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Research Paper

Antioxidant and antimicrobial activity of Rosemary and Clove essential oils and their Nano emulsions on butter quality.

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Abstract

According to losses in fatty foods due to oxidation and microbial contamination, In attendance a worldwide interest on the safety of food among food researchers, food producers and food microbiologist. So, many ideas to improve the food additives are welcomed mostly. Studies confirmed that addition of Essential oils (Eos), declining limitations of artificial additives minimizing their predictable side effects. Furthermore, the application of nanoemulsions (NEs) as food preservatives has raised a considerable concern. According to our research, studied butter samples were supplemented with Clove and Rosemary Eos and their NEs with 1.5% concentration. The prepared NEs was characterized by Zetasizer, Fourier-transform infrared spectroscopy (FTIR) and Transmission Electron Microscope (TEM); the size of prepared Clove and Rosemary NEs by TEM was 87.92 and 77.43 dynamic nanometer (d. nm) respectively. The antimicrobial and antioxidant activities of the EOs and NEs were detected by Minimum inhibitory concentration (MIC) and Free radical scavenging activity (DPPH assay) tests. The physicochemical properties of supplemented butter, including sensory properties, peroxide value (PV), Thiobarbituric value (TBA) and acid value (AV) was detected. Furthermore, the microbial quality was determined with detection of total bacterial count (TBC), psychrotrophic bacterial count, Lipolytic bacterial count and yeast and mold count. The finding indicated that the butter fortified with Clove nanoemulsion exhibited the most favorable outcomes followed by butter enriched with RNE then CEO and finally REO.

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Introduction

Dairy industry which is dealing with multiple encounters as food quality, safety and nutritional distress, is alongside challenged with a demand for novelty to evolving consumer favorites and tolerate markets share (Patvardhan *et al*, 2026). For butter quality attributes include nutritional value, color, desirable texture, *prolonged* shelf life and sensory demand (Timlin *et al*, 2024). In Egypt, butter is not only one of the most popular dairy products but also, highly nutritious; however, it concurrently constitutes a threat to public health. During storage of butter spontaneous oxidation of lipids, triggers to flavor defects under the cold storage conditions, depending on the temperature of storage, quality of milk and the processes involved in butter production. Production of bad tastes is occurred with the creation of hydroperoxides that considered unacceptable to consumers and affect their health (Lan *et al*, 2026).

Trends of natural preservatives usage become a different choice taken by food producers and consumers since they increase safety of food products and the shelf life with no limitations. Herbal extracts and essential oils (EOs) are stated to be an excellent natural food additive (Gadallah *et al*, 2024).

Clove oil (CO) is an essential oil originated from the stems, leaves, flowers of the clove tree, which is a member of the family Myrtaceae (*Eugenia caryophyllata* or *Eugenia aromatica*). Eugenol, a phenylpropanoid, constitutes the main component, a strong antioxidant and antimicrobial agent, which makes CO a powerful natural preservative substitute for the food industry (Bentayeb *et al*, 2014). The phenyl group in eugenol can alter the protein structure and interact with fats in the microbial cell membrane, affecting permeability and ultimately leading to cell death; this is responsible for its antimicrobial effect (Zare *et al*, 2026). Rosemary essential oil (REO) is extracted from Rosemary (*Rosmarinus officinalis*), a perennial herb of the Lamiaceae family that is widely used for the preservation and flavoring of food products (Ramos *et al*, 2024).

In the meantime, eugenols hydroxyl group and Rosemary extracts is associated with powerful activity as an antioxidant, making it equivalent

to synthetic antioxidants used in foods nowadays, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Alanazi *et al*, 2026). Due to the high hydrophobicity and separation of oil in the finished product, EOs dispersion in water-based products is rather difficult (Xu *et al* 2026).

Additionally, the EOs taste affects the sensory characters of food at operative dosages. Therefore, applying EOs in the form of NEs presents a hopeful solution to the challenges encountered in food production. The small size of the NE droplet (from 10 to 100 nm) makes NEs translucent and light, that not alter the origin color of the food. Moreover, NEs characterized by good solubility without any separations or clumping compared to coarse emulsions (Mokhtar 2026).

In this regard, the main objectives of the current studies were to evaluate antimicrobial and antioxidative activity of Clove and Rosemary (EOs) and their nano-emulsions alongside the sensory, microbial and oxidative quality of butter enriched with these oils and their NEs.

Materials and Method

Clove and Rosemary EOs were purchased from the National Research Center, Egypt. Both EOs was dissolved in sterile dimethylsulfoxide (DMSO) (Sigma) and two-fold serial dilutions were carried out to reach a serial concentration from 1 to 0.03 % (V\V) (Abdallah *et al*, 2015).

