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Effects of Adding Clove (Syzygium aromaticum L.) Seed Extract on Oxidative Stability, Microbial Activity, and Sensory Attributes in Beef Patties During Refrigerated Storage

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Abstract: The study investigated the impact of clove seed extract (CSE) at concentrations of 0.20, 0.25, and 0.30% on the oxidative stability, microbial activity, and sensory quality of beef patties stored at 4°C for 10 days. The extract was obtained using ethanol (70%) and incorporated into the patties. Lipid oxidation was assessed through malondialdehyde (MDA) levels, microbial counts (aerobic plate count, coliform, and *Pseudomonas* spp. were recorded and sensory evaluation measured color, taste, and overall acceptance. Results highlighted the effectiveness of 0.30% CSE in significantly reducing MDA levels, with values of 0.606 mg/kg compared to 0.657 mg/kg in the control group on Day 10. Microbial growth was inhibited in a concentration-dependent manner; for instance, aerobic plate counts decreased from 5.31 to 4.68 Log10 colony forming unit (CFU)/g, and coliform counts declined from 2.92 to 2.57 Log10 CFU/g at 0.30% CSE. *Pseudomonas* spp. also showed reduced numbers, stabilizing the patties' microbial quality. Sensory tests revealed that CSE-treated patties retained better color, flavor, and acceptability over time than untreated samples. Overall, the study concluded that 0.30% CSE significantly improved oxidative stability, inhibited microbial activity, and preserved sensory quality, thereby enhancing the shelf life of beef patties. These findings suggest that clove seed extract can serve as a natural, effective alternative to synthetic preservatives, promoting both product safety and consumer appeal.

1. Introduction

Due to the complex composition of meat, it is vulnerable to spoilage due to biochemical and chemical changes. Lipid oxidation, also known as oxidative rancidity, is a major reason for off-flavor development, discoloration, and poor texture of meat products [1]. Synthetic antioxidants, such as butylated hydroxyl anisole, butylated hydroxytoluene, and Tertiary Butylhydroquinone, are used to prevent lipid oxidation in meat products but have raised health concerns; they are linked to potential carcinogenic effects, endocrine disruption, and liver damage in animal studies as well as allergic reactions. These concerns have led to a shift toward natural alternatives [2, 3]. As a result, interest has developed in natural preservatives from plant sources. Herbs and spices are rich in phytochemicals, natural pigments, and antioxidants and offer many other health-promoting properties. These herbs and spices have attracted attention for their antimicrobial and antioxidant activity in the forms of essential oils, oleoresins, and other extracts [4]. Clove is a common dried flower bud used as a flavorant and natural preservative in food industries; it is rich with bioactive compounds having antioxidant and antimicrobial activity [5]. Thus, these bioactive compounds are potential natural preservatives to improve the quality and safety of beef patties during storage [6].

Although many studies have highlighted the antimicrobial activity of clove, specific data on the effect of clove seed extract on spoilage microorganisms in beef patties are still limited. Hence, studying this aspect is both interesting and valuable, given its practical relevance to the meat industry [7]. Natural extracts are potential alternatives for synthetic preservatives, having gained considerable interest in the scientific literature. Historical utilization of substances such as clove in food preservation also supports the rationale [8]. Clove possesses high phenolic compound content, particularly of eugenol, whose antimicrobial and antioxidant activity is extensively documented. Likewise, earlier studies demonstrated that phenolics found in clove seed display antioxidant activity in lipid and protein oxidation that may delay lipid oxidation in fresh beef patties [9]. Therefore, the beneficial role of plant extracts in the sensory experience and shelf-life prolongation of meat products has been widely described in the literature. Volatile compounds from plant extracts have shown good ability to mask/reduce unwanted smell and maintain sensory and shelf-life properties of meat products [10].

