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Improving Bacterial, Oxidative, and Sensory Quality of Meat Steaks Using Cumin, Garlic, and Thyme Essential Oils

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ABSTRACT
The meat industry increasingly considers meat shelf life as a critical problem. Some essential oils contain antibacterial and antioxidant characteristics that help to keep meat safe. Therefore, the purpose of this study was to evaluate the preservation benefits, including antibacterial and antioxidant properties, of cumin, garlic, and thyme essential oils at 1% on chilled beef meat steaks, as well as their effects on pH, total volatile basic nitrogen (TVBN), thiobarbituric acid (TBA), and related sensory aspects (color, odor, appearance, consistency, and overall acceptability). The results of the current study showed that pretreating beef meat steaks with EOs of cumin, garlic, and thyme at a concentration of 1% effectively reduced levels of APC, coliform count, *staph aureus* count, TVBN, and TBA while extending shelf life to 12, 15, and 18 days when stored at 4°C. In terms of antibacterial and antioxidant properties, shelf life, and sensory quality on beef meat steaks, the thyme essential oil group outperformed cumin and garlic essential oils. The current study introduced an effective natural preservative alternative that could replace undesirable synthetic substances in the future while also lowering antibiotic resistance.

Keywords: Coliforms, Cumin, Garlic, Preservation, Shelf life, Thyme

INTRODUCTION

At every stage of processing, meat is contaminated with microorganisms and chemicals. It is believed that meat is the optimum environment for the growth of bacteria that cause food poisoning and spoiling since it is a perishable item with a high nitrogen content and an appropriate pH range (Alizadeh Behbahani et al., 2021). The main causes of beef spoilage are the multiplication of microbes that induce protein disintegration and the release of toxic components (Lorenzo et al., 2018). Meat spoilage is mainly caused by microbial growth, but it is also influenced by oxidation and enzymatic processes, such as the oxidation of lipids, which results in crucial changes to the meat's flavor and nutritional value (Bonilla et al., 2014; Vital et al., 2016). Furthermore, lipid oxidation not only results in organoleptic changes but is also associated with the production of dangerous chemicals, such as aldehydes (Banerjee et al., 2017).

Synthetic preservatives are used in the meat industry to reduce potential toxicity associated with microbial load and lipid oxidation processes. Natural preservatives are plant-based molecules that could extend the shelf life of numerous foods (Atarés and Chiralt, 2016). Natural preservatives, such as phenolic compounds, are gaining popularity in the food sector as effective preservatives (Shin et al., 2017). Natural antioxidants are chosen over synthetic antioxidants because they not only extend product shelf life but also protect nutritional components (Çakmakçı et al., 2014). They are also used in the pharmaceutical and cosmetic sectors (Pateiro et al., 2018). Spices and herbs commonly used in food preparation include peppers, thyme, and cumin (Hayaloglu and Farkye, 2011). Cumin is used in traditional medicine as an astringent and stimulant, as well as to alleviate diarrhea and indigestion (Alizadeh Behbahani et al., 2019). Due to its antibacterial and antioxidant effects, cumin, a plant from the Ranunculaceae family, is a valuable medication used for years (Salem, 2005). Cumin potency is greatly boosted by a plethora of vital vitamins, minerals, and volatile chemicals (Çakmakçı and Çakır, 2011). Thyme (*Thymus vulgaris* L), used as a flavoring ingredient, also has significant antibacterial potential because of its thymol composition (Burt, 2004). *Thymus vulgaris* L. possesses fragrant and therapeutic properties as a chemically polymorphic cuisine (El-Sayed and El-Sayed, 2021b); hence thyme essential oil (TEO) has been researched for its antibacterial activities (Nieto, 2020). Thyme's antibacterial efficacy against food spoilage pathogens has been studied extensively *in vitro*, but rarely in foods (Govaris et al., 2011). Garlic and its extracts are employed as antioxidants due to the presence of several chemicals, such as alliin, allyl cysteine, and allyl disulfide; they also play an essential role as antibacterial agents (Casella et al., 2013). The use of essential oils (EOs) in foods is authorized by the Food and Drug Administration (FDA) in the United States and the European Commission in Europe. Essential oils are not subject to quantitative limits because they are generally recognized as safe (GRAS) by the FDA (FDA, 2016).

