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THE EFFECT OF DIFFERENT MARINADES ON THE MICROBIAL SHELF LIFE OF CHICKEN MEAT

Simona Kunová[1], Ivana Timoracká[1], Lucia Zeleňáková[1], Martina Fikselová[1], Viktória Zachar Lovászová[1], Soňa Felšöciová[2]*

Address(es): doc. Ing. Simona Kunová, PhD.
[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 94976 Nitra, Slovakia.
[2] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Tr. A. Hlinku 2, 94976 Nitra, Slovakia.

*Corresponding author: simona.kunova@gmail.com

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ABSTRACT

The aim of the work was to evaluate the microbiological quality of chicken meat after treatment with various marinades in combination with vacuum packaging. The samples were stored at 4°C. The samples were treated with lemon marinade with rosemary, mustard-honey, greek and sweet and sour marinades. The samples were divided into two groups - aerobically packaged and vacuum packaged samples. Total viable counts (TVC), coliform bacteria (CB), lactic acid bacteria (LAB), and *Pseudomonas* spp. were determined on days 0, 1, 7, and 14. Identification of microorganisms isolated from the meat samples was performed using MALDI-TOF MS Biotyper technology. At the end of the storage period, the highest TVC value (6.14 log CFU/g) was observed in the aerobically packaged control samples, whereas the lowest TVC (4.86 log CFU/g) was recorded in samples treated with a sweet-and-sour marinade combined with vacuum packaging. The highest number of CB was 2.88 CFU/g in the aerobically packaged control group, in marinated vacuum-packed samples, CB values were less than 1 CFU/g. The highest number of LAB was 3.50 log CFU/g in the samples treated with greek marinade in combination in vacuum packaging, and the lowest number of LAB was 2.37 log CFU/g in the samples treated with mustard honey marinade with vacuum packaging. Bacteria of the genus *Pseudomonas* were detected only in the aerobically control group. The most frequently isolated species was *Hafnia alvei*, followed by *Lacticaseibacillus rhamnosus*. The high prevalence of Hafniaceae (25.65%) and Enterobacteriaceae (19.66%) suggests the presence of spoilage-associated microorganisms, which can negatively impact meat quality. These results emphasize the importance of effective preservation strategies and hygiene measures to ensure the safety and shelf life of marinated poultry products.

Keywords: meat, marination, vacuum packaging, microbial quality, inhibitory effect

INTRODUCTION

Chicken meat is one of the most widely consumed protein sources worldwide due to its affordability, versatility, and nutritional benefits. However, poultry products are highly perishable due to their high moisture content and favorable conditions for microbial growth, which can lead to rapid spoilage and pose significant food safety concerns. The microbial quality of raw chicken meat is influenced by several factors, including slaughtering practices, processing conditions, storage temperature, and packaging methods (**Zhang *et al.*, 2021**). Pathogenic and spoilage microorganisms such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Pseudomonas* spp. are commonly associated with poultry products and can contribute to foodborne illnesses and reduced shelf life (**Mead, 2004**).

To mitigate microbial contamination and extend the shelf life of chicken meat, various preservation strategies have been explored, including refrigeration, freezing, vacuum packaging, and the application of antimicrobial agents (**Guo *et al.*, 2018**). Among these, marination has gained considerable attention as a method not only for enhancing the sensory properties of meat but also for improving its microbiological stability.

Marination is a widely used technique in meat processing, primarily aimed at improving flavor, texture, and juiciness. It involves the application of acidic, enzymatic, or oil-based solutions that penetrate the meat tissue, modifying its physicochemical properties (**Alvarado and McKee, 2007**). In addition to improving palatability, certain marinades have been shown to exhibit antimicrobial effects, reducing the growth of spoilage organisms and foodborne pathogens (**Hernández-Hernández *et al.*, 2009**).

Natural ingredients with antimicrobial properties, such as vinegar, citrus extracts, essential oils, and fermented dairy products, have been increasingly incorporated into marinades to enhance food safety (**Ju, 2023**). The ingredients used in marinade formulations play a crucial role in achieving the desired sensory attributes, including flavor and texture, which ultimately define the overall quality of the final product. By significantly lowering the pH of meat postmortem, marinades enhance the natural rate of proteolysis, thereby promoting enzymatic and proteolytic activities during muscle maturation. In addition, they accelerate the tenderization process by shortening the time required for meat aging (**Gómez *et al.*, 2020**). In certain cases, the incorporation of various acids facilitates the breakdown of connective tissue in meat, thereby enhancing tenderness, while seasonings and spices contribute to flavor development. Beyond their impact on sensory attributes, these components also influence enzymatic activity and can inactivate or inhibit pathogenic and spoilage microorganisms, thereby extending the shelf life of meat. Such effects are primarily attributed to the presence of bioactive compounds, including polyphenols, organic acids, ethanol, and other antimicrobial agents (**Lopes *et al.*, 2021**).

Despite the known benefits of marination, the impact of different marinade formulations on the microbial shelf life of chicken meat remains an area of active research. While some studies have demonstrated the effectiveness of specific marinade ingredients against spoilage bacteria, there is still a need to explore the synergistic effects of multiple antimicrobial compounds in different combinations (**Zhang *et al.*, 2021**).

The present study aims to evaluate the microbiological quality of chicken meat treated with various marinades—specifically lemon marinade with rosemary, mustard-honey, Greek, and sweet and sour marinades—in combination with vacuum packaging. By analyzing total viable counts (TVC), coliform bacteria (CB), lactic acid bacteria (LAB), and Pseudomonas spp. at multiple time points during storage at 4 °C, this research seeks to assess the effectiveness of these combined preservation strategies in inhibiting microbial growth and extending the shelf life of marinated chicken meat.

