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Antimicrobial activity of Chitosan nanoparticles against *Escherichia coli and Aspergillus flavus* strain in kareish cheese.

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ABSTRACT

Kareish cheese is the most famous soft cheese in Egypt. The distinctly nutritious nature makes them accurate media for bacterial growth during processing, it is hard to produce milk free from microbes therefore, the microbial content of milk is a crucial feature in figuring out its quality.so the effect of different concentrations of chitosan nanoparticles on some pathogenic microbes like Escherichia coli and *Aspergillus flavus* could be determined. Different concentrations of chitosan nanoparticles (CSNPs) were utilized to improve the bacterial condition of made kareish cheese during storage at cold temperatures (2.5%, 5% and 10%). After being inoculated with *E. coli* and *Aspergillus flavus*, cheese samples were treated with various doses of chitosan nanoparticles and stored at 4°C. According to the findings, (CSNPs) (10%) had the greatest influence on the inoculated microbial count in cheese samples, followed by CSNPs (5%) and (2.5%). It may be inferred that chitosan nanoparticles (CSNPs) have an antimicrobial action against these species, which is amplified by increasing CSNps concentration. As conclusion we suggest using chitosan nanoparticles to preserve kareish cheese

1. INTRODUCTION

Many zoonotic bacteria as E. coli, salmonella, and S. aureus, can be found in dairy products and cause significant illness, especially people with impaired immune systems (Pal, 2007; FAO, 2013).

Kareish Cheese is a curd of milk solids that has been stabilized by casein coagulation and milk fat entrapment in the coagulum (Fernandes, 2008). Microbial contamination of cheese can come from a variety of places, including the cheese handler, the packing material, and the environment (Pal et al., 2014). As a result, chemicals that are efficient against bacteria, particularly E. coli, and improve microbiological conditions must be sought. The main step in preventing foodborne diseases is to track the source of E. coli (liu et al., 2019). In the food sector, significant efforts are being made to improve cleanliness and extend shelf life by inhibiting the multiplication of food-borne viruses so, Nanotechnology has the potential to improve food safety and quality control while also extending the shelf life of foods in numerous sections of the food supply chain (Baltić et al., 2013).

Nanotechnology also affects the development of novel or enhanced flavors, textures, and bioavailability of minerals and supplements, as well as the reduction of preservatives and other unwanted or potentially dangerous compounds in food items (Chaudhry and Castle, 2011). Nanomaterials are now used in food applications in a variety of ways, such as food ingredients or additions, or as part of packaging materials (Rhim et al., 2013).

According to the data, chitosan (DD 65%) was made from crab shell using chitin (Islam et al., 2011).

Demineralization, deproteinization, and deacetylation are the three basic processes in the manufacture of chitosan from crustacean shell. Because of its antibacterial qualities, chitosan is commonly utilized in antimicrobial films to offer edible protective coatings, as well as in the dipping and spraying of food products (Aider et al., 2010). Because of its nontoxicity, biodegradability, and antibacterial characteristics, chitosan has a wide range of uses. It's utilized in biomedical research, agriculture, genetic engineering, the food business, pollution control, water treatment, paper production, and photography, among other things (Cheba, 2011).

Due to its biodegradable and harmless qualities, chitosan has been the material of choice for the creation of nanoparticles in a variety of applications (Phaechamud, 2008). Chitosan nanoparticles develop spontaneously when a polyanion such as tripolyphosphate (TPP) is added to a chitosan solution and stirred continuously. After that, the nanoparticles are harvested and used for gene therapy and drug delivery (De Campos et al., 2001; Jayakumar et al., 2010). Chitosan nanoparticles were able to penetrate fungal cell walls and bind to their DNA. This will stop the generation of vital proteins and enzymes by inhibiting mRNA synthesis (kong et al., 2010).

The purpose of this research is to monitor antimicrobial effect of chitosan nanoparticles against E. coli and *Aspergillus flavus* strains.

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2. MATERIAL AND METHODS

2.1 Kareish cheese manufacturing

Cheese manufacture essentially involves gelation of the casein via enzymatic (rennet) or acidic coagulation. skim milk (0. 5% fat and 8.5% SNF) was heated at 74°C for 15sec. and then rapidly cooled to 40°C. At this point yogurt starter culture was added at level of 1.5% to the base mixture for coagulation. After complete coagulation, the curd was separately transferred into gauze for wheying off in 24 hr., then cut and stored in its pasteurized salted whey (7% salt) for 24 hr. Resultant cheeses were stored at 4°C. (Phelan *et al.* ,1993).

