Journal of Food Safety

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Journal of Food Safety

ORIGINAL ARTICLE OPEN ACCESS

Antimicrobial Activity of Thyme, Olive Oil, and Their Nanoemulsions Against *Cronobacter Sakazakii*: In Vivo Application as Natural Food Preservatives in Tallaga Cheese

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Received: 27 August 2024 | **Revised:** 10 November 2024 | **Accepted:** 30 November 2024

Keywords: antibacterial activity | *C. sakazakii* | MICs | nanoemulsions | olive oil | thyme oil

ABSTRACT

Tallaga cheese is the most popular type of white soft cheese in Egypt. *Cronobacter sakazakii* is an opportunistic foodborne pathogen that poses a threat to the health of almost every age group, with a particular emphasis on neonates and infants. Essential oils (EOs), such as olive oil and thyme oil, exhibit a broad-spectrum antibacterial effect. However, few studies have investigated the antibacterial activity of these EOs and their formulated nanoemulsions (NEs)—olive oil nano-emulsions (ONE) and thyme oil nano-emulsions (TNE)—on *C. sakazakii* in cheese to date. The purpose of this study was to investigate the inhibitory effects of these EOs and their NEs on the development of *C. sakazakii* during the processing and storage of Tallaga cheese. Additionally, the organoleptic properties of the resulting cheese were evaluated. Herein, ONE and TNE were prepared and characterized using a zetasizer and transmission electron microscopy (TEM). Furthermore, the minimum inhibitory concentrations (MICs) of EOs and NEs were determined using well diffusion assay. Fresh buffalo's milk for the elaboration of Tallaga cheese was inoculated with *C. sakazakii* at a concentration of 6 log CFU/mL, along with the addition of MICs of either EOs or NEs. The obtained results showed that the MICs were 15 mg/mL (v/v) for either olive oil, thyme oil, ONE and TNE with diameter inhibition zones of 10.3 ± 0.5, 13.3 ± 1.7, 12.3 ± 0.5, and 16 ± 0.8 mm, respectively. In addition, NEs exhibited high antibacterial activities against *C. sakazakii* in comparison to pure EOs. Particularly, the strongest antibacterial effect toward *C. sakazakii* was reported with TNE during manufacturing and storage of Tallaga cheese. Overall, these results suggest that thyme and its NEs have the potential to prevent bacterial contamination by *C. sakazakii* in the dairy industry.

1 | Introduction

Tallaga cheese is the most popular white soft cheese in Egypt. It is one of the local types of packaged or unpackaged cheeses, its consumption is high due to its desirable flavor and texture, high nutritional value, and pleasant taste with low salt content (average of 3%) (Hamad and Eldin 2021). It can be consumed either fresh or after ripening for 1 month at refrigerator temperature (El-Kholy et al. 2016). Due to the rich nutrient composition and high moisture content (from 58.17% to 66.02%) (Ghada et al. 2004), Tallaga cheese can support the growth of foodborne pathogens during its shelf-life. Among these, *Cronobacter sakazakii* stands out as a particularly hazardous pathogen. It is the predominant species within the *Cronobacter* genus, which comprises seven species: *C. sakazakii*, *Cronobacter malonaticus*, *Cronobacter turicensis*, *Cronobacter muytjensii*, *C. dublinensis*, *C. universalis*, and *Cronobacter condiment* (Healy et al. 2010). Particularly, *C. sakazakii* has been implicated in severe, life-threatening diseases, especially affecting neonates and immunodeficient adults (Iversen and Forsythe 2003).

C. sakazakii is a widespread pathogen previously recovered from different food substrates, including foods of plant origin such as vegetables, herbs, spices, and cereal products, as well as foods of animal origin such as powdered infant formula (PIF), milk, cheese, fish, meat, and meat products (Beuchat et al. 2009; Das et al. 2021; Hayman et al. 2020; Saad and Ewida 2018). Isolates of *C. sakazakii* recovered from different kinds of cheese have been reported in various countries (Adeyemi 2012; Aigbekaen and Oshoma 2010; Casalinuovo et al. 2014; Chaves-López et al. 2006; El-Sharoud et al. 2008; Morales et al. 2004; Restaino et al. 2006; Saad and Amin 2014). In particular, El-Sharoud et al. (2008) reported that *C. sakazakii* can survive for up to 1 month in cheese processed from contaminated milk powder under experimental conditions. Cheese is light and easy to digest, so it is often recommended in the diet of all ages. Consequently, isolation of *C. sakazakii* from cheese produced in an artisan system sheds more light on food contamination by this bacterium and reflects to what extent consumers are exposed to the risk of diseases caused by the microorganism (Casalinuovo et al. 2014).

To avoid and control food contamination with food pathogens as *C. sakazakii*, various strategies have been developed. Physical methods, for instance, were used for microbial control to preserve food such as high temperatures, radiation, filtration, and desiccation (drying). Nevertheless, these methods could induce some undesired changes in food through production of free radicals, off-flavors, off-odors, viscosity diminution, discoloration, and lipid oxidation (Ali et al. 2016). Moreover, chemicals (e.g., salt, sugar, vinegar, and preservatives) and biological preservation (e.g., beneficial microbes, bacteriocins, or bacteriophages) were also applied in food (Abdelhamid and El-Dougdoug 2020). Some of chemical food preservatives pose health hazards to the consumers in the form of headache, allergic reactions, asthma, cancer, and others such as sulfates (Sharif et al. 2017). Recently, synthetic antimicrobial preservatives are widely used in food industry to increase food safety (El-Saber Batiha et al. 2021). However, antimicrobials have gained researchers' attention for their unique advantages. For instance, using natural antimicrobials could reduce the risk of using antibiotics in food which is implicated in a serious problem called antibiotic-resistant pathogens. In addition, the application of such antimicrobials had no potential negative health effects as expected with the synthetic preservatives (Guo et al. 2020). Among natural antimicrobials, plant-derived compounds are considered effective natural antibiotics and/or bactericidal agents because of their safety, nature, and abundance in environment (Farid, Waheed, and Motwani 2023). Essential oils (EOs) have gained more interest from food hygienists because of their antimicrobial effectiveness and their ability to enhance the food flavor (Chang et al. 2021). Several studies previously examined the strong antibacterial activity of EOs against pathogenic and food spoilage bacteria such as *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Staphylococcus aureus*, and *C. sakazakii* (Duda-Chodak, Tarko, and Petka-Poniatowska 2023; Guo et al. 2022; Kim and Rhee 2016; Wang et al. 2022; Yang et al. 2022). However, only a few studies reported the inhibitory effects of olive oil and thyme oil on *C. sakazakii* (Fei et al. 2018).

Olive oil was used for its antimicrobial activity in food industry as a natural food preservative. The antibacterial effect of olive oil was reported toward both Gram-positive and Gram-negative bacteria such as *Helicobacter pylori*, *E. coli*, *L. monocytogenes*, and *Salmonella enteritidis* (Guo et al. 2019; Karaosmanoglu et al. 2010). This powerful antimicrobial effect has been attributed to the high concentration of polyphenols (Nazzaro et al. 2019). On the other hand, thyme (*Thymus vulgaris* L.) is an important medicinal herb that is considered a source of some bioactive substances including EO and thymol (Gedikoğlu 2022). These compounds have a potential role in the microbiological stability of different food matrices (Gedikoğlu 2022; Posgay et al. 2022). Thyme could exhibit the antibacterial effect by inducing the permeability and depolarization of the cytoplasmic membrane of microorganisms according to the previous suggestion by Xu et al. (2008).

On the other hand, nanoemulsions (NEs), which consist of very small droplet-sized emulsions used for encapsulating bioactive components, offer increased stability and have the potential to enhance the biological activity of hydrophobic compounds. This makes NEs suitable for utilization in the food industry (Acevedo-Fani et al. 2015; Moghimi et al. 2016). Edible NEs can be used for coating some food such as dairy products including cheese, meat, vegetables, and fruits to keep it fresh and enhance their storage (Mushtaq et al. 2023). The main advantages of oil-in-water NEs are that they get digested quickly inside the gastrointestinal tract. Besides, they can inhibit gaseous exchange, reduce moisture losses, and oxidation of food matrices (Jafari and McClements 2017). Hence, edible NEs can meet the market's large-scale demand in the dairy industries (Mushtaq et al. 2023). Importantly, new research reported a higher antimicrobial activity of EO NEs (Dávila-Rodríguez et al. 2019; Rinaldi et al. 2021; Touayar et al. 2023). However, data focused on the antibacterial impact of thyme or its NEs against *C. sakazakii* in dairy products are still very scarce (Lee and Jin 2008; Tian et al. 2021). Thus, the objective of the present study was to prepare and characterize NEs of thyme and olive EOs using a zetasizer and transmission electron microscopy (TEM), then determine the inhibitory effect of these EOs and their NEs against *C. sakazakii* in vitro and in vivo during manufacturing and storage of the Egyptian Talaga cheese.

