

The Role of Thyme and Clove Essential Oils Nanoemulsions in Quality Improvement of Soft Cheese

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Abstract:

White soft cheese is extremely vulnerable to contamination from a range of pathogenic and/or spoiling microorganisms during handling, production, and storage. There for this study was aimed to investigate the characterization of NEs of Thyme and Clove EOs, as well as their effects on the sensory and microbiological properties of UF-cheese along the storage period of 50 days at 7 °C. Therefore, the nanoemulsions (NEs) of Thyme and Clove essential oil (EO) were prepared by ultrasonication (as delivery systems) to be utilized as a natural preservative for ultrafiltrated (UF)-soft cheese. The prepared NEs were characterized by Dynamic light scattering (DLS) for particle size. Different concentrations of Thyme NE (0.03, 0.06 and 0.1%) as well as Clove NE (0.06, 0.1 and 0.25%) were used to preserve UF-cheese. The microbiological analysis and sensory evaluation of different UF-cheese treatments were evaluated during cold storage period for 50 days. The results revealed that the droplet size of the prepared NEs was 94.85 and 68.67 nm for Thyme and Clove NE, respectively. According to the sensory analysis, soft cheese fortified with NEs was still acceptable. The highest sensory acceptance was related to the cheeses fortified with 0.03 and 0.06 % Thyme and Clove NE, respectively. The results of microbial groups indicated that NEs of both EOs added to retentate led to decrease the total bacterial count (TBC), compared with the control. The growth of psychrotrophic bacteria, as well as yeast and mold were inhibited by the concentrations of 0.1% and 0.25% of Thyme and Clove NE, respectively, until the end of the storage period. These findings recommend using NEs of Thyme and Clove oils as natural

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preservatives to maintain the quality of soft cheese and prolong its shelf life.

Keywords: UF-soft cheese, Nanoemulsion, Antimicrobial, Essential oils.

Introduction

Cheese is a very nutritious dairy product that is extensively consumed across the world. The variation of cheese products in terms of sensory qualities is attributable to the varied types of milk and production techniques employed (*Boukria et al., 2020*). Ultrafiltration is a technique that concentrates milk before the formation of the curd. So, the draining stage employed in traditional methods to remove most of the whey in the curd is not necessary for UF-cheese production (*Lauzin et al., 2020*). During processing, handling, and distribution, dairy products especially soft cheese may become contaminated with a range of bacteria that might degrade the goods and render them unsafe for human consumption, posing serious threats to food safety (*Fusco et al., 2020*). Synthetic additives are commonly employed in the food industry for preservation, extending the shelf life and improving overall safety and quality. However, due to the drawbacks of synthetic additives and the interest of customers in ecologically friendly, less processed, natural food products, they have been increasingly substituted with natural

ones in recent years (*Nikmaram et al., 2018*).

Essential Oils and extracts from aromatic plants were found to have significant antibacterial and antioxidant activity, so they can be employed as alternative natural food preservatives (*Varghese et al., 2020*). Two of the most popular aromatic plants used in foods and beverages are the Thyme and Clove due to their flavor, aromatic and therapeutic properties (*Jiang, 2019*).

Thyme EO is widely used as a medicinal substance due to its antimicrobial properties (*Vassiliou et al., 2023*). It is commonly known that the primary elements responsible for Thyme's strong antibacterial and antioxidant action are the phenolic compounds, especially Thymol and carvacrol (*Perez et al., 2019*). The Thymol and carvacrol's antibacterial action may be due to the presence of phenolic hydroxyl on its phenolic ring. So, they can kill bacteria by altering the cell membrane's permeability and causing internal material loss (*Saricaoglu and Turhan, 2018*). Clove Oil is the most common EO in the Myrtaceae family (*de Meneses et al., 2019*). According to *Golmakani et al. (2017)*, it contains at least thirty recognized components, with

Eugenol accounting for at least 50 % of the main ingredient, which is a potent antibacterial and antioxidant agent making it an excellent natural preservative in food and pharmaceutical sectors.

However, the application of EOs in various food items is still restricted since they adversely change their organoleptic properties and may interact with food components leading to losing some of their effectiveness, so greater concentrations are required to exhibit their intended action. Furthermore, EOs are difficult to dissolve in aqueous media and are easily broken down by heat, light, and oxidation (*Saeed et al., 2022 and Zheng et al., 2024*).

The restrictions on using EOs as natural additives in the food sector were solved via nanotechnology as nanoemulsion process (NEs).. These processes can impact the physicochemical properties of EOs including stability, release characteristics, antibacterial activity, and sensory properties (*Mukurumbira et al., 2022*). Nanoemulsions are kinetically stable liquid-in-liquid dispersions with nanometer-sized droplets on the order of 100 nm that are employed in a variety of applications such as medicine delivery, food and cosmetics (*Salem and Ezzat, 2019*). It can be prepared in a variety of approaches and is thought to be a stable system for encapsulating bioactive

compounds (*Zambrano-Zaragoza et al., 2020*).

