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Effect of *Jerusalem artichoke* chitosan nanoparticles on shrimp popcorn shelf life and quality

Alaa Eldin M.A. Morshdy[1], Mervat Ayed[2*], Miral Abdelwahab[3,4], Mohamed Hassan[5], Wageh S. Darwish[1], Nabil Beker[6], Eman Ali[2]

[1]*Food Hygiene, Safety, and Technology Department, Faculty of Veterinary Medicine, Zagazig University., Zagazig, Egypt.*
[2]*Food Hygiene, Technology and Food Safety Department, Faculty of Veterinary Medicine, Damanhour University, Egypt.*
[3]*Faculty of Technological Industry and Energy, Thebes Technological University, Thebes, Luxor 85863, Egypt.*
[4]*Pharmaceutical and Fermentation, Industries Development Centre (PFIDC), City of Scientific Research and Technological Applications (SRTA-City), New Borg El Arab 21934, Egypt.*
[5]*Food Hygiene, Technology and Food Safety Department, Faculty of Veterinary Medicine, Banha University, Egypt.*
[6]*Infectious Diseases Department, Faculty of Veterinary Medicine, Damanhour University, Egypt.*

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***Correspondence:**

Corresponding author: Mervat Ayed
E-mail address: marvethussain1122@gmail.com

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ABSTRACT

Jerusalem artichoke (*Helianthus tuberosus* L.) is a crop that basically originated in countries of central-eastern North America. The tuber of the plant contains inulin, a fructose polymer. The degraded product is called oligofructose which is extensively used in the food industry. In recent decades, chitosan nanoparticles (NPs), a potential polymeric and bio-based NP, have attracted a lot of attention. They have considerable promise as functional elements that enhance the application of antimicrobial activity in food packaging, as well as nanocarriers that encapsulate items like medications or active compounds, convey them to a specific region or site, and enable a controlled release. One option for extending the shelf life of food is nanotechnology, which makes it possible to create active food packaging that combines the qualities of antimicrobial agents and external barriers. In this case, we combine chitosan nanoparticles with *Jerusalem artichoke* plant. Therefore, the goal of the current study is to assess, by sensory, chemical, and microbiological tests, the effects of *Jerusalem artichoke* chitosan nanoparticles on the shelf life and quality of shrimp popcorn at different concentrations (0.02, 0.2, and 0.5%). Samples treated with *Jerusalem artichoke* chitosan nanoparticles saw a 12-day shelf-life extension at 4±1°C, but untreated samples only saw a 6-day shelf-life extension at the same temperature. On the other hand, the data obtained showed that during the storage period (0, 3, 6, 9, and 12) days, shrimp popcorn samples treated with 0.5% *Jerusalem artichoke* chitosan nanoparticles significantly reduced the total aerobic bacterial count (TBC), total coliforms count, and total yeast and mould count.

Introduction

Jerusalem artichoke is rich in inulin. Also, it contains some antioxidant compounds such as polyacetylenic derivatives, sesquiterpenes and coumarins (Furlan *et al*., 2014).

Deacetylated chitin, or chitosan, is a biopolymer derived from shellfish that has several benefits, including non-toxicity, biodegradability, and biocompatibility. It has drawn interest as a possible natural food preservative due to its antibacterial activities (Rabea *et al*., 2009; Badawy and Rabea, 2017; Marei *et al*., 2018).

Shrimp is a high-quality protein, lipid, vitamin, water, and mineral-rich meal. Shrimp also includes 2-4 percent lipids, which have a high amount of polyunsaturated fatty acids that are susceptible to oxidation, resulting in off-flavor, colour, taste, and texture alterations, as well as nutritional loss. At tropical conditions, most species become inedible within twelve hours, and deterioration occurs as soon as the shrimp dies (George, 2006). Rancidity is the main cause of shrimp quality degradation, and oxidation is the primary source of shrimp rancidity.

