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Viability of Bacterial and Fungal Pathogens in Soft Cheese Incorporated with Some Essential Oils-Chitosan Nanoparticles

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Abstract

OUR CURRENT study used clove and rosemary essential oils (EOs) and related EOs-chitosan nanoparticles at various concentrations to determine their inhibitory effect on the viability of bacterial and fungal pathogens (*Staphylococcus aureus* and *Pseudomonas fluorescence*), (*Aspergillus flavus* and *Geotrichum candidum*), respectively. Soft cheese was produced as control groups, fortified crude EOs cheese groups (0.1%) and EOs-chitosan nanoparticles groups (0.04%) and examined during refrigeration. EOs-chitosan nanoparticles showed more potent inhibitory effects than crude ones. Rosemary-chitosan nanoparticles reduced *Staphylococcus aureus* growth by 62.57%, while clove-chitosan nanoparticles reduced it by 57.97% after 48 hours of storage. At the end of 1st week of storage, rosemary-chitosan nanoparticles reduced *Pseudomonas fluorescence* growth by 67.43% while clove-chitosan nanoparticles (61.40%). Staphylococcus aureus was completely inhibited in all inoculated cheese samples at the end of 1st week of storage. In contrast, *Pseudomonas fluorescence* was totally inhibited at the end of the 2nd week of storing rosemary-chitosan nanoparticles and clove-chitosan nanoparticles. For their antifungal effect, clove-chitosan nanoparticles had potent antifungal activity followed by rosemary-chitosan nanoparticles, clove then rosemary EO. Clove-chitosan nanoparticles inhibited *Aspergillus flavus* and *Geotrichum candidum* by 90.10 and 87.47% respectively after 48hrs of storage. At the end of 1st week of storage, both fungi were completely inhibited in cheese samples fortified with clove-chitosan nanoparticles, clove EO, while rosemary-chitosan NPs made 100% reduction at end of 1st and 2nd week of storage for *Aspergillus flavus* and *Gortrichum candidum*, respectively. In conclusion, the combined clove or rosemary with chitosan nanoparticles is recommended for soft cheese preservation over crude EOs.

Keywords: Essential oils, Eos-chitosan NPs, Bacterial pathogen, fungal pathogen, soft cheese.

Introduction

One of the most common forms of cheese in the Middle East is soft cheese. Based on the numerous traditional cheese variations, Egypt has a long and rich history of cheese production [1]. Due to cheese constituents, Pseudomonas, Alcaligenes, Achromobacter, and Flavobacterium are examples of psychrotrophic Gram-negative rods that are thought to be significant spoiling organisms for soft cheeses. These organisms can cause unpleasant smells and aromas because of their lipolytic and proteolytic actions [2]. Foodborne disease incidence can be increased by microbial pathogens such as S. aureus (Staphylococcus aureus), *E. coli* (*Escherichia coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and others that may result in spoilage [3]. According to reports, foodborne disease poses a major concern to global public health, affecting around 48 million people and resulting in 3,000 deaths annually in the United States alone [4].

Additionally, fungal spoilage results in either observable or non-observable sensory faults in cheese, including surface-level fungus growth and the generation of metabolites that produce disagreeable flavor, texture, and fragrance alterations, ultimately lowering the final product quality [5]. The fungi that cause issues with cheese production in the dairy sector varied and come from several species, including Aspergillus, Penicillium, Geotrichum, Cladosporium, Mucor, and

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Trichoderma [6, 7]. As well as the significant financial losses brought on by spoiling, certain fungi can produce mycotoxins, which can be dangerous for food safety. The growth of toxigenic fungus like Aspergillus and Penicillium during the preparation and storage of cheese raises the possibility of mycotoxins in the cheese [5]. The primary reasons for the growth of fungus in cheese include their capability to proliferate at cold temperatures, low pH, low oxygen tensions, lipolytic action, and resistance to mild acid preservatives [8]. The air in ripening rooms is one of the primary causes of fungus contamination, which can happen to cheese at different phases of production[9].

The development of food preservation techniques that are effective in eliminating food microbial contamination is one potential strategy to reduce foodborne diseases. Plants that are abundant in bioactive compounds, some of which are widely recognized for having antibacterial qualities have attracted attention as natural material sources. The antibacterial action of these chemicals is largely dependent on their composition and the functional groups found in their essential oils (EOs). Since plant EOs are widely accepted as harmless, they are regarded as a safe, biodegradable natural substitute. It has been demonstrated that EOs and their constituents have antifungal and antiparasitic qualities in addition to their antibacterial ones [10].

Encapsulation is a compelling technology that preserves the physical and chemical characteristics of EOs, preventing unintended modifications and enhancing food preparation. One or extra substances, such as chitosan, gum arabic, maltodextrin, hydroxypropyl methylcellulose, phthalate gelatin, and starch, among others, can be combined or used alone to make the encapsulating materials [11].

Nanoparticles (NPs) are generated naturally when tripolyphosphate aqueous solution is added. Stir the mixture at room temperature for a further 2 hrs. The NPs were next exposed to intense ultrasonication (Cole-Parmer ultrasonic processor) at 35 Hz for at least 30 minutes on an ice bath to prevent temperature rise. The sample was centrifuged at 14000 g for 30 minutes at 4°C using an Eppendorf 5804R benchtop centrifuge. To prepare clove and rosemary-chitosan NPs, 5 mL of each individual oil was combined with 4 mL of TPP solution.

Determination of Zeta Potential Nanoparticles

The zeta potential is the charge on the diffused aqueous layer produced on the NP surface while it is immersed in water. At room temperature, the zeta potential was measured using the Microtrac Zetasizer Wave II (USA).

Characterization of the Nanoparticles

The particle size and relative measurement indices for NPs were examined using Dynamic light scattering (DLS) instrument, Microtrac Zetasizer wave II, USA at room temperature, 250 and 383 nm for clove and rosemary EO-chitosan NPs, respectively were recorded.

Because chitosan has uses in food and pharmaceuticals, it has also attracted attention. Among Chitosan's most intriguing qualities for enhancing food preservation and minimizing the need for chemical preservatives are its antibacterial and antifungal activities [12, 13]. It has been linked with EOs using nano-encapsulation techniques, which could have been used in the food sector. Since light, air, and high temperatures may quickly cause EOs to break down, nanoencapsulation has emerged as a useful method for shielding EOs from oxidation and evaporation [14].

In sight of these facts, the objective of the study was to determine the antimicrobial activity of crude clove and rosemary EOs, and chitosan NPs (Nanoparticles) loaded with such EOs to detect their effect on bacterial pathogens.

Material and Methods

Essential oils

Clove and rosemary EOs (pure, 100% concentration) were purchased from 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 (EOs-chitosan NPs) were made utilizing the process of ionic gelation designated by Piras et al. [15]. Chitosan and tripolyphosphate (TPP) solution were prepared for this purpose. Chitosan solution was made by putting 0.3 g of chitosan in a 1% acetic acid solution (100 mL) with continuous Stir till a clear solution is attained. TPP solution was made by addition of 0.1 g TPP to 10 mL of water. The (TPP) tripolyphosphate solution (4 mL) was added dropwise to the chitosan solution (100 mL) while mixing continuously.

Indicator pathogenic bacteria

The indicator pathogenic bacteria: *S. aureus* (MRSA) ATCC43300 and *P. fluorescence* were obtained from the Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary medicine, Benha University, Egypt .

Activation of bacterial pathogen

Staphylococcus aureus and *P. fluorescence* strains were activated on Tryptone Soya Broth (TSB) at 37°C/24h then three successive cultures were done for each strain. Serial dilutions were then made for the microorganism till obtained the concentration of $7\log_{10}$ CFU/mL based on the method described by Ahmed et al. [16].