Therefore, this work is designated to study the characterization of NEs of Thyme and Clove EOs, as well as their effects on the sensory and microbiological properties of UF-cheese along the storage period of 50 days at 7°C.

Material and Methods

Extraction of EOs, preparation of NEs and their characterization

Thyme and Clove oils were extracted at the Oil Extraction Unit of the National Research Centre (NRC), Giza, Egypt. The NEs of Thyme and Clove oils were prepared using a high-energy method described by *Ghosh et al. (2013)*. Characterization of NE was investigated using Dynamic light scattering (NICOMP 380 ZLS, Dynamic light scattering (DLS) equipment, USA) to measure the NE droplet sizes.

Manufacture of UF-soft cheese

According to *Renner and Abd-El-Salam (1991)*, the ultrafiltration retentate which was obtained from the Dairy Industry Units, Animal Production Research Institute, Ministry of Agriculture, Giza, Egypt was pasteurized at 72°C for 15 seconds, and then quickly cooled to 42°C. The pasteurized UF-retentate was divided into seven portions, with the first serving as a control. The remaining six portions were fortified with 0.03, 0.06 and 0.1% Thyme EO NE (Treatments: T₁, T₂ and T₃) and 0.6, 0.1 and

0.25% Clove EO NE (Treatments: C₁, C₂ and C₃). These concentration of NEs were determined according to the MIC test (data did not shown). Rennet was added to cheese portions, mixed thoroughly and kept at 42°C until coagulation occurred. The cheese batches were stored at 5 ± 2°C for 50 days to be exposed to sensory evaluation and microbiological examination at 10-days intervals.

UF-cheese evaluation during storage period

Sensory evaluation of UF-cheese:

The sensory evaluation of cheese was done according to *Clark et al. (2009)*. About 12 panelists from the staff of the Dairy Department rated the cheese, the maximum score points were (5) for taste, odor, color, body & texture.

Microbiological evaluation of UF-cheese:

The samples were prepared for Total bacterial counts (TBC) according to *APHA (2004)*. TBC was determined using standard plate count agar at 35°C for 48 h (*APHA, 2004*). Psychrotrophic bacterial counts were detected using nutrient agar at 7°C for 10 days (*APHA, 2004*). Yeast and mould counts were enumerated using sabaroud dextrose agar medium of pH 3.5 at 25°C for 5 days (*APHA, 1994*).

Statistical analysis:

Statistical analysis of the data was done using 2-way ANOVA (analysis of variance) using SPSS, 25 for

Windows. Data significance was tested at $P < 0.05$ level.

Results and Discussion

Nanoemulsions were characterized for droplet size by DLS technique and the results obtained were 94.85 and 68.67 nm for Thyme and Clove NE, respectively. It was recorded that the droplet sizes have an impact on EO NE's antibacterial action. The obtained results are in accordance with *Haro-González et al. (2023)*, *Doghish et al. (2023)* and *Hashem et al. (2023)*, who revealed that droplet size was < 100 nm. *da Silva et al. (2023)* reported that EO NE has better antimicrobial properties than pure oil when its droplet sizes are less than 180 nm.

The sensory evaluation of fortified cheese (Figure 1) revealed that the lowest concentration of NE was found to be the most approved sample. The results also indicated that cheese samples treated with Thyme NE were more favorable compared to the samples treated with Clove NE. By increasing the NE concentration, the overall acceptability of UF-cheese was decreased. Similar findings were reported by *El-Sayed and El-Sayed (2021)*, who found that labneh fortified with 0.1 Thyme NE gained the highest total scores.

The microbiological analysis of UF-soft cheese revealed that the TBC in the control samples increased gradually until the 30th day, and then the counts started to gradually decrease until the end

of the storage period. However, the TBC of all treated samples decreased significantly ($p < 0.05$) during the storage period. The reduction rate was increased significantly by increasing NE concentrations. The best treatments for TBC reduction in UF-cheese were T3 and C3 with a 2.08 and 1.74 log reduction, respectively, throughout the storage period (Table 1). This can be due to the antibacterial activity of Thyme and Clove NEs *He et al. (2022)*, *Pilong et al. (2022)*, *Doghish et al. (2023)* and *Haro-González et al. (2023)*.

Regarding the psychrotrophic bacterial counts, the results illustrated in Table (2) demonstrated that they were not detected during the first 20 days of storage in control and treated samples. However, on the 30th day of storage, the count appeared with an average of 2.30 log cfu/g in the control sample. In treated samples, as the concentration of NEs increased, the appearance of the psychrotrophic count was delayed as it began to be detected on the 40th day for T₁ and C₁, and on the 50th day for T₂ and C₂. While in C₃ and T₃, the psychrotrophic bacterial counts were not detected until the end of the storage periods. These results may be attributed to the effectiveness of the added EOs to inhibit the growth of psychrotrophic bacteria as recorded by *Al-Moghazy et al. (2021)*.

