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Impact of Thymus vulgaris Extract with Nano Chitosan in preserving beef meat contaminated with *Staphylococcus aureus* and *Escherichia coli O157:H7*

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Abstract

Beef is one of the most consumed types of food in many countries, whether local or imported, which requires attention to its quality and health safety. Meat and its products contaminated by various types of bacteria which are regarded as a critical concern globally due to their significant role in foodborne illnesses and zoonotic diseases. This research aims to focus on isolating and identifying bacteria *S. aureus* and *E.coli O157:H7* found in local beef meat and investigate the effect of Thymus vulgaris extract, Chitosan nanoparticles and Chitosan nanoparticles loaded with Thymus vulgaris on preserving meat from microbial spoilage due to study the bacterial load and other quality parameters of beef meat under refrigerator temperature at different times 0, 2, 5, 7 and 10 days of storage. This work was carried out from January to October 2025. A total of 100 beef meat samples were collected and cultured on specialized media to isolate and identify bacteria and confirm using the polymerase chain reaction (PCR) method to detect *S. aureus* and E. coli O157:H7. The results of this study identified *S. aureus* in 20 out of 100 (20%) beef meat samples collected, while identified *Escherichia coli O157:H7* in 13 out of 100 (13%) beef meat samples collected. Thymus extract and Chitosan nanoparticles demonstrated high effectiveness in preserving the quality of meat during significantly ($p \leq 0.05$) reduced the total bacterial count, stabilized pH values within normal limits. Also, reduced the Thio barbituric acid reductase levels. In conclusion, Thymus vulgaris extract and Chitosan nanoparticles have ability to inhibit the growth of (*S. aureus* and E. coli O157:H7) and reflecting their role as effective antioxidants. Also, the ability to preserve the meat at refrigerator temperature.

Key words: Thymus, *S. aureus*, E. *coli*, beef meat, Chitosan, nanoparticles

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Introduction

Microbial contamination by *S.aureus* is a significant challenge for the meat manufacturing sector because it accelerates the spoilage of raw meat and meat products. *S. aureus* is Gram-positive bacteria potentially found in foods due to environmental, human, and animal contamination. and it is considered as an important infectious pathogen in health sector and communities (*Thwala et al*., 2021). Annually, numerous individuals suffer from consuming food contaminated with pathogenic E. coli, leading to food poisoning and affecting the food processing, health, and agricultural sectors (*Oliveira et al.,* 2023).

Chitosan, a distinctive biomaterial, has shown potential as an antimicrobial packaging solution, meeting the growing need for safe and eco-friendly packaging (*Zhou et al.,* 2023). The food industry has experienced substantial growth in nanotechnology applications recently (*Tiwari et al.,* 2023).

Thymus vulgaris is recognized as a highly beneficial medicinal plant due to its ability to kill and inhibit the growth of numerous microorganisms. The antimicrobial activity of thyme oil is primarily attributed to its significant concentration of phenolic compounds, including carvacrol and Thymol (*Rota et al.,* 2008). Thyme (Thymus vulgaris) is a herbaceous annual plant found in various regions globally. Known as "Thyme," Thymus vulgaris has been utilized for its culinary, flavoring, and medicinal benefits for centuries. The term thyme comes from the Greek word ‘Thymos,’ which translates to courage or strength. During the first century AD, Thyme was primarily recognized for its medicinal qualities, as noted in the writings of Dioscorides. In the Mediterranean area, it was predominantly used as a spice before making its way around the world (Stahl-Biskup and Venskutonis, 2012). The aim of this study the evolution potential effect of nano Chitosan loaded with Thymus vulgaris extract as preservative materials against bacterial contamination isolates from beef meat with increasing the shelf life.

Materials and Methods

Samples collection and processing

Total of one hundred meat samples were collected randomly from different Baquba areas in Iraq and district during the period of January to October 2025. All samples of meat distribution such that all fresh beef meat was collected exclusively from butchers’ shops of all districts and sub-districts of Baquba city. The collected meat samples are immediately transported to the laboratory in a vacuum-sealed container packed with ice. In the laboratory, the collected meat samples 25 g of the sample was added to 225 ml TSB (Trypton soy broth). It is placed in a special container, and then the samples are placed in an incubator at 37°C for 24 h and then cultured on the appropriate media.

