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Use of Chitosan as Edible Coating on Fruits and in Micro biological Activity - An Ecofriendly Approach.

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Abstract: *Chitin is a biodegradable,long, linear chain polymer found naturally abundantly in the marine and terrestrial environments. In this study, the capability of Chitin to delay the ripening of fruits is proved by coating chitin composites in three concentrations (low0.25%, Medium0.5%, High0.75%) on Apple and Tomato samples. A comparison study was carried out between three groups of samples which were coated with Glucose/Chitosan Medium, Glucose/Chitosan medium added chitinase enzyme and Chitosan Silver Nano composites respectively. Edible Chitosan coating effected positively on the samples and the coated samples showed significant difference in all physiochemical parameters than the control (uncoated). The results showed that all the groups showed similar effects in the quality parameters such as pH, phenolic content and antimicrobial activity of the samples. The third group comprising of the Apples and Tomatoes coated with Chitosan silver Nano composites showed significant time delay of ripening of the fruits in comparison with the other two groups.Chitosan coatings can be used for storage of highly perishable fruits as it had showed increase in the shelf life of the samples used in the study. They significantly control the moisture content between the fruits and the external environment thus proving effective in preventing fungal contamination of the fruits.*

I. Introduction

There is rising concern for food safety and nutrition amongst the consumers. This increase in health consciousness has led to the demand in organic fruits and vegetables along with healthy preservation techniques. According to some reports, 25% to 80% of harvested fresh fruits and vegetables may be lost due to spoilage worldwide [17].

Hence, research is being carried out to increase the shelf-life of the fruits and vegetables without loss in their nutritive content by identifying and studying the cause for the quality deterioration of these commodities and the feasible solution for this ubiquitous problem. Their deterioration is mainly because they are biologically active and carry out transpiration, respiration, ripening and other biochemical activities.[18]

Edible coatings offer a plausible solution to obtain fresh, nutritive comestible products. Theyserve as a semipermeable barrier to gases and water vapour, thereby reducing respiration and water loss. If the edible coatings are modified to be biodegradable, they add yet another advantage to the smart film technology as the coatings can be used directly on the fruits and vegetable.[5] As these films prove to be effective concerning food safety and environmental pollution, they gain high popularity in the industrial sectors[14].

Though there are several technologies for food preservation,there is a need to find the most effective and economical method. Chitosan, from chitin uses the edible coating technology to form a film closely around the fruits and vegetables to preventing food spoilage and contamination.Chitin, a cationic polysaccharide (Figure 1), (1, 4-linked 2-deoxy-2- acetoamido-α-D-glucose) is a major component in the exoskeletons of insects and the shells of crustaceans (crabs, shrimp and crayfishes), making it easy to obtain. Chitosan (poly-beta-1, 4-glucosamine), the deacetylated form (Figure 2) of chitin can be obtained from crustacean shells either by chemical or microbiological processes and is also produced by some fungi (*Aspergillus niger , Mucor rouxii, Penecillium notatum*)[15].

Chitosan presents numerous uses: flocculants, clarifier, thickener, gas-selective membrane, plant disease resistance promoter, wound healing promoting agent and antimicrobial agent. Chitosan is presented as a potential material for edible coatings processing, mainly due its non-toxic nature, biocidal activity and gas barrier properties Chitosan is well known to have an antimicrobial activity against various microorganisms[12]. Studies showed chitosan could be released from the film matrix and inhibit microbial growth.

Chitosan ensures to slow down senescence by preventing respiration and transpiration. This study aims to compare the potency of chitosan as an edible coating between three groups including chitosan with chitinase enzyme added to the substrate, crude chitosan and chitosan – silver nanoparticles on tomatoes and apples. The coatings were tested for three concentrations (0.25g, 0.5g, 0.75g) for all three groups. Tomatoes contain high water content of about 90% of its total weight which makes it easily susceptible to food spoilage. Their nutritive

value decreases as the fruit ripens and it can be noted that the starch content decreases and sugar content increases [11].

