



## **Effect of Chitosan Coating on the Post Harvest Quality of Banana during Storage**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author GV designed the study and wrote the protocol. Author ASR managed the literature searches and author LD managed the analyses of the study. Author SMZ performed the laboratory experiments. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Chitosan-based coating was concerned in recent years owing to its non-toxic, biodegradable, and biocompatible. The objective of the study was to evaluate the effect of Chitosan coatings that were applied by double immersion of fruits in the film-forming solutions for 5 min, depending on treatments: (i) Chitosan at 1.5% (w/v) in lactic acid 1% (v/v); (ii) Chitosan at 1.5% (w/v) in lactic acid 1% (v/v) and Tween 80 at 0.1% (w/v); and (iii) Chitosan at 1.5%(w/v) in acetic acid 1% (v/v). (iv) uncoated. The effectiveness of the treatments in extending fruit shelf-life was evaluated by determining ripening stages, weight loss, firmness, pH, total sugars, reducing sugars, non-reducing sugars.

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The results have proved that the addition of Lactic acid at 1% (w/v based on chitosan) and Tween 80 at 0.1% (v/v) in chitosan solution improved coating properties delaying the ripening stage with lowest weight loss (4.5%), lowest firmness (38%), with no change in pH (4.8-4.9), high total sugars (95%), high reducing sugars (46%) and low non-reducing sugars (45%) in banana.

**Keywords:** *Banana; chitosan; food; fruits; lactic acid; shelf life; vegetable.*

## 1. INTRODUCTION

The physical and chemical characteristics of Chitin and Chitosan influence their functional properties such as solubility, chemical reactivity and biological activities [1] like biodegradability [2,3], which differs depending on the crustacean species and preparation methods [4]. Chitosan, is a natural, non-toxic, biodegradable, high molecular weight polycationic polymer. It has been described as "nature's most versatile biomaterial". Chitosan is composed primarily of glucosamine, or 2- amino-2-deoxy-D-glucose linked together by  $\beta$  (1-4) glycosidic bonds [5].

Chitosan is not native to animal sources and is normally obtained by the deacetylation of chitin (extracted from exoskeleton of prawns) using sodium hydroxide. Most Chitosan is manufactured from shellfish because a large amount of shellfish exoskeleton is available as a by-product of food processing. Plant sources of chitin include algae, commonly known as marine diatoms, protozoa and the cell wall of several fungal species [6].

Edible coatings are traditionally used to improve food conservation and appearance due to their environmentally friendly nature. They are obtained from both animal and vegetable or plant agricultural products. The type and concentration of edible components have important effects on the quality characteristics of coated fruits such as weight loss, pH, firmness, colour, reducing sugars, total sugars and Non reducing sugars.

The Allahabad Safeda guava when performed Post-harvest treatment, the fruits applied with 1% chitosan have delayed the ripening process and prolonged the storage life up to 7 days at ambient conditions (28-32 oC and 32 - 41% RH) [7]. Chitosan coating could prolong fresh-cut Fa-lun mangoes during storage at 6°C for 7 days. Chitosan could reduce weight loss, maintain total soluble solids and retard the growth of microorganisms in fresh-cut Fa-lun mangoes [8] The application of chitosan coating (with optimum concentration 20 g/kg) could be beneficial and considered for commercial

application in extending the shelf-life and maintaining quality and to some extent controlling decay of mushroom. In using Chitosan for decay control we consider that it may be suitable in the treatment of mushroom stored for shorter periods (e.g. 3 days) or for short-distance transport and distribution. However, for longer storage and marketing, Chitosan coating to control [9].

Application of edible Chitosan coatings can increase the shelf-life time of matured melons. The effect was monitored by several physical and chemical techniques. It could be explained in the sense of decreasing of respiration rate and stabilization of the cell wall. Further strengthening of the structure could be achieved by addition of calcium ions that interact with cell pectin to create water insoluble ca-pectate network [10]. The major factors responsible for extending the shelf life of fruits and vegetables include: careful harvesting so as not to injure the product, harvesting at optimal horticultural maturity for intended use, and good sanitation [11].

