GMR
The Original

THE IMPACT OF THYMUS VULGARIS EXTRACT WITH NANO CHITOSAN IN PRESERVING BEEF MEAT CONTAMINATED WITH STAPHYLOCOCCUS AUREUS

Maysaa Gheni Taher[1], Mushtaq Talib Abdulwahid[2]

[1]Department of pathology, College of Medicine, University of Diyala. Baqubah, Iraq.
[2] Department of Public Health, College of Veterinary Medicine, University of Baghdad. Baghdad, Iraq.

ABSTRACT
Beef meat is one of the most consumed types in many countries, whether local or imported, which requires attention to its quality and health safety. These organisms are of critical concern globally due to their significant role in foodborne illnesses and zoonotic diseases. This research aims to focus on isolating and identifying bacteria *Staphylococcus aureus* 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 2025 to October 2025. A total of 100 beef meat samples were collected in various regions of Baquba city and cultured on specialized media to isolate and identify bacteria, then PCR method to Detect S. aureus. This study identified *Staphylococcus aureus* in 20 out of 100 (20%) 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 Thiobarbituric acid reductase levels. In conclusion, Thymus vulgaris extract and Chitosan nanoparticles have ability to inhibit the growth of spoilage microbes (S. aureus) and reflecting their role as effective antioxidants. Also, the ability to preserve the meat at refrigerator temperature.

KEYWORDS: Thymus, Staphylococcus, beef meat, Chitosan, nanoparticles.

1. INTRODUCTION

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. Microbial contamination by *Staphylococcus 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. Also, important infectious pathogen in health sector and communities [1].

Chitosan, a distinctive biomaterial, has shown potential as an antimicrobial packaging solution, meeting the growing need for safe and eco-friendly packaging [2]. The food industry has experienced substantial growth in nanotechnology applications recently [3].

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 [4]. 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 [5]

2. MATERIALS AND METHODS

Area of the Study: This study was conducted in Baqubah city/ Diyala province.

Samples collection and processing

A total of one hundred meat samples were collected randomly from different districts of Baquba areas during the period of January 2025 to October 2025. All samples of meat distribution such that all fresh meat was collected exclusively from butchers’ shops of all 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 hours and then cultured on the appropriate media.

Culture Media Preparation: All culture media (blood agar and mannitol salt 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 C°, cooled to 45 C°, and then stored in a water bath until used [6].

Bacterial isolation and identification: *S. aureus* colonies that were found to be distinct on blood agar and mannitol salt agar 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 [7].
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:
PCR approach has been used to detect S. aureus 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 [8]

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 minutes and centrifuged for 15 minutes. The solution is then kept in an electric furnace at 35 °C until the extract is obtained.[9].

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 [10].
In addition, Zeta- potential test and Scanning Electron Microscopy (SEM) were done to examine the morphology and particle surface charge of the prepared chitosan nanoparticles. These tests were conducted for nano-chitosan in Department of Science and Technology at the Ministry of Higher Education and Scientific Research according to Jha and Mayanovic (2023) [11]..

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 minutes, 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.

Statistical Analysis: SPSS software (version 24.0, IBM SPSS Inc., Chicago: USA) was used for all statistical computations. The normal distribution of the features under investigation was verified using the Shapiro-Wilk test. All data for treatments for each attribute or time were examined using a complete randomized approach. Additionally, the probability threshold $P < 0.05$ was used to determine the least significant difference between group means [12].

RESULTS AND 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 [13].

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[14].

Isolation of *Staphylococcus aureus*:

In this study, the findings of the cultural investigation of 100 samples of beef meat were collected from local markets in the same previously mentioned districts. All samples of fresh beef meat were taken from the thigh of carcasses yielded results for *Staphylococcus aureus* 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 Ali and Alsayeqh (2022),[15] these pathogens can enter the food chain through direct animal infection or contamination during the handling, processing, and retailing of meat as a result of unsanitary surroundings and inadequate personal hygiene.

Table: 1. percentage of contamination with S. aureus in beef meat

sample	Total	Positive
Meat	100	20 (20%)

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 *Staphylococcus aureus*. These *S. aureus* isolates were originally cultivated on blood agar and mannitol salt agar plates under aerobic conditions (Figure 1).

