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Antimicrobial Activity of Some Essential Oils as Natural Preservatives in Minced Meat

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Abstract

This work aimed to investigate the preservation efficiency of some essential oils (thyme, cumin, and rosemary) in minced meat during refrigerated storage. The antimicrobial analysis showed a significant effect of thyme *E. coli O157:H7, Staphylococcus aureus, Bacillus cereus, and Salmonella typhimurium* against compared to cumin and rosemary. Meanwhile, there were no significant differences in the antimicrobial activity between thyme, cumin and rosemary against *Listeria monocytogenes*. These oils were applied with the concentrations chosen as the best concentrations, with a higher concentration being chosen (thyme 0.1 and 0.25%), (cumin 0.3 and 0.6%) and (rosemary 0.3 and 0.6%) on a sample of minced beef, as natural preservatives to determine their effect of shelf life, chemical, and microbial properties of minced meat when been stored at 4 °C for 14 days. Obtained results showed that treated samples revealed decreasing values in chemical, microbial and improving sensory properties than untreated samples (control). Also, thyme oil at 0.25% showed the most effective treatment among other oils at different concentrations.

Keywords: Minced meat, Essential oil, Natural preservatives, Antimicrobial activity, physicochemical properties and freshness tests.

1. Introduction

Meat comprises vital nutrients, particularly proteins, which are distinguished by high digestibility with a corrected amino acid score of 0.92. This protein is necessary for numerous physiological functions in the human body [1, 2]. Meat provides around 15% of the proteins consumed in our diet. It covers almost all essential amino acids as well as several fatty acids and micronutrients (e.g., Fe, Zn, Se, and vitamin B complex) [3]. However, approximately 20% of the initial meat production is lost or wasted annually, representing 304.2 million tons. This portion of meat is wasted essentially due to spoilage characterized by the decreased sensory quality of meat and meat products [4]. Meat spoilage is a complex matter in which chemical and biological activities may interact, making it unacceptable to the consumer and unsafe for consumption. There is a multiplicity of spoilage characteristics in meat products for consumers, such as off-odors, various color deteriorations or changes in aspect (texture, slime or liquid production) [5]. Despite the effect of autolytic enzymatic reactions and lipid oxidation on meat spoilage, microbial activity (essentially bacteria) is the major cause of spoilage. A wide variety of microorganisms can cause spoilage due to the nutritional composition of meat, pH (5.5–6.5) and high moisture content that allows the growth and survival of a large range of microorganisms [6]. Conditions of processing, transportation, preservation, and storage, as well as the animal's physiological state at slaughter, all affect the microbial quality of raw meat. Meat and meat products provide an appropriate environment for spoilage microorganisms or food borne pathogens due to their high water activity and nutrient factors. Foodborne pathogens, including *Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens*, pathogenic *Escherichia coli, Vibrio spp.*, and *Campylobacter spp.* cause a large number of illnesses, with substantial damage to human health and the economy [7]. Contaminated foods cause 600

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million cases of food borne illness and 420,000 deaths worldwide yearly [8].

The multiplication of spoilage and pathogenic microorganisms should be controlled to ensure food safety. The search for new natural products as alternatives to artificial preservatives has increased because of the harmful effects they cause, as is the wide range of effective compounds that can be obtained from plants (e.g., essential oils) [9, 10]. Essential oils are secondary metabolites obtained from some plant families, such as *Lamiaceae, Asteraceae, Lauraceae, Rutaceae, Myrtaceae, Zingiberaceae,* and *Umbelliferae* [11].

Recently, essential oils have received great interest as natural antibacterial agents for pathogenic bacteria, which has become a promising strategy to extend the shelf life of meat and meat products. Most essential oils have undergone verification for their efficacy, safety, toxicity, and mechanism of action. The results of these quality controls have recommended them as Generally Recognized as Safe (GRAS), allowing the use of essential oils as food additives to preserve food and food products [12]. The efficacy of essential oils is correlated to their bacteriostatic (essential oils prevent bacterial growth, then the microbial cells may recuperate their reproductive ability) or bactericide effect (essential oils kill bacterial cells) [13]. The hydrophobicity of essential oils causes the accumulation in the phospholipid bilayer of the cytoplasmic membrane of the bacterial cells, which is consequential in the loss of structure, impermeability, cellular constituents and functions of the membrane [14]. In general, the antibacterial activity of essential oils was not the same against Gram-positive and Gram-negative bacteria, which is attributed to the restriction of the outer membrane of bacteria (Gram-negative) and the composition of essential oils [15]. However, some essential oils showed the same efficacy against both types of bacteria, such as cinnamon, clove and oregano [16]. The higher antimicrobial activity of EOs was related to the presence of hydroxyl groups (alcohol and phenolic compounds) compared to hydrocarbons, with significant antimicrobial activity against Gram-negative bacteria for free terpenes compounds [17].

Therefore, the present work was carried out to evaluate the efficiency of thyme, Cumin and rosemary essential oils as antimicrobial agents in control of *Staphylococcus aureus* and *E. coli* in minced meat to enhance its shelf life during cold storage.

2. Materials and methods

Materials

The raw meat samples (post-rigor lean beef muscles) were obtained from El Abed retrial shop Toukh, Qalyubia Governorate, Egypt.

Essential oils included thyme (*Thymus vulgaris L.),* Cumin (*Cuminumcyminum*) and rosemary (*Rosmarinusofficinalis*) as a core material (99% of purity) were obtained from Nevertary Co., Cairo, Egypt. These oils were stored in sterile bottles at 4°C until use.

Five pathogenic bacteria (*E. coli O157:H7* ATCC 6933, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 20231, *Bacillus cereus* ATCC 33018and *Salmonella typhimurium* ATCC 14028) were obtained from MERCIN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Chemicals and reagents used in this study were of analytical grade and obtained from El-Nasr Co., Cairo Governorate, Egypt. Packaging materials were obtained from food local market in Attaba at Cairo Governorate, Egypt.

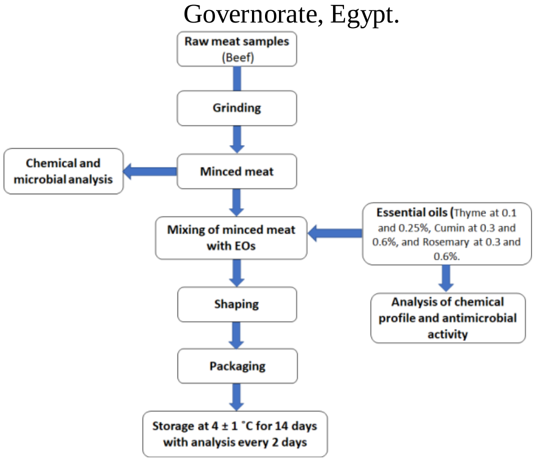

Fig.(1). Flow chart of the experimental procedures

Methods

Experimental design

The flow chart of the experimental procedure is presented in Figur 1. Seven Kg of fresh meat were purchased immediately after slaughter from El Abed retrial shop Toukh, Qalyubia Governorate, Egypt and was directly minced and packed in clean polyethylene bags. Then under the controlled and complete aseptic conditions, the samples were transported to the laboratory within 30 min at about 4 °C. The batch (7 kg) was divided into equal portions (1 Kg each), which included one control sample (untreated sample) and six treated samples. Each group was divided into 8 samples after being mixed with selected Eos (thyme, cumin and rosemary) with different concentrations: thyme at 0.1 and 0.25 mL/100 g, cumin and rosemary at 0.3 and 0.6 mL/100 g. Subsequently, blends were stored at 4 ˚C and examined every 2 days for chemically, microbiologically and sensory. The experiment was conducted in triplicate for 14 days of storage. [18]

Analysis of essential oils

Gas chromatography/mass spectrometry (GC–MS) analyses of thyme, cumin and rosemary essential oil composition:

The chemical composition of different essential oils was performed by the NRC-GC/EI-MS Lab, according to the method described by [19].

