

Effects of Thyme Extract and Essential Oil on the Quality of Thawed Chicken Breast During Refrigerated Storage

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Abstract

The present study evaluated the efficacy of aqueous thyme extract and thyme essential oil as natural preservatives for maintaining the quality of thawed chicken breast meat during refrigerated storage. Five treatments were tested: untreated control, thyme aqueous extract (1%), thyme aqueous extract (2%), thyme essential oil (1%), and thyme essential oil (2%). Physicochemical parameters such as cooking loss, drip loss, water-holding capacity (WHC), pH, total volatile nitrogen (TVB-N), thiobarbituric acid (TBA) and free fatty acids (FFA) plus microbiological parameters (total plate count (TPC) and psychrotrophic bacterial count (PTC) were observed on storage days (0, 3, 6) at refrigeration temperature (4 ± 1 °C). As storage progressed, meat quality gradually decreased; however, the deterioration was significantly lower in thyme-treated samples compared with untreated samples. The treated samples generally exhibited lower cooking loss, drip loss, TVB-N, TBA, and FFA values compared with untreated samples. Microbiological tests also showed that both the thyme formulations effectively reduced the growth of the aerobic and psychrotrophic bacteria during storage. Among the tested treatments, 2% thyme essential oil showed the greatest overall preservation potential during refrigerated storage. The results in this study show that thyme-derived products, especially thyme essential oil (TEO), are potential natural preservative agents that can help to improve the physicochemical and microbiological stability of chilled chicken. Their antioxidant and antimicrobial properties suggest that thyme-derived products may serve as potential natural alternatives to reduce reliance on synthetic preservatives and to prolong the refrigerated shelf life of fresh meat.

Keywords: Thyme extracts, Thyme essential oil, Natural preservatives, Shelf life.

Introduction

Meat has been known as one of the most valuable sources of quality protein, essential amino acids, vitamins, and minerals for

human nutrition. However, immediately after slaughter, fresh meat undergoes rapid changes in quality through biochemical reactions, lipid oxidation, protein degradation, and microbial growth [1]. All

these spoilage processes negatively affect the physicochemical, microbiological, and sensory properties of meat, that is, meat's shelf life and consumer acceptance. Although refrigeration is the most widely used method for preserving fresh meat, low-temperature storage does not prevent but merely slows down the oxidative and microbial changes [2, 3].

The demand for meat products with a low level of synthetic additives and an interest in using natural additives has increased. Bioactive compounds in plant extracts and essential oils possess antimicrobial and antioxidant activities. The incorporation of these natural compounds can inhibit spoilage microorganisms and reduce lipid oxidation in meat products, and help maintain the physicochemical and sensory properties during storage [4].

One of the medicinal plants widely investigated as a natural preservative in meat products is thyme (*Thymus vulgaris* L.). Thyme essential oil contains bioactive volatile compounds, particularly thymol and carvacrol, which contribute to its antimicrobial activity, which affect the cell membranes and interfere with the important cell functions of microorganisms [5, 6]. The extract from thyme is rich in phenolic acids, flavonoids and antioxidants that are effective in preventing oxidative reactions in meat [7, 8]. Thyme essential oil and aqueous thyme extract differ in their chemical composition and bioactive compounds. Essential oil is mainly characterized by volatile phenolic compounds such as thymol and carvacrol, whereas aqueous extracts contain mainly water-soluble phenolic compounds and flavonoids [9].

Previous studies have demonstrated the effectiveness of thyme extracts in improving meat quality and extending shelf life [4].

Thyme extracts (aqueous and alcoholic) were reported to inhibit the growth of bacteria and extend the shelf life of refrigerated meat. The effect of thyme oil on the water holding capacity of frozen minced calf meat and its role in reducing the loss during thawing, dripping and cooking has been investigated by Al-Kinani and Al-Perkhedri. The extract of thyme has also been found to reduce the level of lipids oxidized and the level of microbiological contamination in stored lamb and chicken patties [10]. Research has reported that thyme oil inhibits growth of *Escherichia coli*, *Staphylococcus aureus* and coliform bacteria in minced meat [2].

