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Yasser A Saber
Food Science Department,
College of Agriculture, Tikrit
University, Tikrit, Iraq

Evaluation of the antimicrobial and antioxidant activity of rosemary and thyme extracts and their use as natural preservatives to improve meat quality and safety during refrigerated storage

Yasser A Saber

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Abstract

This research was conducted with the purpose of assessing the antimicrobial and antioxidant effects of rosemary and thyme extract, as well as their potential for being used as natural preservatives to enhance the quality of meat products and safety during refrigerated storage. There was a total of 3 treatment groups: 0.1% rosemary extract group, 0.1% thyme extract group, a mixture of both extracts (0.5% rosemary + 0.5% thyme), and a control group with no additives. These groups were compared by looking at how well they produced antimicrobial or antioxidant properties while stored at 4°C for 1, 6, and 12 days.

The evaluation of changes to the meat involved using various physical, chemical, and microbial methods of testing. Physical methods used to evaluate the samples included the cooking loss from the product as it was cooked, water-holding capacity of the meat sample, and the oxidation index using the Thio barbituric acid reactive substances (TBARS) test. Various chemical tests included the measurement of the pH and the peroxide value of the meat samples. Microbial analyses conducted during this study were to measure total bacterial counts, cold-loving bacterial counts, and yeast and mold counts present on the meat samples. Additionally, sensory analyses were performed on the samples based on odor, taste, and overall acceptability.

All treatments researched in this study affected the physical properties of meat positively by significantly decreasing cooking loss and increasing water-holding capacity, compared with control samples; the greatest effect was with the mixture treatment. Additionally, TBARS and peroxide values were significantly lower, showing that all treatments are effective at inhibiting lipid oxidation processes, with relative pH stability.

Microbial analysis of the samples showed that plant extract-based treatments significantly decreased total, psychrophilic, and yeast and mold counts compared to untreated controls, confirming that these extracts were effective in reducing microbial growth at refrigerated temperatures. Sensory evaluation results indicated that all treatments exhibited improved aroma, flavor, and overall acceptability when compared with untreated controls, with the mixture treatment receiving the highest overall preference score of all treatments.

The results show a synergy between oregano and rosemary extracts (both antioxidants and antimicrobials), leading, together, to improved shelf stability and enhanced quality and safety of refrigerated meats; therefore, they are both innovative natural alternatives to synthetic preservatives.

Keywords: Rosemary, water-holding capacity, peroxide value, cold-loving bacteria

1. Introduction

Meat and the products derived from it contain large amounts of necessary nutrients that have high biological value (e.g., protein); vitamins, minerals, etc. However, meat is also among the most perishable of all types of food because they are susceptible to lipid oxidation and microbial growth during storage under refrigeration, resulting in various detrimental sensory properties and effects on nutritive value in addition to possible composition of harmful by-products that pose health risks for consumers^[1].

Oxidation of lipids will be one of the major reactions affecting meat product quality, as it will generate secondary products that produce rancidity and off-flavor odors in meat products as well. Microbial contamination is another contributing factor to the decreased shelf life of meat products as well as increased risk for consumer health.

Corresponding Author:
Yasser A Saber
Food Science Department,
College of Agriculture, Tikrit
University, Tikrit, Iraq

For this reason, meat products use many synthetic preservatives to maintain quality and increase the shelf life of meat products: nitrates, nitrites, sulfites, and polyphosphates are the most common/most-used preservatives, which provide sensory enhancement and/or protect from quick spoilage of processed meat products; however, there is increasing interest to consume more natural and healthier types of food due to potential health effects from consumption of these preservatives regularly [2]. As a result of this, investigators are looking for natural substitutes that will not be harmful to people's health. One of the main types of these substances is aromatic and medicinal Oils of plants called essential oils, which can be found in other types of products, such as oils, vinegars, and pickles. Some other types of products are candy and chocolate, using essential oils to give them flavor and scent; alcoholic beverages, soft drinks, and ice cream use essential oils for the same purpose. Other uses of essential oil products can be as natural additives like flavoring, colorants, antioxidants, or preservatives [3].

