Role of Chitosan in Post Harvest Disease Management

ABSTRACT

Post-harvest losses due to microbial spoilage remain a significant challenge in agriculture, affecting both food security and economic sustainability. In recent years, chitosan, a biopolymer derived from chitin, has emerged as a promising tool for mitigating post-harvest diseases in fruits and vegetables.This review explores the multifaceted role of chitosan in post-harvest disease management, highlighting its antimicrobial properties, ability to induce plant defense mechanisms, formation of physical barriers, regulation of enzymatic activity, compatibility with biological control agents, and environmental friendliness.Chitosan-based treatments offer an environmentally sustainable approach to prolonging the shelf life of produce by effectively inhibiting microbial growth, enhancing the plant's natural resistance to pathogens, and maintaining post-harvest quality. Understanding the mechanisms underlying the efficacy of chitosan in disease management is essential for optimizing its application and integrating it into integrated pest management strategies in agricultural practices.

Key words: Chitosan, Post-harvest, Disease Management, Eco friendly strategy, Shelf life, Sustainable Agriculture

1.INTRODUCTION

The United Nations (UN) reports that, typically, around 13.8% of globally produced food is wasted during post-harvest stages, including transportation, storage, and processing [1]. Post-harvest diseases refer to the various microbial, fungal, or bacterial infections that affect crops after they have been harvested from the field.The primary contributor to food waste within the supply chain is losses caused by diseases brought on by pathogens, which can happen at any stage from pre-harvest to consumption.

Fruits and vegetables are classified as perishable crops compared to cereals, pulses, and oilseed crops. They typically have high moisture content (around 70-95% water), larger size, higher respiration rates, and soft textures, creating favourable conditions for microbial growth and disease development from harvest to consumption.In less developed nations, there are higher rates of losses because of inadequate methods for handling, transportation, and storage, leading to increased incidents of injuries or damage during harvesting and transportation [2].

As a result of the non judicious use of fungicides and pesticide, residues were determined in various fruits and vegetables [3].The worldwide tendency seems to be moving towards environmentally friendly options, such as using natural compounds, to decrease the spoilage of harvested commodities.Chitosan stands out among these materials for packaging purposes due to its biodegradability, lack of toxicity, ability to form films, chemical stability, and inherent antimicrobial and antioxidant qualities[4].

2. STRUCTURE AND SOURCE OF CHITOSAN

Chitin, a naturally occurring mucopolysaccharide found in crustaceans, insects, and similar organisms, is widely recognized as comprising 2-acetamido-2-deoxy-β-D-glucose units linked via β (1→4) connections. Chitosan is the (1, 4-linked 2-amino-2-deoxy-β-D-glucan), a derivative of chitin that is formed when the deacetylation of chitin reaches about 50%[5].The figures are not cited in the text.

Fig. 1. Chitosan

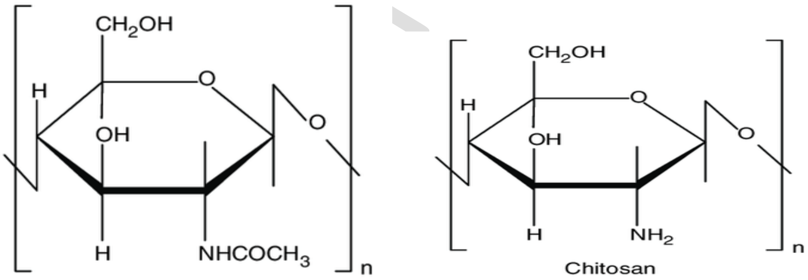

Fig. 2.Structure of chitin and chitosan

Main source for raw material of chitosan production nowadays is based on crab and shrimp shells derived from canning industries. So, chitosan stands out as the most used biopolymer in agriculture [6].Chitosan can create a protective film on the surfaces of fruits and vegetables, lowering their respiration rate by regulating the passage of carbon dioxide and oxygen. Additionally, the -NH3 group in chitosan might inhibit the growth of harmful microorganisms, effectively managing fruit decay.

3. EXTRACTION OF CHITOSAN

For extraction of chitosan, both biological and chemical methods are followed. Chitosan extraction involved three primary stages: demineralization, deproteination, and deacetylation.The chemical method is widely employed in commercially because of its quick processing time. However, there's growing interest in biological extraction, as it's considered a safer and more cost-effective treatment due to its lack of effluents. Nevertheless, its application has so far been restricted to laboratory-scale operations [7].

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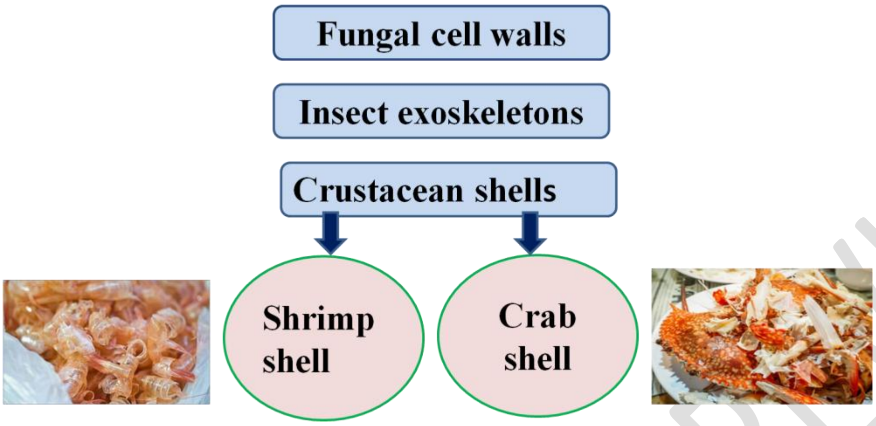

