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Using Some Essential Oils and Chitosan Nanoparticles to Preserve Soft Cheese

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Abstract

OUR CURRENT study focused on utilizing natural preservatives as essential oils (clove, thyme, and rosemary) and EOs-chitosan nanoparticles (NPs) that exhibit antimicrobial effects against several bacteria, yeast, and mold). The electrical charge and particle size of NPs were determined. The EOs and EO-chitosan NPs antimicrobial action against some spoilage, pathogenic bacteria, and fungi were detected for MIC, MBC, and MFC. Then, EOs and EOs-chitosan NPs were inoculated separately in examined soft cheese samples. They were analyzed for titraTable acidity (T.A%), microbiological examination, and sensory evaluation at production time and weekly until signs of spoilage were detected. The EO-chitosan NPs showed higher antimicrobial activity than crude forms, especially clove-chitosan NPs recorded highest, followed by thyme and rosemary EO-chitosan NPs, clove EO was recorded lowest MIC (0.1 mg/mL) as relative to other crude EOs while (0.01 mg/mL) for clove and thyme-chitosan NPs recorded lowest as relative to others. TitraTable acidity increased gradually during storage time. Total coliform, aerobic spore formers, psychotropics, and *listeria monocytogenes* could not be found in every examined sample. The cheese group fortified with rosemary-chitosan NPs at 0.04% showed highest sensory scores with extending cheese shelf life up to 84 days, while cheese groups fortified with clove-chitosan NPs at 0.04% extended their shelf life up to 105 days with accepted organoleptic scores. unlike control and crude oil-fortified cheese groups, EOs-chitosan NPs showed better antimicrobial activity than crude ones and could be applied for soft cheese preservation and extending shelf life without affecting sensory quality.

Keywords: Antibacterial, Antifungal, EOs-chitosan NP, Shelf life, Essential oils.

Introduction

Fungal growth is common in cheese due to its high protein, low pH, salt, and moisture content, and ripening conditions. Penicillium and Aspergillus species are the main contaminants [1]. Fungal spoilage can degrade cheese quality, causing off odors, abnormal colors, and off-flavors. Some fungi produce mycotoxins, a food safety concern. Many fungi can produce toxins harmful to humans and animals[2].

Plant phenolic compounds can suppress the proliferation of several food-borne and food-spoilage microorganisms classi-fied as GRAS (Generally Recognized As Safe) substances [3]. Consumers are increasingly wary of chemical preservatives. Food producers seek natural alternatives like essential oils, which are GRAS-certified and possess antibacterial, antifungal, and antioxidant properties [4].

Essential oils can inhibit the growth of foodborne pathogens like Listeria, *E. coli*, Bacillus, Salmonella, and Aspergillus. Their antimicrobial activity depends on their chemical structure and functional groups [5,6].

Clove is a food flavoring and preservative with antibacterial, antifungal, and antioxidant properties [7]. Thyme is known for its antifungal, antioxidant, and antibacterial qualities. Carvacrol (55–85%) and thymol (0–10%), which make up most of the blend of more than 30 chemicals that make up thyme EO, have the most antibacterial activity due to their phenolic makeup [8, 9]. It has less efficacy against Gram-positive Lactobacillus and Bifidobacterium but is more efficient against Gram-negative bacteria.[10].

Rosemary is a naturally occurring antimicrobial [11]. It was used to inhibit *L. monocytogenes* in mozzarella cheese. It also neutralized free radicals and prevented lipid oxidation [12]. EOs have been shown to have antimicrobial properties and extend food shelf life [13].

The antimicrobial effect of essential oils is limited by their volatility and low water solubility such as eugenol [14]. To overcome these obstacles,

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Nano-encapsulation can protect essential oils from evaporation and oxidation [15], Nano-encapsulation can extend the activity of encapsulated compounds, improve the bioavailability and water solubility of lipophilic compounds, and enhance the stability and antimicrobial activity of unsTable compounds during food processing and storage [16, 17]. Ionic gelation is a simple, eco-friendly method for producing sTable chitosan nanoparticles, ideal for encapsulating sensitive bioactive compounds like essential oils [18].

As a result, the food industry faces a growing problem in developing customer confidence in using nano-food ingredients. As a result, such work aimed to assess the in vitro antimicrobial action of clove, thyme, and rosemary essential oils and their related EOs-chitosan NPs. Furthermore, EOs-chitosan NPs were created to assess their impact on the quality of laboratory-produced soft cheese. Soft cheese's chemical, microbiological, and sensory qualities were evaluated on zero-day storage and then weekly until signs of spoilage appeared.

Material and Methods

Essential oils

Clove, thyme, and rosemary essential oils (pure, 100% concentration) were obtained from the National Research Center, Doki, Cairo, Egypt. Such oils were kept in the refrigerator in a brown glass vial.

Preparation of essential oils-chitosan nanoparticles

Essential oils-Chitosan NPs were made following the instructions for the ionic gelation process by Piras et al. [19]. For preparing clove, thyme, and rosemary-chitosan NPs, 5 mL of each separate oil was taken and diversified with 4 mL of TPP solution.

Determination of zeta potential nanoparticles

The charge on the dispersed aqueous layer is known as the zeta potential, which forms on a nanoparticle's surface when retained in water. The zeta potential was determined via NANOTRAC Zetasizer wave II, USA**.**

Characterization of the nanoparticles

The size of the particle and related measurement indices for NPs were evaluated via dynamic light scattering (DLS) instrument, Microtrac Zetasizer wave II, USA.

Antimicrobial activity of essential oils and their nanoparticles:

Antibacterial activity of essential oils and associated eos-chitosan nps by agar well diffusion

The indicator pathogenic bacteria, *Bacillus subtilis* (Gram-positive) and *E. coli* (Gram-negative), were obtained from the Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Egypt.

Activation of indicator bacterial strains

Indicator strains *B. subtilis* (*Bacillus subtilis)* and *E. coli* (*Esherechia coli*) were activated on Tryptone Soya Broth (TSB). Serial dilution was then made for the microorganism until the concentration of $7\log_{10}$ CFU/mL as performed by ahmed et al. [20]**.**

Antibacterial Activity of essential oils and their NPs was performed by Agar Well Diffusion Assay [21]. Clove, thyme, rosemary, and associated EOs-Chitosan NPs, the phytochemical component dispersed throughout the agar, inhibiting the microbial strain's growth. Following a 24-hour incubation period, the diameter of the inhibition zone was measured in mm [22]. The experiment was performed 3 times.

Typically, it declared that an agent with antimicrobial properties assessed by the disc diffusion technique or agar that posed a halo of inhibition bigger than 13 mm is active [23]**.**

Antifungal activity of essential oils and associated eos-chitosan nps in broth activation of indicator fungal strains

The indicator pathogenic mold, *A. flavus* (*Aspergillus flavus*) (MT645073.1), was purchased from the Food Hygiene Department, Animal Health Research Institute, Egypt. Moreover, spoilage yeast, *Goetricum candidum* (AUMC 226), was purchased from the Mycology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

The indicator pathogenic mold *A. flavus* and spoilage yeast, *Goetricum candidum,* inoculated on glucose peptone yeast extract broth. Serial dilution was then made for the microorganism until the concentration of $6\log_{10}$ CFU/mL as performed by Guichard and Bonnarme [24]**.**

Antifungal activity was performed using the tube method and was tested following the procedure described by Ibrahim et al. [25] for the antibacterial test. The reduction % was calculated according to Harikumar [26].

Cheese manufacture

It has been performed by the method described by Scott et al. [27]. cheese groups were classified into (19 groups):

Sensory evaluation of cheese samples

Eos effects and their NPs on sensory properties of fortified fresh soft cheese were judged based on the approach described by Ahmed [28]. The maximum attainable scores were 60 points out of 100 for flavor, 30 for body and texture, and 10 points out of 100 for appearance. Ten panelists of the staff members at Food Hygiene and Control Department,

Faculty of Veterinary Medicine, Benha University carried out this.

Chemical examination of cheese samples

TitraTable acidity of cheese samples as stated by Fox et al. [29]. The acidity was calculated as lactic acid using a titration technique with 1/9N NaOH.

Microbiological evaluation of examined soft cheese.

Preparation of cheese sample:

In a stomacher for 1 minute, 10 g of every representative sample was aseptically mixed in 90 mL of a sterile 2% sodium citrate solution. Decimal dilutions were prepared in 0.1% sterile peptone water, and appropriate dilutions were employed to count the subsequent dilutions, as expressed by Roberts and Greenwood [30]**.**

Determination of coliform count

As stated by the approach illustrated by Wehr and Frank [31], it was done by serial using Violet Red Bile Agar at 37°C for 48 days.

Determination of total aerobic spore former bacteria count

It was performed according to Wehr and Frank [31] via dextrose tryptone agar medium at 32°C for 72 hours.

Determination of lesteria monocytogenes count.

After preparation of the enrichment culture, the Plates were plated on Oxford Agar medium and checked for characteristic Listeria colonies after 48 hours of incubation at 37°C [32].

Determination of psychrotrophic bacteria

The sample was diluted and plated as in the standard plate count (SPC) for 10 days, and the plates were incubated at 7 ± 1 °C [33].

Total yeasts and molds count

Using the serial dilutions that have already been made, duplicate apparent cultures of Sabaroud Dextrose Agar (SDA) supplemented with chloramphenicol (0.01%) were inoculated using 1 mL of every dilution, and incubation of inoculated plates for 3 to 5 days at 25 °C before being examined [34].

