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Sonochemical treatment of packaging materials for prolonging fresh produce shelf life

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ABSTRACT

Packaging bags made of polyethylene (PE) were sonochemically coated with edible antibacterial nanoparticles of chitosan (CS). In this work, the nanoparticles (NPs) were deposited on the surface of PE packaging bags by applying sonication waves on an acetic solution of chitosan. The characterization of CS NPs and PE bags was conducted by physicochemical techniques. The results showed that the coated bags had longer freshness than the uncoated ones. Furthermore, the characterization of cucumber, mushroom, and garlic placed into coated and uncoated PE bags was conducted by monitoring various parameters such as mass loss, total soluble solids, pH, and visual inspection. The study revealed that the PE bags coated with CS NPs showed a noticeable result in extending the shelf life of fresh produce. Finally, the antibacterial activity of PE bags was evaluated against various bacterial species. Hence, the PE bags coated with CS NPs could be a promising candidate for elongating the shelf life of packaged fresh produce.

1. Introduction

The food preservation primary objective is to prevent the deterioration of food from damaging agents, enhance the shelf life of food, and ensure food safety [1]. Fresh produce is often perishable after postharvest and requires conservation during storage and distribution to maintain a desirable shelf life [2]. Fresh produce can be exposed to infection by bacteria and fungi. Bacteria and fungi can infect fresh produce, leading to undesirable reactions that alter its physical and chemical properties, affecting its shelf life [3].

Food packaging plays a crucial role in protecting food from contamination and environmental factors during storage and transport [4]. Packaging with materials possessing antibacterial properties can delay the growth of microorganisms, prolonging the shelf life of fresh produce [5]. Edible antibacterial coatings, which can be applied as a thin layer onto packaging polymer surfaces, have gained importance in maintaining food quality [6]. Particles made from natural edible materials at varying sizes, ranging from 10 to 1000 nm, show unique physical and chemical properties due to the quantum size effect and surface properties [7]. The nanoparticles size plays a vital role in the antibacterial activity where smaller nanoparticles show larger antibacterial activity during the storage period. This could be due to the larger surface area of nanoparticles and their higher affinity for bacterial cells [8].

Edible coating based on natural biopolymers like lipids, polysaccharides, and proteins can be an alternative route to the existing food preservation technologies [9]. Chitosan (CS) a natural biopolymer (polyβ-(1, 4)-acetyl-D-glucosamine) is considered a cationic polysaccharide in its active form, biodegradable compound, non-toxic for human health and eco-friendly. It occurs as the shell component of crustaceans (crab and shrimp), as the skeletal substance of invertebrates, and as the cell wall constituent of some fungi

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and insects [10]. The most prevalent proposed antibacterial activity of chitosan nanoparticles is by the binding of positively charged amino groups of glucosamine to the negatively charged bacterial cell wall disrupting the cell, followed by attachment to DNA that is causes inhibition of DNA replication, resulting in bacterial cell death [11]. Using chitosan nanoparticles for edible coatings is helpful due to their excellent physicochemical properties, high transmittance values in the visible range, and optically transparent films that enhance the visual appeal of fresh produce [12]. Additionally, CS as a food packaging material has a remarkable ultraviolet (UV) absorbance which helps protect fresh produce from oxidations induced by UV radiation [13].

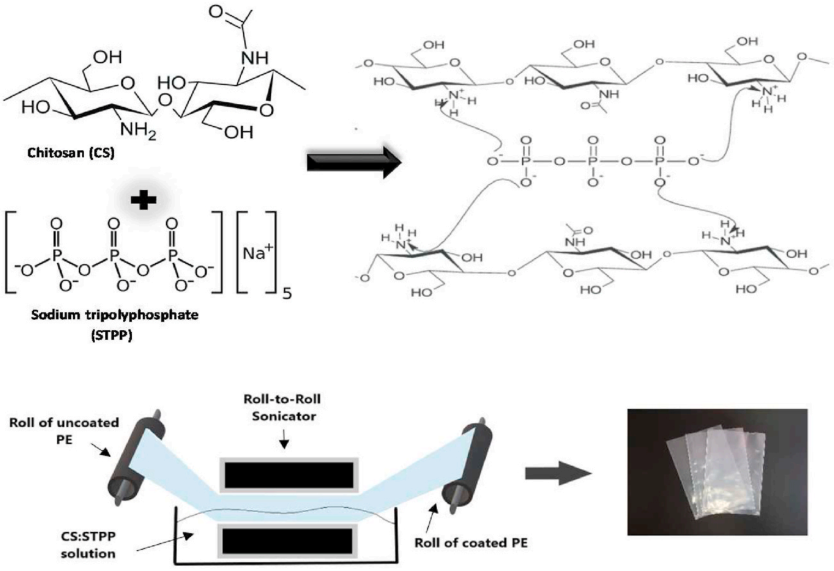
The sonochemical method is a promising technique for the synthesis of CS NPs as it is efficient and can lead to the formation of nanoparticles of the solute [14,15]. Moreover, sonochemistry can also be utilized for the surface coating of functional NPs on various substrates such as: textile [16], ceramic, cotton [17], paper [18], glass [19], polymeric [20], metallic, fabric [21,22] and even fresh produce [23,24]. Moreover, It is interesting to note that other techniques, such as dielectric barrier discharge (DBD) plasma and Atmospheric cold plasma (ACP) treatment, have also been investigated for extending the shelf life of fresh produce [25–27]. These techniques can modify the surface properties of packaging materials or produce itself, thereby reducing microbial growth and oxidative degradation. The combination of these techniques with CS NP-based coatings could potentially lead to even better preservation outcomes for fresh produce. Sonochemistry can significantly enhance and optimize the coating process, for example, CuO NPs coated on Cotton were washed 65 times in Hospital washing machine (75 °C) and 83% of the NPs were found on the surface of the Cotton at the end of this protracted process [17].