We examined how clove seed extract, a natural product, could be used to make beef patties better and safer. Consumers want natural food additives, especially those that improve food safety, as many diseases are spread by meat [11]. Several studies have demonstrated the antimicrobial properties of clove extracts. For example, Bai *et al.* [12] observed that alcohol-based clove essential oil effectively inhibited *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* [12, 13] found that ethanolic clove extract exhibited stronger antibacterial activity against meat spoilage microorganisms than aqueous extracts, which they attributed to its higher phenolic content, particularly of eugenol [13]. These results underscore the potential of clove extracts, particularly alcohol-based, as natural food preservatives. The main goal of this study was to find out how clove seed extracts affect the quality and shelf life of ground beef. This study aimed to evaluate the antioxidant, antibacterial, and sensory properties of the patty. Consumer choices of samples were evaluated in order of preference.

2. Materials and Methods

2.1. Preparation of Clove Seed Extracts

For this study, clove bud powder from a local market in Erbil governorate was used. The extraction was made with clove bud powder (100 g) ground in a frying pan and added to 1000 mL of 70% (v/v) aqueous ethanol and 30% distilled water in a 3 L sealed conical flask. The extraction was done at room temperature, in the dark, for 24 h with a magnetic stirrer without heat. The residue was then re-extracted three more times in similar fashion. The pooled extracts were filtered through fresh cheesecloth, dried, and concentrated using a vacuum oven at 40 °C, followed by storage at −20 °C until analysis [14].

2.2. Meat Sample Collection

Samples of leg meat from beef cattle were gathered from a slaughterhouse in Erbil governorate, Iraq, the day before the experiment. We collected all samples in polyethylene bags according to their respective categories and then transported them to the lab. Fresh patty samples were prepared as described by Ramadan *et al.* [15]. The gathered meat was washed with refined water to evacuate any contaminant particles and minced twice. The mixture was shaped manually using a patty maker (stainless steel, model) to obtain round discs of 10 cm diameter and 0.5 cm thickness with an average weight of 50 g. The beef patties were formulated to contain 0 (control), 0.20, 0.25, and 0.30 grams, respectively, of the clove bud extracts. Beef cattle patties were packaged in polyethylene bags and stored at 4 ˚C for 10 days to evaluate oxidative stability, microbial activity, and sensory attributes at 1, 3, 5, 7, and 10 days.

2.3. Lipid Oxidation Determination

Lipid peroxidation was determined by measuring the concentration of 2-thiobarbituric acid reactive substances (TBARS) according to Aminzade *et al.* [16], with some modifications. About 5 g of samples were homogenized, using a mechanical homogenizer (Ultra-TurraxTerex, Model), with 50 mL of distilled water and 1.25 mL of 4N hydrochloric acid for 2 mins. The mixture was boiled until 25 mL of distillate was obtained. Then, 2.5 mL of distillate was added to 2.5 mL of thiobarbituric acid reagent (15% trichloroacetic acid and 0.375% thiobarbituric acid) and left in a boiling water bath for 35 mins. The temperature was then lowered under running tap water for 10 min, and absorbance was measured at 538 nm using a UV-Vis spectrophotometer (Spectrophotometer Model) with blank correction, which resulted in a colored compound. TBARS values were calculated by multiplying the optical density by 7.843 and expressed as malondialdehyde (MDA) equivalents (mg MDA/kg meat).

2.4. Microbiological Analysis

Microbiological studies were performed on days 1, 3, 5, 7, and 10. An aseptic sampling of 1 g of beef meat in a tube with 9 mL of distilled water was homogenized using a vortex mixer (Model) for 1–2 minutes to ensure uniform mixing and meat particle disintegration. Ten-fold serial dilutions (1:10 diluents in distilled water) of the patty homogenates were spread directly onto the surface of solid media to determine microbial counts. Dilutions were plated in duplicates to enumerate colonies.

