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Antimicrobial Effects of Selenium and Chitosan Nanoparticles on Raw Milk and Kareish Cheese

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ABSTRACT

The contamination of milk and its dairy products with different microorganisms could cause public health hazards. Antibacterial nanoparticles (NPs) are a novel way to ensure that milk and milk products are safe. The present study investigated the effect of chitosan NPs (CS-NPs) and selenium NPs (Se-NPs) on some microorganisms, which consequently affect raw milk and Kareish cheese. Small-sized nanomaterials of Se-NPs and CS-NPs at the size of approximately 20 nm were used in this study. The samples were 700 ml raw milk and 700g Kareish cheese manufactured from 3000 mg milk. The concentrations of used nanoparticles were 0.5%, 1%, and 1.5% for Se-NPs and 2.5%, 5%, and 10% for CS-NPs. They were used to improve the microbial properties of milk and Kareish cheese samples during storage at the refrigerated temperature of 4°C. The aerobic plate count, Enterobacteriaceae count, Staphylococcus count, and mold count were significantly reduced in milk and Kareish cheese samples treated with CS-NPs and Se-NPs. The study has confirmed that CS-NPs and Se-NPs indicated high antimicrobial activity against the studied microorganisms at all concentrations although CS-NPs were more effective than Se-NPs. It can be concluded that these NPs can be used as preservatives in milk and milk products, such as Kareish cheese. In addition, increasing the concentrations of these NPs by 10% for CS-NPS and 1.5% for Se-NPS boosted their effects.

Keywords: Chitosan, *Enterobacteriaceae*, Kareish cheese, Nanoparticle, Selenium, *Staphylococcus aureus*

INTRODUCTION

In many parts of the world, milk and dairy products are perfect media for microorganism growth due to their high nutritional content (Ledenbach and Marshall, 2009). It is impossible to eliminate microbe contamination of milk during the preparation of various dairy products; consequently, the microbiological content of milk is an important factor in its quality from a safety standpoint (Singh et al., 2011). Many zoonotic bacteria, such as *Escherichia coli* (*E. coli*), *Salmonella*, and *Staphylococcus aureus* (*S. aureus*), can be found in dairy products and can cause serious diseases, especially in immunocompromised consumers (Pal, 2007).

Nanotechnology has made its way into improving the quality of food as well as unique food supplements, additives, and nutrients (Huang et al., 2017). It is a new technology that could mark the beginning of the second technological generation. It focuses on the characterization, fabrication, and manipulation of structures or materials smaller than 100 nm (Ozimek et al., 2010). It aims to improve the tastes, textures, and bioavailability of minerals and supplements, as well as extending the shelf life of the products (Chaudhry and Castle, 2011). As a result, nanotechnological advantages have lately been used to tackle food and environmental challenges **(**Jaiswal et al., 2019) by enhancing the quality of micronutrients during processing, storage, and distribution (Chen et al., 2006). Nanomaterials are now used in the food industry for various purposes, such as food ingredients or additives, or as part of packaging materials (Rhim et al., 2013). Selenium nanoparticles (Se-NPs) can be used instead of antibiotics, such as ampicillin, to prevent and treat a variety of bacterial diseases and infections in people. Nano selenium is 60 times more effective than traditional treatments in treating infections caused by *S. aureus*, *E. coli*, and *P. aeruginosa*.

It can improve absorption into plants, animals, people, and microbes and act as an antioxidant with a lower risk of selenium toxicity. Furthermore, one of the most important uses of Se-NPs is chemoprevention via immune activation (Majeed et al., 2018). Chitin is a polysaccharide of animal origin that has a fibrous structure and is abundant in nature. Chitosan (CS) can be made by removing the acetyl groups from the chitin structure that is the major component of the exterior skeleton of insects and crustaceans, such as shrimp, crabs, and lobster (Kumar et al., 2005). Because of its nontoxicity, biodegradability, and antibacterial characteristics (Widnyana et al., 2021), CS is used in biomedical research, agriculture, genetic engineering, as well as the food industry, and water treatment (El-Dahma et al., 2017). Chitosan has a stronger effect on Gram-positive bacteria (*S. aureus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and

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Lactobacillus bulgaris) than Gram-negative bacteria (*E. coli*, *Salmonella typhy* (Coma et al., 2003). In contrast, Chung et al. (2004) found that Gram-negative bacteria are more sensitive to CS than Gram-positive bacteria as they have substantially more hydrophilicity. Chitosan's antifungal effect is thought to be fungistatic rather than fungicidal, with the potential to transmit regulatory changes in both the host and the fungus (Raafat and sahl, 2009).

This study aimed to investigate the way CS-NPS and Se-NPs effectively reduce pathogens in milk and Kareish cheese during cold storage.

MATERIAL AND METHODS

Collection of samples

Fresh raw milk (700ml) used in this study was purchased from dairy shops in El Monofiya Governorate, Egypt. All samples were kept in an ice box, transferred to the laboratory with minimum delay under completely hygienic conditions, and examined as rapidly as possible. The total sample was divided into two parts, one for raw milk examination and the other for manufacturing Kareish cheese for cheese examination. The experiment was repeated three times on different batches of milk dairy shops.

