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# Role of Nano-Chitosan in enhancing the safety of tilapia fillet

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## ABSTRACT

It has been demonstrated that chitosan nanoparticles can preserve fish fillets, which are very perishable, due to its ability to penetrate deep into its meat tissue and significant antimicrobial activity. The current study investigated the effects of chitosan nanoparticles at concentrations of 0.5%, 1%, and 2% on the shelf life of fresh tilapia fish fillets kept refrigerated. Furthermore, relying on hygiene indicators (total bacterial count, total psychrotrophic count, and total mold and yeast counts), the effects of chitosan nanoparticles on enhancing the microbiological quality of tilapia fish fillets were examined. Additionally, the impact of the chitosan nanoparticles on certain foodborne pathogens, including *Aspergillus flavus*, *Salmonella typhimurium* and *Staphylococcus aureus* was investigated. The obtained results indicated that chitosan nanoparticles were effective in reducing the count of *Salmonella typhimurium*, *Staphylococcus aureus and Aspergillus flavus* but not able to eleminate such organisms completely until elapsing some days of storage depending on the used concentration of chitosan nanoparticles used.

## INTRODUCTION

Fish is seen to be a superior choice to natural nutrition sources because of its readily digested protein, large amount of polyunsaturated fatty acids, rich macro and micro mineral content, and low calorie density **(Ameur et al. 2022)**.

Fish have more free amino acids, less connective tissues, and greater enzyme activity than other muscle products **(Shokri et al. 2020)**. As a result, when fish deteriorates, quality degradation such as protein breakdown, lipid oxidation, colour change, formation of an off flavour, and texture softening easily happens **(Çorapci, 2022)**. Since its short shelf life, tilapia (*Oreochromis niloticus*), a freshwater fish species, is widely cultivated around the world and sold in fish markets and general stores. However, preservation of this fish has long been a challenge. Fish and its products are extremely perishable foods that quickly deteriorate due to a variety of chemical, physical, and microbiological changes that occur during harvesting and processing **(Hesham et al. 2023)**.

The microbiological degradation of fish quality and safety may be due to poor transportation facilities, inexperience and irresponsible

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handling **(Hebano et al. 2020).** Furthermore, fish contamination from external sources during processing and storage may introduce harmful bacteria such *Staphylococcus aureus* and *Salmonella* spp. **(Pal et al. 2016),** globally, these main foodborne pathogens cause 42% of foodborne outbreaks **(Yeni et al. 2014)**. A significant public health concern is food poisoning and foodborne illnesses brought on by *Staph aureus*, which is known to produce a broad variety of toxic substances **(Hu et al. 2018)**. *Salmonella typhimurium* cause gastrointestinal infections with symptoms such as stomach discomfort, diarrhoea, mild fever, and vomition **(Shinohara et al. 2008)**.

Aflatoxin produced by *Aspergillus flavus* can result in immediate side effects such as nausea, vomition, stomach discomfort, and convulsions, Furthermore, long-term exposure can also induce consequences including immunotoxicity, teratogenicity, and hepatotoxicity **(Kew, 2012)**. Cold treatment is one of the most often utilized preservation methods in seafood sector. Microorganisms in seafood have been demonstrated to be limited or reduced by cold processing and preservation techniques such as freezing, chilling, super-chilling, and refrigeration, with or without the use of natural or synthetic chemical preservatives **(Tavares et al. 2021).** Synthetic chemical preservatives have been connected to possible risks that might result in major health problems like cancer, neurological impairment, allergic responses, and asthma **(Ebirim, 2020)**.

Because natural preservatives come from nature, there is less likelihood of health problems arising from their use. Fishermen's harvests can be maintained before distribution by using the antibacterial and antioxidant qualities of natural materials **(Lourenco et al. 2019).** Along with producing functional food, organic nanotechnology may enhance food safety, increase shelf life, improve taste and detect toxins/pesticides **(Seabra et al. 2013)**.

Chitosan nanoparticles, or nano-chitosan, are one of the organic by-products of the nanotechnology process, during the storage phase, nanochitosan can be used as an inexpensive and effective food preservative to extend the shelf life of tilapia fish and control their microbiological over growth (**Sorour et al. 2021).** Nano-chitosan exhibits superior absorption ability as an antibacterial and antifungal compared to chitosan, making it highly suitable for use as a natural preservative **(Abdeltwab et al. 2019).** Consequently, the aim of this study was to evaluate the preservative effect of different concentrations of nanochitosan particles on tilapia fillet samples and also study the effect of nanochitosan particles on artificially contaminated tilapia fillet samples with *Staph. aureus*, *Salmonella typhimurium* and *Aspergillus flavus.*

