



Research Article

Chitosan-Based Zinc Oxide and Silver Nanoparticles Coating on Postharvest Quality of Papaya**S. Timilsina^{1*}, R. Adhikari², P.P. Khatiwada³, G. Devkota² and K.C. Dahal¹**¹Institute of Agriculture and Animal Science (IAAS), Tribhuvan University, Nepal²Research Center of Applied Science and Technology (RECAST),
Tribhuvan University, Nepal³Value Chain Development Project (VCDP), Lalitpur, Nepal**Abstract**

The study aimed to investigate the effects of chitosan-based zinc oxide and silver nanoparticles coating on physicochemical properties of papaya and to optimize nanoparticles concentrations for extending its postharvest shelf life. The experiment was conducted in February, 2022 at the Institute of Agriculture and Animal Science (IAAS), Tribhuvan University, Kathmandu, Nepal, using a Completely Randomized Design (CRD) with nine treatments and three replications. The treatments consisted of different concentrations (50 ppm, 100 ppm, 150 ppm, and 200 ppm) of either Zinc oxide and silver nanoparticles, along with a control. Zinc oxide and silver nanoparticles were synthesized from banana peel extract and the average crystalline sizes were found to be 22.30nm and 4.46nm, respectively. Fruits were dipped in 1.5% (w/v) chitosan with 50–200 ppm of zinc oxide and silver nanoparticles and then stored at ambient temperature ($14\pm 1.6^{\circ}\text{C}$) and $\sim 75\%$ RH. The nanoparticles coatings slowed changes in total soluble solids (TSS) and total titratable acidity (TTA), delaying ripening. Treated fruits showed reduced physiological loss in weight and maintained firmness better than control, with firmness decreasing to 4.79 kg in untreated fruits compared to 7.35 kg in coated fruits on 18th day of storage. Coated fruits retained more ascorbic acid, and the storage life increased by 8 and 9 days with 150 and 200 ppm treatments, respectively. However, consumer preference declined for concentrations above 150 ppm. Thus, 100–150 ppm chitosan-based zinc oxide/silver nanoparticles are found to be effective for extending shelf-life and maintaining physiochemical characteristics of papaya. However, its residual and health-related dimensions need to be extensively studied before its commercial application.

Keywords: Chitosan, Coating, Nanoparticles, Postharvest

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Introduction

Papaya (*Carica papaya* L. $2n = 18$) is widely cultivated tropical fruit known for its nutritional and economic importance. It is native to Central America but now grown in tropical and subtropical regions of South America, Africa and Asia (Ming et al., 2008). It is a climacteric fruit, continues to ripen after being harvested, with a high respiration rate and significant ethylene production during the ripening process (Zhu et al., 2019). Perishability of papaya coupled with improper handling, poor storage conditions and its susceptibility to fungal diseases reduces the postharvest life and quality of fruits leading to higher postharvest losses. It has been reported that the average postharvest loss in fruit and vegetables are about 20-40% (El-Ramady et al., 2015), but the postharvest loss in papaya is much higher i.e., 40-100% (Vyas et al., 2014). These losses negatively affect all stakeholders in the supply chain, from growers to consumers (Shiga et al., 2009). Therefore, the shelf-life prolongation of papaya has become an essential issue to be addressed.

Applying safe, edible coatings has shown promise in extending the self-life of fruits. These coating form a protective layer that slows respiration and transpiration rates, reduces deterioration process, and preserves the internal quality (Joshy et al., 2020). Coatings also help to retain structural integrity and firmness, protect volatile compounds responsible for flavor and aroma and improve aesthetic appeal by covering physical damage and enhancing shine (Murmu and Mishra, 2018). Edible coating can be formulated with various materials such as polysaccharides, lipids, protein, and resin (Murmu and Mishra, 2018). Among these materials, polysaccharides like chitosan, alginate, carrageenan, carboxymethyl cellulose and pectin, being widely used due to their renewability, biodegradability and biocompatibility (Zhao et al., 2021). Although, polysaccharide-based coating provides an effective barrier to gaseous exchange, creating a modified atmosphere around the fruit, they typically have weak moisture barriers and limited mechanical strength (Meindrawan et al., 2018). This makes polysaccharide alone less suitable for high moisture contenting fruits like papaya (Yousuf et al., 2018).

Nanoparticles (NPs) can be added to enhance the performance of polysaccharide-based coating. The use of NPs in food science has grown globally in recent years (Bouwmeester et al., 2014). Adding NPs, such as zinc oxide (ZnO) and silver (Ag), to polysaccharides coatings can greatly improve mechanical strength, structural integrity, and barrier properties, while also providing antimicrobial effects (Butu et al., 2019). This combination of NPs and polysaccharide-based coating offers a promising approach for extending papaya's postharvest life, benefiting producers and traders by reducing losses. This study investigates the potential of ZnO and Ag NPs in enhancing the shelf life of papaya.