Preparation of milk sample

The milk samples (700 ml of raw milk) were divided into seven groups, 100 ml per group. The first three groups were treated with Se-NPs at concentrations of 0.5%, 1%, and 1.5%. The second three groups were treated with 2.5%, 5%, and 10% CS-NPs. The seventh group served as control. All samples were kept at 4°C. The analysis of the samples was performed on at days 1, 3, 6, 9, 12, and 15 of storage.

Kareish cheese manufacturing

Cheese manufacture essentially involves the coagulation of casein. Raw milk was heated at 74°C for 15 seconds and then cooled rapidly to 40°C AQt this point, 1.5% yogurt starter culture was added for coagulation. When coagulation had been completed, the curd was transferred into gauze to get rid of whey in 24 hours. In the next step, cut and stored in its pasteurized salted whey (7% salt) for 24 hours. Cheese samples were stored at 4°C (Phelan et al., 1993). In Kareish cheese, the fat level in dry matter and the moisture content should not exceed 10% and 75%, respectively (Egyptian Standard 2000/4-1008). To manufacture 700g of Kareish cheese, 3000 ml of raw milk was used. After that, the cheese was divided into seven groups followed by the addition of Se-NPs (0.5%, 1%, and 1.5%) and CS-NPs (2.5%, 0.5%, and 1%).

Nanomaterials

The Se-NPs and CS-NPs were prepared at the Naqaa Foundation for Scientific Research, Technology, and Development in Giza, Egypt. The Se-NPs were prepared according to the modified method of Qian Li et al. (2010). The Se solution was obtained by adding 100 mM of Sodium selenite to 50 mM ascorbic acid. Varied sodium selenite to ascorbic acid ratios (1:1, 1:2, 1:3, 1:4, 1:5, 1:6) had been reacted from the stock solution. The ascorbic acid was added drop by drop to the sodium selenite under magnetic stirring at various rpm (200, 600, 1000 rpm) at room temperature for 30 minutes. Combos had been allowed to react with each other's in the targeted shape until the shade alternate was observed from colorless to mild orange. Soon after the shad alternate was once determined, the combination used to be diluted to 25 ml with double distilled water.

Chitosan NPs were prepared according to Calvo et al. (1998). Chitosan deacetylation was 75%, with a molecular weight of 200 KDa. The CS solution was made using the ionotropic gelation process, which involved dissolving 100 mg of CS in a 1 percent v/v acetic acid solution and stirring it at room temperature until it turned transparent. A 0.1 molar sodium hydroxide solution was added to the mixture with a pH of 6.5. In a Pyrex glass flask, 10 ml of 0.80 mg/ml tripolyphosphate aqueous solution was added dropwise at room temperature under a magnetic stirrer at 750 rpm. The solution was then sonicated at room temperature for 10 minutes at 80 percent amplitude using the SB-5200 DTD Ultrasonic Cleaner, China (Vaezifar, 2013). The CS-NP solution was filtered by nylon syringe 0.22µm mesh and then freeze-dried for subsequent analysis.

Microbiological assay

Serial dilution was prepared according to ISO 1999; the surface plate method determined aerobic plate count at 35°C (Petran et al., 2015). After that, Baird Parker Agar was used to isolate and differentiate coagulase-positive *staphylococci* in food (FDA, 2001). Colonies appeared in gray-black, and a clear halo was developed around colonies from coagulase-positive *S. aureus*. Enterobacteriaceae counts, *E coli*, and *Salmonella spp.*, were determined according to ISO 21528: 2017. Neutral red colonies resulted in pink colonies due to glucose fermentation resulting from produced acid and decreased PH. Finally, Enumeration and isolation of fungi were done according to International Commission on

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Microbiological Specifications for Foods (ICMSF, 1996) using Sabouraud dextrose agar with chloramphenicol (0.05 mg/ml), which was then incubated at 28-30°C for 2-21 days.

Statistical analysis

Microbiological data were converted into logarithms of the colony number of forming units (CFU/gm). The analysis of variance (ANOVA) was performed in SPSS software (Version 22, SPSS Inc. Chicago, IL, and USA). Means and standard deviations were calculated. By applying Duncan's Multiple Range test, multiple mean comparisons were made to measure the specific differences between pairs of means. Values were statistically significant at the $p \leq 0.05$ level

RESULTS AND DISCUSSION

Selenium has an antibacterial effect at extremely high concentrations (1.5 to 3 mg/kg body weight), which are fatal to living organisms. Therefore, its use is limited to medicinal purposes (Khiralla and El-Deeb 2015). According to some researchers, Se-NPs are better than elemental selenium because they have antibacterial activity at low doses of 20 µg/mL (Huang et al., 2017).