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To evaluate the microbiological and biochemical deterioration of meat products, both traditional and cutting-edge measures are still applied (Luong, 2020). Some of these indicators are required to be included by food safety authorities in some nations, such Egypt, for the accurate evaluation of spoilage. Aerobic plate count (APC), coliform, and *staphylococcus aureus* count are the top microbial spoilage evaluation parameters, whereas pH, total volatile basic nitrogen, and thiobarbituric acid reactive substances (TBARS) are the best for accurate and quick evaluation of meat chemical spoilage (EOS, 2013). The hydrogen potential (pH), at some point during food preparation, processing and storage, influences enzyme reactions and promotes microbial growth. The manufacture of safe and well-preserved food benefits from pH control. Simply put, lower pH values hinder bacterial multiplication, whereas higher pH levels encourage it (Mendes, 2013). Most meat has a pH range between 5.2 to 7, with higher-quality meat often occurring between 5.7 and 6 (Barbut, 2009). Malonaldehyde (MDA), a characteristic biomarker that offers helpful information on the lipid rancidity in meat products, is measured using the thiobarbituric acid reactive substances (TBARS) assay (Tornuk et al., 2015). The third component is the organoleptic evaluation of the meat, regardless of the employed technique—panel or instrumental—because food sensory attributes are thought to have a significant role in shaping customer choices (Fernández-López et al., 2005). Changes in the acceptable aroma, taste, color, and rancid flavor are frequently linked to the development of lipids and myoglobin oxidation in fresh or cooked meat (Pires et al., 2018; Luong, 2020). Off flavors, altered textures, and altered colors associated with spoiling reactions all reduce consumer acceptance and choice (Gong et al., 2010). Therefore, one of the most important factors to take into account is the assessment of changes in the acceptable aroma, taste, color, and flavor (Luong et al., 2020). The antibacterial effectiveness of EOs in various foods has previously been studied. However, studies continue to support the concept that different EOs possess distinct antibacterial and antioxidant properties in different ecosystems. This is primarily because various bacteria in the same environments react differently to the same applied EOs. Another aspect is the variation in EOs chemical composition used in different studies as a result of plant species used, growing area, and extraction procedure (Alexopoulos et al., 2011; Eisenman et al., 2013; Bag and Chattopadhyay, 2015). Thus, more research is required to assess the vulnerability of the microbiota of particular ecosystems to various EOs and to determine whether or not they could effectively replace synthetic preservatives.

This study aimed to investigate the preservation activities of cumin, garlic, and thyme EOs (1%) on chilled beef meat steaks, as well as their impact on associated sensory aspects (color, odor, appearance, consistency, and overall acceptability).

MATERIAL AND METHODS

Fresh beef steaks weighing 4 Kg were purchased from a butcher shop in the Egyptian province of al-Qalyubia. The collected samples were kept in sterile plastic bags and in an ice container as soon as feasible before being transported to the lab.

Preparation of samples

The beef meat steaks were separated equally into four groups, with five replicates in each group (200g per replicate). The first group was established as the control, while the other three groups received interventions at the concentrations of 1% cumin EO, 1% garlic EO, and 1% thyme EO. The experiment was repeated five times for each group, with mean values calculated.

Treatment of sample with the essential oil

On the first day, each sample that was selected to receive EO pretreatment was immersed in the emulsion solution (1% pure essential oil to a final volume of 100 ml of sterile distilled water) for 15 minutes before being allowed to drain completely for 5 minutes on a clean stainless wire mesh screen. 100 cc of sterile, distilled water was used to dip the control group. Steaks that had already undergone pretreatment were individually labeled and wrapped in polyethylene bags. The experiment involved chilling storage for 18 days at 4°C (Barbosa et al., 2009).

All samples from these groups, control and treated, were evaluated for microbiological, chemical, and sensory characteristics at the start of the experiment (within 2 hours after treatment), then on a regular basis every 3 days until decomposition was observed in each group (days 1, 3, 6, 9, 12, 15, and 18).

Bacteriological examination

Determination of aerobic plate count

The aerobic plate count (APC) in beef samples was determined in the same way that it had been done for ground beef products (Sabike et al., 2015). Each sample was prepared as a 10% homogenate, followed by tenfold serial dilutions, and 1 ml from each dilution was loaded onto two separate sterile Petri dishes. The solidified inoculation plates were then incubated at 37°C for 24 hours (ISO, 2013).

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Determination of coliforms count

For the enumeration of coliforms in beef steaks following incubation on a Violet red bile agar at 37°C, ISO 4832:2006 general guidelines were employed (ISO, 2006). As specified in APC, the samples were prepared, homogenized, and decimally diluted. Plating procedures were used to transfer 1 mL aliquots of each dilution (twice) to Petri dishes.

Determination of Staphylococcus aureus count

The count of *Staphylococcus aureus* (*S. aureus*) in beef steaks was determined twice using the surface-plating method on the Baird Parker agar plate, similarly as it had been described earlier for milk (Sabike et al., 2014). One ml from each of the previously prepared serial dilutions was spread over using a sterile bent glass spreader. The plates were kept upright in the incubator for around 10 minutes or one hour until the agar absorbed the inoculums. The infected and control plates were then inverted and incubated for 48 hours at 37°C (Bennett et al., 2001).