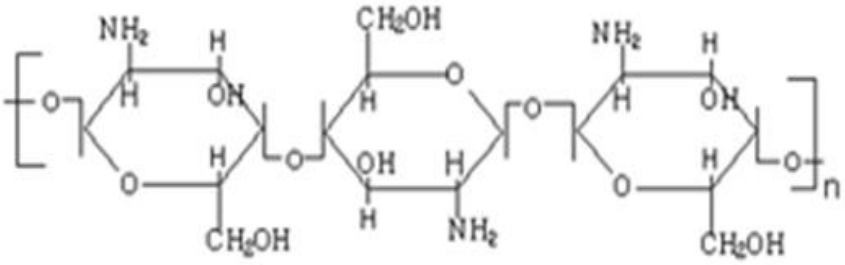

Figure 1 structure of chitin

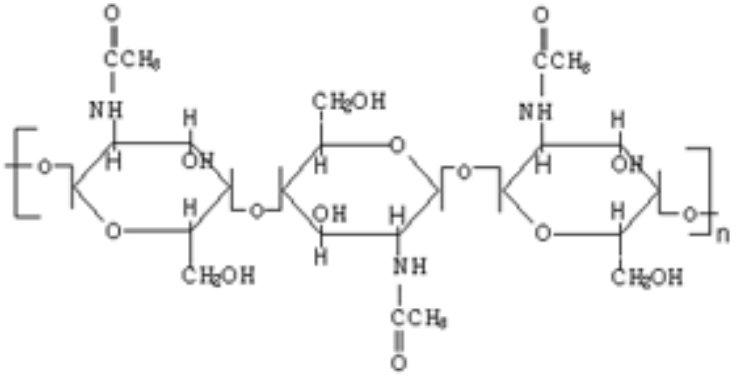

Figure 2 structure of chitosan

Apples are chosen because of their wide availability and nutritive value. Studies suggest that a single coating of chitosan combined with other substances enrich its capacity in forming a well-layered coating on fruits and vegetables and in addition, the respiration rate of fruits and vegetables can be reduced by adjusting the permeability of carbon-di-oxide and oxygen.[4][7] Hence, to make it more economical and effective, a single layered coating was done on the fruits and checked for their weight loss, morphological characteristics, pH , Phenolic content and microbial turbidity.

Considering the biological and environmental risks of the use of silver nanoparticles in comestible goods, an alternative eco-friendly approach has been implemented by the use of silver nanoparticles with chitosan thereby evading any detrimental effects and ensuring increased efficiency of the edible coating as Silver Nanoparticles (AgNPs) have proved to have potential applications such as catalysts, photonic devices, biosensor and antimicrobial activity.[2][10]

Hence, the present study included the green synthesis of silver nanocomposites and its characterization through UV – VIS Spectrophotometer , FTIR & SEM. The synthesized silver nanoparticles along with chitosan were used as edible coatings to access the shelf life period of tomato and apples and and the results indicated that the chitosan silver nanoparticles were effective than the other two groups.

II. Materials And Methods

Coating materials

The materials used to prepare for edible coating solutions were: chitin (HiMedia Mumbai), purifiedchitinase enzyme (CBNR), Glucose as emulsifying agent and Acetic Acid as preservative. In addition to this $AgNO_3$solution was required for the synthesis of Chitosan silver Nano particles.

Preparation of specimens for coating

The materials comprised in this study are non-coated Tomatoes (*Lycopersicum esculentum*) and Apples (*Malus pumila*) purchased from the local market. They were placed on separate sterilized petri plates. Both were selected for their uniformity, size,colour and absence of damage and fungal contamination. Before using,they were isolated in room temperatures and their dry weightswere measured.[2]

Source of Chitin

The chitin used in this study is obtained from the Centre of Bioscience and Nanoscience Research Institute where crude powdered chitin is obtained from Shrimps in coastal areas and preserved for practical purposes.

