

Antimicrobial properties of Chitosan Nanoparticles and their Role in Post-Harvest Shelf Life Extension

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Abstract: Chitosan is the second-most abundant natural biopolymer that possess excellent nontoxic, antimicrobial, biocompatible and biodegradable properties and has been used as a protective finish in food industry because of its proven antimicrobial activity. Various nanocomposites of chitosan exhibited better antimicrobial performance than Native chitosan biopolymer. This article seeks to review extensively the use of nano fillers like metal ions metal oxides, referred to as chitosan nanoparticles, for significant improvement or better use of antimicrobial properties of chitosan in food industry with the help of nanotechnology to increase the shelf life of fruits and vegetables. The article also studies in detail the extended properties of the target, methods of application, its effect and the microorganisms inhibited in the process.

Keywords: chitosan; nanoparticles; antimicrobial; nanofillers;

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I. Introduction

Plant production in the world is estimated at between 486 million and 392 million tonnes, with losses of 30%–40% in industrialized economies as well as >50% in developing countries. This means that a significant portion of the world's total plant production is lost each year. Poor post-harvest practices and microorganisms cause fruit and vegetable spoilage and losses of up to 40% after harvesting. Most of the postharvest degradation in warm and subtropical fruit occurs because of fungal species, such as *Rhizopus*, *Botrytis*, *Colletotrichum* and as well as *Penicillium*, *Alternaria* and *Fusarium*. Even so, there is a massive demand from consumers for plant products that are environmentally benign and contaminants, and the evolution of new systems to fungicide use is an urgent need. Different alternative systems, such as electrolyzed water, ozone, chlorine, hydrogen peroxide, ethanol, and organic acids, have been used to minimize, decrease, or regulate the pathogenic potential of these biotic factors. Food packaging businesses rely heavily on edible coatings for their capacity to keep food fresh and increase the shelf life. On the food surface, biopolymers form a thin layer of edible coating. Edible films ought to have high barrier, thermal, and antibacterial qualities in order to fulfill their roles. Non-toxic, biodegradable, and edible polysaccharides are widely employed in the manufacturing of various edible films.

II. Antimicrobial properties of chitosan and its influencing factors:

Considering its high biodegradability, nontoxicity and antimicrobial properties against a wide range of microorganism, nowadays chitosan is extensively used in food industries as an antimicrobial agent either as a native biopolymer or as a nanocomposite, blended with other nano particle. A wide spectrum of antimicrobial activities of chitosan has been demonstrated against many gram positive and gram negative bacteria, filamentous fungi and yeast, but it showed lower toxicity towards mammalian cell, which enhancing its application for mankind in many ways. (Ming Kong et al) In last two decades, there has been an increasing interest on enhancing antimicrobial properties of chitosan mainly for environment friendly application with high rise in the number of published article. In respect to that several studies have reported that various nanocomposites of chitosan exhibited better antimicrobial performance than Native chitosan biopolymer. There are several review studies on analyzing the antimicrobial activity of chitosan and they conclude that there are some influencing factors that affect the antimicrobial activity of chitosan. They include microbial factors like type of microbial species and cell age. Intrinsic factors like positive charge density, Mw, hydrophilic or hydrophobic characteristics of chitosan, chelating capacity with various metal ions and metal oxide, and some external factors like pH, ionic strength, time and temperature. [1,2,3]

2.1 Microbial factors

Although chitosan exhibit a wide spectrum of antimicrobial activity but several studies showed that it displayed different efficiency on different gram negative and gram positive bacteria and fungi. Since Gram positive and gram negative bacteria exhibit a huge differences in their cell wall structure antimicrobial performance of chitosan differs. A study on bactericidal activity of chitosan film against salmonella spp. concluded that gram-positive bacteria is more sensitive to chitosan than gram-negative bacteria and some other researches concluded the same. But on contrary some other studies revealed antibacterial activities was apparently stronger against gram negative bacteria because of the higher negative charge value on cell surface. Results of some studies said that cell age can also influence the microbial efficiency. For example chitosan showed more efficiency against gram-positive bacteria in late exponential phase than bacteria with mid-exponential or late stationary phase. In contrast some other study referred gram-positive bacterial showed better susceptibility towards chitosan in mid-exponential phase.

2.1.1 Intrinsic factors

- Positive charge density of chitosan : Higher positive charge density confers considerably higher antimicrobial activity through strong electrostatic interaction. Chitosan with high DD showed excellent antimicrobial efficiency against *S.aureus*. furthermore, several literature concluded antimicrobial performace of chitosan significantly increased against *E.coli*, salmonella enteritidis, *L.monocytogenes*, with increasing DD.
- Molecular weight (Mw) of chitosan : The conclusion of several studies on effect of Molecular weight on efficiency of chitosan is contradictory. In one study *S. aureus* exhibit increased susceptibility to chitosan with increased Mw, whereas chitosan with low Mw showed better antimicrobial efficiency against *E.coli* than chitosan with increased Mw. *Tikhonov et al*, emphasized in his study that low Mw chitosan and its derivatives are the excellent antimicrobial agent against bacteria, yeast, fungi.

2.1.2 Extrinsic factors

- pH : Lim and Hudson suggested that antimicrobial activity of chitosan is highly pH dependent not only because chitosan is only soluble in acidic media but also the molecule become polycationic at low PH(<6.3) The result of antimicrobial experiment of chitosan showed better bactericidal activity against *E.coli* and *S.aureus* at pH less than 5. It has proved by several literature that chitosan with increasing pH showed decreased antibacterial efficiency and lost completely at neutral pH.
- Temperature and time : High Relationship between temperature and chitosan influence greatly on efficiency of chitosan for industrial application. Different temperature may also change the viscosity or Mw of chitosan. Studies by Tsai and su mentioned that increased temperature in the range of 4 -37°C exert enhanced efficiency against *E.coli*. another study showed same result against *Aeromonas hydrophila*. Study investigating antibacterial activity against *L.monocytogenes* and *S.aureus* at 4 and 25°C for 15 week storage resulted that chitosan had a better antibacterial efficiency before storage and chitosan solution stored at 25°C showed same or weaker antibacterial performance than 4°C.