Materials and Methods

The experiment was conducted at the Laboratory of the IAAS, Tribhuvan University, Nepal (located at 27°39' latitude and 85°16' longitude, with an altitude of 1300 meters) during February, 2022.

Synthesis and Characterization of Nanoparticles

Preparation of Banana Peel Extract

Two hundred gram of banana peel was heated with 1000 ml of distilled water at 70°C for 60 min on a magnetic stirrer. The extract was cooled and filtered through cheesecloth to remove water-insoluble fractions, then stored at 4°C for further use.

Green Synthesis of Nanoparticles

Zinc Oxide Nanoparticles

A 0.1M zinc acetate dihydrate solution was mixed with banana peel extract in a 9:1 ratio and heated at 70°C under constant stirring at 1000 rpm. A 0.1M sodium hydroxide (NaOH) solution was added dropwise to the mixture until it turned pale yellow and a white precipitate formed. The mixture was then cooled and filtered using Whatman No. 1 filter paper. The residue was washed three times with distilled water, dried at 40°C for 24 hours, and ground into a fine powder.

Silver Nanoparticles

A 0.1M silver nitrate solution was heated to 70°C under constant stirring at 1000 rpm. Banana peel extract was added dropwise until the solution turned brown. The mixture was then incubated in the dark for 48 hours. After incubation, it was centrifuged at 8000 rpm for 50 minutes at 4°C and washed three times with distilled water. The resulting Ag NPs pellets were stored in a desiccator for 72 hours to remove moisture.

Characterization of Nanoparticles

A light-yellow solution with white precipitation indicated the synthesis of ZnO NPs, while a color shift from colorless to yellowish-brown to radish brown signified the formation of Ag NPs (Jain et al., 2009; Ahmad et al., 2003). UV-Spectrophotometer (Model Shimadzu UV-1900i) was used to record the optical absorption spectra from 200–800 nm, with distilled water as a blank. The crystal size was calculated using Scherrer formula as described in Jain et al. (2009).

Fruit Sample Collection

Papaya cv. Red Lady (mature green with less than 10% color development) was taken from Nobel Agriculture Farm in Bharatpur-25, Chitwan, Nepal and transported to the Institute of Agriculture and Animal Science (IAAS) laboratory at night. The fruits were carefully chosen for uniformity in size, shape, maturation stage, color, and free from visible wounds or defects.

Research Design

The experiment was laid out in a completely randomized design (CRD) with nine treatments and three replications. The treatments consisted of different concentrations (50 ppm, 100 ppm, 150 ppm, and 200 ppm) of either ZnO or Ag NPs and a control.

Coating Materials Preparation and Fruit Coating

Firstly, chitosan solution was prepared by dissolving 1.5% (w/v) chitosan in 1% (v/v) acetic acid at 60°C with continuous stirring for 4 hours and then filtered. ZnO and Ag NPs (50-200 ppm) were dispersed in distilled water and added to the chitosan solution, adjusting the pH to 5.0 with 0.1N NaOH. Glycerol (1% v/v) was added to improve wettability of the coating solution. The papaya cv. Red Lady of uniform size were washed in tap water and then in a 2% (v/v) sodium hypochlorite solution. The fruits were dipped in coating mixtures for 3 min, then placed on a grid to drain excess coating. After drying, both coated and control (uncoated) fruits were stored at room temperature (14±1.6°C) and 75% relative humidity.

Evaluation of Fruit Quality Attributes

Physiological loss in weight was measured on the 4th, 8th, 14th, and 18th days of treatment. Firmness was assessed by destructive sampling method using a digital penetrometer (Model FR-5120) with an 8mm probe pressed perpendicularly to the fruit's equator, and was recorded in kg. Total soluble solids (TSS) in the pulp were determined with a digital refractometer (Model HI 96801). TTA was measured as per Ranganna (1991) by grinding 10 g of papaya pulp with distilled water, filtering through Whatman No. 1, and adjusting the volume to 50 ml. After that, three drops of 1% phenolphthalein were added and titrated with 0.1N NaOH until a stable pink color persisted (pH 8.1-8.3). The TTA was expressed as g of citric acid per 100 g fresh weight; this is the predominant organic acid in papaya cv. Red Lady. Ascorbic acid was quantified using the indophenol method (Sadasivam and Balasubraminan, 1987), by extracting 10 g of pulp in oxalic acid, adjusting to 50 ml, and titrating with indophenol dye and compared it with the standard ascorbic acid. The ascorbic acid content was calculated as mg per 100 g of fruit pulp. The shelf life was visually assessed, with five panelists ranking fruit from 1-5 for overall acceptability after 16 days; scores above 3 were deemed marketable. The storage life was calculated by counting the period between the day of treatment and the final marketable stage of the fruits. An organoleptic test was conducted with 20 panelists using a seven-point hedonic scale on the 14th day of storage.

