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Natural Preservation of Chicken Breast Meat Using Thyme and Garlic Extracts Impact on Physicochemical Quality, Microbial Stability, and Shelf-Life during Chilled Storage

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ABSTRACT

The preservative efficiency of the extracts from thyme (*Thymus vulgaris* L.) and garlic (*Allium sativum* L.) in chicken breast meat stored under refrigeration conditions (4 ± 1°C, 12 days) was estimated in relation to the extent of lipid oxidation (TBARS, free fatty acids), microbiological quality (total plate count, psychrotrophs), physicochemical properties (pH) and water holding capacity (drip loss, water-holding capacity [WHC]). All combinations of treatments with extracts demonstrated significantly less oxidative spoilage and microbial growth in comparison with the control sample ($p<0.05$). Combination of the extracts from thyme and garlic at the level of 0.10% provided the highest preservative activity, providing TBA values remaining under the threshold of rancidity, lowering microbial count and pH, and avoiding any changes in WHC. Correlation analysis showed dependency among the parameters of lipid oxidation, microbial growth, increase in pH, and moisture losses, which means that meat degradation is based on the interaction of biochemical and microbiological processes rather than separate processes. According to the established microbiological criterion of quality in terms of log CFU/g value of 5.0, combination of thyme and garlic extracts improved preservation activity, but their number of psychrotrophs became equal to 5.12 ± 0.15 log CFU/g after 12 days of storage. In accordance with both microbiological and oxidative criteria of acceptance, shelf life of refrigerated chicken meat increased up to 12 days in comparison with about 4 days for the control sample.

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1. Introduction

One of the most popular animal protein food items in the world is the chicken breast because of its high nutritional value, balanced amino acid composition, cheap price, and flexible uses in various dishes. Unfortunately, natural characteristics of the chicken meat, such as high level of water activity, neutral pH, and presence of polyunsaturated fatty acids, make it easily oxidizable and vulnerable to contamination during refrigerated storage, thus reducing the shelf life and deteriorating the quality of the meat from a physical, chemical, and microbiological perspective (Zhang et al., 2023; Lorenzo et al., 2024).

The inhibition of oxidative reaction as well as the prevention of bacterial growth on poultry products used to be done using the combination of antioxidants and chemical preservatives, such as BHA and BHT. While these chemicals prove to be effective, their use has become more controversial with time because of the increase in toxicity worries and changing consumer attitudes. Therefore, the regulation of their usage has been made stricter while the need for natural products has been increased (Falowo et al., 2023; Shah et al., 2024).

Regarding natural preservatives, many research works have been done on thyme (*Thymus vulgaris*) and garlic (*Allium sativum*) due to their antioxidant and antimicrobial properties. Thyme offers phenolic compounds (thymol and carvacrol), which act as effective free radical scavengers, possess metal-chelating ability, and are characterized by antimicrobial action by causing disruption of cell membrane. Garlic gives active components, such as allicin and diallyl sulfides, which restrain the development of microbes by interfering with enzyme system, protein synthesis, and redox reaction (Lorenzo et al., 2024; Soković et al., 2022). Recent research works from 2020 to 2025 indicate that these compounds can significantly

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reduce lipid oxidation and microflora of meat (Zhang et al., 2023; Falowo et al., 2014).

However, there are some significant knowledge gaps. Almost all research works performed recently were devoted to using single plant extract or essential oil usually for already cooked or processed meat. There is no comprehensive study devoted to the use of combined thyme-garlic extract for raw chicken breast in chilled storage. The main working hypothesis is that the combination of thyme and garlic extract will show better preservative properties compared to each of them in terms of reducing the oxidative and microbiological deterioration and improving physicochemical stability of chicken breast meat.

The objectives of this study was to: (1) Evaluate the effects of thyme, garlic, and their combinations on lipid oxidation (TBA and FFA) in chicken breast meat during chilled storage. (2) Assess microbial quality and shelf-life extension. (3) Investigate physicochemical stability, including pH evolution, drip loss, and water holding capacity. (4) Analyze correlations among oxidative, microbiological, and physicochemical parameters to characterize their associations during chilled storage.