Antimicrobial activity of essential oils:

The antimicrobial activity of essential oils (thyme, cumin, rosemary) against two Gram-negative strains (*E. coli O157:H7* and *Salmonella typhimurium*) and three Gram-positive strains (*Listeria monocytogenes*, *Staphylococcus aureu* and *Bacillus cereus*) was conducted according to the method described by [20], with slight modifications. Briefly, different strains were activated in Tryptic Soy Broth for 16 h at 37 °C before use. The cell concentration was adjusted using a sterilized medium to reach 106 cfu/ml.

The disc-diffusion method was used to determine the antimicrobial activity of different essential oils against different pathogenic strains. 100 µL of diluted culture (107- 108) was spread onto the NB agar. A paper disc (6 mm) impregnated with 20 µL of the oil was placed on the inoculated plates. After keeping the plates at 4 °C for 2 h, the plates were incubated at 37°C for 24 h. Compared with chloramphenicol (30 µg/mL) as a positive control, the antimicrobial activity was calculated using the inhibition zone (millimeters) of different oils with different pathogenic strains.

Characterization of treated samples:

Chemical analysis:

The moisture content, crude protein, ether extract and ash content were determined according to the method described by [21]. Total carbohydrate was calculated by the differences between one hundred and the summation of the percentage of moisture, protein, fat and ash.

Total volatile nitrogen (TVN):

Total volatile nitrogen (TVN) was measured according to the method described by [22].

Thiobarbituric acid value (TBA):

Thiobarbituric acid value in the treated and untreated samples was determined according to the method described by [22] and the modified method reported by [23].

Physical properties:

pH value:

pH value of treated and untreated samples was determined during storage period based on the procedure reported by [24, 25].

Water holding capacity (WHC) and plasticity:

Water holding capacity (WHC) and plasticity were measured according to the method described by [26, 27].

Microbiological analysis:

Bacteriological counts were determined according to the [28, 26, 29] methodology by placing a 10 g sample in 90 ml of 0.85% NaCl solution and homogenizing it with a stomacher. Other decimal dilutions were prepared and plated in the appropriate media from this dilution.

The pour plate method determined total viable aerobic bacterial counts using plate count agar (PCA, Merk, Darmstadt, Germany). The inoculated plates were incubated at 37 °C for 2 days for total viable counts and at 10 °C for 7 days for psychrotrophic counts. Using violet red bile agar media to enumerate E. coli O157:H7, the inoculated plates were incubated at 37 °C for one day. All counts were expressed as log10 cfu/g and performed in duplicate.

Statistical analysis:

- The statistical analysis was carried out using Two-way ANOVA using SPSS, ver. 22 (IBM Corp.

Released 2013). Data were treated as a complete randomization design [30]. Multiple comparisons were conducted using the Duncun test at a significance level < 0.05.

3. Results and Discussions

Gas chromatography/mass spectrometry (GC–MS) analyses of thyme, cumin and rosemary essential oil composition:

The chemical composition of selected essential oils was analysed by GC-MC, which is described as a useful tool in biological, medical and modern food research aiming to separate and identify different components in organic mixtures. This method has already been applied successfully for analyzing and profiling essential oils [31].

As shown in Tables (1, 2, and 3) and Figures (1, 2, and 3), the most abundant compounds in the thyme oil were thymol (35.01%), 5-methyl-2-(1-methylethyl)-Phenol (16.67%), and isocomene (11.73%), while the α and γ-Terpinene, o-Cymene, and Cyclohexene, 1,5,5-trimethyl-3-methylene were the highest abundant compounds in the cumin oil.

In addition, the most abundant compounds in the rosemary oil were α-pinene, camphor, and eucalyptol, which presented 24.95, 18.01, and 16.84% of the oil composition, respectively. The chemical composition of thyme, cumin and rosemary oils is almost similar to the results of GC-MC analysis in some previous studies [32,33,34,35].

Table (1).Chemical composition of thyme oil analyzed by GC-MS.

Compound Name	R.T.	Area %	Mol. mass	Mol. formula
Cis-Ocimene	8.23	0.49	136	C10H16
Camphene	8.65	0.12	136	C10H16
1,7-Octadien-3-ol	10.25	1.27	126	C8H14O
Linalyl propionate	1.34	0.65	210	C13H22O2
Thymol	11.28	35.01	134	C10H14
2,4-Dimethoxyphenol	11.98	2.70	154	C8H10O3
3,5-Dinitrobenzoic acid	12.49	5.97	416	C22H28N2O6
Linalool	13.82	3.54	154	C10H18O
5-methyl-2-(1-methylethyl)-Phenol	19.46	16.67	150	C10H14O
Isocomene	22.65	11.73	204	C15H24
Caryophyllene oxide	26.48	5.94	220	C15H24O
Fenchone	32.89	0.42	152	C10H16O
Patchoulane	33.17	0.12	206	C15H26
Ferruginol	34.26	0.07	286	C20H30O

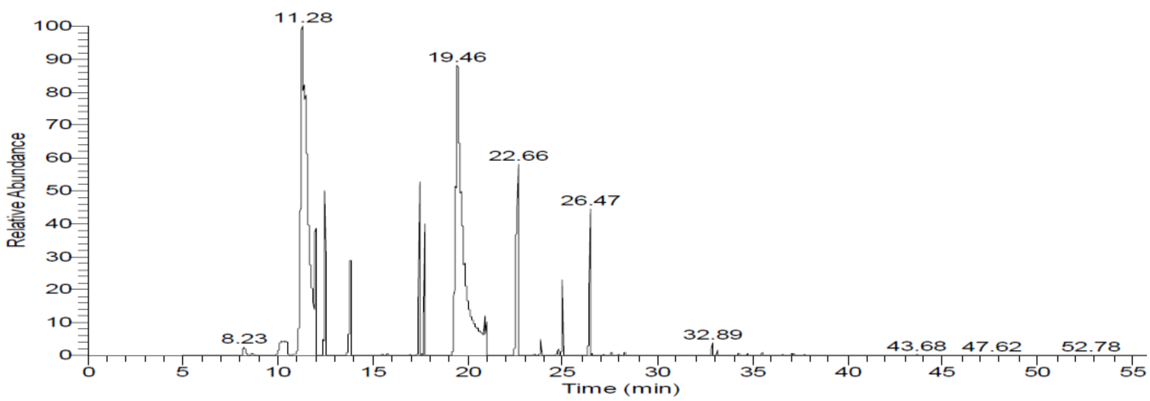

Fig. (1). GC-MS chromatogram of thyme oil.

Table (2).Chemical composition of cumin oil analyzed by GC-MS.

