Influence of Chitosan Coating on Shelf-Life, Biochemical Properties and Nutrient Elements of Carrot (*Daucus carota* L.) during Postharvest Storage

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Author HMZ designed the study, supervise the work, and corrected the final draft of the manuscript. Author SS conducted the experiment, collected samples, data and performed analysis. Author MSR performed data analysis, wrote the first draft of the manuscript and corrected the final draft of the manuscript. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/CJAST/2022/v41i2831796

**ABSTRACT**

Post-harvest loss of different vegetables in Bangladesh is widespread due to the lack of suitable technologies. A laboratory trial was conducted to assess the effect of chitosan coating on weight loss, shelf-life, biochemical qualities and mineral elements of carrots at postharvest storage. At room temperature (=23-25°C), four treatments were selected with different chitosan solutions (0.00%, 0.10%, 0.20% and 0.30%). Matured carrots were collected at 2, 4, 6 and 8 days after postharvest storage (DAPS) for weight loss, while the shelf-life data were measured at 4 and 8 DAPS. On the other hand, carrots were collected at 0 (fresh) and 8 DAPS to determine total sugar, phenolic and mineral contents (Ca, Mg, P, S, Na, and K). Compared to control, the application of 0.30% chitosan coating preserved weight loss by 1.48% at 8 DAPS. The results also showed that applying 0.30% chitosan coating significantly (P ≤ 0.05) extended the shelf-life of carrots up to 35% at 8 DAPS compared to the control. Chitosan coatings also enhanced the loss of total sugars and contents of Mg in carrots during postharvest storage. However, total phenolic contents in carrots
The study concluded that chitosan coating with 0.30% solution might be used to prevent weight loss, extend shelf-life, and improve some nutritional qualities of carrots viz., Ca, Na, K, P and S.

Keywords: Carrot; chitosan; mineral nutrients; storage life; sugar; total phenol.

1. INTRODUCTION

Carrot (Daucus carota L.) is a nutrient-rich root vegetable that belongs to the Apiaceae family. It is one of the widely produced vegetables in winter during the rabi cropping season (September to mid-December) in Bangladesh and is consumed both as salad and cooked vegetables [1]. It contains a greater content of vitamins including carotene (precursor of vitamin A), thiamine and riboflavin, proteins, carbohydrates, fiber, and mineral nutrients such as potassium, sodium and high sugar [2]. It is also rich in phenolic content, which represents the antioxidant compounds in carrots [3]. Due to its high nutritional value, the production and consumption of carrots are increasing gradually both locally and globally. According to the Bangladesh Bureau of Statistics, nationally the carrot was grown on 5085 acres of land with 19246 Metric tonnes of production in the year of 2018 to 2019, whereas the production was 16306 Metric tonnes in the year of 2016 to 2017 [1]. However, to keep up with the pace of the demand throughout the year and to ensure the nutritional status of carrot quality post-harvest storage is inevitable. A daily newspaper reported that “Thousands of tonnes of vegetables and fruits go to waste annually in Bangladesh due to a lack of sufficient technologies and knowledge on post-harvest processes” [4]. The report showed that among the vegetables, postharvest losses in country bean 24.29%, cabbage 24.44% and tomato 27.64% and losses of vegetables at traders’ level are much higher than at farmers’ level.

Besides the degradation during handling, storage and transportation to distribution post; losses due to active metabolism, water transpiration, respiration and spoilage through microorganisms are highly considerable [5]. The loss of water shrinks the vegetables and loss luster and consumer preferences. The microbial infection by several bacteria and fungus causes rotting, discoloration and reduces the market value to a greater extent [6]. Postharvest diseases can be accounted for more than 25% of total yield loss in developed countries and 50% or more in underdeveloped countries [7]. Methods including controlled atmosphere storage, low temperature and edible coating are available to extend the shelf life and maintain the quality of post-harvest vegetables. The consumer acceptability of the addible food coating has increased in recent decades and is used widely around the globe [8-11].

Chitosan is a widely used food coating. Its application has gained popularity for growth and yield of vegetables, and prolonging the post-harvest shelf life of different fruits and vegetables in recent decades [12-16]. After cellulose, chitosan is the second most abundant polysaccharide found in nature which is a deacetylated derivative of chitin [17]. It is known to be biodegradable, easily dissolvable in organic acids, non-toxic, biocompatible, biofunctional, and has strong antifungal and antimicrobial properties [18-21]. Chitosan has also been known to reduce post-harvest water loss from fruits and vegetables by maintaining firmness [22,23]. Study results showed that chitosan coating with a 0.2% solution in tomato fruits extended the shelf-life at postharvest storage by decreasing the weight loss and decay incidence [24]. In vivo investigations have shown that chitosan treatment can delay the postharvest deterioration of fruits and vegetables [24,25].

