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Role of Chitosan in Post Harvest Disease Management

Arun. A.T. [a*], Pramod. R [a], Radhakrishnan N.V [a], Susha S. Thara [a], Reji Rani O. P [b] and Anuradha. T [c]

[a] Department of Plant Pathology, College of Agriculture, Vellayani, Kerala Agricultural University, Thrissur, (Kerala), India.
[b] Department of Entomology, College of Agriculture, Vellayani, Kerala Agricultural University, Thrissur, (Kerala), India.
[c] Department of Plant Biotechnology, College of Agriculture, Vellayani, Kerala Agricultural University, Thrissur, (Kerala), India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Post harvest losses due to microbial spoilage remain a significant challenge in agriculture, affecting both food security and economic sustainability. Chitosan, a biopolymer derived from chitin, showed potential in recent years as a means of reducing post harvest diseases in fruits and vegetables. This review investigates into the complex role that chitosan plays in managing post harvest diseases. It emphasises the antimicrobial qualities, capacity to trigger defence mechanisms in plants, development of physical barriers, regulation of enzymatic activity, compatibility with biological control agents, and ecological sustainability of chitosan. Chitosan based treatments offer an environmentally sustainable approach to prolonging the period of the shelf life of produce by effectively preventing the growth of microbes, 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 disease management strategies in agricultural practices.

**Corresponding author: E-mail: arunkaralmanna55@gmail.com;*

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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.

In contrast to cereals, pulses, and oilseed crops, fruits and vegetables are categorised as perishable crops. They usually contain an excessive amount of moisture (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 is an organic 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. The chitosan is the substance (1, 4-linked 2-amino-2-deoxy-β-D-glucan), a derivative of chitin that is formed when chitin's deacetylation reaches approximately 50 per cent [5].

Fig. 1. Chitosan

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

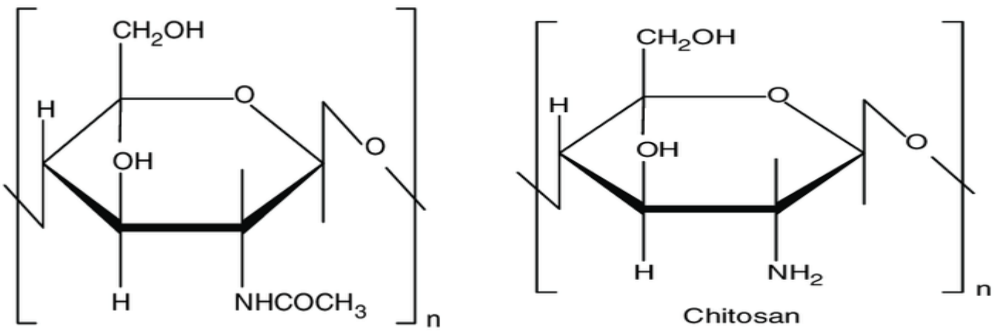

Fig. 2. Chemical structure of chitin and chitosan [5]

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 are growing interest in biological extraction, as it is considered a safer and more cost-effective treatment due to its lack of effluents. Nevertheless, this application has so far been restricted to laboratory scale operations [7].

