



Microbial Spoilage Profiles of Chicken Breast Stored at 4°C Assessed by MALDI-TOF MS

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ABSTRACT

This study investigated the microbiological and physicochemical changes in broiler breast meat stored aerobically under overwrap packaging at 4 °C. During the 13-Day storage period, bacterial counts increased steadily in all four media and exceeded the consumer acceptability threshold after Day 9. While pH and water activity remained largely stable, marked changes were detected in colour parameters. The decline in L^* values reflected the darkening of meat, whereas the rise in b^* values indicated progressive yellowing. MALDI-TOF MS analyses revealed microbial succession during storage: *Acinetobacter* spp. and *Candida* spp. dominated in the early stage, *Pseudomonas* spp. became predominant in the mid-to-late phase, *Serratia* spp. and *Hafnia* spp. acted as secondary spoilers, and lactic acid bacteria increased in the final days. Spearman correlation analysis revealed strong positive associations between bacterial load and both pH and b^* values, as well as a negative correlation with L^* . Moreover, *Serratia* spp. abundance was positively correlated with b^* values. These findings demonstrate that microbial dynamics are closely linked to physicochemical changes and the progression of spoilage. The study provides valuable insights into shelf life prediction and spoilage ecology, offering a foundation for future applications in packaging and sensor-based real-time monitoring systems.

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Introduction

Poultry meat, particularly chicken breast, is one of the most widely consumed animal protein sources worldwide due to its low cost, balanced nutritional profile, and broad cultural acceptance (FAO, 2023; OECD-FAO, 2025). Chicken breast is nutritionally valuable with its high protein content (20–24%) and water content (70–76%); however, these features also make it highly susceptible to microbial spoilage, even under refrigerated storage conditions (Abdullah et al., 2025; Augustyńska-Prejsnar et al., 2024). Spoilage manifests through sensory defects such as off-odours, surface slime formation, and discolouration, which limit shelf life and reduce consumer acceptance (Katiyo et al., 2020; Wang et al., 2017). Refrigeration is the most common method for slowing microbial growth, yet its effectiveness depends on the initial microbial load, hygiene practices, and the maintenance of an uninterrupted cold chain (Balamatsia et al., 2006; Asadi et al., 2025).

Meat and meat products are among the food groups most prone to microbial spoilage. Under aerobic storage conditions, psychrotrophic bacteria are typically dominant in chicken meat. Among these, *Pseudomonas* spp., members of Enterobacteriaceae, *Brochothrix thermosphacta*, and lactic acid bacteria (LAB) play key roles in the spoilage process

(Casaburi et al., 2015; Doulgeraki et al., 2012; Lei et al., 2023). Their proteolytic and lipolytic activities result in the accumulation of volatile compounds, thereby reducing the sensory quality of the product (Snyder et al., 2024). Specifically, *Pseudomonas* spp. are associated with off-odours and slime formation, *Brochothrix thermosphacta* with metabolite production, and Enterobacteriaceae with discolouration and gas production. Furthermore, LAB species have been reported as late-stage indicators of spoilage progression (Pothakos et al., 2015; Saenz-García et al., 2020; Tao et al., 2025). Therefore, accurate and timely identification of spoilage microbiota is crucial for developing effective quality preservation strategies and shelf life prediction models (McMillin, 2017).

Culture-based methods have long been used to monitor microbial quality in chicken meat. These approaches are inexpensive and standardized but only represent the culturable fraction, are labour-intensive, and offer limited taxonomic resolution (Yu et al., 2019). Molecular methods (PCR, qPCR, 16S rRNA sequencing, whole-genome sequencing) provide more comprehensive insights but remain limited by high costs, analytical complexity, and their inability to distinguish between viable and non-viable

cells (Dourou et al., 2021; Söylemez-Milli et al., 2023). Sensor-based technologies, such as electronic nose systems and BME688 gas sensors, offer rapid and non-destructive evaluation by monitoring volatile organic compounds; however, their taxonomic resolution is limited (Milli et al., 2025; Parlak et al., 2025; Söylemez-Milli et al., 2025a).

In recent years, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a powerful tool in food microbiology (Söylemez-Milli et al., 2025c). This technique enables rapid and accurate identification of microorganisms based on ribosomal protein spectra, while offering high-throughput analysis at relatively low cost (Karasu-Yalçın et al., 2021; Höll et al., 2016; Yu et al., 2019). Although its performance is limited by the scope of reference databases and the challenges of differentiating closely related species, MALDI-TOF MS provides a significant advantage in the species-level identification of live spoilage-associated microorganisms (Ertürkmen et al., 2024; Söylemez-Milli et al., 2025b).

The present study aimed to investigate the temporal dynamics of microbial communities in broiler chicken breast packaged aerobically overwrap with polyvinyl chloride film packaging (OWP) and stored at 4 °C to mimic retail-like conditions. MALDI-TOF MS performed species-level identifications on storage days 0, 1, 2, 3, 4, 5, 7, 9, 11, and 13. In addition, physicochemical analyses, including pH, water activity (a_w), and colour parameters, were conducted. This integrated approach allowed for the simultaneous evaluation of microbial succession and the accompanying physicochemical changes, thereby providing a comprehensive understanding of spoilage progression under typical refrigerated retail conditions.