The utilization of ultrasound in food technology for processing, preservation, and extraction evolved to keep the wheel of development rolling [28–30]. Specifically, the study highlights the use of a roll-to-roll sonochemical coating machine to create chitosan nanoparticles and coat them onto a polyethylene surface in a one-step process. This technique was found to be effective in improving the shelf life and quality of fresh produce. This study also emphasizes the importance of edible coatings in preserving the quality of fresh produce during storage and transportation. The combination of sonochemical formation of nanoparticles with sonochemical coating technique to create a new packaging material is a significant advancement in the field and has not been reported previously. (Scheme 1). The bags made from PE-coated CS NPs and PE as a control were filled with three kinds of fresh produce and stored at cold storage. Overall, this research proves the potential of sonochemistry as a useful tool in the food industry for improving the quality and shelf life of food products. The use of sonochemical coating technique for creating packaging materials has the potential to resolve transport problems and improve consumer acceptability of fresh produce.

2. Materials and methods

2.1. Coating compounds

Low molecular weight chitosan (CS), (<100 kDa, high deacetylation degree with good solubility in water). and sodium tripolyphosphate (STPP) were purchased from Sigma-Aldrich. The commercial polyethylene (PE) sheets were supplied by Hanamal Company. Glacial acetic acid (99.7 %) was analytical reagent grade and was purchased from Sigma-Aldrich. Double-distilled deionized water was used for the preparation of all the solutions.



Scheme 1. Sonochemical coating of PE sheets with CS NPs.

2.2. Fresh produce samples

Cucumber (*Cucumis sativus*), mushrooms, and peeled garlic cloves (*Allium sativum*) were purchased from the local market at Bar-Ilan University. The cucumbers were carefully selected with a maturity of about (120 mm length, 20 mm diameter, and dark green color), mushrooms were selected in uniform sizes of 30–35 mm diameter, free damaged, and the peeled garlic cloves were uniformly shaped with medium maturity about 15 mm diameter per one clove, white color and free of any visible defects.The produce was transported to a laboratory and immediately packed in two types of bags: coated PE bags and uncoated bags (control). The fresh produce was divided into two groups: the first group were filled in 6 un-perforated PE bags, and the second group was filled in 6 perforated PE bags. Each group held 3 PE bags coated with CS NPs and 3 PE bags as a control.

2.3. Chitosan nanoparticles formation and deposition on polyethylene (PE) films

CS was dissolved in 1 % aqueous acetic acid solution to obtain concentration of 0.3 % w/v and stirred overnight at room temperature (25 °C). STPP was dissolved in Double-distilled deionized water to obtain a concentration of 2 % w/v (2g/100 ml). STPP solution was added dropwise to the chitosan solution under magnetic stirring at 40 °C in the ratio 5:1 v/v (CS: STPP). The reaction slurry was irradiated for 30 min with roll-to-roll high intensity sonicator in the presence of a 30 cm × 3 m of PE sheet at 45 °C. The machine was coating the PE at a speed of 0.2 m per 1 min and at the end of the process, the chitosan coated PE films were air-dried on the hood at 25 °C for 5 days prior to characterization. The PE bags filled with the fresh produce and were subsequently stored at 5 °C and 75–85 % relative humidity for 21 days at cold storage. The quality parameters were checked at 0, 3,6,9,11,13,15,17,19, and 21 days, respectively. The samples were evaluated at least in triplicate. The chitosan amino groups are cross-linked with phosphoric groups of sodium tripolyphosphate this approach has drawn notable attention because this process is free of organic solvents, comfortable, controllable, and non-toxic [31].

2.4. Characterization of PE sheets and CS NPs

The surface morphology and the size of Edible nanoparticles of the PE sheets were characterized by high-resolution scanning electron microscopy (HR-SEM) at 5 kV. The particle size of CS NPs in the solution after the sonochemical process was measured by dynamic light scattering (DLS) instrument (Malvern, UK) to confirm the formation of nanoparticles in the solution. The analysis was conducted at a scattering angel of 90° at room temperature of 25 °C using CS dispersed in distilled water. The average particle size of NPs and the polydispersity index (PDI) are reported.

2.5. Physical and chemical properties of PE sheets and fresh produce

Mass loss was determined by weighing the PE bags coated CS NPs that filled with the fresh produce, before and during the storage period. In parallel, the same process was conducted for un-coated PE bags. And the percentage of weight loss was calculated according to the following Eq:

$$Weight\ Loss\ \% = \frac{Wi - Wf}{Wi} \times 100$$

W_i is the initial weight of the coated and un-coated bags, and W_f is the weight at time t (with t = 0, 3,6,9,11,13,15,17,19, and 21 days). The total soluble solid content (TSS, ºBrix) of homogenized fresh produce samples was determined. by using a handheld refractometer (REF113 Brix/ATC 0–32 %) which measures the refractive index expressed as ºBrix. The pH analysis was conducted directly on homogenized samples using a pH-meter (Mettler Toledo Sevenmulti) [32,33]. Images of the PE bags coated with CS NPs and un-coated PE bags (control) throughout the storage show the decay rate of those fresh produce [34,35].

2.6. Sensory acceptance

Decay rate a damage that produced by deterioration symptoms such as, mold growth, bacteria, and inhibition of enzymatic processes on fresh produce, were appreciated by visual inspection that based on general visual attraction, color and visible structural completeness, according to the following scale: 1 = no damage; 2 = minor damage below 25 %; 3 = moderate damage between 25 % and 50 %; 4 = drastic damage between 50 % and 75 %; 5 = totally damage between 75 % and 100 %. Fresh produce counted below 2 was considered acceptable.