Figure (1): 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

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) [16]. This disease can infect people all across the world by spreading through water supplies [17]. 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.

Polymerase Chain Reaction (PCR):

Twenty S. aureus 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 trans illuminator. 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 molecular size of 141 bp (figure 2). Likewise, this gene was also isolated by Wang et al., (2015) [18]in most samples of food productions.

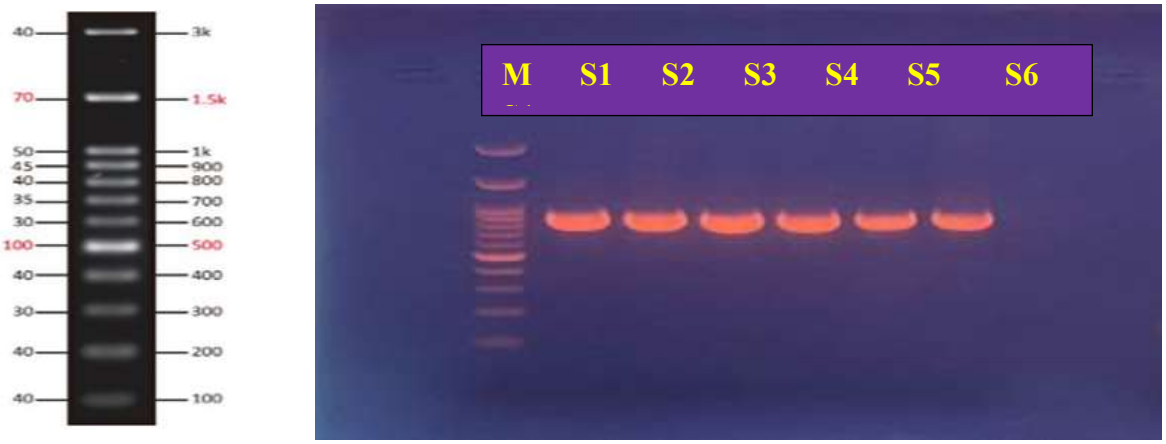

Figure (2): Agarose gel electrophoresis (1.5% agarose,7v/cm$^{2\ for}$ 60 min) for *16sRNA* gene (141 bp amplicon). lane M represent 100bp DNA Ladder, Lane 1 - 6 represent *S. aureus* isolated from meat

Zeta- Potential, Morphology and Practical Size of Nano-Chitosan

Morphology and surface the scanning characteristics of the prepared chitosan nanoparticles and the particle surface charge and stability in aqueous solutions were determined by Electron Microscopy (SEM) and Zeta- potential (figure 3 and 4)