2.2. Effect of nano-metal and metal oxide on Increased Antimicrobial properties of chitosan

In recent years, many studies have reported chemical modification of chitosan either by grafting with other polymer or by reinforcement of different Nano fillers like metal ions metal oxides, for significant improvement or better use of antimicrobial properties of chitosan. Chitosan-zinc nanocomposite showed a broad spectrum of effective antimicrobial activities which was 2-8 times higher than native chitosan, owing to zinc can increase the positive charge on the amino group of chitosan. Oxide form of zinc when combined with chitosan exhibited excellent antimicrobial properties against gram positive bacteria like *E. faecalis*, *Bacillus subtilis*, and gram negative like *E.coli*, *K. planticola* and fungus like *C. albicans*. Chitosan-nano silver composite performed stronger antibacterial activity against *S.aureus* and *E.coli* than chitosan. Silver nanoparticle cause leakage on bacterial membrane protein and essential lipopolysaccharide that eventually results cell death. Antifungal activities of silver/chitosan composite are also resulted better than chitosan. Copper nanoparticle is commonly used to chitosan to improve the antibacterial activity of chitosan commonly against *E. coli*. Different nanocomposite of CuO with chitosan showed a wide range of antimicrobial efficiency against *P. aeruginosa*, *S. aureus*, *E.coli*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *C. albicans*. TiO₂ has been studied a lot as a prominent bactericide due to its attractive properties like high photocatalytic efficiency, non-toxicity. Through photocatalytic reaction TiO₂ can generate reactive oxygen species that change the permeability of bacterial membrane and causes leakage of cytoplasm. TiO₂-chitosan nanocomposite showed considerably higher antimicrobial efficacy against bacteria like *S. aureus*, *E.coli*, *P. aeruginosa*, *S. typhimurium* and fungi like *aspergillus* and *penicillium*.

III. Tools of nanotechnology to increase the shelf life of fruits and vegetables; the role of chitosan nanoparticles as antimicrobial agents

One of the most significant strategies for extending the shelf - life of fruits and vegetables is the use of coverings. It suggests that the use of chitosan nanoparticles in food processing will greatly improve the shelf life of strawberries. For 16 days at 6–2°C and six days at 25–3°C, strawberries coated with CHNPs were able to maintain their quality. Other encouraging findings of a study suggest a viable strategy for dealing with *Botrytis*, grey mould in strawberry plants. When compared to natural fungal chitosan, irradiated chitosan showed a lower molecular weight and higher bioactivity than the latter. As an effective composite for inhibiting *B. cinerea* completely, preventing the growth of strawberry grey mould, and improving the quality of coated strawberries, Nano sized fungal chitosan-Ag might be recommended. Wood, sugarcane, and crab shell nanomaterials, particularly sustainable (SNMs) were developed and used to a variety of fruits. As a case study, the antimicrobial action and quality retention of the SNMs were tested using strawberries as an example. For convenient spraying on fruit, SNMs with their nanocrystalline morphology generate powerful shear-thinning microemulsions. There was a direct correlation between the fruit's surface shape, waxes and cutin monomers, and its surface energy transfer. The surface intermolecular forces and particle shape of SNMs influenced their antifungal activity (crystals vs fibers). chitosan nanofiber and wood nanocrystal were shown to have equivalent antifungal properties to antifungal thiabendazole (80 mg L⁻¹). These chitosan nanofiber and wood nanocrystal coated strawberries had less weight loss and colour change than the control samples, indicating that the coating was successful in keeping fruit freshness [1,2,3].

BLUEBERRY: In current research studies it was noticed that the performance on post-harvest life extension of tomato cherries and blueberries can be increased by adding chitosan thymol nanoparticles generated by ionotropic gelation of chitosan/quinoa protein edible coatings. The colonisation of *Botrytis cinerea* was effectively inhibited by these nanoparticles. Water permeability was dramatically reduced in films with nanoparticles than films using sunflower oil as well as film control without the use of nanoparticles. Films nano carriers put to a polyethylene terephthalate clamshell container were used to keep tomato cherries and blueberries, and the weight loss was measured. Storage at 7 °C and 85 percent relative humidity for 10 days of both meals in modified and unmodified PET clamshells resulted in less weight loss. Hence Chitosan thymol nanomaterials can be used as an antibacterial for fresh fruit preservation and as a vapour pressure barrier in chitosan-quinoa protein films[4]. Using nisin as an antibacterial agent, researchers are looking into the characterization of new chitosan/silica nanoparticle/nisin films for long-term blueberry storage. The membrane's strength and elongation were decreased as a result of the nanomaterials used in the chitosan films. Film-coated blueberries with Chitosan-Silica Nanoparticle/N films had 2.82 log CFU/g of aerobic bacteria, 3.73 log CFU/g of moulds and 3.58 log CFU/g of yeasts, respectively, in comparison to Chitosan/Silica Nanoparticle films. Thus summarise, (CH-SN-N) film is suggested for blueberry preservation in order to extend the shelf life of the fruit while in storage [5].

