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Extending the shelf life of guava fruits using silver nanoparticles infused biopolymer coatings

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Abstract

Guava is a climacteric fruit with a short shelf life (5-8 days). The rapid postharvest deterioration of guava fruits poses a major challenge in increasing longevity and retaining quality. This study examined the impact of silver nanoparticles (AgNPs) infused into different biopolymer edible coatings, including beeswax, gelatin, guar gum, and xanthan gum, to extend the shelf life which is relatively unexplored in guava fruits. The results revealed that AgNPs-coated fruits exhibited significantly prolonged shelf life: beeswax-coated fruits lasted 11 days, gelatin-coated fruits lasted 9.5 days, guar gum-coated fruits lasted 7 days and xanthan gum-coated fruits lasted 6.25 days, compared to the control (4.25 days). The highest PLW (Physiological Loss in Weight) of 70.44 % was noticed in uncoated fruits and the lowest PLW of 38.73 % in beeswax + AgNPs at the 12^{th} day of storage was noticed. The highest firmness of 24.25 kg cm^{-2} was observed in beeswax + AgNPs and the lowest firmness of 6.00 kg cm^{-2} was noted in uncoated fruits on the 12^{th} day of storage. The highest Total Soluble Solids (TSS) content of 16.80° Brix on the 10^{th} day was recorded in the control (uncoated fruits), but the fruits shrunk and lost consumer acceptance. On the contrary, the lowest value of 11.80° Brix was found in fruits coated with beeswax + AgNPs, but there was only a slight decline on 10^{th} day. The fruits retained their form and shape. High vitamin C retention was observed in beeswax + AgNPs coating as 195.2 $mg100g^{-1}$, but a massive reduction was observed in control as 169.6 $mg100g^{-1}$ on the 12^{th} day of observation. The observations taken on the 12th day of storage denote delayed starch conversion with a high percentage of total sugar reduction at 5.51 in beeswax + AgNPs coating. The high percentage of total sugars was observed in control as 9.22. The biopolymer coatings with AgNPs delayed fruit senescence and maintained better physiological and biochemical properties, demonstrating their potential as an effective postharvest treatment for guava fruits. This is the first study of its kind in guava using AgNPs with biopolymers. The results of the study were highly promising, opening the door for more intensive research and the need to scale up this technique to address postharvest loss of guava fruits.

Key words: Edible coating, guava, silver nanoparticles, shelf life, biopolymer, AgNPs, antimicrobial

Introduction

Guava (*Psidium guajava* L.) is referred as the "Apple of the tropics" or "Poor man's apple" and the fruits are rich in fiber (6.9 %) and vitamin C content (260-300 mg $100g^{-1}$ of pulp). India is the leading guava producer globally, with Uttar Pradesh leading among the states in its production. Being a climacteric fruit, guava continues to ripen after harvest at maturity; this is primarily due to its high water content, rapid softening of tissue, and susceptibility to microbial growth resulting in significant postharvest losses. These losses range from 10-15% in developed countries to as high as 20-40% in underdeveloped nations (Omayio *et al.*, 2019). The focus in the food industry is natural and the maximum emphasis is on alternatives to synthetic preservatives. Edible coatings are thin layers applied to the surface of fruits to extend their shelf life by preserving colour, flavour, and nutrients. These coatings also possess anti-browning and antimicrobial properties, protecting the fruit during storage. Additionally, edible coatings help to maintain integrity and firmness by reducing weight loss, lowering respiration rates, ethylene production, and browning reactions. The choice of coating materials depends on the type of fruit and the characteristics of the coating material (Ungureanu *et al.,* 2023). The use of nanoemulsions as edible coatings is a recent concept offering tremendous scope to enhance shelf life. It has been demonstrated that a variety of postharvest methods, such as gaseous treatments, modified and controlled atmospheric packaging, irradiation, edible coatings and silver nanoparticle coatings can decrease postharvest losses and increase the shelf life of several fruit crops (Chen *et al.,* 2024; Kaur et al., 2025).