## MATERIALS and METHODS

### Preparation of Chitosan Nanoparticles (CNPs).

The ionotropic-gelation process was used to create Chitosan Nanoparticles CNPs, which rely on the electrostatic interaction of chitosan's positively charged amino groups with TPP's (tripolyphosphate) negatively charged groups. Chitosan solution was made at 3 g dissolved in 600 ml of acidified distilled water (DW) with 6 ml Glacial acetic acid by vigorous stirring until a transparent solution was observed. The pH of the solution was adjusted up to 4.5-4.8 by using NaOH then the solution was filtered to remove all undissolved particles. TPP was prepared at a concentration of 200 mg/200 ml DW and added drop by drop at a consistent rate of 2 ml/min with a titration pipette under continuous mixing for 2 hours at room temperature before being sonicated for 10 minutes **(Eman et al. 2022).** The solution was centrifuged twice with washing at 12,000 rpm and 4 °C for 15 minutes. After removing the supernatant, the sediment was dialyzed with DW, lyophilized, and finely powdered for use in additional characterisation.

Characterization of Chitosan Nanoparticles CsNp was done through high resolution transmission electron microscopy (HRTEM) imaging JEM 2100F transmission electron microscope with accelerating voltage 200 kV.

### Preparation of foodborne reference microbial isolates:

Reference isolates for *Aspergillus flavus, Salmonella typhimurium* and *Staphylococcus*

*aureus* were taken from the Animal Health Research Institute's Microbiology Laboratory. Every microorganism was refreshed and cultivated on a particular media. *Aspergillus flavus,* was cultivated on Sabouraud's dextrose agar with oxytetracycline and chloramphenicol added as supplements, Xylose lysine deoxycholate (XLD) agar was used to cultivate *Salmonella typhimurium* and Baird parker medium supplemented with egg yolk-tellurite emulsion was used to cultivate *Staph aureus*. Purified bacterial cells were cultivated in brain -heart infusion broth, the bacterial and fungal cells were centrifuged for 15 minutes at 3000 rpm, washed twice in 10 ml of 0.01 phosphate buffered saline (PBS), pH 7.0, and diluted to 1.0 x $10^6$ cfu/ml in phosphate buffered saline for sample inoculation with *Salmonella typhimurium*, *Staph aureus* and diluted to 1.0 x $10^7$ cfu/ml in phosphate buffered saline for sample inoculation with *Aspergillus flavus* **(Govaris et al. 2010)**.

**Collection of samples**

Fillets of Nile tilapia (Oreochromis niloticus) were sampled and stored in sterile polyethylene bags after being collected from fish markets in the El-Menofia governorate. After that, the bags were delivered hygienically in insulated ice containers to the Animal Health Research Institute's microbiological lab for additional processing and examination. In the first part of the study, half of collected fresh tilapia fillet samples were split into four groups. Group 1 was designated as a control group, and it was submerged in distilled water for a duration of 30 minutes, group 2, 3 and 4 were submerged in different concentrations of nanochitosan 0.5%,1.0%, and 2.0% respectively, for 30 minutes. After 30 minutes, the samples were removed from the solution and let to dry for five minutes on the bench at room temperature. Each group was labelled, covered with polyethylene sheets, and kept in storage at 4ºC. Sensory and microbiological analyses were done every two days.

In the second part of this study, the other half of collected fresh tilapia fillet samples were contaminated experimentally with 3 different previously prepared reference isolates of microbes (*Staph. aureus*, *Salmonella typhimurium* and *Aspergillus flavus*). Each microorganism was injected in 4 group of samples, after that the contaminated groups recieved different treatments (control group submerged in distilled water for a duration of 30 minutes, the other 3 groups submerged in different concentrations of nanochitosan (0.5%,1.0%, and 2.0% respectively, for 30 minutes), then given time to dry for five minutes on the bench at room temperature. Each group was labelled, covered with polyethylene sheets, and treatment groups underwent microbiological analysis every 2 days while the samples were refrigerated, according to **APHA (2001)** guidelines.

**Sensory analysis**

Using a five-point rating system that took texture, color, and smell for consideration, the overall approval of the tilapia fish fillet was verified. Sensory characteristics such as color change (score 5 indicates no color change; score 1 indicates exceptional color change), smell (score 5 indicates amazing acceptable; score 1 indicates extremely unacceptable/off-odors), and texture (score 5 indicates rigid; score 1 indicates highly soft) were recorded by specialists (6-member trained panel). Overall approval was the middle for these scores, which were as follows: 5 for much acceptable, 4 for good, 3 for average, 2 for doubtful, and 1 for completely unacceptable. According to shelf life criteria, rejection would occur if the sensory characteristics fell below 4.0 **(Ojagh et al. 2010).**

**Bacteriological examination**.

Samples were prepared in accordance with **APHA (2001).** To create a 1-10 dilution, 10 grammes of each fish fillet sample were taken under sterilized conditions and mixed with 90 millilitres of 0.1% sterile buffered peptone water. Next, for every sample, decimal serial dilutions up to $10^6$were prepared.

**Estimation of total bacterial count (TBC):**

According to **APHA (2001)**, total bacterial counts were counted utilizing the pour platting

method with plate count agar. The plates were incubated for 48 hours at 35 ± 2°C.