Because of its nontoxicity, biodegradability, and antibacterial characteristics, CS is used for various purposes (Widnyana et al., 2021), including food processing (Cheba, 2011). *In vitro* tests have shown that Gram-negative bacteria are more sensitive to CS than Gram-positive bacteria, with higher morphological alterations after treatment (Chen et al., 2002; Simunek et al., 2006; and Eaton et al., 2008). The amount of adsorbed CS is determined by the charge density on the cell surface. Adsorbed CS at higher levels would cause more cell membrane structure and permeability alterations. This indicates that the host-microbe influences the antibacterial method of action (Másson et al., 2008). In the current study, the microbiological changes as aerobic plate count (APC), *Staphylococcus* spp. count, Enterobacteriaceae count, and mold count of milk and cheese samples were estimated throughout the cooling storage at 4°C for 15 days

Aerobic bacterial count

Aerobic bacterial count in milk samples

The initial total bacterial load was reduced over time when CS-NPs and Se-NPs were added to milk samples. During chilling storage of milk samples treated with CS-NPs, APC decreased from 5.71 to 4.2 ~1 $\log_{10}$CFU/ml at a concentration of 2.5%. The microbial effect of CS-NPs against total bacterial count increased by increasing the concentration of CS-NP**s**, so when the concentration reached 10%, the count of total bacterial count significantly decreased from 5.71 to 3.86 (~2 $\log_{10}$) CFU/ml (Table 1, $p \leq 0.05$). In milk samples treated with 0.5% Se-NPs, APC decreased from 5.71 to 4.2 ~1 $\log_{10}$ CFU/ml, but when the concentration reached 1.5%, APC decreased from 5.71 to 3.8 ~2 $\log_{10}$ CFU/ml ($p \leq 0.05$, Table 1). The Egyptian standards for raw milk (ES:154-1/2005) mentioned that the acceptable count of total bacterial count should be less than 200 count /ml (EOS, 2005)**.**

Aerobic bacterial count in Kareish cheese samples

In Kareish cheese samples treated with 10% CS-NPs, the APC count decreased from 4.56 to 2.63 ~2 $\log_{10}$ CFU/ml (Table 2) and the APC count significantly decreased from 4.56 to 3.4 ~1 $\log_{10}$ CFU/ml in cheese samples treated with 1.5% Se-NPS (Table 2, $p \leq 0.05$). According to the obtained results, 10% CS-NPs was the most effective antimicrobial agent (against total bacterial count followed by 1.5% Se-NPs. As Hariharan et al. (2012) stated, the antibacterial activity was related to the concentration of nanoparticles. Gram-positive and Gram-negative bacteria are both inhibited by the antibacterial mechanism of Se-NPs, which is unknown yet. Currently, it is thought that Se-NPs break the bacterial cell wall by interacting with the peptidoglycan layer and damaging the double-stranded DNA structure (Sonkusre et al., 2014). Chitosan NPs have antimicrobial activity due to electrostatic interaction between their positive charge and the negative charge of bacterial membranes leading to cell membrane lysis of bacterial cells (Rabea et al., 2003; Tripathi et al., 2008). Chitosan NPs can also interact with essential microbial nutrients, causing microbial growth disruption and eventually death (Jia et al., 2001; Rabea et al., 2003).

***Staphylococci* bacterial count**

As shown in figures 1 and 2, the antibacterial effect of nanoparticles against staphylococci in milk and Kareish cheese, respectively, was confirmed.

Staphylococci count in milk samples

Staphylococci count decreased in milk samples from 4.66 to 3.36 ~1 $\log_{10}$ CFU/ml at the concentration of 2.5% CS-NPs. Moreover, the microbial effect of CS-NPs against *staphylococci* increased by increasing the concentration of CS-NP**s,** so the count of *staphylococci* decreased from 4.66 to 2.66 (~2 $\log_{10}$) CFU/ml when the concentration reached 10% (Figure 1). In milk samples treated with 0.5% Se-NPs, *staphylococci* decreased from 4.66 to 3.3 ~1 $\log_{10}$ CFU/ml, and when the concentration reached 1.5%, *staphylococci* decreased from 4.66 to 2.7 ~2 $\log_{10}$ CFU/ml (Figure 1).

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Table 1. Effects of different concentrations of chitosan and selenium nanoparticles on the aerobic plate count of the examined milk samples during storage at 4ºC