Statistical analysis

The statistical testing was made via Two-Way ANOVA utilizing SPSS, ver. 27 (IBM Corp. Released 2013). Data were treated as a complete randomization model according to Steel et al. [35]. Multiple comparisons were performed using the Duncan test, and the significance level was < 0.05. The experiment was done 3 times, and the findings were the mean ± Standard error.

Results and Discussion

Cheese spoilage is one of the greatest significant obstacles challenging the food sector. Furthermore, more successful preservation techniques based on numerous components are required to limit food contamination and deterioration due to the growth of resistance to most synthetic antibacterial agents. Due to their complex character, which involves the interaction of many modes of action and synergistic interactions to avoid the promotion of germ resistance, using EOs in the food industry is growing [36].

Food safety is a growing public health involvement since food-borne illnesses are becoming more prevalent. In recent years, a noticeable attempt has been made to use natural antimicrobials, which block bacterial and fungal growth, to improve food product quality and safety. To reduce the unfavorable taste of EOs, disperse EOs throughout the food matrix, and boost EO solubility and antibacterial activity, EOs-chitosan NPs must be prepared. Furthermore, researchers have drawn attention to the claim that EO encapsulation is a green option for food safety [37].

The electrostatic or charge repulsion/attraction among particles is expressed by the zeta potential, which is a most critical influence in facts like dispersion, flocculation, or aggregation and, hence, a key parameter for assessing the stability of dispersions, emulsions, plus suspensions [38].

In the current study, clove EO-chitosan NPs (the most significant value of zeta potential) had a Positive charge (37.3 mV), while the lowest value of zeta potential was recorded in thyme EO-chitosan NPs (Positive charge (26.0 mV) are documented in (Table 1).

Positively charged NPs give higher antibacterial activity, as described by Chandrasekaran et al. [39] can attach to negatively charged bacteria, producing membrane degradation, enhanced membrane permeability, osmotic leakage, and eventually death of bacterial cells.

A study by Hasani et al. [40] found that the zeta potential values for chitosan-Hicap Nanocapsules with lemon EO varied from +10.58 to +44.23 mV. In another study, chitosan NPs and cinnamon EO-chitosan NPs had positively charged surfaces due to amine groups; the zeta potential values were +24.0 mV and +26 to +30.5 mV, respectively. In contrast, chitosan NPs loaded with diverse levels of Summer savory EO showed negative zeta potential values ranging from -7.54 to -21.12 mV, with a substantial decline found as EO concentration increased [41]. These findings suggested that EO and NPs interactions affect the charge and structure of these nanocarriers.

The results of measuring the particle size of EOs-chitosan NPs produced using the ionotropic gelation technique showed the prepared particles 250, 579, and 383 nm size for clove, thyme, and rosemary EO-chitosan NPs, respectively. Such results agreed with the nanoscale structures of the nanocapsules. Low polydispersity index (PDI) values for nanocapsules (0.0923, 0.02612, 0.1930) for clove, thyme, and rosemary EO-chitosan NPs (Fig. 1), which was lower than 0.5, NPs have a limited size distribution, and monodisperse nano-systems can be found in aqueous solution (well homogenous solution).

Higher antibacterial activity was associated with smaller NP sizes, which resulted in a considerable shift in physical attributes compared to their original counterparts. Chitosan NPs share traits of chitosan and essential qualities of NPs, such as tiny size, enhanced surface area, and significant size impacts, resulting in easy cell wall penetration [42].

Jawad Al-Mowsowy and Ahmed salih [43] showed that ultrasound waves influence the lowering of the size of nanoparticles due to the physical bonding between them.

Clove has a high eugenol content (the primary constituent [70% to 90%]). It showed a potent antimicrobial activity through protein denaturation and interaction with cell membrane phospholipids, modifying their permeability with consequent suppression of many Gram-negative as *E. coli,* Gram-positive bacteria such as *S. aureus* and diverse sorts of yeast [44]. Furthermore, the antimicrobial action of thyme oil can be related to its primary components, thymol, and carvacrol, which create functional and structural damage to the cytoplasmic membrane [45].

The results proved that clove, thyme, rosemary essential oils, and their associated EOs-chitosan NPs demonstrated good antibacterial action against the tested pathogens. The results showed inhibitory zones with diameters ranging from 18 to 30 mm (NPs recording higher diameter (Clove-chitosan NPs the highest) against the strains tested, with MIC values of 0.01% and 0.03% for crude essential oils (clove and thyme EO respectively), 0.06% for crude rosemary EO and 0.001% for essential oils-chitosan NPs (clove and thyme EOs) and 0.006% for rosemary chitosan-NPs with various levels of microbial sensitivity (Fig. 2). Variations in the cell wall of bacteria composition could cause this. For example, the Gram-positive bacterial cell wall comprises 90–95% peptidoglycan, enabling hydrophobic compounds to enter the cell and affect both the cell wall and the cytoplasm. Gram-negative bacteria, however, have more complex cell walls. Gram-negative bacteria are extra resistant to EOs and other natural antimicrobial chemicals because their peptidoglycan layer is 2-3 nm thicker than Gram-positive bacteria and is enclosed by an outer membrane containing numerous proteins and lipopolysaccharides (LPS) [46].

Smith-Palmer et al. [47] proved that clove had antibaterial effect versus *L. monocytogenes* and *S. enteritidis* in tryptone soya broth and cheese, which was attributed to the large amount of eugenol found in clove EO that oversaw its significant antimicrobial and biological properties. It is widely recognized that both eugenol and clove EO phenolic components can denature proteins and counter with phospholipids on cell membranes, affecting their permeability and suppressing a large variety of Gram-negative and Gram-positive bacteria in addition to several types of yeast [48].

Carvacrol and thymol's antimicrobial mechanism (major constituents in thyme) can destroy bacterial cells by altering the cell membrane permeability, resulting in cell component leakage [9].

Rosemary sensitizes the pathogenic microorganism's cell membrane and increases its permeability, leaking essential cellular elements. Consequently, the bacterial enzyme system and cell respiration are influenced [12].

The EOs nanoencapsulation demonstrated a significant ability to increase crude EO's antimicrobial capacity versus food-borne bacteria. These findings agreed with the results of Esmaeili and Asgari [49] who showed an enhancement in the antibacterial action of encapsulated Carum copticum essential oil against two of the most significant food-borne pathogens (*S. aureus* and *E. coli*).

Also, the minimum inhibitory concentration (MIC) for EOs-chitosan NPs was lower than in crude form against the tested bacteria (Fig. 2). It is known that nano-sized particles can penetrate the cell wall of bacteria and damage the cell membrane [50]. They are showing a greater antibacterial capability than larger particles [48]. This antibacterial activity can be related to mechanisms such as enhancing bacterial cell membrane permeability and substantial depolarization by chitosan [51].

The current results agreed with those of Feyzioglu and Tornuk [52] who examined the antibacterial action of Summer savory (Satureja hortensis L) EO loaded into chitosan NPs. The mode of action of antibacterial activity in EOs has not been linked to a particular component or mode of action. The EOs hydrophobicity is a critical feature that allows them to break down the bacterial cell membrane lipid, releasingons and other cell components and, hence, cell death [53].

In contrast, a higher MIC value of thyme oil versus *E. coli* was recorded as 0.5% by Ayah and Saad [54], in addition to Carvalho et al. [55] who performed superior MIC of 0.78, 1.56, and 0.78% versus *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213, respectively.

These results were congruent with Carvalho et al. [55] who evidenced the suppressive action of clove oil versus food-borne Gram-positive bacteria (*S. aureus*, *E. faecalis,* and *L. monocytogenes*), Gram-negative bacteria (verotoxin-producing *E. coli* and *Pseudomonas Saeruginosa*).

Also, the current results agreed with that of El-Sayed and El-Sayed [56], who performed that the inclusion of Thyme EO displayed great inhibitory influences against several pathogenic bacteria, with inhibition zone diameter varying from 10 to 36 depending on the concentration of thyme EO.

Essential oils have antifungal activities almost entirely owing to their direct interaction with the fungus. EOs enter and change the fungal cell wall plus cytoplasmic membranes via a permeabilization route, breaking down the membranes of the mitochondria [57].

Fungi are extensively spread in nature and can degrade foods such as cheese since they have a stronger resilience to intrinsic food components [58]. Thyme oil demonstrated good antifungal action, which was approximately consistent with what was reported by Puškárová et al. [59].

The EOs-chitosan NPs had the strongest antifungal activity against tested fungi compared with crude EOs. Clove-chitosan NPs showed the highest fungicidal and fungistatic effect, followed by thyme-chitosan NPs and rosemary-chitosan NPs (Fig. 3).

Clove EOs recorded an minimum fungicidal concentration (MFC) of 0.05% while thyme and rosemary EOs 0.1%; clove-chitosan NPs recorded an MFC of 0.01 while thyme-chitosan NPs and and rosemary-chitosan NPs 0.03%. Also, our results in (Fig. 3) showed that the reduction % of *A. flavus* was higher than that recorded in *Goetricum candidum* by the effect of crude EOs and EOs-chitosan NPs.

These findings demonstrate the EO encapsulation efficacy within a nanoscale system. The Nanocapsules could keep essential oils against degradation events and increase the transport and diffusion processes of essential oils via the cell membrane [60].

