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Development of Low Molecular Chitosan and Its Silver Nanocomposites for the Edible Fruits Coating to Improve the Shelf Life Period.

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ABSTRACT

Chitosan is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine and can be derived by the partial deacetylation of chitin. The present study was concluded that the edible coating affects positively on the physiochemical parameters of tomatoes. The coated sample shows significant difference in almost all parameters as compared to control (uncoated) tomatoes. As far as storage period is concerned as increase the quality parameters like weight loss, pH, and antimicrobial activity of tomatoes and grapes. For this study low molecular chitosan (0.5 %) and synthesized (AgNPs) low molecular weight chitosan were used. The coating of chitosan can modify the internal atmosphere, decrease transpiration, delay ripening fruit while increasing the shelf life of tomatoes and grapes. After applying improved chitosan-based coating, the preserving effects were increased in most of the cases compared with single chitosan coating.
Keywords: Chitosan, Low molecular weight chitosan, Chitosan AgNPs, Tomato, coating Microbial loading.

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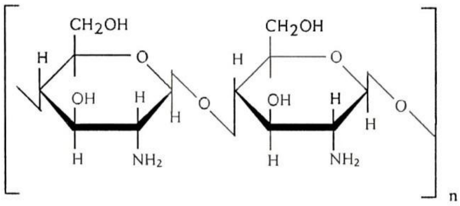
INTRODUCTION

Chitosan is a linear polysaccharide consisting of β-(1→4)-linked 2-amino-2- deoxy-D-glucose residues, originating from deacetylated derivative of chitin, which is the second most abundant polysaccharide in nature after cellulose. It is non-toxic, biodegradable, biofunctional, and biocompatible. Chitosan has strong antimicrobial and antifungal activities that could effectively control fruit decay [1]. Considering the superior properties of chitosan, it has been successfully used in many postharvest fruits and vegetables, such as grape, berry, jujube and fresh-cut lotus root [2- 4]. Though chitosan coating has many advantages to the preservation of postharvest fruits and vegetables, single chitosan coating sometimes demonstrates a certain defect, which includes limited inhibition, to microorganism that leads fruit to decay, and poor coating structure to adjust the permeability of carbon dioxide and oxygen [5]. To effectively apply the chitosan coating, the chitosan was combined with other substances, in addition, the single chitosan coating was often combined with physical methods such as short heating, short gas fumigation, modified atmosphere packaging, and so on it could easily form coating on fruits and vegetables, and the respiration rate of fruit and vegetable was reduced by adjusting the permeability of carbon dioxide and oxygen [6]. Due to its excellent properties such as adsorption, film-forming and antimicrobial properties, chitosan has a wide range of application in many industrial fields such as food, biotechnology, paper manufacture, cosmetics, agriculture, environment and medical fields [7]. Edible film is defined as a thin layer of edible materials formed on food as a coating or a self supporting thin layer placed on or in between the food components, and in both cases consumed along with the food [8]. Edible coating have recently become one of the most effective methods in maintaining the quality of food. Its functions are to extend the shelf life of food and maintain its quality by inhibiting migration of moisture, oxygen, carbon dioxide, aromas, lipid, and solute as well as to solve environment waste problems. In recent year, Silver Nanoparticles (AgNPs) have potential applications, such as catalysts, photonic devices, biosensor and antimicrobial activity [9].

Structure of chitosan



However, there are many concern about the biological and environmental risks of Silver nanoparticles. Silver nanoparticles have same adverse effect as genotoxicity to fish, inhibition of photosynthesis in plants. An alternative and an eco friendly process is the use of chitin / chitosan silver nanoparticles. Use of chitin and chitosan based nanoparticles overcomes the above said environmental risk [10].

The traditional industrial source of chitin is Shell fish waste shrimp, crab, lobster and processing. However problem with seasonal and limited supply, several alternative industrial raw material sources of chitin have been suggested. Use of mycelia of various fungi such as, Ascomycetes, Zygomycetes, Deutromycetes and Basidiomycetes increased the attention of researches and fungi are sees as promising chitosan sources [11].