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Culture Media Preparation: All culture media for *S. aureus* (blood agar and mannitol Salt agar) and for *E. coli O157:H7* (Hicrome™ agar selective media *E. coli O157:H7* and Sorbitol MacConkey agar) were produced in accordance with the company's manufacture instructions, and they were autoclaved at 121 Co. for 15 minutes at a weight of 15 lb per inch2. For the sensitivity test Muller Hinton agar was employed. As directed by the manufacturer, 38 grams of powder were dissolved in one liter of distillation water, autoclaved for fifteen minutes at 121 degrees Celsius, cooled to 45 degrees Celsius, and then stored in a water bath until used (*Wambui et al*., 2018). A single new colony of suspected *E. coli O157:H7* from the SMAC agar was chosen.

Bacterial isolation and identification: *S. aureus* and *E. Coli O157:H7* colonies that were found to be distinct on selective media were closely inspected macroscopically to determine their color, shape, and consistency by using the proper culture conditions, Gram staining, and biochemical assays in accordance with accepted practices. The VITEK2 system was used to confirm bacterial identification for conclusive bacteriology (*Pellissery et al.*, 2020). The typical procedure for examining isolates calls for 0.50 McFarland inoculums. A single loop of culture from the stock agar was cultivated on agar for 24 hours; the bacterial suspension was made with regular saline and contained roughly 1-2 $\times 10^6$ CFU/ml. In a sterile tube set on a specific stand, a single, pure colony of bacteria was extracted and diluted in three milliliters of saline solution. The VITEC 2 DENSICHEK was used to measure the solution in order to determine that the turbidity was equal to the McFarland constant of 0.5, or x 1.5 cells/ml 10^6. The stuck was placed in the gram-negative bacteria VITEK 2 cassette. After inserting the VITEK 2 cassette into the apparatus, 64 biochemical assays were used to diagnose the bacterium**.**

PCR Method to Detect *S. aureus* and *E. Coli O157:H7*.

PCR approach has been used to detect *S. aureus* and *E. Coli O157:H7* from meat samples. Bacterial DNA was extracted from the obtained isolates using standard genomic DNA extraction procedures. The presence of bacterial 16S rRNA gene was detected using the polymerase chain reaction (PCR) technique. Specific primers targeting the 16S rRNA gene were used to amplify the target DNA fragments. After electrophoresis, the gel was stained and visualized under UV illumination. The expected amplicon sizes were 141 bp for Staphylococcus aureus and 850 bp for *E. Coli O157:H7* (*Wei et al.*, 2018).

Preparation of Thymus vulgaris extract:

Thyme (Thymus vulgaris) was ground and stored at a lab temperature until needed. To make an aqueous extract, 40 grams of thyme powder were added to a conical flask that held 200 cm3 of distilled water. The mixture was then combined using a magnetic blender for 30 min and centrifuged for 15 minutes. The solution is then kept in an electric furnace at 35 °C until the extract is obtained. (*Fayad et al*., 2013).

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Preparation of Chitosan nanoparticles:

Using deionized distilled water (DDW) to dissolve Chitosan and then treated with an ultrasonic bath for half an hour to produce nanomaterial via the Solution Gelation (Sol-Gel) method with specialized alterations. NaOH was used to raise the pH to 12, and the rotor was magnetically spun for an hour at the ambient temperature. After using hydrochloric acid (HCL) to get the pH down to 4, the mixture was agitated for an hour at room temperature using a magnetic stirrer. The solution was agitated for 60 minutes at room temperature on a rotor using a magnetic stirrer after the pH was adjusted to 7 with NaOH (*Ghadi et al*., 2014).

Preparation of Chitosan nanoparticles loaded with Thymus vulgaris: According to Agarwal et al. (2018), gel was added to the CNPs solution at a molar ratio of 1:1, swirled for approximately an hour at room temperature, and then ultrasonicated for a total of forty five minutes.

Impact of extract on bacteria and quality parameters of meat: After isolating and identifying the bacteria, 80 meat samples were collected from local markets and divided into four groups of 20 samples each. The first group was considered the control group and was contaminated with bacteria and left untreated. 2nd group (20) samples of beef meat were contaminated with *S. aureus* and treated with Nano chitosan by dipping for 5 minute. 3rd group (20) samples of beef meat were contaminated with *S. aureus* and treated with thyme extract by dipping for 5 minutes, while 4th group (20) samples of beef meat were contaminated with *S. aureus* and treated with Nano chitosan and thyme extract by dipping for 5 min, then studying the Bacterial load and other quality parameters under refrigerator temperature at different times 0, 2, 5, 7 and 10 days. The quality parameters included pH, **TVB-N,** TBARs, Hydrogen Peroxide value of meat and Total bacterial count. The same experiment was repeated with the same number of samples to study the effect on *E. coli* bacteria in order to compare the effects of the substances on the two bacterial species.