Materials and Methods

Sample Collection and Packaging

Fresh chicken breast fillets (*Pectoralis major*) were obtained from a commercial poultry processing plant (Erpiliç, Bolu, Türkiye). Samples that had completed the rigor mortis stage were transported to the Microbiology Laboratory of the Scientific, Industrial and Technological Application and Research Center (SITARC), Bolu Abant İzzet Baysal University, under cold chain (4 °C) within 1 h of collection. Each fillet was aseptically divided into 100 g portions, placed on foam plates, and individually overwrapped in polyvinyl chloride film (OWP) under aerobic conditions. A total of 10 packages were prepared for each sampling day (0, 1, 2, 3, 4, 5, 7, 9, 11, and 13 days) and stored at 4 ± 0.5 °C in a temperature-controlled refrigerator. Temperature was monitored continuously using a calibrated digital data logger.

Microbiological Analysis

Ten grams of chicken breast meat were aseptically weighed and homogenised with 90 mL Maximum Recovery Diluent (Merck, Germany) using a stomacher (Smasher, BioMérieux, France) at 200 rpm for 2 min. From the initial suspension, serial decimal dilutions were prepared up to 10⁻⁶ using the same diluent. The spread plate technique was used for inoculation onto Plate Count Agar (PCA; Merck, Cat. No. 1.05463) for the enumeration of Total Aerobic Mesophilic Bacteria (TAMB) at 30 °C for

48 h, de Man, Rogosa and Sharpe Agar (MRS; Merck, Cat. No. 1.10660) for lactic acid bacteria (LAB) at 30 °C for 72 h, Potato Dextrose Agar (PDA; Merck, Cat. No. 1.10130) for yeasts and moulds at 28 °C for 5 days, and Violet Red Bile Agar (VRBA; Merck, Cat. No. 1.10275) for Enterobacteriaceae at 37 °C for 24 h. The results were expressed as log₁₀ colony-forming units per gram (log CFU/g) (Wang et al., 2017; Yu et al., 2019).

Identification of Microbiota by MALDI-TOF MS

For the determination of the microbiota composition by MALDI-TOF MS, single colonies (<75 colonies per plate) were collected from each agar type (PCA, MRS, VRBA, and PDA) and dilution step. Identification was performed using an Autoflex Speed MALDI-TOF MS system (Bruker Daltonics GmbH, Germany) according to the manufacturer's "on-target extraction" protocol. Briefly, each colony was smeared onto an MTP 384 Ground Steel Target plate and air-dried. Subsequently, 1 µL of 70% formic acid was applied and allowed to dry, followed by the addition of 1 µL of a saturated α-cyano-4-hydroxycinnamic acid (α-CHCA; 10 mg/mL) matrix solution. After the matrix had dried, the samples were analysed. Method calibration (MBT_FC.par) was carried out using the Bruker Bacterial Test Standard (BTS; Bruker, Germany), containing eight reference mass spectral peaks ranging from 2000 to 20,000 Da. Mass spectra were acquired in linear positive ion mode with laser intensity set between 30% and 40%, and processed using Biotyper 3.4 software (Bruker Daltonics GmbH, Germany). Identifications were based on the Biotyper score values, and only isolates with scores ≥ 1.999 were considered reliable (Höll et al., 2016; Karasu-Yalçın et al., 2021; Söylemez-Milli et al., 2025c).

Physicochemical Analyses

The a_w of chicken breast samples was measured in duplicate at 25 ± 0.1 °C using a LabMaster- a_w device (Novasina AG, Switzerland) as described by Abdullah et al. (2025). For pH determination, 10 g of sample was homogenised with 90 mL of distilled water for 2 min using a Waring Commercial Blender (USA), and pH was measured with a calibrated digital pH meter (Thermo Scientific Orion Star A211, USA). The colour values (CIE L^* , a^* , and b^*) was determined using a colourimeter (Chroma Meter CR-210; Minolta, Japan) calibrated with a white standard plate. Colour values were recorded in triplicate from inner-outer surface points of each sample (Albrecht et al., 2019).

Statistical Analysis

One-way ANOVA was used to analyse the data obtained from the study, utilising the IBM SPSS Statistics 26.0 software (IBM Corp., Armonk, NY, USA). Tukey's HSD Multiple Comparison Test was then conducted to identify differences among the significant sources of variation (Tukey, 1949). Additionally, the relationships between microbial load, the abundance of dominant genera (*Pseudomonas* spp. and *Serratia* spp.), and various physicochemical parameters (pH, a_w , L^* , a^* , and b^*) were assessed using Spearman correlation analysis, with a significance level set at $p < 0.05$ (Spearman, 1904).

Results and Discussion

Microbial Load

The results of the culturable microbiota count analyses of OWP chicken breast meat samples are given in Table 1. The TAMB, yeast-moulds, LAB, and Enterobacteriaceae count in samples increased steadily from the initial values throughout the storage period ($p < 0.05$) (Table 1). The spoilage threshold reached an acceptable value of 10^7 CFU/g (Katiyo et al., 2020; Wang et al., 2017) for TAMB on Day 9, yeast-mould on Day 7, and Enterobacteriaceae on Day 11 of storage. LAB was measured as 5.88 log CFU/g on the last day of storage (Day 13). The fact that the yeast-mould count reached the spoilage threshold before TAMB is due to the dominance and abundance of Debaryomycetaceae and Moraxellaceae family, especially on Days 0, 1, and 2 of storage (Figures 1 and 2). These findings suggest that spoilage begins on Day 7 and that the limit of consumer acceptability is exceeded by the end of Day 9. Similar research has indicated that the microbial load in chicken meat reaches the critical limit under cold storage conditions within approximately 7 to 10 Days (Abdullah et al., 2025; Augustyńska-Prejsnar et al., 2024).