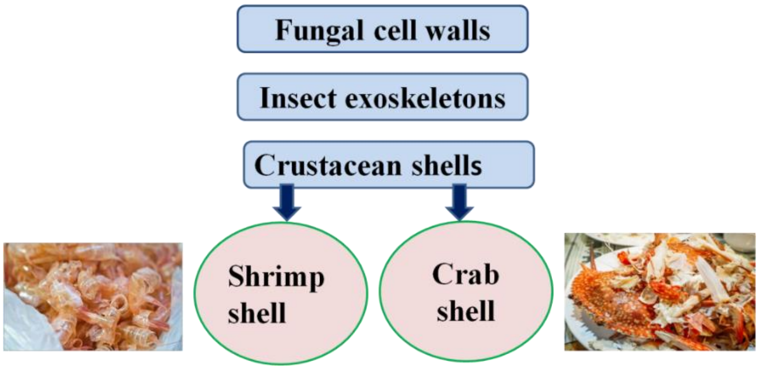

Fig. 3. Chitosan's sources

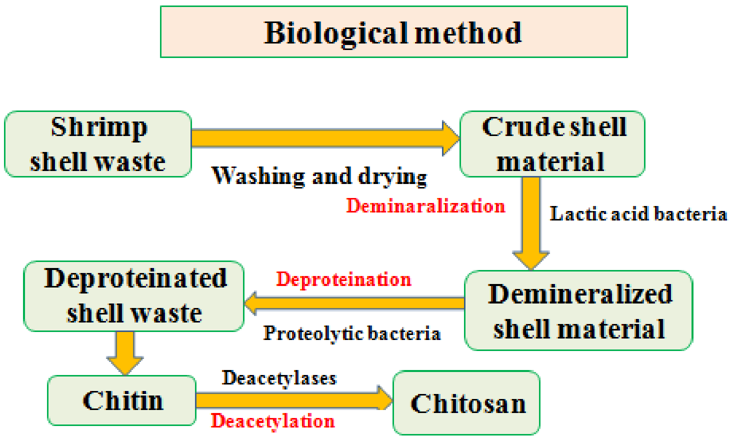

Fig. 4. Biological method of extraction of chitosan

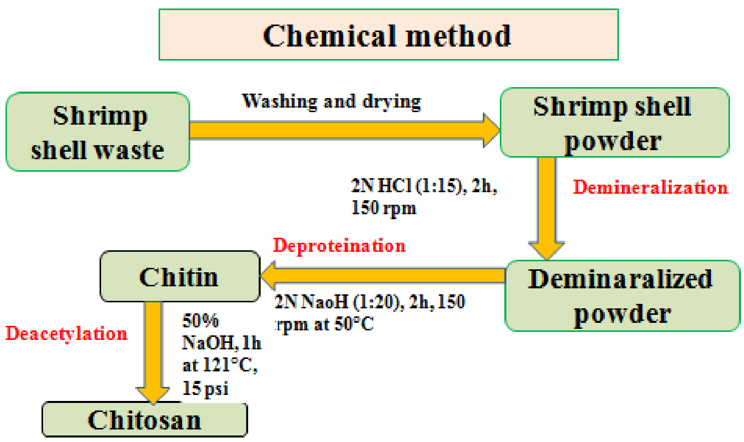

Fig. 5. Chemical method of extraction of chitosan

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 domains include biology, dentistry, ophthalmology, bioimaging, hygiene products, veterinary medicine, packaging, agrochemicals, chromatography, functional fabrics, aquaculture, catalysis, beverages, biotechnology, sludge dewatering and wastewater treatment [8].

5. BENEFITS OF CHITOSAN IN AGRICULTURE

Chitosan derived from waste in the seafood industry and characterized by their non-toxic, biocompatible, and biodegradable nature, provides effective pathogen control by stimulating the plant immune system across various pathogens. The various attributes, specifically the degree of deacetylation and the molecular weight, affect the substance's physicochemical qualities (such as viscosity and solubility) and directly impact the biological properties of the material as well as its impacts on pathogens and plants. Because of all these qualities, chitosan is highly beneficial for a variety of industrial uses, including those in food, medicine, pharmacology, cosmetics, biotechnology, and, more recently, agriculture. With remarkable results, it has been applied to agriculture as fertiliser, sprays, and coatings for seeds, leaves, fruits, and vegetables. Chitosan helps boost plant productivity in addition to protecting plants from microbes that are harmful. Chitosan has been successfully applied to a variety of post harvest fruits, vegetables, and their fresh-cut samples because of these exceptional qualities. Because of its antibacterial qualities and ability to trigger plant defences against infections, chitosan is regarded as the perfect fruit and vegetable coating in the post harvest period. Fresh fruit and vegetable items treated with chitosan are safe for the environment and consumers. The United States Food and Drug Administration (USFDA) has approved chitosan as a "Generally Recognised as Safe" (GRAS) food additive [9].

6. ANTIMICROBIAL EFFECTS OF CHITOSAN AGAINST POST HARVEST PATHOGENS

Chitosan antibacterial properties are closely linked with its physicochemical characteristics, structure, and surrounding environment in addition to the reactive hydroxyl groups at positions C-3 and C-6 [10]. Depending on the precise location of its antibacterial activity, chitosan's mode of action against microorganisms can be classified as extracellular effects, intracellular effects, or a combination of both [11]. As high molecular weight (HMW) chitosan typically cannot penetrate cell walls and membranes, their 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]. Chitosan antibacterial action differs greatly according on the kind of microbe it is intended to target. Chitosan dual action, affecting both its host and the pathogen, is crucial for managing post harvest diseases in agricultural products.

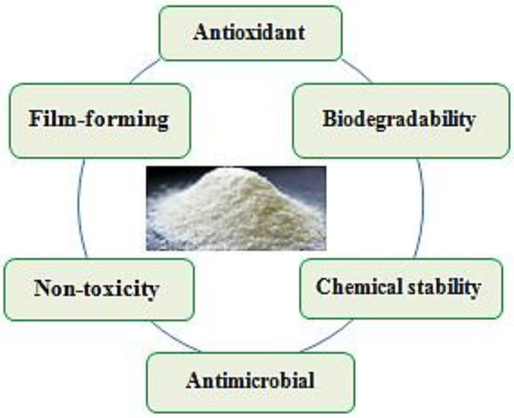

Fig. 6. Important charecteristics of chitosan

Table 1. Commercial products of chitosan [9]

Sr. No	Trade name of the product	Company (Nation)	Formulation of product	Active ingredient (%)
1.	Armour-Zen	Botry-Zen Limited (Dunedin, New Zealand)	Liquid	14.4
2.	Biochikol 020 PC	Gumitex (Lowics, Poland)	Liquid	2
3.	Biorend	Bioagro S.A. (Chile)	Liquid	1.25
4.	ChitoClear	Primex ehf (Siglufjordur, Iceland)	Powder	100
5.	Chito plant	ChiPro GmbH (Bremen, Germany	Powder	99.9
6.	Chito plant	ChiPro GmbH (Bremen, Germany)	Liquid	2.5
7.	Chitosan 87%	Korea Chengcheng Chemical Company (China)	TC (Technical material)	87
8.	FreshSeal	BASF Corporation (Mount Olive, NJ, United States)	Liquid	2.5
9.	Kadozan	Lytone Enterprise, Inc. (Shanghai Branch, China)	Liquid	2
10.	KaitoSo	Advanced Green Nanotechnologies Sdn Bhd (Cambridge, United Kingdom)	Liquid	12.5
11.	Kendal cops	Valagro (Atessa, Italy)	Liquid	4
12.	Kiforce	Alba Milagro (Milan, Italy)	Liquid	6
13.	OII-YS	Venture Innovations (Lafayette, LA, United States)	Liquid	5.8

