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# The impact of chitosan and guava leaf extract as preservative to extend the shelf-life of fruits

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**Abstract**

This research evaluates the effectiveness of chitosan and guava leaf extract (GLE) as preservativesto increase the storage life of fruits. Chitosan was prepared from chitin extracted from shrimp shells. GLE was extracted from mature green leaves by the hot water (80°C) extraction method. Chitosan with the solid concentrations of 1.0, 1.5 and 2.0%, and GLE with 0.5% were sprayed on banana, carambola and tomatoto create a surface coating and were stored at 25±2°C temperature and 85±5% relative humidity for 12 days. To evaluate the effectiveness of chitosan and GLE, moisture content, weight loss percentage (WLP), pH value of juice from fruits, carbohydrate content, protein contents and percentage disease index (PDI) were measured after 12 days storage. People's perceptions were also assessed for identifying the effectiveness of the preservatives. From the results, it was found that the application of 2% chitosan showed the lowest weight loss 6.58% for banana compared to the control samples. Protein and carbohydrate content were the highest 1.96 and 14.97% respectively when there was 2% chitosan application whereas GLE also showed similar results. The PDI was also lower 87.23 and 86.52% for banana coated with 2% chitosan and 0.5% GLE, respectively. Therefore, it is concluded that the chitosan and GLE can be applied as bio-preservative to extend the shelf life of the fruits without compromising their quality.



## Introduction

Food products are naturally perishable and it needs protection against microorganisms during preparation, storage and distribution. As they are sold far distances from the production site, large amount of food damage occurred due to microbial activity and physiological activity of the food itself during transportation and storage (Ofor, 2011). From ancient time, drying, freezing and application of antimicrobial components are commonly used for food preservation techniques (Rasooli, 2007; Latip *et al*., 2013). Recently, chemical preservatives are used to extend the shelf life of food products for fulfilling the consumer demand. However, consumers lose their interest on chemical preservatives because of its toxicity induced negative impact on health (Zaman *et al*., 2007; Mohammed *et al*., 2017). Thus, bio-preservatives are recently used to extent the shelf life, enhance the hygienic quality and maintain the nutritional properties of food products (Stiles, 1996). Different components of the plants like barks, fruits, flowers, roots, leaves, etc. may provide extracts with antimicrobial activity and many of them have been enjoyed generally recognized-as-safe (GRAS) status (Negi, 2012). Many researchers are now working on bio-preservatives extracted from plants and marine resources (eg. chitosan from crab or shrimp) to lengthen the shelf-life of food (Rasooli, 2007; Negi, 2012).

Chitosan is a deacetylated derivatives of chitin, which is one of the naturally most abundant mucopolysaccharides and supporting materials of crustaceans and insects. It is non-toxic, biodegradable, biofunctional, and biocompatible. Chitosan can effectively control fruit decay through its antimicrobial and antifungal activities (Aider, 2010). It can successfully form coating on fruits and vegetables, and reduce the respiration rate by inhibiting penetration of carbon dioxide and oxygen (Elsabee and Abdou, 2013). The ability of chitosan as food preservative has been studied by different researchers worldwide (Xing *et al*., 2010; dos Santos *et al*., 2012; Perdones *et al*., 2012; Shi *et al*., 2013). Chitosan could be an ideal preservative because of its film forming properties, biochemical properties and inherent antifungal properties (Li and Yu, 2000). Guava leaves extract has several chemical constituents such

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as comarins, essential oils, ellagitannins, flavonoids in particular triterpenes, quercetin, saponins, tannins, alkaloids anthraquinones, phlobatannins, cardiac glycosides (Biswas *et al*., 2002; Chang *et al*., 2013) which are known to have antimicrobial properties. The extracts are broadly classified as terpenoids, phenolics and alkaloids. In several studies, guava leaves extract showed significant antibacterial activity against different bacteria like *Staphylococcus* species, *Shigella* species, *Salmonella* species, *Bacillus* species, *E. coli, Clostridium* species and food spoilage bacteria like *Pseudomonas* species (Lutterodt, 1989; Jaiarj *et al.*, 1999; Abdelrahim *et al.*, 2002; Joseph, 2011). The antifungal compounds are mainly tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols. Much of the guava therapeutic activity is attributed because of these flavonoids. The extract also shows antioxidant properties which are attributed to the polyphenols found in the leaves. However, there are very limited works to apply these extracts for the preservation of fruits. Thus, the purpose of this study was to apply chitosan from shrimp shell chitin and guava leaves extrat for the preservation of tomato *(Solanum lycopersicum)*, carambola *(Averrhoa Carambola)* and banana *(Musa acuminata)* fruits.

