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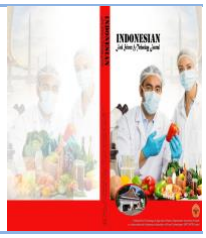

Biosynthesis of ZnO Nano-particle and its quality evaluation on the shelf life extension of fruit

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***Abstract*— Consumers worldwide desire fruits of high quality that are free from chemical preservatives and boast an extended shelf life. Edible films and coating received a considerable amount of attention in recent years because they are beneficial over synthetic packaging. Prolonging of shelf life of food is an important goal to be attained. Many storage techniques have been adapted to extend the marketing distance and holding periods for commodities after harvest. Edible coatings are thin layers of edible material applied to the product surface to provide a barrier to moisture, oxygen, and solute movement for food. The purpose of this study was to produce bio-synthesized ZnO Nano-particles from spinach. The coating solution is prepared by mixing Nano-particles with chitosan-acetic acid solution and evaluates the shelf-life after treatment as a coating. The study showed that coated fruit maintained its quality up to 28 days of the study period. Thus, it can be concluded that ZnO Nano-particles can be used as a coating for increasing shelf-life.**

***Keywords*— Orange (*Citrus sinensis*), spinach (*Spinacia oleracea*) leaves, coating, polyphenols**

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I. INTRODUCTION

Bangladesh produces a huge amount of fruits and vegetables every year as we know that Bangladesh is an agricultural country. Directly or indirectly more than 80 % of people depend on the agricultural sector. Fruits and vegetables are one of the important sectors in Bangladesh. But they are highly perishable due to high water content. So they are required to be properly handled after harvesting. Many kinds of fruits and vegetables are produced in Bangladesh. Post-harvest losses of fruits and vegetables are huge and it is almost 18 to 44 percent dependent on varieties. The number of losses is 30 %, 24 %, and 20 % for the banana, pineapple, and orange respectively [1]. Vitamin-C content of fruits and vegetables is reduced by 18-21 % after 96 h of harvesting [1]. Daily consumption of fruit and vegetables is 126 g/day/capita which is lower than the daily minimum intake limit of 400 g/day/capita in Bangladesh [2]. Post-harvest losses of fruits are caused by mishandling. Spoilage, pest infestation. Approximately 25 % of fruits are lost after harvesting. If post-harvest losses are reduced, then sufficient food is available. Reducing post-harvest losses and maximizing the shelf life can distribute the food evenly. Many methods are used to control post-harvest losses. They are cold storage temperature, controlled or modified atmosphere storage, irradiation, chemical treatment, and coating [3].

One of the citrus fruits is orange which is grown in some parts of Bangladesh. Orange belongs to the family Rutaceae. Orange is enriched with vitamin C, calcium, magnesium, etc. Orange is also spoiled by delayed selling (2.1 %) and spoilage (2.1 %) which is noticeable. Based on the retail price post-harvest losses

of oranges are approximately 1.12 crore taka. Many types of oranges are cultivated in Bangladesh. They are Mandarin, Chinese, and some local variables such as Sylheti.

The coating is used to delay ripening, better appearance, retard moisture migration, and retard respiration [4, 5]. In recent years, attention has been given to the study of biopolymer films like polysaccharides to reduce crop losses and regulate the quality of fresh fruits for a prolonged shelf life. Chitosan is one of the biopolymers which is used for coating because of its biodegradability, biocompatibility, and non-toxicity. Chitosan has been remarked as safe by the Food and Drug Administration (FDA) [6]. Chitosan is a polysaccharide that is obtained from the deacetylation of chitin. Chitin is the second most abundant polysaccharide after cellulose. It is found in many sources [7]. Chitosan is non-toxic, film-forming, and easily detroit. It has antimicrobial properties and can form a semi-permeable film. Prolonged shelf life has been observed in fruits and vegetables coated by chitosan [8]. Commercial chitosan is easily blended with water [9]. Other than that Chitosan can easily be mixed with other polymers and antimicrobial agents such as Gallic acid [10], and silver nanoparticles [11]. Premanathan [12] found that metal oxides such as zinc oxide (ZnO) and magnesium oxide (MgO) have higher antibacterial properties and higher stability compared to other antimicrobial agents.