2 | Materials and Methods

2.1 | Bacterial Strain and Culture Conditions

C. sakazakii (ON197907) was previously isolated using standard culture methods on Cronobacter Chromogenic Isolation agar (Oxoid, Basingstoke, UK) from dairy products in our laboratory at the Department of Hygiene, Safety and Technology of Food, Faculty of Veterinary Medicine, Assiut University, Egypt, and identified with conventional biochemical methods, PCR and gene sequencing techniques. Bacterial isolates were stored in tryptone soya broth (TSB) with 20% glycerol (v/v) at −80°C. Before each experiment, stock cultures were streaked on

Cronobacter Chromogenic isolation agar and grew at 37°C for 18 h. Then a loopful of each strain was inoculated into 30 mL TSB and incubated for 18 h at 37°C. Before inoculation in milk, the inoculum was washed twice in phosphate-buffered saline (PBS) (Oxoid), then resuspended in skim milk and kept at refrigerator temperature until further use.

2.2 | Preparation of NEs

NE of thyme (TNE) was carried out according to Ghosh, Mukherjee, and Chandrasekaran (2012) with minor modifications. Briefly, 75% w/w of deionized water was mixed with 10% (w/w) of EOs and 15% (w/w) of Tween 80 at a total volume of 100 mL for the solution, respectively. The mixture was homogenized at 800 rpm with a magnetic stirrer for 30 min. The obtained emulsion was sonicated for 20 min using a 25-kHz ultrasonic homogenizer (USH650, max power of 650 W). On the other hand, olive oil nanoemulsion (ONE) was prepared as previously described by Karami, Khoshkam, and Hamidi (2019) with some modifications. In brief, span 80 and span 20 were mixed in deionized water, then olive oil was added to the mixture in a ratio of 6:9:2, respectively. Furthermore, 0.01 g of NaCl was added to the mixture to increase the stability of the obtained solution. Finally, the mixture was placed on a magnetic stirrer at a high speed of 1.500 rpm/min for 6 h with a stirring rotor inside. The resulting emulsion was then sonicated for 20 min using a 25-kHz ultrasonic homogenizer (USH650, max power: 650 W). At last, the obtained NEs (TNE and ONE) were stored at refrigerator temperature for further use as a natural food biopreservatives during manufacturing cheese.

2.3 | Characterization of the EOs′ NEs

2.3.1 | Particle Size Measurement for TNE

The diameter of thyme NE droplets was measured using dynamic light scattering (DLS, NICOMP 380 ZLS, DLS instrument, USA) by photon correlation spectroscopy. Samples were diluted in purified water prior to analysis to avoid multiple scattering effects (Lu et al. 2017).

2.3.2 | Fourier-Transform Infrared Spectroscopy (FTIR) Spectral Analysis

FTIR analysis was performed in the analytical chemistry-accredited laboratory, Chemistry Department, Faculty of Science, Assiut University, Egypt. This step was used to identify the functional groups with their means of attachment and the fingerprint of the molecule. In FTIR, samples were prepared by employing a suitable method called Nujol mulls method. Briefly, a small amount of the sample was mixed with a suitable liquid paraffin or Nujol to form a thick paste. Furthermore, the Nujol-sample mixture was spread onto a transparent salt plate or a suitable infrared-transparent window. The mixture was left to evaporate forming a thin film of the sample. Then, the salt plate with the Nujol mull was placed in the sample compartment of the FTIR instrument. Finally, samples were scanned by the FTIR spectrometer at wave numbers ranged from 4000 to 500 cm^{-1} (Mikhailova 2022).

2.3.3 | TEM

TEM analysis was carried out to confirm the size measurements and determine the morphological characteristics of the obtained NEs using JEOL JEM-1230 TEM (JEOL, Japan) (Gruskiene et al. 2018). The sample was diluted with deionized water (100 times), then one drop of the diluted sample was placed on a 200-mesh film grid and dried using Whatman filter paper at room temperature. Furthermore, samples were stained with uranyl acetate and allowed to dry for 10 min before observation with the electron microscope.

2.4 | Calculation of the Minimum Inhibitory Concentrations (MICs)

The MICs of thyme, olive oils, and their NEs against *C. sakazakii* were determined using the well diffusion method as previously described by Suresh et al. (2016). In brief, the culture was initially diluted with sterile peptone water (0.1% w/v) to achieve an inoculum of approximately 10^6 CFU/mL. Then, 0.1 mL aliquots were placed onto the surface of Mueller Hinton agar (Oxoid). The plates were allowed to dry for 20 min at room temperature. 6-mm wells were made using a sterile cork borer into each of the petri plates of Mueller Hinton agar. Then, 5 μL of the different concentrations (5, 10, 15, 20, 25, 30 mg/mL) of EOs (thyme oil and olive oil) and their NEs (TNE and ONE) were added to the wells. Sterile saline solution (0.85 v/v) was used as a negative control. The plates were incubated at 37°C for 24 h, then the inhibition zones were observed and measured in millimeters. All experiments were conducted in triplicate.

2.5 | Laboratory Manufacturing of Tallaga Cheese

Tallaga cheese was manufactured according to Abdel-Salam (2010) (Figure 1). Fresh buffalo's milk was standardized to have 5% fat followed by a pasteurization at 72°C for 15 s. and cooling to 38°C–40°C. Calcium chloride, sodium chloride, and commercial rennet were added at the ratios of 0.02%, 4% and 0.05% (w/v), respectively. The obtained milk was divided into six equal portions for further use as follows: (1) the first group used as negative control which free from EOs and pathogenic bacteria under study, (2) plain sample contained EOs or NEs for sensory evaluation, (3) samples inoculated with *C. sakazakii* without EOs or NEs (considered a positive control cheese), (4) *C. sakazakii* + 15 mg/mL olive oil, (5) *C. sakazakii* + 15 mg/mL ONE, (6) *C. sakazakii* + 15 mg/mL thyme oil, and (7) *C. sakazakii* + 15 mg/mL TNE. *C. sakazakii* was inoculated in milk at a concentration of 10^6 CFU/g in all treatments apart from the negative control group. All cheese milk was kept coagulating at room temperature. The curds were whey out, and the obtained cheeses were stored in refrigeration at 7°C ± 2°C. Samples were collected and *C. sakazakii* was enumerated immediately after elaboration, and after 12, 24, 72, and 120 h of storage in Cronobacter Chromogenic Isolation Agar.

2.6 | Organoleptic Assay of Tallaga Chesse

Cheeses were processed with the addition of EOs or NEs, and without inoculation of pathogen, then used for sensorial evaluation just after processing and during storage of cheese (after 24,