Physicochemical Properties

Significant changes were observed in pH, a_w , and colour parameters (L^* , a^* , b^*) during storage ($p < 0.05$) (Table 2). The initial pH value was 6.17, decreasing to 5.84 on Day 2, then trending upward again, reaching 6.84 on Day 13 (Table 2). This initial decrease can be explained by lactic acid accumulation resulting from glycogen

breakdown (Balamatsia et al., 2006). The subsequent increase is associated with alkaline metabolites resulting from microbial proteolysis and amino acid decarboxylation (Casaburi et al., 2015; Doulgeraki et al., 2012). The a_w values remained within a narrow range from 0.911 to 0.925 throughout storage (Table 2), indicating that the product's moisture status remained essentially constant with OWP and that these slight fluctuations were related to the homogeneous structure of the meat matrix (Mudalal et al., 2014).

Significant changes were observed in colour values during storage (Table 2). On the outer surface, L^* values decreased from 58.59 to 47.27, indicating a loss of gloss and darkening of the meat (Table 2). On the inner surface, L^* values started higher (54.79) and decreased to 48.49 on Day 13. The a^* values fluctuated during storage on both surfaces, but generally increased, peaking on Day 11 and then decreasing. This change may be related to both oxidative processes and microbial activities. The b^* values showed a significant increase on the outer surface (3.58 → 10.23), suggesting that the yellowing tendency strengthened during storage. A similar increase in b^* values was observed on the inner surface, which was associated with lipid oxidation and microbial metabolism (*Serratia* spp., *Pseudomonas* spp.) (Doulgeraki et al., 2012; Indriani et al., 2025; Han et al., 2025).

However, the fluctuations observed in L^* , a^* , and b^* values measured during storage are thought to be related to structural heterogeneity originating from the anatomical regions (cranial, central, caudal) of the breast meat (Muñoz-Lapeira et al., 2024; Rocha et al., 2022).

Table 1. Enumeration of chicken breast during storage culturable microbiota (Log CFU/g)

Storage Day	TAMB	Yeast-moulds	Lactic Acid Bacteria	Enterobacteriaceae
0	1.48 ± 0.12 ^a	1.74 ± 0.17 ^a	1.40 ± 0.61 ^a	ND
1	2.34 ± 0.18 ^b	1.81 ± 0.14 ^b	1.30 ± 0.31 ^b	ND
2	3.32 ± 0.21 ^c	2.41 ± 0.05 ^c	1.81 ± 0.05 ^c	3.19 ± 0.06 ^a
3	4.04 ± 0.25 ^d	3.66 ± 0.21 ^d	1.98 ± 0.16 ^d	1.85 ± 0.26 ^b
4	4.91 ± 0.27 ^e	4.45 ± 0.16 ^e	1.65 ± 0.20 ^e	2.52 ± 0.02 ^c
5	6.01 ± 0.22 ^f	5.50 ± 0.09 ^f	3.10 ± 0.07 ^f	3.89 ± 0.32 ^d
7	6.78 ± 0.20 ^g	7.10 ± 0.07 ^g	4.63 ± 0.04 ^g	4.80 ± 0.03 ^e
9	6.85 ± 0.24 ^h	7.33 ± 0.11 ^h	5.30 ± 0.02 ^h	5.22 ± 0.02 ^f
11	8.78 ± 0.30 ⁱ	8.72 ± 0.06 ⁱ	5.08 ± 0.03 ⁱ	7.84 ± 0.16 ^g
13	8.38 ± 0.28 ^j	8.39 ± 0.09 ^j	5.88 ± 0.02 ^j	6.11 ± 0.06 ^h

Values are expressed as mean ± standard deviation (SD). Different superscript letters (a-j) within the same column indicate significant differences among storage days ($p < 0.05$). ND (<1 log CFU/g)