Synthesis of Chitin composites

The coating solution Glucose /Chitosan medium were prepared in three concentrations as 0.25%, 0.5% and 0.75% initially. 0.25g, 0.5g and 0.75g of Chitin was measured was taken in three vials respectively and named accordingly. Then, it was then dissolved in 4ml of 0.5% Acetic acid and mixed well with a magnetic stirrer for 10 minutes at room temperature (27^{o}C). After homogenizing, 6ml of 1% Glucose was added. Glucose is used as an emulsifying agent and the ratio of Acetic acid:Glucose added in the vials was 2:3. The medium prepared was then sterilized at 121^{o}C for 15 minutes. The three concentrations were considered as low, medium and high concentrations and comparison of their potency to preserve the Apple and tomato samples was carried out.. The second group underwent coating of the Glucose/Chitosan medium added Chitinase enzyme. For obtaining purified Chitinase enzyme, equal volume of Pre cooled acetone was added to 0.75 ml of Chitinase in threeeppendrof tube separately.The tubes were centrifugeed at 10,000rpm for 10 minutes to get white precipitate. The supernatants which was the purified enzyme were transferred to sterilized Eppendorf tubes from which 1 ml was taken and added to the vials containing three concentrations of Glucose/Chitosan medium respectively. The medium was then sterilized at 121^{o}C for 15 minutes in autoclave.

Synthesis of Chitin silver Nanocomposites

A third group of threeApple and Tomato samples each were displayed for coating with Chitosan silver Nanocomposite solution. The Glucose/Chitosan medium was prepared as described as above for varying concentration (0.25%, 0.5% and 0.75%) and added a constant volume of silver nitrate solution constant (5ml of 1mM $AgNO_3$).The medium is sterilized at 121^{o}C for 15 minutes in autoclave.The mixture was then gradually cooled to room temperature. Carbon Paper was bound to the vials and they were incubated in a dark room for 24 hours to facilitate the synthesis of Chitosan silver Nanocomposites.The apple and tomato samples were then swabbed with the above prepared medium and given four coatings.

III. Characteristics Of Glucose/Chitosan AgNPs [13]

UV-VIS studies

The silver Nanoparticles stabilized in Chitosan solution was analysed by UV-vis absorbance spectroscopy. UV-vis spectroscopic measurements were made at room temperature using a SL-159 UV-vis spectrophotometer. UV-vis spectra was recorded in the range of 200-600nm.

Fourier Transform Infrared (FTIR)

Fourier Transform Infrared spectra were recorded using an FTIR spectrophotometer. (SL-159 FTIR).The scan was performed in the range of .

Scanning Electron Microscopy (SEM)

The scanning electron microscopy was performed utilizing the instrument to study the morphology of the prepared Chitosan silver Nanoparticles.

Edible Coating[3]

The Apples and Tomatoes weredisplayed separately in three groups of three samples each. The first group of tomatoes and Apples underwent 4 coatings of the Glucose/Chitosan Medium by swabbing the medium on the samples under sterilized environmental conditions. The controls were taken for each group and isolated separately at room temperature (25^{o}C). The second group of tomatoes and Apples were displayed separately and coated with Glucose/Chitosan added chitinase enzyme by similar procedure as above. The third group of Tomatoes and Apples were coated with Chitosan silver Nanoparticles by swabbing the prepared Glucose/Chitosan medium added AgNO3 solution 4-5 times.

Microbiological growth (Turbidity Method)

The invitro Antibacterial property of Chitosan edible coating on the sample fruits was evaluated using the Turbidity method. 40ml of Nutrient broth medium was prepared by dissolving 0.52g Nutrient broth in 40 ml distilled water for each group of samples separately. The medium was then sterilized in autoclave at 121^{o}C for 15 minutes. The medium was left to cool and then distributed in equal volumes in 7 sterilized test tubes for three groups respectively. The tomato and Apple samples were swabbed gently using cotton swabs over their surfaces and then dipped in the test tubes containing the Nutrient broth medium. The test tubes were incubated overnight and the turbidity was compared visually with the control which contains only the nutrient broth medium. The Turbidity test is carried out primarily for all the samples without Chitin composite coatings. Later after coating the samples of all three groups with the respective mediums, the Turbidity test was carried out after 10 days incubation. The results were compared with the initial noncoated turbidity visualization.

