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Effects of Different Concentrations of Chitosan on Shelf Life and Quality of Banana Fruit

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ABSTRACT
The experiment was aimed to investigate the effect of different concentrations of chitosan on shelf life and quality of banana fruit. The single factor experiments were laid out in a completely randomized design with three replications. Experimental treatments included Control, 0.50%, 0.75%, 1.0%, 1.5%, 2.0% chitosan. Sabri Banana cultivar is one of the most popular commercial cultivars of banana in Bangladesh. Chitosan treatment caused highly significant variation in respect of the physico-chemical parameters such as color changes, firmness, weight loss, disease severity and shelf life of banana during storage. Considering the effects of different concentrations of chitosan, 0.75% and 2.0% chitosan were found to be the best in respect of all the parameters investigated. Fruits treated with the above concentrations of chitosan, had the longest shelf life (9 days), whereas fruits under control had the shortest shelf life (8 days). The application of chitosan treatment in fresh crops is safe for consumers and for the environment. It can be used to improve postharvest quality and to extend shelf life of banana.

Keywords: Banana, chitosan, postharvest, shelf life.

Introduction
Banana (Musa sp.) is the most communal fruit crop in tropical and sub-tropical regions of the world. Banana is the largest among the herbaceous flowering plant (Picq et al., 2000). It belongs to the family Musaceae. The genus Musa contains about 40 species which probably originated in the Assam, Myanmar and Thailand (Khader et al., 1996). Banana constitutes the fourth most important global food commodity after rice, wheat and maize in terms of gross value of production (Arias et al., 2003). It is a popular, delicious, nutritious and affordable priced table fruit having a consumption rate higher than any other fruit in Bangladesh. It contains carbohydrate, crude fiber, protein, fat, ash, phosphorus, calcium, iron, ß-carotene, riboflavin, niacin and ascorbic acid (Mondal and Rouf, 2011). In 2015-16, Bangladesh produces 798012 tonnes of

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bananas from 47412.56 ha of land (BBS, 2016). Maintenance of the freshness of fruits and vegetables is one of the most important aspects of fruits and vegetables production in the tropical regions (Bachmann and Earles, 2000). Losses in fruits and vegetables are of about 40 to 50% in the tropical and subtropical regions (Mejia, 2003). Banana is a highly perishable fruit that is attributed to adverse physiological changes namely loss of weight due to respiration and transpiration, softening of flesh and lack of resistance capacity against microbial attack. Such spoilage causes considerable economic losses to both traders and retailers. In Bangladesh, a considerable amount of banana is spoiled every year due to prevailing high temperature and humidity. Here, lack of appropriate storage facilities and poor postharvest management practices among the farmers make the situation even worse. The enormity of postharvest losses of banana in Bangladesh ranges from 25-40% and it is only 5-25% in developed countries (Kader, 1992). Hassan (2010) reported that the postharvest loss of banana is 24.62% annually. An edible film (e.g. chitosan) is defined as a thin layer of material which can be eaten by the consumer, functions as barriers against transmission of gases, vapors and solutes and provide mechanical protection (Wu et al., 2002). Chitosan could be an absolute preservative coating because of its film forming properties, biochemical properties, inherent antifungal properties and elicitation of phytoalexins (Kittur et al., 1998; Jiang and Li, 2001). Lower doses of chitosan can be used as a natural preservative of fruits substitute to hazardous formalin (Sakif et al., 2016). Chitosan coating minimized weight loss of stored apples, and its combination with heat treatment showed significantly reduced pH value, increased titratable acidity content, and the lowest respiration rate (Shao et al., 2012). 2% (w/w) chitosan with deacetylation of 80% was proved to be the most suitable coating among the others for reducing the weight loss and vitamin C content and desirable sensory analysis (Suseno et al., 2014). Coated pears with chitosan showed a significantly reduced weight loss (Zhou et al., 2008). The combined treatment of chitosan-based edible coating and 1-methylcyclopropene could be used to extend the commercial life of bananas for up to four more days (Baez-Sanudo et al., 2009). Mature green Cavendish banana were coated with 1% chitosan and placed inside the food storage chamber can delay ripening process (Pratiwi et al., 2015). Coating with chitosan and chitosan + gibberellic acid has the potential to control decay percentage, prolong the shelf life, and preserve valuable attributes of banana (Gol and Rao, 2011). The possibility of using 10% (w/v) GA combined with 0.75% (w/v) chitosan as an alternative strategy to control post-harvest anthracnose disease in banana fruit (Maqbool et al., 2010). Chitosan can be used as an antimicrobial film which acts as water-binding agent, inhibiting various enzymes and activates several defense processes in the host tissue (Tharanathan and Kittur, 2003). The controlled release of chitosan from film matrix likes to effectively inhibit bacterial growth if the amount of chitosan in film matrix is sufficient to inhibit the recovery of microbial growth (Park et al., 2002). Therefore, prolongation of banana shelf life is an utmost need to minimize the postharvest losses. Rao and Rao (1979) opined that for reducing the postharvest losses, banana fruits should be harvested at appropriate stage of maturity for the transport, handling and storage envisaged. It is necessary to delay ripening for distant markets and then enhance ripening for retail sale. Extension of shelf life may be done by coating material such as chitosan. The application of chitosan treatment in fresh crops is safe for consumers and the environment. Chitosan has been approved by the United State Food and Drug

