Hindawi
Journal of Food Quality
Volume 2024, Article ID 5822690, 11 pages
https://doi.org/10.1155/2024/5822690

Research Article

Improvement of Quality Characteristics and Shelf Life Extension of Raw Chicken Meat by Using Black Mulberry Leaf (*Morus nigra* L.) Extracts

Yasemin Çelebi

Department of Food Processing, Eşme Vocational School, Uşak University, Uşak 64000, Türkiye

Correspondence should be addressed to Yasemin Çelebi; yasemincelebisezer@gmail.com

Received 15 January 2024; Revised 10 March 2024; Accepted 27 March 2024; Published 24 April 2024

Academic Editor: Ali Akbar

The objective of this study was to examine the impact of different concentrations of black mulberry leaf extract (BMLE) on the microbial quality, lipid oxidation, biogenic amine content, color stability, and sensory attributes of raw chicken meat during a 12-day chilled storage period. The raw chicken meat was treated with 0.1% BHT (positive control), 0.1%, 0.3%, and 0.5% BMLE, and the outcomes were then compared to the results obtained from raw chicken meat with no additive (control). In comparison to the control group, the inclusion of BMLE resulted in a decrease ($P < 0.05$) in pH and thiobarbituric acid reactive substances (TBARS), as well as an improvement in redness (a^*) ($P < 0.05$). The addition of BMLE significantly extended the shelf life of raw chicken meats compared to the control, as it limited microbiological development and lipid oxidation during storage ($P < 0.05$). Additionally, the BMLE exhibited the most potent inhibitory impact on the buildup of these four BAs (tyramine, cadaverine, histamine, and tyramine) in raw chicken samples at the 12-day storage period ($P < 0.05$). Despite the 0.5% BMLE groups' lowest results for microbial counts, TBARS, and biogenic amines, the concentration of 0.3% BMLE proved to be the most advantageous in terms of sensory acceptability. These findings suggested that BMLE, rather than artificial chemicals, could be utilized in raw chicken products as a promising natural antioxidant and antibacterial agent.

1. Introduction

Poultry meat is one of the most widely consumed foods in the world, and its production and consumption have increased significantly in the past several decades. The appeal of poultry meat stems from the fact that it is the most affordable and readily available meat source, and unlike beef or hog, in terms of culture or religion, there are no restrictions on it [1]. Its low-fat content has led to the designation of poultry meat as a low-calorie food. However, because of its high degree of unsaturation, the lipids in its muscles are pretty vulnerable to oxidation. Modern countries prefer poultry due to its accessibility, ease of use in further processed dishes, and healthier profile [2]. One of the main reasons meat quality deteriorates is lipid oxidation, which can lead to rancidity and the development of unwanted flavors and aromas. These effects reduce the functional, sensory, and nutritional value of meat products and their acceptance by consumers [3]. Internal factors such as iron content and antioxidant enzymes, along with external factors like feeding with oxidized foods, stress, slaughtering procedures, temperature, additional processing processes, and storage conditions, primarily influence the oxidation of poultry meat [2]. Poultry meat products can spoil due to either chemical deterioration or microbial growth. The main form of chemical deterioration is oxidative rancidity, which can cause significant changes in flavor, color, and protein structure and a loss of freshness that may discourage repeat purchases by consumers [4]. Synthetic antioxidants, including propyl gallate (PG), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroxyquinone (TBHQ), can be added to poultry meat to delay, lessen, or avoid oxidative degradation [5, 6]. However, due to the potentially harmful

effects of synthetic additives, consumers reacted negatively, prompting producers to turn to natural antioxidant sources [7]. In meat and meat products, edible extracts from plants and fruits high in phenolics have recently replaced synthetic substances to slow the oxidation of lipids and proteins, lessen discoloration, and inhibit the growth of microorganisms [8, 9].

Black mulberry leaves (*Morus nigra* L.) have been used as herbal medicines in China since ancient times and have recently become the most popular form of herbal medicine. The variations in nutritional components of mulberry leaves across different studies can be attributed to different factors such as varieties, genetics, environments, ecologies, and plant harvest conditions. Research findings indicated that dried mulberry leaf powder consists of moisture (ranging from 5.11% to 7.24%), crude protein (15.31% to 30.91%), total ash (14.59% to 17.24%), neutral detergent fiber (NDF) (27.50% to 36.66%), crude fat (2.09%), carbohydrates (9.70% to 29.74%), and energy content (113 to 224 kcal/100 g). Its bioactive compounds and phenolic substances contribute to its high antioxidant activity. The black mulberry leaves contain several primary phenolic acids, including caffeic acid, vanillic acid, chlorogenic acid, hydroxybenzoic acid, p-coumaric acid, sinapic acid, and ferulic acid. Furthermore, black mulberry leaves have demonstrated antimicrobial properties [10]. Despite these features, there is a significant lack of research on the use of black mulberry leaves in poultry meat. Hence, exploring the possible use of black mulberry leaves as a natural source of antioxidants in poultry products is crucial for enhancing their quality properties. So far as we are aware, no study is available to determine how black mulberry leaves affect the qualitative attributes of raw chicken meat. The objective of the investigation was to assess the impact of different concentrations of black mulberry leaf extract (BMLE) on the microbiological quality, lipid oxidation, biogenic amine content, color stability, and sensory attributes of raw chicken meat over a 12-day refrigerated storage period.

2. Material and Method

2.1. Materials and Chemicals. For this study, armless and skinless chicken thigh flesh was shipped in ice boxes to the laboratory from a nearby poultry meat processing facility (Gedik Pilic Co).

2.2. Preparation of Black Mulberry Leaf Extract (BMLE). Fresh black mulberry leaves (*Morus nigra*) were collected from diverse locations in Uşak, Turkey, using clean, dry, and sterilized plastic containers. Subsequently, these leaves were dried in the shade at room temperature in the clean, dry laboratory and were finely ground into a powder with a grinder for 2 minutes. The resulting powder was stored at −20°C to prevent enzymatic degradation. Black mulberry leaf extract was extracted following the procedure outlined by Martin-Garcia et al. [11]. Approximately 5 grams of black mulberry leaf powder were added to 25 mL of ethanol (96%) and water (50/50, V/V). The product blend was subjected to ultrasonication at 60°C for 45 minutes using an ultrasonic water bath (WB11, Daihan Scientific, Korea). Then, the mixture was centrifuged and filtered through the Whatman No: 1 filter paper. The liquid that passed through the filter was concentrated using a rotary evaporator (IKA, HB4 basic; RV 05 basic, Germany) in a vacuum at 40°C. After that, 20 mL of the condensed extract was put into 90 mm-diameter Petri dishes. These dishes were frozen at −40°C for 24 hours and subsequently subjected to lyophilization at 50°C.

2.3. Analyzing the Characteristics of BMLE. A pH measurement was conducted using a digital pH meter (Hanna Instruments, pH210, USA). A chromameter (Konica Minolta CR-410 from Osaka, Japan) was used to measure color values. Total phenolic content (TPC) was assessed using the Folin–Ciocalteu method, as detailed by Singleton et al. [12]. The DPPH radical scavenging activity, denoted as DPPH-RSA, was assessed through the methodology outlined by Blois [13] for measuring the capability to neutralize the DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical.