7. ANTIMICROBIAL ACTIVITIES REGARDING BACTERIA

Gram-positive (G +ve) and Gram-negative (G -ve) bacteria have distinct cell wall compositions. The 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]. Additional negatively charged cell surfaces enhance cationic chitosan binding to phospholipids in environments with pH values below 6.5.

G +ve bacteria have negatively charged teichoic acids due to the presence of phosphate groups in their structure [15]. Disrupting the teichoic acid synthesis path led to *Staphylococcus aureus* developing heightened resistance to chitosan, suggesting that chitosan mechanism of action involves more than just basic electrostatic interactions. Moreover, G +ve bacteria have a thicker cell wall than G -ve bacteria, which may prevent chitosan from directly attaching to the cell membrane. However, some oligomers of chitosan (<5 kDa) are able to get through the cell wall and affect the production of proteins or

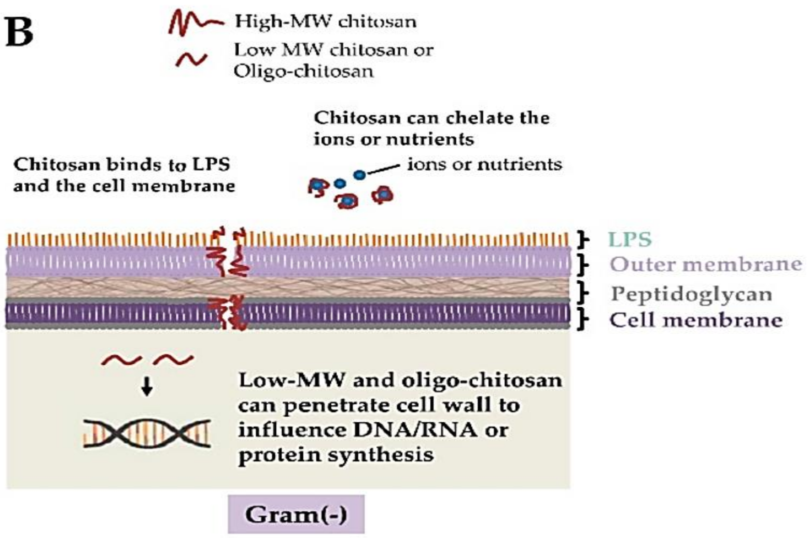

Fig. 7. Antimicrobial activity against G -ve bacteria [14]

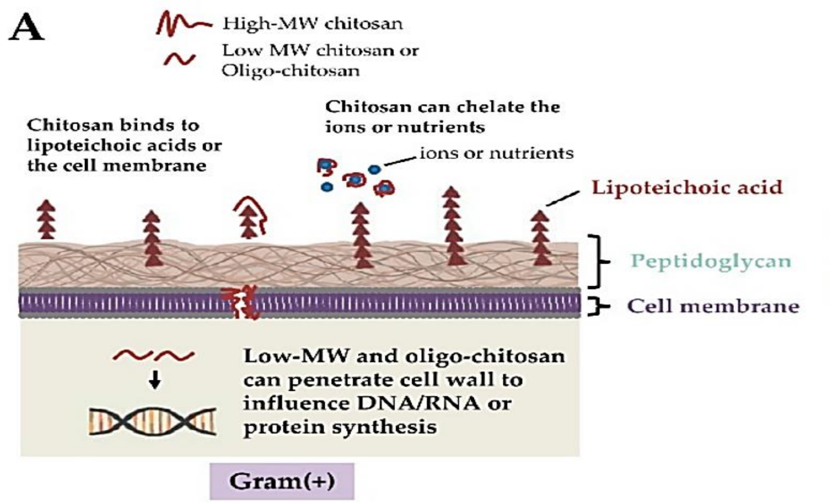

Fig. 8. Antimicrobial activity against G +ve bacteria [14]