Administration (USFDA) as a Generally Retained as Safe (GRAS) food additive (Knorr, 1986). The specific objectives of the present study were to standardize the concentrations of chitosan for shelf life extension of banana.

Materials and methods

Experimental location

The experiment was conducted in the laboratory of the Department of Horticulture, Bangladesh Agricultural University, Mymensingh. It was carried out in the month of July, 2017.

Experimental materials

Green matured banana cv. Sabri of uniform size, shape and color was used. Banana cv. Sabri was collected from a commercial banana orchard of Badarganj Upazila under Rangpur district. Pericarp of Sabri is medium thick and pulp of the ripe fruit is soft with mild to distinct aroma.

Experimental treatments

T_0: Control

T_1: 0.50% Chitosan

T_2: 0.75% Chitosan

T_3: 1.0% Chitosan

T_4: 1.5% Chitosan

T_5: 2.0% Chitosan

Experimental design

For this experiment single factor experiment was laid out in completely randomized design (CRD) with 3 replications. Three bananas were used at each replication. In total, 54 bananas were used for this experiment.

Application of experimental treatments

The selected fruits were randomly assigned to the postharvest treatments. After treatments, the fruits were kept on a brown paper previously placed on the table in the post-graduate laboratory room at normal temperature. The procedures of applying the postharvest treatments to the fruits of each variety were as follows.

(A). Control

Three bananas were randomly selected and kept on the brown paper placed on the table in the laboratory at ambient conditions (28±2 °C and 66 to 74% RH).

(B). Chitosan treatment

Five concentrations of chitosan (0.50, 0.75, 1.0, 1.5 and 2.0% solution) were prepared. Laboratory-prepared chitosan was obtained from Department of Agricultural Chemistry, BAU, Mymensingh.

Fig. 1. Chitosan powder

Preparation of chitosan

Fresh shrimps were collected at first step. Shrimp head and skin were separated from shrimp using sharp knife. The collected shrimp shell wastes were then washed with tap water and crushed with mortar and pestle. Crushed shrimp shell wastes were kept in a polyethylene bags at ambient temperature (28±2 ^{o}C) for 24 hours for partial autolysis to facilitate chemical extraction of chitosan and to improve the quality of chitosan (Toan, 2009). There were mainly 3 steps, namely demineralization, deproteinization and deacetylation were followed for the isolation of chitosan. The procedure of isolating chitosan from fresh shrimp shell is given below (Fig 1, 2).