Although thyme extracts and thyme essential oil have been widely investigated as natural preservatives for meat products, most previous studies have focused on fresh meat or evaluated only a single thyme preparation. Information comparing the preservative efficacy of aqueous thyme extract and thyme essential oil under identical experimental conditions remains limited, particularly for frozen chicken breast meat after thawing and subsequent refrigerated storage. Thawing accelerates physicochemical deterioration and microbial proliferation due to cellular disruption and drip loss, making effective natural preservation strategies particularly important. Therefore, evaluating different thyme preparations under these conditions is essential to identify the most effective natural preservative for maintaining the quality and safety of thawed refrigerated meat. Accordingly, the present study compared the effectiveness of aqueous thyme extract and thyme essential oil at two concentrations (1% and 2%) in maintaining the physicochemical and microbiological quality of thawed chicken breast meat during refrigerated storage.

Materials and Methods

Raw materials

Frozen skinless chicken breast meat (pectoralis major) was purchased from a local commercial market in Sulaymaniyah, Iraq. Samples were transported to the laboratory under refrigerated conditions and stored at -18°C until analysis. The experiment was conducted using 40 independent samples obtained from 8 batches. Before sample preparation, visible fat and connective tissues were removed by trimming to obtain uniform chicken breast portions. The Frozen meat samples were transported to the laboratory under refrigerated conditions and stored at -18°C until used. Samples were thawed at $4 \pm 1^{\circ}\text{C}$ for approximately 24 h before treatment. After thawing, samples were drained and the exudate was removed before applying treatments. To minimize variability among treatments, the thawed meat was cut into uniform pieces of similar weight and size.

Fresh thyme leaves (*Thymus vulgaris* L.) were obtained from a trusted local supplier in Sulaymaniyah, Iraq. Thyme essential oil was purchased from a trusted supplier and stored in amber glass bottles at 4°C until use to minimize oxidation and loss of volatile compounds.

Preparation of aqueous thyme extract and thyme essential oil treatments

The fresh leaves of the thyme (*Thymus vulgaris* L.) were washed with distilled water, shade dried, finely powdered and stored in airtight containers. The aqueous thyme extract was prepared according to

Alu'datt et al [11] with slight modification. Thyme powder was extracted with distilled water at a ratio of 1:10 (w/v). The extraction process was performed under controlled heating conditions, followed by filtration and concentration. The mixture was filtered through muslin cloth and Whatman No. 1 filter paper, then concentrated under vacuum and reduced pressure at 40°C in a rotary evaporator and dried to get the extract which was stored at 4°C . The extract was reconstituted in sterile distilled water to 1% and 2% (w/v) prior to use to create the treatments. Thyme essential oil was obtained from a trusted local supplier and stored in amber glass bottles at 4°C until use.

Experimental design

After thawing, chicken breast samples were immersed in thyme treatment solutions for 30 min at $4 \pm 1^{\circ}\text{C}$. The ratio between meat sample weight and treatment solution volume was maintained at 1:2 (w/v). Meat portions were immersed in the prepared thyme aqueous extract or thyme essential oil solutions (1% and 2%) for 30 min at $4 \pm 1^{\circ}\text{C}$ to ensure uniform contact between the treatment solution and meat surface. The samples treated were then drained under sterile conditions to remove excess solution, individually packed in sterile polyethylene bags, and stored at $4 \pm 1^{\circ}\text{C}$ for subsequent analyses. Untreated samples were considered as the control group and received the same handling procedure without thyme treatment. Aqueous thyme extract and thyme essential oil effects on the physicochemical and microbiological quality of chicken breast during refrigerated storage were assessed in the experiment. Five treatments: untreated control (C), 1% thyme extract, 2% thyme extract, 1% thyme essential oil, and 2% thyme essential oil. Following treatment,

the samples were placed in individual sterile food grade polyethylene bags and were stored at $4 \pm 1^\circ\text{C}$ for six days. Analyses were performed on days 0, 3, and 6. Physicochemical and microbiological tests were performed at the beginning (day 0) and on the 3rd & 6th day of storage.

Physical characteristics of meat

pH: Five grams of chicken breast sample were homogenized with 45 mL of distilled water for 1 min. The homogenate was filtered, and pH was measured using a calibrated digital pH meter [12].