Compound Name	R.T.	Area %	Mol. mass	Mol. formula
cis-Ocimene	8.23	0.62	136	C10H16
α and γ-Terpinene	9.73	44.81	136	C10H16
o-Cymene	11.35	10.53	134	C10H14
Benzene, Methyl(1-methylethyl)-	11.67	3.38	134	C10H14
Cyclohexene, 1,5,5-trimethyl-3-methylene	12.45	13.77	136	C10H16
3-Octen-5-yne, 2,7-dimethyl-	13.01	0.98	136	C10H16
1,3-Cyclohexadiene-1-methanol,4-(1-methylethyl)-	16.41	3.36	152	C10H16O
Benzene, 1-ethyl-4-(1-methylethyl)-	18.00	7.61	148	C11H16
Ethanone, 1-(3,4-dimethylphenyl)-	18.58	0.82	148	C10H12O
Benzaldehyde, 4-(1-methylethyl)-	19.11	1.33	148	C10H12O
2-Arachidonoyl-glycerol	19.59	6.93	3.78	C23H38O4
p-Cymen-7-ol	20.42	0.90	150	C10H14O

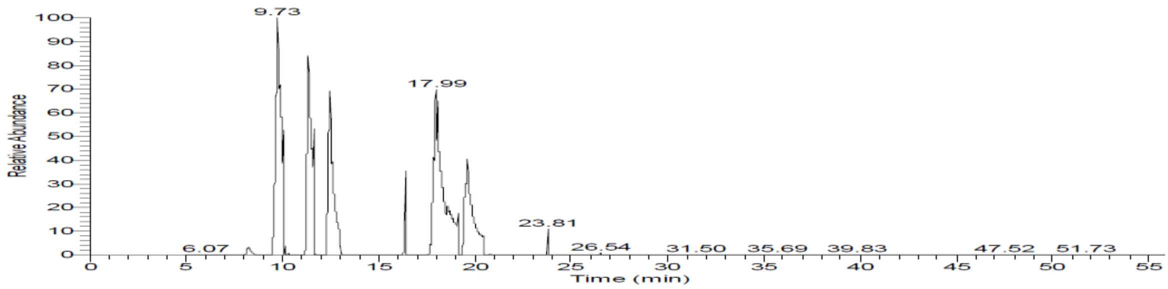

Fig.(2). GC-MS chromatogram of cumin oil.

Table (3).Chemical composition of rosemary oil analyzed by GC-MS.

Compound Name	R.T.	Area %	Mol. mass	Mol. formula
α-Pinene	8.897	24.95	136	C10H16
Camphene	9.321	4.14	136	C10H16
2,4(10)-thujadien	9.506	1.13	134	C10H14
β -Pinene	10.016	0.86	98	C10H16
β-Myrcene	10.306	1.14	136	C10H16
β-Ocimene	10.749	0.62	136	C10H16
1-Octen-3-ol	10.835	0.48	128	C8H16O
Limonene	11.235	4.52	136	C10H16
o-Cymene	11.335	2.22	134	C10H14
Eucalyptol	11.478	16.84	154	C10H18O
Linalool	13.53	5.11	154	C10H18O
Chrysanthenone	14.354	0.3	150	C10H14O
Camphor	14.84	18.01	152	C10H16O
Terpinen-4-ol	15.221	0.98	154	C10H18O
Isoborneol	15.354	6.34	154	C10H18O
α-Terpineol	15.673	1.18	154	C10H18O
Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl-, (1S)-	16.654	4.36	150	C10H14O
Bornyl acetate	17.387	1.82	196	C12H20O2

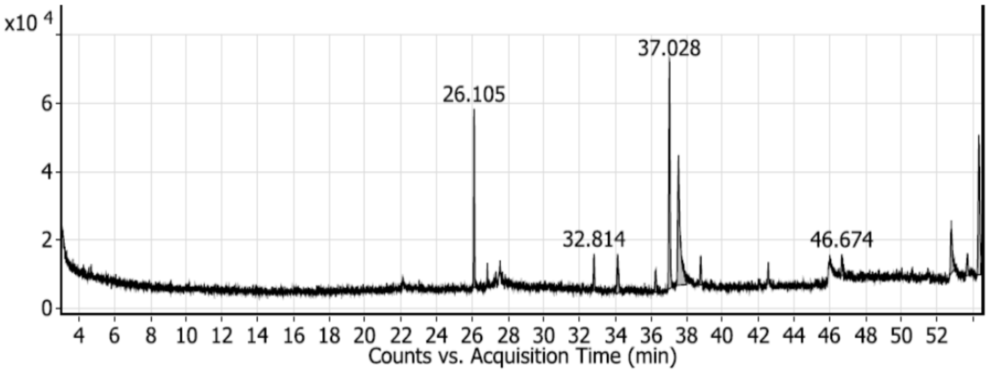

Fig. (3). GC-MS chromatogram of rosemary oil

Antimicrobial activity of essential oils:

Recently, there has been increased interest in using natural substances with antimicrobial activity in foods as an alternative to antibiotics that have many health risks. Essential oils represent one of the most promising natural materials as safe antimicrobial substances. The antimicrobial activity of thyme, cumin, and rosemary against different pathogenic bacteria is presented in Table (4). The results showed a significant inhibition of all essential oils against different pathogenic strains used in this test. The results showed no significant differences between different essential oils against listeria monocytogenes. In contrast, thyme at 0.1% showed a significant inhibition against *E. coli, Staphylococcus aureus, Salmonella typhimurium, and Bacillus cereus* compared to cumin and rosemary at 0.3%. These results agree with those mentioned in previous studies for the antimicrobial activity of thyme [36, 37], cumin [38, 39], and rosemary [40, 32, 41].

Chemical composition of raw minced meat:

The results of the approximate chemical composition of raw minced meat used in this study are presented as shown in Table (5). The moisture content was 72.31%, crude protein 20.40%, ether extract 5.17%, ash 1.03%, and total carbohydrate 1.09%. These results were similar to the chemical composition of beef studied by [26, 42, 43], who found that the moisture, protein, and ash content in beef was 72.12, 21.07 0.92%, respectively.

Table (4): Antimicrobial activity of essential oils.

Bacterial strains	Inhibition zone (mm)		
	Thyme (0.1%)	Cumin (0.3%)	Rosemary (0.3%)
listeria monocytogenes	8.67 ± 4.04^{a}	8.33 ± 11.02^{a}	6.67 ± 0.58^{a}
E. coli O157:H7	17.33 ± 1.15^{a}	6.67 ± 9.81^{b}	4.67 ± 0.58^{b}
Staphylococcus aureus	37.00 ± 2.00^{a}	8.33 ± 0.58^{b}	10.33 ± 1.53^{b}
Salmonella typhimurium	24.00 ± 3.46^{a}	8.67 ± 7.37^{b}	8.00 ± 1.73^{b}
Bacillus cereus	16.33 ± 4.04^{a}	9.33 ± 7.57^{ab}	4.33 ± 0.58^{b}

The values are expressed as the mean ± SD (n = 3). Means with different small letters superscripts in the same row represent significant differences ($p< 0.05$)

Characterization of treated minced meat:

WHC of minced meat treated with essential oils:

The water holding capacity (WHC) of meat and meat products indicates its freshness and maintains its properties during storage periods. Compared with untreated samples, all treated minced meat samples showed a significant increase in WHC, particularly during storage for a long period. The samples treated with rosemary at 0.3 and 0.6% showed the lowest values of WHC compared to those treated with thyme and cumin at different concentrations (Table 6).

These results agree with the results obtained by [44], who found that the WHC in the meat samples treated with thyme was higher than that in the control sample during storage, which reached 61.36% in treated samples compared to 52.6% in the control sample. In addition, [45] found that the WHC in the beef hamburger treated with cumin after chilled storage for 7days was 72.37% compared to 65.66% in untreated samples.

Table (5): Chemical composition of raw minced meat

Component (%)	Moisture	Crude protein	Ether extract	Ash	Total carbohydrate
	72.31 ± 0.469	20.40 ± 2.65	5.17 ± 0.176	1.03 ± 0.045	1.09 ± 0.608

Table (6): Changes in WHC in treated minced meat during storage.