Statistical analysis: Data were calculated by SPSS for windows TM version 24.0. Statistical analysis of data was performed using T test (Steel and Torrie, 1980).

RESULTS

Isolation of *Staphylococcus aureus* and *E.coli*:

In this study, the findings of the cultural investigation of 100 samples of beef meat were collected from local markets in the same previous region. All samples of fresh beef meat were taken from the thigh of carcasses yielded results for *Staphylococcus aureus* and E.coli species as shown in tables 1. Meat can harbor a variety of infections, depending on the health of the animals and the hygienic conditions of the meat processing facilities. According to Ijaz *et al*. (2012), these pathogens can enter the food chain through direct animal infection or contamination during the

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handling, processing, and retailing of meat as a result of unsanitary surroundings and inadequate personal hygiene.

Table 1: percentage of contamination with *Staph. aureus* and *E. coli* in beef meat

sample	Total	Positive	Negative
E. coli	100	20 (20%)	80 (80%)
Staph. aureus	100	13 (13%)	87 (87%)

Isolation and Identification of Bacterial:

Bacteriological culturing demonstrated a range of morphological forms and hues. The samples were cultured and analyzed using suitable culture medium, followed by the identification of *Staph. aureus*.

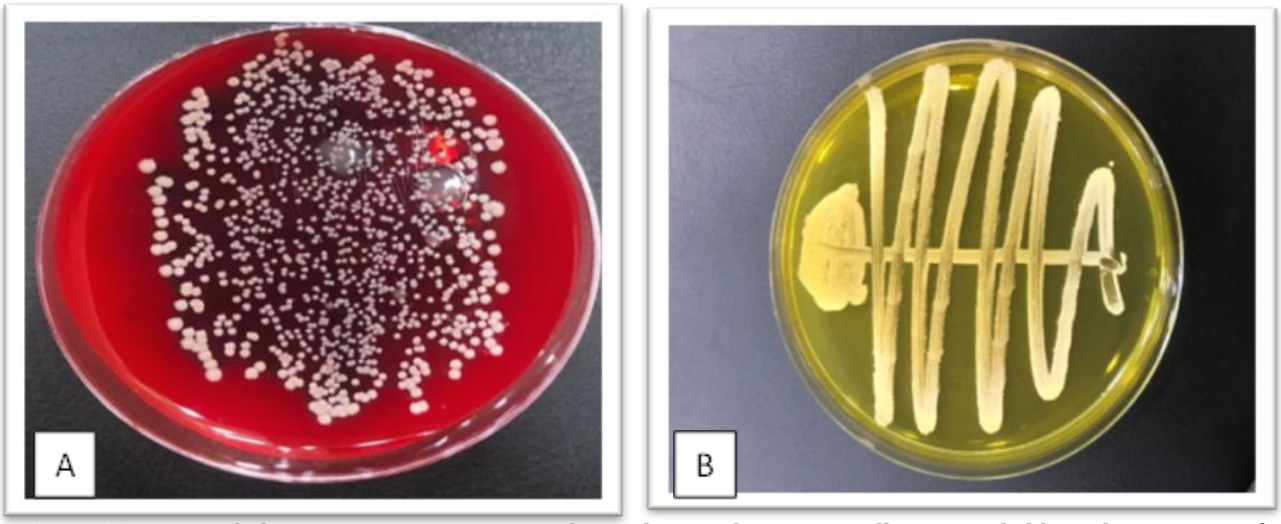

Figure (2): A- *Staphylococcus aureus* appear as white colonies, characteristically surrounded by a distinct zone of beta-hemolysis (clear, complete destruction of red blood cells) B- The *Staphylococcus aureus* on mannitol agar, colonies appeared yellow, indicating mannitol fermentation

These *S. aureus* isolates were originally cultivated on blood agar and mannitol salt agar plates under aerobic conditions. Also, *E. coli* isolates were originally cultivated on blood agar and MacConkey agar plates under aerobic conditions.(Figure 2, 3 and 4).