By partially covering stomata and fruit surface lenticels, edible coatings increase shelf life while improving quality metrics like TSS, total sugars, and firmness and decreasing respiration and water loss. These coverings also lessen deterioration, lower lipid oxidation, and shield fruits from microbial diseases (Suhag *et al.,* 2020). Biopolymer coatings are an ideal alternative to synthetic coatings because of their eco-friendly and biodegradable nature. Silver nanoparticles (AgNPs) have become increasingly important in the food industry because of their strong antimicrobial properties. Numerous microorganisms, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Fusarium oxysporum*, *Curvularia lunata*, *Rhizopus arrhizus*, *Aspergillus niger*, and *Aspergillus flavus* are effectively combatted by AgNPs (Savithramma *et al.,* 2011).

Fruits coated with silver nanoparticles exhibit prolonged shelf life and enhanced quality in various crops, such as mangoes, grapes, strawberries, apricots, kinnow, loquat, papaya and Murcott mandarin, as reported by different researchers.

The work of Magami (2015) on food coatings focuses on pure or mixed forms such as antioxidants, preservatives and antimicrobial agents. The recent reviews of Ungureanu *et al.* (2023) on bio-coatings and Pillai *et al.* (2024) on biopolymer based coatings reveal the lack of studies on guava - a highly perishable tropical fruit. The lone reference on guava available in alginate coatings with black cumin extract as an active ingredient shows the wide gap in the knowledge and huge potential for such studies (Hasan *et al.*, 2022).

Hence, this study was undertaken to find viable eco-friendly alternatives and the possibility of use of silver nanoparticles as antimicrobial properties combined with natural coatings.

Materials and methods

The experiment was carried out in the Horticulture Laboratory and the Nanotechnology Central Laboratory at Karunya University, Coimbatore. Uniform-sized, high-quality fruits of guava (cv. Lucknow-49) were procured from a local orchardist in Ayakudi, Tamil Nadu. The selected fruits were free from insects, pests, and other contaminants. Gelatin powder, beeswax, xanthan gum powder and guar gum powder were employed as biopolymers to coat the fruits.

Green synthesis of silver nanoparticles: 20 g of neem leaves were collected, cleaned and allowed to air dry. The chopped neem leaves were taken in a beaker along with distilled water (100mL), the preparation was boiled in a water bath at 80^0 C for 45 minutes and the leaf extract solution was prepared. In the meantime, silver nitrate was solubilized in distilled water and l mM $AgNO_3$ solution was prepared. Crude solution was filtered using Whatman filter paper No.1, to collect leaf extract. The neem leaf extract and $AgNO_3$ solution were combined in a proportion of 1:9 and heated for 30 minutes. The colour changes from yellow to dark brown confirmed the formation of silver nanoparticles. The obtained solution was kept for 2 days, and after that, the deposited particles were shifted to a centrifuge tube. The tube was centrifuged thereafter for ten minutes at 3000 rpm. The centrifuged particles were subjected for lyophilization to obtain AgNPs in powder form.

The characterization of nanoparticles was confirmed by using the techniques *viz.*, UV-visible spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and Zeta potential measurement (Ananth *et al.*, 2023).

Preparation of coating materials: The AgNPs solution was added dropwise into the biopolymer solutions at 1:9 ratio using a burette and stirred on a magnetic stirrer for uniform distribution. The gelatin solution was prepared by dissolving 5% gelatin powder and 1.5 mL glycerol as a plasticizer in distilled water (Kowsalya *et al.,* 2021). While 2 g of guar gum powder was dissolved in 100 mL of distilled water to create the guar gum solution (Malik *et al.,* 2022), 4 g/L of xanthan gum powder and 2 mL glycerol were combined with distilled water for xanthan gum (García-Betanzos *et al.,* 2017). The coating was applied using the dip method, after which the coated fruits were placed on paper plates and air-dried at ambient temperature.

The various postharvest treatments included in the study were T_1: Control fruits, T_2: Beeswax + AgNPs, T_3: Gelatin + AgNPs, T_4: Guar gum + AgNPs and T_5: Xanthan gum + AgNPs. The fruits washed with distilled water served as the control.

The biopolymers were selected based on their cost-effectiveness, local and ready availability, proven efficacy, ease in application and serving the dual purpose of preventive and interceptive treatments instead of their richness in essential metabolites as proven in other fruit crops (Ungureanu *et al.*, 2023; Pillai *et al.*, 2024). Because of their antibacterial, anti-inflammatory, and antioxidant properties—attributable in part to the presence of thicker wax coats—beeswax and other bee-derived products have been widely used in both traditional and modern medicine.