An extracellular chitinase (ChiA-65) was produced and purified from a newly isolated *Bacillus licheniformis* LHH100. Pure protein was obtained after heat treatment and ammonium sulphate precipitation followed by Sephacryl S-200 chromatography. Based on matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) analysis, the purified enzyme is a monomer with a molecular mass of 65,195.13 Da. The sequence of the 27 N-terminal residues of the mature ChiA-65 showed high homology with family-18 chitinases. Optimal activity was achieved at pH 4 and 75 °C. Among the inhibitors and metals tested, p-chloromercuribenzoic acid, N-ethylmaleimide, Hg<sup>2+</sup>, and Hg<sup>+</sup> completely inhibited enzyme activity. Chitinase activity was high on colloidal chitin, glycol chitin, glycol Chitosane, chitotriose, and chito-oligosaccharide [12].

In the food processing industry shrimp shells (*Parapenaeus longorostris*) have great commercial value because they are rich in chitin

(24 wt%), protein (40 wt%), lipids, pigments and flavor compounds. In the present study protein recovery by ultrafiltration was examined during isolation of chitin from shrimp shell *P. longirostris*. Up to 96 wt% of the proteins could be removed by deproteinization from the shrimp shells by incubating them in NaOH (2 N) over 2 h, at T = 45°C and with solid to solvent ratio of 1:2 (w/v). A solute rejection coefficient (R<sub>0</sub>) of 97% was obtained to recover proteins from deproteinized shell waste water by ultrafiltration process. The protein concentration process was carried out at beyond the critical flux of 380 L/h.m<sup>2</sup>, at a trans-membrane pressure of 3 bars and tangential velocity of 5 m/s, was found to reduce the hydrolysate volume by a factor of 2.4. Due to a reduction in organic matter in the effluent, the chemical oxygen demand (COD) of the permeate was reduced by 87% [13].

Evaluation of modified Algerian clay as mineral adsorbent was done for its adsorbing capacity on copper (Cu) and Zinc (Zn) cations. The results obtained show a rapid kinetic adsorption for both metals (less than 2 h) following the pseudo-second order model with high elimination rates of 67.2 and 61.8% for Cu and Zn respectively. The adsorption isotherms analyzed with Langmuir model revealed a correlation with the experimental values. The use of obtained Chitosan as flocculent and coagulant at room temperature accelerates the decantation of the colloidal particles in suspension. This was generated after the adsorption process [14].

The effect of coating tomato fruit (*Lycopersicon esculentum*) with shrimp shell Chitosan, a deacetylated form of chitin, and a Chitosan derivative, i.e. N,O-carboxymethyl Chitosan (NOCC) on postharvest preservation was studied. The effects of various Chitosan and NOCC concentrations on fruit ripening as well as fruit physiochemical characteristics were evaluated during storage at room temperature i.e., at 25–30 °C. Coating the fruit with 2 % (w/v) Chitosan or NOCC solutions was found to be more effective in extended its storage life than coating with 0.5 % (w/v) solutions. The tomatoes which are covered were firmer, higher in titratable acidity and exhibited less red pigmentation than the control uncoated fruits at the end of the storage. The results suggest that the suitability of Chitosan and its derivative NOCC as an alternative agents for preserving the fresh fruits [15].

Edible coatings as affected by Chitosan extraction processes were used to preserve the quality of strawberries (*Fragaria × ananassa*) during their storage at ambient temperature (20–25 °C). Three different Chitosans were prepared from shrimp shell and were designated as the following, C1 by classical method, C2 without the decoloration step and C3 without the decoloration and deproteinization steps. To study the effectiveness of coatings, changes in physical and chemical parameters along with mold spoilage were studied. Chitosan coatings had no significant effects on titratable acidity, pH and soluble solids content (SSC) of strawberries throughout the storage. The SSC content of control fruits increased with the storage time. In contrast it was observed that Chitosan coatings delayed changes in weight loss and the appearance of fungal infection in strawberries. Coated fruits had greater visual acceptability than had the untreated fruits. By visual analysis, it was possible to verify that the best quality was maintained until the day 12, for the strawberries coated with C3 (1 %) as reported by [16].