## Materials and Methods

*Collection and preparation of materials*

Freshly harvested mature banana *(Musa acuminata)*, carambola *(Averrhoa Carambola)* and tomato *(Solanum lycopersicum)* were collected from the commercial farms around Khulna city, Bangladesh. A total 36 of each type of fruits were collected during the period of October to November, which were spotless.The fruits were almost uniform in shape and color. Average weight of the selected banana, carambola and tomatowere 105±0.86, 112±0.56 and 120±0.76 g, respectively.

*Extraction of chitosan from chitin*

The chitin was collected from the Forestry and Wood Technology Lab., Khulna University, Bangladesh. In order to prepare the chitosan, firstly the acetyl groups were removed from the chitin by deacetylation process (Puvvada *et al*., 2012). The purified chitin was further treated in a hot water bath at 100°C for 2 hrs with 50% NaOH solution where the mixing ratio of chitin and NaOHwere 1:25 ($gmL^{-1}$). Afterwards, the resulted chitosan washed with water and conversion of chitosan from chitin was completed by dissolving the chitosan into ethanol. To form the slurry, chitosan dispersed in water having a concentration of 1, 1.5 and 2 wt%. Then a high-speed blender (Vita-Mix Blender) was used to blend the suspension for 10 minutes with 37000 rpm rotating speed and thus the chitosan was prepared.

*Extraction of extracts from guava leaves*

Mature green guava leaves were collected from a commercial guava farm around Khulna city, Bangladesh. The leaves were washed with distilled water to remove the impurities. Afterwards, 250 g guava leaves were transferred to 2000 ml water and boiled at 80°C for 3 hours. Then, the temperature of the solution wasmaintained at 70°C to evaporateit until 0.5% concentration of extracted solution (Seo *et al*.,2014).

*Preparation and application of preservatives*

Three different concentrations, viz. 1.0, 1.5 and 2.0% of chitosan were prepared by adding water in it to apply on fruits as preservative (Herna´ndez-Mun˜oz *et al*.,2008). The extracted guava leaves extract having 0.5% concentration were also prepared earlier. These four solutions were sprayed uniformly and separately on the collected fruits with a hand pump sprayer. The fruits were then allowed to dry at room temperature. Untreated samples were taken as control. All the fruits were stored in an open and safe place which is free from insects. The storage duration was determined by considering the complete perishtime of the control fruits.

*Evaluation of the fruit quality*

Collected each type of fruits were grouped into fresh (6), control (6) and remaining 24 for the treatments. Fresh fruit samples were analyzed immediately after collection while remaining samples analyzed after 12 days of storage to compare the effectiveness of the treatments.

*Peel color, flavor and firmness*

Peel color, flavor, firmness and overall acceptability of the fruits were assessed by a panel consists of 10 members. The members were selected randomly from the faculty members of Life Science, School of Khulna University, Bangladesh. The fruits were assessedby comparing with the original color, flavor and firmness and the panel membersfilled a questionnaire (Brishti *et al*.,2013).