2.7. Antibacterial activity

The antibacterial activity of the PE bags coated CS NPs and un-coated PE bags was done with three bacterial species: Gram-positive *Staphylococcus aureus*, *Listeria*, and Gram-negative *Escherichia coli*. The bacterial tissue culture was grown in a nutrient broth (NB) medium in a shaking incubator at 37 °C at 250 rpm for 24 h. The concentration of bacteria was adjusted to be between 10^5 and 10^6 colony forming units per ml (CFU/ml) [8]. For each bacterial strain, PE control and PE coated CS NPs were inoculated with the dilute bacterial suspension. Each bacterial suspension was added dropwise to the PE to allow it to be fully contacted to the surface. The bacterial growth was monitored and calculated by viable bacterial cell count and expressed in CFU/ml [15].

2.8. Statistical analysis

The data presented for the physical and chemical properties of fresh produce, were conducted in triplicate ($n = 3$). Significant differences in means were calculated by using one-way analysis of variance (ANOVA) which reflects edible coating treatment at $P \leq 0.05$. The data are expressed as the mean ± SD.

3. Results and discussion

3.1. Characterization of CS NPs and coated PE

3.1.1. High resolution scanning electron microscopy (HR-SEM)

The morphology of the PE sheets coated with CS NPs were examined by high resolution scanning electron microscopy (HR-SEM) after the performance of the sonochemical coating.

In parallel, the morphology of the un-coated PE was characterized as a control sample. The results clearly prove that the sonochemical coating process successfully deposited CS NPs onto the surface of the PE sheets, resulting in a rougher and more textured surface compared to the smooth surface of the uncoated PE (Fig. 1(a, b, and c)). In contrast, the coated PE exhibit significant difference, the nanoparticles size of CS NPs that were distributed on the surface of PE packaging was between 100 and 700 nm as shown in Fig. 1d, e, f, g, h, i. The HR-SEM results suggest that the sonochemical coating process is an effective method for depositing CS NPs onto the surface of PE sheets, which could improve the barrier properties of the packaging material and enhance its performance in food packaging applications.

3.2. Dynamic light scattering (DLS)

The average particle size of CS NPs was measured by DLS. The aim is to confirm the formation of the NPs in the solution and estimate their size. The DLS measurement was performed before and after the sonochemical process. The results show that the particle size of CS NPs in the solution before sonication was above 1 μ, indicating the presence of larger particles or aggregates. Fig. 2a, After sonication, the particle size of CS/STPP ranged between 60 and 700 nm, indicating the successful formation of smaller nanoparticles shown in Fig. 2b. Smaller nanoparticles have a larger surface area to volume ratio, which can enhance their reactivity and surface

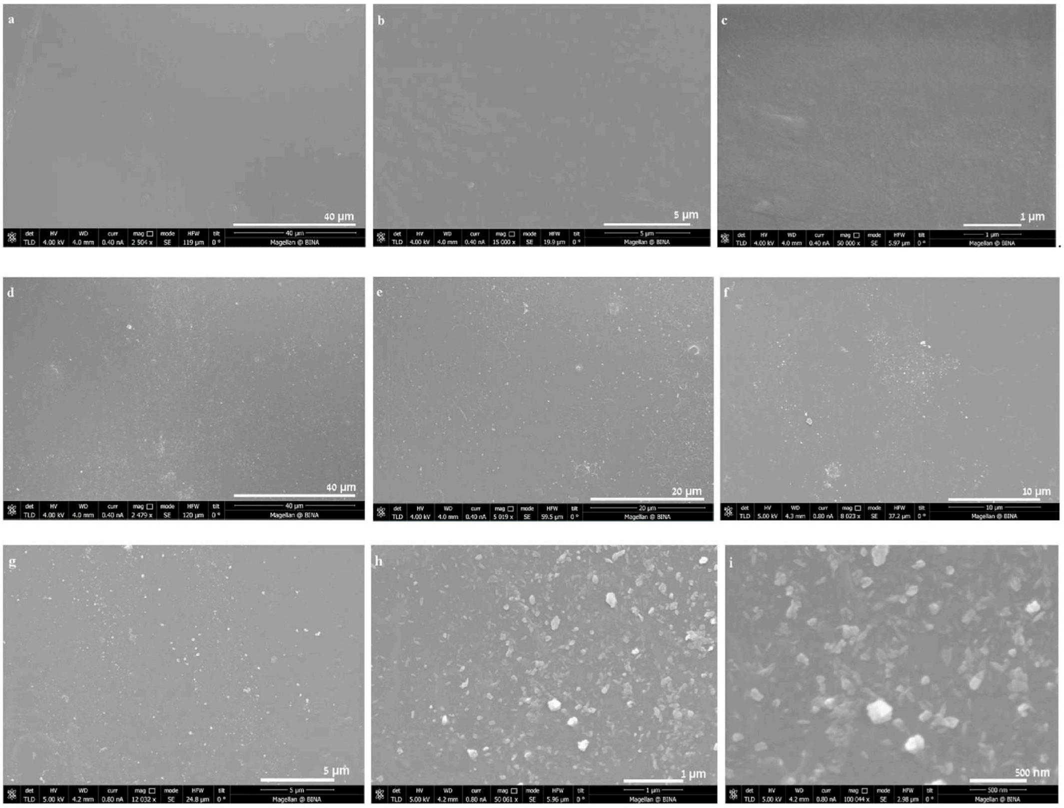


Fig. 1. HR-SEM of un-coated PE bags (control) before the sonochemical process, (a) low magnification, (b–c) high magnification. Versa PE bags coated CS NPs after the sonochemical process, (d–f) low magnification, (g–i) high magnification.