In the present work Chitosan coating of the banana were performed by using three different solutions i.e., Lactic acid, Acetic and Tween 80. The banana were kept under observation for 10 days by testing physical and chemical parameters every day and graphs were plotted to analyze the delay of the fruit ripening. The results show that Chitosan coating with Lactic Acid and Tween 80 is better to use for fruit storage and to delay the ripening stages.

## 2. MATERIALS AND METHODS

### 2.1 Materials and Chemicals Used

Banana, All the chemicals were obtained from Hi Media. Sodium Hydroxide, Lactic acid, Tween 80, Acetic acid, Alcohol, Phenol, Sulphuric acid, dinitrosalicylic acid, pH Meter (Systronics), Penetrometer (Aimil Universal) Chitin & Chitosan was prepared in our lab.

The replication number for the experiments was in duplets and the average values were taken for all the parameters.

### 2.2 Preparation of Chitosan

The process was carried out by adding 50% sodium hydroxide to the obtained chitin sample on a hot plate and boiling it for 2 hrs at 100°C.

The sample was then allowed to cool at room temperature for 30 minutes. Then they were washed continuously with 50% sodium hydroxide. The sample obtained is filtered and oven-dried for 6 hrs at 110°C to obtain Chitosan [17].

## 2.3 Bananas

Long life banana fruits of nearly 80 number were purchased from a local market. Fruits were selected, based on uniformity of size, ripening stage, absence of physical damage and fungal infection.

## 2.4 Edible Coating Formulations

A Chitosan aqueous solution (1.5%, w/v) was prepared dissolving Chitosan powder in a solution of lactic acid (1%, v/v) and acetic acid (1% v/v) at 40°C, since Chitosan is only soluble in an acidic medium. Then, Tween 80 at 0.1% (v/v) was added for improving wettability for 24 h. After wards, Chitosan solution was added into pretreated Lactic acid solutions The resulting mixture was stirred vigorously with heating using a magnetic stirrer during 60 min until Chitosan was dissolved. After the Chitosan was dissolved, the solutions were filtered to remove foam and any undissolved impurity.

## 2.5 Coating Applications

80 Bananas were randomly distributed into four groups. Three groups were assigned to one of three treatments whilst the fourth group provided the untreated control. Coatings were applied by double immersion of fruits in the film-forming solutions for 5 min, depending on treatments: (i) Chitosan at 1.5% (w/v) in lactic acid 1% (v/v); (ii) Chitosan at 1.5% (w/v) in lactic acid 1% (v/v) and Tween 80 at 0.1% (w/v); and (iii) Chitosan at 1.5% (w/v) in acetic acid Fruits were allowed to dry by natural air for 1 h at 25°C and were subsequently stored for future use.

## 2.6 Physical Parameters-quality Attributes

### 2.6.1 Classification according to ripening stages

The bananas were classified according to their ripening stage using a visual scale proposed by [18]. These changes during ripening period (loss of greenness and increase in yellowness) may

occur as breakdown of the chlorophyll in the peel tissue. Maximum polyphenol oxidase activity was observed at maturity stage four days which was gradually decreased as ripening progressed. The bananas were expressed as the predominant ripening stage in each treatment. The bananas were observation of ripening stage different treatment for every day.

## 2.7 Weight Loss

The selected 80 bananas, corresponding to each treatment, were weighed at the beginning, just after coating and air-drying, and thereafter each sampling days during the storage. Weight loss was expressed as the percentage loss of the initial total weight and of every day weight.

$$\% \text{Weight loss} = \left( \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \right) \times 100$$

## 2.8 Firmness and Texture Analysis

The firmness of a banana is linked to the state of maturity and ripeness and may be influenced by the variety as well as the region of production and the growing conditions. The Penetrometer instrument used consists of a cone, set in position so that the rake of the dial touches the upper end of the stick. The dial gauge is set on "0" position by a small knob. Now arm with dial and cone set is lowering till the tip of cone touch the surface of banana the botton of arm is pressed for 5 seconds and the cone can down in to the banana. Four replicates in individual banana were done for each treatment. Each banana was measured in the central point and both sides' points. Firmness was measured as the maximum penetration distance reached during penetration time.

