Middle East Journal of Agriculture Research
Volume: 15 | Issue: 02| April – June| 2026

EISSN: 2706-7955 ISSN: 2077-4605
DOI: 10.36632/mejar/2026.15.2.9
Journal homepage: www.curresweb.com
Pages: 124-135

A New Technology in the Manufacture of Appetizers Using Nanotechnology for Thyme Oil

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Received: 01 June 2026 **Accepted:** 20 June 2026 **Published:** 30 June 2026

ABSTRACT

In recent years, consumer demand for products with natural preservatives has increased. Therefore, a modern trend in the food manufacturing sector has been to produce high-quality food while eliminating the need for artificial preservatives. In the current study, the using of both thyme oil and nano thyme oil (NTO) in manufacture of mayonnaise was evaluated for their effect. The TO was converted to NTO by using ultrasonic treatment which characterized by nanoscale=10nm and zeta potential= -14. The mayonnaise samples were processed by using 1,2 and 3% of TO and NTO, each one separately. Two mayonnaise control samples were processed, the first control sample (C1) was processed without TO and NTO, whilst the second control sample (C2) was processed by using 0.3% sodium benzoate. Zeta potential, antioxidant activity, sensory evaluation was estimated for all mayonnaise samples under investigation. Also, all mayonnaise samples were asset for pH and acidity during storage for 0, 2, 4 and 6 months, whilst for total count and yeast and molds for 0 and 4 months. Results showed that, the zeta potential yielded an average surface charge of -14.0 mV which made the formulation remained highly stable and showed no signs of phase separation. Both TO and NTO samples recorded the best values for DPPH, IC_{50} as compared with both C1 and C2 samples. Concerning the sensory properties, both TO1, TO2, NTO1 and NTO2 samples recorded a good overall acceptability. Likewise, at the end of storage period the TO and NTO samples exhibit an accepted pH, acidity values and had a longer shelf life. So, using TO and NTO with 1 and 2% in mayonnaise manufacture is recommend.

Keywords: Functional mayonnaise, thyme oil, nanotechnology, organic preservatives, ultrasonication.

1. Introduction

Food producers have recently attempted to utilize product labels to assert that they can formulate their goods using natural components free of chemical additions, such as ascorbic acid, benzoic acid and their salts which are utilized as preservatives in the foods and drinks (Piper and Piper, 2017). Consuming these substances has been linked to chromosome abnormalities, DNA damage, pseudo-allergy in susceptible individuals, and hyperactivity in kids (Pongsavee, 2015 and Piper and Piper, 2017). Pregnant women should avoid eating the food containing sodium benzoate as an additive because their showed damages genomic in the cells of liver in pregnant rats (Saatci *et al.*, 2016). WHO, (2016) recommended 0–5 mg/kg body weight/day for benzoic acid and its salts and 0–25 mg/kg body weight/day for sorbic acid and its salts (Chaleshtori *et al.*, 2018).

Mayonnaise (oil in water emulsions) is considering one of the likely food products which have market demand (Alvarez-Sabatel *et al.,* 2018). Production of a useful mayonnaise is a problem in the food science and technology, so that some investigators suggested new functional mayonnaise with adding of antioxidants, probiotics and prebiotics (Mirzanajafi-Zanjani *et al*., 2019). Some other investigators utilized organic preservatives to generate sorbate-benzoate free mayonnaise (Rafiee *et al.*, 2018).

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Thyme herb (*Thymus vulgare* Linn) is considered the highest levels of antioxidant among herbs. It has vitamins and minerals that are important for good health. Its leaves are riche sources of iron, potassium, manganese, magnesium, calcium and selenium. The main phenolic compound is mainly responsible for antioxidant is thymol. Thyme essential oil prevents the growth of both gram-negative and gram-positive species, including *Salmonella Typhimurium, Salmonella Enteritidis, Escherichia coli O157:H7, Yersinia enterocolitica, Listeria monocytogenes, Shigella flexneri, Shigella sonnei, Escherichia coli, and Staphylococcus aureus*, with inhibition zones ranging from 19.6 to 45.0 mm, (Posgay *et al.*, 2022).

Nanotechnology is a one of the new preservation methods. In food systems, oil-in-water (o/w) nano-emulsions are regarded as an active antimicrobial agent carrier. Because of their small size, nano-emulsion droplets can migrate and adhere to bacterial cell walls, disrupting the lipid components of the cell membranes (Hashem *et al.*, 2024).

Ultrasound is a good technology and new conservation techniques. It can eliminate microbial activity, rupture cells, and denature enzymes, while keeping nutritional value and organoleptic characteristics (texture, color, and taste). The process has some benefits such as accelerating the rate of food sterilization, significant energy savings, a competitive cost, environmental friendliness, a high degree of safety, and greater homogeneity. The mechanism of the antimicrobial function is thinning the cell membranes and disrupting cell walls of microbes, localized heating, and production of free radicals (Wang *et al.*, 2020).