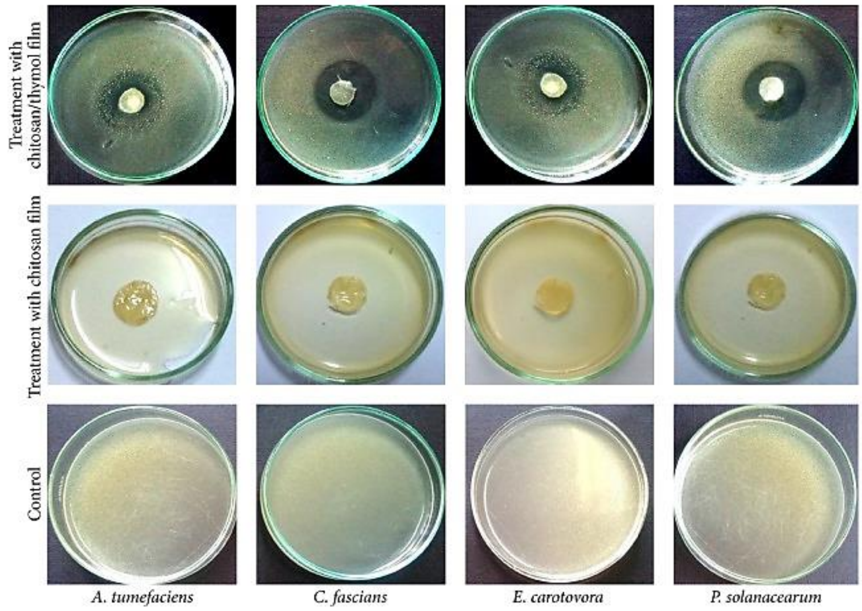

Fig. 9. *Agrobacterium tumefaciens*, *Corynebacterium fascians*, *Erwinia carotovora* and *Pseudomonas solanacearum* growing in vitro on nutrient agar plates with chitosan film added and enhanced with 0.5 per cent thymol [15]

DNA/RNA. It is interesting to note that research has revealed chitosan (≤ 50 kDa) can penetrate the cell wall and prevent DNA transcription. Consequently, the structure of chitosan, rather than its molecular weight, determines whether it has extracellular, intracellular, or combined antimicrobial action, whereas its molecular size plays an important role for targeting.

8. ANTIMICROBIAL ACTIVITIES REGARDING FUNGI

It has been demonstrated that chitosan exerts fungicidal effects on a variety of fungal diseases that affect plants [16]. The antifungal properties of chitosan are mostly caused by the way it interacts with the cell wall or membrane. But the minimum inhibitory concentrations (MICs) of chitosan against fungi vary greatly and are very dependent on things like the chitosan molecular weight and degree of deacetylation (DDA), the solvent pH, and the specific type of fungus that is being targeted [17]. Moreover, the existence of unsaturated fatty acids in higher concentrations in the membrane may be linked to a cell susceptibility to chitosan since they promote greater membrane fluidity and a negatively charged cell membrane. For example, the

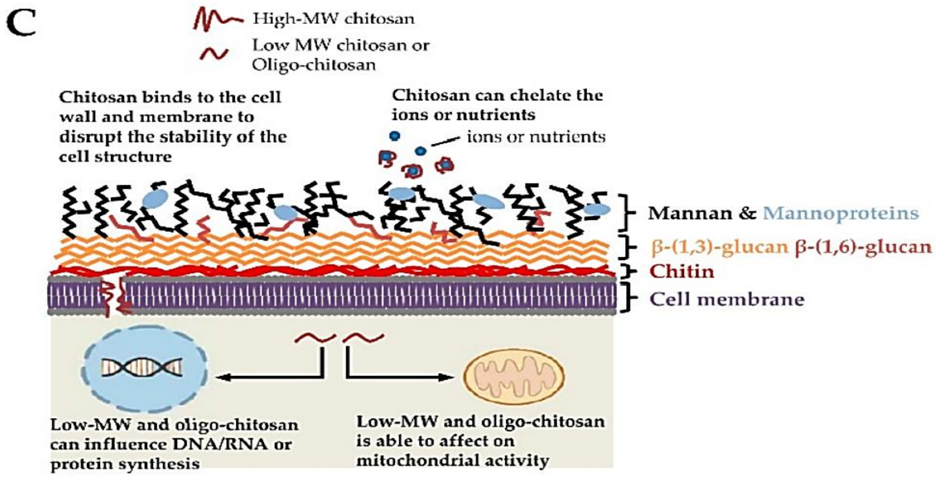

Fig. 10. Chitosan antimicrobial action against fungus [14]

distinct properties of *Neurospora crassa* strains that are chitosan sensitive and chitosan resistant are associated with variations in the quantity of unsaturated fatty acids in their cell membranes. Similarly, LMW chitosan can penetrate the cell wall and cell surface in addition to its extracellular antifungal qualities, which prevents DNA/RNA and protein synthesis [18].

9. CHITOSAN MODE OF ACTION IN RELATED TO POSTHARVEST DISEASES

Because of the positive charge on the C2 of the glucosamine monomer at pH values lower than 6, chitosan is more soluble and has superior antibacterial properties than chitin [19]. Hence, when discussing the potential mechanisms for controlling post harvest decay of fruits, much emphasis is placed on chitosan. Various approaches have been suggested, however the precise antimicrobial properties of chitin, chitosan, and their derivatives remain enigmatic [20].

9.1 The Direct Impact of Chitosan and Chitin on Pathogenic Fungi

The antibacterial properties of chitosan are still a subject of discussion, with two primary explanations put forward: (1) Polycationic chitosan attaches to negatively charged areas on the surfaces of cells, changing the ability of the cells to let substances to pass through. This leads to the release of electrolytes and proteins from inside the cells. (2) Chitosan enters fungal cells and absorbs vital nutrients, so hindering or reducing the production of mRNA and proteins, ultimately blocking their synthesis [21]. Many studies have shown that chitosan can directly obstruct the processes of spore germination, germ tube elongation, and mycelium growth in a variety of plant diseases, such as *Fusarium solani*, *Penicillium* spp., *Sclerotium rolfsii*, *Botrytis cinerea* and *Rhizopus stolonifer* [22]. Chitosan exerted a 100% inhibitory effect on the germination of *Penicillium expansum* spores at a concentration of 0.5%, and on *Botrytis cinerea* spores at a concentration of 1% [23]. Fungal pathogens such as *Botrytis cinerea*, *Penicillium* spp., *Colletotrichum* spp., and *Alternaria* spp. were found to grow differently in vitro when 1% chitosan was added [24].