Chemical examination

Determination of pH

Approximately 10 g of the beef flesh sample was blended with 10ml of sterilized distilled water in a blender. The homogenate was shaken continuously at room temperature for 10 minutes. An electrical pH meter was used to determine the pH value (Bye model 6020, USA). The pH meter calibration used two buffer solutions with known pH (alkaline pH 7.01, acidic pH 4.01). As a result, after adjusting the temperature correction system, the pH electrode was cleaned with neutralized water and inserted into the homogenate (Kirk and Sawyer, 1991).

Determination of total volatile basic nitrogen

Total volatile basic nitrogen (TVBN) in beef meat steaks was evaluated by distillation (Micro-Kjeldahl technique; Vapodest 30 S distillation unit, Gerhardt, Germany), as described in previous studies (Pearson, 1968; El-Bahr et al., 2021). TVN/l00g = (ml$_s$ $H_2 So_4$ n 0.1 for sample – ml $H_2 So_4$ n 0.1 for Blank) x 14

Determination of Thiobarbituric Acid Number

The spectrophotometric method was used to determine the Thiobarbituric Acid Number (TBA) in beef meat steaks, as defined by Egyptian organization standards (EOS, 2006).

TBA value = absorbance of sample x 7.8 (malonaldehyde (mg/Kg)

Sensory evaluation

Six member panels that had received the necessary training and testing in sensory sensitivity assessed the sensory qualities of raw meat samples. Training sessions were declared complete when panelists were satisfied with the evaluation procedure and individual scores did not deviate more than one unit from the mean score. Randomly chosen representative meat samples were served in the laboratory on porcelain plates (open area). The panelists evaluated the freshness grade using a 5-point scale, with each attribute receiving a score between 1 and 5 points based on the sensory quality requirements. The qualities of color, odor, appearance, and consistency were all assessed. The beef steaks were rated as very good, good, acceptable, unacceptable, and bad in line with the overall sensory quality scores of 5, 4, 3, 2, and 1, respectively (4.5- 5: Very good, 4-4.5: Good, 3-4: Acceptable, 2-3: Unacceptable and less than 2: Bad; Fik and Leszczyńska-Fik, 2007).

Statistical analysis

The obtained data were subjected to one-way variance analysis (ANOVA) with SPSS Version 22 (SPSS Inc. Chicago, IL, USA) and are provided as means with standard errors of the mean. The statistical model includes Duncan's multiple range tests was used to compare the means of treatments. Significant differences were observed at $p < 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

This study aimed to determine the antibacterial and antioxidant benefits of 1% Cumin, garlic, and thyme EOs on the shelf-life and sensory attributes of chilled beef steaks. Meat spoilage is almost generally triggered by microbial pathways, in addition to lipid oxidation, and enzyme reactions (Iulietto et al., 2015). The characterization of microbial populations using conventional culture techniques has been the gold standard for assessing food shelf life (Luong et al., 2020). The effect of cumin, garlic, and thyme EOs on the APC of beef meat steaks chilled at 4°C is shown in Table 1. There were significant variations in APC between the control group and the beef steak groups treated with 1% Cumin, garlic, and thyme EOs and chilled at 4°C ($p < 0.05$). Compared to spoiled control beef steak on day 9 of chilling, the results showed that cumin oil pretreated beef steak had a longer shelf life on day 12.

Furthermore, garlic and thyme-treated groups exhibited the best keeping quality until the 15th and 18th days of chilling, respectively. Compared to control and cumin essential oil-treated beef steaks, groups given garlic and thyme treatments were able to keep APC below the spoiling level of 10^6 cfu/g previously defined by the Egyptian standards organization (EOS (Egyptian standards), 2004; EOS, 2005; EOS (Egyptian standards), 2013) for fresh and frozen beef.

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The current study results are consistent with previous research that supports the antibacterial activity and potential of EOs to improve the shelf life of meat products (Kalemba and Kunicka, 2003; García-Díez et al., 2017; Nieto, 2020).

