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Research Article

Comparing the Effects of Thyme (*Zataria multiflora*) and Rosemary (*Rosmarinus officinalis*) Essential Oils on Microbiological, Physicochemical, and Sensory Properties of Vacuum-Packaged and Refrigerated Chicken Breast

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This study aimed to compare the effects of thyme and rosemary essential oils (EOs) with vacuum packaging on shelf life extension and organoleptic properties of chicken breast meat. For this purpose, two concentrations of 0.1% and 0.3% (v/w) from thyme and rosemary EOs were added individually to fresh chicken breast samples, packed under vacuum conditions, and stored at 4°C for 12 days. All the samples were evaluated for microbiological, physicochemical, and sensory properties at 3-day intervals. The results showed that the thyme EO treatments, especially at 0.3% concentration, significantly reduced both total and psychrotrophic colony counts, and they were more effective in reducing microbial counts compared to rosemary EO treatments. Moreover, thyme EO significantly controlled *Escherichia coli* growth in treated samples. TBA values in treated samples with thyme EOs were significantly less than in rosemary EOs and control samples. Results indicated that rosemary EO treatments had the lowest pH during 12 days. Colorimetric and organoleptic analysis of the samples showed statistical differences on various days. In the EOs-treated chicken breasts, the color and texture of the samples improved compared to controls, but the taste and odor were not desired at the higher concentration of thyme and rosemary EOs.

1. Introduction

Vacuum packaging is a technique for prolonging the shelf life of fresh chicken meat, which can minimize the oxidative deteriorative reactions and reduce aerobic bacterial growth [1, 2]. However, due to the respiration of fresh meat and the metabolism of microorganisms, which change the gaseous atmosphere inside the package, the shelf life of the packaged product cannot be extended for a long time. Therefore, combining vacuum packaging with antimicrobial and antioxidant compounds of plant extracts/essential oils could be more effective in reducing microbial load and lipid oxidation [3, 4].

The antibacterial and antioxidant activities of spices and their essential oils (EOs) have been known for a long time, and different kinds of plant EO were investigated in chicken meat and its products in many studies [5–7]. Among studies, thyme and rosemary EOs have received more attention and shown acceptable effects on the microbial and oxidative properties of chicken meat [8–11]. Furthermore, thyme and rosemary EOs were successfully used in vitro as well as in other types of meat and meat products for microbial control and shelf life extension [12, 13].

Moreover, the use of thyme and rosemary EOs in both nonencapsulated and encapsulated forms has been effective in the control of oxidation and microbial growth. It was reported that nonencapsulated rosemary EO had stronger antioxidant activity than the encapsulated EO and greater antimicrobial effect against *E. coli*, but the encapsulated thyme EO had better antioxidant and antibacterial effects against *Enterobacteriaceae*, *Staphylococcus aureus*, yeasts, and molds in comparison with the free EO [14, 15].

Carvacrol and thymol are the most representative compounds in thyme EO, which are the main constituents for their antimicrobial and antioxidative activity [11]. The remarkable compounds responsible for the antibacterial activity of rosemary EO are α-pinene, bornyl acetate, camphor, and 1,8-cineole, whereas carnosic acid and carnosol are related to their antioxidant capacity [16].

As mentioned above, the appropriate combination of preservation methods such as vacuum packaging as well as effective EOs could be very promising to achieve microbial and chemical stability and safety of chicken meat. The antimicrobial and antioxidant properties of thyme and rosemary EOs have been well documented in the literature, but due to many factors such as application forms, nonencapsulated or encapsulated, and the type of packaging accompanied with EOs, their effects can be different in the products. Accordingly, the present study was conducted to compare the effects of thyme and rosemary EOs as natural preservatives combined with vacuum packaging on the shelf life extension and sensory properties of refrigerated fresh chicken breast.

2. Materials and Methods

2.1. Materials. The fresh chicken breast meat was randomly collected from chicken carcasses in a local slaughterhouse (Tehran, Iran) and transported immediately to the laboratory in a cold chain. Thyme (*Zataria multiflora*) and rosemary (*Rosmarinus officinalis*) EOs with a density of 0.922 and 0.906 g/mL at 20°C, respectively, were provided by Barij Essence Pharmaceutical Co. (Kashan, Iran), with specified composition analyzed by gas chromatography-mass spectrometry which is shown in Table 1. The analysis of the composition of EOs was conducted according to the method described by Hosseini et al. [7], using a gas chromatograph (Agilent 7890A, CA, USA) coupled to a mass spectrometer (Agilent 5975C, CA, USA) and equipped with a 5% phenyl methylpolysiloxane capillary column (HP-5MS, J&W Scientific Inc., Folsom, CA, USA; 30 m × 0.25 mm ID × 0.25 μm film thickness). All culture media were purchased from Liofilchem S.r.l. (Italy) and other chemicals from Merck Chemical Co. (Darmstadt, Germany).

2.2. Sample Preparation and Storage. Skinless chicken breast fillets were cut in about 4 cm × 4 cm × 1 cm dimensions at ambient temperature with a sterile knife. These chicken cuts were divided equally into six experimental groups. Thyme and rosemary EOs were individually prepared in two different concentrations of 0.1% and 0.3% v/w and added in appropriate volumes to four groups of chicken breast samples using a micropipette so as to achieve mentioned final concentrations of EOs. In order to prepare the concentration of EOs, the required percentages of each EO were calculated based on the ratio of the volume of EO (mL) to the final weight (gram) of the chicken breast samples. Then, all samples were massaged and marinated at 15°C for 5 minutes as treatments with EO. Also, distilled water was added to two other groups as controls without EO. Subsequently, all thyme EOs (T1 and T2) and rosemary EOs (R1 and R2) treatments and one of the control samples (C2) were individually placed in sterile polyamide bags and packaged using a vacuum packaging machine (R5200, MULTIVAC, Germany) and the other control sample air-packaged (C1). Then, all prepared samples were labeled and stored at 4°C in the refrigerator for 12 days, and microbiological, physicochemical, and sensory properties were analyzed in three-day intervals (0, 3, 6, 9, and 12 days). All examinations were carried out in three replications.