Zinc oxide is less toxic than other nanoparticles such as silver nanoparticles. In recent years, Zinc oxide has become popular. ZnO nanoparticles can inhibit microbial growth. It is now widely used in the food industry. It is approved by the U.S. Food and Drug Administration [13]. ZnO is obtained in two ways. One of them is commercial ZnO which is available on the market and another is biosynthesized ZnO nanoparticles. ZnO can be biosynthesized from many sources such as Aloe Vera, spinach, etc. In this context, the objective of this research is to biosynthesis of ZnO nanoparticles from spinach and incorporated with chitosan and applied on the orange and the effect on its quality and to determine the shelf life of the treated and control samples.

II. MATERIAL AND METHODS

A. Sample collection

Freshly harvested orange (*Citrus sinensis*) is collected from a local wholesale market of Sylhet. Visually blemished, diseased, damaged oranges had been removed for their vulnerability to microorganisms. For treatment, 5 samples were used.

B. Preparation for Biosynthesis of ZnO Nano-particle

The procedure of biosynthesis of ZnO is followed by a previous work [14]. Fresh spinach (*Spinacia oleracea*) leaves are collected from the market. Leaves are washed thoroughly to remove dirt and kept in the freezer for 24 hours. Leaves are dried in the freeze-drieder for 6 hours. Dried leaves are pulverized in the blender. Separate the powder by their size using a mesh analyzer. 5 g powder (100 mesh size) is dissolved in 100ml ethanol and kept for 24 hours. The solution is filtered by filter paper (Whatman No.1). 1Mm Zinc nitrate hexahydrate was prepared using distilled water as a precursor and added to leaf extract. The solution is boiled for 30 minutes or till the color changes. The solution was centrifuged at 3000 rpm for 15 min and the supernatant was collected.

C. Identification of ZnO nanoparticle

ZnO nanoparticles were identified by two methods. One of them is color changes and another is wavelength using a UV-visible spectrophotometer. Wavelength is generally varied from 370 to 400 nm.

D. Preparation of coating

The procedure was followed by previous works [14]. 5 g chitosan is dissolved in 300ml of 1% acetic acid. The solution is boiled at 60 degrees Celsius for 6 hours. The solution is filtered by using filter paper. 5 % ZnO nanoparticle solution is added. The coating solution was subjected to ultra-sonication for 30 min.

E . Coating of ZnO nanoparticles on orange

The collected samples were washed in water. Then the selected fruits were dipped in 100 ml prepared solution for coating. The treated and untreated (control) are kept at room temperature for further experiments.

F. Physiochemical Analysis

The physicochemical properties of samples were measured as described below:

1. *pH*

The choice of the pH was made by preparing a buffer at pH 7.0 and the temperature adjusted to 28 °C, the glass electrode was standardized with a standard buffer solution with the electrode was rinsed with distilled water before inserting it into the sample solution and pH.

2. *Total Soluble Solid (TSS)*

TSS of sample juice was determined by using a hand refractometer and the data were recorded as degree Brix.

3. *Weight loss*

The physiological loss of mass was calculated according to the procedure [15]. Weight loss was determined considering the fresh weight at harvest using a balance with an accuracy of 0.0001 g. Weight loss was then calculated from the weight of each sample measured before storage and after 7,10,13 days.

$$\% \text{ Weight loss} = \frac{\text{initial weight} - \text{Final weight}}{\text{initial weight}} \times 100\%$$

4. *Color*

The color of the sample peel was measured using a hunter color spectrophotometer (model-PCE-CSM4). The color parameters L* indicates the degree of lightness, a* indicates the degree of redness/greenness, and b* indicates the degree of yellowness/blue. The instrument was standardized before use. After standardized using instrument L*, a* and b* values were measured by [16].

G Determination of bioactive components

Total Phenolic Content (TPC)

The total phenolic content which is in the sample extraction was evaluated using the Folin–Ciocalteau assay [17] with a bit of modification. For the experiment, 20 µL of extract, Gallic acid standard which is a blank solution were taken in different test tubes and 1.58 mL of distilled water was added in each of them, then 100 µL of Folin–Ciocalteau reagent, blended well and in between 8 min, 300 µL of sodium carbonate was mixed. Then samples were vortexed at once and the samples were kept in the dark place for 30 min and temp. should be. The absorbance was taken at 765 nm in the UV-Vis spectrophotometer (Model-UV-1800, Shimadzu Scientific Instruments, Japan). Results were calculated in mg/Gallic acid and calculation was done using the Gallic acid standard curve.