Fig. 3.Sources of Chitosan

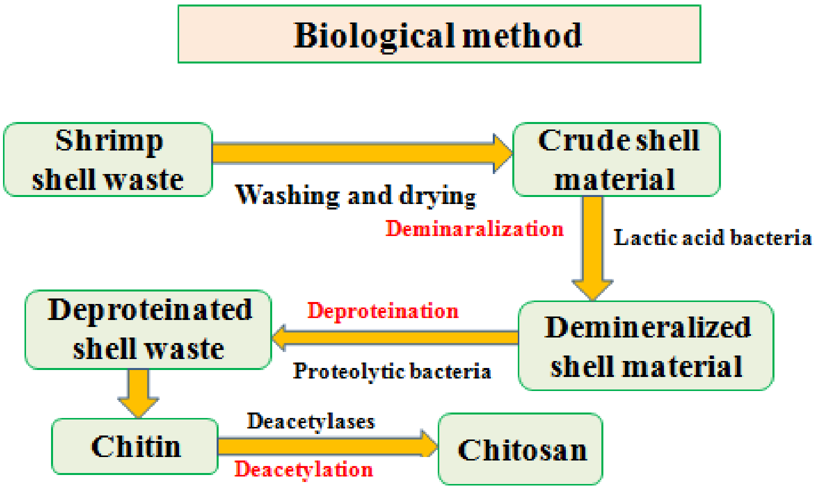

Fig. 4.Biological method of extraction of chitosan

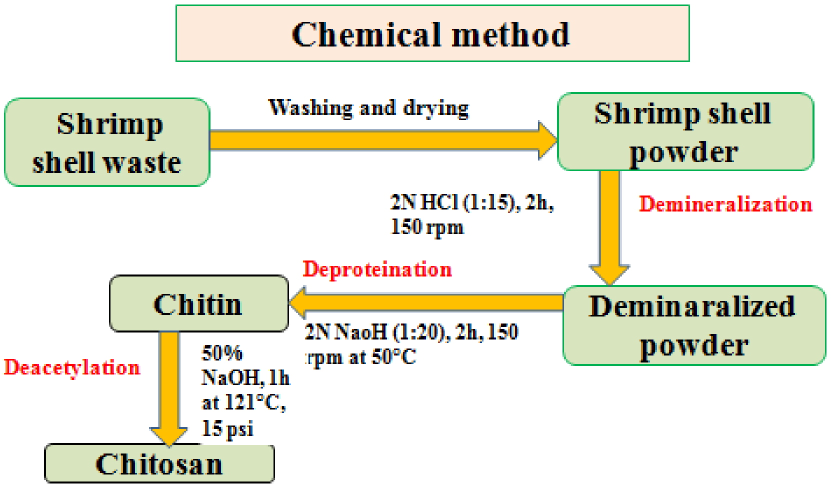

Fig. 5.Chemical method of extraction of chitosan

4. VARIOUS APPLICATIONS OF CHITOSAN

Chitosan and its derivatives find diverse uses across several industries including food, agriculture, pharmacy, medicine, cosmetics, textiles, and paper production, as well as in various chemical applications. Recently, chitosan has garnered significant interest in fields such as dentistry, ophthalmology, biomedicine, bioimaging, hygiene products, veterinary medicine, packaging, agrochemicals, aquaculture, functional textiles, catalysis, chromatography, beverages, wastewater treatment, sludge dewatering, and biotechnology[8].

5. BENEFITS OF CHITOSAN IN AGRICULTURE

Chitosan derived from waste in the seafood industry and characterized by its non-toxic, biocompatible, and biodegradable nature, provides effective pathogen control by stimulating the plant's immune system across various pathogens.The different characteristics, in particular the degree of deacetylation and the molecular weight, influence the physicochemical properties (including viscosity and solubility), and they have a direct influence on the biological properties of the substance and the effects in plants and pathogens. All these characteristics make chitosan very useful for several industrial applications, namely cosmetology, food, biotechnology, pharmacology, medicine and, more recently, agriculture. In agriculture, it has been used in seed, leaf, fruit and vegetable coatings, sprays, and as fertilizer with astounding results. Chitosan not only protects plants against harmful microorganisms but it helps to increase plant productivity. Considering these superior properties of chitosan, it has been successfully used in many post harvested fruits, vegetables or their fresh-cut samples. In postharvest chitosan is considered as an ideal coating for fruit and vegetables for its antimicrobial properties, and role it plays as an elicitor of plant defenses against pathogens. Chitosan treatment on fresh products of fruits and vegetables are safe for consumer and environment.Chitosan has been granted approval by the United States Food and Drug Administration (USFDA) as a "Generally Recognized as Safe" (GRAS) food additive[9].

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Fig. 6.Important charecteristics of chitosan

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Table.1 Commercial products of chitosan

Trade name of the product	**Company (Country)**	**Formulation**	**Active ingredient (%)**
Chito plant	ChiPro GmbH (Bremen, Germany	Powder	99.9
Chito plant	ChiPro GmbH (Bremen, Germany)	Liquid	2.5
OII-YS	Venture Innovations (Lafayette, LA, United States)	Liquid	5.8
KaitoSo	Advanced Green Nanotechnologies Sdn Bhd (Cambridge,	Liquid	12.5

	United Kingdom)		
Armour-Zen	Botry-Zen Limited (Dunedin, New Zealand)	Liquid	14.4
Biorend	Bioagro S.A. (Chile)	Liquid	1.25
Kiforce	Alba Milagro (Milan, Italy)	Liquid	6
FreshSeal	BASF Corporation (Mount Olive, NJ, United States)	Liquid	2.5
ChitoClear	Primex ehf (Siglufjordur, Iceland)	Powder	100
Bioshield	Seafresh (Bangkok, Thailand)	Powder	
Biochikol 020 PC	Gumitex (Lowics, Poland)	Liquid	2
Kadozan	Lytone Enterprise, Inc. (Shanghai Branch, China)	Liquid	2
Kendal cops	Valagro (Atessa, Italy)	Liquid	4
Chitosan 87%	Korea Chengcheng Chemical Company (China)	TC (Technical material)	87

6. ANTIMICROBIAL EFFECTS OF CHITOSAN AGAINST POST HARVEST PATHOGENS

The antimicrobial attributes of chitosan are closely linked to its structure, physicochemical traits, and environmental factors, alongside the reactive hydroxyl groups located at the C-3 and C-6 positions [10].The mechanism through which chitosan acts against microbes can be categorized into extracellular effects, intracellular effects, or a combination of both, depending on the specific site targeted by its antimicrobial actions[11].As high molecular weight (HMW) chitosan typically cannot penetrate cell walls and membranes, its antimicrobial effects primarily involve chelating essential metals, hindering nutrient uptake extracellularly, and modifying cell permeability. Conversely, low molecular weight (LMW) chitosan exhibits both extracellular and intracellular antimicrobial activity, influencing RNA, protein synthesis, and mitochondrial function [12].The antimicrobial mechanism of chitosan varies significantly depending on the specific type of microorganism being targeted.Chitosan's dual action, affecting both its host and the pathogen, is crucial for managing post-harvest diseases in agricultural products.