These results were congruent with Carvalho et al. [55] who demonstrated the inhibitory action of clove oil against Aspergillus and Candida fungus. Furthermore, our acquired results agreed with those of Kapustová et al. [61] who performed that Essential Oil Nano Capsules suppress the growth of tested fungi at MIC values varying from 0.125 to 0.25 mg/mL. These concentrations were 2 to 4 times lower than those in crude EOs. For both EO-NCs, an MIC of 0.125 mg/mL was potent for reducing the growth of *Aspergillus fumigatus*, *cladosporium* aggregatocicatricatum, *cladosporium* herbarum, and *Pleurotus eryngii*. Also, this concentration for Origanum vulgare Nano Capsules (OV-NCs) was sufficient to suppress the fungus.

In addition, our current results came in alignment with that of Kapustová et al. [61] who reported that MIC and MFC for thyme EO and thyme EO NPs were 0.25 and 0.5 mg/ mL for *Aspergillus flavus,* respectively, in addition to 0.5 and 1 mg/mL for *Goetricum candidum*. Also, Hasheminejad et al. [62] informed that Clove EO encapsulated in chitosan NPs had much higher antifungal action against *A. niger* than unloaded chitosan NPs and free clove EO.

The findings were in accordance with those of Da Silva and Cabral-Albuquerque [63] who evaluated the antifungal and anti-afla toxigenic action of rosemary EO against *A. flavus*. (MIC) Moreover, (MFC) were both 500 µg/mL. At a 250 µg/mL concentration, rosemary EO inhibited *A. flavus* mycelial development by 15.3%. The findings acquired from scanning electron microscopy (SEM) displayed a drop in the size of conidiophores and the thickness of hyphae in *A. flavus* produced by treatment with rosemary EO (250 µg/mL). Ergosterol generation and mycelium biomass decreased as the rosemary EO treatment concentration rose. The results showed that rosemary EO can be used as a substitute for synthetical fungicides.

In addition, the present results were in alignment with those obtained by Yuan et al. [64] who found that Rosemary EO could suppress the growth of *Colletotrichum gloeosporioides* fungi with MIC and MFC concentrations of 15.625 µL/mL and 31.25 µL/mL, respectively.

The main ingredients, thymol and carvacrol, were generally responsible for the growth-inhibiting effects; however, the bioactive monoterpene hydrocarbons, p-cymene, and γ-terpinene, which exist in both EOs in non-negligible amounts, may have made a significant influence on the antifungal qualities [65].

Chitosan nanoparticle-encapsulated clove prevented the growth of *A. niger*, isolated from destroyed pomegranate, at a concentration of 1.5 mg/mL [66, 67]. As an alternative against *A. flavus*, Ismael and Hadi [68] informed us that the MIC value of 300 mg/L was achieved using a chitosan benzoic acid nanogel with thyme essential oil under conserved restrictions. Also, Devecioglu et al. [69] proved that the lowest MFC value was acquired from the findings of clove at a concentration of 1000 µg/mL.

In the same context, the current results were nearly agreed with those of Yuan et al. [64] who demonstrated how rosemary could hinder the growth of *Colletotrichum gloeosporioides* with minimum inhibitory (MIC) and fungicidal (MFC)

concentrations of 15.625 µL/mL and 31.25 µL/mL, respectively. Moreover, clove essential oil had strong antifungal activities with a MIC value of 1000 µg/mL against *A. flavus* DDS6, and its highest MIC value was 1500 µg/mL for *Aspergillus niger* DDS7.

The sensory characteristics for the soft cheese treatments (the control in addition to inoculated samples) were displayed in (Fig. 4).

The acquired results indicated no significant difference in appearance scores during the storage time of the skimmed white soft cheese treatment samples in addition to the control. In contrast, there was a significant difference between the control and other inoculated groups throughout storage time from the 7th to the 35th day (Fig. 4).

The current results agreed with El-Sayed and El-Sayed [70] and found no significant appearance changes between diverse preserved cheese treatments by nanoemulsion of cumin EO throughout the storage period.

The storage duration had a statistically significant impact on the flavor score across the control and treatment groups. Additionally, a statistically significant correlation was noted between the various fortified cheese samples and EOs during the storage period (Fig. 5).

The acquired results indicated a significant difference in flavor scores with exceeding storage time for the skimmed white soft cheese treatment samples and the control. The control group was found to retain the highest flavor score for up to 21 days compared to other inoculated groups, and then it decreased until signs of spoilage appeared.

Also, there was a significant difference across the control and other inoculated groups throughout storage time. The group inoculated with 0.2% clove EO recorded the lowest flavor score compared to other inoculated and control groups till the end of storage time up to 84 days. In contrast, the 0.01% rosemary NPs containing group recorded the highest flavor score compared to other inoculated groups till the end of storage time up to 77 days (Fig. 5).

In addition, the current results showed that samples containing low-concentration EOs, and EOs-chitosan NPs recorded higher scores than high-concentration crude EOs (Fig. 5); this may be due to nanoencapsulation technique covering the undesirable flavor of crude EOs.

Unfortunately, EOs are lipophilic substances readily broken down by temperature, moisture, light, and oxygen. One workable solution to get around these challenges is nanoencapsulation. Using this technique, essential oils could be shielded from the effects of heat and light, making them more soluble in water, reducing their flavor, and enhancing their bioavailability and accessibility [71].

The current results were in agreed with those of Momeni Sarvestani and Lashkari [72] who stated that the shelf-life duration of concentrated yogurt could be extended by adding essential oils of clove and rosemary (6 weeks at 5 ± 1°C) with respecTable texture, flavor and taste; however, the strong flavors that the clove and rosemary oils imparted did not sit well with some panelists.

In addition, these results were in alignment with those of Kavas et al. [73] who established that kashar cheese samples coated with an edible film containing WPI-based thyme essential oil (WPIOF) and edible film having WPI-based clove essential oil (WPISF) were recorded to be advanced titraTable acidity than others including the control sample during storage. This was related to certain components found in the formulation of the clove and thyme EOs.

Ismael and Hadi [68] revealed that the chemical, microbiological, and sensory characteristics of Iraqi soft white cheese are significantly influenced by the essential oils of thyme and rosemary. These oils improve sensory qualities, especially overall acceptability, and lower the microbiological load of the cheese, which helps it last longer in the refrigerator. Treatment (treated with 10 mL/kg) displayed the best maintenance of both microbiological and sensory characteristics throughout 21 days at 1 ± 5°C of cooled storage period.

On the other hand, Ayah and Saad [54] found that evaluated white soft cheese fortified with thyme oil received an overall acceptability score of B until the 2nd weekend, then C in the 3rd and 4th weeks. The flavor, which had a slightly bitter almond flavor, received a taste score of 35 out of 40 until the end of the 2nd week, 32 in the 3rd week, and 29 in the 4th week of storage.

The results indicated that the control group recorded a higher score (29.33) at zero days, then decreased gradually till it reached 25 scores up to 35 days. Generally, body and texture decreased gradually with extending storage periods in all groups. There were no significant differences among inoculated groups in addition to the control group up to 21 days, but there were significant differences between the control group and other inoculated groups at the 28th and 35th day of storage (Fig. 6).

The current results aligned with those of El-Sayed and El-Sayed [70], who found no significant changes in body and texture between different preserved cheese treatments by cumin EO nanoemulsion during storage. In addition, our results agreed with those of El-Sayed and El-Sayed [56] who found that body and texture varied slightly between all the treatments and throughout the storage period of labneh preserved by 0.1, 0.2, 0.3% thyme EO nanoemulsion after eight weeks of storage and This could reflect how total solids and acidity

content influence the labneh body and texture as useful variables.

Therefore, compared to cheese samples of the control group produced and kept under the same conditions, the inclusion of lower quantities of EOs into cheese seemed to increase general acceptability as well as sensory qualities, notably the flavor score, somewhat. Some research suggested using EOs sparingly due to their impact on the organoleptic qualities of cheese, particularly flavor [75].

In addition, these results agreed with those of El-Sayed and El-Sayed [70] who observed that the lowest total scores were recorded to cheeses well-preserved with elevated levels of Cumin EO nanoemulsion solution (0.1%), also found that the greatest flavor scores were recorded to white soft cheese kept by cumin EO nanoemulsion (0.5%) throughout 60 days of storage.

TitraTable acidity is crucial because it encourages syneresis or the evacuation of moisture from the cheese curd. This process significantly impacts the cheese composition, particularly its moisture content. Additionally, lactic acid affects the activity of enzymes throughout the ripening process, which impacts the flavor and quality of cheese. Lastly, acidity aids in limiting or stopping the development of spoilage or pathogenic microbes [76].

Values change in the TA of various skimmed soft cheese samples throughout storage (TABLE 2). As storage extended, there was a significant increase in the titraTable acidity results gradually for each sample evaluated.

TitraTable acidity may have increased after storing due to non-pathogenic microorganisms like lactic acid bacteria that were not removed through pasteurization processes. The lactic acid bacteria's development and reproduction of the concentration of phosphate ions increases their metabolic efficiency; otherwise, the buildup of lactate or further organic acids could have been produced [68].

The TA of all cheeses increased progressively during the storage period (Table 2). Fresh cheese of the control group had the lowest TA (0.60%), while the rosemary 0.2% group had the highest value of TA either when fresh or stored for up to 63 days (0.65 and 1.27%, respectively) (Table 2). This could be attributed to the inhibition of the growth of cheese microflora by adding essential oils, particularly at the extra concentrations. These findings were agreed with those of Abd-alla et al. [77]. Statistically, TA was significantly influenced by treatments, the pickling period, and the interaction between treatments and the pickling period ($P<0.05$). Adding inoculated material to cheese milk, especially at high concentrations, increased the resultant cheese's TA values compared to the control group (TABLE 2).