Groups	First day	Third day	Sixth day	Ninth day	Twelfth day	Fifteenth day
Control	5.71 ± 0.05^{a}	5.84 ± 0.089^{b}	6.48 ± 0.094 ^{d}	6.87 ± 0.061^{d}	7.51 ± 0.047^{d}	7.88 ± 0.04^{d}
2.5% chitosan	5.71 ± 0.05^{a}	5.57 ± 0.02^{ab}	5.25 ± 0.031^{ab}	4.84 ± 0.01^{e}	4.61 ± 0.02^{e}	4.2 ± 0.03^{f}
5% chitosan	5.71 ± 0.05^{a}	5.43 ± 0.03^{ab}	5.19 ± 0.02^{ab}	4.72 ± 0.01^{e}	4.27 ± 0.01^{f}	3.86 ± 0.04^{fg}
10% chitosan	5.71 ± 0.05^{a}	5.11 ± 0.035^{ab}	4.86 ± 0.03^{c}	4.5 ± 0.01^{f}	3.74 ± 0.04^{fg}	3.17 ± 0.05^{g}
0.5% selenium	5.71 ± 0.05^{a}	5.67 ± 0.01^{ab}	5.46 ± 0.06^{ab}	4.91 ± 0.03^{e}	4.80 ± 0.02^{e}	4.62 ± 0.02^{f}
1% selenium	5.71 ± 0.05^{a}	5.58 ± 0.01^{ab}	5.43 ± 0.01^{ab}	4.79 ± 0.02^{e}	4.53 ± 0.01^{f}	4.39 ± 0.01^{fg}
1.5% selenium	5.71 ± 0.05^{a}	5.38 ± 0.03^{ab}	5.17 ± 0.02^{ab}	4.67 ± 0.01^{e}	4.38 ± 0.01^{f}	3.8 ± 0.07^{fg}

The values represented as mean ± standard deviation of three experiments. $^{a, b,c, d,e, f, g}$ means superscript letters within a column are significantly different ($p \leq 0.05$).

Table 2. Effect of different concentrations of chitosan and selenium nanoparticles on the aerobic plate count of the examined cheese samples during storage at 4ºC

Groups	First day	Third day	Sixth day	Ninth day	Twelfth day	Fifteenth day
Control	4.56 ± 0.3^{a}	4.76 ± 0.3^{b}	4.98 ± 0.3^{e}	5.39 ± 0.13^{e}	5.56 ± 0.13^{g}	6.3 ± 0.4^{g}
2.5% chitosan	4.56 ± 0.3 ^{a}	4.37 ± 0.2^{ab}	4.22 ± 0.15^{c}	3.85 ± .02^{d}	3.45 ± 0.1^{d}	3.33 ± 0.11^{d}
5% chitosan	4.56 ± 0.3^{a}	4.31 ± 0.11^{ab}	4.19 ± 0.11^{cd}	3.63 ± 0.1^{d}	3.4 ± 0.17^{d}	3.32 ± 0.16^{d}
10% chitosan	4.56 ± 0.3 ^{a}	4.24 ± 0.2^{ab}	4.15 ± 0.21^{d}	3.42 ± 0.15^{d}	3.25 ± 0.14^{f}	2.63 ± 0.2^{h}
0.5% selenium	4.56 ± 0.3 ^{a}	4.45 ± 0.15^{ab}	4.37 ± 0.2^{c}	4.25 ± 0.2^{c}	4.13 ± 0.1^{c}	3.82 ± 0.13^{d}
1% selenium	4.56 ± 0.3 ^{a}	4.4 ± 0.1^{ab}	4.28 ± 0.1^{cd}	4.13 ± 0.3^{c}	3.85 ± 0.3^{d}	3.6 ± 0.11^{d}
1.5% selenium	4.56 ± 0.3 ^{a}	4.32 ± 0.2^{ab}	4.22 ± 0.1^{cd}	3.91 ± 0.2^{cd}	3.61 ± 0.1^{d}	3.41 ± 0.20^{d}

The values represented as mean ± standard deviation of three experiments. $^{a, b, c, d, e, f, g}$ means superscript letters within a column are significantly different ($p \leq 0.05$).