7. ANTIMICROBIAL ACTIVITY AGAINST BACTERIA

Gram-positive (G +ve) and Gram-negative (G -ve) bacteria display notable distinctions in their cell wall composition, with G +ve bacteria possessing thicker peptidoglycan layers, whereas G -ve bacteria are characterized by a higher abundance of lipopolysaccharides (LPS)[13].Variations in the cellular surface architecture of these bacterial categories result in differing sensitivities to chitosan. Specifically, G -ve bacteria exhibit a higher negative charge compared to G +ve bacteria due to the frequent attachment of LPS to phosphorylated groups [14].More negatively charged cell surfaces allow the binding of cationic chitosan to phospholipids when the environmental pH is below 6.5.

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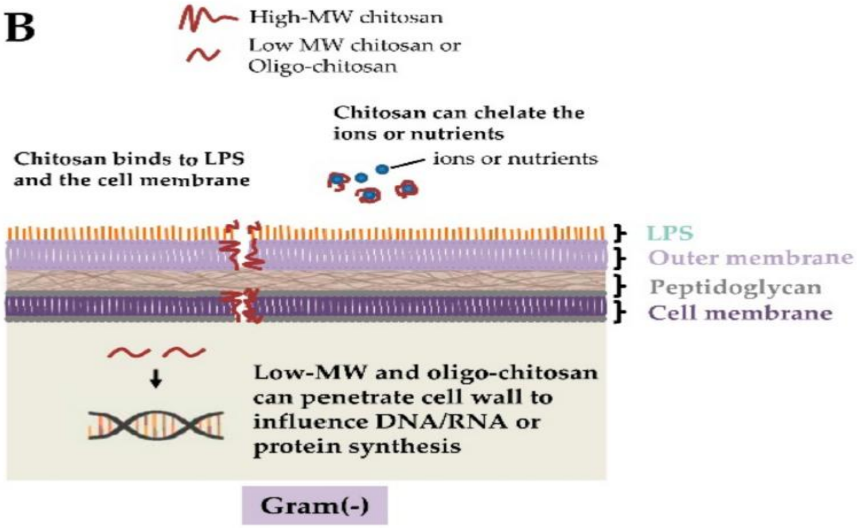

Fig. 7.Antimicrobial activity against G -ve bacteria

Teichoic acids in G +ve bacteria are also negatively charged due to the presence of phosphate groups in their structure[15].The elimination of the teichoic acid biosynthesis pathway in *Staphylococcus aureus* led to heightened resistance to chitosan, suggesting that chitosan's mechanism of action is more intricate than simple electrostatic interactions. Furthermore, unlike ~~Gram negative (~~G -ve~~)~~ bacteria, ~~Gram positive (~~G +ve~~)~~bacteria possess a thick cell wall that may hinder direct binding of chitosan to the cell membrane. However, certain chitosan oligomers (<5 kDa) can penetrate the cell wall and impact DNA/RNA or protein synthesis. Interestingly, studies have shown that chitosan (≤ 50 kDa) is capable of traversing the cell wall and inhibiting DNA transcription. Hence, while the molecular size of chitosan is crucial for its targeting, its structure rather than its molecular weight also dictates it’s extracellular, intracellular, or combined antibacterial activity.