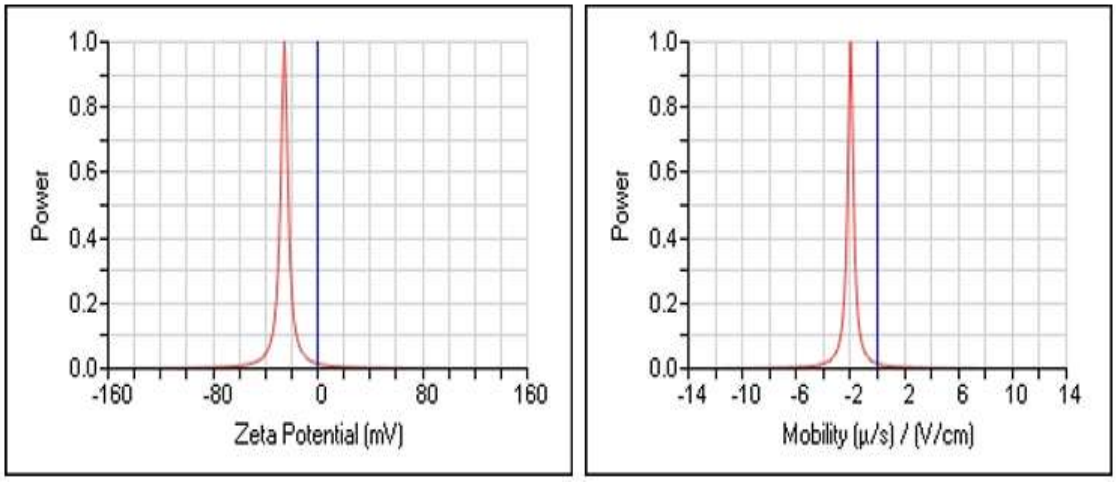

Figure (3): Zeta- potential test and Mobility

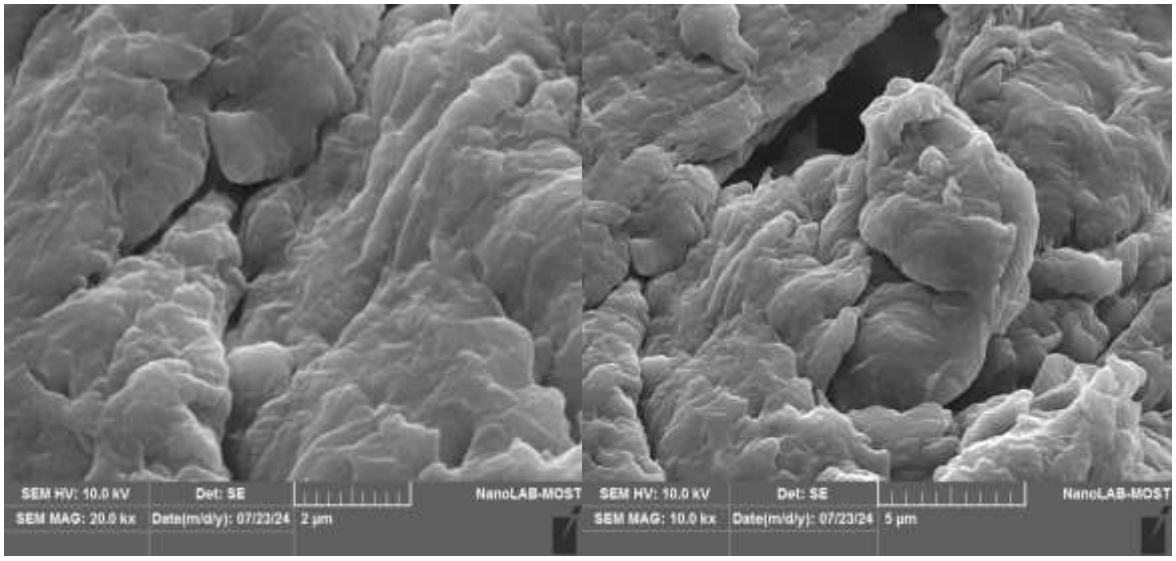

Figure (4): Scanning electron microscopy (SEM) shapes of chitosan nanoparticles (CH-NPs)

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

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 [19]

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. The effect was significant ($p < 0.05$) on the Thiobarbituric acid value in group of chitosan nanoparticles loaded with Thymus vulgaris (G4), chitosan nanoparticles (G3) and Thymus vulgaris extract (G2) after 5, 7, and 10 days of refrigeration.

At five days of storage, the highest TBA values were recorded in control (G1) compared with treatment groups at values of (0.94 ± 0.14) Malonaldehyde (MDA) mg/kg, while the lowest TBAs values were shown in G4 at (0.50 ± 0.12) mg MDA/kg.

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 [20] 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 [21]

Table 2: TBARs results of contamination meat with *S. aureus* at refrigerator temperature

Time / Groups	0 day	2 days	5 days	7 days	10 days
G1	0.34 ± 0.08 Da	0.67 ± 0.03 Ca	0.94 ± 0.14 Ba	1.33 ± 0.15 Aa	1.53 ± 0.16 Aa
G2	0.40 ± 0.07 Ba	0.44 ± 0.05 Bb	0.56 ± 0.13 Bb	0.61 ± 0.14 ABb	0.82 ± 0.15 Ab
G3	0.32 ± 0.12 Ca	0.44 ± 0.12 Cb	0.53 ± 0.09 BCb	0.67 ± 0.12 ABb	0.90 ± 0.08 Ab
G4	0.37 ± 0.06 Ba	0.42 ± 0.11 Bb	0.50 ± 0.12 ABb	0.65 ± 0.10 ABb	0.75 ± 0.19 Ab

A significant difference ($p < 0.05$) exists between the means that have a different small letter in the same column.
A significant difference ($p < 0.05$) exists between the means that have a different capital letter in the same row.
G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus

Total bacterial count of contamination meat with *S. aureus* 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. this count was significantly increased (P≤ 0.05) through the preservative period, reaching 6.10 ± 2.94log CFU/g. after 2 day and 7.49 ± 2.73 log CFU/g after 5 days and 14.35 ± 3.09 log CFU/g. after 10 days of experiment.