Quality parameter of fruits

Physiological loss in weight (%): The weight of the fruits was recorded regularly at a fixed interval of two days, and the weight loss percentage was assessed through the following formula suggested by Sar and Dhutraj (2023).

Firmness (kg cm^{-2}): Firmness of fruit was tested with a penetrometer (Model: V-TECH).

Shelf life (Days): The duration of fruit shelf life was measured by counting the days from imposition of treatment until 50% of the fruits exhibited shrivelling (Malik *et al.,* 2015).

Total Soluble Solids (^{o}Brix): TSS was analyzed using a digital hand refractometer (Model: MA871).

Total sugars (%): Total sugars were estimated by using the method of Association of Official Agricultural Chemists (AOAC, 1980).

Vitamin C content (mg 100g^{-1}): The vitamin C content was measured by using 2,6-Dichloro Phenol Indophenol (DCPIP) dye visual titration method (Ranganna, 1986).

Data were recorded at 2, 4, 6, 8, 10, and 12-day intervals following the coating.

Statistical analysis: The five treatments were repeated four times using a Completely Randomized Design (CRD). The data were statistically evaluated employing ANOVA technique (Panse and Sukhatme, 1985).

Results and discussion

Physiological loss in weight (PLW): The highest PLW was observed in the control at 70.44%, while the lowest PLW was recorded in beeswax + AgNPs at 38.73%, on the 12th day of storage (Table 1). Fruit surfaces retain water effectively with the help of edible coatings, which form a protective barrier that prevents water transfer, mechanical damage, and dehydration, thereby minimizing physiological weight loss. In addition, they reduce transpiration, respiration rates, and fruit surface moisture loss (Patil *et al.*, 2023).

Firmness: The beeswax + AgNPs coated fruits showed the highest firmness at 24.25 kg cm^{-2} on the 12th day of storage, while the lowest firmness was observed in control at 6.00 kg cm^{-2} (Table 1). The rapid loss of firmness in the control is attributed to lipid disintegration, which accelerates fruit softening and leads to quick deterioration (Paliyath and Subramanian, 2008). These findings are consistent with the results of Triyono *et al.* (2020) for beeswax-coated fruits.

Shelf life: The guava fruits coated with beeswax + AgNPs showed the longest shelf life which extended up to 11 days, while there was a significantly shorter shelf life of just 4.5 days for uncoated fruits (Table 1). The extended shelf life is because of the antimicrobial effect of AgNPs on the coated fruits. The shelf life increased to 11 days because of AgNPs + beeswax fruit coating. This coating may have contributed to limiting gaseous exchange because it formed a physical barrier that covers the stomatal apertures on the fruit surface. The nano-membrane created by the coatings reduces respiration and acid utilization during the fruit's metabolic processes, leading to enhanced shelf life (Zhang *et al.,* 2018). Additionally, fruit coatings help maintain tissue rigidity over time, which reduces respiration rates and transpiration levels, ultimately delaying oxidation and the ripening process (Elsabee and Abdou, 2013). There are no reports on this aspect in guava fruit; there is an identical work in apple where fruits wrapped in AgNPs-impregnated paper extended their shelf life by up to 15 days (Bhople *et al.,* 2016).

Total soluble solids (TSS): The TSS levels of fruits increased initially until the 10th day of storage, after which it decreased across all the treatments. The highest TSS level of 16.80° Brix was observed among uncoated fruits on the 10th day, while the lowest value of 11.80° Brix was exhibited by fruits coated with beeswax + AgNPs. The maximum TSS of 15.17° Brix was once more noted in the uncoated fruits on the twelth day of storage, whereas the minimum TSS content of 11.12° Brix was observed in the beeswax + AgNPs coated fruits (Table 2). The early rise in TSS during storage can be ascribed to fruit dehydration and the transformation of pectin, starch, hemicellulose and other polysaccharides into soluble sugars. TSS levels continue to rise until starch reserves are depleted for conversion into soluble sugars; thereafter, a decrease in TSS is expected as the starch reserve is exhausted and also the sugars are utilized in respiration. The degradation of complex organic compounds into simpler molecules or the hydrolysis of starch into sugars during storage might have contributed to the rise in TSS levels. When starch is completely metabolized, sugar availability ceases and there is a decrease in these parameters. This is typical because they, along with other organic acids, serve as essential substrates for respiration. Furthermore, the delay in metabolic processes, such as the hydrolysis of starch into sugars has been shown due to edible coatings (Thakur *et al.,* 2019), which accounts for retention of TSS in coated fruits.