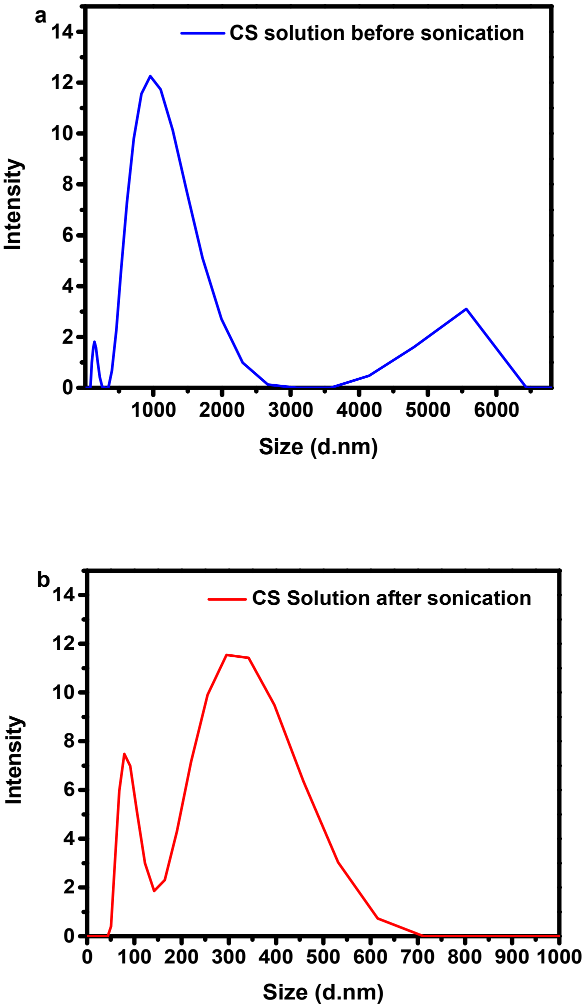


Fig. 2. Dynamic light scattering (DLS) of (a) CS: STPP solution before the sonochemical process, (b) CS: STPP solution after the sonochemical process.

properties. Overall, the DLS results confirm the successful formation of CS NPs in the solution and supply information about their size distribution, which can be useful for perfecting their performance in in food packaging applications.

3.3. Physical and chemical properties of PE sheets and fresh produce

The mass loss of fresh produce is an important index that reflects the respiration rate and the moisture content. Significant variation in mass loss was seen in fresh produce samples packed in PE coated with CS NPs compared to uncoated PE bags after 21 days of storage. As shown in Fig. 3 (a, b) samples of fresh produce stored in the coated bags show 2–5% mass loss, while the fresh produce that was stored in the control bags (un-coated PE) shows values of 16–32 % mass loss after the same 21 days of cold storage.

The pH reflects the changes in acidity of fresh produce during the storage period. The pH values of fresh cucumbers stored in PE bags coated with CS NPs were higher than those stored in uncoated PE bags, while the pH values of garlic and mushroom stored in PE bags coated with CS NPs were lower than those stored in uncoated PE bags. The results exhibited that fresh produce stored in coated PE bags showed slight differences in pH values, while uncoated PE bags showed remarkable and extremely significant differences in pH values. as shown in Fig. 4. It is interesting to note that the pH changes were more pronounced in the samples stored in uncoated PE bags, indicating that the use of coated PE bags may help to maintain a more stable pH environment during storage. Overall, these findings suggest that the type of packaging material used can have an impact on the acidity of fresh produce during storage.

Total soluble solid (TSS) content is considered as an important parameter for detecting deterioration of fresh produce after the postharvest during the storage period. Fresh produce filled in PE coated CS NPs bags shows noticeable effect on total soluble solid content which maintained constant values throughout the storage period. TSS increased during storage in garlic gloves, whereas TSS values decreased in the case of cucumber and mushroom during the storage as shown in Fig. 5. This could be due to differences in the composition of the fresh produce. The use of PE-coated CS NPs bags has been shown to maintain the TSS values of fresh produce at a

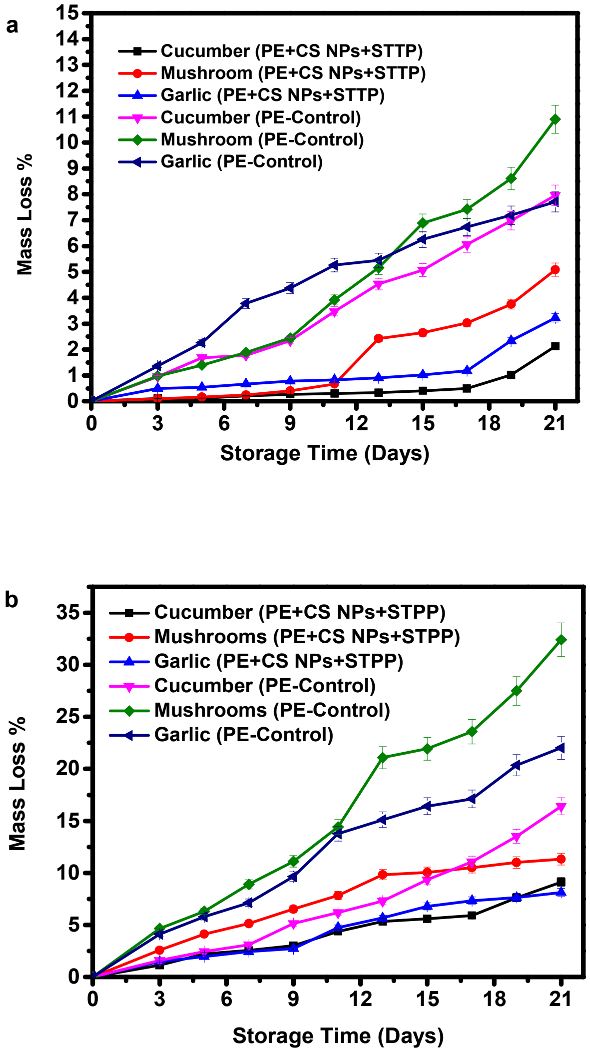


Fig. 3. Mass Loss of (a) un-perforated PE bags, (b) perforated PE bags coated CS NPs versa uncoated PE bags (Data are mean ± SD, n = 3).

constant level throughout the storage period. This is likely because the coated bags supply a protective barrier that helps to reduce the rate of moisture loss and prevents the entry of oxygen and other gases that can lead to spoilage.