In contrast, the meat samples treated with Thymus vulgaris extract, nanoparticle chitosan and nanoparticle chitosan nanoparticle-loaded Thymus extract demonstrated a significant decrease in TBC during two days of refrigerator at 4°C. The lowest bacterial count was observed in the samples treated with Thymus vulgaris extract

(G2) and chitosan nanoparticles loaded with Thymus vulgaris extract (G4), which recorded 4.37 ± 1.35 and 4.81 ± 0.29 log CFU/g. respectively, after 2 days of refrigerator. 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).

The effect of preservation duration on all groups was significant. Furthermore, bacterial growth disappeared by day five and throughout the end of the experiment in the meat samples 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, also 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 [22]

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 [23].

Because of its antimicrobial action, chitosan can fight against foodborne pathogens that affect the shelf life and quality of the food [24,25]

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 to satisfy customer expectations for foods free of artificial preservatives and more environmentally friendly [26].

Table 3: Total bacterial count of contamination meat with S.aureus at refrigerator temperature. (log^{10} CFU/gm)

Time / Groups	0 day	2 days	5 days	7 days	10 days
G1	4.15 ± 1.76 Da	6.10 ± 2.94 Ca	7.49 ± 2.73 Ca	10.51 ± 4.19 Ba	14.35 ± 3.09 Aa
G2	3.82 ± 0.27 Ba	4.37 ± 1.35 Ab	2.16 ± 1.20 Cc	0.21 ± 0.03 Cc	ND
G3	4.17 ± 2.04 Ba	5.85 ± 2,03 Aa	4.03 ± 0.47 Bb	3.06 ± 0.45 Cb	1.50 ± 0.80 Db
G4	3.65 ± 1.08 Ba	4.81 ± 0.29 Ab	2.20 ± 0.83 Cc	0.92 ± 0.07 Cc	ND

A significant difference ($p < 0.05$) exists between the means that have a different small letter in the same column.
A significant difference ($p < 0.05$) exists between the means that have a different capital letter in the same row.
G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus

Nanomaterials significantly improve the operation of metal, ceramic, polymeric, or mixtures, which serve as the basis for both nanosized materials and nanotechnology. Nanomaterials are currently used to produce several items in our everyday lives. Nanotechnology, a crucial innovation of the twenty-first century, finds applications in various scientific domains, including chemistry, biology, biomedicine, pharmacy, agriculture, electronics, and bioengineering [27]

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 [28] 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 [29] and by its direct use in animal products, including veal [30] and lamb [31]. 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 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 [32]. 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 [33].

TVB-N of contamination meat with *Staphylococcus aureus*

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 (4) 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.

On the second days, the highest TVB-N values showed in control (G1) and chitosan nanoparticles (G3), were 4.22 ± 1.03 and 4.13 ± 0.49 mg/100 g, respectively, whereas the smallest TVB-N level showed in Thymus extract, chitosan nanoparticles loaded with Thymus extract, G2 and G4 at 4.10 ± 1.12 mg/100 g and 3.85 ± 0.67 mg/100 g respectively, indicated a significant reduction in TVB-N. The control meat samples were notable a spoilage after 10 days of refrigerator storage. In contrast, meat coated with chitosan nanoparticles had an acceptance value of 6.82 ± 0.72 mg/100 g simultaneously. Furthermore, chitosan nanoparticles loaded with Thymus extract (G2 and G4) recorded a low score of 5.25 ± 0.34 and 5.15 ± 0.43 mg/100 g which demonstrated a longer shelf life than control untreated sample.

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 [39] 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 [40]. 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.