**Estimation of Total psychrophilic count (TPsC)**

According to **APHA (2001)**, Total psychrophilic count were counted using plate count agar using the pour platting method. The plates were incubated for 10 days at 7°C **(Greer, 1982).**

**Estimation of Total mould and yeast count (TMYC)**

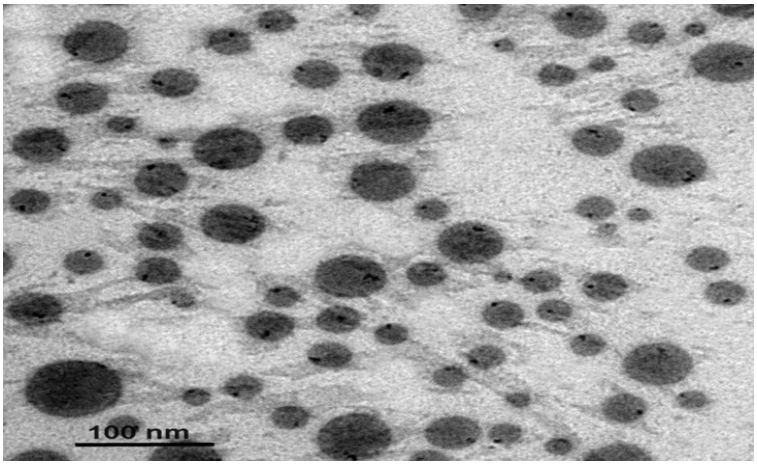
According to **APHA (2001)**, using Sabouraud's dextrose agar formulated with oxytetracycline and chloramphenicol, the pour platting method was used to estimate the total mold and yeast counts. The plates were incubated at 25°C for 7 days.

**Statistical analysis**

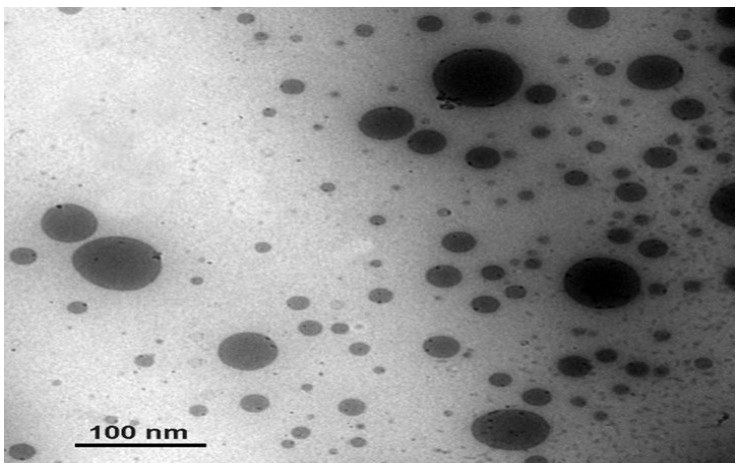
The results were statistically analyzed using the SPSS program using one-way ANOVA test.

**RESULTS**

Fig. (1) High resolution transmission electron microscopy( HRTEM ) of chitosan nanoparticles:




(A)




(B)

A and B showed nano sphere shape, no aggregation and size between 25.85 -36.86 nm. (Central lab. in NRC)

Table 1. Effect of Different concentrations of Chitosan Nano particles on the sensory attributes of Tilapia fish fillet.