*Determination of moisture content*

Moisture content of the fruits were determined by oven dry method (AOAC, 2005). The moisture content of fruits was calculated by using the equation 1.

$$\text{Moisture content}(\%) = \frac{\text{Weight}_{\text{initial}} - \text{Weight}_{\text{dry}}}{\text{Weight}_{\text{dry}}} \times 100 \quad \text{Eq.(1)}$$

Where, Weight $_{initial}$ = Initial weight of fruit (g)
Weight $_{dry}$ = Constant weight after drying (g)

*Measurement of weight loss*

Weight loss is considered as an indicator of quality of the fruits which was measured in every four days interval by using a digital balance (Brishti *et al*.,2013).The fruits were subjected to air dry for 12 days and finally, cumulative weight loss were calculated. Weight loss of fruits was calculated by using equation 2.

$$\text{Weight loss}(\%) = \frac{\text{Weight}_{\text{initial}} - \text{Weight}_{\text{final}}}{\text{Weight}_{\text{final}}} \times 100 \quad \text{Eq.(2)}$$

Where, Weight $_{initial}$ = Initial weight of a fruit (g)
Weight $_{final}$ = Weight after 4 days (g)

*Measurement of pH of the fruit juice*

To determine the pH value of the fruit juice, the sample frutis were peeled and blended separately by a digital blender (Vita-Prep 3®) having 37000 rpm to prepare juice. The pH meter (Benchtop pH meter) was used to measure the pH value of the fruit juice(Brishti *et al*., 2013).

*Determination of total carbohydrate content*

Total carbohydrate content of the fruits were measured separately by using the Lane-Eynon method (Okoye *et al.,* 2008).The fruit juice (carbohydrate solution) was gradually poured to a flask containing copper sulfate solutionand a methylene blue indicator. The carbohydrate solution reacts with the copper sulfate present in the flask. Once all the copper sulfate solution was reacted, any further addition of reducing sugars causes the indicator to change from blue to white. The volume of sugar solution required to reach the end point was recorded and total carbohydrate was determined.

*Determination of protein content*

The protein content of fruit samples were estimated by Lowry's method using a standard curve of Bovine Serum Albumin (BSA) solution (20-100 Mg/ml) and absorbance at wavelength of 660 nm using double beam UV-Visible spectrophotometer (Lowry *et al*., 1951).

*Measurement of percentage disease index (PDI)*

PDI was used to observe the effectiveness of preservatives on fruit samples in retarding fruit disease. Among the three different fruits, only bananawas used for PDI because of its noticeable appearance at outer surface and thus, for easy detection of color change. PDI was assessed by a scanner (Canon DR M140) and Adobe Photoshop (Version CS6) software (Hossain *et al.,* 2010). The bananapeel was scanned and analyzed the percentage of yellow and black portion, which were theresults for PDI.

*Statistical analysis*

Analysis of the data was carried out by using SAS system software (version 8.1) at 95% confidence level. The significance of differences among treatment means was determined by analysis of variance (one-way ANOVA) followed by least significant difference (LSD) test.

## Results and Discussion

*Peel color, flavor and firmness*

Visual assessment is one of the major determinant and a key feature to choose the fruit as well as to assess the quality of fruit. The modified atmosphere created by the edible coating (spray) which retarded the ethylene production rate, therefore, delay ripening, chlorophyll degradation and carotenoid synthesis thus, ultimately delaying color change of fruits. Figure 1 illustrates the condition of fruits including banana, carambola and tomato after 12 days. People's perception regarding color, flavor and firmness areshowed in Figure 2. Color, flavor and firmness of the control samples of banana, carambola and tomato were almost deteriorated after 12 days of storage. According to 90% respondents, banana samples coated with 0.5% GLEshowed as usual color, flavor and firmnessfollowed by 2% chitosan.In addition, 90% of the respondents found that the carambola samples coated with 0.5% GLE showed as usual color, flavor and firmness followed by 2% chitosan. Finally, Tomato coated with 0.5% GLE showed as usual color, flavor and firmness as mentioned by the 90%, respondents which was followed by 2% chitosan.Similar outcomes are also described by Cortez-Vega *et al.* (2004) and Xing *et al*. (2010) that showed coating did not significantly affect the firmness of fruits. Fungal chitosans, due to their influential antimicrobial activity, were successfully applied for the preservation of many foodstuffs through the formation of edible coatings (Mohammed *et al.*, 2017).

