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# Effect of peppermint essential oil and ultrasonication on microbiology evaluation and quality parameters of stored chicken meat

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Chicken meat is a highly perishable food item that provides an ideal environment for microbial growth, which can affect its quality under refrigeration. The objective of this study was to determine the effects of peppermint essential oil (PEO) and ultrasonication (US) on the quality of broiler chicken meat. A total of seven chicken meat samples ($T_0$–$T_6$) were used, where $T_0$ served as the control sample and $T_1$–$T_6$ were treated with different PEO concentrations (0.5 and 1%). These samples were subjected to US at a frequency of 37 kHz (for durations of 2, 4, and 6 min) and a power level of 600 W. Then, the samples were vacuum-packed in polyethylene bags and stored at refrigerator temperature for 12 days. Physicochemical analysis [including thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen (TVBN), pH, and water holding capacity (WHC)], texture profile analysis (cohesiveness, hardness, and chewiness), and microbial analysis (TPC, *E. coli, Salmonella,* and *coliform*) were conducted at 0, 3, 6, 9, and 12 days. Compared to untreated samples, all treated samples exhibited a significant ($p < 0.05$) decrease in microbial counts during storage. Ultrasonication for 6 min combined with 1% peppermint oil resulted in a significant decrease in TVBN and TBARS, along with an increase in WHC and cooking yield of chicken meat during storage. The treated group showed a significant decrease in total plate count, *Salmonella, coliform,* and *E. coli,* with counts decreasing from 1.53–3.76 CFU/g, 1.21–1.99 CFU/g, 1.08–1.48 CFU/g, and 1.95–2.99 CFU/g, respectively. In contrast, the untreated group showed counts of 2.4–7.71 CFU/g, 3.56–5.61 CFU/g, 1.87–4.41 CFU/g, and 4.47–7.23 CFU/g, respectively, over 12 days of refrigeration at 4°C. The highest cooking yield was observed in $T_6$ (74.86%), while the lowest was in the control sample $T_0$ (72.45%) after 12 days of storage. These findings indicate that the T6 sample showed significantly enhanced quality attributes of treated chicken meat samples. These results showed that PEO and US were effective natural methods of

meat preservation that ultimately benefit the meat industry in terms of improved product quality and safety.

KEYWORDS

peppermint essential oil, ultrasonication, microbial analysis, physicochemical, chicken meat quality

## Introduction

Meat is an essential component of the human diet and provides a substantial source of protein, vitamin B, lipids, and minerals. Higher-quality protein and other essential nutrients for optimal body function can be readily obtained from chicken meat (Gallo et al., 2018). The United States Department of Agriculture (USDA) reports that the overall production of chicken meat in 2022–2023 was 102.2 million metric tons, reaching 103.83 million metric tons in 2023–2024, a yearly increase of 2%. Global meat consumption is expected to rise from 42.4 kg in 2021 to 43.7 kg in 2030, according to the data from the Organization for Economic Co-operation and Development (OECD) and Food and Agriculture Organization Food and Agriculture Organization (FAO) in 2021 (Outlook, 2021). Microbial growth and physicochemical variations are the primary reasons for the decline in the freshness or quality of chilled chicken meat. Approximately 20% of the initial global meat production (304.2 million tons) is lost each year due to spoilage (Luong et al., 2020). The metabolic activities of microorganisms produce metabolites that lead to chemical and physical changes in chicken meat, such as unpleasant smells, discoloration, and slime formation (Katiyo et al., 2020). The use of food additives (preservatives and antioxidants) has raised global concerns in the last few decades (Aguiar Campolina et al., 2023). In response, numerous plant-based essential oils have been effectively used in meat as natural alternatives to synthetic food additives, due to their antimicrobial properties (Shaltout, 2024). The recent trend is to decrease synthetic additives, which have been vastly used because of the growing concern among consumers about their serious effects on human health (Khaled et al., 2016). Researchers are focusing on studying the effectiveness of natural substitutes to increase the shelf life of raw chicken. Essential oils, originating from plants and their derivatives, are considered natural alternatives in this scenario. In addition to prolonging the freshness of meat and related products, essential oils (EOs) can also effectively treat a number of human diseases (Dakhlaoui et al., 2022). EOs display their antimicrobial action against pathogenic microorganisms. Due to their high concentration of phenolic components along with additional active ingredients that are capable of preventing oxidative processes, natural antioxidants have an advantage over synthetic alternatives (Azadeh et al., 2019).

Plant extracts are used as natural preservatives in meat because they prevent lipid oxidation and the formation of harmful microorganisms (Sobri et al., 2023). The peppermint (*Mentha piperita* L.) plant is an aromatic perennial herb first grown in Egypt (Abdel-Wareth and Lohakare, 2014). Multiple advantageous attributes are studied, including hepatoprotective, antiviral, antibacterial, antioxidant, and antiulcer properties (Kaur et al., 2020). Menthol makes up 30–55% of the total components in peppermint essential oil, and it is primarily responsible for the antibacterial and antioxidant properties in food systems (Kamatou et al., 2013).

The rising consumer concerns for healthy and high-quality food products and the increasing demand for these items are driving advancements in food processing worldwide. Technological innovations are seen as the most effective solution. Innovations such as microfiltration, high-pressure processing, and ultrasonication have been specifically developed to enhance flexibility, energy efficiency, cost-effectiveness, and sustainability in food production (Al-Hilphy et al., 2020). An ultrasound (US) is a non-invasive and non-ionizing kind of mechanical energy (Alarcon-Rojo et al., 2019). The ultrasound waves cause the formation of fast-moving microbubble streams. In subsequent US cycles, bubbles expand until they become unstable, and they burst and produce high pressure and temperatures (Gavahian et al., 2022). Such phenomena may significantly influence biological tissues at the micro level, potentially improving food quality (Alarcon-Rojo et al., 2015). US is an emerging technique that is used for improving the biochemical and functional properties of meat products (Altemimi et al., 2017). The US is frequently employed to enhance quality, textural, and microbial inhibition, which are key attributes of consumer acceptability (Boateng and Nasiru, 2019).

The combination of peppermint essential oil and ultrasonication can be used as a natural preservative to improve the quality of chicken meat at an industrial level. The current research is designed to investigate the effect of peppermint oil and ultrasonication on the physicochemical and microbial properties of chicken meat and also to improve the storage stability of chicken meat at refrigerator temperature (4°C).

## Materials and methods

### Procurement of raw material

The chicken breast and peppermint leaves are procured from the local market of Faisalabad, Pakistan. The fresh chicken breast meat (Broiler) was vacuum-packed in polyethylene bags after being cooled to 4°C and weighed. Then, they were brought to the meat lab in an insulated iced container within 20 min. The research was conducted in the meat laboratory (University of Agriculture, Faisalabad, Pakistan). All of the standards, chemicals, and reagents used during the present research were supplied by Sigma-Aldrich (St. Louis, MO, USA).

### Isolation of essential oil

Peppermint oil was extracted using the method of Jayakumar et al. (2019). A Soxhlet apparatus was used to extract peppermint oil using hexane as a solvent. A 25 mg sample of peppermint leaves, oven-dried at 30°C for 5 days and grounded, was taken in filter paper and stapled to make a sachet. Then, samples were placed in an extraction chamber attached to a condenser. Hexane (250 mL) was taken in a flask, placed on a heating source, and turned on the condenser and heating supply.

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To extract the oil from the sample, hexane was boiled (69°C) and dripped onto the sachet. The process should run for a total of 16 h. The hexane was then eliminated using a rotating evaporator operating under vacuum.

## Treatment of chicken meat using peppermint oil and ultrasonication

Elma E 60 H (Germany), a laboratory-scale ultrasonic instrument with a 37-kHz frequency at power levels of 600 W, was used. The chicken meat was cut into pieces (20 mm x 20 mm x 20 mm). Then, chicken meat was treated by spraying the peppermint oil on the surface of the meat with different percentages, specifically 0.5 and 1%. After treatment, the samples were vacuum-packed. To transmit acoustic waves, every sample was placed at the bottom of an ultrasonic bath filled with distilled water at 30°C. After that, the samples were subjected to ultrasound for 2, 4, and 6 min at a frequency of 37 kHz and a power level of 600 W. As indicated in Table 1, a total of seven distinct treatment combinations were employed in this investigation. Following each treatment, the samples were vacuum-packed in low-density polythene bags for 12 days at refrigerator temperature. Unprocessed meat is used as a control sample.