MATERIAL AND METHODS

Preparation and Packaging of chicken meat with marinades

Chicken breast samples of chicken meat were used in this experiment. The meat was bought from an authorised store. The meat samples were transported under hygienic conditions in a cleaned refrigerator to the microbiological laboratory, where they were stored at temperature 4°C until the analysis was performed. Under sterile conditions, the meat was cut and weighed into 100 g samples. The samples were then marinated for 12 hours. The marinades consisted of (g/100 g meat): M1:

Lemon marinade with rosemary - 22 g lemon juice, 6 g fresh rosemary, 16 g olive oil, 6 g garlic, 3 g salt, 2 g ground white pepper. M2: Mustard-honey marinade – 21 g mustard, 20 g honey, 6 g garlic, 6 g dried onion, 2 g salt. M3: Greek marinade - 15 g olive oil, 5 g garlic, 10 g mustard, 10 g Greek yogurt, 5 g honey, 2 g dried thyme, 2 g dried oregano, 3 g parsley, 3 g lemon juice. M4: Sweet and sour marinade 20 g pineapple, 11 g soy sauce, 11 g olive oil, 6 g cane sugar, 4 g grated ginger, 3 g dried garlic. There was 55 g marinade used/100 g meat. Subsequently, the samples were packed in polyethylene bags. A vacuum packer (Concept, Choceň, Czech Republic) was used to pack the samples in a vacuum manner. A total of 160 samples were analyzed. The samples were stored at temperature 4°C. Each sample of chicken meat (100 g) was packaged individually. The methods of meat packaging are shown in Table 1.

Table 1 Preperation of marinated chicken meat samples

Control group	C	Samples of chicken meat packaged under aerobic conditions and stored at temperature 4°C
Control vacuum-packed group	CV	Vacuum-packed chicken meat, stored at 4°C
Samples treated with lemon marinade with rosemary	M1	Samples of chicken meat treated with lemon marinade with rosemary, stored under aerobic conditions, at a temperature of 4°C
Vacuum-packed samples of treated with lemon marinade with rosemary	M1V	Samples of chicken meat treated with lemon marinade with rosemary, packaged in polyethylene bags, stored under under vacuum, at temperature of 4°C
Samples treated with mustard-honey marinade	M2	Samples of chicken meat treated with mustard-honey marinade, stored under aerobic conditions, at a temperature of 4°C
Vacuum-packed samples treated with mustard-honey marinade	M2V	Samples of chicken meat treated with mustard-honey marinade, packaged in polyethylene bags, stored under under vacuum, at temperature of 4°C
Samples treated with greek marinade	M3	Samples of chicken meat treated with greek marinade, stored under aerobic conditions, at a temperature of 4°C
Vacuum-packed samples treated with greek marinade	M3V	Samples of chicken meat treated with greek marinade, packaged in polyethylene bags, stored under under vacuum, at temperature of 4°C
Samples treated with sweet and sour marinade	M4	Samples of chicken meat treated with sweet and sour marinade, stored under aerobic conditions, at a temperature of 4°C
Vacuum-packed samples treated with sweet and sour marinade	M4V	Samples of chicken meat treated with sweet and sour marinade, packaged in polyethylene bags, stored under under vacuum, at temperature of 4°C

Samples Cultivation

Microbiological analyses were conducted on days 0, 1, 7, and 14 of storage at 4 °C. Meat samples (5 g) were diluted in 45 mL of 0.1% sterile saline solution and homogenized using a shaker (GFL 3005, Berlin, Germany) for 30 minutes. Serial dilutions ranging from 10^{-2} to 10^{-4} were then prepared. The following microbial groups were enumerated: total viable counts (TVC) on Plate Count Agar (PCA; Oxoid, Basingstoke, UK) incubated at 30 °C for 48–72 h; coliform bacteria (CB) on Violet Red Bile Lactose Agar (VRBL; Oxoid, Basingstoke, UK) incubated at 37 °C for 24–48 h; lactic acid bacteria (LAB) on De Man, Rogosa, and Sharpe Agar (MRS; Oxoid, Basingstoke, UK) incubated in 5% CO_2 at 37 °C for 48–72 h; and *Pseudomonas* spp. on Pseudomonas Agar (Oxoid, Basingstoke, UK) incubated at 35 °C for 48 h.

Microorganism Identification Using MALDI-TOF MS

Identification of microorganisms isolated from chicken meat samples was carried out using MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) Biotyper (Bruker Daltonics, Bremen, Germany) by comparing the obtained spectral profiles with reference libraries.

Preparation of MALDI Matrix Solution

A stock organic reagent was prepared comprising 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid (TFA). Accordingly, 1 mL of stock solution contained 500 µL acetonitrile, 475 µL distilled water, and 25 µL TFA. An aliquot (250 µL) of this stock solution was subsequently combined with the α-cyano-4-hydroxycinnamic acid (HCCA) matrix in an Eppendorf tube.