The most prominent examples of natural alternatives are rosemary and thyme because both plants have unique medicinal/effective qualities. Rosemary (*Rosmarinus officinalis*) is an evergreen-aromatic plant that is part of the family Lamiaceae. Some of the active components in rosemary are Rosmarinus acid, carnosic acid, and carnosol, along with volatile oils. These active types of oils are known for their high antioxidant capabilities by acting against free radicals and slowing down the oxidation process of lipids, as well as acting to prevent microbial growth [4].

Thyme (*Thymus vulgaris*) is another aromatic herb in the same family and contains two of the most potent phenolic compounds with antimicrobial properties: thymol and carvacrol. These compounds attack the outer membranes of microbial cells, disrupting their growth or killing them. Additionally, thyme has antioxidant properties that prevent fat and protein degradation in food products [5].

It has been demonstrated that the use of extracts from both rosemary and thyme, alone or in combination, produces a synergistic effect resulting in enhanced inhibitory capacity on both oxidation as well as microbial growth, which will thus contribute to longer shelf lives of foods, especially chilled meats. These extracts are also considered relatively safe for human consumption, which supports the current trend toward natural foods [6].

Subject to the above-stated objectives, the purpose of this research project is to evaluate the antimicrobial or antioxidant properties of rosemary and thyme extracts and determine their potential application as natural preservatives to enhance meat quality and safety during storage in a refrigerated environment throughout the storage time period by observing physical, chemical, microbial, and sensory changes throughout the storage duration.

2. Materials and Methods

This experiment was performed in the laboratories of the Food Science Department at the University of Agriculture in Tikrit. The materials (chicken breast meat) used for this experiment were bought from Al-Taj Store in Salah al-Din Governorate (Tikrit). The meat was kept at a temperature of 4°C until testing samples were taken every day for 1-3 days after purchase (testing was performed at 1, 6, and 12 days). Both rosemary and thyme leaves used for this study were purchased in local markets located in Salah al-Din

Governorate and dried for extraction. An alcoholic solution was made with rosemary and thyme using ethanol (70-80%) and distilled water. Both plants were pulverized to a fine powder, then 50 g of powdered plant matter was weighed out, and 500 ml of 70% ethanol was added (1:10 ratio). Next, the mixture is thoroughly mixed/combined and placed in a container at room temperature for 48-72 hours. After that, the mixture is filtered to remove solid material and evaporated using a water bath at 40-45°C to remove the leftover alcohol.

The treatments were carried out by immersing chicken breast pieces in the alcoholic extract according to Table 1.

Table 1: Proportions of materials used in the experiment

Treatment	chicken	Rosemary extract	Thyme extract
Control	500 gr	---	---
Rosemary extract	500 gr	(0.1%)	---
Thyme extract	500 gr	---	(0.1%)
Mix	500 gr	(0.5%)	(0.5%)

2.1 Determination of Active Compounds

Total Phenolics Content (TPC) was estimated using the Folin-Ciocalteu method [7], then expressed as mg of gallic acid equivalents (GAE) per gram of extract. Total Flavonoids Content (TFC) was estimated using the aluminum chloride method [8], then expressed as mg of quercetin equivalents (QE) per gram of extract. The antioxidant activity of each plant extract was evaluated using the DPPH free radical scavenging assay [9], which measures percent inhibition.

2.2 Physical Tests

The measurement of cooking loss and meat's water-holding capacity was measured using the methods described in reference [10] (cooking loss) and (water-holding capacity), respectively [11].

2.3 Chemical Analyses

The determination of the TBARS value, peroxide value, and pH of meat were made using the methods described in [12], [13], and [14], respectively.

2.3 Microbiological Testing

The total bacterial, yeast, and mold count was determined using reference [15], while cold-loving bacteria were counted according to the method described in reference [16]. Note: The microbial counts have been converted to log₁₀ CFU/G (Log cfu/g).

2.4 Sensory Evaluation

Samples were evaluated by a semi-trained panel with 5-to-10 members. Raters rated aroma, flavor, and overall acceptability of the samples using a 9-point "likability" scale (9 = "like extremely," 1 = "dislike very much"). Samples were assigned a random 3-digit number and presented to the panel in random order to avoid bias. Evaluations were performed under controlled laboratory conditions (room temperature, sufficient light). Panellists were given water to rinse their mouths after each sample and evaluated the sensory characteristics of the samples at 0, 6, and 12 days of refrigerated storage at 4°C according to the methods outlined above (17).