Storage period (day)	Treatment						
	Control	Thyme (%)		Cumin (%)		Rosemary (%)	
		0.1	0.25	0.3	0.6	0.3	0.6
WHC							
0	7.90 ± 0.00^{aA}	7.85 ± 1.20^{aA}	8.10 ± 1.70^{aA}	8.10 ± 0.99^{aA}	8.30 ± 0.42^{aA}	8.05 ± 0.64^{aA}	7.95 ± 1.48^{aA}
2	7.15 ± 0.07^{abA}	7.70 ± 0.71^{aA}	7.85 ± 1.06^{aA}	7.50 ± 0.42^{abA}	7.60 ± 0.71^{abA}	7.30 ± 0.42^{abA}	7.35 ± 0.49^{abA}
4	6.20 ± 0.99^{bcA}	7.45 ± 1.48^{aA}	7.50 ± 0.85^{aA}	7.25 ± 0.78^{abA}	7.35 ± 0.64^{abA}	6.95 ± 0.64^{abA}	7.05 ± 0.64^{abcA}
6	5.60 ± 0.14^{cdA}	7.10 ± 0.99^{aA}	7.20 ± 1.13^{aA}	7.00 ± 0.57^{abA}	7.05 ± 1.20^{abA}	6.65 ± 0.64^{abA}	6.80 ± 1.70^{abcA}
8	5.10 ± 0.28^{cdeB}	6.95 ± 0.35^{aA}	7.00 ± 0.85^{aA}	6.55 ± 1.20^{abAB}	6.70 ± 0.42^{abAB}	6.0 ± 0.42^{bcAB}	6.20 ± 0.57^{abcAB}
10	4.70 ± 0.85^{deB}	6.65 ± 0.92^{aAB}	6.75 ± 1.06^{aA}	6.45 ± 0.07^{abAB}	6.55 ± 1.06^{abAB}	5.80 ± 0.57^{bcAB}	5.95 ± 0.35^{abcAB}
12	4.10 ± 0.57^{eA}	6.25 ± 0.07^{aA}	6.50 ± 0.85^{aA}	5.85 ± 1.06^{abA}	6.00 ± 1.56^{abA}	4.90 ± 1.41^{cA}	5.30 ± 0.28^{bcA}
14	N.D	6.00 ± 0.99^{aA}	6.30 ± 0.85^{aA}	5.35 ± 1.34^{bA}	5.65 ± 0.78^{bA}	4.45 ± 0.21^{cA}	4.70 ± 0.99^{cA}

WHC = Water holding capacity. N.D = None Determined. The values are expressed as the mean ± SD (n = 3). Means with different small letters superscripts in the same column represent significant differences (p< 0.05).Means with different capital letters superscripts in the same row represent significant differences (p< 0.05

pH of minced meat treated with essential oils:

The pH value of meat is an essential indicator of meat spoilage during meat storage. The increase in pH reflects a degree of meat spoilage through protein breakdown for the production of free amino acids, leading to the formation of NH3 and amines, compounds of alkaline reaction. [46] stated that meat shows signs of spoilage when the pH rises higher than 6.0. Table (7) shows the changes in pH values in the control and treated samples during cold storage at 4º C for 14 days. Most treated and untreated meat samples maintained the pH value without significant changes until day 8 of storage, and then there was a fluctuation in pH during storage for longer periods. The increase of pH value during chilled storage of meat is correlated with an increase of free amino acids on the 6th and 8th days of storage, which may result from proteolysis bacteria, particularly Pseudomonas that commonly implicated in the spoilage of meat at chill temperature [47].

Table (7): Effect of storage period on pH value of minced meat

Storage period (day)	Treatment (mL/100 g minced meat)						
	Control	Thyme		Cumin		Rosemary	
		0.1	0.25	0.3	0.6	0.3	0.6
0	5.65 ± 0.01^{cA}	5.65 ± 0.01^{bA}	5.62 ± 0.00^{cCD}	5.64 ± 0.00^{cAB}	5.64 ± 0.00^{cAB}	5.63 ± 0.00^{cBC}	5.62 ± 0.01^{dD}
2	5.72 ± 0.01^{cA}	5.66 ± 0.01^{bC}	5.68 ± 0.01^{cB}	5.67 ± 0.00^{cBC}	5.67 ± 0.00^{cBC}	5.69 ± 0.00^{cB}	5.73 ± 0.01^{cA}
4	5.75 ± 0.01^{bcA}	5.69 ± 0.01^{bC}	5.69 ± 0.01^{cC}	5.69 ± 0.01^{cC}	5.69 ± 0.00^{cBC}	5.71 ± 0.01^{cB}	5.75 ± 0.01^{cA}
6	5.79 ± 0.01^{bcD}	5.90 ± 0.01^{aABC}	5.92 ± 0.02^{abAB}	5.87 ± 0.02^{abC}	5.91 ± 0.01^{bABC}	5.87 ± 0.01^{abBC}	5.92 ± 0.03^{bA}
8	5.83 ± 0.08^{bcC}	6.07 ± 0.01^{aA}	5.96 ± 0.03^{aB}	5.93 ± 0.04^{aB}	5.99 ± 0.02^{aAB}	5.93 ± 0.00^{aB}	6.01 ± 0.01^{aAB}
10	6.02 ± 0.24^{abA}	6.06 ± 0.09^{aA}	5.85 ± 0.01^{bA}	5.91 ± 0.03^{abA}	5.95 ± 4.17^{abA}	5.86 ± 0.04^{abA}	5.91 ± 0.00^{bA}
12	6.19 ± 0.22^{aA}	6.01 ± 0.09^{aAB}	5.91 ± 0.01^{abB}	5.76 ± 0.04^{bcB}	5.89 ± 0.06^{bB}	5.88 ± 0.07^{abB}	5.91 ± 0.03^{bB}
14	N.D	5.99 ± 0.19^{aA}	5.96 ± 0.09^{aA}	5.96 ± 0.18^{aA}	5.93 ± 0.00^{abA}	5.83 ± 0.07^{bA}	5.88 ± 0.07^{bA}

N.D = None Determined. The values are expressed as the mean ± SD (n = 3). Means with different small letters superscripts in the same column represent significant differences ($p < 0.05$).Means with different capital letters superscripts in the same row represent significant differences ($p < 0.05$).

All treated samples showed a low pH value after storage for 14 days (ranging from 5.87 to 5.99) compared with control samples, which reached 6.19 on day 12 of storage. These results agree with the results obtained by [48], who reported that the changes in pH values in minced meat during chilled storage may be due to the activation effect of microbial load, which may cause protein hydrolysis with the appearance of alkaline groups, leading to increase of pH value. The impact of different treatments on the pH values of meat during storage is close to the results reported by [23], who reported that the pH value in the chilled meat treated with 0.1 thyme increased by 0.2 degrees of pH after storage for nine days. Our findings indicate the effect of essential oils used in maintaining the pH of minced meat in the appropriate range due to their antimicrobial activity..

Total volatile nitrogen (TVN) of minced meat treated with essential oils:

The results of total volatile nitrogen (TVN) in the treated and untreated minced meat are presented in Table (8). With the progression of cold storage, TVN rapidly increased for all investigated treatments with different rates depending on the initial treatment. The Egyptian Standard recommended the maximum level of TVN in minced meat, which is 20 mg/100 grams, and any increase in TVN indicates spoilage of meat [49]. Control samples showed the highest incremental rate compared to other treatments and had TVN of about 21.1 mg/100 g at 6 days. Among all treatments, the samples treated with thyme 0.25% was the most effective treatment, which maintained the TVN level below the maximum value till day 12 of storage (19.6 mg/100g). Whereas, the samples treated with cumin at 0.3 and 0.6% were below the acceptable TVN value till day 8 (18.2 and 17.5 mg/100g, respectively). In addition, the samples treated with rosemary showed the lowest ability to maintain the TVN value under the acceptable value for long storage time (6th day in the samples treated with 0.3% and 8th day in the samples treated with 0.6% of rosemary oil) (Table 8). The increase in TVN during cold storage of minced meat might be attributed to the breakdown of nitrogenous substances because of microbial activity and any autolytic enzymes found naturally in meat tissues. The achieved results seemed comparable to the results of some previous studies that evaluated the antimicrobial and antioxidant activity of certain EOs and their effect on reducing the value of TVN [48, 50, 18]. Moreover, the results also nearly agree with the results obtained by [51], who mentioned that preserved minced beef at 4 °C after 6 days of storage recorded the highest rate of TVN values at control samples, while the sample treated with 1.5% of thyme oil was more effective in decreasing TVN values than sample treated with 0.5% thyme oil, due to the role of thyme oil on microbial population and bacterial growth as antimicrobial agents.