Sample Preparation and Identification

Eight colonies per plate were analyzed. The biological material was transferred from a Petri dish into an Eppendorf flask containing 300 µL of distilled water, mixed, and supplemented with 900 µL of ethanol. The mixture was then centrifuged at 10,000× g for 2 minutes (Eppendorf AG, Hamburg, Germany). After discarding the supernatant, the pellet was allowed to dry at 20°C. Next, 30 µL of 70% formic acid and 30 µL of acetonitrile were added to the pellet, followed by another centrifugation at 10,000× g for 2 minutes. A 1 µL aliquot of the supernatant was pipetted onto a MALDI plate, left to dry, and subsequently overlaid with 1 µL of MALDI matrix solution. Once dried, the samples were ready for microorganism identification using a MALDI-TOF mass spectrometer (Bruker, Daltonics, Bremen, Germany).
Mass spectra were automatically generated using the microflex LT MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) operated in the linear positive mode within a mass range of 2000–20,000 Da. The instrument was calibrated using the Bruker bacterial test standard. Results of mass spectra were processed with the MALDI Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany). The identification criteria used were a score of 2.300 to 3.000 indicated highly probable identification on species level; a score of 2.000 to 2.299 secure genus identification with probable species identification; a score of 1.700 to 1.999 probable identification to the genus level; <1.700 was considered as unreliable identification.
Mass spectral data were analyzed using MALDI Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany). Identification scores were interpreted according to the manufacturer's criteria: scores between 2.300 and 3.000 indicated highly probable species-level identification; scores from 2.000 to 2.299 corresponded to secure genus identification with probable species identification; scores between 1.700 and 1.999 suggested probable genus-level identification; and scores below 1.700 were considered unreliable for identification.

Statistical analysis

Analyses and measurements were performed in triplicate. Standard deviation (SD) and mean were calculated using Microsoft Excel software.

RESULTS AND DISCUSSION

Results of microbiological analyses of marinated meat during storage

The microbial quality of chicken meat is a crucial factor influencing food safety, shelf life, and public health. The microbial load in chicken meat is affected by various factors, including farming conditions, slaughtering processes, storage, and handling practices (**Rouger *et al.*, 2017**). The average values of individual groups of microorganisms in the control sample are shown in table 2.

Table 2 Average values of microorganisms in fresh meat

	TVC	CB	LAB	Pseudomonas
C	2.92 ±0.01	1.49 ± 0.02	2.20 ± 0.02	1.30 ± 0.01

C – control group of samples, TVC – total viable counts, CB – coliform bacteria, LAB – lactic acid bacteria.

The average value of total viable counts (TVC) ranged from 2.81±0.02 log CFU/g in the sample treated with marinade M2 to 3.13 ±0.02 log CFU/g in the control sample in aerobic packaging after 1st day of storage. During storage, there is an increase in the microbial population in all samples. After 14 days, the highest increase in TVC is in the control sample in aerobic packaking (6.14 log ±0.02 CFU/g) and the lowest in the sample treated with marinade M3 in combination with vacuum packaging (4.78 ±0.02 log CFU/g), indicating that Greek marinade in combination with vacuum packaging (M3V) has the strongest inhibitory effect on microbial growth (figure 1).
This finding aligns with previous studies that indicate that marinades rich in acids and antioxidants can significantly extend the shelf life of food and limit the growth of undesirable microorganisms (**Rahman *et al.*, 2023**). Vacuum packaging, on the other hand, minimizes oxygen exposure, which is also a crucial factor in inhibiting aerobic bacteria, as confirmed by the results of this study.
In the study of **Karam *et al.* (2019)** the use of essential oils in combination with vacuum packaging, retarded the growth of spoilage microbiota and resulted in a significant reduction of about 2.9–3.1 log CFU/g and a microbiological shelf-life extension of marinated chicken.
In the study by **Augustyńska-Prejsnar et al. (2021)**, marination of turkey meat in buttermilk or whey, compared with marination in lemon juice and the untreated control, resulted in a reduction in the counts of mesophilic aerobic bacteria,

Pseudomonas spp., and members of the Enterobacteriaceae family, as well as a decrease in the number of identified mesophilic aerobic bacterial isolates.

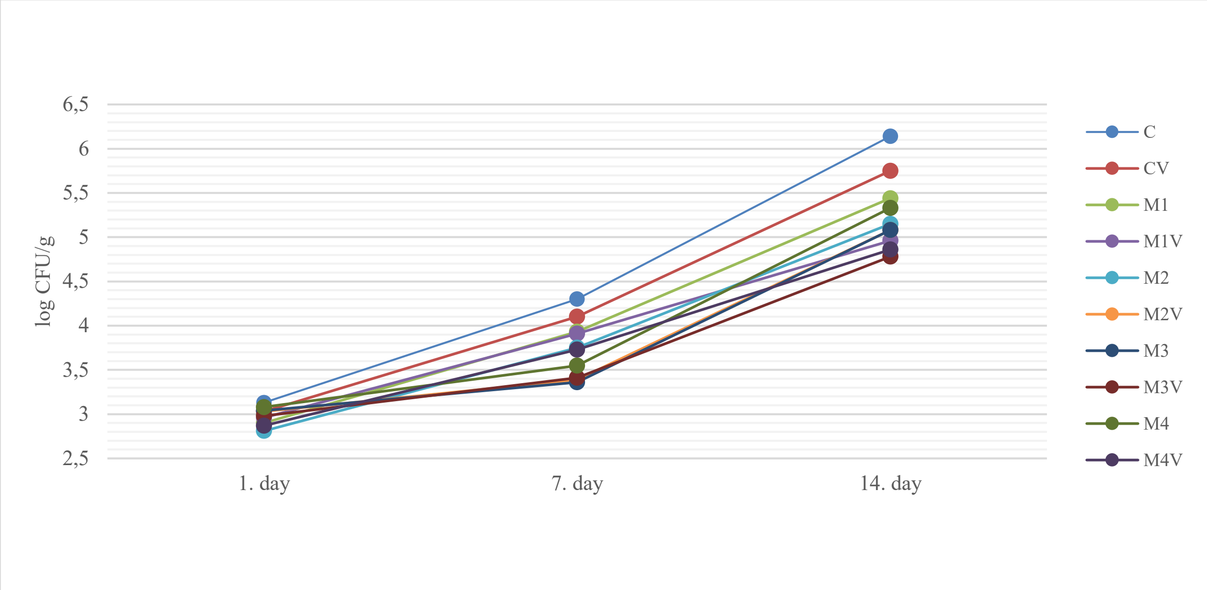

Figure 1 Average values of total viable counts in meat samples during storage
C – control group; CV – control vacuum-packed group; M1 – samples treated with lemon marinade with rosemary; M1V - samples treated with lemon marinade with rosemary in vacuum packaging; M2 – samples treated with mustard-honey marinade; M2V - samples treated with mustard-honey marinade in vacuum packaging; M3 – samples treated with greek marinade; M3V - samples treated with greek marinade in vacuum packaging; M4 – samples treated with sweet and sour nmarinade; M4V - samples treated with sweet and sour nmarinade in vacuum packaging