Table 4. TVB-N of contamination meat with *S. aureus* at refrigerator temperature. (mg/100 g)

Groups	0 day	2 days	5 days	7 days	10 days
G1	3,72 ± 0.22 Ea	4.22 ± 1.03 Da	5.42 ± 0.88 Ca	6.71 ± 1.29 Ba	7.96 ± 1.62 Aa
G2	3.79± 0.45 Ca	4.10 ± 1.12 Cab	4.51 ± 0.93 Bc	5.20 ± 0.58 Ac	5.25 ± 0.34 Ac
G3	3.88 ± 0.19 Da	4.13 ± 0,49 Dab	4.89 ± 0.55 Cb	5.93 ± 0.95 Bb	6.82 ± 0.72 Ab
G4	3.65 ± 0.32 Ca	3.85 ± 0.67 Cb	4.33 ± 0.64 Bc	5.11 ± 0.81 Ac	5.15 ± 0.43 Ac
A significant difference ($p < 0.05$) exists between the means that have a different small letter in the same column. A significant difference ($p < 0.05$) exists between the means that have a different capital letter in the same row. G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus					

pH result of contamination meat with *Staphylococcus aureus*:

The results in table (5) referred to the pH change in meat samples at refrigerator storage. The control (G1) group exhibited the most excellent pH value of 5.75 ± 0.20 after 2 days of storage, also in the groups treated without significant differences at refrigerator storage.

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 [41]. Furthermore, because dietary pH promotes the solubilization of hydrophobic essential oils, it affects these substances [42].

The pH of the untreated (control) group showed a significant increase ($P \leq 0.05$) on days 5, 7 and 10, compared with the pH level in samples treated with (G2, G3 and G4) 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 environment [43]. Also, the sample treated with Thyme Extract exhibited a statistically significant elevation in pH values compared to the groups containing CNPs and CNPs loaded with Thyme Extract.

Table 5: pH results of contaminated meat with S.aureus at refrigerator temperature

Time / Groups	0 day	2 days	5 days	7 days	10 days
G1	5.55 ± 0.16 Ba	5.75 ± 0.20 Ba	6.99 ± 0.91 Aa	7.35 ± 0.56 Aa	8.10 ± 0.76 Aa
G2	5.69 ± 0.56 Aa	5.73 ± 0.92 Aa	5.99 ± 0.38 Aa	6.07 ± 0.86 Ab	6.12 ± 0.14 Ab
G3	5.52 ± 0.18 Aa	5.64 ± 0.14 Aa	5.89 ± 0.59 Aa	6.16 ± 0.38 Ab	6.24 ± 0.17 Ab
G4	5.65 ± 0.15 Aa	5.72 ± 0.62 Aa	5.81 ± 0.29 Ab	5.95 ± 0.71 Ab	6.08 ± 0.24 Ab

A significant difference ($p < 0.05$) exists between the means that have a different small letter in the same column.
A significant difference ($p < 0.05$) exists between the means that have a different capital letter in the same row.
G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus

The results in Table 6 showed that treated samples with chitosan nanoparticles significantly reduced the hydrogen peroxide value, which improved the quality of meat.

Untreated meat samples deteriorated within 5 and 10 days at refrigerator temperatures, while the sample treated with thymus extract and chitosan nanoparticles loaded with thymus extract maintained low levels of Hydrogen Peroxide throughout the storage period.

At five days of storage, the highest Hydrogen Peroxide values were recorded in control (G1) compared with treatment groups at values of (2.61 ± 0.06) meq O_2/Kg, while the lowest Hydrogen Peroxide values were shown in groups of thymus extract and chitosan nanoparticles loaded with thymus extract (G2 and G4) at (1.88± 0.21) and (1.83 ± 0.28) meq O_2/Kg, also In the Ten days of the preservative period significant differences were observed across all groups.

Nanotechnology plays a key role in the food business across three main domains: increasing and improve the quality of foods, inhibiting food degradation that is caused by microbial infection and identifying food deterioration.

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 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* [29].

Table 6: Peroxide value of contaminated meat with S. aureus at refrigerator temperature. (meq O_2/Kg)

Time / Groups	0 day	5 days	10 days
G1	1.54 ± 0.16 Ba	2.61 ± 0.06 Aa	3.16 ± 0.27 Aa
G2	1.63 ± 0.19 Aa	1.88± 0.21 Ab	2.11 ± 0.22 Ab
G3	1.68 ± 0.24 Ba	2.20 ± 0.38 ABab	2.57 ± 0.73 Aab
G4	1.59 ± 0.27 Aa	1.83 ± 0.28 Ab	2.03 ± 0.94 Ab

A significant difference ($p < 0.05$) exists between the means that have a different small letter in the same column. A significant difference ($p < 0.05$) exists between the means that have a different capital letter in the same row. G1=Control, G2= Thymus extract, G3= Nano Chitosan, G4= Nano Chitosan loaded Thymus

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.