Table (8): Effect of storage period on TVN content of minced meat

Storage period (day)	Treatment (mg/100 g minced meat)						
	Control	Thyme		Cumin		Rosemary	
		0. 1	0. 25	0.3	0.6	0.3	0.6
0	9.80 ± 3.96^{dA}	9.10 ± 0.99^{dA}	8.40 ± 3.96^{eA}	8.40 ± 5.94^{cA}	9.10 ± 2.97^{dA}	8.40 ± 1.98^{cA}	9.10 ± 0.99^{dA}
2	18.20 ± 3.96^{cdA}	11.90 ± 0.99^{cdAB}	10.50 ± 0.99^{deB}	15.40 ± 1.98^{bcAB}	14.70 ± 0.99^{cAB}	17.50 ± 2.97^{bA}	16.80 ± 3.96^{cAB}
4	19.60 ± 1.98^{cdA}	14.70 ± 2.97^{bcdA}	13.30 ± 0.99^{cdA}	16.80 ± 3.96^{abcA}	16.10 ± 0.99^{cA}	18.20 ± 0.00^{bA}	18.20 ± 3.96^{bcA}
6	Rejected	16.10 ± 2.97^{abcdA}	15.40 ± 1.98^{bcA}	17.50 ± 4.95^{abA}	16.80 ± 1.98^{cA}	19.60 ± 1.98^{abA}	18.90 ± 0.99^{bcA}
8	Rejected	16.80 ± 1.98^{abcB}	16.10 ± 0.99^{bcB}	18.20 ± 3.96^{abB}	17.50 ± 0.99^{bcB}	Rejected	19.60 ± 1.98^{bcB}
10	Rejected	Rejected	17.50 ± 0.99^{abcC}	Rejected	Rejected	Rejected	Rejected
12	Rejected	Rejected	19.60 ± 1.98^{abB}	Rejected	Rejected	Rejected	Rejected
14	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected

The values are expressed as the mean ± SD (n = 3). Means with different small letters superscripts in the same column represent significant differences (p< 0.05).Means with different capital letters superscripts in the same row represent significant differences (p< 0.05

Thiobarbituric acid (TBA) of minced meat treated with essential oils:

In Table (9), the content of thiobarbituric acid (TBA) in treated and non-treated meat samples was reported. TBA test is normally conducted to measure the value of lipid oxidation in meat and meat products [52].

Oxidation of lipids can occur during the storage of meats and other fat-containing foods such as milk, oils, and nuts. Lipid oxidation is mainly associated with food quality deterioration, including rancidity and off-flavor formation, as well as the formation of potentially toxic and carcinogenic products, which could impair the nutritive value and safety [53].

The incremental rate of TBA was rapidly increased for the control sample, which was higher than the treated sample during most storage periods. After 6 days of chilled storage, the control sample had TBA value of about 1.240 mg MDA/kg, which was higher than the value of TBA (0.9 mg MDA/Kg) recommended by [49] as a maximum acceptable value of TBA in minced meat.

All treated samples had an acceptable value of TBA during storage for up to 14 days, except for the samples treated with rosemary 0.3%, which had an acceptable value of TBA for up to 10 days only (Table 9). Generally, samples treated with high concentrations of

EOs showed a better effect in reducing the oxidation of lipids during minced meat storage compared to low concentrations. The lowest values of TBA were shown in the samples treated with thyme (ranging from 0.312 to 0.289 mg MDA/Kg) and cumin (ranging from 0.507 to 0.429 mg MDA/Kg).

These results showed the superiority of thyme and cumin over rosemary, as well as compared them with the results reported by [18].

for the meat samples treated with lettuce oil, marjoram oil, and cumin oil. Furthermore, these results showed a high similarity with some previous studies, which found that treated meat samples with thyme oil at different concentrations showed a lowering in TBA values than control samples, while increasing concentrations of thyme led to a decrease in the TBA values. [54, 50].

Such findings may be attributed to the high antioxidant effect of thyme essential oil, which is related to the scavenger nature of its flavonoids and phenolic content.

Table (9): Effect of storage period on TBA content of minced meat.

Storage period (day)	Treatment (mg MDA/Kg minced meat)						
	Control	Thyme		Cumin		Rosmery	
		0.1	0.25	0.3	0.6	0.3	0.6
0	0.207 ± 0.03^{deA}	0.125 ± 0.01^{cA}	0.121 ± 0.06^{cA}	0.16 ± 0.02^{cA}	0.168 ± 0.04^{cA}	0.203 ± 0.07^{cA}	0.215 ± 0.05^{dA}
2	0.383 ± 0.03^{cdeB}	0.141 ± 0.08^{bcD}	0.121 ± 0.01^{cD}	0.246 ± 0.02^{bcC}	0.211 ± 0.02^{bcCD}	0.593 ± 0.03^{bA}	0.363 ± 0.05^{cB}
4	0.663 ± 0.13^{bcdA}	0.168 ± 0.02^{abcC}	0.125 ± 0.03^{bcC}	0.262 ± 0.03^{bBC}	0.226 ± 0.01^{bcC}	0.612 ± 0.06^{bA}	0.386 ± 0.01^{cB}
6	0.812 ± 0.36^{bcA}	0.215 ± 0.02^{abcC}	0.141 ± 0.02^{bcC}	0.297 ± 0.01^{bBC}	0.234 ± 0.09^{bcC}	0.632 ± 0.12^{bAB}	0.426 ± 0.03^{bcBC}
8	Rejected	0.254 ± 0.07^{abcC}	0.156 ± 0.01^{bcC}	0.30 ± 0.05^{bBC}	0.258 ± 0.03^{bcC}	0.659 ± 0.01^{bB}	0.457 ± 0.02^{abcBC}
10	Rejected	0.262 ± 0.08^{abcC}	0.191 ± 0.12^{abcC}	0.332 ± 0.04^{bBC}	0.277 ± 0.02^{bC}	0.663 ± 0.04^{bB}	0.488 ± 0.05^{abcBC}
12	Rejected	0.281 ± 0.09^{abD}	0.254 ± 0.00^{abD}	0.457 ± 0.04^{aCD}	0.386 ± 0.01^{aCD}	Rejected	0.534 ± 0.05^{abC}
14	Rejected	0.312 ± 0.02^{aC}	0.289 ± 0.06^{aC}	0.507 ± 0.08^{aB}	0.429 ± 0.03^{aBC}	Rejected	0.577 ± 0.10^{aB}

The values are expressed as the mean ± SD (n = 3). Means with different small letters superscripts in the same column represent significant differences (p< 0.05).Means with different capital letters superscripts in the same row represent significant differences (p< 0.05).

Microbial analysis of minced meat treated with essential oils:

Total viable bacterial count (TVBC) was measured to determine the impact of different EOs on reducing the bacterial growth in refrigerated minced meat, as shown in Table (10). It is evident that during the initial period of storage, the increase in TVBC was lower in treated samples, probably because of the presence of the active compounds in the oils which acted as a hurdle for the growth of microbes, and later due to lipid oxidation, an increase in microbial growth occurred. These results agree with the results obtained by [48], who reported that a reduction in TVBC in refrigerated minced beef owing to the antibacterial impact of marjoram and cumin oils, meanwhile, cumin oil had an antibacterial effect at 2 and 3% for 6th and 7th days, respectively. As well as they reported that the essential oils caused sudden lethal effects for microorganisms.