2.4.1. Aerobic Plate Bacteria Count

Aerobic plate count (APC) was determined by counting colonies on Standard Methods Agar (Neogen®, Lansing, Michigan, United States) plates, which were incubated at 32 °C for 3 days because this temperature is optimal for the growth of mesophilic aerobic bacteria commonly found in meat. Enumeration of *Pseudomonas* spp. was conducted on *Pseudomonas* Isolation Agar (Neogen®, Lansing, Michigan, United States) after 48 hours of incubation at 25 °C, according to Ghollasi-Mood *et al.* [17]. Microbial growth counts were represented as log10 values.

2.4.2. Coliform Bacteria Count

Serial dilutions were conducted as previously detailed to enumerate coliform bacteria. Coliform bacteria were cultured using MacConkey agar medium (MAC). Inoculated plates were incubated at 37 °C for 48 hours. After incubation, the coliform counts were again assessed by estimating the red colonies using the approach described by Vanderzant and Splittstoesser [18].

2.5. Sensory Evaluation

The sensory characteristics of beef patties, such as color, flavor, and overall acceptability, were evaluated by a 10-member trained sensory panel with expertise in meat technology and processing, following the procedure described by Teixeira *et al.* [19]. Panelists were chosen based on their sensory evaluation experience and their familiarity with the products that they would be evaluating. The 5-point hedonic scale (1 = least desirable attribute, such as poor color, flavor, or overall acceptability, and 5 = most desirable attribute--excellent color, flavor, or overall acceptability) was used. The sensory analysis was carried out in a sensory laboratory under controlled conditions. To minimize bias, random three-digit numbers were used to code each sample, and samples were presented to panelists in random order. Panelists were provided water with which to cleanse their palates between samples in order to avoid carryover effects.

2.6. Statistical Analysis

The mean values ± standard errors for the results of the sensory evaluation. The experiment included three biological replicates to ensure statistical significance and account for biological variability. The Statistical Analysis system [20] software package was used to analyze the variables using the General Linear Model method. Differences between means were identified using Duncan's test, with a p value of 0.05.

3. Results

The results of this study demonstrate the effects of clove seed extract (CSE) on lipid oxidation, microbial growth, and the sensory evaluation of beef patties during postmortem aging over a 10-day storage period.

Table 1 illustrates the MDA content (mg kg^{-1} meat) in beef patties treated with CSE. MDA is a widely accepted indicator of lipid oxidation, which significantly influences meat quality and shelf life. The results indicate that increasing the concentration of CSE led to a statistically significant reduction ($p < 0.05$) in MDA levels compared to the control (0 g CSE) at all storage intervals, corroborating the antioxidant efficacy of CSE. Statistical analysis confirmed that both storage duration and CSE concentration significantly influenced MDA levels. Further, table 1 provides substantial evidence that CSE is an effective natural antioxidant for enhancing the oxidative stability of beef patties. These results were obtained under controlled experimental conditions through the quantification of MDA levels at specific intervals using validated analytical methodologies.

Table 1: Changes in malondialdehyde (mg kg^{-1}meat) content of beef patties marinated with different CSE levels of during postmortem aging.

Storage times (days)	CSE Treatments			
	Control (0gram)	**0.20 gram**	**0.25 gram**	**0.30 gram**
1	0.399 ± 0.002^{aE}	0.292 ± 0.002^{bE}	0.281 ± 0.002^{cE}	0.278 ± 0.002^{cE}
3	0.496 ± 0.003^{aD}	0.398 ± 0.002^{bD}	0.387 ± 0.002^{cD}	0.379 ± 0.002^{cD}
5	0.576 ± 0.003^{aC}	0.542 ± 0.002^{bC}	0.529 ± 0.002^{cC}	0.516 ± 0.002^{dC}
7	0.625 ± 0.004^{aB}	0.604 ± 0.003^{bB}	0.587 ± 0.003^{cB}	0.572 ± 0.002^{dB}
10	0.657 ± 0.002^{aA}	0.629 ± 0.002^{bA}	0.617 ± 0.002^{cA}	0.606 ± 0.002^{dA}