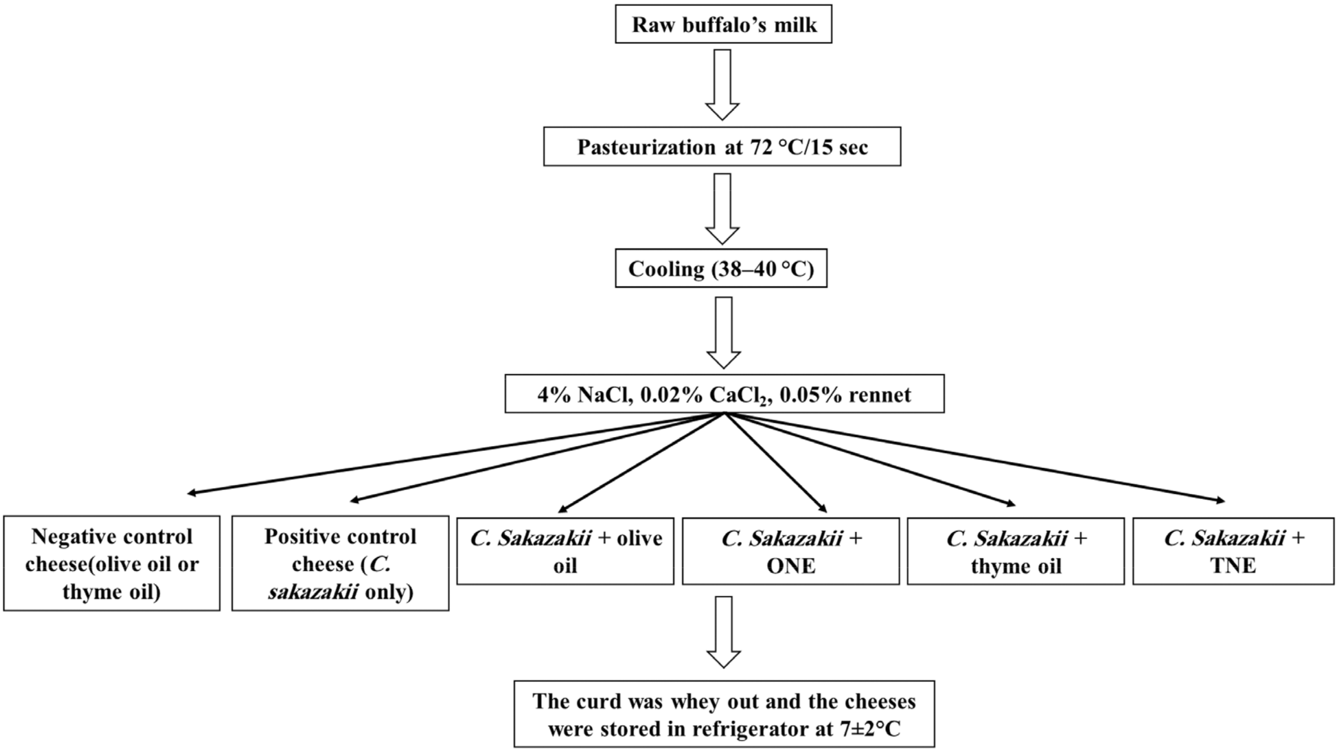

FIGURE 1 | Flow diagram of Tallaga cheese making; negative control cheese; and positive control cheese: *C. sakazakii* + olive oil, *C. sakazakii* + ONE, *C. sakazakii* + thyme oil, and *C. sakazakii* + TNE.

48, 72, and 120 h). Thirty-five panelists were selected in teams of different ages, gender, and education. Sensorial attributes such as color, flavor, mouthfeel, appearance, and overall acceptability of the prepared cheese samples were assessed. The scale points were excellent (5); very good (4); good (3); acceptable (2); and poor (1) (Elsherif et al. 2024).

2.7 | Statistical Analysis

Experiments were done in triplicate to better capture microbial variability. Descriptive statistics were calculated from the observed data with MS Excel (Microsoft Corporation). The statistical analysis performed consisted of means comparison tests, univariate analysis of variance (ANOVA) followed by Tukey post hoc test ($p < 0.05$) to evaluate significant differences in the levels of *C. sakazakii* in cheese. The SPSS v22.0 software (Chicago, Illinois, USA) was used for statistical analyses.

3 | Results

3.1 | Characterization of the EOs′ NEs

3.1.1 | Particle Size Measurement of ONE and TNE Using Photon Correlation Spectroscopy

In the current study, the freshly prepared ONE had an average droplet size of 534.2 ± 181.9 nm and the polydispersity index (PDI) was 0.280 using a zetasizer. The diameter of thyme nanoemulsion (TNE) was 924.4 ± 172.0 nm with a PDI equal to 0.113.

3.1.2 | FTIR Spectral Analysis

Herein, the nature of the biomolecules involved in the reduction and formation of NEs was examined by FTIR (Figure 2). Several highlighted peaks can be observed in the FTIR spectra of which six were the main ones. These primary bonds were located at the following coordinates: 3423.72 (OH vibration of thyme oil hydroxyl group), 2962.12, and 2871.12 cm^{-1} (C–H stretching of methyl and isopropyl groups on the phenolic ring that may be related to the two main bioactive compounds in thyme oil, carvacrol, and thymol), and 1458.17, 1421.41, and 808.10 cm^{-1} (C–C in the ring of aromatics group). The peaks centered between 1640 and 1736.16 cm^{-1} wavenumbers were related to the C═C bonds, which were related to the thymol. However, a smooth and enlarged peak at 3358.58 cm^{-1} was observed, indicating the hydrophilic interaction between the hydrophilic group in Tween and oil. On the other hand, the olive oil peak at 2924.77 cm^{-1} indicated C–H stretching due to alkenes, and pure olive oil showed two prominent IR bands at around 1746.6 and 1456 cm^{-1}, respectively. These bands are caused by the C═O stretching of acid halides and the C═N stretching of amines. While in NE formulations, the peak at 1744.63–1465 was almost there, although there was a slight shift to a lower wavelength (Figure 2). It was also clear that when olive oil was encapsulated, some small and significant peaks were created (Figure 2).

3.1.3 | TEM

TEM was carried out to determine the morphology and size of the biosynthesized NEs of olive oil (ONE) and thyme (TNE).

The TEM image of NEs (Figure 3) showed that the particles are spherical in shape, and the size of the ONE was found to be in the range from 85 to 97 nm. On the other hand, the size of TNE was in the range of (95:123) nm (Figure 3).

3.2 | Determination of the MICs

MICs were examined to evaluate the antibacterial properties of two EOs and their NEs against *C. sakazakii* (Table 1). After determining the antibacterial effect of different concentrations (5, 10, 15, 20, 25, and 30 mg/mL) of the studied EOs, 15 mg/mL was the MIC that could inhibit the *C. sakazakii* strains for both olive oil and thyme oil, but the diameter of the inhibitory zone was wider in the case of thyme oil in comparison with that of olive oil (13.3 ± 1.2 and 10.3 ± 0.3 mm, respectively). The obtained MICs of ONE and TNE were 10 and 5 mg/mL, respectively, and the largest inhibition diameters were shown with TNE (Table 1).

Overall, the obtained results showed a dose-dependent increase in the diameters of the inhibitory zones against *C.*

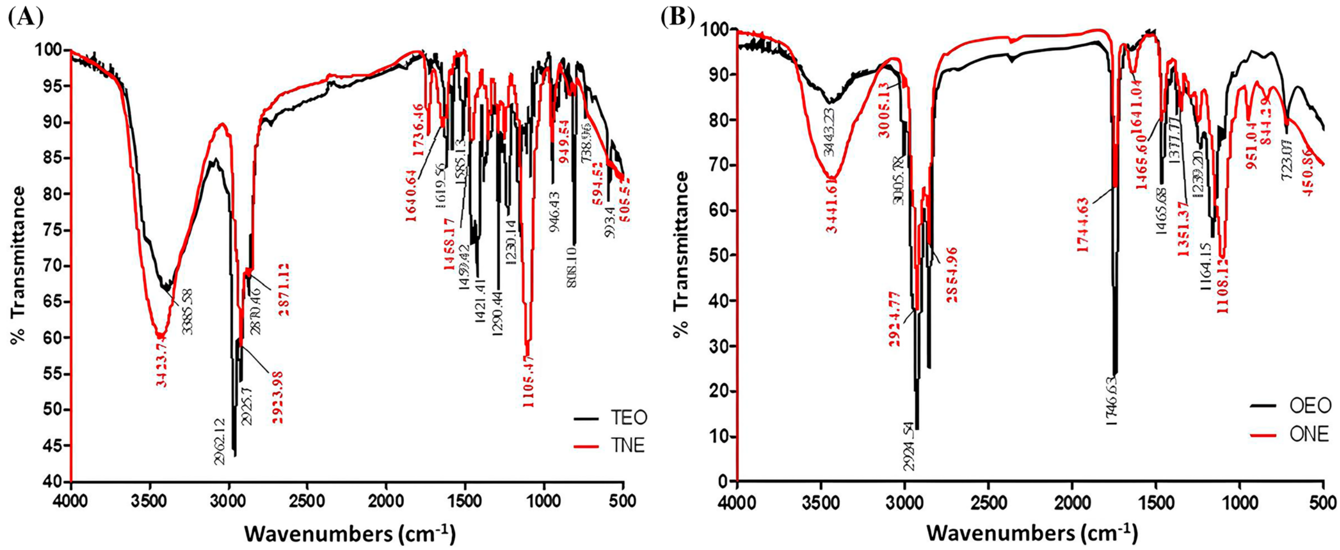

FIGURE 2 | Fourier-transform infrared (FTIR) spectra of (A) pure thyme oil (TEO) and thyme nanoemulsion (TNE); and (B) pure olive oil (OEO) and olive oil nanoemulsions (ONEs).