Water-Holding Capacity (WHC): WHC was established through a protocol that was already outlined by [13] Concisely, 2g of chicken samples were put in a 15ml conical tube lined with gauze. Then the tubes were centrifuged at 4°C , 10 min at 3000 rpm. To calculate WHC, the mass of samples before and after centrifugation was compared, and the equation below was used:

$$\text{WHC (\%)} = (W_2 / W_1) \times 100$$

W 1: initial weight of chicken breast meat sample (g)

W 2: weight of chicken breast meat sample after centrifugation (g)

Drip loss: The samples were weighed, then wrapped in waterproof plastic film in polyethylene trays and stored for 48 hours under retail conditions. After this time, the exudate was removed, and the samples were returned to an analytical balance. The drip loss (%) was then calculated as the ratio of the initial weight to the end weight, with the difference between the two [14].

$$\text{Drip loss (\%)} = (W_1 - W_2) / W_1 \times 100$$

W₁: initial weight of breast sample (g)

W₂: final weight of breast sample (g)

Cooking loss: The cooking procedure was carried out as described by Xia et al [15] with some modifications. Cooled breast meat samples were then cooked by a water bath method at 80°C until the samples' internal core temperature reached 80°C , and then reweighed at room temperature for 30 min. The cooking loss (%) was determined using the following equation:

$$\text{Cooking loss} = (W_1 - W_2) / W_1 \times 100$$

W₁: weight of raw breast sample (g)

W₂: weight of cooked breast sample (g)

Chemical analysis of meat

Thiobarbituric Acid Reactive Substances (TBARS): TBARS were determined using the method as described by Malle and Poumeyrol [15]. Meat was ground with 7.5% trichloroacetic acid (TCA), filtered, and 10 grams of meat was collected. The filtrate was then made to react with thiobarbituric acid (TBA) reagent and then heated at a boiling water bath for 30min. Absorbance was measured following the cooling process at a wavelength of 532nm by UV-Vis Spectrophotometer, and the results were reported as mg malondialdehyde (MDA)/kg meat and calculated as follows:

$$\text{TBA} = (A_{532} / w) \times 9.48$$

Determination of Total Volatile Basic Nitrogen (TVB-N): It was determined according to the method described by Malle and Poumeyrol [16]. Briefly, 20 g of minced meat samples were mixed with 40 mL of

7.5% trichloroacetic acid (TCA) and homogenized thoroughly, filtered, and 12.5 ml of the filtrate was distilled with 2.5 ml of 10% NaOH using a macro-Kjeldahl apparatus. The distillate was collected in 7.5 ml of 4% boric acid and titrated with 0.05 N H₂ SO₄ using methyl red–red-bromocresol green as an indicator. The TVB-N content was calculated and expressed as mg nitrogen per 100 g of meat (mg N/100 g) as follows:

$$TVN(mg /100 gm) = \frac{V * 14 * \left(100 + \left(\frac{M}{100}\right) * 100\right)}{12.5 * 50}$$

Where: V=ml of 0.05 of H₂ SO₄,
M=moisture content

Determination of Free fatty acids (FFA):

Weights of 10 gm of the sample were placed in a mechanical blender and 25ml of chloroform was added, the mixture was blended for 2-3 min and filtered immediately through a large filter paper. This was then re-filtered through a paper containing a small amount of anhydrous sodium sulfate [17]. Portions of the filtrate were used for the determination of free fatty acids as follows: Five ml of 95% ethanol neutralized with drops of 0.1 N NaOH after adding phenolphthalein. The solution was added to 5 ml of the filtered and the mixture was titrated with 0.1 N NaOH until the pink color persists for 15 seconds. FFA was calculated as oleic acid percentage of the sample, following this equation:

$$FFA\% = \frac{\text{ml of 0.1 NaoH} \times 0.0282 \times \text{dilution factor}}{\text{sample weight}} \times 100$$

Microbiological Tests

Total Bacterial Count: The samples were homogenized and serial decimal dilutions (10^{-1} – 10^{-6}) were prepared in sterile 0.1% peptone water. Appropriate dilutions were pour-plated using Plate Count Agar. Once the agar had set, the plates were incubated for 48 h in an incubator under aerobic conditions at 37°C and the results were expressed in log CFU/g [18].

Psychrotrophic Bacteria Count: To determine the psychrotrophic bacterial count, 1mL of the appropriate dilution of each culture was plated on plate count agar (PCA). The plates were kept for 10 days at 7 °C. Each colony was counted daily and the conclusive counts were presented as log₁₀ CFU/g, in line with the protocol for [3].

Sensory Evaluation

Samples were cooked under identical conditions and presented in a randomized order. Each sample was evaluated for colour, flavour, tenderness, juiciness, and overall acceptability using a five-point hedonic scale, where a score of 5 indicated excellent quality and 1 indicated poor quality, following the guidelines of [19, 20].

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) to determine the effects of treatment, storage period, and their interaction. Differences among means were compared using Duncan's multiple range test at a significance level of $p < 0.05$.