*Data are presented as ^{a,d} mean ± S.E. Different superscripts (p<0.05) identify significant differences in values within rows.
*Different superscripts in the same column also indicate significant (p<0.05) differences between ^{A,D} mean values. To analyze the difference between measured parameters, statistical comparisons were performed.
CSE = Clove Seed Extract

Table 2 presents the total aerobic count (Log10 CFU/g) of beef patties marinated with CSE during refrigerated storage. This parameter serves as an indicator of the microbial load and spoilage potential of the meat. The objective of the experiment was to evaluate the antimicrobial efficacy of CSE on microbial proliferation during storage.

Table 2: Changes in the microbiological count of beef patties marinated with different levels of CSE during postmortem aging.

Parameter	Storage time (days)	CSE Treatments			
		Control (0g)	**0.20 g**	**0.25 g**	**0.30 g**
Total aerobic count (Log10 CFU/g)	1	4.46 ± 0.02^{aE}	4.34 ± 0.02^{bE}	4.24 ± 0.01^{cD}	4.14 ± 0.01^{dC}
	3	4.59 ± 0.01^{aD}	4.48 ± 0.02^{bD}	4.29 ± 0.02^{cD}	4.17 ± 0.02^{dC}
	5	4.75 ± 0.03^{aC}	4.64 ± 0.03^{bC}	4.59 ± 0.02^{cC}	4.52 ± 0.01^{cB}
	7	4.96 ± 0.03^{aB}	4.79 ± 0.04^{bB}	4.66 ± 0.04^{cB}	4.57 ± 0.03^{cB}
	10	5.31 ± 0.03^{aA}	4.91 ± 0.01^{bA}	4.78 ± 0.02^{cA}	4.68 ± 0.02^{dA}
Coliform Count (Log10 CFU/g)	1	1.95 ± 0.01^{aE}	1.77 ± 0.01^{bE}	1.64 ± 0.01^{cE}	1.53 ± 0.02^{dE}
	3	2.11 ± 0.01^{aD}	1.96 ± 0.03^{bD}	1.86 ± 0.01^{cD}	1.73 ± 0.03^{dD}
	5	2.39 ± 0.02^{aC}	2.27 ± 0.02^{bC}	2.19 ± 0.01^{cC}	2.12 ± 0.01^{dC}
	7	2.75 ± 0.03^{aB}	2.66 ± 0.03^{aB}	2.55 ± 0.02^{bB}	2.37 ± 0.02^{cB}
	10	2.92 ± 0.01^{aA}	2.81 ± 0.01^{bA}	2.70 ± 0.01^{cA}	2.57 ± 0.02^{dA}
***Pseudomonas* spp. (Log10 CFU/g)**	1	2.71 ± 0.03^{aE}	2.65 ± 0.04^{abE}	2.56 ± 0.04^{bcD}	2.48 ± 0.02^{cD}
	3	2.88 ± 0.02^{aD}	2.77 ± 0.01^{bD}	2.62 ± 0.02^{cD}	2.51 ± 0.01^{dD}
	5	3.11 ± 0.02^{aC}	3.00 ± 0.02^{bC}	2.88 ± 0.02^{cC}	2.75 ± 0.02^{dC}
	7	3.48 ± 0.03^{aB}	3.40 ± 0.03^{abB}	3.36 ± 0.03^{bcB}	3.26 ± 0.01^{cB}
	10	3.92 ± 0.02^{aA}	3.70 ± 0.02^{bA}	3.64 ± 0.03^{bA}	3.53 ± 0.03^{cA}

Data are presented as^{a,d} mean ± S.E. Different superscripts (p<0.05) identify significant differences in values (within rows).
*The different superscripts in the same column also indicate significant (p<0.05) differences between ^{A,D} mean values. To analyze the difference between measured parameters, statistical comparisons were performed.
CSE = Clove Seed Extract, CFU= colony forming unit.