## 2.9 Chemical Parameters

**pH:** After firmness analysis, banana were cut into small pieces and homogenized in a grinder, and 10 g of ground banana was suspended in 100 mL of distilled water and then filtered. The pH samples were assessed using a pH meter (SYSTRONICS).

**Titrateable Acidity (TA):** After firmness analysis, banana were cut into small pieces and homogenized in a grinder, and 10 g of ground Banana was suspended in 100 mL of distilled water and then filtered. The titrable acidity of the samples was titrated using 0.1 N NaOH. Titrable acidity was expressed as grams of citric acid per 100 g of banana weight.

## 2.10 Extraction and Determination of Total Sugar and Reducing and Non Reducing Sugar from Banana Pulp

Extraction of sugar from banana pulp was done by using [19] method. Four banana pulps were cut into small pieces and immediately plunged into boiling ethyl alcohol and were allowed to boil for 5 to 10 minutes (10 to 20 ml of alcohol was used per gm of pulp). The extract was filtered through the two layers of cheese cloth and the ground tissue was re-extracted for 3 minutes in hot 80% alcohol, using 2 to 3 ml of alcohol per gm of tissue. The second extraction was ensured complete removal of alcohol suitable substances. The extract was cooled and passed through the two layers of cheese cloth. Both extracts were filtered through Whatman No.41 filter paper.

The volume of the extract was evaporated to about 25% of the volume over steam bath and cooled. This reduced volume of extract was transferred to a 100 ml volumetric flask and it was made up to the mark with distilled water. Total sugar content of banana pulp was determined by phenol sulphuric acid method. Reducing sugar content of banana pulp was determined by dinitrosalicylic acid method [20] and non reducing sugar was calculated by subtracting reducing sugar from the total sugar.

## 3. RESULTS AND DISCUSSION

Classification according to ripening stages:

The Figs. 1 - 8 show the changes in ripening stages of uncoated (Control) and coated banana.



(A) Uncoated banana (Control)



(B) Coated banana with Solution (i) (ii) (iii)

Fig. 1A. Effect of Chitosan coating on ripening stages of (control) Banana during storage (Represent on day 1)

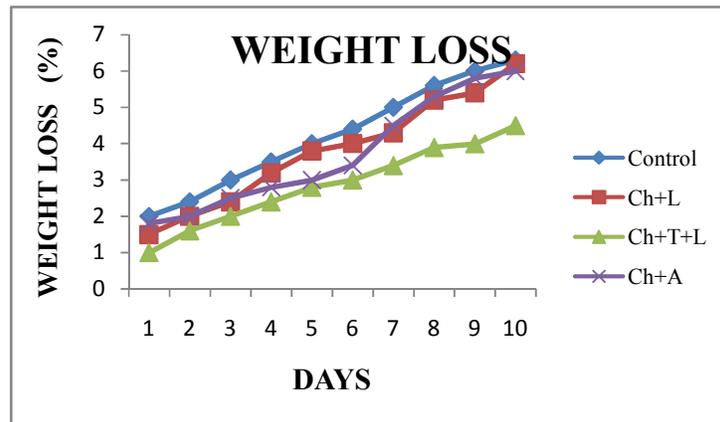


(A) UnCoated banana (control)