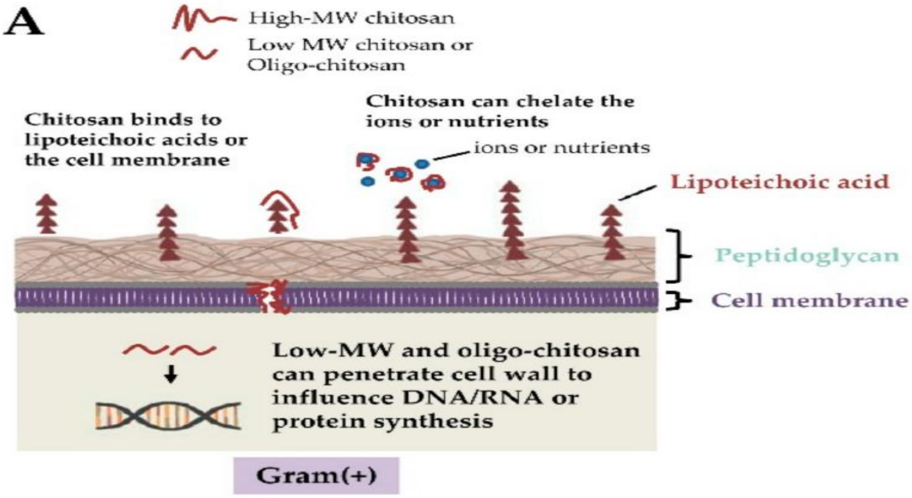

Fig. 8.Antimicrobial activity against G +ve bacteria

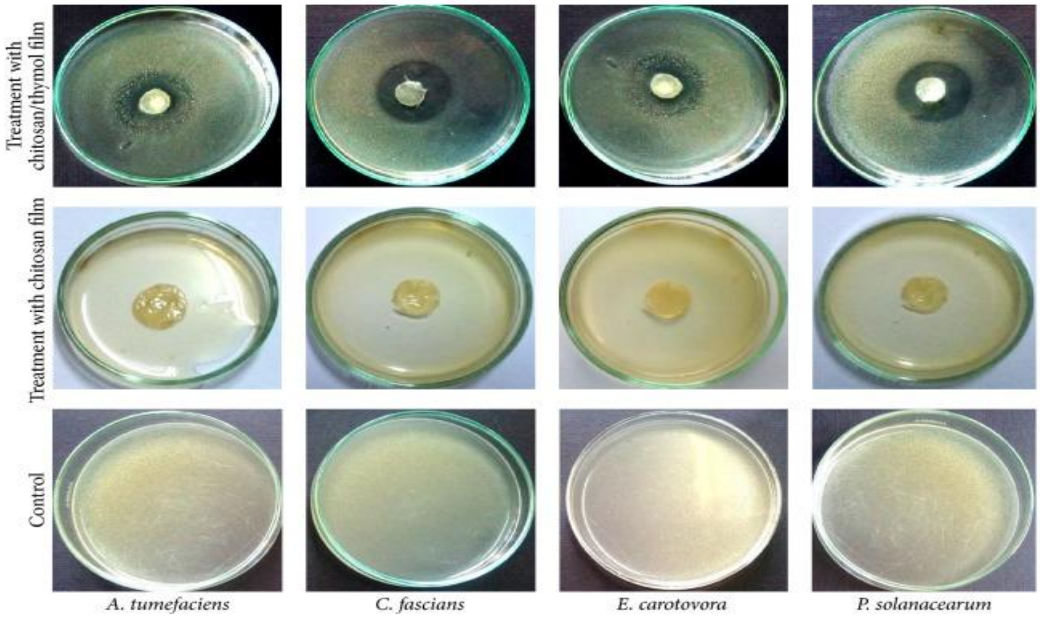

Fig.9.*In vitro* growth of *Agrobacterium tumefaciens,Corynebacterium fascians*, *Erwinia carotovora* and*Pseudomonas solanacearum* in Nutrient Agar plates incorporated with chitosan film enriched with thymol (0.5%). Does this figure belong to the authors?

8. ANTIMICROBIAL ACTIVITY AGAINST FUNGI

Chitosan has been demonstrated to exhibit fungicidal properties against various fungal pathogens in plants [16].Its antifungal properties are mainly related to the interaction of chitosan with the cell wall or cell membrane.However, the minimum inhibitory concentrations (MICs) of chitosan against fungi differ significantly and are closely linked to factors such as the molecular weight and degree of deacetylation (DDA) of chitosan, the pH of the solvent, and the specific type of fungus being targeted [17].Moreover, the presence of unsaturated fatty acids in the cell membrane could be linked to susceptibility to chitosan, as higher levels of unsaturated fatty acids result in enhanced membrane fluidity and a more negatively charged cell membrane. For instance, the contrasting features between chitosan-sensitive and chitosan-resistant strains of *Neurospora crassa* are associated with variations in unsaturated fatty acid content in their cell membranes. Similarly, aside from its extracellular antifungal properties, ~~low molecular weight (~~LMW~~)~~ chitosan has the capability to permeate the cell wall and cell surface, thereby hindering DNA/RNA and protein synthesis [18].

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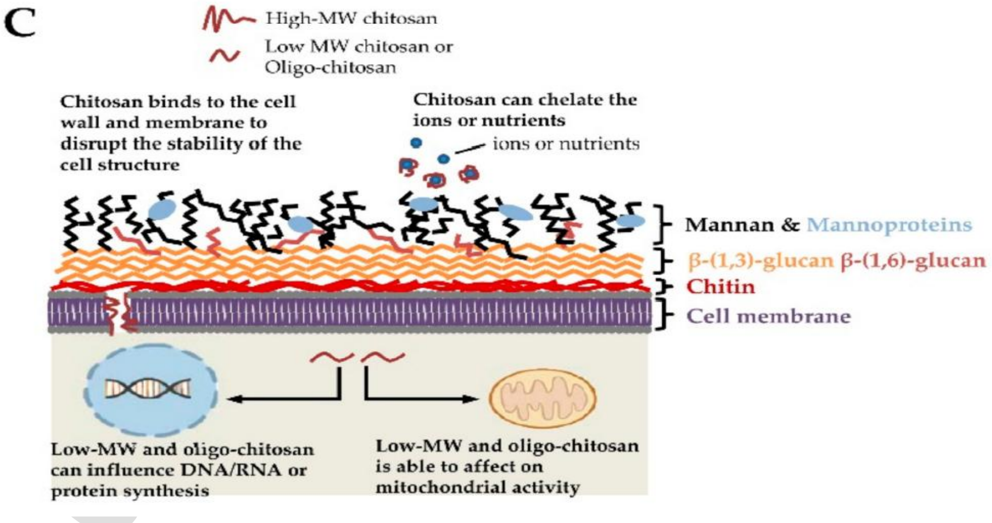


Fig.10.Antimicrobial activity of chitosan against fungi. Does this figure belong to the authors?

9. MODE OF ACTION OF CHITOSAN AGAINST POSTHARVEST DISEASES

Due to the positive charge present on the C2 of the glucosamine monomer when the pH is below 6, chitosan exhibits increased solubility and demonstrates superior antimicrobial properties compared to chitin [19].Hence, when discussing the potential mechanisms for controlling postharvest decay of fruits, much emphasis is placed on chitosan. While the precise antimicrobial mechanisms of chitin, chitosan, and their derivatives remain incompletely understood, various mechanisms have been suggested [20].

The direct effect of chitin and chitosan on fungal pathogens

The antimicrobial nature of chitosan is still a topic of debate, with two main hypotheses proposed: (1) Polycationic chitosan binds to electronegative charges on cell surfaces, altering cell permeability, thereby causing leakage of intracellular electrolytes and proteins. (2) Chitosan penetrates fungal cells, leading to adsorption of essential nutrients, consequently inhibiting or slowing down mRNA and protein synthesis[21].Numerous studies have shown that chitosan could directly inhibit spore germination, germ tube elongation and mycelial growth of many phytopathogens, such as *Botrytis cinerea* , *Fusarium solani*, *Penicillium*spp.,*Sclerotium rolfsii* and*Rhizopus stolonifer*[22].Chitosan completely inhibited spore germination of *Penicillium expansum* at 0.5% and *Botrytis cinerea* at 1% [23]. The application of 1% chitosan was observed to affect the *in vitro* mycelial growth of fungal pathogens such as *Botrytis cinerea*, *Penicillium* spp., *Colletotrichum* spp., and *Alternaria* spp.[24].