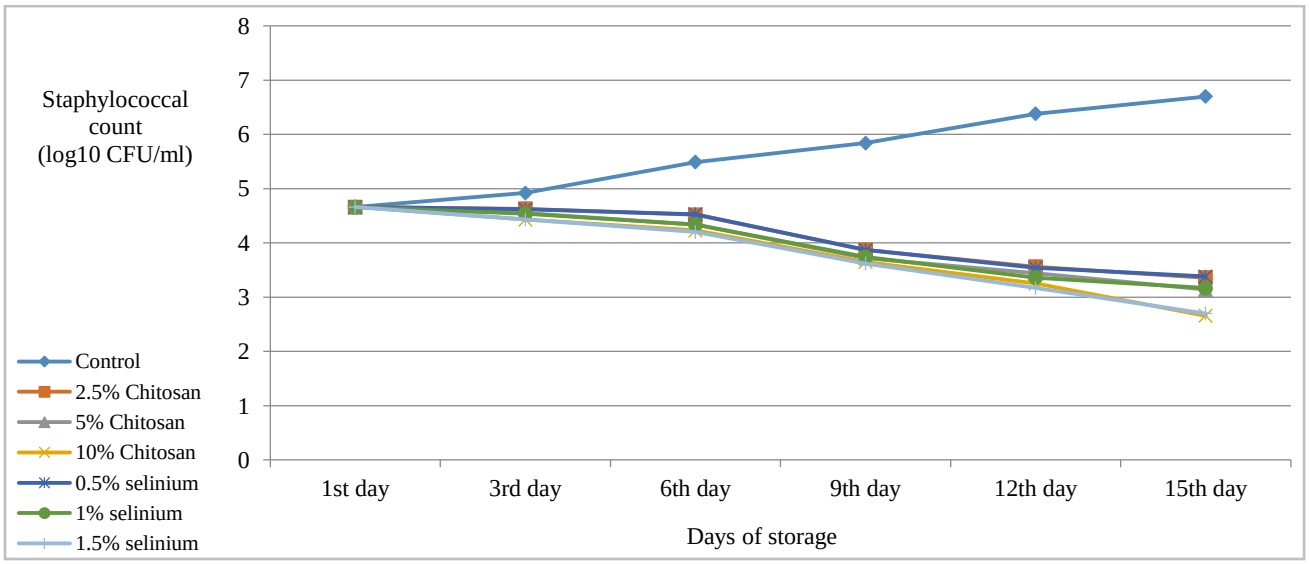

Figure 1. Effect of different concentrations of chitosan and selenium nanoparticles on *Staphylococci* count of the examined milk samples during storage at 4ºC

Staphylococci count in Kareish cheese samples

Staphylococci count decreased in cheese samples from 4.43 to 3.24 ~1 $\log_{10}$CFU/ml using 2.5% CS-NPs. The microbial effect of CS-NPs against *staphylococci* increased by increasing the concentration of CS-NP**s,** so when the concentration reached 10%, the count of *staphylococci* decreased from 4.43 to 2.58 ~2 $\log_{10}$ CFU/ml (Figure 2). In cheese samples treated with Se-Nps at the concentration of 0.5%, *staphylococci* decreased from 4.43 to 3.63~1 $\log_{10}$ CFU/ml, and when the concentration reached 1.5%, *staphylococci* decreased from 4.43 to 3.2 ~1 $\log_{10}$ CFU/ml as seen in Figure 2. Similar to the findings of Qi et al. (2004), Ro-drigus-Nunez et al. (2012), Salmabi and Seema (2013), Van Toan et al. (2013), Younes et al. (2014), and Widnyana et al. (2021), the *S. aureus* was inhibited by CS. Moreover, the antimicrobial effect of Se-NPs recorded by Khiralla and El-Deeb (2015) indicated that the inhibition zone increased with an increase in the concentration of Se-NPs. The Se-NPs were reported to be a potent antimicrobial agent against *S. aureus* (Chudobova et al., 2014). According to Phong et al. (2011), the proportion of live *S. aureus* decreased in the presence of Se-NPs at 7.8, 15.5, and 31 g/mL after 3, 4, and 5 hours. The Egyptian standards for Kareish cheese (No.1008/2000) mentioned that *S. aureus* (coagulate-positive) was absent in 1 g (EOS, 2000).

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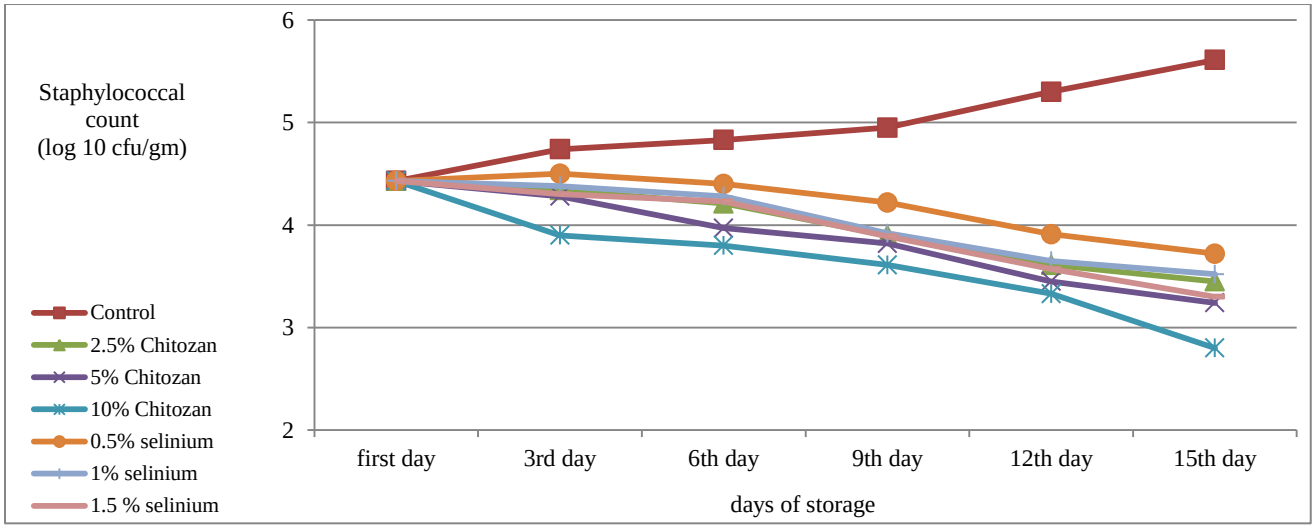

