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Effect of shrimp chitosan coating on postharvest quality of banana (*Musa sapientum* L.) fruits

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Abstract

To reduce the post-harvest loss and extension of shelf-life of banana, green mature bananas were coated with 0.5%, 0.75% and 1% chitosan, respectively. Following treatments, bananas were stored at 26±2^{0}C and 85 ± 5% RH. The effectiveness of the treatments in extending fruit's shelf-life was evaluated by determining total weight loss, colour, total soluble solid, titratable acidity, disease incidence and disease severity during the storage period. The chitosan coating reduced respiration activity, thus delaying ripening and the progress of decay due to senescence. Chitosan coatings delayed changes in weight loss, total soluble solids, titratable acidity and external colour compared to untreated samples. Bananas coated with 1% chitosan exhibited less weight loss and reduced darkening than other treatments and control sample. Disease incidence and disease severity was remarkably reduced by chitosan coating application. Chitosan coating extended banana up to the shelf life of more 3 to 4 days. This study showed that 1% chitosan was more suitable in prolonging the shelf-life and quality of banana during ripening and storage.

Introduction

Huge post-harvest losses of fruits and vegetables are a matter of grave concern for any country whose economy is agriculture based. But this is a general phenomenon happening in almost every developing country. Fruits and vegetables are highly perishable commodities that require to be handled with much care to minimize losses. Because of the high moisture content, horticultural crops are inherently more liable to deteriorate especially under tropical conditions (Thumula, 2006). They are biologically active and carry out transpiration, respiration, ripening and other biochemical activities, which result in quality deterioration.

Banana is one of the most favoured tropical fruits and is very popular worldwide. It is one of the prime fruit in Bangladesh occupying huge area for production among the fruits. The total production of banana is about 746000 metric tons from 122000 acres of land in 2011-12 (BBS, 2013). It is the only fruit crop which is available throughout the year and its consumption is higher than any other fruits. Banana fruits are climacteric in nature which ripen rapidly and soften after harvest. Banana fruit are usually harvested at mature green stage and stored either at ambient or at low temperature. Due to its high nutritive value, banana is susceptible to diseases caused by microorganisms. In addition, banana is also sensitive to low temperature storage (Malmiri *et al*., 2011). All these mentioned factors limit the handling, storage, distribution and marketing potentials of banana fruit.

Postharvest loss of banana is one of the major problems globally. In a developing country like Bangladesh the scenario is much worse. In Bangladesh huge amount of Banana is spoiled due to prevailing high temperature, humidity, inappropriate post-harvest handling and due to sub optimal knowledge in the field of post-harvest technology. The spoilage of the fruit attributed to adverse physiological changes, namely loss of weight due to respiration and transpiration, loss of flesh hardness and loss of resistance to microbial attack. Such spoilage can occur either during transportation and/or in the market resulting considerable economic loss to farmer, importer and retailers. Moreover, there is no known technique to the growers/traders of Bangladesh, in particular to extend the shelf life of banana. As a result, a considerable quantity of harvested bananas goes waste due to its perishable nature. The extents of postharvest losses of banana in Bangladesh from harvesting to consumption of banana were recorded as 26.63% (Molla *et al*., 2012).

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Edible coatings may be applied on fruits and vegetables to improve appearance, delay ripening, reduce water loss and decay, and extend shelf life, but may also change flavour (Saucedo-Pompa *et al*., 2007). An edible film is defined as a thin layer of material which can be eaten by the consumer, may be applied on or within the food by wrapping, dipping, brushing or spraying and act as barriers against transmission of gases, vapours and solutes and provide mechanical protection (Wu *et al*., 2002). Coatings can be formulated from different components such as hydrocolloids (polysaccharides and proteins), lipids (waxes and resins) and synthetic polymers. These edible materials have different barrier properties against gases and physico-chemical and mechanical characteristics. Therefore, most coatings are made of more than one material with the addition of low molecular weight molecules including sorbitol, polyols or glycerol that serves as plasticizers (Olivas and Barbosa-Canovas, 2005). Current research on edible films aims at facilitating formation of films using new materials with improved properties. The films might be formed into stand-alone packaging or coatings on foods. Even more than thermoplastic materials, edible films may have the potential for incorporation of further functional entities such as antimicrobials, antioxidants, flavors, and nutrients (Brody, 2005).