The purpose of this study was to evaluate the effect of using nanotechnology for thyme oil on the manufacture of mayonnaise. Where mayonnaise was processed by different percentages of thyme oil and nano thyme oil without adding any artificial preservative.

2. Materials and Methods

2.1. Materials

Thyme grass (*Thymus vulgare* Linn), from El Qantara Sharq research station, Desert Research Center, Cairo, Egypt. Tween 80, nutrient agar and potato dextrose was obtained from Al-Gomhorya Company for Chemicals, Cairo, Egypt. All other chemicals and reagents were of analytical grade. Guar gum was obtained from Alpha Zyme Company, El-Asher of Ramadan, Sharkiya governorate, Egypt. Sunflower oil, thyme oil, whole egg, sugar, salt, mustard, vinegar and starch from local market, Cairo, Egypt. Citric acid and sodium benzoate from El-Gomhorya Company, El-Amiria, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of thyme oil

Thyme essential oil was obtained according to Julio *et al.* (2019) and Otero *et al.* (2020), where 50 g of leaf powder placed in a Soxhlet apparatus with 250 ml of ethanol for 6 hrs. period at 40°C.

2.2.2. Preparation of thyme essential oil nano-emulsion

The thyme oil nano emulsion (NTO) was prepared by using a spontaneous emulsification-ultrasonication method as referred to Hamdy *et al.* (2026) and Danjolli-Hashani and Selen-Işbilir, (2022). Briefly, the aqueous phase was prepared by adding 1% (v/v) Tween 80 into distilled water. The organic phase (TO) was then introduced dropwise into the aqueous phase under continuous magnetic stirring (1000 rpm, 15 min, room temperature) to yield the emulsion. To achieve nanoscale droplet fragmentation, the mixture was subjected to probe ultrasonication (20 kHz, 100 W) for 10 min. with 30 sec. pulses and 10 sec. rest intervals. The resulting formulation was cooled and stored in amber vials at 4 °C.

The TO emulsion was characterized for particle size and surface charge using Dynamic Light Scattering and Electrophoretic Light Scattering, employing a Zetasizer Nano ZS. A 2 mg sample was re-dispersed in water, sonicated, and analyzed at 25 °C. The measured particle size ranged from 10 to less than 100 nm, confirming that it is a nanoscale formulation. Additionally, the zeta potential values were evaluated, indicating good stability due to electrostatic repulsion (Lunardi *et al.,* 2021).

2.2.3. Preparation of mayonnaise samples

The preparation of mayonnaise samples according to Tavakoli *et al.* (2021) were presented in table (1). Mayonnaise samples were processed by using 1,2 and 3% of TO and NTO each on separately and coded as TO1, TO2, TO3, NTO1, NTO2 and NTO3, respectively. Also, two control mayonnaise samples were processed, the first control sample (C1) was processed without TO and NTO, whilst the second control sample (C2) was processed by using 0.3% sodium benzoate. All mayonnaise samples were made in 150g batch for each treatment and stored at room temperature for 4 months. Three replicates of each treatment were prepared.

Table 1: Formulation of mayonnaise samples

Ingredients (%)	**Mayonnaise samples**							
	C1	**C2**	**TO1**	**TO2**	**TO3**	**NTO1**	**NTO2**	**NTO3**
Water	43.98	43.68	43.98	43.98	43.98	43.98	43.98	43.98
Oil	40	40	39	38	37	39	38	37
Thyme oil	-	-	1	2	3	-	-	-
Nano thyme oil	-	-	-	-	-	1	2	3
Whole egg	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47
Sugar	4.65	4.65	4.65	4.65	4.65	4.65	4.65	4.65
Salt	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Mustard	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vinegar	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Citric acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Guar	1	1	1	1	1	1	1	1
Starch	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sodium benzoate	-	0.3	-	-	-	-	-	-

C1: mayonnaise control sample without sodium benzoate, C2: mayonnaise control sample with 0.3% sodium benzoate, (TO1): mayonnaise sample with 1% thyme essential oil, (TO2): mayonnaise sample with 2% thyme essential oil, (TO3): mayonnaise sample with 3% thyme essential oil, (NTO1): mayonnaise sample with 1% nano thyme essential oil, (NTO2): mayonnaise sample with 2% nano thyme essential oil and (NTO3): mayonnaise sample with 3% nano thyme essential oil.