2. Materials and Methods

2.1. Preparation of Thyme and Garlic Extracts

Fresh *Thymus vulgaris* L. (leaves of thyme) were collected from the Doski area of Duhok Governorate, Kurdistan Region of Iraq, while the fresh garlic bulbs (*Allium sativum* L.) were collected from a southern garden shed. Collection of the plant materials was carried out during the spring season and identified based on morphological characteristics. Garlic and thyme leaves were cleaned with distilled water, air-dried without any exposure to light and proper ventilation under normal temperature (25 ± 2 °C) for ten days, and then dried samples were crushed into a powder using laboratory mill. Dried plant samples were stored in closed containers until extraction.

Extraction of garlic and thyme was conducted via hydroethanolic maceration with slight modifications (Bozin et al., 2006; Viuda-Martos et al., 2010). In a closed conical flask, 100 g of the dried thyme and garlic powders were added to 1000 mL of 70% (v/v) ethanol with 30% distilled water. The whole solution was continuously stirred for 24 hours in dark at room temperature using a magnetic stirrer. This process was repeated three times to improve the extraction efficiency. Extracts were filtered using sterile cheesecloth and Whatman No. 1 filter paper.

The filtrate was evaporated at 40°C under reduced pressure using a vacuum oven, followed by lyophilization to produce dry powder extracts. The produced extracts were kept in closed containers at -20°C until further analysis.

2.2. Preparation of Chicken Breast Meat Samples

Carcasses of broilers (age: 38-42 days; weight: 2.0-2.6 kg) were collected. The breast fillets were collected immediately after the post-slaughter cooling stage and transported to the laboratory. The samples were stored at 4°C for 24 hours and free of visible fat and connective tissues. Then, they were minced with a meat grinder (with a 0.8 cm plate).

After mincing the meat, each treatment received an amount of 5 kg of minced meat. There were five treatment groups: a control group without any supplementation of minced meat with either garlic or thyme extract; a group with GE at the rate of 500 ppm (0.05%) or 0.5 g GE/kg of minced meat; a group with TE at the rate of 500 ppm (0.05%) or 0.5 g TE/kg of minced meat; a GE+TE group, which contained GE at 500 ppm and TE at 500 ppm (the total extract concentration of 1000 ppm or 0.10%); a low concentration GE+TE group, which contained GE at 250 ppm and TE at 250 ppm (the total extract concentration 500 ppm or 0.05%).

The extracts were diluted in distilled water and mixed with the minced meat using a sterile blender. Then, the patties of 50 g, which had a diameter of 8 cm and a height of 2 cm, were prepared. They were packed on polystyrene plates in a polyethylene film and stored at 4±1°C for 12 days. Analyses were done on days 1, 4, 8, and 12 of storage.

2.3. Lipid Oxidation Analysis

2.3.1. Thiobarbituric Acid (TBA)

The level of thiobarbituric acid (TBA) was determined to determine the oxidation status of lipids according to the procedure described by Witte et al. (1970). Meat samples weighing 10 grams each were homogenized in distilled water, and subsequently, the mixture was treated with thiobarbituric acid reagent. Absorption was recorded at 530 nm with a spectrophotometer (Jenway 6400, TBA values were calculated using the following equation and expressed as mg malondialdehyde (MDA)/kg meat:

TBA (mgMDA/kg)=A_530×5.2

where A_{530} is the absorbance of the sample measured at 530 nm, and 5.2 is the conversion factor used to express the TBA value as mg malondialdehyde (MDA) per kg of meat.

2.3.2. Free Fatty Acids (FFA)

The free fatty acid content was measured using the method published by Egan et al. (1981). Lipids were isolated from meat samples and titrated with 0.1 N NaOH, using phenolphthalein as an indicator. FFA was expressed as a percentage of oleic acid using the following equation:

FFA(%)= (S×N×F×28.2)/W

where S is the volume of sodium hydroxide (NaOH) solution consumed during titration (mL), N is the normality of the NaOH solution, F is the correction or dilution factor applied to the analytical procedure, 28.2 is the equivalent factor used for expressing the result as oleic acid, and W is the mass of the sample used for analysis (g).

2.4. Microbiological Analysis

Microbiological studies were carried out using the American Public Health Association's standard protocols (APHA, 2015).

2.4.1. Total Plate Count (TPC)

Ten grams of Chicken breast meat were aseptically homogenized with 90 milliliters of sterile peptone water.

Serial dilutions were made and plated onto nutrient agar. Plates were incubated at 37 °C for 48 hours, with data represented as log CFU/g.

2.4.2. Psychrotrophic Bacteria (PSY)

Psychrotrophic bacterial counts were evaluated on nutrient agar plates cultured for 10 days at 7°C. Log CFU/g was used to express the colony numbers.