2.3. Microbiological Analysis. Standard procedures were followed in analyzing the microbial profile of the samples. First, 10 g of each chicken breast sample was blended and homogenized with 90 mL peptone solution (0.1%) in a sterile stomacher bag for 1–2 minutes. Then, serial dilutions were prepared, and standard microbiological methods were used for the enumeration of total viable counts as follows. Total aerobic mesophilic colony counts were carried out by the pour plate technique in plate count agar (PCA) at 30°C for 72 h incubation according to ISO 4833-1 [17]. In addition, the effects of EOs on the maximum growth rate of the mesophilic microorganisms were evaluated through the DMFit function of ComBase online freeware, by fitting the microbial growth data obtained to the Baranyi–Roberts model, as automatically proposed by software [18]. In another analysis, dilutions were plated on the same medium and were incubated at 7°C for 10 days for the enumeration of total aerobic psychrotrophic colony counts [19]. All these results were reported as colony-forming units per gram (CFU/g) and log-transformed (log CFU/g). For the enumeration of presumptive *E. coli*, the most probable number (MPN) technique was used according to ISO 7251 [20] in lauryl sulfate broth and EC broth which were incubated at 37°C for 24–48 h and 44°C for 24–48 h, respectively, and in the following steps, peptone water incubated at 44°C for 48 h, and the indole test was conducted by adding Kovacs' reagent.

2.4. Physicochemical Analysis. Following preparation, samples were analyzed for pH, color (L^*, a^*, b^*), and lipid oxidation (TBA reactive substances; TBARs) on marinated chicken breast. For measurement of pH, 15 g of each sample was homogenized with 200 mL distilled water for 1 min. Then, the pH value was measured at room temperature by using a pH meter (PTR79, Zagchemie, Iran) calibrated at pH 4.0 and 7.0 according to ISO 2917 [21].

To evaluate the susceptibility to oxidation of lipids, the thiobarbituric acid (TBA) value was determined by the distillation technique using 2- thiobarbituric acid 0.02 M reagents according to the method described by Kilinc et al. [22]. The absorbance at 538 nm was measured using a UV/VIS spectrometer (UV 2100, Unico, USA). The TBA content was expressed as mg of malondialdehyde (MDA) per kg of meat.

Table 1: Chemical composition of thyme and rosemary EOs used in this study.

Compound	%
Thyme EO composition	
Carvacrol	23
Thymol	17.7
γ-Terpinene	9
p-Cymene	7.9
Carvacrol methyl ether	5.9
α-Pinene	5.2
β-Caryophyllene	4.8
Linalool	3.6
α-Terpinene	3.2
Myrcene	2.9
Carvacrol acetate	1.7
Terpinene-4-ol	1.5
3-Octanone	1.4
α-Thujene	1.4
Limonene	1.2
Thymol methyl ether	1.1
Eugenol	1
β-Pinene	1
1,8-Cineole	0.9
Aromadendrene	0.8
α-Terpineol	0.6
Thymol acetate	0.5
3-Octanol	0.5
α-Phellandrene	0.5
Viridiflorene	0.5
Terpinolene	0.3
Camphene	0.3
cis-Sabinene hydrate	0.3
α-Humulene	0.3
Spathulenol	0.2
Caryophyllene oxide	0.2
Total	99.4
Rosemary EO composition	
Verbenone	11.42
α-Pinene	10.44
Camphor	9.31
Borneol	8.37
1,8-Cineole	7.38
Bornyl acetate	5.14
Myrcene	4.7
Camphene	4.4
Terpinen-4-ol	4.37
3-Octanone	4.01
Limonene	3.94
α-Terpineol	3.71
Linalool	3.33
Linalyl acetate	2.47
p-Cymene	1.48
γ-Terpinene	1.36
(E)-Caryophyllene	1.02
(E)-Verbenol	0.95
(E)-β-Ocimene	0.75
(E)-Pinocamphone	0.61
Verbenene	0.55
Octen-3-ol	0.54
α-Terpinene	0.49
Dihydro carveol acetate	0.32
2,6-Dimethyl phenol	0.30
Tricyclene	0.22
Neryl acetone	0.21
α-Phellandrene	0.19
α-Terpinyl acetate	0.17
α-Humulene	0.15
Total	92.3

The color of each sample, including the L^* (lightness), a^* (redness), and b^* (yellowness) values, was measured according to the method described by Petracci and Baéza [23], using a HunterLab Colorimeter (A60-1005-654 45/0, Colorflex, USA).

2.5. Sensory Evaluation. Seven experienced panelists, trained according to the ISO 8586-1 [24] protocol, were asked to evaluate the sensory properties of samples including color, taste, odor, and texture after 0, 3, 6, 9, and 12 days of storage at 4°C in the refrigerator (1700, Pars, Iran). Sensory attributes were evaluated using a 3-point hedonic scale in which 1 referred to "satisfactory quality without significant change," 2 referred to "acceptable quality with little change," and 3 referred to "unacceptable quality with significant change." The flavor attribute was evaluated after cooking breast samples at 80°C for 1 h in the oven (FS560, Kenwood, China).

2.6. Statistical Analysis. All the assays of samples were carried out in triplicates, and the results were expressed as the mean and standard deviation (SD). Differences between the variables were tested for significance by analysis of variance (ANOVA) using SPSS V22.0, and differences at $p < 0.05$ were considered significant. The mean differences were determined using Duncan's multiple range test.