Table 2. Physicochemical and color values in chicken breast during storage

SD	pH	a_w	Outer surface			Inner surface		
			L^*	a^*	b^*	L^*	a^*	b^*
0	6.17 ± 0.00 ^c	0.924 ± 0.000 ^a	58.59 ± 0.73 ^h	2.33 ± 0.08 ^c	3.58 ± 0.42 ^c	54.79 ± 1.11 ^c	2.69 ± 0.06 ^d	2.89 ± 0.35 ^a
1	6.13 ± 0.01 ^d	0.923 ± 0.001 ^a	59.45 ± 0.95 ⁱ	3.91 ± 0.20 ⁱ	1.92 ± 0.63 ^a	57.70 ± 0.21 ^h	3.19 ± 0.18 ^g	3.04 ± 0.59 ^b
2	5.84 ± 0.02 ^a	0.925 ± 0.000 ^a	57.60 ± 0.09 ^g	4.89 ± 1.44 ⁱ	2.77 ± 1.32 ^b	58.83 ± 3.58 ⁱ	3.78 ± 0.17 ^h	1.45 ± 0.03 ^a
3	5.95 ± 0.08 ^b	0.917 ± 0.001 ^b	55.23 ± 2.81 ^d	3.71 ± 0.68 ^h	6.78 ± 1.96 ^e	59.45 ± 1.42 ^j	4.35 ± 0.07 ^j	1.85 ± 1.22 ^a
4	6.07 ± 0.04 ^e	0.911 ± 0.001 ^c	57.44 ± 1.00 ^f	2.81 ± 0.57 ^e	7.43 ± 0.38 ^g	57.94 ± 0.62 ⁱ	4.08 ± 0.38 ⁱ	5.68 ± 0.83 ^c
5	6.28 ± 0.02 ^f	0.911 ± 0.001 ^c	59.23 ± 1.32 ⁱ	2.84 ± 1.15 ^f	8.28 ± 1.29 ⁱ	54.89 ± 2.81 ^f	3.50 ± 0.02 ^f	5.47 ± 0.45 ^d
7	6.30 ± 0.07 ^g	0.919 ± 0.001 ^b	55.77 ± 0.61 ^e	3.02 ± 0.48 ^g	5.28 ± 4.40 ^d	54.68 ± 1.42 ^f	3.07 ± 0.24 ^c	5.32 ± 0.34 ^c
9	6.50 ± 0.04 ^h	0.919 ± 0.001 ^b	54.45 ± 2.13 ^c	2.38 ± 0.06 ^d	6.84 ± 1.88 ^f	50.48 ± 1.38 ^c	2.93 ± 0.15 ^b	6.38 ± 1.30 ^f
11	6.72 ± 0.08 ⁱ	0.922 ± 0.002 ^a	49.99 ± 0.96 ^b	2.08 ± 0.65 ^a	7.60 ± 1.42 ^h	50.20 ± 4.70 ^b	2.76 ± 0.49 ^a	6.55 ± 0.27 ⁱ
13	6.84 ± 0.35 ^j	0.917 ± 0.001 ^b	47.27 ± 1.40 ^a	2.15 ± 0.90 ^b	10.23 ± 4.59 ^j	48.49 ± 1.61 ^a	1.46 ± 0.01 ^a	6.04 ± 0.13 ^e

SD: Storage Day; Values are expressed as mean ± standard deviation (SD). Different superscript letters (a-j) within the same column indicate significant differences among storage days ($p < 0.05$).

Table 3a. MALDI TOF MS results of species isolated from chicken breast meat samples

Family	Species	Number of Isolates	Day										
			0	1	2	3	4	5	7	9	11	13	
Aeromonadaceae	<i>Aeromonas bestiarum</i>	2				+					+		
Aeromonadaceae	<i>Aeromonas salmonicida</i>	6					+			+	+		
Aeromonadaceae	<i>Aeromonas sp.</i>	5								+	+		
Bordetellaceae	<i>Bordetella avium</i>	1		+									
Carnobacteriaceae	<i>Carnobacterium maltaromaticum</i>	5				+	+					+	
Comamonadaceae	<i>Comamonas terrigena</i>	1			+								
Comamonadaceae	<i>Delftia acidovorans</i>	12			+								
Corynebacteriaceae	<i>Corynebacterium variabile</i>	1	+										
Corynebacteriaceae	<i>Corynebacterium xerosis</i>	1	+										
Debaryomycetaceae	<i>Candida zeylanoides</i>	74	+	+	+	+	+	+	+	+	+	+	
Debaryomycetaceae	<i>Debaryomyces hansenii</i>	1			+								
Enterobacteriaceae	<i>Citrobacter gillenii</i>	1					+						
Enterobacteriaceae	<i>Buttiauxella gaviniae</i>	13			+		+	+		+			
Enterobacteriaceae	<i>Citrobacter koseri</i>	1					+						
Enterobacteriaceae	<i>Enterobacteriaceae</i>	1										+	
Enterobacteriaceae	<i>Escherichia coli</i>	2	+										
Enterobacteriaceae	<i>Lelliottia amnigena</i>	1										+	
Erwiniaceae	<i>Pantoea agglomerans</i>	3				+				+			
Hafniaceae	<i>Hafnia alvei</i>	47			+	+	+	+	+	+	+	+	
Lactobacillaceae	<i>Latilactobacillus curvatus</i>	22		+					+	+	+	+	
Lactobacillaceae	<i>Fructilactobacillus fructivorans</i>	1										+	
Lactobacillaceae	<i>Latilactobacillus fuchuensis</i>	13								+	+	+	
Lactobacillaceae	<i>Lactobacillus johnsonii</i>	2		+									
Lactobacillaceae	<i>Latilactobacillus sakei</i>	22							+	+	+	+	
Lactobacillaceae	<i>Lactobacillus sp.</i>	1										+	
Listeriaceae	<i>Brochothrix thermosphacta</i>	11					+			+			
Micrococcaceae	<i>Kocuria rhizophila</i>	2	+										
Micrococcaceae	<i>Rothia nasimurium</i>	2		+									
Moraxellaceae	<i>Acinetobacter johnsonii</i>	3	+		+								
Moraxellaceae	<i>Acinetobacter junii</i>	35		+	+								
Moraxellaceae	<i>Acinetobacter parvus</i>	1		+									
Moraxellaceae	<i>Moraxella osloensis</i>	1	+										
Morganellaceae	<i>Providencia rustigianii</i>	3	+										
Pseudomonadaceae	<i>Pseudomonas abietaniphila</i>	1		+									
Pseudomonadaceae	<i>Pseudomonas antarctica</i>	6				+	+		+		+	+	
Pseudomonadaceae	<i>Pseudomonas azotoformans</i>	4			+	+					+		
Pseudomonadaceae	<i>Pseudomonas brassicacearum</i>	1					+						
Pseudomonadaceae	<i>Pseudomonas brenneri</i>	1						+					
Pseudomonadaceae	<i>Pseudomonas brenneri</i>	8				+	+			+			
Pseudomonadaceae	<i>Pseudomonas chlororaphis</i>	4							+	+	+		
Pseudomonadaceae	<i>Pseudomonas chlororaphis</i>	1					+						
Pseudomonadaceae	<i>Pseudomonas coagulans</i>	1	+										
Pseudomonadaceae	<i>Pseudomonas corrugata</i>	3			+			+		+			
Pseudomonadaceae	<i>Pseudomonas extremorientalis</i>	6				+	+		+		+		
Pseudomonadaceae	<i>Pseudomonas fluorescens</i>	4					+	+		+			
Pseudomonadaceae	<i>Pseudomonas fragi</i>	76				+	+	+	+	+	+	+	
Pseudomonadaceae	<i>Pseudomonas frederiksbergensis</i>	1				+							
Pseudomonadaceae	<i>Pseudomonas fuscovaginae</i>	1				+							
Pseudomonadaceae	<i>Pseudomonas gessardii</i>	43				+	+	+	+	+	+	+	
Pseudomonadaceae	<i>Pseudomonas grimontii</i>	3		+				+	+				
Pseudomonadaceae	<i>Pseudomonas jessenii</i>	1				+							
Pseudomonadaceae	<i>Pseudomonas kilonensis</i>	1				+							
Pseudomonadaceae	<i>Pseudomonas koreensis</i>	17			+	+	+	+	+	+	+	+	
Pseudomonadaceae	<i>Pseudomonas libanensis</i>	9		+		+			+			+	
Pseudomonadaceae	<i>Pseudomonas lundensis</i>	62				+	+	+	+	+	+	+	
Pseudomonadaceae	<i>Pseudomonas marginalis</i>	2					+		+				
Pseudomonadaceae	<i>Pseudomonas mucidolens</i>	1						+					
Pseudomonadaceae	<i>Pseudomonas poae</i>	2			+	+							
Pseudomonadaceae	<i>Pseudomonas proteolytica</i>	2								+	+		

