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Improvement of yoghurt safety by addition of thyme-oil and thyme oil nanoemulsion as antibacterial agent

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ABSTRACT

Throughout the years, consumer demand for healthy, proper food has increased significantly. To cater the consumer demands, present trends in the food manufacturing are shifting to natural, high-quality products with decreasing processed additives changing them with plant-based substitutes. In the current study, the antibacterial activity of thyme essential oil and thyme oil-nanoemulsion (NE) was detected against *E. coli* O157, *S.* Typhimurium, *S. aureus* and *L. monocytogenes* in manufactured yoghurt. The antibacterial activities of TEO and TNE were evaluated using disc diffusion and resazurin microdilution methods. The droplets size of the TNE was 135.7±23.04 nm also, showed a greater reduction in MIC and a greater increase in zone of inhibition (ZOI) against *E. coli* O157 (0.04%; 36 mm), *S.* Typhimurium (0.01%; 41.5 mm), *S. aureus* (0.08%; 45 mm), and *L. monocytogenes* (0.01%; 31 mm). A scanning electron microscope was applied to perceive the morphological changes in the selected bacterial cells. Damage in bacterial cells following the addition of the nanoemulsion was observed. Yoghurt inoculated with the tested bacteria was supplemented with TEO and TNE at different concentrations (0.5%, 1.0%, and 2.0%) to enhance its quality. The supplementation of yoghurt with TEO nanoemulsion could represent a promising natural preservative for enhancing the microbiological safety and maintaining the sensory properties of yoghurt.

Introduction

Consumers are progressively prioritizing the health effect of products when making purchasing choices. They favor foods beverages with high nutritional value, fresh flavors and natural appearance (Napiórkowska *et al.*, 2024).

Milk acts as a basic food for mankind and an important source of the wealth of human as it contains the ingredients that make it self-sufficient containing carbohydrates, fat, protein, lactose, vitamins, as well as mineral salts. A fermented milk product with a somewhat sour taste and a soft texture is called yogurt. It is regarded as one of the most important dairy products for the continuation of life (Rifky *et al.*, 2023). Yogurt is a dairy product that is well-liked all over the world because of its enticing characteristics and consequent health benefits (Khalaf *et al.*, 2021). Bacterial infections and outbreaks through food are enduring global threats. It is believed to represent a serious public health hazard worldwide, with 420,000 fatalities happening each year (Lee and Yoon, 2021).

Because of the known effect of industrial food preservatives on human health and the rise in pathogens spread through processed foods especially foods including dairy products it become important to find alternatives to their use. Recently, there has been a focus on obtaining bioactive components from natural sources. Production of healthy yoghurt has become popular as a result of the recent rise in demand for food that is both delicious and of high quality (Harshitha *et al.*, 2024).

Oils originated from plant materials (essential oils) are volatile highly aromatic water-insoluble fluids. They belong to a class of terpenoids and other optional metabolic products that are vital to the phyto-defense mechanism and include potent antibacterial agent (Adetuyi *et al.* 2024). Additionally, some components of essential oils display health-enhancing effects, such as anticancer properties of cumin-aldehyde, the anti-inflammatory effects of eugenol, the analgesic properties of menthol, and digestive benefits. Thyme essential oil, originated from *Thymus vulgaris* L., contains predominantly thymol (45%), carvacrol (approximately 25–60%), linalool (about 8%) and borneol (8–15%) (Wróblewska-Łuczka, 2021). The antimicrobial properties of essential oils have aroused interest for their potential as an alternative to the use of chemical preservatives in foods (de Almeida *et al.*, 2023).

Consequently, a relevant question about whether consumers will accept the use of essential oils to food. According to a study by Vital *et al.* (2018), 82.2% of respondents believe that essential oils are natural compounds, and 70% of respondents are familiar with them. Additionally, 57.8% of participants indicated that they would be prepared to pay more for food enhanced with essential oils (EOs). However, application of essential oils in food is hindered by many factors, as their chemical composition, which can vary widely depending on many factors such as plant species, agricultural practices, geographical origin and extraction methods (Shetta *et al.*, 2024). Furthermore, the interaction between essential oils and other food additives is poorly understood (Yammine *et al.*, 2024), which makes using them in food matrices more difficult. Essential oils have shown promise in their ability to combat bacteria, yeast, and oxidative stress resolving these issues is crucial to the broader use in food preservation and improvement (Valdivieso-Ugarte *et al.*, 2021).