GRAPES: Many advantages, such as biodegradability or the availability of renewable resources, are prompting increased interest in biopolymer-based packaging materials. It is possible that the synergistic effect of packaging multiple antimicrobial agents will lead to improved antibacterial properties against a wider range of deteriorative agents or a reduced amount of antimicrobials being necessary. A recyclable antimicrobial film which can be used to package food has been developed in this study. Casting was used to create films with chitosan as the biodegradable polymer and Ag and ZnO nanoparticles as the filler/antimicrobial agents. Citronella Essential Oil (CEO) was added to the nanoparticles in order to boost their antimicrobial properties. To demonstrate the synergistic effect, antimicrobial results were compared to those obtained with chitosan films containing only CEO or CEO films containing only CEO and Ag films. These formulations, according to the systematic review and preliminary studies, are suitable for coating fruits. For the bacterial (*S. aureus* and *E. coli*) and fungal (*C. albicans*) strains, the results show that the synergistic effect of all components can guarantee significant antimicrobial activity against with the two bacterial strains, with inhibition diameters of between 30 and 40 millimeters. These findings suggest that this membrane has broad antimicrobial activity and could be used to coat fruits, as the CEO scent is compatible with such foods [6]. Another study shows that Chitosan edible coatings could be used to extend the shelf life of fruit after harvest. Nanoparticles of chitosan can enhance the properties of the material. There were two storage temp used in this experiment: 12°C, and 25°C, and researchers wanted to see how gel + fungal chitosan nanoparticles affected in vitro antimicrobial activity and sensory properties of grapes stored at both temperatures (titratable acidity, dissolved solids, pH, decline in sugar, and moisture content). Microdilution tests were used to determine the MIC and MBC of chitosan nanoparticles for food-borne pathogenic bacteria using chitosan nanoparticles. It is therefore possible to improve post-harvest quality of grapes by using edible films comprising chitosan nanoparticles at various concentrations (MIC/2, MIC, 2MIC)[7].

CHERRY: The goal of this research is to use an ion gelation method to create *Eryngium Campestre Essential Oil* (ECEO) entrapped chitosan nanoparticles (CHNPs). In aspects of particle size, zeta potential, and

polydispersity index, the CHNP-ECEO dispersions were characterized. Sweet cherries have been coated with a layer of CHNP-ECEO in order to extend their shelf life. At 4 degrees Celsius, cherries were stored for 21 days to determine their physical and chemical properties (weight loss; pH; titratable acidity; respiration; firmness; antioxidant activity; and total phenolic content); as well as the growth of microbes. All of the CHNP-ECEO-coated cherries in the study had significantly lower microbial counts than the control cherries. Finally, it was shown that the CHNPs and ECEO coatings were effective at controlling cherry microbial growth and that they can be applied to other kinds of fruits to similar compositions in a similar manner as well [8].

AVOCADO: Studies have shown that chitosan (CS) micro and nanoparticles have an antifungal effect on filamentous fungi that are important in food products, according to recent research. Using *Colletotrichum gloeosporioides* as a test organism, this research examined the antifungal effects of chitosan nanoparticles, essential oil of pepper tree (PEO), and chitosan biocomposites infused with PEO. The CS-PEO biocomposite showed a greater (P 0.05) inhibitory action on radial growth, sporulation, and viability of such spores at an intensity of 0.160 mg/mL, which is a healthier alternative to its use of fungicidal synthetic chemicals [9,10].

PINEAPPLE: Pineapple growers and handlers face significant challenges due to yield loss and quality degradation. In recent years, chitosan nanoparticle coatings on fruit's outer surface are becoming increasingly important in order to extend the fruit's shelf life after harvest. Chitosan-based composite coatings with ZnO nanoparticles were coated with aloe vera gel as a natural antioxidant. Freshly harvested pineapples were treated with the new formulation. Antimicrobial ZnO nanoparticles were used. There were significant weight losses of about 5%, and oxidative decay of about 10%, when the fruit was coated with polyethylene glycol rather than uncoated. Reduced post-harvest losses and increased shelf life of pineapples are two of the primary goals of the newly developed coating formulation [11].

GUAVA: In a recent study researchers used chitosan (100 percent Q), alginate (100 percent A), and 50/50 Q/A blends, as well as 90/10 Q and 90/10 A/10 Q nanoZnO blends, to create nanostructured coatings for guavas. Fruits were coated and stored for 15 days at 21 °C and 80 percent RH after application. Coatings were found to be effective in preventing the appearance of rot in all samples, which is consistent with the antibacterial properties of nanoZnO. According to the results, ZnO nanomaterials coatings with 100 percent Q or 90 percent Q–10 percent A can extend the shelf life of guavas by up to 20 days compared to seven days for uncoated fruits [12].

FIG FRUITS: A coating of chitosan nanoparticles along with propolis nanoparticles + propolis increased antioxidant activity by about 30% while having no effect on fruit maturation over the course of 12 days of storage. Under science lab and semi-commercial conditions, figs addressed with CS + CSNPs + PNPs + P inhibited fungus growth by 20% to 30%. Under science lab and semi-commercial environments, the coatings reduced dramatically the presence of *A. flavus* and the production of aflatoxin [13].

BANANA: Antibacterial ZnO nanoparticle supplements have been widely used in the food business world, – particularly as edible coatings, to protect foods from the deterioration caused by bacteria, viruses, and mould. ZnO nanoparticles are non-toxic inorganic oxides. SEM and X-ray diffraction were used to examine the properties of hydrothermally synthesised ZnO nanoparticles. *Staphylococcus aureus*, *E. coli*, as well as *Bacillus subtilis* have all been successfully eradicated by the ZnO nanoparticles created in this study. The antibacterial properties of ZnO nanoparticles in a chitosan, gum arabic (CH/GA) chitosan coating were investigated in the context of banana storage. Banana quality, such as fruit firmness, loss of weight, reduced sugar, as well as titratable acidity, could be preserved for a significantly longer time with the coating. CH/GA/ZnO-coated bananas showed more than 17 d of freshness compared to less than 13 d with the control bananas at 35 °C and 54 percent RH [14].

CARROTS: Microbiological tests on minimally processed carrots were used to evaluate the effectiveness of a safe –to- eat antimicrobial coating depending on a starch–chitosan matrix. There were two types of coatings prepared: one based on 4 percent yam starch (w/w) plus two percent glycerol (w/w) and another that included chitosan in 0.5 and 1.5 percent concentrations. These coatings were applied to slices of minimally processed carrots. Expanded polystyrene trays were used to hold the samples, which were then coated throughout polyvinyl chloride film as well as stored at 10 °C/15 days. Using antibacterial agent's yam starch as well as chitosan coating to control microbiological expansion throughout minimally processed carrots is a viable alternative [15].