The images of the coated PE bags show significant difference compared with the un-coated PE bags (control), suggesting that the fresh produce in the PE bags coated CS NPs will not be damaged by mold growth whereas the decay rate significantly increased in un-coated PE bags. Moreover, the PE bags sonochemically coated CS NPs y exhibited an ability to inhibit the severity of mold growth on the fresh produce during 21 days of cold storage at 4 °C (Fig. 6). Although the un-coated PE bags filled with fresh produce show a noticeable increment in mold growth (Fig. 7). Additionally, unperforated bags coated with CS NPs showed a slight difference in preserving the quality of the fresh produce during storage, leading to a prolonged shelf life as shown in Fig. 6a and b whereas perforated and unperforated uncoated PE bags showed significant deterioration in the quality of the fresh produce during storage as shown in Fig. 7a and b. Overall, the results describe the effect of sonochemically coated chitosan nanoparticles (CS NPs) on inhibiting mold growth and preserving the quality of fresh produce during cold storage.

3.4. Sensory acceptance

The Decay rate reflects the occurrence and severity of mold. An initial evaluation of the fresh produce filled in PE bags coated with CS NPs and uncoated PE (control) presented a source of 1, suggesting that the fresh produce will not be damaged by molds and enzymatic processes. Nevertheless, the decay rate increased significantly in the fresh produce stored in uncoated PE bags control with a decay rate of 4.7 for mushrooms, likewise, cucumbers stored in control PE bags, show an increasing at decay rate (decay rate = 4) as compared with cucumber stored in PE coated CS NPs (decay rate = 2). It is worth mentioning that the fresh produce stored in PE bags coated with CS NPs stays stable throughout the storage period. Moreover, CS NPs edible coating showed a significant ability to inhibit

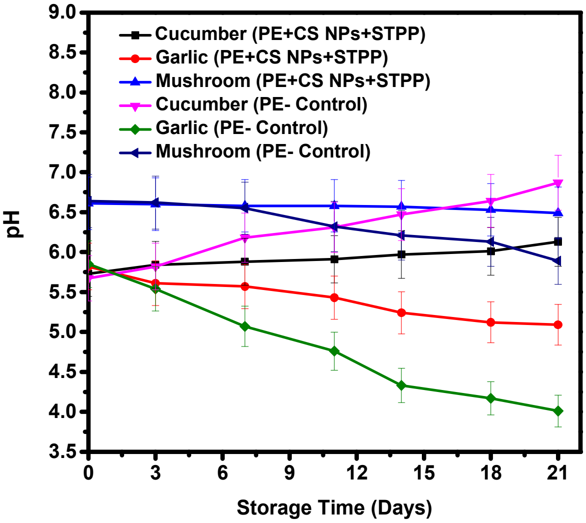


Fig. 4. Effect of PE coated CS NPs on the pH of fresh produce at cold storage (Data are mean ± SD, n = 3).

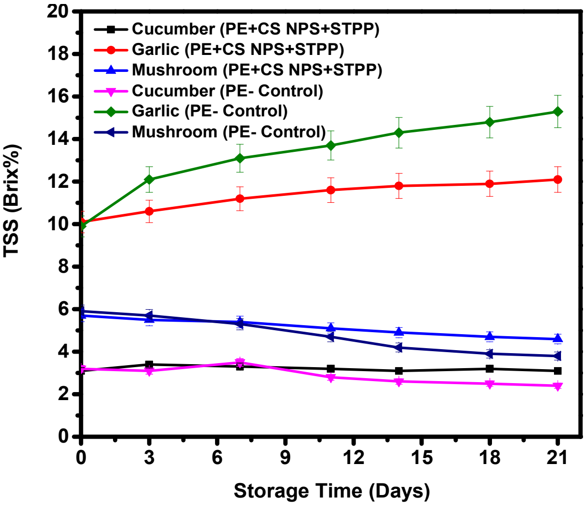


Fig. 5. Changes in total soluble solid (TSS) of fresh produce during storage period (Data are mean ± SD, n = 3).

mold growth during 21 days of storage as shown in Fig. 8.

3.5. Antibacterial test

Antibacterial activity is a crucial factor in evaluating the efficiency of the packaging used for food preservation. The sonochemical coated surfaces showed good antibacterial activity by inhibiting fungal growth and reducing the number of bacteria in the packed fresh produce during the storage period. The sonochemical chitosan coated PE bags showed an excellent antibacterial activity against *E. coli.* In this case, a greater than 3 log reduction in bacterial numbers on CS NPs coated PE compared to un-coated PE control samples was observed (Fig. 9a). After being used with the fresh produce, the bags were washed with water repeatedly and then filled again with new fresh produce. The same results were obtained in the second run (Fig. 9b). Also, after reusing the coated PE bags the antibacterial activity against *E. coli.* were more than 3 log reductions as compared to the control. The antibacterial activity levels of the PE films against the Gram positive – bacterium *S. aureus*, and *Listeria*, were not as good as against *E. coli* which is a Gram-negative bacterium.