Vitamin C content: The fruits coated with beeswax + AgNPs showed the highest vitamin C content at 195.15 mg 100g^{-1} on the 12th day of storage, while the lowest vitamin C content was observed in the uncoated fruits at 169.55 mg 100g^{-1} (Table 2). The edible coating may reduce oxygen diffusion, thus delaying respiration and leading to the preservation of vitamin C in fruits. Additionally, guava fruits coated with beeswax have shown an increase in vitamin C content (Sharma and Kotiyal, 2022).

Total sugars: The overall sugar content had increased until the 8th day of storage and then decreased in all treatments. The highest total sugar level on the 8th day was observed in the uncoated fruits at 9.61%, and the lowest was observed in beeswax + AgNPs at 7.15%. Maximum total sugars (9.22%) were seen in uncoated fruits on the 12th day of observation, whereas the lowest total sugar content in beeswax + AgNPs dropped to 5.51% (Table 2). The hydrolysis of polysaccharides into simpler sugars may have contributed to the initial surge in sugar levels during the storage period. The rise in sugar levels accelerates ripening and enhances palatability, but reduces the shelf life. Thus leading to accelerated deterioration and senescence. This study proves that beeswax +

Table 1. Effect of biopolymer coating with AgNPs on physiological loss in weight, firmness and shelf lifeof guava cv. Lucknow 49

Treatments	Physiological Loss in Weight (%)						Firmness (kg cm^{-2})						Shelf life (Days)
	Storage intervals (Days)						Storage intervals (Days)						
	2	4	6	8	10	12	2	4	6	8	10	12	
T_1 (Control)	11.07	24.39	36.00	47.73	58.10	70.44	27.50	18.75	15.25	11.5	8.00	6.00	4.50
T_2 (Beeswax + AgNPs)	1.06	10.78	20.9	28.65	33.96	38.73	44.50	39.00	35.00	30.5	27.25	24.25	11.00
T_3 (Gelatin + AgNPs)	7.12	19.11	31.20	40.51	50.04	58.71	39.50	31.75	28.00	24.5	21.00	17.25	9.50
T_4 (Xanthan gum + AgNPs)	8.67	18.70	30.70	42.31	56.74	64.53	37.80	29.25	25.00	21.5	17.75	13.75	6.25
T_5 (Guar gum + AgNPs)	7.79	21.28	30.50	40.24	51.79	60.56	35.00	26.25	21.75	16.75	13.25	11.25	7.00
C.D (5%)	0.77	5.46	5.92	6.50	5.71	4.61	4.57	3.49	3.75	2.85	3.29	2.804	1.02
SE(m)	0.25	1.79	1.94	2.13	1.88	1.12	1.50	1.14	1.23	0.93	1.08	0.922	0.34

Table 2. Effect of biopolymer coating with AgNPs on vitamin C content, total sugars and total soluble solids of guava cv. Lucknow 49

Treatments	Vitamin C content (mg100g^{-1})						Total sugars (%)						Total Soluble Solids (^{0}B)					
	Storage intervals (Days)						Storage intervals (Days)						Storage intervals (Days)					
	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12
T_1 (Control)	233.1	214.7	202	187.8	178.3	169.6	7.04	7.71	8.64	9.61	9.46	9.22	10.77	13.2	14.22	15.60	16.80	15.20
T_2 (Beeswax + AgNPs)	240.1	230.2	222	213.8	203.6	195.2	6.37	6.69	6.94	7.15	6.29	5.51	9.50	10.00	10.52	11.17	11.80	11.10
T_3 (Gelatin + AgNPs)	241.4	229.6	217	204.9	194.4	186.4	6.61	6.90	7.21	7.56	7.36	7.14	9.55	10.50	11.70	12.60	13.47	12.20
T_4 (Xanthan gum + AgNPs)	239.5	226.2	208	198.6	188.6	180.2	6.86	7.42	8.11	8.80	8.66	8.53	10.50	11.10	11.57	12.07	12.80	11.70
T_5 (Guar gum + AgNPs)	239.8	224.2	206	191.3	184.4	173.8	6.58	6.93	7.41	7.73	7.59	7.38	9.62	10.90	12.35	13.45	14.35	12.90
C.D (5%)	4.14	7.36	7.72	5.22	3.84	4.29	0.2	0.31	0.23	0.31	0.37	0.43	0.58	0.60	0.89	0.88	1.18	1.17
SE(m)	1.36	2.42	2.54	1.71	1.26	1.41	0.07	0.10	0.07	0.10	0.12	0.14	0.19	0.19	0.29	0.29	0.39	0.38