Indicator pathogenic fungi

The indicator pathogenic mold; *A. flavus* (MT645073.1) was purchased from Food Safety Department, Animal Health Research Institute, Egypt. While spoilage yeast; *Goetrichum candidum* (AUMC 226) was purchased from Mycology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Activation of fungal strains

The indicator pathogenic mold *A. flavus* and spoilage yeast; *Geotricum candidum* were inoculated on glucose peptone yeast extract broth at 25°C/3-5 days then three successive cultures were made to activate them. Serial dilution was then made for the microorganism till obtained the concentration of $6\log_{10}$ CFU/ mL [17] .

Cheese-making with inoculated with some pathogens and fortified by some EOs and EOs-chitosan NPs :

Cheese manufacture has been performed using the method designated by Scott et al. [18]. Skimmed milk (fat not more than 0.5%) has been pasteurized (62.7°C for 30 min), cooled to 37.8-40.0°C in a 5-L bucket, NaCl (8% wt/wt), and CaCl2 (0.2% wt/wt) were added to milk before rennet addition (at 38°C) (11 ml rennet per 45.5 kg milk). The rennet was diluted at 1:40 with water before adding milk. After the addition of rennet (at 38°C), milk was stirred and before being left to develop a curd the bacterial and fungal pathogen (separately) were inoculated in soft cheese fortified by crude EOs (0.1) and EOs-chitosan nanoparticles (0.04%) (ten groups of cheese (for bacterial and fungal pathogen) were developed as follow as shown in tables 1 &2:

Then the whey was drained, and the curd ladled into small cylindrical metal molds lined with cheesecloths and pressed lightly overnight. The following day, the curd was cut into small cubes (ca. 5.5 x 3.5 x 2.0 cm) and stored in the collected whey at 4°C in sterile foil cups. The curd was examined till signs of spoilage appeared.

Cheese manufacturing was performed 3 times and samples were tested at zero-time, 48hrs, and every week throughout the refrigerating storage period (1 month).

Statistical analysis

The statistical analysis was conducted using Two-Way ANOVA using SPSS, ver. 27 (IBM Corp. Released 2013). Data were treated as a complete randomization design according to Steel et al. [19]. Multiple comparisons were done out using Duncun test at the significance level < 0.05. The experiment was conducted three times, and the results were the mean ± Standard error.

Results and Discussion

The primary causes of cheese spoiling are microorganisms (bacteria, yeasts, and molds) that can proliferate throughout production development or the shelf life, altering sensory qualities, structure, aroma, and color. Clostridia are the primary reason for deterioration in long-ripened hard cheeses [20, 21], The spoiling microbiota in fresh cheeses is primarily composed of psychotropic Gram-negative bacteria [22], Frequently causes gas generation and paste problems with slime production and foul flavor, so in our study we tried to control of such spoilage by using of clove, clove chitosan-NPs, rosemary and rosemary chitosan-NPs and detect their inhibitory effect on some bacterial and fungal pathogen which inoculated by specific concentration in cheese samples.

Recently, much emphasis has been paid to the usage of EOs and their primary bioactive constituents as replacements for synthetic chemicals in agriculture and food industries. Along with being classified as "GRAS" (generally recognized as safe) by the United States Food Administration (FDA), the usage of EOs by plant-based enterprises is predicted to greatly rise shortly, particularly with growing "green consumerism" and "sustainable food security" needs [23].

The particle sizes were recorded at 250 and 383 nm for clove and rosemary EO-chitosan NPs, respectively (the data not shown). These high values indicate that the particle surface is positively charged, stable, and has potential functionality. The rise in this value could be attributed to the interactions between the OH groups of the EO and the amino groups of the chitosan[24].

Typically, the polydispersity index (PDI) is used to assess particle size dispersal in suspensions. A lower PDI suggests a more homogeneous particle size distribution, and so imitates diameter homogeneity [25]. Our data indicated that the PDI of samples was in the range of 0.02612 to 0.1930 (Data not shown) which suggests a uniformly distributed, monodisperse, and stable particle population[26] .

Smaller NP sizes were linked to higher antibacterial activity, and these NPs showed a marked shift in their physical characteristics when contrast to their original complement. Chitosan NPs share qualities with chitosan and significant properties of NPs, such as tiny size, enhanced surface area, and quantum size effects which result in cell wall penetration easily[27] .

The studied NPs showed positive charge (Data not shown), such positively charged NPs giving higher antibacterial activity as described by Chandrasekaran et al. [28] who confirmed that positively charged chitosan may bind to negatively charged bacteria, producing membrane damage, increased membrane permeability, osmotic leakage, and eventually bacterial cell death.

Similarly, Cava et al. [29] found that, when compared to skim milk, the antibacterial activity of clove oil was reduced in high-fat milk. Furthermore, outside variables that affect the natural antimicrobial efficacy include the type of microbe, packaging, initial inoculum concentration, and storage temperature.[30]

Conversely, Feyzioglu and Tornuk [31] performed that the zeta potential values of chitosan NPs loaded with diverse concentrations of Summer savory EO varied from -7.54 to -21.12 mV; a notable decrease in zeta potential value was noted with increasing EO concentration. Their findings suggested that the structure and charge of these nanocarriers are influenced by interactions between EO and NPs.

The reduction % differed significantly between all inoculated groups at 48hrs. examination time. Also, the acquired results indicated a significant difference between 48hrs and other examination times within every inoculated group.

The current results revealed that rosemary-chitosan NPs (0.04%) showed more potent inhibitory activity (62.57% reduction) than others, with the reduction % for *S. aureus* reached 25, 57.97, 26.30, and for clove 0.1%, clove-chitosan NPs 0.04% and rosemary 0.1%, respectively. While the reduction % reached 100% at the end of 1st week for all inoculated cheese samples, the control group showed an increase in the staphylococcal count during the storage period (Table. 3 and Graph. 1).

Chitosan NPs that have been encapsulated in rosemary oil can offer a controlled release, guaranteeing long-term exposure to the antibacterial agent. Furthermore, chitosan nanoparticles have a larger contact area and higher penetration into bacterial cells due to their smaller size compared to clove oil droplets. Moreover, there are differences in the mechanisms by which chitosan and rosemary oil inhibit infections. Rosemary oil has the power to harm bacterial cell membranes, whilst chitosan can prevent the creation of cell walls. Combining these two compounds can result in stronger antibacterial action (Synergistic action) [24].

Our current findings were substantially like Kavas et al. [32] who reported that throughout the 60-day storage of Kashar Cheese, S. aureus levels enhanced in the control sample but reduced in samples coated with WPISF .

Chitosan NPs (500 µg/mL) and NPs of chitosan with S. molle (250 and 500 µg/mL) were found to reduce the proliferation of *S. aureus* by 35.0% [33]. Also, Chitosan NPs containing carvacrol showed antibacterial efficacy against *S. aureus*, *B. cereus*, and *E. coli* when contrasted to chitosan NPs[34].

Our outcomes agreed with Hadidi et al. [26] who described that chitosan NPs loaded with cloves had strong antibacterial activity against *S. aureus*, with an inhibitory (diameter of the halo): 4.80–4.78 cm. The Potential to increase the antibacterial action of pure Clove against foodborne germs was greatly enhanced by the nanoencapsulation of clove oil. These results agree with the outcomes of Esmaeili and Asgari [35] who proved an improvement in encapsulated Carum copticum EO's (CEO) antibacterial activity against *S. aureus* and *E. coli*, two of the most significant foodborne pathogens.

Essential oils having a high phenolic components percentage, such as thymol, carvacrol, and eugenol, have the greatest antibacterial activity against spoilage and foodborne pathogens and disrupt bacterial metabolic actions [36] .