It was found that the psychrotrophs are rarely detected in freshly drawn

milk and these populations appeared to grow during the cold storage reducing the normal refrigerated shelf-life (*Samaržija et al., 2012*).

The same behavior of preservative action was observed for the yeast and mold during the storage period. Yeast and mold counts were first recorded after 30 days of storage in control samples with an average count of 2.46 log cfu/g, and then the count was raised gradually until the storage period ended (Table 3). Also, the growth of yeast and mold was inhibited by the NEs at higher concentrations (T₃ and C₃) till the end of the storage period (50 days) compared to lower concentrations. The results obtained are in agreement with those reported by *Hassanin et al. (2021)* and *ABDEL KADER et al. (2024)*, who found that the Thyme and Clove EOs had potential antifungal activity resulting in extending the shelf-life of cheese.

The yeast and mold count were considered the most important factors in determining cheese shelf-life and quality. Their development causes detectable quality and organoleptic changes in dairy products such as off-flavor, texture, and color defects (*Mutlu-Ingok et al., 2020*).

The results of the bacteriological quality assessment of UF-cheese revealed that the Thyme and Clove EOs in NE form can suppress the bacterial growth in UF-cheese during cold storage and enhance its

qualities. These findings are in agreement with *Al-Obaidi and Hussein (2019)*, *Saleh et al. (2020)* and *ramzy (2023)*.

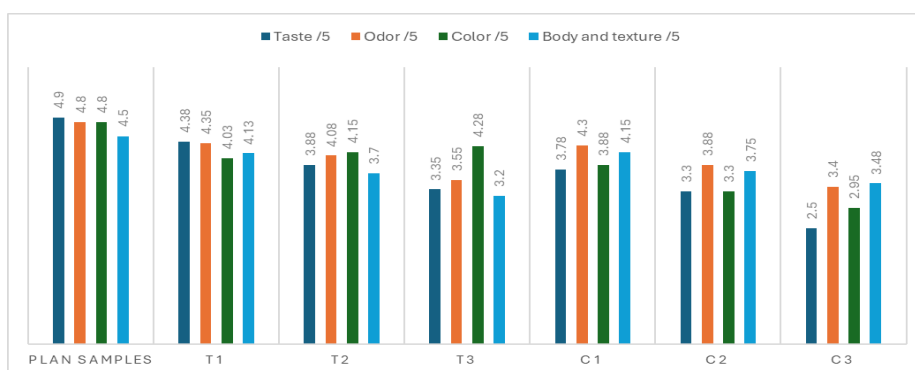
Conclusion

The obtained results clearly concluded that the addition of

Thyme and Clove oils NEs, as natural preservatives, to UF-soft cheese would improve the microbiological quality that assists in prolonging the shelf-life, with acceptable sensory quality.

Figure (1): Sensory evaluation results of examined samples.

Table (1): The effect of NE concentrations of Thyme and Clove oils on the TBC (log cfu/g) in UF-soft cheese throughout the storage periods



Storage periods	Control	Thyme NE Concentrations			Clove NE Concentrations		
		T ₁	T ₂	T ₃	C ₁	C ₂	C ₃
0-day	4.89 ^a	4.83 ^b	4.90 ^b	4.86 ^b	4.86 ^b	4.79 ^{bc}	4.68 ^d
10 th	5.64 ^a	4.54 ^b	4.55 ^b	4.29 ^c	4.64 ^b	4.49 ^c	4.27 ^c
20 th	5.99 ^a	4.17 ^b	4.21 ^b	3.91 ^{cd}	4.32 ^b	3.99 ^{bc}	3.81 ^d
30 th	6.45 ^a	3.90 ^b	3.867 ^b	3.49 ^c	3.99 ^b	3.84 ^{bc}	3.67 ^c
40 th	6.15 ^a	3.62 ^b	3.47 ^{cd}	3.17 ^e	3.85 ^c	3.40 ^{cde}	3.27 ^{de}
50 th	5.83 ^a	3.42 ^b	3.29 ^c	2.78 ^d	3.65 ^b	3.36 ^c	2.94 ^d

N.B:

1. Data expressed as the means of three replicates. Means between columns (effect of treatments) showing the same letters are not significantly different ($p \leq 0.05$).
2. Control: cheese without NE, (T₁, T₂ and T₃) are cheese with NE solution containing (0.03, 0.06 and 0.1%) Thyme EO, respectively. While (C₁, C₂ and C₃) cheese with NE solution contains 0.06, 0.1 and 0.25%) Clove EO, respectively.