Family	Species	Number of Isolates	Day											
			0	1	2	3	4	5	7	9	11	13		
Pseudomonadaceae	<i>Pseudomonas putida</i>	2			+									
Pseudomonadaceae	<i>Pseudomonas rhodesiae</i>	5			+		+		+			+		
Pseudomonadaceae	<i>Pseudomonas synxantha</i>	1					+							
Pseudomonadaceae	<i>Pseudomonas taetrolens</i>	13			+	+	+	+						
Pseudomonadaceae	<i>Pseudomonas taetrolens</i>	21				+	+			+		+		+
Pseudomonadaceae	<i>Pseudomonas thivervalensis</i>	1							+					
Pseudomonadaceae	<i>Pseudomonas tolaasii</i>	9		+		+		+			+	+		
Pseudomonadaceae	<i>Pseudomonas umsongensis</i>	1						+						
Pseudomonadaceae	<i>Pseudomonas vancouverensis</i>	1							+					
Pseudomonadaceae	<i>Pseudomonas veronii</i>	1				+								
Shewanellaceae	<i>Shewanella baltica</i>	1				+								
Staphylococcaceae	<i>Staphylococcus capitis</i>	5		+	+					+				
Staphylococcaceae	<i>Staphylococcus epidermidis</i>	1			+									
Staphylococcaceae	<i>Staphylococcus sp.</i>	7								+				
Staphylococcaceae	<i>Staphylococcus warneri</i>	1		+										
Xanthomonadaceae	<i>Stenotrophomonas maltophilia</i>	26			+									
Yarrowiaceae	<i>Yarrowia lipolytica</i>	5								+	+	+		
Yersiniaceae	<i>Ewingella americana</i>	3												+
Yersiniaceae	<i>Rahnella aquatilis</i>	2												+
Yersiniaceae	<i>Serratia fonticola</i>	13					+	+	+		+	+		
Yersiniaceae	<i>Serratia liquefaciens</i>	47				+	+	+	+	+	+	+	+	+
Yersiniaceae	<i>Serratia proteamaculans</i>	8						+	+		+	+	+	+
Yersiniaceae	<i>Serratia quinivorans</i>	1				+								
Yersiniaceae	<i>Yersinia enterocolitica</i>	4						+					+	
Yersiniaceae	<i>Yersinia pseudotuberculosis</i>	2											+	
Total isolates		739												

“+” indicates the presence of a bacterial isolate on the analyzed day during storage days.

These changes in pH, L^* , a^* , and b^* generally support the progression of the spoilage process in parallel with the increase in microbial load. The pH increase and colour changes observed after Day 9, in particular, coincide with a period of decreased consumer acceptability.