Microbial counts exhibited an increase over time across all treatments. However, patties treated with higher CSE concentrations (0.25 g and 0.30 g) demonstrated significantly lower counts compared to the control (0 g CSE) at all time points, thus confirming the antimicrobial effect of CSE. Results are presented as mean ± standard error (S.E.), with superscript letters denoting significant differences ($p < 0.05$). The table 2 elucidates CSE's potential as a natural antimicrobial agent to mitigate microbial growth and enhance the microbiological stability of beef patties.

Table 3 presents the sensory evaluation of beef patties treated with CSE during refrigerated storage, assessing color, flavor, and overall acceptance utilizing a 5-point hedonic scale. Sensory scores decreased over time due to natural quality deterioration; however, patties treated with higher CSE concentrations (0.25 g and 0.30 g) maintained significantly higher scores compared to the control (0 g). Results are presented as mean ± S.E., with superscripts indicating significant differences ($p < 0.05$) within rows and columns. The data demonstrate CSE's potential to preserve sensory attributes and delay quality deterioration in beef patties during storage.

Table 3: Changes in color, flavor, and overall acceptance of beef patties marinated with different levels of CSE during postmortem aging.

Parameter	Storage time (days)	CSE Treatments			
		Control (0gram)	0.20 gram	0.25 gram	0.30 gram
Color	1	$4.30 \pm 0.08^{b A}$	4.45 ± 0.09^{abA}	4.70 ± 0.11^{aA}	4.55 ± 0.11^{abA}
	3	$4.15 \pm 0.07^{c A}$	$4.30 \pm 0.08^{bc AB}$	4.60 ± 0.10^{aA}	$4.55 \pm 0.11^{a bA}$
	5	$4.05 \pm 0.11^{a A}$	$4.15 \pm 0.07^{a B}$	$4.25 \pm 0.08^{a B}$	$4.05 \pm 0.08^{a B}$
	7	$2.50 \pm 0.12^{a B}$	$2.60 \pm 0.14^{a C}$	$2.75 \pm 0.11^{a C}$	$2.65 \pm 0.10^{a C}$
	10	$2.05 \pm 0.07^{b C}$	$2.35 \pm 0.07^{ab C}$	$2.50 \pm 0.07^{a C}$	$2.55 \pm 0.09^{a C}$
Flavor	1	$3.90 \pm 0.12^{b A}$	$4.05 \pm 0.13^{b A}$	4.50 ± 0.10^{aA}	4.45 ± 0.11^{aA}
	3	$3.80 \pm 0.08^{c A}$	$4.05 \pm 0.11^{bc A}$	4.40 ± 0.06^{aA}	$4.25 \pm 0.08^{ab A}$
	5	$3.60 \pm 0.12^{b A}$	$3.75 \pm 0.08^{ab A}$	$3.90 \pm 0.10^{ab B}$	$3.95 \pm 0.11^{a B}$
	7	$2.35 \pm 0.10^{a B}$	$2.45 \pm 0.08^{a B}$	$2.60 \pm 0.10^{a C}$	$2.50 \pm 0.10^{a C}$
	10	$2.15 \pm 0.07^{c B}$	$2.25 \pm 0.08^{bc B}$	$2.45 \pm 0.08^{ab C}$	$2.55 \pm 0.08^{a C}$
Overall acceptance	1	4.25 ± 0.15^{aA}	4.35 ± 0.13^{aA}	4.50 ± 0.12^{aA}	4.40 ± 0.12^{aA}
	3	4.05 ± 0.15^{aA}	$4.15 \pm 0.13^{a AB}$	$4.25 \pm 0.11^{a AB}$	4.15 ± 0.07^{aAB}
	5	3.90 ± 0.12^{aA}	$4.00 \pm 0.10^{a B}$	$4.10 \pm 0.10^{a B}$	$4.00 \pm 0.10^{a B}$
	7	$2.45 \pm 0.11^{a B}$	$2.55 \pm 0.08^{a C}$	$2.65 \pm 0.13^{a C}$	$2.50 \pm 0.10^{a C}$
	10	$2.10 \pm 0.07^{b B}$	$2.30 \pm 0.11^{ab C}$	$2.30 \pm 0.08^{ab D}$	$2.45 \pm 0.08^{a C}$