Statistical Analysis

Data were analyzed using one-way ANOVA and the source of variation was treatment only. Mean comparisons were conducted with Fisher-protected least significant differences at $p \leq .05$. Statistical analysis was performed using Excel and GenStat (18th edition).

Results and Discussion

Characterization of Nanoparticles

Characterization of Zinc Oxide Nanoparticles

The spectrum showed a characteristic absorption peak at 369 nm (Figure 1a),

confirming ZnO NPs synthesis. X-ray diffraction revealed the characteristic ZnO NPs peak, with an average crystalline size of 22.3 nm (Figure 1b).

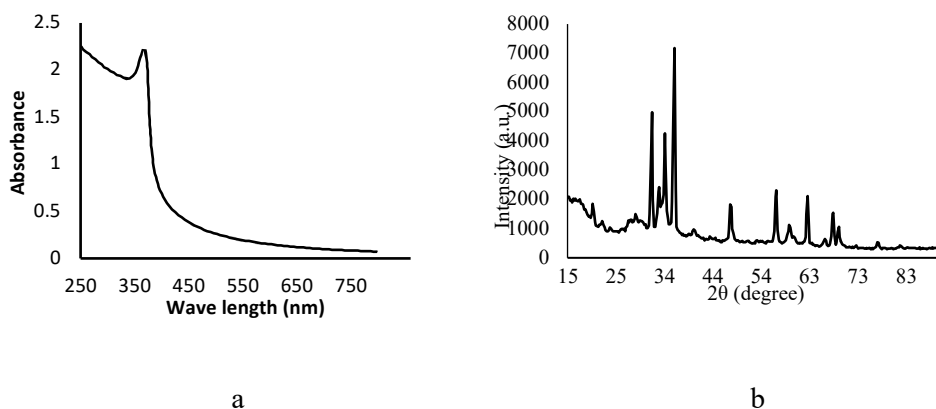


Fig. 1. UV-Vis's absorption spectra (a) and X-RD pattern (b) of green synthesized zinc oxide NPs.

Characterization of Silver Nanoparticles

The absorbance peak was found at 422 nm, conformed the synthesis of Ag NPs (Figure 2a). The synthesis of Ag NPs was further supported by X-Ray Diffraction (X-RD) pattern and the average crystalline size was found to be 4.46 nm (Figure 2b).

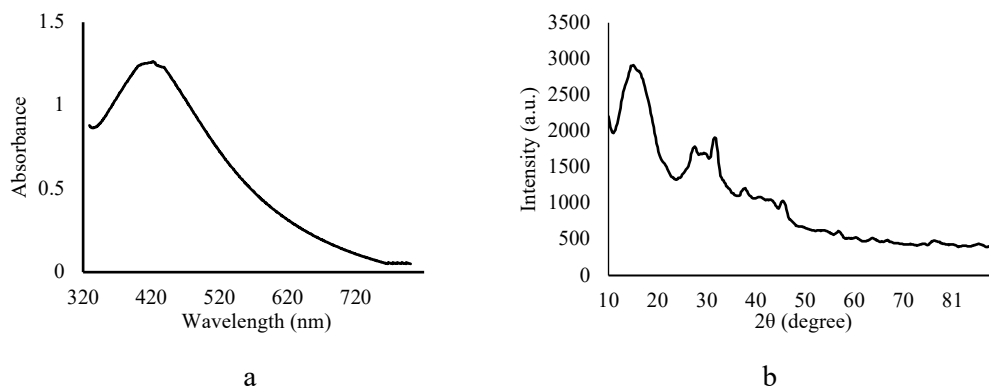


Fig. 2. UV-Vis's absorption spectra (a) and X-RD pattern (b) of green synthesized silver NPs.

Evaluation of Fruit Quality Attributes

Physiological Loss in Weight (PLW)

Weight loss is a key factor in determining the storage life and quality of fruits, as well as the effectiveness of coatings for preserving papaya (Ali et al., 2011). On the 4th day, PLW was significantly ($p \leq .05$) higher on untreated fruits compared to

treated, though no significant difference was observed among the treated fruits (Figure 3). On 8th day of storage, fruits treated with 200ppm Ag NPs had the least PLW statistically at par with 200ppm ZnO NPs and 150ppm Ag NPs, 150ppm ZnO NPs and 100ppm Ag NPs. On the 18th day, the lowest PLW was observed with 200ppm Ag NPs treated fruits, which is statistically ($p \leq .05$) at par with 150ppm Ag NPs, 200ppm ZnO NPs and 100ppm Ag NPs treated fruits.