As it forms semi permeable films, chitosan can modify the internal atmosphere (by altering the permeability to water, oxygen and carbon dioxide), thereby decreasing the transpiration loss, reducing respiration rate and delay ripening in fruits, maintaining the quality of harvested fruits and reducing mold growth (Li and Yu, 2000). Chitosan is good for fresh-cut fruits and vegetables when it is in close contact with the tissue. Chitosan could be an ideal preservative coating because of its film forming properties, biochemical properties, inherent antifungal properties and elicitation of phytoalexins (Li and Yu, 2000). The chitosan coating against a wide variety of microorganisms including fungi, algae and some bacteria had been reported. Chitosan coating had been successfully applied to prolong storage life and control decay of many fruits (Shi *et al*., 2013). The specific objectives of this research are to extract chitosan from shrimp shell waste and to investigate the prospect of using chitosan coating for extending shelf life of banana after harvesting.

Materials and Methods

The research has been carried out in the laboratory of the Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Preparation of chitosan

Fresh shrimp was collected from local market. Shrimp head and skin was separated from shrimp using sharp knife. The collected shrimp wastes were then washed with tap water and crushed with mortar pastle. Crushed shrimp waste was 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). Then isolation of chitosan was carried out using the following 3 (three) steps, namely Demineralization, Deproteinization and Deacetylation.

Demineralization of shrimp shell has been carried out with 3% HCI at ambient temperature (28±2^{o}C) with a solid to solvent ratio 1:5 (w/v) for 16 hours (Toan, 2009). The residue was washed and soaked in tap water until neutral pH. Deproteinization of shrimp shell was done with 4% NaOH at ambient temperature (28±2^{o}C) with a solid to solvent ratio 1:5 (w/v) for 20 hours (Toan, 2009). The residue was washed and soaked in tap water until neutral pH. Then purified chitin was dried until it was become crispy. Chitin flakes was grounded to small particle to facilitate deacetylation (Removal of acetyl groups from chitin). Deacetylation was experimented using four different concentration of NaOH (30%, 40%, 50%, 60%) at 65^{0}C temperature with a solid to solvent ratio 1:10 (w/v) for 20 hours (Toan, 2009). The residue was washed until neutral pH with tap water. The resulting chitosan was then dried at cabinet dryer for 4 hours at 65±5^{0}C and subsequently used for coating purposes.

Collection and preparation of banana for coating

'Amritasagar' banana (*Musa sapientum* cv. Amritasagar) of green mature stage, were purchased from local market. The banana hands were carefully selected to be uniform in appearance (weight, shape and colour). Then the latex allowed drying for one hour at ambient temperature (26±2°C and 85±5% relative humidity).

Bananas were then disinfected by immersing in 250 ppm chlorinated water for 5 min, washed and air-dried (Gómez-López *et al*., 2009). Bananas were selected randomly for each treatment. Glycerol (87%) and Chlorinated water were used from laboratory stock.

Application of chitosan coating on banana

Shrimp shell Chitosan solutions (0.5%, 0.75%

and 1% solutions, which are termed as film forming solutions) were prepared using 0.6% acetic acid, adding 25% glycerol (w/w chitosan) as plasticizer (Park *et al*., 2004). Each of the solutions was thoroughly mixed, filtered and the pH was adjusted to 5.6 using 1M sodium hydroxide (Abd-AllA and Haggag, 2010). This resulted in a set of treatments as: T_0=Control (Uncoated); T_1=0.5% chitosan; T_2=0.75% chitosan; T_3=1% chitosan

Bananas were dipped into the prepared coating emulsions for 1 min and then drained. Uncoated bananas as control samples were immersed in a 0.6% glacial acetic acid solution at pH 5.6 for the same duration of time. The treated and control banana samples were dried in ambient conditions (26±2°C and 40-50% relative humidity) for 2 hours. After setting a thin layer of edible coating on the surface of treated samples, control and coated banana samples were stored at ambient conditions in the laboratory.

Observation of banana during storage

Weight loss

For determining the weight loss, five fruits in each replication for each treatment were marked before storage and weighed using a digital balance. The same fruits were weighed at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage loss of initial weight.

Colour

Days required to reach different stages of colour during storage and ripening was determined objectively using numerical rating scale of 1-7, where (1 = 0 to < 10% yellow, 2 = 10 to < 30% yellow, 3 = 30 to < 50% yellow, 4 = 50 to < 70% yellow, 5 = 70 to< 90% yellow, 6 = 90 to 100% yellow and 7 = blackened / rotten).

TSS and titratable acidity

Titratable acidity and Total soluble solid was determined as per methods of Ranganna (2011).

Disease severity

Disease severity represents the percentage diseased portion of infected fruit and was measured based on eye estimation. Disease severity was scored as per the method described by Sivakumar *et al.* (2002) using the scale (1= 0% of fruit surface rotten; 2 = 1-25%; 3 = 26-50%; 4 = 51-75% and 5 = 76-100%).