The reduction, as well as a prolonged increase in APC count in beef steak pretreated with EO extracts, may be attributable to Eos's ingredients that have significant antibacterial activity (Kalemba and Kunicka, 2003; García-Díez et al., 2017). The total phenolic content of EOs is among the components most closely related to biological properties, such as antibacterial and antioxidant activity, and they have been demonstrated to be effective in protecting meat products from foodborne pathogens (Daglia, 2012; Papuc et al., 2017; Pateiro et al., 2018; Stan et al., 2021). Cumin essential oils are a valuable source of 16 major bioactive chemicals, such as cumin aldehyde, γ-terpinene, p-coumaric acid and β-pinene, with antibacterial activities (Bag and Chattopadhyay, 2015; Fang et al., 2018; El-Sayed and El-Sayed, 2021a). Moreover, in vitro tests using foodborne pathogens as well as in situ tests using total bacterial count and other spoilage indicators showed antioxidant and antimicrobial activity for TEO (Ghabraie et al., 2016; Sharma et al., 2017; Radünz et al., 2020; Huang et al., 2021). The primary bioactive antioxidant and antibacterial components in TEO derive from phytochemicals such as carvacrol thymol, p-cymene, carvacrol, and γ-terpinene (Borugă et al., 2014). Oil-soluble organosulfur compounds, such as allicin, ajoenes, and allyl sulphides, are the main phytochemicals in garlic that have antibacterial properties (Bhatwalkar et al., 2021). Significant inhibitory effects, mainly thyme, shown in the current study are consistent with past research, indicating the potential for EOs application as a natural preservative in a variety of meat products (Zhang et al., 2017a; Huang et al., 2021).

Table 2 highlights the impact of cumin, garlic, and thyme EOs on the coliform levels of beef meat steaks chilled at 4°C. Coliforms are not always pathogens; yet, fecal coliforms, such as *Escherichia coli* (*E. coli*) continue to be the usual indicator organisms indicating direct or indirect feces contamination caused by poor handling and sanitation measures throughout the meat production chain (Halkman and Halkman, 2014). Compared to a high coliform level of 4.74 log in control beef steaks after the sixth day of storage, TEO was the most effective coliform inhibitor, keeping the coliform count of beef steaks refrigerated at 4°C below four log cfu/g until day 15 of storage. However, the succeeding treatments, garlic, and cumin essential oil, delayed this count back until days 9 and 12, respectively. According to previous research, Gram-negative organisms are slightly less vulnerable to the antibacterial activity of EOs than Gram-positive bacteria (Burt, 2004). Citrobacter, Enterobacter, Hafnia, Klebsiella, and *E. coli* are examples of coliforms, which comprise a significant subfamily of the *Enterobacteriaceae* family. Thyme essential oil was more potent than other EOs due to their phytochemicals being more effective under the present studied ecosystem conditions. In diverse food habitats such as horse and chicken sausage, TEO was an efficient inhibitor of Gram-negative *Enterobacteriaceae* members, such as Coliform count, *E. coli*, and *Salmonella enterica* (Sharma et al., 2017; Huang et al., 2021). Additionally, earlier research showed that EOs have a quorum-sensing inhibitive effect on specific microorganisms, particularly unfavorable ones like gram-negative bacteria, delaying their capacity to adapt to their environment (Zhang et al., 2016; Zhang et al., 2017a).

Table 3 shows the effect of thyme, cumin, and garlic EOs (1%) on naturally occurring *Staphylococci aureus* (*S. aureus*) in beef meat steaks chilled at 4°C. The results revealed that pretreatment of beef meat steaks chilled at 4°C with thyme, garlic, and cumin EOs (1%) resulted in a substantial decrease in *S. aureus* count as compared to the control group ($p > 0.05$). It was observed that thyme and garlic EOs could keep *S. aureus* counts below 4 logs until day eighteen of storage, while cumin essential oil (1%) slowed it down until day fourteen. Essential oils and phytochemicals, particularly polyphenols, have been found to suppress microbial growth and possibly even kill bacteria in various ways. Among these, EOs can interact with bacterial cell wall components and the bacterial cell membrane, destabilizing the bacterial lipid bilayer and causing bacterial death. Along with that, EOS has the potential to inhibit microbial enzymes, disrupt protein regulation, deprive bacterial cell enzymes of substrates and metal ions, and reduce a variety of microbial virulence factors (Daglia, 2012; Papuc et al., 2017; Radünz et al., 2020).

Table 1. The influence of cumin, garlic, and thyme essential oils on the aerobic plate count of beef meat steaks chilled at 4°C

Treatment / Storage time (day)	Control Count (log cfu/g)	Cumin oil 1%		Garlic oil 1%		Thyme oil 1%	
		Count (log cfu/g)	R (%)[1]	Count (cfu/g)	R (%)	Count (cfu/g)	R (%)
1	3.87 ± 0.95^a	3.86 ± 0.95^a	-	3.86 ± 0.95^a	-	3.86 ± 0.95^a	-[3]
3	$5. 76 \pm 1.77^b$	4.91 ± 0.84^c	44.44	4.81 ± 0.4^c	49.74	4.58 ± 0.3^c	61.90
6	$6.46 \pm 5.6^{\mathbf{b}}$	5.54 ± 0.46^d	35.14	5.23 ± 0.1^d	47.10	4.88 ± 0.4^e	60.62
9	S[2]	$5.76 \pm 0.0.47^d$	-	5.46 ± 0.3^d	--	5.26 ± 0.11^e	-
12	S	5.97 ± 0.5^d	-	5.72 ± 0.5^d	-	5.48 ± 0.25^e	-
15	S	6.41 ± 0.1^f	-	5.92 ± 0.5^d	-	$5.77 \pm 0.0.33^d$	-
18	S	S	-	6.18 ± 0.1^e	-	5.77 ± 0.7^f	-