9.2 Enhancing the Disease Resistance of Fruits with the Use of Chitin and Chitosan

Chitinase activity is typically triggered when chitin is present, with potential diverse biological functions, including exerting antifungal effects [25]. Chitosan, acting as an exogenous elicitor, can enhance the host resistance by boosting the functions of various enzymes involved in defence mechanisms. For example, it promotes the activity of chitinase and β-1, 3-glucanase in oranges, strawberries, and raspberries, and increases the activity of phenylalanine ammonialyase (PAL) in table grapes and strawberries [26].

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

10.1 Reduction in Weight Loss

Transpiration and substrate consumption during respiration have an impact on post harvest fruits

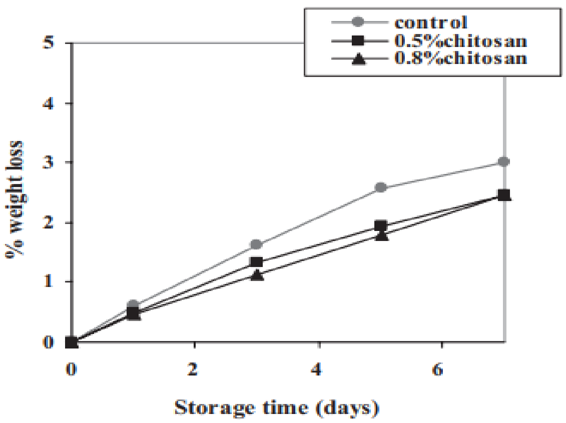

Fig. 11. Weight loss variability of refrigerated fresh-cut mangoes at 6°C [28]

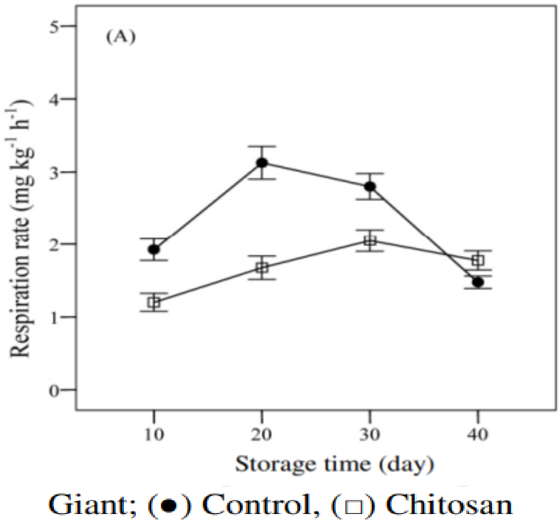

Fig. 12. Respiration rate fluctuations in chitosan-treated plum fruits during cold storage [30]

and vegetables weight loss. Approximately 80% of the overall reduction in weight can be attributable to the loss of water. After undergoing dehydration, the texture of fruits and vegetables transitions from being firm and crunchy to becoming tender and less firm. Furthermore, their taste declines, and their resistance to several physical and microbiological diseases lowers [27]. Applying a chitosan coating to fruits and vegetables resulted in increased moisture retention within the tissue of the produce [28].Therefore, the favourable quality and marketability of post-harvest fruits and vegetables are effectively preserved.

10.2 Respiration

Aerobic respiration is crucial for maintaining the unique properties of post harvest fruits and vegetables. Nutrients function as substrates for the process of respiration. As nutrient levels decline, both the nutritional and commercial value decreases accordingly. Properly regulating the respiration rate can effectively prolong the storage time of harvested fruits and vegetables [29]. Slowing down the respiration rate is possible in an environment with a lower ratio of oxygen to carbon dioxide. Applying a coating to the surface of fruits and vegetables enables control over the rate at which oxygen enters the produce tissue or carbon dioxide is discharged into the air as a result of respiration [30]. Nevertheless, it is crucial for the chitosan coating to have an appropriate thickness. Insufficient adjustment of permeability occurs when the coating is too thin, while excessive accumulation of dioxide carbonate happens when the coating is excessively thick. High concentrations of carbon dioxide can trigger anaerobic respiration, resulting in the production of ethanol, which can spoil post-harvest fruits and vegetables.

10.3 Firmness

The crispness, which is strongly linked to firmness, is a crucial sensory characteristic of fresh fruits and vegetables. During storage, the texture of post harvest fruits rapidly deteriorates as they soften, leading to the loss of their crispness. During the storage period of fresh fruits and vegetables, their firmness decreases as a result of numerous variables such as dehydration, pectin degradation, nutrient consumption, and other factors [31]. Applying a chitosan coating reduces transpiration, leading to increased water retention. Consequently, the cells of fruits and vegetables maintain higher swelling pressure and exhibit greater firmness. Hence, the use of chitosan coating can partially mitigate the reduction in firmness of fruits and vegetables after harvesting [32].