Shrimp popcorn is made from minced shrimp flesh; adding various additives to the mincing flesh allows for the removal of unpleasant smells and aromas. However, during storage, Shrimp and Shrimperies products can experience unfavourable alterations (Tokur *et al*., 2006).

Several synthetic additives are used to maintain quality and prevent the deterioration of seafood items. However, utilization of synthetic additives has raised the consumer awareness. Therefore, scientists all around the world are seeking for natural preservatives with high antioxidant and antibacterial activity that can help extend the shelf life of seafood items (Olatunde and Benjakul, 2018).

The present study investigated the effects of the *Jerusalem artichoke* chitosan nanoparticles on the sensory and microbiological qualities of the shrimp popcorn during chilled storage.

Materials and methods

Preparation of Jerusalem artichoke water extract

With minor adjustments, the tested plant's extract was made in accordance with Vongsak *et al*. (2013). Samples of leaves and rhizomes were dried, and fifty grams of the plant powder was mixed in deionized water (1:20 W/V) for an hour at 45°C. After that, it was steeped using a magnetic stirrer for 24 hours at 245°C. The residues were weighed after being centrifuged and filtrated, and the extraction yield of plant material was calculated after lypholization by Vacuum freeze dryer and stored at -20ºC until further analysis & we made three different concentrations (0.02, 0.2 and 0.5%).

Formulation and Characterization of Jerusalem artichoke Chitosan Nanoparticles (JA-NPS)

Formulation of *Jerusalem artichoke* -loaded Chitosan Nanoparticles (JA-NPs)

TPP (0.5 mg/ml, w/v) was dissolved in triple distilled water, while chitosan (0.5 mg/ml, w/v) was dissolved in a 2% v/v acetic acid solution. 15 ml of the room-temperature CS solution and 6 ml of the TPP solution were combined while being stirred magnetically at 600 rpm. For creating CS/TPP nanoparticles, the volume ratio of CS/TPP was maintained at 2.5:1 (v/v).15 ml of the CS acidic solution with various concentrations of

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*Jerusalem artichoke*s (0.02, 0.2, and 0.5 mg/ml) was mixed with 6 ml of the TPP aqueous solution. Using a cooling centrifuge, the JA-NPs were extracted from the opalescent suspension by centrifugation at 20000 xg for an hour at 4°C.

Characterization of *Jerusalem artichoke*-loaded Chitosan Nanoparticles (JA-NPs) by Transmission electron microscopy

The morphology and elemental analysis of the JA-NPs were detected by transmission electron microscopy (TEM) (JEOL, JEM 1400, Tokyo, Japan) (El-Zahaby *et al*. 2016).

Determination of total phenol contents (TPC) of Jerusalem artichoke extract

Using the Folin-Ciocalteu reagent, the total phenolic content was determined (Singleton *et al*., 1999; Dewanto *et al*., 2002). Total phenol contents (TPC) were expressed as Gallic acid equivalent (GAE)/mg of dry weight.

Determination of total flavonoid contents (TFC) of Jerusalem artichoke extract

The plant extract's total flavonoid content was ascertained by a modified colorimetric approach outlined by Sakanaka *et al.* (2005), which employed catechol as a reference at concentrations ranging from 20 to 200 µg/ml. Total flavonoids content was expressed as catechol equivalent (CE).

DPPH radical scavenging activity of Jerusalem artichoke extract

With some adjustments, the DPPH method as described by Brand-Williams *et al*. (1995) was used to assess the plant extract's capacity to scavenge free radicals. Mg of ascorbic acid equivalent (AAE)/g of dried material was used to express the DPPH radical scavenging activity. The following formula was used to determine the proportion of DPPH radical-scavenging activity: % inhibition $=Abs_{Control}- Abs_{Sample}/Abs_{Control} \times 100$

Antibacterial activity of the Jerusalem artichoke extract

An agar well diffusion essay was used to evaluate the antibacterial activity of each sample extract (Kadaikunnan *et al*., 2015). Two of the microbial species that are known to be dangerous are Gram's positive bacteria (*S. aureus* ATCC25923) and Gram's negative bacteria (*E. coli* ATCC25922). The zone of inhibition was calculated by measuring the diameter of the inhibition zone surrounding the well (mm), including the diameter of the well.