GARLIC CLOVES: Edible active coatings' main advantages are that they are edible, biodegradable, and improve food safety. Agar-agar (1%) coatings with chitosan (0.2%) and acetic acid (0.2%) were tested for their coating properties and effects on highly processed garlic cloves in this study. Garlic cloves coated with acetic acid and chitosan antimicrobial compounds inhibited filamentous fungi and aerobic mesophilic spores for a period of six days [16].

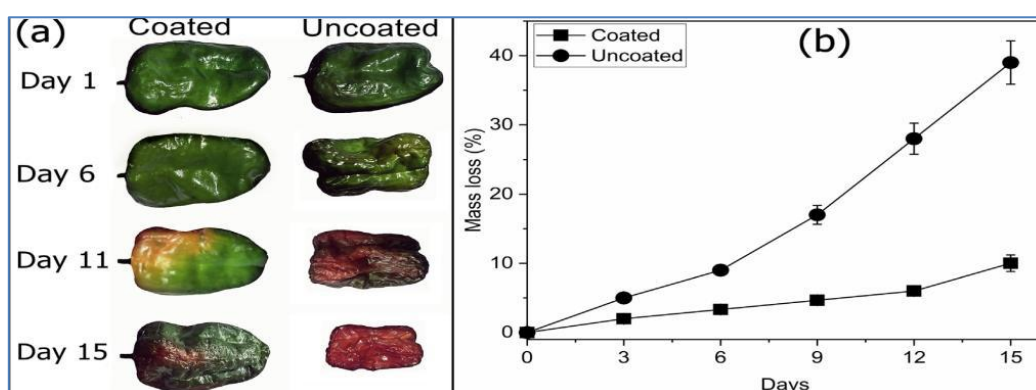
MUSHROOMS: It was investigated how chitosan coating affected new mushroom restoration, including microbiological and enzyme activities, colour characteristics, and chemical quality characteristics. However, the use of chitosan nanoparticles to control enzyme activity and maintain the quality of fresh-cut

mushrooms was studied. Researchers found that increasing the chitosan coating concentration increased the positive effects of chitosan nanocomposite on shelf life extension and fresh-cut mushroom quality maintenance [17].

TOMATO: The impacts of chitosan on *Botrytis cinerea* as well as *Penicillium expansum*-caused grey and blue mould on tomatoes preserved at 25 and 2 °C were examined. Both pathogens of tomatoes kept at 25 & 2 °C were effectively controlled by chitosan [18].

OKRA: Vegetables such as okra were tested for the preservation of quality by using a nanocomposite ZnO anti - microbial polyethylene packaging film. Okra samples were packaged in low density polyethylene films (LDPE) encased to chitosan-ZnO nanocomposites at room temperature (25 °C). Chitosan-ZnO nanocomposite coatings have been shown in this study to inhibit microbial and fungal growth while also maintaining the quality of packed okra. As a result, a coating made of chitosan-ZnO nanocomposite can be used for active food packaging [19].

BELL PAPER: There are several pepper cultivars within *Capsicum annuum L.* and bell pepper is one of them that is notable for its high vitamin and antioxidant content, as well as its antioxidant and antimutagenic characteristics. It has been discovered that edible coatings (ECs), which are made up of a thin, continuous matrix of proteins, lipids, or polysaccharides, can extend the storage life of these items by limiting gas exchange and altering their permeability. EC is typically made with chitosan, a biopolymer that helps keep bell peppers fresh and nutritious even after they've been stored for a long time. *C. gloeosporioides* and other organisms can be reduced by using this polymer's broad antibacterial spectrum and ability to incorporate functional ingredients such as antioxidants, plant extracts and essential oils. The following coatings were created: coating to chitosan nanoparticles (ENC), which was composed of 0.1 percent v/v of edible canola oil, 0.3 percent v/v of glycerol (Mallinckrodt Baker, Mexico), 44.6 percent v/v of 0.05 percent chitosan, and 55% v/v of CNPs; and coating with nanomaterials of chitosan and nanche isolate (Both coatings were homogenized for 30 minutes at 0.33 Hz with a design 45 homogenizer at 0.33 Hz. Carotenoids and antioxidant activities in the postharvest bell pepper were greatly raised and improved by edible coating applications in this stage with the extension of shelf life[3] Another study by preparing edible coating of Pullulan solution (2% concentration) which was dissolved in distilled water, whereas chitosan solution (2% concentration) was dissolved in a 0.5 percent citric acid aqueous solution. A magnetic stirrer was used to homogenise both solutions at room temperature (231oC) for 60 minutes. After that, a homogenizer was used to combine and homogenise the chitosan and pullulan solutions in a 50:50 ratio for 10 minutes at 9000 rpm . In the chitosan and pullulan composite coating material, glycerol (1%) and pomegranate peel extract (5%) were included as plasticizers. A magnetic stirrer was used to gently mix and swirl the composite mixture for an hour. Green bell peppers were treated with a chitosan-pullulan composite covering that had been developed. This edible coating, composed of 50% chitosan and 50% pullulan, managed to maintain the shelf life and total soluble solids, acid value and pH with significant reduction in physiological weight loss and color browning [20,21,22]. Similarly, some case studies achieved minimal water vapor permeability in edible coatings based on Carboxymethyl cellulose and chitosan biguanidine hydrochloride. During 15 days of treatment the treated and untreated pepper were monitored for mass loss. On day 15, uncoated pepper lost over 40% of its bulk. From the other end, the coated pepper lost only 10% of its mass on day 15. Significant resistance to mass loss is caused by reduced water barrier characteristics and also increased shelf life [23,24].