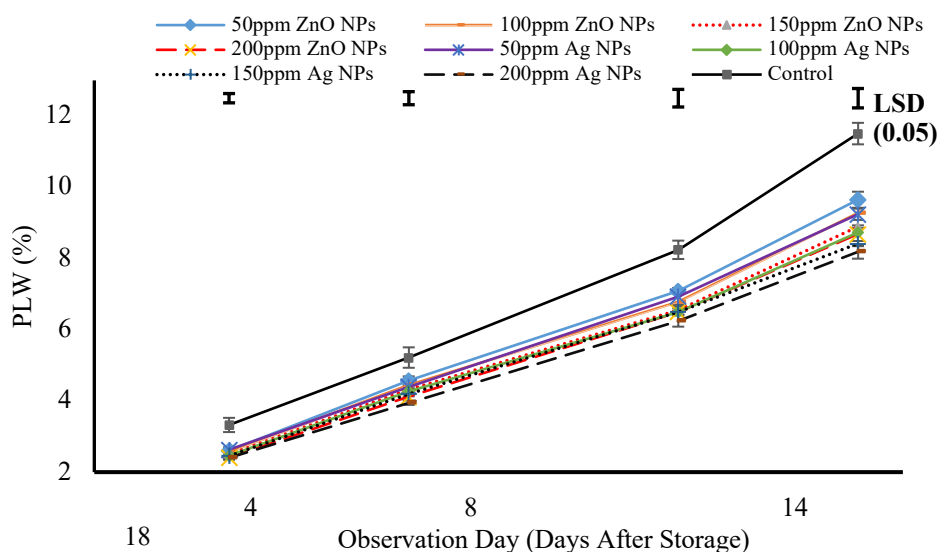


Fig. 3. Effect of ZnO and Ag NPs coatings on PLW in papaya cv. Red Lady stored at $14 \pm 1.6^\circ\text{C}$ and $\sim 75\%$ RH.

Physiological loss in weight of the harvested fruits is mainly due to water loss from transpiration and carbon loss from respiration (Sogvar et al., 2016). Chitosan-based ZnO and Ag NPs create a thin barrier on the fruit, reducing moisture loss and slowing respiration by limiting gas exchange (Ali et al., 2020; Anugrah et al., 2020). This reduced respiration slows carbohydrate breakdown, minimizing PLW (Arowora et al., 2013). The reason for the reduction in PLW with the increase in the concentration of NPs might be due to the improvement of the coating's barrier properties, such as an increasing barrier to moisture and gaseous exchange with the increased concentration.

Hmmam et al. (2021), Saekow et al. (2019), and Taha et al. (2022) studies showed that the coating of polysaccharide-based coatings with ZnO and Ag NPs function as a partially permeable barrier to water and gas, effectively reducing moisture loss and weight loss. Additionally, Svagan et al. (2009) concluded that nanomaterials create crisscross structure within the coating layer, which prevents the exchange of oxygen and carbon dioxide and further minimizes moisture loss.

Fruit firmness

Firmness is a key fruit quality attribute for handling, consumer acceptability, transportability, marketability, and postharvest life (Ali et al., 2011). Firmness decreased during storage in all treatment, but higher concentrations of ZnO and Ag NPs slowed the decline (Figure 4). On the 4th day, NPs-treated fruits had significantly ($p \leq .05$) higher firmness than untreated fruits, though no significant difference was found among the treated fruits. On the 8th day, 200ppm ZnO NPs-treated fruits had the highest firmness, followed by 200ppm Ag NPs, 150ppm Ag NPs and 150ppm ZnO NPs all significantly ($p \leq .05$) higher than 50ppm ZnO, 50ppm Ag, and untreated fruits.