Preparation of shrimp popcorn samples

Twelve kilograms of fresh shrimp samples were collected from a local market in Damanhour, El Behiera governorate, Egypt during july 2023 .The shrimp used for this study had a weight and total length of 8.0±6.0 g and 10.0±5.0 cm, respectively.

Shrimp popcorn was processed in accordance with Egyptian Standards 3495 (2005) for products containing quick-frozen shrimp. The samples were sprayed with individual *Jerusalem artichoke* chitosan nanoparticles. Samples of fresh prawns were peeled and then chopped. Samples were created using a blend of the following spices: 25% black pepper, 25% cumin, 15% cardamom, 15% ginger, 10% red pepper, and 10% paprika. Before combining, each kind of spice was ground into a powder. To create samples of shrimp popcorn, the meat from the minced prawns was divided into five equal pieces. Negative control (NC) was the first portion that was left unaltered; positive control (PC) was the second portion that was made with 0.02% butylated hydroxytoluene (BHT) as a chemical preservative; and the remaining three portions were made with three different concentrations of plant extract *Jerusalem artichoke* (0.02, 0.2, and 0.5%). Each treatment's shrimp meat was molded into shrimp popcorn (1±0.5 cm in diameter and 2±0.5g on average). Every treatment was put into foam plates, covered with polyethylene sheets, and kept at 4±1°C in a sterile and clean refrigerator.

Determination of pH values of shrimp popcorn Samples treated with chitosan nano Jerusalem artichoke smart packaging

The pH values were determined according to EOS 63/11 (2006).

Determination of Water Holding Capacity (WHC) of shrimp popcorn samples treated with chitosan nano Jerusalem artichoke smart packaging

According to Mehri *et al*. (2015), the centrifugation assay was used to determine the shrimp popcorn sample's water holding capacity (WHC). WHC was determined by applying the subsequent formula:
WHC(%)= (weight before centrifugation/weight after centrifugation)×100

Determination of Thiobarbituric acid reactive substances (TBARS) value of shrimp popcorn samples treated with chitosan nano Jerusalem artichoke smart packaging

Thiobarbituric acid reactive compounds (TBARS) were detected in shrimp popcorn samples using the technique outlined in (EOS63/10, 2006). Milligrammes of malonaldehyde per kilogram of beef was the unit of measurement for TBARS.

Permissible limit not to go over 0.9 Mg MDA/Kg according to Egyptian Standards.

Determination of Total Volatile Basic Nitrogen (TVBN) using a distillation process of shrimp popcorn samples treated with chitosan nano Jerusalem artichoke smart packaging

This method was tested for its ability to qualitatively evaluate fishery products by (EOS 63/9, 2006). In order to perform a protein precipitation. The TVBN level is equal to n x 16.8 mg of nitrogen per 100 g of shrimp.

Microbiological analysis of shrimp popcorn samples treated with chitosan nano Jerusalem artichoke smart packaging (American Public Health Association APHA, 1992)

Preparation of samples for microbiological examination of shrimp popcorn samples treated with chitosan nano *Jerusalem artichoke* (ICMSF, 1978)

Shrimp popcorn samples were microbiologically analyzed at days; 0, 3, 6, 9, 12 of cold storage at 4°C.

Serial dilution was prepared (1 ml aliquots from 10^{-1} to10^{-6} dilutions). The prepared samples were subjected to the following examination.

Determination of aerobic bacterial count of shrimp popcorn samples treated with chitosan nano *Jerusalem artichoke* smart packaging (Cruickshank *et al*., 1975; Mailoa *et al*., 2017)

Standard plate count agar medium (PCA) (Conda, Spain) was used for the experiment. Plates with a total aerobic bacterial count of 30–300 colonies were the only ones that were counted and recorded as CFU/g.