Shrimp shell waste
↓
Autolysis (28±2 ^{o}C, 24 h)
↓
Demineralization
(4%, 3%, 2% HCl sol^{n}, 28±2 ^{o}C,16 h)
↓
Deproteinization
(4% NaOH sol^{n}, 28±2 ^{o}C, 20 h)
↓
Chitin → Characterization
↓
Deacetylation → Characterization
(30%,40%,50%,60% NaOH sol^{n},65 °C, 20 h)
↓
Chitosan

Fig. 2. Flow chart showing the production of chitin and chitosan from shrimp (modified from Toan, 2009)

Preparation and application of chitosan solution

Using shrimp shell, chitosan of 0.50, 0.75, 1.0, 1.5 and 2.0% concentrations were prepared using 0.6% acetic acid, adding 25% glycerol (w/w chitosan) as plasticizer. Each of the solutions was thoroughly mixed, filtered and the pH was adjusted to 5.6 using 1M sodium hydroxide. Washed and air dried banana fingers were dipped into the solution for five min ensuring that enough quantity of the solution was being absorbed. Uncoated bananas (control samples) were immersed in a 0.6% glacial acetic acid solution at pH 5.6 for the same period of time. The treated and controlled banana samples were dried in ambient conditions (27±2 °C) and 84-87% relative humidity. Then, the treated samples (control and coated bananas) were kept at ambient conditions in the laboratory.

Data collection

Changes in different parameters namely color, firmness, weight loss, disease severity and shelf life were investigated.

Physico-chemical parameters

The methods of studying the afore-mentioned parameters are discussed below.

Color

Days required to reach different stages of color during storage and ripening were determined objectively using numerical rating scale of 1-7, where

1 = 0 – <10% yellow; 2 = 10 – <30% yellow; 3 = 30 – <50% yellow; 4 = 50 – <75% yellow; 5 = 75 – <100% yellow; 6 = 100% yellow; 7 = blackened/rotten. N.B. The remaining percentages are green color for 1-6 numeric scale.

Firmness

Days required to reach different stages of firmness during storage and ripening were determined objectively using numerical rating scale of 1-5, where 1 = hard green, 2 = sprung, 3 = between sprung and eating ripe, 4 = eating ripe, and 5 = over ripe. Similar rating scale was used by Hassan (2006).

Determination of percentage of total weight loss

The banana hands used in this study were weighed using an electric balance and kept for storage. Percentage of total weight loss was calculated daily by using the following formula:

Percentage of total weight loss (WL) = $\frac{IW-FW}{IW} X\ 100$

where,
% WL = Percentage total weight loss
IW= Initial fruit weight and
FW= Final fruit weight

Disease severity

Disease severity represents the percentage of diseased portion of infected fruit. The infected fruits of each replication under treatment were selected to determine percentage of fruit area infected, and was measured based on eye estimation.

Shelf life of banana

Shelf life of banana fruits as influenced by different postharvest treatments was calculated by counting the days required to ripe fully as to retaining optimum marketing and eating qualities.

Statistical analysis

The collected data on various parameters were analyzed statistically using MSTAT statistical package to find out the variation resulting from experimental treatments following F variance test. The significance of difference between the pair of means was compared by LSD test at 1% and 5% level of probability (Gomez and Gomez, 1984).

Results

In the present study, the data were recorded every day on selected physical, chemical and microbial properties and shelf life of banana.

Changes in color

Results revealed that significant variation was observed in respect of color changes of banana during storage and ripening. The changes in color were faster (scores 1.0, 1.33, 1.33, 5.00, 6.00, 6.00, 6.33, and 7.00) in bananas coated with 0.5% chitosan, whereas the changes were slower in those bananas coated with 0.75% (scores 1.00, 1.00, 1.00, 3.33, 6.00, 6.00, 6.33 and 6.33) chitosan and 2.0% (scores 1.00, 1.00, 1.00, 5.00, 6.33, 6.33, 6.33 and 6.33) chitosan during eight days after storage (Fig. 3).

Changes in fruit firmness

Significant variation was observed in respect of firmness of banana during storage. Faster rates of firmness changes (scores 1.00, 1.00, 2.33, 3.67, 4.00, 4.33, 5.67 and 5.67) were found in bananas coated with 1% chitosan. On the contrary, the rates of firmness changes were slower in banana fruits coated with 0.75% (scores 1.00, 1.00, 1.67, 1.67, 2.67, 3.67, 4.00 and 4.67) and 2.0% (Scores 1.00, 1.00, 2.00, 2.33, 3.33, 4.33, 4.67 and 5.00) chitosan during eight days after storage (Table 1).

