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# Influence of selected essential oils on some pathogenic microorganisms in white soft cheese

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**Abstract :** This study was designed to show the effect of antimicrobial properties of some Essential Oils (EOs); cinnamon and thyme oils for four weeks on *S.aureus* and *E.coli* microorganisms in white soft cheese. The higher sensory score of cheese samples was gained at first two weeks with cinnamon oil. *S.aureus* and *E.coli* count of cinnamon and thyme oils cheese samples significantly decreased ($P<0.05$) through 3 and 4 weeks of storage. The cinnamon and thyme oils had the different degrees of antibacterial effect on the selected pathogenic microorganisms during the storage of cheese.
**Key words:** Cinnamon oil, thyme oil, *S.aureus*, *E.coli*, white soft cheese.

## Introduction

The most one of delicious cheeses consumed in Egypt is white soft cheese[1]. Although it is properly produced and stored under hygienic conditions, cheese is unstable because of its dynamic biologic and biochemical structure [2]. Previously, cheese was classified under "safe foods", but after 1980, Poisoning related to the consumption of cheese with pathogenic microorganisms and/or their toxins have been reported [3]. Need for natural substitutional is due to consumers preference for fewer chemicals in foods. EOs have been considered as powerful alternatives and contain bioactive compounds which have antioxidant activity and antimicrobial activity against food-borne pathogen[4,5,6,7,8].

Pathogens related to cheese outbreaks included (six outbreaks) pathogenic *Escherichia coli*, (four outbreaks) *Staphylococcus aureus* [9]. *Staphylococcus aureus* produces heat-stable enterotoxins that cause vomiting and nausea. Enteropathogenic *Escherichia coli* strains cause severe diarrhoea among infants and have been identified from a great range of milk products[10,11].

Several studies on application of EOs as antimicrobials have been proceeded to increase the safety & shelf life and sensory quality of food products [4,12]. Essential oils use has been described with the phenol coefficients of a variety of essential oils [13]. Recently, it has been elucidated that thyme oil showed a significant activity against some Gram-positive and Gram-negative bacteria[14]. Cinnamon Essential Oil (EO) and its constituents, which are known to possess several antimicrobial activities[15]. This research has developed towards the use of naturals alternatives due to the unwanted consumer perception against chemicals. The antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant and carcinopreventive actions of EOs has been reported [16,17].

The aim of this study was to evaluate the acceptability of cinnamon and thyme oils white soft cheese to consumers and investigate the antimicrobial activity of these EOs against *S.aureus* and *E.coli* microorganisms.

## Materials and Methods

### Preparation of bacterial strains

Stock cultures of two pathogenic bacterial strains; *Staphylococus aureus* (ATCC43300) and *Escherichia coli* (ATCC35150) were maintained on Nutrient Broth (Oxoid CM0001) at 4°C. Microorganisms inocula were prepared in Nutrient Broth for 24 h at 37°C. Cell suspensions were diluted with Peptone Water (Oxoid CM0009) to provide a count of about $10^9$ CFU/ml for each strain.

### Determination of Minimum Inhibitory Concentration (MIC) according to[18]

The MIC was estimated by the broth dilution method in Brain-Heart infusion broth (Oxoid CM1135). Each EO was first diluted in dimethylsulfoxyde; 40% for Cinnamon and 80% for the thyme oil. Serial dilutions of EOs were carried out with concentrations ranging from 0.25 % to 1%. One milliliter of a *S. aureus* and *E.coli* inoculum ($10^6$ CFU/ml) and one tenth ml of each EO dilution were added to 2.9 ml of Brain Heart infusion broth. After 24 h at 37°C, MIC was determined as the lowest EO concentration inhibiting visible growth of bacteria.

### Cheese preparation

Buffaloe's milk was obtained from a Faculty of Agriculture, Cairo University, Egypt. The fat % of milk was 7.2, which was determined according to [19]. Microbial rennet (Reniplus NG) was provided by Caglio Star, Murcia, Spain, corresponded to a thermolabile enzyme obtained by *Mucor miehei* fermentation. Calcium chloride anhydrous (C1016) and cinnamon oil (W229105) were obtained from Sigma-Aldrich. The thyme oil was obtained from Nubassa Gewurzwerk, Vierenheim-Germany.