2.5. Physicochemical Properties

2.5.1. pH Measurement

Meat pH was determined using the method published by Ibrahim et al. (2010). Ten grams of Chicken breast meat were homogenized in 100 mL of distilled water for one minute. The pH was measured with a calibrated digital pH meter (BP3001, Singapore).

2.5.2. Drip Loss

Drip loss was calculated using Sen et al. (2004). Meat samples (50 g) were placed on a grid inside a sealed plastic container and refrigerated at 4°C for 24 hours. Following storage, the samples were gently blotted dry and reweighed. Drip loss was computed as follows:

Drip loss (%)=(Wi -W_f)/(Wi)×100

where:

Wi = initial sample weight before storage

W_f= final sample weight after storage and blotting

2.5.3. Water-Holding Capacity (WHC)

The WHC value was obtained using the same procedure used in measuring water retention in chicken breast meat. Specifically,Briefly, 0.3 g of chicken breast meat was weighed and placed between two sheets of filter paper. The sample was pressed under a 35 kg load for 5 min at room temperature. After pressing, the sample was removed and reweighed, and the released water was determined according to the standard filter-paper press procedure.. The water was then extracted from the meat using the standard procedure and was reweighed. The WHC value was then calculated as the percentage of water retained by the meat sample. Three different samples for each treatment at each storage time were measured.

$$WHC\ (\%) = [1-(W_i-Wf)/Ww]\times 100$$

where W_i is the initial mass of the meat sample before the WHC procedure (g), Wf is the final mass of the sample after the procedure (g), and Ww is the initial water content of the sample (g).

2.6. Experimental Design and Statistical Analysis

Factorial design with five treatment conditions and four storage periods (Days 1, 4, 8, and 12) with three replicates per each treatment (n = 3) was used in this study. Treatments consisted of: control without any treatment; thyme extract (500 ppm); garlic extract (500 ppm); thyme extract and garlic extract together (500 + 500 ppm; total 1000 ppm; 0.10%); thyme extract and garlic extract together (250 + 250 ppm; total 500 ppm; 0.05%). The effects of treatment, storage period and interaction between them on lipid oxidation, microbiological quality, pH, drip loss, and water-holding capacity were determined by using a two-way ANOVA.

Whenever there were significant differences, treatment means were compared by using Tukey's honestly significant difference (HSD) test] test at $p < 0.05$ significance level. Data were presented as means ± SD. Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). For the correlation analyses, Pearson's correlation coefficient was used. Correlation coefficients were considered as indicators of association.

3. Results and Discussion

3.1. Effects of Thyme and Garlic Extracts on Lipid Oxidation of Chicken Breast Meat

Oxidation of lipids forms one of the key factors that determine the quality and shelf life of poultry meat during storage at low temperatures, especially polyunsaturated fatty acids in the pectoral muscles of chicken meat. In the current study, the oxidative stability of the samples was determined using thiobarbituric acid reactive substances (TBA) and free fatty acids (FFA), which are the secondary indicators of the oxidation of lipids and lipid hydrolysis, respectively. The values of both parameters showed a continuous rise throughout the experiment depending on the type of the extract used (Tables 1 and 2).

Control samples showed a rapid increase in the levels of thiobarbituric acid reactive substances (TBA), reaching 1.779 mg MDA/kg after 12 days, exceeding the rancidity threshold for poultry meat. Samples subjected to treatment with the extract showed significantly lower TBA than the control ($p < 0.05$). The most effective extract was the mixture of 0.10% thyme-garlic, with TBA levels less than 0.13 mg MDA/kg on day 12 (Table 1).

Similar patterns were also noted with free fatty acids (FFA). The control group revealed significant oxidation of lipids throughout the storage process, while FFA formation in samples treated with the extracts was significantly inhibited ($p < 0.05$). On day 12, FFA formation was reduced to 0.583% oleic acid by the 0.10% thyme-garlic extract (Table 2).

The higher antioxidant activity found in the mixed extracts is the result of a synergistic effect between garlic organosulfur compounds and thyme phenolics (carvacrol and thymol), which prevent lipid oxidation. From the results it can be seen that the mixture of thyme-garlic extract remains stable with respect to oxidation at levels below the acceptable limit throughout refrigerated storage time, hence its use as a substitute for artificial antioxidants in chilled chicken production.