Results and Discussion

Physical Analysis

Chicken breast meat quality gradually deteriorated during refrigerated storage. The increase in quality deterioration indicators was significantly greater in untreated control samples compared with thyme-treated samples. Cooking loss increased significantly during refrigerated storage, increasing from 23.33% at day 0 to 38.64% at day 6 in the untreated control samples

(Table 1). Samples treated with thyme essential oil showed lower cooking loss values compared with untreated control samples, with lowest cooking loss obtained in the sample treated with 2% thyme essential oil (31.91%). Thyme extract and essential oil resulted in moisture loss reduction during cooking, overall. However, thyme essential oil showed greater effectiveness than aqueous extract, particularly at higher concentration.

Table 1: The effect of thyme extracts and thyme essential oil at different concentrations on the cooking loss (%) of chicken breast meat

Storage days	Tretments					
	Control	Extract 1%	Extract 2%	Essential 1%	Oil Essential 2%	Oil
0	23.33 ± 2.52g	22.81 ± 1.96g	23.10 ± 2.62g	23.54 ± 1.65g	23.51 ± 2.11g	
3	32.91 ± 3.92cd	29.47 ± 2.69e	26.62 ± 2.45f	28.80 ± 2.78e	26.53 ± 2.39f	
6	38.64 ± 3.89a	35.513 ± 3.18b	32.71 ± 3.63cd	± 34.27 ± 3.91bc	31.91 ± 3.18d	

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

Cooking loss increased progressively in chicken meat during refrigerated storage, whereas thyme-treated samples, particularly those treated with 2% thyme essential oil, exhibited significantly lower values than the control. Similar reductions in cooking loss were reported by [10] in lamb patties treated with thyme extract and by [21] in chicken breast treated with thyme essential oil, where the treatments effectively preserved muscle proteins and reduced moisture loss during storage. Likewise, [22] reported that thyme essential oil improved the quality characteristics of refrigerated chicken breast, with the higher concentration showing greater effectiveness in reducing lipid oxidation and microbial growth compared with untreated samples. The agreement among these studies suggests that these effects may be attributed to the high

phenolic content of thyme, particularly thymol and carvacrol, which are the major active components of thyme [5]. They are natural compounds that can help prevent muscle proteins from being damaged by oxidation during refrigerated storage [23]. Proteins oxidized undergo structure changes and lose the power to keep water. Consequently, a greater amount of moisture is lost when cooking [24].

All samples experienced a gradual increase in drip loss during refrigerated storage, with all thyme-treated samples having a less dramatic increase. The drip loss of control samples of chicken meat increased from approximately 2% at day 0 to 6.25% at day 6 (Table 2). In contrast, the 2% thyme essential oil treatment only reduced the increase to 4.01%. Overall, increasing thyme

concentration resulted in greater reduction of drip loss during storage. Thyme essential oil showed higher effectiveness than aqueous

thyme extract in reducing drip loss and thus proved to be more effective in maintaining moisture during refrigerated storage.

Table 2: The effect of thyme extracts and thyme essential oil at different concentrations on the drip loss (%) of chicken breast meat

Storage days	Tretments				
	Control	Extract 1%	Extract 2%	Essential Oil 1%	Essential Oil 2%
0	1.94 ± 0.07i	1.96 ± 0.05i	1.95 ± 0.09i	1.94 ± 0.08i	1.97 ± 0.09i
3	4.08 ± 0.25e	3.55 ± 0.33f	3.21 ± 0.29g	3.03 ± 0.26gh	2.86 ± 0.25h
6	6.25 ± 0.34a	5.31 ± 0.58b	4.76 ± 0.38c	4.33 ± 0.35d	4.01 ± 0.35e

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

Drip loss increased significantly in chicken meat throughout refrigerated storage, whereas all thyme-treated samples showed lower values than the control, with 2% thyme essential oil exhibiting the greatest reduction. Similar findings were reported by [10], who observed that thyme extract effectively reduced drip loss and improved moisture retention in refrigerated meat products. Likewise, [21] demonstrated that thyme essential oil preserved muscle integrity and reduced exudate formation during storage. The possible explanation for reduced drip losses of thyme treated samples might be related to the enhanced protection of muscle cells during storage. Thyme bioactive compounds may help preserve cellular membrane integrity by reducing oxidative damage and helps to retard the decay of structural proteins. Lipid oxidation can lead to damage of the membrane and water may easily escape from the cell.