Induced disease resistance of fruits by chitin and chitosan

Chitinase activity is typically triggered when chitin is present, with potential diverse biological functions, including exerting antifungal effects[25].As an exogenous elicitor, chitosan can induce resistance in the host by increasing the activities of several defense-related enzymes, such as chitinase and β-1, 3-glucanase in oranges, strawberries, raspberries and phenylalanine ammonialyase (PAL) activity in strawberries and table grapes [26].

10. EFFECT OF CHITOSAN COATING ON PHYSIOLOGICAL QUALITY PARAMETERS OF FRUITS AND VEGETABLES

Weight loss: The factors contributing to the weight reduction of post-harvest fruits and vegetables involve transpiration and substrate consumption during respiration. Around 80% of the total weight loss is attributed to water loss. Following dehydration, the texture of fruits and vegetables shifts from crisp to soft. Additionally, their flavour diminishes, and their resilience against various physical and microbial diseases decreases. [27].Upon application of chitosan coating onto the surface of fruits and vegetables, more moisture within the tissue of the produce was retained [28].Therefore, the favourable quality and marketability of post-harvest fruits and vegetables are effectively preserved.

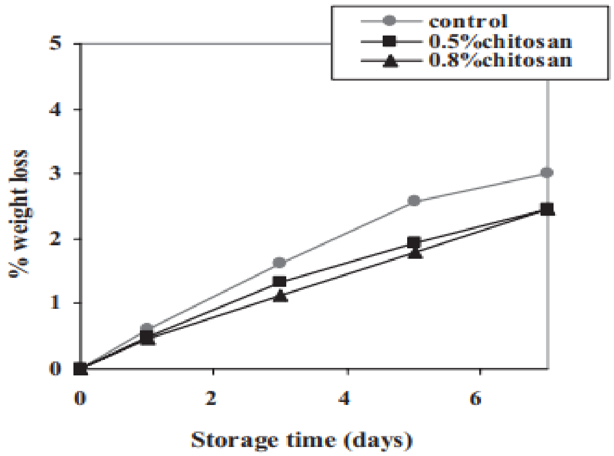
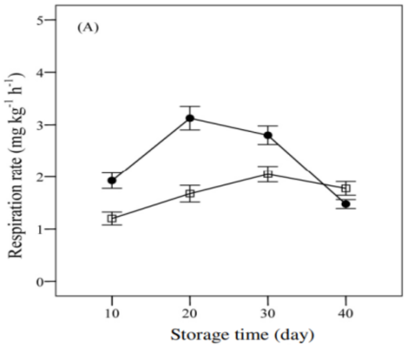


Fig. 11.Variation in weight reduction of fresh-cut mangoes stored at 6°C. Does this figure belong to the authors?

Respiration:Aerobic respiration is essential for post-harvest fruits and vegetables to retain their inherent qualities. Nutrients serve as substrates for respiration. As nutrient levels decline, both the nutritional and commercial value decreases accordingly. Effectively controlling the respiration rate can help extend the shelf life of post-harvest fruits and vegetables [29].Slowing down the respiration rate is possible in an environment with a lower ratio of oxygen to carbon dioxide. Coating the surface of fruits and vegetables allows for adjustment of the permeability through which oxygen from the air enters the produce tissue or carbon dioxide generated from respiration is released into the air. Consequently, this adjustment leads to a decrease in respiratory rate [30].However, the thickness of chitosan coating needs to be suitable. The permeability cannot be effectively adjusted if the coating is too thin, or the dioxide carbonate was accumulated if the coating is much thicker andhigh concentrations of carbon dioxide can induce anaerobic respiration, leading to the production of ethanol, which could spoil the post-harvest fruits and vegetables.



Giant; (●) Control, (□) Chitosan **Does this figure belong to the authors?**

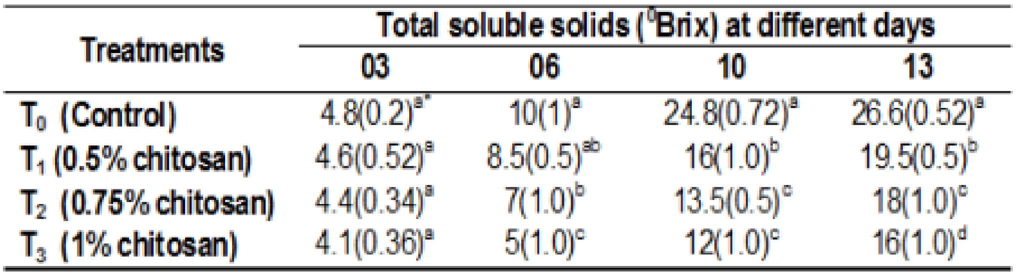
Fig. 12.Variations in the respiration rate of plum fruits treated with chitosanduring cold storage

Firmness:The crispness, closely associated with firmness, is a vital sensory attribute of fresh fruits and vegetables. As post-harvest fruits soften over time during storage, their crisp texture gradually diminishes and eventually fades away. Throughout the storage duration of fresh fruits and vegetables, their firmness diminishes due to factors such as water loss, degradation of pectin, nutrient utilization, and other factors [31].The application of chitosan coating helps to reduce transpiration, resulting in the retention of more water. Consequently, the cells of fruits and vegetables maintain higher swelling pressure and exhibit greater firmness. Therefore, chitosan coating can partially mitigate the decrease in firmness of post-harvest fruits and vegetables [32].

Fungal decay: The application of chitosan reduced the deterioration of strawberries during storage. Samples coated with either 1.5% chitosan or chitosan calcium gluconate (Cs-CaGlu) showed no apparent decay throughout the storage period [33].

Total soluble solid and pH: Over the course of cold storage, both the total soluble solids and pH levels of strawberries increased. However, there were no notable differences in these measurements between the coated groups during cold storage. The coating influenced the total soluble solids and pH levels throughout the storage period compared to the control samples. Notably, the control fruit exhibited higher total soluble solid and pH values than the treated samples. The rise in pH during storage may be attributed to water loss, with the control fruit experiencing more significant water loss compared to the coated fruit. Furthermore, variations in total soluble solids and pH may also be associated with the ripeness stage [34].