Antioxidant activity (DPPH radical Scavenging)

The antioxidant activity of each sample was analyzed by DPPH scavenging activity and the stable DPPH radical-scavenging activity was calculated using the little bit modified method which is described by [18]. Briefly. 2 ml of 0.2 mg methanol DPPH solutions were mixed into 2 ml of extract solution in different concentrations and then the contents were mixed vigorously for 15 s. After that solutions were kept at a dark place for 10 min for at room temperature for the reaction to occur. And then when 10 min is over. The absorbance was measured of a blank solution at 517 nm with a UV-Vis spectrophotometer. The percentage of DPPH radical-scavenging characteristics of each sample solution was calculated as:

DPPH radical-scavenging activity (%I) = $\frac{A_{\circ}-A}{A_{\circ}} \times 100$

Where $A_{\circ}$ = absorbance of the blank; A = absorbance of the sample.

Ascorbic Acid (AA)

This experimental system was used to calculate the vitamin C or Ascorbic acid in the orange juice sample. This procedure was proposed by [19]. The orange sample 1ml was homogenized with 10 ml 0.056 M sodium oxalate for 2 minutes. The mixture of extraction was left resting for 5 min. Then the mixture was filtered and then 0.5 mL was diluted to 5mL with sodium oxalate. Absorbance was taken at 266nm at $25^{o}c$ in UV-Vis spectrophotometer. The blank solution is made of 0.056 M sodium oxalate. L-ascorbic acid was taken as a standard calibration curve.

H. Statistical analysis

SPSS software (SPSS Inc., Chicago, IL, USA) was used for analyzing the results and reported as the mean ± standard deviation (SD).

III. Result and Discussion

A. Identification of nanoparticles

Nanoparticles can be identified by color and another one is a UV-visible spectrum. Initially, the color solution is green, and after giving heat sometimes the color changes to yellow [14]. The bands of zinc oxide nanoparticles were observed at 374.3 nm. Zinc oxide nanoparticles band were observed at 374 nm and in another case band were observed at 368 nm.

B. Effect of coating on physicochemical characteristics

The effect of the coating on physicochemical characteristics is determined by a different factor such as pH, TSS, Weight loss, and Color loss shown in **Table 1**.

C. Effect of coating on physicochemical characteristics

From the result, Coated fruits were observed to have a lower pH than the control fruits. The same results were observed in papaya (*Carica papaya*) [20]. Coating fruits were shown to maintain a lower pH than the control fruits. Increasing pH value can be due to respiration due to the breakup of acids during storage [21]. Fruits that are coated moderate the change of pH. Moderating the change of pH effectively increased the product's shelf life.

TABLE 1.
EFFECT OF COATING ON PHYSICOCHEMICAL PROPERTIES

Days	Coating treatment	pH	TSS	Weight loss	L*	a*	b*
0	Control	3.58±0.03	5.2±0.06	-	43.26±2.17	11.68±0.43	38.04 ± 1.48
7	Control	4.09±0.02	5.1±0.05	5.31±0.09	42.67±2.07	13.81±0.42	37.02 ± 1.83
	Coating	4.01±0.01	5.2±0.07	4.84±0.05	37.15±1.87	17.11±0.53	31.57 ± 1.17
10	Control	4.91±0.04	5.2±0.02	7.19±0.11	39.78±1.78	15.88±0.72	35.06 ± 1.38
	Coating	4.86±0.03	5.4±0.06	5.19±0.03	31.54±1.06	18.92±0.87	29.41 ± 0.96
13	Control	5.46±0.06	5.25±0.03	10.64±0.13	36.14±1.24	17.56±0.71	34.49 ± 1.29
	Coating	5.02±0.05	5.50±0.07	6.18±0.07	30.08±0.92	20.07±0.94	26.72 ± 0.78

Values in the table presented as mean ± Standard deviation of triplet evaluation.

Total soluble solids (TSS)

Coated fruits were observed to have a higher TSS than the control fruits. Similar results found in Orange (*Citrus*

sinensis) [22] and Mango (*Mangifera indica*) [5]. The increasing rate of TSS because of the dissolving of some components. Among of dissolving components, sugar content is high. Because carbohydrate converts into sugar which is the result of increased respiration rate and using Metabolites [14].