1. Preparation of Nanoemulsions (Ghosh *et al*, 2013).

Clove and Rosemary oil-in-water NEs were prepared by adding 1:1 v/v % of Tween 80 and Tween 20 in deionized water at room temperature. The oil added was 10% concentration within the NE. A magnetic stirrer was used for shaking the mixture for 10 minutes to reach a homogenous solution. After that, we added the EOs slowly to a direct driven stirrer (Hotplate stirrer, DAIHAN Scientific Co., Ltd, Korea) for 20 min, then, the resulted solution was sonicated using a 25 kHz ultrasonic Homogenizer (USH650, max power: 650 watt) for 15 min. The resulted NEs were stored at 25 °C.

1.1. Characterization of the prepared Nanoemulsions formulation (Kabri *et al*, 2011 & Gurpreet and Singh, 2018 &

Shakeel *et al.*, 2019).

A zeta-sizer (3000 HS, Malvern Instrument, Malvern, UK) were used for analyzing the Droplet light scattering and PDI (at the nano-technology unit, Al-Azhar University, Assiut Branch, Egypt). To analyze the functional groups with their means of attachments and the fingerprint of each molecule we used Fourier-transform infrared spectroscopy (FTIR, NICOLET, iS10, Thermo Fisher Scientific). This test was in Chemistry Department, Faculty of Science, Assiut University. Finally, the morphological identification was applied in the Electronic Microscopic Unit, Assiut University, Egypt by TEM (TEM, JEOL-100CX II).

1.2. Determination of the minimum inhibitory concentration (MIC) of Clove and Rosemary Essential oils and their Nanoemulsions (El-Shenawy *et al.*, 2015)

Clove and Rosemary EOs and their NEs were diluted in sterile dimethylsulfoxide (DMSO) to reach range concentrations from 1 to 0.03% (V/V). 100µl of the fresh *E. coli* and *Staph. aureus* broth with 10^8 cfu/ ml were cultivated on Muller Hinton agar plates. 100µl of EOs and their NEs concentrations (1, 0.5, 0.25, 0.125, 0.06 and 0.03%) were put into agar wells. Then, incubation of the plates at 35 ± 2 °C for 24 hours. The resulted zones of inhibition were measured to detect the MIC.

1.3. Antioxidants activity assay (Free radical scavenging activity) using DPPH (2,2-diphenyl-1-picrylhydrazyl assay) (Behbahani *et al.* 2019).

3ml of 0.1mM ethanolic solution of DPPH was added to 1 ml of EO, NE at concentration 100 µl /ml. The absorbance was measured against a blank at 517 nm 0-, 30-, 60- and 120-min. Inhibition of free radical DPPH in percent was calculated.

2. Evaluation of Essential Oils and their Nanoemulsions effect on butter quality

2. 1. Butter manufacturing (Deosarkar *et al.*, 2016).

Butter was manufactured by the traditional churning method using buffalo milk without any additions or salt. The resulted butter was divided into 5 equal parts (negative control, 2 parts for with 1.5% Clove oil, with 1.5% Clove NE, with 1.5% Rosemary EO and with 1.5% RM NE). Then a sample was taken from each part at zero time for sensory, chemical and microbiological evaluation. Butter was stored at 5°C samples were taken for examination after 24 hours then weekly till deterioration of butter.

2. 2. Sensory analysis (IS, 2003).

Butter samples were examined at room temperature in the morning immediately after receipt, after 24 hours and every week for some exterior characteristic: color, odor, taste, surface, shape, form and structure. Nine panelists from the Animal Health Research Institute, Assiut branch, merged to justice the tested samples in a scale sheet.

Table (1). Quality Grading Based on Sensory Scores

Score	Quality	Grade
90 or more	Excellent	A
80-90	Good	B
60-79	Fair	C
59 or below	Poor	D

2.3. Microbiological Analyses

Ten folds serial dilutions were prepared according to (ISO 6887-1:2017), then aerobic mesophilic microorganism (AMM) according to (ISO 16297:2020), Psychrotrophic microorganisms according to (ISO 17410:2019), lipolytic microorganism (Gubash, 1991) finally, total yeasts and molds (Bailey and Scott, 1998). Analyses were carried out at 0-day, then 1-day and 1-week intervals up to the 5th week at 4°C.

2.4. Determination of Lipid Oxidation

Peroxide value (IDF, 2006), Thiobarbituric acid reactive substances assay (TBARS)(Ke and Woyewoda, 1979) and Acid value (ISO 660:2005) were applied for detecting the extent of lipid oxidation occurred to the examined samples.

3. Statistical analysis (Lantz *et al* 2016)

All the analysis were carried out in triplicate and the experimental results obtained were expressed as means $\pm$ standard deviation. The statistical program Graph pad Prism 5 (version 5.01) was used for data analysis (Prism 5, 2007). The differences between groups were detected using the Dunnett Multiple Comparison test ($P \leq 0.05$).

Results and Discussion

Milk fat deterioration, mainly through lipolysis and oxidation, adversely affects flavor, reduces nutritional value, and limits storage stability. Oxidative rancidity in butter is a major challenge in the dairy industry, primarily resulting from the oxidation of unsaturated glycerides, leading to peroxide formation, and increases free fatty acid levels (Özkanlı and Kaya, 2007).