Table.2Effect of chitosan coating on TSS of banana fruitDoes this table belong to the authors?The tableis not cited in the text.



Treatments	Total soluble solids (°Brix) at different days			
	03	06	10	13
T_0 (Control)	$4.8(0.2)^{a*}$	$10(1)^{a}$	$24.8(0.72)^{a}$	$26.6(0.52)^{a}$
T_1 (0.5% chitosan)	$4.6(0.52)^{a}$	$8.5(0.5)^{ab}$	$16(1.0)^{b}$	$19.5(0.5)^{b}$
T_2 (0.75% chitosan)	$4.4(0.34)^{a}$	$7(1.0)^{b}$	$13.5(0.5)^{c}$	$18(1.0)^{c}$
T_3 (1% chitosan)	$4.1(0.36)^{a}$	$5(1.0)^{c}$	$12(1.0)^{c}$	$16(1.0)^{d}$

11. METHOD OF APPLICATION OF CHITOSAN

Chitosan edible films were prepared by dissolving 1.5-2.5 % w/v of chitosan in a stirred mixture of 1 % (v/v) acetic acid aqueous solution containing 0.5-1.0 % w/v glycerol.Within the fruits and vegetables industry, edible bio-based coatings present numerous benefits due to their capacity to carry antimicrobial agents, like essential oils, which combat pathogenic microorganisms. Consequently, chitosan oil coatings have been developed by integrating them with various essential oils[35].

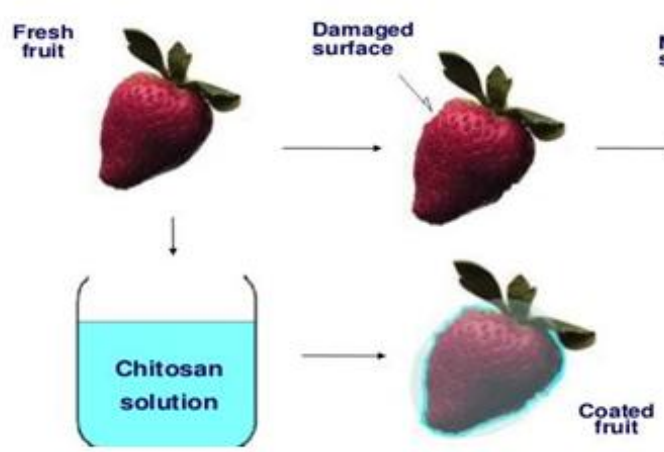


Fig. 13.Dipping method of chitosan application The figure is not cited in the text.

12. Factors determining chitosan's microbial activity

When utilizing chitosan to manage post-harvest diseases, it's important to consider the factors influencing its effectiveness against microbes. Various factors contribute to chitosan's microbial activity.

pH value

Chitosan, being a polycationic polysaccharide at pH levels below 6, readily interacts with negatively charged molecules like phospholipids, proteins, and fatty acids because of the abundance of amino groups on its structure. The pH of chitosan plays a critical role in its ability to penetrate pathogen cells. Studies indicate that for optimal antimicrobial effectiveness, the pH of chitosan should be maintained below its pKa value [36].Furthermore, as the solution pH decreases, solubility increases, resulting in a higher positive charge on the -NH3 groups of chitosan and enhancing its antimicrobial efficacy[37].

Concentration of chitosan

Chitosan sprays applied before harvest effectively suppressed postharvest decay of strawberry fruit caused by *Botrytis cinerea* during storage at 3 and 13 °C, with decay decreasing as the concentration of chitosan increased[38].Chitosan shows its highest effectiveness in suppressing microbial growth within the concentration range of 0 to 5%.Moreover, the higher the chitosan concentration, the greater its antimicrobial activity [39].At lower concentrations, chitosan readily attaches to the cell surface membrane, disrupting it and leading to cell leakage, ultimately resulting in cell death. However, at higher concentrations, it binds to the cell membrane, preventing the leakage of intracellular components [40].

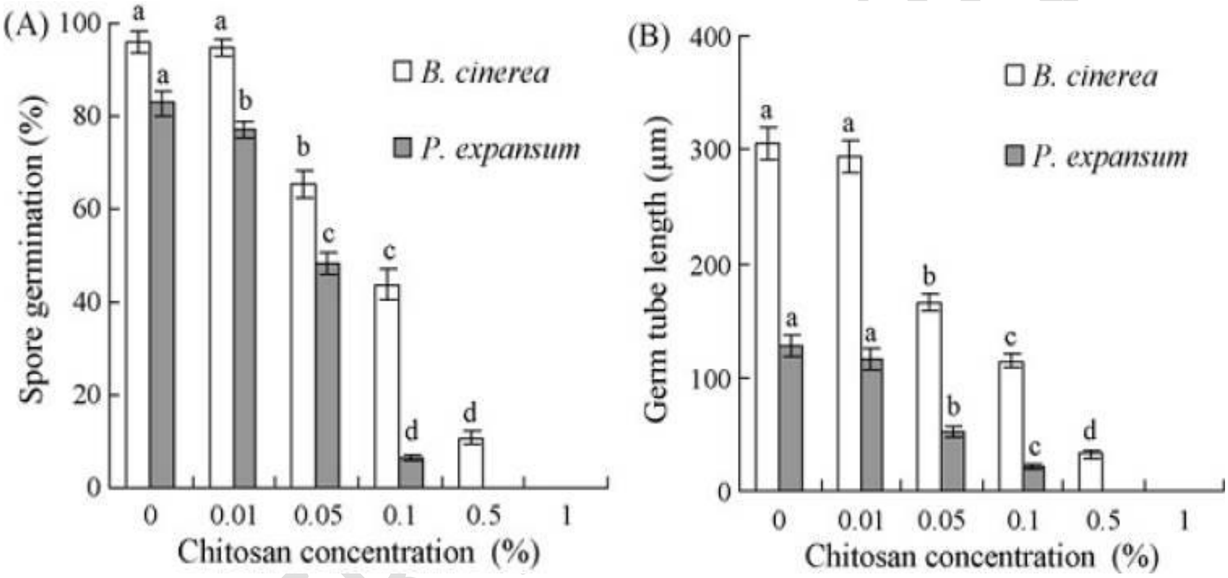


Fig. 14.The impact of varying chitosan concentrations on spore germination (A) and germ tube elongation (B) of *Botrytis cinerea* and *Penicillium expansum* 12 hours after incubation at 25°C.Does this figure belong to the authors?The figure is not cited in the text.