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

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the Ministry of Higher Education, university of Baghdad, college of veterinary medicine, department of veterinary public health for supporting this study. The authors also extend their appreciation to the college of Veterinary Medicine, University of Diyala, for providing the facilities and assistance that made this research possible.

CONFLICT OF INTEREST

The authors have declared no conflict of interest

REFERENCES

1. Agarwal M, Agarwal MK, Shrivastav N, Pandey S (2018). Preparation of Chitosan Nanoparticles and their In-vitro Characterization. Int. J Life-Sci Res, 4(2):1713–1720.
2. Ali S. and Alsayeqh A.F. (2022). Review of major meat-borne zoonotic bacterial pathogens. Front. Public Health 10:1045599. doi: 10.3389/fpubh.2022.1045599
3. Bassirou N., Mamoudou H. D. & Colin G. (2025). Exploring the spoilage microbiota of raw and processed meats under different storage environments and its related profiling methods: a review, Cogent Food & Agriculture, 11:1, DOI: 10.1080/23311932.2025.2562177
4. Bastiaens L, Soetemans L, D'Hondt DK (2019). ElstSources of Chitin and Chitosan and Their Isolation, Chitin and Chitosan, pp. 1-34, 10.1002/9781119450467.ch1
5. Chi W, Cao L, Sun G, Meng F, Zhang C, Li J, Wang L (2020). Developing a highly pH-sensitive 115 κ-carrageenan-based intelligent film incorporating grape skin powder via a cleaner process. J. Cleaner Prod., 244,118862
6. Donsi F, Annunziata M, Seesa M, Ferrari G (**2010).** Nanoencapsulation of essential oils to enhance their antimicrbial activity in foods. Food Sci. Technol., 44, 1908–1914.
7. Fayad NK, AL- Obaidi OHS, Al-Noor TH, Ezzat MO (2013). Water and Alcohol Extraction of Thyme Plant (Thymus Vulgaris) and Activity Study Against Bacteria, Tumors and Used As Anti-Oxidant In Margarine. Innovative Systems Design and Engineering. Vol.4, No.1.
8. Ge SUN, Tongxuan WU, Yanwei MAO (2024). Advances in Understanding the Mechanism of Red Meat Spoilage Caused by Bacteria 38(8): 63-71.
9. Ghadi A, Mahjoub S, Tabandeh F, Talebnia F (2014). Synthesis and optimization of chitosan nanoparticles: Potential applications in nanomedicine and biomedical engineering. Caspian J. Int. Med., 5(3):156.
10. Halat D, Krayem M, Khaled S, Younes S (2022). A focused insight into thyme: biological, chemical, and therapeutic properties of an indigenous Mediterranean herb.
11. Han J, Dong P, Holman BWB, Yang H, Chen X, Zhu L, Luo X, Mao Y, Zhang Y (2024). Processing interventions for enhanced microbiological safety of beef carcasses and beef products: A review. *Critical Reviews in Food Science and Nutrition*, *64*(8), 2105–2129. https://doi.org/10.1080/10408398.2022.2121258
12. Harpaz S, Glatman L, Drabkin V & Gelman A (2003). Effects of Herbal Essential Oils Used To Extend the Shelf Life of Freshwater-Reared Asian Sea Bass Fish (Lates calcarifer) J. Food Prot.;66:410–417. doi: 10.4315/0362-028X-66.3.410.
13. Hemmingsen L.M., Škalko-Basnet N. and Jøraholmen M.W. (2021). The Expanded Role of Chitosan in Localized Antimicrobial Therapy. Mar. Drugs 2021, 19, 697. https://doi.org/10.3390/md19120697
14. Ijaz M, Yar MK, Badar IH, Ali S, Islam MS, Jaspal MH (2021). Meat production and supply chain under COVID-19 scenario: current trends and future prospects. Front Vet Sci. 8:660736. 10.3389/fvets.660736
15. Jha R., & Mayanovic R.A. (2023). A Review of the Preparation, Characterization, and Applications of Chitosan Nanoparticles in Nanomedicine. Nanomaterials, 13(8), 1302. https://doi.org/10.3390/nano13081302