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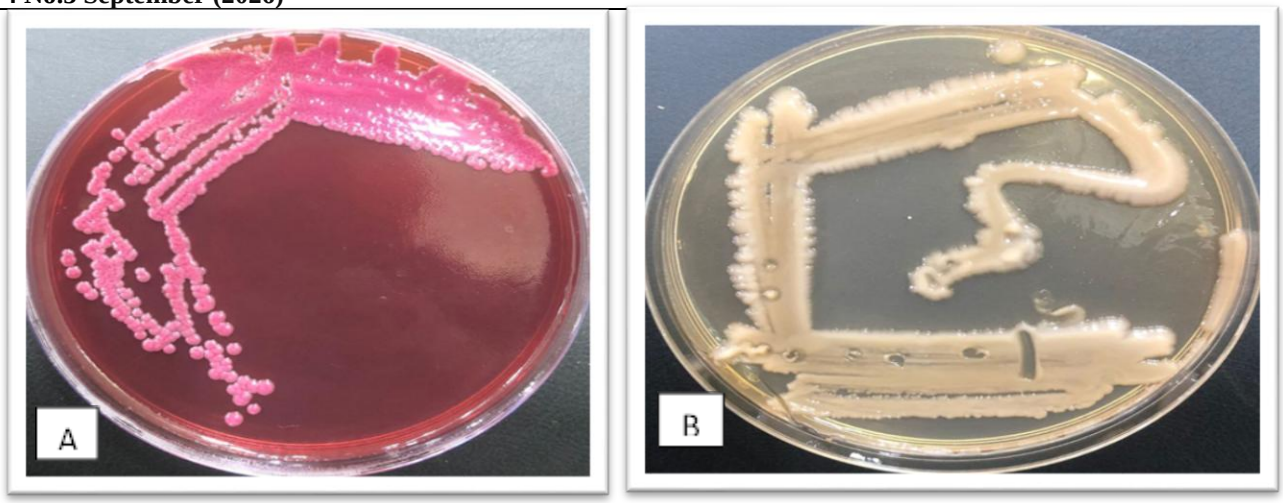

Figure 3: *E. coli O157:H7* on A- MacConkey agar EC O157:H7 as pink to red colonies B- Sorbitol agar EC

Figure 4: *E. coli O157:H7* on Hicrome™ EC O157:H7 Selective Agar as pink to red colonies

Polymerase Chain Reaction (PCR):

Twenty *Staph. aureus* and *E. coli O157:H7* isolates had their genomic DNA effectively retrieved. The isolated genomic DNA was 100% visible when the DNA bands were found on an agarose gel and examined under a UV transilluminator. The 16sRNA gene, a putative pathogenic factor, was found in S. aureus using polymerase chain reaction (PCR) in the present investigation. The 16sRNA gene was serially amplified in each of the 20 positive samples. The presence of the gene was verified by electrophoresis on a 1.5% agarose gel, which produced a clear band with a

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molecular size of 141 bp for S. aureus and 850 bp for Escherichia coli *O157:H7* (figure 3). Likewise, this gene was also isolated by Wang et al., (2015) in most samples of food productions.

Figure (5): Agarose gel electrophoresis (1.5% agarose,7 v/cm^2 for 60 min) for *16sRNA* gene (850 bp amplicon). lane M represent 100bp DNA Ladder, lanes 1-5 represent *E. coli* bands , Lan 6 represent *S. aureus* e bands. lane NC represent Negative control, lanes SN1 represent *S. aureus* positive bands *16sRNA* gene (141 bp amplicon)isolated from meat.

TBARs results of contamination meat with *S. aureus* at refrigerator temperature:

The results as showed in table (2) presents the effect of Thymus vulgaris extract, chitosan nanoparticles and chitosan nanoparticles loaded with thymus vulgaris on the Thiobarbituric acid (TBAs) value of meat during refrigerator storage. There are significant differences in control groups at day 2 between two types of bacteria. The thiobarbituric acid values were significant lower ($p < 0.05$) in groups of chitosan nanoparticles loaded with Thymus vulgaris (G4), chitosan nanoparticles (G3) and Thymus vulgaris extract (G2) at all periods of experiment in refrigeration temperature. At ten days of storage, the lowest TBA values were recorded in group of chitosan nanoparticles loaded with Thymus vulgaris (G4) compared with all treatment groups at values of (0.57 ± 0.13) Malonaldehyde (MDA) mg/kg, while the highest TBAs values were shown in G1 at (1.53 ± 0.16) mg MDA/kg.