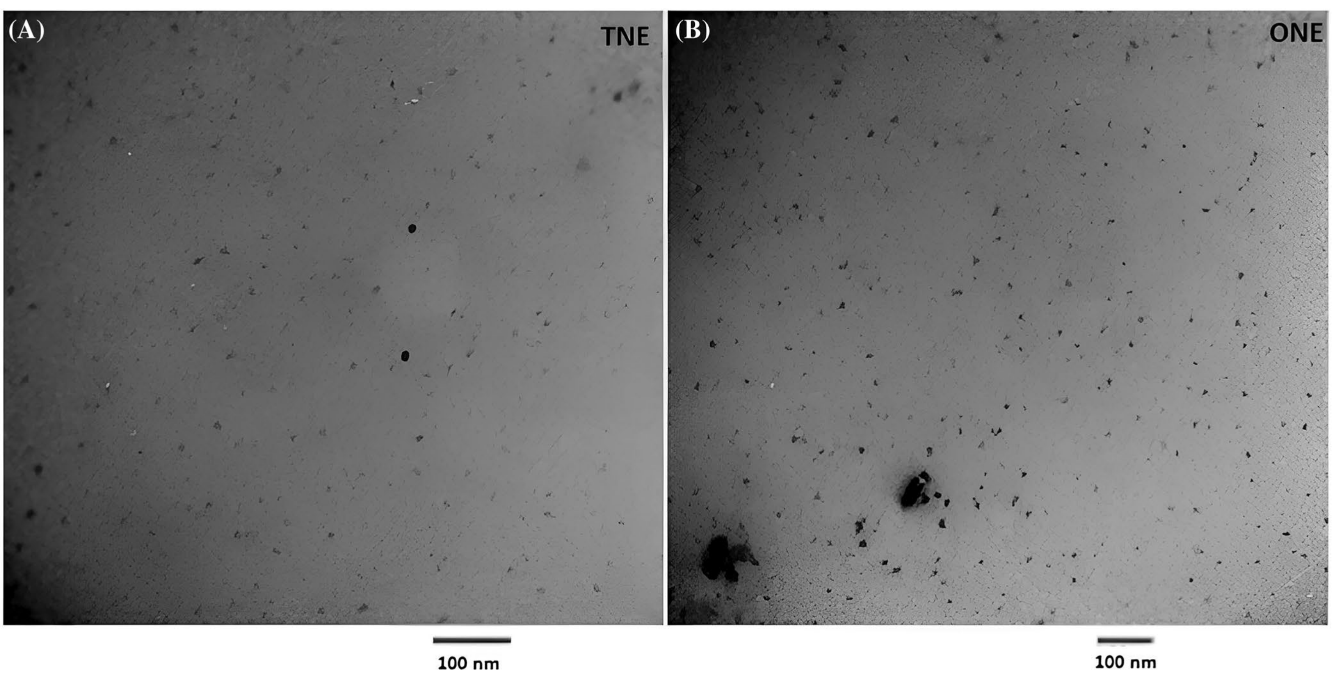

FIGURE 3 | Transmission electron microscopy images of the nanoemulsions prepared with the ratio of (A) thyme essential oil (TEO), Tween 80, and water in 2:3:15; and (B) olive essential oil (OEO), Span 80, Span 20 in 2:6:9 in deionized water; (w/w/w; scale bar is 100 nm).

TABLE 1 | Inhibition zone diameter (mm) of pure essential oils (thyme oil and olive oil) and their nanoemulsions (TNE and ONE) against *C. sakazakii* using well diffusion assay.

Concentrations of EOs or NEs (mg/mL)	Thyme oil	TNE	Olive oil	ONE
30	21.5 ± 0.5^{b}	27.5 ± 0.5^{a}	18.0 ± 1.0^{b}	21.5 ± 1.5^{b}
25	17.5 ± 0.5^{ab}	22.5 ± 2.5^{a}	14.5 ± 0.5^{b}	19.0 ± 0.0^{ab}
20	15.3 ± 1.2^{b}	19.3 ± 0.3^{a}	12.3 ± 0.9^{b}	17.3 ± 0.3^{ab}
15	13.3 ± 1.2^{ab}	16.0 ± 0.6^{a}	10.3 ± 0.3^{b}	12.3 ± 0.3^{b}
10	5.7 ± 2.8^{b}	9.7 ± 0.9^{a}	2.7 ± 2.7^{b}	5.0 ± 2.5^{b}
5	—	5.3 ± 2.7^{a}	—	—

Note: Means with different superscripts denote significant differences (p value < 0.05) between the different treatments at the same concentration (different columns within the same row). — indicates absence of inhibition zone.

sakaakii in all treatments (Table 1). However, there were significant differences ($p < 0.05$) in the inhibition diameters produced by TNE against the examined pathogen in comparison to other treatments (thyme oil, olive oil, and ONE) at concentrations of 5, 10, 15, 20, and 30 mg/mL (Table 1). Notably, the inhibitory effect of TNE was higher ($p < 0.05$) than that induced by thyme oil toward *C. sakazakii*. Both olive oil and ONE exert similar antibacterial activity ($p > 0.05$) against such organisms in the current study.

3.3 | Antibacterial Activity of EO (Olive Oil and Thyme Oil) and Their NEs (ONE and TNE) Against *C. sakazakii* During Manufacturing and Storage of Tallaga Cheese

Herein, Figure 4 illustrates the antibacterial effect of olive oil and ONE on the studied foodborne pathogen during manufacturing and storage of Tallaga cheese. There was a significant reduction in the mean count of *C. sakazakii* in olive oil and ONE cheeses in comparison to control cheese ($p < 0.05$). There was rapid reduction in the level of *C. sakazakii* in cheeses formulated with ONE (3.91 ± 0.23 log CFU/g) after 12 h of cheesemaking when compared with control and olive oil cheeses (4.95 ± 0.37 and 4.41 ± 0.22 log CFU/g, respectively) (Figure 4). Interestingly, ONE could induce the highest inhibitory effect ($p < 0.05$) against *C. sakazakii* as the organism failed to be detected at the end of the experiment (after 120 h of storage) in comparison to other cheese formulations (Figure 4).

On the other hand, the periodical examination of Tallaga cheese supplemented with the MIC of 15 mg/mL of either thyme (thyme oil) or TNE for their antibacterial activity on *C. sakazakii* showed that there was a drastic decrease in the number of bacterial cells during manufacture and ripening of cheeses versus to control samples ($p < 0.05$). In other words, *C. sakazakii* counts were reduced directly after manufacturing (a few hours after getting the finished product) thyme oil and TNE Tallaga cheese (Figure 5). After 12 h of storage time, the population of *C. sakazakii* decreased by about 1.5 log CFU/g (3.64 ± 0.45 log CFU/g) in thyme oil Tallaga cheese, while nearly 2 log reduction (3.15 ± 0.78 log CFU/g) was observed in the case of TNE group in comparison to initial time and control cheese (4.96 ± 0.26 log CFU/g). Additionally, *C. sakazakii* rapidly decreased ($p < 0.05$) during cheese ripening until became undetectable (< 10 CFU/g) at 120 h of storage in both thyme oil and TNE Tallaga cheeses (Figure 5).

Altogether, the microbial kinetics of *C. sakazakii* in control Tallaga cheese compared with pure EOs (olive oil and thyme oil) and their NEs (ONE and TNE) Tallaga cheeses is shown in Figure 6. Free oils and NEs prevented the increase of *C. sakazakii* from the first step of manufacture with undetectable levels at 120 h of cheese ripening with thyme oil, ONE, and TNE groups (Figure 6). After cheesemaking, the initial concentration of *C. sakazakii* remained at the same levels in the control samples till 24 h of storage (4.85 ± 0.87 log CFU/g), while a slight decrease ($p > 0.05$) was found for cheese sample with the addition of olive oil (3.37 ± 0.25 log CFU/g) at the same time (Figure 6). On the other hand, a significant reduction ($p < 0.05$) in the average count of *C. sakazakii* was found in Tallaga cheeses treated with ONE, thyme oil, or TNE from 24 h of cheese making till the end of the experiment (Figure 6). Moreover, there was a significant log reduction ($p < 0.05$) of *C. sakazakii* strains in the presence of ONE, thyme oil, and TNE in comparison to control and olive oil during making and storage of Tallaga cheese as shown in Figure 7.

3.4 | Sensorial Evaluation of Tallaga Cheeses Inoculated With EOs and Their NEs

Sensorial evaluation of Tallaga cheeses was carried out at different time points till the end of the storage period (120 h). The panelists found that cheeses containing pure EOs (thyme or olive oils) were unpalatable with strong smells. On the other hand, Tallaga cheeses with NEs (TNE or ONE) had high scores of general acceptability parameters (smell, odor, taste, and flavor) (Figure 8). There were no significant differences between TNE and ONE cheeses comparing with the control group until the end of the experiment. However, the acceptability of all cheeses was reduced with the progress of storage period (Figure 8).