Chitosan nanocoating with EOs was beneficial against certain foodborne bacteria. Incorporating both EOs (clove and argan) into chitosan-based coatings produced a synergistic antibacterial impact . [38 ،37]

However, Abdullah et al. [39] found that clove essential oil (EO) showed somewhat greater antibacterial activity than rosemary oil; for *S. aureus*, MICs ranged from 0.312% (v/v) to 1.25% (v/v), whereas MICs for rosemary oil ranged from 0.312% (v/v) to 5% (v/v).

The rosemary and clove (chitosan NPs (0.04%)) had a reduction % for *P. fluorescence* reached 67.43 and 61.40 at the end of 1st week respectively; and reached 100% reduction for both examined cheese samples groups at the end of 2nd week of storage (Fig.1 and Graph. 1).

Cheese samples inoculated with clove by 0.1% reduced the growth of *P. fluorescence* growth by 63.50% at the end of the storage period, rosemary by 0.1% had a reduction % reached 65.23 at the end of the storage time (end of 4th week), while control cheese samples showed an increase in the count with storage (Fig.1 and Graph. 1).

The current results disagreed with that of Wilkinson et al. [40] who stated that *P. aeruginosa* was less sensitive to Backhousia citriodora EO, this may be because the bacteria *P. aeruginosa* are Gram-negative. Gram-negative bacteria have an extra outer membrane that can function as a barrier to shield them from external agents like essential oils, whereas gram-positive bacteria have a thicker cell wall that is more easily pierced by essential oils [41, 42]. Furthermore, Pseudomonas species have been reported to possess effective efflux pumps, which may aid in their ability to withstand the impacts of essential oils[43] .

However, it nearly agreed with Ahmed et al. [44] who recorded that 50% reduction of *P. aeruginosa* by the end of the storage period (1 month at 4˚C) fortified with 0.1% ginger oil. In addition, Srivastava

et al. [45] found that the MIC of rosemary is 1% (v/v) for *P. aeruginosa* in broth.

As well, our findings were like Araby and El-Tablawy [46] who reported that rosemary has antibacterial activity against multidrug-resistant *P. aeruginosa* isolates in vitro, Which might be due to its negative impact on several virulent features like as biofilm formation, motility, and pyocyanin synthesis.

The least effective antimicrobial of rosemary encapsulated on chitosan was observed on *P. aeruginosa* (0.78 mg/ mL) [47].

Hellali and Ayachi [48] concluded that the study conducted on the encapsulation of rosemary within rosemary-chitosan NP and Alginate/Chitosan NPs rosemary represents an intriguing and potential attempt. Rosemary has remarkable antibacterial action, particularly against *S. aureus*, which is known to cause common diseases. Additionally, it demonstrated significant antifungal action against Candida strains, including *Candida albicans*.

Our current results were nearly parallel to those of Elsherif and Talaat AL Shrief [49] who found that carvacrol NE (nano-emulsion) shown excellent antibacterial action without any cheese sensory harm. It decreased *Listeria monocytogenes* by 99% (reduction %) after 7 days and after 3 weeks of refrigerated Talaga cheese storage. Additionally, discovered that in week 4, clove and its NEs had an inhibitory action against Salmonella. flexneri, with a drop in percentage in chilled Talaga cheese ranging from 98% to 100%.

The hydrophobic property of EOs permits them to easily go through the lipid membrane of bacteria and disrupt their cell walls, making them more susceptible to EOs. As a result, the proton pumps and ion channels are damaged, the membrane potential is lowered, and cellular components coagulate, resulting in bacterial death[24].

Mold growth in cheese is both a quality and food safety issue, resulting in large economic losses. Several mold genera may harm cheese. Penicillium is the dominant genus, followed by Aspergillus. Some mycotoxins produced by cheese-contaminating mold species, including ochratoxin A, cyclopiazonic acid, and sterigmatocystin, are stable throughout normal processing[50].

Clove-chitosan NPs showed a potent antifungal effect followed by clove, rosemary-chitosan NPs, and rosemary. Clove-chitosan NPs 0.04% made inhibition to *A. flavus* by 90.10% after 48hrs then complete inhibition at the end of 1st week of storage. While clove 0.1%, rosemary-chitosan NPs 0.04%, and rosemary 0.1% had reduction % reached 69.50, 62.10 and 49.27%, then 100% at the end of 1st week for clove 0.1% and rosemary-chitosan NPs 0.04% and end of 2nd week of storage for rosemary 0.1%, while control group there was an increase in *A. flavus* count during storage period (Fig.2 and Graph. 2) .

Boukaew et al. [51] confirmed that clove oil had potent antifungal activity against ten isolates of *A. flavus*. All strains, except *A. flavus* PSRDC-2, were greatly inhibited by clove. Also, Ali et al. [33] verified that clove's primary ingredient, eugenol, has potent anti-*C. albicans* effects in vivo. therefore, has the potential to be therapeutic.

When compared to free EO, the encapsulated EO performed better against *A. flavus*. This is likely because the encapsulated EO's regulated release of protected volatile oil from chitosan NPs during the trial had a stronger inhibitory impact in addition to the chitosan NPs' inherent inhibitory effect. Considering that clove EO-chitosan NPs exhibit remarkable antifungal activity[52].

Nano-encapsulated clove (with chitosan) displayed greater effectiveness against *A. niger*, isolated from rotten pomegranate, as compared to chitosan NPs and free oil [53], Hence, this study demonstrated that clove EO-chitosan NPs can be employed as a potential natural fungicide in the agriculture and food industries.

Unlike those of Hasheminejad and Khodaiyan [53] who demonstrated that crude clove EO did not entirely prevent the mycelial development of *A. niger*, even at concentrations as high as 3 mg/mL. However, after loading the oil into chitosan NPs, the encapsulated clove EO totally inhibited the fungus progress at 1.5 mg/mL.

Our obtained results agreed with those of Oliveira et al. [54] who found that exposing *A. flavus* to clove and rosemary EOs caused major apoptosis-like cell death and downregulation of the laeA, lipA, and metP genes provide new insights into the cellular and molecular antifungal processes of EOs against mycotoxigenic species. While both EOs have antifungal activities against *A. flavus*, clove EO reduced mycelial development extra effectively than rosemary EO. At the lowest tested EO concentrations (0.1 and 0.25 µL/mL), clove EO suppressed 75 and 85% of mycelial development ($p < 0.05$), respectively, but rosemary EO had no inhibition at the same concentrations. Clove EO at 0.5 µL/mL totally inhibited mycelial development (0 mm/day), while rosemary EO at all dosages did not entirely suppress *A. flavus* growth. Essential oils can cause programmed cell death (PCD) in fungus, which is defined as self-destruction in an adverse environment such as environmental stressors and exposure to hazardous metabolites [55]. The phenomenon is called by a sequence of morphological shifts that begin with nuclear compression, followed by plasma membrane blebbing, and the creation of apoptotic bodies, which are then consumed by phagosomes[56].

Clove-chitosan NPs 0.04% made potent inhibitory effect on *Geotrichum candidum* reached 87.47% followed by 66.47, 60.00, and 47.27 for clove 0.1, rosemary-chitosan NPs 0.04% and rosemary 0.1%, respectively. Then 100% reduction % at the end of 1st week of storage for cheese samples fortified with clove-chitosan NPs 0.04% and clove 0.1%. While 100% reduction % at the end of 2nd week of storage for cheese samples fortified with rosemary-chitosan NPs 0.04%, and at the end of 3rd week of rosemary 0.1%, *Geotrichum candidum* counts increased in the control group over the storage period (Table 4 and Graph. 2).