The average value of coliform bacteria in the control sample in the aerobic packaging was 3.55 ±0.01 log CFU/g after 1 day of storage, but this number decreased to 2.88 ±0.01 log CFU/g after 14 days of storage. In samples treated with marinade M1 and marinades combined with vacuum packaging, the levels of coliform bacteria were less than 1, indicating that these marinades can effectively suppress the growth of coliform bacteria. In the case of samples treated with marinades M2, M3, and M4 packaged with air access, the number of coliform bacteria gradually increased slightly but remained at relatively low levels compared to the control samples (figure 2).
These findings are consistent with other authors reports stating that the use of marinades can reduce the number of pathogens in meat. In the study of **Lopes *et al.* (2021)** found that using marinades on meat not only prevents pathogen growth but also inactivates them. Most marinades were able to reduce pathogen counts by less than 3 log CFU/g, with *Vibrio* species populations showing the highest reductions (more than 4 log CFU/g). Additionally, vacuum packaging is effective in extending the shelf life of meat by reducing oxidation and the growth of aerobic microorganisms. Onaverage, marinades lead to a pathogen reduction of about 1.0 to 2.0 log10 CFU/g (**Meneses and Teixeira, 2022**).

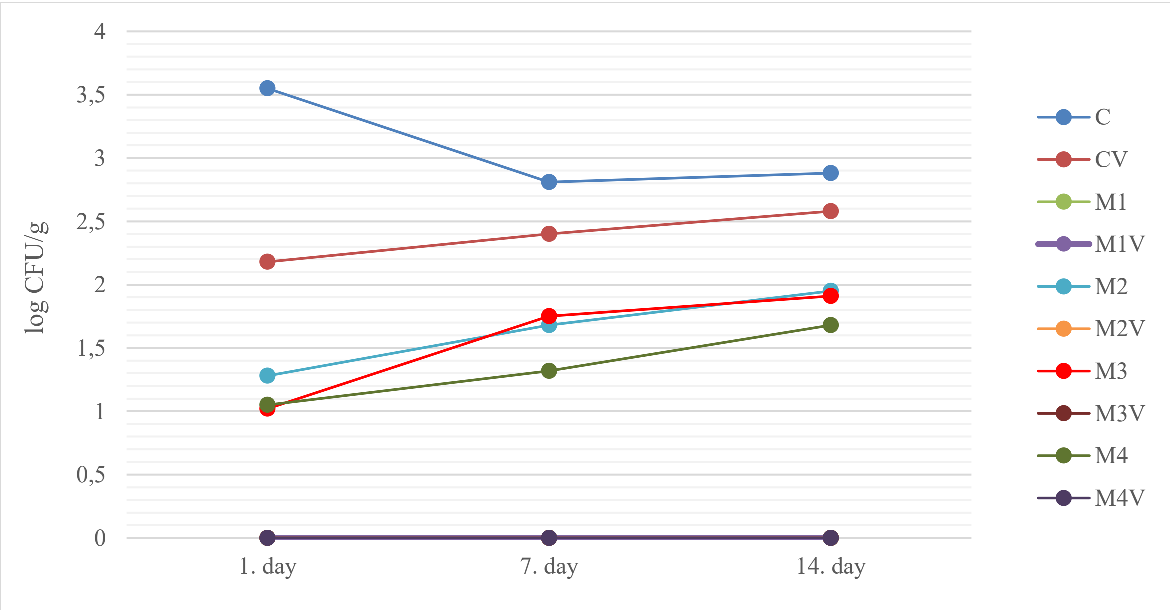

Figure 2 Average values of coliform bactera in meat samples during storage
C – control group; CV – control vacuum-packed group; M1 – samples treated with lemon marinade with rosemary; M1V - samples treated with lemon marinade with rosemary in vacuum packaging; M2 – samples treated with mustard-honey marinade; M2V - samples treated with mustard-honey marinade in vacuum packaging; M3 – samples treated with greek marinade; M3V - samples treated with greek marinade in vacuum packaging; M4 – samples treated with sweet and sour nmarinade; M4V - samples treated with sweet and sour nmarinade in vacuum packaging

In the control samples with aerobic packaging, the LAB count slightly increased during storage from 2.24 ±0.01 to 2.60 ±0.01 log CFU/g. The highest LAB count was recorded in the sample treated with marinade M3 (3.19 ±0.02 log CFU/g) and marinade M3V (3.50 ±0.01 log CFU/g) after 14 days of storage. Most samples showed an increase in LAB during storage, which is natural since lactic acid bacteria can grow in marinated meat and contribute to its fermentation. In some cases, meat samples treated with marinades in combination with vacuum packaging had higher LAB values, which may be related to conditions that promote their growth (figure 3).