| groups / storage period | Control | 0.5% chitosan (NPs) | 1 % chitosan (NPs) | 2 % chitosan(NPs) |
|---|---|---|---|---|
| colour 1st day | 4.8 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ |
| 3rd day | 3.5 ± 0.2$^{a}$ | 4.5 ± 0.1$^{b}$ | 4.7 ± 0.2$^{bc}$ | 4.8 ± 0.1$^{b}$ |
| 5th day | 2.5 ± 0.4$^{a}$ | 4.1 ± 0.2$^{b}$ | 4.5 ± 0.1$^{c}$ | 4.6 ± 0.2$^{c}$ |
| 7th day | S | 3.9 ± 0.3$^{a}$ | 4.1 ± 0.2$^{b}$ | 4.4 ± 0.1$^{c}$ |
| 9th day | S | 3.2 ± 0.2$^{a}$ | 3.9 ± 0.1$^{b}$ | 4.2 ± 0.2$^{c}$ |
| 11th day | S | 2.9 ± 0.1$^{a}$ | 3.3 ± 0.2$^{b}$ | 3.6 ± 0.3$^{c}$ |
| 13th day | s | 2.5 ± 0.2$^{a}$ | 3 ± 0.2$^{b}$ | 3.3 ± 0.1$^{c}$ |
| 15th day | s | s | 2 ± 0.2$^{a}$ | 2.6 ± 0.2$^{b}$ |
| odour 1st day | 4.8 ± 0.1$^{a}$ | 4.8 ± 0.1$^{a}$ | 4.8± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ |
| 3rd day | 3.7 ± 0.2$^{a}$ | 4.2 ± 0.2$^{b}$ | 4.6 ± 0.2$^{c}$ | 4.7 ± 0.2$^{d}$ |
| 5th day | 2.5 ± 0.2$^{a}$ | 4 ± 0.3$^{b}$ | 4.3 ± 0.1$^{c}$ | 4.5 ± 0.1$^{d}$ |
| 7th day | S | 3.6 ± 0.2$^{a}$ | 4 ± 0.2$^{b}$ | 4.3 ± 0.2$^{c}$ |
| 9th day | S | 3 ± 0.2$^{a}$ | 3.8 ± 0.1$^{b}$ | 4 ± 0.2$^{c}$ |
| 11th day | S | 2.7 ± 0.2$^{a}$ | 3.1 ± 0.2$^{b}$ | 3.6 ± 0.1$^{c}$ |
| 13th day | s | 2.5± 0.3$^{a}$ | 2.9 ± 0.1$^{b}$ | 3.3 ± 0.1$^{c}$ |
| 15th day | s | S | 2.5 ± 0.2$^{a}$ | 2.7 ± 0.3$^{b}$ |
| texture 1st day | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ |
| 3rd day | 4.2 ± 0.2$^{a}$ | 4.5 ± 0.2$^{b}$ | 4.6 ± 0.1$^{c}$ | 4.8 ± 0.1$^{d}$ |
| 5th day | 2.8 ± 0.4$^{a}$ | 4.3 ± 0.1$^{b}$ | 4.5 ± 0.2$^{c}$ | 4.6 ± 0.2$^{d}$ |
| 7th day | S | 3.8 ± 0.2$^{b}$ | 4.1 ± 0.1$^{a}$ | 4.4 ± 0.1$^{b}$ |
| 9th day | S | 3.4 ± 0.1$^{b}$ | 3.7 ± 0.1$^{a}$ | 4 ± 0.2$^{b}$ |
| 11th day | S | 3.1 ± 0.2$^{b}$ | 3.5 ± 0.2$^{a}$ | 3.7 ± 0.2$^{a}$ |
| 13th day | S | 2.6 ± 0.3$^{b}$ | 3 ± 0.3$^{b}$ | 3.3 ± 0.1$^{b}$ |
| 15th day | S | S | 2.5 ± 0.2$^{b}$ | 2.7 ± 0.3$^{b}$ |
| Overall acceptability 1st day | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ | 4.9 ± 0.1$^{a}$ |
| 3rd day | 3.9 ± 0.2$^{a}$ | 4.1 ± 0.1$^{b}$ | 4.5 ± 0.1$^{c}$ | 4.7 ± 0.1$^{d}$ |
| 5th day | 2.5 ± 0.4$^{a}$ | 3.8 ± 0.2$^{b}$ | 4.1 ± 0.1$^{c}$ | 4.5 ± 0.2$^{d}$ |
| 7th day | S | 3.4 ± 0.1$^{a}$ | 3.8 ± 0.2$^{b}$ | 4.2 ± 0.1$^{c}$ |
| 9th day | S | 3.1 ± 0.2$^{a}$ | 3.4 ± 0.1$^{b}$ | 3.9 ± 0.1$^{c}$ |
| 11th day | S | 2.9 ± 0.1$^{a}$ | 3.1 ± 0.2$^{b}$ | 3.6 ± 0.2$^{c}$ |
| 13th day | s | 2.5 ± 0.3$^{a}$ | 2.8 ± 0.2$^{b}$ | 3.2 ± 0.2$^{c}$ |
| 15th day | s | s | 2.5 ± 0.3$^{a}$ | 2.7 ± 0.4$^{b}$ |

S= Spoiled
The results are considered significant ($p< 0.01$) when the same row contained different superscripted small letters.

Table 2. Effect of varying chitosan nano particles concentrations on the total bacterial count of non contaminated experimentally tilapia fish fillet.

| groups / storage period | Control | 0.5% chitosan (NPs) | 1 % chitosan (NPs) | 2 % chitosan (NPs) |
|---|---|---|---|---|
| 1st day | 6.14 ± 0.21[a] | 6.08 ± 0.10[a] | 5.94 ± 0.11[a] | 5.90± 0.11[a] |
| 3rd day | 6.95 ± 0.11[a] | 5.64 ± 0.20[b] | 5.52 ± 0.12[c] | 5.32 ± 0.10[d] |
| 5th day | 7.53 ± 0.22[a] | 5.03 ± 0.11[b] | 4.88 ± 0.14[c] | 4.57 ± 0.15[d] |
| 7th day | S | 4.66 ± 0.15[a] | 4.31 ± 0.12[b] | 4.07 ± 0.12[c] |
| 9th day | S | 4.08 ± 0.10[a] | 3.62 ± 0.11[b] | 3.34 ± 0.14[c] |
| 11th day | S | 3.87 ± 0.11[a] | 3.27 ± 0.09[a] | 3.01 ± 0.12[b] |
| 13th day | S | 4.02 ± 0.11[a] | 2.81 ± 0.11[b] | 2.27 ± 0.14[c] |
| 15th day | S | S | 3.48 ± 0.15[a] | 3.05 ± 0.15[b] |

S= Spoiled The results are considered significant ($p< 0.01$) when the same row contained different small letters.