2.4. Preparation of Raw Chicken Meat. To achieve this goal, around 1 kg of chicken thigh meat without skin or bones was soaked in 2 L of distilled water with varying levels of BMLE for 5 minutes, such as control (0% BMLE, without extract), 0.1% BMLE, 0.3% BMLE, and 0.5% BMLE. The other group dipped in a 0.1% BHT solution. Each piece of chicken meat from the extract solution was drained through a sieve for five minutes, then placed in polystyrene trays and covered with polyethylene film. Following packaging, the samples were kept at 4°C for 12 days, and analyses were conducted on days 0, 3, 6, 9, and 12. The complete research comprised two independent trials with three measurements for each analysis at distinct manufacturing times.

2.5. Analysis of Raw Chicken Meat

2.5.1. Approximate Composition. The analytical methods indicated by Gökalp et al. [14] were utilized to approximate the chicken flesh samples' ash, moisture, fat, and crude protein content.

2.5.2. pH. About 10 g of chicken meat were combined with 100 mL of distilled water and blended for 25–30 seconds utilizing an ultra-turrax. The pH levels were carried out at a temperature of about 20°C using a pH meter (Hanna Instruments, pH210, USA).

2.5.3. Color Analysis. The color of the samples was evaluated using a chroma meter (CR-410, Konica Minolta, Osaka, Japan) equipped with an 8 mm-diameter aperture and the standard illuminant D50 at an observed angle of 10 degrees. In the CIE Lab color system, the L^*, a^*, and b^* values correspond to black-white, red-green, and yellow-blue color characteristics, respectively. The L^* value denotes the

lightness ranging from black to white, the a^* value indicates the presence of red or green hues, and the b^* value represents the degree of yellowness or blueness in the color. The white standard plate was used to calibrate the instrument before color readings were taken.

2.5.4. Microbiological Analyses. Samples weighing ten grams of chicken meat were extracted from each pack and placed in an aseptic stomacher pouch. The samples were then homogenized using a stomacher for 90 seconds after adding 90 mL of pepton water. Following the decimal homogenate dilutions, a duplicate plate was constructed to count the microbes for each dilution using the surface spreading method. Petri plates were then incubated under aerobic conditions at 30°C for 2-3 days for total mesophilic aerobic bacteria (TMAB) and at 10°C for 5–7 days for total psychrotrophic aerobic bacteria (TPAB). After being cultured on MRS (de Man-Rogosa-Sharpe) and VRBD (Violet red bile dextrose) agars for three days at 30°C, respectively, lactic acid bacteria (LAB) and *Enterobacteriaceae* were enumerated. Colony-forming units, or log CFU, were used to express the results per gram of sample [15].

2.5.5. Determination of 2-Thiobarbituric Acid Reactive Compounds (TBARS). The spectrophotometric technique calculated the samples' TBARS values [16, 17]. Samples were taken from both the surface and interior for TBARS analysis. Two grams of homogenized samples were double-extracted using 10 mL of 0.4 M perchloric acid each time. The volume of extracts was completed to 25 mL with 0.4 M perchloric acid, followed by centrifugation (1790 × g for 5 min) (LAB 312 R, TD5, Turkey). Subsequently, 1 mL of the supernatant was transferred to a test tube with a glass stopper, and 5 mL of TBA reagent was added. The supernatant was then heated in a boiling water bath for 35 min. After cooling, the absorbance was measured at 538 nm (Spektrofotometre, Biochrom, Libra S70, England). The calibration curve was established using 1, 1, 3, 3- tetraethoxypropane (TEP).

2.5.6. Biogenic Amine Analyses. The determination of BA content was conducted using the HPLC chromatographic method, following the method outlined by Bulut et al. [18] and Çelebi et al. [17] with some adjustments. Two grams of chicken meat were treated with 25 mL of 0.4 M perchloric acid and centrifuged (LAB 312 R, TD5, Turkey) at 1500 × g for 5 min. Then, 1 mL of the supernatant was alkalized using 200 μL of 2 N NaOH; 300 μL of saturated sodium bicarbonate was added as a buffer. 2 mL of dansyl chloride solution was then added, and the sample was incubated at 40°C for 75 min. 100 μL of 25% ammonia was added to suspend the residual dansyl chloride. After 30 min of incubation at room temperature, the sample was diluted to 5 mL with acetonitrile and then centrifuged at 1500 × g for 5 min. The supernatant was filtered through a sterile microfilter (0.45 μL). A gradient elution program was employed with mobile phases of acetonitrile (solvent A) and 0.4 M ammonium formate (solvent B), starting at 50% solvent A and 50% solvent B and concluding at 90% solvent A and 10% solvent B after 20 min. The temperature of the column was 40°C, and the flow rate was 1 mL/min^{-1}.

2.5.7. Sensory Analysis. All samples were prepared by cooking them in a heated oven at 175°C until the internal temperature of the meat samples reached roughly 70°C. The panelists were allowed to evaluate a range of attributes of the cooked chicken meats, including color, smell, flavor, texture, and overall acceptability, using a hedonic scale ranging from 9 to 1 (with 9 representing extreme liking, 5 representing moderate liking, and 1 representing dislike). The panelists were provided with water and a galette between samples to cleanse their palates and remove residual flavors [19]. Ten pre-informed and trained panelists carried out the sensory evaluation of chicken meats.

2.6. Statistical Analysis. The data of analyses were evaluated using the SPSS-20 (Armonk, NY, USA) package program. pH, color, TBARS, BAs, and microbiological counts data were subjected to multivariate analysis of variance (MANOVA) using the general linear model (GLM). Duncan's multiple comparison test was used to determine whether there were differences between the groups. Additionally, the results of BAs were treated with GraphPad Prism 10 Software, and the significance levels were indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

3. Results and Discussion

3.1. BMLE's Physicochemical Characteristics and Antioxidant Capacity. Table 1 lists a few of the physicochemical (pH, L^*, a^*, b^*) and antioxidant potential characteristics (TAC, TPC, TFC, DPPH-RSA) of BMLE. The phenolic compounds found in MLs can differ depending on factors such as variety, how they are grown, how long they are allowed to mature, and how they are processed [20]. Our findings are similar to the TPC (0.54–0.76 mg GAE/g) and TFC (105.33–143.94 mg QE/g) reported by Bülbül [21]. The physicochemical and antioxidant results of BMLE (Table 1) are consistent with information found in the literature about black mulberry leaves [22]. The primary naturally occurring active component of mulberry leaves (MLs) is polyphenol, an extremely potent antioxidant that may scavenge free radicals of oxygen, hydrogen peroxide, hydroxyl, etc [20]. These results suggest that BMLE may be a good source of antioxidants for fresh poultry items susceptible to oxidative processes that cause rancidity and discoloration.

3.2. Approximate Composition of Raw Chicken Meat. Table 2 displays the approximate composition (moisture, protein, ash, and fat) of chicken meat products containing varying amounts of BMLE (0.1%, 0.3%, and 0.5%) and BHT (0.1%). The moisture, fat, protein, and ash values of the raw chicken meat samples were not significantly affected by the BMLE application ($P > 0.05$).

Table 1: Physicochemical properties and antioxidant activities of BMLE.

	TAC (mg/L)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH-RSA (μg TE/mg)	pH	L^*	a^*	b^*
BMLE	20.89 ± 0.15	0.83 ± 0.01	165.13 ± 1.90	52.13 μg ± 1.59	4.88 ± 0.03	49.52 ± 0.67	13.87 ± 2.64	17.11 ± 0.31

All values are expressed as mean ± SD of three replicates. TPC: total phenolic content; TAC: total anthocyanin content; TFC: total flavonoid content; DPPH-RSA: DPPH radical scavenging activity; GAE: gallic acid equivalent; QE: quercetin equivalent; TE: trolox equivalent.