The current study explained the inhibitory effect of Clove and the inhibitory effect of

Clove and Rosemary EOs and their NEs on the hygiene of butter during storage. Patently, adding of CEO, REO NEs to butter could induce high antimicrobial and antioxidative effect on butter than the addition of the oils with high consumer accept-ability. Accordingly, the NEs may be an effective natural preservative used in dairy products. Furthermore, our study showed that NEs was prepared by a novel and safe method with natural substances as tween 80 that can be safely added to food

Characterization of the prepared nanoemulsions

The DP (intensity-average diameter) and PDI (polydispersity index) of the NE were measured using a Zeta-sizer (Table 1). For Clove nanoemulsion, results of the NEs droplets size were resembled the size obtained by Sikhao *et al* (2026) and higher than results of Zare *et al* (2026), Regarding the Rosemary nanoemulsion, results were in acceptance with study of Kaur *et al* (2026) but less than Elsherif *et al* (2022).

There are many factors affecting the mean droplet size of NEs as conditions of preparation type of oil, type of surfactant, the ratio of surfactant-to-oil and co-solvent or co-surfactant addition (Mittal *et al* 2023). Smaller PDI values indicate narrow particle size distribution (Sharif *et al*, 2017). As, PDI lower than 0.25 shows a very narrow particle size distribution, while PDI higher than 0.5 indicates large particle size distribution (Gibis *et al*, 2013).

Table (2). Droplet size distribution of Clove and Rosemary nanoemulsions

Nanoemulsion	*PDI	d.nm $\pm$ st. dev (d.nm)**
Clove nanoemulsion	0.52	131.6 $\pm$ 99.65
Rosemary nanoemulsion	0.36	116.1 $\pm$ 48.63

* Polydispersity Index, **dynamic nanometer

FTIR analysis was conducted to identify potential molecular interactions among the functional groups of essential oils, tween 80. The FTIR spectra of Clove and Rosemary EOs and NEs were displayed in Fig. 1 and 2.

As illustrated in the figure 1 (Clove oil and NE), These main bonds were centered at 3385.72 cm^{-1} (OH vibration of hydroxyl group of Clove EO), 2971.06 and 2931.89 cm^{-1} (C-H stretching of methyl and isopropyl groups on the phenolic ring C-H stretching vibration of benzene, 1375 cm^{-1} was the C—H deformation vibration of eugenol methyl that could be related to the main bioactive compound. The characteristic absorbance peaks of phenolic O-H and C-H stretching in aromatic ring at 3385.712 cm^{-1} and 2971 cm^{-1} respectively were observed that were attributed to O-H group and aromatic structure of eugenol.

Interestingly, nanoemulsion of Clove oil exhibits a small peak at wavenumber 1734.87 cm^{-1} , this peak resulted from the -C=O stretch in acetate attached to aromatic ring. A deep peak at 1457.58 cm^{-1} corresponding to the bending vibrations of OH and CH (**Semwal and Randeep 2014**). Similar results were recorded by **De Oliveira and Tavares (2022)**.

The CNE spectra showed a broad peak at 3422 cm^{-1} due to overlapping and stretching of NH_2 and -OH. Transmittance peak appeared at 2872 cm^{-1} owing to symmetric stretching of the CH group of the pyranose structure. Deep peaks appeared at 1636 cm^{-1} due to deformational vibrations of C-O group of primary amines. A peak at 1515.65 cm^{-1} is due to the bending of NH_2 groups. A deep peak at 1457.58 cm^{-1} corresponded to the bending vibrations of OH and CH. In the ATR-FTIR spectrum of Rosemary EO (Figure 2), the spectrum revealed distinct absorption bands corresponding to key functional groups: C=C bending ($1700\text{-}1500\text{ cm}^{-1}$), aromatic C-H stretching ($3150\text{-}3050\text{ cm}^{-1}$) and aromatic carboxylic acid O-H stretch spanning $3000\text{-}2500\text{ cm}^{-1}$. The bands confirm 1,8-cineole as rosemary oil's primary constituent, assigned to $\text{CH}_3(\text{CO})$, C-O-C, and CH_2 vibrations (**Schulz et al, 2005**) the same findings were detected by (**Chandu et al, 2020**).