The lactic acid bacteria (LAB) species most commonly associated with meat spoilage include heterofermentative *Lactobacillus* spp., *Leuconostoc* spp., *Carnobacterium* spp., homofermentative *Lactobacillus* spp., and *Pediococcus* spp. Excessive proliferation of these microorganisms results in the production of metabolites such as lactic acid, carbon dioxide, ethanol, acetic acid, butanoic acid, and acetoin, which contribute to spoilage defects including off-odors and the formation of ropy slime. Lactic acid bacteria and *Brochothrix thermosphacta* have been major concerns in spoiled meats under anaerobic conditions (**Iulietto *et al.*, 2015**).

Lundström and Björkroth (2007) analyzed LAB present in marinades commonly used in Finland for modified-atmosphere-packaged poultry products. LAB concentrations varied from less than 100 to 8.0 × 10^5 CFU/ml. The predominant LAB identified was *Lactiplantibacillus* ***plantarum***.

Sharma *et al.* (2022) explored the preservation effects of LAB and essential oils on chicken and seafood products. The study highlighted that LAB can inhibit spoilage microorganisms and pathogens, thereby extending the shelf life of these products.

The presence of these compounds may enhance microbial activity, potentially contributing to the development of desirable sensory attributes such as mild acidity and improved tenderness (**Hugas and Monfort, 1997**).

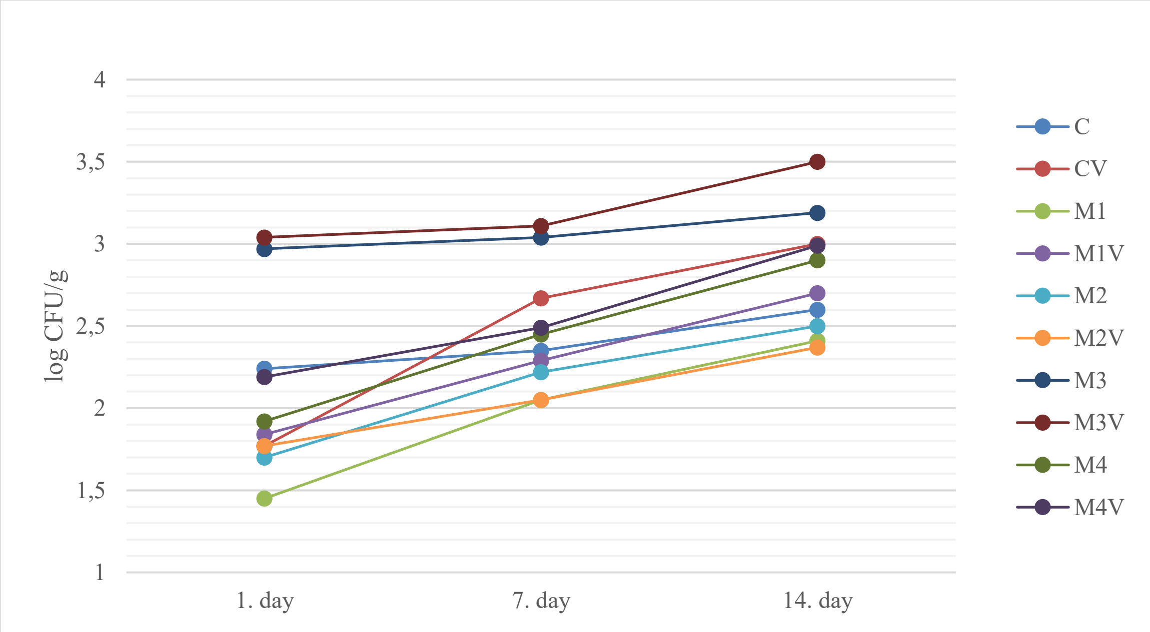

Figure 3 Average values of lactic acid bacteria in meat samples during storage
C – control group; CV – control vacuum-packed group; M1 – samples treated with lemon marinade with rosemary; M1V - samples treated with lemon marinade with rosemary in vacuum packaging; M2 – samples treated with mustard-honey marinade; M2V - samples treated with mustard-honey marinade in vacuum packaging; M3 – samples treated with greek marinade; M3V - samples treated with greek marinade in vacuum packaging; M4 – samples treated with sweet and sour nmarinade; M4V - samples treated with sweet and sour nmarinade in vacuum packaging

The number of *Pseudomonas* increased from 2.30 ±0.02 to 2.87 ±0.02 log CFU/g in the control samples with aerobic packaging. In control vacuum packed samples and in all marinated samples (M1, M2, M3, M4) and their combinations with vacuum packaging (M1V, M2V, M3V, and M4V), the *Pseudomonas* count remained below 1 log CFU/g throughout the entire storage period (Figure 4). This result suggests that marinades can effectively suppress the growth of *Pseudomonas* bacteria, which are typical spoilage microorganisms in meat. Marination generally had a positive effect on reducing the growth of undesirable bacteria, such as coliform bacteria and *Pseudomonas*. Overall, the results suggest that properly selected marinades can extend the shelf life of meat and limit the growth of certain pathogenic or spoilage bacteria.