Clove EO has been proven to have powerful antifungal properties against both fungi, including A. flavus and *Geotrichum candidum*, but less so against *Geotrichum candidum*. This difference in potency can be due to the major component of clove EO, eugenol, which is a powerful antifungal agent. Its mechanism of action involves destroying fungal cell membranes, resulting in cell death. Furthermore, the nature of the fungal cell wall can affect its susceptibility to clove EO. Aspergillus flavus may have a cell wall composition that makes it more vulnerable to eugenol's disruptive effects than *Geotrichum candidum*. Furthermore, some fungi, such as *Geotrichum candidum*, may have efflux pumps that actively remove foreign compounds, including antifungal medicines. This can lower the effective concentration of clove essential oil within the fungal[57] .

Rosemary has the most potent action against potato *C. albicans*. The highest efficient concentration in all food forms was 500 µL. L-1. Rosemary officinalis has a variety of antifungal processes, as well as antioxidant and antibacterial properties. The results reveal that the plant EO can limit *C. albicans* adherence via cell structure denaturation and hence change membrane permeability. One study recommends that rosemary may even help to inhibit fungal biofilm formation. Nanoparticles of rosemary EO cover the structure, creating a nano biosystem that can dramatically reduce the adhesion and therefore biofilm of Candida fungal strains [58]. Several studies have indicated that rosemary inhibits the pathogen's growth as *S. aureus* [59, 60]. The optimized Cinnamomum verum EO/chitosan NPs showed significant antifungal efficacy against *C. albicans* infections (CMI = 125 µg/mL) [61] .

Bolouri *et al*. [47] showed that the antimicrobial action of rosemary can be attributed to phenolic chemicals, camphor, 1.8 cineole, and borneol derivatives, or the synergistic action of the complex or part of its components. Encapsulating rosemary increased its antifungal efficacy against *A. flavus* pathogenic fungus when compared to free EO. The reduction of bioactive chemicals to the nanoscale, which reduces their size and increases their surface area, will boost their efficacy [62]. Additionally, Bolouri *et al.* [47] concluded that free rosemary had the strongest antibacterial activity against the pathogenic fungus *C. Albicans* (0.009 mg/mL).

Conclusion

These findings concluded that clove and rosemary loaded on chitosan NPs could be used as antimicrobial agents in the dairy industry. Rosemary-chitosan NPs 0.04% reduced *S. aureus* growth by 62.57% and 100% inhibition at the end of 1st week of storage for all inoculated cheese samples. Also, we found 100% inhibition of *P. fluorescence* growth at the end of 2nd week of storage for rosemary-chitosan NPs and clove-chitosan NPs. In addition, clove-chitosan NPs had potent antifungal activity followed by rosemary-chitosan NPs, clove then rosemary EO. clove-chitosan NPs made inhibition to *A. flavus* and *Geotrichum candidum* by 90.10 and 87.47% respectively after 48hrs of storage. Clove-chitosan NPs and clove EO 0.1% made 100% reductions at the end of 1st week while rosemary-chitosan NPs made 100% reduction at end of 1st and 2nd week of storage for *A. flavus* and *Gortrichum candidum*, respectively. This study found that chitosan NPs containing clove and rosemary were efficient against various foodborne pathogens and appeared to have a positive influence on microbial reduction.

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Declaration of Conflict of Interest

The authors have declared that no conflict of interest exists.

Authors' contributions

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

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.

TABLE 1. Bacterial pathogens viability in soft cheese fortified by crude EOs and EOs-chitosan NPs: Cheese groups were divided into 10 groups as follows:

Groups	Group description
G1	Staphylococcus aureus inoculated group ($7\log_{10}$ CFU/mL)
G2	Pseudomonas fluorescence inoculated group ($7\log_{10}$ CFU/mL)
G3	Clove 0.1% + *S. aureus* ($7\log_{10}$ CFU/mL)
G4	Clove-chitosan NPs 0.04% +*S. aureus* ($7\log_{10}$ CFU/mL)
G5	Rosemary 0.1% + *S. aureus* ($7\log_{10}$ CFU/mL)
G6	Rosemary-chitosan NPs 0.04% +*S. aureus* ($7\log_{10}$ CFU/mL)
G7	Clove 0.1% + *P. fluorescence* ($7\log_{10}$ CFU/mL)
G8	Clove-chitosan NPs 0.04% + *P. fluorescence* ($7\log_{10}$ CFU/mL)
G9	Rosemary 0.1% + *P. fluorescence* ($7\log_{10}$ CFU/mL)
G10	Rosemary-chitosan NPs 0.04% + *P. fluorescence* ($7\log_{10}$ CFU/mL)

TABLE 2. Fungal pathogens viability in soft cheese fortified by crude EOs and EOs-chitosan NPs: Cheese groups were divided into 10 groups as follows:

Groups	Groups description
T1	Aspergillus flavus inoculated group ($6\log_{10}$ CFU/mL)
T2	Geotrichum candidum inoculated group ($6\log_{10}$ CFU/mL)
T3	Clove 0.1% + *A. flavus* ($6\log_{10}$ CFU/mL).
T4	Clove-chitosan NPs 0.04% + *A. flavus* ($6\log_{10}$ CFU/mL)
T5	Rosemary 0.1% ($6\log_{10}$cfu/ml) + *A. flavus* ($6\log_{10}$ CFU/mL)
T6	Rosemary-chitosan NPs 0.04% + *A. flavus* ($6\log_{10}$ CFU/mL)
T7	Clove 0.1% + *Geotrichum candidum* ($6\log_{10}$ CFU/mL)
T8	Clove-chitosan NPs 0.04% + *Geotrihcum candidum* ($6\log_{10}$cfu/ml)
T9	Rosemary 0.1% + *Geotrichum candidum* ($6\log_{10}$ CFU/mL)
T10	Rosemary-chitosan NPs 0.04 + *Geotrichum candidum* ($6\log_{10}$ CFU/mL)

TABLE 3. Statistical analysis of reduction percentage (%) of *Staphylococcus aureus* in soft cheese fortified by different concentrations of crude EOs and EOs-chitosan NPs during refrigeration storage.

Groups	Storge period				
	48 hrs.	**1st week**	**2nd week**	**3rd week**	**4th week**
Clove 0.1%	25.00 ± 0.58^{dB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Clove-chitosan NPs 0.04%	57.97 ± 0.15^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Rosemary 0.1%	26.30 ± 0.17^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Rosemary-chitosan NPs 0.04%	62.57 ± 0.15^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}

a, b and c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B and C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

TABLE 4. Statistical analysis of reduction percentage (%) of *Geotrichum candidum* in soft cheese fortified by different concentrations of crude EOs and EOs-chitosan NPs during refrigeration storage.

Groups	Storge period				
	48 hrs.	**1st week**	**2nd week**	**3rd week**	**4th week**
Clove 0.1%	66.47 ± 0.24^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Clove-chitosan NPs 0.04%	87.47 ± 0.26^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Rosemary 0.1%	47.27 ± 0.18^{dD}	64.37 ± 0.20^{cC}	70.30 ± 0.12^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Rosemary-chitosan NPs 0.04%	60.00 ± 0.12^{cC}	88.17 ± 0.12^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}

a, b and c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B and C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