Table 2: Approximate composition of raw chicken meat samples.

Samples	Composition (%)			
	Moisture	Protein	Ash	Fat
Control	52.75 ± 0.56	19.2 ± 0.08	1.55 ± 0.04	15.14 ± 0.09
BMLE 0.1%	52.45 ± 0.48	19.22 ± 0.04	1.6 ± 0.05	15.19 ± 0.33
BMLE 0.3%	52.89 ± 2.67	19.25 ± 0.02	1.52 ± 0.02	15 ± 0.70
BMLE 0.5%	52.98 ± 0.73	19.29 ± 0.05	1.55 ± 0.03	15.23 ± 0.60
BHT 0.1%	52.12 ± 1.58	19.28 ± 0.04	1.57 ± 0.03	15.28 ± 0.35
Significance	NS	NS	NS	NS

Values are expressed as mean ± SD. NS: nonsignificant, $^*P < 0.05$. BMLE: black mulberry leaf extract.

3.3. pH and Color Parameters of Poultry Meat. The pH levels of raw chicken meat indicate no statistical difference ($P > 0.05$) (Table 3) in the A ∗ S interaction. The pH levels of all raw chicken meats applied with BMLE were lower than those of the control samples, with a significance level of $P < 0.05$ (Table 4). This drop could be explained by BMLE's average pH value of 4.88 (Table 1). The pH levels of the raw chicken meats rose during storage, with the most notable rises occurring in the control group (Table 4). Turan and Şimşek [23] found that throughout the storage period, the pH of beef patties with *Morus nigra* leaf extract progressively increased in all samples. After storage, the pH values of the 0.1%, 0.3%, and 0.5% BMLE groups were 0.31, 0.40, and 0.46 units lower than those of the control samples. The rise in pH levels in stored samples results from bacterial activity in meat, leading to the accumulation of microbial by products. When stored glucose is depleted, bacteria consume the amino acids generated during the breakdown of proteins, and ammonia from the breakdown of amino acids builds up and raises pH [24]. The presence of large quantities of *Enterobacteriaceae* microorganisms with proteolytic activity can also contribute to higher pH levels in meats [25].

The incorporation of BMLE had no effect ($P > 0.05$) on the L^* levels of samples at storage days (Table 4). The study by Zhang et al. [22] yielded similar findings to our research, as they also observed no discernible trend in the changes of L^* and b^* values in the color of raw ground beef when treated with mulberry leaf extracts and stored in refrigeration. However, the L^* levels of raw chicken meats significantly decreased ($P < 0.05$) during storage. Adding BMLE made the thighs slightly darker, leading to lower L^* values (Table 4). Lower L^* values during storage may be caused by the dipping solution containing BMLE, which has naturally occurring dark color pigments and a darker hue than the purified water and 0.1% BHT used in the control groups. Several studies have demonstrated that the inclusion of natural antioxidants led to a decrease in the L^* values of chicken meat samples [26, 27]. According to Turan and Şimşek [23]; using black mulberry water extract in packaging beef patties can decrease their lightness values. The extract's initial color values and high anthocyanin content are believed to cause this change. The a^* levels of the chicken meats were not significantly different on days 0, 3, 6, and 9. However, on day 12, a statistically significant difference was observed in redness between the samples (Table 4). Myoglobin oxidation caused a reduction in the a^* values of all treatments from day 0 to day 3. Iron atoms can oxidize or denature myoglobin molecules during oxidation, which results in a negative color change in the products and the conversion of myoglobin to methemoglobin [26]. Throughout storage, the TBARS levels of the groups in this investigation rose, correlated with decreasing a^* values, consistent with other studies in the literature [26, 28]. Following the storage time, the control samples showed the lowest a^* value, whereas the 0.5% BMLE samples showed the greatest a^* value. At the end of storage (day 12), the order of a^* values was as follows: 0.5% BMLE > 0.3% BMLE > 0.1% BMLE > 0.1% BHT > Control. These findings suggest that the extracts successfully maintained the red color of the meat. Turan and Şimşek [23] observed that beef patties with 0.2% black mulberry water extract had increased a^* compared to the control group. Additionally, the patties treated with 0.2% extract had higher a^* values than those treated with 0.4% after being stored aerobically for 15 days. Additionally, Zhang et al. [22] stated that the incorporation of mulberry leaf extracts caused a decrease in the a^* values of unprocessed ground beef. In the current investigation, yellowness (b^*) was found to be significantly influenced ($P < 0.001$) by A ∗ S interaction (Table 3). The yellowness values of chicken samples on days 0, 3, and 9 were not statistically ($P > 0.05$) different. Nevertheless, there were notable variations between the treatments on the remaining days ($P < 0.05$). b^* values of samples are influenced differentially by the extracts' color, which might vary from light green to dark yellow. The storage had a notable impact ($P < 0.05$) on the b^* values of the treatments except for the % 0.5 BMLE group. Generally, the b^* values of all samples

Table 3: The effects of application (A), storage day (S), and correlation of A ∗ S on pH, TBARS, color, biogenic amine values, and microbial counts of raw chicken meats.

Effect	pH	Color values			Microbiological analyses				TBARS	Biogenic amines (Bas)					
		L^*	a^*	b^*	TMAB	TPAB	LAB	*Enterobacteriaceae*		HIS	CAD	PUT	TYR	SPD	SPM
Application (A)	***	**	*	***	***	***	***	***	***	***	***	***	***	NS	NS
Storage days (S)	***	***	***	***	***	***	**	***	***	***	***	***	***	NS	NS
A ∗ S	NS	NS	NS	***	*	***	NS	*	***	***	***	***	***	NS	NS

NS: not significant, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; spermine: SPM, spermidine: SPD, tyramine: TYR; putrescine: PUT, cadaverine: CAD, histamine: HIS.

Table 4: pH and color values of raw chicken samples during chilled storage.