3. Results and Discussion

3.1. Microbial Analysis of Chicken Breast Meat. The effects of thyme and rosemary EOs on microbial properties of vacuum-packaged (VP) chicken breast samples are reported in Table 2. Total colony counts (TCC) showed that adding different concentrations of EOs on day 0 decreased the microbial load of the chicken breast compared to control samples, and all treatments had significantly lower TCC by the 6th day ($p < 0.05$). The initial number of TCC of chicken breasts ranged from 3.95 log CFU/g in T2 to 5.95 log CFU/g in air-packaged chicken breast samples (C1) which was increased progressively during storage in all controls and treatments that are in accordance with similar studies [4, 7]. On day 12th, the use of VP alone (C2) and its combination with 0.3% concentration of thyme (T2) or rosemary (R2) EOs resulted in a reduction in TCC by 0.66, 1.1, and 0.47 log CFU/g, respectively, compared to C1. Both thyme and rosemary EOs can effectively inhibit the growth of total

Table 2: Effect of thyme and rosemary EOs on microbial counts of vacuum-packaged chicken breast samples during refrigeration (mean ± SD).

Microbial tests	Samples	Days 0	3	6	9	12
Total colony counts (log CFU/g)	C1	$5.95 \pm 0.01^{A*d**}$	8.01 ± 0.03^{Ac}	8.29 ± 0.03^{Ab}	8.01 ± 0.06^{Ac}	8.37 ± 0.04^{Aa}
	C2	5.90 ± 0.01^{Be}	7.29 ± 0.01^{Bd}	8.15 ± 0.01^{Ba}	7.59 ± 0.01^{Ec}	7.71 ± 0.02^{Db}
	T1	5.17 ± 0.02^{De}	6.43 ± 0.02^{Ed}	7.22 ± 0.04^{Ec}	7.90 ± 0.01^{Cb}	7.98 ± 0.02^{Ca}
	T2	4.95 ± 0.01^{Ee}	5.96 ± 0.00^{Fd}	6.78 ± 0.01^{Fc}	7.59 ± 0.02^{Ea}	7.27 ± 0.05^{Eb}
	R1	5.83 ± 0.01^{Cd}	7.11 ± 0.01^{Cc}	8.08 ± 0.04^{Ca}	7.95 ± 0.02^{Bb}	8.05 ± 0.05^{Ba}
	R2	5.82 ± 0.01^{Cd}	6.95 ± 0.00^{Dc}	7.70 ± 0.01^{Db}	7.70 ± 0.01^{Db}	7.90 ± 00.02^{Ca}
E. coli counts (MPN/g)	C1	110^{A*a**}	110^{Aa}	46^{Ab}	9^{Bc}	9^{Bc}
	C2	110^{Aa}	110^{Aa}	4^{Bb}	4^{Cb}	0^{Dc}
	T1	110^{Aa}	1^{Cb}	0^{Cb}	0^{Eb}	0^{Db}
	T2	9^{Ca}	0^{Db}	0^{Cb}	0^{Eb}	0^{Db}
	R1	110^{Aa}	110^{Aa}	46^{Ab}	15^{Ad}	24^{Ac}
	R2	46^{Ba}	29^{Bb}	46^{Aa}	1^{Dc}	1^{Cc}
Psychrotrophic colony counts (log CFU/g)	C1	$6.95 \pm 0.01^{A*e**}$	7.84 ± 0.01^{Ad}	8.64 ± 0.03^{Aa}	8.19 ± 0.04^{Bb}	7.95 ± 0.01^{Ac}
	C2	5.90 ± 0.01^{Bd}	6.11 ± 0.03^{Bc}	7.62 ± 0.02^{Ea}	7.10 ± 0.05^{Eb}	7.11 ± 0.07^{Db}
	T1	4.78 ± 0.00^{Cd}	5.48 ± 0.03^{Cc}	8.39 ± 0.03^{Ca}	8.37 ± 0.03^{Aa}	7.53 ± 0.00^{Cb}
	T2	4.78 ± 0.00^{Ce}	5.31 ± 0.04^{Dd}	6.78 ± 0.01^{Fc}	7.40 ± 0.04^{Da}	6.89 ± 0.04^{Eb}
	R1	5.91 ± 0.01^{Be}	7.84 ± 0.02^{Ac}	8.47 ± 0.02^{Ba}	8.12 ± 0.05^{Bb}	7.66 ± 0.02^{Bd}
	R2	5.90 ± 0.00^{Bd}	7.86 ± 0.01^{Aa}	7.67 ± 0.02^{Db}	7.70 ± 0.03^{Cb}	7.59 ± 0.02^{Cc}

*A–F: mean values with different superscript letters in a column are significantly different ($p < 0.05$). **a–e: mean values with different superscript letters in a row are significantly different ($p < 0.05$). (C1: air-packaged, C2: VP without EO, T1: VP + 0.1% thyme EO, T2: VP + 0.3% thyme EO, R1: VP + 0.1% rosemary EO, and R2: VP + 0.3% rosemary EO).

aerobic flora [25]. However, in the present study, thyme EO treatments were more effective than rosemary EO treatments and led to 1 log CFU/g reduction in TCC by the 6th day.