R^1 (%): Reduction percentage was calculated using following equation: $[((C^{(a)} - C^{(b)}) – (T^{(a)} - T^{(b)}))/ (C^{(a)} - C^{(b)})] X 100$
$C^{(a)}$: Control new count (at storage day 3 or 6), $C^{(b)}$: Control initial count, $T^{(a)}$: EOs new count (at storage day 3 or 6), $T^{(b)}$: EOs initial count, S [2]: spoilage. -[3]: With the exception of the first day, this means that the control count for the tested day point became spoiled, making it difficult to calculate the reduction percentage. Mean values with different superscripts in the same rows are significantly different at $p < 0.05$. EOS (2005) established that APC of meat should not exceed 10^6/g, otherwise it is unfit for human consumption.

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Table 2. The impact of the essential oils of thyme, cumin, and garlic on the coliform of beef meat steaks chilled at 4°C

Storage time (day) \ Treatment	Control Count (log cfu/g)	Cumin oil 1%		Garlic oil 1%		Thyme oil 1%	
		Count (log cfu/g)	R[1] (%)	Count (log cfu/g)	R (%)	Count (log cfu/g)	R (%*)
1	3.34 ± 0.11[a]	3.34 ± 0.11[a]		3.32 ± 0.13[a]	-	33.2 ± 0.11[a]	-[3]
3	4.23 ± 0.11[b]	3.69 ± 0.14[c]	60.67	3.57 ± 0.01[c]	71.91	3.51 ± 0.13[c]	78.65
6	4.74 ± 0.12[b]	3.90 ± 0.16[c]	60.00	3.72 ± 0.17[c]	71.43	3.66 ± 0.14[cd]	75.71
9	S[2]	3.96± 0.11[c]	-	3.84± 0.12[d]	-	3.79± 0.15[e]	-
12	S	4.26± 0.2[b]	-	3.95±0.14[c]	-	3.89± 0.03[d]	-
15	S	4.49± 0.16[d]	-	4.04± 0.11[e]	-	3.95± 0.12[f]	-
18	S	S	-	4.46± 0.16[e]	-	4.20± 0.12[g]	-

R[1] (%): Reduction percentage was calculated using following equation: [((C $^{(a)}$ - C $^{(b)}$) – (T $^{(a)}$ - T $^{(b)}$))/ (C $^{(a)}$ - C $^{(b)}$)] X 100.
C $^{(a)}$: Control new count (at storage day 3 or 6), C $^{(b)}$: Control initial count, T $^{(a)}$: EOs new count (at storage day 3 or 6), T $^{(b)}$: EOs initial count, S [2]: spoilage. -[3]: With the exception of the first day, this means that the control count for the tested day point became spoiled, making it difficult to calculate the reduction percentage. Mean values with different superscripts in the same rows are significantly different at $p < 0.05$. EOS (2005) established that *S. aureus* count should not exceed 10^2/g.

Table 3. The effect of essential oils of thyme, cumin, and garlic on naturally occurring *Staphylococci aureus* in beef meat steaks chilled at 4°C

Storage time (day) \ Treatment	Control Count (log cfu/g)	Cumin oil 1%		Garlic oil 1%		Thyme oil 1%	
		Count (log cfu/g)	R %	Count (log cfu/g)	R %	Count (log cfu/g)	R %*
1	2.7 ± 0.11[a]	2.7 ± 0.12[a]	-	2.7 ± 0.11[a]	-	2.6 ± 0.12[a]	-[3]
3	3.61 ± 0.2[a]	3.40 ± 0.13[a]	23.08	3.28 ± 0.12[a]	36.26	3.15 ± 0.14[a]	39.56
6	4.08 ± 0.22[ab]	3.76 ± 0.15[b]	23.19	3.57 ± 0.2[b]	36.96	3.46 ± 0.21[b]	37.68
9	S[2]	3.85 ± 0.11[ab]	-	3.69 ± 0.19[c]	-	3.6 ± 0.09[c]	-
12	S	3.95 ± 0.14[d]	-	3.79 ± 0.11[e]	-	3.71 ± 0.12[e]	-
15	S	4.04 ± 0.4[d]	-	3.9 ± 0.15[d]	-	3.8 ± 0.11[e]	-
18	S	S	-	3.95 ± 0.16[d]	-	3.88 ± 0.1[f]	-

