Effect of a chitosan edible-coating enriched with *Citrus limon* peel extracts and *Ocimum tenuiflorum* leaf extracts on the shelf-life of bananas

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Abstract: In recent years, the need for the capability of decelerating the ripening process has increased significantly, especially in countries such as India where the post-harvest loss is ~630 billion dollars annually. The ripening process primarily starts with transpiration and respiration through microscopic pores present on the fruit. Accordingly, edible coatings can act as barriers, reducing the acceleration of these biochemical processes and enhance the shelf-life of the fruit. For this study, 2% chitosan enriched with peel extracts from *Citrus limon* and leaf extracts from *Ocimum tenuiflorum* were used as an edible coating and the quality of bananas was evaluated over time. The ripening process was found to be delayed for a significant amount of time. The study also revealed increased anti-oxidant activity based on absorbance changes of 2,2-diphenyl-1-picrylhydrazyl, which correlated with the phytochemical content of the fruit.

1 Introduction

The most widely consumed tropical fruit across India is the banana (*Musa spp.*), with a per capita consumption of 19.0 kg. The health-promoting effects of bananas, related to their content of ascorbic acid, carbohydrates, vitamins, potassium, and phenolic compounds, have been previously demonstrated in a research study [1]. According to research findings reported to date, it has been determined that without proper storage facilities, the shelf-life and quality of bananas deteriorate significantly due to acceleration of the ripening processes. As a result, the acidity and levels of phytochemicals decrease over time and there is a noticeable loss of dry weight. The initial signs of ripening can generally be observed by visual inspection with the naked eye with changes occurring in the colour of the fruit peels [2]. Among the many global sustainability challenges listed by the United Nations [3], the mitigation of post-harvest loss is of prime importance. There are many factors influencing post-harvest loss, but the primary cause is the inefficacy of cold chain logistics. Cold storage is one of the most efficient ways to sustain the quality of food products for extended periods of time [4]. However, the major problem associated with cold storage is its high energy consumption, making it a non-viable option for many states in India, as well as many countries around the world. To combat this problem, significant research has been performed regarding edible coatings. Edible coatings are an innovative and unique approach for lengthening the shelf-life of fruits and vegetables by attacking the root cause of post-harvest loss, accelerated ripening rates.

In recent studies, it has been found that the physiological processes involved with the ripening process, such as respiration and transpiration, can be or slowed by using edible coatings that somewhat block the pores of fruits for a significant period of time, thereby extending the ripening period. Increase in the ripening period is directly proportional to increases in the shelf-life of fruit, which in-turn reduces the chances of post-harvest losses [5]. Edible films and coatings generated from biological compounds, such as polysaccharides, alginates, chitosan, proteins, and lipids, are currently in commercial use and therefore, have the potential to be applied for preserving the quality of fruits for a significant period of time [6].

Chitosan is widely used for extending the shelf-life of fruits by reducing the rate of the ripening process (respiration + transpiration) [7]. Lengthening the ripening process helps to maintain the quality of the fruit. Chitosan is applied over the fruit as an ambigous permeable membrane or coating. Numerous laboratory and field studies have reported the significance of reducing the ripening rate of strawberries [6], cherries [8], carambola [9], papaya [10], Cavendish bananas [11], mango [12], mushrooms [13], grapes [14], citrus [15], and tomatoes [16].

A wide variety of natural extracts and oils have been used to enhance the shelf-life of the fruit by impeding the rate of ripening [17]. These include chitosan enriched with extracts of olive leaves which have been used on sweet cherries [18], hairy fig extracts, which have been used on oranges [19], and moringa leaf extracts, which have been used on guava and avocado [20].