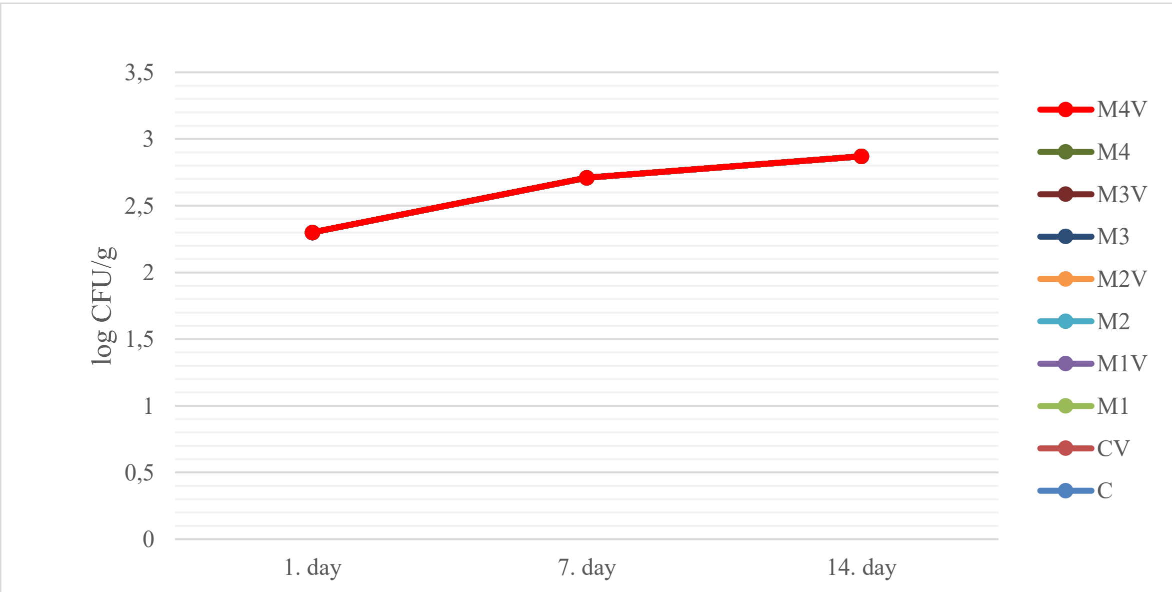

Figure 4 Average values of *Pseudomonas* in meat samples during storage
C – control group; CV – control vacuum-packed group; M1 – samples treated with lemon marinade with rosemary; M1V - samples treated with lemon marinade with rosemary in vacuum packaging; M2 – samples treated with mustard-honey marinade; M2V - samples treated with mustard-honey marinade in vacuum packaging; M3 – samples treated with greek marinade; M3V - samples treated with greek marinade in vacuum packaging; M4 – samples treated with sweet and sour nmarinade; M4V - samples treated with sweet and sour nmarinade in vacuum packaging

The observed increase in *Pseudomonas* counts in the control sample with air access aligns with previous research, which identifies *Pseudomonas* as one of the dominant spoilage bacteria in aerobic meat storage conditions (**Remenant *et al.*, 2015**). These bacteria are psychrotrophic, thrive in refrigerated environments, and play a significant role in meat spoilage due to their ability to degrade proteins and lipids, leading to off-odors and discoloration (**Li *et al.*, 2014**).
In contrast, the significantly reduced *Pseudomonas* counts in all marinated samples, regardless of packaging conditions, suggest that the marinades effectively inhibited their proliferation. Organic acids like acetic and lactic acid can reduce the pH and disrupt bacterial metabolism, while plant-derived phenolics from herbs and spices can interfere with bacterial cell membranes, leading to growth suppression (**Lorenzo *et al.*, 2013**).
Furthermore, the combination of marinades with vacuum packaging likely contributed to enhanced microbial control. Vacuum packaging is known to limit oxygen availability, which is essential for *Pseudomonas* proliferation, thus further inhibiting their growth (**Nychas *et al*., 2008**).

Identification of isolated microorganisms

The results of the identification of microorganisms from the control meat sample are shown in Table 3.
resh chicken meat is highly perishable due to its intrinsic physical and chemical characteristics. Properties such as high water-holding capacity and the abundance of nitrogenous compounds, lipids, carbohydrates, and vitamins create a favorable environment for microbial proliferation **(Odeyemi et al., 2020)**. The presence and subsequent overgrowth of microorganisms in meat lead to sensory deterioration, rendering chicken products unacceptable for consumption typically within one week post-slaughter **(Nychas et al., 2016; Rouger et al., 2017)**. The bacterial communities most frequently detected on fresh poultry include *Acinetobacter*, *Pseudomonas*, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus*, *Micrococcus*, lactic acid bacteria (LAB), and members of the *Enterobacteriaceae* family. Among these, a subset of the initial microbiota—known as specific spoilage organisms—predominates during spoilage and is mainly responsible for product deterioration **(Odeyemi et al., 2020)**. In addition to pathogenic bacteria, chicken meat harbors spoilage microorganisms such as *Pseudomonas* spp., *Brochothrix thermosphacta*, and LAB, which contribute to undesirable changes in color, texture, and odor, thereby reducing both shelf life and consumer acceptability **(Casaburi et al., 2015)**.

Table 3 Number of identified isolates from control sample

Microorganisms	**C**
Serratia proteamaculans	2
Pseudomonas gessardii	1
Pseudomonas brenneri	2
Hafnia alvei	3
Yersinia frederiksenii	1
Pantoea agglomerans	1
Yersinia ruckeri	1

After 1st week of storage, a total of 68 isolates were isolated from samples of marinated meat. The most frequently isolated bacterium was *Kocuria rhizophila* (15 isolates), followed by *Hafnia alvei* (14 isolates), *Lacticaseibacillus rhamnosus* (9 isolates), and *Klebsiella oxytoca* (7 isolates) **(table 4).**
The presence of *Kocuria rhizophila* suggests that this bacterium might be part of the natural microbiota of marinated meat, while *Hafnia alvei* has been associated with meat spoilage due to its proteolytic activity (**Shen *et al.*, 2021**). A study analyzing marinades used for modified atmosphere packaged broiler chicken meat products found that while marinades contained high numbers of LAB, these were not necessarily the species responsible for meat spoilage. This indicates that the presence of LAB in marinades does not directly correlate with spoilage risks (**Lundström and Björkrothfound, 2007**). On the other hand, the presence of *Klebsiella oxytoca*, an opportunistic pathogen, emphasizes the importance of strict hygiene measures in processing and storage (**Crippa *et al*., 2023**).