*Moisture content*

Moisture content of banana, tomato and carambola arepresented in Table 1. It is observed

Figure 1. Changes in color of banana, carambola and tomato (A fresh; $B_1$ control at 1st day, $B_2$ control after 12 days; $C_1$ treated with 1% chitosan at 1st day; $C_2$ treated with 1% chitosan after 12 days; $D_1$ treated with 1.5% chitosan at 1st day; $D_2$ treated with 1.5% chitosan after 12 days; $E_1$ treated with 2% chitosan at 1st day; $E_2$ treated with 2% chitosan after 12 days; $F_1$ treated with 0.5% GLE at 1st day; $F_2$ treated with 0.5% GLE after 12 days) after 12 days of treatment with chitosan and GLE

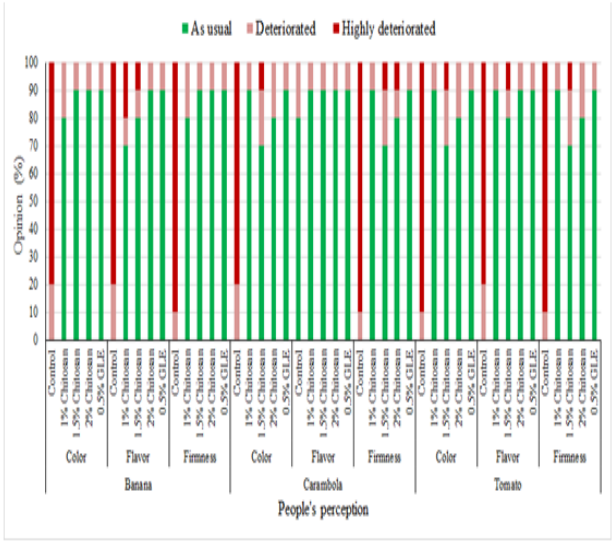


Figure 2. People's perception regarding the quality of banana, carambola and tomato

that moisture content variesamong fresh, control and coated banana, carambola and toamto. Moreover, the highest moisture content (83.54%) was found for the fresh banana while the lowest moisture content was found in control banana (55.71%). For Carambola, the highest (93.35%) and the lowest (42.57%) moisture content were found in coated with 0.5% GLE andcontrol samples, respectively. Besides, for Tomato, the highest (89.89%) and the lowest (52.2%) moisture content were found in samples coated with 1.5% chitosan and control samples, respectively. The variation in moisture content might be because of the preservative applications as there were same temperature and humidity in the storage place. This statement is supported by the study of Lanka *et al.* (2011) where they have also found variation of moisture content because of the preservative applications.From statistical analysis, it was observed that there was significant difference ($P < 0.05$) in moisture content among the fruit samples (Table 1).

After 12 days of storage, the cumulative weight loss percentage of three type of fruits were lower compared to the control fruits of same type (Table1). Moreover, after 12 days of storage, control samples of banana showed the highest weight loss (25.56%),while samples coated with 2% chitosan showed the lowest weight loss (6.58%). For carambola, control sample showed 12.36% weight loss, which was the highest, compared to the treated fruit samples and fruits coated with 1% chitosan showed the lowest weight loss (6.75%).Regardless of the treatment, it was also observed that the rate of weight loss increasedwith the increase of storage time. This variation might be due to the loss of water from the fruits during the storage period. Zagory and Kader (1988) reported that the weight loss mainly occurred due to transpiration and loss of carbon reserved due to respiration. Similar results were reported by Zhang and Quantick (1997) for litchi coated with chitosan-based edible coating. The results of this study also complied with the results of the study by Salvador *et al.* (1999) where chitosan was used on avocado fruits to increase their storage life. Furthermore, statistical analysis showed that there was significant different ($P<0.05$) in WLP among the fruit samples(Table 1).