Name:		Age:		Date:	
Samples	odor	Flavor	Overall Acceptability	Comments	
T ₁					
T ₂					
T ₃					
T ₄					

								
I don't enjoy it very much	I don't enjoy it much	I don't enjoy it to a certain degree	I don't enjoy it slightly	I neither liked it nor disliked it	I enjoyed it slightly	I enjoyed it to a certain degree	I enjoyed it a lot	I enjoyed it greatly
1	2	3	4	5	6	7	8	9

Fig 1: Sensory evaluation score form for pastrami discs ^[18]

2.5 Statistical Analysis

A complete randomized design was utilized to analyze the data to determine the impact of the treatments on the traits studied in this analysis. Duncan's Multiple Range Test ^[19] was used to compare the differences between treatments and was significant at the level of 0.05. Statistical analyses were performed using Statistical Analysis System (SAS, 2010); the following mathematical model was used in the analysis of the data:

$$Y_{ij} = \mu + T_i + e_{ij}$$

3. Results and Discussion

3.1 Estimation of Effective Components

The combination of thyme and rosemary recorded the highest amount of total phenolics (TPC) (158.4 mg GAE/g) when compared to either extract alone; rosemary was second highest (145.2 mg), and thyme was third (132.5 mg). The increase of phenolics in the combination is due to the

synergetic activity of the phenolic compounds of thyme (thymol) and rosemary (rosmarinic acid, carnosic acid) on total phenolic levels ^[20].

A similar trend was observed for TFC, with the thyme/rosemary combination having the highest concentration (45.20 mg QE/g) compared to rosemary (42.60 mg) and thyme (38.40 mg). Increase in flavonoid concentrations can be attributed to the variety of flavonoids, the increase in extractability due to combining the two plants, and the positive interaction between the compounds in the combined extract (synergism) ^[21].

The antioxidant activity (DPPH) indicated that the mixture had the highest inhibition activity (88.50%) compared to rosemary (82.40%) and thyme (78.60%) and this was supported by the TPC and TFC values being significantly high, and there is a positive correlation between antioxidant activity and phenolic content, so as the amount of free radical scavenging capacity increases with increased phenolic content ^[22].

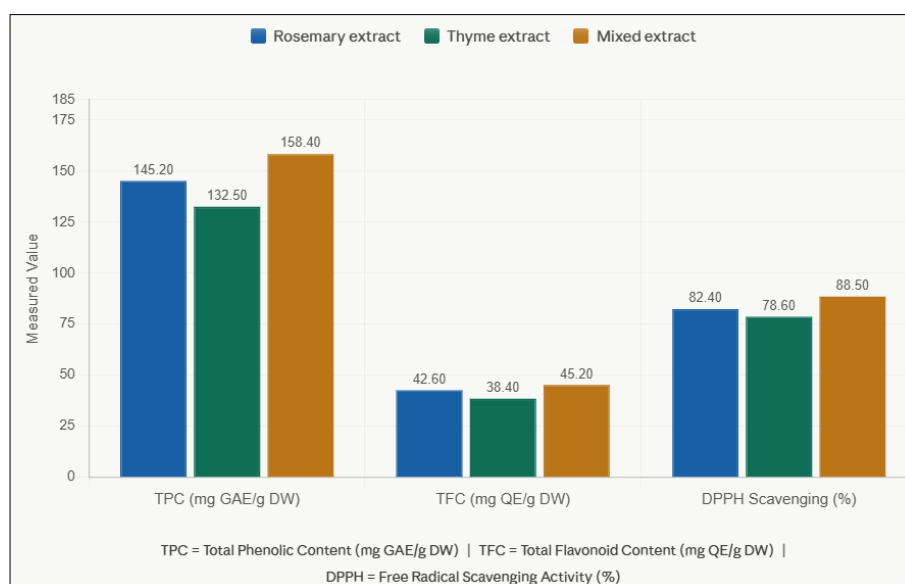


Fig 2: Active compounds and antioxidant activity of rosemary, thyme, and mixed extracts. Values are means $\pm$ SD (n = 3)