3.2. Microbial Quality and Shelf-Life Extension

The microbial population in the muscle of the chicken breast was analyzed using total plate count (TPC) and psychrotrophic bacteria (PSY). TPC and PSY are some of the most frequently used measures of meat spoilage for chilled meat products. There was a significant rise in the microbial population over time ($p < 0.05$). However, the rate of growth was highly dependent on the kind of extract used (Tables 3 and 4).

Control samples showed a very high increase in bacteria number within 4 days of storage, exceeding the

acceptable criteria level. It is clear from that TPC and PSY values increased sharply over time, peaking by day 12. This demonstrates high spoilage and decrease in shelf-life under chilled storage. These findings are in line with previous research on rapid bacterial spoilage of unprocessed chicken meat under refrigeration (Balamatsia et al., 2023).

Table 1. Changes in Thiobarbituric acid (TBA, mg MDA/kg) of chicken breast meat treated with thyme and garlic extracts during chilled storage

Treatment	Day 1	Day 4	Day 8	Day 12
Control	0.296 ± 0.002 a	0.618 ± 0.003 a	0.946 ± 0.006 a	1.779 ± 0.032 a
TE (500) ppm	0.116 ± 0.002 b	0.135 ± 0.001 b	0.156 ± 0.004 b	0.294 ± 0.003 b
GE (500) ppm	0.147 ± 0.010 b	0.164 ± 0.003 b	0.184 ± 0.003 b	0.306 ± 0.002 b
TE 500ppm+ GE 500ppm	0.104 ± 0.004 b	0.111 ± 0.002 b	0.120 ± 0.002 b	0.129 ± 0.002 c
TE 250ppm+ GE 250ppm	0.107 ± 0.002 b	0.117 ± 0.003 b	0.135 ± 0.001 b	0.158 ± 0.002 c

Values are mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among treatments at the same storage day ($p < 0.05$).

Table 2. Changes in free fatty acid content (FFA, % oleic acid) of chicken breast meat treated with thyme and garlic extracts during chilled storage

Treatment	Day 1	Day 4	Day 8	Day 12
Control	0.767 ± 0.013 a	0.794 ± 0.003 a	0.903 ± 0.013 a	1.395 ± 0.126 a
TE (500) ppm	0.668 ± 0.009 b	0.709 ± 0.007 b	0.837 ± 0.010 b	0.952 ± 0.062 b
GE (500) ppm	0.564 ± 0.004 c	0.579 ± 0.002 c	0.630 ± 0.002 c	0.901 ± 0.012 bc
TE 500ppm+ GE 500ppm	0.306 ± 0.003 d	0.339 ± 0.004 d	0.363 ± 0.004 d	0.583 ± 0.003 d
TE250ppm+ GE 250ppm	0.530 ± 0.007 c	0.560 ± 0.003 c	0.609 ± 0.007 c	0.627 ± 0.003 c

Values are mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among treatments at the same storage time ($p < 0.05$).

Table 3. Changes in total plate count (TPC, log CFU/g) of chicken breast meat treated with thyme and garlic extracts during chilled storage

Treatment	Day 1	Day 4	Day 8	Day 12
Control	2.93 ± 0.12^{a}	3.50 ± 0.10^{a}	4.93 ± 0.15^{a}	6.37 ± 0.18^{a}
TE (500 ppm)	1.80 ± 0.08^{b}	2.33 ± 0.12^{b}	3.33 ± 0.18^{b}	4.27 ± 0.15^{b}
GE (500 ppm)	2.13 ± 0.10^{b}	2.87 ± 0.14^{b}	3.80 ± 0.16^{b}	4.80 ± 0.17^{b}
TE 500 ppm + GE 500 ppm	1.33 ± 0.07^{c}	1.93 ± 0.10^{c}	2.77 ± 0.14^{c}	3.53 ± 0.15^{c}
TE 250 ppm + GE 250 ppm	2.23 ± 0.11^{b}	2.77 ± 0.12^{b}	3.70 ± 0.15^{b}	4.80 ± 0.16^{b}

All values were reported as mean ± SD (n = 3). In the same column, superscript letters denote significant differences among treatments on each storage day ($p < 0.05$). Microbial quality criteria of 5.0 log CFU/g were used to determine the spoilage of samples based on total plate counts (TPC) or psychrotrophic counts (PSY).