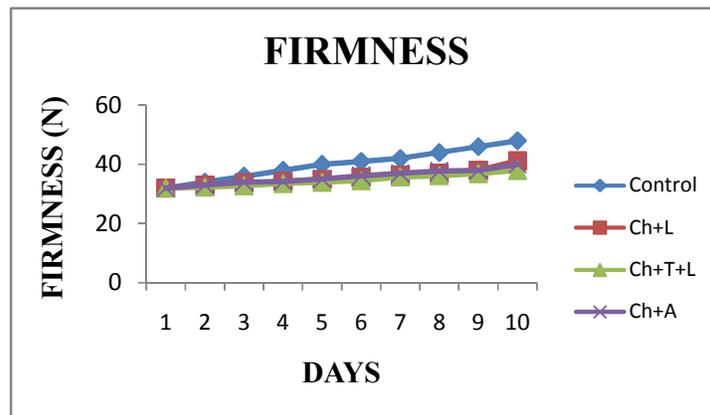


(B) Coated banana with solution (i) (ii) (iii)

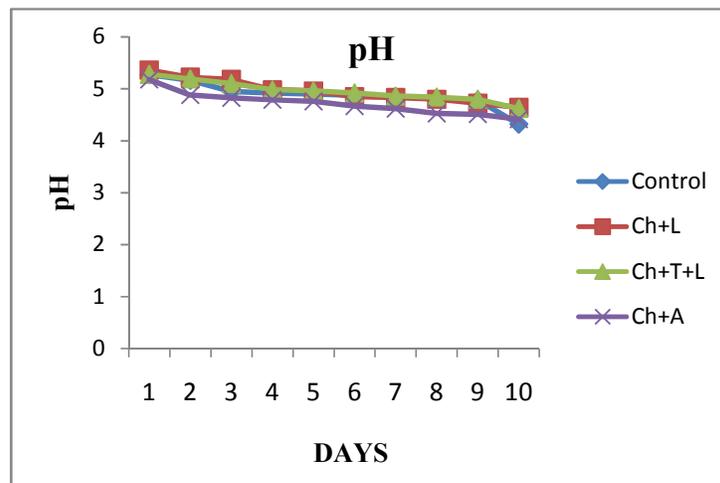
Fig. 1B. Effect of Chitosan coating on ripening stages of banana during storage (Represent after day 10)



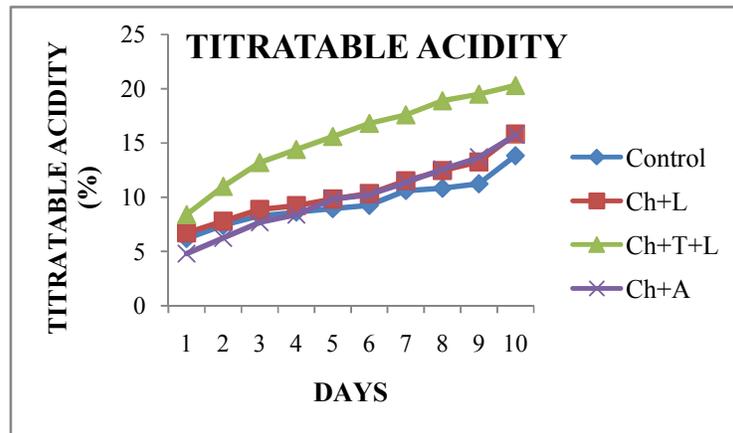
**Fig. 2. Effect of Chitosan coating on weight loss of banana during storage**  
 (Ch+L = Chitosan +Lactic Acid, Ch+T+L = Chitosan+Tween 80+Lactic Acid, Ch+A = Chitosan+Acetic Acid)



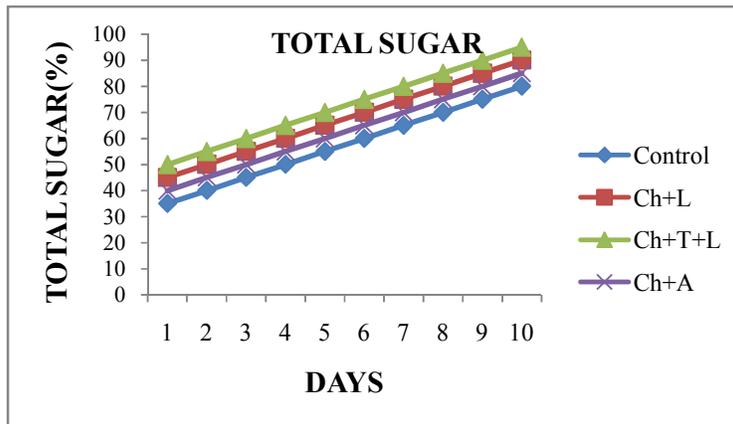
**Fig. 3. Effect of Chitosan coating on firmness of banana during storage**  
 (Ch+L = Chitosan +Lactic Acid, Ch+T+L = Chitosan+Tween 80+Lactic Acid, Ch+A = Chitosan+Acetic Acid)