Samples	0 day	3 day	6 day	9 day	12 day
			pH		
Control	5.9b,B ± 0.1	6.12b,A,B ± 0.25	6.23a,A,B ± 0.24	6.46a,A ± 0.02	6.51a,A ± 0.02
BMLE 0.1%	5.75b,c,C ± 0.07	5.87b,c,B ± 0.01	5.93b,B ± 0.04	6.1b,A ± 0.02	6.2b,A ± 0.03
BMLE 0.3%	5.7c,D ± 0.02	5.78c,C,D ± 0.02	5.88b,B,C ± 0.02	6b,A,B ± 0.14	6.11b,c,A ± 0.01
BMLE 0.5%	5.78b,c,D ± 0.04	5.88b,c,C ± 0.01	5.94b,B,C ± 0.01	6b,A,B ± 0.07	6.05c,A ± 0.01
BHT 0.1%	6.35a,C ± 0.04	6.42a,B,C ± 0.02	6.46a,A,B,C ± 0.02	6.5a,A,B ± 0.02	6.55a,A ± 0.07
			L^*		
Control	55.83a,A ± 1.17	53.65a,b,A,B ± 0.49	51.52a,B,C ± 0.67	48.99a,C,D ± 2.81	47.04a,D ± 1.35
BMLE 0.1%	54.98a,A ± 1.21	52.98a,b,A,B ± 0.02	50.85a,B ± 1.21	48.32a,C ± 0.96	46.37a,C ± 0.88
BMLE 0.3%	54.00a,A ± 0.02	52.88a,b,A ± 0.03	50.75a,B ± 1.06	48.22a,C ± 1.10	46.27a,C ± 1.02
BMLE 0.5%	53.56a,A ± 0.79	52.50b,A,B ± 0.24	50.37a,B ± 1.94	47.84a,C ± 0.22	45.89a,C ± 0.15
BHT 0.1%	55.00a,A ± 1.41	54.74a,A ± 1.75	52.61a,A,B ± 1.95	50.08a,B,C ± 0.11	48.13a,C ± 1.22
			a^*		
Control	5.87a,C ± 0.09	5.40a,C ± 0.14	5.88a,B,C ± 0.02	6.25a,A,B ± 0.35	6.66c,A ± 0.22
BMLE 0.1%	5.87a,C ± 0.02	5.25a,C ± 0.70	6.20a,B,C ± 0.14	7.14a,A,B ± 0.19	7.89a,b,A ± 0.55
BMLE 0.3%	5.82a,C ± 0.02	5.18a,C ± 1.01	6.18a,B,C ± 0.25	7.30a,A,B ± 0.28	8.23a,A ± 0.17
BMLE 0.5%	5.80a,D ± 0.08	5.49a,C,D ± 0.72	6.55a,B,C ± 0.15	7.55a,A,B ± 0.07	8.33a,A ± 0.46
BHT 0.1%	5.85a,A ± 0.07	5.45a,A ± 0.63	6.00a,A ± 0.59	6.78a,A ± 1.10	7.00b,c,A ± 0.62
			b^*		
Control	12.38a,C ± 0.07	11.27a,b,D ± 0.22	12.85a,B,C ± 0.40	13.59a,b,B ± 0.62	14.5b,A ± 0.04
BMLE 0.1%	12.37a,A ± 0.14	10.95a,b,B ± 0.21	10.85c,B ± 0.21	12.13b,A ± 0.55	11.88b,A ± 0.04
BMLE 0.3%	12.30a,A ± 0.02	10.81b,B ± 0.20	11.00c,B ± 0.35	12.78a,b,A ± 0.58	11.15d,B ± 0.03
BMLE 0.5%	12.32a,A,B ± 0.17	11.46a,B ± 0.22	11.5b,c,B ± 0.70	12.88a,b,A ± 0.59	11.45c,B ± 0.04
BHT 0.1%	12.37a,C ± 0.07	11.37a,b,D ± 0.23	12.48a,b,C ± 0.39	13.88a,B ± 0.63	14.88a,A ± 0.05

A–E: in the same samples, the difference between the values expressed in different capital letters in the same rows on different storage days is statistically significant ($P < 0.05$). a-b: The difference between values expressed with different lowercase letters in different samples in the same column on the same storage days is statistically significant ($P < 0.05$).

fluctuated throughout the storage. At the end of storage, the b^* values of the control and BHT groups increased compared to their initial values. However, there was a notable difference in the yellowness of the samples with BMLE addition ($P < 0.05$). The use of BMLE in this study resulted in a decrease in the b^* values of chicken meat compared to control groups by the end of the storage period. These findings indicate that BMLE had a noticeable impact on the yellowness. In this study, the preservation of desired color parameters of chicken meat during chilled storage was achieved by BMLE. The reason for the enhancement in color values compared to the control can be attributed to its high amount of antioxidants and low pH level.

3.4. Microbial Counts of Raw Chicken Meats. The results presented in Table 3 demonstrate that the interaction of A ∗ S had significant $P < 0.05$, $P < 0.001$, and $P < 0.05$ effects on the counts of TMAB, TPAB, and *Enterobacteriaceae* in raw chicken meat products, respectively. The variations in microbial counts that occur when chicken meats are stored, both with and without BMLE, are displayed in Figures 1(a)–1(d). No differences in TMAB numbers as of day 0 were observed among the control, 0.1%, 0.3%, 0.5% BMLE, and 0.1% BHT treatments ($P > 0.05$) (Figure 1(a)). The TMAB and TPAB counts of chicken meats with BMLE added were considerably ($P < 0.05$) lower than those of the control groups (Figures 1(a) and 1(b)). During the storage period, there was a continuous increase in the TMAB levels of chicken meats. It was found that the control groups (7.46 log CFU/g) and 0.1% BHT incorporated groups (7.25 log CFU/g) surpassed the limit value on day 9 in chicken meats (Figure 1(a)). Additionally, the TMAB counts of the BMLE and BHT-added chicken meats stayed within acceptable limits throughout the 9-day storage period (Figure 1(a)). The shelf life of raw chicken meats stored aerobically in refrigerated conditions is approximately 5 days, based on conditions for hygiene and preservation [29]. The current investigation showed a significant increase ($P < 0.05$) in TPAB counts as the storage time increased. The chicken