The major post harvest losses of tomatoes, fresh vegetables are due to fungal infection. Physical disorder many tech have been studied in order to extend the shelf life of fresh products. However, they have advantages and disadvantages. Quality maintenance of fresh products is still a major challenge for the food industry. Nanotechnology will facilitate the development of light and more precise food manufacturing equipment. Nonpolluting as well as cheaper packaging techniques. Chitin/ chitosan based (extra from fungi) nanoparticles coating on fruits save better research ie., long storage & antimicrobial activity against spectrum of microbes [12].

In the present study, the chitosan based low molecular glucose –chitosan molecule and its AgNPs were synthesized , characterized, and used for the edible fruit coating on tomato and grapes. The weight loss, pH, firmness and antimicrobial load of coated fruits were also determined.

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MATERIALS AND METHODS

Tomatoes (*Lycopersicume sculentum)* and grapes (*Vitis vinifera)* were purchased from local farm. Fruits with uniform size and shapes, without damage and fungal activity, washed twice water and used for further analysis.

Chemicals and reagent

All the chemicals were purchased from the Himedia, Mumbai, India

Preparation of low molecular weight chitosan [13]

0.5g chitosan was added to 10ml of 2% acetic acid, and mixed well, then kept it in water bath at 42.8^{0}C for 3to 5 hrs. After the reaction 10%NaOH was added to neutralize the solution, filtered to remove the residues and the two fold volume of ethanol , then the crystal of water soluble chitosan was collected, after air drying in hot air oven at 80^{O}C for 20 minutes and used for further analysis.

***Preparation of Ag NPs* [14]**

A suspension of size-controlled Ag NPs was prepared as previously described [14]. Briefly, 0.50 g of silver-containing glass powder was dispersed in 50mL of an aqueous solution of 0.5% glucose in a 100mL glass vial. The mixture was autoclaved. The mixture was then gradually cooled to room temperature and centrifuged at 3000 rpm for 10min. The supernatant containing the Ag NP suspension was removed and stored in the dark at 4^{O}C and used for fruit coating.

Fruit coating [15]

Fruits were coated in low molecular chitosan solution and low molecular chitosan AgNPs, by using sterile cotton swab thrice. Each coating has been done by after drying of fruits in equal interval of time period, and stored at room temperature.

Samples (coated and uncoated fruit-control) were stored in aseptic condition for 28 days for further analysis.

Weight loss [15]

Three replicates of fruits were used for each treatment. Every week (Tomato - four week ; Grape – Eight days), a sample of fruits were weighed regularly to determine weight loss.

Determining the pH

pH was determined using a pH meter (ELICO L1 617) as described in [16].

Tomatoes firmness [17]

The firmness changes of fresh and stored tomatoes fruits were measured using a Fruit firmness tester controlling the penetration depth by interesting an appropriate penetrometer tip into the fruit pulp.

Characterization of low molecular weight chitosan AgNPs [18]

The formation of AgNPs was monitored by visual inspection of the solution, as well as by periodical recording of the ultraviolet (UV)-VIS spectra of the reaction mixture. The UV-VIS spectroscopy measurements were recorded on a UV visible ELICO SL 159 nanodrop spectrophotometer. The aqueous filtrate containing AgNPs and their controls was subjected to Fourier transform infrared **(FTIR)** spectrum using a Thermo Nicolet, Avatar 370. The aqueous solution of AgNPs synthesized was freeze dried and used for scanning electron microscopy (SEM).

RESULTS AND DISCUSSION

Weight loss

Three replicate of fruit were used for each treatment, every week a sample of fruit was removed from each treatment. The fruit were weighed regularly to determine the weight loss. Weight loss depends upon transpiration of the fruits. Permeability of coating material is another factor that could reduce the weight loss of tomatoes (Table 1).