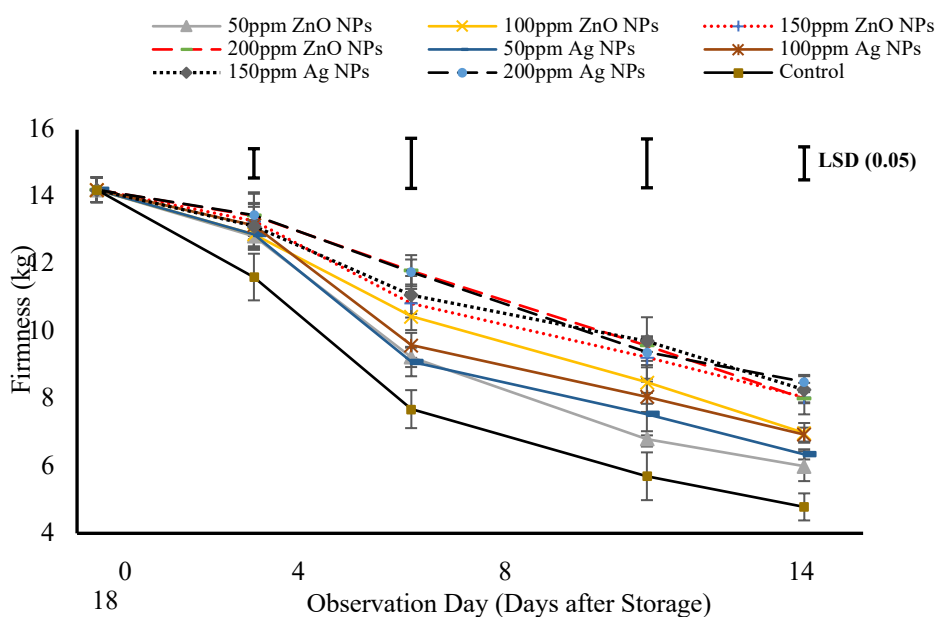


Fig. 4. Effect of ZnO and Ag NPs coatings on firmness in papaya cv. Red Lady stored at $14 \pm 1.6^\circ\text{C}$ and $\sim 75\%$ RH.

On the 18th day, 200ppm Ag NPs maintained the highest firmness, followed by 150ppm Ag, 150ppm ZnO, and 200ppm ZnO, all significantly ($p \leq .05$) firmer than other treatments. The fruit firmness was 1.78, 1.72, 1.67 and 1.68 fold more in fruit treated with 200ppm Ag NPs, 150ppm Ag NPs, 200ppm ZnO NPs and 150ppm ZnO NPs, respectively, compared to control on the 18th day of storage. While the firmness of untreated fruit decreased to 4.79 kg, the average firmness of treated fruit remained significantly higher at 7.35 kg on the same day. The reduced internal oxygen in NP-treated fruits slows cell wall-degrading enzymes like polygalacturonase, pectin methylesterase, and cellulase, preserving firmness longer by slowing carbohydrate conversion (Yahia et al., 2019). These findings are similar to Batool *et al.* (2022) and

La *et al.* (2021) which demonstrates that nano-ZnO NPs and nano-silver effectively maintain the firmness of fruits.

Total Soluble Solids (TSS)

Total soluble solids (TSS) is one of the vital parameters for assessing postharvest storage and fruit quality. TSS was 7.83 °Brix before coating and increased up to 11.03 °Brix on 18th day (Figure 5). On the 4th day of storage, untreated fruits had significantly higher TSS than those treated with NPs. On the 8th day, untreated fruits still had higher TSS, which is statistically at par with 50ppm Ag NPs treated fruits. After this, no significant differences in TSS were observed between coated and uncoated fruits. The result indicates that TSS increased more slowly in treated fruits, with slower increases at higher NPs concentration.

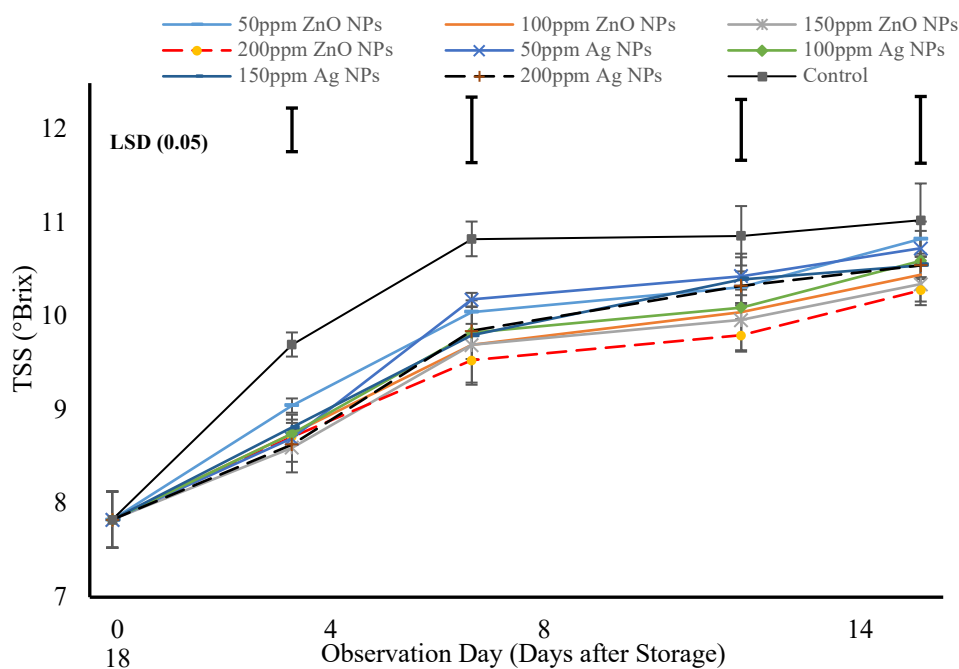


Fig. 5. Effect of ZnO and Ag NPs coatings on TSS in papaya cv. Red Lady stored at $14 \pm 1.6^\circ\text{C}$ and $\sim 75\%$ RH.