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Table 2: Compare TBARs results of contamination meat with *Staph. aureus and E. coli* at refrigerator temperature

Groups	Time bacteria	0 day	2 days	5 days	7 days	10 days
G1	*E. coli*	0.28 ± 0.12 A	0.35 ± 0.17 B	0.88 ± 0.09 A	1.20 ± 0.28 A	1.45 ± 0.38 A
	Staph. aureus	0.34 ± 0.08 A	0.67 ± 0.03 A	0.94 ± 0.14 A	1.33 ± 0.15 A	1.53 ± 0.16 A
G2	*E.coli*	0.30 ± 0.14 B	0.37 ± 0.04 B	0.45 ± 0.06 B	0.56 ± 0.17 A	0.72 ± 0.22 B
	Staph. aureus	0.40 ± 0.07 A	0.44 ± 0.05 A	0.56 ± 0.13 A	0.61 ± 0.14 A	0.82 ± 0.15 A
G3	*E. coli*	0.25 ± 0.07 B	0.37 ± 0.08 B	0.54 ± 0.16 A	0.70 ± 0.26 A	0.81 ± 0.30 B
	Staph. aureus	0.32 ± 0.12 A	0.44 ± 0.12 A	0.53 ± 0.09 A	0.67 ± 0.12 A	0.90 ± 0.08 A
G4	*E. coli*	0.26 ± 0.10 B	0.32 ± 0.15 B	0.43 ± 0.21 A	0.45 ± 0.09 B	0.57 ± 0.13 B
	Staph. aureus	0.37 ± 0.06 A	0.42 ± 0.11 A	0.50 ± 0.12 A	0.65 ± 0.10 A	0.75 ± 0.19 A

The averages that carry a different capital letter in the same group between the two types of bacteria differ greatly (p. < 0.05).G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus

Total bacterial count of contamination meat with *Staph. aureus* and *E. coli* at refrigerator temperature:

Accordingly to the results in table (3) the bacterial count of meat stored at refrigerator temperature for 0, 2, 5, 7 and 10 days. The mean of Total Bacterial Count (TBC) in the control group (untreated meat) samples was initially recorded as 4.15 ± 1.76 log CFU/g and 2.73 ± 1.20 log CFU/g in meat contaminated with E.coli and S. aureus respectively. These counts increased through the preservative period, reaching 13.36 ± 4.56 log CFU/g and 14.35 ± 3.09 log CFU/g. after 10 days of experiment in meat contaminated with E.coli and S. aureus respectively.

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Table 3: Compare bacterial count results of contamination meat with *Staph. aureus* and *E.coli* at refrigerator temperature

Groups	Time bacteria	0 day	2 days	5 days	7 days	10 days
G1	*E. coli*	2.73 ± 1.20 B	6.30 ± 0.86 A	8.79 ± 1.13 A	11.48 ± 3.83 A	13.36 ± 4.56 A
	Staph. aureus	4.15 ± 1.76 A	6.10 ± 2.94 A	7.49 ± 2.73 B	10.51 ± 4.19 A	14.35 ± 3.09 A
G2	*E. coli*	2.45 ± 0.89 B	4.61 ± 1.48 A	ND	ND	ND
	Staph. aureus	3.82 ± 0.27 A	4.37 ± 1.35 A	2.16 ± 1.20	0.21 ± 0.03	ND
G3	*E. coli*	2.64 ± 1.68 B	4.43 ± 1.55 B	1.51 ± 0.61 B	1.22 ± 0.76 B	ND
	Staph. aureus	4.17 ± 2.04 A	5.85 ± 2,03 A	4.03 ± 0.47 A	3.06 ± 0.45 A	1.50 ± 0.80
G4	*E. coli*	2.55 ± 1.12 B	3.17 ± 0.75 B	ND	ND	ND
	Staph. aureus	3.65 ± 1.08 A	4.81 ± 0.29 A	2.20 ± 0.83	0.92 ± 0.07	ND
The averages that carry a different capital letter in the same group between the two types of bacteria differ greatly (p. < 0.05).G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus						

TVB-N of contamination meat with *Staph. aureus:*

The results as showed in table (4) presents the effect of Thymus vulgaris extract, chitosan nanoparticles and chitosan nanoparticles loaded with thymus vulgaris on the TVB-N values of meat during refrigerator storage. There are significant differences in control groups at day 7 and 10 between two types of bacteria. The TVB-N values were significant lower ($p < 0.05$) in groups of chitosan nanoparticles loaded with Thymus vulgaris (G4) and Thymus vulgaris extract (G2) at all periods of experiment in refrigeration temperature.