Weight loss

Coated fruits were observed to have a lower weight loss than the coated fruits. The same result was observed in coated orange founded by [22]. The coated material is acted as a semi permeable layer probably which is the reason to lose less weight loss. Weight is lost due to the transmission of oxygen, carbohydrate, water, and respiration [22]. Fresh kiwi fruits which are coated with ZnO Nano-particles have observed to reduce gas exchange and the same results as this experiment [23].

Color loss

The value of L*, a*, and b* were recorded. It was observed that L* and b* values decreased and a* values were increased. Reduction of chlorophyll can be the reason of decreasing value of L* and b* and increasing value of a*. The same type of result was found on papaya (*Carica papaya*) fruits [24] and mango (*Mangifera indica*) fruits [25]. Thus, coating decreased the respiration rate.

C. Effect of coating on bioactive components

The effect of the coating on bioactive components is determined by a different factor such as TPC, DPPH, RSA and Ascorbic acid shown in **Table 2**.

Total Phenolic Content (TPC)

Phenol is one of the important ingredients which is found in an orange. Phenol has become important due to having a prevention capability of various human diseases. Synthesis of a phenolic compound is started during the maturation and the process is going after harvesting [26]. From the result, it is clearly observed that TPC is decreasing is day by day. Decreasing of total phenolic content is due to the braking of structural components of cells. A similar result has been shown in the experiment on orange (*Citrus sinensis*) [27]. Other than orange, same result was observed in fruits [28].

TABLE 2
EFFECT OF COATING ON TPC, DPPH RSA AND ASCORBIC ACID

Days	Coating treatment	TPC (mg/100g)	Changes (%)	DPPH RSA (%)	Changes (%)	Ascorbic acid (mg/100g)	Changes (%)
0	Control	70.09 ± 2.19	-	75.17 ± 2.12	-	48.25 ± 1.79	-
7	Control	20.61 ± 0.96	0.00	67.11 ± 2.06	0.00	35.67 ± 1.51	0.00
	Coating	46.21 ± 1.26	124.21	69.21 ± 2.43	3.13	46.66 ± 1.06	30.81
10	Control	15.13 ± 0.87	0.00	65.15 ± 2.03	0.00	33.24 ± 1.12	0.00
	Coating	45.21 ± 1.76	198.81	67.5 ± 2.34	3.61	42.28 ± 1.43	27.2
13	Control	7.38 ± 0.41	0.00	60.24 ± 2.16	0.00	31.25 ± 1.28	0.00
	Coating	40.25 ± 1.68	445.39	64.09 ± 2.18	6.39	36.64 ± 1.56	17.25

Values in the table presented as mean ± Standard deviation of triplet evaluation. TPC- Total phenolic content (mg/100g); RSA- Radical Scavenging Activity (%); Ascorbic acid (mg/100g).

DPPH radical scavenging activity (RSA)

An antioxidant is responsible for the freshness of the human cells. DPPH is seen on different days. DPPH is changed and having a lower concentration than previous days. Changing of DPPH in control is bigger than the coating. DPPH or antioxidant activity is observed to decrease with ripening were found in [29].

Ascorbic Acid (AA)

In fresh orange, the amount of AA is the highest. With passing time, the freshness is reduced and AA is also reduced. Ascorbic acid is reduced with time. The edible coating is acting as an active barrier for fruits. The coating prevents the fruits to expose in the sunlight and oxygen which leads to less decreasing AA of coated fruits than control fruits [30]. A similar result was observed in orange (*Citrus sinensis*) [22]. In another study, similar results were also observed on coated mango (*Mangifera indica*) [3].

D. Visual representation of shelf life of orange

Visual observations are done at different days in **Fig. 1**. From the observation, it has been clearly seen that coated fruits have a glossy appearance which makes the fruits appealing. A similar observation is found on mango (*Mangifera indica*) [5]

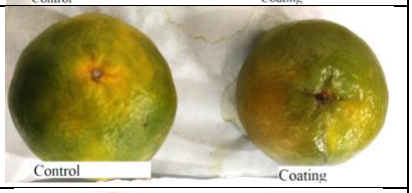
Days	Control vs Coating
7	
10	
13	
28	

Fig. 1 Visual representation of shelf life of orange

IV. CONCLUSION

This study found that coated fruits reduce weight loss, reduce color change, reduce microbial attack, reduce total soluble solids compared to uncoated fruits. Thus, it is concluded that using nanoparticle zinc oxide can be beneficial for maintaining quality during storage and reducing post-harvest loss of orange.

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