Table 3. Effect of varying chitosan nano particles concentrations on Total psychrotrophic count of non contaminated experimentally Tilapia fish fillet.

| groups / storage period | Control | 0.5% chitosan (NPs) | 1 % chitosan (NPs) | 2 % chitosan (NPs) |
|---|---|---|---|---|
| 1st day | 5.29 ± 0.12[a] | 5.22 ± 0.11[a] | 5.19 ± 0.02[a] | 5.17 ± 0.04[a] |
| 3rd day | 5.99 ± 0.15[a] | 4.68 ± 0.10[b] | 4.45 ± 0.05[c] | 4.31 ± 0.06[d] |
| 5th day | 6.58 ± 0.17[a] | 4.01 ± 0.05[b] | 3.81 ± 0.06[c] | 3.62 ± 0.03[d] |
| 7th day | S | 3.89 ± 0.07[a] | 3.25 ± 0.07[b] | 3.05 ± 0.04[c] |
| 9th day | S | 3.45 ± 0.12[a] | 3.01 ± 0.05[b] | 2.81 ± 0.08[c] |
| 11th day | S | 3.11 ± 0.14[a] | 2.99 ± 0.10[a] | 2.45 ± 0.07[b] |
| 13th day | S | 3.99 ± 0.13[a] | 2.51 ± 0.00[b] | 2.11 ± 0.05[c] |
| 15th day | S | S | 3.11 ± 0.11[a] | 2.45 ± 0.04[b] |

S= Spoiled The results are considered significant ($p< 0.01$) when the same row contained different superscripted small letters.

Table 4. Effect of Different concentrations of Chitosan Nano particles on Mould and Yeast count of non contaminated experimentally Tilapia fish fillet.

| groups / storage period | Control | 0.5% chitosan (NPs) | 1 % chitosan (NPs) | 2 % chitosan (NPs) |
|---|---|---|---|---|
| 1st day | 5.14 ± 0.09[a] | 4.10 ± 0.14[a] | 4.06 ± 0.08[a] | 4.01 ± 0.06[a] |
| 3rd day | 5.22 ± 0.11[a] | 3.88 ± 0.12[b] | 3.75 ± 0.07[bc] | 3.66 ± 0.08[c] |
| 5th day | 6.41 ± 0.14[a] | 3.42 ± 0.09[b] | 3.29 ± 0.05[c] | 3.09 ± 0.07[d] |
| 7th day | S | 3.07 ± 0.11[a] | 2.95 ± 0.09[b] | 2.72 ± 0.05[c] |
| 9th day | S | 2.75 ± 0.08[a] | 2.60 ± 0.07[b] | 2.41 ± 0.06[c] |
| 11th day | S | 2.11 ± 0.11[a] | 1.95 ± 0.12[a] | 1.70 ± 0.08[b] |
| 13th day | S | 2.85 ± 0.10[a] | 1.72 ± 0.13[b] | 1.45 ± 0.07[c] |
| 15th day | S | S | 2.15 ± 0.14[a] | 1.99 ± 0.06[b] |

S= Spoiled The results are considered significant ($p< 0.01$) when the same row contained different superscripted small letters

Table 5. Effect of varying chitosan nano particles concentrations on *Staph aureus* count of experimentally contaminated Tilapia fish fillet (log cfu/g).

| groups / storage period | Control | 0.5% chitosan (NPs) | 1 % chitosan (NPs) | 2 % chitosan (NPs) |
|---|---|---|---|---|
| 1st day | 6.63 ± 0.12[a] | 6.51 ± 0.13[a] | 6.02 ± 0.11[b] | 5.54 ± 0.13[c] |
| 3rd day | 6.98 ± 0.16[a] | 6.23 ± 0.14[b] | 5.32 ± 0.10[c] | 4.02 ± 0.18[d] |
| 5th day | 7.35 ± 0.14[a] | 5.75 ± 0.12[b] | 4.63 ± 0.10[c] | 3.11 ± 0.16[d] |
| 7th day | S | 5.12 ± 0.15[a] | 3.21 ± 0.12[b] | 2.18 ± 0.10[c] |
| 9th day | S | 4.07 ± 0.17[a] | 1.45 ± 0.11[b] | ND* |
| 11th day | S | 3.62 ± 0.10 | ND* | ND* |
| 13th day | S | 4.25 ± 0.12 | ND* | ND* |
| 15th day | S | S | ND* | ND* |

S= Spoiled ND=Not Detected The results are considered significant ($p< 0.01$) when the same row contained different superscripted small letters

Table 6. Effect of varying chitosan nano particles concentrations on *Salmonella typhimurium* count of experimentally contaminated Tilapia fish fillet (log cfu/g).