The results showed that significant weight loss was observed in 22.2% in chitosan coated fruits followed by chitosan AgNPs , which showed less weight loss (9.09%). The transpiration rate of tomatoes and grapes depended on the thickness of film. The fruits coated with 0.5% chitosan showed biggest weight loss when compared with chitosan based AgNPs during storage at room temperature (28^{O}C). This results are in line with [19] and[20].

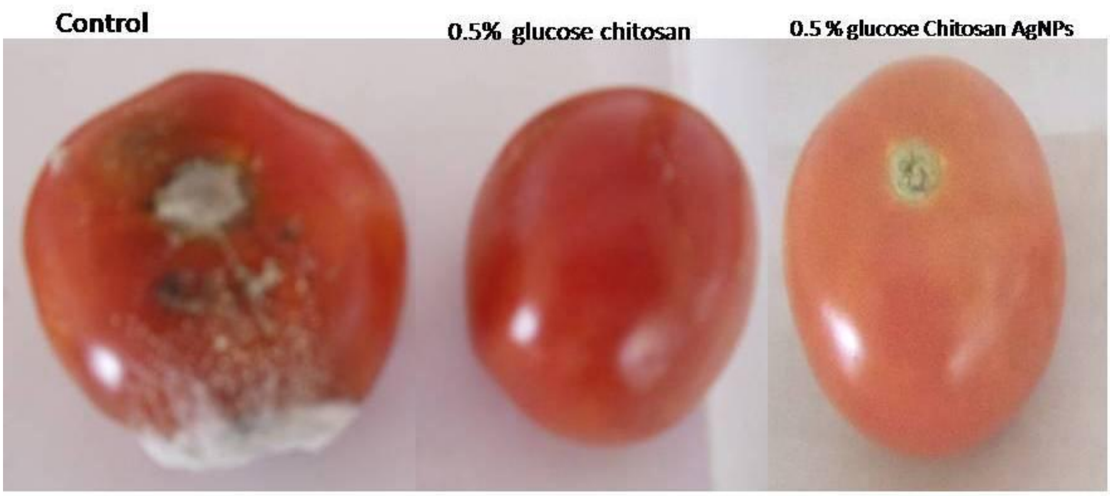
Determination of pH

The results revealed that there is not that much variation in pH in control and edible coated fruits. This may be due to coating of chitosan on the surface of the fruits.

Table 1: Weight loss of fruits before and after edible coating

Fruits	Coating type	Storage period (week)					% of weight loss
		0	1	2	3	4	
		Weight in grams					
Tomatoes	Control (without coating)	40	37	33	29	26	35.0
	Low molecular weight chitosan	45	44	42	40	35	22.2
	Synthesized low molecular weight chitosan	44	44	42	41	40	9.09
Grapes	Coating type	Storage periods (days)					
		0	2	4	6	8	% of weight loss
		Weight in grams					
	Control (without coating)	2.6	2.0	1.8	0.9	0.2	92.3
	Low molecular weight chitosan	2.8	2.6	2.4	1.6	1.2	57.1
	Synthesized low molecular weight chitosan	3.1	3.0	2.9	2.6	2.2	22.6

Figure 1: Tomato coated 0.5% glucose chitosan & chitosan AgNPs



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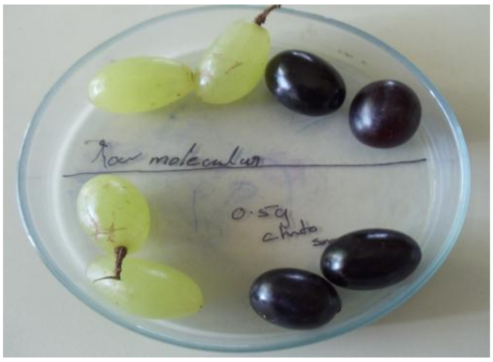
Coated with chitosan **Coated with chitosan based nanoparticles**



Tomatoes firmness

Among the different fruit of tomatoes and grapes suffers a loss of firmness during senescence which contribute greatly to its short postharvest life and susceptibility to microbial contamination. Change in firmness between control coated and Ag NPs coated fruits samples during four week of storage at room temperature have been studied. Initial firmness values were similar for control and all coated samples. On the second week of storage, uncoated tomatoes began to show a gradual loss of firmness. After second week of storage samples have significant difference in firmness were noted in all the samples. With regard to coated samples, chitosan coated fruit samples followed by 0.5% (AgNPs) was more effective in preparation, decrease of fruit firmness than other treatments.