## Physicochemical analysis

### Thiobarbituric acid reactive substances (TBARS)

The TBARS value of the meat sample treated with ultrasonication and peppermint oil was examined through a methodology of Sun et al. (2021). First, minced meat was diluted with 25 mL water. After adding 25 mL (v/v) trichloroacetic acid (TCA), the mixture was flapped for 3 min using a homogenizer. After filtering, the supernatant was mixed with 5 mL of TCA, heated at 80°C (40–45 min), and cooled to room temperature. The test TBARS absorbance was evaluated at 532 nm.

### Total volatile basic nitrogen (TVBN)

TVBN was estimated using a method of Bekhit et al. (2021). The TVBN was measured using the titration method. Ten grams of chicken flesh were dispersed in 100 mL of distilled water. All meat tissues were homogenized to break them down and then filtered. Next, 5 mL of magnesium oxide (MgO) and 5 mL of the filtrate were added to a distillation tube. This tube was connected to the distillation assembly for sample distillation. The distillation assembly contained 20 mL of boric acid solution and a few drops of indicator in a separate beaker to confine the distilled liquid. Finally, the endpoint of the titration was determined using 0.1 N hydrochloric acid.

TABLE 1 Treatment plan of chicken meat treated with peppermint essential oil and ultrasonication.

| Treatments | Peppermint oil (%) | Ultrasonic frequency (kHz) | Time (min) |
|---|---|---|---|
| $T_{o\ (control)}$ | 0 | 0 | 0 |
| $T_1$ | 0.5 | 37 | 2 |
| $T_2$ | 1 | 37 | 2 |
| $T_3$ | 0.5 | 37 | 4 |
| $T_4$ | 1 | 37 | 4 |
| $T_5$ | 0.5 | 37 | 6 |
| $T_6$ | 1 | 37 | 6 |

### pH of chicken meat

A pH meter was used to analyze the pH of chicken meat samples following the method mentioned by AOAC (2023). The samples were blended with distilled water. The electrode of the pH meter was used to calculate the pH by immersing it in the homogenized solution of the chicken meat.

### Water holding capacity (WHC)

The WHC was assessed using the centrifugation method (AOAC, 2023). A 15 g minced chicken meat sample was placed in centrifuge tubes. Then, 22.5 mL of sodium chloride (NaCl) (0.6 M) was added to the sample and mixed thoroughly. The mixture was then centrifuged for 16 min at 10,000 rpm at a temperature of 4°C. Finally, the volume of the supernatant was measured.

$$\text{WHC\%} = \frac{\text{Final volume} - \text{Initial volume}}{\text{Weight of sample}} \times 100$$

### Cooking yield

The raw sample was weighed and then placed into a polythene bag. The polythene bag was placed in a water bath. Then, it was cooked at 90°C for 30 min. After cooling for 30 min under running tap water, the bags containing the sample were weighed and the cooking yield was calculated, as stated by Shin et al. (2022).

$$\text{Yield}\,(\%) = \frac{\text{Edible Product}}{\text{Actual Product}} \times 100$$

### Texture profile analysis

The texture profile of the chicken meat samples was evaluated using the Lamy Rheology TX-700 texture analyzer (Champagne at Mont d'Or France), as described by Aguirre et al. (2018). The samples were cooked at 90°C, cut into cubes, and placed under the texture analyzer. The procedure was initiated once the equipment was properly set up. Both graphical and digital readings were obtained. The texture analyzer needle was allowed to penetrate the sample at an average speed of 80 mm per minute, perpendicular to the muscle fibers. The measured variables were the hardness, adhesiveness, and cohesiveness of the chicken meat.

### Microbiological analysis

Media (Nutrient agar, *Salmonella*-Shigella agar, MacConkey agar, and Eosin-methylene blue agar) was prepared for total plate count, "*Salmonella,*" "*Escherichia coli,*" *and* "*coliform* count" and autoclaved at 121°C. Aliquots of 1 mL were prepared from the dilutions and plated onto agar plates using a sterilized pipette. The prepared Petri plates were inverted and incubated for 24 h at 37°C. The inoculate was spread by sterile glass spreaders over the agar plates. Zhang et al. (2021) calculated the total number of colonies per gram by multiplying the count obtained from a specific dilution by a dilution factor.

$$\text{Total viable count} = \frac{\text{Average no of colonies} \times \text{Dilution factor}}{\text{Volume factor}} \times 100$$

### Sensory analysis

The sensory evaluation was conducted using a 9-point hedonic scale, as described by Civille et al. (2024). Panelists with extensive training and expertise in food sensory evaluation were selected for each group to examine the flavor, texture, and color of raw chicken meat. Additionally, each chicken group was oven-cooked for 15 to 20 min at 180 to 200°C and evaluated for color, texture, and flavor. Each panelist was instructed to assign a single numerical grade between 1 (unacceptable) and 9 (extremely like). The panelists performed the sensory evaluation three times.

## Statistical analysis

The Montgomery (2017) method was used to statistically analyze the data. Factorial analysis under two-way ANOVA was conducted at a significance level of $p < 0.05$ to check the effect of storage and different treatments. Tukey's *post-hoc* test was used to compare means and find the difference between storage and their interaction among different treatments. The sample size was 7, which was performed in triplicate. To find the significant variations between mean values and standard deviation, Statistix 10 software was used.

# Results and discussion

## Physicochemical analysis

### TBARS analysis

The meat quality degradation and the meat stability index were determined using the TBARS analysis (Wang et al., 2021). The TBARS values of each treated sample throughout a 12-day storage period are shown in Figure 1. Chicken meat had TBARS values between 0.33 and 1.04 mg/kg in controls, 0.30 and 0.76 mg/kg in PEO 0.5% + US 2 min, 0.26 and 0.63 mg/kg in PEO 1% + US 2 min, 0.24 and 0.81 mg/kg in PEO 0.5% + US 4 min, 0.2 and 0.6 in PEO 1% + US 4 min, 0.21 and 0.56 in PEO 0.5% + US 6 min, and 0.12 and 0.45 in PEO 1% + US 6 min samples over 12 days at 4°C. Similar TBAR values were observed by Nath et al. (2024), who noticed that TVBN values for samples treated with clove and bamboo essential oils nano-emulsion were lower than those for untreated samples and were lower until 15 days of storage. The results demonstrated that the untreated sample ($T_0$) had higher TBARS values than the treated samples. It means that PEO and US are beneficial in retarding lipids oxidation and preventing the aldehyde and ketone formation. PEO lessens TBARS values from increasing gradually and plays a role in meat stability (Pavelková et al., 2016). At 0 days, the lowest TBARS value was found in $T_6$ (0.12 mg MDA/kg) and the highest value in the untreated sample (0.33 mg MDA/kg). In 3, 6, and 9 days, there were increases in TBARS values of the $T_0$ sample, which were 0.46, 0.61, and 0.78 mg MDA/kg, respectively, compared to 0 days. These findings demonstrated that there were significant synergistic effects in retarding lipid oxidation when the US+PEO were combined. As the concentrations of PEO and US increased, the combination of PEO + US treatment showed a more favorable effect than each treatment individually. Lipid oxidation causes microbial spoilage, which makes the meat deteriorate due to oxidations, ultimately leading to aldehydes and ketones formation, which are responsible for the deterioration of meat quality. Similar results were described by Sharma et al. (2017) and Jaspal et al. (2021). Sharma et al. (2017) found a significant rise in TBARS values of chicken sausages subjected to various essential oils throughout storage days, although the increasing rate was relatively slower in oil-treated samples, indicating greater oxidative stability of the treated products. An increase in TBARS in stored products may be due to increased lipid oxidation and the release of volatile metabolites under aerobic storage. The decrease of TBARS in the treated group may be linked to the polyphenols included in essential oils, which possess antioxidant properties.