Despite the many beneficial effects of chitosan on the post-harvest quality of vegetables only a few studies have been conducted so far on the effect of chitosan on the post-harvest quality of vegetables and fruits in Bangladesh [24,26-28]. Moreover, studies are very limited regarding the effect of chitosan on the post-harvest shelf life, biochemical properties and nutrient content of carrots in Bangladesh for its successful application as a vegetable coating. Therefore, this study was designed to assess the influence of chitosan application on the postharvest storage of carrots in Bangladesh and determine its biochemical and nutritional properties.

2. MATERIALS AND METHODS

2.1 Experimental Site

The present investigation was conducted at the Laboratory of Plant Nutrition and Environmental
Chemistry, Dept. of Agricultural Chemistry, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh, from February to June 2020. The main objective of this study was to find out the most suitable application dose of chitosan for postharvest storage of carrots in Bangladesh conditions as well as to study its effect on major biochemical and nutritional properties of carrots.

2.2 Collection and Sorting of Carrots

Twelve (12) kg of fully matured carrots were collected from the farmer’s field of the Sadar Upazila of Mymensingh district. After that, all the carrot samples were transported to the Laboratory of Plant Nutrition and Environmental Chemistry, Dept. of Agricultural Chemistry, BAU, Mymensingh, Bangladesh. Then sorting/screening of carrots was done manually based on their shape, size, and colour. Decomposed and pest-infested carrots were discarded at this stage. Finally, almost similar shapes, sizes, and coloured carrots were chosen for the laboratory trial.

2.3 Treatments and Their Preparation

Chitosan was obtained from Research-Lab Fine Chem Industries, Maharashtra, India (CAS No. 9012-76-4; Deacetylation >80%). Four treatments were selected with different chitosan solutions Control (no chitosan), 0.10%, 0.20% and 0.30% following the method [24] to prepare the treatment solutions.

2.4 Postharvest Application of Chitosan

A total of 5-6 carrots were dipped for 30 seconds in each chitosan treatment, and the same number of carrots were likewise dipped in distilled water with a pH of 5.0 (control). At a temperature of 25°C, all treated carrots were left to air dry for 1 hour. One group was considered a replicate with three replications. There were 12 (4×3) groups of carrots in this experiment. The treated and control carrots were placed in zip-lock bags to keep the relative humidity (RH) between 90 to 95% and then stored at room temperature (25°C).

2.5 Data Recorded at Postharvest Storage

Data on the shelf-life of carrots were recorded at 4 and 8 days after storage (DAS), while the weight loss data of carrots were measured at 2, 4, 6 and 8 days after post-harvest storage. For chemical analyses, two carrots from each replication were randomly selected at 8 DAS. However, the shelf-life of carrot was measured by static testing, where carrot samples were left on a dark shelf to rot and were then visually counted until 40-50% of a carrot was rotten at the respective storage time (in days).

\[ \text{Shelf life (\%) = } \frac{\text{No.of carrot decays at storage}}{\text{Total no.of carrots in storage}} \times 100 \]

2.6 Measurement of Biochemical Quality of Carrots

Two (2) carrots samples from each replication were collected at 8 DAS to determine total sugar and phenol contents. Total phenol estimation in carrots was carried out with the Folin-Ciocalteau reagent [29]. The concentration of phenols in carrots was calculated against the catechol standard curve and expressed as mg phenols/100 g material.

Total sugar content in carrots was estimated by determining the volume of the unknown sugar solution required to reduce a measured volume of Fehling’s solution [29]. Representative carrot slice was extracted using methanol solution at a 1:10 ratio and distilled water was used to make the final volume of 50 mL. Then 1.0 mL of extract was evaporated using a water bath near to dryness. Exactly 50 mL of distilled water was then added to dilute and dissolve the remaining content. 4 mL anthrone reagent was added to a test tube containing 1.0 mL of sample solution and heated for 5 minutes in a boiling water bath. Then a UV-spectrophotometer was used to record absorbance at 620 nm wavelength [29]. The external standard was sourced from glucose, and gram glucose equivalents per 100 g of fresh carrot (g/100 g FW) were used to express the total sugar content.

2.7 Measurement of Mineral Elements of Carrots

To determine different mineral elements (Ca, Mg, Na, K, P, and S), collected carrots samples were chopped into small pieces with a sharp stainless-steel knife and dried for roughly 72 hrs in an electric oven at 50°C temperature. The samples were then pulverized in a grinding mill and utilized to make an extract by wet oxidation technique using a di-acid mixture [30]. Among the mineral elements, Ca and Mg were assessed titrimetrically, P and S were measured by
spectrophotometry, and Na and K were estimated by flame photometry [30].