Data are presented as^{a,d} mean ± S.E. Different superscripts ($p<0.05$) identify significant differences in values (within rows).
*The different superscripts in the same column also indicate significant ($p<0.05$) differences between ^{A,D} mean values. To analyze the difference between measured parameters, statistical comparisons were performed.
CSE = Clove Seed Extract

4. Discussion

The data presented in Table 1 show the Malondialdehyde content changes ($mgkg^{-1}$ meat) in beef patties marinated with different concentrations of CSE during postmortem aging over a period of 10 days. Malondialdehyde is a lipid-oxidation marker that serves as an indicator of meat quality and shelf life [21]. Malondialdehyde levels gradually increased over the period of storage for all treatments, increasing from 0.399 $mgkg^{-1}$ on day 1 to 0.675 $mgkg^{-1}$ on day 10, indicating that lipid oxidation continues during postmortem aging. This trend corresponds with previous research indicating that oxidative breakdown of lipids is a significant issue in meat products during cold storage, especially in the absence of efficient antioxidants or packaging measures [22, 23]. The oxidation of lipids leads to the formation of off-flavors and odors, rendering the meat less appealing to consumers. The increase in Malondialdehyde levels during storage is due to the heightened vulnerability of unsaturated fatty acids to peroxidation at refrigeration temperatures, facilitating interactions between reactive oxygen species and lipids. A link has also been shown between prolonged storage durations and elevated oxidative indicators in diverse beef products [22, 24].

Lipid oxidation affects the taste qualities of beef patties and poses safety risks due to the generation of secondary oxidation products, as noted by Domínguez *et al.* [22]. Significantly lower malondialdehyde levels were observed in beef patties with added clove seed extract than in the control's overall storage times, and the higher the concentration of clove seed extract the greater its antioxidant activity. The malondialdehyde amounts were significantly higher on day 1 in control groups (0.399 $mg \cdot kg^{-1}$) than treated groups (0.292, 0.281, and 0.278 $mg \cdot kg^{-1}$ for 0.20, 0.25, and 0.30% clove seed extract, respectively). The trend of declining malondialdehyde level with increasing clove seed extract concentration continued over the storage period. This suggests that clove seed extract possessed a strong antioxidant capacity throughout the post-slaughter aging period. Even the minimum tested concentration, 0.20%, significantly decreased malondialdehyde concentration [7]. This result is in agreement with other studies that also observe lower malondialdehyde concentrations in the presence of clove seed extract in beef patties [9]. Increasing the clove seed extract concentration caused a gradual decrease in malondialdehyde level. Correspondingly, Liñán-Atero *et al.* [25] added 800 parts per million (ppm) clove extract containing the lowest malondialdehyde concentration.

Because of its high antioxidant content, particularly its eugenol and flavonoid components, clove seed extract is also a key factor in preventing lipid oxidation in meat during post-mortem aging [26]; this study revealed that 0.30% clove seed extract treatment produced the lowest malondialdehyde values in each storage period and therefore the most antioxidant effect among the treatments tested. In particular, use of 0.30% clove seed extract altered the initial and shelf-life-period malondialdehyde levels to 0.278 $mg \cdot kg^{-1}$ to 0.606 $mg \cdot kg^{-1}$, suggesting that the lipid oxidation process was delayed compared with other treatments, 0.20% and 0.25%, respectively. The results show a gradual increase in malondialdehyde levels, indicating that the 0.30% clove seed extract concentration is able to preserve antioxidant activity and reduce oxidative degradation over time.