The results in Table (10) observed that the control sample always had high TVBC compared with all treated samples. Furthermore, the samples treated with thyme oil had low TVBC compared with other treated samples during shilled storage. The results obtained in this study were comparable with the results reported by [18], who found that the control sample always had high TVBC compared with different samples treated with EOs. In addition, the sample treated with cumin oil had low TVBC compared with other treated meat samples during cold storage. The effect of thyme to reduce the TVBC found in this study agree with the results reported by [50], who found that the samples treated with different concentrations of thyme showed the lowest count of TVBC compared to untreated samples and that high concentrations of thyme showed more effectiveness. Some previous studies also indicated the same results that indicated the best effect of thyme against TVBC was found at high concentrations of up to 1.5% [51, 55, 56].

All essential oils selected in this study showed a great antimicrobial effect against psychrophilic bacteria (Ps. B) during refrigeration storage of minced meat, as shown in Table 10. Compared with the control samples, all treated minced meat showed the lowest count of Ps. B at any storage period. Whereas the initial count of Ps. B was lower than the initial count of TVBC, the Ps. B count gradually increased at a higher rate than the TVBC, which may be ascribed to optimum temperature conditions in chilling storage for Ps. B. compared to TVBC. These results are consistent with the results reported by [48].

Table (10): Microbiological quality of minced meat during refrigerated storage.

Storage period (day)	Treatments (%)						
	Control	Thyme		Cumin		Rosmery	
		0.1	0.25	0.3	0.6	0.3	0.6
TVBC							
0	1.97×10^5	1.35×10^5	1.36×10^5	1.55×10^5	1.46×10^5	1.65×10^5	1.61×10^5
2	2.46×10^6	1.02×10^5	9.05×10^4	1.27×10^5	1.12×10^5	1.52×10^5	1.32×10^5
4	3.15×10^6	8.6×10^4	8.0×10^4	1.18×10^5	1.11×10^5	1.34×10^5	1.25×10^5
6	Rejected	4.9×10^5	4.4×10^5	5.3×10^5	5.03×10^5	2.03×10^6	1.28×10^6
8	Rejected	3.4×10^6	3.06×10^6	8.1×10^6	5.9×10^6	Rejected	Rejected
10	Rejected	4.6×10^6	3.2×10^6	8.7×10^6	7.3×10^6	Rejected	Rejected
12	Rejected	8.01×10^6	6.6×10^6	Rejected	Rejected	Rejected	Rejected
14	Rejected	9.1×10^6	7.03×10^6	Rejected	Rejected	Rejected	Rejected
Ps. B							
0	6.7×10^3	6.7×10^3	5.8×10^3	6.1×10^3	6.2×10^3	6.3×10^3	6.03×10^3
2	5.5×10^4	5.7×10^3	5.6×10^3	5.9×10^3	5.8×10^3	6.1×10^3	5.9×10^3
4	3.66×10^5	5.1×10^3	4.7×10^3	5.7×10^3	5.5×10^3	8.8×10^4	7.5×10^4
6	4.24×10^6	5.5×10^4	4.7×10^4	8.03×10^4	6.4×10^4	3.3×10^5	3.2×10^5
8	Rejected	4.03×10^5	3.4×10^5	6.5×10^5	5.8×10^5	1.53×10^6	1.21×10^6
10	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected
12	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected
14	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected
CG							
0	1.32×10^3	1.13×10^3	5.9×10^2	9.6×10^2	1.05×10^3	1.13×10^3	1.11×10^3
2	1.17×10^4	5.8×10^2	5.2×10^2	9.3×10^2	9.2×10^2	1.08×10^3	1.02×10^3
4	1.66×10^4	3.8×10^2	2.3×10^2	8.5×10^2	4.6×10^2	1.35×10^3	1.27×10^3
6	Rejected	2.2×10^2	1.6×10^2	1.34×10^3	1.05×10^3	2.07×10^3	1.82×10^3
8	Rejected	1.06×10^3	3.2×10^2	2.5×10^3	1.18×10^3	Rejected	1.11×10^4
10	Rejected	1.23×10^3	8.2×10^2	1.36×10^4	1.10×10^4	Rejected	2.04×10^4
12	Rejected	1.26×10^4	1.01×10^4	1.53×10^4	1.15×10^4	Rejected	Rejected
14	Rejected	1.35×10^4	1.21×10^4	2.45×10^4	1.61×10^4	Rejected	Rejected

(TVBC) total bacterial count, (Ps.B) psychrophilic bacteria, and (CG) coliform group

The impact of different EO on the growth of coliform group (CG) bacteria in minced meat was studied during storage at 4 ± 0.5 °C, and the results were presented as shown in Table (10). The results showed that the count of CG in the untreated samples was higher compared to different treated samples at the end of the storage period, which indicates the antimicrobial activity of the EOs used in this study against CG. The CG count reached 5.22×10^4 in the untreated sample on day 10 of storage compared to 1.23×10^3 and 8.2×10^2 in the samples treated with 0.1% and 0.25% of thyme, respectively. The high concentration of thyme (0.25%) showed superior antimicrobial activity against CG compared to the low concentration (0.1%), as well as compared to cumin and rosemary at two concentrations (0.3% and 0.6%). These results agree with the results found by [50], who found that samples treated with different concentrations of thyme oil showed a decreasing of coliform bacterial count compared to control samples, and the high concentration of thyme oil (2%) was more effective in decreasing this count than the lower concentration (1%). The results were almost similar to some previous studies that studied the effect of different EOs on the coliform bacterial count in refrigerated meat products [57, 58, 59, 60]. In addition, [61] reported that the thymol showed high effectiveness in destroying coliforms when used at concentrations of 250, 500 and 750 mg/kg of beef minced patties and stored for 16 days under ordinary or modified packaging at refrigeration temperature, also mentioned that covering the surface of meat products by 0.8 % thymol, the count of coliforms decreased by

2-3 log units. Similarly to our findings, [51] stated that the highest concentration of thyme (1.5%) showed more effectiveness in decreasing coliform count, especially on the third and sixth day of the storage period compared with the lower concentration (0.5%) minced beef at refrigeration temperature.

4. Conclusion

The results obtained from this study presented a superior antimicrobial activity of essential oils (thyme, cumin, and rosemary) against pathogenic bacteria, including listeria monocytogenes, E. coli O157:H7, *Staphylococcus aureus*, *Salmonella typhimurium* and *Bacillus cereus*. According to the quality measurement of minced meat treated with different essential oils, the samples treated with thyme showed a high ability to maintain their quality during refrigerated storage. In addition, the thyme treatment significantly decreased bacterial growth during minced meat storage, including total bacterial count, psychrophilic group, and coliform group. The obtained results in this study concluded that the essential oils can be used as natural meat preservatives with antimicrobial activities against food-borne pathogens and therefore may be useful in the meat industry to enhance safety and shelf life by controlling food poisoning bacteria

References

1] Pereira, P. M. d. C. C. and Vicente, A. F. d. R. B. (2013). Meat nutritional composition and nutritive role in the human diet. Meat science, 93(3), 586-592. https://doi.org/10.1016/j.meatsci.2012.09.018.

[2] Elmadfa, I. and Meyer, A. L. (2017). Animal proteins as important contributors to a healthy human diet. Annual review of animal biosciences, 5, 111-131. https://doi.org/10.1146/annurev-animal-022516-022943.

[3] Lee, H. J.; Yong, H. I.; Kim, M.; Choi, Y. S. and Jo, C. (2020). Status of meat alternatives and their potential role in the future meat market—A review. Asian-Australasian journal of animal sciences, 33(10), 1533. doi: 10.5713/ajas.20.0419.

[4] Bekhit, A. E. D. A.; Holman, B. W. B.; Giteru, S. G. and Hopkins, D. L. (2021). Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. Trends in Food Science and Technology, 109, 280-302. https://doi.org/10.1016/j.tifs.2021.01.006.

[5] Casaburi, A.; Piombino, P.; Nychas, G. J.; Villani, F. and Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. Food Microbiology, 45, 83-102. https://doi.org/10.1016/j.fm.2014.02.002.