| groups / storage period | Control | 0.5% chitosan (NPs) | 1 % chitosan (NPs) | 2 % chitosan (NPs) |
|---|---|---|---|---|
| 1st day | 6.54 ± 0.10[a] | 6.43 ± 0.11[a] | 6.21 ± 0.11[b] | 5.73 ± 0.12[c] |
| 3rd day | 6.83 ± 0.12[a] | 6.02 ± 0.12[b] | 5.72 ± 0.12[c] | 3.24 ± 0.11[d] |
| 5th day | 7.24 ± 0.21[a] | 5.92 ± 0.17[b] | 4.87 ± 0.14[c] | 2.93 ± 0.13[d] |
| 7th day | S | 5.27 ± 0.12[a] | 3.38 ± 0.16[b] | 1.34 ± 0.12[c] |
| 9th day | S | 4.30 ± 0.15[a] | 2.07 ± 0.15[b] | ND* |
| 11th day | S | 3.74 ± 0.18 | ND* | ND* |
| 13th day | S | 4.05 ± 0.14 | ND* | ND* |
| 15th day | S | S | ND* | ND* |

S= Spoiled ND=Not Detected The results are considered significant ($p< 0.01$) when the same row contained different superscripted small letters

Table 7. Effect of varying chitosan nano particles concentrations on count of *Aspergillus flavus* in experimentally contaminated Tilapia fish fillet(log cfu/g).

| groups / storage period | Control | 0.5% chitosan (NPs) | 1 % chitosan (NPs) | 2 % chitosan (NPs) |
|---|---|---|---|---|
| 1st day | 7.35 ± 0.13[a] | 7.28 ± 0.15[ab] | 7.15 ± 0.12[b] | 7.02 ± 0.11[b] |
| 3rd day | 7.98 ± 0.14[a] | 6.12 ± 0.14[b] | 5.42 ± 0.14[c] | 4.51 ± 0.16[d] |
| 5th day | 8.44 ± 0.18[a] | 5.67 ± 0.16[b] | 3.78 ± 0.13[c] | 3.31 ± 0.11[d] |
| 7th day | S | 4.81 ± 0.15[a] | 2.08 ± 0.14[b] | 1.55 ± 0.12[c] |
| 9th day | S | 3.98 ± 0.14 | ND* | ND* |
| 11th day | S | 3.11 ± 0.13 | ND* | ND* |
| 13th day | S | 3.95 ± 0.17 | ND* | ND* |
| 15th day | S | S | ND* | ND* |

S= Spoiled ND=Not Detected The results are considered significant ($p< 0.01$) when the same row contained different superscripted small letters.

## DISCUSSION

Due to its distinctive composition and limited oxidative stability, fish fillet is susceptible to fat oxidation and microbiological degradation. Protective coatings appear to be a useful strategy for improving fish quality and extending its shelf life by suppressing microbial development of the fish's surface **(Pabast et al. 2018).**

As shown in Table 1, The treated samples with nanochitosan 2% had the highest overall acceptance, followed by samples treated with nanochitosan 1% and, lastly, samples treated with nanochitosan 0.5%, according to a significant difference ($p<0.01$) between groups. In contrast to the control group, which was rejected on the seventh day of storage based on sensory evaluation, nanochitosan (1% and 2%) improved the sensory qualities of tilapia fish fillet, allowing it to be accepted until the fifteenth day of storage.The most used microbiological test for determining the hygiene in the food sector is the total bacterial count. A maximum allowable level was established by the **Egyptian Organization for Standardization (EOS, 2020)** for total bacterial count is $10^6$ cfu/g. A significant difference ($p<0.01$) was seen between the groups receiving treatment and control groups.

From result tabulated in table 2 The data reveled that total bacterial count (TBC) in 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 15th days of storage were lower in samples treated with 2% chitosan nanoparticles (NPs) than samples treated with 0.5% and 1% as compared with control samples. Moreover the control samples were spoiled at 7th day of storage, but samples treated with 0.5% chitosan (NPs) spoiled at 15th day of storage. On the other hand samples treated with 1 and 2% chitosan (NPs) were still sound till the 15th day of storage. Meaning that nanochitosan 2% had good reducing action on Aerobic plate count of treated samples than 0.5 and 1.0% nanochitosan. These findings are compliant with **Elkassas et al. (2020)** who found that 2% chitosan nanoparticles can be used as a natural preservative for preserving quality and extend the shelf life of tilapia fish slices during refrigerated storage. Chitosan nanoparticles could reduce TBC in the treated groups, particularly nanochitosan 1%, 2% till the 13th day at chilling temperature, while nanochitosan 0.5% reduce TBC till the 11thday then the count increase because appearance of deterioration signs. These clear antimicrobial properties of chitosan nanoparticles. Chitosan nanoparticles have a bigger surface area, a stronger attraction for bacterial cells, and exhibited higher antibacterial activity during product storage **(Ramezani et al. 2015)**. It's possible that the inhibitory effect of nanochitosan coatings on microorganisms results from their capacity to block oxygen, which prevents the entry of oxygen required for microbial respiration **(Abdel-Wahab et al. 2020).**