Determination of coliforms count of shrimp popcorn samples treated with chitosan nano *Jerusalem artichoke* smart packaging (Ray and Speck, 1978; ICMSF, 1996)

Using Violet Red Bile agar medium (VRB) (Conda, Spain), the pour

plate method was used. The average number of colonies was then calculated by counting all the dark red colonies. CFU/g, the Coliforms count, was computed.

Determination of yeast and molds count of shrimp popcorn samples treated with chitosan nano *Jerusalem artichoke* smart packaging (Copetti *et al*., 2009)

Using Potato Dextrose Agar (PDA) medium (Himedia, India), the pour plate method was used. Yeast and mould were cultured at 28°C for 72 hours and five days, respectively. The CFU/g of yeast and mould was computed.

Sensory analysis of shrimp popcorn samples treated with chitosan nano Jerusalem artichoke smart packaging

Every test was conducted in a controlled environment. To clear the palette in between tastes, tap water was available. According to Fernández-López *et al.* (2006), shrimp popcorn from each recipe was cooked in an oven at 180°C until the core temperature reached 75°C. The oven was then kept warm until the test was completed in 3–8 minutes. Each panelist was asked to rate the overall acceptability of the provided sample on a scale of 1 (strongly dislike) to 9 (strongly like). A score of 6 was regarded as the bottom limit of acceptability. The scale points were as follows: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (initial off-odor, off-taste development), 6. Once the first unpleasant taste or smell appeared, a product was deemed unsuitable.

Statistical Analysis

The data was analyzed using SPSS® version 16.0 using Duncan's one-way analysis of variance (ANOVA). A statistical probability (p value) less than 0.05 indicated a statistically significant difference between the groups. Steel and Torrie (1980) provided the data as the average ± standard deviation of three duplicates.

Results and Discussion

Antioxidant activity of Chitosan nano Jerusalem artichoke

Result showed that chitosan nano *Jerusalem artichoke* showed high antioxidant activity with 60.31±0.15 mg GAE/g total phenolic content; while total flavonoids content was 28.38±0.18 mg/g which is slightly lower than that reported by Mashkor (2015), while the DPPH activity showed IC50= 25.31±0.03 µg/ml. The obtained results emphasized the high antioxidant activity of Chitosan nano *Jerusalem artichoke*.

Flavonoids and phenolic chemicals abound in *Helianthus tuberosus* L. leaves and tubers. We therefore postulated that there might be antioxidant potential in both extracts. The potential of *Helianthus tuberosus* L. tuber and leaves extract to reduce free radicals was assessed using the DPPH scavenging assay, which measures the colour change of the free radical solution after it has been incubated with the substances under investigation. The amount of DPPH that forms when tested chemicals are added to the radical solution is directly related to the decreasing absorption values (Alam *et al*., 2013).

Antibacterial activity of Chitosan nano Jerusalem artichoke

With minimum inhibitory concentrations (MIC) of 50 mg/ml for gram negative and 25 mg/ml for gram positive bacteria, the obtained results demonstrated a remarkable antimicrobial activity for Chitosan nano *Jerusalem artichoke*s against gram-positive strains (Staphylococcus aureus EMCC1451) and gram-negative strains (Escherichia coli ATCC25922) (Table 1). As shown in Table 2, it is likely that chitosan nano *Jerusalem artichoke*s will lower mould and counts. Furthermore, it has the ability to decrease TVBN values in a concentration-dependent way (Table 3). The action and makeup of phenolic compounds may be the cause of variations in plant extracts' antibacterial agent efficacy. Plant extract may have antibacterial properties because phenolic chemicals can attach to the cell wall of bacteria and stop them from proliferating and dividing. These results matched with Perez *et al.* (1990) who observed the antimicrobial activity of chicory (roots and leaves), Jerusalem (leave and tuber) extracts using agar well diffusion method.

Table 1. Aqueous extract of *Jerusalem artichoke* against bacterial strains.