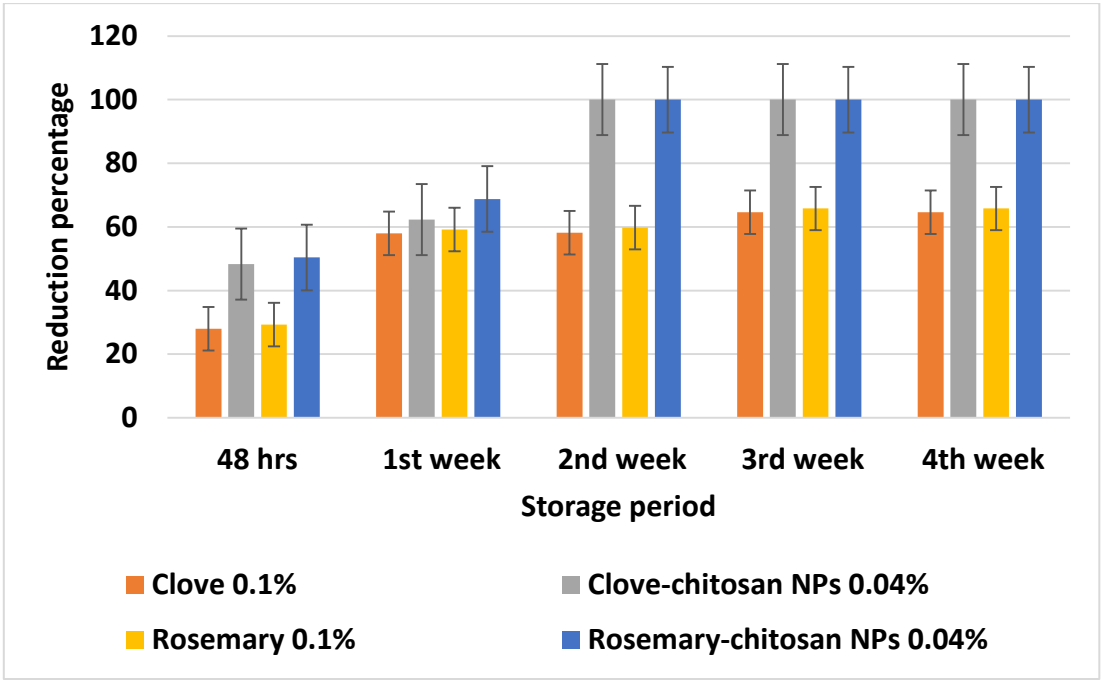


Fig. 1. Reduction percentage (%) of *Pseudomonas fluorescence* in soft cheese fortified by different concentrations of crude EOs and EOs-chitosan NPs during refrigeration storage.

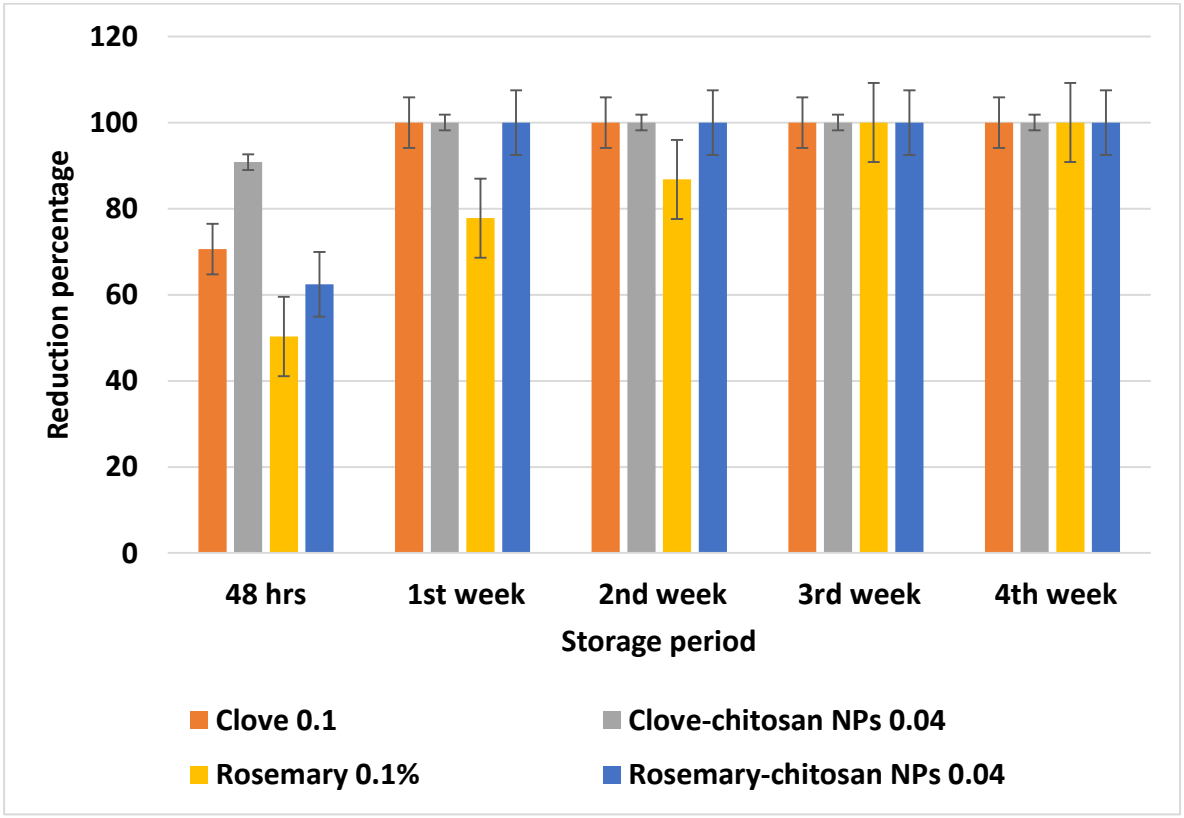
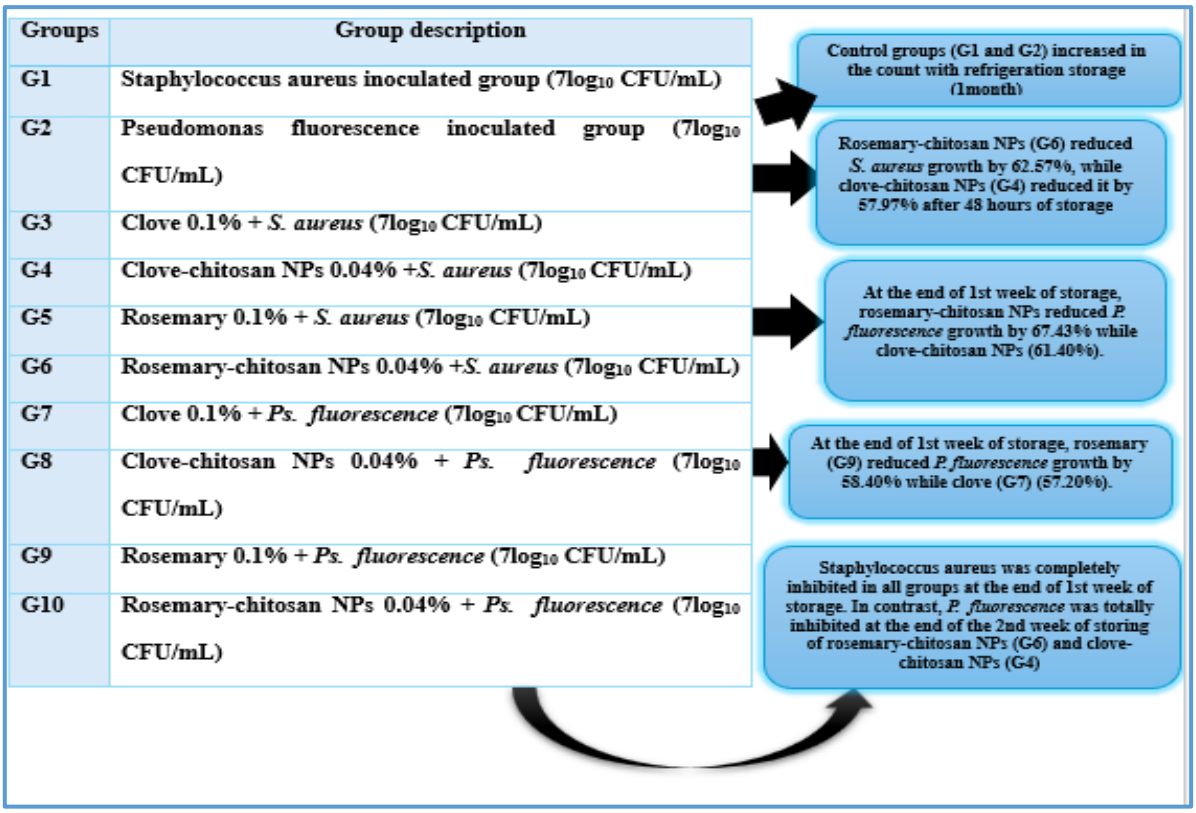
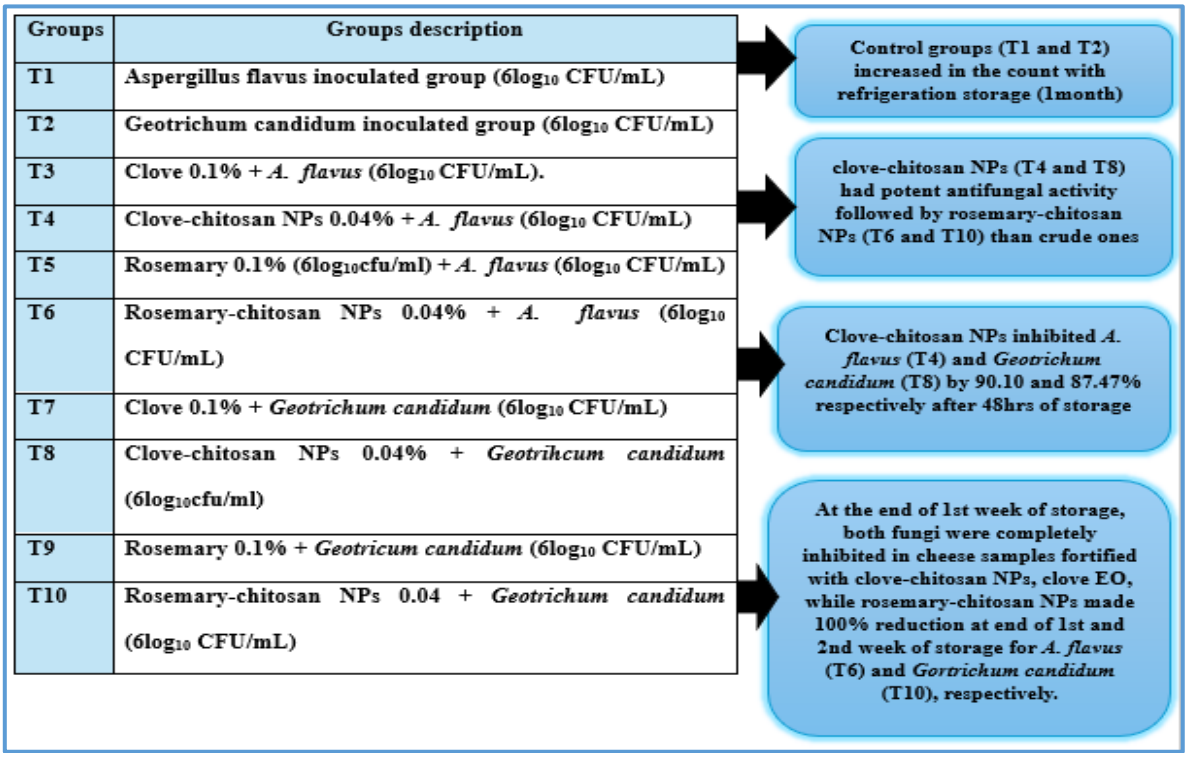