Figure 2: Firmness of Tomato after 4 weeks of incubation

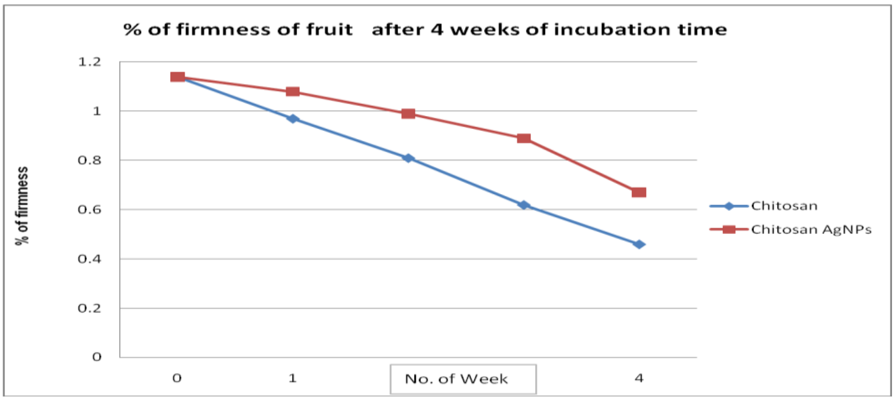
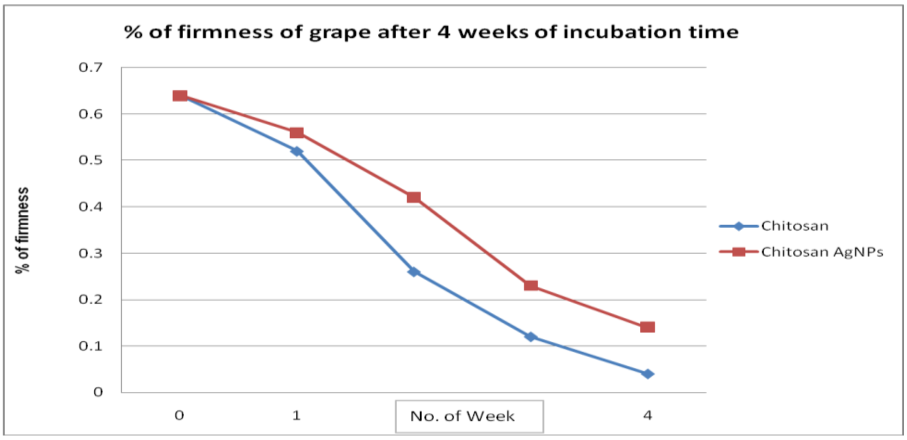


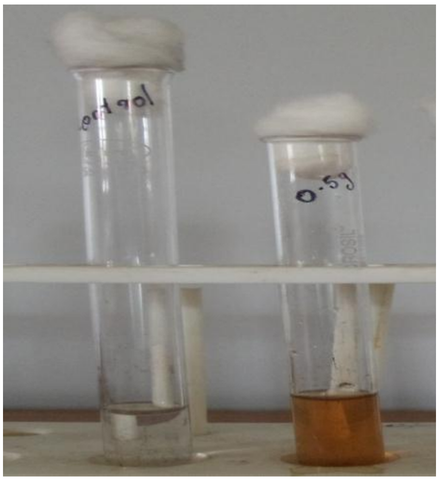
Figure 3: Firmness of grape after 4 weeks of incubation



Synthesis of Chitosan AgNPs.