*Citrus limon* peels are widely known to be a potential source of several anti-oxidants and have disinfectant and anti-microbial properties [21]. In addition to being strongly acidic, *C. limon* can be used to stop bacterial and insecticidal infestations of fruits for a significant period of time. The presence of the wide range of phenolic compounds makes it an ideal choice to be used in combination with the *Ocimum tenuiflorum* extracts. Some previous studies have reported that *O. tenuiflorum* leaves possess the quality of slowing the enzymatic secretion of beta-carotene, one of the primary agents responsible for initiating the ripening process in bananas [22]. Therefore, the oil extracted from a mixture of *C. limon* peels and *O. tenuiflorum* leaves may enhance the shelf-life of bananas. A number of field studies have reported using oil extract from *C. limon* in edible coatings, but reports in the literature specific to the use of both *C. limon* peels and *O. tenuiflorum* leaves is currently unavailable.

In this study, we hypothesised that *C. limon* peel extracts and *O. tenuiflorum* leaf extracts would not only enhance the phytochemical properties of bananas but also be a significant catalyst in improving the chemical properties of chitosan, the edible coating used. The study entailed an evaluation of the storage of bananas for a period of 10 days without refrigeration. The results from this research should be helpful in determining steps that can be taken to improve the shelf-life of bananas in open-markets in India and throughout the world.
2 Experimental methodology and materials

2.1 Natural extracts and experimental design

Bananas (2 kg) were purchased from local farms of Shekerkot Village, Tripura, India and the different chemicals required during the experiment were all bought from the company named Scientific and Chemical Supply Co, based in Kolkata in India. Pieces of the fruit were chosen according to being of similar size and colour (more yellow than green), commercial maturity, and quality of freshness, including being free from external injuries or insecticidal infestations. The entire 2 kg of the fruit was rigorously washed with water and 2% hypochlorite salts of chlorine and then soaked in the solution for ~20 min. Following this, the bananas were then taken out from the solution and air-dried. After drying, these fruits were sorted into five groups and each group immersed in one of the following solutions: (i) 3% chitosan (C), (ii) 1% chitosan (C)+2% C. limon (CL) peel extract, (iii) 1% chitosan (C)+2% O. tenuiforum (OT) leaf extract, (iv) 2% chitosan (C)+2% C. limon (CL) peel extract +2% O. tenuiforum (OT) leaf extract, and (v) normal tap water or NTW (controlled amount). The NTW group was used considering the water generally available in the open-markets of India, in contrast to distilled water at ambient temperature and relative humidity. The fruit was packaged at desired quantities in plastic boxes at an ambient temperature of ~30 ± 5°C and relative humidity of ~75 ± 5%. Pieces of fruit were analysed in quadruple at 4-day intervals for a total of 16 days or until the fruit was no longer in a condition acceptable for consumption. The fruit at the end of the study period was blacker in colour than yellow.

2.2 Synthesis of the edible coating

Approximately 1 kg of each of the CL peels and OT leaves were bought. The leaves and peels were ground separately using an electric automatic feed processing type grinding machine (BHARATH ENGINEERS 08APKPV8265MIZT) and then mixed. After grinding, the mixture was then dissolved in 1 l of distilled water at ambient temperature and relative humidity. The mixture then underwent the ‘oil–steam distillation’ process [23]. The mixture was immersed in water and after being heated to ~95°C for a period of 20 min, was converted into steam (water + oil). The steam was then passed through the condenser section of the apparatus. The resultant compound obtained at the end of the process yielded a water–oil mixture of ~800 ml. To extract and separate the oil from the water, 10 ml of hexane was added to the mixture, resulting in ~500 ml of pure edible oil (Fig. 1).

To obtain proper mixing and dispersion, 1 g of C (Sigma-Aldrich) was added to 100 ml of an aqueous solution of glacial acetic acid (0.5% v/v) with constant agitation at room temperature for ~50 min [24]. The solution was maintained at approximately pH 6 with 1 M NaOH and glycerol (0.75%) was then added as a plasticiser. The OT leaf extract was prepared by mixing 2 g of leaves with 100 ml of 30% ethanol at 60°C for ~90 min. The mixture was centrifuged at 3000 rpm for 5 min. The extract was concentrated using a rotary evaporator at 42°C. The extract was then mixed with 2% C solution for use in subsequent experiments. The C solution was adjusted according to the requirements of the experiments and the percentage of input OT leaf extract and CL peel extract.