R[1] (%): Reduction percentage was calculated using following equation: [((C $^{(a)}$ - C $^{(b)}$) – (T $^{(a)}$ - T $^{(b)}$))/ (C $^{(a)}$ - C $^{(b)}$)] X 100.
C $^{(a)}$: Control new count (at storage day 3 or 6), C $^{(b)}$: Control initial count, T $^{(a)}$: EOs new count (at storage day 3 or 6), T $^{(b)}$: EOs initial count, S [2]: spoilage. -[3]: With the exception of the first day, this means that the control count for the tested day point became spoiled, making it difficult to calculate the reduction percentage. Mean values with different superscripts in the same rows are significantly different at $p < 0.05$. EOS (2005) established that *S. aureus* count should not exceed 10^2/g

Table 4. The influence of thyme, cumin, and garlic essential oils on the pH of beef meat steaks chilled at 4°C

Storage time (day) \ Treatment	Control	Cumin oil 1%	Garlic oil 1%	Thyme oil %
1	5.69 ± 0.01[a]	5.68 ± 0.01[a]	5.67 ± 0.01[a]	5.67 ± 0.01[a]
3	6.14 ± 0.02[a]	5.80 ± 0.01[b]	5.77 ± 0.01[b]	5.73 ± 0.01[b]
6	6.82 ± 0.02[a]	6.01 ± 0.03[b]	5.86 ± 0.01[bc]	5.79 ± 0.01[c]
9	S[1]	6.22 ± 0.02[a]	6.03 ± 0.03[b]	5.91 ± 0.02[b]
12	S	6.39 ± 0.01[a]	6.19 ± 0.02[b]	6.04 ± 0.02[b]
15	S	6.57 ± 0.03[b]	6.33 ± 0.1[c]	6.20 ± 0.01[c]
18	S	S	6.61 ± 0.03[d]	6.42 ± 0.02[e]

S[1], means spoilage. Mean values with different superscripts in the same rows are significantly different at $p < 0.05$.

Chemical quality criteria

pH

The length of time that a product will retain its good quality is known as its shelf life (Iulietto et al., 2015). The pH is one of the intrinsic elements influencing meat shelf life; the pH of beef muscle is approximately 7.0 at the moment of slaughter and drops to 5.3 to 5.8 for 18 to 40 hours. The growth of spoilage bacteria is accelerated by a higher pH (Hazards and Panel, 2016). Table 4 shows the impact of garlic, cumin, and thyme EOs (each at 1%) on the pH of beef steaks refrigerated to 4°C. Within 6 days of chilling, the pH of the control steaks rose to an adverse level of 6.82, making steaks unfit for consumption. Pretreating steaks with 1% EOs of cumin, garlic, and thyme minimized pH fluctuations and preserved their viability for 12, 15, and 18 days of chilling, respectively ($p < 0.05$). This is most likely due to antioxidant and antibacterial components of EOs preventing chemical, microbiological, and physical lipid/protein damage, hence preventing nitrogen and high alkaline volatile base generation such as ammonia creation and minimizing pH increase (Esmer et al., 2011; Badee et al., 2014). Furthermore, the susceptibility of bacteria to the antimicrobial activity of EOs tends to increase as the pH of the food decreases. At low pH, an EO's hydrophobicity rises, allowing it to dissolve more easily in the lipids of the target bacteria's cell membrane (Alizadeh Behbahani et al., 2021).

Total volatile basic nitrogen

Total volatile basic nitrogen is among the chemical indicator for assessing the microbiological quality of meat (Luong et al., 2020). Table 5 displays the effect of thyme, cumin, and garlic EOs (1% each) on the TVBN content of chilled beef meat steaks at 4°C. TVBN accumulations of more than 20 mg N/100g in raw samples, according to Egyptian

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Standards, indicate the unfitness of chilled beef products due to associated decomposition (Egyptian standards, 2013). Pretreating beef steaks with 1% EOs of cumin, garlic, and thyme reduced TVBN accumulation below the critical spoiling level and retained beef quality for 12, 15, and 18 days ($p < 0.05$). Nitrogen and high alkaline volatile base formation, such as ammonia generation, is connected with chemical, microbiological, and physical lipid/protein degradation (Luong et al., 2020). The results of this investigation support previous findings that EOs, particularly TEO, are effective at suppressing undesirable microbiological and chemical alterations like TVBN in examined beef products (Ghabraie et al., 2016; Sharma et al., 2017; Huang et al., 2021). The differences in EO composition may account for the variations in pH, TVBN, and TBA among EOs containing beef steaks (Sharma et al., 2017).