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Target	Chitosan type used	Extended Properties	Application Method Details	Effects	Microorganism(s) inhibited	Ref.
STRAWBERRY	Chitosan/CHNPs	coated strawberry showed extended shelf life upto 8 days	Coated with chitosan at (0.5 and 1%) or CHNPs at (0.5 and 1%). some kept in room temp (25±3°C) and some in fridge condition (6 ±2°C).	Result indicated strawberries coated with CHNPs showed better antimicrobial properties with lowest TBC, mould and yeast count 3.88,3.56 log CFU/g compared to uncoated one with 6.65 and 5.82 Log CFU/G.	mould and yeast count	[1]
	Nano-Ag reinforced into chitosan.	Coated sample showed significantly improved antifungal properties against gray mould	Inoculated strawberries dipped into Nano Ag-CTS Composite in 1% acetic acid solution at room temp. for 30 second and stored at 5±2°C with relative humidity 70±5%.	Nano Ag-CTS composite exhibited remarkable antifungal activity against B.cinerea with MIC of 125µg/ml and lowered mw. No grown colonies observed even after 21 days from treatment.	<i>Botrytis cinerea</i>	
	Coating with chitin, cellulose, chitosan nanomaterial	Coated sample significantly reduce the case of pear diseases by performing antifungal actions.	Aqueous coating suspensions were homogenized using microfluidizer and diluted later for use. Coated strawberries are incubated at 25°C and 75% RH	Showed significant antifungal properties against <i>Botrytis cinerea</i> . And reduce the count of disease causing fungus <i>penicillium expansum</i>	<i>Botrytis cinerea</i> .	
BLUEBERRY	Chitosan-thymol nanoparticle (CTNPs)	Coated blueberries significantly improve the shelf life by reducing the case of black mold or black rot disease	CTNPs were prepared by adding 300mg of chitosan(LMWC) into diluted solution of 1.9g citric acid and 100ml of 1mg/ml thymol and ultimately dried into film.coated blueberries were stored at 7±1°C for 10 days with 85% RH	Prepared coating had greater inhibitory effect against salmonella typhimurium, staphylococcus aureus, and listeria innocua. And also effective against mycelia growth of <i>Botrytis cinera</i>	<i>Salmonella typhimurium, Staphylococcus aureus, and Listeria innocua.</i>	
	Silica/ nisin/ chitosan nanoparticle	Coating	Film prepared using 1% chitosan powder with 1 % acetic acid and 0.5% glycerol. Then 1% SiO2 and nisin is dispersed and sonicated. blueberries are coated with this solutuion and studied for 9 days during storage	Aerobic bacteria reported lowest counts in blueberries coated with chitosan/SiO2/N film with 2.82 log CFU/g comparison to untreated and native chitosan sample with 4.23 and 3.90 log CFU/g respectively. Lowered yeast and mould count at coated sample with 3.58 logCFU/g comparison to highest with untreated sample at 4.62logCFU/g.	yeast and mould count	
GRAPES	ZnO/AgNP/citronella essential oil/chitosan	Coated green grapes showed acceptable visual appearance even	Using solvent casting method chitosan blend is prepared. ZnO/Ag NPs suspension were added to chitosan	Chitosan/Zno film showed highest efficiency against E.coli and increased inhibition over	<i>C. albicans</i>	[6]

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		after 14 days with minimum rusty spot and no microbial damage.	solution where 0.66g chitosan was dissolved in 100ml of 1% acetic acid. Film were stored at 20°C and 60% humidity for 14 days	C.albicans. chitosan/Zn /AgNp film showed higher resistance to S.aureus with inhibition diameter being 30mm.		
	Chitosan-nanoparticle (CHNPs)	Produced nano film showed significant inhibitory effect against various pathogenic food borne bacteria and maintain the shelf life for more than 12 days.	20g/L -1 CH added in 1% acetic acid to form chitosan gel.grapes are coated and left dry for 30 minutes and stored at room temp(25°C) for 12 days and at refrigerated temp (12°C) for 24 days	Coated sample showed highest inhibitory properties against S.aureus with lowest MIC of 2 gL-1=. It also showed significant inhibition against other gram positive bacteria like L.monocytogenes and gram negative bacteria like E.coli, P.aeruginosa, Salmonella. With MIC 3gL-1.	<i>S.aureus, E.coli, P.aeruginosa, Salmonella.</i>	[7]
CHERRY	Eryngium campestre essential oil(ECEO)/Na no-chitosan	Even after 21 days of storage coated cherries had considerably lowered bacterial count than uncoated one.	CHNP-ECEO Coating is prepared using 0.32gm chitosan powder into 100ml of acetic acid and adding diff. concentration of ECEO into dispersion. Then homogenized, centrifuged and freeze dried at - 40°C for 24h. Cherries were fully immersed into the dispersion and stored at 4±1°C with relative humidity of 70%.	After 21 days, In cherries coated with CHNPs showed, reduced bacterial count to 4.8 logCFU/g. cherries treated by CHNP-ECEO had a significant reduction in the population of aerobic bacteria with TBC around 4log CFU/g.while in the control group bacterial count increased to 5.5log CFU/g.	bacterial count	[8]
AVOCADO (Persea Americana)	Chitosan nanoparticle and chitosan biocomposites with pepper tree essential oil	Increase shelf life of avocado by preventing fungal infection	Film were prepared following the instruction proposed by Chavez Magdalendo et al[] Avocados were dipped in the solution for 1 min and then dried at 25°C with 38% RH for 60 min, and stored for 10 days	Showed Effective performance against Colletrotichum gloeosporioides, the microorganism causes fungal infectons on avocado	<i>Colletrotichum gloeosporioides</i>	[9,10]
PINEAPPLE	Aloevera gel(AVG)/ ZnONP/ Chitosan composite	Pineapple coated with CH/ZnONPs/ AVG composite showed acceptable visual appearance with no visible damage after 15 days.	700ml CH solution (1%w/v)was mixed into 1% acetic acid solution.thereafter diff % of AVG and ZnONP added to formulate coating.coated fruits are fan dried for 3 hr in 25°C and 65%RH	Significantly lowered decay index observed in coated pineapples. Fruits coated with CH and 50%(w/w) AVG showed no decay(0 Aa) comparison to control with (1.33±0.2 cC)Fresh appearance and maintained colour were also observed with no damages in internal tissue even after 15 days of storage.	damages in internal tissue	
GUAVA (Psidium guajava L.)	Alginate/Na no-Zno/ chitosan	Increase storage life allowing upto 20 days with favourable condition.	5%(m/v) of chitosan added into 0.5%(v/v) acetic acid solution,5%(m/v) sodium alginate	Showed effective inhibition against microorganism causing post-harvest guava fruit rot,Phyllosticta	<i>Phyllosticta psidicola</i>	[12]