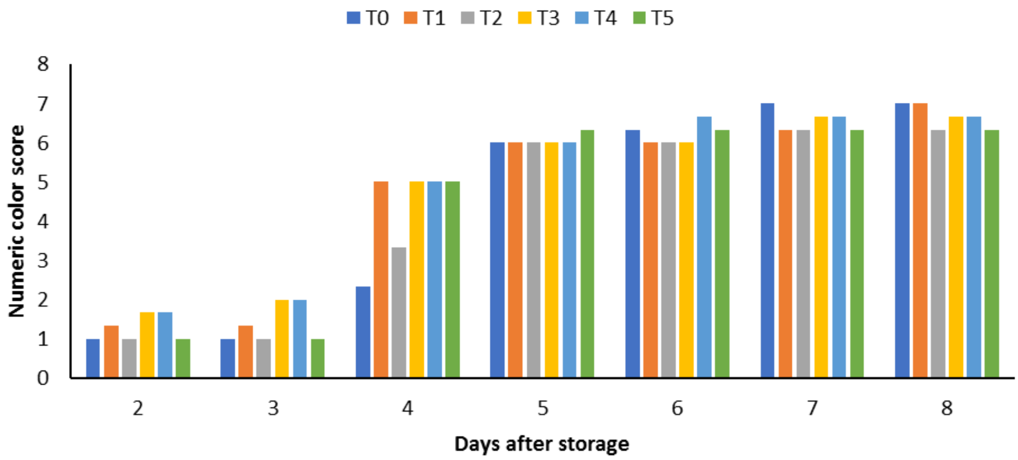

Fig. 3. Effects of chitosan treatments on color change of banana (cv. Sabri) during different days after storage. Color scores: 1 = 0 – <10% yellow; 2 = 10 – <30% yellow; 3 = 30 – <50% yellow; 4 = 50 – <75% yellow; 5 = 75 – <100% yellow; 6 = 100% yellow; 7 = Blackened/rotten. Vertical bar shows standard deviations. T_0: Control, T_1: 0.50% Chitosan, T_2: 0.75% Chitosan, T_3: 1.0% Chitosan, T_4: 1.5% Chitosan, T_5: 2.0% Chitosan

Table 1. Effects of chitosan treatments on firmness of banana (cv. Sabri) during 8 days after storage

Concentration of chitosan (%)	Firmness scores[A] of banana during storage							
	1	2	3	4	5	6	7	8
T_0	1.00	1.00	1.33	1.33	2.33	4.00	5.67	6.00
T_1	1.00	1.00	2.00	2.67	3.67	4.00	5.33	6.00
T_2	1.00	1.00	1.67	1.67	2.67	3.67	4.00	4.67
T_3	1.00	1.00	2.33	3.67	4.00	4.33	5.67	5.67
T_4	1.00	1.00	2.33	3.33	4.00	4.67	5.67	5.67
T_5	1.00	1.00	2.00	2.33	3.33	4.33	4.67	5.00
$LSD_{0.05}$			0.04	0.27	0.22	0.21	0.16	0.24
$LSD_{0.01}$			0.06	0.39	0.31	0.29	0.22	0.33
Level of significance	-	-	0.46**	2.50**	1.47**	0.37**	1.44**	0.90**

[A]Firmness scores: 1 = hard green, 2 = sprung, 3 = between sprung and eating ripe, 4 = eating ripe, and 5 = over ripe, 6 = Blackened / rotten. ** Significant at 1% level of probability. T_0: Control, T_1: 0.50% Chitosan, T_2: 0.75% Chitosan, T_3: 1.0% Chitosan, T_4: 1.5% Chitosan, T_5: 2.0% Chitosan

Total weight loss

Results showed that significant variation in respect of total weight loss was observed in banana during storage. Higher rates of weight loss (2.11, 3.99, 5.86, 7.51, 10.80, 12.94 and 18.14) were found in bananas treated with 1.5% chitosan. By contrast, the rates of weight loss were slower in bananas treated with 0.5% (1.27, 2.96, 5.07, 6.55, 8.67, 10.36 and 14.39) chitosan and 2.0% (1.88, 4.02, 5.19, 7.79, 9.39, 11.34 and 13.94) chitosan during storage (Fig. 4).