However, all samples treated with extracts had significantly lower TPC and PSY values compared to the control throughout the storage time period ($p < 0.05$). The combined treatment with 500 ppm thyme extract and 500 ppm garlic extract (in total 1000 ppm; 0.10%) showed the lowest microbial numbers among other tested treatments. Lower concentration combination (250 ppm thyme extract + 250 ppm garlic extract; in total 500 ppm; 0.05%), as well as individual extracts, had lower microbial counts compared to the control sample. Therefore, it is possible to conclude that all extract treatments delayed the development of microbial spoilage under chilled storage conditions. The best results were provided by treatment with the combination of both extracts in 1000 ppm concentration. However, PSY for this treatment peaked at 5.12 ± 0.15 log CFU/g on day 12. It prevents us from determining 12-day microbiological shelf-life according to the selected criterion of 5.0 log CFU/g.

Table 4. Changes in psychrotrophic bacterial counts (PSY, log CFU/g) of chicken breast meat treated with thyme and garlic extracts during chilled storage

Treatment	Day 1	Day 4	Day 8	Day 12
Control	2.77 ± 0.15^{a}	3.67 ± 0.15^{a}	5.56 ± 0.15^{a}	6.73 ± 0.20^{a}
TE (500) ppm	2.33 ± 0.06^{b}	3.10 ± 0.15^{b}	4.32 ± 0.20^{b}	5.57 ± 0.15^{b}
GE (500) ppm	2.60 ± 0.10^{b}	3.23 ± 0.10^{b}	4.44 ± 0.15^{b}	5.56 ± 0.18^{b}
TE 500ppm+ GE 500ppm	2.12 ± 0.06^{c}	2.70 ± 0.20^{c}	4.21 ± 0.25^{c}	5.12 ± 0.15^{c}
TE250ppm+ GE 250ppm	2.75 ± 0.06^{b}	3.38 ± 0.12^{b}	4.50 ± 0.18^{b}	5.85 ± 0.15^{b}

The data are presented as mean ± SD (n = 3). In the same column, different superscripts indicate the statistically significant differences between treatments at the particular storage time ($p < 0.05$). The microbiological criterion of 5.0 log CFU/g was applied to assess the microbial spoilage based on total plate count (TPC) or psychrotrophic count (PSY).

Both thyme and garlic extracts possess antibacterial properties as a result of their complementary active components. Organosulfur compounds, particularly allicin, are responsible for the ability of garlic to destroy cell membrane and block enzymes that take part in bacterial metabolism. Meanwhile, thyme phenolic compounds such as thymol and carvacrol are known for their ability to increase permeability of cell membrane and affect cellular energy metabolism. The combination of both treatments is more effective than using separate extracts (Burt, 2004; Soković et al., 2010; Shah et al., 2014). Therefore, one may conclude that the use of thyme and garlic extracts improves the microbiological qualities and shelf life of chilled chicken breast meat. The differences between the data prove that the combined treatment shows better performance of preserving microorganisms in comparison with separate extracts in the particular experimental conditions. The lowest values of TPC and PSY were noted for the 500 ppm thyme + 500 ppm garlic treatment (total 1000 ppm; 0.10%) treatment, while the 250 ppm thyme + 250 ppm garlic treatment (total 500 ppm; 0.05%) showed an intermediate effect. However, no mechanistic or synergistic interaction of extracts was determined in the present research.

3.3. Changes in pH During Chilled Storage

However, the pH value of chicken breast meat showed a consistent rise during the refrigerated storage period for all the treatment groups (see Table 5) due to the formation of products such as ammonia and biogenic amines from the metabolism by microorganisms (Rosa et al., 2013; Jay et al., 2005). The control group showed a rise in the pH value from 5.87 on day 1 to 6.19 on day 12, which is an indication of high microbial metabolism and proteolysis. These results are consistent with earlier findings that show the relationship between pH and the quality of raw poultry meat.

Table 5. Changes in pH of chicken breast meat treated with thyme and garlic extracts during chilled storage

Treatment	Day 1	Day 4	Day 8	Day 12
Control	5.87 ± 0.01 a	5.92 ± 0.01 a	6.02 ± 0.01 a	6.19 ± 0.02 a
TE (500) ppm	5.83 ± 0.02 b	5.88 ± 0.02 b	5.92 ± 0.02 b	5.98 ± 0.01 b
GE (500) ppm	5.85 ± 0.02 ab	5.89 ± 0.02 ab	5.96 ± 0.04 ab	6.03 ± 0.32 b
TE 500ppm+ GE 500ppm	5.81 ± 0.01 c	5.84 ± 0.01 c	5.90 ± 0.01 c	5.93 ± 0.02 c
TE250ppm+ GE 250ppm	5.85 ± 0.02 ab	5.90 ± 0.01 ab	5.98 ± 0.01 ab	6.17 ± 0.15 a

Values are mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among treatments at the same storage day ($p < 0.05$).