White soft cheese was made from previously heated 45 liters buffaloe's milk (75°C for 15 second, cooled to 43°C) as described in[20]. Calcium chloride and rennet were added at the ratio of 0.1% and 0.4 gram / 4 liter respectively, stirred well and the mixture was divided into nine equal portions as follow: **(I)**; control without any essential oils or bacterial strains, **(II)**; *S.aureus* strain only, **(III)**; *E.coli* strain only (count in milk after inoculation about $10^5$ CFU/ml for each), **(IV)**; *S.aureus* with cinnamon oil, **(V)**; *S.aureus* with thyme oil, **(VI)**; *E. coli* with cinnamon oil, **(VII)**; *E.coli* with thyme oil. Two portions of cinnamon and thyme oils were prepared for sensoy evaluation (0.5% for each oil according to MIC results), then set for 2 hours, finally whey drainage. Cheeses from different treatments were stored into tightly closed plastic containers and covering with whey at refrigerator (6±1°C) for four weeks.

### Examination of cheese samples

Cheese samples were taken when fresh and after 1, 2, 3 and 4 weeks for sensory evaluation, *S. aureus* and *E.coli* counting. All experiments were performed in three replicates and mean values were recorded. Samples were sensory evaluated according to the scheme of [21,22] , a panel test of 3 panelists (each sample) of staff members of Food Hygiene & Control Department, Faculty of Veterinary Medicine, University of Cairo. Samples were evaluated for flavor (40 points), body & Texture (40 points), color & appearance (10 points), salts (5 points) and style (5 points).

Ten gram of cheese sample was aseptically transferred with a sterile pipette to 90 ml of diluent 2% sodium citrate (Sigma-Aldrich,W302600) for preparation the cheese homogenate, then 1ml of primary dilution was transferred to nine ml of diluents to obtain decimal serial dilutions [23]. One tenth ml of the prepared decimal dilutions was transferred onto duplicate plates of Baird-Parker medium (Lab M, LAB085) for *S.aureus* and Levine Eosin Methylene Blue Agar (Oxoid, CM0069) for *E.coli*. The plates were incubated at 37°C for 24-48 hours. Typical colonies of *S.aureus* and *E.coli* were counted and recorded according to [24,25]. The analysis of variance (ANOVA) test was conducted to analyze the possible significance ($P \leq 0.05$) between mean values of parameters using Fishers Least Significance Difference (LSD).

## Results

**Table (1): The Minimum Inhibitory Concentrations of the two selected essential oils on pathogenic bacteria.**

| Essential oil | Concentration (%) | *S.aureus* | *E.coli* |
|---|---|---|---|
| Cinnamon | Control* | ++ | ++ |
| | 0.25 | + | + |
| | 0.5 | - | - |
| | 0.75 | - | - |
| | 1.00 | - | - |
| Thyme | Control | ++ | ++ |
| | 0.25 | + | + |
| | 0.5 | - | - |
| | 0.75 | - | - |
| | 1.00 | - | - |

*Essential oils absence., + Growth present., - Growth absent.

**Table (2): Effect of adding EOs on sensory parameters of the examined cheese samples.**

| Treatments | Control (I) | Cinnamon oil | Thyme oil |
|---|---|---|---|
| Items | Storage period (zeroday) | | |
| Flavor (40) | 32 | 35 | 35 |
| Texture (40) | 34 | 35 | 35 |
| Color (10) | 9 | 9 | 9 |
| Salts (5) | 3 | 5 | 4 |
| Style (5) | 3 | 4 | 4 |
| Total (100) | $81^{a}$ | $88^{b}$ | $87^{b}$ |
| | Storage period (1 week) | | |
| Flavor (40) | 36 | 37 | 35 |
| Texture (40) | 33 | 35 | 35 |
| Color (10) | 9 | 9 | 9 |
| Salts (5) | 3 | 4 | 4 |
| Style (5) | 4 | 4 | 4 |
| Total (100) | $85^{a}$ | $89^{a}$ | $87^{a}$ |
| | Storage period (2 weeks) | | |
| Flavor (40) | 36 | 37 | 35 |
| Texture (40) | 34 | 35 | 35 |
| Color (10) | 9 | 9 | 9 |
| Salts (5) | 3 | 4 | 4 |
| Style (5) | 3 | 4 | 4 |
| Total (100) | $85^{a}$ | $89^{a}$ | $87^{a}$ |
| | Storage period (3 weeks) | | |
| Flavor (40) | 36 | 34 | 32 |
| Texture (40) | 34 | 35 | 33 |
| Color (10) | 9 | 9 | 8 |
| Salts (5) | 3 | 4 | 3 |
| Style (5) | 4 | 4 | 3 |
| Total (100) | $86^{a}$ | $86^{a}$ | $79^{b}$ |
| | Storage period (4 weeks) | | |
| Flavor (40) | 35 | 32 | 29 |
| Texture (40) | 35 | 33 | 33 |
| Color (10) | 7 | 7 | 6 |
| Salts (5) | 3 | 4 | 3 |
| Style (5) | 3 | 4 | 3 |
| Total (100) | $83^{a}$ | $80^{a}$ | $74^{b}$ |