Figure 2. Effect of different concentrations of chitosan and selenium nanoparticles on *Staphylococci* count of the examined cheese samples during storage at 4ºC

Enterobacteriaceae count

Tables 3 and 4 showed that results obtained from treated samples significantly differed from the control result ($p \leq 0.05$). The antibacterial and antibacterial assays of CS-NP**s** against Gram-negative and Gram-positive bacteria were applied at different concentrations. The inhibition increased by increasing the concentration of CS-NP**s**. Gram-negative bacteria were more sensitive to CS-NP**s** than gram-positive bacteria (Coma et al., 2003).

Enterobacteriaceae count in milk samples

Enterobacteriaceae count has been decreased in milk samples from 4.57 to 3.27 ~1 $\log_{10}$CFU/ml at a concentration of 2.5%, and the microbial effect of CS-NPs against Enterobacteriaceae increased by increasing the concentration of CS-NP**s**. Enterobacteriaceae were not detected at the highest concentration of chitosan, 10% (Table 3). In milk samples treated with 0.5% Se-Nps, Enterobacteriaceae decreased from 4.57 to 3.39 ~1 $\log_{10}$ CFU/ml, but when the concentration reached 1.5%, *staphylococci* decreased from 4.57 to 2.65 ~1 $\log_{10}$ CFU/ml (Table 3). The Egyptian standards for raw milk (ES:154-1/2005) declared that the total coliforms are less than 10 count/ml *E. coli* and *Salmonella* and other pathogens absent in 1 ml (EOS, 2005).

Enterobacteriaceae count in Kareish cheese samples

Enterobacteriaceae count significantly decreased in cheese samples from 3.44 to 2.63 ~1 $\log_{10}$CFU/ml in different concentrations of CS-NP**s** and Se-Nps ($p \leq 0.05$, Table 4). The results agreed with Balicka-Ramisz et al. (2005), Liu et al. (2006), and Chung and Chen (2008), who reported that CS had antibacterial activity against *E. coli*. Balicka-Ramisz et al. (2005) and Benhabiles et al. (2012) recorded the antibacterial activities of CS against *Salmonella* sp. Results agreed with Hassanien and Shaker (2020), who used CS-NPs at a 30 µg/mL concentration. Chitosan NPs exerted a high bactericidal effect on isolates, such as *E. coli O157:H7* recovered from Kareish cheese samples, which significantly increased with an increase in concentration. Khiralla and El-Deeb (2015) evaluated the effect of Se-NPs against foodborne pathogens, such as *E. coli* and *S. aureus*. They found that the inhibition zone increases with increasing Se-Nps concentration. According to Shrestha et al. (2010) and Khurana et al. (2019), CS-NPs and Se-NPs have antibacterial effects against *Enterococcus faecalis*. Selenium NPs were highly effective against *E. faecalis* biofilm at the concentration of 1mg/ml (Sanjay et al., 2021). The Egyptian standards for Kareish cheese (No.1008/2000) mentioned that the total coliforms should be less than 10 CFU/g, *E. coli* should be absent in 1g Kareish cheese, *Salmonella* and other pathogens absent in 25 g (EOS, 2000).

Molds count

The antibacterial action of nanoparticles against molds was demonstrated by the fact that the counts of treated and control samples were significantly different ($p \leq 0.05$)**.** Molds were not detected at high concentrations of CS-NPs (10%) and Se-NPs (1.5%) in milk samples (Table 5). However, the mold count significantly decreased by ~1 $\log_{10}$ CFU/ml in cheese samples at high concentrations CS-NPs (10%) and Se-NPs (1.5%), respectively ($p \leq 0.05$, Table 6). Antifungal activity in the current study agreed with that of Yien et al. (2012), indicating that the CS-NP**s** were observed to be natural antifungal agents when used in concentrations of 1-3 mg/ml against *Candida albicans*, *Aspergillus niger*, and *Fusarium solani* pathogenic strain isolated from clinical specimens. Moreover, a study by Shakibaie et al. (2015) indicated the anti-biofilm activity of biologically generated (Se-NPs) in concentrations ranging from 10 to 200 mg/mL against the biofilm

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produced by clinically isolated fungus strains, such as *Aspergillus fumigatus* and *Candida albicans*. The obtained results of the current study agreed with those of Rasha et al. (2019), indicating that the use of CS-NPS in concentrations of 0.25% and 0.5% before or after manufacturing Kareish cheese could prolong safe preservation as the nano-chitosan have the antimicrobial potential against several bacteria and fungi, such as *Aspergillus flavus*. Furthermore, Elsharawy et al. (2019) revealed that the mold counts in Kareish cheese treated with 1% CS were lower than untreated cheese samples during the storage period. Since direct contact with CS causes hyphae to weaken and swell, the fungistatic characteristics of CS are linked to its ability to induce morphological changes in the cell wall (Rabea et al., 2003). The Egyptian standards for Kareish cheese (No.1008/2000**)** mentioned that yeasts and molds should be less than 10 CFU/g (EOS, 2000).