During this time, muscle protein is also being oxidised which further helps in losing fluid. The antioxidants in thyme can help to reduce such changes and help to retain water in the muscle [23, 25, 26].

The trend of cooking and drip losses was opposite to that of water-holding capacity (WHC). WHC gradually decreased during refrigerated storage for chicken meat (Table 3). However, a general trend was observed in all the thyme-treated samples, which had a greater water content compared to the control. Control samples saw the WHC of chicken meat drop from about 40% on day 0 to 24.16% on day 6. The 2% thyme essential oil treatment had a much higher value of 30.71% in contrast. The WHC of samples treated with thyme stayed significantly higher than the control throughout the storage period, particularly in samples treated with 2% thyme extract and 2% thyme essential oil.

Table 3: The effect of thyme extracts and thyme essential oil at different concentrations on the WHC (%) of chicken breast meat

Storage days	Tretments					
	Control	Extract 1%	Extract 2%	Essential Oil 1%	Essential Oil 2%	
0	39.84 ± 1.89a	39.92 ± 2.39a	39.71 ± 1.33a	39.84 ± 2.37a	39.97 ± 1.33a	
3	29.23 ± 1.37cde	32.33 ± 1.43bcd	33.14 ± 1.94bc	31.75 ± 2.23bcde	34.82 ± 2.16b	
6	24.16 ± 1.14f	27.41 ± 1.33ef	30.32 ± 1.32cde	28.63 ± 1.33de	30.71 ± 1.51bcde	

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

WHC gradually decreased during refrigerated storage; however, thyme-treated samples maintained significantly higher values than the untreated control. These results agree with those reported by Al-Kinani and Al-Perkhedri, who found that thyme essential oil improved the water-holding capacity of frozen minced calf meat. Similar observations were also reported by [27], who concluded that natural antioxidants preserve the functional properties of myofibrillar proteins and improve water retention in meat products.

The higher WHC for the thyme treated samples is in line with lower cooking and drip losses during storage. This indicates that Thyme treatments may help preserve and 6.33. The results indicate that thyme treatments delayed the increase in pH

muscle protein functionality during storage. This essential oil was more effective than the aqueous extract. This difference may be related to the higher concentration of lipophilic bioactive compounds present in thyme essential oil, which have more antioxidant activity and better interaction with cell membranes [25].

Changes in pH were observed during refrigerated storage. In chicken meat, the pH slowly rose with time [28], with a slow increase in both thyme-treated samples as compared to the control (Table 4). The untreated samples of chicken meat had a pH of 5.87 and increased to 6.68 after 6 days. Thyme-treated samples, on the other hand, were generally low, between 6.20 associated with meat spoilage during storage.

Table 4: The effect of thyme extracts and thyme essential oil at different concentrations on the pH of chicken breast meat

Storage days	Tretments				
	Control	Extract 1%	Extract 2%	Essential Oil 1%	Essential Oil 2%
0	5.87 ± 0.17c	5.89 ± 0.27c	5.88 ± 0.57c	5.85 ± 0.71c	5.81 ± 0.72c
3	6.15 ± 0.12bc	6.00 ± 0.57bc	5.95 ± 0.47bc	6.01 ± 0.57bc	5.99 ± 0.37bc
6	6.68 ± 0.27a	6.20 ± 0.84bc	6.33 ± 0.58ab	6.33 ± 0.43ab	6.21 ± 0.57bc

Values are expressed as mean $\pm$ SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

The increase in pH value during refrigerated storage is mainly attributed to microbial degradation of proteins to alkaline compounds like ammonia, volatile nitrogen compounds etc. The lower pH values in the thyme treated samples are an indication of the ability of thyme to inhibit microbial growth, protein breakdown and the accumulation of these alkaline metabolites. This is in line with the antimicrobial activity of thyme, as the results of pH and TVB-N are very close. These results are in agreement with [4], who reported that thyme extract effectively delayed pH changes in refrigerated meat. Similar findings were also described by [29], who suggested that the antimicrobial constituents of thyme inhibit spoilage bacteria responsible for producing alkaline metabolites. Consequently, the lower pH values observed in the present study indicate delayed microbial spoilage and improved preservation of meat quality.