3.2 Physical Properties

Table 2 demonstrates the impact of using alcohol extracts from rosemary and thyme to enhance the physical properties of meat. The extract treatments had less cooking loss than the control treatment; the control treatment had a cooking loss of 22.76, compared to extract treatments T₂ (22.30) and T₃ (22.41), while the minimum cooking loss was found in T₄ at 22.11. The lesser cooking loss of the extract treatments is attributed to reduced oxidation and protection of muscle protein integrity. This finding agrees with those reported by [23].

With respect to the interaction between treatment and storage, the results indicated that the cooking loss% of samples stored for different lengths of time differed, with the maximum cooking loss seen in the control treatment (22.76% to 37.26%) after 12 days of storage, while T₄ had a larger percentage of weight loss (22.11% to 26.46%). The percentage increase in cooking loss was gradually increased throughout the storage period for all four treatments; however, there still existed a statistically significant difference between the control group and the treatments with extracts of thyme and rosemary. The capacity of alcohol extracts of rosemary and thyme to hinder oxidation of muscle fibers was reflected in the ability of the extracts to maintain the integrity of muscle proteins and to retain moisture, which led to an increase in water-holding capacity and decreased fluid loss during cooking. This conclusion is in agreement with [24].

As shown in Table 2, the vegetable extracts of rosemary and thyme had significant effects ($P < 0.05$) on the water-holding capacity of the meat, with the water-holding capacity being increased (as compared to the control) by the addition of either extract. The largest improvement in water-holding capacity was produced with treatment T₄, with an improvement of 65.63% as compared with the control; whereas the least improvement was produced with treatment T₁, with a value of 65.11%. The increases in WHC attributed to the rosemary and thyme extracts may be due to the ability of these extracts to slow oxidation of myofibrillar proteins and to preserve myofibril and cell membrane integrity, thus decreasing degradation of muscle fibres and keeping the muscle tissue available for binding with water when it is cooked. These results were also supported by [25]. Storage duration displayed that there was interaction between treatment and descending amounts of stored item, with reference to water-holding capacity, with the largest decrease occurring with T₁ (from 65.11 to 42.13), and the least amount of storage loss would occur with T₄ (from 65.63 to 58.31) at the end of the study. There was a minor decline in the water holding capacity of the extract-treated samples (rosemary + thyme) over time, most likely due to the presence of phenolic component antioxidants acting in a synergistic manner to inhibit lipid oxidation and protect muscle proteins, which determine the capacity for binding water. This corresponds with [26].

Table 2: Changes in the physical properties of chicken meat stored at 4 °C

Transaction impact		Characteristics	
		Cooking Loss	Water holding capacity
Transactions	T ₁	29.73±2.10 A	53.02±3.32 D
	T ₂	25.34±0.95 B	59.64±1.63 B
	T ₃	25.72±1.03 B	59.14±1.73 C
	T ₄	24.03±0.07 C	62.02±1.06 A
Morale		*	*
Storage periods	1	22.39±0.09 C	65.33±0.07 A
	6	25.71±0.09 B	58.12±1.15 B
	12	30.52±1.23 A	51.91±1.81 C
Morale		*	*
T ₁	1	22.76±0.22 f	65.11±0.17 h
	6	29.16±0.37 b	51.83±0.21 i
	12	37.26±0.56 a	42.13±0.21 j
T ₂	1	22.30±0.11 f	65.33±0.08 a b
	6	24.86±0.14 d	59.61±0.15 d
	12	28.86±0.24 b	54.01±0.11 g
T ₃	1	22.41±0.11 f	65.26±0.11 a b
	6	25.26±0.14 d	58.93±0.08 e
	12	29.51±0.21 b	53.23±0.14 h
T ₄	1	22.11±0.11 f	65.63±0.08 a
	6	23.53±0.17 e	62.13±0.14 c
	12	26.46±0.21 c	58.31±0.11 f
Morale		*	*

* Indicates significant differences within a single column at the ($p \leq 0.05$) level between the effects of treatments

N.S indicates no significant differences at the ($p > 0.05$) level between the effects of treatments.