[6] Pellissery, A. J.; Vinayamohan, P. G.; Amalaradjou, M. A. R. and Venkitanarayanan, K. (2020). Spoilage bacteria and meat quality Meat quality analysis (pp. 307-334): Elsevier. https://doi.org/10.1016/B978-0-12-819233-7.00017-3.

[7] Yu, H. H.; Chin, Y. W. and Paik, H. D. (2021). Application of natural preservatives for meat and meat products against foodborne pathogens and spoilage bacteria: A review. Foods, 10(10), 2418. https://doi.org/10.3390/foods10102418.

[8] Lee, H. and Yoon, Y. (2021). Etiological agents implicated in foodborne illness world wide. Food science of animal resources, 41(1), 1. doi: 10.5851/kosfa.2020.e75.

[9] Alirezalu, K.; Pateiro, M.; Yaghoubi, M.; Alirezalu, A.; Peighambardoust, S. H. and Lorenzo, J. M. (2020). Phytochemical constituents, advanced extraction technologies and techno-functional properties of selected Mediterranean plants for use in meat products. A comprehensive review. Trends in Food Science and Technology, 100, 292-306. https://doi.org/10.1016/j.tifs.2020.04.010.

[10] Munekata, P. E. S.; Rocchetti, G.; Pateiro, M.; Lucini, L.; Domínguez, R. and Lorenzo, J. M. (2020). Addition of plant extracts to meat and meat products to extend shelf-life and health-promoting attributes: an overview. Current Opinion in Food Science, 31, 81-87. https://doi.org/10.1016/j.cofs.2020.03.003.

[11] Wińska, K.; Mączka, W.; Łyczko, J.; Grabarczyk, M.; Czubaszek, A. and Szumny, A. (2019). Essential oils as antimicrobial agents—myth or real alternative? Molecules, 24(11), 2130. https://doi.org/10.3390/molecules24112130.

[12] FDA. (2020). Essential Oils, Oleoresins (Solvent-Free), and Natural Extractives (Including Distillates). Substances Generally Recognized as Sa. https://www.law.cornell.edu/cfr/text/21/182.20.

[13] Falleh, H.; Ben Jemaa, M.; Saada, M. and Ksouri, R. (2020). Essential oils: A promising eco-friendly food preservative. Food Chemistry, 330, 127268. https://doi.org/10.1016/j.foodchem.2020.127268.

[14] Jayasena, D. D. and Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. Trends in Food Science & Technology, 34(2), 96-108. https://doi.org/10.1016/j.tifs.2013.09.002.

[15] Bharti, S. K.; Pathak, V.; Alam, T.; Arya, A.; Singh, V. K.; Verma, A. K. and Rajkumar, V. (2020). Materialization of novel composite bio-based active edible film functionalized with essential oils on antimicrobial and antioxidative aspect of chicken nuggets during extended storage. Journal of Food Science, 85(9), 2857-2865. https://doi.org/10.1111/1750-3841.15365.

[16] Aminzare, M.; Hashemi, M.; Hassanzad Azar, H. and Hejazi, J. (2016). The use of herbal extracts and essential oils as a potential antimicrobial in meat and meat products; a review. J Hum Environ Health Promot, 1(2), 63-74.

[17] Guimarães, A. C.; Meireles, L. M.; Lemos, M. F.; Guimarães, M. C. C.; Endringer, D. C.; Fronza, M. and Scherer, R. (2019). Antibacterial

activity of terpenes and terpenoids present in essential oils. Molecules, 24(13), 2471. https://doi.org/10.3390/molecules24132471.

[18] Mahmoud, A. (2019). Effect of lettuce, marjoram and cumin essential oils on the quality and shelf life of minced meat during refrigerated storage. Zagazig Veterinary Journal, 47(3), 288-297. DOI: 10.21608/zvjz.2019.13680.1047.

[19] Pilon, A. C.; Del Grande, M.; Silvério, M. R.; Silva, R. R.; Albernaz, L. C.; Vieira, P. C.; Lopes, J. L. C.; Espindola, L. S. and Lopes, N. P. (2022). Combination of GC-MS molecular networking and Larvicidal effect against Aedes aegypti for the discovery of bioactive substances in commercial essential oils. Molecules, 27(5), 1588. https://doi.org/10.3390/molecules27051588.

[20] Ghavam, M.; Manca, M. L.; Manconi, M. and Bacchetta, G. (2020). Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of Salvia hydrangea DC. ex Benth. Scientific reports, 10(1), 15647. https://doi.org/10.1038/s41598-020-73193-y.

[21] A.O.A.C. (2016). "Official Methods of Analysis" Associatation Official Analytical Chemists of the 19th Ed. International,Gaithersburg, MD, USA.

[22] Arboud, N. H.; Hussein, M. A.; Bkear, N. M. and Ali, E. H. (2023). Effect of Cranberry Essential Oil on Quality Parameters of Chilled Minced Meat. Journal of Advanced Veterinary Research, 13(6), 1124-1130.

[23] Karabagias, I.; Badeka, A. and Kontominas, M. (2011). Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat science, 88(1), 109-116. https://doi.org/10.1016/j.meatsci.2010.12.010.

[24] Zhang, B.; Liu, Y.; Peng, H.; Lin, Y. and Cai, K. (2023). Effects of ginger essential oil on physicochemical and structural properties of agar-sodium alginate bilayer film and its application to beef refrigeration. Meat Science, 198, 109051.

[25] Saleh, W. A. M. (2010). Studies on Texture of some Foods(Doctoral dissertation, M. Sc., Thesis, Food Sci. Dept., Fac. Agric., Benha Univ., Egypt).

[26] Hussein, K. N.; Friedrich, L.; Dalmadi, I. and Kiskó, G. (2023). Evaluation of In Vitro Antimicrobial Activity of Bioactive Compounds and the Effect of Allyl-Isothiocyanate on Chicken Meat Quality under Refrigerated Conditions. Applied Sciences, 13(19), 10953. https://doi.org/10.3390/app131910953.

[27] American Public Health Association (1992): Compendium of methods for the microbiological examination of foods. A.P.H.A. Inc. Washington D.C.

[28] İlknur, U. Ç. A. K. and Afreen, M. (2023). Determination of Microbiological Quality of Fish Burgers Enriched with Orange Peel Extract. Eurasian Journal of Food Science and Technology, 7(1), 51-58.

[29] Oyom, W.; Mahmud, N.; Islam, J.; Valizadeh, S.; Awuku, R. B.; Ibrahim, S. A. and Tahergorabi, R. (2024). Enhancing the oxidative stability, physicochemical and sensory quality of deep-fat fried chicken nuggets using thyme essential oil-loaded oleogel coatings. Progress in Organic Coatings, 186, 107977. https://doi.org/10.1016/j.porgcoat.2023.107977.

[30] Ayirezang, F. A.; Arhin, R. E.; Whyte, B. K.; Antwi, E. O. and Boateng, B. N. A. (2023). Shelf-Life feasibility study of moringa oleifera seasoned beef. Journal of Food Quality, 2023. https://doi.org/10.1155/2023/5346409.

[31] Delazar, A.; Reid, R. and Sarker, S. (2004). GC-MS analysis of the essential oil from the oleoresin of Pistacia atlantica var. mutica. Chemistry of Natural Compounds, 40, 24-27.

[32] Jiang, Y.; Wu, N.; Fu, Y. J.; Wang, W.; Luo, M.; Zhao, C. J.; Zu, Y. G. and Liu, X. L. (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environmental toxicology and pharmacology, 32(1), 63-68. https://doi.org/10.1016/j.etap.2011.03.011.