2.2 Preparation of microbial strain

The antibacterial activity of the prepared chitosan nanoparticles was tested against. *Escherichia coli (*ATCC 25923*)* and *Aspergillus flavus* strain that obtained from Media Unite, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt. The cell count was adjusted to 10^4 CFU/ml for *E. coli* (Barbosa et al., 2009) with tube dilution methods. *Aspergillus flavus* reference strain with adjusted concentration (10^5 CFU/g) as recorded by Chang and Kim (2007). 2.5ml of a spore suspension ($1.5\times l0^5$ spores/ml) of *Aspergillus flavus,* grown on sabouraud dextrose agar, was inoculated to the medium, and incubated at 25ºc for 10 days .

2.3 Nano materials preparation

Chitosan nanoparticles have been prepared at Naqaa foundation for scientific research technology and development, Giza, Egypt according to Vaezifar et al. (2013).

2.4 Assessment of antimicrobial activity of nanomaterials in kareish cheese

In a sterile bag, cheese was inoculated with *E. coli* (~ 4 $\log_{10}$ CFU/g) and *Aspergillus flavus* (~ 5 $\log_{10}$ CFU/g). Then, it was mixed thoroughly by gently squeezing the bags by hand till even distribution of microbe occurred, and left for 30 min for complete attachment between inoculated strains and cheese.

Then cheese sample was divided into 12 groups (200 g each).

Group 1 (treated with *E. coli* and *Aspergillus. flavus*), as a positive control samples Group 2 (CSNps 2.5% + *E. coli*), (CSNps 2.5% + *A. flavus*), Group 3 (CSNps 5% + *E. coli*), (CSNps 5% + *A. flavus*), Group 4 (CSNps 10% + *E. coli*), (CSNps 10% + *A. flavus)*. Packed samples were labeled and kept at 4°C.

2.4.1 E. coli count

100 µl from each previously prepared serial dilution was accurately spread over duplicated plates of EMB agar (OXOID, CM0 069). then incubated for 24 hours at 37°C. The suspected colonies of *E. coli* were greenish metallic colonies. these colonies were enumerated and expressed as $\log_{10}$ CFU/g of sample (FDA, 2001)

2.4.2 Aspergillus flavus count

According to (ICMSF, 1978) Sabouraud dextrose agar (SDA) with chloramphenicol (0.05mg/ml), which then incubated at 28-30 °C for 2 days-3weeks.

2.5 Statistical analysis

All data were subjected to analysis of variance (ANOVA) at $P \leq 0.05$ were indicated as significant different (Feldman et al., 2003) Means ± standard error from triplication of the design were conducted to remove diversity of results.

3. RESULTS

As presented in Table (1), the Count of *Escherichia coli* in manufactured kareish cheese samples increased in the control sample from 4.48 to 6.19 $\log_{10}$ CFU/g about 2 $\log_{10}$ CFU/g, during the storage period of cheese. Count was significantly different from all treated samples $P \leq 0.05$. Samples treated with CSNPS in concentration (2.5%) exhibited antibacterial effect and it decreased the count from 4.48 to 2.81 log CFU/g about 2 $\log_{10}$ CFU/g, that was differed significantly from that treated by CSNPS (5%) which decreased the count from 4.48 to 1.53 $\log_{10}$ CFU/g and when the concentration of CSNPS increased to (10 %) *E. coli* could not be detected at that concentration at 12th and 15th day of storage.

Table 1 Effect of different concentrations of CSNPs on *E. coli* count of the examined cheese samples during storage at 4ºC.

Groups	zero day	3rd day	6th day	9th day	12th day	15th day
Control	4.48±0.05^{a}	4.75±0.06^{a}	5.57±0.094^{d}	5.80±0.07^{d}	5.95±0.03^{d}	6.19±0.02^{d}
Chitosan (2.5%)	4.48±0.05^{a}	4.34±0.02^{ab}	3.91±0.031^{b}	3.78±0.01^{b}	3.68±0.02^{c}	2.81±0.01^{b}
Chitosan (5%)	4.48±0.05^{a}	4.23±0.03^{ab}	3.80±0.02^{b}	3.60±0.01^{c}	3.36±0.01^{e}	1.53±0.01^{e}
Chitosan (10%)	4.48±0.05^{a}	3.90±0.06^{b}	3.63±0.03^{c}	3.36±0.01^{e}	ND*	ND*