The application of essential oils in nanoemulsion form has become a promising solution to the challenges encountered in food manufacturing, including their incorporation into yogurt. Nanoemulsions, characterized by particle diameters ranging from 10 to 100 nm, offer unique physicochemical properties conducive to food applications (Prado *et al.*, 2024). The small size of the NE droplet gave nanoemulsions light-transmitting and translucent characters, which prevent color change of food. Moreover, nanoemulsions display enhanced resistance to gravitational separation and particle aggregation compared to coarse emulsions (Napiórkowska *et al.*, 2024).

Nanoemulsions (oil-in-water o/w) are considered an active carrier of antimicrobial agent in food systems. The small droplet size enables

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the migration and attachment of nanoemulsion droplets to bacterial cell walls, causing the disruption of lipid fractions of the cell membranes. From the above we exclude the cause of enhancement of the antimicrobial activity of essential oils encapsulated in nanoemulsions, thereby addressing the challenges associated with their practical application in food production (Hashem *et al*., 2024).

In this work, we studied the antibacterial activity Thyme (EOs) and thyme (NE) against strains of *Staphylococcus aureus*, *Listeria* sp, *Escherichia coli* and *Salmonella* sp. that contaminate yogurt and cause Foodborne Diseases (FBD).

Materials and methods

Preparation of Thyme nano-emulsions (TNEs)

Thyme Essential oil was purchased from National research center, Egypt and stored at 7±1°C. At room temperature (25ºC), The oil-in-water NEs were prepared by dissolving Tween 80 2 v/v% in double-distilled water. The non-ionic surfactant was preferred due to its favorable oil-in-water (O/W) characteristic. Tween 80 displays efficient solubility with essential oils, and effectively minimizes droplet diameter by adhering to the droplet's surface, improving the overall stability of O/W emulsion system. The mixture was shaken using a magnetic type of stirrer for 10 minutes to obtain a homogenous solution. Then, the TEOs were added slowly and mixed with a direct driven stirrer (25 kHz, 650 W) (Moradi and Barati, 2019) for 15 min, then sonication of the resulting emulsion using a 25 kHz ultrasonic Homogenizer (USH650, max power: 650 watt) was done for 20 minutes. The nano-emul¬sions preparation was in the Nanotechnology Research Unit, Animal Health Research Institute, Assiut, Egypt.

Characterization of the prepared nanomaterials

The particle size and PDI of nano-emulsions were measured at 25.0±0.2°C by Zeta-sizer (3000 HS, Malvern Instruments, Malvern, UK), Zeta-sizer® software (version 7) was used to gather and evaluate the data (Hassanien *et al*., 2021). Then morphology of TNE was detected in the Electronic Microscope Unit, Assiut University using Transmission Electron Microscope (TEM) (Shakeel *et al*., 2019).

Determination of the minimum inhibitory concentrations (MICs) of thyme oil /thyme oil-nanoemulsion

Four bacterial strains; *Staphylococcus aureus* (ATCC No. 6538), *Escherichia coli* O157:H7 (ATCC No. 25922), *Salmonella* Typhimurium (ATCC No.14028) and *Listeria monocytogenes* (ATCC No. 7644) were obtained from High Quality Media unit (HQM), (Animal Health Research Institute, Dokki, Egypt). All cultures were reactivated, the reactivated strains were adjusted at a concentration of 108 cfu /ml using 0.5 McFarland standard (Sanchez *et al*., 2010). The obtained growths were used as an inoculum for the antimicrobial assay.

The minimum inhibitory concentration (MICs) of thyme oil /thyme oil-nanoemulsion were detected with 2 methods: agar well diffusion method (Hossain, 2024) and Resazurin-based 96-well plate micro-dilution method (Elshikh *et al*., 2016).

Scanning electron microscopy imaging of bacterial strains treated with thyme oil /thyme oil-nanoemulsion

Detection of the morphology and intracellular structure alteration of the tested bacterial cells after addition the TEO and TNE, scanning electron microscopy (SEM) were performed. This part was carried out in the Electronic Microscope Unit, Assiut University, Egypt.

Bacterial cell suspensions from each treatment were centrifuged at 8000 rpm for ten minutes at 4°C, then washed twice with 0.85% sterile saline solution. The supernatant was discarded, and the cells were fixed with 2.5% glutaraldehyde overnight at 4°C, followed by dehydration with a graded ethanol series (30, 50, 70, 80, 90, 100%). After dehydration, each sample was fixed on SEM support and observed with SEM.