Determination of Total Phenols

Phenol reacts with phosphomolybdic acid in folin ciocalteau reagent in alkaline medium and produce blue coloured complex. Total phenolic content of the samples was determined by folin's-ciocalteau reagent method. 0.5 ml of extract sample was mixed with 0.5 ml of folins phenol reagent and 0.5ml of sodium carbonate. It was allow to incubate at 45^{o}C. After incubation, the absorbance of reaction mixture was measured at765nm.

Determination of pH content[1]

The samples of the three groups were separately checked for their pH. The tomatoes and Apples were smashed using mortar and pestle and the juice was filtered using filter paper. The extract was homogenized by continuous stirring and the pH was then measured using pH meter (ELICO.LI 617)

IV. Results And Discussion

In the present study , Chitin/Chitosan AgNO3 were synthesized ,characterised and used as edible fruit coating. The synthesized Nano composites were characterized under UV-vis spectroscopy, Fourier-Tranform Infra Red spectroscopy and Scanning Electron Microscopy.

UV-vis spectra

The UV-vis spectra of the chitin Nano composite reactionsolution were recorded (Fig 3). 1-2 ml of the solution was pipetted and taken in a cuvette and scanned on a UV-visible spectrophotometer. The absorbance band (plasmon peak) was observed ataround 442nm indicating the presence of synthesized chitin silver nanoparticles. This is in accordance with the results of Honary *et al* in which the surface plasmon peak was obtained in the range of 400-420nm indicating formation of silver nanocomposites with chitosan.[9]

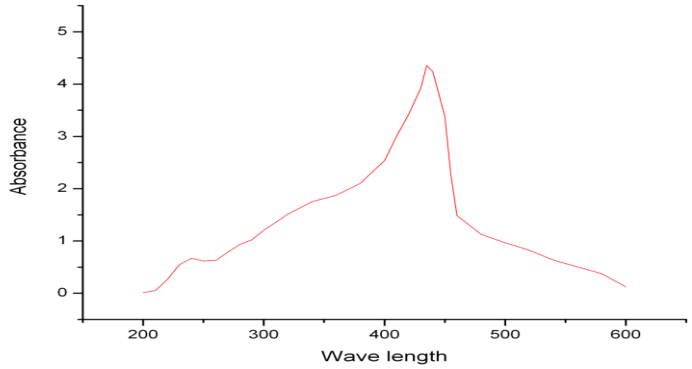

Figure 3 UV-vis spectra of chitin Nano composite in the reaction volume of 2ml.

Fourier Transform Infrared (FTIR)

The Figure 4 illustrates the FTIR spectra of chitosan-silver nanoparticles .The merged bands for OH and NH_2 bands are explicitly seen as the broad absorbtion peak at 3298.61 cm^{-1}. The C-N bending is found at 2824.36 cm^{-1} . The absorbtion band near 1643cm^{-1} indicates the presence of the $CONH_2$.The shifts in the peaks when compared with the FTIR spectra of only Chitosan confirmed the synthesis of nanoparticles. These FTIR results are found to line with the finding of [13]. Further, SEM analysis is done in order to measure the size of the particle (Fig.4).