2.3. Analytically methods

2.3.1. Physical properties of nano thyme essential oil

The NTO emulsion was characterized for particle size and surface charge using Dynamic Light Scattering and Electrophoretic Light Scattering, employing a Zetasizer Nano (ZS). A 2 mg sample was re-dispersed in water, sonicated, and analyzed at 25 °C. The measured particle size ranged from 10 less than 100 nm, confirming that it is a nanoscale formulation. Additionally, the zeta potential values were be evaluated, indicating good stability due to electrostatic repulsion (Lunardi *et al.*, 2021)

2.3.2. Evaluation of antioxidant activity of mayonnaise samples

Free radical scavenging activity of different extracts of mayonnaise samples were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) according to Shekhar and Anju (2014). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (125, 250, 500, 1000 µg/ml). Here, only those extracts are used which are solubilize in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS milton roy). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:
DPPH (%) = A0 - A 1 / A0 × 100.

Where: A0: was the absorbance of control reaction.
A1: was the absorbance in presence of test or standard sample.

2.3.3. Sensory evaluation

The sensory evaluation of prepared mayonnaise samples was conducted organoleptically. Ten panelists in the desert research center were requested to evaluate the most acceptable samples for sensory attributes of mayonnaise with TO and NTO percentages according to Said *et al.* (2019), whereas every quality received a score ranging from 1 (poor) to 10 (excellent).

2.3.4. The pH measurement

The pH values of all mayonnaise samples were measured at zero, 2, 4 and 6 months of storage period at room temperature, using a pH meter (Model: SenTix 41WTW Bench7110, Germany), during the storage period (Isiri, 2017).

2.3.5. Acidity measurement

The acidity of mayonnaise samples was measured at zero, 2, 4 and 6 months of storage period at room temperature, where two hundred ml of distilled water was added to 15g of the mayonnaise samples, then mixed with 3–4 drops of phenolphthalein and titrated with NaOH (0.1N) until the first color change to violet and calculated with the corresponding formula following the National Standard of Iran (Isiri, 2017):

$$\text{Acidity (\%)} = \frac{V \times N \times Meq \times 100}{W}$$

V: Volume of sodium hydroxide (NaOH) consumed during titration (in mL).
N: Normality of the consumed NaOH (e.g., 0.1 N).
Meq: Milliequivalent weight of the predominant acid. For mayonnaise and sauces, it is typically 0.06 g (Acetic acid).
W: Weight of the food/test sample (in grams).

2.3.6. Micro-organisms determination

The total number of colonies (total micro-organisms count), mold and yeast was carried out at zero time and after 4 months according to Yörük, (2018) with some modifications. 1 g of sample was added to a sterile test tube containing 9 ml of Ringer’s solution, and sample was mixed thoroughly by shaking 10 serial dilutions. Each dilution was aseptically transferred into triplicate sets of disposable sterile petri dishes that contained nutrient agar and potato dextrose. The petri dishes were mixed and gently shaken, and allowed to form a solid. These were incubated at 37°C for 72 hrs. for total number of colonies count, incubated at 25°C for 120 hrs. for molds and yeast.

2.4. Statistical Analysis

The collected data were analyzed using the SPSS (Statistical Program for Sociology Scientists) Statistics Version 20 for computing the mean values, LSD, ANOVA ($p < 0.05$) and Duncan Multiple Range test (Armonk, 2011).

3. Results and Discussion

3.1. The particle size distribution of the thyme essential oil system

Data represented in figure (1) demonstrated that the DLS analysis revealed an ultra-small droplet size strictly centered at approximately 10 nm with a sharp, monomodal distribution peak. This substantial reduction in droplet size is attributed to the optimized homogenization energy and appropriate surfactant selection, which effectively minimized the interfacial tension between the aqueous phase and the hydrophobic thyme oil constituents (Hamdy *et al.*, 2026). The narrow width of the distribution peak indicates a low Polydispersity Index (PDI), which serves as a critical parameter in suppressing Ostwald ripening (droplet coarsening). In highly monodisperse systems, the absence of extreme size variations prevents the diffusion-driven mass transfer from smaller droplets to larger ones, thereby preserving the physical integrity of the colloid over prolonged storage periods (Lunardi *et al.*, 2021).