The values represented Mean ±SD of three experiments. Means with in a column followed by different letter (a, b, c, d, e) are significantly different ($P \leq 0.05$). ND*= not detected

The Count of *Aspergillus flavus* in manufactured kareish cheese samples *increased* in the control sample from 5.86 to 7.92 $\log_{10}$ (CFU/g) about 2 $\log_{10}$ CFU/g during the storage period of cheese Count was significantly different from all treated samples $P \leq 0.05$. Counts of these microbes in CSNPS treated samples decreased throughout 15 day of storage indicating antibacterial activity of these nanoparticles. CSNPS (2.5%) exhibited antibacterial effect and it decreased the count from 5.86 to 4.45 $\log_{10}$ (CFU/g) about 1.4 $\log_{10}$ (CFU/g) during the storage period 15 days, the results indicated significance difference between samples affected by CSNPS (5%) which decreased the count from by 5.86 to 4.36 $\log_{10}$ (CFU/g) and when the concentration of CSNPS increased to (10 %) *Aspergillus flavus* could not be detected at that concentration at 15th day (Table 2).

Table 2 Effect of different concentrations of Chitosan nanoparticles on *Aspergillus flavus* count of the examined cheese samples during storage at 4ºC.

Groups	zero day	3rd day	6th day	9th day	12th day	15th day
Control	5.86±0.6^{a}	5.96±0.9^{a}	6.43±0.4^{d}	6.76±0.6^{d}	6.92±0.7^{d}	7.92±0.4^{h}
Chitosan (2.5%)	5.86±0.6^{a}	5.70±0.2^{b}	5.41±0.3^{c}	5.20±0.4^{c}	4.80±0.2^{e}	4.54±0.3^{f}
Chitosan (5%)	5.86±0.6^{a}	5.49±0.3^{bc}	5.17±0.2^{c}	4.91±0.3^{e}	4.73±0.3^{e}	4.36±0.6^{g}
Chitosan (10%)	5.86±0.6^{a}	5.17±0.5^{c}	4.81±0.3^{e}	4.5±0.5^{f}	4.36±0.04^{g}	ND*

The values represent Mean ±SD of three experiments. Means within a column followed by different letter (a, b, c, d, e, f, g, h) are significantly different ($P \leq 0.05$). ND*= not detected

4- DISCUSSION

It was clear that, CSNPS has an antibacterial action against *E. coli*, in a dose dependent manner according to our findings. These findings were similar to those previously published by Saharan et al. (2013), Sarwar et al. (2014), Xing et al. (2016), Nguyen et al. (2016), Divya et al. (2017). According to Hassanien and Shaker (2020), Chitosan nanoparticles had a high bactericidal effect on isolates such as *E. coli* O157:H7 isolated from kareish cheese samples, the effect was improved with increasing concentrations. They used Chitosan nanoparticles at concentrations of (30µg/mL,). The antibacterial effect of chitosan nanoparticle is mediated in different ways. The negatively charged phospholipid of the plasma membrane interacts with the positively charged chitosan, altering cellular permeability and causing cell death. Chitosan nanoparticle possesses a metal ion chelating property, which could explain its antibacterial properties. Chitosan nanoparticle has also been shown to permeate the cell wall and bind to DNA, preventing mRNA production (Hernandez-Lauzardo et al., 2011).

The results came in agreement with Kaur et al. (2012) who studied on the fungicidal effects of nano size chitosan against Aspergillus flavus. Also, Resmila and Rinto (2017) employed a lower dose and found that a 2% treatment can limit A. flavus growth by more than 50%. The findings matched those of Sayed-Elahl et al. (2019) who found that cheese coated with CSNPs prevents mold infection and growth for a longer period than cheese not coated with chitosan nanoparticles.

Food and drug administration of the United States approved chitosan as a food component in 1983.Because it is a Generally Recognized as Safe (GRAS) component, chitosan has been widely used as a functional food, in environmental protection, and as a safe biotechnology product to promote human and animal health (Katiyar et al., 2014).

5. CONCLUSION

According to the findings, it can be inferred that chitosan nanoparticles has an antibacterial effect in kareish cheese against *E. coli* and *Aspergillus flavus*, in a dose dependent manner It was reported that (CSNPS 10%) had the greatest effect against the both microorganisms tested, followed by (CSNPS 5%) then (CSNPS 2.5 %). These chitosan nanoparticles have been demonstrated to inhibit microbial development and extend the shelf life of kareish cheese.

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