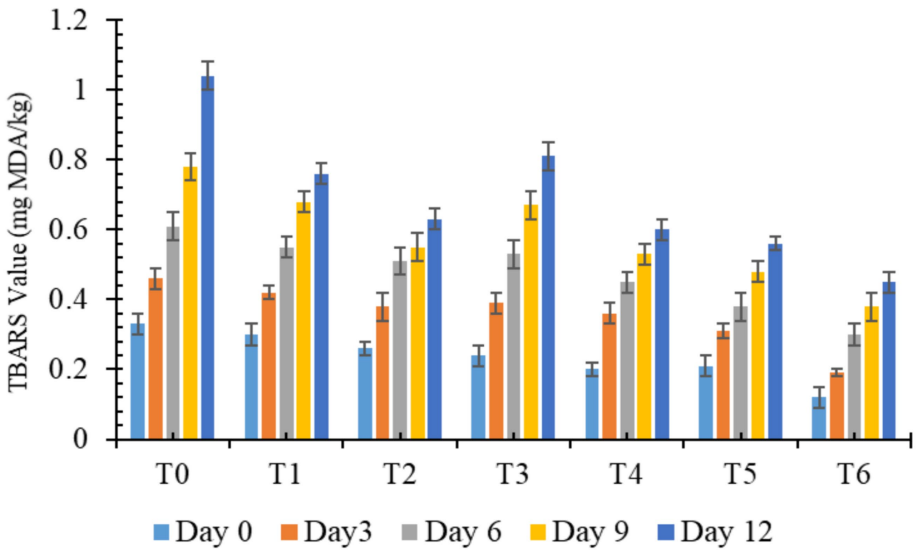


FIGURE 1
The impact of PEO + US treated samples on TBAR value.

## TVBN of meat

The results demonstrated that the untreated sample ($T_0$) had a high TVBN value (32.59 mg/100 g), while the $T_5$ and $T_6$ samples had minimal values (21.57 mg/100 g and 19.78 mg/100 g) at day 12, respectively. TVBN of chicken meat decreases with increased PEO concentration (Figure 2). All treated samples substantially influenced TVBN (total volatile basic nitrogen) levels in chicken meat. Similar results were found by Musalem et al. (2024), who examined how adding 1% Nanoparticles of chitosan and ginger (NCHG) to chicken fillet significantly reduced its TVBN values while maintaining the rate of protein oxidation from 0 to 28 days of storage. During the 12–28 days storage period, TVBN values were reduced by NCHG 1% instead of by NCHG 0.5%. At 0 days, $T_0$ showed the highest mean value of TVBN (6.85 mg/100 g) and $T_6$ showed the lowest value (6.05 mg/100 g). There was a significant ($p < 0.05$) increasing trend in TVBN values from 0 to 12 days of storage. $T_6$ showed a minimum increase in the TVBN value (8.93 mg/100 g) on day 3 of storage. The synergistic effect of PEO and ultrasonication significantly decreases the TVBN value and prevents the meat from rancidity because sometimes the lipid structure is disrupted due to ultrasonication, which can lead to oxidation. However, peppermint essential oil has strong antioxidant properties that prevent meat lipid peroxidation. Hence, in combination, the phenomenon of oxidation is controlled while ensuring microbial safety and improving the overall quality of meat (Khare et al., 2014). The TVBN values increased significantly during the refrigerated storage temperature. However, the increasing pattern varied significantly among the different treatment samples. The breakdown of proteins and other non-protein nitrogenous substances, such as free amino acids and nucleotide catabolizes, by spoilage bacteria and endogenous enzymes is mostly responsible for the increased TVBN content (Bekhit et al., 2021). Our results showed that PEO and US might lower TVBN values on day 12 of storage; the breakdown of protein due to bacterial activity and their proteolytic enzymes may be the source of the increase in TVN values in the meat (Ea Hassan, 2011). These findings were found to be similar to those mentioned by Khaled et al. (2023), in which the chicken meat was treated with basil oil at different storage intervals. In meat treated with BEO at concentrations of 1 and 1.5%, TVN values increased less significantly in comparison to untreated samples at days 0, 2, 4, and 6 of storage due to several bacteria and their proteolytic enzymes.

## pH

pH is an essential criterion to determine the quality of meat. The peppermint oil and ultrasonication significantly ($p < 0.05$) affected the chicken meat pH value over 12 days. The pH value of chicken meat decreases gradually from 0 to day 12 of storage, as demonstrated in Figure 3. The sample treated with 1% peppermint essential oil ($T_6$) exhibited a lesser decline in pH (5.58) than the untreated sample (4.76) during 12 days of storage. Our findings were consistent with the results of Iulietto et al. (2015). They found that beef meat samples treated with 2% of thyme oil had a lower pH value than those treated with 1% of thyme essential oil. Hamoen et al. (2013) found that the samples treated with 1.5% thyme oil showed lower pH effects. The gradual decrease may be due to the antimicrobial and antifungal effects of phenolic compounds found in peppermint oil. Additionally, the scavenging activity of phenolic and flavonoid compounds in peppermint oil results in high antioxidant activity. According to Pösö and Puolanne (2005), the pH of the sample decreased during storage, which indicates the deterioration and spoilage of meat. These findings were comparable to those of Sobri et al. (2023), who studied the impact of US and oil palm frond extract on the quality attribute of marinated goat, where pH value decreased significantly during the storage period.

## Water holding capacity (WHC)

The combined effect of PEO + US significantly affected the WHC of the chicken over 12 days (Table 2). The water holding capacity for the control samples varied from 58.61 to 57.15%, while the values for treated samples ($T_1$, $T_2$, $T_3$, $T_4$, $T_5$, and $T_6$) ranged from 59 to 58.37%, 59.12 to 59.81%, 59.19 to 60.15%, 59.28 to 60.5%, 59.36 to 60.9% and

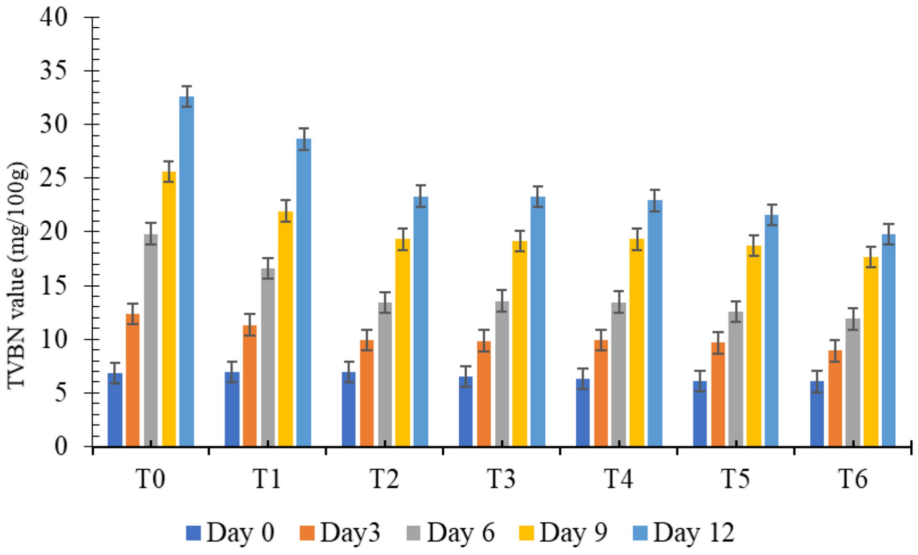


FIGURE 2
The impact of PEO + US treated samples upon TVBN value.