The coliform bacteria logarithmic numbers of the control and inoculated samples could not be detected during storage (Data not shown).

The current results agreed with those obtained by El-Kholy et al. [78] who could not find those coliform bacteria in any of the cheese samples used in the experiment. This might also prove the antimicrobial action of EOs tested in the current work. Among ingredients, phenolic composites were the effective antimicrobial substances in the essential oil extract.

Instead, the obtained results disagreed with that of Ismael and Hadi [68] who detected those coliform bacteria logarithmic numbers of the control sample at the 0, 7, 14, and 21 days were 1.75, 3.54, 5.41, and 6.75 CFU /g of cheese, respectively, Rising with the length of storage. While the cheese samples containing thyme or rosemary oil in the storage periods of 0, 7, 14, and 21 days reached (1.4, 1.48, 2.10, 2.88) and (1.54, 1.76, 2.88, 3.31) CFU /g of cheese, respectively, at the same time, the cheese treatment with lemongrass oil could not exhibit any coliform progress during the period.

In addition, Metry et al. [79] found all fresh cheese samples prepared from goats' milk with or without spice cardamom, thyme, and clove EOs contained coliforms; the numbers ranged between 0.14 to 0.45 x 10^2 cfu/gm cheese. This could be attributed to recontamination during the manufacturing processes. On the other hand, coliforms were not noticed in cheese made from buffalo milk, either fresh or during the pickling.

The present study showed that aerobic spore former failed to be detected in all treatments, including the control group (Data not shown). These results were constant with Hanson et al. [80] who informed that there was no finding of bacillus in the examined milk samples heat treated at 82˚C for 30 min, but bacillus existed in samples after 14 days of storage, proving that the time needed for damage and recuperation came before growth.

The current investigation revealed that *L. monocytogenes*, including the control group, could not be found in any treatments (Data not shown). Such results were in agreed with those of Polat Yemiş et al. [81] who found that nanoemulsion coatings having 1.0% and 2.0% myrtle essential oil decreased the population of *L. monocytogenes* in cheese throughout the storing by 0.42 and 0.88 $\log_{10}$ cfu/g, respectively. In addition, Elsherif and Talaat Al-Shrief [82] who proved that nanoemulsions of carvacrol, clove, and cumin EOs significantly lowered the inoculated pathogens counts from 8.2

log_{10}cfu/g to 1.5 log_{10}cfu/g after 2 to 3 weeks related to EOs, which dropped them after 4 to 5 weeks.

El-Sayed and El-Sayed [56] concluded that the inclusion of thyme exposed high inhibitory influences against *L. monocytogenes* in which inhibition zone diameter varied from 10 to 36 according to diverse concentrations of thyme (0.1: 3.0% concentration).

The current study found that Psychrophilic failed to be detected in all treatments, including the control group (Data not shown); these results were agreed with Jawad Al-Mowsowy and Ahmed salih [43] who proven complete eradication of Psychrophilic bacteria in treatments m3 and m4, which added 0.5 and 1% from (Nigella Sativa oil particles was prepared by encapsulating oil with chitosan nanoparticle) (NSP) to their curd.

The current results disagreed with those of Metry et al. [79] who detected that Psychrophilic in buffalos' and goats' white soft cheese untreated and treated with cardamom, thyme, and clove essential oils at different concentrations throughout the pickling period at 6±1°C and there were no clear differences among treatments at zero time. In addition, Psychrophilic in all treated cheese samples significantly ($P \leq 0.05$) decreased as the pickling period extended up to 30 days after that increased at the end of the pickling process.

Total yeast and mold are represented in (TABLE 3). They could not be detected in the control group throughout the storage period of up to 7 days, while the group that contained (0.04 clove EOs-chitosan NPs) could not detected for up to 21 days. They started to appear on the 24^{th} and then increased gradually till spoilage appeared up to 105 days. There was a significant difference between each crude oil-associated Nanoparticles. This may be the protective role, and the synergistic effect of chitosan is used in the encapsulation technique.

Total yeast and mold in the group inoculated with clove EOs-chitosan NPs 0.02% could not be detected up to 21 days, then appear at 24^{th}, then increase gradually till spoilage up to 98 days. They could not be detected in groups inoculated with clove EOs-chitosan NPs (0.02 and 0.01) % and thyme EOs-chitosan NPs (0.04 and 0.02) % were not detected up to 21 days then started to appear at 24^{th} then increased gradually till spoilage appeared up to up to 105 days, up to 98 days and up to 91 days for clove EOs-chitosan NPs (0.04, 0.02 and 0.01) % respectively. In addition, up to 85 days and up to 77 days for thyme EOs-chitosan NPs (0.04 and 0.02) % respectively (Table 3).

These findings show that additional EOs effectively inhibit yeast and mold growth. Yeasts and molds were found in the control cheese samples after 14 days of storage. The results found in the current study agreed with those informed by El-Kholy [78] who concluded that essential oils, mostly from thyme and and rosemary, had possible antifungal activity.

The thyme EO-nanoemulsion 0.1% has been confirmed to extend the shelf life of up to 6 weeks of UF labneh at cold storage, with tolerable taste and aroma [56]. Also, the addition of coating of Nigella sativa oil with chitosan NPs to soft cheese in two percentages, 0.5 and 1%, assisted in increasing the preservation ability of the cheese so that it might be stored for up to 14 days [43].

Conclusion

As using EOs in food items is challenging owing to their poor solubility in water, encapsulation approaches have been presented in current years as an effective method to enhance their capacity to disperse in watery media. In the current work, chitosan NPs are loaded with clove, thyme, and rosemary EO separately. EOs-chitosan NPs led to an excellent compatibility between chitosan and the essential oil. The EOs-chitosan NPs illustrated stronger antibacterial against (*E. coli* and *B. subtilius*) and antifungal (against *A. flavus* and *Goetricum candidum*) activities than crude EO. Clove EO-chitosan NPs were the most potent, followed by (thyme and rosemary EO-chitosan NPs). In addition, the Cheese group fortified with rosemary-chitosan NPs at a concentration of 0.04% showed the highest sensory scores with an extended cheese shelf life of up to 84 days, while cheese groups fortified with clove-chitosan NPs encapsulated form at a concentration of 0.04% extended their shelf life up to 105 days with accepted organoleptic scores. The outcomes of this study prove that EO could be loaded into chitosan NPs, and this approach could be recommended as a novel method for applying active packaging in the dairy industry.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This research was carried out following the guidelines of the ethics committee of Faculty Veterinary Medicine, Benha University with ethical ID: BUFVTM 14-08-24.

The study was approved by the ethics committee of Faculty Veterinary Medicine, Benha University with ethical ID: BUFVTM 14-08-24.

Informed written constant was obtained from all individual participants included in the study.

TABLE 1. Shows Zeta Potential of EOs-chitosan NPs (Electrical charge)

Clove-chitosan NPs	Positive charge (37.3 mV)
Thyme-chitosan NPs	Positive charge (26.0 mV)
Rosemary-chitosan NPs	Positive charge (31.3 mV)

Electrical charge of nanoparticles were determined using NANOTRAC Zetasizer wave II