16. Joseph T.M., Kar Mahapatra D., Esmaeili A., Piszczyk Ł., Hasanin M.S., Kattali M, Haponiuk J. and Thomas S. (2023). Nanoparticles: Taking a Unique Position in Medicine. Nanomaterials (Basel). 2023 Jan 31;13(3):574. doi: 10.3390/nano13030574. PMID: 36770535; PMCID: PMC9920911.
17. Jalil S, Nazir MM, Eweda MA, Zulfiqar F, Ahmed T, Noman M, Asad MAU, Siddique KHM, Jin X. Zinc oxide application alleviates arsenic-mediated oxidative stress via physio-biochemical mechanism in rice. Environ Sci Pollut Res Int. 2024 May;31(23):34200-34213. doi: 10.1007/s11356-024-33380-0. Epub 2024 May 3. PMID: 38702484.
18. Kamalipooya S, Nasrabadi D, Abtahi H, Golmohammadi M, Fahimirad S (2025). Chitosan quaternary ammonium salt-stabilized cerium oxide nanoparticles green-synthesized using *Thymus vulgaris* extract: multifunctional antibacterial, anticancer, and wound healing applications. *Journal of Biomaterials Science, Polymer Edition*, 1–27. https://doi.org/10.1080/09205063.2025.2528934
19. Kathayat D, Lokesh D, Ranjit S, Rajashekara G (2021). Avian pathogenic escherichia coli (APEC): an overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens.;10:467. doi: 10.3390/pathogens10040467.
20. Liu H, Sun X, Liao X, Gänzle M (2020). Control of pathogenic and spoilage bacteria in meat and meat products by high pressure: Challenges and future perspectives. Rev Food Sci Food Saf. 19(6):3476-3500.
21. Lohith KDH, Sarkar P (2018). Encapsulation of bioactive compounds using nanoemulsions. Environ Chem Lett., 16,59–70.
22. Manaswini BGVS, BhagyaRaj KKD, Rafeeya S (2024). A thorough evaluation of chitosan-based packaging film and coating for food product shelf-life extension, Journal of Agriculture and Food Research,Volume 16,2024,101164 ISSN 2666-1543, https://doi.org/10.1016/j.jafr.2024.101164
23. Mogessie H, Legesse M, Hailu AF, Teklehaymanot T, Alemayehu H, Abubeker R, Ashenafi M (2024). Vibrio cholerae O1 and Escherichia coli O157:H7 from drinking water and wastewater in Addis Ababa, Ethiopia. BMC Microbiol. Jun 20;24(1):219. doi: 10.1186/s12866-024-03302-8.
24. Muñoz-Tebar N, Pérez-Álvarez JA, Fernández-López J, Viuda Martos, M (2023). Chitosan Edible Films and Coatings with Added Bioactive Compounds: Antibacterial and Antioxidant Properties and Their Application to Food Products: A Review. Polymers, 15, 396. https://doi.org/10.3390/polym1502039669.
25. Nieto G (2020). A Review on Applications and Uses of *Thymus* in the Food Industry. *Plants*, *9*(8), 961. https://doi.org/10.3390/plants9080961
26. Odeyemi OA, Alegbeleye OO, Strateva M, Stratev D (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, *19*(2), 311–331. 2, https://doi.org/10.1111/1541-4337.12526
27. Oh H, Lee J (2024). Psychrotrophic bacteria threatening the safety of animal-derived foods: Characteristics, contamination, and control strategies. *Food* Science of Animal Resources, 44(5), 1011–1027. https://doi.org/10.5851/kosfa.2024.e70
28. Pellissery S, Mathew S, Ninan G (2020). Nanotechnology in food processing, preservation, and safety: A review. Food Research International, 132, 109056
29. Peshawa Y, Aziz L, Shokhan H, Nabaz HH, Yousif TH, Hevar NA (2022). Antibacterial Activity Evaluations of Thymus vulgaris Essential Oil Extract Against Clinically Isolated Gram-Positive and Gram-Negative Pathogens Euphrates Journal of Agriculture Science-14 (2): 192-201.
30. Ricardo R.S., Rouxinol M.I., Agulheiro-Santos A.C., Potes M.E., Laranjo M. and Elias M. The Antioxidant and Antibacterial Potential of Thyme and Clove Essential Oils for Meat Preservation—An Overview. Appl. Biosci. 2024, 3, 87–101. https:// doi.org/10.3390/applbiosci3010006
31. Sahoo M., Vishwakarma S., Panigrahi C. and Kumar J. (2021). Nanotechnology: Current applications and future scope in food. Food Frontiers. 2021;2:3–22. https://doi.org/10.1002/fft2.58
32. Sateriale D., Forgione G., De Cristofaro G.A., Pagliuca C., Colicchio R., Salvatore P., Paolucci M. and Pagliarulo C. (2023). Antibacterial and Antibiofilm Efficacy of Thyme (Thymus vulgaris L.) Essential Oil against Foodborne Illness Pathogens, Salmonella enterica subsp. enterica Serovar Typhimurium and Bacillus cereus. Antibiotics 2023, 12, 485. https://doi.org/10.3390/ antibiotics1203048
33. Shin CS, Kim DY, Shin WS (2019). Characterization of chitosan extracted from Mealworm Beetle (Tenebrio molitor, Zophobas morio) and Rhinoceros Beetle (Allomyrina dichotoma) and their antibacterial activities Int. J. Biol. Macromol., 125 pp. 72-77
34. Shuaifei L., Wanying H., Peng W.,Honglei T., Jingzhang G., Ping Z., and Yonghuan L. (2025). Effects of thyme (*Thymus vulgaris L.*) polyphenol addition on the flavor profiles of stewed lamb meat using SPME-GC-MS and E-nose. Journal of Agriculture and Food Research 24 (2025) 102476
35. Steel RGD, Torrie JH (1980). Principles and procedures of statistics. A biometrical approach, 2nd Edition, McGraw-Hill Book Company, New York.20-90.
36. Thwala T, Madoroba E, Basson A, Butaye P (2021). Prevalence and Characteristics of Staphylococcus aureus Associated with Meat and Meat Products in African Countries: A Review. Antibiotics (Basel). 2021 Sep 14;10(9):1108. doi: 10.3390/antibiotics10091108. .