Disease incidence

Disease incidence means percentage of banana infected with diseases. The diseased fruits were identified symptomatically. The disease incidence of banana was calculated by using the following formula (Sivakumar *et al*., 2002):

$$\text{Disease incidence } (\%) = \frac{\text{Number of banana infected}}{\text{Total Number of banana}} \text{ X } 100$$

Shelf life

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

All the above determinations were carried out in triplicate and average values as well as standard deviations were reported. Mean separations were analyzed using the ANOVA and Fisher's least significant difference (LSD) procedure at $\alpha = 0.05$ using statistical software (StatGraphics, 1999).

Results and Discussion

Properties of prepared chitosan

Demineralization and Deproteinization were done to remove protein and minerals from shrimp shell respectively to get chitin. Experiment showed that 3% HCl can effectively reduce the ash content of chitin up to 0.48%. A high quality grade of chitosan should have less than 1% of ash content (No and Meyers, 1995). Chitin is not soluble but chitosan the deacetylated product of chitin is soluble. To observe the properties of chitosan prepared in this investigation, the selected parameters were evaluated as per standard methods, e.g., moisture and ash content by (AOAC, 2005), solubility by (Fernandez-Kim, 2004), degree of deacetylation (DD) by (Khangtragool *et al*., 2008) and the results are presented in Table 1.

Based on the Table 1, it is indicated that the degree of deacetylation and solubility are influenced by NaOH concentration. In this case, the increase of NaOH concentration addressed to enhance the deacetylation grade where the highest deacetylation grade (81.24%) could be reached at NaOH concentration of 60%.The solubility of chitosan is one of important parameter for quality of chitosan, where higher solubility will produce a better chitosan. The solubility, however, is controlled by the degree of deacetylation and it is estimated that deacetylation must be at least 85% complete in order to achieve the desired solubility (No and Meyers, 1995). The solubility of chitosan

Table 1. Effect of NaOH concentration at deacetylation step on the characteristics of chitosan

NaOH concentration	Parameters			
	Moisture (%)	Ash (%)	Solubility (%)	DD (%)
30	8.25(0.29)^{a*}	0.3(0.06)^{a}	48.3(2.01)^{a}	45.5
40	7.69(0.32)^{ab}	0.29(0.02)^{a}	71.27(1.25)^{b}	61.24
50	8.32(0.36)^{b}	0.26(0.01)^{a}	97.02(0.56)^{c}	79.57
60	7.96(0.06)^{b}	0.27(0.02)^{a}	97.65(0.29)^{c}	81.24

*Numbers in parentheses are standard deviations (±). Means with different letters in each column are significantly different ($p < 0.05$).

obtained in this study was ranged from 48.3% to 97.65% with significant difference with respect to NaOH concentration. Shrimp shell chitosan samples treated with 50% and 60% NaOH were found to have excellent solubility ranging from 96.01% to 97.2% with no significant difference (Table 1). Although 60% NaOH treatment produced highest deacetylated chitosan with maximum solubility but from economic point of view, 50% NaOH could be used to get high quality chitosan with 79.57% degree of deacetylation and 97.02% solubility.

Post-harvest changes of banana during storage

Total weight loss

Different treatments have been used in the present investigation showed a marked effect on total weight loss of banana during storage. Statistical significant difference was observed on percent weight loss due to application of various chitosan coating treatments (Table 2). At 3rd day after storage maximum weight loss (6.7%) was found in untreated fruit where minimum weight loss (5.1%) was observed in 1% chitosan coated fruits. Similarly, untreated banana noticed the maximum weight loss (10.5%, 19.2%, 24.5%) and 1% chitosan coated banana showed the minimum weight loss (8.3%, 15.8%, 21.1%) at 6th, 10th and 13th day of storage, respectively.

Shao *et al.* (2012) reported that chitosan coating minimized weight loss of stored apples and its combination with heat treatment showed the lowest respiration rate. Dang *et al.* (2010) also reported that chitosan formed a coating film on the outside surface of the sweet cherries that effectively retarded the loss of water.