2.8 Statistical Analysis

The data were statistically analyzed using the software package Minitab 17 following analysis of variance (ANOVA) and a general linear model. The least significant differences (LSD) were used to separate treatment means at the 5% level of probability at a specific time of data collection [31].

3. RESULTS AND DISCUSSION

3.1 Weight Loss of Carrots at Storage

The addition of chitosan had no significant effect on reducing the weight loss of carrots most of the time compared to the control except by 0.30% chitosan solution at 2 DAPS (Fig. 1). The addition of 0.30% chitosan solution significantly (P ≤ 0.05) reduced the weight of carrots compared to control at 2 DAPS. In general, the addition of chitosan reduced the weight loss of the carrot and prolonged the post-harvest storage time ranging from 4.61% weight loss to 25.09% compared to control which always had the highest percentage of weight loss ranging from 5.81% to 25.09% most of the time except by 0.10% chitosan solution at 2 DAPS. Among all the chitosan treatments the rate of weight loss of carrots decreased as the rate of chitosan increased from 0.10% to 0.30%. At all times, the lowest weight loss was recorded by the 0.30% chitosan solution ranging from 4.61% to 23.61% weight loss at 2 to 8 DAPS, respectively. An almost similar reduction in weight loss has been reported after the addition of chitosan in tomato and grapefruit stored at room temperature [24,32]. Chien et al. [33] found that citrus fruits covered with low molecular weight chitosan reduced weight loss considerably. They also reported that postharvest water retention prevents quick deterioration of fruits due to shriveling and that postharvest water loss may modify metabolism and, in certain cases, increase fruit ripening before shriveling becomes visible. As a result, limiting water loss from the fruit during storage or ripening aids in preserving fruit quality. However, the present study results inferred that postharvest application of chitosan coating could be used to prevent the weight loss of carrots.

![Fig. 1. Effect of chitosan application on weight loss (%) of carrots on different days after post-harvest storage (DAPS) at room temperature. Each value is the mean for three replicates, and the vertical bar indicates the standard error. Red-colored bars are indicating the least significant difference (LSD) value at P-value ≤ 0.05]
3.2 Shelf-life of Carrots at Storage

Compared to the fresh carrots (0 DAPS), the shelf life of all the carrots with all the treatments decreased as the time of storage progressed (Fig. 2). However, the addition of chitosan significantly (P ≤ 0.05) reduced the loss of shelf-life of carrots compared to the control most of the cases except by 0.20% chitosan solution at 4 DAPS (Fig. 2). At 4 DAPS the loss of shelf-life of carrots showed no significant difference compared with control. The control had the highest loss of shelf-life of carrots at both the 4 and 8 DAPS recording 94.30% and 41.70% shelf-life, respectively whereas the lowest loss of shelf-life was observed by 0.10% chitosan solution at 4 DAPS (99.30% shelf-life) and by 0.20% chitosan solution at 8 DAPS (88.70% shelf-life). However, no significant difference in shelf-life of carrots was observed among 0.10%, 0.20% and 0.30% chitosan solution treatments at both 4 DAPS and 8 DAPS. Sultana et al. [24] reported a similar finding that the addition of chitosan during the postharvest storage increased the shelf-life of the tomatoes. According to Liu et al. [34], gray mold (caused by Botrytis cinerea) and blue mold (caused by Penicillium expansum) in tomato fruits stored at room and refrigeration temperature have been greatly reduced by the application of 0.5 and 1% chitosan solution. Furthermore, Romanazzi et al. [35] stated that both preharvest and postharvest application of chitosan had shown promising effects in disease control. They also stated that chitosan has a dual mechanism of action on pathogens and plants. According to their findings, chitosan inhibits the growth of decay-causing fungus and foodborne pathogens and induces resistance reactions in the tissues of the host plant. Thus, the study results suggest that chitosan coating could be utilized to improve the shelf-life of carrots during postharvest storage, which could be owing to chitosan’s ability to suppress postharvest damage of carrots due to various pathogens/microbes.