Chemical Analysis

Protein quality indicators deteriorated during refrigerated storage of chicken breast meat, increasing TVB-N values. The increase was, however, very low in the thyme-treated samples as compared to the control sample (Table 5). In chicken meat, TVB-N rose from 8.72 mg N/100 g on day 0 to 22.91 mg N/100 g in control after six days. The 2% thyme essential oil treatment only reached 13.42 mg N/100 g, in comparison. By the end of the storage period, TVB-N values increased in all treatments during refrigerated storage; however, thyme-treated samples showed lower increases compared with untreated controls, indicating delayed protein degradation.

Table 5: The effect of thyme extracts and thyme essential oil at different concentrations on the TVB-N (mg N/100 g) of chicken breast meat

Storage days	Tretments				
	Control	Extract 1%	Extract 2%	Essential Oil 1%	Essential Oil 2%
0	8.72 $\pm$ 0.63h	8.65 $\pm$ 0.73h	8.54 $\pm$ 0.83h	8.47 $\pm$ 0.94h	8.39 $\pm$ 0.48h
3	14.86 $\pm$ 0.83d	13.21 $\pm$ 0.98de	11.82 $\pm$ 0.93ef	10.91 $\pm$ 0.98fg	9.86 $\pm$ 0.93gh
6	22.91 $\pm$ 0.93a	18.94 $\pm$ 1.33b	16.63 $\pm$ 1.19c	14.95 $\pm$ 1.03cd	13.42 $\pm$ 0.83de

Values are expressed as mean $\pm$ SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

TVB-N values increased in all treatments during refrigerated storage, although the increase was significantly lower in thyme-treated samples, particularly those treated with 2% thyme essential oil. Therefore, it is suggested that microbial growth and natural breakdown of muscle proteins during

refrigerated storage is the primary cause of the gradual increase in TVB-N, which is generally correlated with the accumulation of ammonia, trimethylamine, dimethylamine and other volatile bases commonly used as indicators of freshness loss and protein spoilage. Comparable results were reported

by [4], who found that thyme extracts effectively delayed the accumulation of volatile nitrogen compounds in refrigerated meat. Similarly, [29] attributed the antimicrobial activity of thyme essential oil to thymol and carvacrol, which disrupt bacterial cell membranes and suppress the growth of spoilage microorganisms. Therefore, the lower TVB-N values observed in the present study likely reflect reduced microbial proteolysis and delayed protein degradation.

During refrigerated storage, the TBA values increased in all treatments, reflecting the

increase in lipid oxidation. This means that secondary oxidation products are continually formed over time. The TBA values for all the thyme treated samples, however, were always lower than the control (Table 6). The TBA content of chicken meat was raised from 0.53 to 0.97 mg MDA/kg in the control after 6 days. Lower values of 0.74 mg MDA/kg and 0.78 mg MDA/kg respectively, were obtained for extract 2% and oil 2%. Thyme treatments appeared to retard lipid oxidation and preserve meat quality during refrigerated storage as shown by these results.

Table 6: The effect of thyme extracts and thyme essential oil at different concentrations on the TBA (mg MDA/kg) of chicken breast meat

Storage days	Tretments					
	Control	Extract 1%	Extract 2%	Essential 1%	Oil 2%	Essential Oil 2%
0	0.53 ± 0.03d	0.51 ± 0.03d	0.55 ± 0.09cd	0.52 ± 0.02d	0.53 ± 0.03d	
3	0.83 ± 0.09ab	0.78 ± 0.05ab	0.71 ± 0.06bcd	0.77 ± 0.05abc	0.69 ± 0.04bcd	
6	0.97 ± 0.08a	0.83 ± 0.06ab	0.74 ± 0.06abcd	0.87 ± 0.07ab	0.78 ± 0.06ab	

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

The lower TBA values observed in thyme-treated samples may be attributed to the antioxidant activity of bioactive compounds such as thymol, carvacrol, rosmarinic acid, and flavonoids in thyme. These compounds have the potential to remove free radicals, disrupt the chain reaction of lipid oxidation, and bind metals that are pro-oxidants, reducing the formation of malondialdehyde and other products of lipid oxidation during refrigerated storage. These findings are consistent with those of [27], who reported that plant-derived antioxidants effectively inhibit lipid oxidation in meat products.

Similarly, [22] concluded that phenolic compounds from aromatic plants scavenge free radicals and interrupt lipid peroxidation, thereby preserving meat quality during storage. The lower TBA values observed in this study indicate the potential antioxidant effect of thyme treatments, particularly when applied as essential oil.