Molecular weight of chitosan

The molecular weight of chitosan dictates its ability to penetrate the cell surface and exert intracellular antimicrobial effects. Additionally, the polysaccharides and some proteins constituting the complex layers of the cell wall in bacteria and fungi serve crucial functions in pathogenesis, adhesion to biotic and abiotic surfaces, immune response induction, as well as providing mechanical strength and environmental barrier [41].Chitosan can be categorized into high molecular weight (HMW), typically 100 kDa and above, and low molecular weight (LMW), usually ranging from 2 to 99 kDa. HMW chitosan primarily binds to the cell membrane rather than penetrating it, thus obstructing nutrient intake and leading to cell death. On the other hand, LMW chitosan enters the cell, binds to DNA, and impedes protein synthesis. Additionally, when the carboxylic content of chitosan increases, its molecular weight decreases, consequently affecting its antimicrobial effectiveness.Reports indicate that low molecular weight chitosan (LMWC) has shown effectiveness in managing postharvest diseases in citrus fruit [40].The findings demonstrated that LMWC significantly suppressed the decay of citrus fruit caused by *Penicillium digitatum, Penicillium italicum, Botrydiplodia lecanidion*, and *Botrytis cinerea* following a 14-day storage period at 25°C, surpassing the effectiveness of both TBZ (Thiabendazole) and high molecular weight chitosan (HMWC). Additionally, the application of LMWC coating positively impacted the firmness, total soluble solid content, titratable acidity, ascorbic acid content, and water content of citrus fruit after 56 days of storage at 15°C.

Chitosan binding affinity and uptake capacity decreases with decreasing polymer molecular weight and degree of deacetylation (DD). Uptake fell by 26% when MW was decreased from 213 to 10 kDa, and by 41% when DD was lowered from 88 to 46% [43].Certain research indicates that decreasing the molecular weight of chitosan can enhance its antimicrobial activity [44].

Derivatives of chitosan

One of the primary benefits of chitosan is its versatility in generating various derivatives. Among these derivatives, carboxymethyl chitosan and quaternized carboxymethyl chitosan are well-established examples [45].

The antimicrobial efficacy of chitosan is constrained due to the relatively weak positive charge centers formed by its amino groups. Oligochitosan was observed to be more effective than chitosan in suppressing the mycelial growth of *Phytophthora capsici*[46].Hence, enhancing the functionality of chitosan requires the addition of further positive charge groups to it [47].

Degree of deacetylation

The primary functional group of chitosan is the amino group (-NH2), and its performance in various applications is affected by the degree of deacetylation (DD) [48].The degree of deacetylation (DD) is quantified by the ratio of glucosamine to N-acetyl glucosamine units within the copolymer chain, representing the percentage of glucosamine units present. This parameter dictates the abundance of free amino groups in chitosan, impacting its applicability across various fields. The electrostatic interaction between chitosan and negatively charged phospholipids is a significant factor in its mechanism of action. Typically, a higher number of amino groups results in increased solubility in acidic environments, thereby enhancing microbial activity [49].The effectiveness of chitosan against fungi enhances with higher degrees of deacetylation and lower molecular weights. This was demonstrated through experiments involving chitosan samples of varied molecular weights and degrees of deacetylation, tested against *Fusarium oxysporum*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, and *Candida albicans*[50].Increasing the degree of deacetylation (DD) resulted in improved water barrier properties, tensile strength, and antimicrobial effectiveness. Consequently, the antibacterial capabilities of chitosan films against *Listeria innocua* and *Escherichia coli* were enhanced with increased DD [51].

Type of organism

Chitosan exhibits varying effects on different types of microorganisms. Fungi possessing thick cell walls tend to resist chitosan penetration more than those with thinner cell walls. Additionally, chitosan demonstrates greater inhibition against gram-positive bacteria compared to gram-negative ones [52].Chitosan was found to exhibit varied antimicrobial effects on different bacteria strains. For instance, *Listeria monocytogenes* experienced a 6 log viable cell reduction, while *Bacillus cereus* and *Salmonella enterica* saw a 3–5 log viable cell reduction. In contrast, *Staphylococcus aureus* showed less than a 1 log reduction in cell count [53].Based on the available evidences, it seems that bacteria are typically less responsive to chitosan's antimicrobial effects compared to fungi [54].

Sources of chitosan

Currently, there are two primary sources of chitosan production: crustaceans and fungi. Recent research has revealed that certain fungi, such as zygomycetes, can also produce chitosan, leading to investigations into its production. Some studies have indicated that fungal chitosan demonstrates lower antimicrobial activity against *E. coli*, *Klebsiella pneumoniae*, and *S. aureus* compared to chitosan derived from crustacean shells. However, fungal chitosan closely resembling chitosan from crab shells has shown promising inhibitory effects on gram-positive bacteria compared to gram-negative ones [55].

Chitosan complexes

Some research suggests that to enhance the antimicrobial effectiveness of chitosan, it can be coated with natural bioactive substances like essential oils (EOs) [56].Recent research indicates that various essential oils (EOs) like lemongrass, clove, and oregano, when coated with chitosan, enhance its antimicrobial efficacy. Moreover, recent studies demonstrated that employing ~~low molecular weight (~~LMW~~)~~ chitosan, sodium sulfate as a cross-linker, and achieving a particle size below 300 nm through 20 minutes of sonication resulted in the highest antimicrobial activity. Chitosan nanoparticles produced under these conditions effectively eradicated pathogenic *E. coli* O157:H7 [57].