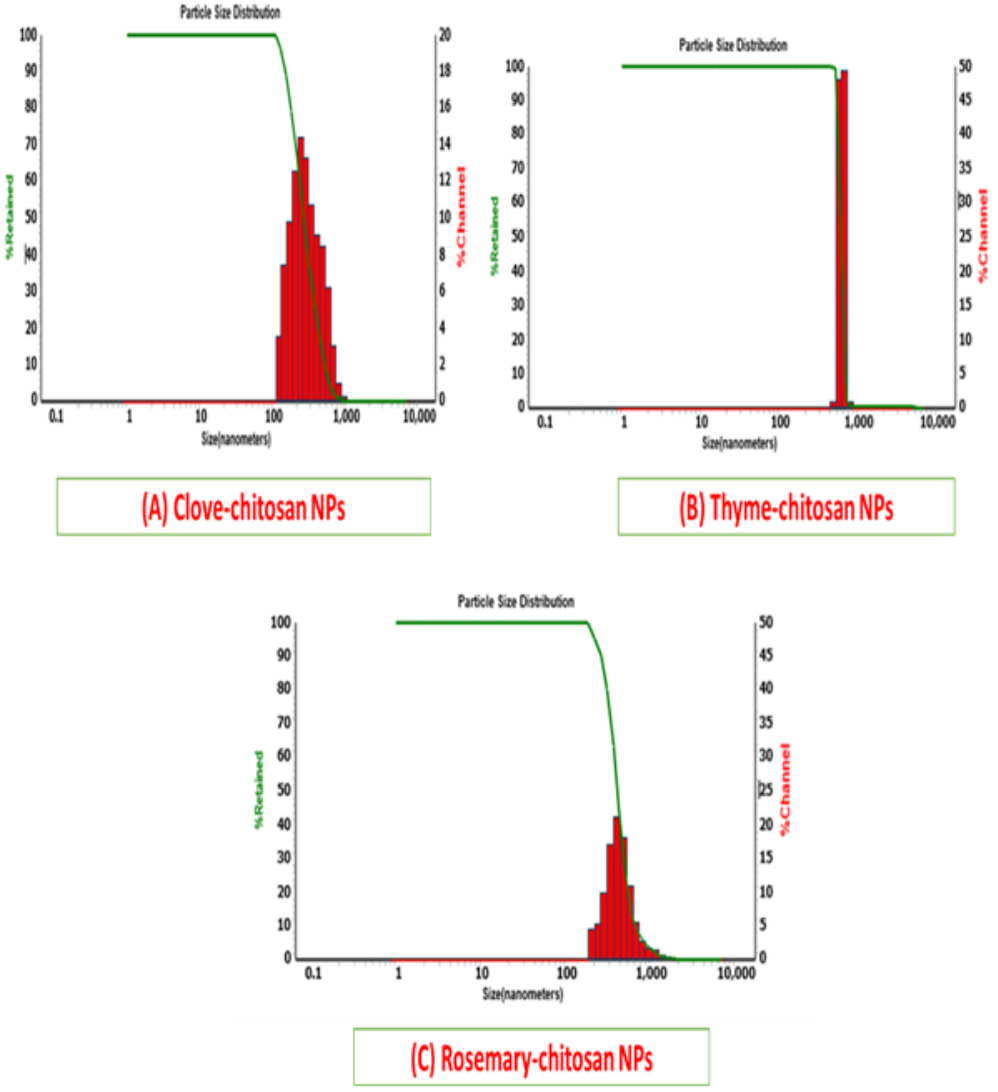
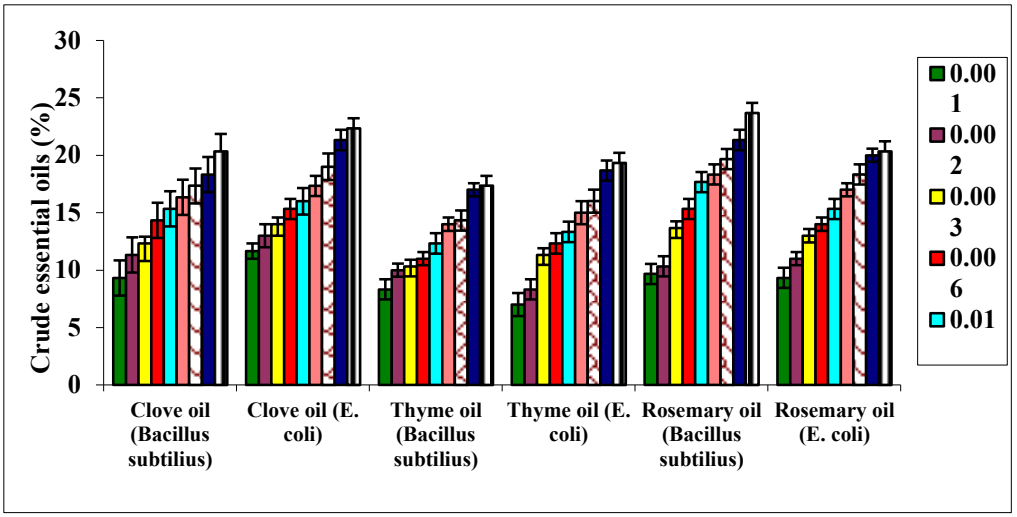
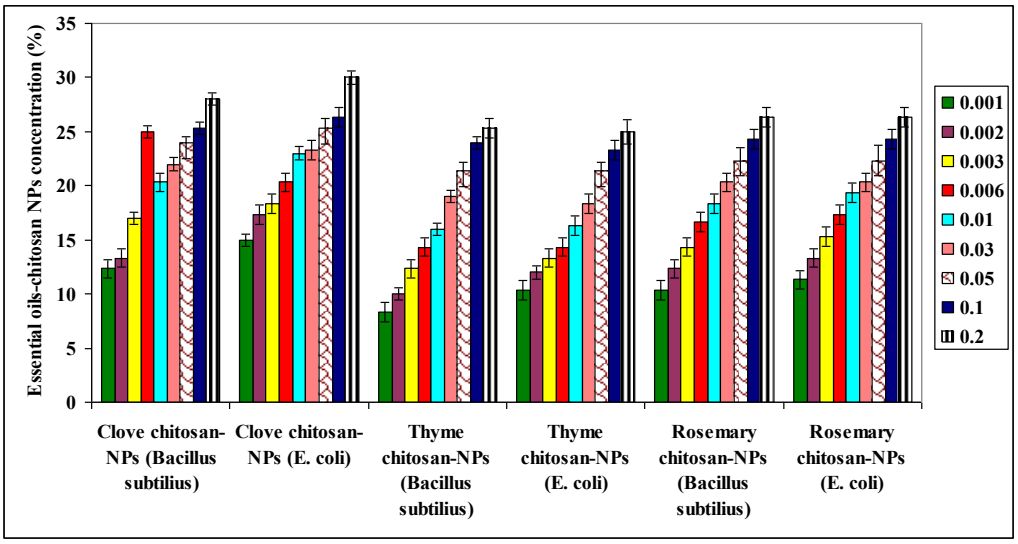


Fig. 1. Graph representing particle size of EOs-chitosan NPs (A): Clove-chitosan NPs, (B): Thyme-chitosan NPs, (C): Rosemary-chitosan NPs.

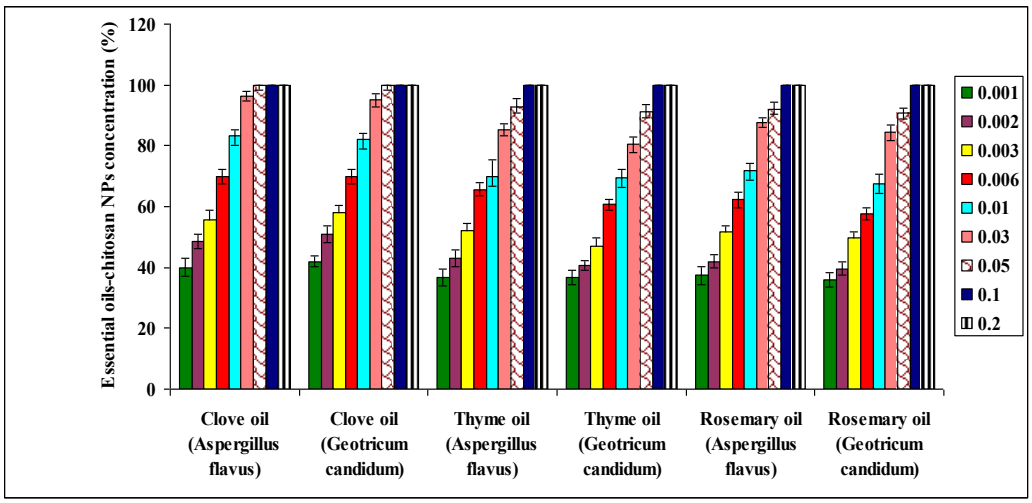


(A)

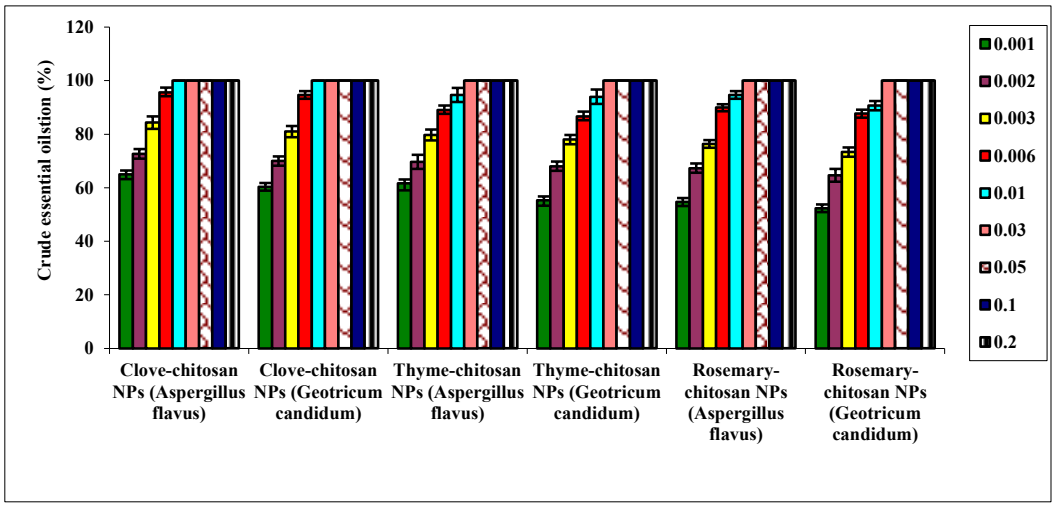


(B)

Fig. 2. Graph representing antibacterial activity of crude EOs and their EOs-chitosan NPs by agar well against *B. subtilius* and *E. coli* (A): Crude EOs, (B): EOs-chitosan NPs.



(A)



(B)

Fig. 3. Graph representing antifungal activity crude EOs and their EOs-chitosan NPs by tube method against *A. flavus* and *Goetricum candidum* by tube method (A): Crude EOs (B): EOs-chitosan NPs.