T₁: Control, T₂: Rosemary 0.1%, T₃: Thyme 0.1%, T₄: Mix (0.5+0.5)%

3.3 Chemical Analyses

Physical characteristics are presented for the possibility of using rosemary and thyme alcohol extracts on chicken meat in Table 3. Analysis of each treatment showed that there was an effect of treatment applied to chicken on the peroxide ($P \leq 0.05$) and TBARS ($P \leq 0.05$) values, with control being the highest amount, recording 0.43 for

peroxide and 0.16 for TBARS, followed by T₄ being the lowest amount, at 0.39 for peroxide and 0.14 for TBARS. The higher values of peroxide and TBARS for the control group were due to significant lipid oxidation and subsequent by-products such as malondialdehyde forming; whereas, the lower amount of these compounds in the extract-treated group was due to the presence of phenolic compounds as

antioxidants, which donate a hydrogen atom and scavenge free radicals. The results from [27] supported these findings. For the interactions of treatment and duration, it was found that the peroxide and TBARS measurements changed and increased in the control group and each treatment during the 12-day storage period. The control treatment had the greatest amount of peroxide at the first measurement (0.43) and last measurement (6.61) at the end of the study. The T₄ treatment had the least increase in peroxide, with the first amount equal to 0.39 and the last measurement equal to 1.73. The TBARS also rapidly increased throughout the length of storage as time passed across every treatment as a function of increasing degradation, due to increased oxidation of fat and protein, and the increase in microorganisms; however, treatment with the extracts significantly inhibited the above-mentioned deterioration. These data were consistent with [28].

A statistically significant difference ($P \leq 0.05$) in pH between the four treatments was observed at the conclusion of this study. Treatment T₁ had a pH of 5.56, whereas treatments T₂, T₃, and T₄ had a pH of 5.54, 5.55, and 5.52, respectively. This agrees with [29].

Table 3: Changes in the chemical analysis of chicken meat stored at 4 °C

Transaction impact		Characteristics		
		TBARS	pH	Peroxide
Transactions	T ₁	1.25±0.33 A	6.30±0.22 A	3.33±0.89 A
	T ₂	0.51±0.11 C	5.85±0.01 C	1.36±0.31 C
	T ₃	0.55±0.11 B	5.91±0.11 B	1.53±0.35 B
	T ₄	0.39±0.07 D	5.75±0.07 D	1.01±0.19 D
Morale		*	*	*
Storage periods	0	0.15±0.01 C	5.54±0.01 C	0.41±0.02 C
	6	0.62±0.09 B	5.91±0.06 B	1.59±0.24 B
	12	1.25±0.22 A	6.41±0.12 A	3.41±0.56 A
Morale		*	*	*
T ₁	1	0.16±0.01 h	5.56±0.11 h	0.43±0.01 h
	6	1.15±0.03 b	6.25±0.04 b	2.95±0.07 b
	12	2.45±0.05 a	7.12±0.02 a	6.61±0.11 a
T ₂	1	0.15±0.06 h	5.54±0.02 h	0.41±0.12 h
	6	0.48±0.11 f	5.82±0.03 f	1.19±0.04 f
	12	0.92±0.02 d	6.21±0.01 c	2.48±0.14 c
T ₃	1	0.16±0.01 h	5.55±0.05 h	0.42±0.11 h
	6	0.52±0.15 f	5.88±0.21 e	1.43±0.18 e
	12	0.98±0.09 c	6.28±0.03 b	2.83±0.05 b
T ₄	1	0.14±0.05 h	5.52±0.05 h	0.39±0.11 h
	6	0.35±0.11 g	5.71±0.11 g	0.89±0.18 g
	12	0.68±0.15 e	6.05±0.04 d	1.73±0.15 d
Morale		*	*	*

* Indicates significant differences within a single column at the ($p \leq 0.05$) level between the effects of treatments

N.S indicates no significant differences at the ($p > 0.05$) level between the effects of treatments.