Meanwhile in case of *Rosemary oil* NE, FTIR results showed a slight difference in number

and intensity of peaks. At 2924.52 cm^{-1} , a peak was found related to methyl C-H stretch (-CH₃). A sharp strong peak presented at 1108.88 cm^{-1} indicating presence of -OH. Also, a strong peak of aldehyde was observed at 2856 cm^{-1} followed by another aldehyde peak at 1736 cm^{-1} . At 1637 cm^{-1} , a peak was found related to C=C stretching. Then, 2 peaks were observed at range of $1350\text{-}1458\text{ cm}^{-1}$ related to O-H bending. A peak of C-O stretch (alkyl-substituted ether) and finally, a peak at 950 cm^{-1} was found indicating C-H stretching. FTIR spectrum results was in consistent with that of **De Oliveira and Tavares (2022)**. Herein, that the peak of -OH stretching of Clove and Rosemary NEs was stronger than of EO, that indicated a stronger hydrogen bonding formation NEs. According to CNE, there were deep peaks at 1636 cm^{-1} due to deformational vibrations of C-O group of primary amines. Interestingly, nanoemulsion of Clove oil exhibits a small peak at wavenumber 1734.87 cm^{-1} , this peak may result as the -C=O stretch in acetate attached to aromatic ring. A peak at 1515.65 cm^{-1} is from the NH_2 group bond. FTIR confirmed no chemical interaction between the essential oils and the materials during NE formation. This proves the method's efficacy in producing chemically stable, small sized nanoemulsions.

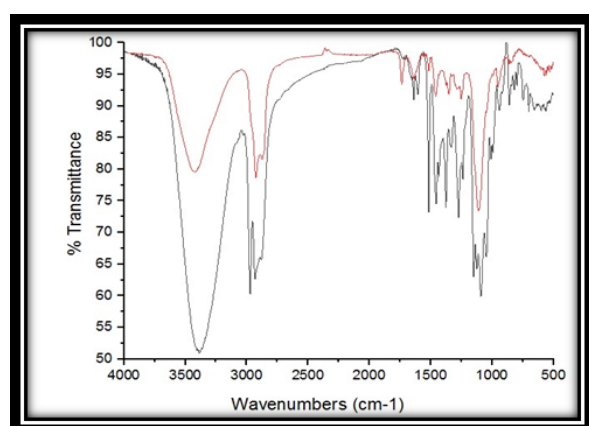


Figure (1). FTIR spectrum of clove EO and its NE scanned at 4000-500 cm⁻¹

Transmission Electron Microscope (TEM) studies showed spherical shaped of nanoemulsion droplets. The droplet size of distribution recorded by TEM typically lower than droplet size distribution recorded with the Zetasizer which was 87.92 nm in Clove nanoemulsion (CNE) and 77.43 nm in Rosemary nanoemul-

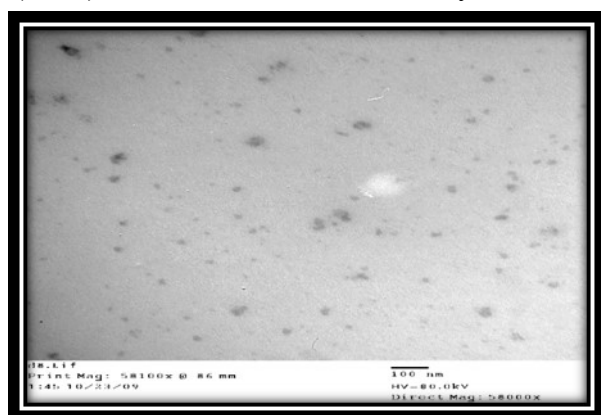


Photo. (1). Transmission Electron Microscope for clove NE with an average size 87.92 nm

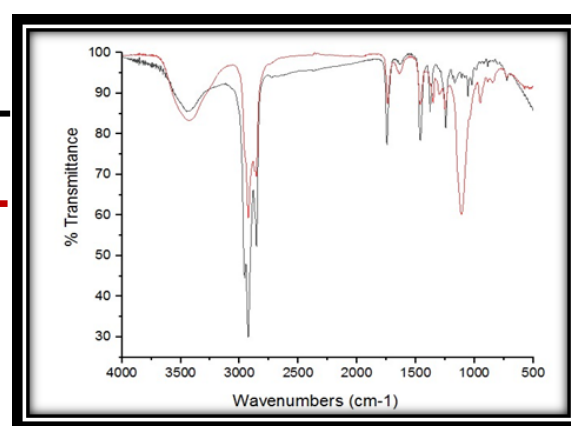


Figure (2). FTIR spectrum of rosemary EO and its NE scanned at 4000-500 cm⁻¹

sion (RME) (Photo 1, 2). CNE were with diameter higher than that was detected by **Sharma et al. (2017)** but lower than **de Almeida et al. (2025)** while TEM results of RNE resemble the result of **Mutlu and Mor (2025)**.

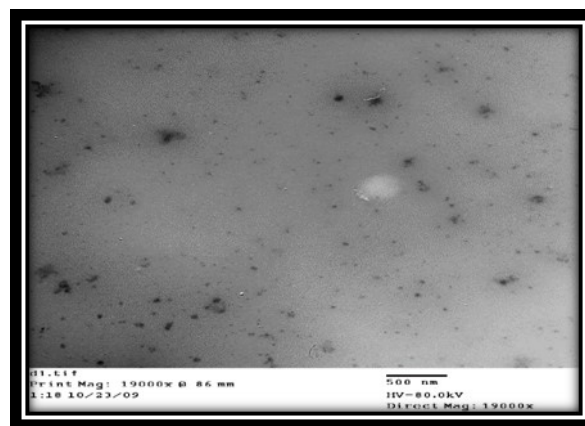


Photo. (2). Transmission Electron Microscope for rosemary NE with an average size 77.43 nm

Minimum Inhibitory Concentration of Essential oils and their Nanoemulsions against *Staph aureus* and *E. coli*.