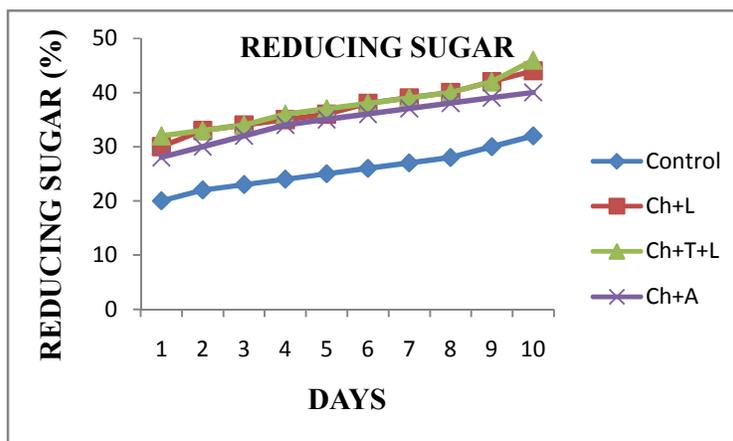
**Fig. 4. Effect of Chitosan coating on pH of banana during storage**  
 (Ch+L = Chitosan +Lactic Acid, Ch+T+L = Chitosan+Tween 80+Lactic Acid, Ch+A = Chitosan+Acetic Acid)



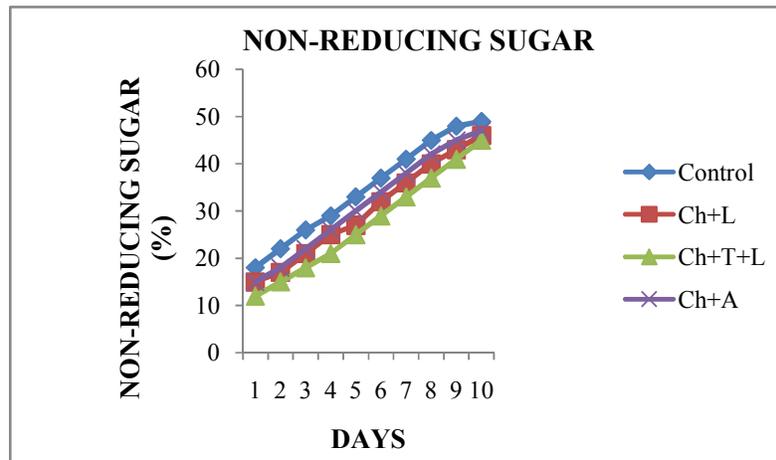
**Fig. 5. Effect of Chitosan coating on Titratable Acidity of banana during storage**  
(Ch+L = Chitosan +Lactic Acid, Ch+T+L = Chitosan+Tween 80+Lactic Acid, Ch+A = Chitosan+Acetic Acid)



**Fig. 6. Effect of Chitosan coating on Total Sugars of banana during storage**  
(Ch+L = Chitosan +Lactic Acid, Ch+T+L = Chitosan+Tween 80+Lactic Acid, Ch+A = Chitosan+Acetic Acid)



**Fig. 7. Effect of Chitosan coating on Reducing Sugar of banana during storage**  
(Ch+L = Chitosan +Lactic Acid, Ch+T+L = Chitosan+Tween 80+Lactic Acid, Ch+A = Chitosan+Acetic Acid)