The colour change occurred from yellow color indicated the synthesis of nanoparticles. ([18] It has been reported that upon addition of silver ions into cell free filtrates in dark changed in color from almost colorless to brown with intensity increasing during the period of incubation. [21] also reported the change of color from pale yellow to brown (fig.5)

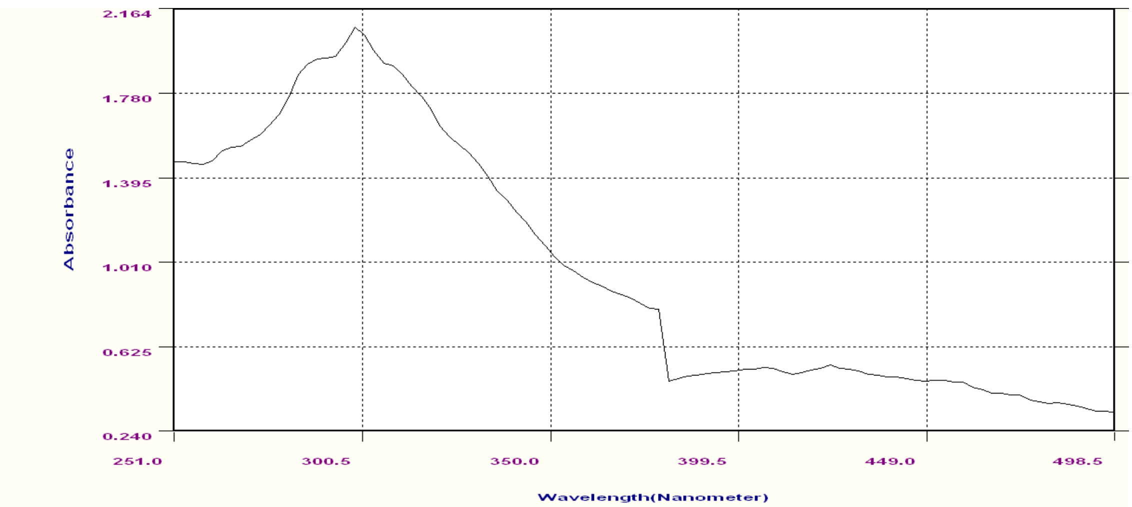
Figure 6: Synthesis of Chitosan AGNPs



Characterization of chitosan –AgNPs – UV- Visible Nanodrop spectrum

The UV-Vis spectra of the aqueous reaction mixture were recorded (Figure 2). Aliquots of the reaction mixture were withdrawn at 12 hrs time interval and scanned on a UV-visible spectrophotometer. The absorbance band was observed at 298 nm in our study [14] observed the Plasmon peak at 390.5nm (Fig.6)

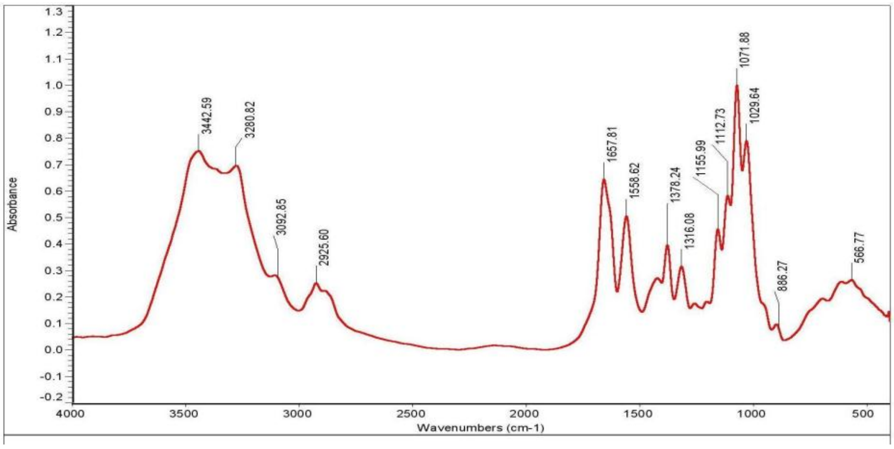
Figure 7: UV- VIS study of Chitosan AgNPs