On the other hand, the samples treated with thyme and garlic extracts showed decreased pH value through the storage time ($p < 0.05$). On the twelfth day of storage, the combination of 0.10% thyme and garlic had the highest efficacy in preserving the pH value of 5.93 (Table 4). Additionally, compared with the control group, garlic and thyme extracts also slowed down the increase in pH. The regulation of pH in extract-treated samples might be associated with the low activity of microorganisms; this assumption fits with the proven antimicrobial properties of thyme and garlic components (Soković et al., 2010; Viuda-Martos et al., 2010).

There were found significant influence of treatment ($p = 0.0026$, $\eta^2 = 0.148$) and highly significant influence of storage time on the development of pH ($p < 0.001$, $\eta^2 = 0.473$) (Table 5). There was no significant interaction between treatment and time of pH development ($p = 0.616$), which means that the effectiveness of extracts in regulating pH was stable across the storage time. This result coincides with the previous researches proving that natural extracts can maintain the biochemically stability of meat, inhibiting proteolytic activity and pH elevation caused by bacteria (Shah et al., 2014; Lorenzo et al., 2018). Overall, the results show the antibacterial and anti-spoilage effects of thyme and garlic extracts, which are demonstrated by slowing down the pH elevation. The use of the extracts as natural preservative to prolong the shelf life and quality of chicken breast meat was proved by the pH regulation in extract-treated samples.

3.4. Water Retention Properties and Physicochemical Quality

The water holding capacity, whose ability depends on the stability of muscle proteins while in storage, forms an important factor in determining the quality and commercial acceptability of meat. In this study, drip loss and WHC were affected by refrigeration as both drip loss and WHC were reduced significantly in all treatment groups (Table 6). The decrease in WHC was from 73.60% to 65.70%. Control had the greatest loss in drip loss from 0.45% on the first day of storage to 2.86% on the twelfth day.

Table 6. Effect of thyme and garlic extracts on drip loss (%) of chicken breast meat during chilled storage at 4 ± 1 °C

Treatment	Day 1	Day 4	Day 8	Day 12
Control	0.45 ± 0.02 a	1.06 ± 0.02 a	1.99 ± 0.02 a	2.86 ± 0.02 a
TE (500) ppm	0.42 ± 0.02 b	0.83 ± 0.02 b	1.55 ± 0.03 b	2.07 ± 0.03 b
GE (500) ppm	0.40 ± 0.02 bc	0.81 ± 0.02 bc	1.44 ± 0.03 bc	2.06 ± 0.12 b
TE 500ppm+ GE 500ppm	0.38 ± 0.01 c	0.72 ± 0.02 c	1.43 ± 0.03 c	1.96 ± 0.02 c
TE250ppm+ GE 250ppm	0.43 ± 0.01 b	0.86 ± 0.02 b	1.59 ± 0.01 b	2.09 ± 0.01 b

Values are mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among treatments at the same storage day ($p < 0.05$). Percentage improvement was calculated relative to the control.

These results are similar to previous reports in studies on chilled chicken (Jaing et al.,2018;Honikel,1998).In contrast, WHC was higher and drip loss was significantly reduced in extract-treated samples during storage ($p<0.05$) (Table 7). The effect of thyme extract alone, garlic extract alone, and 250ppm combination treatment were intermediate, while the combination of thyme and garlic extract in 0.10% was more efficient in terms of preventing drip loss and maintaining high WHC. In particular, the minimum drip loss was recorded as 1.96% on day 12 for the 0.10% combination treatment, whereas maximum WHC was 70.37% on day 12. The extract-dependent patterns are confirmed by a highly significant treatment x time interaction ($p<0.001$) (Table 7).

It has been found that The greater WHC observed in extract-treated samples may be consistent with reduced oxidative deterioration of muscle proteins, although protein oxidation and microstructural changes were not directly measured in the present study. Reduced drip loss is directly correlated with juiciness and sensory quality of meat for consumers (Balamatsia et al., 2006).