The rise in TSS during storage primarily results from carbohydrate hydrolysis, particularly the conversion of starch into simple sugars driven by increased respiration and metabolic activity (Cordenunsi and Lajolo, 1995). Studies by Li *et al.* (2019) and Nguyen *et al.* (2021) revealed that polysaccharide-based coatings with ZnO and Ag NPs effectively slow the ripening process and reduce the rate of TSS accumulation. In contrast, Taha *et al.* (2022) found that starch-based Ag NPs coatings

had no significant effect on the TSS content of strawberries, likely due to the fruit's non-climacteric nature. Thus, concluded that chitosan-based NPs coatings have been shown to slow the increase in TSS in climacteric fruits.

Total titratable acidity (TTA)

TTA was 0.20 g citric acid/100 g at the start and decreased with storage time, regardless of treatments. However, the decrease was slower in coated fruits compared to uncoated ones (Figure 6). Among the coated fruits, the reduction in TTA slowed down as the concentration of both NPs increased.

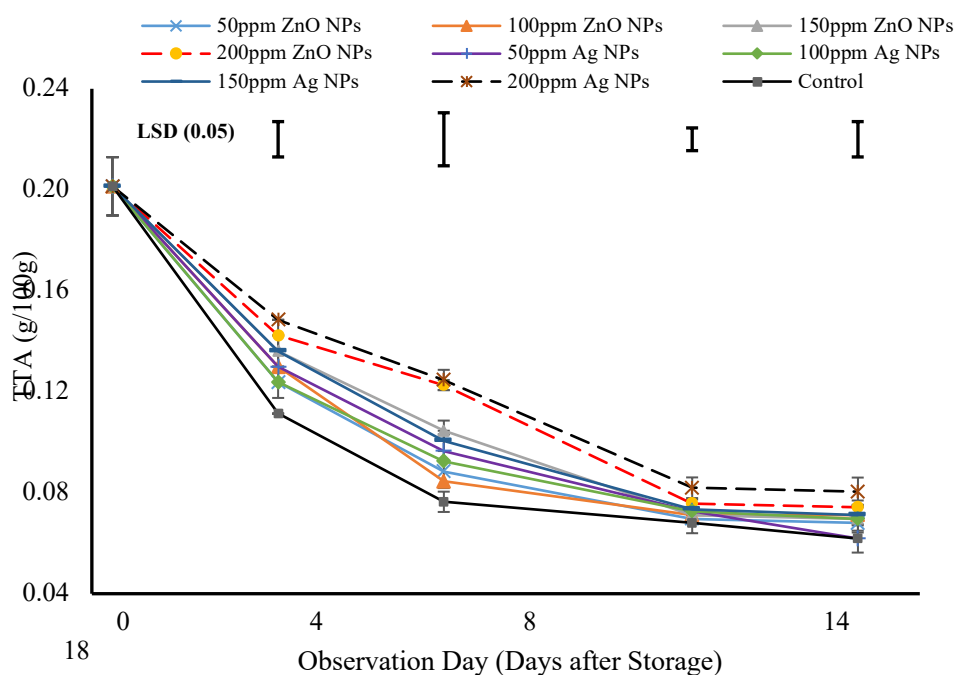


Fig. 6. Effect of ZnO and Ag NPs coatings on TTA in papaya cv. Red Lady stored at $14 \pm 1.6^\circ\text{C}$ and $\sim 75\%$ RH.

On the 4th day of storage, TTA was found as the highest with 200ppm Ag NPs treated fruits that was statistically ($p \leq .05$) at par with 150ppm Ag NPs, 200ppm ZnO NPs and 150ppm ZnO NPs treated fruits. On the 8th day of storage, TTA was found higher on 200ppm Ag NPs treated fruits that are statistically ($p \leq .05$) at par with 200ppm ZnO NPs and 150 ppm ZnO NPs treated fruits. After that, though TTA was higher at increased NPs concentration, there was no significant ($p \leq .05$) difference among all treatments.

The reduction in fruit respiration rate slows the consumption of organic acids, primarily citric and malic acid (Xu et al., 2018), which act as substrates in the

enzymatic reactions of respiration (Hazrati et al., 2017). The slower decline in TTA with increased NPs concentration is largely attributed to the formation of a strong barrier to gas exchange, thereby reducing internal metabolic reactions. This observation is consistent with findings from Basumatary et al. (2021) and Himmam et al. (2021), who showed that NPs coatings on fruits mitigate the loss of TTA.