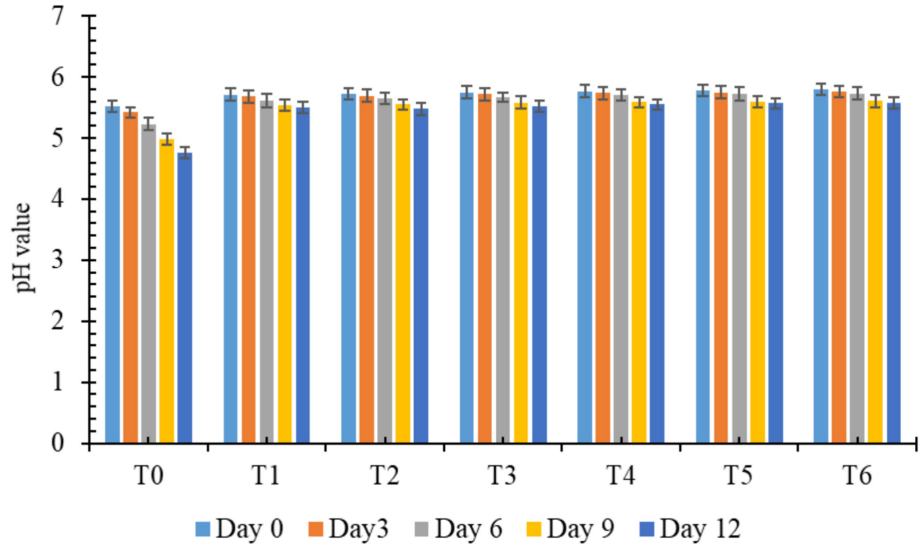


FIGURE 3
The impact of PEO + US treated samples on pH value.

TABLE 2 The impact of PEO + US treated samples on water-holding capacity.

| Treatments | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 |
|---|---|---|---|---|---|
| $T_0$ | 58.61 ± 0.05$^{o}$ | 58.53 ± 0.04$^{op}$ | 58.48 ± 0.06$^{pq}$ | 57.65 ± 0.05$^{r}$ | 57.15 ± 0.03$^{s}$ |
| $T_1$ | 59 ± 0.03$^{lm}$ | 58.96 ± 0.06$^{mn}$ | 58.86 ± 0.04$^{n}$ | 58.84 ± 0.07$^{n}$ | 58.37 ± 0.05$^{q}$ |
| $T_2$ | 59.12 ± 0.06$^{kl}$ | 60.08 ± 0.05$^{ef}$ | 60.13 ± 0.05$^{e}$ | 59.96 ± 0.03$^{f}$ | 59.81 ± 0.04$^{g}$ |
| $T_3$ | 59.19 ± 0.05$^{jk}$ | 60.41 ± 0.03$^{d}$ | 60.42 ± 0.07$^{d}$ | 60.39 ± 0.06$^{d}$ | 60.15 ± 0.06$^{e}$ |
| $T_4$ | 59.28 ± 0.03$^{ij}$ | 60.87 ± 0.05$^{c}$ | 60.86 ± 0.03$^{c}$ | 60.79 ± 0.04$^{c}$ | 60.5 ± 0.07$^{d}$ |
| $T_5$ | 59.36 ± 0.05$^{i}$ | 61.36 ± 0.04$^{b}$ | 61.35 ± 0.06$^{b}$ | 61.29 ± 0.03$^{b}$ | 60.9 ± 0.04$^{c}$ |
| $T_6$ | 59.49 ± 0.04$^{h}$ | 61.88 ± 0.03$^{a}$ | 61.85 ± 0.04$^{a}$ | 61.80 ± 0.05$^{a}$ | 61.76 ± 0.05$^{a}$ |

59.49 to 61.76%, respectively, during 0–12 days of refrigerated storage. The results demonstrated that the $T_0$ (untreated sample) had a low WHC value (low rate of water activity), while $T_5$ and $T_6$ had maximum WHC values. WHC of chicken meat increases with the increase of PEO concentration. At 0 days, $T_0$ showed the lowest mean value of WHC (58.61%) and $T_6$ showed the highest mean value (59.49%). There was a significant decreasing trend in WHC values from day 0 to 12 days of storage period. The $T_6$ sample showed an increasing WHC value (61.88%) than other treatments on the third day of storage. The increased WHC values are likely due to an increase in pH and the amount of protein in meat. This enhances meat's capacity to retain a considerable amount of water (Bowker and Zhuang, 2015). These results can be related to the active chemicals in these oil-treated samples that can prevent cellular membranes from degradation, thereby protecting proteins from breakdown and water loss from protein–water interactions. These findings were comparable to those of Al-Zaidi and Ahmed (2020), who investigated the impact of essential oils on beef sausages at different storage intervals. Treatment $T_4$ (rosemary leaf oil) significantly enhances water holding capacity (WHC) relative to the untreated sample. The results can be related to the active chemicals in these treatments that prevent cellular membranes from degradation in order to keep proteins from decomposition and prevent the loss of water that remains bound to protein–water interactions (Viuda-Martos et al., 2015).

## Cooking yield

Cooking yield is the amount of food product at the end of the cooking process (Ozilgen, 2019). Chicken meat had cooking yield values between 74.6 and 72.45% in controls, 74.75 and 73.69% in PEO 0.5% + US 2 min, 75.01 and 73.01% in PEO 1% + US 2 min, 74.98 and 72.93% in PEO 0.5% + US 4 min, 75.25 and 74.25% in PEO 1% + US 4 min, 75.10 and 74.42% in PEO 0.5% + US 6 min, and 75.51 and 74.86% in PEO 1% + US 6 min samples over 12 days at refrigerator temperature (4°C). The highest cooking yield was observed in $T_6$ (74.86%), while the lowest was in $T_0$ (72.45%) during the 12 days of storage. The PEO + US combined treated samples had more cooking yield than the untreated sample. The treated samples significantly ($p < 0.05$) decreased the cooking yield value during storage than that of the untreated sample (Table 3). These findings were similar to those of Jama et al. (2008), who studied the effect of cooking losses on beef. They found that cooking losses increased in cattle meat stored at 4°C, resulting in moisture losses and deterioration of meat compounds. However, the edible-coated meat reduces moisture losses during storage, prevents spoilage, and may increase the yield.

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TABLE 3 The impact of PEO + US treated samples upon cooking yield.

| Treatments | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 |
|---|---|---|---|---|---|
| $T_0$ | $74.6 \pm 0.07^{gh}$ | $74.02 \pm 0.04^{k}$ | $73.45 \pm 0.05^{n}$ | $73.08 \pm 0.06^{p}$ | $72.45 \pm 0.05^{r}$ |
| $T_1$ | $74.75 \pm 0.06^{ef}$ | $74.4 \pm 0.04^{i}$ | $74.02 \pm 0.05^{k}$ | $73.85 \pm 0.04^{l}$ | $73.69 \pm 0.03^{m}$ |
| $T_2$ | $75.01 \pm 0.05^{c}$ | $74.22 \pm 0.05^{j}$ | $73.66 \pm 0.04^{m}$ | $73.35 \pm 0.04^{no}$ | $73.01 \pm 0.04^{Pq}$ |
| $T_3$ | $74.98 \pm 0.06^{cd}$ | $74.45 \pm 0.03^{i}$ | $74.25 \pm 0.05^{j}$ | $73.23 \pm 0.04^{o}$ | $72.93 \pm 0.05^{q}$ |
| $T_4$ | $75.25 \pm 0.05^{b}$ | $74.95 \pm 0.05^{cd}$ | $74.71 \pm 0.06^{fg}$ | $74.52 \pm 0.05^{hi}$ | $74.25 \pm 0.05^{j}$ |
| $T_5$ | $75.19 \pm 0.06^{b}$ | $75.01 \pm 0.03^{c}$ | $74.81 \pm 0.05^{ef}$ | $74.61 \pm 0.05^{gh}$ | $74.42 \pm 0.04^{i}$ |
| $T_6$ | $75.51 \pm 0.05^{a}$ | $75.42 \pm 0.05^{a}$ | $75.24 \pm 0.06^{b}$ | $75.06 \pm 0.05^{c}$ | $74.86 \pm 0.05^{de}$ |