10.4 Fungal Decay

Chitosan treatment reduced the spoilage of strawberries during storage. Samples treated with either 1.5% chitosan or chitosan calcium gluconate (Cs-CaGlu) did not exhibit any visible deterioration during the entire storage duration [33].

Table 2. Impact of chitosan coating on total soluble solids (TSS) of banana fruit [34]

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)	$4.1(0.36)^{a}$	$5(1.0)^{c}$	$12(1.0)^{c}$	$16(1.0)^{d}$

10.5 Total Soluble Solid and pH

During the period of cold storage, the strawberries had a rise in both their total soluble solids and pH levels. However, there were not any noticeable disparities in these parameters across the coated groups during the period of cold storage. The coating had a significant impact on the total soluble solids and pH levels during the storage period, in comparison to the control samples. Significantly, the control fruit displayed greater levels of total soluble solids and pH values compared to the treated samples. The increase in pH during storage can be attributed to dehydration, with the control fruit exhibiting more pronounced dehydration compared to the coated fruit. Furthermore, variations in total soluble solids and pH may also be associated with the ripeness stage [34].

11. METHOD OF APPLICATION OF CHITOSAN

The preparation of chitosan edible films involved dissolving chitosan at a concentration of 1.5-2.5% w/v in a mixture of 1% (v/v) acetic acid aqueous solution containing 0.5-1.0% w/v glycerol, with stirring. 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].

12. FACTORS DETERMINING CHITOSAN MICROBIAL ACTIVITY

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

12.1 pH value

Chitosan, a polycationic polysaccharide with an abundance of amino groups, rapidly interacts with negatively charged molecules such as phospholipids, proteins, and fatty acids at pH values below 6. The pH level of chitosan is crucial in determining its capacity to penetrate pathogen cells. Studies indicate that for optimal antimicrobial effectiveness, the pH of chitosan should be maintained below its pKa value [36]. Moreover, when the pH of the solution lowers, the solubility of chitosan increases, leading to a greater positive charge on the -NH3 groups of chitosan. This, in consequently, improves the antimicrobial activity of chitosan [37].

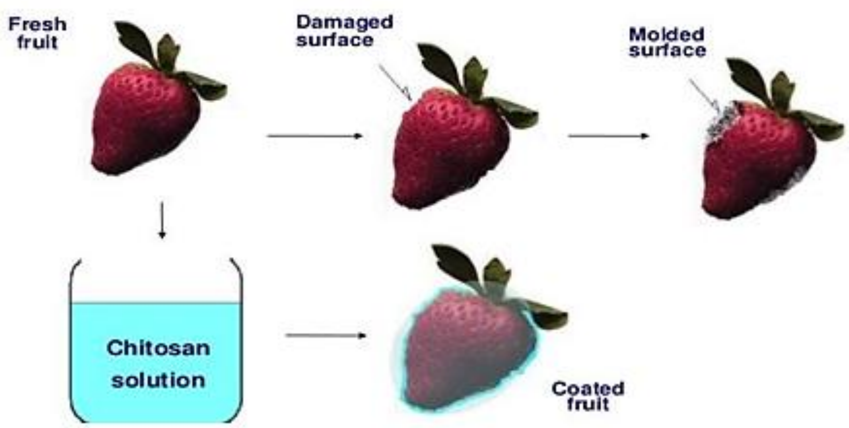

Fig. 13. Dipping method of chitosan application [35]

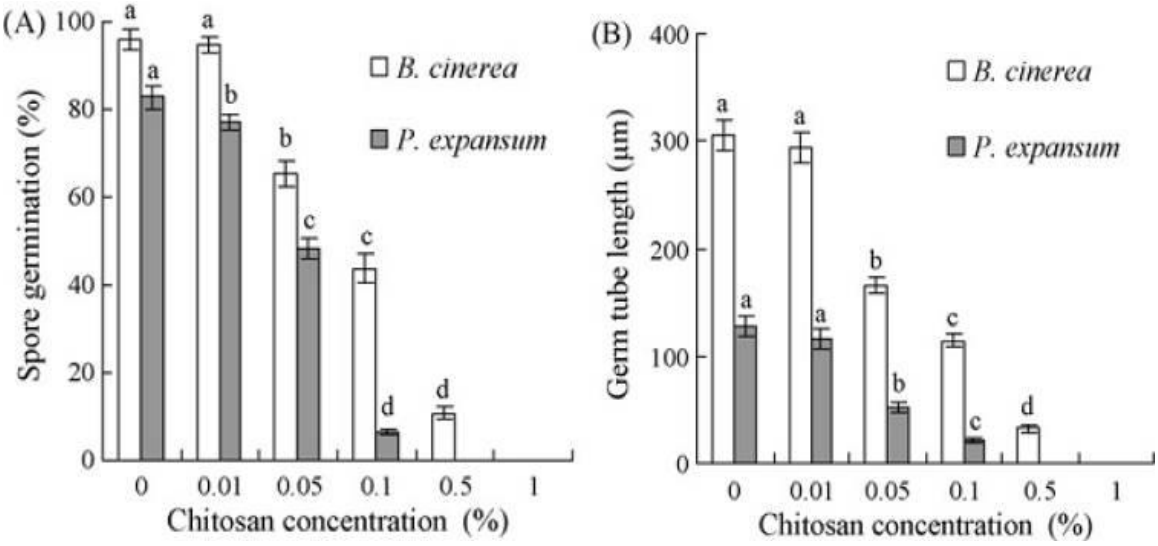

Fig. 14. The effects of different concentrations of chitosan on the germination of spores (A) and the elongation of germ tubes (B) of *Botrytis cinerea* and *Penicillium expansum* were observed 12 hours after incubation at 25°C [40]

