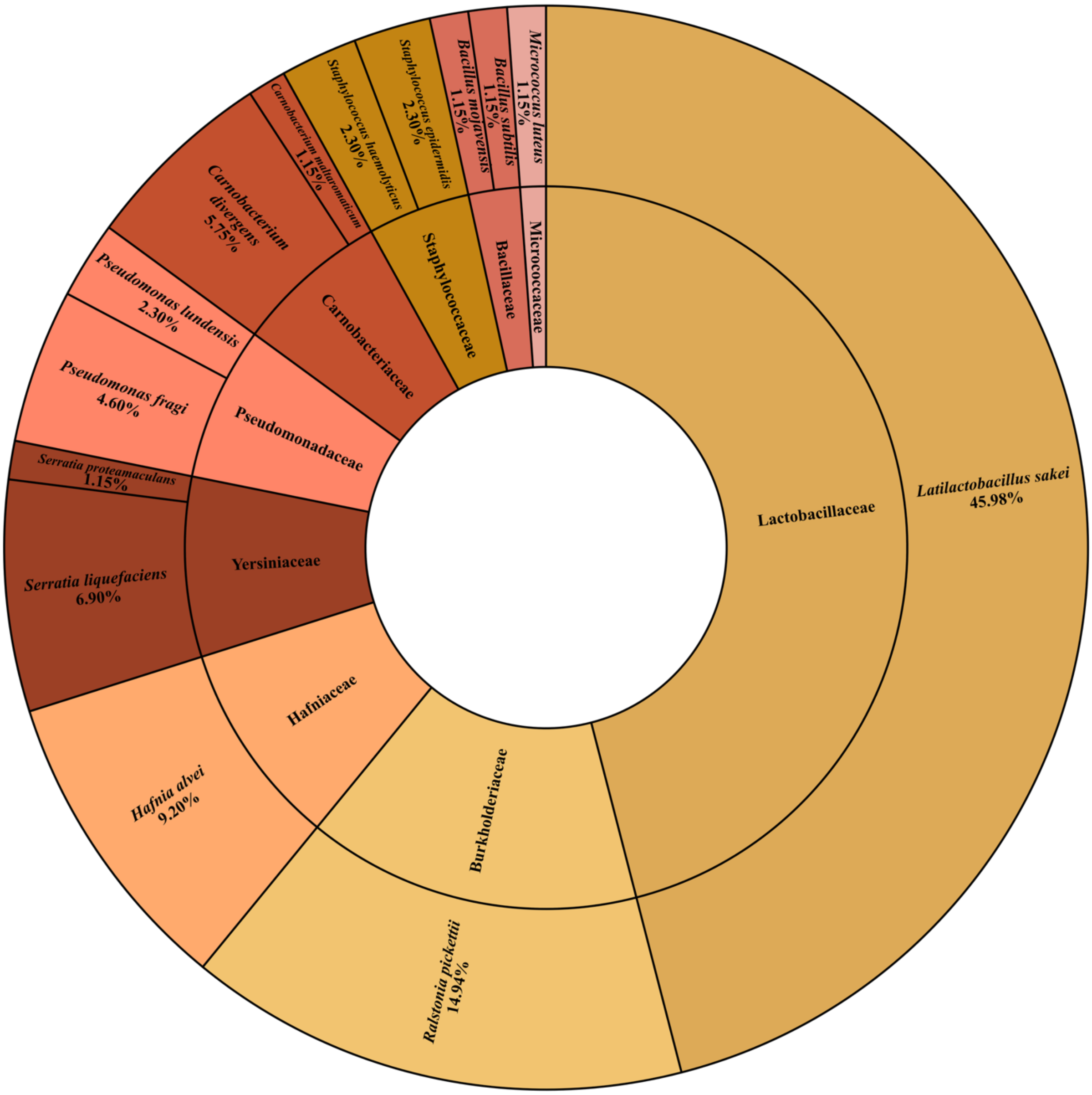

Figure 1 Structure of bacterial community of samples

DISCUSSION

The results of the present study suggest that the combination of yogurt marinade and EOs from coriander and basil can improve the microbiological quality of vacuum-packed beef during refrigerated storage. The beneficial effects of coriander EO (**Michalczyk *et al.*, 2012; Rattanachaikunsopon & Phumkhachorn, 2010; Silva & Domingues, 2017**) and basil EO (**Befa Kinki *et al.*, 2024; Gorzin *et al.*, 2024; Suppakul *et al.*, 2003**) in inhibiting spoilage-causing and pathogenic microorganisms in food, particularly in beef meat products, are quite well known. Vacuum packaging has also been countlessly proven to be highly effective in prolonging the shelf life of meat (**Conte-Junior *et al.*, 2020; Luzardo *et al.*, 2024; Narasimha Rao & Sachindra, 2002**). However, there is a lack of scientific literature that specifically examines the microbiological quality of beef marinated in yogurt with the incorporation of EOs. Nevertheless, there are limited studies that explore different marinades, different EOs and their concentrations, different air exposure storage conditions, or different types of meats to compare with. For example, marinating raw beef in a soy sauce or red wine marinade with the addition of 0.5% oregano EOs can effectively inhibit microbial spoilage (**Kargiotou *et al.*, 2011**). The vacuum-packed pork loin meat, marinated in oil, beer, and lemon with oregano, rosemary and juniper EOs, demonstrates a decreased presence of primary spoilage microorganisms (**Siroli *et al.*, 2020**). A hawthorn vinegar-based marinade, which itself contains bioactive substances, also has a positive effect on the microbiological quality and safety of beef (**Karatepe *et al.*, 2023**). Some other natural antimicrobials (thymol, cinnamaldehyde, allyl isothiocyanate, citric acid, ascorbic acid, rosemary extract, and grapefruit seed extract) demonstrate inhibitory effects on bacterial growth *in vitro*. However, when added to vacuum-packed pork, they do not have a significant impact on total bacterial growth, despite their inhibitory effects observed *in vitro* (**Schirmer & Langsrud, 2010**).