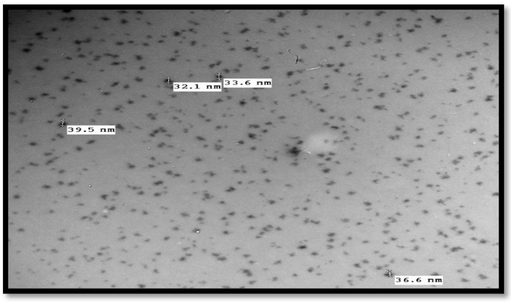

Fig. 1. TEM of TNE with average size 35.45nm.

Detection of the antibacterial effect of TEO and TNE against S. aureus, E. coli O157, S. Typhimurium *and L. monocytogenes in experimentally manufactured yogurt (food model) during refrigerated storage*

Preparation of yogurt

Fresh buffalo milk was heated at 90°C for 20 minutes then homogenized. Following heating, the milk was cooled to 40°C to add the starter culture 2% (1:1 mixtures of *Streptococcus thermophiles* and *Lactobacillus bulgaricus*). After cooling some milk was moved and kept in a sterile jar to be a negative control group (without bacteria or treatments). After that, the inoculated milk was divided into 25 parts to add the different treatments as following: part 1 is the positive control of *S. aureus* (inoculated milk without any treatment), part 2 (with 0.5% of TEO), part 3 (with 1% of TEO) and part 4 (with 2% of TEO). Part 5 (with 0.5% of TNE), part 6 (with 1% TNE) and part 7 (with 2% TNE). Part 8 is the positive control of *E. coli* (inoculated milk without any treatment), part 9 (with 0.5% TEO), part 10 (with 1% TEO) and part 11 (with 2% TEO). Part 12 (with 0.5% TNE), part 13 (with 1% TNE) and part 14 (with 2% TNE). Part 15 is the positive control of *S.* Typhimurium (inoculated milk without any treatment), part 16 (with 0.5% TEO), part 17 (with 1% TEO) and part 18 (with 2% TEO). Part 19 (with 0.5 % TNE), part 20 (with 1 % TNE) and part 21 (with 2 % TNE). Part 22 is the positive control of L. monocytogenes (inoculated milk without any treatment), part 23 (with 0.5 % TEO), part 24 (with 1% TEO) and part 25 (with 2 % TEO). Part 26 (with 0.5 % TNE), part 27 (with 1 % TNE) and part 28 (with 2 % TNE). The yogurt was packed in sterile jars and stored at 4°C and were analyzed at zero time, after curdling, after 24 h then after 1, 3, 5, 7, 10 days. As well as sensory evaluation was performed periodically on the control negative.

Organoleptic examination of manufactured yoghurt samples

The manufactured yogurt was examined for sensory characteristics by 35 panelists (varying ages, genders, and educational backgrounds) immediately following processing and throughout storage (after 1, 3, 5, 7, 10 days). Samples of yogurt were organoleptically scored for color, odor, flavor, body and texture, appearance and general acceptance according to score card (Excellent 5, very good 4, good 3, acceptable 2, and bad 1) were the scale points suggested by Mosiyani *et al*. (2017).

Microbiological examination of manufactured yoghurt samples

Sample of yogurt (1 g) was diluted in 9 ml saline solution yielding a 10-1 dilution then serial dilutions were prepared afterward. After performing the serial dilution, a 0.1 mL of the desired dilutions was cultured. *Escherichia coli* O157:H7 was cultivated on EMB agar (HiMedia, India) at 37°C for 24 h according to ISO 16649-1:2001(ISO, 2013), *S. aureus* was cultivated on Mannitol salt Agar (HiMedia, India) at 37°C for 48 h ISO

6888-1:1999/Amd (ISO, 2018), *Salmonella Shigella* Agar medium (HiMedia, India) was used for *S.* Typhimurium at 37°C for 24 h according to ISO 6579-1:2017/Amd1:2020 and *Listeria* Selective Agar medium for *Listeria monocytogenes* 37°C for 24 h according to ISO 12290-1:2017(ISO, 2017). Three replicates were conducted.

Statistical analysis

Obtained results were analyzed by variation-statistical methods in ANOVA. The differences between treatments were considered significant at $p < 0.05$. and SPSS® 22.0 was used to perform a one-way analysis of variance on the means of three replicates.