Another way to quantitatively compare the effects of treatments is the Baranyi–Roberts no-lag mathematical model which highlights differences in the μmax (maximum specific growth rate) values between groups for the microbial population [18]. In the present study, the values were 0.030, 0.019, 0.014, 0.013, 0.018, and 0.015 log CFU/h for TCC in C1, C2, T1, T2, R1, and R2, respectively. These results showed that the combination of vacuum packaging with both EOs, especially in the higher concentration, lowered the maximum growth rate of the microbial population. However, the thyme treatments showed a more decrease in the maximum growth rate in comparison with the rosemary treatments. Consistent with our results, the greater antibacterial activity of thyme EO compared to rosemary EO has been proven in foods [26]. Among all treatments, T2 (VP with 0.3% thyme EO) had more effect on the reduction of microbial load during 12 days of storage. In addition, C2 (VP without EO) had significantly lower TCC compared to C1 (air-packaged) on all days of the study, and also, from the 9th day, it had lower TCC compared to treatments containing EOs except for T2 ($p < 0.05$). Mathew et al. [1] reported that the vacuum-packed chicken, stored at 4°C, had a shelf life of 15 days, and the TCC was found as 42×10^5 CFU/ml which is less than the counts obtained in C2 in the present study. It is reported that oxygen removal, from the headspace of the packages, affects microbial metabolism and subsequently decreases aerobic microbial growth rate during the log phase [3, 4].

In this study, thyme and rosemary EOs contained nine and thirteen components that make up 80.3% and 80.5% of the total contents of the EOs, respectively (Table 1), most of which have shown great potential as antimicrobial agents in meat [26]. Previous studies [27–29] showed similar constituents of thyme and rosemary EOs to those observed in the present study. The antibacterial activity of spices is still under investigation, but flavonoids and phenols' function in the chelation of metals and the destruction of cell membranes, respectively, have been discussed as possible mechanisms that control microbial growth [30]. Our results on the synergistic effect of EOs and VP are in line with Kačániová et al. [10] and Karam et al. [31], who described the inhibitory effects of sage and rosemary EOs and active components of thyme EO (carvacrol and thymol) on microbial growth in chicken meat, respectively, especially in the highest concentration. The upper microbiological acceptable limit of TCC is considered as the value of 7 log CFU/g [32], which was reached on day 3 for the control samples and R1 (VP with 0.1% rosemary EO), on day 6 for the T1 (VP with 0.1% thyme EO) and R2 (VP with 0.3% rosemary EO) treatments, and on day 9 for T2 (VP with 0.3% thyme EO) treatment. The findings were in accordance with Majdinasab et al. [11], who reported that TCC of coated chicken fillet samples with summer savory and Shirazi thyme EOs increased during storage and remained below the acceptable limit until the 8th day of storage. However, Khorshidi et al. [2] reported that the total number of aerobic mesophilic bacteria was below 6 log CFU/g in all treatment groups of chicken drumsticks until the 12th day of storage which might be due to the lower initial bacterial load in addition to the combination of their studied coatings with

chitosan that enhanced the antibacterial effects of phenolic compounds of used EO.

The highest enumerations of *E. coli* (110 MPN/g) were found on days 0 and 3 in control samples and R1, which indicated that the effects of air-packaging and VP alone are similar to each other and similar to the low concentration of rosemary EO. *E. coli* was found in T2 only on day 0 (9 MPN/g), and it was not detected on other days of the study, whereas *E. coli* counts in R2 were from 1 MPN/g to 46 MPN/g during storage (Table 2). In other words, T2 and R2 inhibited *E. coli* growth more effectively compared to C1 and C2 samples. The absence of *E. coli* in T2 (on days 3 to 12) and T1 (on days 6 to 12) indicated that thyme EO is more effective than rosemary EO in the reduction of *E. coli*. It has been shown that thyme EO effectively inhibited *E. coli* growth, and at low concentrations of less than 0.3% exhibited both bacteriostatic and bactericidal effects on *E. coli* O157:H7 [27]. Furthermore, it has been reported that thyme EO displayed the highest inhibition zone and antimicrobial activity against *E. coli* O157:H7 in vitro compared to rosemary EO [13]. The observed effect of rosemary EO in the present study agreed with Kahraman et al. [16], who showed that rosemary EO in the concentration of 0.2% was not effective in the reduction of pathogenic bacteria in chicken meat packaged in MAP in the refrigerator but led to a significant reduction in higher concentrations (0.3 to 1%) of rosemary EO. Moreover, Karam et al. [31] reported lower *E. coli* counts in marinated chicken with carvacrol and thymol compared to the control, but it increased in all treatments and control during 21 days of storage at 4°C which was not in agreement with our findings. This may be related to the synergistic effect of the amount and type of components in the studied EO that could affect antimicrobial efficiency.

The initial number of psychrotrophic colony counts (PCC) of chicken breasts increased in all treatments during storage by the 6th day. However, there was no consistent trend in the samples. PCC varied from 6.95 log CFU/g on day 0 to 8.64 log CFU/g on day 6 in C1 but from 5.9 log CF CFU/g to 7.62 log CFU/g on the same days in C2, which indicated the effectiveness of VP in control of psychrotrophic microorganisms. Similarly, Naveena et al. [33] found that both total and psychrotrophic counts remained lower in vacuum-packaged and refrigerated chicken meat products. According to the findings, the lowest PCC in treated samples during 12 days of storage was observed in treatments containing thyme EO, especially in T2 which showed 1 to more than 2 log CFU/g reduction in PCC compared to C1 by the 6th day (Table 2). A similar result was reported that coating VP chicken drumsticks with *Elettaria cardamomum* essential oils caused 2.5 logs CFU/g reduction in psychrotrophic counts compared to the control group on the 6th day [2]. Treatments containing thyme EOs and C2 were more effective than treatments containing rosemary EOs on PCC and had significant differences with each other throughout 12 days of storage ($p < 0.05$). This could be due to the lower minimum inhibitory concentration (MIC) of thyme EO than rosemary EO for inhibiting the growth of some psychrotrophic bacteria such as *Brochothrix thermosphacta* which is one of the typical spoilage bacteria in vacuum-packed refrigerated meat [34]. Generally, in VP samples, the final psychrotrophic counts decreased by approximately 0.3 to 1 log compared to the air-packaged control sample. PCC reached the upper acceptability limit of 7 log CFU/g for fresh meat [3] on day 3 for C1, R1, and R2 treatments, whereas C2 and T1 exceeded this limit on the 6th day, and T2 reached this level on the 9th day of storage.