12.2 Concentration of Chitosan

Pre-harvest application of chitosan sprays effectively inhibited postharvest decay of strawberry fruit caused by *Botrytis cinerea* during storage at temperatures of 3 and 13 °C. The level of decay decreased as the concentration of chitosan increased [38]. Chitosan shows its highest effectiveness in suppressing microbial growth within the concentration range of 0 to 5%. Furthermore, as the concentration of chitosan increases, its antimicrobial effect also increases [39]. Chitosan easily binds to the cell surface membrane at lower concentrations, causing disruption and leading to the leakage of cells, eventually resulting in their death. Nevertheless, when present in larger concentrations, it adheres to the cell membrane, so impeding the dissolution of intracellular constituents [40].

12.3 The Molecular Weight of Chitosan

The molecular weight of chitosan determines its capacity to enter the cell membrane and exert antimicrobial actions within the cell. Moreover, the polysaccharides and certain proteins that make up the intricate layers of the cell wall in bacteria and fungi have vital roles in pathogenicity, attachment to living and non-living surfaces, stimulation of the immune response, and also provide structural support and act as a barrier against the environment [41]. Chitosan can be classified into two categories based on its molecular weight: high molecular weight (HMW), which is typically 100 kDa and above, and low molecular weight (LMW), which normally falls between the range of 2 to 99 kDa. HMW chitosan predominantly adheres to the cell membrane rather than penetrating it, hence impeding nutrient absorption and resulting in cellular death. However, LMW chitosan has the ability to penetrate the cell, bind to DNA, and hinder the process of protein synthesis. In addition, an increase in the carboxylic content of chitosan leads to a decrease in its molecular weight, which in turn affects its ability to inhibit the growth of microorganisms. According to reports, low molecular weight chitosan (LMWC) has shown efficacy in controlling postharvest diseases in citrus fruit [42]. The results showed that LMWC effectively inhibited the deterioration of citrus fruit caused by *Penicillium digitatum*, *Penicillium italicum*, *Botrydiplodia lecanidion*, and *Botrytis cinerea* after being stored for 14 days at 25°C. LMWC performed better than both TBZ (Thiabendazole) and high molecular weight chitosan (HMWC) in terms of effectiveness. Furthermore, the utilisation of LMWC coating had a beneficial effect on the hardness, total soluble solid content, titrable acidity, ascorbic acid content and moisture content of citrus fruit after being stored for 56 days at a temperature of 15°C.

The binding affinity and absorption capacity of chitosan decrease as the molecular weight and degree of deacetylation (DD) of the polymer decrease. The uptake decreased by 26% when the molecular weight (MW) was reduced from 213 to 10 kDa, and by 41% when the degree of dilution (DD) was decreased from 88 to 46% [43]. Some studies suggest that reducing the molecular weight of chitosan can improve its ability to kill microorganisms [44].

12.4 Chitosan Derivatives

Chitosan is highly advantageous due to its ability to produce a wide range of derivatives. Carboxymethyl chitosan and quaternized carboxymethyl chitosan are two well-established examples of derivatives in this category [45].

The antimicrobial efficacy of chitosan is limited because of the comparatively weak positive charge centres produced by its amino groups. Observations revealed that oligochitosan had superior efficacy compared to chitosan in inhibiting the mycelial growth of *Phytophthora capsici* [46]. Hence, enhancing the functionality of chitosan requires the addition of further positive charge groups to it [47].

12.5 Degree of Deacetylation

The amino group (-NH2) is the main functional group of chitosan, and its effectiveness in various uses is influenced by the degree of deacetylation (DD) [48]. The degree of deacetylation (DD) is determined by calculating the ratio of glucosamine to N-acetyl glucosamine units in the copolymer chain, which indicates the proportion of glucosamine units in the chain. This parameter determines the concentration of unbound amino groups in chitosan, which affects its suitability for use in different areas. The electrostatic interaction between chitosan and phospholipids with a negative charge plays a crucial role in its mechanism of action. Generally, a greater quantity of amino groups leads to better solubility in acidic conditions, which in turn boosts microbial activity [49].The effectiveness of chitosan against fungi enhances with higher degrees of deacetylation and lower molecular weights. This was illustrated by assays conducted on chitosan samples with different molecular weights and degrees of deacetylation, which were evaluated against *Fusarium oxysporum*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, and *Candida albicans* [50].Enhancing the degree of deacetylation (DD) led to enhanced water barrier characteristics, tensile strength, and antibacterial efficacy. As a result, the ability of chitosan films to kill Listeria innocua and Escherichia coli bacteria was improved as the degree of deacetylation (DD) increased [51].