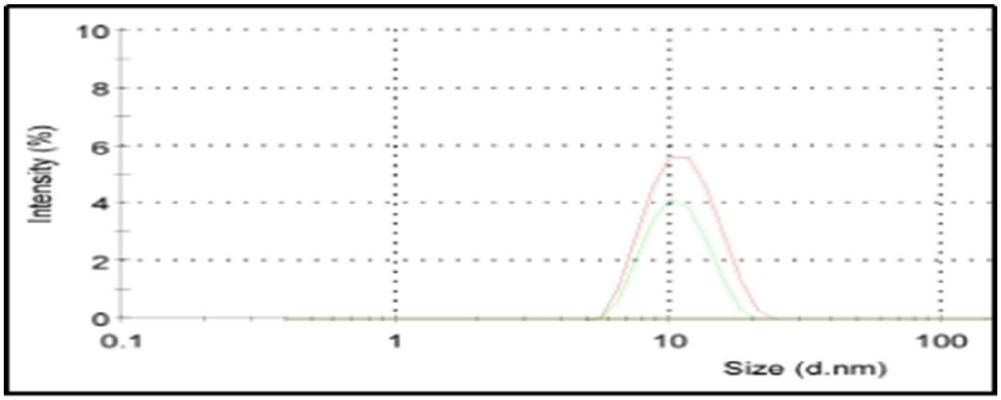

Fig. 1: The particle size distribution of the thyme essential oil system

3.2. The surface charge and physical stability of the thyme essential oil nano-emulsion:

Complementing the size data, the zeta potential report generated a 'Good' quality result as displayed in figure (2), yielding an average surface charge of -14.0 mV. The distribution profile demonstrated a multimodal pattern dominated by a primary peak (Peak 1) at -23.5 mV, accounting for 55.1% of the total area. This prevalent negative charge arises from the ionized functional groups of thyme oil's primary bioactive constituents, or the longitudinal alignment of the surfactant headgroups at the oil-water interface (Tadros, 2013). While an overall zeta potential of -14.0 mV usually suggests moderate electrostatic stability, the formulation remained highly stable and showed no signs of phase separation. This stability can be attributed to steric stabilization; the dense surfactant layer surrounding the 10 nm oil droplets creates a strong physical barrier (steric hindrance) that prevents droplet coalescence, even when the electrostatic repulsive forces are less than optimal (Pavoni *et al.*, 2020).

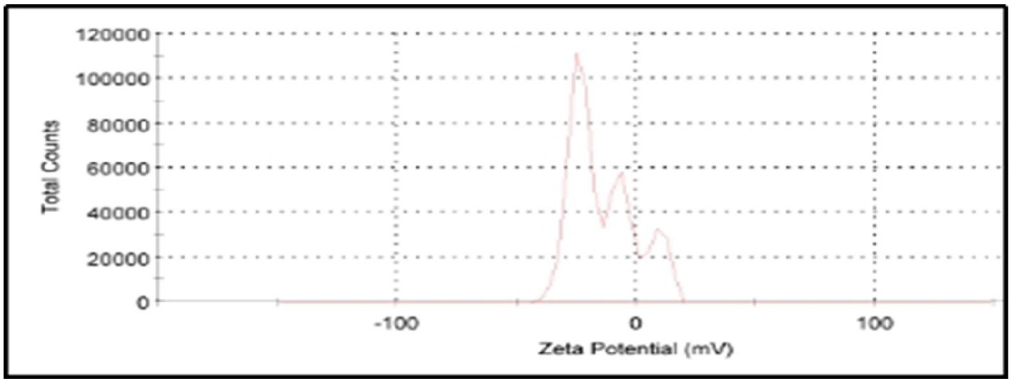

Fig. 2: The surface charge and physical stability of the thyme essential oil nano-emulsion.

From a food science perspective, the intersection of these two parameters provides profound functional advantages for the final food product. Retaining the thyme essential oil within a 10 nm threshold guarantees absolute optical clarity (transparency), preventing light scattering and undesirable turbidity, which makes it ideal for fortifying clear functional beverages, liquid, or semi-liquid food matrices (Pavoni *et al.*, 2020). Additionally, the nanoscale droplet fragmentation considerably maximizes the exposure of thyme oil's volatile compounds. This enormous surface-to-volume ratio facilitates the rapid mass transfer and penetration of thymol across microbial cellular membranes, deeply enhancing its antimicrobial and antioxidant efficacy as a natural preservative at lower operational doses, thereby mitigating any adverse strong sensory impacts on the food's flavor profile (Silva, 2011 and Özoğul *et al.,* 2020).

3.3. Antioxidant activity of mayonnaise samples

Antioxidant activity of mayonnaise samples under study were evaluated by determine the DPPH free radical scavenging. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable free radical that reduced in the presence of antioxidants, resulting in color changing from purple to yellow (Shekhar and Anju, 2014). Data showed in Figure (3) display that, the highly significant DPPH values were observed with all the TO and NTO samples as compared with both C1 and C2 at all concentration. Farthermore, it was noticed that, the DPPH values were increased as the concentration of both TO and NTO in prepared mayonnaise samples increased, taking into consideration that, the highest DPPH values were found with the mayonnaise samples that prepared by using NTO followed by mayonnaise samples that prepared by using TO.