*pH value*

Variation in pH value of the juice produced from the fruits which were fresh, control and coated with chitosan and 0.5% GLE illustrated in Table 1. The average pH value of fresh banana, carambola and tomato juice were lower compared to the control and coated fruits except for carambola coated with 1.5% chitosan and 0.5% GLE. Moreover, regardless of treatment control samples showed the highest value of pH for all fruits.The highest pH value (6) was found for control samples of banana while the lowest was found for carambola coated with 0.5% GLE. Therefore, samples sprayed with 1 and 1.5% chitosan and 0.5% GLE slowed the changes in pH compared to the control samples. The changes in pH value after 12 days storage may be due to the metabolic processes of the fruit that resulted in a decrease of organic acids. The results complied with the results reported by Coseteng and lee (1987). Statistical analysis showed a significant difference ($P<0.05$) in variation in pH value of the fresh, control and coated fruits forbanana, carambola and tomato (Table 1).

Table 1. Analysis of fresh and treatment Banana, Carambola and Tomato with 1, 1.5 and 2% Chitosan and 0.5% GLE samples

| Quality | Fruits | Fresh | Control | 1% Chitosan | 1.5% Chitosan | 2% Chitosan | 0.5% GLE |
|---|---|---|---|---|---|---|---|
| Moisture content (%) | Banana | $83.54^{A}$ (0.03) | $55.71^{F}$ (0.03) | $78.49^{B}$ (0.02) | $75.18^{C}$ (0.05) | $74.04^{E}$ (0.05) | $74.39^{D}$ (0.03) |
| | Carambola | $93.35^{C}$ (0.05) | $42.58^{F}$ (0.04) | $93.43^{B}$ (0.03) | $92.08^{E}$ (0.06) | $93.29^{D}$ (0.04) | $93.70^{A}$ (0.04) |
| | Tomato | $89.89^{D}$ (0.04) | $52.20^{F}$ (0.02) | $90.65^{C}$ (0.040 | $90.88^{A}$ (0.03) | $90.73^{B}$ (0.02) | $89.34^{E}$ (0.04) |
| Weight loss (%) | Banana | - | $25.56^{A}$ (0.03) | $10.31^{B}$ (0.04) | $8.86^{D}$ (0.02) | $6.58^{E}$ (0.01) | $8.94^{C}$ (0.05) |
| | Carambola | - | $12.35^{A}$ (0.05) | $6.75^{E}$ (0.03) | $10.42^{B}$ (0.04) | $9.30^{C}$ (0.03) | $8.64^{D}$ (0.03) |
| | Tomato | - | $20.70^{A}$ (0.02) | $9.42^{D}$ (0.02) | $11.02^{B}$ (0.04) | $7.71^{E}$ (0.03) | $10.53^{C}$ (0.04) |
| pH value | Banana | $4.01^{D}$ (0.05) | $6.01^{A}$ (0.06) | $5.55^{B}$ (0.03) | $5.01^{C}$ (0.04) | $6.01^{A}$ (0.03) | $5.01^{C}$ (0.05) |
| | Carambola | $4.01^{B}$ (0.03) | $5.01^{A}$ (0.05) | $4.02^{B}$ (0.05) | $3.91^{C}$ (0.03) | $4.02^{B}$ (0.05) | $3.51^{D}$ (0.04) |
| | Tomato | $4.02^{C}$ (0.05) | $5.02^{A}$ (0.04) | $4.50^{B}$ (0.05) | $4^{C}$ (0.04) | $4.02^{C}$ (0.04) | $4.51^{B}$ (0.04) |
| Carbohydrate content (%) | Banana | $22.65^{A}$ (0.10) | $11.29^{F}$ (0.07) | $15.46^{D}$ (0.05) | $17.99^{C}$ (0.06) | $14.97^{E}$ (0.07) | $19.66^{B}$ (0.05) |
| | Carambola | $9.63^{A}$ (0.04) | $5.20^{F}$ (0.03) | $6.62^{E}$ (0.06) | $7.93^{C}$ (0.05) | $8.27^{B}$ (0.04) | $7.84^{D}$ (0.05) |
| | Tomato | $2.38^{B}$ (0.05) | $0.14^{B}$ (0.02) | $0.76^{B}$ (0.03) | $0.85^{B}$ (0.02) | $12.71^{A}$ (5.54) | $1.65^{B}$ (0.04) |
| Protein content (%) | Banana | $2.17^{A}$ (0.03) | $0.65^{F}$ (0.02) | $1.35^{E}$ (0.03) | $1.64^{D}$ (0.02) | $1.96^{C}$ (0.03) | $2.04^{B}$ (0.02) |
| | Carambola | $0.54^{A}$ (0.04) | $0.17^{D}$ (0.02) | $0.33^{C}$ (0.02) | $0.42^{B}$ (0.02) | $0.42^{B}$ (0.03) | $0.41^{B}$ (0.03) |
| | Tomato | $0.80^{A}$ (0.03) | $0.22^{E}$ (0.02) | $0.56^{D}$ (0.02) | $0.65^{C}$ (0.03) | $0.73^{B}$ (0.01) | $0.71^{B}$ (0.02) |