In addition, the antimicrobial activity of CS derives from the presence of amino groups as CS functional groups, which undergoes a chemical modification under acidic condition and becomes a positive charge carrier [36]. The positively charged amino groups of CS function as antimicrobials through the following mechanism: adsorption and diffusion, binding, disruption of cell membrane, and releasing the cell constituents that eventually leads to the death of bacterial cell as shown in Fig. 10. Electrostatic interaction between the positively charged amino group on CS toward negatively charged components present on the bacterial surface. However. The

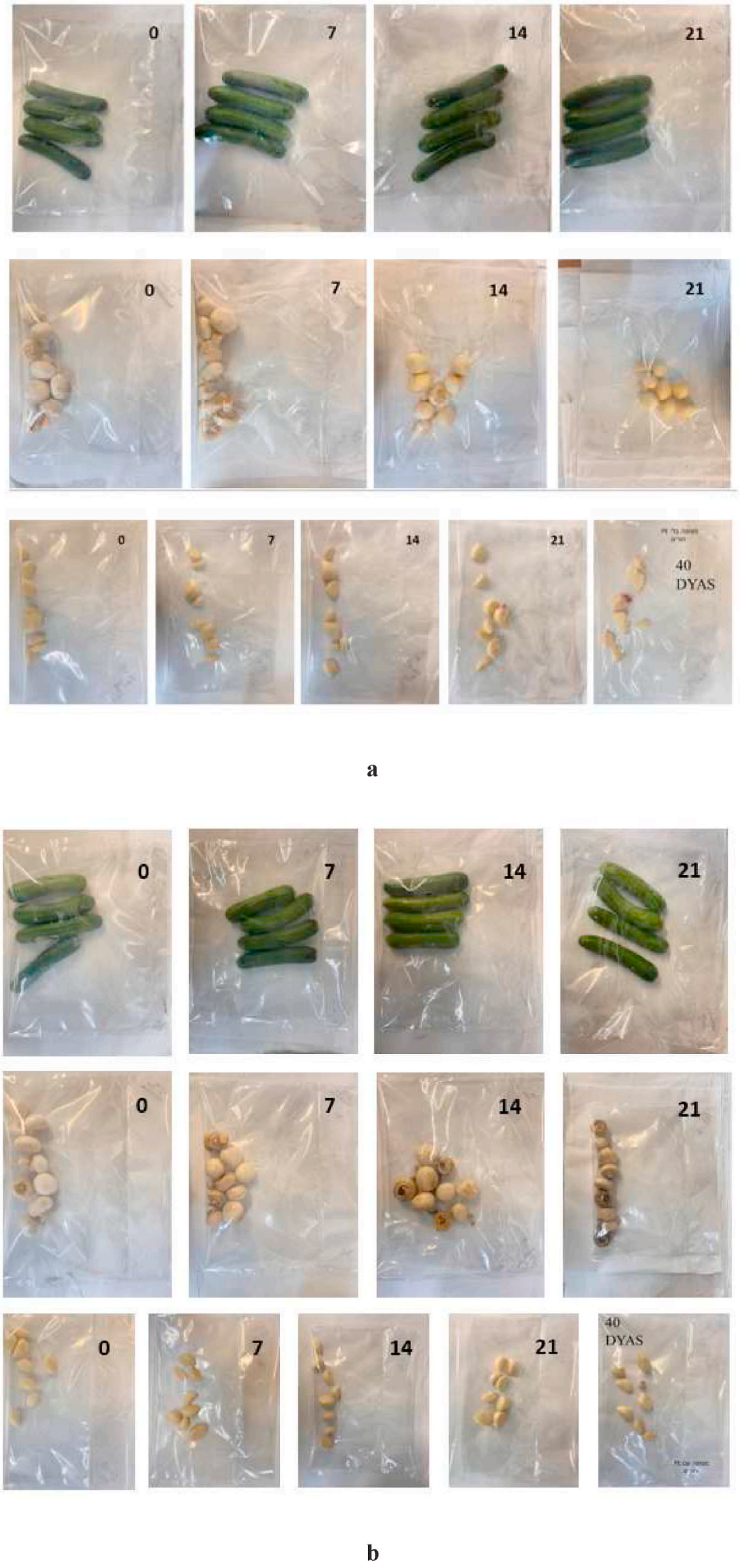


Fig. 6. a Images of un-perforated PE bags coated CS NPs filled with fresh produce. b: Images of perforated PE bags coated CS NPs filled with fresh produce.