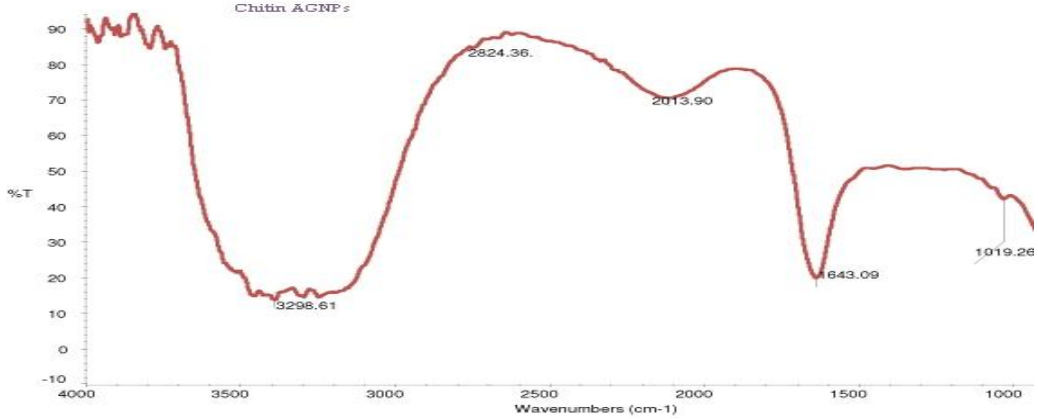

Figure 4 FTIR study of Chitin $AgNO_3$ Nanocomposites

Scanning Electron Microscopy

SEM determination of the freeze dried sample showed formation of AgNPs (Fig. 5). The morphology of the nanoparticles was uniform and spherical. The particles are nanosized and well dispersed with the size range of 100nm.

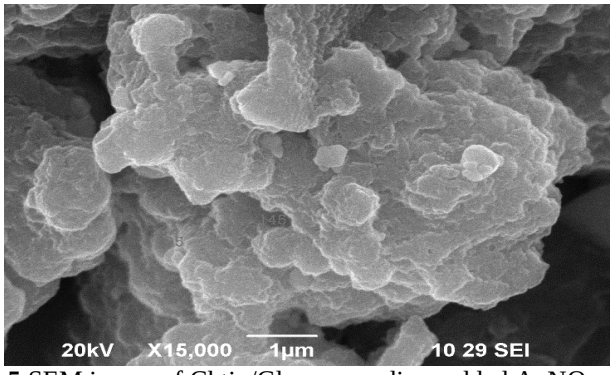

Figure 5 SEM image of Chtin/Glucose medium added $AgNO_3$ solution

Determination of weight loss

The weights of the samples were measured for the periods of 3 days to 12 days of incubation at room temperature (without refrigeration). The results(Figure 9 and 10) indicated that the weight loss is minimum in the coated Apples and tomatoes of all the three groups when the controls showed drastic reduction in weight. The controls exhibit changes in morphology as well as fungal and bacterial contamination while the coated samples exhibited no change in morphology and absence of contamination due to the antibacterial property of Chitosan. This confirms with the findings of [5].

Figure 6(i) Edible coating usingGlucose/Chtin medium of three concentrations (0.25%, 0.5% and 0.75%) on Apples.

Figure 6(ii) Edible coating using Glucose/Chitin coating of three concentrations (0.25%, 0.5% and 0.75%) on Tomatoes.

Figure 7(i) Edible coating usingGlucose/Chtin medium with added chitinase enzyme of three concentrations (0.25%, 0.5% and 0.75%) on Apples.

Figure 7(ii) Edible coating using Glucose/Chitin coating with added added Chitinase Enzyme of three concentrations (0.25%, 0.5% and and 0.75%) on Tomatoes.

Figure 8(i) Edible coating of Chtin AgNPs on Apples with three concentrations(0.25%,0.5% and 0.75%)

Figure 8(ii) Edible coating of Chtin AgNPs on Tomatoes with three concentrations(0.25%,0.5% and 0.75%)