These results agree with prior studies that have revealed the anti-oxidative potential of various clove extracts in preventing lipid peroxidation and enhancing quality traits of meat [27]. Clove extracts also effectively alleviate meat oxidative stress, resulting in preservation of shelf-life and qualitative characteristics such as tenderness and taste. Awad *et al.* [7] reported that clove-extract supplementation reduced malondialdehyde levels during meat storage, a key marker of lipid oxidation and spoilage. These findings support our results, highlighting the role of clove extracts in preventing meat quality degradation caused by oxidative damage. The residual antioxidant activity observed in the 0.30% clove seed extract treatment is consistent with previous reports and confirms that clove extracts can be utilized in meat products as a natural preservative. clove seed extract improves the sensory properties and nutritional quality of meat by slowing lipid oxidation, increasing the shelf life. These findings contribute to the increasing evidence for food preservation using natural antioxidants, such as clove extracts, and their promise as alternatives to synthetic additives generally regarded as unsafe for human health.

Microbiological counts of beef patties marinated with different concentrations of clove seed extract were evaluated at postmortem aging (days 1, 3, 5, 7, and 10). As detailed in Table 2, both storage time and clove seed extract concentration exerted significant effects on APC, coliform, and *Pseudomonas* spp. counts. The APC increased significantly ($p<0.05$) during storage across all treatments, with the highest counts observed on day 10. However, patties treated with clove seed extract exhibited significantly lower total aerobic count compared to the controls, with the reduction being more pronounced as the clove seed extract concentration increased. For instance, on day 10, the total aerobic count for the control group was 5.31 Log10 colony forming unit (CFU)/g, while the counts for the 0.20, 0.25, and 0.30% CSE treatments were 4.91, 4.78, and 4.68 Log10 CFU/g, respectively. This indicates that clove seed extract effectively inhibits microbial growth in a dose-dependent manner. Numerous studies have highlighted the antibacterial properties of clove oil against food borne pathogens, spoilage microorganisms, and fungi [28-30].

These findings align with previous research that has investigated the effects of clove seed extract on beef patty preservation using APC numbers as an indicator; also, the impact of clove seed extract on beef patties is evident in the general reduction of bacterial growth [9, 31, 32]. Like the total aerobic count, the total coliform counts observed in the study were higher than the acceptable standard levels. According to International Commission on Microbiological Specifications for Foods [33], acceptable coliform levels in ready-to-eat foods should not exceed 2 Log10 CFU/g. In comparison, the coliform count in the control group increased significantly ($p< 0.05$) over time, reaching 2.92 Log10 CFU/g on day 10, which is above the acceptable limit. However, the CSE-treated samples showed a progressive decline in coliform counts, with values of 2.81 Log10 CFU/g for 0.20% CSE, 2.70 Log10 CFU/g for 0.25% CSE, and the lowest count, 2.57 Log10 CFU/g for 0.30% CSE. Although these values were still higher than the acceptable limit, the reduction in coliform counts can be attributed to the phenolic compounds present in clove extract, such as eugenol. Eugenol is known for its antibacterial properties, as it penetrates and disrupts bacterial membranes, inhibiting the growth of microorganisms, particularly coliform [34]. A similar inhibitory effect was observed for *Pseudomonas* spp., a key spoilage microorganism in meat products. On day 10, *Pseudomonas* spp. CFU/g reached3.92 Log10 UC in the control group, while in clove seed extract samples, counts lowered to 3.70, 3.64, and 3.53 Log10 UC/g with treatments of 0.20, 0.25, and 0.30% clove seed extract, respectively. These results confirm the antimicrobial potential of clove extract on spoilage microorganisms, as previously reported. For instance, Burt [34] highlighted the ability of eugenol, the main bioactive component of clove, to display broad-spectrum antimicrobial activity by contributing to the growth inhibition of *Pseudomonas* spp. and other spoilage bacteria. Similarly, Ali *et al.* [35] indicated that with an increased clove extract concentration, microbial inhibition in meat products also increased, confirming the dose-dependent tendencies seen in the present study. Microbial counts were observed to increase over time as anticipated (instead of ambient condition of storage medium used to make them as favorable condition for microbial proliferate). Nevertheless, the clove seed extract -treated patties exhibited a slower rate of microbial growth, indicating that clove extract is able to inhibit spoilage significantly. This is in agreement with the findings of Seydim and Sarikus [36], who discovered that natural extracts of plants such as clove improve the shelf life of meat products by reducing microbial growth during storage. The phenolic compounds present in clove extract are most likely a key factor in this phenomenon, since they obstruct both microbial metabolism and degradation of cell integrity, reducing spoilage [37].