Fig. 2. Reduction percentage (%) of *Aspergillus flavus* in soft cheese fortified by different concentrations of crude EOs and EOs-chitosan NPs during refrigeration storage



Graph 1. Conclusion of viability of bacterial pathogens in fortified cheese samples.



Graph 2. Conclusion of viability of fungal pathogens in fortified cheese samples.

References

1. Deeb, M.M.A., Aman, I.M. and Ahmed, H.F. Bacteriological quality of Kareish cheese and trial to control Staphylococcus aureus in cheese. Alexandria journal of veterinary sciences, **21**(2), 514–523(2004).
2. Farkye, N.Y. and Vedamuthu, E.R., 2002. Microbiology of soft cheeses. Dairy microbiology handbook: the microbiology of milk and milk products, No. **Ed.3**, 479-513(2002).
3. Miladi, H., Zmantar, T., Chaabouni, Y. and Fedhila, K., Bakhrouf, A., Mahdouani, K. and Chaieb, K. Antibacterial and efflux pump inhibitors of thymol and carvacrol against food-borne pathogens. *Microbial Pathogenesis*, **99**, 95–100 (2016).
4. Silva, F. and Domingues, F.C. Antimicrobial activity of coriander oil and its effectiveness as food preservative. *Critical Reviews in Food science and Nutrition,* **57**(1), 35-47(2016).
5. Kure, C.F. and Skaar, I. The fungal problem in cheese industry. *Current Opinion in Food Science*, **29**, 14–19(2019).
6. Garnier, L., Valence, F. and Mounier, J. Diversity and control of spoilage fungi in dairy products: An update. Microorganisms, **5**(3), 42(2017).
7. Hymery, N., Vasseur, V., Coton, M. and Mounier, J., Jany, J.L., Barbier, G. and Coton, E. Filamentous fungi and mycotoxins in cheese: A review. *Comprehensive Reviews in Food Science and Food Safety,* **13**(4), 437–456(2014).
8. Pitt, J. Penicillium and Related Genera; Food Spoilage Microorganisms Woodhead Publishing Series in Food Science, Technology and Nutrition, 437-450(2006).
9. Kandasamy, S., Park, W.S., Yoo, J. and Yun, J., Kang, H.B., Seol, K.H., Oh, M.H. and Ham, J.S. Characterisation of fungal contamination sources for use in quality management of cheese production farms in Korea. *Asian-Australasian Journal of Animal Sciences,* **33**(6), 1002(2020).
10. Elsabee, M.Z. and Abdou, E.S. Chitosan based edible films and coatings: A review. *Materials Science and Engineering C,* **33**(4), 1819-1841(2013).
11. Perna, A., Simonetti, A. and Gambacorta, E. Phenolic content and antioxidant activity of donkey milk kefir fortified with sulla honey and rosemary essential oil during refrigerated storage. *International Journal of Dairy Technology*, **72**(1), 74–81(2019).
12. Liu, Q., Meng, X., Li, Y., Zhao, C.-N., Tang, G.Y. and Li, H.B. Antibacterial and antifungal activities of spices. *International Journal of Molecular Sciences*, **18**(6), 1283(2017).
13. Abdeltwab, W.M., Abdelaliem, Y.F., Metry, W.A. and Eldeghedy, M. Antimicrobial effect of chitosan and nano-chitosan against some pathogens and spoilage microorganisms. *Journal of Advanced Laboratory Research in Biology,* **10**(1), 8–15(2019).
14. Beyki, M., Zhaveh, S., Khalili, S.T. and Rahmani-Cherati, T., Abollahi, A., Bayat, M., Tabatabaei, M. and Mohsenifar, A. Encapsulation of Mentha piperita essential oils in chitosan-cinnamic acid nanogel with enhanced antimicrobial activity against Aspergillus flavus. *Industrial Crops and Products*, **54**,310-319(2014).
15. Piras, A.M., Maisetta, G., Sandreschi, S., Gazzarri, M., Bartoli, C., Grassi, L., Esin, S., Chiellini, F. and Batoni, G. Chitosan nanoparticles loaded with the antimicrobial peptide temporin B exert a long-term antibacterial activity in vitro against clinical isolates of Staphylococcus epidermidis. *Frontiers in Microbiology*, **6**,372(2015).
16. Ahmed, L.I., Sabry, D., Ragaa, H. and Abeer, A. Influence of yoghurt starter culture on viability of some pathogenic microorganisms in yoghurt. *International Journal of Dairy Science*, **9**(3),82–88(2014).
17. Guichard, H. and Bonnarme, P. Development and validation of a plate technique for screening of microorganisms that produce volatile sulfur compounds. *Analytical Biochemistry*, **338**(2),299–305(2005).
18. Scott, R., Robinson, R.K. and Wilbey, R.A. Cheesemaking Practice, Springer Science & Business Media, 1998.
19. Steel, R., Torrie, J. and Dickey, D. Principles and procedures of Statistics: A Biometrical Approach., McGraw-Hill, New York, NY, 1997.
20. [Bassi, D., Puglisi, E. and Cocconcelli, P.S. Understanding the bacterial communities of hard cheese with blowing defect. *Food Microbiology*, **52**,106–118(2015).
21. Ávila, M., Gómez-Torres, N., Delgado, D., Gaya, P. and Garde, S. Industrial-scale application of Lactobacillus reuteri coupled with glycerol as a biopreservation system for inhibiting Clostridium tyrobutyricum in semi-hard ewe milk cheese. *Food Microbiology*, **66**,104–109(2017).
22. Machado, S.G., Baglinière, F., Marchand, S., Van Coillie, E., Vanetti, M.C., De Block, J. and Heyndrickx, M. The biodiversity of the microbiota producing heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy products. *Frontiers in microbiology*, **8**,302(2017).
23. Prakash, B., Kedia, A., Mishra, P.K. and Dubey, N.K. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities–Potentials and challenges. *Food Control*, **47**, 381–391(2015).