Disease severity

Chitosan treatments during the storage of banana had significant effect on the levels disease severity. Higher rates of disease severity (6.67, 20.00, 51.67, 61.67, 98.33 and 100.00) were found in 1.5% chitosan, whereas lower rates were found in 0.75% (0.00, 0.00, 6.67, 10.00, 15.00 and 35.00) and 2.0% (10.00, 20.00, 30.00, 33.33, 41.67 and 53.33) chitosan during storage (Table 2).

Shelf life of banana

Shelf life is the period from harvesting to the last edible stage. The effects of chitosan coating were significant on extension of the shelf life of banana. The longest shelf life (9 days) was observed in 0.75% and 2.0% chitosan (Fig. 5).

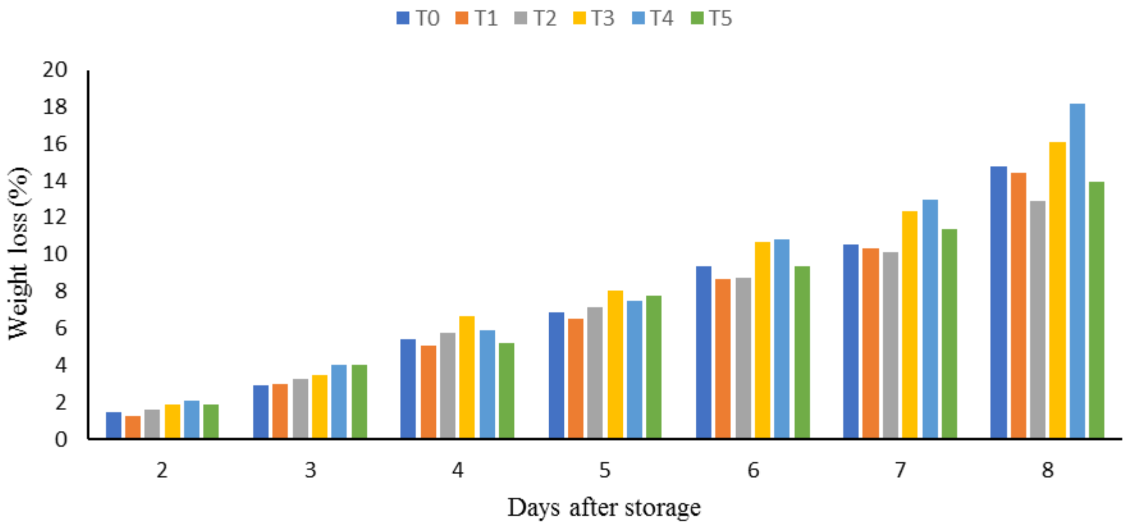

Fig. 4. Effects of chitosan treatments on percent weight loss of banana (cv. Sabri) at different days after storage. T_0: Control, T_1: 0.50% Chitosan, T_2: 0.75% Chitosan, T_3: 1.0% Chitosan, T_4: 1.5% Chitosan, T_5: 2.0% Chitosan

Table 2. Effect of chitosan treatments on disease severity of banana (cv. Sabri) during storage