Results

Particle size and polydispersity index (PDI) of TNE

The polydispersity index (PDI) (0.251) and average dynamic size of the nanoemulsion (TNE). The distribution and uniformity of the particle sizes within the sample are shown by the average dynamic size and its standard deviation. High-resolution visualization of the TNE particles was made possible by the TEM pictures, which verified their homogeneity and nanoscale size. The well-defined particle size distribution indicated by the average size of 35.45 nm is crucial for the nanoemulsion's planned uses.

Minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) of thyme oil /thyme oil-nanoemulsion

The four bacterial strains (*E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, and *L. monocytogenes*) were examined for their susceptibility to Eos and Eos nanoemulsion. The results reported in Table1, show that thyme oil and thyme oil nanoemulsion exhibited a remarkable MIC and zones of inhibition for all strains. This antibacterial effect increased by increasing the concentration of the extract.

Table 1. The minimum inhibitory concentration and ZOI of Thyme oil and Thyme nano against tested bacterial strains.

Bacterial strains	*Thyme EO*		*Thyme NE*	
	MIC%	ZOI (mm)	MIC (%)	ZOI (mm)
E. coli	0.1	32	0.04	36
S. Typhimurium	0.1	39	0.01	41.5
S. aureus	0.2	44	0.08	45
L. monocytogenes	0.1	28	0.01	31

MIC: Minimum inhibitory concentrations; ZOI: Zone of inhibition

Microbiological examination of Yoghurt

In the present study we tested the contamination of yoghurt by 4 food-borne pathogens (*E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, and *L. monocytogenes*) and evaluated the effect of 3 (0.5, 1 and 2%) different concentrations of thyme oil and it's nanoemulsion against the aforementioned bacteria.

It is clear in Figures 4-7, that the total viable bacterial count (TVBC) cfu /ml of the four tested bacteria during storage interval varied in the control and thyme treatments whereas, control sample had the highest bacterial counts and thyme oil and thyme oil nanoemulsion treatments showed significant decrease ($p \leq 0.05$).

The yoghurt prepared with thyme oil and thyme oil nanoemulsion lead to retardation of bacteria growth. As thyme oil has inhibitory effects on the bacteria in general, with increasing the added thyme and its encapsulation concentration the bacterial total counts decreased. High significant differences were found among thyme oil and thyme oil nanoemulsion concentrations at all storage periods.

The current results showed that *E. coli* O157:H7 was not detected on days 7 & 15 in the yoghurt treated with both Thyme oil and thyme-oil nanoemulsion.

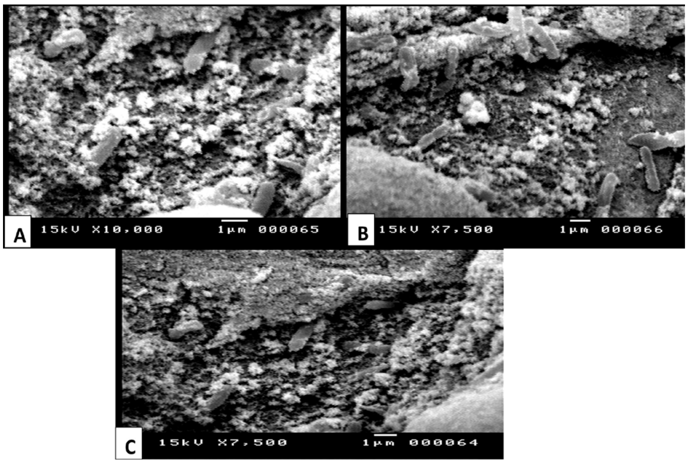

Figure 2. A, B, C: Scanning electron microscopy imaging of *S.* Typhimurium treated with TEO and TNE. A: Control, B: TEO treated, C: TNE treated.

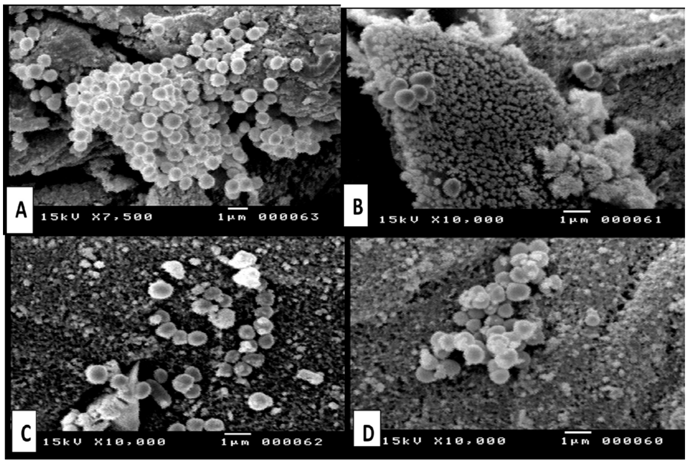

Figure 3. SEM imaging of *S. aureus* treated with TEO/TNE: A: control B: Thyme treated C, D: TNE treated.