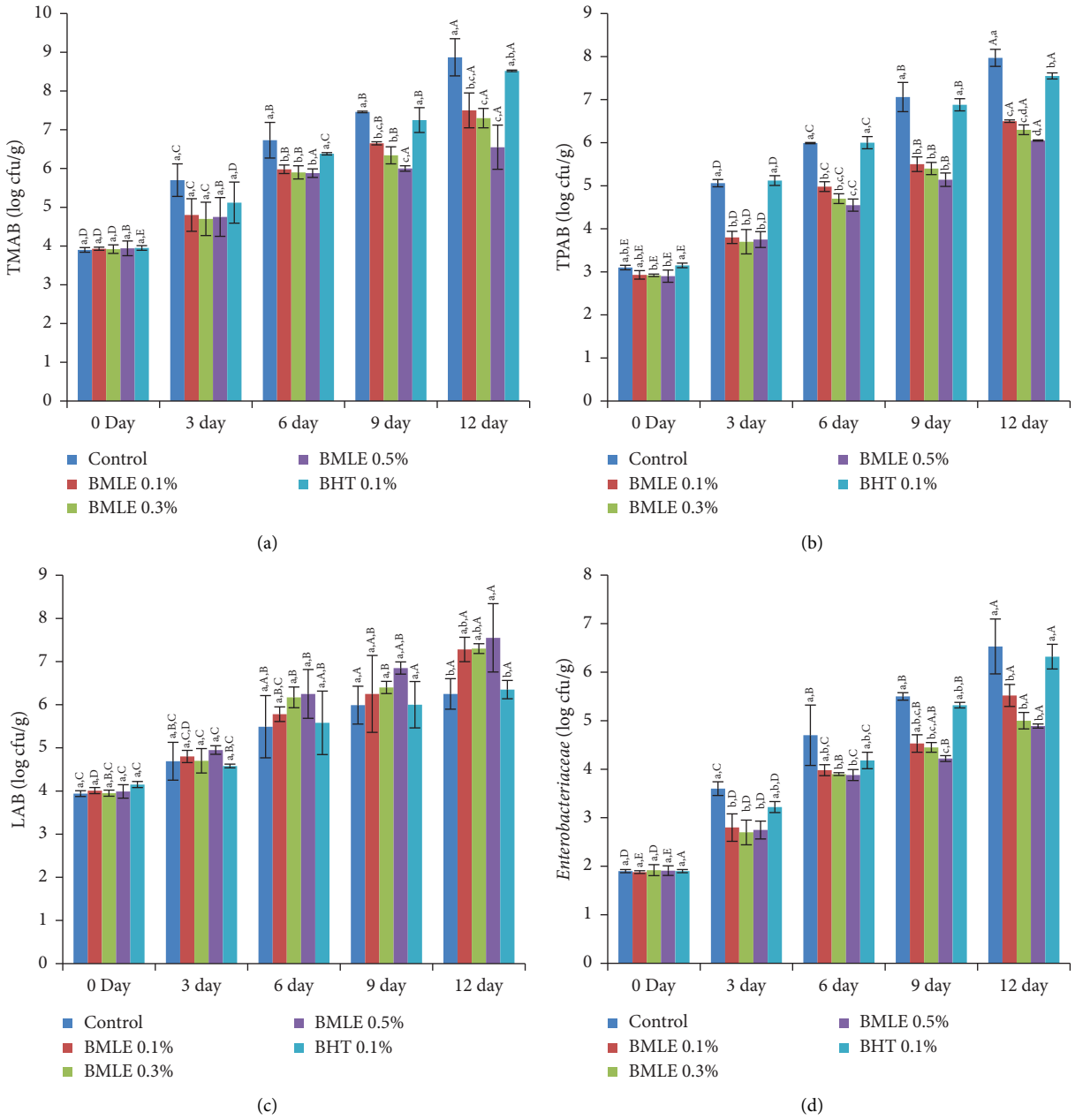

Figure 1: Microbial counts of raw chicken meat groups during cold storage (log CFU/g). Bar charts with different letters (a–d) and (A–D) indicate significant differences between treatments and storage days, respectively ($P < 0.05$, Duncan's test).

meats with 0.5% BMLE had the lowest TPAB counts, whereas the control samples had the highest values ($P < 0.05$) (Figure 1(b)). Throughout the storage period, LAB numbers increased continuously in treatments ($P < 0.05$). The LAB proliferation of chicken meats was promoted by the addition of BMLE, and this effect depended on the level of extract on day 12 of storage ($P < 0.05$). The groups containing 0.3% and 0.5% BMLE, respectively, had the highest LAB number ($P < 0.05$) at the end of storage (Figure 1(c)). The promotion of LAB development by BMLE was likely due to its ability to reduce pH and the potential stimulating impact of its components on LAB [23]. Despite initial counts ranging from 1.88 to 1.92 log CFU/g, there were no significant differences ($P > 0.05$) in *Enterobacteriaceae* numbers on day 0 across all treatments studied. The counts of *Enterobacteriaceae* significantly increased ($P < 0.05$) during storage. Chicken meat with 0.5% BMLE had the lowest counts ($P < 0.05$) (Figure 1(d)). Turan and Şimşek [23] found that the initial levels of *Enterobacteriaceae* in aerobically packaged beef patties, whether or not lyophilized black mulberry water extract was applied, were between 2.43 and 2.52 log CFU/g. They also observed that the increasing number of *Enterobacteriaceae* in control groups (from 2.52 to 5.01) was higher than in groups with 0.4% BMWE extract (from 2.45 to 4.91) over a 15-day storage period. The inclusion of BMLE in samples resulted in a significant

($P < 0.05$) decrease in microbial numbers (apart from LAB), indicating that BMLE positively affected the shelf life and microbial quality of chicken meat.

3.5. Lipid Oxidation. The quantity of secondary lipid oxidation products, mostly aldehydes (or carbonyls), that give oxidized meat and meat products an unpleasant flavor is represented by the TBARS values. The degree of lipid oxidation in meat samples during storage can be monitored using these measurements [30]. The results presented in Table 3 indicate that A ∗ S interactions had an important ($P < 0.001$) impact on the TBARS levels of chicken meats. Including BMLE in chicken meat resulted in a beneficial impact on reducing TBARS levels (Figure 2). The chicken meat with BMLE exhibited a noticeably ($P < 0.05$) reduced TBARS content compared to the control groups. The progressive rise in TBARS values over time is consistent with previous research findings that suggest an increasing formation of TBARS during storage [22, 23, 26]. The findings from chicken meat in Figure 2 demonstrate that TBARS values in the control group reached threshold values (<1 mg/kg) on the sixth day. There were values exceeding the limit on day 9 in the groups with 0.1% BMLE (1.12 mg/kg), 0.3% BMLE (1.00 mg/kg), and 0.1% BHT (1.24 mg/kg) ($P < 0.05$). However, the TBARS value of the 0.5% BMLE group was 1.21 mg/kg and exceeded the threshold value on day 12 ($P < 0.001$) (Figure 2). As a result, it was discovered that the application of 0.1–0.3% and 0.5% BMLE led to a delay in chicken meat lipid oxidation by 3 and 6 storage days, respectively, compared to the control groups. The TBARS values of chicken meat on day 12, which were incorporated with 0.1%, 0.3%, or 0.5% BMLE and 0.1% BHT, were 43.22%, 54.16%, 83.47%, and 18.08% lower ($P < 0.001$), respectively, compared to the control sample (Figure 2). These findings suggest that BMLE may be a natural antioxidant to prevent lipid oxidation rather than synthetic antioxidants. Furthermore, it was discovered that 0.5% BMLE was superior to 0.1% and 0.3% BMLE in both packing techniques for postponing lipid oxidation. This extract's substantial phenolic and antioxidant content is responsible for BMLE's preventive activity against lipid oxidation. Phenolic compounds exhibit vigorous antioxidant activity using mechanisms such as transition-free radical scavenging activity and single-oxygen quenching capacity [31]. According to Turan and Şimşek [23], the beef patties infused with black mulberry water extract exhibited lower and more consistent TBARS levels throughout the 15-day storage duration compared to the control group. In the same way, mulberry leaf extracts reduced the TBARS level of ground beef compared to the control sample [22].

3.6. Biogenic Amine Analysis. The high amount of protein in poultry meat leads to an increased breakdown of proteins and the release of amino acids. This, along with the presence of bacteria that can break down amino acids, speeds up the spoilage of meat and leads to higher levels of substances produced by microorganisms, such as biogenic amines [32]. Six biogenic amines (Bas)—cadaverine, putrescine, tyramine, histamine, spermine, and spermidine—were found and measured during storage. The results presented in Table 3 indicate that A ∗ S interactions had an important ($P < 0.001$) impact on the histamine, tyramine, cadaverine, and putrescine levels of chicken meats. Additionally, Bas (without spermine and spermidine) in the samples with and without BMLE increased at the end of storage ($P < 0.05$) (Figure 3). However, spermine and spermidine, crucial for cell division and growth, remained unaffected ($P > 0.05$) by storage conditions, unlike the other biogenic amines analyzed in all samples. Notably, in addition to their role in cellular processes, spermine and spermidine also serve as a nitrogen source for bacteria [33]. The levels of spermine and spermidine vary between 12.80 and 13.30 mg/kg and 23.98–25.00 mg/kg, respectively, throughout the 12-day storage period ($P > 0.05$) (Figures 3(a) and 3(b)). Decarboxylase-positive contaminating bacteria convert lysine into cadaverine, which can be utilized as a food hygiene indication. The cadaverine content in the control and sample groups with percentages of 0.1 BMLE, 0.3 BMLE, 0.5 BMLE, and 0.1 BHT increased to 6.11, 4.55, 3.50, 2.80, and 4.77 mg/kg at 12 days of storage ($P < 0.05$). This could be attributed to the bacteria that produce cadaverine proliferating in raw chicken meats. Chicken meats treated with BMLE had a lower cadaverine content than the control group ($P < 0.001$), suggesting that BMLE can effectively prevent cadaverine from building up (Figure 3(c)). According to Renes et al. [34], consuming too much putrescine might increase the toxicity of histamine and tyramine, in addition to causing poisoning. As seen in Figure 3(d), the putrescine in the control groups grew more quickly than in the other treatments, reaching 20.79 mg/kg at the end of storage ($P < 0.05$; $P < 0.01$; $P < 0.001$). The addition of BMLE may inhibit putrescine accumulation, notably 0.5% BMLE, which at 12 days significantly decreased putrescine production by 32.65% ($P < 0.001$) compared to the control. The most harmful BA found in food is histamine, which, when consumed in excess, might result in symptoms including headaches and diarrhea [35, 36]. Histamine formation was not observed in all samples at the beginning of storage, and their formation increased to 4.55, 3.11, 3.00, 2.79, and 3.90 mg/kg at 12 days for the control and samples with 0.1% BMLE, 0.3% BMLE, 0.5% BMLE, and 0.1% BHT, respectively ($P < 0.01$, Figure 3(e)). BMLE exhibited effective inhibition of histamine. The histamine content in chicken meats with 0.5% BMLE was 34.06% lower than that of the control ($P < 0.0001$) at 12 days (Figure 3(e)). The changes in the contents of tyramine exhibited a similar pattern, as indicated in Figure 3(f). The levels of these substances were significantly higher in the control groups compared to the samples treated with BMLE and BHT at the end of storage ($P < 0.05$). Additionally, the group treated with BMLE demonstrated the greatest inhibition ($P < 0.0001$). The BMLE exhibited the most potent inhibitory impact on the buildup of these four BAs in raw chicken samples at the end of storage. This could be due to its ability to suppress the growth of TMAB, TPAB, and *Enterobacteriaceae* (BA-positive bacteria) in chicken meats, as demonstrated in Figure 2 since BAs are predominantly produced by