T₁: Control, T₂: Rosemary 0.1%, T₃: Thyme 0.1%, T₄: Mix (0.5+0.5)%

3.4 Microbiological Tests

Table 4 summarizes the effect of thyme and rosemary alcohol extracts on the microbiological analysis of chicken meat. The findings from the experiments showed that there

were significantly fewer microbial counts found among all the treatments that contained either thyme or rosemary extracts versus the control sample during refrigeration at 4°C. A significantly different ($P \leq 0.05$) total bacterium count was measured among the treatments, with the control having the highest count (3.25 CFU), followed closely by treatment T₃ (3.22 CFU), and the least number of bacteria being seen in treatment T₄ (3.18 CFU). The presence of active compounds in the rosemary and thyme extracts with antimicrobial and antioxidant activity is the likely explanation for this result. This finding supports previous findings reported from [30]. When examining the interaction between treatments and storage time, it was discovered that there were significant differences ($P \leq 0.05$) in bacterial counts for all of the treatments as time increased. The largest increase in bacterial counts occurred in treatment T₁, going from 3.25 CFU at the time of storage to 5.81 CFU and 8.41 CFU after 6 and 12 days of refrigeration, respectively; these findings are consistent with those reported by [31].

According to Table 4, there are statistically significant ($P \leq 0.05$) differences in the total number of cold-loving bacteria across each of the samples and throughout all of the tested storage periods (T₁, T₂, T₃, and T₄) as a result of both storage and treatment. Although the treatment with the highest cold-loving bacteria count was T₁ at 2.18 CFU, the number of cold-loving bacteria decreased in all three treatment replacement groups (T₂, T₃, and T₄) by 2.10, 2.15, and 2.06 CFU, respectively. The decrease in cold-loving bacteria in the treatments that contained alcoholic extracts of rosemary and thyme is believed to be a result of a synergistic effect between the two extracts that enhanced their antibacterial activity [32]. There was a statistically significant ($P \leq 0.05$) effect of storage duration on the total number of cold-loving bacteria across all of the samples. As the duration of storage increased, the total number of cold-loving bacteria in each of the treatment groups also increased (e.g., in T₁, the total number of cold-loving bacteria increased from a 2.18 CFU count immediately after the start of storage to 8.28 CFU after 12 days of storage), whereas T₄ had the lowest overall increase from the start to the end of storage, with total counts of cold-loving bacteria of 2.06 and 4.55, respectively. These findings confirm previous work by [33].

Table 4 presents data for yeast and mold counts when employing alcohol extracts of thyme and rosemary during microbiological analysis of chicken meat. Significant differences ($P \leq 0.05$) between treatments T₁-T₄ for yeast and mold counts were noted (1.20, 1.12, 1.15, 1.07 CFU, respectively). Yeast and mold counts decreased due to the presence of compounds like thymol in thyme and carnosic acid from rosemary, which both inhibit the growth of molds. The results are also supported by [34]. Concerning the interactions of treatments and storage, there were significant differences ($P \leq 0.05$) in yeast and mold counts across treatments. Treatment T₁ resulted in vast increases in yeast and mold counts, going from 1.20 CFU (start of storage) to 4.56 CFU (12 days in storage); these results also concur with those found in [35].

Table 4: Changes in microbial tests of chicken meat stored at 4 °C

Transaction impact		Characteristics		
		Total number of bacteria (log CFU/g)	Cold-loving bacteria (log CFU/g)	Yeasts and Molds (log CFU/g)
Transactions	T ₁	5.81±0.74 A	5.32±0.88 A	2.88±0.48 A
	T ₂	4.31±0.35 C	3.63±0.46 C	1.78±0.19 C
	T ₃	4.45±0.39 B	3.80±0.41 B	1.85±0.21 B
	T ₄	3.99±0.25 D	3.21±0.36 D	1.53±0.13 D
Morale		*	*	*
Storage periods	0	3.21±0.11 C	2.12±0.09 C	1.13±0.15 C
	6	4.51±0.23 B	3.92±0.28 B	1.97±0.16 B
	12	6.22±0.39 A	5.92±0.42 A	2.92±0.29 A
Morale		*	*	*
T ₁	0	3.25±0.28 h	2.18±0.44 h	1.20±0.02 h
	6	5.81±0.05 b	5.51±0.06 b	2.88±0.44 b
	12	8.41±0.07 a	8.28±0.09 a	4.56±0.06 a
T ₂	0	3.20±0.25 h	2.10±0.02 h	1.12±0.01 h
	6	4.10±0.28 f	3.48±0.44 f	1.73±0.04 f
	12	5.65±0.05 d	5.31±0.06 c	2.48±0.44 c
T ₃	0	3.22±0.20 h	2.15±0.03 h	1.15±0.17 h
	6	4.25±0.36 f	3.68±0.04 e	1.79±0.04 e
	12	5.90±0.04 d	5.56±0.06 b	2.63±0.04 b
T ₄	0	3.18±0.15 h	2.06±0.24 h	1.07±0.14 h
	6	3.85±0.02 f	3.01±0.06 g	1.48±0.41 g
	12	4.95±0.36 d	4.55±0.07 d	2.03±0.04 d
Morale		*	*	*