The results revealed that the inhibitory effect of the combination of VP and studied EOs against mesophilic microorganisms was more effective than psychrotrophic microorganisms which are consistent with those reported by Dalvandi et al. [4] for chicken meat. Petrová et al. [3] and Zhang et al. [30] reported that rosemary essential oil (2%) and extract have a less preventive effect on *Pseudomonas* spp. growth in raw and VP chicken samples which is one the most important psychrotrophic bacteria responsible for chicken meat degradation. However, the main constituents of thyme EO including thymol and carvacrol have shown inhibitory effects against *Pseudomonas* spp., as well as lactic acid bacteria and *B. thermosphacta* in the marinated chicken samples, and the use of these components along with VP reduced the microbial spoilage [31]. Our results were not in accordance with Ntzimani et al. [5] and Can and Şahin [12] who reported a 7–9 day shelf life extension in VP chicken meat and chicken meatball treated by 0.2 and 0.3% rosemary EO, which could be due to the initial lower microbial counts of chicken meat used in their study.

3.2. Physicochemical Analysis of Chicken Breast Meat. The effects of thyme and rosemary EOs on the physicochemical properties of VP chicken breast are reported in Table 3. The pH of the chicken breast at the beginning of the study was found to be 5.73–5.82 which is in line with the previous studies [2]. However, the pH values in C1 showed a significant difference compared to C2 and treated samples with EOs during the 12 days of storage ($p < 0.05$). The higher pH values were mostly observed in C1 and T2 during 0–6 and 6–12 days of storage, respectively. However, lower pH values were determined in rosemary EOs treatments (R1 and R2) during days 3 to 12 of storage which might be related to the pH of rosemary EO and also growth inhibition of some microorganisms against substrate decomposition by this EO. Moreover, despite the results of some research [11], that pH values have increased during the storage period, in our study, pH values decreased in all treatments and control samples by the 9th day and slightly increased on the 12th day except for C1. The decrease in pH could be related to the dominance of lactic acid bacteria over time which leads to the accumulation of acidic products in the extracellular environment [7]. Our results are in agreement with previous studies that control samples had significantly higher pH than EO-treated meat products [6, 28]. However, the results were not in accordance with Rimini et al. [9] and Can and Şahin [12] who had reported no significant effect on the pH of marinated chicken breast samples with thyme and orange EOs and coated chicken meat with rosemary EO, respectively, compared to

Table 3: Effect of thyme and rosemary EOs on physicochemical properties of vacuum-packaged chicken breast samples during refrigeration (mean ± SD).