Average values with different alphabetical superscripts within row are significantly different at $P<0.05$.

**Table (3): The effect of adding EOs on the bacterial count (log CFU/g) in the different examined portion samples.**

| Storage period | Portion (II) | Portion (IV) | Portion (V) |
|---|---|---|---|
| | ***S.aureus* count** | | |
| zeroday | 3.85[a] | 3.61[a] | 3.68[a] |
| 1 week | 3.39[a] | 3.17[a] | 3.32[a] |
| 2 weeks | 3.23[a] | 2.69[a] | 2[a] |
| 3 weeks | 4.34[a] | 1.69[b] | 2[b] |
| 4 weeks | 3.30[a] | 1.30[b] | 1.30[b] |
| | **Portion (III)** | **Portion (VI)** | **Portion (VII)** |
| | ***E.coli* count** | | |
| zeroday | 5.43[a] | 4.44[a] | 3.84[a] |
| 1 week | 5.23[a] | 4.30[a] | 5.17[a] |
| 2 weeks | 6.04[a] | 4[b] | 4.30[b] |
| 3 weeks | 6[a] | 3.47[b] | 3.30[b] |
| 4 weeks | 7.30[a] | 2.69[b] | 3.30[b] |

Average values with different alphabetical superscripts within row are significantly different at $P<0.05$.

## Discussion

The data illustrated in **(Table 1)** revealed that the Minimum Inhibitory Concentrations (MICs) value of the cinnamon and thyme oils were 0.5% against both selected bacterial strains. [26]recorded the MIC of cinnamon essential oil against some pathogenic bacteria and found that the highest MIC values (0.5%) were obtained for *S. aureus* and *E. coli*. The MICs of thyme oil for *E. coli* and *S. aureus* were in line with the values reported by[27]. Results of *E.coli* obtained for both oils MIC values are inaccordance to this recorded by [28], while lower figures of cinnamon and thyme oils MIC for *S. aureus* were recorded (0.04% & 0.03%) by the same investigators respectively. Lower result of *S.aureus* for thyme oil was obtained by [29], also higher result (1.56 %) of *E.coli* was obtained by the same researchers.

In **(Table 2)**; a higher flavor score was obtained in cinnamon and thyme oil cheese samples at zeroday. At first and second week of storage, a higher flavor score than control samples was obtained in cinnamon oil cheese samples, while a lower flavor score was recorded in thyme oil cheese samples from first to third week of storage with slightly almond (bitter) flavor in fourth week. Addition of EOs, produced no significant effect on the average texture score. The total score of cheese samples was significant ($P<0.05$) increase at zeroday with cinnamon and thyme oils. There was a significant ($P<0.05$) decrease in cheese samples score with thyme oil as compared to cinnamon oil cheese and **(I)** portion of cheese samples at 3 and 4 weeks of storage. A higher sensory score treatment was recorded in cheese samples with cinnamon oil at first two week of storage compared to control and thyme oil treatment, while the lowest score was reported in case of thyme oil cheese samples at 4 weeks (74). White cheese fortified with essential oils had softer texture than control; as the presence of EOs in cheese enhanced the enzymatic activity, so produced softer texture[30].