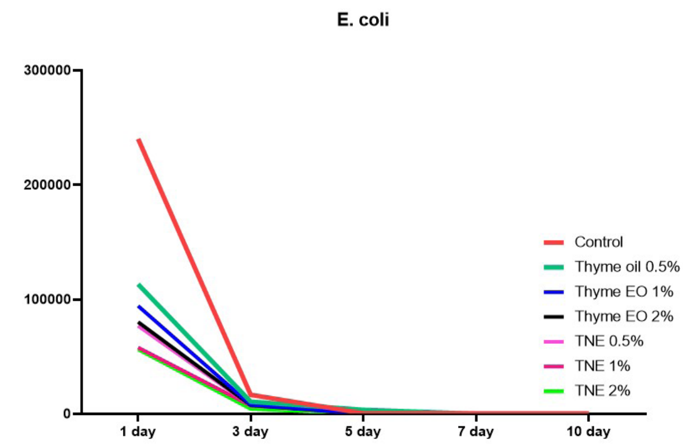

Figure 4. Effect of TEO and TNE on *E. coli* O157:H7 strain (CFU/g) during storage of yogurt sample.

Regarding treatment with Thyme oil, the tested bacteria *S.* Typhimurium and *L. monocytogenes* and *S. aureus* were still isolated till day 10 but in decreasing the total count. Interestingly, thyme-oil nanoemulsion had more effective antimicrobial activity against the four tested microorganisms (*E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, and *L. monocytogenes*) as these bacteria were not detected at day 7 and 10. The results are summarized in Figures 4-7.

From the achieved results, the addition of thyme oil nanoemulsion at concentration of 2.0% is relatively more effective than other concentration of both thyme oil and thyme oil nanoemulsion.

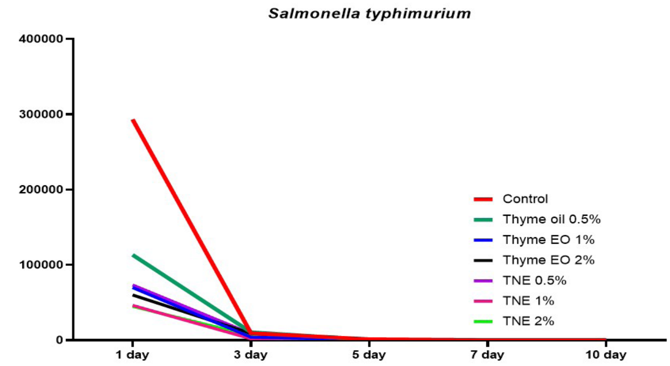

Figure 5. Effect of TEO and TNE on *Salmonella* Typhymurium strain (CFU/g) during storage of yogurt sample.

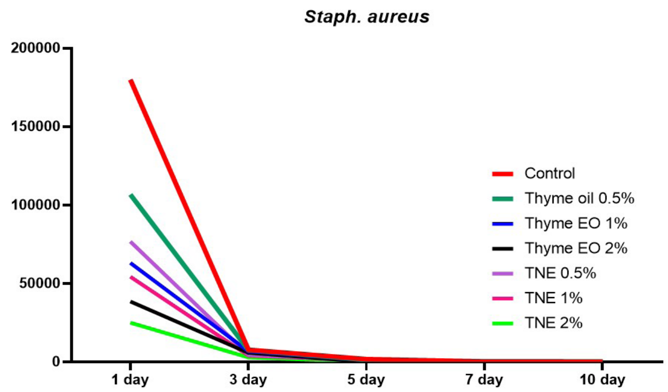

Figure 6. Effect of TEO and TNE on *S. aureus* (CFU/g) during storage of yogurt sample.