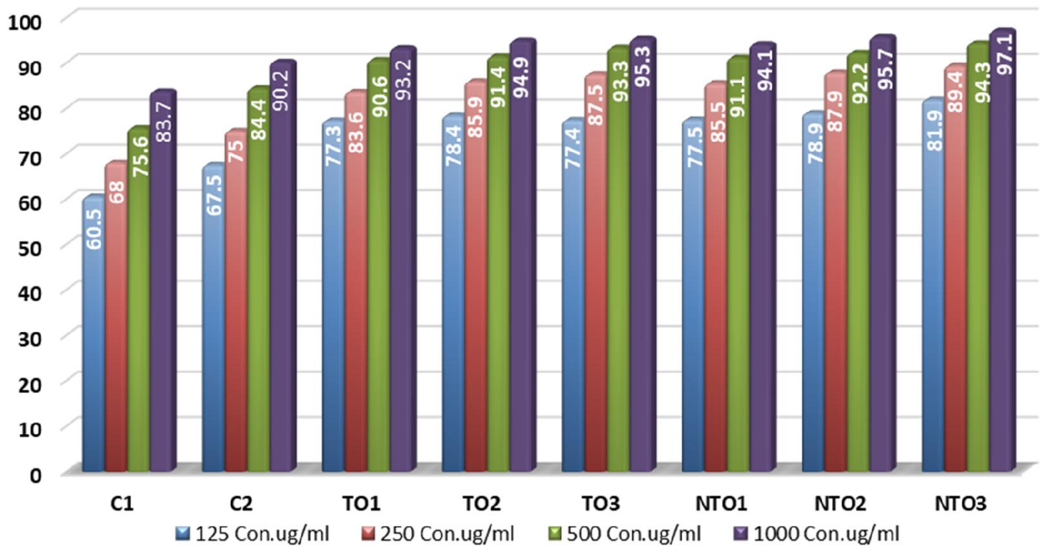

Fig. 3: The DPPH values of mayonnaise samples

These results agree with Işcan *et al.* (2012), Alsaraf *et al.* (2020) and Mammdouh *et al.* (2021), who reported that increasing level of essential oils resulted in increasing antioxidant activity. This may be due to thyme oil compounds; thymol (47.36 %), p-cymene (26.38 %) and carvacrol (3.24 %), which have great antioxidant effects (Walker *et al.,* 2017).

Moreover, the IC_{50} which mean measures the concentration of a substance required to inhibit 50% of free radicals was determined. In antioxidant assays, the IC_{50} value is inversely proportional to the antioxidant efficacy, thus, Lower IC_{50} values indicate stronger antioxidant capacity. Data represented in table (2) showed that the C1 sample, which contained no preservatives, exhibited the weakest antioxidant capacity with the highest IC_{50} (+ 57.66 µg/ml). In contrast, the addition of synthetic sodium benzoate in C2 showed a moderate IC_{50} value of 27.02 µg/ml. Addition of TO into the matrix resulted in a dose-dependent increase in radical scavenging activity across all treated groups. For TO mayonnaise samples, increasing the concentration from 1% to 3% progressively lowered the IC_{50} values from 13.65 µg/ml to 10.89 µg/ml. This strong antioxidant capability of TO is mainly attributed to its high phenolic content, which acts as a key antioxidant by donating hydrogen atoms to unstable free radicals, thereby halting radical chain reactions (Amiri, 2012).

The NTO groups showed lower IC_{50} values than their conventional counterparts at all concentrations tested. The NTO3 formulation achieved an impressive IC_{50} of 7.54 µg/ml, nearing the effectiveness of pure ascorbic acid and outperforming the 3% conventional oil (10.89 µg/ml). This enhancement in nano-formulations is due to the reduction of oil droplets to a nano-scale (~10 nm), which increases the specific surface area and improves the dispersion and availability of the phenolic compounds for interaction with free radicals (Sallam *et al.*, 2022). Second, the structural encapsulation within the surfactant film (Tween 80) successfully shields the volatile and highly reactive components from premature ambient oxidation during storage, thereby preserving their native functional integrity and amplifying their radical trapping efficiency (Kong *et al.*, 2020). These metrics demonstrate that nano-encapsulating to effectively transform it into a highly efficient bioactive ingredient that can suppress lipid oxidation and enhance the chemical shelf-life of the developed food product.

Table 2: The IC$_{50}$ values of mayonnaise samples

Samples	**IC$_{50}$ value (µg/mL)**
Ascorbic Acid (control standard)	2.78
C1	57.66
C2	27.02
TO1	13.65
TO2	12.57
TO3	10.89
NTO3	12.91
NTO2	11.10
NTO3	7.54

C1: mayonnaise control sample without sodium benzoate, C2: mayonnaise control sample with 0.3% sodium benzoate, (TO1): mayonnaise sample with 1% thyme essential oil, (TO2): mayonnaise sample with 2% thyme essential oil, (TO3): mayonnaise sample with 3% thyme essential oil, (NTO1): mayonnaise sample with 1% nano thyme essential oil, (NTO2): mayonnaise sample with 2% nano thyme essential oil and (NTO3): mayonnaise sample with 3% nano thyme essential oil.