Microbial Community Dynamics and Spoilage Progression (MALDI-TOF MS)

Analyses performed with MALDI-TOF MS revealed a significant succession in microbial communities throughout storage at the family and genus levels (Figures 1 and 2). All the isolated species from all analyzed days are shown Table 3a, b. A heterogeneous flora was detected on Day 0, with *Candida zeylanoides* (36.8%; 28 isolates), *Corynebacterium variabile* (10.5%; 1 isolate), *Escherichia coli* (10.5%; 2 isolates), *Kocuria rhizophila* (10.5%; 2 isolates), *Providencia rustigianii* (15.8%; 3 isolates), and a low percentage of *Pseudomonas* spp. (5.3%; 1 isolate). This diversity reflects the initial microbiota, which may be associated with slaughter hygiene and environmental contamination (Haleem et al., 2013; Yu et al., 2019). As of Day 1, *Acinetobacter junii* became the dominant species (41.5%; 35 isolates), followed by *Candida zeylanoides* (30.2%; 25 isolates), and *Pseudomonas* spp. (11.3%; 9 isolates), and, to a lesser extent, *Staphylococcus* spp. (5.7%; 5 isolates). The early dominance of *Acinetobacter* species has been previously reported in cold-stored poultry meat, particularly in relation to protein and lipid metabolism (Saenz-García et al., 2020; Indriani et al., 2025). Microbial succession became evident during the early storage period (Days 2–3), with *Stenotrophomonas maltophilia* (34.2%; 26 isolates) and *Delftia acidovorans* (15.8%; 12 isolates) being the most prominent.

Pseudomonas spp. during the middle period, Enterobacteriaceae, such as *Hafnia alvei* and *Serratia* spp., began to increase rapidly at this stage (66.2%; 50 isolates, Day 3). During the middle period, members of the Enterobacteriaceae, such as *Hafnia alvei* and *Serratia* spp., also joined the system. These findings are consistent with previous reports that Enterobacteriaceae act as secondary spoilers in aerobic storage (Doulgeraki et al., 2012; Casaburi et al., 2015). In the middle period of storage (Day 4–7), *Pseudomonas* spp. remained dominant (44.9–67.9%), but species such as *Serratia liquefaciens* (16.3%; 12 isolates, Day 4) and *Brochothrix thermosphacta* (10.2%; 8 isolates, Day 4) also became prominent. *Brochothrix thermosphacta* has previously been reported to alter the odour profile of meat through the production of volatile metabolites (Casaburi et al., 2015; Han et al., 2025). *Serratia* spp. has been observed to contribute to the increase in b^* (yellowness) values, particularly through the production of pigments and metabolites; indeed, its presence was detected as early as Day 2 in this study. In the late storage period (Days 9–13), the microbiota became less diverse, with species such as *Pseudomonas fragi*, *P. lundensis*, and *P. gessardii* remaining dominant. However, a significant increase in LAB was noted in the late storage period, with *Latilactobacillus curvatus* and *L. sakei* reaching abundances exceeding 30%, particularly on Days 11 and 13. The late-stage spoilage process is accompanied by LAB, which can be explained by their metabolic activity leading to sour taste and aroma changes (Pothakos et al., 2015; Augustyńska-Prejsnar et al., 2024). This sequence is consistent mainly with typical spoilage patterns reported in the literature. For example, *Pseudomonas* spp. is the predominant spoilage agent in aerobically packaged

chicken meat (Wang et al., 2017; Saenz-García et al., 2020), while members of Enterobacteriaceae, which act as spoilage-associated microorganisms (Doulgeraki et al., 2012; Casaburi et al., 2015), have also been reported in various studies. It was observed that *Brochothrix thermosphacta* triggered sensory changes by producing volatile organic compounds. At the same time, *Serratia* spp. was associated with changes in colour parameters (increase in b^* values) (Han et al., 2025). Furthermore, the late-stage prominence of LAB species in this study is parallel to that of Augustyńska-Prejsnar et al. (2024) and Pothakos et al. (2015), who discussed the role of psychrotrophic bacteria in fresh chicken meat. Supporting these findings, Zhu et al. (2022) emphasised that spoilage is a complex ecological process governed not by a single

species but by multiple interactions among microorganisms, and that this process leads to meat quality loss through mechanisms such as pigment production, mucus formation, biofilm, and volatile organic compounds release (Zhu et al., 2022). The success of the MALDI-TOF MS method in this comprehensive evaluation demonstrated that this technology can be used for the rapid and reliable identification of both spoilage microorganisms and pathogens in meat products (Höll et al., 2016, Höll et al., 2019; Karasu-Yalçın et al., 2021; Yu et al., 2019). Recent studies have demonstrated that MALDI-TOF MS offers high species-level accuracy compared to 16S rRNA sequencing and metagenomics approaches, making it a practical tool for the food industry (Asadi et al., 2025).