Table 3. Effect of different concentrations of chitosan and selenium nanoparticles on Enterobacteriaceae count of the examined milk samples during storage at 4ºC

Groups	First day	Third day	Sixth day	Ninth day	Twelfth day	Fifteenth day
Control	$4.57 \pm 0.0\ 2^{a}$	4.76 ± 0.09^{a}	4.97 ± 0.04^{a}	5.53 ± 0.06^{a}	5.89 ± 0.05^{e}	6.2 ± 0.04^{g}
2.5% chitosan	$4.57 \pm 0.\ 02^{a}$	4.17 ± 0.02^{a}	3.92 ± 0.031^{b}	3.79 ± 0.01^{c}	3.65 ± 0.02^{c}	3.27 ± 0.03^{bc}
5% chitosan	$4.57 \pm 0.\ 02^{a}$	4.34 ± 0.03^{a}	3.73 ± 0.02^{c}	3.11 ± 0.01^{bc}	3.14 ± 0.01^{dc}	2.75 ± 0.04^{f}
10% chitosan	$4.57 \pm 0.\ 02^{a}$	4.19 ± 0.04^{b}	3.36 ± 0.03^{bc}	2.82 ± 0.01^{d}	2.74 ± 0.04^{f}	*ND
0.5% selenium	$4.57 \pm 0.0\ 2^{a}$	4.49 ± 0.01^{a}	4.17 ± 0.06^{b}	3.9 ± 0.03^{c}	3.87 ± 0.02^{c}	3.39 ± 0.02^{c}
1 % selenium	$4.57 \pm 0.\ 02^{a}$	4.36 ± 0.01^{a}	3.76 ± 0.01^{c}	3.44 ± 0.02^{c}	3.57 ± 0.01^{dc}	2.95 ± 0.01^{f}
1.5 % selenium	4.57 ± 0.02^{a}	4.23 ± 0.03^{b}	3.57 ± 0.02^{c}	3.35 ± 0.01^{d}	2.9 ± 0.01^{f}	2.65 ± 0.07^{f}

The values represented as mean ± standard deviation of three experiments. $^{a, b, c, d, e, f, g}$ means superscript letters within a column are significantly different ($p \leq 0.05$). *ND: Not detected

Table 4. Effect of different concentrations of chitosan and selenium nanoparticles on Enterobacteriaceae count of the examined cheese samples during storage at 4ºC.

Groups	First day	Third day	Sixth day	Ninth day	Twelfth day	Fifteenth day
Control	3.44 ± 0.1^{a}	3.56 ± 0.3^{a}	3.98 ± 0.1^{e}	4.26 ± 0.13^{e}	4.56 ± 0.13^{f}	4.73 ± 0.2^{f}
2.5% chitosan	$3.44 \pm 0.1\ ^{a}$	3.33 ± 0.2^{ab}	3.27 ± 0.1^{b}	$3.12 \pm .02^{c}$	2.91 ± 0.1^{cd}	2.63 ± 0.11^{d}
5% chitosan	$3.44 \pm 0.1\ ^{a}$	3.28 ± 0.1^{ab}	3.21 ± 0.1^{b}	2.85 ± 0.2^{cd}	2.66 ± 0.2^{d}	2.42 ± 0.14^{d}
10% chitosan	3.44 ± 0.1^{a}	3.2 ± 0.1^{ab}	3.15 ± 0.1^{c}	2.69 ± 0.15^{d}	2.52 ± 0.14^{d}	2.32 ± 0.2^{g}
0.5% selenium	3.44 ± 0.1^{a}	3.4 ± 0.2^{ab}	3.33 ± 0.2^{b}	3.22 ± 0.2^{c}	3.12 ± 0.1^{c}	2.83 ± 0.15^{cd}
1% selenium	3.44 ± 0.1^{a}	3.35 ± 0.1^{ab}	3.29 ± 0.1^{b}	3.13 ± 0.3^{c}	2.84 ± 0.1^{cd}	2.64 ± 0.21^{cd}
1.5% selenium	3.44 ± 0.1^{a}	3.3 ± 0.2^{ab}	3.25 ± 0.1^{b}	2.98 ± 0.2^{cd}	2.72 ± 0.1^{cd}	2.58 ± 0.23^{d}

The values represented as mean ± standard deviation of three experiments. $^{a, b, c, d, e, f, g}$ means superscript letters within a column are significantly different ($p \leq 0.05$).

Table 5. Effect of different concentrations of chitosan and selenium nanoparticles on molds count of the examined milk samples during storage at 4ºC.