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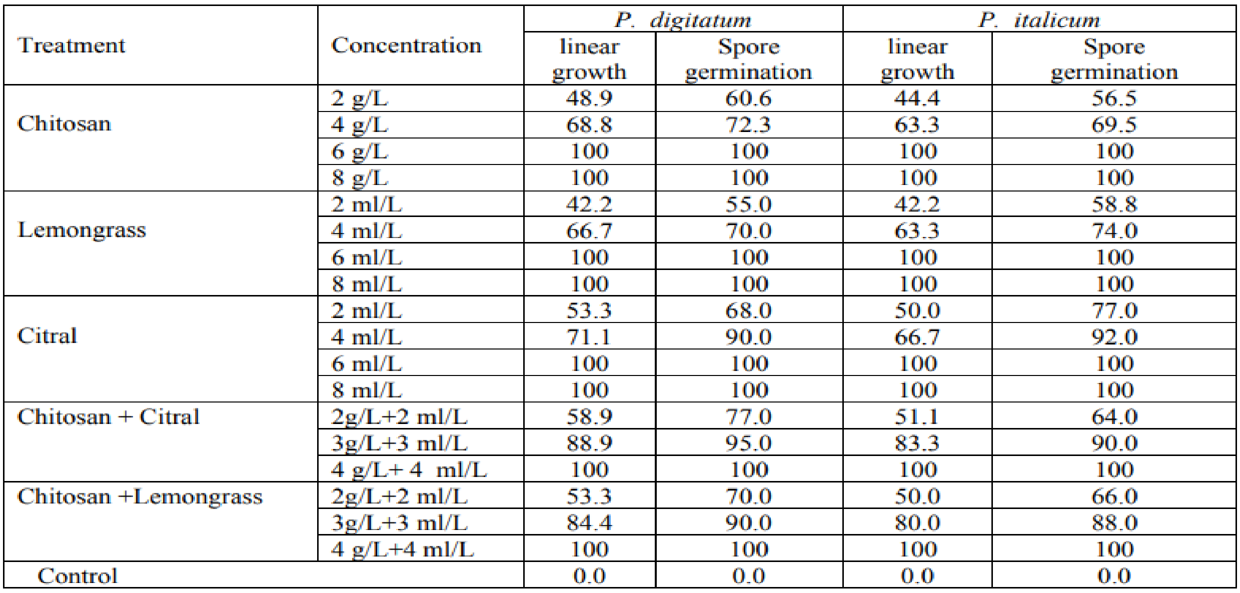
Table.3Decreased linear growth and spore germination percentages of *Penicillium digitatum* and *P.italicum* in citrus fruits were observed when exposed to various concentrations of chitosan, lemongrass, and citral essential oils on PDA medium



Treatment	Concentration	*P. digitatum*		*P. italicum*	
		linear growth	Spore germination	linear growth	Spore germination
Chitosan	2 g/L	48.9	60.6	44.4	56.5
	4 g/L	68.8	72.3	63.3	69.5
	6 g/L	100	100	100	100
	8 g/L	100	100	100	100
Lemongrass	2 ml/L	42.2	55.0	42.2	58.8
	4 ml/L	66.7	70.0	63.3	74.0
	6 ml/L	100	100	100	100
	8 ml/L	100	100	100	100
Citral	2 ml/L	53.3	68.0	50.0	77.0
	4 ml/L	71.1	90.0	66.7	92.0
	6 ml/L	100	100	100	100
	8 ml/L	100	100	100	100
Chitosan + Citral	2g/L+2 ml/L	58.9	77.0	51.1	64.0
	3g/L+3 ml/L	88.9	95.0	83.3	90.0
	4 g/L+ 4 ml/L	100	100	100	100
Chitosan +Lemongrass	2g/L+2 ml/L	53.3	70.0	50.0	66.0
	3g/L+3 ml/L	84.4	90.0	80.0	88.0
	4 g/L+4 ml/L	100	100	100	100
Control		0.0	0.0	0.0	0.0

Time of application

Fruits harvested from plants treated with chitosan spray exhibited increased firmness and underwent a slower ripening process, as evidenced by anthocyanin content and titratable acidity, compared to berries from untreated plants [58].

13. Problems associated with the usage of chitosan

The primary challenge in utilizing chitosan lies in its solubility. One approach to address this issue involves producing chitosan derivatives or combining chitosan with natural compounds such as essential oils. Other concerns related to chitosan use include its characteristics and variability. As there is no consensus regarding the relationship between molecular weight and degree of deacetylation, the biological activity of chitosan remains poorly understood. Additionally, when chitosan is applied to control post-harvest diseases, insufficient data exists on its varied effects on fungi affecting fruits or vegetables, making it difficult to assess its inhibitory effects on different fungal strains. Certain fungi, like *Pochonia chlamydosporia*, possess saturated free fatty acids that provide protection against chitosan permeability [59].The influence of temperature and pH was examined across various molecular weight variants of chitosan. Findings revealed that chitosan's antimicrobial efficacy heightened with elevated temperatures and decreased pH levels [60].Another concern regarding chitosan involves the ambiguity related to low molecular weight chitosan and oligochitosan [61].Environmental conditions such as moisture, pH, and temperature have been demonstrated to impact the antimicrobial effectiveness of chitosan. Considering the challenges outlined above, it's evident that utilizing chitosan as an antimicrobial agent requires thorough comprehension, as numerous intrinsic and extrinsic factors can influence its microbial activity.

14. Conclusion

Chitosan, a naturally occurring compound with broad-spectrum antimicrobial properties and plant innate immunity elicited activities, has potential in agriculture regarding controlling plant diseases. Its use could help reduce reliance on chemical pesticides, at least to some extent.The polysaccharide chitosan represents a renewable source of natural biodegradable polymers and meet with the emergence of more and more food safe problems.

15. Future perspective

Regarding the antimicrobial mechanism of chitosan, future research should focus on identifying the specific target molecule on the cell surface or within the cell. Creating gene mutant strains could provide valuable insights into antimicrobial mechanisms. Integrating transcriptome and proteome analyses of essential defense genes and proteins will advance our comprehension of the chitosan-mediated signaling pathway, leading to a better understanding of its antimicrobial effects.

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