24. Barrera-Ruiz, D.G., Cuestas-Rosas, G.C., Sánchez-Mariñez, R.I., Álvarez-Ainza, M.L., Moreno-Ibarra, G.M., López-Meneses, A.K., Plascencia-Jatomea, M. and Cortez-Rocha, M.O. Antibacterial activity of essential oils encapsulated in chitosan nanoparticles. *Food Science and Technology*, **40**, 568-573(2020).

25. Hasani, S., Ojagh, S.M. and Ghorbani, M. Nanoencapsulation of lemon essential oil in Chitosan-Hicap system. Part 1: Study on its physical and structural characteristics. *International Journal of Biological Macromolecules*, **115**,143–151(2018).

26. Hadidi, M., Pouramin, S., Adinepour, F., Haghani, S. and Jafari, S.M. Chitosan nanoparticles loaded with clove essential oil: Characterization, antioxidant and antibacterial activities. *Carbohydrate Polymers*, **236**, 116075(2020).

27. Kassem, A., Ayoub, G.M. and Malaeb, L. Antibacterial activity of chitosan nano-composites and carbon nanotubes: A review. *Science of The total Environment*, **668**,566–576(2019).

28. Chandrasekaran, M., Kim, K.D. and Chun, S.C. Antibacterial activity of chitosan nanoparticles: A review. *Processes,* **8**(9),1173(2020).

29. Cava, R., Nowak, E., Taboada, A. and Marin-Iniesta, F. Antimicrobial activity of clove and cinnamon essential oils against Listeria monocytogenes in pasteurized milk. *Journal of Food Protection*, **70**(12), 2757–2763(2007).

30. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods - A review. *International Journal of Food Microbiology,* **94**(3), 223–253(2004).

31. Feyzioglu, G.C. and Tornuk, F. Development of chitosan nanoparticles loaded with summer savory (Satureja hortensis L.) essential oil for antimicrobial and antioxidant delivery applications. *Lwt,* **70**, 104-110(2016).

32. Kavas, G., Kavas, N. and Saygili, D. The Effects of Thyme and Clove Essential Oil Fortified Edible Films on the Physical, Chemical and Microbiological Characteristics of Kashar Cheese. *Journal of Food Quality*, **38**(6),405–412(2015).

33. Ali, B.M., Ibrahim, O.M.S. and Ali, M. Antifungal Activity of Clove (Syzygium aromaticum) Essential Oil Extract against Induced Topical Skin Infection by Candida albicans in Mice in vivo. *The Egyptian Journal of Hospital Medicine*, **91**(1)3855-3861(2023).

34. Keawchaoon, L. and Yoksan, R. Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids and surfaces B: *Biointerfaces,* **84**(1)163-171 (2011).

35. Esmaeili, A. and Asgari, A. In vitro release and biological activities of Carum copticum essential oil (CEO) loaded chitosan nanoparticles. *International Journal of Biological Macromolecules*, **81**,283-290(2015).

36. Zhang, J., Wang, Y., Pan, D.D., Cao, J.X., Shao, X.F., Chen, Y.J., Sun, Y.Y. and Ou, C.R. Effect of black pepper essential oil on the quality of fresh pork during storage. *Meat Science*, **117**, 130-136 (2016).

37. Munteanu, B.S., Sacarescu, L., Vasiliu, A.-L., Hitruc, G.E., Pricope, G.M., Sivertsvik, M., Rosnes, J.T. and Vasile, C. Antioxidant/antibacterial electrospun nanocoatings applied onto PLA films. *Materials*, **11**(10),1973(2018).

38. Stoleru, E., Vasile, C., Irimia, A. and Brebu, M. Towards a bioactive food packaging: Poly(lactic acid) surface functionalized by chitosan coating embedding clove and argan oils. *Molecules*, **26**(15)4500 (2021).

39. Abdullah, B.H., Hatem, S.F. and Jumaa, W. A comparative study of the antibacterial activity of clove and rosemary essential oils on multidrug resistant bacteria. *Pharmaceutical and Biosciences Journal*, **3**(1)18-22(2015).

40. Wilkinson, J.M., Hipwell, M., Ryan, T. and Cavanagh, H.M.A. Bioactivity of Backhousia citriodora: antibacterial and antifungal activity. *Journal of Agricultural and Food Chemistry*, **51**(1),76–81(2003).

41. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R. and De Feo, V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals,* **6**(12), 1451-1474(2013).

42. Angane, M., Swift, S., Huang, K., Butts, C.A. and Quek, S.Y. Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods,* **11**(3), 464(2022).

43. Coşeriu, R.L., Vintilă, C., Pribac, M., Mare, A.D., Ciurea, C.N., Togănel, R.O., Cighir, A., Simion, A. and Man, A. Antibacterial effect of 16 essential oils and modulation of mex efflux pumps gene expression on multidrug-resistant Pseudomonas aeruginosa clinical isolates: is cinnamon a good fighter?. *Antibiotics,* **12**(1), 163(2023).

44. Ahmed, L.I., Ibrahim, N., Abdel-Salam, A.B. and Fahim, K.M. Potential application of ginger, clove and thyme essential oils to improve soft cheese microbial safety and sensory characteristics. *Food Bioscience*, **42**, 101177(2021).

45. Srivastava, A., Kumar, V. and Agarwal, V. Antimicrobial Activity of Some Essential Oils against Pseudomonas aeruginosa. In conference BioSangam 2022: Emerging trends in Biotechnology (BIOSANGAM 2022), Atlantis Press, 27–34(2022).

46. Araby, E. and El-Tablawy, S.Y. Inhibitory effects of rosemary (Rosemarinus officinalis L.) essential oil on pathogenicity of irradiated and non-irradiated Pseudomonas aeruginosa. *Journal of Photochemistry and Photobiology B: Biology,* **159**,24–32(2016).