Pathogenic strain	Herbal infusion (mm)xx					
	100 ×	75×	50×	25×	12.50×	MIC
E. coli ATCC25922	15	13	10	ND	ND	50
Staph aureus ATCC25923	20	17	13	11	ND	25

×: Concentrations of extracts and MIC are in mg/ml; ND; Not detected; MIC: Minimum inhibition concentration; ××: Diameter include 5 mm well diameter.

Table 2. Effect of Chitosan nano *Jerusalem artichoke* smart packaging on Yeast and Mould of Shrimp popcorn samples during storage at 4 °C for 12 days.

Treatment	CFU/g (Storage time (Days))				
	Zero	3	6	9	12
Control negative	ND	$3x10^{Aa}$	7×10^{Ab}	1.1×10^{2Ac}	3.6×10^{2Ad}
Control positive	ND	ND	$2x10^{Ca}$	5×10^{Cb}	9×10^{Cc}
Jerusalem artichoke 0.02%	ND	ND	4×10^{Ba}	8×10^{Bb}	1.3×10^{2Bc}
Jerusalem artichoke 0.2%	ND	ND	$1x10^{Da}$	$3x10^{Db}$	4×10^{Dd}
Jerusalem artichoke 0.5%	ND	ND	ND	ND	$2x10^{Ea}$

Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$). Upper case letters for columns and lower case letters for rows.

Table 3. Effect of Chitosan nano *Jerusalem artichoke* smart packaging on Total Volatile Basic Nitrogen (TVBN) of Shrimp popcorn samples during storage at 4°C for 12 days.

Treatment	TVB-N Content (mg/100g)	
	Zero	12
Control negative	6.08 ± 0.00^{Aa}	34.21 ± 0.01^{Ab}
Control positive	5.96 ± 0.00^{Aa}	30.58 ± 0.00^{Bb}
Jerusalem artichoke 0.02%	6.03 ± 0.01^{Aa}	28.11 ± 0.01^{Cv}
Jerusalem artichoke 0.2%	5.84 ± 0.00^{Aa}	25.23 ± 0.00^{Dd}
Jerusalem artichoke 0.5%	6.00 ± 0.00^{Aa}	21.13 ± 0.00^{Ee}

Means in the same column followed by different letters are significantly different ($p<0.05$). Means in the same raw followed by different letters are significantly different ($p<0.05$). Means with similar letters are not significantly different at ($P<0.05$). Upper case letters for columns and lower case letters for rows.

Characterization of Jerusalem artichoke-loaded Chitosan Nanoparticles (JA-NPs) by ransmission electron microscopy (TEM)

TEM scans revealed that Ch NPs appear to be tiny and distinct; larger particles are due to the aggregation of single small particles that tend to fuse generating a larger entity. A highly quick fusion (seconds/minutes) of individual particles into one entity was seen during TEM examination when magnifying CSNPs aggregation as much as possible. This was made feasible by the heat of the electron beam promoting intermolecular connections because the gel-network's aqueous environment was still present. This behaviour was induced by a linking agent; even when the beam was left on multiple nearby things for extended periods of time, their structures remained unchanged, and no fusion took place. Chitosan nano Jerusalem could keep the pH at the acidic level and reduce the water holding capacity (Figs. 1, 2).

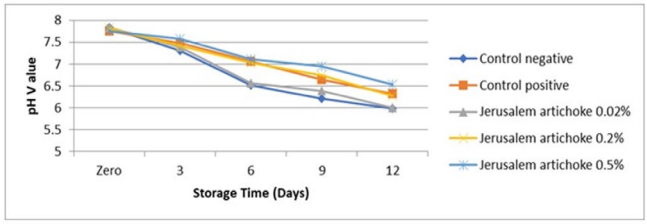

Figure 1. Effect of Chitosan nano *Jerusalem artichoke* smart packaging on pH values of shrimp popcorn samples during storage at 4°C for 12 days.