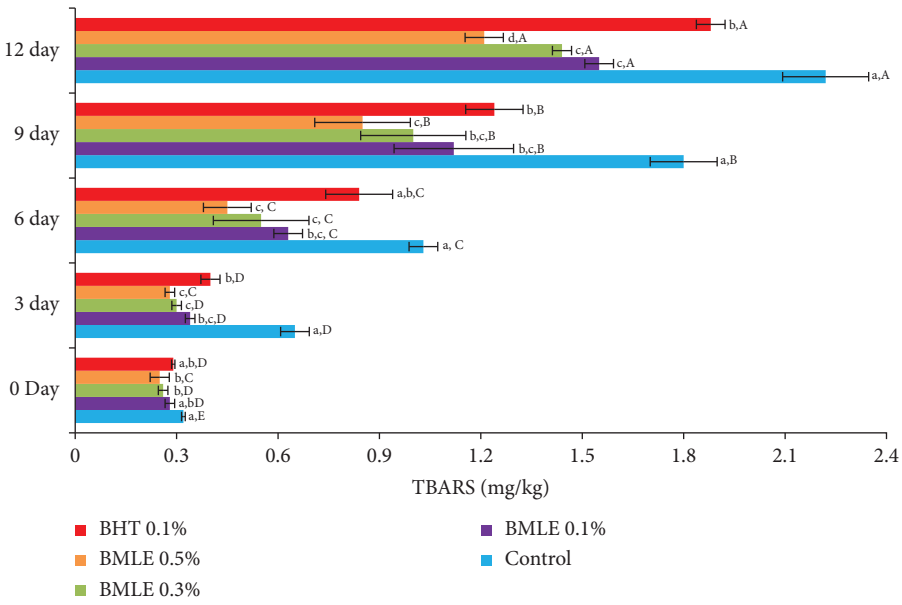

Figure 2: TBARS levels of raw chicken meat groups during cold storage (mg/kg). Bar charts with different letters (a–d) and (A–E) indicate significant differences between treatments and storage days, respectively ($P < 0.05$, Duncan's test).

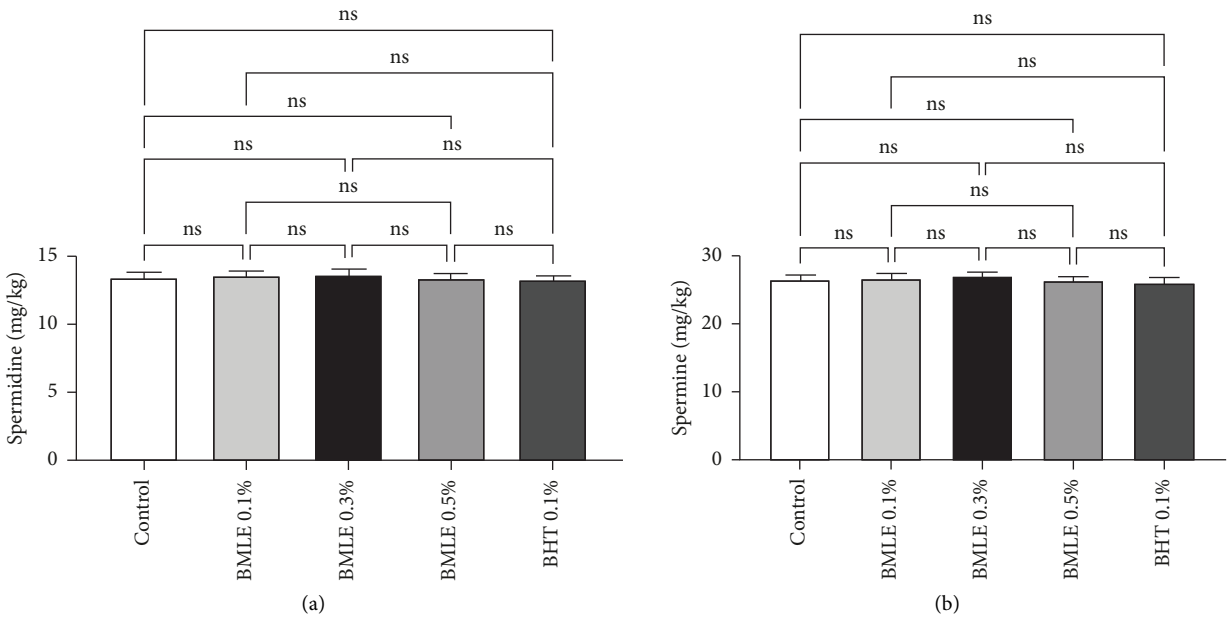

Figure 3: Continued.