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		Effective against fruit rot.	solubilized in deionized water. 1% (v/gel) nano ZnO added to them. Finally coated guava were stored in 21±1°C with 80% RH for 20 days	psidicola, amorphous form of <i>Guignardia psidii</i> .		
	Chitosan/ZnO/CuO	Coated sample showed effectiveness against some pathogenic bacteria.	Chitosan-NP composite film developed by casting technique where 1% (w/v) chitosan is used. films were allowed to set and dried at 60°C and ultimately stored at ambient temp with 60% RH.	Showed maximum performance against <i>Campylobacter</i> followed by against salmonella and <i>S.aureus</i>	<i>Salmonella</i> and <i>S.aureus</i>	[12]
FIG FRUITS	Chitosan and propolis nano-particle (CSN Ps-PNPs)	Coated sample showed reduced presence of fungus, <i>A.flavus</i> , hence significantly reduce the production of aflatoxin. And maintain the visual appearance of figs even upto 12 days of storage.	During coating preparation propolis extract and glycerol at the concentration of 0.3% (v/v) were added to chitosan preparation. CSNPs and PNPs then added to the preparation after some certain steps and homogenized. Coated figs were dried at 4±1°C for 12 days	Application of coating based on chitosan and propolis reported 100% inhibition in the production of aflatoxin. This is believed to happen due to ability of chitosan to adsorb aflatoxin B1	aflatoxin	[13]
BANANA (Musa Acuminata L.)	Chitosan/gum Arabic/ ZnO nanoparticle	Coated banana was observed to maintain freshness for more than 17 days in comparison with less than 13 days for uncoated banana	2gm chitosan dissolved in 100 ml of distilled water and 20 gm of GA powder dissolved into 100 ml of water mixed together with various concentration of ZnO nanoparticle. After coating bananas were dried and stored in 35°C with 54% RH for 17 days.	Exhibited significant antimicrobial performance against <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , and <i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , and <i>Bacillus subtilis</i>	[14]
Carrots	chitosan in yam starch coating	Up to 2.5 log inhibition during storage for 15 days	sliced Samples immersed in 0.5–1.5 % chitosan in yam starch coating	Viable alternative for shelf life extension of carrots	<i>S. aureus</i> , <i>E. coli</i> , lactic acid bacteria, molds, yeasts	[15]
Garlic cloves	Agar agar and chitosan	Improvement in garlic quality during 6-day storage	Agar-agar coatings incorporated with chitosan	Coatings reduced respiration rate and water loss	Counts of filamentous fungi and yeasts	[16]
Mushrooms	Mushrooms that had been freshly cut and treated with an aqueous solution that contained 5, 10, and 20 grammes of chitosan/1 litre were stored at 4°C.	Microbial plate counts; oxidative enzyme assays	fresh-cut Chitosan solutions, 0.5–2 %	Inhibited microbial growth and discoloration /Extended quality and shelf life	Total bacterial growth and yeast counts	[17]
Tomato fruit	Chitosan solution 2% in HCl solution of	At 25 and 2 °C, Chitosan was able to	Petri dish plating	fungi growth was inhibited / tomato fruit developed	<i>Botrytis cinerea</i> , <i>Penicillium expansum</i>	[18]

	1%	effectively control both pathogens of tomato fruit.		resistance to blue and grey mould		
Okra	chitosan- ZnO nanocompo site	After dissolving 2 grammes of Chitosan in a solution of 0.5 percent Acetic Acid, the solution was brought to a volume of 2 percent.	35–45nm-sized commercial ZnO nanoparticulates were incorporated into the chitosan solution.	for bacteria and fungi growth reduction after 12 storage days, the nanocomposite coatings were found to be effective	bacteria and fungi	[19]
Bell paper	nanomaterials of chitosan and nanche isolate	coating to chitosan nanoparticles (ENC), which was composed of 0.1 percent v/v of edible canola oil, 0.3 percent v/v of glycerol (Mallinckrodt Baker, Mexico), 44.6 percent v/v of 0.05 percent chitosan, and 55% v/v of CNPs	coating with nanomaterials of chitosan and nanche isolate (Both coatings were homogenized for 30 minutes at 0.33 Hz with a design 45 homogenizer at 0.33 Hz	Carotenoids and antioxidant activities in the postharvest bell pepper were greatly raised and improved by edible coating applications in this stage with the extension of shelf life	<i>C. gloeosporioides</i>	[20,21,22]
	Carboxymethyl cellulose and chitosan biguanidine hydrochloride	During 15 days of treatment the treated and untreated pepper were monitored for mass loss	minimal water vapor permeability in edible coatings based on Carboxymethyl cellulose and chitosan biguanidine hydrochloride. During 15 days of treatment	Significant resistance to mass loss is caused by reduced water barrier characteristics and also increased shelf life	fungi and mould	[23,24]