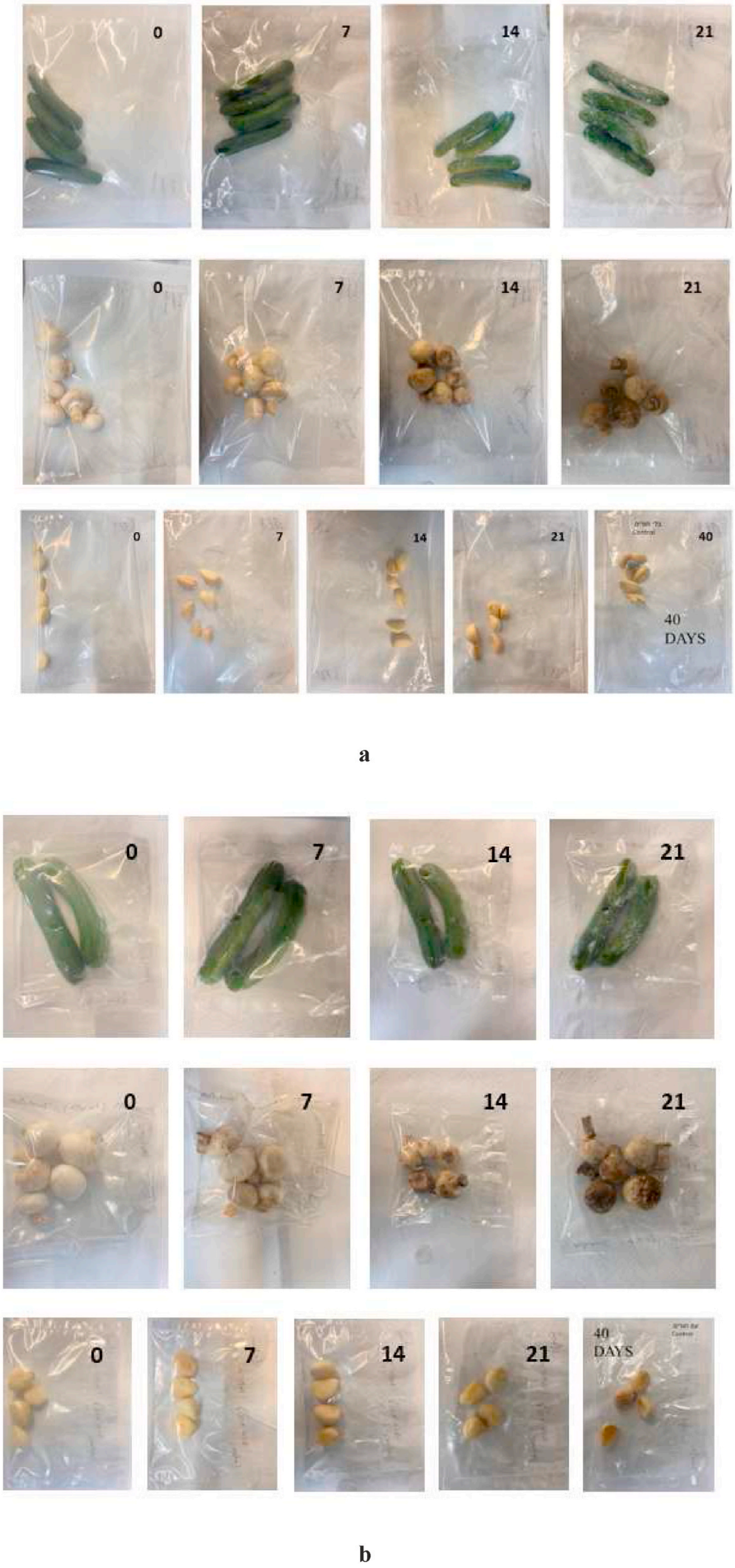


Fig. 7. a Images of un-perforated PE bags (control) filled with fresh produce. b: Images of perforated PE bags (control) filled with fresh produce.

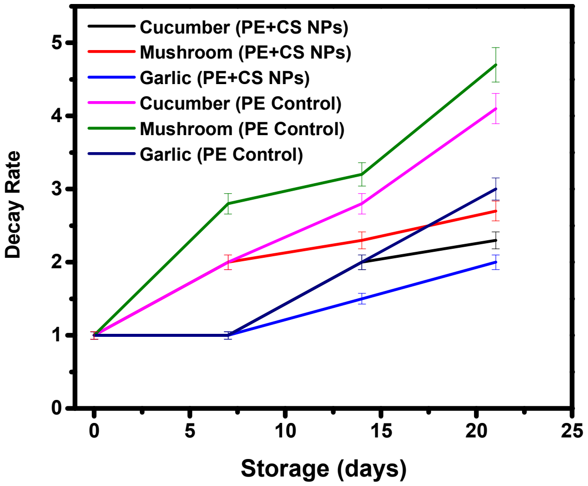


Fig. 8. Effect of CS NPs edible coating on decay rate of fresh produce stored at 4 °C. (Data are mean ± SD, n = 3).

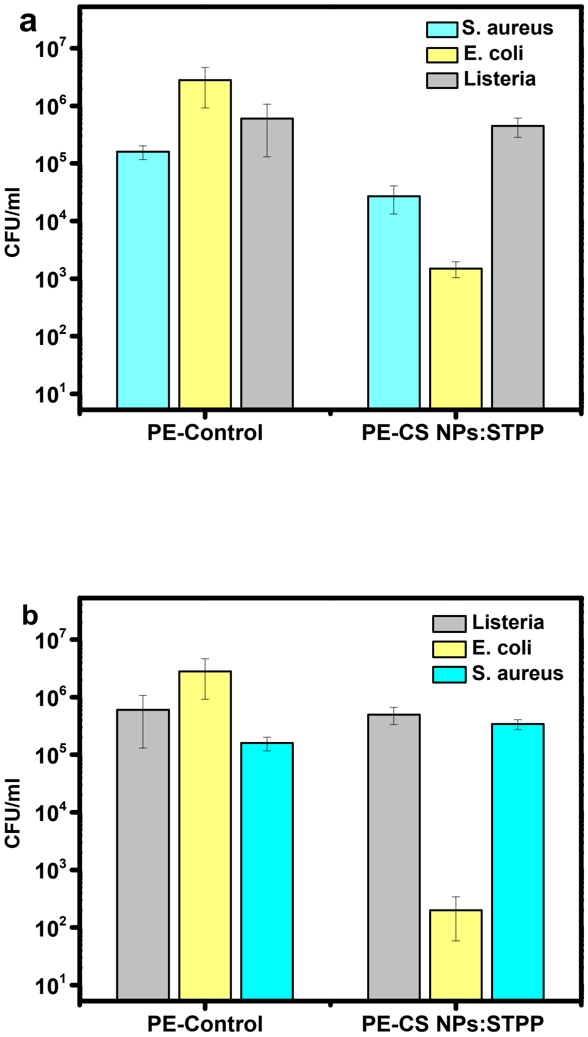


Fig. 9. Antibacterial activity of (a) PE coated CS NPs first run, (b) second run, against *E. coli*, *S. aureus* and *Listeria*.