3.4. Sensory evaluation of mayonnaise samples

The sensory evaluation of mayonnaise samples presented in table (3). Data showed that no significant differences between all samples in color, texture and aroma. Meanwhile there are significant decreases in TO3 sample (7.75±1.17, 8.08±1.20, 7.75±1.17, respectively) and NTO2 (8.00±0.66, 6.67±0.52 and 7.00±0.63) samples, whereas NTO3 recorded the lowest values in taste, flavor and over all acceptability (6.67±0.52, 6.67±0.52 and 7.00±0.63, respectively) compared to C2 sample (8.92±0.66, 8.67±1.03 and 9.17±0.75, respectively). This may be due to the increasing in thyme oil and Nano thyme oil percentage which affected on some parameters such as taste, flavor and over all acceptability. Meanwhile, Ghasemi *et al*. (2025), found that using thyme extract 2% concentration led to a good taste and overall acceptability and was like better: Although the increasing in thyme extract led to a lower score for color of mayonnaise, but the smell, taste, texture and overall acceptability of mayonnaise samples were better than control sample with sodium benzoate. Also, Salem *et al.* (2010) reported that the sensory evaluations of beef samples through cold storage (4°C) were increased by treatments (0.5, 1 and 1.5%) of thyme oil compared to control samples and treatment contain thyme oil 1.5% produced the best sensory evaluation. Added to Shaltout *et al.* (2017) who revealed that meat samples including 2% thyme oil showed the highest increment of sensory evaluation, meanwhile the samples containing 1% of thyme oil showed the lower.

Table 3: Sensory evaluation of mayonnaise samples

Samples	**Color (10)**	**Texture (10)**	**Aroma (10)**	**Taste (10)**	**Flavor (10)**	**Overall acceptability (10)**
C1	9.00±0.89^{a}	8.67±0.82^{a}	8.58±0.80^{a}	8.25±0.99^{a}	8.50±1.05^{a}	8.67±0.82^{ab}
C2	9.17±0.75^{a}	9.00±0.63^{a}	8.92±0.66^{a}	8.92±0.66^{a}	8.67±1.03^{a}	9.17±0.75^{a}
TO1	9.00±0.89^{a}	8.67±0.82^{a}	8.83±0.75^{a}	8.25±1.54^{a}	8.33±0.82^{a}	8.58±0.92^{ab}
TO2	9.00±0.89^{a}	8.50±1.05^{a}	8.17±0.75^{a}	8.00±1.41^{a}	8.00±0.89^{a}	8.25±1.17^{ab}
TO3	9.17±0.75^{a}	8.50±1.05^{a}	8.33±1.03^{a}	7.75±1.17^{ab}	8.08±1.20a	7.75±1.17^{bc}
NTO1	9.00±0.89^{a}	8.17±1.17^{a}	8.33±0.82^{a}	8.08±1.10^{a}	8.17±0.98^{a}	8.17±0.98^{ab}
NTO2	9.17±0.75^{a}	8.67±0.82^{a}	8.00±1.10^{a}	8.00±0.66^{ab}	7.67±0.82^{ab}	8.08±0.66^{abc}
NTO3	9.33±0.82^{a}	8.33±1.37^{a}	7.83±1.17^{a}	6.67±0.52^{b}	6.67±0.52^{b}	7.00±0.63^{c}

C1: mayonnaise control sample without sodium benzoate, C2: mayonnaise control sample with 0.3% sodium benzoate, (TO1): mayonnaise sample with 1% thyme essential oil, (TO2): mayonnaise sample with 2% thyme essential oil, (TO3): mayonnaise sample with 3% thyme essential oil, (NTO1): mayonnaise sample with 1% nano thyme essential oil, (NTO2): mayonnaise sample with 2% nano thyme essential oil and (NTO3): mayonnaise sample with 3% nano thyme essential oil. Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at $p < 0.05$.

3.5. The pH values of mayonnaise samples during storage

The pH measurement of mayonnaise is an important chemical factor, it should not be greater than 4.1 (Ghasemi *et al.*, 2025). pH values of mayonnaise samples at 0, 2, 4 and 6 months are presented in table (4). Data showed that the pH of all samples among storage period was found less than 4.1 except both C1 and C2. The lowest pH values were observed with the NTO3 sample followed by NTO2 then NTO1 samples. Also, the same trend for pH results were found with the TO samples whereas the pH value decrease as the concertation of TO increase at the end of storage period (6 months). Meanwhile, C1 followed by C2 recorded the highest pH values after 6 months of storage (4.60±0.02, 4.48±0.02, respectively). Decreasing in pH may be described due to the breakage of somewhat ester groups and their transformation into acidic groups. As well, Lactobacillus (acid-resistant bacteria) can also influence this process. It was observed decreasing in pH, increasing in acidity value over time. Acidification and esterification of fatty acids, along with the growth of lactobacilli (nonpathogenic acid-resistant bacteria) may contribute to this impact (Gavahian *et al.*, 2013; Stephen and Phillips, 2016 and Ghasemi *et al.*, 2025).