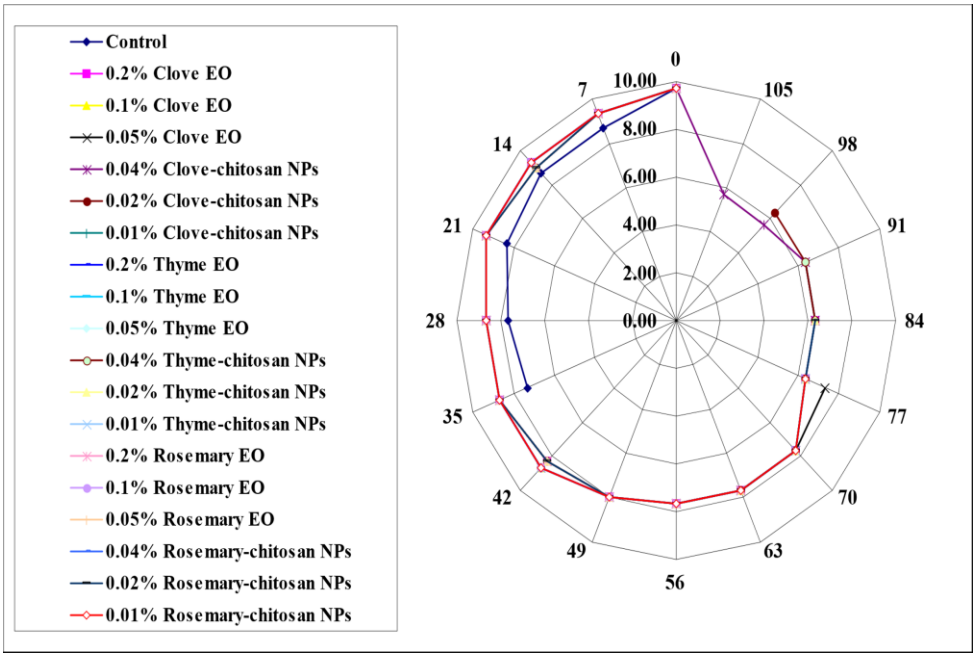


Fig. 4. Graph representing sensory evaluation (Appearance (10)) of cheese samples fortified with different concentrations of crude EOs and their EOs-chitosan NPs during the refrigerating storage period.

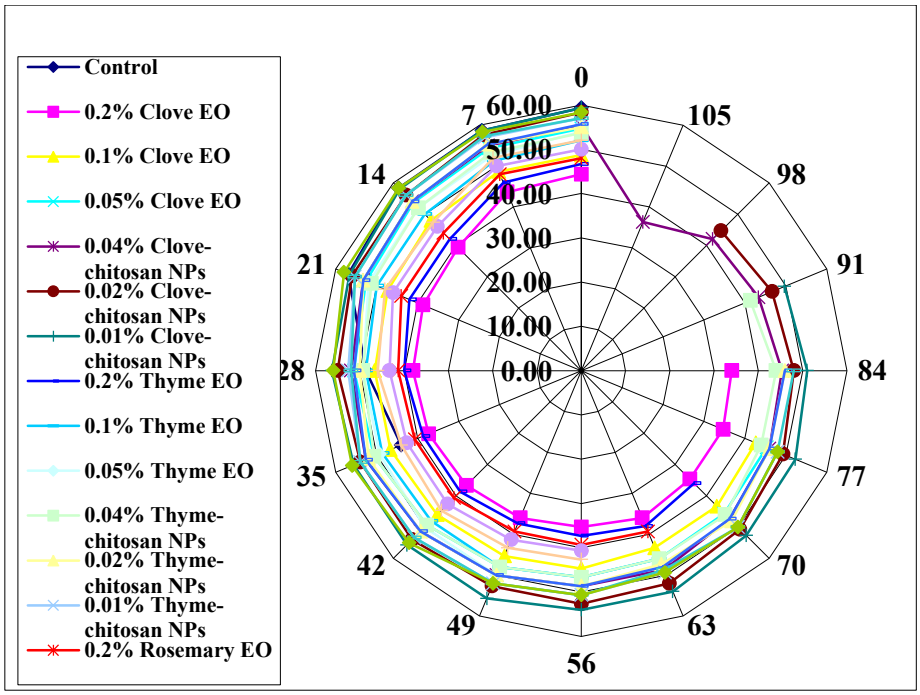


Fig. 5. Graph representing sensory evaluation (Flavor (60)) of cheese samples fortified with different concentrations of crude EOs and their EOs-chitosan NPs during the refrigerating storage period.

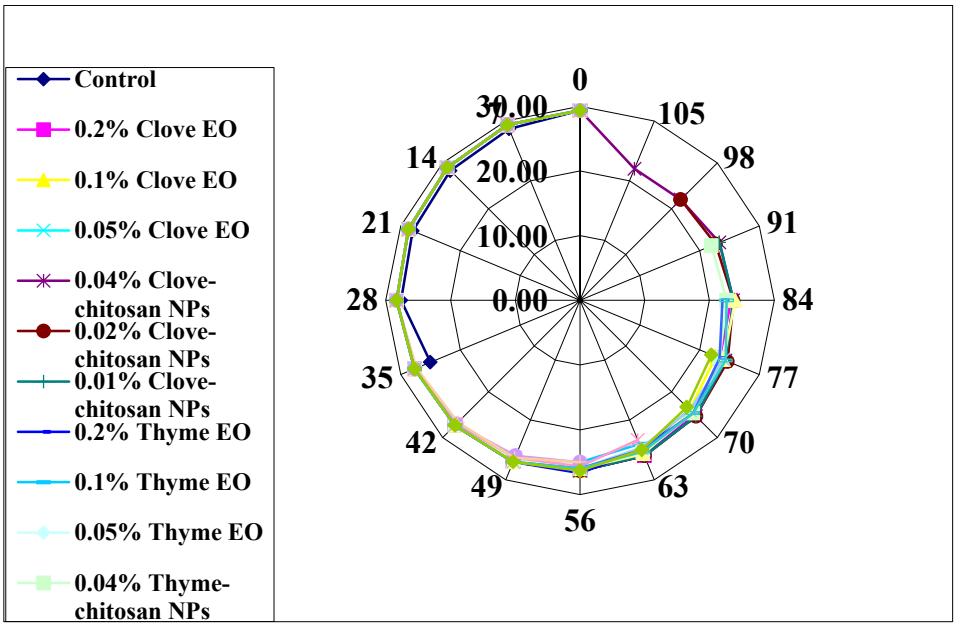


Fig. 6. Graph representing sensory evaluation (Body and texture (30)) of cheese samples fortified with different concentrations of crude EOs and their EOs-chitosan NPs during the refrigerating storage period.

TABLE 2. Statistical analysis of cheese samples TA of different groups fortified with different concentrations of crude EOs and their EOs-chitosan NPs during refrigerated storage.