3.3 Biochemical Properties of Carrots at Storage

3.3.1 Total sugar content

The total sugar content of the carrots decreased over time ranging from 2.02% to 2.19% during storage compared to the fresh carrot (2.62%) (Table 1). Chitosan addition at the rate of 0.10% and 0.20% was recorded with a higher sugar content ranging from 2.14 to 2.19% compared to the control (2.05%) whereas 0.30% chitosan solution was recorded with a slightly lower sugar content (2.02%). However, no significant difference was observed in the sugar content of carrots among any of the treatments. The degradation of sugar during post-harvest storage of carrots has also been reported in previous studies [36-37]. Similarly, the addition of chitosan was also reported to reduce sugar loss in pears compared to the control [38]. Respiration has been accounted as the main reason for the loss in sugar content during storage in carrots. Sugar is utilized as a source of energy during respiration and other metabolic activities [36]. However, the chitosan coating is responsible for reducing respiration and other metabolic activities in carrots during storage [39] which was also may be the case in this study with the addition of chitosan.

3.3.2 Total phenolic content

Similar to sugar content, the phenolic content of the carrots decreased over time ranging from 0.48 to 0.78 mg/100 g in all treatments compared to fresh carrots (0.90 mg/100 g) (Table 1). All the chitosan treatments significantly (P ≤ 0.05) reduced the phenolic content of carrots compared with control at 8 DAPS. There was no significant difference in phenolic content of carrots observed between the 0.10% and 0.20%; 0.20% and 0.30% chitosan solution. The lowest phenolic content was recorded in 0.30% chitosan solution (0.48 mg/100 g) and was significantly (P ≤ 0.05) different compared to control and 0.10% chitosan solution. The decrease of phenol content in carrots during storage has also been reported by Haq and Prasad [3]. Similar results were also reported for long-time storage of beans by Coelho et al. [40]. In addition to storage time, the phenolic content of vegetables during storage depends on the storage environment, methods, processing and genotypes. The storage of carrots with low oxygen has been reported to reduce phenolic content [41]. Thermal processing reduced the number of phenolic compounds, tannins, and antioxidant activity in vegetables [42]. Beninger et al. [43] reported that the total phenolic content of several pinto bean genotypes fluctuated significantly during storage. Thus, it can be inferred that several factors, viz. storage, genotypes, processing methods, and environmental conditions, play a vital role in the total phenolic contents of carrots.

3.4 Mineral Element Contents of Carrots at Storage

The addition of chitosan coating increased the nutrient contents (Ca, Mg, Na, K, P and S) in
carrots at 8 DAPS compared to the fresh carrots (0 DAPS) (Table 2). The chitosan coating significantly (P ≤ 0.05) increased the Ca content in carrots compared to the control whereas showed no significant difference in the case of P and S. The addition of 0.30% chitosan solution recorded significantly lowest Mg contents in carrots at 8 DAPS whereas significantly highest in case of Na and K content. According to Leclerc et al. [44], the carrot contains 0.34-0.37% Ca, 0.12-0.17% Mg, 2.80-3.21% K and 0.30-0.34% P depending on the types of fertilization. The nutrient content in carrots was slightly different in this study compared to the report by Leclerc et al. [44] which may be due to the difference in the genotype and variety. A similar finding was reported by Khazaei and Vandenberg [45], where they stated that mineral nutrient contents in the bean were different depending on the genotype, environmental variation, and their interactions. The present study also showed minimum changes in the nutrient content of the carrot with chitosan treatments which may also be evident that chitosan can be safely added to the carrots during post-harvest storage as a food coating. Besides, various types of chitosan from different manufacturers have varying physicochemical properties and perhaps from multiple sources, which might contribute to the increase of nutrient contents upon addition to the carrots/vegetables.

3.5 Correlations among the Nutritional Qualities of Carrots

Pearson correlation coefficients among the nutritional qualities of carrots harvested at 8 DAPS are summarized in Table 2. Total phenol content of carrots showed a significant negative correlation with the Ca, Na and K. Thus, it can be inferred that total phenol contents in carrots were negatively affected by the contents of Ca, Na and K at 8 DAPS. On the other hand, the total sugar content of the sugar carrots showed no correlation with any of the nutrient elements determined in this study. Among the nutrient elements Ca showed a significant positive correlation with Na, K and P. In addition, Na showed a significant positive correlation with K, P and S; K showed a significant positive correlation with P and S; P showed a significant positive correlation with S. Magnesium showed a negative relationship with all other nutrient elements of carrots, and among those, the relationship in between Mg and S was significantly negative (r= -0.562*). However, such inverse relationships indicated that the content of these mineral elements is moved in the reverse direction, i.e., when the content of any one of these elements is increased, the other is decreased with the same magnitude and vice-versa.