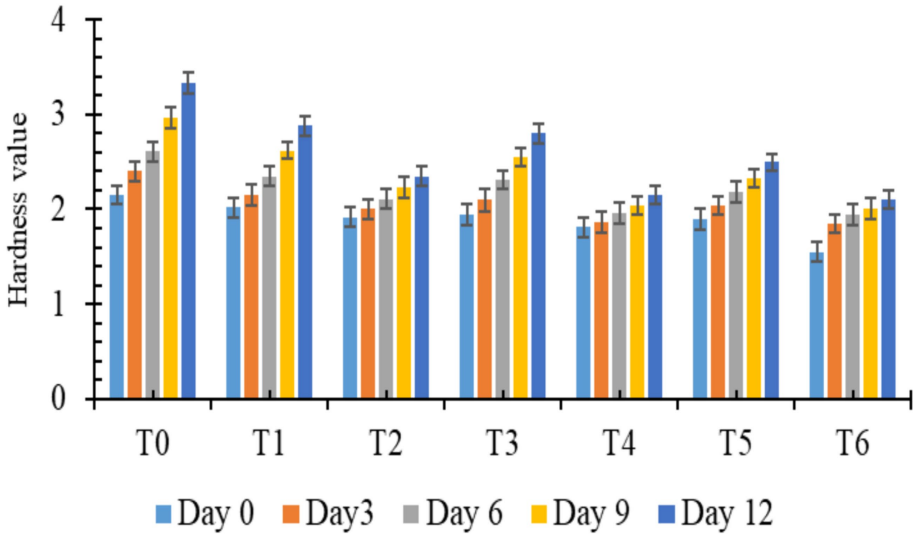


FIGURE 4
The impact of PEO + US treated samples on hardness value.

## Texture profile analysis

Texture analysis is important in determining meat tenderness, flavor, and juiciness (Warner et al., 2021).

## Hardness

The PEO and US significantly ($p < 0.05$) affected the hardness value of the chicken over 12 days, as shown in Figure 4. During day 0, the hardness values of chicken meat varied between 1.55 and 2.15 among all the treated samples. The highest hardness value was observed in the $T_0$ sample (3.33), while the lowest was in $T_6$ (2.1) during the 12 days of storage. It was observed that using the PEO and US techniques decreased the hardness values during storage. The antioxidant activity of plant extracts in chicken meat helps to prevent textural variations compared to the control meat sample (Ferreira et al., 2017). These findings were comparable to those of Carvalho et al. (2020), who studied the combined impact of active packaging and oregano oil in beef burgers in which the hardness of meat decreased when oil was applied to the samples.

## Chewiness

The US+ PEO showed significant variations in the chewiness of all treated samples. During day 0, the chewiness values of chicken meat varied between 1.88 and 2.13 among all the treated samples. The chewiness of chicken meat varies from 1.99 to 2.32 among all treatments during 6 days, with the maximum value of chewiness in $T_0$ (2.62) and the minimum value in $T_6$ (2.08), as shown in Figure 5. These findings were comparable to those of Chang et al. (2012), who studied the effect of characteristic changes in beef during ultrasound processing. The chewiness of meat is determined by the level of fiber expanding, which is impacted by the breakdown of myosin. The development of gels on the meat surface due to heat or additional pressure may account for the enhanced chewiness. Roobab et al. (2024) observed an initial decrease in textural parameters, followed by an increase with higher intensity or longer treatment time.

## Cohesiveness

The cohesiveness values of chicken meat among all treatments on 0 days varied from 1.15 to 1.75, and the maximum value of cohesiveness was observed in $T_6$ while the minimum value was observed in $T_0$, as shown in Figure 6. Roobab et al. (2024) found that samples exposed to ultrasound treatment of 800 W for 30 min exhibited improved cohesiveness relative to untreated samples. The development of protein gels on the meat's surface due to heat may account for enhancing cohesiveness. Xiong et al. (2020) studied the combined application of oregano oil and nano-emulsion coating, which significantly retained the meat texture better during storage

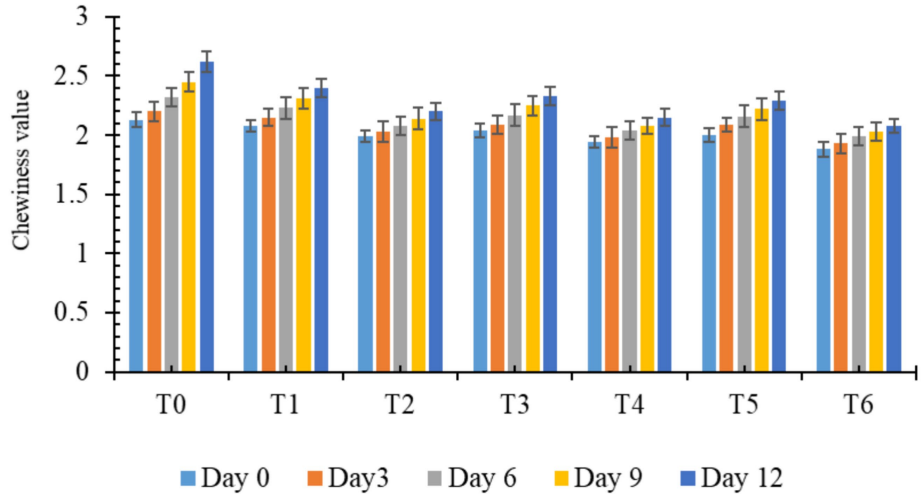


FIGURE 5
The impact of PEO + US treated samples on the chewiness of meat.

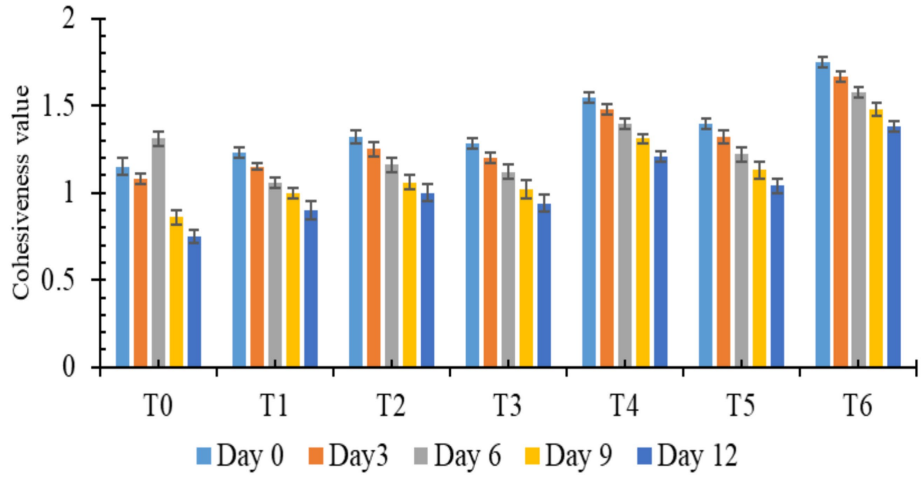


FIGURE 6
The impact of PEO + US treated samples on the cohesiveness of meat.

than untreated due to essential oil and nano-emulsion coating that prevents protein degradation.