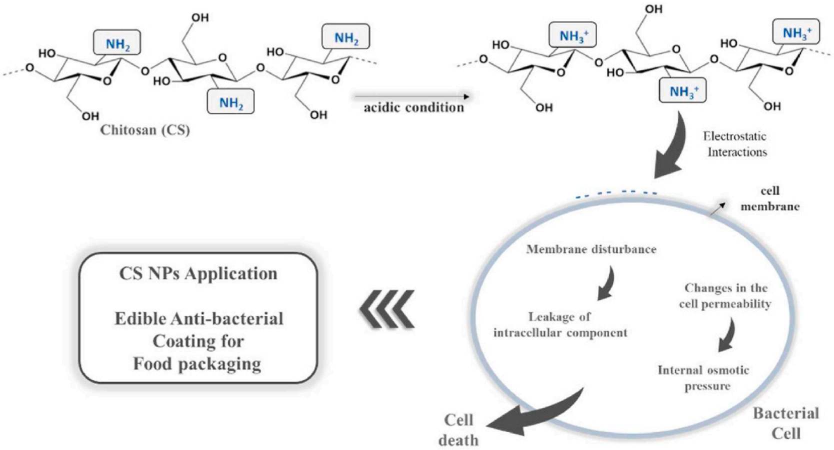


Fig. 10. Schematic diagram of Preparation and Antibacterial activity mechanism of CS NPs.

interaction runs differently according to the type of bacterial species. In the case of Gram-positive bacteria, the peptidoglycans in the cell wall which help protect bacterial cells from environmental stress are hydrolyzed, that leads to leakage of intracellular part. And in the case of Gram-negative bacteria, the CS causes changes in the permeability of the outer envelope that prevent the transportation of nutrients into the bacterial cell and cause an internal osmotic pressure [37]. Therefore, the mechanism involving changes in the permeability of the outer envelope, which prevents the transportation of nutrients into the bacterial cell and causes internal osmotic pressure, was more prominent in this specific case.

The current study has been designed to compare the effects of the bags and coatings on the preservation of the produce, with the hypothesis that the coated bags would have a better preservation effect. Our research manifests the usefulness of the sonochemical coating of packaging by embedding edible antibacterial NPs on the surface of PE sheets to elongate the shelf life of fresh produce placed into PE bags coated CS NPs and preserve the quality of fresh produce. The PE bags proved their effectiveness in elongating the shelf life of fresh produce significantly in the second run since the CS NPs tightly embedded in the PE, which make it sustainable for reuse [38]. The HR-SEM images show the difference in the morphology between PE bags coated CS NPs and uncoated bags. Moreover, the sonochemical process helps on controlling and reducing the particle size of CS from micron to NPs, which will grant more affinity to the particles on the PE surface, leading to more coated surface area, which improve the antibacterial activity. Applying the sonochemical method as an excellent technique for edible antibacterial coating reduced the mass loss and delayed the mold growth on the fresh produce which was placed into PE bags coated CS NPs. These findings are related to the fact that the sonochemical coating mechanism is based on physical interaction of the coated material with the substrate and is not based on chemical interactions, also without any modification to the PE sheets before the sonochemical process, as a validation of what has been done in previous studies [25,26]. Edible coating helps at maintaining the pH values and TSS of fresh produce more efficiently compared to the control (un-coated PE bags). The shelf life of fresh produce placed into PE bags coated CS NPs has been extended for more than 21 days at cold storage. Furthermore, edible antibacterial coating of CS NPs significantly increases the antibacterial activity against *E. coli* compared to the control. This is associated to the particles size of CS which play an essential role in increasing the antibacterial activity of PE sheets [39]. Moreover, low molecular weight of CS has a significant effect on increasing the antibacterial activity because of the small chins of CS [40]. The ionic interaction, attraction, and mobility of the CS NPs with the bacterial species occurs according to our assumption through the direct contact with the bacteria on the PE surface [31]. While the study presented promising results regarding the benefits of sonochemical coating with edible antibacterial nanoparticles on PE sheets, it is important to acknowledge its limitations to provide a balanced perspective: Limited scope of produce types, Lack of real-world conditions, Limited bacterial strains evaluated, Lack of consumer acceptance analysis, Cost-effectiveness analysis.

4. Conclusions

The current study investigates the effects of bags and coatings on the preservation of fresh produce, specifically focusing on the application of sonochemical coating with edible antibacterial nanoparticles on polyethylene (PE) sheets, making them an ideal solution for transport problems, and improving consumer acceptability. The study highlights the potential of sonochemical coating technology in developing food packaging materials with enhanced antibacterial properties. The combination of sonochemical formation of nanoparticles and sonochemical coating techniques to create packaging materials is a significant advancement in the field and has the potential to revolutionize the food industry. The results show that the antibacterial activity of PE bags coated with CS NPs was significantly increased. Furthermore, the application of the sonochemical coating method effectively reduces mass loss and delays mold growth on fresh produce stored in the coated PE bags. The edible antibacterial coating assists in maintaining optimal pH values and Total Soluble Solids (TSS) of the produce, outperforming uncoated PE bags in this aspect. Future studies should highlight the use of various edible antibacterial compounds and their combination to have broader antibacterial activity and elongating the shelf life of fresh produce.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Belal Abu Salha: Conceptualization, Methodology, Software, Writing – original draftWriting – original draft. **Ilana Perelshtein:** Data curation, Formal analysis, Writing – review & editingWriting – review & editing, Software. **Aharon Gedanken:** Funding acquisition, Methodology, Project administration, Supervision, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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