4 | Discussion

The present study elucidates for the first time the inhibitory effect of EOs (olive oil and thyme) and their formulated NEs (ONE and TNE) on *C. sakazakii* during the manufacturing and storage of Tallaga cheese in Egypt. Interestingly, thyme and TNE

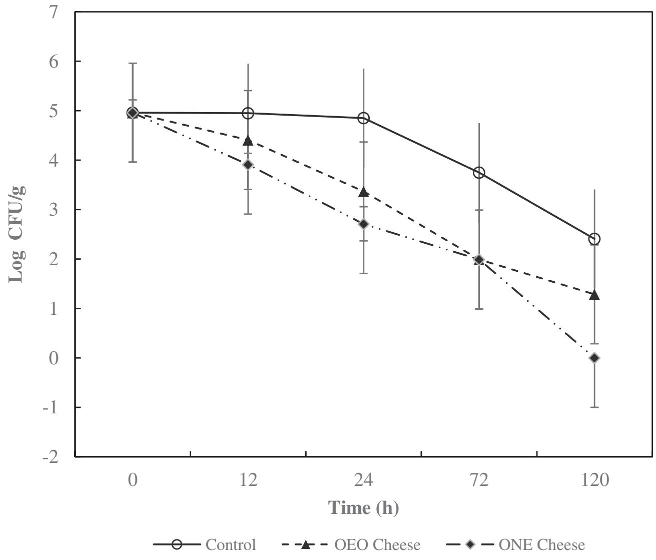

FIGURE 4 | *C. sakazakii* survival during Tallaga cheese ripening for 120 h storage after the addition of pure olive oil (15 mg/mL) or olive nanoemulsion (ONE; 15 mg/mL) in comparison to control group (inoculated with *C. sakazakii* only).

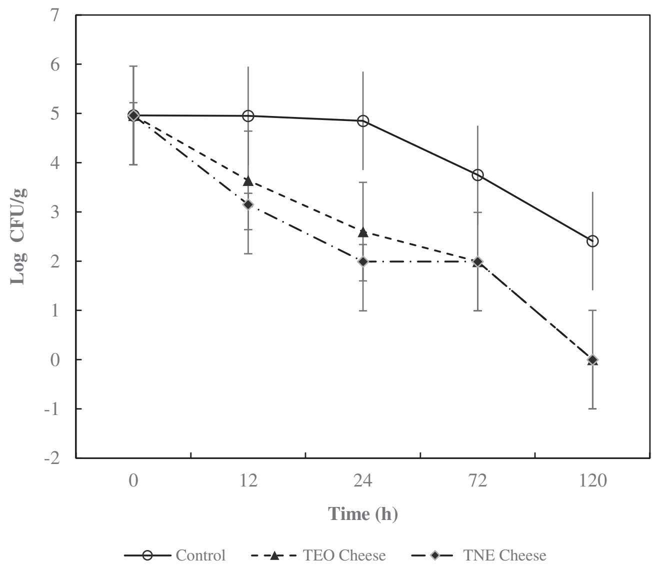

FIGURE 5 | *C. sakazakii* survival during Tallaga cheese ripening for 120 h storage after the addition of pure thyme oil (15 mg/mL) or thyme nanoemulsion (TNE; 15 mg/mL) in comparison to control group (inoculated with *C. sakazakii* only).

exhibited the highest antibacterial activity against *C. sakazakii* during the initial steps of Tallaga cheese making in comparison to olive oil and its NE. Moreover, NEs did not change the sensorial properties of Tallaga cheese. Consequently, TNE followed by ONE could play a crucial role as biopreservatives against *C. sakazakii* in the dairy industry.

PDI can be defined as the ratio of standard deviation to the mean particle diameter (Jaiswal, Dudhe, and Sharma 2015). The higher value of PDI indicates the lower uniformity of droplet size of NE. The current findings showed that reasonable small-sized particles of ONE and TNE were obtained. Notably, the small size

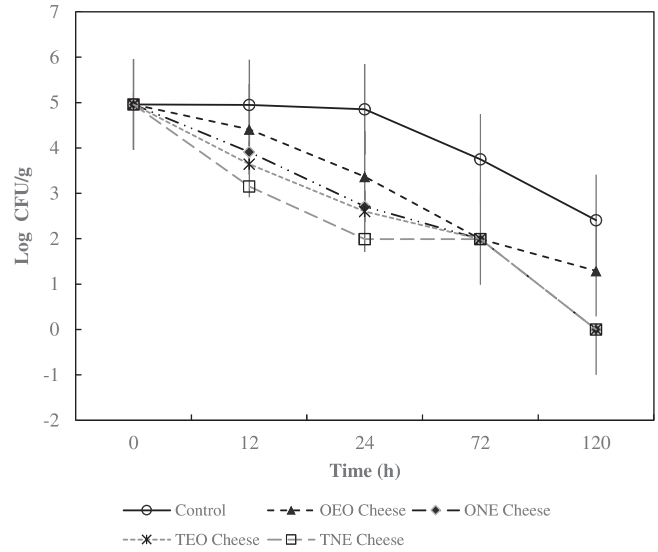

FIGURE 6 | Growth of *C. sakazakii* during Tallaga cheese maturation for 120 h of storage at 7°C ± 2°C. Cheese was treated with pure essential oils (olive oil or thyme oil) or their nanoemulsions (ONE or TNE) in comparison to control group cheese (inoculated with *C. sakazakii* only).

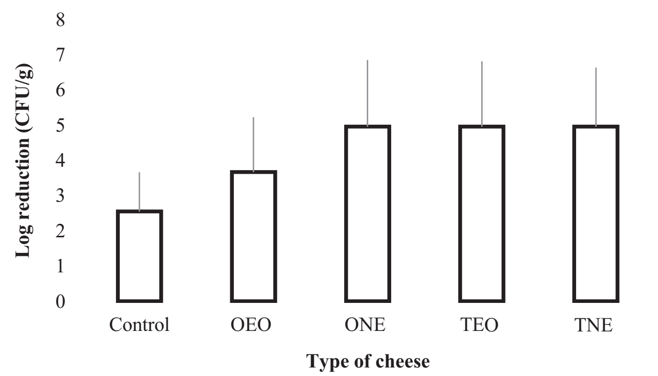

FIGURE 7 | Comparison between the inactivation levels of essential oils and their nanoemulsions (ONE and TNE) on the count of *C. sakazakii* versus control during manufacture and storage of Tallaga cheese.

of the prepared NEs and the small range of PDI (from 0.2 to 0.4) indicated a good stability of the prepared NEs. The morphology and size of the obtained NEs were measured using TEM. The droplets had uniform spherical shape and size that was in agreement with the findings reported by Moradi and Barati (2019). Keeping this in view, FTIR spectra were studied for determining any chemical change in ONE and TNE. Besides, the obtained results specifying that ONE and TNE were chemically stable (Chen, Zhang, and Zhong 2015; Wu, Luo, and Wang 2012).

The antimicrobial activity of olive oil and thyme oil showed a dose-dependent inhibitory pattern against *C. sakazakii*. However, the inhibitory effect of thyme oil was stronger than those noticed with olive oil. This may be due to the higher antibacterial activity of olive oil against Gram-positive and, to a lesser extent, Gram-negative bacteria as previously reported in the literature (Gutierrez et al. 2008; Švarcová et al. 2022). These results are in line with those obtained by Abdollahzadeh, Rezaei, and Hosseini (2014) and Mazzarrino et al. (2015) whose

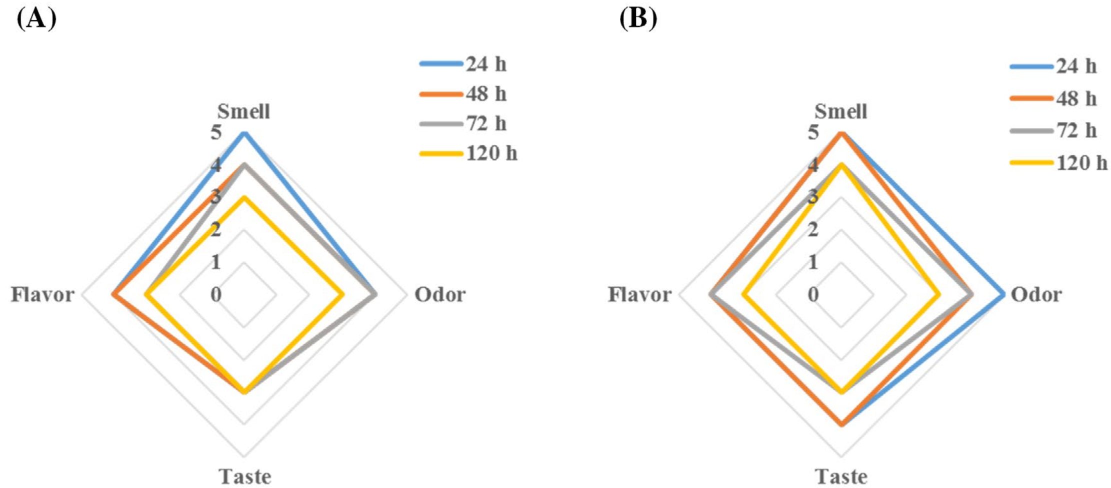

FIGURE 8 | Sensorial evaluation of Tallaga cheeses inoculated with: (A) thyme nanoemulsion (TNE) and (B) olive oil nanoemulsion (ONE) after 24, 48, 72, and 120 h of cheese storage.

reported that thyme oil had the highest antimicrobial activity among several types of EOs.