## Microbial analysis

### Total plate count (TPC) of meat

The PEO and US techniques significantly ($p < 0.05$) influenced the TPC value of the chicken over 12 days (Figure 7). The TPC for $T_0$ (control) varied from 2.4 to 7.71 log CFU/g, while the treated samples ($T_1$, $T_2$, $T_3$, $T_4$, $T_5$, and $T_6$) varied from 2.18 to 6.37, 2.07 to 6.67, 1.95 to 5.52, 1.72 to 5.18, 1.58 to 4.29, and 1.53 to 3.36 log CFU/g, respectively, during 0–12 days of storage. A similar TPC trend was observed by Nath et al., who studied the effect of clove and bamboo essential oil nano-emulsion in meatballs to increase their shelf life in which the TPC value in the control sample was 2.18–5.87 CFU/g and in the essential oil-treated sample was 2.42–5.09 CFU/g during 15 days of storage. TPC values increase during storage, while lower counts and slower increases in essential oil-treated meatballs compared to controls are likely due to the antimicrobial properties of these treatments. The results demonstrated that the untreated sample ($T_0$) had a high total plate count (TPC) value, whereas the minimum values were observed in the $T_4$ and $T_6$ samples. On day 0, $T_0$ exhibited the highest TPC value (2.40 CFU/g), while $T_6$ showed the lowest value (1.53 CFU/g). The PEO + US samples have a significant decline in microbial populations compared to the treatment individually. The synergistic effect of US+PEO is greater on microbial reduction because ultrasonication leads to the increase in microbial cell permeability, and peppermint essential oil penetrates more easily or quicker into cells, which results in a greater reduction of microbes as compared to the use of these techniques alone, similar to studies reported by

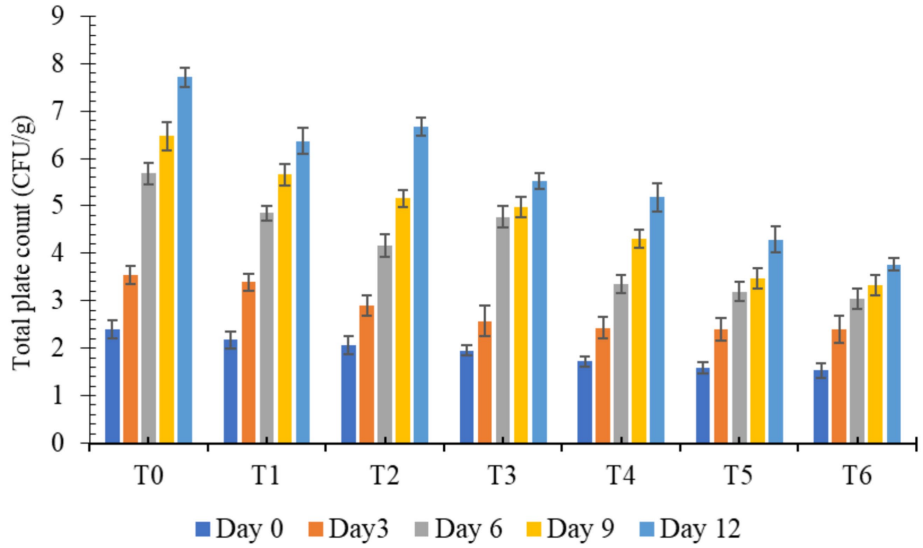


FIGURE 7
The impact of PEO + US treated samples on Total plate count.

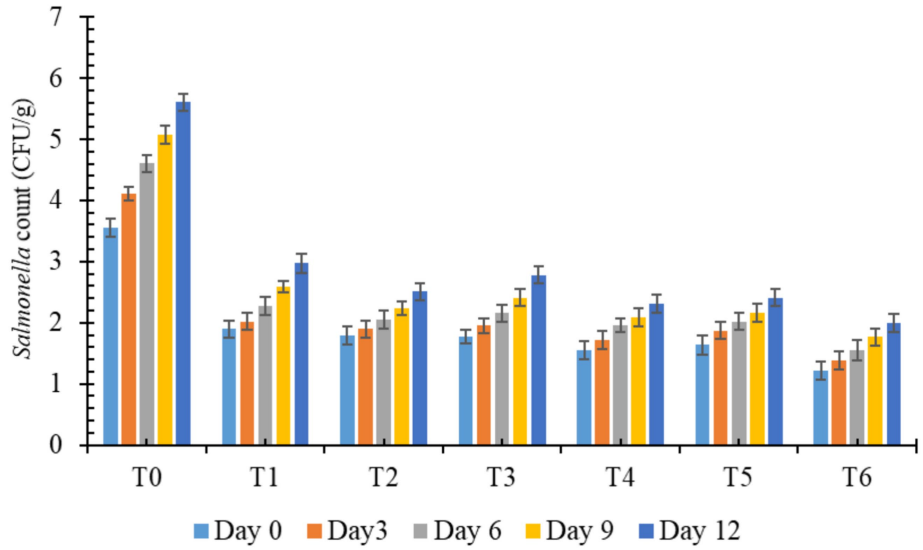


FIGURE 8
The impact of PEO + US treated samples on *Salmonella* count.

Sun et al. (2021). Millan-Sango et al. (2015) demonstrated that using oregano essential oil and ultrasound could improve the inactivation of microbes on lettuce leaf surfaces. There was an increasing trend in TPC values from day 0 to the 12th day of storage. On the 12th day, $T_6$ showed a less increasing trend of TPC (3.76 CFU/g) compared to $T_0$ (7.71 CFU/g). $T_6$ showed the minimum microbial spoilage compared to other treatments because high-frequency sound waves would rupture the cell membranes, release their intracellular contents, and kill microbes (Mustapha et al., 2024). These results supported Yu et al. (2023), who demonstrated that the bacterial loads significantly increased during storage while total bacterial counts in the thyme essential oil samples were lower than those in the control sample of mutton patties.

## *Salmonella* count

*Salmonella* infection commonly occurs when one consumes raw or undercooked meat, poultry, eggs, or egg products (Wessels et al., 2021). The combined effect of US and PEO showed a significant ($p < 0.05$) effect on the degradation of Salmonella in meat, as demonstrated in Figure 8. The *Salmonella* counts in the chicken meat samples at 0 days were 3.56, 1.9, 1.79, 1.77, 1.55, 1.64, and 1.21 CFU/g for control ($T_0$), $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, and $T_6$ treated samples, respectively. Similar results were reported by Farokhzad et al. (2023), who studied the chitosan and rosemary essential oil effect in chicken burgers during storage. As rosemary oil has antibacterial properties, the combination of chitosan and rosemary essential oil reduces the growth of microorganisms. During the

storage period, the number of microbes increased significantly among all samples. On day 0, the Salmonella growth of the untreated sample was significantly higher at 3.56 (CFU/g) compared to $T_6$ (1.21 CFU/g). On day 12, among the treated samples, $T_0$ showed the highest value of *Salmonella* count (5.61 CFU/g), while $T_6$ showed the lowest count (1.99 CFU/g). These findings were found to be similar to those of Haughton et al. (2012) and Joo et al. (2020), who studied how ultrasound treatment and peroxy-acetic significantly affected the inhabitation rate of *Salmonella and Campylobacter jejuni*. The $T_6$ treatment showed the highest decrease in *Salmonella* count because the combined PEO and US effect was more effective than individual treatment. Similar results were reported by Shu et al. (2021), who studied the synergistic effect of ultrasound and Mannosylerythritol Lipids-A (MEL-A) in which the antibacterial effect was more effective than a separate treatment.

## *Escherichia coli* (*E. coli*) count

The combination of PEO and US significantly ($p < 0.05$) affected the *E. coli* value of the chicken during the 12 days, as shown in Figure 9. The $T_0$ (control) sample values varied from 4.47 to 7.23 log CFU/g, while the treated sample values ($T_1$, $T_2$, $T_3$, $T_4$, $T_5$, and $T_6$) ranged from 2.98 to 3.7, 2.63 to 3.25, 2.8 to 3.58, 2.51 to 3.13, 2.63 to 3.32, and 1.95 to 2.99 log CFU/g, respectively, during 0–12 days of storage. Similar results were reported by Badee et al. (2013), who studied the significant reduction of microbial population in chicken meat treated with the Marjoram essential oil. The number of *Escherichia coli* in treated samples slightly increased during storage. The $T_4$ (3.13 CFU/g) and $T_6$ (2.99 CFU/g) samples have longer periods of exposure and a high percentage of PEO, which showed a significant decrease in *E. coli* during 12 days of storage. Various secondary metabolites found in PEO can reduce microbial growth. Due to their combined antimicrobial effect, PEO applications and ultrasonication treatment decrease the *E. coli* count (Roby et al., 2013). The combined utilization of cold plasma and lemongrass essential oil resulted in a synergistic antimicrobial effect against food-borne microorganisms (Cui et al., 2016). These results supported Kačániová et al. (2019), who investigated the combined impact of essential oils and vacuum packaging, which led to a significant reduction in *Enterobacteriaceae* count compared with the control in chicken thighs. Essential oils and their constituents are responsible for their antibacterial properties. Hanková et al. (2023) investigated the impact of heat treatment, thyme, oregano, and lemongrass essential oils, as well as traditional preservatives, on the survival and development of virulent *Escherichia coli* within vegetable sauces. In comparison to the other treatments, lemongrass essential oil exhibited quite notable outcomes on the fifth day of storage, reducing 1.9 logs CFU/g. These findings offer helpful perspectives into the possible use of essential oils as alternative preservatives in vegetable sauces and their influence on consumer approval.