At ten days of storage, the lowest TVB-N values were recorded in group of chitosan nanoparticles loaded with Thymus vulgaris (G4) compared with all treatment groups at values of (4.70 ± 0.29) while the highest TVB-N values were shown in G1 at (8.56 ± 1.08).

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Table 4: Compare TVB-N result of contamination meat with *S.aureus* and *E.coli* at refrigerator temperature

Groups	Time bacteria	0 day	2 days	5 days	7 days	10 days
G1	*E. coli*	3.93 ± 0.31 A	4.85 ± 0.77 A	5.73 ± 0.75 A	7.26 ± 1.17 A	8.56 ± 1.08 A
	Staph. aureus	3,72 ± 0.22 A	4.22 ± 1.03 A	5.42 ± 0.88 A	6.71 ± 1.29 B	7.96 ± 1.62 B
G2	*E. coli*	3.84 ± 0.47 A	4.65 ± 0.52 A	4.69± 0.22 A	4.85 ± 0.57 B	5.08 ± 0.46 A
	Staph. aureus	3.79± 0.45 A	4.10 ± 1.12 A	4.51 ± 0.93 A	5.20 ± 0.58 A	5.25 ± 0.34 A
G3	*E. coli*	3.81 ± 0.84 A	4.73 ± 0.63 A	5.21 ± 0.72 A	5..98 ± 0.18 A	6.34 ± 0.83 A
	Staph. aureus	3.88 ± 0.19 A	4.13 ± 0,49 A	4.89 ± 0.55 A	5.93 ± 0.95 A	6.82 ± 0.72 A
G4	*E. coli*	3.97 ± 0.25 A	4.28 ± 0.19 A	4.39 ± 0.92 A	4.47 ± 0.48 B	4.70 ± 0.29 A
	Staph. aureus	3.65 ± 0.32 A	3.85 ± 0.67 A	4.33 ± 0.64 A	5.11 ± 0.81 A	5.15 ± 0.43 A
The averages that carry a different capital letter in the same group between the two types of bacteria differ greatly (p. < 0.05).G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus						

pH result of contaminated meat with *E. coli* and *Staph. aureus* respectively:

The results in table (5) referred to the pH change in meat samples at refrigerator storage. All groups exhibited the lowest significant differences in pH value after 10 days of storage in contaminated meat with E.coli compare with pH value in contaminated meat with *Staph. aureus.*

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Table 5: Compare pH result of contamination meat with *Staph. aureus* and *E. coli* at refrigerator temperature

Groups	Time bacteria	0 day	2 days	5 days	7 days	10 days
G1	*E. coli*	5.83 ± 0.29 A	6.49± 0.84 B	6.96 ± 0.33 A	7.08 ± 0.87 A	7.19 ± 0.55 B
	Staph. aureus	5.55 ± 0.16 A	5.75 ± 0.20 A	6.99 ± 0.91 A	7.35 ± 0.56 A	8.10 ± 0.76 A
G2	*E. coli*	5.45 ± 0.23 A	5.57 ± 0.33 A	5.70 ± 0.74 A	5.72 ± 0.72 A	5.90 ± 0.08 A
	Staph. aureus	5.69 ± 0.56 A	5.73 ± 0.92 A	5.99 ± 0.38 A	6.07 ± 0.86 A	6.12 ± 0.14 B
G3	*E. coli*	5.51 ± 0.84 A	5.60 ± 0.34 A	5.90 ± 0.45 A	6.35 ± 0.33 A	6.63 ± 0.35 A
	Staph. aureus	5.52 ± 0.18 A	5.64 ± 0.14 A	5.89 ± 0.59 A	6.16 ± 0.38 A	6.24 ± 0.17 B
G4	*E. coli*	5,61 ± 0.15 A	5.65 ± 0.83 A	5.70 ± 0.28 A	5.67 ± 0.62 B	5.72 ± 0.79 B
	Staph. aureus	5.65 ± 0.15 A	5.72 ± 0.62 A	5.81 ± 0.29 A	5.95 ± 0.71 A	6.08 ± 0.24 A
The averages that carry a different capital letter in the same group between the two types of bacteria differ greatly (p. < 0.05).G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus						

DISCUSSION

During the slaughter, carcass deboning and cutting processes, the raw beef meat can be contaminated by different microorganisms. The microbial contamination of the freshly cut meat as well as its composition and diversity are dependent on both the microbial ecosystem of the host organism and hygiene practices during slaughter, carcass deboning and cutting processes (Han *et al.*, 2024). The storage conditions of raw and processed meat have been abundantly studied in the past years and until now in order to prevent their early spoilage and contamination by microbes (Oh & Lee, 2024 and Odeyemi *et al.*, 2020), Spoilage occurs when contaminating microorganisms reach a high level to induce enough organoleptic changes on the tissue,(Hultman *et al.*, 2015).