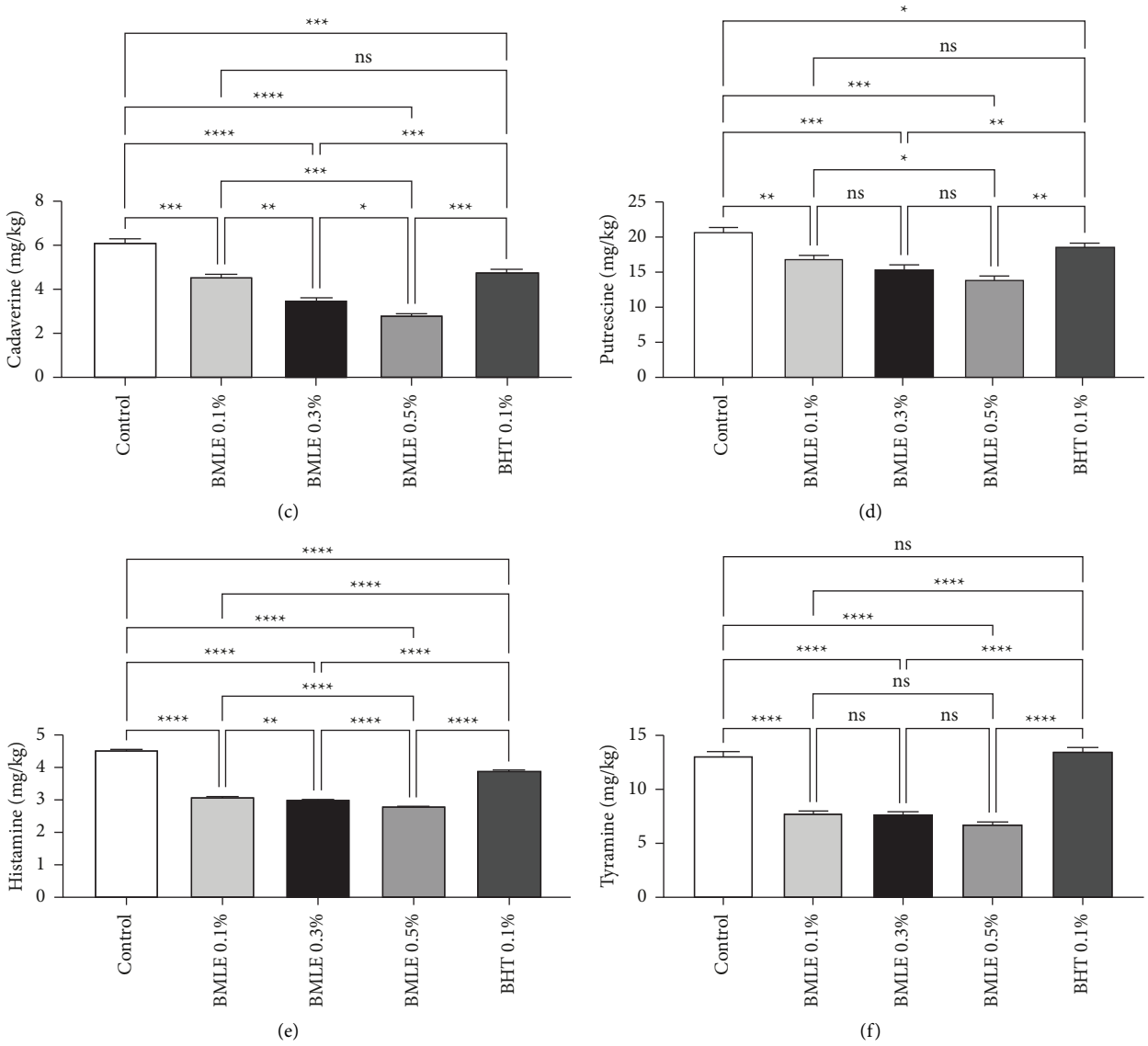

Figure 3: Biogenic amine levels of raw chicken meat groups at the end of cold storage (mg/kg). ns: not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

uncontrolled microbial enzymatic activity. On the other hand, it is believed that the BMLE group, having a higher lactic acid bacteria (LAB) count, can suppress the formation of biogenic amines (BAs).

3.7. Sensory Analyses. Figure 4 displays the sensory scores of the cooked chicken samples on day 5. No significant differences ($P > 0.05$) existed between the control and BMLE-added chicken meats, even though flavor scores were highest in the 0.3% BMLE-added chicken meats. The color and texture of cooked chicken meats were enhanced ($P < 0.05$) by adding 0.3% BMLE. The control groups and those with 0.1%, 0.3%, and 0.5% BMLE's smell scores were not statistically significant ($P > 0.05$). Similarly, Turan and Şimşek [23] showed that the addition of 0.1% and 0.2% black mulberry water extract did not impact the color, texture, smell, and flavor ratings of cooked beef patties. The samples

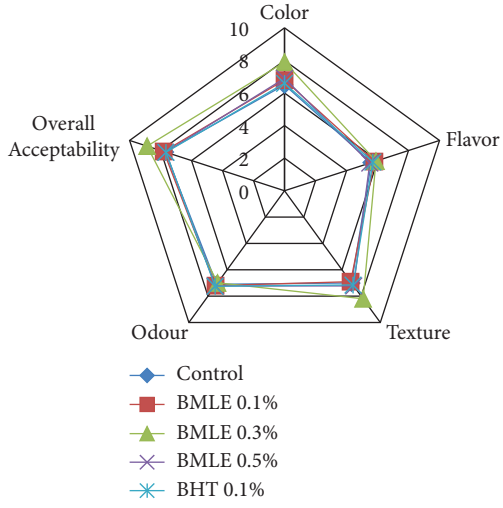

Figure 4: Sensory evaluation of cooked chicken meat samples.

with 0.3% BMLE added had the highest overall acceptance scores for cooked chicken meats ($P < 0.05$). The addition of 0.3% BMLE can be regarded as the most appropriate quantity to avoid adversely affecting the sensory qualities of cooked chicken meats, taking into account all sensory features that have been studied. However, the results for lipid oxidation and microbial count indicated a more favorable effect with 0.5% BMLE. So, the suggested recommendation is to utilize 0.5% black mulberry leaf extract (BMLE) for raw meat preservation.

4. Conclusion

The findings suggest that incorporating BMLE significantly enhances the microbial quality, lipid oxidation, biogenic amine content, color stability, and sensory properties of chicken meat. The results demonstrate a substantial enhancement in microbial quality, lipid stability, and color retention, particularly at specific concentrations of BMLE. Additionally, the extract exhibits inhibitory effects on biogenic amine accumulation, contributing to raw chicken meat's overall freshness and safety. These outcomes underscore the potential of black mulberry leaf extract as a valuable natural ingredient for raw chicken products during refrigerated storage, preserving and enhancing their overall quality. Additionally, the application of BMLE is recommended not only for enhancing the quality of raw chicken meat but also for improving processed chicken products such as nuggets, schnitzel, and chicken patties.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The author declares that there are no conflicts of interest.

References

[1] Z. Khiari, Z. Pietrasik, N. J. Gaudette, and M. Betti, "Poultry protein isolate prepared using an acid solubilization/precipitation extraction influences the microstructure, the functionality and the consumer acceptability of a processed meat product," *Food Structure*, vol. 2, pp. 49–60, 2014.

[2] M. Estévez, "Oxidative damage to poultry: from farm to fork," *Poultry Science*, vol. 94, no. 6, pp. 1368–1378, 2015.

[3] A. B. Falowo, P. O. Fayemi, and V. Muchenje, "Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: a review," *Food Research International*, vol. 64, pp. 171–181, 2014.

[4] J. G. Sebranek, V. J. H. Sewalt, K. L. Robbins, and T. A. Houser, "Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage," *Meat Science*, vol. 69, no. 2, pp. 289–296, 2005.

[5] Q. Guo, S. Gao, Y. Sun, Y. Gao, X. Wang, and Z. Zhang, "Antioxidant efficacy of rosemary ethanol extract in palm oil during frying and accelerated storage," *Industrial Crops and Products*, vol. 94, pp. 82–88, 2016.

[6] C. Caleja, L. Barros, A. L. Antonio, M. B. P. P. Oliveira, and I. C. F. R. Ferreira, "A comparative study between natural and synthetic antioxidants: evaluation of their performance after incorporation into biscuits," *Food Chemistry*, vol. 216, pp. 342–346, 2017.

[7] M. Aziz and S. Karboune, "Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: a review," *Critical Reviews in Food Science and Nutrition*, vol. 58, no. 3, pp. 486–511, 2018.