FTIR

A new peak at 1071.88-1 cm-1 was found to have been appeared confirmed the synthesis of nanoparticles. These FTIR results are found to line with the finding of [18]. Though the shift in peak confirms that there is a formation of silver oxide by the reduction of silver nitrate, and then it is necessarily to be subjected to SEM analysis to measure the size of the particle (Fig.7).

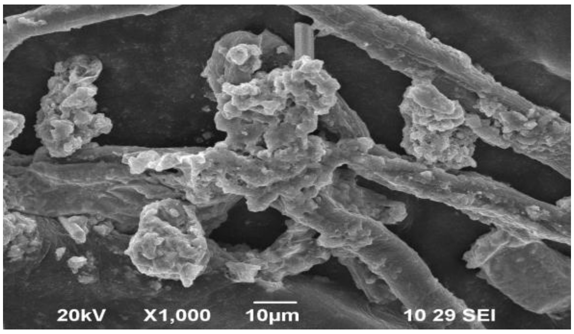
Figure 8: FTIR study of Chitosan AgNPs



SEM

SEM determination of the freeze dried sample showed formation of AgNPs (Fig. 8). The morphology of the nanoparticles was highly variable. The morphology of the nanoparticles was uniform and spherical. The particles are nanosized and well dispersed with the size range of 120nm.

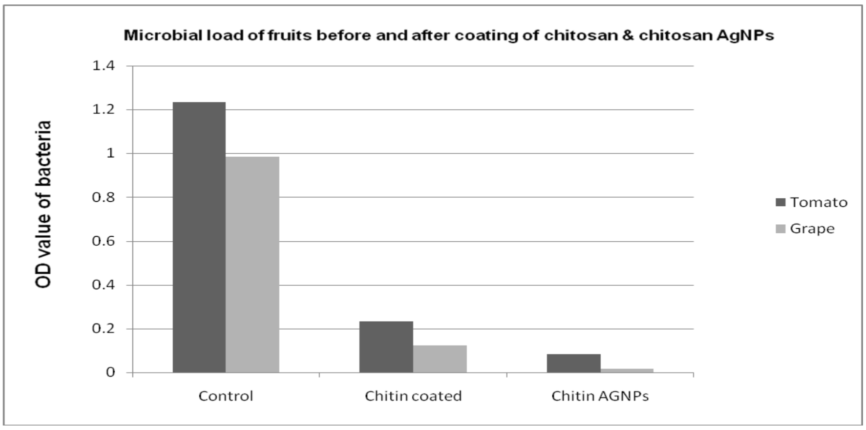
Figure 9: SEM image of Scanning electron microscope



Microbial count

The initial growth of the bacterial population was high in both the fruits tried , but after applying the chitosan and chitosan AGNPs the bacterial populations were reduced significantly(Fig. 8). Our results are on line with the findings of [22] who found that chitosan based coating reduced microorganisms level in fish coated with chitosan.

Figure 10: Microbial load of fruits before coating with chitosan



CONCLUSION

The present study is to circumvent the negative factor of the fruit coating and introduce technologies to attain the objective of prevention of water losses to avoid shrinking of the fruit surfaces and other negative features that co along with that, and that with the eco friendly safe product like chitosan. The edible coated samples shows significant differences in almost all parameters like weight loss, pH, firmness and antimicrobial activity of fruits. The texture of the coated fruits could be improved after 4 weeks of incubation time. It is further hypothesized the chitosan AgNPs have the greater spread ability and reduced the residential microbial flora and non hazardous to humans and thereby contributing in improving the shelf life of the fruits at storage level.

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