37. Tiwari BK, Valdramidis VP, O'Donnell CP (2023). Emerging applications of nanotechnology in food processing, packaging, and safety. Comprehensive Reviews in Food Science and Food Safety, 22(2), 1285–1310
38. Tri U, Andi F, Mohammad MS, Rina W, Teguh W (2022). Nanoemulsion application in meat product and its functionality: review. J. Anim. Sci. Technol., 65(2):275-292. ISSN: 2672-0191, eISSN: 2055-0391
39. Wambui J, Lamuka P, Karuri E, Matofari J, Njage PMK (2018). Microbial contamination level profiles attributed to contamination of beef carcasses, personnel, and equipment: case of small and medium enterprise slaughterhouses. J Food Prot. 81:684–91. doi: 10.4315/0362-028X.JFP-17-402.
40. Wang L., Ye C., Xu H., Aguilar Z.P., Xiong Y., Lai W. & Wei H. (2015). Development of an SD-PMA-mPCR assay with internal amplification control for rapid and sensitive detection of viable Salmonella spp., shigella spp. and Staphylococcus aureus in food products. Food Control 2015. 57, 314 – 320.
41. Wei C., Zhong J., Hu T. & Zhao X. (2018). Simultaneous detection of E.coli O157: H7, Staphylococcus aureus and Salmonella by multiplex PCR in milk. 3 Biotech.2018, 8.76.
42. Yan D, Li Y, Liu Y, Li N, Zhang X, Yan C (2021). Antimicrobial Properties of Chitosan and Chitosan Derivatives in the Treatment of Enteric Infections. Molecules. 26. 7136. 10.3390/molecules26237136.
43. Zhou Y, Li Y, Zhang M, Wang S (2023). Applications of nanotechnology in food safety and quality: A review. *Trends in Food Science & Technology*, 134, 58–72