Physicochemical properties	Samples	Days 0	3	6	9	12
pH	C1	$5.82 \pm 0.01^{A*a**}$	5.75 ± 0.01^{Ab}	5.70 ± 0.02^{Ac}	5.57 ± 0.02^{Bd}	5.57 ± 0.01^{Bd}
	C2	5.75 ± 0.02^{Ba}	5.62 ± 0.01^{Cb}	5.46 ± 0.01^{Cd}	5.41 ± 0.01^{Ce}	5.58 ± 0.01^{Bc}
	T1	5.73 ± 0.01^{Ca}	5.69 ± 0.00^{Bb}	5.62 ± 0.02^{Bc}	5.58 ± 0.02^{Bd}	5.73 ± 0.03^{Aa}
	T2	5.76 ± 0.03^{Ba}	5.70 ± 0.01^{Bc}	5.70 ± 0.02^{Ac}	5.65 ± 0.02^{Ad}	5.74 ± 0.03^{Ab}
	R1	5.75 ± 0.02^{Ba}	5.61 ± 0.02^{Cb}	5.47 ± 0.01^{Cc}	5.36 ± 0.01^{Dc}	5.55 ± 0.02^{Cb}
	R2	5.82 ± 0.01^{Aa}	5.51 ± 0.01^{Db}	5.43 ± 0.03^{Dc}	5.40 ± 0.01^{Cc}	5.51 ± 0.01^{Db}
TBARs (mg MDA/kg meat)	C1	$0.25 \pm 0.01^{A*a**}$	0.21 ± 0.01^{Ab}	0.17 ± 0.01^{Bc}	0.20 ± 0.01^{Db}	0.25 ± 0.01^{Ca}
	C2	0.14 ± 0.01^{Be}	0.15 ± 0.00^{Bd}	0.18 ± 0.01^{Bc}	0.22 ± 0.01^{Cb}	0.26 ± 0.02^{Ca}
	T1	0.13 ± 0.01^{Ce}	0.14 ± 0.01^{Cd}	0.15 ± 0.01^{Cc}	0.19 ± 0.01^{Eb}	0.22 ± 0.01^{Da}
	T2	0.09 ± 0.01^{Dd}	0.09 ± 0.01^{Dd}	0.11 ± 0.01^{Dc}	0.13 ± 0.01^{Fb}	0.14 ± 0.01^{Ea}
	R1	0.12 ± 0.01^{Ce}	0.16 ± 0.01^{Bd}	0.20 ± 0.01^{Ac}	0.31 ± 0.00^{Ab}	0.39 ± 0.02^{Aa}
	R2	0.13 ± 0.01^{Ce}	0.15 ± 0.01^{Bd}	0.17 ± 0.00^{Bc}	0.26 ± 0.01^{Bb}	0.33 ± 0.01^{Ba}
Color L^* values	C1	$54.16 \pm 0.01^{B*e**}$	55.64 ± 0.02^{Bc}	57.19 ± 0.01^{Ba}	56.02 ± 0.06^{Db}	54.62 ± 0.06^{Cd}
	C2	51.35 ± 0.02^{De}	53.85 ± 0.03^{Dd}	56.36 ± 0.07^{Dc}	57.52 ± 0.09^{Cb}	58.78 ± 0.16^{Ba}
	T1	53.71 ± 0.13^{Cb}	49.95 ± 0.03^{Fd}	46.27 ± 0.09^{Fe}	50.92 ± 0.03^{Fc}	55.54 ± 0.02^{Ca}
	T2	61.16 ± 0.02^{Aa}	58.58 ± 0.05^{Ac}	56.14 ± 0.11^{Ed}	58.50 ± 0.06^{Bc}	60.94 ± 0.09^{Ab}
	R1	48.51 ± 0.04^{Fe}	52.62 ± 0.04^{Ec}	56.88 ± 0.12^{Ca}	53.60 ± 0.02^{Eb}	50.24 ± 0.01^{Dd}
	R2	49.69 ± 0.01^{Ed}	54.60 ± 0.01^{Cc}	59.66 ± 0.13^{Aa}	58.92 ± 0.03^{Ab}	58.86 ± 0.12^{Bb}
Color a^* values	C1	$3.36 \pm 0.01^{D*e**}$	4.72 ± 0.02^{Ec}	5.98 ± 0.02^{Aa}	4.95 ± 0.02^{Cb}	3.81 ± 0.07^{Ed}
	C2	5.26 ± 0.01^{Ba}	5.03 ± 0.02^{Cb}	4.83 ± 0.01^{Fc}	4.81 ± 0.01^{Dc}	4.78 ± 0.02^{Bd}
	T1	3.32 ± 0.01^{De}	4.14 ± 0.03^{Fd}	4.98 ± 0.02^{Ea}	4.73 ± 0.02^{Eb}	4.47 ± 0.02^{Cc}
	T2	4.11 ± 0.05^{Ce}	4.95 ± 0.03^{Dc}	5.95 ± 0.04^{Ba}	5.21 ± 0.02^{Bb}	4.48 ± 0.01^{Cd}
	R1	6.29 ± 0.03^{Aa}	5.91 ± 0.02^{Bb}	5.55 ± 0.02^{Dc}	5.01 ± 0.05^{Cd}	4.34 ± 0.05^{De}
	R2	6.30 ± 0.02^{Aa}	6.04 ± 0.02^{Ab}	5.80 ± 0.07^{Cd}	5.64 ± 0.05^{Ae}	5.93 ± 0.01^{Ac}
Color b^* values	C1	$15.06 \pm 0.01^{A*a**}$	13.93 ± 0.03^{Ac}	12.91 ± 0.03^{Ce}	13.64 ± 0.01^{Ad}	14.38 ± 0.04^{Ab}
	C2	8.74 ± 0.01^{Fe}	11.82 ± 0.02^{Ed}	14.96 ± 0.02^{Aa}	13.61 ± 0.04^{Ab}	12.19 ± 0.08^{Cc}
	T1	11.72 ± 0.07^{Da}	10.33 ± 0.03^{Fc}	8.97 ± 0.04^{Fe}	10.18 ± 0.01^{Ed}	11.42 ± 0.04^{Db}
	T2	10.82 ± 0.01^{Ee}	12.40 ± 0.01^{Dc}	13.95 ± 0.05^{Ba}	12.71 ± 0.01^{Bb}	11.40 ± 0.03^{Dd}
	R1	13.41 ± 0.07^{Ba}	13.02 ± 0.01^{Bb}	12.51 ± 0.02^{Dc}	12.50 ± 0.02^{Cc}	12.26 ± 0.08^{Bd}
	R2	13.21 ± 0.04^{Ca}	12.45 ± 0.02^{Cb}	11.72 ± 0.01^{Ee}	11.96 ± 0.04^{Dd}	12.23 ± 0.04^{Bc}

*A–F: mean values with different superscript letters in a column are significantly different ($p < 0.05$). **a–e: mean values with different superscript letters in a row are significantly different ($p < 0.05$). (C1: air-packaged, C2: VP without EO, T1: VP + 0.1% thyme EO, T2: VP + 0.3% thyme EO, R1: VP + 0.1% rosemary EO, and R2: VP + 0.3% rosemary EO).

control samples during storage. These differences could be due to the variety of herbs, the extraction method, composition, and concentration of used EOs.

TBARS is an indicator of lipid oxidation of unsaturated fatty acids in meats which is increased during storage [6]. The results indicate that air-packaged chicken breast (C1) showed higher values of TBARs than VP breast samples by the 3rd day, but during 6–12 days of the study did not show any differences compared to C2 and some other treatments, and even lower values were observed especially compared to rosemary EO treatments. In addition, the combination of VP and rosemary EO (in 0.3%) showed the same template of oxidation inhibition as VP alone by the 6th day. Dalvandi et al. [4] showed that the TBARs values of chicken breasts in VP were lower than those in air-packaged during the entire storage time which was in accordance with the results obtained in the present study. Can and Şahin [12] found that TBARs values were lower in rosemary EO-coated VP chicken meatballs compared to VP control without EO which was not in agreement with our findings, and it might be related to the higher amount of EO (0.5%) used in that study.