Essential oils (EO) are increasingly valued as natural antimicrobials for food preservation, a movement propelled by today's consumer demand for cleaner, more natural food option **Niu et al. (2023)**.

The efficacy of the EOs and prepared NEs against *Staph. aureus* and *E. coli* was investigated using agar well diffusion assay (Table 2,3). EOs and their NEs exhibited forceful antibacterial effect against *Staph. aureus* than *E.*

coli. It is obvious that the diameter of the zone of inhibition was proportional to the concentration of essential oils and nanoemulsions. The zones of inhibition of the nanoemulsions were higher than the essential oils. Regarding the inhibitory effect on *Staph aureus*, concentration 0.25% didn't have an inhibitory effect except Clove NE, whereas concentration 0.5% of the used nanoemulsions and Clove EO had the same effect while Rosemary EO didn't have an inhibitory effect.

The results also showed that *Staph. aureus* (gram +ve bacteria) was affected by EOs and

their NEs than *E. coli* (gram -ve bacteria). These results concur with the fact that Gram positive bacteria do not have lipopolysaccharide (LPS) protection from hydrophobic compounds **Mutlu and Mor (2025)**. **Teimour *et***

***al.* (2026)** study was conducted the antimicrobial effect of REO against foodborne pathogens.

Table (3). Minimum inhibitory concentration of Clove and Rosemary Essential Oils and their nanoemulsions.

	Conc./ Well	Zone of inhibition (mm)			
		Clove		Rosemary	
		EO	NE	EO	NE
<i>Staph. aureus</i>	2.5%	15	17	14	16
	2%	11	13	10	13
	1.5%	9	10	8	9
	1%	6	7	5	6
	0.5%	5	5	0	5
	0.25%	0	5	0	0
<i>E. coli</i>	2.5%	12	14	10	11
	2%	10	12	9	9
	1.5%	7	10	6	7
	1%	5	9	0	5
	0.5%	0	6	0	0
	0.25%	0	0	0	0

Antioxidant Activities of essential oils and their Nanoemulsions

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is one of the most widely used substrates for rapid evaluation of antioxidant activity due to its stability and simplicity of analysis. The principal of this assay is built on the measurement of the scavenging activity of the extract toward the stable radical DDPH. The results in table 3 viewed that the RSA for Rosemary EO and its NE was found to be higher than that of Clove EO and its NE. The same results were detected by **(Gadallah *et al.* 2024 and Rui *et al.* 2024)**. Due to their richness in phenolic acids and terpenoids, essential oils demonstrate potent antioxidant properties,

making them effective natural agents for improving food preservation **(Ahmed *et al.* 2021)**.

Table (4). The DPPH free radical scavenging activity of Clove and Rosemary Essential oils and their nanoemulsions.

DPPH Free Radical Scavenging Activity %	Clove		Rosemary	
	EO	NE	EO	NE
	92	64.3	95.4	74.5

The most important feature of a new functional product is sensory properties. Because the physical and chemical properties of the product no matter how good if the sensory characteristics of the product are not good this product is not consumed.

However, EOs have strong odor and flavor, the samples enriched with NEs showed an overall acceptability better than samples enriched with EOs within the same concentration through the experiment. Regarding the sensory characters; CEO, REO and their NEs were transparent and not viscous so they were no changes in color, texture and consistency after their addition in butter. However, according to the flavor, odor (overall acceptability) of butter before and after addition of EO and their NE table 5 showed that after the addition of CEO, CNE, REO and RNE to the butter there were a significant difference between control sample and treated samples except in butter sample with Rosemary nanoemulsion where both had grade excellent which made it more acceptable to the panelists. The best Flavor of butter which was the closest to the control was butter with RNE followed by butter with butter with REO and the least flavor score was recorded with butter with CEO.

During the first and second weeks of the experiment, there is no changes in overall acceptability between control and treated groups, except butter sample containing CEO. However, from the third week onward, significant differences emerged between the control and most treated samples, with the exception of the CEO-treated sample.

By the end of the storage period, the overall acceptability of the control, CEO-treated, and Clove nanoemulsion (CNE)-treated samples declined to a poor level, whereas samples containing REO and RNE maintained a fair quality. Additionally, the general appearance of butter samples became dull, with visible mold

growth, and a pronounced nutty odor was particularly evident in the control, CEO, and CNE samples.

Antimicrobial effect of EOs and their NEs during manufacturing and storage of butter

In terms of microbiological quality of control butter sample, the results showed that there was a significant difference within total bacterial, psychrotrophic, lipolytic, yeast and mold count (tables 6, 7, 8, 9, 10) in control sample starting from the first week till the complete deterioration at the 5th week.