12.6 Type of Organism

Chitosan demonstrates diverse impacts on different categories of microorganisms Fungi with thicker cell walls exhibit greater resistance to chitosan penetration compared to fungi with thinner cell walls. In addition, chitosan exhibits stronger suppression against G +ve bacteria in comparison to G –ve bacteria [52]. Chitosan shown diverse antibacterial properties on several species of bacteria. As an example, *Listeria monocytogenes* had a significant decrease of 6 log in viable cell count, but *Bacillus cereus* and *Salmonella enterica* suffered a reduction of 3-5 log in viable cell count. Conversely, *Staphylococcus aureus* had a cell count drop of less than 1 log [53].Based on the available evidences, it seems that bacteria are typically less responsive to chitosan's antimicrobial effects compared to fungi [54].

12.7 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. Numerous studies have shown that fungal chitosan exhibits reduced antibacterial efficacy against *E. coli*, *Klebsiella pneumoniae*, and *S. aureus* when compared to chitosan obtained from crustacean shells. However, fungal chitosan, which closely resembles chitosan derived from crab shells, has demonstrated encouraging inhibitory effects on gram-positive bacteria in comparison to G –ve bacteria [55].

12.8 Chitosan Complexes

Some research indicates that the antibacterial activity of chitosan can be improved by applying a coating of natural bioactive compounds such as essential oils (EOs) [56]. Various essential oils (EOs) like lemongrass, clove, and oregano, when coated with chitosan, enhance its antimicrobial efficacy. Furthermore, recent research has shown that using LMW chitosan, sodium sulphate as a cross-linker, and reducing particle size to below 300 nm through 20 minutes of sonication led to the most potent antibacterial effect. Chitosan nanoparticles produced under these conditions effectively eradicated pathogenic *E. coli* O157:H7 [57].

Table 3. Exposure to different concentrations of chitosan, lemongrass, and citral essential oils on PDA media resulted in reduced linear growth and spore germination percentages of *Penicillium digitatum* and *Penicillium italicum* in citrus fruits [57]

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	2ml/L	42.2	55.0	42.2	58.8
	4ml/L	66.7	70.0	63.3	74.0
	6ml/L	100	100	100	100
	8ml/L	100	100	100	100
Citral	2ml/L	53.3	68.0	50.0	77.0
	4ml/L	71.1	90.0	66.7	92.0
	6ml/L	100	100	100	100
	8ml/L	100	100	100	100
Chitosan + Citral	2 g/L + 2ml/L	58.9	77.0	51.1	64.0
	3 g/L + 3ml/L	88.9	95.0	83.3	90.0
	4 g/L + 4ml/L	100	100	100	100
Chitosan +Lemongrass	2 g/L + 2ml/L	53.3	70.0	50.0	66.0
	3 g/L + 3ml/L	84.4	90.0	80.0	88.0
	4 g/L + 4ml/L	100	100	100	100
Control		**0.0**	**0.0**	**0.0**	**0.0**

12.9 Time of Application

The application of chitosan spray on plants resulted in fruits that displayed enhanced firmness and experienced a slower ripening phase. This was demonstrated by higher levels of anthocyanin content and titratable acidity in comparison to berries from untreated plants [58].

13. CHALLENGES RELATED TO THE UTILISATION OF CHITOSAN

The primary challenge in utilizing chitosan lies in its solubility. One method to resolve this problem involves producing chitosan derivatives or combining chitosan with natural components like essential oils. Other concerns related to chitosan use include its characteristics and variability. Due to the lack of scientific consensus on the correlation between molecular weight and degree of deacetylation, our understanding of the biological activity of chitosan is limited. Furthermore, the lack of appropriate data on the diverse impacts of chitosan application in managing post-harvest diseases on fruits or vegetables complicates the evaluation of its inhibitory effects on various fungal strains. *Pochonia chlamydosporia* and similar fungi have saturated free fatty acids that offer defence against chitosan permeability [59]. A study was conducted to assess the impact of temperature and pH on different molecular weight forms of chitosan. The findings indicated that the antimicrobial efficacy of chitosan increased with higher temperatures and lower pH values [60]. Another issue regarding chitosan is to the uncertainty around low molecular weight chitosan and oligochitosan [61]. The antimicrobial activity of chitosan has been shown to be influenced by environmental factors such as moisture, pH, and temperature. 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 wide ranging antimicrobial characteristics and the ability to activate plant innate immunity, shows promise in agriculture for the management of plant diseases. Its use could help reduce reliance on chemical pesticides, at least to some extent. The polysaccharide chitosan is a sustainable and biodegradable polymer that may be derived from natural sources. It is particularly relevant in addressing the growing concerns over food safety.

15. FUTURE PERSPECTIVE

In order to better understand the antimicrobial mechanism of chitosan, it is important for future study to concentrate on finding the precise target molecule located on the cell surface or within the cell. Generating gene mutant strains could offer useful insights into the mechanisms of antimicrobial activity. Analysing the transcriptome and proteome of essential defence genes and proteins would enhance our understanding of the chitosan mediated signalling pathway, hence improving our knowledge of its antimicrobial properties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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