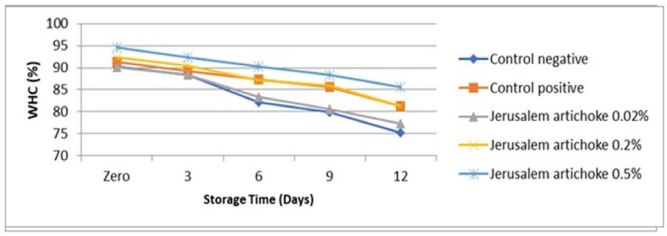

Figure 2. Effect of Chitosan nano *Jerusalem artichoke* smart packaging on Water holding capacity (WHC) of shrimp popcorn samples during storage at 4°C for 12 days.

Evaluation of shrimp popcorn samples treated with Chitosan nano Jerusalem artichoke

Effect of Chitosan nano *Jerusalem artichoke* on Thiobarbituric acid reactive substances (TBARS) of Shrimp popcorn Samples during storage at 4°C for 12 days

The TBARS value increased significantly in all samples over the course of the 12-day storage period, reaching 1.20, 0.92, and 0.63 Mg MDA\ Kg for the negative control, 0.5% chitosan nanojersulum artichoke, and 0.5% for the positive control, respectively (Fig. 3). This indicates a significant retardation of lipid oxidation and kept the level of TBARS below the allowable limit, which is not to exceed 0.9 Mg MDA/ Kg in accordance with Egyptian Standards 3495 (2005). These intriguing results demonstrated the examined mixture of natural extracts' capacity to delay lipid oxidation in shrimp popcorn and avert off-flavor.

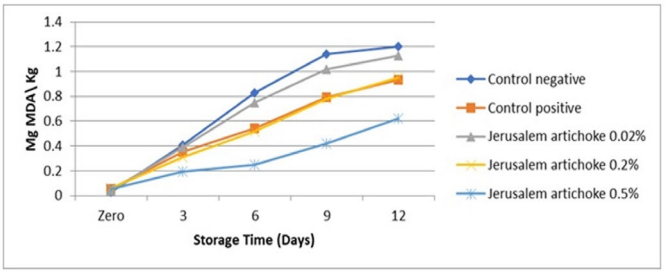

Figure 3. Effect of Chitosan nano *Jerusalem artichoke* smart packaging on Thiobarbituric acid reactive substances (TBARS) of Shrimp popcorn samples during storage at 4°C for 12 days.

Effect of Chitosan nano *Jerusalem artichoke* on Total bacterial count (TBC) of Shrimp popcorn Samples during storage at 4°C for 12 days

During the storage period, the bacterial count grew significantly in all samples; the positive and negative controls climbed from ($1.5x10^2$ and $1.3x10^2$ cfu/g) at day zero to ($2.1x10^7$ and $2.5x10^6$ cfu/g) at day 12. At the same time the TBC of treated samples with 0.5% of nanoformulation were increased from ($1.3x10^2$ cfu/g) at day zero to ($3.2x10^5$ cfu/g) at day 12 (Fig. 4). The maximum recommended bacterial count for fish products is $5x\ 10^5$ cfu/g (ICMSF, 1998).

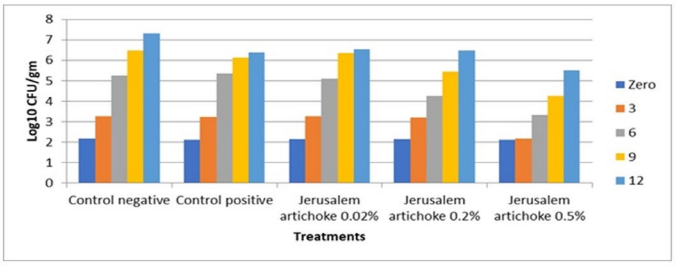

Figure 4. Effect of Chitosan nano *Jerusalem artichoke* smart packaging on aerobic bacterial count of Shrimp popcorn samples during storage at 4°C for 12 days.