Table 4: pH values of mayonnaise samples during storage

Samples	**Values of pH**			
	Zero	**2 months**	**4 months**	**6 months**
C1	3.88 ± 0.01^{h}	4.04 ± 0.01^{g}	4.55 ± 0.01^{g}	4.60 ± 0.02^{g}
C2	3.85 ± 0.02^{g}	4.00 ± 0.01^{f}	4.18 ± 0.01^{f}	4.48 ± 0.02^{f}
TO1	3.59 ± 0.02^{a}	3.71 ± 0.02^{e}	3.73 ± 0.02^{e}	3.81 ± 0.01^{e}
TO2	3.61 ± 0.02^{c}	3.64 ± 0.02^{c}	3.67 ± 0.01^{c}	3.75 ± 0.05^{d}
TO3	3.65 ± 0.01^{e}	3.68 ± 0.02^{d}	3.70 ± 0.01^{d}	3.73 ± 00.01^{c}
NTO1	3.77 ± 0.01^{f}	3.62 ± 0.08^{c}	3.66 ± 0.01^{c}	3.73 ± 0.01^{c}
NTO2	3.63 ± 0.01^{d}	3.65 ± 0.02^{b}	3.67 ± 0.01^{b}	3.71 ± 0.05^{b}
NTO3	3.60 ± 0.02^{b}	3.61 ± 0.09^{a}	3.64 ± 0.01^{a}	3.67 ± 0.01^{a}

C1: mayonnaise control sample without sodium benzoate, C2: mayonnaise control sample with 0.3% sodium benzoate, (TO1): mayonnaise sample with 1% thyme essential oil, (TO2): mayonnaise sample with 2% thyme essential oil, (TO3): mayonnaise sample with 3% thyme essential oil, (NTO1): mayonnaise sample with 1% nano thyme essential oil, (NTO2): mayonnaise sample with 2% nano thyme essential oil and (NTO3): mayonnaise sample with 3% nano thyme essential oil. Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at $p< 0.05$.

3.6. Acidity of mayonnaise samples during storage

Acidity is most important characteristic of mayonnaise to determine the growth and the survival of pathogenic bacteria. Still, they have a reactive effect with acetic acid in vinegar on decrease the growth of foodborne pathogens (Aganovic *et al*., 2018). Acidity of mayonnaise samples with TO and NTO are presented in table (5). Data revealed that NTO3, NTO2, TO3 and C2 samples had the same highest value of acidity (0.74) followed by NTO1 and TO2 (0.73), then TO1 (0.72) compared to C1 (0.66) at zero time. The increment in acidity values may be due according to the using of both TO and NTO, noted that the rate of increment from the beginning of the storage period to the end of the storage period was low. These data agree with Jalilzadeh *et al.* (2018) who reported that the acidity value of all cheese samples treated by ultrasonic was higher than control sample on 60 days, also, mentioned that the increasing in acidity values may be ascribed to the consequent increase of free fatty acids and the hydrolysis of triglycerides (Uluko *et al.*, 2015).

3.7. Antimicrobial and preservative efficacy of mayonnaise samples during storage

The antimicrobial performance of mayonnaise samples was observed over a 4-month storage period by calculating the total plate count (TPC) alongside Yeast and Mold counts table (6). Initially, the control group revealed a TPC of 5 CFU/g (Colony-Forming Unit per gram), which increased to 19 CFU/g after 4 months, confirming predictable microbial proliferation in the absence of preservative agents. The combination of the chemical benzoate limited TPC growth to 7 CFU/g, highlighting its effective preservative action (Prescott *et al*., 2002).

Remarkably, both TO and NTO samples demonstrated notable inhibitory effects that either matched the chemical benzoate control. At day zero, TO3 achieve absolute sterility (0.5 CFU/g) for TPC, showing superior direct decontamination compared to their TO1 and TO2 (1 CFU/g). This immediate bactericidal effect is directly explained by the ultra-small droplet size (~10 nm) previously observed in the dynamic light scattering (DLS) analysis. The nano-scale configuration significantly increases the surface-area-to-volume ratio, enabling rapid mass transfer and allowing TO's active constituents to penetrate bacterial cellular membranes, leading to cellular leakage and immediate death (Donsì and Ferrari, 2016).