Table 7. Effect of thyme and garlic extracts on water holding capacity (WHC %) of chicken breast meat during chilled storage at 4 ± 1 °C

Treatment	Day 1	Day 4	Day 8	Day 12
Control	73.60 ± 0.20 d	71.87 ± 0.06 d	68.23 ± 0.12 d	65.70 ± 2.14 d
TE (500) ppm	75.00 ± 0.10 b	74.00 ± 0.10 b	71.87 ± 0.15 b	69.90 ± 0.10 b
GE (500) ppm	75.23 ± 0.15 b	74.27 ± 0.15 b	72.20 ± 0.10 b	70.10 ± 0.10 b
TE 500ppm+ GE 500ppm	75.40 ± 0.10 a	74.60 ± 0.10 a	72.43 ± 0.15 a	70.37 ± 0.15 a
TE250ppm+ GE 250ppm	73.60 ± 0.44 c	71.50 ± 0.61 c	68.87 ± 0.15 c	68.07 ± 0.06 c

Values are mean ± SD (n = 3). Different letters in the same column indicate significant differences ($p < 0.05$). % improvement calculated versus control.

The findings are in accordance with previous studies related to the efficacy of natural extracts obtained from plants, especially when used in combination, for preserving the physicochemical characteristics of meat during cold storage. Studies by Shah et al. (2014) and Viuda-Martos et al. (2010) have established the use of herbs as reducing drip loss and preserving the water holding capacity (WHC) of poultry and red meats due to the synergetic effect of phenolic and organosulfur components contained in the herbs. Moreover, the increase in WHC found in extract-added samples compared to the control group is consistent with the decrease in pH value and microbial activity discussed in Sections 3.2 and 3.3.

To conclude, extracts of thyme and garlic reduced water loss and improved WHC in the course of chilled storage of chicken breast. The combination of thyme and garlic in equal quantities (at 500 ppm each, or total 1000 ppm; 0.10%) revealed the best preservation effectiveness among other applied treatment options. Since the microstructure of muscle tissue was not directly investigated in this study, it cannot be considered evidence of preservation of muscle structure.

3.5. Correlation Analysis Between Quality Parameters

Correlation coefficients depicting the relationships between different personas were calculated for all treatments and storage periods (n = number of observations). Correlation analysis was used to study the relationships between the variables associated with lipid oxidation, microbial growth, physicochemical characteristics, and water retention in chilled chicken breast meat. The strong correlations ($|r| > 0.70$) between the major quality parameters, suggesting biologically relevant relationships.

TBA correlated positively with the amount of dripping ($r = 0.523$) (Figure 1). The above-mentioned results suggest that higher values of lipid oxidation correlate with increased water loss during storage. There is evidence in the literature about the relationship between oxidative degradation and changes in the water-retention characteristics of meat. However, the relationship found in the current study does not imply causality.

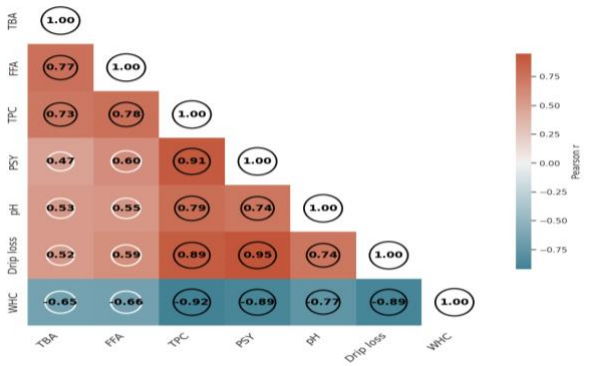


Figure 1. Heatmap of Pearson correlation coefficients among physicochemical, oxidative, and microbiological parameters of chicken breast meat during chilled storage.

The correlation between microbial load and pH depends on the extent of acidity generated by microbial metabolism during storage. There was a positive correlation between TPC and pH (TPC-pH: $r = 0.794$) and between PSY and pH (PSY-pH: $r = 0.737$). This is in agreement with the simultaneous increase in pH and microorganisms during storage. This could be because of the generation of alkaline metabolites that may occur simultaneously with microbial growth, but the metabolites were not measured in this study (Arshad et al., 2023).