Although olive oil and thyme oil have been reported to be effective against a variety of microbial species, few studies have been focused on *C. sakazakii*. Notably, Tian et al. (2021) examined the antibacterial effect of thymol and suggested that thymol has the potential to prevent bacterial contamination by *C. sakazakii* in the food industry. In contrast, Fei et al. (2018) indicated that olive oil polyphenols extract (OOPE) played an effective antibacterial activity against *C. sakazakii*. Importantly, the strong antimicrobial activity of thyme oil may be due to its richness with phenolic compounds such as thymol and carvacrol (Guo et al. 2022).

Several studies have examined the antimicrobial effect of some natural antimicrobial compounds of plant origin against *C. sakazakii* strains. For instance, Lee and Jin (2008) found that the MIC of thymol was 0.19 mg/mL. However, the MICs of olive oil against *C. sakazakii* strains were ranged from 0.625 to 1.25 mg/mL (Fei et al. 2018). While in this study, The MIC of olive oil and thyme oil was 15 mg/mL. Herein, the obtained MIC is higher than those reported in the previous studies, which is possible due to the differences in the origin of EOs and/or the examined pathogens. Although there are many studies, with different outcomes, evaluating the antimicrobial effect of pure EOs on foodborne pathogens, little is known about the inhibitory activity of NEs on spoilage and pathogenic microorganisms. The present study suggests that the antibacterial potential of TNE was higher than ONE in vitro. In line with our findings, He et al. (2022) studied the antibacterial effect of thyme EO NEs on *E. coli* O157:H7 and *S. aureus* finding out a higher bacteriostatic and bactericidal activities when compared with control samples. Additionally, Liu et al. (2022) found that garlic EO in water NE is a useful alternative to inhibit methicillin-resistant *S. aureus* species. In particular, our findings confirms that the antibacterial efficacy of EOs increased when utilized in the NE form as previously reported by Pagán et al. (2017).

During the processing and storage of Tallaga cheese in the present study, the inhibitory effect of thyme oil was higher than olive oil against *C. sakazakii*. The application of thyme oil reduced the concentration of *C. sakazakii* to undetectable levels (≤10 CFU/g) after 72 h of Tallaga cheese storage. Similarly, the inhibitory effect of EOs against different pathogens has been proven in other food products. For instance, Al-Nabulsi et al. (2020) investigated the inhibitory effect of thyme oil against *E. coli* O157:H7 in Tahini. Furthermore, thyme oil also inhibited the growth of *Salmonella* and mesophilic aerobic bacteria in hummus and chickpea dip, being microbial counts constant until the end of storage at 4°C (Olaimat et al. 2019). On the contrary, thyme oil was ineffective against *S. enteritidis* in the full-fat cheese and showed antibacterial activity toward *S. enteritidis* from Day 4 of storage in low-fat cheese (Smith-Palmer, Stewart, and Fyfe 2001). Despite the ability of thyme oil to extend the shelf-life of food, it leads to an unpleasant odor and taste (Nieto 2020). Hence, we used NEs that did not change the color, texture, appearance, flavor, and other sensory characteristics of Tallaga cheese.

It is worth mentioning that the current study represents the first record of utilizing NEs as in vivo food biopreservatives in the dairy industry. Herein, we investigated that the addition of TNE at a concentration of 15 mg/mL (v/v) to Tallaga cheese reduced the average counts of *C. sakazakii* after 12 h of cheese making, and the drastic decrease was continued till became below the detection limit after 72 h of storage at 7°C ± 2°C. Using NEs in the food industry is still elusive; however, our findings provide a theoretical possibility that TNE and ONE can be used as natural antimicrobials to reduce the food safety problems caused by *C. sakazakii* strains in the dairy industry. However, further studies are required to investigate the mechanism of action of these NEs in cheese, as well as the impact of cheese components on the antimicrobial activity of NEs against *C. sakazakii*. Altogether, when comparing the antibacterial effect of EOs and NEs on *C. sakazakii* during Tallaga cheese making, it was obvious that NEs (TNE and ONE) were more effective. Consequently, this study could be useful for cheese makers and stakeholders to use specific natural additives as novel preservation technologies to inhibit bacterial growth in soft cheeses aiming to extend their shelf-life.

5 | Conclusions

Small-sized particles of ONE and TNE with good stability were developed, characterized, and utilized in Tallaga cheese to evaluate their antibacterial activity toward *C. sakazakii*. The obtained MIC in the present study was 15 mg/mL (v/v) for either EOs or their NEs. NEs of olive oil and thyme oil could exert potent antibacterial effects against *C. sakazakii* in Tallaga cheeses stored at 7°C ± 2°C in comparison to pure EOs. In addition, the strongest antibacterial effect toward *C. sakazakii* was reported with TNE during the manufacturing and storage of Tallaga cheese. The sensory evaluation of cheeses containing pure EOs (olive oil or thyme oil) revealed the unpalatability of such cheeses due to the strong smells of oils. Tallaga cheeses with ONE and TNE had high scores of general acceptability parameters (smell, odor, taste, and flavor). Overall, these insights develop more sustainable and efficient methods for ensuring the safety and quality of cheese in the dairy industry.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Abdelhamid, A. G., and N. K. El-Dougdoug. 2020. "Controlling Foodborne Pathogens With Natural Antimicrobials by Biological Control and Antivirulence Strategies." *Heliyon* 6, no. 9: e05020. https://doi.org/10.1016/j.heliyon.2020.e05020.

Abdel-Salam, A. M. 2010. "Functional Foods: Hopefulness to Good Health." *American Journal of Food Technology* 5: 86–99. https://doi.org/10.3923/ajft.2010.86.99.

Abdollahzadeh, E., M. Rezaei, and H. Hosseini. 2014. "Antibacterial Activity of Plant Essential Oils and Extracts: The Role of Thyme Essential Oil, Nisin, and Their Combination to Control *Listeria monocytogenes* Inoculated in Minced Fish Meat." *Food Control* 35, no. 1: 177–183. https://doi.org/10.1016/j.foodcont.2013.07.004.

Acevedo-Fani, A., L. Salvia-Trujillo, M. A. Rojas-Graü, and O. Martín-Belloso. 2015. "Edible Films From Essential-Oil-Loaded Nanoemulsions: Physicochemical Characterization and Antimicrobial Properties." *Food Hydrocolloids* 47: 168–177. https://doi.org/10.1016/j.foodhyd.2015.01.032.

Adeyemi, P. 2012. *The Incidence of Enterobacter sakazakii (Cronobacter spp.) in Unripened Soft Cheese ("wara") Sold in Odeda Local Government*. Abeokuta, Nigeria: Department of Food Science and Technology, Federal University of Agriculture.

Aigbekaen, B., and C. Oshoma. 2010. "Isolation of *Enterobacter sakazakii* From Powdered Foods Locally Consumed in Nigeria." *Pakistan Journal of Nutrition* 9, no. 7: 659–663.

Ali, S., A. Amin Mohamed, M. Sameeh, O. Darwesh, and T. M. El-Razik. 2016. "Gamma-Irradiation Affects Volatile Oil Constituents, Fatty Acid Composition and Antimicrobial Activity of Fennel (*Foeniculum vulgare*) Seeds Extract." *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 7: 524–532.

Al-Nabulsi, A., T. Osaili, A. Olaimat, et al. 2020. "Inhibitory Effect of Thyme and Cinnamon Essential Oils Against *E. coli* O157:H7 In Tahini." *Ciência e Tecnologia de Alimentos* 40, no. 4: 885–893. https://doi.org/10.1590/fst.21619.

Beuchat, L. R., H. Kim, J. B. Gurtler, L. C. Lin, J. H. Ryu, and G. M. Richards. 2009. "*Cronobacter sakazakii* In Foods and Factors Affecting Its Survival, Growth, and Inactivation." *International Journal of Food Microbiology* 136, no. 2: 204–213. https://doi.org/10.1016/j.ijfoodmicro.2009.02.029.

Casalinuovo, F., P. Rippa, L. Battaglia, and N. Parisi. 2014. "Isolation of Cronobacter spp. (*Enterobacter Sakazakii*) From Artisanal Mozzarella." *Italian Journal of Food Safety* 3, no. 1: 1526. https://doi.org/10.4081/ijfs.2014.1526.

Chang, Y., M. Xing, X. Hu, et al. 2021. "Antibacterial Activity of Chrysanthemum Buds Crude Extract Against Cronobacter Sakazakii and Its Application as a Natural Disinfectant." *Frontiers in Microbiology* 11: 632177. https://doi.org/10.3389/fmicb.2020.632177.

Chaves-López, C., M. De Angelis, M. Martuscelli, A. Serio, A. Paparella, and G. Suzzi. 2006. "Characterization of the Enterobacteriaceae Isolated From an Artisanal Italian ewe's Cheese (Pecorino Abruzzese)." *Journal of Applied Microbiology* 101, no. 2: 353–360. https://doi.org/10.1111/j.1365-2672.2006.02941.x.