Essential Oils and their Nanoemulsions had stronger effect on total bacterial count with a slight superiority to RNE followed by REO the same manner was shown with psychrotrophic count (table 6 and 7). The same effect of RNE was observed by **Huang *et al.* (2020)**.

Table (5). Sensory evaluation of butter treated with *Clove* and *Rosemary* Essential oils and their Nanoemulsions.

Storage period	Control		Clove				Rosemary			
			Clove EO		Clove NE		Rosemary EO		Rosemary NE	
	Quality	Score	Quality	Score	Quality	Score	Quality	Score	Quality	Score
0	Excellent	96	Fair	78	Good	88	Good	83	Excellent	95
1 st day	Excellent	96	Fair	78	Good	88	Good	83	Excellent	95
1 st w	Good	85	Fair	74	Good	85	Good	82	Excellent	90
2 nd w	Fair	76	Fair	72	Fair	77	Good	80	Good	87
3 rd w	Fair	63	Fair	69	Fair	73	Good	79	Good	81
4 th w	Poor	52	Poor	50	Fair	69	Fair	75	Fair	78
5 th w	Poor	32	Poor	45	Poor	55	Fair	70	Fair	75

Table (6). Effect of *Clove* and *Rosemary* Essential oils and their *Nanoemulsions* on total bacterial count during storage of butter.

Storage period	Control	Clove				Rosemary			
		Clove EO		Clove NE		Rosemary EO		Rosemary NE	
		Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %
zero time	7x10 ³	7x10 ³	0	7x10 ³	0	7x10 ³	0	7x10 ³	0
24 h	3x10 ⁵	4.8x10 ⁴	84	2x10 ⁴	93.33	1x10 ⁵	66.66	3.6x10 ⁴	88
1 st wk	9.3x10 ⁵	7x10 ^{4*}	92.47	1x10 ^{4*}	98.92	9x10 ^{4*}	90.32	3x10 ^{4*}	96.77
2 nd wk Rosemary % cloveil	1.84x10 ⁶	5x10 ^{3*}	99.72	9x10 ^{2*}	99.95	4x10 ^{4*}	97.82	1x10 ^{3*}	99.94
3 rd wk	2.7x10 ⁶	1.1x10 ^{3*}	99.5	8x10 ^{2*}	99.97	2.7x10 ^{4*}	99	9.1x10 ^{2*}	99.96
4 th wk	4.5x10 ⁶	2x10 ^{4*}	99.55	1.7x10 ^{3*}	99.96	9.8x10 ^{4*}	97.82	5x10 ^{3*}	99.88
5 th wk	9x10 ⁶	2.8x10 ^{5*}	96.88	3x10 ^{4*}	99.66	4.7x10 ^{5*}	94.77	3x10 ^{4*}	99.66

*Significant difference between the mean of total bacterial count of treated butter samples and the

Maurya *et al.* (2021) explained that Eos and NEs affect microbial cell through alterations in the histology of the microbial cell, and cell membrane permeability causing further exit of cellular nucleic acids and proteins. Also, **Yousef *et al.* (2019)** observed that encapsulated Clove NE act as a strong antimicrobial against lipolytic bacteria when compared to non-nanoemulsion structures. More studies have reported that the fungicide effect of EO nano-encapsulated are resulted from altering the microstructure of the cell wall induced by the higher surface area of nanocomposites and changes in permeability of ions of the fungal cell membrane. With the same view **Mahdi *et al.* (2021)** reported that CEO and CNE had a

strong antifungal effect more than the common synthetic fungicide thiabendazole.

Entering the EOs and NEs into the microbial cell lead to contraction of the lipid bilayer of the cell membrane which results in damage of the integrity of it and changes in the membrane permeability; So, loss of vital intracellular components (Ca_2C , KC, and Mg_2C) and mitochondrial electron transport system inhibition and inhibit the proton pump and loss of ATP which end with apoptosis (**Singh *et al.*, 2020**).

Table (7). Effect of *Clove* and *Rosemary* EOs and their Nanoemulsions on *psychrotrophic* count during storage of butter

Storage Period	Control	Clove				Rosemary			
		Clove EO		Clove NE		Rosemary EO		Rosemary NE	
	Count Average CFU/g	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count average CFU/g	Red %	Count Average CFU/g	Red %
Zero Time	2×10^4	2×10^4	0	2×10^4	0	2×10^4	0	2×10^4	0
24 hours	1.6×10^5	1.15×10^5	28.12	1×10^5	37.5	1.3×10^5	18.75	1×10^5	37.5
1st wk	5×10^5	2×10^5 *	60	8×10^4 *	84	3×10^5 *	40	9×10^4 *	82
2nd wk	2.6×10^6	3.6×10^4 *	98.61	8×10^3 *	99.69	5×10^4 *	99.8	8×10^3 *	99.69
3rd wk	6×10^6	2.3×10^3 *	99.96	3×10^2 *	99.99	4×10^3 *	99.93	8×10^2 *	99.98
4th wk	1.4×10^7	5×10^3 *	99.96	1.1×10^3 *	99.99	3.5×10^4 *	99.75	2.2×10^3 *	99.99
5th wk	4×10^7	1×10^5 *	99.75	3×10^4 *	99.92	2×10^5 *	99.5	3.5×10^4 *	99.91

*Significant difference between the mean of psychrotrophic bacterial count of treated butter samples and the control sample.