IV. Conclusion

There are some influencing factors like microbial factors and intrinsic factors that affect the antimicrobial activity of chitosan. From the studies demonstrated, the use of chemically modified chitosan either by grafting with other polymer or by reinforcement of different Nano fillers like metal ions metal oxides remains a challenge. Chitosan-ZnO or chitosan/copper complexes attracted great interests for their potential use in preserving vegetables and fruits. CS–TiO₂ have been reported on different cell lines, which supports their use in food industries. Nano sized fungal chitosan-Ag has been recommended for few vegetables and fruits. Wood, sugarcane, and crab shell nanomaterials, were developed and used to a variety of fruits. Citronella Essential Oil (CEO) was added to the nanoparticles in order to boost their antimicrobial properties. *Eryngium Campestre Essential Oil* (ECEO) entrapped chitosan nanoparticles (CHNPs) also showed significantly lower microbial counts. A coating of chitosan nanoparticles, propolis nanoparticles and propolis increased antioxidant activity by a considerable amount, while having no effect on fruit maturation. The choice of nanotechnology to be used depends mainly on the type of food, for their respective outcomes.

References:

- [1]. Taha, I., Shahat, M., Mohamed, M., & Osheba, A. (2020). Improving the quality and shelf-life of strawberries as coated with nano-edible films during storage. *Al-Azhar Journal of Agricultural Research*, 45(2), 1-14. https://ajar.journals.ekb.eg/article_149403_e45d63c8aa0277975d4a2221f942fe6c.pdf
- [2]. Moussa, S. H., Tayel, A. A., Alsohim, A. S., & Abdallah, R. R. (2013). Botryticidal activity of nanosized silver-chitosan composite and its application for the control of gray mold in strawberry. *Journal of food science*, 78(10), M1589-M1594. <https://onlinelibrary.wiley.com/doi/abs/10.1111/1750-3841.12247>
- [3]. Sun, X., Wu, Q., Picha, D. H., Ferguson, M. H., Ndukwe, I. E., & Azadi, P. (2021). Comparative performance of bio-based coatings formulated with cellulose, chitin, and chitosan nanomaterials suitable for fruit preservation. *Carbohydrate Polymers*, 259, 117764. <https://www.sciencedirect.com/science/article/pii/S014486172100151X>
- [4]. Medina, E., Caro, N., Abugoch, L., Gamboa, A., Díaz-Dosque, M., & Tapia, C. (2019). Chitosan thymol nanoparticles improve the antimicrobial effect and the water vapour barrier of chitosan- quinoa protein films. *Journal of Food Engineering*, 240, 191-198. <https://www.sciencedirect.com/science/article/pii/S0260877418303121>