**Fig. 8. Effect of Chitosan coating on Non-Reducing Sugar of banana during storage**  
(Ch+L = Chitosan +Lactic Acid, Ch+T+L = Chitosan+Tween 80+Lactic Acid, Ch+A = Chitosan+Acetic Acid)

The highest weight loss was observed in control fruit (6.3%). The lowest weight loss was observed in coated fruits with the Chitosan, Tween 80 and Lactic acid (4.5%). There are no particular changes in coated fruits with Chitosan and Lactic acid (6.2%), Chitosan and Acetic acid (6%) coatings. Chitosan was found to be more effective at delaying weight loss (Fig. 2).

The highest firmness was observed in control fruit (48%). The lowest firmness was observed in coated fruits with the Chitosan, Tween 80 and Lactic acid (38%). There is no particular difference in coated fruits with Chitosan and Lactic acid (41.2%), Chitosan and Acetic acid (40%). Chitosan was found to be more effective at delaying firmness (Fig. 3).

The lowest pH was observed in uncoated fruit (4.84). There is no particular difference in coated fruits with Chitosan, Lactic acid and Tween 80 (4.92) Chitosan and Lactic acid (4.87), Chitosan and Acetic acid (4.89). Chitosan was found to be more effective at delaying pH concentration (Fig. 4).

The highest titratable acidity was observed in coated fruit with Chitosan, Tween 80 and lactic acid (20.3%). There are no particular changes in coated fruits with Chitosan and Lactic acid (15.83%), Chitosan and Acetic acid (15.75%). The lowest titratable acidity was observed in uncoated fruit (13.80%). At the beginning of the ripening process the sugar/acid ratio is low because of low sugar content and high fruit acid content. When the ripening process started the

sugar/acid ratio is high because of high sugar content and low fruit acid content (Fig. 5).

The total sugar content was gradually increased and reached the pick point at day of storage in both coated and uncoated fruits. The highest total sugar content was observed in coated fruit with Chitosan and Tween 80 and lactic acid (95%). There is no difference in coated fruits with Chitosan and Lactic acid (90%), Chitosan and Acetic acid (85%). Chitosan was found to be more effective at delaying of total sugar contents. The lowest total sugar content was observed in uncoated fruit (80%) (Fig. 6).

The effect of Chitosan coated on banana was observed in reducing sugar content gradually which increased among the fruits of uncoated and coated during storage. The increase in reducing sugar with the progress of ripening as well as storage time was due to the degradation of starches to glucose and fructose by the activities of amylase and maltase. The highest reducing sugar content was observed in coated fruit of Chitosan and Tween 80 and lactic acid (46%). There is no difference in coated fruits with Chitosan and Lactic acid (43%), Chitosan and Acetic acid (40%). Chitosan was found to be more effective at delaying reducing sugar contents. The lowest reducing sugar content was observed in uncoated fruit (control) (32%) (Fig. 7).

The effect of Chitosan coated on banana were observed in non-reducing sugar which gradually increased in the fruits of uncoated and coated during of storage. The maximum non-reducing

sugar contents were found in uncoated fruit (49%). The lowest non-reducing sugar content was observed in Chitosan and Tween 80 and lactic acid (45%). There is no difference in coated fruits with Chitosan and Lactic acid (46%), Chitosan and Acetic acid (47%) (Fig. 8).

#### 4. CONCLUSION

The application of Chitosan coating with different combinations of solutions was performed. The work is carried on important physicochemical parameters. It was observed that the lowest Weight loss and Firmness is low in coated banana. The control shows early ripening stage, pH decreased in uncoated banana. Total sugars levels gradually increased in both coated and uncoated bananas. Reducing sugars levels increased only in coated bananas, whereas Non-reducing sugars levels decreased in coated banana. However the study does not demonstrate the parameters like enzymatic studies, antioxidant activity, Membrane damage, which done for future study. Our results proves that Chitosan solution containing Tween 80 and Lactic acid is a valuable product for the fruit preservation and viable for commercial applications.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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