2.3 Fruit weight loss

The bananas were visually inspected at each time point prior to the other analyses. Representative visual results from day 4 are shown in Fig. 2. Fruit weight loss was used to determine the level of bacterial or insecticidal infestation of the fruit during the experimental period of time. The weight of the fruit was measured using standard laboratory instruments. Fruit weight loss was calculated according to the difference in initial and final weights of the fruit and was expressed as a percentage relative to the initial weight. The equation used to calculate weight loss is as follows:

$$\text{Weight Loss ()} = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

where $W_i$ was the initial weight of the fruit and $W_f$ was the final weight of the fruit over the experimental period of time.

2.4 Determination of ascorbic acid content

The AOAC Method 967.21 is a standard test for determining levels of ascorbic acid of a fruit and was used in this study. The AOAC method uses the 2,6-dichloroindophenol titrimetric method. The standard titration process yielded a typical rose/pink colour at the 19th s of the assay. The ascorbic acid content (mg) was expressed per 100 g of fresh weight sample.

2.5 Determination of the antioxidant response and levels of phenolics

Antioxidant activity of the extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [25]. For the assay, 10 mg of extract was added to 40 ml of methanol. The mixture

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**Fig. 1** Extracted edible oil

**Fig. 2** Visual inspection of bananas with and without the extracted edible coating (day 4 post-treatment)
was incubated at 3°C and the homogeneity maintained by placing it in an ultrasonic bath for 20 min. The mixture was then centrifuged and the supernatant removed and filtered. A 0.2 ml aliquot of the extract supernatant was added to 4 ml of DPPH and incubated for 50–60 min. The absorbance of the mixture was then measured at 500 nm using a ultraviolet–visible (UV–vis) double-beam spectrophotometer.

The methanol extract used to determine the antioxidant response of the fruit was also used to evaluate the phenolic content using the Folin–Ciocalteu method. Briefly, 0.5 ml of 1 N Folin–Ciocalteu reagent was mixed with 0.1 ml of the methanol extract and then 2 ml of distilled water and 1.9 ml of 10% Na2CO3 were added. The mixture was incubated for 20 min in a hot water bath at 40°C. The same instrument used for determining the absorbance for the antioxidant property is used here as well. The absorbance of the solution was then measured at 720 nm using the UV–vis double-beam spectrophotometer. The amount of phenolics was determined according to the amount of gallic acid (mg) per 100 g of fresh weight sample. The results are expressed as the mean ± S.D. To determine the effect the extract had on the fruit, a comparison was done using bananas with and without the coating. The results were compared using ANOVA tests with p<0.03 considered to be statistically significant.

2.6 Analysis of soluble solid content and titratable acidity

After extraction of the extract using the process described above, the gross amount of soluble solid content was measured using an Abbe 3-L refractometer. The results were expressed as degrees Brix (°Brix). The acidity was then measured by titration with 0.2 N NaOH. The titratable acidity content was expressed as malic acid (g) per 100 g of fresh weight sample.

3 Results and discussion

3.1 Percentage weight loss

Results of the weight loss analysis for the 16-day period of the study revealed differences among the different groups of bananas treated with the various extracts. The 3% C group had a 7.76±0.3% weight loss. The 1% C + 2% C and CL peel extract group had a 6.97±0.2% weight loss. The 1% C + 2% OT leaf extract group had a 6.3±0.03% weight loss. The 2% C + 2% CL peel extract + 2% OT leaf extract group had the lowest recorded weight loss of 4.9±0.19%. Finally, the NTW group had a 6.23±0.03% weight loss. This is the first time that such low weight-loss percentages have been encountered using edible coatings. These experimental results suggested that the transpiration and respiration rates were significantly impeded and reduced, resulting in the ripening rate being slowed.

3.2 Ascorbic acid content

The component that reduces the rate of ripening is ascorbic acid. Therefore, ascorbic acid plays a vital role in determining the freshness quality of the fruit. Studies performed by Ma et al. [26] and Zam [27] illustrated the functional characteristics of the ascorbic acid in terms of the oxygen permeability and enzymatic secretions which are some of the important parameters governing a fruit’s quality. Therefore, this chemical test was crucial for determining the performance of the extracted edible coating on the bananas. The ascorbic acid content over a period of 16 days for different concentrations of the extracts is shown in Fig. 3.