* Indicates significant differences within a single column at the ($p \leq 0.05$) level between the effects of treatments

N.S indicates no significant differences at the ($p > 0.05$) level between the effects of treatments.

T₁: Control, T₂: Rosemary 0.1%, T₃: Thyme 0.1%, T₄: Mix (0.5+0.5)%

4.5 Sensory Evaluation

Table 5 shows the effect of using alcohol extracts of rosemary and thyme on the sensory evaluation of chicken meat. The results showed no significant differences ($P \leq 0.05$) in odor evaluation among the treatments, with T₁, T₂, T₃, and T₄ recording 8.71, 8.60, 8.62, and 8.80, respectively. The results were consistent with [36].

Regarding how treatments and storage interact, the results show that as storage time increases, storage time has an effect on odor attribute decreases, especially for the control sample, which shows rapid loss of odor at increasing storage durations, decreasing from 8.71 at storage initiation to 2.11 at the end of storage due to the presence of unwanted compounds produced during microbial activity and oxidation. For the treated samples, however, the odor has been retained over a longer period of time. T₄ exhibited the highest odor rating, decreasing from an initial rating of 8.80 to an end rating of 7.30. The reason for this is associated with phenolic compounds inhibiting the growth of bacteria that produce unwanted odors through microbial activity and oxidation of fats that produce rancid odors. This is supported by [37].

The same pattern was demonstrated by differences in flavor between treatments, as the statistical results ($P \leq 0.05$) differed significantly between all samples (except T₄), which showed the highest mean acceptability of 8.76 compared to an average of 8.60 for T₁, 8.51 for T₂, and 8.66 for T₃. These results agree with those of [38].

The results of the study concerning the interaction of treatments and storage duration show that as time passes, the flavor of the control sample deteriorates significantly with an increase in the amount of storage time (from 8.6 to 4.6, 1.34 at six and twelve days, respectively). On the contrary, extracts produced similar results while retaining exceptional flavors longer than the control. Treatment T₄ had a unique

flavor that was very well received by all participants; thus, it balanced acceptable flavor levels with microbial inhibition. The combination of aromatic compounds present in thyme, such as thymol, provides additional flavor enhancement as well as protection from the breakdown of flavor-enhancing compounds through the use of antioxidants.

According to Table 5, there were significant effects ($P < 0.05$) due to using alcohol extracts of rosemary and thyme on the sensory evaluation of chicken. The results of the sensory evaluations showed that the highest overall acceptability score (8.1) was found for treatment T₄, and therefore, this treatment was ranked first by the evaluators. Next, the evaluations ranked T₂ (8.61) second, T₃ (8.06) third, and T₁ (8.70) fourth overall acceptability. The overall acceptability scores were found to be similar to those previously reported [39].

In terms of interaction between treatment and storage time, the study indicates that the acceptability of the control sample decreased rapidly during storage, while samples treated with extracts of thyme and rosemary retained relatively high levels of acceptability. The treatment with the highest acceptability rating was T₄, displaying an initial overall acceptability value of 8.81 and a final overall acceptability value of 7.3, demonstrating that both greater aroma/flavor quality and reduced spoilage contributed to this treatment's higher acceptability ratings than for the control treatments. The higher acceptability of the extract-treated samples was due to the reduction of growth of microorganisms that produce undesirable compounds and consequent rancid odors and flavors, and the inhibition of oxidation within the extract-treated samples, resulting from the traditional aromatic compounds found in thyme and rosemary. The results presented herein support the results found by [40].