Table 3 depicts the color, flavor, and overall acceptance changes of beef patties marinated with various levels of clove seed extract over postmortem aging. Sensory attributes significantly ($p< 0.05$) decreased with storage time for all treatments, but the extent of decline was influenced by clove seed extract concentration. The color scores of control group decreased abruptly from the 1st day (4.30) to the 10th day (2.05), whereas the patties marinated with 0.25 g and 0.30 g clove seed extract retained higher color scores during the entire study period. For example, the 0.25 clove seed extract treatments remained at a score of 4.25 on day 5 versus 4.05 in the control and were at 2.50 and 2.05, respectively, on day 10. This is likely because clove seed extract has powerful antioxidant activity as phenolic compounds, especially eugenol, and help maintain color stability by inhibiting lipid oxidation and preserving myoglobin structure [25, 38, 39]. These results are in line with previous studies [10, 39, 40] reporting that treatment with natural plant extracts containing phenolic compound results in increased retention of color in meat products. In addition, our study, in accordance with the study of Bestoon *et al.* [43], found that the amount of MDA in beef and mutton marinated with crud fenugreek leaves was considerably decreased at days 7 and 10 postmortem, respectively.

The flavor scores exhibited a similar trend, significantly decreasing ($p<0.05$) in all treatments during storage. Flavor scores in the control group diminished from 3.90 on day 1 to 2.15on day 10; however, those of beef patties treated with 0.25 g and 0.30 g clove seed extract were significantly better, recording flavor scores of 3.90 and 3.95 on day 5 and 2.45 and 2.55 on day 10, respectively. In addition, the antimicrobial and antioxidant properties of clove seed extract led to reduced lipid oxidation and microbial spoilage in the clove seed extract-treated samples, consequently leading to improved flavor stability as well as confirming [40-42], who reported earlier that phenolic compounds such aseugenol were effective against oxidative degradation of flavor compounds. Likewise, the overall acceptance scores decreased significantly ($p< 0.05$) throughout the storage period; by day 10, the control groups had the lowest scores (2.10). Overall acceptability scores were significantly higher for clove seed extract -treated patties; scores for 0.25 g and 0.30 g clove seed extract treatments were 4.50 and 4.40 on day 1 and 2.30 and 2.45 on day 10, respectively. The higher total acceptance of clove seed extract -treated samples compared to controls may reflect the retained desirable sensory attributes (such as color and flavor) thanks to the antioxidant and antimicrobial properties of clove seed extract seen in earlier studies. For instance, it has been emphasized that the addition of natural antioxidants to meat products could remarkably improve overall sensory quality during storage [9, 27], which are consistent with the findings of this study.

5. Conclusions

In conclusion, the results demonstrated that clove seed extract can effectively serve as a natural agent for preserving the sensory quality of beef patties during storage. Specifically, concentrations of 0.25 g and 0.30 g were effective in maintaining the color, flavor, and overall acceptability of the beef patties. These findings align with previous studies that have highlighted the functionality of natural plant-derived phenolic compounds in enhancing the stability and quality of meat products. This suggests the potential of clove seed extract as a natural additive to extend the shelf life and improve the overall acceptability of meat products.

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