Colour

Mean score for colour began to increase appreciably from the 3rd days of storage. At 10th and 13th days of storage chitosan coated sample showed significantly lower colour score indicating lower colour development than control sample (Table 2). Among all the treatments 1% chitosan coating delayed

Table 2. Effect of chitosan coating on weight loss (%) and colour(score) of banana

Treatments	Weight loss (%) at different days			
	03	06	10	13
T_0 (Control)	6.7(0.26)^{a*}	10.5(0.26)^{a}	19.2(0.60)^{a}	24.5(0.7)^{a}
T_1 (0.5% chitosan)	5.8(0.1)^{b}	10.2(0.3)^{a}	17.8(0.36)^{b}	22.8(0.75)^{b}
T_2 (0.75% chitosan)	5.4(0.2)^{bc}	9.8(0.43)^{a}	16.8(0.62)^{c}	22.2()0.3^{b}
T_3 (1% chitosan)	5.1(0.26)^{c}	8.3(0.62)^{b}	15.8(0.43)^{d}	21.1(0.26)^{c}
Treatments	Colour (score) at different days			
	03	06	10	13
T_0 (Control)	1	2.9(0.17)^{a*}	5.6(0.34)^{a}	6.5(0.2)^{a}
T_1 (0.5% chitosan)	1	2.2(0.2)^{b}	3.3(0.2)^{b}	4.4(0.26)^{b}
T_2 (0.75% chitosan)	1	1.9(0.17)^{b}	2.8(0.17)^{c}	4(0.17)^{c}
T_3 (1% chitosan)	1	1.5(0.2)^{c}	2.3(0.26)^{d}	3.2(0.2)^{d}

*Numbers in parentheses are standard deviations (±). Means with different letters in each column are significantly different ($p < 0.05$).

the colour development of banana to a greater extent.

Kittur *et al.* (2001) stated that during storage, chitosan coating delayed color changes in banana. Ali *et al.* (2011) noticed that the delay of color development in the papaya fruit treated with higher concentrations of chitosan could be attributed to the slow rate of respiration and reduced ethylene production, leading to a delayed fruit ripening and senescence.

TSS

The effect of chitosan concentration on TSS is shown in Table 3. In general, TSS of banana increased during the ripening process. TSS includes carbohydrates, organic acids and amino acids of fruit. In most ripe fruits, including banana, sugar forms the main component of soluble solids (Cano *et al*., 1997).

Chitosan coating as postharvest treatment on banana showed significant effect on total soluble solids at 6th, 9th and 10th days of storage (Table 3). The lowest TSS value (16 ^{0}Brix) and the highest TSS value were recorded in 1% chitosan coating treatment and control samples, respectively at 13th days of storage.

Results showed that increase in chitosan concentration successfully retard the increase of TSS. This is due to chitosan coatings could provide a semipermeable film around the fruit surface, which modifies the internal atmosphere by reducing oxygen and/or elevating carbon dioxide levels, which decrease the fruit respiration level and metabolic activity. Hence, retards the fruit ripening and senescence process (Vargas *et al*., 2008). A suppressed respiration rate slows down the synthesis and the use of metabolites, resulting in lower soluble solids due to the slower hydrolysis of carbohydrates to sugars (Das *et al.*, 2013). Similar views were expressed by Ali *et al.* (2011) who observed that chitosan coating effectively delayed changes in soluble solids

Table 3. Effect of chitosan coating on TSS and titratable acidity of banana

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]
Treatments	**Titratable acidity (%) at different days**			
	03	06	10	13
T_0 (Control)	0.37(.01)[a]	0.41(.01)[a]	0.44(.01)[a]	0.46(.01)[a]
T_1 (0.5% chitosan)	0.38(.01)[a]	0.39(.01)[b]	0.41(.01)[b]	0.42(.01)[b]
T_2 (0.75% chitosan)	0.37(.01)[a]	0.38(.01)[b]	0.39(.01)[b]	0.4(.01)[c]
T_3 (1% chitosan)	0.35(.01)[b]	0.36(.01)[c]	0.38(.01)[c]	0.39(.01)[c]

*Numbers in parentheses are standard deviations (±). Means with different letters in each column are significantly different ($p < 0.05$).

concentration of papaya during 5 weeks of storage.

Titratable acidity

The effect of chitosan concentration on titratable acidity is shown in Table 3. As the ripening of banana proceeds there was increase in titratable acidity at different days of storage. There was a significant increase in acidity in the uncoated bananas (Table 3). The coating brought about less increase in acidity in the treated bananas, especially in the 1% chitosan coated samples. This shows that 1% chitosan does not completely inhibit metabolic changes in the fruits though the rate of change was slow.

Kittur *et al.* (2001) stated that polysaccharide-based coatings applied on banana fruit displayed reduced carbon dioxide evolution, loss in weight and titratable acidity. Moreover, the reducing sugar content and total soluble solids of coated fruit were lower than uncoated, suggesting that the former synthesized reducing sugars at a slower rate, having slowed down the metabolism.