Table 5: Acidity values of mayonnaise samples during Storage

Samples	**Acidity (%)**			
	Zero	**2 months**	**4 months**	**6 months**
C1	0.66±0.2^{d}	0.66±0.27^{f}	0.59 ±0.08^{e}	0.59±0.46^{g}
C2	0.74±0.05^{a}	0.70±0.23^{e}	0.65±0.45^{d}	0.65±0.27^{f}
TO1	0.72±0.18^{c}	0.71±0.18^{d}	0.69±0.37^{c}	0.66±0.36^{e}
TO2	0.73±0.1^{b}	0.71±0.27^{d}	0.70±0.22b^{c}	0.67±0.18^{d}
TO3	0.74±0.05^{a}	0.73±0.45^{b}	0.70±0.18b^{c}	0.68±0.27^{c}
NTO1	0.73±0.19^{c}	0.70±0.27^{e}	0.72±0.27^{b}	0.69±0.13^{b}
NTO2	0.74±0.08^{a}	0.72±0.36^{c}	0.73±4.88^{a}	0.70±0.18^{b}
NTO3	0.74±0.04^{a}	0.74±0.35^{a}	0.73±0.14^{a}	0.73±0.31^{a}

C1: mayonnaise control sample without sodium benzoate, C2: mayonnaise control sample with 0.3% sodium benzoate, (TO1): mayonnaise sample with 1% thyme essential oil, (TO2): mayonnaise sample with 2% thyme essential oil, (TO3): mayonnaise sample with 3% thyme essential oil, (NTO1): mayonnaise sample with 1% nano thyme essential oil, (NTO2): mayonnaise sample with 2% nano thyme essential oil and (NTO3): mayonnaise sample with 3% nano thyme essential oil. Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at $p < 0.05$.

Table 6: Total plate count and yeast and mold count of mayonnaise samples during storage

Samples	**Total Plate Count (Initial)**	**Total Plate Count (After 4 Months)**	**Yeast & Mold (Initial)**	**Yeast & Mold (After 4 Months)**
C1	5±0000^{c}	16.5±2.74^{d}	1±0	1±0
C2	2±0000^{b}	6±1.100^{bc}	0±0	0±0
TO1	1±0000^{a}	6.5±0.55^{bc}	0±0	0±0
TO2	1±0000^{a}	6±00000^{bc}	0±0	0±0
TO3	0.5±0.55^{a}	5.5±0.55^{ab}	0±0	0±0
NTO1	0.5±0.55^{a}	8.5±0.550^{c}	0±0	0±0
NTO2	1±1.100^{a}	7±1.1000^{c}	0±0	0±0
NTO3	1±1.100^{a}	5±00000^{a}	0±0	0±0

C1: mayonnaise control sample without sodium benzoate, C2: mayonnaise control sample with 0.3% sodium benzoate, (TO1): mayonnaise sample with 1% thyme essential oil, (TO2): mayonnaise sample with 2% thyme essential oil, (TO3): mayonnaise sample with 3% thyme essential oil, (NTO1): mayonnaise sample with 1% nano thyme essential oil, (NTO2): mayonnaise sample with 2% nano thyme essential oil and (NTO3): mayonnaise sample with 3% nano thyme essential oil. Each value represents the mean of three replicates (Mean ± SD). Same letters in each column represents the insignificant difference at $p < 0.05$.

After 4 months of storage, the NTO3 sample had the lowest overall microbial load at 5 CFU/g, compared to the benzoate group at 7 CFU/g and the TO3 sample at 6 CFU/g. This improved long-term preservation results from the properties of the nano-emulsion. The surfactant film surrounding the 10 nm nano-droplets protects volatile contents from oxidation and primary volatilization, ensuring a sustained and controlled release of antimicrobial compounds throughout their shelf life (Ribes *et al.*, 2017). Regarding yeast and mold populations, complete inhibition (0 CFU/g) was sustained across all oil-treated and benzoate groups after 4 months, proving that TO acts as a potent broad-spectrum antifungal agent even at lower strategic doses (Özoğul *et al.,* 2020). In conclusion, these findings confirm that NTO can successfully serve as a highly stable, green-label alternative to synthetic chemical preservatives in developed food products.

4. Conclusion

Chemical preservatives in food carry dangers, especially considering how widely mayonnaise is consumed worldwide. Therefore, it is crucial to use natural substitutes for these preservatives, particularly natural substances with antimicrobial qualities, such as thyme oil and nano thyme oil, whose efficacy was investigated in this study. Whereas, the mayonnaise samples that containing 1 and 2% of thyme oil or nano thyme oil had the more overall acceptability and longer shelf life without the need for chemical preservatives, also, had a high antioxidant stability with accepted acidity values at the end of storage period. Therefore, it is recommended to use both NTO or TO in mayonnaise processing.

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