Table 5: Sensory evaluation of chicken meat stored at 4 °C

Transaction impact		Characteristics		
		Odor	Flavor	Overall acceptability
Transactions	T ₁	5.34±0.96 D	4.85±1.05 D	5.41±0.95 D
	T ₂	7.54±0.33 B	7.64±0.27 B	7.61±0.31 B
	T ₃	7.33±0.37 C	7.36±0.33 C	7.53±0.35 C
	T ₄	8.06±0.21 A	8.13±0.25 A	8.10±0.19 A
Morale		*	*	*
Storage periods	0	8.67±0.35 A	8.61±0.36 A	8.67±0.35 A
	6	7.13±0.32 B	7.03±0.42 B	7.17±0.32 B
	12	5.41±0.67 C	5.35±0.78 C	5.50±0.67 C
Morale		*	*	*
T ₁	0	8.71±0.57 a	8.60±0.11 a b	8.70±0.01 a b
	6	5.33±0.08 g	4.63±0.88 h	5.41±0.07 h
	12	2.11±0.12 h	1.34±0.08 i	2.10±0.55 i
T ₂	0	8.60±0.06 a	8.51±0.57 c	8.61±0.05 b
	6	7.71±0.05 c	7.82±0.51 d	7.70±0.10 d
	12	6.33±0.88 e	6.63±0.08 c	6.51±0.57 f
T ₃	0	8.62±0.05 a	8.66±0.05 a b	8.06±0.57 b
	6	7.52±0.15 d	7.04±0.01 f	7.14±0.10 e
	12	6.01±0.10 f	6.11±0.57 g	6.10±0.11 g
T ₄	0	8.80±0.05 a	8.76±0.33 a	8.81±0.51 a
	6	8.01±0.70 b	8.31±0.11 g	8.02±0.51 c
	12	7.30±0.05 d	7.33±0.88 d	7.03±0.15 e
Morale		*	*	*

* Indicates significant differences within a single column at the ($p \leq 0.05$) level between the effects of treatments

N.S indicates no significant differences at the ($p > 0.05$) level between the effects of treatments.

T₁: Control, T₂: Rosemary 0.1%, T₃: Thyme 0.1%, T₄: Mix (0.5+0.5)%

4. Conclusion

The results presented in this study demonstrate that the use of both thyme and rosemary alcoholic extracts is an effective strategy for improving meat quality and safety during refrigerated storage at 4°C for 1, 6, and 12 days. All extract-treated samples demonstrated physical property enhancement as evidenced by increased water holding capacity and decreased cooking loss as compared to the control sample for each extracted treatment, thereby indicating greater stability of the protein structure and protein stability in this study. Additionally, samples treated with thyme and rosemary extracts had significantly lower levels of oxidation (as measured by peroxide values and TBARS), while exhibiting relatively stable pH levels, supporting the use of these extracts as effective natural antioxidants in preventing lipid degradation during storage. Thyme and rosemary extracts demonstrated the ability to reduce the growth (rate) of microorganisms, such as total bacteria, cold-loving bacteria, yeasts, and molds. This was most pronounced with the combination of both extracts having the greatest inhibition when compared with each extract individually, indicating that there is a synergistic effect of thyme and rosemary on inhibition of microbial growth.

In regard to sensory properties, the samples treated with extracts have longer-lasting odor, flavor, and overall acceptability than those of the control. The samples treated with extracts were the most acceptable based on sensory criteria due to their ability to strike a balance between delaying the deterioration of the product caused by microbial and oxidative activity and improving sensory characteristics.

Thyme and rosemary extracts (especially in combination) are very promising as natural alternatives to chemical preservatives and improvements in safety and quality of meat products with no negative effects on sensory

characteristics. It is recommended that these extracts be applied to meat products on a commercial level, and future studies should be conducted to identify optimal concentrations for use in a variety of meat products.

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