Effect of Chitosan nano *Jerusalem artichoke* on Coliform count of Shrimp popcorn Samples during storage at 4°C for 12 days

Egyptian Standards 3495 (2005) state that the maximum coliform count that should be present is 10^2 cfu/g. All samples showed a considerable increase in the coliform count throughout the course of the 12-day storage period. On day three, the control sample ($1.2x10^2$ cfu/g) exceeded the legal limit, whereas the positive control ($1.1x10^2$ cfu/g) with BHT exceeded the permissible limit. However, the treated sample with the 0.5% nanoformulation achieved the allowable limit ($1.1x10^2$ cfu/g) on day six (Fig. 5). One may argue that the 0.5% concentration of nanoformulation shown a strong ability to stabilize the coliform count for a period of 12 days, making it a more effective natural preservative than BHT.

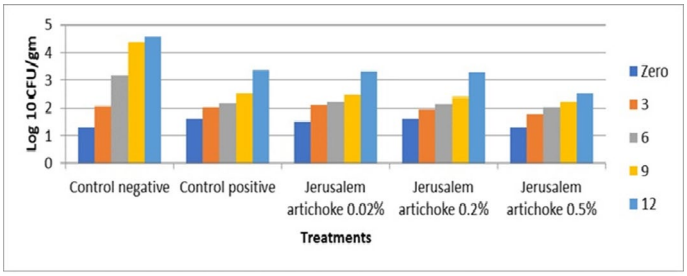

Figure 5. Effect of Chitosan nano *Jerusalem artichoke* smart packaging on Coliform count of Shrimp popcorn samples during storage at 4°C for 12 days.

Effect of Chitosan nano *Jerusalem artichoke* on sensory characteristics of shrimp popcorn samples during storage at 4°C for 12 days

The results showed that samples treated with chitosan nano *Jerusalem artichokes* had higher sensorial scores. This suggests that the effects of treatment on maintaining the sensory qualities of shrimp popcorn are primarily because of negatively charged macromolecules like sodium tripolyphosphate, which have been the subject of numerous studies.

Additionally, coating chitosan nanoparticles with chemicals or natural antibacterial agents, antioxidants, enzymes, or active ingredients is a possibility (Abd El Tawab *et al*., 2019). Since nano-chitosan has a bigger surface area and a stronger attraction for bacterial cells than chitosan, it exhibited higher antibacterial activity during product storage than chitosan (Ramezani *et al*., 2015).

The ionic gelation process is then used to convert chitosan from the deacetylation stage into nano-chitosan. Ionic gelation is a commonly employed technique due to its simplicity, effectiveness, and ease of control. After dispersing chitosan in acetic acid and adding polyanions, nanoparticles spontaneously form while being constantly stirred (Qonintannisa *et al*., 2020). The antibacterial activity test used the disc diffusion method to find out if nano-chitosan could stop the growth of *S. aureus* and *E. coli* bacteria. A clear or inhibitory zone encircling the paper disc indicates a positive outcome when using disc diffusion to test an extract or chemical for antibacterial activity (Magaldi *et al*., 2004). The highly powerful positive charge of nano-chitosan may be responsible for its antibacterial qualities as it draws negatively charged amino acid molecules, which are needed by bacteria to produce proteins. The membrane suffers a leaky

pressure as a result of the electrostatic interaction between these positive and negative charges. Microbes are unable to proliferate because of this imbalance in the osmotic pressure inside the cell. Furthermore, microbial cells die because of internal hydrolysis processes in the cell wall that liberate cell electrolytes (Sarwono, 2010).

Conclusion

This study revealed the positive impact of Chitosan nano *Jerusalem artichoke* smart packaging with different concentrations (0.02, 0.2, and 0.5 %) on shrimp popcorn samples at chilled storage (0, 3, 6, 9, and 12) days via improving the microbiological and chemical parameters with preserving the sensory characteristics of the shrimp popcorn samples and extending the shelf life for 12 days.

Conflict of interest

The authors declare that they have no conflict of interest.

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