Several studies have investigated the impact of yogurt marinades on the microbiological quality of beef. For instance, the objective of the study conducted by **Birk & Knøchel (2009)** was to investigate the impact of red wine and yogurt marination of pork meat on pathogenic bacteria (*Brochotrix thermosphacta*, *Carnobacterium maltaromaticum*, *Listeria monocytogenes*, and *Campylobacter jejuni*). While red wine demonstrated greater efficacy in reducing the viability of the tested bacteria compared to yogurt, it is worth noting that *Campylobacter jejuni* showed sensitivity to yogurt marination. In a similar work yogurt-based marinade with added 1% and 2% EOs components (thymol, carvacrol, cinnamaldehyde) inhibited *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. in camel meat during cold storage (4°C and 10°C) (**Osaili *et al.*, 2021**). **Osaili *et al.* (2023)** conducted an adjacent study on the impact of EO components (0.5% or 1% eugenol, vanillin, or β-resorcylic acid) on camel meat contaminated with the same pathogens as in the previous study. The results showed that the EO components, either alone or in combination with the marinade, were effective in reducing the pathogens at both concentrations. However, the addition of marinade alone was not as effective in decreasing pathogen levels, just like in our case. The study conducted by **Hafez *et al.* (2024)** involved marinating beef in a yogurt marinade and monitoring its microbiological quality during cold storage. In contrast to using EOs, the researchers opted for the addition of papain and bromelain, which similarly proved to have a beneficial impact on prolonging the shelf life of the beef. These studies demonstrate that various natural antimicrobials enhanced marinades can effectively improve the microbiological quality of meat, with some variations in efficacy depending on the specific marinade, natural antimicrobials, and meat types used.

Considering the counts and the results of species identification, LAB was the most common group of bacteria in our samples, suggesting that LAB were a significant contributor to spoilage. However, in our scenario the TVCs likely decreased not only due to the addition of EOs but also due to the presence of the yogurt marinade. This is supported by the lower TVCs observed in the marinated treatments (MM, MV, MB, MBV, MC, MCV) compared to the untreated (C and CV) over the time points studied. To deepen the knowledge, comparison of microbial counts in meat treated with EOs without the addition of marinade would also be beneficial. This finding is consistent with the research conducted by **Masoumi *et al.* (2022)**, who concluded that both regular yogurt and probiotic yogurt containing *Latilactobacillus casei* were effective in inhibiting microbial growth on chicken fillets during storage at 4°C for 9 days. However, according to **Björkroth (2005)**, marinating poultry products generally does not extend their shelf life and can actually promote the growth of new types of LAB spoilage bacteria. LAB and Enterobacteriaceae were also identified as the primary spoilage bacteria present in beef treated with spice-based (cumin, fennel, and pepper) marinade by researchers **Gopu & Shetty (2016)**. In our study, we did not observe coliform bacteria (CB), a subset of the Enterobacteriaceae family, and *Pseudomonas* spp. in the treated samples. They were present in the control group (C). Based on this, it can be concluded that these particular groups of bacteria did not play a significant role in causing spoilage of the treated samples. Increased levels of LAB in our samples treated with marinade (MM, MV, MB, MBV, MC, MCV), as opposed to the untreated controls (C and CV), could be attributed to the presence of microorganisms found in the yogurt. It would be beneficial to compare LAB counts in yogurt during storage with LAB counts in marinade-treated meat. Regardless of this, the high presence of LAB and specifically the Lactobacillaceae family cannot be solely attributed to the yogurt marinade. LAB, with predominant representative *L. sakei,* are consistently found in treatments without marinade (C and CV). This aligns with **Schirmer *et al.* (2009)**, who examined non-yogurt marinated vacuum-packed pork meat, both laboratory-marinated and commercially produced, at the time of expiration. They found that LAB, including the representative *L. sakei*, were dominant in all samples just like in our case. Equivalently **Björkroth *et al.* (2005)** conducted a study of LAB contaminants that lead to spoilage in non-yogurt marinated broiler legs and concluded that *L. sakei* is frequently found bacteria in marinated meat products.

CONCLUSION

Based on the results, our research has shown that utilizing a yogurt marinade infused with basil or coriander essential oils, in combination with vacuum packaging, can significantly enhance the microbiological quality of beef meat. Through our study, we have confirmed that vacuum packaging plays a crucial role in prolonging the shelf-life of beef meat, with vacuum-packed samples consistently outperforming those exposed to aerobic conditions. The antimicrobial efficacy of the yogurt marinade and mentioned essential oils could not be confidently attributed. Exploring microbial counts in beef treated solely with basil and coriander essential oils, without the addition of marinade, could offer further understanding of their individual impact on the microbial quality of beef. Additionally, it would be appropriate to carry out a sensory evaluation of beef meat treated in this way.

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