## *Coliform* count

The combined effect of PEO and ultrasonication technique significantly ($p < 0.05$) affects *coliform* count during the 12 days of storage (Figure 10). The *coliform counts* of the chicken meat samples range from 1.87 to 4.41 in $T_0$, 1.55 to 2.58 in $T_1$, 1.46 to 1.88 in $T_2$, 1.37 to 1.69 in $T_3$, 1.24 to 1.59 in $T_4$, 1.11 to 1.55 in $T_5$, and 1.08 to 1.48 CFU/g in $T_6$ during 0–12 days of storage. During 12 days of storage, there was a gradual decreasing trend among all treatments. $T_0$ exhibited the highest mean value across all treatments over the 12-day storage duration. The minimum value was found for the $T_6$ sample (1.08 CFU/g) on day 0 compared to the $T_0$ sample (1.87 CFU/g), indicating a significant effect of the PEO and US techniques on microbial growth. The PEO may break down the lipid bilayers of cell membranes, causing a leak down of cell material, thus losing cell integrity and structure and leading to cell death (Ahmad et al., 2018). Ultrasonication causes cavities in the cell membranes, forming bubbles in the cell membranes and thus causing cell bursts (Saleem et al., 2015). The findings were similar to Bagheri et al. (2016), who studied the fennel extract (FE) on the shelf life of minced common kilka. During the storage period, the total viable count of the 0.5% encapsulated fennel extract samples was relatively low. Microbial study of fennel extract samples showed significant differences

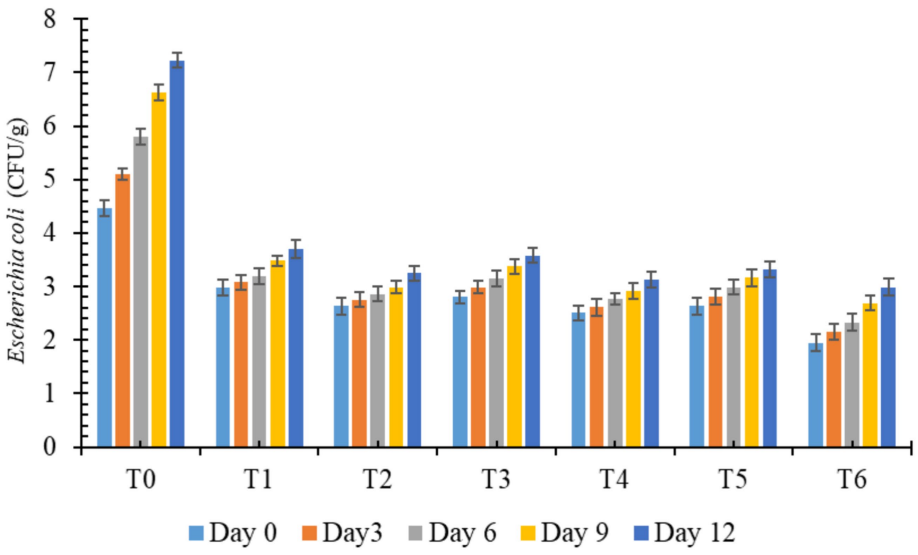


FIGURE 9
The impact of PEO + US treated samples on *E. coli* count.

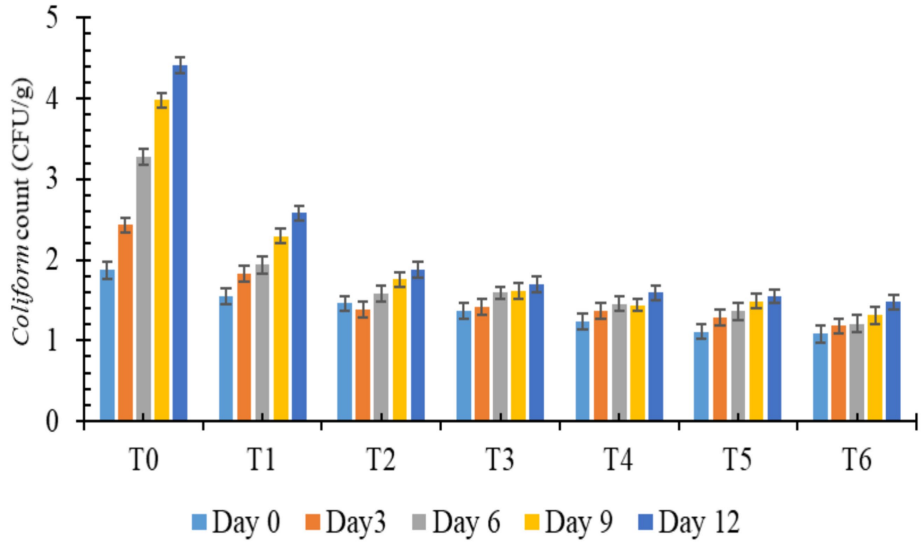


FIGURE 10
The impact of PEO + US treated samples on *coliform* count.

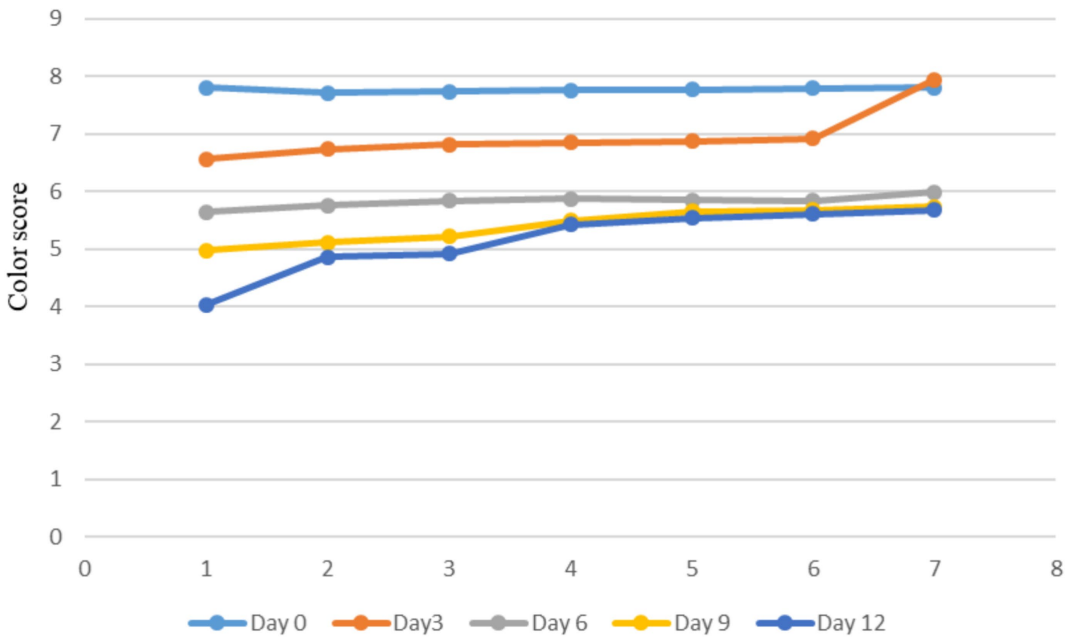


FIGURE 11
The impact of PEO + US treated samples on color.