Thibarbituric acid

Lipid oxidation is a type of meat deterioration that causes meats to gradually lose sensory and nutritional quality, impacting customer acceptance. It happens when polyunsaturated fatty acids interact with reactive oxygen species, causing a cascade of secondary events that end in lipid breakdown and the development of oxidative rancidity (Amaral et al., 2018). To assess rancidity or the onset of decomposition in chilled beef meat, TBARS must be tested and must not exceed 0.9 mg/kg, or the meat will be judged unfit for consumption of EOS (Egyptian standards, 2013). The effect of 1% garlic, cumin, and thyme EOs on the oxidative stability (TBA) of chilled beef meat steaks at 4°C is shown in Table 6. Some oils are more antimicrobial than others when used on meat. High-fat content appears to reduce the efficacy of EOs in meat products significantly. Depending on the type of oil applied, the amount of fat provides varying degrees of protection to the bacterial cells (Burt, 2004). When compared to Egyptian TBARS standards (Egyptian standards, 2013), the results demonstrate that the control chilled beef meat steaks are unsatisfactory on day 6 of chilling.

In contrast, those treated with 1% cumin, garlic, and thyme EOs remain acceptable till days 12, 15, and 18, respectively. The strong antioxidant activity of EOs derived from their phenolic diterpene structure, which inhibits free radical formation, might explain the lower TBRS progression in steaks treated with EOs. A few plant phenolic compounds also can act as singlet O2 quenchers and metal chelators (Shahidi et al., 1992). Nonetheless, the organosulfur compounds alliin, allylsulfide, propylsulfide, and diallylsulfide, as well as tert-butylhydroquinone (TBHQ), which are active in garlic, are responsible for its potent antioxidant and prevent malonaldehyde generation (Dewi et al., 2010; GHEISARI and RANJBAR, 2012; Nurwantoro et al., 2015).

Sensory evaluation

Sensory assessments conducted by a panel of multiple members were recognized as the most often utilised methods for assessing meat products' organoleptic quality and spoilage judgments (Luong et al., 2020). Table 7 shows the sensory characteristics of control and treated beef steak with cumin, garlic oil, and thyme EOs at a concentration of 1% stored at 4°C. According to sensory analysis, beef steaks treated with cumin, garlic oil, and thyme EOs at a concentration of 1% considerably maintained their acceptable quality until days 9, 12, and 18 of chilling, respectively ($p \leq 0.05$). In contrast, control beef steaks had poor sensory features on day 6 of chilling. These findings reflect the effects of applied EOs' antibacterial and antioxidant properties on the sensory quality of beef steaks (Sirocchi et al., 2017). Changes in lipid and pigment oxidation, as well as fatty liberation, are considered to be the primary variables influencing product appearance and flavor scores during storage (Sharma et al., 2015). In some situations, natural preservatives may alter the original flavor of the product, which is unsatisfactory to some consumers (Zhang et al., 2017b). Fortunately, the organoleptic influence would have the least detrimental effects on foods that are frequently treated with herbs, spices, or other seasonings. Nevertheless, specific EO components, such as 0.3% TEO can also give food a unique flavor (Boskovic et al., 2017), with some of them being recognized as food flavorings (Burt, 2004). Thyme oil up to 0.9 % had no negative impacts on the flavor or appearance of a coating for cooked shrimp, but when applied at 1.8%, the shrimp were much less appealing (Ouattara et al., 2001).

Table 5. The effect of thyme, cumin, and garlic essential oils on the total volatile basic nitrogen of chilled beef meat steaks at 4°C

Treatment / Storage time (day)	Control (mg/100g)	Cumin oil 1% (mg/100g)	Garlic oil 1% (mg/100g)	Thyme oil 1% (mg/100g)
1	2.07 ± 0.09^{a}	2.03 ± 0.09^{a}	1.97 ± 0.09^{a}	1.94 ± 0.09^{a}
3	12.95 ± 0.78^{b}	5.76 ± 0.12^{c}	4.92 ± 0.11^{c}	4.08 ± 0.09^{c}
6	27.61 ± 1.10^{d}	9.21 ± 0.53^{e}	7.57 ± 0.29^{e}	6.39 ± 0.22^{e}
9	S	14.56 ± 0.69^{f}	11.84 ± 0.47^{g}	9.72 ± 0.41^{h}
12	S	18.17 ± 0.80^{de}	15.26 ± 0.52^{f}	12.98 ± 0.59^{g}
15	S	23.64 ± 0.96^{g}	19.40 ± 0.71^{h}	16.05 ± 0.63^{i}
18	S	S	24.15 ± 1.04^{g}	19.61 ± 0.65^{h}

S[1] means spoilage. Mean values with different superscripts in the same rows are significantly different at ($p < 0.05$).