[8] R. Prommachart, T. S. Belem, S. Uriyapongson, P. Rayas-Duarte, J. Uriyapongson, and R. Ramanathan, "The effect of black rice water extract on surface color, lipid oxidation, microbial growth, and antioxidant activity of beef patties during chilled storage," *Meat Science*, vol. 164, Article ID 108091, 2020.

[9] M. I. Aksu and E. Turan, "Effects of lyophilized black carrot (*Daucus carota* L.) water extract on the shelf life, physicochemical and microbiological quality of high-oxygen modified atmosphere packaged (HiOx-MAP) ground beef," *Journal of Food Science and Technology*, vol. 58, no. 9, pp. 3514–3524, 2021.

[10] E. M. Sánchez-Salcedo, M. Tassotti, D. Del Rio, F. Hernández, J. J. Martínez, and P. Mena, "(Poly)phenolic fingerprint and chemometric analysis of White (*Morus alba* L.) and black (*Morus nigra* L.) mulberry leaves by using a non-targeted UHPLC-MS approach," *Food Chemistry*, vol. 212, pp. 250–255, 2016.

[11] B. Martín-García, M. J. Aznar-Ramos, V. Verardo, and A. M. Gómez-Caravaca, "The establishment of ultrasonic-assisted extraction for the recovery of phenolic Compounds and evaluation of their antioxidant activity from Morus alba leaves," *Foods*, vol. 11, no. 3, p. 314, 2022.

[12] V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventós, "Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent," *Methods in Enzymology*, vol. 299, pp. 152–178, 1999.

[13] M. S. Blois, "Antioxidant determinations by the use of a stable free radical," *Nature*, vol. 181, no. 4617, pp. 1199-1200, 1958.

[14] H. Y. Gökalp, M. Kaya, Y. Tülek, and Ö. Zorba, "Guide for quality control and laboratory application ofMeat products," *Ataturk Uni,Erzurum, Turkey*, pp. 30–93, Erzurum, Turkey, Middle East, 4th edition, 2010.

[15] O. Erkmen, *Basic Methods for the Microbiological Analysis of Foods*, University of Gaziantep, Gaziantep, Türkiye, 2000.

[16] Y. Sezer, "Effects of different temperatures on the quality characteristics of Turkey sausage and changes during storage time," *Journal of the Hellenic Veterinary Medical Society*, vol. 73, no. 3, pp. 4433–4440, 2022.

[17] Y. Çelebi, E. Kavrut, M. Bulut et al., "Incorporation of hydrogen-producing magnesium into minced beef meat protects the quality attributes and safety of the product during cold storage," *Food Chemistry*, vol. 448, Article ID 139185, 2024.

[18] M. Bulut, Y. Çelebi Sezer, M. M. Ceylan, D. Alwazeer, and M. Koyuncu, "Hydrogen-rich water can reduce the formation of biogenic amines in butter," *Food Chemistry*, vol. 384, Article ID 132613, 2022.

[19] G. Yıldız-Turp and M. Serdaroglu, "Effects of using plum puree on some properties of low fat beef patties," *Meat Science*, vol. 86, no. 4, pp. 896–900, 2010.

[20] W. J. Lee and S. W. Choi, "Quantitative changes of polyphenolic compounds in mulberry (*Morus alba* L.) Leaves in relation to varieties, harvest period, and heat processing,"

Preventive. Nutrition and Food Science, vol. 17, no. 4, pp. 280–285, 2012.

[21] F. Z. Akay Bülbül, "*Kara dut (Morus nigra) yaprağı etanol ekstraktının DNA glikasyonuna etkısi*," Master's thesis, Fen Bilimleri Enstitüsü, 2019.

[22] D. Y. Zhang, Y. Wan, J. Y. Xu, G. H. Wu, L. Li, and X. H. Yao, "Ultrasound extraction of polysaccharides from mulberry leaves and their effect on enhancing antioxidant activity," *Carbohydrate Polymers*, vol. 137, pp. 473–479, 2016.

[23] E. Turan and A. Şimşek, "Effects of lyophilized black mulberry water extract on lipid oxidation, metmyoglobin formation, color stability, microbial quality and sensory properties of beef patties stored under aerobic and vacuum packaging conditions," *Meat Science*, vol. 178, Article ID 108522, 2021.

[24] P. Singh, J. Sahoo, M. K. Chatli, and A. K. Biswas, "Shelf life evaluation of raw chicken meat emulsion incorporated with clove powder, ginger and garlic paste as natural preservatives at refrigerated storage (4±1°C)," *International Food Research Journal*, vol. 21, no. 4, 2014.

[25] M. I. Aksu, H. Alinezhad, and E. Erdemir, "Effect of lyophilized water extract of U rtica dioica L. On the shelf life of vacuum-packaged beef steaks," *Journal of Food Processing and Preservation*, vol. 39, no. 6, pp. 3059–3066, 2015.

[26] O. Özünlü, H. Ergezer, and R. Gökçe, "Improving physicochemical, antioxidative and sensory quality of raw chicken meat by using acorn extracts," *Lwt*, vol. 98, pp. 477–484, 2018.

[27] S. K. Devatkal, P. Thorat, and M. Manjunatha, "Effect of vacuum packaging and pomegranate peel extract on quality aspects of ground goat meat and nuggets," *Journal of Food Science & Technology*, vol. 51, no. 10, pp. 2685–2691, 2014.

[28] K. Radha krishnan, S. Babuskin, P. Azhagu Saravana Babu et al., "Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat," *International Journal of Food Microbiology*, vol. 171, pp. 32–40, 2014.

[29] E. Chouliara, A. Karatapanis, I. N. Savvaidis, and M. G. Kontominas, "Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C," *Food Microbiology*, vol. 24, no. 6, pp. 607–617, 2007.

[30] Y. Y. Qin, Z. H. Zhang, L. Li et al., "Antioxidant effect of pomegranate rind powder extract, pomegranate juice, and pomegranate seed powder extract as antioxidants in raw ground pork meat," *Food Science and Biotechnology*, vol. 22, no. 4, pp. 1063–1069, 2013.

[31] D. M. Reddy, G. V. B. Reddy, and P. K. Mandal, "Application of natural antioxidants in meat and meat products-a review," *Food and Nutrition J*, vol. 7, no. 3, 2018.

[32] H. M. Ibrahim, R. A. Amin, N. Z. Eleiwa, and N. M. Ahmed, "Estimation of some biogenic amines on chicken meat products," *Benha Veterinary Medical Journal*, vol. 32, no. 1, pp. 23–28, 2017.

[33] S. Bardócz, "Polyamines in food and their consequences for food quality and human health," *Trends in Food Science & Technology*, vol. 6, no. 10, pp. 341–346, 1995.

[34] E. Renes, I. Diezhandino, D. Fernandez, R. E. Ferrazza, M. E. Tornadijo, and J. M. Fresno, "Effect of autochthonous starter cultures on the biogenic amine content of Ewe's milk cheese throughout ripening," *Food Microbiology*, vol. 44, pp. 271–277, 2014.

[35] V. Šimat and P. Dalgaard, "Use of small diameter column particles to enhance HPLC determination of histamine and other biogenic amines in seafood," *LWT-Food Science & Technology*, vol. 44, no. 2, pp. 399–406, 2011.

[36] L. Maintz and N. Novak, "Histamine and histamine intolerance," *American Journal of Clinical Nutrition*, vol. 85, no. 5, pp. 1185–1196, 2007.