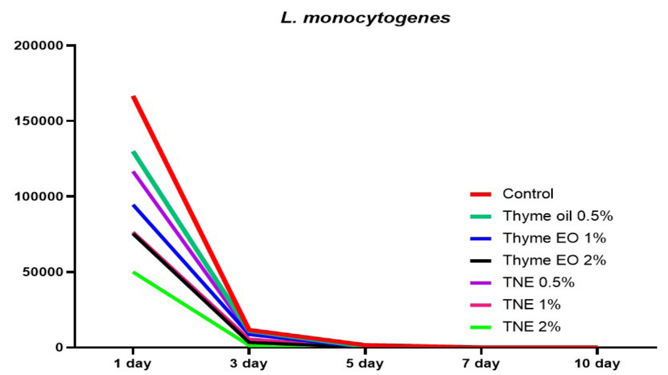

Figure 7. Effect of TEO and TNE on *L. monocytogenes* strain (CFU/g) during storage of yogurt sample.

Sensorial evaluation of yoghurt inoculated with thyme nanoemulsions

Yoghurt was subjected to sensory evaluation at various intervals until the end of the 10 days storage period. The panelists discovered that yoghurt with pure essential oils smelled strongly and was not appetizing. However, overall acceptability parameters (taste, flavor, smell, and odor) scored highly for yogurt containing nanoemulsions (TNE). But as the yoghurt's storage duration extended, its acceptance decreased (Table 2).

Discussion

The food-borne illnesses have expanded worldwide at an anomalous rate and are linked to the emergence of social and economic problems. This emphasizes the necessity for consistently developing novel antimicrobial agents with distinct mechanisms of action (Yu *et al*., 2021). Fermented dairy products are appreciated worldwide because of the benefits they bring to the consumer's health (Iglesia, *et al*., 2020). Yogurt was chosen as a representative product for consumers and supplemented it with thyme oil and thyme oil nanoemulsion to solve the different problems of the volatile oils added to the food and highlight the effect of it against some food borne pathogens (*E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, and *L. monocytogenes*) that must not be detected in the yogurt as coined by the Egyptian standards.

Nanoparticles have received significant attention worldwide because of their antibacterial activity and their great physical and chemical stability. These properties are predominated in the food science for enhancing the overall quality, shelf life, taste, flavor, process-ability, etc., of the food (Kumar *et al*., 2020). Also, the strong antimicrobial activity of nano-emulsified version of essential oils has been demonstrated against a broad spectrum of pathogens and spoilage Mos in various food systems (McClements *et al*., 2021).

One of the Characterization methods of nanomaterials is assessment of their droplet size and PDI by Zeta sizer. Polydispersity index (PDI) is an important parameter, which represents the particle size distribution of the droplets. The value of PDI is calculated by the ratio of the mean standard deviation of the droplet size to the average droplet diameter (Schober *et al*., 2024) the mean droplet diameter of TNE was 35.45 nm with PDI of 0.251 (table 1). The same results were in the study of Hashem *et al*. (2024). The PDI results indicated stability and good homogeneity of the prepared Nanoemulsion, as it was lower than 0.5, as a ratio of surfactant used in TNE was used prevent the coalescence at room temperature and for a long period of storage. In addition, the greater value of PDI showed the lower uniformity of droplet size, as PDI represents the homogeneity of droplet size in a nano-emulsion (Yuliani *et al*., 2018).

The morphology and size by TEM of TNE in Fig. 1, showed separate nano-micelles with average size 35.45 nm. The size of the TNE measured by TEM was smaller than the results measured by Zeta sizer (refer to the hydrodynamic diameters of nanoparticles in a solution); this difference was attributed to the nanoparticles shrinking during the drying process of the TEM sample (Mittal *et al*., 2014).

According to our results in Table 2, the MIC and ZOI of free thyme oil against *E. coli* O157: H7, *S.* Typhimurium, *S. aureus* and *L. monocytogenes* showed to be (0.1% ;32 mm) (0.1% ;39 mm) (0.2% ;44mm) (0.1% ;28 mm) respectively. Similarly, Previous study reported antibacterial effect of thyme oil in milk products against *E. coli* O157:H7 (Simsek, *et al*., 2007 and Botsoglou, *et al*., 2011). Lower ZOI against *E. coli* and *S.* Typhimurium were detected in various literature studies (Gedikoˇ glu, *et al*., 2019; Sateriale *et al*., 2022).

Regarding, the Thyme oil nanoemulsion antibacterial effect was illustrated in Table 2, showed marked increase in the MIC and ZOI compared to TEO against the tested bacteria *E. coli* O157: H7 (0.04%; 36 mm), *S.* Typhimurium (0.01% ;41.5 mm), *S. aureus* (0.08%; 45 mm) and *L. monocytogenes* (0.01%; 31 mm). these result in accordance with the recent results

Table 2. Organoleptic evaluation of thyme NE in manufactured yoghurt.