Concentration of chitosan (%)	Disease severity (%) of banana during storage							
	1	2	3	4	5	6	7	8
T_0	0.00	0.00	0	10.00	33.33	45.00	65.00	85.00
T_1	0.00	0.00	0	0.00	11.67	23.33	53.33	98.33
T_2	0.00	0.00	0	0.00	6.67	10.00	15.00	35.00
T_3	0.00	0.00	0	3.33	20.00	33.33	67.00	91.67
T_4	0.00	0.00	6.67	20.00	51.67	61.67	98.33	100.00
T_5	0.00	0.00	10.00	20.00	30.00	33.33	41.67	53.33
$LSD_{0.05}$			0.63	1.14	1.41	1.59	1.67	2.49
$LSD_{0.01}$			0.89	1.59	1.98	2.23	2.34	3.50
Level of significance	-	-	58.89 **	262.24 **	805.50 **	945.72 **	2330.50 **	2152.29 **

** Significant at 1% level of probability. T0: Control, T1: 0.50% Chitosan, T2: 0.75% Chitosan, T3: 1.0% Chitosan, T4: 1.5% Chitosan, T5: 2.0% Chitosan

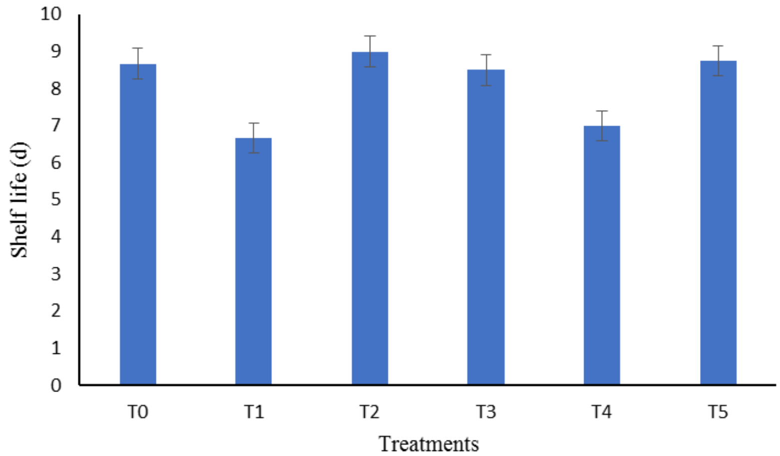

Fig. 5. Effect of postharvest chitosan treatments on shelf life of banana (cv. Sabri). Vertical bar shows Standard Error. T_0: Control, T_1: 0.50% Chitosan, T_2: 0.75% Chitosan, T_3: 1.0% Chitosan, T_4: 1.5% Chitosan, T_5: 2.0% Chitosan

Correlation and regression studies

At 8th day of storage, the relationship between color and firmness, color and weight loss, color and disease severity, firmness and disease severity of banana has been found out significant at 1% level of probability. The positive slopes exhibited positive relationship.

Color and disease severity

From the results of experiment, it is observed that disease severity showed significantly positive correlation with its color (r = 0.797692**). The regression equation of Y (disease severity) vs (color) was found to be Y = 71.305x – 398.14 (Fig. 6). It means that an increase in color scale will lead to an increase in disease severity. The present experiment was undertaken with some objectives.

Firmness and disease severity

The relationship between disease severity and firmness of banana was studied (Fig. 7). It is shown from the result that there was a direct significant and positive relationship at 1% level of significance between disease severity and firmness. The correlation co-efficient was r = 0.924793** and the regression line of y (disease severity) on firmness having Y = 45.275x – 171.86. The positive slope indicates that disease severity (%) and firmness are directly correlated, i.e. increase in disease severity simultaneously increase in firmness scale.