Table (8). Effect of *Clove* and *Rosemary* Essential Oils and their *Nanoemulsions* on *lipolytic* count during storage of butter

Storage Period	Control	Clove				Rosemary			
		Clove EO		Clove NE		Rosemary EO		Rosemary NE	
	Count Average CFU/g	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %
Zero Time	1.7x10 ³	1.7x10 ³	0	1.7x10 ³	0	1.7x10 ³	0	1.7x10 ³	0
24 hours	1.9x10 ⁵	4.9x10 ⁴	74.21	2x10 ⁴	89.47	5x10 ⁴	73.68	2.7x10 ⁴	85.78
1st wk	9x10 ³	6x10 ³	33.33	1.7x10 ³	81.11	7x10 ³	22.22	1.2x10 ³ *	86.66
2nd wk	1.4x10 ⁵	4x10 ⁴	71.42	1.5x10 ⁴	89.28	6x10 ⁴	57.14	1.7x10 ⁴ *	87.85
3rd wk	5x10 ⁵	1.1x10 ⁵	78	4.7x10 ⁴ *	90.6	2.5x10 ⁵	50	7x10 ⁴ *	86

*Significant difference between the mean of lipolytic bacterial count of treated butter samples and the control samples

Table (9). Effect of *Clove* and *Rosemary* Essential oils and their *Nanoemulsions* on *yeast count* during storage of butter.

Storage Period	Control	Clove				Rosemary			
		Clove EO		Clove NE		Rosemary EO		Rosemary NE	
	Count Average CFU/g	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %
Zero Time	1.7x10 ³	1.7x10 ³	0	1.7x10 ³	0	1.7x10 ³	0	1.7x10 ³	0
24 hours	1.9x10 ⁵	4.9x10 ⁴	74.21	2x10 ⁴	89.47	5x10 ⁴	73.68	2.7x10 ⁴	85.78
1st wk	9x10 ³	6x10 ³	33.33	1.7x10 ³	81.11	7x10 ³	22.22	1.2x10 ³ *	86.66
2nd wk	1.4x10 ⁵	4x10 ⁴	71.42	1.5x10 ⁴	89.28	6x10 ⁴	57.14	1.7x10 ⁴ *	87.85
3rd wk	5x10 ⁵	1.1x10 ⁵	78	4.7x10 ⁴ *	90.6	2.5x10 ⁵	50	7x10 ⁴ *	86
4th wk	6.6x10 ⁶	3.5x10 ⁵ *	94.69	1x10 ⁵ *	98.48	5x10 ⁵ *	92.42	1x10 ⁵ *	98.48
5th wk	7.1x10 ⁶	4x10 ⁵ *	94.36	1.7x10 ⁵ *	97.6	8x10 ⁵ *	88.73	6x10 ⁵ *	91.54

*Significant difference between the mean of yeast count of treated butter samples and the control samples

Table (10). Effect of *Clove* and *Rosemary* Essential oils and their Nanoemulsions on mold count during storage of butter.

Storage Period	Control	Clove				Rosemary			
		Clove EO		Clove NE		Rosemary EO		Rosemary NE	
	Count Average CFU/g	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %	Count Average CFU/g	Red %
Zero Time	4x10 ⁴	4x10 ⁴	0	4x10 ⁴	0	4x10 ⁴	0	4x10 ⁴	0
24 h	2x10 ⁴	1x10 ⁴	50	2x10 ³	90	1.7x10 ⁴	15	3x10 ³	85
1 st wk	1x10 ⁴	7x10 ³	30	1.6x10 ^{2*}	98.4	9x10 ³	10	6x10 ^{2*}	94
2 nd wk	2x10 ⁵	1.2x10 ^{2*}	99.94	3x10 ^{2*}	99.85	2x10 ^{3*}	99	3x10 ^{2*}	99.85
3 rd wk	1x10 ⁶	8x10 ^{3*}	99.2	2x10 ^{3*}	99.8	8x10 ^{3*}	99.2	4x10 ^{3*}	99.6
4 th wk	2x10 ⁷	3x10 ^{5*}	98.5	1x10 ^{5*}	99.5	5x10 ^{5*}	97.5	2x10 ^{5*}	99
5 th wk	5x10 ⁷	5x10 ^{6*}	90	4x10 ^{5*}	99.2	7x10 ^{6*}	86	5x10 ^{5*}	99

*Significant difference between the mean of mold count of treated butter samples and the control samples

Oxidative stability during storage

Lipid oxidation is undesirable in most foods due to quality deterioration that includes changes in appearance, texture, shelf life and nutritional profiles, and development of off-flavors and potentially toxic reaction products (Lim *et al.* 2015). peroxide values, secondary oxidation products of polyunsaturated lipids (TBA value) and Acid value are the most used parameters to determine these processes (Flakelar *et al.* 2015).