- [5]. Sami, R., Soltane, S., & Helal, M. (2021). Microscopic image segmentation and morphological characterization of novel chitosan/silica nanoparticle/nisin films using antimicrobial technique for blueberry preservation. *Membranes*, 11(5), 303. <https://www.mdpi.com/2077-0375/11/5/303>
- [6]. Motelica, L., Ficaí, D., Ficaí, A., Truşcă, R. D., Ilie, C. I., Oprea, O. C., & Andronescu, E. (2020). Innovative Antimicrobial Chitosan/ZnO/Ag NPs/Citronella Essential Oil Nanocomposite—Potential Coating for Grapes. *Foods*, 9(12), 1801. <https://www.mdpi.com/913480>
- [7]. Melo, N. F. C. B., de MendonçaSoares, B. L., Diniz, K. M., Leal, C. F., Canto, D., Flores, M. A., ... & Stamford, T. C. M. (2018). Effects of fungal chitosan nanoparticles as eco-friendly edible coatings on the quality of postharvest table grapes. *Postharvest Biology and Technology*, 139, 56-66. <https://www.sciencedirect.com/science/article/pii/S0925521417308487>
- [8]. Arabpoor, B., Yousefi, S., Weisany, W., & Ghasemlou, M. (2021). Multifunctional coating composed of *Eryngium campestre* L. essential oil encapsulated in nano-chitosan to prolong the shelf-life of fresh cherry fruits. *Food Hydrocolloids*, 111, 106394. <https://www.sciencedirect.com/science/article/pii/S0268005X20315629>
- [10]. Chávez-Magdaleno, M. E., Luque-Alcaraz, A. G., Gutiérrez-Martínez, P., Cortez-Rocha, M. O., Burgos-Hernández, A., Lizardi-Mendoza, J., & Plascencia-Jatomea, M. (2018). Effect of chitosan- pepper tree (*Schinus molle*) essential oil biocomposites on the growth kinetics, viability and membrane integrity of *Colletotrichum gloeosporioides*. *Revista Mexicana de Ingeniería Química*, 17(1), 29-45. <http://www.rmiq.org/ojs311/index.php/rmiq/article/view/15>
- [12]. Chávez-Magdaleno, M. E., González-Estrada, R. R., Ramos-Guerrero, A., Plascencia-Jatomea, M., & Gutiérrez-Martínez, P. (2018). Effect of pepper tree (*Schinus molle*) essential oil-loaded chitosan bio-nanocomposites on postharvest control of *Colletotrichum gloeosporioides* and quality evaluations in avocado (*Persea americana*) cv. Hass. *Food science and biotechnology*, 27(6), 1871-1875. <https://link.springer.com/article/10.1007/s10068-018-0410-5>
- [14]. Basumatary, I. B., Mukherjee, A., Katiyar, V., Kumar, S., & Dutta, J. (2021). Chitosan-Based Antimicrobial Coating for Improving Postharvest Shelf Life of Pineapple. *Coatings*, 11(11), 1366. <https://www.mdpi.com/1348166>
- [15]. Arroyo, B. J., Bezerra, A. C., Oliveira, L. L., Arroyo, S. J., de Melo, E. A., & Santos, A. M. P. (2020). Antimicrobial active edible coating of alginate and chitosan add ZnO nanoparticles applied in guavas (*Psidium guajava* L.). *Food chemistry*, 309, 125566. <https://www.sciencedirect.com/science/article/pii/S0308814619316905>
- [16]. Aparicio-García, P. F., Ventura-Aguilar, R. I., del Río-García, J. C., Hernández-López, M.,
- [17]. Guillén-Sánchez, D., Salazar-Piña, D. A., ... & Bautista-Baños, S. (2021). Edible chitosan/propolis coatings and their effect on ripening, development of *aspergillus flavus*, and sensory quality in fig fruit, during controlled storage. *Plants*, 10(1), 112. <https://www.mdpi.com/952992>
- [18]. La, D. D., Nguyen-Tri, P., Le, K. H., Nguyen, P. T., Nguyen, M. D. B., Vo, A. T., ... & Nguyen, D. D. (2021). Effects of antibacterial ZnO nanoparticles on the performance of a chitosan/gum arabic edible coating for post-harvest banana preservation. *Progress in Organic Coatings*, 151, 106057. <https://www.sciencedirect.com/science/article/pii/S0300944020312686>
- [20]. Durango, A. M., Soares, N. F. F., & Andrade, N. J. (2006). Microbiological evaluation of an edible antimicrobial coating on minimally processed carrots. *Food control*, 17(5), 336- 341. <https://www.sciencedirect.com/science/article/pii/S0956713505000022>
- [21]. Geraldine, R. M., Soares, N. D. F. F., Botrel, D. A., & de Almeida Gonçalves, L. (2008). Characterization and effect of edible coatings on minimally processed garlic quality. *Carbohydrate polymers*, 72(3), 403-409. <https://www.sciencedirect.com/science/article/pii/S0144861707004699>
- [22]. Eissa, H. A. (2008). Effect of chitosan coating on shelf-life and quality of fresh-cut mushroom. *Polish Journal of Food and Nutrition Sciences*, 58(1). <http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.agro-article-b7d9f548-3575-4dca-9d41-331390683b19>
- [23]. Liu, J., Tian, S., Meng, X., & Xu, Y. (2007). Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biology and Technology*, 44(3), 300-306. <https://www.sciencedirect.com/science/article/pii/S0925521407000026>
- [24]. Al-Naamani, L., Dutta, J., & Dobretsov, S. (2018). Nanocomposite zinc oxide-chitosan coatings on polyethylene films for extending storage life of okra (*Abelmoschus esculentus*). *Nanomaterials*, 8(7), 479. <https://www.mdpi.com/310356>
- [25]. Lin, W. C., & Saltveit, M. (2012). *Greenhouse production* (pp. 57-71). CABI Publishing: Wallingford. <https://books.google.com/books?hl=en&lr=&id=dA5mkzfmaTEC&oi=fnd&pg=PA57&ots=wdNIEiBXQx&sig=0tbPBY5mYoXZWWuMU1bXn9wUcgo>
- [26]. Poverenov, E., Zaitsev, Y., Arnon, H., Granit, R., Alkalai-Tuvia, S., Perzelan, Y., ... & Fallik, E. (2014). Effects of a composite chitosan-gelatin edible coating on postharvest quality and storability of red bell peppers. *Postharvest Biology and Technology*, 96, 106- 109. <https://www.sciencedirect.com/science/article/pii/S0925521414001410>
- [27]. Hu, X., Saravanakumar, K., Sathiyaseelan, A., & Wang, M. H. (2020). Chitosan nanoparticles as edible surface coating agent to preserve the fresh-cut bell pepper (*Capsicum annuum* L. var. grossum (L.) Sendt). *International Journal of Biological Macromolecules*, 165, 948-957. <https://www.sciencedirect.com/science/article/pii/S0141813020345359>
- [28]. Kumar, N., Ojha, A., Upadhyay, A., Singh, R., & Kumar, S. (2021). Effect of active chitosan- pullulan composite edible coating enrich with pomegranate peel extract on the storage quality of green bell pepper. *LWT*, 138, 110435. <https://www.sciencedirect.com/science/article/pii/S0023643820314237>
- [29]. Salama, H. E., & Aziz, M. S. A. (2020). Optimized carboxymethyl cellulose and guanidinylated chitosan enriched with titanium oxide nanoparticles of improved UV-barrier properties for the active packaging of green bell pepper. *International Journal of Biological Macromolecules*, 165, 1187-1197. <https://www.sciencedirect.com/science/article/pii/S0141813020346158>
- [31]. Divya, K., Vijayan, S., George, T. K., & Jisha, M. S. (2017). Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. *Fibers and polymers*, 18(2), 221- 230. Retrieved on 17th January from <https://link.springer.com/content/pdf/10.1007/s12221-017- 6690-1.pdf>
- [32]. García-Rincón, J., Vega-Pérez, J., Guerra-Sanchez, M. G., Hernandez-Lauzardo, A. N., Peña- Díaz, A., & Velazquez-Del Valle, M. G. (2010). Effect of chitosan on growth and plasma membrane properties of *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. *Pesticide Biochemistry and Physiology*, 97(3), 275-278. <https://www.sciencedirect.com/science/article/pii/S0048357510000453>
- [33]. Liu, H., Du, Y., Wang, X., & Sun, L. (2004). Chitosan kills bacteria through cell membrane damage. *International journal of food microbiology*, 95(2), 147-155. <https://www.sciencedirect.com/science/article/pii/S0168160504001102>
- [34]. Yien, L., Zin, N. M., Sarwar, A., & Katas, H. (2012). Antifungal activity of chitosan nanoparticles and correlation with their physical properties. *International journal of Biomaterials*, 2012. <https://www.hindawi.com/journals/IJBM/2012/632698/>
- [35]. Roller, S., & Covill, N. (1999). The antifungal properties of chitosan in laboratory media and apple juice. *International journal of food microbiology*, 47(1-2), 67- 77. <https://www.sciencedirect.com/science/article/pii/S0168160599000069>