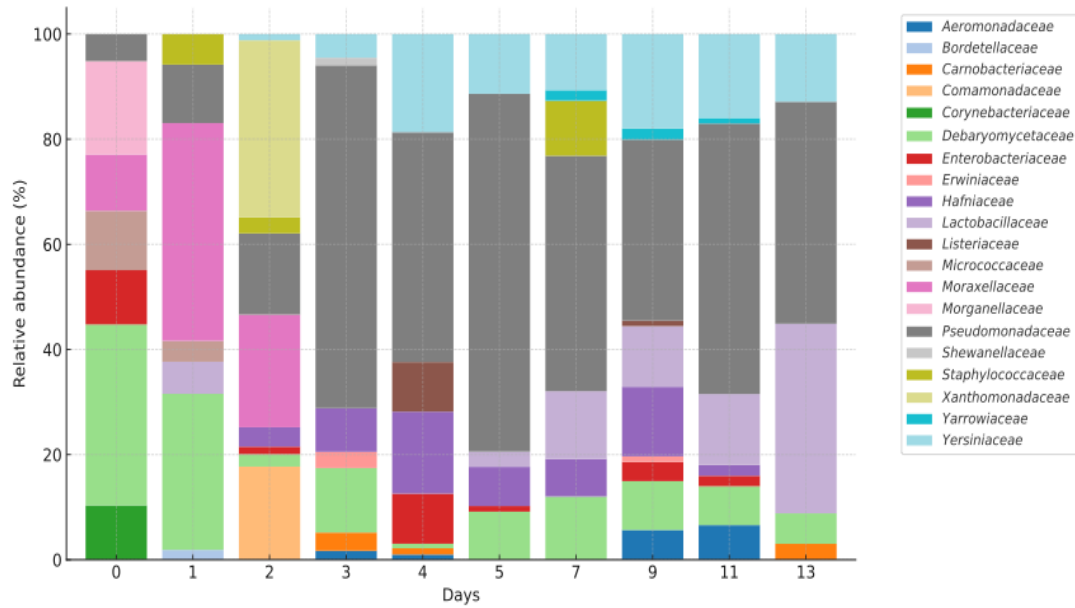


Figure 1. Relative abundance of microbiota in chicken breast at the family level

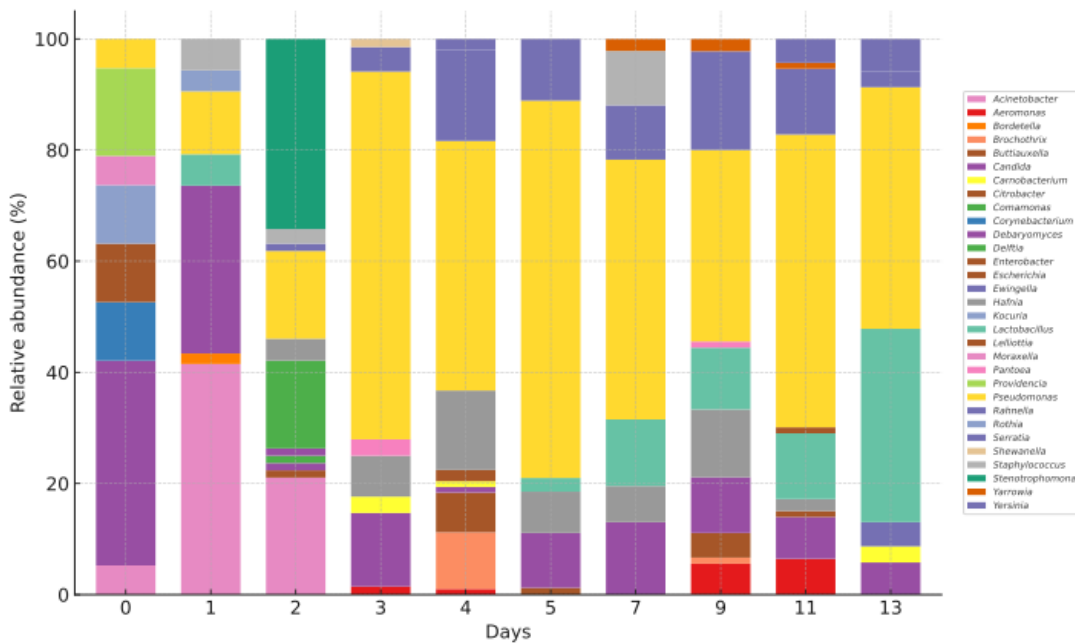


Figure 2. Relative abundance of microbiota in chicken breast at the genus level

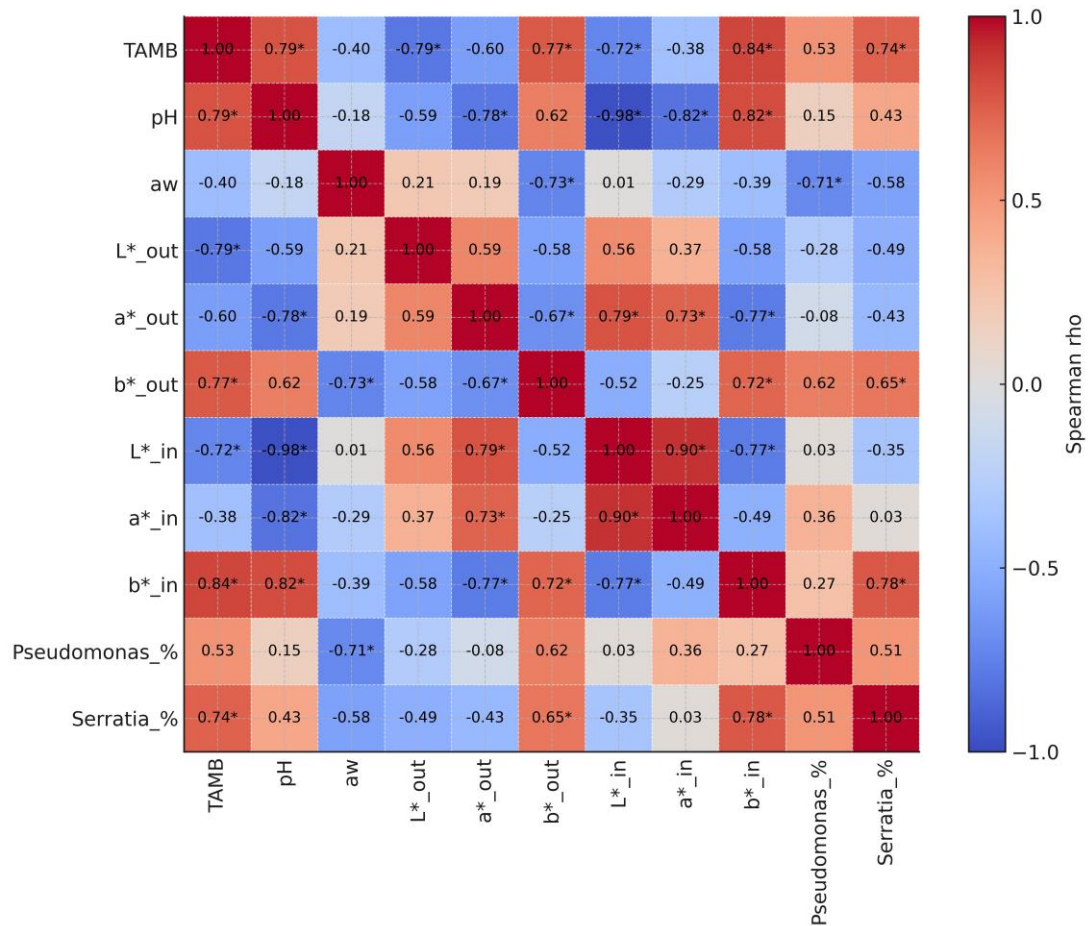


Figure 3. Spearman correlation heatmap between microbial load (log CFU/g), dominant genera (*Pseudomonas* spp., *Serratia* spp.), and physicochemical parameters (pH, a_w , L^* , a^* , b^*) in chicken breast samples during refrigerated storage. Values represent Spearman's rho (ρ). Asterisks (*) indicate statistically significant correlations ($p < 0.05$).

Correlation Analysis

Spearman correlation analysis revealed significant relationships ($p < 0.05$) between microbial load, abundances of dominant species (*Pseudomonas* spp. and *Serratia* spp.), and some physicochemical parameters (pH, a_w , L^* , a^* , b^*) (Figure 3). A combined evaluation of significant positive and negative correlations indicates that spoilage is driven simultaneously by microbial metabolism, pigment oxidation, and oxidative colour changes. Strong negative correlations between TAMB and L^* values (outer surface $r = -0.79$; inner surface $r = -0.72$) suggest darkening due to microbial growth (Katiyo et al., 2020; Wang et al., 2017), while the positive correlation of TAMB with b^* values (outer $r = +0.77$; inner $r = +0.84$) is consistent with the accumulation of yellow-brown oxidative products (Casaburi et al., 2015). Positive relationships between color parameters ($L^*_{in}-a^*_{out}$ $r = +0.79$; $L^*_{in}-a^*_{in}$ $r = +0.90$; $a^*_{out}-a^*_{in}$ $r = +0.73$; $b^*_{out}-b^*_{in}$ $r = +0.72$) indicate that lightness, redness, and yellowness change synchronously during storage and this is explained by myoglobin redox transformations (Balamatsia et al., 2006; Kızıllırmak Esmer et al., 2011). Negative relationships of pH increase with L^*_{in} ($r = -0.98$), a^*_{out} ($r = -0.78$), and a^*_{in} ($r = -0.82$) indicate that increasing pH reduces myoglobin stability and accelerates metmyoglobin formation (Kızıllırmak Esmer et al., 2011). The positive relationship between *Serratia* spp. abundance and outer ($r = +0.65$) and inner surface ($r = +0.78$) b^* values is