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Table 6. The impact of garlic, cumin, and thyme essential oils on the oxidative stability of chilled beef meat steaks at 4°C

Storage time (day) \ Treatment	Control (mg/Kg)	Cumin oil 1% (mg/Kg)	Garlic oil 1% (mg/Kg)	Thyme oil 1% (mg/Kg)
1	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.05 ± 0.01^{a}
3	0.57 ± 0.04^{b}	0.18 ± 0.02^{c}	0.15 ± 0.01^{c}	0.13 ± 0.01^{c}
6	1.19 ± 0.06^{e}	0.47 ± 0.03^{d}	0.31 ± 0.01^{f}	0.24 ± 0.01^{f}
9	S[1]	0.60 ± 0.05^{d}	0.44 ± 0.03^{df}	0.32 ± 0.02^{df}
12	S	0.79 ± 0.05^{g}	0.58 ± 0.03^{f}	0.40 ± 0.03^{df}
15	S	1.05 ± 0.08^{e}	0.81 ± 0.07^{g}	0.67 ± 0.04^{h}
18	S	S	0.98 ± 0.06^{i}	0.83 ± 0.05^{g}

S[1] means spoilage. Mean values with different superscripts in the same rows are significantly different at ($p < 0.05$

Table 7. Sensory characteristics of control and treated beef steak with cumin oil, garlic oil, and thyme oil at a concentration of 1% stored at 4°C

Storage time (day) \ Trait	Color (5)	Odor (5)	Appearance (5)	Consistency (5)	Overall (5)	Grade
Control						
1	4.8	4.7	4.8	4.8	4.8	Very good
3	3.5	2.9	3.1	3.3	3.2	Acceptable
6	1.8	1.4	1.5	1.4	1.5	Bad
9	S[1]	S	S	S	S	Spoiled
12	S	S	S	S	S	Spoiled
15	S	S	S	S	S	Spoiled
18	S	S	S	S	S	Spoiled
Cumin oil 1%						
1	4.9	4.8	4.9	4.8	4.9	Very good
3	4.0	4.0	4.2	4.0	4.0	Good
6	3.6	3.8	3.8	3.6	3.7	Acceptable
9	3.4	3.2	3.1	3.1	3.3	Acceptable
12	2.4	1.8	2.4	2.2	2.2	Unacceptable
15	S	S	S	S	S	Spoiled
18	S	S	S	S	S	Spoiled
Garlic oil 1%						
1	4.9	5	4.9	4.8	4.9	Very good
3	4.4	4.0	4.2	4.6	4.3	Good
6	4.0	3.8	4.0	4.2	4.0	Good
9	3.4	3.2	3.6	3.6	3.5	Acceptable
12	3.2	2.8	3.4	3.2	3.2	Acceptable
15	2.6	2.4	2.6	2.4	2.5	Unacceptable
18	S	S	S	S	S	Spoiled
Thyme oil 1%						
1	4.8	5	5	4.8	4.9	Very good
3	4.6	4.8	4.5	4.7	4.6	Very good
6	4.2	4.4	4.2	4.4	4.3	Good
9	4.0	4.2	4.2	4.0	4.1	Good
12	3.8	3.8	4.0	3.6	3.8	Acceptable
15	3.4	3.4	3.6	3.2	3.4	Acceptable
18	3.0	3.2	3.2	3.0	3.1	Acceptable

4.5- 5: Very good, 4-4.5: Good, 3-4: Acceptable, 2-3: Unacceptable, less than 2: Bad. S[1] means spoilage.

CONCLUSION

According to the current study findings, pretreatment of beef meat steaks with 1% cumin, garlic, and thyme EOs effectively diminished APC, coliform count, staph aureus count, TVBN, and TBARS levels, increasing shelf life up to 12, 15, and 18 days under chilling 4°C. Thyme essential oils surpassed garlic and cumin essential oils in terms of antibacterial and antioxidant activities, as well as shelf life and sensory quality on beef meat steaks. The current work introduces efficient natural preservative alternatives that, in the future, could replace undesired synthetic compounds while also reducing antibiotic resistance.

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Competing interests

The authors declare that they have no competing interests.

Author contributions

Abo baker Edris and Islam Sabeq designed the plan of work, and Sabah Tawfick, wrote the manuscript. Islam Sabeq revised and confirmed the statistical results. All authors confirmed the final revised articles.

Ethical consideration

The authors checked for ethical concerns, such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publishing and/or submission, and redundancy.

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