Table (2): *The effect of NE concentrations of Thyme and Clove oils on the psychrotrophic bacterial count (log cfu/g) in UF-soft cheese throughout the storage periods.*

Storage periods	Control	Thyme NE Concentrations			Clove NE Concentrations		
		T ₁	T ₂	T ₃	C ₁	C ₂	C ₃
0-day	N. D	N. D	N. D	N. D	N. D	N. D	N. D
10 th	N. D	N. D	N. D	N. D	N. D	N. D	N. D
20 th	N. D	N. D	N. D	N. D	N. D	N. D	N. D
30 th	2.30 ^a	N. D	N. D	N. D	N. D	N. D	N. D
40 th	2.64 ^a	1.23 ^b	N. D	N. D	1.34 ^b	N. D	N. D
50 th	3.08 ^a	2.72 ^b	1.29 ^c	N. D	2.76 ^b	1.45 ^c	N. D

N.B:

1. Data expressed as the means of three replicates. Means between columns (effect of treatments) showing the same letters are not significantly different ($p \leq 0.05$).
2. N.D: Non detected = (< 10 cfu/g)
3. See Table 1

Table (3): *The effect of NE concentrations of Thyme and Clove oils on the total yeast and mould count (log cfu/g) in UF-soft cheese throughout the storage periods.*

Storage periods	Control	Thyme NE Concentrations			Clove NE Concentrations		
		T ₁	T ₂	T ₃	C ₁	C ₂	C ₃
0-day	N. D	N. D	N. D	N. D	N. D	N. D	N. D
10 th	N. D	N. D	N. D	N. D	N. D	N. D	N. D
20 th	N. D	N. D	N. D	N. D	N. D	N. D	N. D
30 th	2.45 ^a	N. D	N. D	N. D	N. D	N. D	N. D
40 th	3.36 ^a	1.08 ^b	N. D	N. D	2.16 ^b	N. D	N. D
50 th	4.15 ^a	2.32 ^c	1.38 ^c	N. D	3.24 ^b	1.72 ^d	N. D

N.B

- 1: Data expressed as the means of three replicates. Means between columns (effect of treatments) showing the same letters are not significantly different ($p \leq 0.05$).
- 2: N.D: Non detected = (< 10 cfu/g)
- 3: See Table 1

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دور المستحلبات النانوية لزيت الزعتر والقرنفل الأساسية في تحسين جودة الجبن الطري

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الملخص العربي

يتعرض الجبن الأبيض الطري بشكل كبير للتلوث بالعديد من الميكروبات المسببة للفساد الغذائي وربما للأمراض أثناء الإنتاج والتداول والتخزين. لذلك، تم تحضير مستحلبات نانوية من كل من زيت الزعتر والقرنفل باستخدام الموجات فوق الصوتية لاستخدامها كمادة طبيعية حافظة للجبن الأبيض الطري المعامل بالترشيح الفائق. وقد تم تحليل المستحلبات النانوية المحضرة لمعرفة حجم الجسيمات. ومن ثم إضافة تركيزات مختلفة من المستحلبات النانوية لكل من زيت الزعتر (0.03، 0.06 و 0.1%) وزيت القرنفل (0.06، 0.1 و 0.25%) إلى اللبن المعامل بالترشيح الفائق أثناء تصنيع الجبن. وقد تم فحص الجبن لمعرفة تأثير تلك المستحلبات على الخصائص الحسية والميكروبية للجبن أثناء فترة التخزين عند درجة حرارة 7°C لمدة 50 يومًا. وقد أظهرت النتائج أن حجم الجسيمات لكل من زيت الزعتر والقرنفل كانت 94.85 و 68.67 نانومتر لكلا الزيتين على التوالي. وقد أظهر التقييم الحسي أن الجبن المحتوي على المستحلبات النانوية ظل مقبولاً، حيث ارتبط أعلى قبول حسي بالعينات المحفوظة بالمستحلبات النانوية لكل من زيت الزعتر والقرنفل بنسبة 0.03 و 0.06%، على التوالي. وقد أدي إضافة المستحلبات النانوية لكل من زيت الزعتر إلى انخفاض العدد الكلي للبكتيريا مقارنة بالجبن الضابط. كما أن مستحلبات النانو لكل من زيت الزعتر والقرنفل بنسبة 0.1 و 0.25% -على التوالي- أدت إلى منع نمو البكتيريا المحبة للبرودة وكذلك الخميرة والعفن حتى نهاية فترة التخزين. وتؤكد النتائج المتحصل عليها أن إضافة المستحلبات النانوية لزيت الزعتر والقرنفل كمادة حافظة طبيعية تؤدي إلى تحسين الجودة الميكروبية للجبن الأبيض الطري والتي تؤدي إلى إطالة العمر الافتراضي مع كونه يظل مقبولا بالنسبة للجودة الحسية.