Chen, H., Y. Zhang, and Q. Zhong. 2015. "Physical and Antimicrobial Properties of Spray-Dried Zein–Casein Nanocapsules With Co-Encapsulated Eugenol and Thymol." *Journal of Food Engineering* 144: 93–102. https://doi.org/10.1016/j.jfoodeng.2014.07.021.

Das, S. K., S. H. Kumar, B. B. Nayak, and M. Lekshmi. 2021. "Isolation and Identification of Cronobacter spp. From Fish and Shellfish Sold in Retail Markets." *Current Microbiology* 78, no. 5: 1973–1980. https://doi.org/10.1007/s00284-021-02447-3.

Dávila-Rodríguez, M., A. López-Malo, E. Palou, N. Ramírez-Corona, and M. T. Jiménez-Munguía. 2019. "Antimicrobial Activity of Nanoemulsions of Cinnamon, Rosemary, and Oregano Essential Oils on Fresh Celery." *LWT- Food Science and Technology* 112: 108247. https://doi.org/10.1016/j.lwt.2019.06.014.

Duda-Chodak, A., T. Tarko, and K. Petka-Poniatowska. 2023. "Antimicrobial Compounds in Food Packaging." *International Journal of Molecular Sciences* 24, no. 3: 2457. https://doi.org/10.3390/ijms24032457.

El-Kholy, W., A. El-Khalek, S. Mohamed, M. Tawfeek, and J. Kassem. 2016. "Tallaga Cheese as a New Functional Dairy Product." *American Journal of Food Technology* 11: 182–192. https://doi.org/10.3923/ajft.2016.182.192.

El-Saber Batiha, G., D. E. Hussein, A. M. Algammal, et al. 2021. "Application of Natural Antimicrobials in Food Preservation: Recent Views." *Food Control* 126: 108066. https://doi.org/10.1016/j.foodcont.2021.108066.

El-Sharoud, W. M., M. Z. El-Din, D. M. Ziada, S. F. Ahmed, and J. D. Klena. 2008. "Surveillance and Genotyping of *Enterobacter sakazakii* Suggest Its Potential Transmission From Milk Powder Into Imitation Recombined Soft Cheese." *Journal of Applied Microbiology* 105, no. 2: 559–566. https://doi.org/10.1111/j.1365-2672.2008.03777.x.

Elsherif, W. M., A. A. Hassanien, G. M. Zayed, and S. M. Kamal. 2024. "Natural Approach of Using Nisin and Its Nanoform as Food Bio-Preservatives Against Methicillin Resistant *Staphylococcus aureus* and *E. coli* O157:H7 in Yoghurt." *BMC Veterinary Research* 20, no. 1: 192. https://doi.org/10.1186/s12917-024-03985-1.

Farid, N., A. Waheed, and S. Motwani. 2023. "Synthetic and Natural Antimicrobials as a Control Against Food Borne Pathogens: A Review." *Heliyon* 9, no. 6: e17021. https://doi.org/10.1016/j.heliyon.2023.e17021.

Fei, P., M. A. Ali, S. Gong, et al. 2018. "Antimicrobial Activity and Mechanism of Action of Olive Oil Polyphenols Extract Against *Cronobacter sakazakii*." *Food Control* 94: 289–294. https://doi.org/10.1016/j.foodcont.2018.07.022.

Gedikoğlu, A. 2022. "The Effect of Thymus Vulgaris and Thymbra Spicata Essential Oils and/or Extracts in Pectin Edible Coating on the Preservation of Sliced Bolognas." *Meat Science* 184: 108697. https://doi.org/10.1016/j.meatsci.2021.108697.

Ghada, Z. A. A., M. H. Alia, A.-S. Soha, N. A. Magdy, and F. S. Mohammed. 2004. "Chemical, Nutritional and Microbiological Evaluation of Some Egyptian Soft Cheeses." *Egyptian Journal of Hospital Medicine* 17, no. 1: 44–57. https://doi.org/10.21608/ejhm.2004.18155.

Ghosh, V., A. Mukherjee, and N. Chandrasekaran. 2012. "Mustard Oil Microemulsion Formulation and Evaluation of Bactericidal Activity." *International Journal of Pharmacy & Pharmaceutical Sciences* 4, no. 4: 497–500.

Gruskiene, R., T. Krivorotova, R. Staneviciene, D. Ratautas, E. Serviene, and J. Sereikaite. 2018. "Preparation and Characterization of Iron Oxide Magnetic Nanoparticles Functionalized by Nisin." *Colloids and Surfaces B: Biointerfaces* 169: 126–134. https://doi.org/10.1016/j.colsurfb.2018.05.017.

Guo, D., S. Wang, J. Li, et al. 2020. "The Antimicrobial Activity of Coenzyme Q0 Against Planktonic and Biofilm Forms of *Cronobacter sakazakii*." *Food Microbiology* 86: 103337. https://doi.org/10.1016/j.fm.2019.103337.

Guo, L., Q. Sun, S. Gong, et al. 2019. "Antimicrobial Activity and Action Approach of the Olive Oil Polyphenol Extract Against *Listeria monocytogenes*." *Frontiers in Microbiology* 10: 1586. https://doi.org/10.3389/fmicb.2019.01586.

Guo, X., Y. Hao, W. Zhang, et al. 2022. "Comparison of Origanum Essential Oil Chemical Compounds and Their Antibacterial Activity Against *Cronobacter sakazakii*." *Molecules* 27, no. 19: 6702.

Gutierrez, J., G. Rodriguez, C. Barry-Ryan, and P. Bourke. 2008. "Efficacy of Plant Essential Oils Against Foodborne Pathogens and Spoilage Bacteria Associated With Ready-To-Eat Vegetables: Antimicrobial and Sensory Screening." *Journal of Food Protection* 71, no. 9: 1846–1854. https://doi.org/10.4315/0362-028x-71.9.1846.

Hamad, M. N.-E., and M. Eldin. 2021. "Attempting to Produce Egyptian Tallaga Cheese With International Specifications." *Open Access Journal of Science and Technology* 1: 8–23. https://doi.org/10.30574/oarjst.2021.1.1.0020.

Hayman, M. M., S. G. Edelson-Mammel, P. J. Carter, et al. 2020. "Prevalence of Cronobacter spp. and Salmonella in Milk Powder Manufacturing Facilities in the United States." *Journal of Food Protection* 83, no. 10: 1685–1692. https://doi.org/10.4315/jfp-20-047.

He, Q., L. Zhang, Z. Yang, et al. 2022. "Antibacterial Mechanisms of Thyme Essential Oil Nanoemulsions Against *Escherichia coli* O157:H7 and *Staphylococcus aureus*: Alterations in Membrane Compositions and Characteristics." *Innovative Food Science & Emerging Technologies* 75: 102902. https://doi.org/10.1016/j.ifset.2021.102902.

Healy, B., S. Cooney, S. O'Brien, et al. 2010. "Cronobacter (*Enterobacter sakazakii*): An Opportunistic Foodborne Pathogen." *Foodborne Pathogens and Disease* 7, no. 4: 339–350. https://doi.org/10.1089/fpd.2009.0379.

Iversen, C., and S. Forsythe. 2003. "Risk Profile of *Enterobacter sakazakii*, an Emergent Pathogen Associated With Infant Milk Formula." *Trends in Food Science & Technology* 14, no. 11: 443–454. https://doi.org/10.1016/S0924-2244(03)00155-9.

Jafari, S. M., and D. J. McClements. 2017. "Nanotechnology Approaches for Increasing Nutrient Bioavailability." *Advances in Food Nutrition Research* 81: 1–30.

Jaiswal, M., R. Dudhe, and P. K. Sharma. 2015. "Nanoemulsion: An Advanced Mode of Drug Delivery System." *3 Biotech* 5, no. 2: 123–127. https://doi.org/10.1007/s13205-014-0214-0.

Karami, Z., M. Khoshkam, and M. Hamidi. 2019. "Optimization of Olive Oil-Based Nanoemulsion Preparation for Intravenous Drug Delivery." *Drug Research* 69, no. 5: 256–264. https://doi.org/10.1055/a-0654-4867.

Karaosmanoglu, H., F. Soyer, B. Ozen, and F. Tokatli. 2010. "Antimicrobial and Antioxidant Activities of Turkish Extra Virgin Olive Oils." *Journal of Agricultural and Food Chemistry* 58, no. 14: 8238–8245. https://doi.org/10.1021/jf1012105.