AgNPs treatment significantly reduces the process of ripening and contributes to the decline in sugar levels. During respiration, cells consume simple sugars converting them to carbon dioxide and water leading to the reduction in total sugars (Alberts *et al.,* 2002). The coated fruits in the study exhibited a decline in total sugars similar to the findings of Bashir and Goukh (2003) in guava and Hmmam *et al.* (2021) in mango. The process of ripening generally involves the conversion of starch into sugars, the breakdown of pectin, and development of flavour and aroma in the fruits. These processes are essential but if they reach their maturity peak earlier will continue and lead to over-ripening and loss of quality. In juicy fruits like guava, the fruit respires more quickly and the quality of fruit weakens rapidly (Fonseca *et al.*, 2002).

Consuming fresh fruits is no doubt the best way to relish their taste and palatability but preservation makes its availability out of seasons. Thus, preserving and ensuring continuous supply is an undaunting challenge in perishable fruits. Broadly the preservation techniques can be classified under a) Physical – heat regulation, humidity adjustment, regulated atmosphere treatments, and radiation therapy (Zhang *et al.*, 2020), each having its own disadvantages. b) Chemical preservatives - using chemical preservatives such as fungicides and sterilizing agents leading to residues and health hazards. Another aspect is use of ethylene inhibitors.

Waxy coatings both a byproduct of petroleum and natural sources later came into use and waxy coatings have several challenges arising from the concentration, thickness, and preservatives incorporated to change coating properties. Some of them are hazardous and damage internal organs (Pashova, 2023). Edible coatings enriched with natural substances are the best eco-friendly and wholesome approach for preserving fruits. In this group comes beeswax, the most efficient treatment in the study. Its importance has also been emphasized from the point of scalability, cost-effectiveness and regulatory considerations (Samir *et al.*, 2022). Beeswax is a complete mixture of compounds such as fatty acids, alcohols, esters, and hydrocarbons which are stable and insoluble in water protecting the fruits and making it safe to consume. Gums have additionally been recognized as promising edible coatings for fruits but were less effective in this study. Plant-based waxes namely rue wax have already been approved by the Food and Drug Administration (Sabale *et al.*, 2009).

Nanomaterials have now occupied the prime position in food packaging because of the numerous benefits over conventional packaging and the improvement that has come with natural coatings. The distinct advantages of nanomaterials are homogenous dissemination, biodegradability, mechanical strength, barrier and optical characteristics. In contrast, bio-nanomaterials are more advanced, as they regulate temperature, and stability, and alter barrier properties without affecting quality. This is the reason for the results obtained in the study.

Honey bee products have been acknowledged for their therapeutic and health-benefiting properties along with their ease of access and bioavailability. The novelty of the study is that beeswax is highly effective in the green synthesis of NPs. It has already been reported that their components (carbohydrates, flavonoids and proteins) serve as capping and reducing agents. In addition, using beeswax is cost-effective; eco-friendly, and a natural resource for NPs have specific uses in medicinal and pharmaceutical applications (Radwan *et al.*, 2024). Another major aspect is that, this is the first report of scientific study in this direction on guava and opens out the door for future lines of research on the possible utilization of honey bee products in the green synthesis of nanoparticles to harness their distinct properties while minimizing the negative impacts of synthetic substances.

Fruit perishability is a major challenge faced during storage because a large quantity of fruits is wasted due to poor postharvest management and microbial infection. India ranks second in the production of fruits after China and a study by Anand and Baruah (2022) stated that a loss of 70 percent of production occurs due to substandard storage facilities. This study is of immense practical application as the research findings emanating from the study has proved beyond doubt that beeswax as biopolymer coating with silver nanoparticles extends the shelf life of guava fruits, thereby enhancing food safety and reducing postharvest losses.

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