Cheese groups	Storage period (day)															
	0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105
Control	0.60 ±0.01^{FL}	0.69 ±0.01^{bcE}	0.74 ±0.01^{cdD}	0.85 ±0.04^{aC}	1.00 ±0.02^{aB}	1.47 ±0.09^{aA}	S	S	S	s	s	s	s	s	s	s
Clove 0.2 %	0.64 ±0.01^{abM}	0.69 ±0.01^{bcL}	0.75 ±0.01^{bcK}	0.83 ±0.01^{bcJ}	0.88 ±0.01^{cdI}	0.94 ±0.01^{cdH}	1.03 ±0.02^{Ag}	1.10 ±0.02^{aF}	1.18 ±0.01^{aE}	1.25 ±0.02^{bD}	1.32 ±0.01^{bC}	1.34 ±0.01^{aB}	1.70 ±0.06^{aA}	s	s	s
Clove 0.1 %	0.62 ±0.01^{cdL}	0.66 ±0.01^{Ek}	0.71 ±0.01^{fJ}	0.76 ±0.01^{fI}	0.83 ±0.01^{gH}	0.89 ±0.01^{fG}	0.96 ±0.01^{eF}	1.05 ±0.02^{bE}	1.13 ±0.19^{bD}	1.23 ±0.01^{cC}	1.30 ±0.01^{cB}	1.33 ±0.01^{abA}	s	s	s	s
Clove 0.05 %	0.61 ±0.01^{deL}	0.64 ±0.01^{fK}	0.68 ±0.01^{gJ}	0.73 ±0.01^{gI}	0.81 ±0.01^{hH}	0.86 ±0.01^{gG}	0.90 ±0.02^{fF}	1.01 ±0.01^{eE}	1.10 ±0.01^{cD}	1.18 ±0.01^{dC}	1.26 ±0.01^{dB}	1.32 ±0.01^{bcA}	s	s	s	s
Clove-chitosan NPs 0.04 %	0.60 ±0.00^{eP}	0.63 ±0.00^{fgO}	0.66 ±0.01^{hN}	0.71 ±0.01^{hiM}	0.76 ±0.01^{iL}	0.79 ±0.01^{hiK}	0.84 ±0.01^{hiJ}	0.88 ±0.01^{fgI}	0.93 ±0.01^{fgH}	0.98 ±0.01^{efG}	1.05 ±0.01^{eF}	1.11 ±0.01^{efE}	1.18 ±0.01^{cdD}	1.24 ±0.01^{bC}	1.29 ±0.01^{aB}	1.55 ±0.03^{aA}
Clove-chitosan NPs 0.02	0.61 ±0.01^{deN}	0.62 ±0.00^{ghN}	0.65 ±0.01^{hgM}	0.70 ±0.01^{ijL}	0.75 ±0.01^{jK}	0.78 ±0.01^{ijJ}	0.83 ±0.01^{ijI}	0.87 ±0.01^{ghH}	0.92 ±0.01^{ghG}	0.97 ±0.01^{fgF}	1.03 ±0.01^{fE}	1.10 ±0.01^{fgD}	1.17 ±0.01d^{eC}	1.23 ±0.01^{bcB}	1.28 ±0.01^{aA}	s
Clove-chitosan NPs 0.01	0.61 ±0.00^{deM}	0.61 ±0.00^{hM}	0.64 ±0.^{01gL}	0.69 ±0.01^{jK}	0.74 ±0.01^{iJ}	0.77 ±0.01^{jI}	0.82 ±0.01^{jH}	0.86 ±0.01^{hG}	0.91 ±0.01^{hF}	0.96 ±0.01^{gE}	1.02 ±0.01^{fD}	1.09 ±0.01^{gC}	1.16 ±0.01^{eB}	1.22 ±0.01^{cA}	s	s
Thyme 0|2%	0.64 ±0.01^{abK}	0.70 ±0.01^{abJ}	0.76 ±0.01^{abI}	0.84 ±0.01^{abH}	0.89 ±0.01^{bcG}	0.95 ±0.01^{bcF}	1.01 ±0.01^{bcE}	1.05 ±0.00^{bD}	1.11 ±0.01^{cC}	1.26 ±0.02^{abB}	1.37 ±0.01^{aA}	s	s	s	s	s
Thyme 0.1%	0.62 ±0.01^{cd}	0.69 ±0.01^{bcE}	0.74 ±0.01^{cd}	0.82 ±0.01^{de}	0.88 ±0.01^{cd}	0.94 ±0.01^{cd}	1.00 ±0.00^{cd}	1.02 ±0.01^{deC}	1.10 ±0.01^{cdB}	1.25 ±0.02^{bA}	s	s	s	s	s	s
Thyme 0.05%	0.61 ±0.01^{deI}	0.67 ±0.01^{deH}	0.72 ±0.02^{efG}	0.81 ±0.02^{deF}	0.86 ±0.01^{efE}	0.93 ±0.01^{deD}	0.99 ±0.01^{dC}	1.01 ±0.01^{eB}	1.07 ±0.01^{eA}	S	s	s	s	s	s	s
Thyme-chitosan NPs 0.04%	0.62 ±0.00^{cN}	0.64 ±0.00^{fM}	0.66 ±0.01^{hL}	0.72 ±0.01^{ghK}	0.77 ±0.01^{iJ}	0.80 ±0.01^{hI}	0.85 ±0.01^{gH}	0.89 ±0.01^{fG}	0.94 ±0.01^{fF}	0.99 ±0.01^{eE}	1.06 ±0.01^{eD}	1.12 ±0.01^{eC}	1.19 ±0.01^{cB}	1.27 ±0.01^{aA}	s	s
Thyme-chitosan NPs 0.02%	0.61 ±0.00^{deM}	0.63 ±0.00^{fgL}	0.65 ±0.01^{hgK}	0.71 ±0.01^{hiJ}	0.76 ±0.01^{ijI}	0.79 ±0.01^{hiH}	0.84 ±0.01^{ghG}	0.88 ±0.01^{fF}	0.93 ±0.01^{fgE}	0.98 ±0.01^{efD}	1.05 ±0.01^{eC}	1.11 ±0.01^{efB}	1.17 ±0.02^{deA}	s	s	s
Thyme-chitosan NPs 0.01%	0.60 ±0.00^{eL}	0.62 ±0.00^{gK}	0.64 ±0.01^{gJ}	0.70 ±0.01^{iI}	0.75 ±0.01^{jH}	0.77 ±0.01^{aG}	0.82 ±0.01jF	0.86 ±0.01^{efE}	0.92 ±0.01^{ghD}	0.96 ±0.01^{gC}	1.03 ±0.01^{fB}	1.10 ±0.01^{fgA}	s	s	s	s
Rosemary 0.2%	0.65 ±0.01^{aJ}	0.71 ±0.01^{aI}	0.77 ±0.01^{aH}	0.84 ±0.01^{abG}	0.90 ±0.01^{bF}	0.96 ±0.01^{bE}	1.02 ±0.01^{abD}	1.05 ±0.02^{bC}	1.13 ±0.01^{cB}	1.27 ±0.02^{aA}	s	s	s	s	s	s
Rosemary 0.1%	0.63 ±0.01^{bcI}	0.69 ±0.00b^{cH}	0.75 ±0.00^{bcG}	0.83 ±0.01^{bcF}	0.88 ±0.01^{cdE}	0.94 ±0.01^{cdD}	1.01 ±0.01^{bcC}	1.03 ±0.01^{cdB}	1.13 ±0.01^{bA}	S	s	s	s	s	s	s
Rosemary 0.05%	0.62 ±0.01^{cI}	0.68 ±0.00c^{dH}	0.74 ±0.00^{cdG}	0.81 ±0.01^{deF}	0.87 ±0.02^{deE}	0.92 ±0.00^{eD}	1.00 ±0.00^{cdC}	1.03 ±0.00^{cdB}	1.10 ±0.01^{cdA}	S	s	s	s	s	s	s
Rosemary-chitosan NPs 0.04%	0.63 ±0.01^{bcN}	0.69 ±0.01^{bcM}	0.75 ±0.01^{bcK}	0.82 ±0.01^{cdJ}	0.88 ±0.01^{cdI}	0.95 ±0.01^{bcH}	1.01 ±0.01^{bcG}	1.05 ±0.01^{bF}	1.11 ±0.01^{cE}	1.26 ±0.02^{abD}	1.32 ±0.01^{bC}	1.32 ±0.01 ^{bcB}	1.39 ±0.01^{bA}	s	s	s
Rosemary-chitosan NPs 0.02%	0.62 ±0.01^{cL}	0.67 ±0.01^{deK}	0.74 ±0.01^{cdJ}	0.81 ±0.01^{deI}	0.86 ±0.01^{efH}	0.94 ±0.01^{cdG}	1.00 ±0.01^{cdF}	1.04 ±0.01^{bcE}	1.10 ±0.01^{cdD}	1.25 ±0.02^{bC}	1.30 ±0.01^{cB}	1.31 ±0.01^{cdA}	s	s	s	s
Rosemary-chitosan NPs 0.01%	0.61 ±0.02d^{eL}	0.66 ±0.01^{eK}	0.73 ±0.01d^{eJ}	0.80 ±0.01^{eI}	0.85 ±0.01^{fH}	0.93 ±0.01^{deG}	0.99 ±0.01^{dF}	1.03 ±0.01^{cdE}	1.09 ±0.01^{dD}	1.23 ±0.01^{cC}	1.29 ±0.01^{cB}	1.30 ±0.01^{dA}	s	s	s	s

a, b, and c: No significant difference (P>0.05) exists between any two means within the same column with the same superscript letter.

A, B, and C: No significant difference (P>0.05) exists between any two means within the same row with the same superscript letter.

s: spoiled sample.

TABLE 3. Statistical analysis of total yeast and mold count (log_{10} CFU/g) in fortified cheese samples with different concentrations of crude EOs and their EOs-chitosan NPs during the refrigerating storage period.