Psychrotrophic bacterial contamination of fish can cause a variety of off flavours, such as fruity, stale, bitter, rotten, and rancid, as well as physical defects **(Jay, 2000)**. From the results illustrated in table 3, there was a significant difference ($p<0.01$) between the treated and control groups. Total psychrotrophic counts in 1st, 3rd, 5th, 7th, 9th, 11th , 13th and 15th days of storage were lower in samples treated with 2% chitosan nanoparticles (NPs) than samples treated with 0.5%, 1 % and control samples. Also we found control samples were spoiled at 7th day of storage, while samples treated with 0.5% chitosan (NPs) spoiled at 15 th day of storage. On the other hand samples treated with 1 and 2% chitosan (NPs) weren't spoiled till the 15th day of storage. Such results agreed with **Halimeh and Ainaz (2018)** who found that sample treated with nanochitosan have a lower psychrotrophic count than the control fillets samples. Otherwise, samples treated with 1 and 2% chitosan (NPs) weren't spoiled till the 15th day of storage. Chitosan nanoparticles have various biological uses such as medical sector, DNA, proteins, and antigens; as antimicrobial against bacteria and fungi so it can be used as food preservative to extend its shelf life and improve food quality **(Aider, 2010).**

Using a mould and yeast count to assess fungal contamination of fish can help determine the fish's quality and shelf life

**(Grigorakis et al. 2003).** In table 4, Total Mould and Yeast Counts of treated samples with different concentrations of chitosan (NPs), a significant difference ($p<0.01$) was seen between the group receiving treatment and control groups. Counts in 1st, 3rd, 5th, 7th, 9th, 11th 13th and 15th days of storage were lower in samples treated with 2% chitosan nanoparticles (NPs) than samples treated with 0.5%, 1% and control samples. Control samples spoiled after 7 days of storage but samples treated with 0.5% chitosan (NPs) spoiled at 15th day of storage. Otherwise samples treated with 1 and 2% chitosan (NPs) weren't spoiled till the 15th day of storage. Mould infection might be a sign of poor sanitation practices at the moment of capture. The environment that exists in freezers, chilling boxes, and clothes and hands of fishermen is ideal for the growth of fungal spores **(Reij and Den 2004).** Higher findings were noted by **Samar et al. (2022)** who found that mean mould and yeast count of examined (*Mugil cephalus*) fish steaks treated with 2% chitosan nanoparticles was (3.68 log cfu/g). Fungal infection in fish can cause spoiling and the formation of mycotoxins, which can pose health risks to humans such as cancer, liver illness, and organ damage **(Darwish et al. 2014).** When compared to regular chitosan, chitosan- nanoparticles (CSNP) exhibit a more potent antifungal activity, most likely as a result of superior cellular absorption, larger surface area, and greater surface-to-charge density in comparison to chitosan **(Xing et al. 2021).**

*Staph aureus* induced food poisoning received a lot of attention **(Lv et al. 2021).** Chemical antimicrobials appear to be a good option for controlling the risks associated with *S. aureus* contamination; however, there are drawbacks, including the possibility for human health risks and the ease with which drug-resistant strains can developed **(Jones and Joshi, 2021).** Our goal is to find a natural chemical that inhibits *Staph aureus* and both safe and dependable.

From the results tabulated in table 5 illustrated *Staph aureus* count in the treated and control groups, the counts in 1st, 3rd, 5th days of storage were lower in samples treated with 2% chitosan nanoparticles (NPs) than samples treated with 0.5%, 1 % and control samples**.** Also lower in 7th day in samples treated with 2% chitosan (NPs) than samples treated with 0.5% and 1 %. While counts were lower in 9th day in samples treated with 1% than 0.5%. On the other hand *S. aureus* was not detected at 9th till 15th day of storage in samples treated with 2% chitosan (NPs). Also *S. aureus* was not detected at 11th, 13th and 15th day of storage in samples treated with 1% chitosan (NPs).

Otherwise spoilage of control samples occurred at 7th day of storage. While samples treated with 0.5% chitosan (NPs) spoiled at 15th day of storage.

Fish samples submerged in a 2% nanochitosan coating were negative for *Staph. aureus* during all over the storage period, according to **Elkassas et al. (2020).** Thus, by using 2% chitosan nanoparticles as a natural preservative, tilapia fish slices' shelf life can be extended and their quality attributes preserved by refrigerated storage. According to **Youssef and EL.Masry (2018)** when *Staph aureus* contaminated chicken meat, samples were immersed for 30 seconds in a solution including 0.5%, 1%, and 2% nanochitosan particles, the growth rate of *Staph. aureus* cfu/ g at the beginning was significantly reduced.