After 3 days of storage, the TBARs values of all chicken breast samples increased significantly ($p < 0.05$), except for T2 treatment in which TBARs increased after 6 days. In general, VP chicken breast treated with thyme EO was less susceptible to lipid oxidation compared with rosemary EO and control samples. Saricaoglu and Turhan [13] reported that rosemary EO has a higher EC50 value (204 μg oil/mL) or lower antioxidant capacity than thyme EO (83 μg oil/mL) which could explain our findings. Total polyphenol content is highly correlated with the radical-scavenging antioxidant activity which led thyme EO to act as one of the strongest antioxidant agents among studied EOs [7]. Thymol is one of the most important antioxidative EO phenolic constituents, which is found in added thyme EO in a significant amount of 17.7%. Previous studies [9, 11] have shown that adding antioxidants such as thyme EO to chicken meat reduced DPPH radical formation and also lipid susceptibility to

oxidation in the meat. Similar research has shown that although vacuum packaging can be somewhat effective in controlling oxidation in chicken meat, its combination with some herbal extracts and essential oils has a synergistic effect [2]. On the 12th day of the study, TBARs values of all chicken breast samples ranged between 0.14 (T2) and 0.39 (R1) mg MDA/kg meat which were much lower than the 2 mg MDA/kg level reported as the threshold for suitability of rancidity [6].

Color values of VP chicken breast samples showed slight variation in all the samples during the storage time (Table 3). After marinating with EO, samples showed a significant difference in lightness (L^*) compared with control samples ($p < 0.05$). L^* values increased in a higher concentration of thyme and rosemary EOs which could be related to the reduction of the oxidation process in these treatments. Among all treatments, T2 had more effect on increasing of L^* values of chicken breast meat, and the least L^* values were measured in T1 and R1 (with 0.1% EOs) during the storage days.

Moreover, significant differences were observed in EOs and control groups in redness (a^*) and yellowness (b^*) ($p < 0.05$). Redness increased in thyme and rosemary EOs, with the exception of lower a^* values for T1, but b^* values decreased in these treatments compared with control samples during the storage days. However, rosemary treatments were significantly redder and yellower than thyme treatments in equal concentrations ($p < 0.05$). Moreover, the lower a^* values and the higher b^* values were measured in C1 on days 0 and 12 of the study. These results proposed that a^* and b^* values were affected somewhat by the color of the added EO during the storage days.

The results from the present study showed partial agreement with Kahraman et al. [16], who found that adding 0.2% rosemary EO to poultry fillets significantly decreased the L^* value and increased the a^* value of samples. Our findings were not in accordance with Rimini et al. [9], who reported no differences in color L^*, a^*, and b^* values in marinated chicken with thyme and orange EOs blend compared to control groups. Furthermore, Al-Hijazeen et al. [35] reported significant positive effects of oregano on L^* values of the ground chicken breast comparing the control but found no significant difference in treatments on a^* values during storage. These differences among the results could be related to the variation of used EOs including the different types, concentration, and composition, as well as differences in the oxidation pattern, muscle type, and light intensity.

3.3. Sensory Evaluation of Chicken Breast Meat. The effects of thyme and rosemary EOs on the organoleptic properties of VP chicken breast meat are given in Table 4. The effects of VP and all EO-coating combinations were significant for the mean values of all sensory attributes ($p < 0.05$). On days 0 and 3, control samples received the best taste and odor scores compared to other treatments, and T2 and R2 had significantly lower taste and odor scores ($p < 0.05$). This might be related to the strong pungent smell imparted by EOs to meat. These results are similar to other studies [2, 7, 12] that have reported better sensory scores in control samples than samples containing EOs in the early days of the study. Although a higher concentration than MIC of EOs is needed to attain an adequate antimicrobial effect in the food [36], higher concentrations could lead to unacceptable organoleptic changes. Our findings indicated that cooked chicken breast samples with a higher concentration of thyme and rosemary EOs had an unacceptable and unpleasant taste and odor quality according to panelists' opinions. Hence, a lower concentration of thyme and rosemary EOs (0.1% v/w) would be a more suitable level to apply in the chicken samples. Besides, as high concentrations of EOs could lead to undesirable sensorial changes in chicken meat, it is suggested that a higher concentration of EOs be added to the edible films or packaging materials [34] or alternatively use the active constituents of an EO rather than the EO itself [31]. Moreover, the encapsulation of EOs is recommended as a commercially available technology that minimizes the undesirable sensory changes in meat products without reducing their effects [15].

The lowest acceptability score of 3 was obtained for taste and odor after 6 days for T2, R2, air-packaged, and VP control samples, whereas this score was obtained for T1 and R1 treated samples with the lower concentration of thyme and rosemary EOs after 9 days. No significant differences were observed between the samples after 9 days, and the evaluated quality of taste and odor were unacceptable ($p > 0.05$). The findings are consistent with Hosseini et al. [7] that reported unacceptable quality of chicken breast treated with higher concentrations of clove and lemon EOs. In another study, Karam et al. [31] reported lower odor scores of marinated chicken with higher active EOs concentration, and the best sustainability was found in samples treated with the lowest concentration of carvacrol or thymol. Similarly, in another sensory analysis, Karabagias et al. [28] found that thyme and oregano EOs at the higher concentration (0.3% v/w) in lamb meat gave a strong undesirable odor and taste and were not further used.

All the studied samples had satisfactory color and texture attributes without significant changes in quality by the 3rd day. However, lower acceptability scores of 2 to 3 were reached for color and texture after 6 days for air-packaged and VP control samples which indicates unacceptable quality with significant changes (Table 4). Nevertheless, VP chicken control had significantly better scores than air-packaged chicken control on the 6th day of storage ($p < 0.05$). All EO-treated samples had acceptable color and textural quality with little change by the 9th day; however, treatments containing thyme EO received better scores than treatments containing rosemary EO on days 9 and 12 of storage.