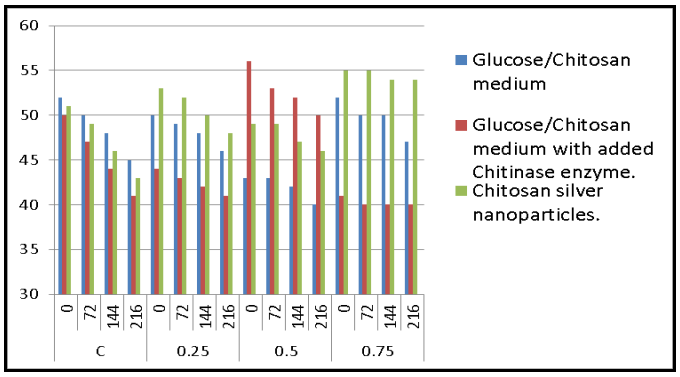

Figure 9 Weight Loss determination for three groups of tomatoes

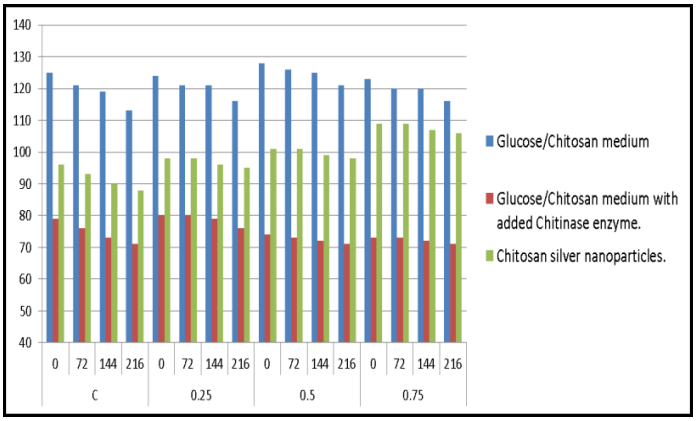

Figure 10 Weight Loss determination for three groups ofApples

Total Phenolic Content

The phenol content in the samples was determined using the absorbance measurements by SL-159 UV-vis Spectrophotometer at 765nm. The results indicated that the coated samples of all three groups showed minimum reduction in the phenolic content than the control after 12 incubation days.

Table 1 Phenolic content of all three groups of coated samples.

CONCENTRATION (%)	TOMATO			APPLE		
	Glucose/ Chitin (mg)	Glucose/ Chitinase (mg)	Glucose/ Nanoparticles (mg)	Glucose/ Chitin (mg)	Glucose/ Chitinase (mg)	Glucose/ Nanoparticles (mg)
0.25	1.78	1.76	1.81	0.169	0.166	0.169
0.50	1.77	1.77	1.76	0.167	0.165	0.171
0.75	1.72	1.76	1.75	0.166	0.167	0.168

Determination of pH

pH was determined using a pH meter (ELICO L1 617) for all the three groups of the coated samples. The negligible reduction in the pH shown in the results proved that the edible coating are effective in their function.

Table 2 pH measurements of three groups of coated samples.

CONCENTRATION	TOMATO			APPLE		
	Glucose/ chitosan	Glucose/ chitinase	Glucose/ nanoparticles	Glucose/ chitosan	Glucose/ chitinase	Glucose/ nanoparticles
0.25	4.3	3.9	4.0	3.4	3.3	3.5
0.50	4.5	4.1	3.8	3.9	3.7	3.8
0.75	4.2	4.2	4.1	3.8	3.7	3.5

Microbiological growth (Turbidity test)

The initial growth of the bacterial population was high in the three groups of both the fruits, but after applying the chitin coating and the Chitin composites, bacterial populations were reduced significantly (Fig. 11 and Fig 12). Our results are on line with the findings of [6] who found that chitosan based coating reduced microorganisms level in fish coated with chitosan.

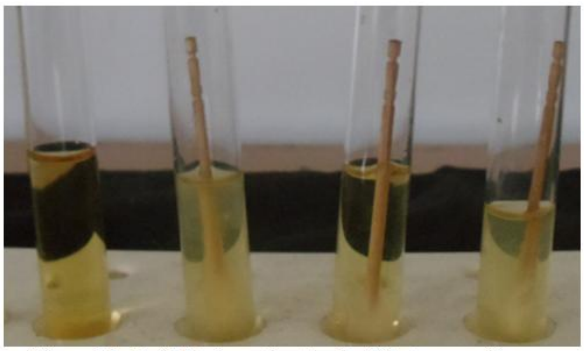

Figure 11a Turbidity in nutrient broth of first group of coated Tomatoes.