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The type of meat, how it is processed, how it is distributed, and how it is stored all have a significant impact on the microbiological quality of post-slaughter meat. It is possible for spoilage associated bacteria to cross contaminate contaminated slaughter equipment, workers, and ambient elements (such as soil, water, and air) (Wambui *et al.*, 2018). This disease can infect people all across the world by spreading through water supplies (Mogessie et al., 2024). Meat is a perfect medium for the growth and spread of both pathogenic and non-pathogenic microbes since it is high in proteins, lipids, carbs, vitamins, and pigments.

The Thiobarbituric acid (TBAs) analysis is utilized to assess the extent of lipid oxidation by measuring the secondary oxidative product, malonaldehyde (MDA). The interaction of MDA and thiobarbituric acid produces a red color, which is detectable calorimetrically. MDA is one of the most extensively researched intermediate products of meat decomposition because of its pathogenic and carcinogenic properties resulting from its interaction with DNA and proteins (Chi *et al.,* 2020).

The research that has been done thus far shows that adding thyme's natural antioxidants directly can prevent many meals from becoming rancid. In this regard, thyme's preservation qualities have been documented in a number of studies (Nieto, 2020). In Seven and Ten days of the preservative period, the highest TBA levels were shown in G1, while by day 10, significant differences were observed across all groups.

Chitosan is frequently utilized as an antibacterial agent, either alone or in combination with other natural polymers, due to its high biodegradability, nontoxicity, and antimicrobial qualities (Kong et al., 2010). Also, Chitosan and chitosan derivatives can kill microbes by neutralizing negative charges on the microbial surface. Additionally, chemical changes improve the water solubility and antibacterial properties of chitosan derivatives (Yan *et al.,* 2021).

The total bacterial counts of E.coli were demonstrated a significant decrease in the meat samples treated with Thymus vulgaris extract, nanoparticle chitosan and nanoparticle chitosan loaded Thymus extract compare with The total bacterial counts of S. aureus. Chitosan-based films are helpful for packaging perishable food items because they may increase shelf life by reducing microbial contamination because chitosan's antibacterial properties naturally restrict the growth of germs. The film's mechanical strength and flexibility can be altered by adding new ingredients or changing the chitosan content. Chitosan coatings can be applied to surfaces to enhance characteristics like water resistance, barrier properties, and activity against bacteria (Manaswini *et al.,* 2024).

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Furthermore, bacterial growth disappeared by day five and throughout the end of the experiment in meat contaminated with E.coli treated with Thymus vulgaris extract and nanoparticle chitosan loaded Thymus extract. Nanotechnology has been utilized as a novel method to enhance the quality and safety of food due to the growing need for safe and clean products (Chaudhry et al., 2008). Nanotechnology can enhance both the quantity and quality of meat and meat products while also improving their hygiene and safety. It can achieve this by extending food shelf life and inhibiting microbial contamination more effectively than traditional methods (Duncan et al., 2011). Chitosan and Thymus vulgaris (thyme) extract are combined in various applications to leverage their combined antimicrobial, antioxidant, and other beneficial properties. These combinations are used to create active food packaging films, enhance crop resilience against stress, and develop nanoparticles for drug delivery and other biotechnological uses. Research has shown that this combination is effective against bacteria and fungi, improves plant growth, and can increase the quality and yield of thyme essential oil (Kamalipooya *et al.,* 2025). Because of its antimicrobial action, chitosan can fight against food borne pathogens that affect the shelf life and quality of the food (Bastiaens *et al.,* 2019 and Shin *et al.,* 2019).

The deacetylated form of chitin, known as chitosan, is one of the most prevalent polymers. Because of its qualities, chitosan, either by itself or in conjunction with bioactive materials, is becoming more and more popular for use in the food industry to produce biodegradable films and coatings. A wide range of plant extracts have been added to chitosan to improve its antibacterial and antioxidant qualities in order to satisfy customer expectations for foods free of artificial preservatives and more environmentally friendly (Muñoz-Tebar *et al.,* 2023).