Table 4 Number of identified isolates from samples after 1st day of storage

Microorganism	**C**	**CV**	**M1**	**M1V**	**M2**	**M2V**	**M3**	**M3V**	**M4**	**M4V**	Total
Buttiauxella gaviniae		1									**1**
Hafnia alvei	5	5						2		2	**14**
Ralstonia pickettii			1	1			1				**3**
Kocuria rhizophila		1	3	2	1	1	2	1	3	1	**15**
Klebsiella oxytoca	2					1			4		**7**
Micrococcus luteus						1					**1**
Sphingobacterium multivorum					1						**1**
Bacillus subtilis					1						**1**
Pseudomonas taetrolens							1				**1**
Empedobacter falsenii	1										**1**
Serratia proteamaculans	1										**1**
Rahnella aquatilis	1										**1**
Lacticaseibacillus rhamnosus							3	2	2	2	**9**
Latilactobacillus sakei	2										**2**
Enterobacter asburiae						2		2			**4**
Enterobacter hormaechei						1					**1**
Enterobacter cloacae						1					**1**
Serratia liquefaciens	2									2	**4**
Total	14	7	4	3	3	7	7	7	9	7	**68**

C – control group; CV – control vacuum-packed group; M1 – samples treated with lemon marinade with rosemary; M1V - samples treated with lemon marinade with rosemary in vacuum packaging; M2 – samples treated with mustard-honey marinade; M2V - samples treated with mustard-honey marinade in vacuum packaging; M3 – samples treated with greek marinade; M3V - samples treated with greek marinade in vacuum packaging; M4 – samples treated with sweet and sour nmarinade; M4V - samples treated with sweet and sour nmarinade in vacuum packaging

After seven days of storage, significant changes in the microbial composition of the meat were observed, accompanied by a reduction in the total number of isolates, with a final count of 22. The most frequently isolated species was *Hafnia alvei*; however, its prevalence declined to eight isolates compared to day one. *Lacticaseibacillus rhamnosus* was successfully isolated in three cases. Additionally, the microbial community was dominated by *Priestia megaterium, Rothia amarae*, *Klebsiella ornithinolytica*, and *Pseudomonas lundensis*, species known for their involvement in protein and lipid degradation (table 5).
In poultry meat, the most common *Pseudomonas* species include ***Pseudomonas fragi***, ***Pseudomonas lundensis***, and ***Pseudomonas fluorescens***. Among the Enterobacteriaceae family, the most frequently represented genera are *Hafnia* (***Hafnia alvei*** and ***Hafnia paralvei***), *Serratia* (***Serratia fonticola***, ***Serratia grimesii***, ***Serratia liquefaciens***, ***Serratia proteamaculans***, and ***Serratia quinivorans***), *Rahnella*, *Yersinia,* and *Buttiauxella*. ***Brochothrix thermosphacta*** is also a commonly present bacterial species in poultry meat (**Harada *et al.*, 2016**).
After 14 days of storage, the total number of isolates decreased; however, the proportion of proteolytic bacteria increased. The most frequently isolated species was *Hafnia alvei* (5 isolates), followed by *Lacticaseibacillus rhamnosus* (3 isolates). Additionally, the microbial community included *Klebsiella oxytoca, Enterobacter hormaechei, Enterobacter asburiae, Serratia liquefaciens, Priestia megaterium, Pseudomonas proteolytica*, and *Latilactobacillus sakei* (table 6).
The results of microbial identification indicate that the diversity of microorganisms in the samples changes during storage, accompanied by a significant reduction in the total number of isolates. Spoilage-associated microorganisms were present throughout the entire storage period, particularly in samples M3 and M3V, which may be related to the composition of the marinades.
In the study by **Johansson et al. (2020)**, a total of 268 unique genera were identified on day 0, whereas only 153 genera remained detectable by day 120. At the end of the storage period, seven genera—*Pseudomonas*, *Serratia*, *Carnobacterium*, *Dellaglioa*, *Leuconostoc*, *Yersinia*, and *Brochothrix*—dominated the meat microbiome regardless of treatment. These cold-tolerant bacterial groups, capable of surviving, proliferating, and prevailing during storage, are classified as specific spoilage organisms and constitute only a small fraction of the initial microbiota.

Table 5 Number of identified isolates from samples after 7th day of storage

Microorganism	C	CV	M1	M1V	M2	M2V	M3	M3V	M4	M4V	Total
Priestia megaterium									2	1	**3**
Kocuria rhizophila							1				**1**
Rothia amarae			1								**1**
Hafnia alvei	1	6					1				**8**
Enterobacter asburiae										2	**2**
Klebsiella ornithinolytica								1			**1**
Pseudomonas lundensis	1										**1**
Lelliottia amnigena		1									**1**
Pseudomonas brenneri	1										**1**
Lacticaseibacillus rhamnosus								3			**3**
Total	3	7	1				2	4	2	3	**22**

C – control group; CV – control vacuum-packed group; M1 – samples treated with lemon marinade with rosemary; M1V - samples treated with lemon marinade with rosemary in vacuum packaging; M2 – samples treated with mustard-honey marinade; M2V - samples treated with mustard-honey marinade in vacuum packaging; M3 – samples treated with greek marinade; M3V - samples treated with greek marinade in vacuum packaging; M4 – samples treated with sweet and sour nmarinade; M4V - samples treated with sweet and sour nmarinade in vacuum packaging

Zhang *et al.* (2021) investigated the microbial composition of chicken meat samples stored at 4°C and identified 145 bacterial genera. The dominant bacterial genera in the samples were *Brochothrix* spp., *Carnobacterium* spp., *Photobacterium* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Serratia* spp., *Kurthia* spp., *Shewanella* spp. and *Obesumbacterium* spp.