Within the present study (tables 11, 12, 13), Both of primary and secondary oxidation products were significantly changed. The levels of PV, TBA, AV raised steadily as storage time progressed. Both the type of butter and the length of storage, as well as the combined effect these two factors, were found to significantly influence these values ($p < 0.01$) resembling the results of Kahyaoğlu and Çakmakçı (2018).

There was increase in oxidative parameter values of butter samples (PV, TBA and acid value) which were significant during storage period, in addition there was a significant difference between butter samples with EOs and butter samples with their NEs. The increase in ox-

idative parameters of butter samples with NEs were lower than those with EOs.

Addition of EOs in butter exhibited prooxidant effect. Many studies mentioned polyphenols in EOs play as a prooxidants that lead to accelerate the oxidation in butter samples with EOs than those with NEs (Chaubal *et al.*, 2005). Gramza-Michalowska *et al.* (2007) and Sert *et al.* (2015) resulted that extraction of Clove and Rosemary modify the butter stability.

Table (11). Effect of *Clove* and *Rosemary* Essential Oils and their Nanoemulsions on *peroxide value* during storage of butter samples.

Storage Period	Control	Clove		Rosemary	
		Clove EO	Clove NE	Rosemary EO	Rosemary NE
	PV mEqO ₂ /kg				
Zero Time	0.23	0.23	0.23	0.23	0.23
24 h	0.35	0.3	0.24	0.35	0.26
1 st wk	0.45	0.39*	0.32*	0.42*	0.36*
2 nd wk	0.52	0.46*	0.39*	0.48*	0.42*
3 rd wk	0.68	0.55*	0.45*	0.58*	0.49*
4 th wk	0.82	0.63*	0.55*	0.67*	0.58*
5 th wk	1.25	0.8*	0.63*	0.9*	0.68*

*Significant difference between the mean PV of treated butter samples and the control samples.

Treated butter samples had generally lower TBA values than the control group. The lowest value of TBA was detected in the samples comprising CNE, CEO, RNE and REO. During storage there was significant increase ($P<0.01$) in the TBA value. The control group showed the highest TBA, but after addition of EO and their NEs, the TBA values decreased significantly. The same results of CNE effect were found by **Sert *et al.* (2015)** and for RNE **Huang *et al.* (2020)** recorded the same results. The acid value increment through storage peri-

od caused by the proliferation of psychrotrophic lipolytic microorganisms in butter plus the action of lipolytic enzymes. These causes promote the gradual hydrolysis of milk fat and release of free fatty acids (**Nadeem *et al.* 2013**). The preventive effect of polyphenols against formation of free fatty acids is likely due to their antimicrobial activity, which has been reported in many literatures (**Ayar *et al.* 2006**).

Table (12). Effect of *Clove* and *Rosemary* Essential oils and their Nanoemulsions on *TBA* during storage of butter

Storage Period	Control	Clove		Rosemary	
		Clove EO	Clove NE	Rosemary EO	Rosemary NE
	mg MDA /kg				
Zero Time	0.007	0.007	0.007	0.007	0.007
24 h	0.01	0.01	0.009	0.013	0.009
1 st wk	0.15	0.025*	0.019*	0.047*	0.028*
2 nd wk	0.22	0.08*	0.03*	0.12*	0.05*
3 rd wk	0.35	0.23*	0.17*	0.25*	0.19*
4 th wk	0.49	0.35*	0.27*	0.39*	0.3*
5 th wk	0.78	0.42*	0.35*	0.56*	0.44*

*Significant difference between the mean TBA of treated butter samples and the control samples.

Table (13). Effect of *Clove* and *Rosemary* Essential oils and their Nanoemulsions on *Acid value* during storage of butter

Storage Period	Control	Clove		Rosemary	
		Clove EO		Rosemary EO	
	mg KOH/g				
Zero Time	0.33	0.33	0.33	0.33	0.33
24 h	0.53	0.49	0.38	0.5	0.43
1 st wk	0.69	0.53*	0.42*	0.56*	0.48*
2 nd wk	0.82	0.64*	0.5*	0.68*	0.54*
3 rd wk	0.91	0.72*	0.59*	0.77*	0.65*
4 th wk	0.98	0.81*	0.67*	0.89*	0.73*
5 th wk	1.6	0.9*	0.82*	0.95*	0.85*

*Significant difference between the mean acid value of treated butter samples and the control samples.

Conclusion

It could be concluded that Clove, Rosemary EOs and their NEs can participate in keeping a good hygienic quality of butter during storage with the superiority of NEs, which makes them recommended for potential applications in the food industry. Considering to growing demand application of safe preservatives for the stabilization of fat-containing foodstuffs; it is recommended to apply further studies on the nano-emulsions and food components of milk products and their response on the health of the people.

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