In the recent years, many EOs possess antimicrobial activity has been proved by investigations. The type and ideal concentration of EO depend on the product used and against which species of pathogen it is to be used. But if EOs are expected to be widely applied as antibacterial, the sensorial impact should be considered as the use of EOs can alter the taste of food or exceed acceptable flavour thresholds. Therefore, research in this area should be focused on the optimization of EO uses to obtain optimal antimicrobial activity at sufficiently low concentrations and not to adversely influence the organoleptic acceptability of the cheese [31]. A alternative is to try to use some of the most active components, rather than the whole oil. This would reduce changes to organoleptic properties and keeping antimicrobial activity[32].

**Table (3)** indicated that *S.aureus* has a high ability for survival in control cheese samples, while *E.coli* could grow and survive to reach 7.30 log CFU/g. *S.aureus* count of cinnamon and thyme oils cheese samples significantly decreased ($P<0.05$) through 3 and 4 weeks of storage. *E.coli* count of cinnamon and thyme oils cheese samples significantly decreased ($P<0.05$) at 2nd, 3rd and 4th weeks of storage. This suggests that the added cinnamon and thyme oils had antimicrobial effect on *S.aureus* and *E.coli*. Cinnamon oil and thyme oil had the non significant decreased ($P>0.05$) effect on *S.aureus* and *E.coli* counts at zeroday and 1st week of

storage. Statistical analysis revealed that, there is a significant differences ($P<0.05$) in *S.aureus* and *E.coli* count as affected by added EOs.

The use EOs at higher concentrations in food than in vitro may be due to the more complex growth environment in food, which protects microbial cells from antimicrobial substances. [33]suggested that the fat in food could form a protective coat (serving as barrier) around bacteria. These researchers also suggested that the lipid portion of food absorbs the antimicrobial substance, thus decreasing the concentration in the aqueous phase and its bactericidal action. Also the reduced water content in food compared to laboratory media could prevent the transfer of antimicrobial agents in the cellular pathogens. The effect of fat % appeared to be most pronounced with thyme oil, which was shown to have a very weak inhibitory effect against *S. enteritidis* in full fat cheese [32,12]. The significant increased antimicrobial action of EOs was recorded at advanced storage periods may be attributed to lipolysis of triglycerides in cheese.

Gram-negative bacteria cell wall is more resistant (greater intricacy of the double membrane-containing cell envelope) to the EOs. The *E.coli* cell wall does not permit for the entrance of hydrophobic molecules as readily as *S.aureus*; thus, EOs are less able to affect the cellular growth of the Gram-negative bacteria (*E.coli*). The mechanisms of action of the EOs include the degradation of the cellular wall, damaging cytoplasmic membrane, destruction membrane proteins, decreased adenosine triphosphate synthesis, increased permeability leading to ions leakage, other cellular contents and death[4, 34-36].

The action rank of EO components is as follows: phenols (in cinnamon and thyme oils) > aldehydes (in cinnamon oil) > alcohols (in cinnamon and thyme oils) > hydrocarbons (in thyme oil)[37]. [38] concluded that the EO mechanism of action against *Staphylococcus aureus* were the leakage of the intracellular potassium ($K^+$) ion from cells of bacteria and a significant reduction in metabolic activity.

Thyme oil is proven to be highly effective against food borne pathogens, including *E. coli* and *S. aureus* [28]. [39] recorded bacteriostatic and bactericidal activities of thyme oil against *E. coli* O157:H7. [32] reported thyme oil as an effective inhibitor of pathogenic microorganisms in soft cheese. Antibacterial characters of thyme oil against *S. aureus* and *E. coli* was reported by[40-43].

[44] found that the *S. aureus* and *E. coli* were sensitive to thyme oil and showed a significant bactericidal effect. A number of essential oils have been registered by European Commission and FDA for their use in food to control pathogens, which means that FDA has classified these EOs as Generally Recognized As Safe (GRAS) [45].

## Conclusion

The adding of the investigated EOs to white soft cheese had a higher significant and non significant effect on organoleptic quality at zeroday and first two weeks respectively compared to control samples. Lower significant effect with thyme oil at 3 and 4 weeks of storage was recorded. A significant antimicrobial effect for both oils on selected pathogenic microorganisms was noticed at 3& 4 weeks of storage , also these EOs can be used as natural preservative agents for maximizing safety and extending the shelf life of white soft cheese throughout storage periods. The data in this study will serve as assistance information for cheese processing.

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