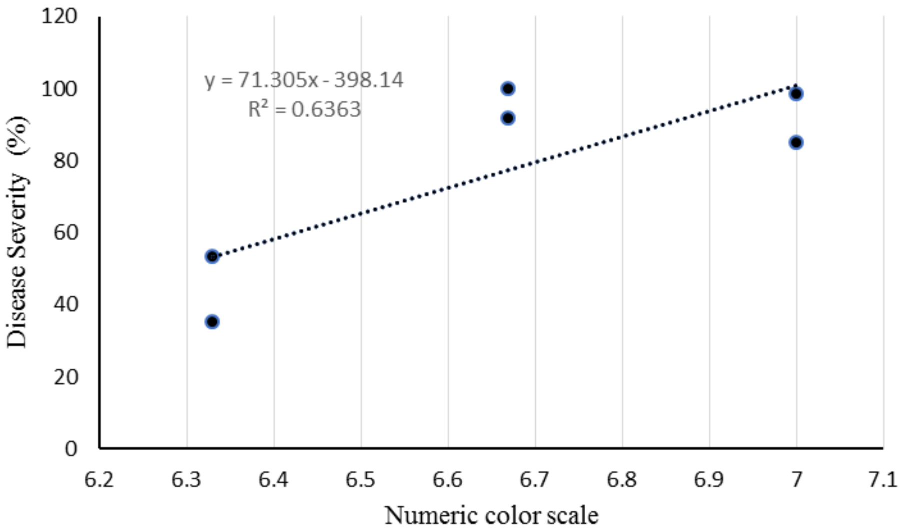

Fig. 6. Relation between color and disease severity (%) of banana cv. Sabri at postharvest

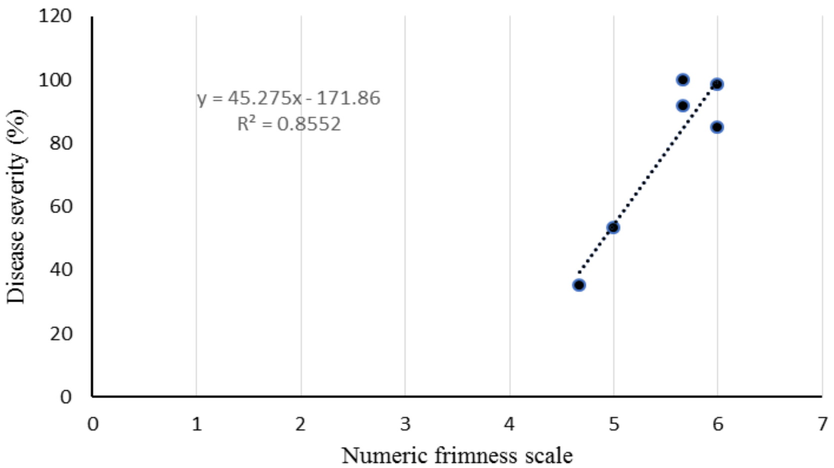

Fig. 7. Relation between firmness and disease severity (%) of banana cv. Sabri at postharvest

Discussion

Treatment with chitosan (1%) is helpful in improving the shelf-life and postharvest quality of banana (Hosseini et al, 2018) when compared to control treatment, banana fruits coated with chitosan nanoparticles (0.2%) has a slower skin discoloration by 2-3 days (Esyanti et al., 2019). Edible coatings including cellulose, chitosan, starch, bees, gum (polysaccharides) that evidences good barrier properties without residue taste or odor impairment (Dhall, 2013; Martin-Belloso and Fortuny, 2010). Edible coating reduces senescence, respiration and enzyme activity; slow down moisture losses; conserves against mechanical damage and microbial growth; protect texture, color and flavor; thereabout, keeps freshness, active volatile ingredients and plant antioxidants (Mahajan et al., 2014). Dull, grey skin color symptom development in banana is dependent on time, exposure temperature and susceptibility depends actually on cultivar (Nakasone and Paull, 1999). At the time of color change pulp become softer and sweeter as the ratio of sugars to starch increases and the characteristics aroma is produced (Robinson, 1996). Color of banana is one of the most important criteria for consumers during buying from the market. During the storage period, the color of banana changes from green to yellow. The changes of peel color involve chlorophyll degradation or qualitative and quantitative alternations of the green pigments into other pigments as reported by Salvador et al. (2007). The late color change in chitosan treated bananas implied significant effect of the treatments. Considering the effects of different concentrations of chitosan, T_2 (0.75% chitosan) and T_5 (2.0% chitosan) showed better result than the other concentrations. Though, the rate of changes of peel color in T_0 (control) was slow up to day 4, it suddenly changed and got rotten in the consecutive days which may be due to the rapid activity of enzymes that are responsible for the color changes of banana and also the impact of pathogens. On the other hand, chitosan coating treatments showed steady color changes. The impact of coatings may be attributed to the reduction of different chemical changes, especially chlorophyll breakdown.