Storage period	Thyme NE											
	Color		Odor		Texture		Flavor		Appearance		General acceptance	
1st day	Excellent	5	Very good	4	Good	3	Very good	4	Very good	4	Very good	4
3rd day	Very good	4	Very good	4	Good	3	Very good	4	Very good	4	Very good	4
5th day	Very good	4	Very Good	4	Good	3	Good	3	Good	4	Very good	4
7th day	Very good	4	Good	3	Good	3	Good	3	Good	3	Good	3
10th day	Good	3	Good	3	Good	3	Good	3	Good	3	Good	3

found that TEO nanoemulsion exhibited strong antimicrobial activities against *E. coli*, *S.* Typhimurium, *Listeria innocua*, and *S. aureus* (Medina *et al*., 2019; El-Sayed *et al*., 2021).

It's concluded that TNE restricted the growth and multiplication of all tested food born bacteria due to its killing action on initial bacterial inoculum exposed to it, this could be correlated with its monodispersed nature and lower droplet diameter along with antibacterial substance content present in CEO. Higher MIC values were obtained by Hashem *et al*. (2024).

From the results of Table 2, the effectiveness of TEO and TNE in inhibiting the growth of *E. coli*, *S. aureus*, *S.* Typhimurium and *L. monocytogenes* was evaluated using the minimum inhibitory concentration (MIC). The nanoemulsion of the thyme oil showed higher antimicrobial activity than thyme oil itself demonstrated the highest bacteriostatic activity indicating its superior activity. it was shown that TEO had good inhibitory effects for the bacteria submitted to the analysis, with MIC values 0.1% for *E. coli*, *S.* Typhimurium and *L. monocytogenes* and 0.2% for *S. aureus*. In the case of TNE MIC values were 0.01 for *S.* Typhimurium and *L. monocytogenes* 0.04 for *E. coli* and 0.078 for *S. aureus*. The same results were investigated by Mohan and Purohit (2020) and Al-Asmari *et al*. (2024).

From this point of the results in Figures 4 and 7 showed that TEO and TNE treatments resulted in reductions of *E. coli* O157:H7 and *L. monocytogenes* counts during the storage period and absence of bacterial growth on the 5th day of storage in yogurt treated with TEO 1%, TEO 2%, TNE 1% and TNE 2%. Although in control sample the growth continued till the end of the experiment. Furthermore, in results of Figures 5 and 6, there were absence in *S.* Typhimurium and *S. aureus* bacterial load from day 7 in case of yogurt treated with TEO, TNE 1% but in yogurt samples with TEO, TNE 2% the bacterial load disappeared on day 5.

Generally, treatment of yogurt with TEO and TNE showed strong antibacterial efficacy against tested bacterial strains. Essential Oils interact with structural and biochemical features of bacteria causing disorders in the functioning of cell membrane. These disorders lead to outflow of the cytoplasm, proteins, and enzymes associated with normal cellular functions, subsequently disrupting the normal metabolic processes required for cellular functioning, leading to cell death (Al-Asmari *et al*., 2024). Nasra *et al*. (2024) also revealed that Thymus essential oil nanoemulsions exhibited potent antibacterial properties. The nanoemulsion had ten times more antibacterial activity than the pure essential oil. They felt that encapsulating the essential oil in the nanoemulsion allowed the hydrophobic molecules of the essential oil to make more contact with the surface of *E. coli* cell membranes, allowing for rupture and leaking of cellular components. Because of its hydrophobicity, the pure essential oil did not easily interface with the cell membrane surface.

The scanning electron microscopy (SEM) imaging of bacterial strains treated with thyme essential oil (TEO) and its nanoemulsion provides a visual confirmation of the antimicrobial effects observed in vitro. The methodology employed fixation, dehydration, critical point drying, and conductive coating ensures that the bacterial morphology is well preserved for detailed SEM analysis. This approach allows for the observation of the physical impact of TEO and its nanoemulsion on the bacterial cells.

The SEM images likely reveal structural damage to the bacterial cell walls and membranes, which are consistent with the potent antimicrobial activity demonstrated by TEO, particularly in its nanoemulsion form. The nanoemulsion form of TEO appears to have a significantly enhanced antimicrobial effect, as evidenced by the increased minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) measurements. The superior efficacy of the TEO nanoemulsion against pathogens like *E. coli* O157, *S.* Typhimurium, *S. aureus*, and *L. monocytogenes* is highlighted by these imaging results.