Table 6 Number of identified isolates from samples after 14th day of storage

Microorganism	C	CV	M1	M1V	M2	M2V	M3	M3V	M4	M4V	Total
Hafnia alvei	4	1									**5**
Enterobacter hormaechei									1		**1**
Enterobacter asburiae	2										**2**
Klebsiella oxytoca	1							1			**2**
Priestia megaterium										2	**2**
Serratia liquefaciens		1									**1**
Pseudomonas proteolytica	1										**1**
Lacticaseibacillus rhamnosus							3				**3**
Latilactobacillus sakei	2										**2**
Total	10	2					3	1	1	2	**19**

C – control group; CV – control vacuum-packed group; M1 – samples treated with lemon marinade with rosemary; M1V - samples treated with lemon marinade with rosemary in vacuum packaging; M2 – samples treated with mustard-honey marinade; M2V - samples treated with mustard-honey marinade in vacuum packaging; M3 – samples treated with greek marinade; M3V - samples treated with greek marinade in vacuum packaging; M4 – samples treated with sweet and sour nmarinade; M4V - samples treated with sweet and sour nmarinade in vacuum packaging

The findings of this study demonstrate that the microbial composition of marinated chicken meat undergoes significant changes during storage, with a notable shift in dominant bacterial populations. The prevalence of *Hafniaceae* (25.65%) and *Enterobacteriaceae* (19.66%) highlights the presence of spoilage-associated microorganisms, which can negatively impact the sensory and microbiological quality of the meat (table 7).

Members of the Enterobacteriaceae family, such as *Escherichia coli, Klebsiella* spp., and *Proteus* spp., are commonly found in raw meats and are known to contribute to spoilage under certain conditions. A study assessing the presence of Enterobacteriaceae in raw meat and handlers in Egypt identified *Proteus* spp. (60.0%) as the most abundant, followed by *Escherichia coli* (38.7%) and *Klebsiella* spp. (17.3%) (**Gwida *et al.*, 2014**). The presence of these bacteria in marinated chicken meat suggests potential spoilage issues, especially if storage conditions are not adequately controlled.

The storage environment plays a crucial role in shaping the microbial communities in meat products. Factors such as temperature, packaging atmosphere, and the presence of marinades influence which bacterial populations dominate during storage. For instance, studies have shown that the use of plant-derived antimicrobial extracts in marinades can inhibit the growth of spoilage-associated bacteria, thereby extending the shelf life of marinated chicken meat (**Alakomi *et al.*, 2017**).

Table 7 Classification of microorganisms into families

Microorganism	**Family**	**Percentage representation (%)**
Serratia proteamaculans *Serratia liquefaciens*	Yersiniaceae	6.85
Pseudomonas gessardii *Pseudomonas brenneri* *Pseudomonas taetrolens* *Pseudomonas lundensis* *Pseudomonas proteolytica*	Pseudomonadaceae	5.98
Hafnia alvei	Hafniaceae	25.65
Yersinia frederiksenii *Yersinia ruckeri* *Rahnella aquatilis*	Yersiniaceae	2.56
Pantoea agglomerans	Erwiniaceae	0.85
Buttiauxella gaviniae *Klebsiella oxytoca* *Enterobacter asburiae* *Enterobacter hormaechei* *Enterobacter cloacae* *Klebsiella ornithinolytica* *Lelliottia amnigena*	Enterobacteriaceae	19.66
Kocuria rhizophila *Micrococcus luteus* *Rothia amarae*	Micrococcaceae	15.38
Sphingobacterium multivorum	Sphingobacteriaceae	0.85
Bacillus subtilis *Priestia megaterium*	Bacillaceae	5.13
Empedobacter falsenii	Weeksellaceae	0.85
Lacticaseibacillus rhamnosus *Latilactobacillus sakei*	Lactobacillaceae	16.24
Serratia proteamaculans *Serratia liquefaciens*	Yersiniaceae	6.85
Pseudomonas gessardii *Pseudomonas brenneri* *Pseudomonas taetrolens* *Pseudomonas lundensis* *Pseudomonas proteolytica*	Pseudomonadaceae	5.98
Hafnia alvei	Hafniaceae	25.65
Yersinia frederiksenii *Yersinia ruckeri* *Rahnella aquatilis*	Yersiniaceae	2.56
Pantoea agglomerans	Erwiniaceae	0.85
Buttiauxella gaviniae *Klebsiella oxytoca* *Enterobacter asburiae* *Enterobacter hormaechei* *Enterobacter cloacae* *Klebsiella ornithinolytica* *Lelliottia amnigena*	Enterobacteriaceae	19.66

CONCLUSION

This study demonstrated the impact of various marinades in combination with vacuum packaging on the microbiological quality of marinated chicken meat during storage at 4°C. The results showed that vacuum packaging significantly reduced the total viable count (TVC) and coliform bacteria (CB) compared to aerobically packaged samples. The presence of *Lacticaseibacillus rhamnosus* and the high prevalence of *Hafnia alvei* and Enterobacteriaceae indicate notable changes in the microbial composition, with spoilage-associated microorganisms being more prevalent in the aerobically packaged control group. Moreover, the use of specific marinades, such as sweet and sour, in combination with vacuum packaging, was found to help inhibit the growth of spoilage microorganisms, highlighting the potential of this approach to extend the shelf life of marinated chicken meat. These findings underline the importance of selecting effective preservation methods and maintaining proper hygiene to ensure the safety and quality of marinated poultry products.

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