Group	Storage period (day)															
	0	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105
Control	0 ± 0^{aE}	0 ± 0^{aE}	1.59 ± 0.06^{Ad}	1.77 ± 0.04^{aC}	1.99 ± 0.02^{aB}	2.58 ± 0.04^{aA}	S	S	S	S	S	s	s	s	s	s
Clove 0.2 %	0 ± 0^{aH}	0 ± 0^{aH}	0 ± 0^{bH}	0 ± 0^{eH}	1.10 ± 0.1^{bG}	1.36 ± 0.06^{eF}	1.52 ± 0.02^{iE}	1.56 ± 0.01^{iE}	1.62 ± 0.01^{kD}	1.66 ± 0.01^{fD}	1.89 ± 0.02^{fC}	2.17 ± 0.06^{eB}	2.45 ± 0.03^{cA}	s	s	s
Clove 0.1 %	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{bF}	0 ± 0^{eF}	1.20 ± 0.10^{gE}	1.58 ± 0.02^{klD}	1.63 ± 0.01^{gD}	1.76 ± 0.01^{ijC}	1.79 ± 0.01^{iC}	1.81 ± 0.01^{eC}	1.93 ± 0.02^{efB}	2.40 ± 0.05^{cdA}	s	s	s	s
Clove 0.05 %	0 ± 0^{aH}	0 ± 0^{aH}	0 ± 0^{bH}	0 ± 0^{eH}	1.52 ± 0.04^{eG}	1.68 ± 0.01^{ijF}	1.74 ± 0.02^{fE}	1.80 ± 0.01^{iD}	1.85 ± 0.01^{hiD}	1.91 ± 0.01^{dC}	2.28 ± 0.05^{dB}	2.65 ± 0.02^{aA}	s	s	s	s
Clove-chitosan NPs 0.04 %	0 ± 0^{aK}	0 ± 0^{aK}	0 ± 0^{bK}	0 ± 0^{eK}	0 ± 0^{iK}	1.16 ± 0.16^{oJ}	1.38 ± 0.19^{jI}	1.65 ± 0.02^{kH}	1.70 ± 0.01^{jH}	1.81 ± 0.02^{eG}	1.89 ± 0.03^{fF}	1.96 ± 0.02^{gE}	2.17 ± 0.05^{eD}	2.33 ± 0.04^{cC}	2.46 ± 0.03^{bB}	2.54 ± 0.04^{aA}
Clove-chitosan NPs 0.02	0 ± 0^{aJ}	0 ± 0^{aJ}	0 ± 0^{bJ}	0 ± 0^{eJ}	0 ± 0^{iJ}	1.03 ± 0.03^{pI}	1.60 ± 0.03^{ghH}	1.81 ± 0.02^{iG}	1.89 ± 0.01^{hF}	1.94 ± 0.01^{dEF}	1.98 ± 0.01^{eE}	2.08 ± 0.02^{fD}	2.31 ± 0.02^{dC}	2.45 ± 0.02^{bB}	2.53 ± 0.03^{aA}	s
Clove-chitosan NPs 0.01	0 ± 0^{aH}	0 ± 0^{aH}	0 ± 0^{bH}	0 ± 0^{eH}	0 ± 0^{iH}	1.51 ± 0.14^{mG}	1.88 ± 0.05^{eF}	2.06 ± 0.03^{ghE}	2.25 ± 0.02^{efgD}	2.34 ± 0.02^{bcC}	2.34 ± 0.03^{cdC}	2.36 ± 0.03^{dC}	2.46 ± 0.01^{cB}	2.54 ± 0.02^{aA}	s	s
Thyme 0.2%	0 ± 0^{aG}	0 ± 0^{aG}	0 ± 0^{bG}	0 ± 0^{eG}	1.42 ± 0.06^{fF}	1.52 ± 0.041^{mE}	1.55 ± 0.04^{hiE}	1.72 ± 0.02^{jD}	1.86 ± 0.01^{bC}	2.38 ± 0.06^{bB}	2.61 ± 0.02^{aA}	s	s	s	s	s
Thyme 0.1%	0 ± 0^{aG}	0 ± 0^{aG}	0 ± 0^{bG}	0 ± 0^{eG}	1.69 ± 0.05^{cF}	1.97 ± 0.03^{dE}	2.21 ± 0.02^{bD}	2.30 ± 0.00^{cC}	2.46 ± 0.36^{dB}	2.62 ± 0.03^{aA}	S	s	s	s	s	s
Thyme 0.05%	0 ± 0^{aG}	0 ± 0^{aG}	0 ± 0^{bG}	1.26 ± 0.14^{dF}	1.78 ± 0.09^{bE}	2.04 ± 0.02^{cD}	2.28 ± 0.01^{aC}	2.37 ± 0.03^{bB}	2.53 ± 0.02^{cA}	S	S	s	s	s	s	s
Thyme-chitosan NPs 0.04%	0 ± 0^{aH}	0 ± 0^{aH}	0 ± 0^{bH}	0 ± 0^{eH}	0 ± 0^{iH}	1.62 ± 0.09^{ijkG}	1.86 ± 0.05^{eF}	2.01 ± 0.01^{hE}	2.20 ± 0.05^{gD}	2.30 ± 0.00^{cC}	2.40 ± 0.01^{bcB}	2.42 ± 0.67^{bcdB}	2.45 ± 0.02^{cB}	2.57 ± 0.02^{aA}	s	s
Thyme-chitosan NPs 0.02%	0 ± 0^{aH}	0 ± 0^{aH}	0 ± 0^{bH}	0 ± 0^{eH}	0 ± 0^{iH}	1.79 ± 0.06^{ghG}	2.00 ± 0.03^{dF}	2.10 ± 0.01^{fgE}	2.22 ± 0.04^{fgD}	2.33 ± 0.01^{bcC}	2.36 ± 0.01^{cC}	2.43 ± 0.01^{bcB}	2.61 ± 0.05^{aA}	s	s	s
Thyme-chitosan NPs 0.01%	0 ± 0^{aI}	0 ± 0^{aI}	0 ± 0^{bI}	0 ± 0^{eI}	1.20 ± 0.10^{gH}	1.83 ± 0.07^{gG}	2.07 ± 0.05^{cF}	2.22 ± 0.01^{dE}	2.29 ± 0.01^{eD}	2.37 ± 0.01^{bC}	2.45 ± 0.03^{bB}	2.64 ± 0.02^{aA}	s	s	s	s
Rosemary 0.2%	0 ± 0^{aG}	0 ± 0^{aG}	0 ± 0^{bG}	1.26 ± 0.14^{dF}	1.71 ± 0.03^{cE}	1.90 ± 0.03^{efD}	2.28 ± 0.01^{aC}	2.45 ± 0.02^{aB}	2.57 ± 0.01^{bcA}	2.61 ± 0.00^{aA}	S	s	s	s	s	s
Rosemary 0.1%	0 ± 0^{aG}	0 ± 0^{aG}	0 ± 0^{bG}	1.43 ± 0.22^{cF}	1.74 ± 0.02^{bcE}	1.94 ± 0.04^{deD}	2.29 ± 0.01^{aC}	2.48 ± 0.02^{aB}	2.60 ± 0.00^{abA}	s	S	s	s	s	s	s
Rosemary 0.05%	0 ± 0^{aG}	0 ± 0^{aG}	0 ± 0^{bG}	1.62 ± 0.09^{bF}	2.03 ± 0.02^{aE}	2.15 ± 0.01^{bD}	2.30 ± 0.01^{aC}	2.47 ± 0.03^{aB}	2.65 ± 0.01^{aA}	s	S	s	s	s	s	s
Rosemary-chitosan NPs 0.04%	0 ± 0^{aI}	0 ± 0^{aI}	0 ± 0^{bI}	0 ± 0^{eI}	1.46 ± 0.09^{efH}	1.73 ± 0.09^{hiG}	$1.940.03^{dF}$	2.14 ± 0.07^{efE}	2.27 ± 0.02^{efD}	2.33 ± 0.01^{bcC}	2.38 ± 0.00^{cC}	2.47 ± 0.01^{bB}	2.53 ± 0.00^{bA}	s	s	s
Rosemary-chitosan NPs 0.02%	0 ± 0^{aI}	0 ± 0^{aI}	0 ± 0^{bI}	0 ± 0^{eI}	1.62 ± 0.09^{dH}	1.86 ± 0.05^{fG}	2.00 ± 0.03^{dF}	$2.19\pm0.05d^{eE}$	2.29 ± 0.01^{eD}	2.35 ± 0.01^{bcC}	2.39 ± 0.00^{bcC}	2.48 ± 0.01^{bB}	2.62 ± 0.03^{aA}	s	s	s
Rosemary-chitosan NPs 0.01%	0 ± 0^{aG}	0 ± 0^{aG}	0 ± 0^{bG}	0 ± 0^{eG}	1.97 ± 0.17^{aF}	2.07 ± 0.11^{cE}	2.12 ± 0.10^{cE}	2.23 ± 0.07^{dD}	$2.30\pm1\pm0.03^{eC}$	2.36 ± 0.01^{bcB}	2.39 ± 0.00^{bcB}	2.64 ± 0.02^{aA}	s	s	s	s

a, b & c: No significant difference (P>0.05) exists between any two means within the same column with the same superscript letter.
A, B & C: No significant difference (P>0.05) exists between any two means within the same row with the same superscript letter.
S: spoiled sample.

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استخدام بعض الزيوت العطرية وجزيئات الشيتوزان النانوية لحفظ الجبن الطري

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قسم الرقابة الصحية علي الأغذية - كلية الطب البيطري- جامعة بنها - مصر.

الملخص

ركزت دراستنا الحالية على استخدام المواد الحافظة الطبيعية مثل الزيوت الأساسية (القرنفل والزعتر والروزماري) وجسيمات النانو من الزيوت العطرية والشيتوزان (NPs) التي تظهر تأثيرات مضادة للميكروبات ضد العديد من البكتيريا والخميرة والعفن). تم تحديد الشحنة الكهربائية وحجم جزيئات النانو. تم الكشف عن التأثير المضاد للميكروبات للزيوت العطرية وجسيمات النانو من الزيوت العطرية واالشيتوزان ضد بعض التلف والبكتيريا المسببة للأمراض والفطريات من حيث MIC وMBC وMFC. بعد ذلك، تم حقن الزيوت العطرية وجسيمات النانو من الزيوت العطرية واالشيتوزان بشكل منفصل في عينات الجبن الطري المفحوصة. تم تحليلها من حيث الحموضة القابلة للقياس (T.A٪) والفحص الميكروبيولوجي والتقييم الحسي في وقت الإنتاج وأسبوعيًا حتى تم اكتشاف علامات التلف. أظهرت جزيئات الزيوت العطرية-الشيتوزان النانونية نشاطًا مضادًا للميكروبات أعلى من الأشكال الخام، وخاصة جزيئات القرنفل-الشيتوزان النانوينة التي سجلت أعلى مستوى، تليها جزيئات -الشيتوزان النانونية التي سجلتها مع الزعتر والروزماري وسجلت جزيئات القرنفل أدنى مستوى تركيز مثبط (0.1 مجم/مل) بالنسبة للزيوت العطرية الخام الأخرى بينما سجلت جزيئات الشيتوزان النانونية التي سجلتها مع القرنفل والزعتر أدنى مستوى بالنسبة للآخرين. زادت الحموضة القابلة للقياس تدريجيًا أثناء وقت التخزين. لم يتم العثور على إجمالي القولونيات ومكونات الجراثيم الهوائية والمؤثرات العقلية والليستيريا المستوحدة في كل عينة تم فحصها. أظهرت مجموعة الجبن المدعمة بجزيئات الروزماري-الشيتوزان بنسبة 0.04٪ أعلى الدرجات الحسية مع إطالة مدة صلاحية الجبن حتى 84 يومًا، بينما مددت مجموعات الجبن المدعمة بجزيئات القرنفل-الشيتوزان بنسبة 0.04٪ مدة صلاحيتها حتى 105 أيام بدرجات حسية مقبولة. على عكس مجموعات الجبن المدعمة بالزيت الخام والتحكم، أظهرت جزيئات الزيوت العطرية-الشيتوزان نشاطًا مضادًا للميكروبات أفضل من الجزيئات الخام ويمكن تطبيقها لحفظ الجبن الطري وإطالة فترة الصلاحية دون التأثير على الجودة الحسية.

الكلمات الدالة: الزيوت العطرية، جزيئات الزيوت- الشيتوزان النانوية، مسببات الأمراض البكتيرية، مسببات الأمراض الفطرية، الزيوت العطرية.