*Total carbohydrate content*

The carbohydrate content of the fresh banana, carambola and tomato were 22.65, 9.63 and 2.38%, respectively. It was observed that the control samples of banana, carambola and tomato showed the highest change in carbohydrate content whereas minimal change was noticed in values of carbohydrate content for samples coated with 0.5% GLE (Table 1). Spraying of preservatives might be slowed the changes of carbohydrate content by effectively delaying fruit senescence. This was due to the semi-permeable coating on the fruit surface, which modified the internal atmosphere. This statement is supported by the study of Martinez-Romero *et al.* (2006).The change in carbohydrate content was significantly different ($P<0.05$) after 12 days storage(Table 1).

*Protein content*

Table 1 shows the protein content of three different fruits at fresh, control and treated condition. The protein content of the fresh banana was 2.17% whereas minimal change was noticed for samples coated with 0.5% GLE after 12 days storage. For carambola, fresh samples contain 0.54% of protein where minimal changes were noticed for samples coated with chitosan 2% (0.42%). Moreover, for tomato, the protein content of fresh samples were 0.79% while the lowest change was observed for samples coated with2% chitosan (0.73%). This might be happened due to the semi-permeable coating on the fruit surface, which modified the internal atmosphere. The study of Jiang *et al*. (2005) support the results of this research. From the statistical analysis, it was observed that the results of protein content among the three different fruits varied significantly ($P<0.05$) (Table 1).

*Percentage Disease Index*

Figure 3 illustrates the PDI of the banana treated with different concentration of chitosan and 0.5% guava leaf extract. After 12 days of storage,57.6% disease incidence was observed in control sample, while the banana samples sprayed with different concentration of chitosan (1.0, 1.5 and 2.0%) and 0.5% guava extractshowed lowerdisease incidence. Moreover, no sign of disease were observed forthe first 7 days of storage period. This might be due to the anti-microbial potentiality of coated materials i.e., chitosan and guava leaf extract. The results of this study complies with the results reported by Serrano *et al*. (2005) where vena gel based coating was used in reduction of microorganism proliferation in sweet cherries. Statistical analysis showed that the PDI index of banana for different treatments are statistically different ($P<0.05$).Chitosan has been used at concentrations of 5–20 $gl^{-1}$ to coat detached fruits such as tomato, strawberry, cucumber, pepper, peach, Japanese pear, kiwi, apple and longan. The coating affects gas exchange, decreases transpiration losses, delays mat-uration and senescence, and maintains the quality of harvested fruits (Fornes *et al*., 2005; Mohammed *et al*., 2017).Chitosan has been stated to elicit diverse host defense responses, offering protection against infection in a variety of

host plants against their respective pathogens(Guilli *et al.*, 2016).

**Conclusion**

In this study, the effectiveness of different concentration of chitosan and 0.5% GLE as preservative for increasing the storage life of banana, carambola and tomato were investigated. Based on the results, it can be concluded that Chitosan and GLE found effective to extend the storage life of banana, carambola and tomato.Effectiveness of chitosan varies depending on the concentration applied for preservation of fruits.The lowest change in carbohydrate and protein content was observed for fruits, which were coated with 2% chitosan and 0.5% GLE although the different concentration of chitosan and 0.5% GLE showed promising results. It would be better to optimize the concentration of the preservatives to maximize the storage life of the fruits.This researchconveys scientific understanding to further study the antimicrobial values and explore other pharmacological properties of chitosan and guava leaf extract.

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