In turn, TBA ($r = −0.653$), TPC ($r = −0.919$), PSY ($r = −0.888$), and pH ($r = −0.765$) showed a negative correlation with WHC. In other words, the higher the values of these indices (which indicate greater levels of

oxidative and microbiological degradation) the lower is the water holding capacity in the stored meat. Such findings are consistent with previous literature that established an association between meat oxidative damage, microbial growth, and water holding capacity. Still, the direct impact of protein oxidation and muscle structure changes could not be assessed in the current experiment NPJ Science of Food. (2024).

Positive correlations between microbial characteristics (TPC–PSY: r = 0.906) show that spoilage microorganisms evolve in unison while storing chicken meat under refrigeration conditions, which is in agreement with the earlier findings revealing simultaneous increments in the number of mesophilic and psychrotrophic bacteria during cold chain storage (Arshad et al., 2023). Dependencies between the development of microorganisms, oxidative stress, pH value, and water-holding capacity (WHC) illustrate the interrelatedness of the processes contributing to chicken flesh degradation.

Overall, correlation matrix proves the connections between the oxidation, microbiology, physicochemical, and water retention factors during chilled storage. Despite the correlations proving that all these quality factors change in concert with one another, they do not imply causality between any of the parameters. Therefore, correlation analysis proves the integral approach to understanding quality degradation, yet it is impossible to explain the biochemical and microbiological processes involved without conducting experiments.

3.6. Overall Shelf-Life Evaluation

The shelf-life estimation was based on the predefined microbiological quality criterion of 5.0 log CFU/g of TPC or PSY along with the quality changes related to oxidative and physicochemical aspects of product quality. Microbial deterioration occurred faster in the control sample without any treatments, while the treated samples showed lower microbial counts compared to the control one. The combination of 500 ppm thyme extract and 500 ppm garlic extract (total concentration 1000 ppm; 0.10%) demonstrated the lowest counts of microorganisms among all the treatment variants studied during the period of refrigerated storage.

Nonetheless, the PSY count for the 500 + 500 ppm treatment variant increased to 5.12 ± 0.15 log CFU/g on day 12. In other words, the 12-day microbiological shelf-life cannot be confirmed based on the predefined criterion of 5.0 log CFU/g. Since the microbial measurements have been conducted on days 1, 4, 8, and 12, it is impossible to define the exact day when the chosen criterion was exceeded (ICMSF, 2011; Jay et al., 2005; Viuda-Martos et al., 2020; Zhang et al., 2023). It can be concluded that both the thyme and garlic extracts delayed the microbial spoilage in comparison to the control samples under defined conditions of chilled storage.

It is necessary to point out that the microbiological measurements in this study were used to estimate the quality of the products rather than to conduct food safety evaluations directly related to the presence of pathogens in the product. In other words, exceeding the chosen criterion was considered to indicate the lowered quality of the microbiological parameter rather than the possible food safety issues. The same conclusion can be applied to the oxidative changes.

4. Conclusion

The extracts of thyme and garlic improved the oxidative, microbiological, and physicochemical characteristics of chicken breast meat during the refrigeration storage in the conditions examined in the current work. The extract treatment with a combination of 500 ppm thyme extract and 500 ppm garlic extract (the total 1000 ppm; 0.10%) had the lowest microbial levels and the least oxidative changes, which were accompanied by lower drip loss and higher water-holding capacity compared to the control group. Therefore, the extracts treatments slowed down the process of chicken breast meat deterioration during the chilled storage. Still, the microbiological data cannot prove that the shelf life of the treated meat is 12 days based on the predetermined quality criterion (5.0 log CFU/g), because the level of psychrotrophic bacteria (PSY) for the 500 + 500 ppm treatment was 5.12 ± 0.15 log CFU/g on the 12th day. Moreover, the microbiological tests in the current study can serve as quality and spoilage indicators rather than pathogens food-safety measurements. The analysis of correlations between oxidative, microbiological, physicochemical, and water retention parameters demonstrated their relationship; still, these relationships cannot indicate causality or particular molecular pathways. Further studies including sensory evaluation and pathogen-specific microbiological testing together with protein oxidation and muscle microstructure measurements could provide useful information about the shelf life and safety limits for thyme- and garlic-treated meat.

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Author contributions

The experimental work, data collection and analysis were done by Hozan. M. Anwar. The research was conducted under the supervision of Mrs. Nazdar. M. Ahmed who also gave advice on the design of the experiment and helped in the interpretation of the findings. The writing and revision of the manuscript was done by both authors.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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