As shown in Table 1, the smallest changes in ascorbic acid levels were for observed in the 2% C + 2% CL peel extract + 2% OT leaf extract group. In contrast, the greatest changes in ascorbic acid levels were seen in the NTW group.

3.3 Phenolic content

The variation in phenolic content of the fruit samples during the 16-day period of the study are shown in Fig. 4 and the units of the phenolic content which is mg/100 g can also be expressed in the form of percentages (%). The phenolic activity was significantly higher for all the five batches of the fruits during the initial 4-day period at ~65%. The phenolic activity differed between the samples with and without the coating. The banana samples with a coating also showed lower variation in phenolic activity, which was possibly a contributing reason for the lower

Table 1  Ascorbic acid content (mg/100 g sample) at the different time points evaluated

| Sample   | 3% C | 1% C + 2% CL | 1% C + 2% OT | 2% C + 2% CL + 2% OT | NTW |
|----------|------|--------------|--------------|----------------------|-----|
| 0th Day  | 35   | 35           | 35           | 35                   | 35  |
| 4th Day  | 32.8 | 26.9         | 31.7         | 31.9                 | 31.9 |
| 8th Day  | 28.2 | 28.2         | 28.2         | 28.2                 | 27.9 |
| 12th Day | 28.2 | 25.8         | 30           | 31.8                 | 24  |
| 16th Day | 26.3 | 24.9         | 27.3         | 30                   | 20  |
Table 2 Phenolic content (mg/100 g) at different time intervals

| Sample                | 3% C | 1% C + 2% CL | 1% C + 2% OT | 2% C + 2% CL + 2% OT | NTW |
|-----------------------|------|--------------|--------------|----------------------|-----|
| 0th day               | 50   | 50           | 50           | 50                   | 50  |
| 4th day               | 70   | 70           | 70           | 70                   | 70  |
| 8th day               | 80   | 83           | 80           | 86.8                 | 29.9|
| 12th day              | 58   | 79           | 60.6         | 90.01                | 24.7|
| 16th day              | 30   | 70.02        | 57.9         | 90.76                | 20  |

Table 3 Soluble solid content and titratable acidity of bananas treated with various edible coatings

| Samples                  | Days | Soluble solid content, °Brix | Titratable acidity, mg/100 g |
|--------------------------|------|-----------------------------|------------------------------|
| 3% C                     | 0    | 21.98 ± 0.98                | 0.45 ± 0.03                  |
|                          | 8    | 22.46 ± 0.14                | 0.41 ± 0.08                  |
|                          | 16   | 22.56 ± 1.03                | 0.37 ± 0.02                  |
| 1% C + 2% CL             | 0    | 21.96 ± 0.98                | 0.45 ± 0.03                  |
|                          | 8    | 22.30 ± 0.79                | 0.41 ± 0.04                  |
|                          | 16   | 22.49 ± 0.05                | 0.37 ± 0.02                  |
| 1% C + 2% OT             | 0    | 21.98 ± 0.98                | 0.45 ± 0.03                  |
|                          | 8    | 22.30 ± 0.96                | 0.41 ± 0.05                  |
|                          | 16   | 22.56 ± 0.98                | 0.37 ± 0.04                  |
| 2% C + 2% CL + 2% OT     | 0    | 21.98 ± 0.98                | 0.45 ± 0.03                  |
|                          | 8    | 22.30 ± 0.96                | 0.41 ± 0.05                  |
|                          | 16   | 22.56 ± 0.98                | 0.37 ± 0.04                  |
| NTW                     | 0    | 21.98 ± 0.98                | 0.45 ± 0.03                  |
|                          | 8    | 22.30 ± 0.96                | 0.41 ± 0.05                  |
|                          | 16   | 22.56 ± 0.98                | 0.37 ± 0.04                  |