consistent with the yellowing-increasing effect of aldehydes and ketones formed as a result of lipolytic and proteolytic activity (Demirok Soncu, 2020; Han et al., 2025; Tao et al., 2025). These correlation findings indicate that spoilage is not limited to microbial load and species diversity but is also closely related to pH changes and sensory critical colour parameters such as L^* , a^* , and b^* .

Conclusion

This study comprehensively demonstrated the microbiological and physicochemical changes that occurred during the storage of broiler chicken breast meat overwrap packaged in under aerobic conditions at 4 °C. The microbial loads indicated that spoilage began on Day 7 and reached the consumer acceptability limit of 10^7 CFU/g by Day 9. Physicochemical analyses revealed distinct colour changes, particularly characterised by decreased lightness (L^*) and increased yellowness (b^*). The presence of *Serratia* species positively correlated with these changes and played a significant role in the colour deterioration resulting from pigment production and lipid oxidation. MALDI-TOF MS analyses detailed the microbial succession during storage. *Acinetobacter* spp. and *Candida* spp. dominated the early stages, while *Serratia* spp. and *Hafnia* spp., along with members of the Pseudomonadaceae, emerged as secondary spoiler species in the mid-stage. In the late stage, the dominance of

Pseudomonas species continued, and a significant increase in LAB was observed. These findings systematically demonstrate the dominant role of Pseudomonadaceae, the concomitant contributions of Enterobacteriaceae and *Brochothrix thermosphacta*, and the involvement of LAB in the late stages. The results demonstrate that a holistic understanding of spoilage ecology can be achieved by integrating classical microbiological analyses, physicochemical parameters, and MALDI-TOF MS-based microbial identification. This multifaceted approach allows for the identification of both early spoilage indicators and late-stage spoilage species. Future studies should comparatively evaluate the effects of different packaging systems on spoilage microbiota, and integrate MALDI-TOF MS data with next-generation sequencing and sensor-based methods to improve shelf life prediction models and reduce food waste.

Declarations

Conflicts of Interest

The author declare no conflict of interest.

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