Ascorbic Acid

Ascorbic acid in papaya increases during ripening and then begins to decline (Chen and Paull, 1986). Ascorbic acid increased in all the treatments on 4th day of storage (Figure 7). The lowest increase was observed with 200 ppm Ag NPs (43 mg/100 g to 55.94 mg/100 g), statistically similar ($p \leq .05$) to 150 ppm Ag NPs and 100-200 ppm ZnO NPs. These results indicate that fruit coating slows ascorbic acid synthesis during ripening, with higher NPs concentrations further inhibiting this process. Consequently, treated fruits exhibited smaller increase in ascorbic acid compared to untreated ones.

On the 8th day of storage, the highest ascorbic acid was obtained in fruits treated with 150ppm Ag NPs, statistically ($p \leq .05$) at par with 200ppm Ag NPs and 200ppm ZnO NPs. On the 14th day, ascorbic acid was recorded as the highest in 200ppm ZnO NPs fruits, statistically ($p \leq .05$) similar to 200ppm and 150ppm Ag NPs treated fruits. The slower decline in ascorbic acid in coated fruits after the 4th day of storage likely results from a barrier that limits oxygen entry, delaying vitamin C oxidation (Ayranci and Tunc, 2004). On the 18th day, there was no significant difference in the ascorbic acid content between the coated and uncoated fruits.

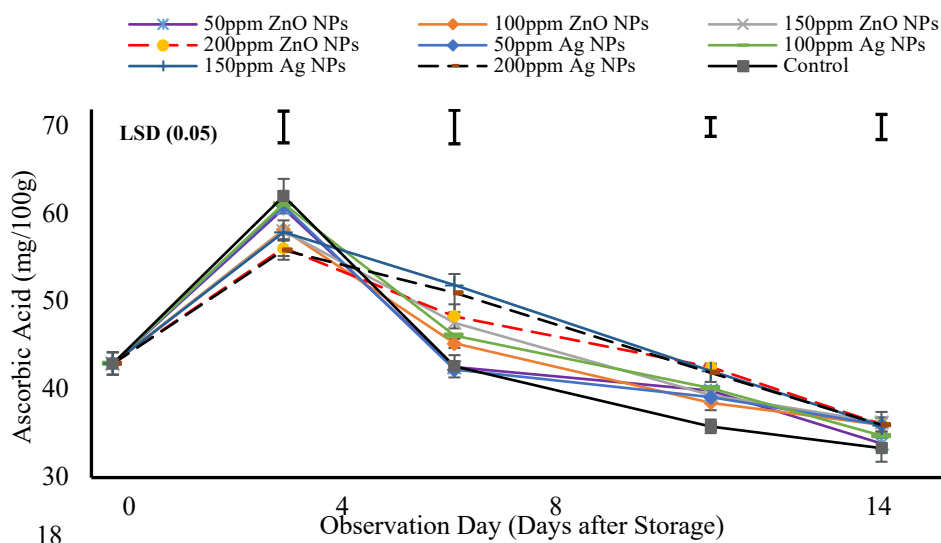


Fig. 7. Effect of ZnO and Ag NPs coatings on ascorbic acid in papaya cv. Red Lady stored at $14 \pm 1.6^\circ\text{C}$ and $\sim 75\%$ RH.

Ezen et al. (2017) observed that half-ripe papayas contain the highest vitamin C levels, followed by ripe and unripe fruits. This aligns with findings that vitamin C increases during ripening but decreases during senescence due to ascorbic acid oxidation (Kamelia, 2019). Ascorbic acid is synthesized in the mitochondria through several proposed pathways, including the L-galactose pathway and the D-galacturonate pathway. The L-galactose pathway is the primary route for ascorbic acid synthesis, primarily active in fruits that are still attached to the parent plant (Wheeler et al., 1998). Consequently, most ascorbic acid in fruits is synthesized prior to detachment from the mother plant. However, after harvest, the D-galacturonate pathway becomes a viable route for ascorbic acid synthesis (Fenech et al., 2019). This pathway may explain the initial increase in ascorbic acid observed in papaya post-harvest. Additionally, a study by Badejo et al. (2012) demonstrated that supplementing red-ripe tomatoes with D-galacturonate increased ascorbic acid content, though genetic evidence supporting the D-galacturonate pathway's role in ascorbic acid synthesis remains limited.