The characteristics of the food (to which thyme is added) such as its composition, storage circumstances, and microbe types can affect the bioactivity of components and their preservation qualities. The presence of interfering substances, such as antioxidants, preservatives, additives, and nutrients in meals, can also lower the microorganism's bioactivity. All of these factors are connected to their preservation qualities. Thyme's ability to stabilize lipids in meat and meat products has been the subject of several researches. Thyme EO was added to minced beef by Harpaz et al., 2003 in order to assess its antioxidant capacity; consequently, they observed a reduction in oxidation in the supplemented samples when compared to control samples. As mentioned earlier, thyme's antibacterial and antioxidant properties have been demonstrated in vitro (Peshawa *et al.,* 2025) and by its direct use in animal products, including veal (Skandamis et al., 2002) and lamb (Moñino *et al.,* 2008). Thyme extract can be used to improve functional foods and as a supplemental food preservative in food systems such milk products, dressings, meat, and oils. Other factors, such thyme's non-toxicity, accessibility, and affordability, are also significant and support its use in the food business. These chemicals are classified as GRAS (generally regarded

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as safe) by the FDA (United States Food and Drug Administration). Vaccines, probiotics, antimicrobial organic acids, and medicinal plants with antibacterial qualities are some of the potential strategies that have been studied (Kathayat *et al.,* 2021). Among these, therapeutic plants like common thyme (Thymus vulgaris) seem especially promising. Thyme's antibacterial and anti-inflammatory properties have made it valuable in conventional medical treatment for generations (Halat *et al.,* 2022).

Lipid oxidation processes occur through a process of free radical reactions, including reactive oxygen species (ROS) and fatty acid compounds, resulting in a type of oxidative breakdown known as rancidity. In the final analysis of data in table (5) showed the effect of Thymus extract, chitosan nanoparticles loaded with Thymus extract and chitosan nanoparticles on meats that Significant effects ($p. \leq 0.05$) in TVB-N were observed in all groups on the second days to final day of storage.

Nanomaterials are utilized in meat products to preserve sensory quality, avoid oxidation, eradicate pathogenic bacteria, and enhance flavor. Multiple natural extracts have been used to protect meat products (Lohith and Sarkar, 2018). Nanotechnology inhibits the pathogen microbe in several ways, including damage to cell membrane integrity, damage to DNA as well as transcriptional and translational function, ribosome dysfunction in protein synthesis, inducing reactive oxygen species (ROS), and interfering with mitochondrial function (Tri *et al.,* 2022). The results showed TVB-N values in all samples containing the extract and Chitosan, showing the efficacy of. TVB-N and. TVB-N loaded with Thymus extract in preserving meat.

Food's immediate composition is crucial, and there are a number of interactions of phenolic compounds and protein that lessen the function of bioactive compounds. Similarly, the content of fat has been shown to have a protective effect on bacteria (Liu *et al.,* 2020). Furthermore, because dietary pH promotes the solubilization of hydrophobic essential oils, it affects these substances (Donsi *et al.,* 2010).

The pH of the untreated (control) meat showed an increased significantly ($P \leq 0.05$) on days 5, 7 and 10 compared with the pH level in meat samples contaminated meat with E.coli and S. aureus and treated with (G2, G3 and G4) that was decreased. CNPs and LNPs help maintain a more stable pH over time in meat by preventing excessive microbial growth and enzymatic activities that would otherwise lead to the production of alkaline compounds, thereby helping maintain a more pH stable (Ge *et al.,* 2024).

Thymus vulgaris (thyme) extract has antimicrobial effects against both Gram-positive and Gram-negative bacteria, although some studies indicate a stronger effect on Gram-positive bacteria. Its

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essential oil, particularly components like thymol, can kill bacteria and inhibit their growth, with some research showing significant antibacterial activity against both types of bacteria in laboratory settings. Studies have evaluated its effectiveness in inhibiting the growth of various bacteria including Staphylococcus aureus, Streptococcus pneumoniae and Escherichia coli (Peshawa and Shokhan *et al.*, 2022).

CONCLUSIONS:

In conclusion Thymus vulgaris extract might be provided as an efficient and effective antibacterial agent against gram-positive, furthermore increasing the storage period of meat sample dipping by Thymus vulgaris extract contrasted to samples without treatment. Also, evidence illustrates that CNPs have good biological activities, including antibacterial activity, and have demonstrated good potential in the field of meat preservation. The effective antibacterial agent of extract against *E. coli* more than *S. aureus.*

Ethical approval: The study protocol was approved by the College of Veterinary Medicine, University of Baghdad, Iraq.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest

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