FFA values gradually increased in chicken breast meat during refrigerated storage, indicating continuous lipid hydrolysis. However, the increase in the samples treated was much less as compared to control (Table

7). The control group had increased FFA levels in the chicken meat from 3.22% on day 0 to 6.84% on day 6. However, the 2% thyme essential oil treatment inhibited the increase to 4.93%. In general, it can be

concluded that both thyme extract and thyme essential oil were effective in providing protection against lipid hydrolysis and lipid stability in the refrigerated storage.

Table 7: The effect of thyme extracts and thyme essential oil at different concentrations on the FFA (%) of chicken breast meat

Storage days	Tretments				
	Control	Extract 1%	Extract 2%	Essential Oil 1%	Essential Oil 2%
0	3.22 ± 0.25h	3.26 ± 0.29h	3.30 ± 0.32h	3.28 ± 0.33h	3.22 ± 0.21g
3	5.61 ± 0.58cd	4.52 ± 0.38fg	4.19 ± 0.39g	4.58 ± 0.41efg	4.31 ± 0.29g
6	6.84 ± 0.62a	6.23 ± 0.53b	5.81 ± 0.42bc	5.12 ± 0.53de	4.93 ± 0.47ef

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

The rise in FFA levels during refrigerated storage is attributed to the continuous hydrolysis of triglycerides by the action of endogenous lipases and microbes. Also, the lower FFA values in thyme treated samples indicate that thyme inhibited this process by inhibiting microbial growth and by protecting the cell membranes from oxidative damage which reduced lipid hydrolysis. Similar observations were reported by [10], who demonstrated that thyme extract reduced lipid hydrolysis in refrigerated lamb patties. The reduced FFA accumulation observed in this study may be explained by the combined antimicrobial and antioxidant properties of thyme, which inhibit microbial lipases and protect membrane lipids against oxidative degradation [25].

Microbial Analysis

Initial total bacterial counts were comparable among all treatments, indicating similar microbiological quality before storage. This means that all samples were in a similar microbiological state prior to storage (Table 8). The numbers of bacteria grew in all the groups as the storage proceeded owing to the proliferation of the spoilage bacteria. However, the increase was much faster in the control samples. The counts of the samples treated with thyme were reduced, particularly at higher concentrations, after 6 days compared with the control which had reached 5.48 log CFU/g at that time. Thyme essential oil treatment (oil 2%) had the highest inhibitory effect with a final number of 4.84 log CFU/g.

Table 8: The effect of thyme extracts and thyme essential oil at different concentrations on the total bacterial count (log CFU/g) of chicken breast meat

Storage days	Tretments					
	Control	Extract 1%	Extract 2%	Essential Oil 1%	Essential Oil 2%	Oil
0	3.82 ± 0.32fg	3.79 ± 0.33fg	3.75 ± 0.29g	±	3.73 ± 0.31g	3.70 ± 0.29g
3	4.71 ± 0.39cd	4.22 ± 0.42ef	3.93 ± 0.24fg	±	4.46 ± 0.39de	4.11 ± 0.36efg
6	5.48 ± 0.51a	5.18 ± 0.49ab	4.95 ± 0.36bc	±	5.04 ± 0.44bc	4.84 ± 0.43bcd

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

The lower counts of bacteria in thyme-treated samples may be primarily due to the compounds thymol and carvacrol, which affect bacterial cell membranes, inhibit essential cellular functions, and suppress the growth of microorganisms. Their antimicrobial mechanisms may contribute to inhibition of bacterial growth during refrigerated storage. Moreover, thyme preserves muscle tissue by halting lipid and protein oxidation, which limits the availability of nutrients for bacterial growth. Similar antibacterial effects of thyme essential oil against spoilage microorganisms have been reported by [29, 30]. Likewise, [2] demonstrated that thyme essential oil effectively inhibited bacterial growth in minced meat. The present findings further confirm that the high phenolic content of thyme essential oil enhances its

antibacterial activity, thereby improving the microbiological stability of refrigerated meat.

The psychrotrophic bacteria also had a similar pattern as the total aerobic bacteria during refrigerated storage. The number of these bacteria has increased in all treatments as they are able to grow at low temperatures. But there was definitely a lag in their growth when it came to chicken meat when treated with thyme (Table 9). In the case of chicken meat, the psychrotrophic counts rose from 2.40 logCFU/g at the initial time to 4.92 logCFU/g in the control after six days. The 2% thyme essential oil treatment only achieved 4.11 log CFU/g, in contrast to the other treatments. This means that during storage, Thyme oil 2% was the most effective at inhibiting psychrotrophic bacteria.