Storage Life

The storage life of fruit treated with 150 ppm and 200 ppm of both NPs was extended by 8 to 9 days compared to untreated fruits (Figure 8). Increasing NPs concentration enhances fruit storage life by reducing respiration rate and improving barrier properties, which help maintain physicochemical quality. Similar results were observed with ZnO and Ag NPs in fruits like banana, guava, strawberry and mango (Arroyo et al., 2020; La et al., 2021; Meindrawan et al., 2018; Sogvar et al., 2016).

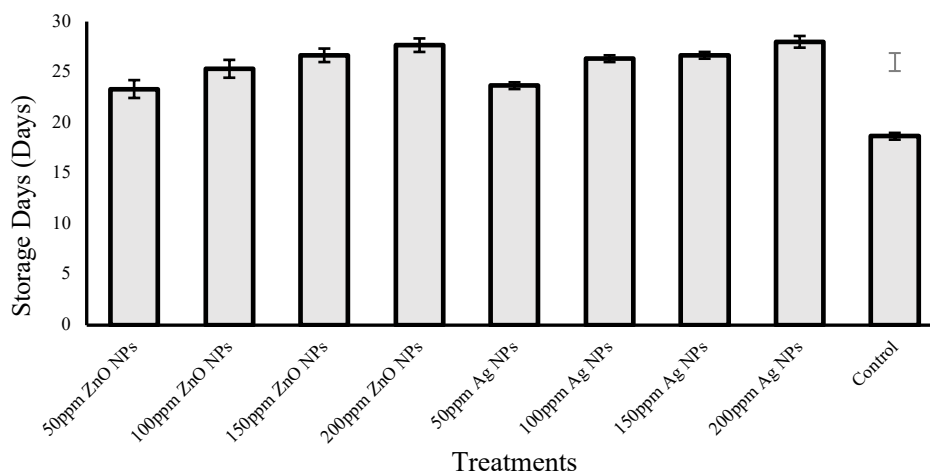


Fig. 8. Effect of ZnO and Ag NPs coatings on Storage life of papaya cv. Red Lady stored at $14 \pm 1.6^\circ\text{C}$ and $\sim 75\%$ RH.

Organoleptic Test

The untreated papaya fruit was preferred (like slightly or like moderately or like very

much) by 70% of the respondent (Table 1). Fruits treated with 200 ppm ZnO and Ag NPs were disliked by 50% and 35% of respondents, respectively. Preference decreased as NPs concentration increased. It was evident that the most liked sample had a highest TSS (10.87 °Brix), highest weight loss, and lowest TTA, indicating that consumers prefer higher sugar content.

Table 1. Frequency of organoleptic test of papaya cv. Red Lady on the 14th day of storage at 14±1.6°C and ~75% RH.

| Treatments | Frequencies | | | | | | |
|----------------|-------------------|--------------------|------------------|--------------------------|---------------|-----------------|----------------|
| | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much |
| 50ppm ZnO NPs | | | 1(5) | 7(35) | 6(30) | 3(15) | 3(15) |
| 100ppm ZnO NPs | | 1(5) | 1(5) | 7(35) | 8(40) | 3(15) | |
| 150ppm ZnO NPs | | 1(5) | 4(20) | 7(35) | 6(30) | 2(10) | |
| 200ppm ZnO NPs | | 3(15) | 7(35) | 10(50) | | | |
| 50ppm Ag NPs | | | 2(10) | 4(20) | 3(15) | 6(30) | 5(25) |
| 100ppm Ag NPs | | 1(5) | 2(10) | 3(15) | 8(40) | 5(25) | 1(5) |
| 150ppm Ag NPs | | | 4(20) | 5(25) | 7(35) | 4(20) | |
| 200ppm Ag NPs | | 1(5) | 6(30) | 8(40) | 2(10) | 3(15) | |
| Control | | | 1(5) | 5(25) | 3(15) | 7(35) | 4(20) |

Figure in the parenthesis indicates percentage

The organoleptic test indicated that panelists preferred fruits with lower concentrations of Ag NPs, as higher concentrations led to reduced acceptance. Although there was no significant difference in TSS among the concentrations tested (50–200 ppm) on the day of the organoleptic assessment, the reduced preference for higher NPs concentrations may be due to diminished aroma and flavor, a trend also observed in guava fruits coated with ZnO NPs (Arroyo et al., 2020).

Conclusions

Coating papaya with chitosan-based zinc oxide and silver nanoparticles enhances postharvest life by reducing physiological loss in weight, maintaining firmness and delay the ripening process by inhibiting the respiration rate. This study concludes that coatings with 100-150 ppm concentration of zinc oxide and silver nanoparticles extent the postharvest life and preserve the fruit quality. Further research is needed to

explore technology across various temperature regimes as well as the residual effects of nanoparticles on consumer health. A multi-dimensional assessment of nanoparticles should be considered before commercial application.

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