![Fig. 2. Effect of chitosan application on shelf-life (%) of carrots on different days after post-harvest storage (DAPS) at room temperature. Each value is the mean for three replicates, and the vertical bar indicates the standard error. Red-colored bars are indicating the least significant difference (LSD) value at P-value ≤ 0.05](image-url)
Table 1. Effect of chitosan application on biochemical properties (total sugar and total phenol) and concentration (±SE) of different mineral elements of carrot samples (Ca, Mg, Na, K, P, S) at 8 days after post-harvest storage (DAPS) at room temperature. Different letters indicating statistical significance at P-value ≤ 0.05

| Treatments (Chitosan solution) | Biochemical properties | Nutrient content (%) in carrots | Ca | Mg | Na | K | P | S |
|-------------------------------|-----------------------|--------------------------------|----|----|----|----|----|----|
|                               | Total Sugar (%)       | Total Phenol (mg/100 g)       |----|----|----|----|----|----|
| Control                       | 2.05± 0.30a           | 0.78± 0.02a                   | 0.26± 0.01c | 0.26± 0.01a | 0.61± 0.02c | 1.09± 0.07b | 0.23± 0.01a | 0.093± 0.003a |
| 0.10%                         | 2.14± 0.24a           | 0.56± 0.02b                   | 0.35± 0.02b | 0.26± 0.01a | 0.70± 0.03bc | 1.14± 0.05b | 0.23± 0.01a | 0.096± 0.003a |
| 0.20%                         | 2.18± 0.15a           | 0.51± 0.03bc                  | 0.40± 0.01a | 0.27± 0.01a | 0.77± 0.03ab | 1.36± 0.06a | 0.24± 0.01a | 0.097± 0.006a |
| 0.30%                         | 2.02± 0.18a           | 0.47± 0.02c                   | 0.41± 0.01a | 0.22± 0.01b | 0.86± 0.03a  | 1.50± 0.07a | 0.27± 0.02a | 0.095± 0.005a |
| LSD*                          | 0.64                  | 0.068                         | 0.037 | 0.026 | 0.082 | 0.182 | 0.043 | 0.012 |
| Fresh (0 DAPS)                | 2.62± 0.12            | 0.90± 0.02                    | 0.28± 0.01 | 0.22± 0.01 | 0.54± 0.04 | 1.02±0.05 | 0.22±0.01 | 0.020±0.003 |

LSD: Least significant difference at P-value ≤ 0.05

Table 2. Pearson correlation coefficients for nutritional qualities of carrots collected at 8 days after postharvest storage (DAPS) (n=12)

| Total Sugar | Total Phenol | Ca | Mg | Na | K | P | S |
|-------------|-------------|----|----|----|----|----|----|
| Total Sugar | -0.078*     | 0.113* | 0.153* | 0.938 | -0.285* | 0.968*** | 0.853*** |
| Ca          | -0.927***   | 0.003* | 0.871*** | -0.212* | 0.968*** | 0.504* | 0.542* |
| Mg          | 0.322*      | 0.201* | -0.153* | 0.968*** | 0.504* | 0.542* | 0.733*** |
| Na          | -0.821***   | 0.008* | 0.871*** | -0.212* | 0.968*** | 0.504* | 0.542* |
| K           | -0.705***   | 0.008* | 0.871*** | -0.212* | 0.968*** | 0.504* | 0.542* |
| P           | -0.429*     | 0.008* | 0.871*** | -0.212* | 0.968*** | 0.504* | 0.542* |
| S           | -0.195*     | -0.114* | 0.610* | -0.076** | 0.796*** | 0.853*** | 0.733*** |

* = non-significant; ** indicating significant at P<0.01, *** significant at P<0.05, * significant at P<0.1
4. CONCLUSION

The application of chitosan coating at different doses showed a remarkable positive effect to preserve weight loss and increase the shelf-life of carrots. Similarly, chitosan coatings also reduce the loss of total sugar contents in carrots during postharvest storage. Furthermore, the study found that chitosan coatings have the potential for enhancing some mineral nutritional aspects. However, total phenolic contents in carrots decreased considerably at storage, and the application of chitosan was unable to protect them. So, it can be inferred from this study that chitosan coating with 0.30% solution may be used to prevent weight loss, extend shelf-life, and improve some nutritional qualities of carrots. However the acceptability of chitosan coating is limited among the people and farmer of developed and developing countries. Consumer acceptance of such coated fruits and vegetables will need to be investigated in the future, and ‘chitosan coating is non-toxic and safe’ - a message must be circulated through print and electronic media.

ACKNOWLEDGEMENT

This work was financially supported by the Bangladesh Agricultural University Research System (BAURES), Mymensingh-2202, Bangladesh, for 2019-21 under Project no. 2019/10/BAU.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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