Fig. 5 Variation in antioxidant activity in bananas treated with different edible coatings expressed as DPPH%. The five groups were treated either with 3% chitosan, 1% chitosan + 2% chitosan and C. limon, 1% chitosan + 2% O. tenuiflorum, 2% chitosan + 2% chitosan and C. limon + 2% O. tenuiflorum, and NTW

Table 4 Antioxidant activity (%) at different times of storage

| Sample          | 3% C | 1% C + 2% CL | 1% C + 2% OT | 2% C + 2% CL + 2% OT | NTW |
|-----------------|------|--------------|--------------|----------------------|-----|
| 0th day         | 74.8 | 74.8         | 74.8         | 74.8                 | 74.8|
| 4th day         | 80.08| 80.08        | 80.08        | 80.08                | 80.08|
| 8th day         | 67.88| 70.07        | 70.07        | 75                   | 70.02|
| 12th day        | 65   | 60           | 60           | 71.6                 | 64.8|
| 16th day        | 50   | 60.05        | 59.9         | 70.08                | 50  |

3.4 Soluble solid content

The soluble solid content of the bananas was assessed during the 16 days of storage. Reasonable assumptions were made for variables such as dehydration and other activities following the ripening process, including decreases in the respiration and transpiration rates and secretion of enzymes, especially hydrolytic enzymes. At the initial time point of the study, the °Brix was measured to be 21.98 ± 0.98. This value did significantly change over the 16 days of storage. Samples of fruit treated with C enriched with extracts from CL and OT had the lowest levels of soluble solid content with an average °Brix value of 22.34 ± 0.34. Meanwhile, the NTW group had an average °Brix value of 23.49 ± 0.67. The titratable acidity was measured to be 0.45 ± 0.03 mg/100 g of the sample during the initial stage of the experiment. These findings were consistent with previous studies of papaya [10–12].

The amount of soluble solids of the bananas during the storage period are shown in Table 3. The analyses were performed at the start of the experiment (day 0) and on days 8 and 16 of storage. The results provide insight into how levels of organic acid decrease over time after the fruit is harvested, which was primarily due to the loss of organic substrates during the process of respiration, transpiration, or any other known metabolic activities. As demonstrated by the data presented in Table 3, there was a significant reduction in acidity in both the coated and non-coated fruit. However, the reduction was less in the coated fruit (C enriched with extracts from CL peels and OT leaves) and greater in the uncoated fruit.

3.5 Antioxidant activity

The antioxidant activity in the fruit was also analysed with the anti-oxidant property being expressed as the percentage as DPPH (Fig. 5). The results were similar to those of the phenolic activity. The samples exhibited an increase in antioxidant activity at day 4 of storage compared to that at day 0. This was then followed with a significant decrease through the end of the experimental period. It is clear from the graph that the antioxidant activity was highest in the 2% C + 2% CL + 2% OT group and lowest in the NTW group. As shown in Table 4, the highest antioxidant activity was observed in the bananas coated with 2% C + 2% CL + 2% OT with an antioxidant activity of 70.08 ± 0.4%. This was followed by the bananas coated with 1% C + 2% CL, which had an activity of 60.05 ± 0.23%. The results support the hypothesis that a coating of C enriched with CL peel extracts and OT leaf extracts could reduce the rate of ripening over a significant period of time, thereby decreasing the degradation and increasing the shelf-life of fruit.

Based on the results regarding ascorbic acid content, phenolic content, antioxidant activity, amount of soluble solids, and...
The results of this study, the optimal composition of the edible coating on bananas with and without the coating. Dramatic differences in the magnitude of values indicate the effectiveness of the edible coating with respect to decelerating the rate of ripening of fruit.

4 Conclusion

In addition to cold storage, which is used in the cold-chain industry, edible coatings are an innovative and unique approach to maintain the quality of fruits and vegetables for extended periods of time. This study demonstrated that a significant increase in the amount of antioxidant activity, ascorbic acid content, soluble solids, and retention of phenolic compounds occurred in bananas coated with the edible coating compared with that of non-coated bananas. Further research can be conducted to determine the levels of anthocyanins and to evaluate additional types of extracts.

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