47. Bolouri, P., Pınar, S.E.N. and Sahin, F. Antimicrobial Properties of Free and Encapsulated-essential Oil into Chitosan of Rosemary. *Research Square*, 1-15(2023) doi.org/10.21203/rs.3.rs-2646496/v1

48. Ayachi, N. Nanoencapsulation of Rosmarinus officinalis essential oil into Chitosan crosslinked to Tripolyphosphate nanoparticles and Alginate/Chitosan nanoparticles: Formulation, characterization, in vitro release study, and in vivo evaluation. *Journal of Drug Delivery and Therapeutics,* **13**(11), 45–55(2023).

49. Elsherif, W.M. and Talaat AL Shrief, L.M. Effects of three essential oils and their nano-emulsions on Listeria monocytogenes and Shigella flexneri in Egyptian Talaga cheese. International Journal of Food Microbiology, **355**,109334 (2021).

50. Kure, C.F. and Skaar, I. The fungal problem in cheese industry. *Current Opinion in Food Science*, **29**,14–19(2019).

51. Boukaew, S., Prasertsan, P. and Sattayasamitsathit, S. Evaluation of antifungal activity of essential oils against aflatoxigenic Aspergillus flavus and their allelopathic activity from fumigation to protect maize seeds during storage. *Industrial Crops and Products*, **97**,558–566(2017).

52. Hasheminejad, N., Khodaiyan, F. and Safari, M. Improving the antifungal activity of clove essential oil encapsulated by chitosan nanoparticles. *Food Chemistry*, **275**,113–122(2019).

53. Hasheminejad, N. and Khodaiyan, F. The effect of clove essential oil loaded chitosan nanoparticles on the shelf life and quality of pomegranate arils. *Food Chemistry*, **309**,125520 (2020).

54. Oliveira, R.C., Carvajal-Moreno, M., Mercado-Ruaro, P., Rojo-Callejas, F. and Correa, B. Essential oils trigger an antifungal and anti-aflatoxigenic effect on Aspergillus flavus via the induction of apoptosis-like cell death and gene regulation. *Food Control,* **110**, 107038(2020).

55. Sharon, A., Finkelstein, A., Shlezinger, N. and Hatam, I. Fungal apoptosis: function, genes and gene function. *FEMS Microbiology Reviews,* **33**(5),833–854(2009).

56. Ramsdale, M. Programmed cell death in pathogenic fungi. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, **1783**(7),1369–1380(2008).

57. Qi, X., Chen, B. and Rao, J. Natural compounds of plant origin in the control of fungi and mycotoxins in foods. *Current Opinion in Food Science,* **52**, 101054(2023).

58. Chifiriuc, C., Grumezescu, V., Grumezescu, A.M., Saviuc, C., Lazăr, V. and Andronescu, E. Hybrid magnetite nanoparticles/Rosmarinus officinalis essential oil nanobiosystem with antibiofilm activity. *Nanoscale Research Letters*, **7**, 1-7(2012).

59. Marinas, I., Grumezescu, A.M., Saviuc, C. Chifiriuc, C., Mihaiescu, D. and Lazar, V. Rosmarinus officinalis essential oil as antibiotic potentiator against Staphylococcus aureus. *Biointerface Research in Applied Chemistry*, **2**, 271–276(2012).

60. Gachkar, L., Yadegari, D. and Rezaei, M.B., Taghizadeh, M., Astaneh, S. A. and Rasooli, I. Chemical and biological characteristics of Cuminum cyminum and Rosmarinus officinalis essential oils. *Food Chemistry*, **102**(3), 898–904(2007).

61. Essid, R., Ayed, A., Djebali, K. and Saad, H., Srasra, M., Othmani, Y., Fares, N., Jallouli, S., Abid, I., Alothman, M.R. and Limam, F. Anti-Candida and anti-leishmanial activities of encapsulated Cinnamomum verum essential oil in chitosan nanoparticles. *Molecules,* **28**(15), 5681(2023).

62. Vanlalveni, C., Lallianrawna, S., Biswas, A. and Selvaraj, M., Changmai, B. and Rokhum, S.L. Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. *RSC Advances,* **11**(5), 2804–2837(2021).

قابلية بقاء مسببات الأمراض البكتيرية والفطرية في الجبن الطري الممزوج ببعض الزيوت العطرية - جزيئات نانوية من الكيتوزان

سميرة سعيد مكي، هند احمد البربري، اقبال محمد عادل ابراهيم وحمدي عبدالسميع محمد

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الملخص

في عملنا الحالي، استخدمنا زيوت القرنفل و الروزماري العطرية وجسيمات النانو من الزيوت العطرية والكيتوزان (مواد حافظة طبيعية للأغذية) بتركيزات مختلفة للكشف عن تأثيرها المثبط على قابلية بقاء مسببات الأمراض الميكروبية (*Staphylococcus aureus* و*Pseudomonas fluorescence*) مسببات الأمراض البكتيرية (*Aspergillus flavus* و*Geotricum candidum*) مسببات الأمراض الفطرية. تم إنتاج الجبن الطري كمجموعات تحكم، محصنة (مجموعات الجبن الخام من الزيوت العطرية (0.1٪) ومجموعات الجسيمات النانوية من الزيوت العطرية والكيتوزان (0.04٪). تم فحصها أثناء التخزين في الثلاجة. أظهرت الجسيمات النانوية من الزيوت العطرية والكيتوزان تأثيرًا مثبطًا قويًا من الجسيمات النانوية الخام. قللت الجسيمات النانوية من الروزماري والكيتوزان من نمو المكورات العنقودية الذهبية بنسبة 62.57٪ بينما قللت الجسيمات النانوية من القرنفل والكيتوزان (57.97٪) بعد تخزين 48 ساعة. في نهاية الأسبوع الأول من التخزين، قللت جزيئات الروزماري والكيتوزان من نمو*Ps. fluorescence* بنسبة 67.43٪ بينما (61.40٪) جزيئات القرنفل والكيتوزان. تم تثبيط المكورات العنقودية الذهبية تمامًا في جميع عينات الجبن الملقحة في نهاية الأسبوع الأول من التخزين، بينما تم تثبيط *Ps. fluorescence* تمامًا في نهاية الأسبوع الثاني من التخزين لجزيئات الروزماري والكيتوزان وجزيئات القرنفل والكيتوزان. بالنسبة لتأثيرها المضاد للفطريات، كان لجزيئات القرنفل والكيتوزان نشاط مضاد للفطريات قوي تليها جزيئات الروزماري والكيتوزان، والقرنفل ثم زيت الروزماري العطري. قامت جزيئات القرنفل والكيتوزان بتثبيط *A. flavus* و *Geotricum candidum* بنسبة 90.10 و 87.47٪ على التوالي بعد 48 ساعة من التخزين. في نهاية الأسبوع الأول من التخزين، تم تثبيط كلا الفطرين تمامًا في عينات الجبن المدعمة بجسيمات النانو القرنفلية والكيتوزان وزيت القرنفل العطري، بينما في نهاية الأسبوع الأول والثاني بالترتيب قامت جسيمات الروزماري والشيتوزان بتثبيط كلا من *A. flavus* و *Geotricum* في نهاية الاسبوع الاول والثاني من تخزين الجبن . وفي الختام، يوصى بالشكل المركب من القرنفل أو الروزماري مع جسيمات النانو الكيتوزان لحفظ الجبن الطري أكثر من الزيوت العطرية الخام. • في حين أظهرت جزيئات النانو المكونة من إكليل الجبل والكيتوزان انخفاضًا بنسبة 100% في نهاية الأسبوع الأول والثاني من التخزين لفطريات Aspergillus flavus وGortrichum candidum على التوالي. وفي الختام، يوصى باستخدام جزيئات النانو المكونة من القرنفل أو إكليل الجبل والكيتوزان لحفظ الجبن الطري بدلًا من الزيوت العطرية الخام.

الكلمات الدالة: مضاد للبكتيريا، مضاد للفطريات، الكيتوزان، الزيوت الأساسية، مدة الصلاحية، الجبن الطري.