During storage, the firmness of banana pulp is usually changed from mature hard to eating ripe stage. Softening of fruits is assigned to the change of cell wall component and degradation of starch (Seymour, 1993). The firmness changes were due to the conversion of starch to sugars. There were no significant changes in the firmness of the fruits until day 2, and the changes appeared at the later stage of storage. Chitosan coating caused significant effect on changes of firmness of banana during ripening and storage. At day 7, 0.75% chitosan and 2.0% chitosan showed the lowest scores (4.00 and 4.67, respectively) and the final day 0.75% was the best scorer (4.67). The rapid firmness changes were found in bananas under control in day 4 to day 8. The results of the present study are supported by the findings of Siriboon and Banlusilp (2004) and Hernandez et al. (1999).

Banana is subjects to shriveling, weight loss and its optical quality, if it is kept under conditions of low humidity (Esguerra and Rolle 2018). Chitosan treated bananas showed lower weight loss than the control. Banana fingers lost weight during storage under all treatments. The loss of weight occurred due to the loss of water from the fruits, microbial decay and variation in storage environment, especially temperature, and humidity. In case of weight loss, 0.75% chitosan and 2.0% chitosan showed best performance at later stages. At day 8, T_2 (0.75% chitosan) and T_5 (2.0% chitosan) got the lowest weight loss (12.88% and 13.94, respectively) whereas weight loss of bananas kept in control was 14.79%. Lower weight loss in treated fruits may be the result of forming an impermeable layer of chitosan around the fruit surface. The result of the present study could be supported by the findings of Sarkar et al. (1997).

Rough handling of bananas can create wounds that could serve as entry points for harmful microorganisms (Esguerra and Rolle, 2018). The diseases in banana surface were greatly influenced by postharvest treatments. Chitosan treated bananas got less disease percentage than the control. This may be due to the formation of impermeable coating on the outer surface of banana. Disease percentage of the fruits was zero until day 2. The appearance of diseases was from day 3 at two treated samples which were negligible. From day 4, huge variation in disease severity was found among the treatments. The lowest disease level was found in 0.75% and 2.0% chitosan coating at day 8. All the treatments except T_4 showed good results. The less disease percentage of the treated fruits may be caused by the antimicrobial action of chitosan. The results are in agreement with the findings found by Ploetz and Galam (1998). Mechanical damage and subsequent wastage due to microbial attack can be minimized by Proper handling during harvesting (Wills et al., 1998). Most food processing functions, minimally processed fruits results in onward perishability and susceptibility to pathogenic and spoilage microorganisms (Alvarez et al., 2015).

Shelf life is the period from harvesting up to the last edible stage. Storage of banana fruit in relatively low concentration of O_2 and high concentration of CO_2 can prolong storage life (Manzur et al., 2007). The longest shelf life (9 d) was observed in 0.75% chitosan and 2.0% chitosan. The other concentrations showed poor results. The results of prolonged shelf life due to chitosan were possibly attributed to the slowing down of the postharvest physiological processes and reduction of microbial decay. The results could be supported by the findings of Suseno et al. (2014). Slower physiological process (e.g. respiration) and weight loss associated with suppressed microbiological activity may possibly contribute to the extended shelf life of banana. These results are in agreement with the findings of Mondal et al. (2011). Sensory, microbiological and nutritional shelf life of minimally processed fruit is normally around 7 days (Siddiqui et al., 2011).

Conclusions

The results of present study clearly indicate that chitosan can significantly extend the shelf life of banana, and its concentration at 0.75% performed the best among the treatments.

Authors' Contributions

Tareque Aziz conducted the whole experiment and collected data. Md. Kamrul Hassan provided the experimental design and supervised in the whole experiment. Fakhar Uddin Talukder contributed in data presentation, analyzing data and finally manuscript processing and writing of this article. Md. Sohanur Rahman contributed in data analysis and journal searching for publication.

Conflict of Interest: The authors indicate no conflict of interest for this work.

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