Groups	First day	Third day	Sixth day	Ninth day	Twelfth day	Fifteenth day
Control	3.8 ± 0.04^{a}	3.96 ± 0.01^{a}	4.73 ± 0.09^{e}	4.95 ± 0.061^{e}	5.67 ± 0.04^{g}	5.92 ± 0.04^{g}
2.5% chitosan	3.8 ± 0.04^{a}	3.51 ± 0.02^{a}	3.35 ± 0.031^{ab}	3.22 ± 0.01^{c}	2.81 ± 0.02^{d}	2.3 ± 0.03^{d}
5% chitosan	3.8 ± 0.04^{a}	3.36 ± 0.03^{ab}	3.21 ± 0.02^{ab}	2.7 ± 0.01^{cd}	2.63 ± 0.01^{d}	*ND
10% chitosan	3.8 ± 0.04^{a}	3.11 ± 0.03^{b}	2.9 ± 0.03^{c}	2.59 ± 0.01^{d}	2.35 ± 0.04^{f}	*ND
0.5% selenium	3.8 ± 0.04^{a}	3.75 ± 0.01^{a}	3.67 ± 0.06^{ab}	3.41 ± 0.03^{c}	3.11 ± 0.02^{cd}	2.7 ± 0.02^{d}
1% selenium	3.8 ± 0.04^{a}	3.68 ± 0.01^{a}b	3.56 ± 0.01^{ab}	3.28 ± 0.02^{cd}	2.94 ± 0.01^{d}	*ND
1.5% selenium	3.8 ± 0.04^{a}	3.59 ± 0.03^{ab}	3.34 ± 0.02^{ab}	2.91 ± 0.01^{cd}	2.55 ± 0.01^{d}	*ND

The values represented as mean ± standard deviation of three experiments. $^{a, b, c, d, e, f, g}$ means superscript letters within a column are significantly different ($p \leq 0.05$). *ND: Not detected.

Table 6. Effect of different concentrations of chitosan and selenium nanoparticles on molds count of the examined cheese samples during storage at 4ºC

Groups	First day	Third day	Sixth day	Ninth day	Twelfth day	Fifteenth day
Control	3.54 ± 0.3^{a}	3.73 ± 0.2^{a}	3.92 ± 0.21^{e}	4.34 ± 0.13^{e}	4.57 ± 0.2^{f}	5.35 ± 0.3^{f}
2.5% chitosan	3.54 ± 0.3^{a}	3.43 ± 0.14^{ab}	3.33 ± 0.15^{b}	3.22 ± 0.2^{c}	2.83 ± 0.12^{cd}	2.63 ± 0.12^{d}
5% chitosan	3.54 ± 0.3^{a}	3.36 ± 0.12^{ab}	3.26 ± 0.11^{b}	3.13 ± 0.11^{cd}	2.72 ± 0.15^{d}	2.53 ± 0.1^{d}
10% chitosan	3.54 ± 0.3^{a}	3.24 ± 0.2^{ab}	3.16 ± 0.14^{c}	2.77 ± 0.12^{d}	2.53 ± 0.13^{d}	2.42 ± 0.14^{g}
0.5% selenium	3.54 ± 0.3^{a}	3.48 ± 0.13^{ab}	3.4 ± 0.21^{b}	3.32 ± 0.14c	3.13 ± 0.1^{c}	2.76 ± 0.11^{cd}
1% selenium	3.54 ± 0.3^{a}	3.39 ± 0.11^{ab}	3.31 ± 0.11^{b}	3.15 ± 0.20^{c}	2.92 ± 0.14^{cd}	2.68 ± 0.2^{cd}
1.5% selenium	3.54 ± 0.3^{a}	3.3 ± 0.21^{ab}	3.25 ± 0.2^{b}	2.91 ± 0.21^{d}	2.65 ± 0.1^{cd}	2.52 ± 0.13^{d}

The values represented as mean ± standard deviation of three experiments. $^{a, b, c, d, e, f, g}$ means superscript letters within a column are significantly different ($p \leq 0.05$).

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CONCLUSION

Biocompatible Se-NPs and CS-NPs had high antimicrobial activity against pathogenic and spoilage Gram-positive and Gram-negative bacteria, as well as molds that affect raw milk and Kareish cheese. According to this study, nanoparticles can be employed as a preservative in milk and Kareish cheese to extend their shelf life. Further studies should be conducted on the effectiveness of nanotechnology and nanoparticles on dairy products, their prevention of microbial contamination, and the limitation of mold excretions like aflatoxins.

DECLARATIONS

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Authors' contribution

Hend Ahmed Elbarbary and Hamdy Abd El Samea Mohamed created the study plan and revised the research article. Shimaa Nabil Mohamed and Nahla Abo EL-Roos examined the data and conducted laboratory experiments. Shimaa Nabil did the statistical analysis and wrote the paper. Nahla Abo EL-Roos, who also revised the research manuscript, provided the experimental instruments. All authors read and approved the final version of the manuscript for publishing in the present journal

Competing interests

There are no conflicts of interest declared by the authors.

Ethical consideration

The author checked the manuscript for ethical issues, such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publishing and/or submission, and redundancy.

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