Table 9: The effect of thyme extracts and thyme essential oil at different concentrations on the psychrotrophic bacteria count (log CFU/g) of chicken breast meat

Storage days	Tretments					
	Control	Extract 1%	Extract 2%	Essential 1%	Oil	Essential 2% Oil
0	2.40 ± 0.17i	2.38 ± 0.27i	2.35 ± 0.18i	2.33 ± 0.21i		2.30 ± 0.22i
3	3.88 ± 0.31def	3.66 ± 0.35fg	3.41 ± 0.33gh	3.72 ± 0.34efg		3.18 ± 0.33h
6	4.92 ± 0.39a	4.79 ± 0.44ab	4.23 ± 0.43cd	4.45 ± 0.42bc		4.11 ± 0.51cde

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

The reduction of psychrotrophic bacteria is of special concern since they are primarily responsible for the occurrence of spoilage during refrigerated storage. The growth inhibition of thyme resulted in reduced degradation of protein and lipids, as observed in lower values for TVB-N, TBA, and FFA, whereas there were better water-holding capacity and reduced cooking and drip loss. These findings agree with those reported by [30], who highlighted the effectiveness of essential oils against cold-tolerant spoilage bacteria. Similarly, [2] observed that thyme essential oil significantly reduced the growth of refrigeration-associated bacteria in meat products. Thyme EO at 2% had the most potent antimicrobial activity, probably due to its phenolics (lipophilic) that are more effective in penetrating the bacterial membranes. Thyme treatments were able to lower psychrotrophic bacterial counts in chicken breast meat, presenting a wide preservative potential during refrigeration [31].

The present study demonstrated that thyme treatments significantly improved the sensory attributes of chicken breast meat compared with untreated samples (Table 10).

These findings highlight the potential role of thyme essential oil as a natural preservative in maintaining meat quality. The observed improvement in sensory characteristics may be attributed to the antioxidant and antimicrobial activities of thyme bioactive compounds, which can reduce lipid oxidation and microbial deterioration, thereby contributing to the preservation of desirable flavour, aroma, and overall acceptability of meat products.

Thyme essential oil at 1% showed the highest overall acceptability, suggesting that this concentration provided a balance between preservative efficiency and sensory acceptance. In contrast, the slightly lower flavour scores observed for 2% thyme essential oil may be related to the higher intensity of volatile phenolic compounds, particularly thymol and carvacrol, which can impart a strong herbal aroma when present at elevated concentrations.

Sensory evaluation

Table 10: The effect of thyme extracts and thyme essential oil at different concentrations on sensory evaluation of chicken breast meat

Treatment	Colour	Flavour	Tenderness	Juiciness	Overall acceptability
Control	3.05 ± 0.32c	2.85 ± 0.36c	3.00 ± 0.30c	2.90 ± 0.34c	2.90 ± 0.31c
Extract 1%	3.55 ± 0.28b	3.45 ± 0.31b	3.60 ± 0.27b	3.50 ± 0.29b	3.53 ± 0.26b
Extract 2%	3.85 ± 0.30ab	3.75 ± 0.33ab	3.90 ± 0.29ab	3.85 ± 0.31ab	3.84 ± 0.28ab
Essential Oil 1%	4.20 ± 0.25a	4.05 ± 0.28a	4.25 ± 0.24a	4.15 ± 0.27a	4.16 ± 0.23a
Essential Oil 2%	4.30 ± 0.24a	3.85 ± 0.32ab	4.35 ± 0.23a	4.25 ± 0.25a	4.10 ± 0.24a

Values are expressed as mean ± SD. Different letters indicate significant differences among treatment and storage-day combinations (p < 0.05).

Similar findings have been reported, where thyme essential oil improved the sensory quality of refrigerated chicken breast, while excessive concentrations affected flavour preference due to the strong aromatic characteristics of essential oil components [22, 25].

The results indicate that an appropriate concentration of thyme essential oil is essential to achieve maximum preservation benefits without negatively affecting consumer acceptability.

Conclusion

In general, both aqueous thyme extract and thyme essential oil showed potential preservative effects for thawed chicken breast meat during refrigerated storage, as they effectively inhibited the microbial population, delayed protein and lipid deterioration and enhanced the technological properties. Of the treatments, 2% thyme essential oil was the most effective preservative treatment, followed by 2% thyme extract, suggesting that the higher the concentration of thyme bioactive compounds, the better the protection. Thyme treatments delayed quality deterioration in chicken breast meat during the six-day refrigerated storage period.

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