Disease severity

Table 4 shows the effect of different chitosan coating on disease severity at different days after storage. At 3rd day T_2 and T_3 treatments results significantly low scores than treatments T_0 and T_1. At 6th, 10th and 13th day control sample scored significantly high indicating high percentage diseased portion of infected fruit. At 13th days of storage chitosan coated fruit showed significantly lower score than control fruit (Table 4).The 1% chitosan samples scored better than the rest. Even after 13 days of storage fungal spoilage were minimum in the treated bananas. The protective effect of the film and might have helped maintain the quality of bananas. Li and Yu (2000) reported that 0.5% and 0.1% chitosan reduced significantly the incidence of brown rot caused by *Monilinia fructicola* in peach stored at 23°C and

Table 4. Effect of chitosan coating on disease severity (score) and disease incidence of banana

Treatments	Disease severity (score) at different days			
	03	06	10	13
T_0 (Control)	1.3(0.1)[a*]	2(0.5)[a]	3.5(0.34)[a]	4.8(0.26)[a]
T_1 (0.5% chitosan)	1.2(0.1)[a]	1.7(.02)[ab]	2.5()0.26[b]	3(0.17)[b]
T_2 (0.75% chitosan)	1(0)[b]	1.4(0.36)[ab]	1.6(0.2)[c]	2.1(0.17)[c]
T_3 (1% chitosan)	1(0)[b]	1.2(0.26)[b]	1.3(0.26)[c]	1.6(0.26)[d]
Treatments	**Disease incidence (%) at different days**			
	03	06	10	13
T_0 (Control)	6.6	20(2)[a]	46.6(1.50)[a]	100(0)[a]
T_1 (0.5% chitosan)	-	13.3(1.05)[b]	40(4.35)[b]	60(4.35)[b]
T_2 (0.75% chitosan)	-	13.3(1.05)[b]	33.3(2.78)[c]	40(2.64)[c]
T_3 (1% chitosan)	-	6.6(1.01)[c]	13.3(1.05)[d]	20(3.6)[d]

*Numbers in parentheses are standard deviations (±). Means with different letters in each column are significantly different ($p < 0.05$).

delayed the development of disease compared with the untreated fruits. Similarly, application of 1% chitosan reduced the postharvest disease of sweet cherry (Feliziani *et al*., 2013).

Disease incidence

A significant variation was found on disease incidence in banana fruits during storage period (Table 4). Among the postharvest treatments only control sample of banana showed the 100% disease incidence at 13th days of storage where the lowest disease incidence (20%) was found with the treated fruits with 1% chitosan coating application.

The disease level was maintained lower in those fruits that are coated with chitosan (Table 4). Chitosan coating successfully reduced disease incidence of banana fruits during storage. Meng *et al*. (2010) stated that treatments with chitosan and oligochitosan reduced the disease incidence caused by *Alternaria kikuchiana* and *Physalospora pyricola* inhibited the lesion expansion of the two fungi in pear fruit stored at 25°C.

Shelf life

Shelf life of banana fruits was significantly affected by the different postharvest treatments of chitosan concentration (Table 5). The maximum shelf life (16.6 days) of banana was recorded in T_3 treated fruits followed by (14.6 days) T_2 treated fruits. The shortest shelf life (12.4 days) of Banana was observed from the untreated fruits. The above results led to the conclusion that chitosan coating influenced on the shelf life of banana. Among the treatment 1% chitosan coating is more suitable for extending the shelf life of banana fruits.

Table 5. Effect of chitosan coating on shelf life (days) of banana

Treatments	Shelf life (days)
T_0 (Control)	12.4(1.08)[a]
T_1 (0.5% chitosan)	13.4(0.78)[ab]
T_2 (0.75% chitosan)	14.6(0.6)[b]
T_3 (1% chitosan)	16.6(0.52)[c]

*Numbers in parentheses are standard deviations (±). Means with different letters in each column are significantly different ($p < 0.05$).

Conclusion

In this study it is seen that the shrimp chitosan extended the shelf life of banana fruits. The fact that the coated banana samples had lower weight loss, total colour difference, TA and TSS values as compared to control samples. Chitosan coating also protected the banana fruits from disease attack. This research recommends chitosan as the edible coating material that is very effective in improving the overall quality of banana fruits during storage. In Bangladesh, it is about a new approach to extract chitosan and to apply it as a coating material for banana to extend shelf life. As the outcome of this research is promising and optimistic, it will not be confined only to banana, but also for other fruits like Mango, Orange, Litchi and similar fruits.

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