Kim, S. A., and M. S. Rhee. 2016. "Highly Enhanced Bactericidal Effects of Medium Chain Fatty Acids (Caprylic, Capric, and Lauric Acid) Combined With Edible Plant Essential Oils (Carvacrol, Eugenol, β-Resorcylic Acid, Trans-Cinnamaldehyde, Thymol, and Vanillin) Against *Escherichia coli* O157:H7." *Food Control* 60: 447–454. https://doi.org/10.1016/j.foodcont.2015.08.022.

Lee, S. Y., and H. H. Jin. 2008. "Inhibitory Activity of Natural Antimicrobial Compounds Alone or in Combination With Nisin Against *Enterobacter sakazakii*." *Letters in Applied Microbiology* 47, no. 4: 315–321. https://doi.org/10.1111/j.1472-765x.2008.02432.x.

Liu, M., Y. Pan, M. Feng, et al. 2022. "Garlic Essential Oil in Water Nanoemulsion Prepared by High-Power Ultrasound: Properties, Stability and Its Antibacterial Mechanism Against MRSA Isolated From Pork." *Ultrasonics Sonochemistry* 90: 106201. https://doi.org/10.1016/j.ultsonch.2022.106201.

Lu, P.-J., W.-E. Fu, S.-C. Huang, et al. 2017. "Methodology for Sample Preparation and Size Measurement of Commercial ZnO Nanoparticles." *Journal of Food and Drug Analysis* 26: 628–636. https://doi.org/10.1016/j.jfda.2017.07.004.

Mazzarrino, G., A. Paparella, C. Chaves-López, et al. 2015. "Salmonella Enterica and *Listeria monocytogenes* Inactivation Dynamics After Treatment With Selected Essential Oils." *Food Control* 50: 794–803. https://doi.org/10.1016/j.foodcont.2014.10.029.

Mikhailova, E. O. 2022. "Green Synthesis of Platinum Nanoparticles for Biomedical Applications." *Journal of Functional Biomaterials* 13, no. 4: 260. https://doi.org/10.3390/jfb13040260.

Moghimi, R., L. Ghaderi, H. Rafati, A. Aliahmadi, and D. J. McClements. 2016. "Superior Antibacterial Activity of Nanoemulsion of Thymus Daenensis Essential Oil Against *E. coli*." *Food Chemistry* 194: 410–415. https://doi.org/10.1016/j.foodchem.2015.07.139.

Moradi, S., and A. Barati. 2019. "Essential Oils Nanoemulsions: Preparation, Characterization and Study of Antibacterial Activity Against *Escherichia coli*." *International Journal of Nanoscience and Nanotechnology* 15, no. 3: 199–210.

Morales, P., I. Feliu, E. Fernández-García, and M. Nuñez. 2004. "Volatile Compounds Produced in Cheese by Enterobacteriaceae Strains of Dairy Origin." *Journal of Food Protection* 67, no. 3: 567–573. https://doi.org/10.4315/0362-028x-67.3.567.

Mushtaq, A., S. Mohd Wani, A. R. Malik, et al. 2023. "Recent Insights Into Nanoemulsions: Their Preparation, Properties and Applications." *Food Chemistry: X* 18: 100684. https://doi.org/10.1016/j.fochx.2023.100684.

Nazzaro, F., F. Fratianni, R. Cozzolino, et al. 2019. "Antibacterial Activity of Three Extra Virgin Olive Oils of the Campania Region, Southern Italy, Related to Their Polyphenol Content and Composition." *Microorganisms* 7, no. 9: 321. https://doi.org/10.3390/microorganisms7090321.

Nieto, G. 2020. "A Review on Applications and Uses of Thymus in the Food Industry." *Plants* 9, no. 8: 961.

Olaimat, A. N., M. A. Al-Holy, M. H. Abu Ghoush, A. A. Al-Nabulsi, T. M. Osaili, and R. A. Holley. 2019. "Inhibitory Effects of Cinnamon and Thyme Essential Oils Against Salmonella spp. in Hummus (Chickpea Dip)." *Journal of Food Processing and Preservation* 43, no. 5: e13925. https://doi.org/10.1111/jfpp.13925.

Pagán, E., D. Berdejo, L. Espina, D. Garcia-Gonzalo, and R. Pagán. 2017. "Antimicrobial Activity of Suspensions and Nanoemulsions of Citral in Combination With Heat or Pulsed Electric Fields." *Letters in Applied Microbiology* 66: 63–70. https://doi.org/10.1111/lam.12815.

Posgay, M., B. Greff, V. Kapcsándi, and E. Lakatos. 2022. "Effect of *Thymus vulgaris* L. Essential Oil and Thymol on the Microbiological Properties of Meat and Meat Products: A Review." *Heliyon* 8, no. 10: e10812. https://doi.org/10.1016/j.heliyon.2022.e10812.

Restaino, L., E. W. Frampton, W. C. Lionberg, and R. J. Becker. 2006. "A Chromogenic Plating Medium for the Isolation and Identification of *Enterobacter sakazakii* From Foods, Food Ingredients, and Environmental Sources." *Journal of Food Protection* 69, no. 2: 315–322. https://doi.org/10.4315/0362-028x-69.2.315.

Rinaldi, F., L. Maurizi, A. L. Conte, et al. 2021. "Nanoemulsions of *Satureja montana* Essential Oil: Antimicrobial and Antibiofilm Activity Against Avian *Escherichia coli* Strains." *Pharmaceutics* 13, no. 2: 134. https://doi.org/10.3390/pharmaceutics13020134.

Saad, N., and W. Amin. 2014. "Occurrence of Cronobacter Species in Kareish and Domiati Cheeses." *Assiut Veterinary Medical Journal* 60, no. 3: 69–74.

Saad, N. M., and R. Ewida. 2018. "Incidence of *Cronobacter sakazakii* in Dairy-Based Desserts." *Journal of Advanced Veterinary Research* 8: 16–18.

Sharif, Z. M., F. A. Mustapha, J. Jai, and N. A. Zaki. 2017. "Review on Methods for Preservation and Natural Preservatives for Extending the Food Longevity." *Chemical Engineering Research Bulletin* 19: 145. https://doi.org/10.3329/cerb.v19i0.33809.

Smith-Palmer, A., J. Stewart, and L. Fyfe. 2001. "The Potential Application of Plant Essential Oils as Natural Food Preservatives in Soft Cheese." *Food Microbiology* 18, no. 4: 463–470. https://doi.org/10.1006/fmic.2001.0415.

Suresh, S., S. Karthikeyan, P. Saravanan, and K. Jayamoorthy. 2016. "Comparison of Antibacterial and Antifungal Activities of 5-Amino-2-Mercaptobenzimidazole and Functionalized NiO Nanoparticles." *Karbala International Journal of Modern Science* 2, no. 3: 188–195. https://doi.org/10.1016/j.kijoms.2016.05.001.

Švarcová, K., L. Hofmeisterová, B. Švecová, and D. Šilha. 2022. "In Vitro Activity of Water Extracts of Olive Oil Against Planktonic Cells and Biofilm Formation of Arcobacter-Like Species." *Molecules* 27, no. 14: 4509. https://doi.org/10.3390/molecules27144509.

Tian, L., X. Wang, R. Liu, et al. 2021. "Antibacterial Mechanism of Thymol Against *Enterobacter sakazakii*." *Food Control* 123: 107716. https://doi.org/10.1016/j.foodcont.2020.107716.

Touayar, M., R. Zayani, C. Messaoud, and H. Salman. 2023. "Influence of Droplet Size on the Antibacterial Efficacy of Citral and Citronella Oil Nanoemulsions in Polysaccharide Coated Fresh-Cut Apples." *Scientific Reports* 13, no. 1: 10460. https://doi.org/10.1038/s41598-023-37528-9.

Wang, H., Y. Li, Z. Li, et al. 2022. "Inhibition of *Cronobacter sakazakii* by *Litsea cubeba* Essential Oil and the Antibacterial Mechanism." *Food* 11, no. 23: 3900.

Wu, Y., Y. Luo, and Q. Wang. 2012. "Antioxidant and Antimicrobial Properties of Essential Oils Encapsulated in Zein Nanoparticles Prepared by Liquid–Liquid Dispersion Method." *LWT- Food Science and Technology* 48, no. 2: 283–290. https://doi.org/10.1016/j.lwt.2012.03.027.

Xu, J., F. Zhou, B. P. Ji, R. S. Pei, and N. Xu. 2008. "The Antibacterial Mechanism of Carvacrol and Thymol Against *Escherichia coli*." *Letters in Applied Microbiology* 47, no. 3: 174–179. https://doi.org/10.1111/j.1472-765X.2008.02407.x.

Yang, Y., S. Ma, K. Guo, et al. 2022. "Efficacy of 405-nm LED Illumination and Citral Used Alone and in Combination for the Inactivation of *Cronobacter sakazakii* in Reconstituted Powdered Infant Formula." *Food Research International* 154: 111027. https://doi.org/10.1016/j.foodres.2022.111027.