($p < 0.05$). This suggested that TVC growth might be significantly inhibited by encapsulated fennel extract. These findings were also comparable with Diao et al. (2020), who studied garlic aqueous extracts and carboxymethyl chitosan US coating solutions to increase the shelf life of ready to eat (RTE) meat. The TVC values of the treated group were lower for their antibacterial activity than the untreated sample.

## Sensory analysis

The results demonstrate that PEO and US significantly ($p < 0.05$) affect sensory attributes, including the color, texture, and flavor of the chicken meat, as shown in Figures 11–13. The combined effect of storage and treatments also showed a significant effect on the sensory attributes of the chicken meat. The color intensity decreases significantly with the increase of storage time. The $T_6$ sample showed a declining score of color from 7.81 to 5.68 over 12 days at refrigeration temperature. The untreated treatment $T_0$ (control) had the lowest color score due to the enzymatic and chemical alterations in chicken meat during storage. The highest scoring of meat texture was observed in $T_6$ (7.86) at 0 day. However, $T_0$ (control) had the lowest score of 4.95 compared to $T_6$ (5.69) during 12 days of storage.

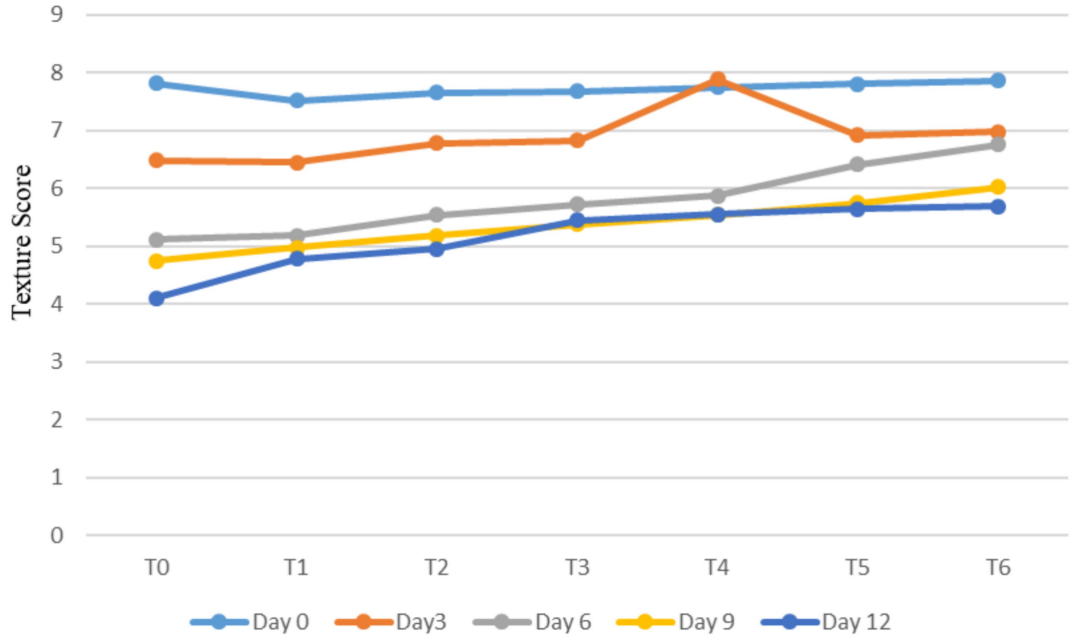


FIGURE 12
The impact of PEO + US treated samples on the texture of meat.

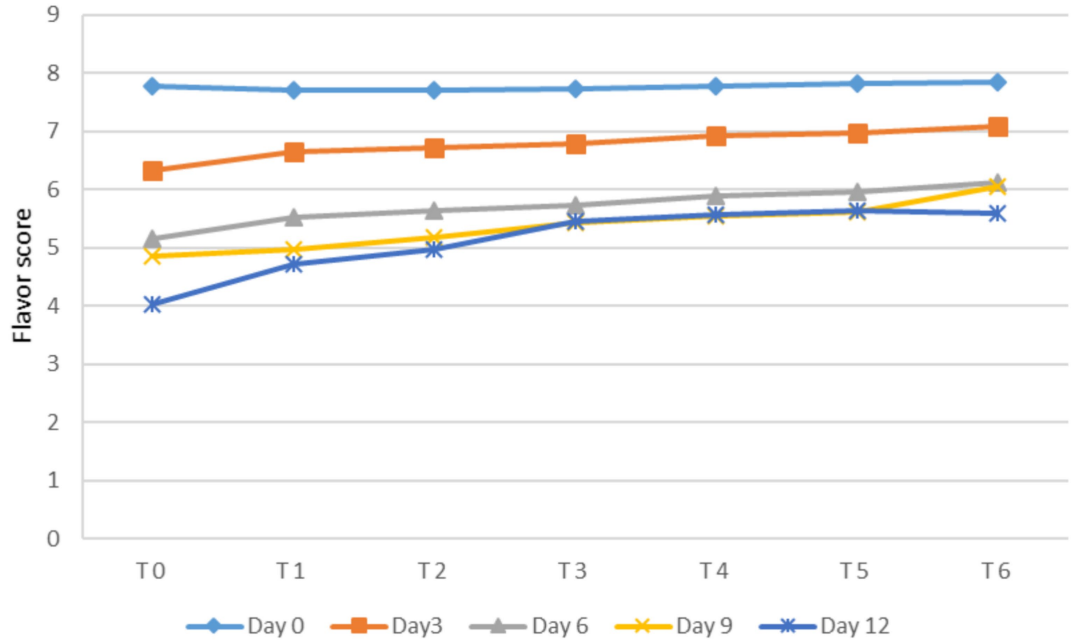


FIGURE 13
The impact of PEO + US treated samples on the flavor of meat.

The improved tenderness of the cooked marinated meat could be due to the disintegration of myofibrils integrity and structural proteins (Shi et al., 2017). The $T_6$ treatment had the highest flavor score (7.84) at 0 days of storage, while $T_0$ (control) had the lowest score (7.77). A significant decline in flavor was observed as storage days increased. However, the control treatment $T_0$ had the lowest flavor score of 4.02 compared to $T_6$, which scored 5.64 after 12 days of storage. Our findings were similar to those of Lee et al. (2023), who studied that muscle samples treated with US showed increased tenderness on days 0, 3, and 7, which resulted in enhanced sensory qualities overall. During storage, ultrasonication treatment significantly improved the umami flavor of cooked beef.

## Conclusion

The findings showed that PEO and US have antimicrobial qualities that considerably reduce microbiological development, delayed pH changes, and lower amounts of TBARS and TVBN in chicken meat throughout 12 days of storage at room temperature. PEO and US treatment also greatly delayed the textural changes and improved the quality of chicken meat. Ultrasound, an emerging technique in food safety, denatures microbial cell membranes and decreases microbial load. The result indicates that $T_6$ treatment (PEO 1% + US 6 min) reduces the growth of TPC, *E. coli*, *Salmonella,* and *coliform* during 12 days of storage study and enhances the chicken meat quality over 12 days at refrigerator temperature. Additional investigation is necessary to determine the modifications in the nutritional components and investigate the effects of PEO + US on the antioxidant properties of chicken items prepared using various cooking techniques. To maximize the best use of plant components and other potent substances and replace chemical preservatives, these natural preservatives can be employed in food processing industries. Future research will determine the effects of PEO and US on meat and seafood commodities to ensure consumer acceptance rates remain unaffected.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

NA: Methodology, Validation, Writing – original draft. ZA: Investigation, Methodology, Writing – review & editing. RS: Methodology, Project administration, Writing – review & editing. AA: Funding acquisition, Software, Writing – original draft. WA: Resources, Visualization, Writing – original draft. RB: Formal Analysis, Supervision, Writing – original draft. RK: Conceptualization, Data curation, Writing – original draft. AA-Z: Supervision, Validation, Writing – review & editing. SA-D: Project administration, Software, Writing – original draft. SA: Conceptualization, Visualization, Writing – original draft. MH: Investigation, Visualization, Writing – original draft.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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