These findings are in line with Guo *et al*. (2020), He *et al*. (2021) and Al-Asmari *et al*. (2024) who have also reported strong antimicrobial activities of TEO nanoemulsion against similar bacterial strains. The enhanced antimicrobial effect can be attributed to the improved dispersion and stability of the essential oil in the nanoemulsion, which facilitates better interaction with bacterial cells. This results in more effective disruption of bacterial membranes and ultimately leads to cell death.

The SEM images complement the quantitative data by providing a visual representation of the bacterial cell damage, thereby reinforcing the conclusion that TEO nanoemulsion is a highly effective natural antimicrobial agent. This makes it a promising candidate for applications in food preservation, particularly in combating microbial contamination in dairy products like cheese and yoghurt. It also provides insight into the mode of action of thyme essential oil (TEO) and its nanoemulsion against various bacterial pathogens, including *E. coli* O157, *S.* Typhimurium, *S. aureus*, and *L. monocytogenes*. The differences observed in the structural integrity of the bacteria after treatment with TEO and its nanoemulsion suggest multiple mechanisms by which these agents exert their antimicrobial effects through cell membrane disruption, cell wall integrity compromise, intracellular content leakage, synergistic effects in nanoemulsion form, and impact on different bacterial types.

As shown in Figs. 2 and 3, both TEO and its nanoemulsion cause significant damage to bacterial cell membranes (Cell membrane disruption). The essential oil's lipophilic components, such as thymol and carvacrol, integrate into the lipid bilayer of bacterial membranes, disrupting their structure and function. This disruption leads to increased membrane permeability, which can be visualized as irregularities or perforations in the bacterial cell surface. These damages compromise the cell's ability to maintain homeostasis, leading to leakage of cellular contents, loss of vital molecules, and ultimately, cell death these result in accordance with Zhou *et al*. (2019) and Li *et al*. (2020). Moreover, TEO and its nanoemulsion caused visible disruptions or thinning of the cell wall, particularly in Gram-positive bacteria (*S. aureus*), making them more susceptible to lysis (Cell wall integrity compromise). The nanoemulsion form of TEO, with its smaller particle size and higher surface area, likely enhances the penetration of active compounds into the cell wall and membrane, leading to more extensive damage (Fig. 3).

Intracellular content leakage was manifested in the collapse of bacterial cells, indicating a loss of internal turgor pressure due to the leakage of intracellular contents. This can be attributed to the essential oil's action on the cell membrane, leading to the outflow of cytoplasmic material The same results were explained by Posgay *et al*. (2022). The damage observed in the SEM images (Figs. 2 and 3) corresponds with the increased zones of inhibition (ZOI) and reduced minimum inhibitory concentrations (MIC) for the TEO nanoemulsion, indicating its potent bactericidal effect. On the other hand, synergistic effects in nanoemulsion form reflected in the significantly larger ZOI observed with the nanoemulsion compared to the pure oil, particularly against *S.* Typhimurium (41.5 mm) and *S. aureus* (45 mm). The improved dispersion of the nanoemulsion ensures that the active components of TEO interact more efficiently with the bacterial cells, leading to more pronounced antimicrobial effects. The nanoemulsion's enhanced effectiveness is likely due to its ability to deliver the active compounds of TEO more efficiently, resulting in more extensive structural damage to the bacteria These findings are in line with Ozogul *et al*. (2020) and Sateriale *et al*. (2023). These findings underscore the potential of TEO nanoemulsions as a powerful natural antimicrobial agent for controlling pathogenic bacteria in various applications, including food preservation and safety.

Conclusion

Although, the high antimicrobial results of the treatment of yogurt with TEO, the sensory analysis of addition of TEO showed low acceptance. The panelists discovered that yoghurt with pure essential oils smelled strongly and was not accepted. However, overall acceptability parameters (taste, flavor, smell, and odor) scored highly for yogurt containing nanoemulsions (TNE). But as the yoghurt's storage duration extended, its acceptance decreased. The SEM images provide crucial evidence that thyme oil, particularly in its nanoemulsion form, exerts its antimicrobial effects by targeting and disrupting bacterial cell membranes and walls, leading to cell death.

Conflict of interest

The authors have no conflict of interest to declare.

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