[33] Cutillas, A. B.; Carrasco, A.; Martinez-Gutierrez, R.; Tomas, V. and Tudela, J. (2018). Thyme essential oils from Spain: Aromatic profile ascertained by GC–MS, and their antioxidant, anti-lipoxygenase and antimicrobial activities. Journal of food and drug analysis, 26(2), 529-544. https://doi.org/10.1016/j.jfda.2017.05.004.

[34] Gotmare, S. and Tambe, E. (2018). Chemical characterization of cumin seed oil (Cuminum cyminum) by Gcms and its comparative study. Int. J. Sci. Res. in Biological Sciences, 5, 3.

[35] Boga, M.; Ozkan, E. E.; Ersoy, E.; Tuncay, E.; Canturk, Y. Y.; Cinar, E.; Kara, E. M. and Zengin, G. (2021). Identification and quantification of phenolic and volatile constituents in five different Anatolian Thyme species using LC–MS/MS and GC-MS, with biological activities. Food Bioscience, 43, 101141. https://doi.org/10.1016/j.fbio.2021.101141.

[36] Xue, J.; Davidson, P. M. and Zhong, Q. (2015). Antimicrobial activity of thyme oil co-nanoemulsified with sodium caseinate and lecithin. International journal of food microbiology, 210, 1-8. https://doi.org/10.1016/j.ijfoodmicro.2015.06.003.

[37] Sakkas, H. and Papadopoulou, C. (2017). Antimicrobial activity of basil, oregano, and thyme essential oils. Journal of microbiology and biotechnology, 27(3), 429-438. https://doi.org/10.4014/jmb.1608.08024.

[38] Ramadan, M. F.; Asker, M. M. S. and Tadros, M. (2012). Antiradical and antimicrobial properties of cold-pressed black cumin and cumin oils. European Food Research and Technology, 234, 833-844. DOI 10.1007/s00217-012-1696-9.

[39] Forouzanfar, F.; Bazzaz, B. S. F. and Hosseinzadeh, H. (2014). Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. Iranian journal of basic medical sciences, 17(12), 929.

[40] Fu, Y.; Zu, Y.; Chen, L.; Shi, X.; Wang, Z.; Sun, S. and Efferth, T. (2007). Antimicrobial activity of clove and rosemary essential oils alone and in

combination. Phytotherapy research, 21(10), 989-994. https://doi.org/10.1002/ptr.2179

[41] Nieto, G.; Ros, G. and Castillo, J. (2018). Antioxidant and antimicrobial properties of rosemary (Rosmarinus officinalis, L.): A review. Medicines, 5(3), 98. doi:10.3390/medicines5030098.

[42] Alamin, S. A.; Ahmed, D. A. and Ahmed, H. E. (2014). A Comparative Study on the Chemical Composition and Cholesterol Content of Fresh Camel, Beef and Goat meat. Journal of Science and Technology, 15.

[43] Hammad, H.; Ma, M.; Damaka, A.; Elkhedir, A. and Jin, G. (2019). Effect of freeze and re-freeze on chemical composition of beef and poultry meat at storage period 4.5 months (SP4. 5). J. Food Process. Technol, 10(2). DOI: 10.4172/2157-7110.1000791.

[44] Abdel-Wareth, A. A.; Taha, E. M.; Südekum, K. H. and Lohakare, J. (2018). Thyme oil inclusion levels in a rabbit ration: Evaluation of productive performance, carcass criteria and meat quality under hot environmental conditions. Animal Nutrition, 4(4), 410-416. https://doi.org/10.1016/j.aninu.2018.02.004.

[45] Hemmatkhah, F.; Zeynali, F. and Almasi, H. (2020). Encapsulated cumin seed essential oil-loaded active papers: Characterization and evaluation of the effect on quality attributes of beef hamburger. Food and bioprocess technology, 13, 533-547. https://doi.org/10.1007/s11947-020-02418-9.

[46] Kameník, J. (2013). The microbiology of meat spoilage: a review. Maso International—Journal of Food Science and Technology.P, 1-9.

[47] Agunbiade, S. O.; Akintobi, O. A. and Ighodaro, O. M. (2010). Some biochemical and organoleptic changes due to microbial growth in minced beef packaged in alluminium polyethylene trays and stored under chilled condition. Life Science Journal, 7(2), 47-51.

[48] El-Desouky, A. I.; Bahlol, H. E. and Sharoba, A. M. (2006). Effect of some essential oils and preservatives on the growth of E. coli O157: H7 and quality of refrigerated minced meat. Annuals of Agric. Sci. Moshtohor, 44(4), 1675-1695.

[49] Egyptian-Standards. (2005). Minced meat. Egyptian Organization for standardization and quality control, No. 1694.

[50] Shaltout, F. A. and Koura, H. A. (2017). Impact of some essential oils on the quality aspect and shelf life of meat. Benha Veterinary Medical Journal, 33(2), 351-364. DOI: 10.21608/bvmj.2017.30503.

[51] Salem-Amany, M.; Amin-Reham, A. and Afifi-Gehan, S. (2010). Studies on Antimicrobial and Antioxidant Efficiency of Some Essential Oils in Minced Beef. J. American Sci, 6, 691-700.

[52] Gray, J. I. and Monahan, F. J. (1992). Measurement of lipid oxidation in meat and meat products. Trends in Food Science and Technology, 3, 315-319. https://doi.org/10.1016/S0924-2244(10)80019-6.

[53] Ramli, A. N. M.; Badrulzaman, S. Z. S.; Hamid, H. A. and Bhuyar, P. (2021). Antibacterial and antioxidative activity of the essential oil and seed extracts of Artocarpus heterophyllus for effective shelf-life enhancement of stored meat. Journal of Food Processing and Preservation, 45(1), e14993. DOI: 10.1111/jfpp.14993.

[54] Kassem, G.; Atta-Alla, O. and Ali, F. (2011). Improving the quality of beef burger by adding thyme essential oil and jojoba oil. Archivos de zootecnia, 60(231), 787-795.

[55] Elatabany, A. I.; Darwish, W. S.; El-Bayomi, R. M. and Hebeshy, R. M. (2016). Using of some volatile oils for improving the quality of some poultry meat products. Paper presented at the 8th International Toxicology Symposium in Africa.

[56] Salem, A. M. (2017). Efficiency of some essential oils in control of methicillin resistant Staphylococcus aureus (MRSA) in Minced Beef. Benha Veterinary Medical Journal, 32(1), 177-183.

[57] Solomakos, N.; Govaris, A.; Koidis, P. and Botsoglou, N. (2008). The antimicrobial effect of thyme essential oil, nisin, and their combination against Listeria monocytogenes in minced beef during refrigerated storage. Food Microbiology, 25(1), 120-127. https://doi.org/10.1016/j.fm.2007.07.002.

[58] Krisch, J. (2010). Antimicrobial effects of commercial herbs, spices and essential oils in minced pork. Acta Biologica Szegediensis, 54(2), 131-134.

[59] Boskovic, M.; Zdravkovic, N.; Ivanovic, J.; Janjic, J.; Djordjevic, J.; Starcevic, M. and Baltic, M. Z. (2015). Antimicrobial activity of thyme (Tymus vulgaris) and oregano (Origanum vulgare) essential oils against some foodborne microorganisms. Procedia Food Science, 5, 18-21. https://doi.org/10.1016/j.profoo.2015.09.005.

[60] Hawashin, M. D.; Al-Juhaimi, F.; Ahmed, I. A. M.; Ghafoor, K. and Babiker, E. E. (2016). Physicochemical, microbiological and sensory evaluation of beef patties incorporated with destoned olive cake powder. Meat science, 122, 32-39. https://doi.org/10.1016/j.meatsci.2016.07.017.

[61] Emiroğlu, Z. K.; Yemiş, G. P.; Coşkun, B. K. and Candoğan, K. (2010). Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat science, 86(2), 283-288. https://doi.org/10.1016/j.meatsci.2010.04.016.