In general, the sensory quality of all samples decreased during the storage time due to the increase of microbial load, fat oxidation, and decomposition of proteins. However, taste, odor, and texture changes were slower in T1 and R1 compared to other treatments and control samples which were in accordance with similar studies [2, 6]. In addition, color sensory changes were slower in T2 and R2 compared to

Table 4: Effect of thyme and rosemary EOs on sensory properties' scores of vacuum-packaged chicken breast samples during refrigeration (mean ± SD).

Properties	Samples	Days 0	3	6	9	12
Taste	C1	$1 \pm 0.00^{C*b**}$	1 ± 0.00^{Db}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	C2	1 ± 0.00^{Cb}	1 ± 0.00^{Db}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	T1	1.43 ± 0.53^{Bc}	2.29 ± 0.49^{Bb}	2.71 ± 0.49^{Bb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	T2	2.86 ± 0.37^{Ab}	2.86 ± 0.37^{Ab}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	R1	1.57 ± 0.53^{Bd}	2 ± 0.58^{Cc}	2.57 ± 0.53^{Cb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	R2	2.71 ± 0.49^{Aa}	2.71 ± 0.76^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
Odor	C1	$1 \pm 0.00^{C*b**}$	1 ± 0.00^{Eb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	C2	1 ± 0.00^{Cb}	1 ± 0.00^{Eb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	T1	1.71 ± 0.49^{Bd}	2.29 ± 0.49^{Cc}	2.86 ± 0.37^{Bb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	T2	2.57 ± 0.53^{Ac}	2.86 ± 0.37^{Ab}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	R1	1.71 ± 0.49^{Bd}	1.86 ± 0.38^{Dc}	2.43 ± 0.53^{Cb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	R2	2.57 ± 0.53^{Ac}	2.71 ± 0.49^{Bb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
Color	C1	$1 \pm 0.00^{A*c**}$	1 ± 0.00^{Ac}	2.57 ± 0.53^{Ab}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	C2	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	2.14 ± 0.37^{Bb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	T1	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	1 ± 0.00^{Dc}	1.29 ± 0.49^{Db}	2.29 ± 0.49^{Da}
	T2	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	1 ± 0.00^{Dc}	1.29 ± 0.49^{Db}	2 ± 0.57^{Ea}
	R1	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	1 ± 0.00^{Dc}	2 ± 0.53^{Bb}	2.71 ± 0.53^{Ca}
	R2	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	1.14 ± 0.38^{Cc}	1.71 ± 0.00^{Cb}	2.57 ± 0.53^{Ba}
Texture	C1	$1 \pm 0.00^{A*b**}$	1 ± 0.00^{Ab}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	C2	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	2.29 ± 0.49^{Bb}	3 ± 0.00^{Aa}	3 ± 0.00^{Aa}
	T1	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	1 ± 0.00^{Dc}	1.29 ± 0.49^{Db}	2.29 ± 0.49^{Da}
	T2	1 ± 0.00^{Ac}	1 ± 0.00^{Ac}	1 ± 0.00^{Dc}	1.43 ± 0.53^{Cb}	2.57 ± 0.53^{Ca}
	R1	1 ± 0.00^{Ad}	1 ± 0.00^{Ad}	1.14 ± 0.38^{Cc}	2 ± 0.58^{Bb}	2.29 ± 0.49^{Da}
	R2	1 ± 0.00^{Ad}	1 ± 0.00^{Ad}	1.14 ± 0.38^{Cc}	2 ± 0.58^{Bb}	2.71 ± 0.49^{Ba}

*A–F: mean values with different superscript letters in a column are significantly different ($p < 0.05$). **a–e: mean values with different superscript letters in a row are significantly different ($p < 0.05$). (C1: air-packaged, C2: VP without EO, T1: VP + 0.1% thyme EO, T2: VP + 0.3% thyme EO, R1: VP + 0.1% rosemary EO, and R2: VP + 0.3% rosemary EO).

other treated and untreated samples, which were in accordance with the results of color lightness values obtained in the present study. Based on sensory evaluations, T1 and R1 containing 0.1% of thyme and rosemary EOs had the highest positive effect on the shelf life extension of fresh chicken breast.

4. Conclusion

The shelf life of chicken meat depends on several factors, such as initial microbial loads, lipid oxidation, storage temperature, and the type of packaging. This study showed that the untreated air-packaged chicken sample was most subjected to spoilage and had more TCC and PCC compared to VP and treated chicken samples with thyme and rosemary EOs. The findings indicated that the application of thyme EO as a natural antimicrobial agent was more effective than rosemary EO in the reduction of *E. coli,* and it also improved the microbial shelf life of chicken breast meat. TBARs analysis showed that thyme EO had a synergistic effect in combination with vacuum packaging to reduce the rate of lipid oxidation in the chicken breast samples. As a result, thyme EO can be considered a more effective natural antioxidant compared to rosemary EO in chicken breast meat. Furthermore, the results showed that rosemary EO treatments had lower pH values and mostly lower L^* values but higher a^* and b^* values in the colorimetric analysis compared to thyme EO treatments. However, the sensory results showed that the control chicken breast samples, as well as high concentrations of thyme and rosemary EOs, were not favored by the panelists, and lower concentrations of thyme EO could be selected for further experiments. Although our results showed that the combined use of vacuum packaging and thyme EO can effectively reduce the deterioration of chicken breast meat and extend its chemical and microbial shelf life up to 9 days at +4°C, with regard to its unsatisfactory effects on some sensory attributes (taste and odor), further investigation is recommended to improve the organoleptic properties.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Sahar Piruz was involved in conceptualization, methodology, investigation, formal analysis, data curation, and resources and wrote the original draft. Mohammadreza Khani was involved in conceptualization, supervision,

methodology, validation, formal analysis, visualization, project administration and wrote the original draft as well as conducted writing, reviewing, and editing.

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