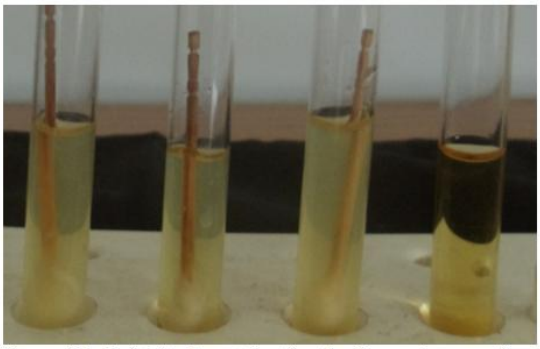

Figure 11b Turbidity in nutrient broth of second group of coated tomoatoes

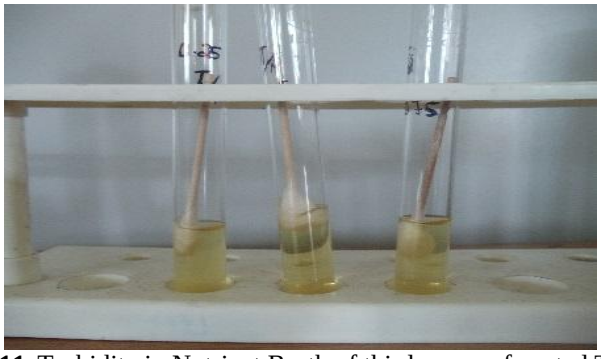

Figure 11c Turbidity in Nutrient Broth of third group of coated Tomato.

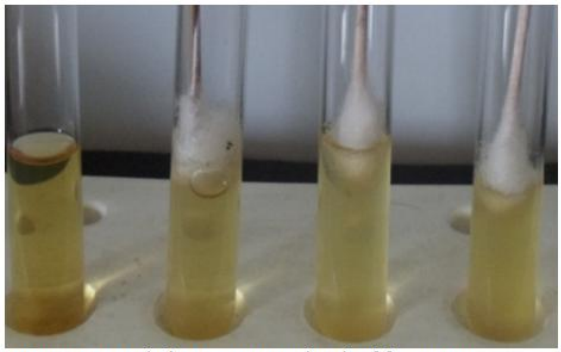

Figure 12a Turbidity in nutrient broth of first group of coated Apples.

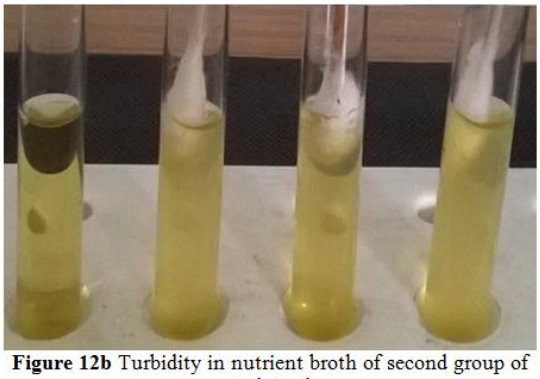

Figure 12b Turbidity in nutrient broth of second group of coated Apples.

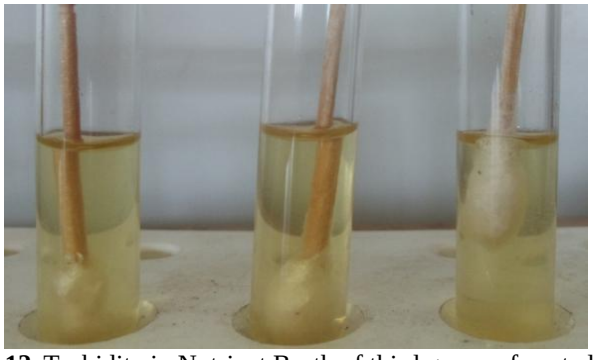

Figure 12c Turbidity in Nutrient Broth of third group of coated apples

V. Conclusion

The chitosan was used as edible fruit coating in the present investigation. The synthesised chitosan silver nanoparticles were characterised using UV- Vis, FTIR and SEM study. The coated fruits exhibited more shelf life period when compared with the control . We concluded that the chitosan based edible fruits coating is an eco friendly approach and showed the less microbial growth in room temperature .

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