In accordance with **Sorour et al. (2021),** both chitosan and its nanoparticles form effectively decreased *S. aureus* counts, especially chitsoan nanoparticles with particle size of 37 nm, that results further confirmed that chitosan nanoparticles were useful for extending the shelf life of tilapia.

Numerous studies have found that chitosan coatings can have a beneficial antioxidant and antibacterial effect on fish preservation **(Abdollahzadeh et al. 2023**; **Homayonpour et al. 2021; Mehdizadeh et al. 2022).**

Nanoemulsion has been investigated for its possible application as a food preservation layer to suppress spoilage or pathogenic bacteria, maintain freshness of food, and prolong its shelf life **(Qiu et al. 2022).**

In table 6 illustrated *Salmonella typhimurium* count of treated samples with different concentrations, there was a significant difference ($p<0.01$) between the treated and control groups. The data revealed that counts in 1st, 3rd, 5th days of storage were lower in samples treated with 2% chitosan nanoparticles (NPs) than that treated with 0.5% , 1 % and control samples**.** Also lower in 7th day in samples treated with 2% chitosan (NPs) than samples treated with 0.5% and 1 %. While counts were lower in 9th day in samples treated with 1% than 0.5%. On the other hand, *Salmonella typhimurium* was not detected at 9th till 15th day of storage in samples treated with 2% chitosan (NPs). Also *Salmonella typhimurium* was not detected at 11th,13th and 15th day of storage in samples treated with 1% chitosan (NPs). Otherwise, spoilage of control samples occurred at 7th day of storage. While samples treated with 0.5% chitosan (NPs) spoiled at 15th day of storage.

**Youssef and EL.Masry (2018)** found that growth of *Salmonella typhmurium* at the beginning was considerably decreased in all experiments when *Salmonella typhmurium* contaminated chicken meat samples were submerged for 30 seconds in a solution holding 0.5%, 1%, and 2% nanochitosan particles.

Because of their unique characteristics, chitosan nanoparticles have been shown to exhibit stronger antibacterial properties against *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli* than conventional chitosan. They probably have a wider field of interaction and possess more attraction for the microorganisms, resulting in increased antimicrobial effect **(Qi et al. 2004)**.

On the other hand, **Du et al. (2009**) found that *Salmonella choleraesuis, Staph aureus,* and *Escherichia coli,* all were more susceptible to the antibacterial effects of chitosan tripolyphosphate nanoparticles loaded with different metal ions. Chitosan is a natural polysaccharide derived from chitin via deacetylation **(Rhazi et al. 2000).** The size of chitosan particles is reduced to nano-chitosan, which boosts its antibacterial action.

The effectiveness of chitosan and nanochitosan coatings in preserving silver carp fillets under refrigeration was validated by **Ramezani et al. (2015).** Nevertheless, during the storage period, nanochitosan had more antibacterial activity than chitosan. Nanochitosan coating is therefore more effective in prolonging the shelf life and delaying the spoilage of fresh silver carp fillets during cold storage.

Because of its distinct physicochemical characteristics, biodegradability, and biocompatibility, chitosan has a potent antifungal impact **(Terkula Iber et al. 2022).**

When compared to regular chitosan, chitosan-derived nanoparticles (CSNP) exhibit a more potent antifungal activity. Most likely as a result of its superior cellular absorption, larger surface area, and higher surface-to-charge density in comparison with conventional chitosan **(Xing et al. 2021).**

Results in table 7 revealed that *Aspergillus flavus* Counts in 1st, 3rd, 5th and 7th days of storage were lower in samples treated with 2% chitosan nanoparticles (NPs) than samples treated with 0.5% ,1% and control samples. There was a significant difference ($p<0.01$) between the treated and control groups. Control samples were spoiled at 7th day of storage, also samples treated with 0.5% chitosan (NPs) spoiled at 15th day. On the other hand *Aspergillus flavus* were not detected in samples treated with 1% and 2% chitosan (NPs) at 9th day till 15th day of storage.

When compared to the control group, **Sorour** (2021) demonstrated that chitosan and its nanoparticles may dramatically lower *Aspergillus flavus* numbers. Additionally, chitosan at 1.5% suppressed *Aspergillus flavus* growth at 50%, according to **Rinto and Resmila (2017)**.

## CONCLUSION

The recorded results in the current study revealed that CSNP at concentrations of (0.5%, 1%, and 2%) could reduce the count of *Salmonela typhimurium, Staph. aureus,* and *Aspergillus flavus* but was not able to eliminate such organisms completely

until elapsing 13, 9, and 7 days from storage, respectively. Meaning that treated fillet in such manner could not be consumed unless the aforementioned periods of product storage has passed. This is considered illogical as it may result in economic losses for producer and health risk for consumers if consumed before passing these periods, as the product loses some of its physico-chemical properties by time. Therefore, it can be concluded that subsequent experiments in the same field should use higher concentrations than that used in the current research to be able to eleminate contamination as quickly as possible or to use other preservatives capable of eliminating contamination rabidly.

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