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Efficacy of Herbal Extracts-Based Nano-Formulations in Extending Guava Fruit Shelf-Life

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Abstract: The guava (*Psidium guajava* L.), known as 'Apple of the Tropics' is a rich source of polyphenols, pectin, dietary fiber, and carotenoids. Guava comes under climacteric types of fruits; due to its high transpiration and respiration rate it experiences high post-harvest losses. Giloy leaf and ajwain seed herbal extracts-based nano-formulations (NFs) were synthesized using biopolymer sodium alginate and $CaCl_2$, viz., T1 (Alginate: $CaCl_2$), T2 (Alginate: $CaCl_2$: Ajwain extract), and T3 (Alginate: $CaCl_2$: Giloy extract). Antibacterial and antioxidant activity of the NFs were examined to check their efficacy as antibacterial agents, which was checked against *E. coli*, *P. aeruginosa* and *B. cereus* bacterial culture. The zone of inhibition against bacteria ranged from 6 ± 0.42 mm to 14 ± 0.92 mm. Antioxidant activity was 93.27%, 71.67%, and 67.04% for T2, T3 and T1 NFs and 89.90% and 67.05% for ajwain and giloy extracts. NFs treated fruits showed minimum loss in physiological weight, firmness, and color change compared to control fruit (uncoated). Physiological loss in weight ranged from 3.16 to 17.21% and 3.23 to 15.57% and fruit firmness ranged from 4.47 to 8.41 kg/cm^2 and 4.84 to 8.37 kg/cm^2 during storage at 25 ± 2 °C (incubation) and 32 ± 2 °C (room temperature), respectively. Among NFs, T2 showed the best results in preventing ripening and maximum loss of quality was observed in control (uncoated) fruits. Thus, NFs are an effective method of extending the shelf-life of fruits and ajwain based NFs increased shelf life of guava from 4–5 days to 7–8 days. Fruits storage at 25 ± 2 °C showed better results compared to storage at 32 ± 2 °C. Thus, NFs treated fruits storage at lower temperature controls the ripening related changes maximally.

Keywords: nanoformulations; guava; antibacterial; shelf-life

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1. Introduction

The guava (*Psidium guajava* L.) is a resilient, easily adaptable, and evergreen tree of the *Myrtaceae* family that is grown in tropical and subtropical climatic regions. It is planted for its edible fruit [1]. India ranks first in the production of guava and mangoes (including mangosteens) (45.89%), papayas (44.05%) and bananas (26.08%) as per FAO data in 2020. India's export of fresh fruits has surged in recent years and guava export has shown a record growth of 260% since 2013, rising from 0.58 million in 2013–2014 to 2.09 million in 2021–2022. As per Statista Research Department data, the estimated guava production in India was 4.43 million metric tons (MMT) in 2021 compared to 4.36 MMT in 2020. The total area under guava cultivation was 304 thousand hectares in 2021. The major production areas of the country are state of Maharashtra, Andhra Pradesh, Tamil Nadu, Gujarat, and Karnataka. Guavas are nutrient rich fruit having a high content of vitamin A, vitamin C, antioxidants and minerals and consumed in both processed and fresh form [2]. They are climacteric in nature and are highly perishable which leads to high post-harvest losses [3]. Post-harvest loss of horticulture crops in India ranges between 5–39%, as per the Association of Social and Economic Transformation (ASET) reports. For guava, the post-harvest loss stands at 20–40% of the produce. This loss occurs due to improper storage of fruit and lack of packaging amenities. There is high loss in the fruit firmness, physiological weight and change in ascorbate, antioxidants, and total soluble solids (TSS) during fruit

storage [4]. Microbial infestation is also a major reason for post-harvest losses. Guava has high moisture content and is prone to high microbial infestation.

The use of antitranspirants [5], wax coatings [6], ethylene inhibitor and irradiation [7] can increase the longevity of harvested fruits. However, the use of chemicals such as antitranspirants and ethylene inhibitors has a negative impact on human health and results into poor consumer acceptability. Wax coatings' main limitation is that they create an anaerobic environment inside fruits by limiting oxygen due to which fermentation takes place. Wax coated fruits appear good but sometimes smell alcoholic due to anaerobic fermentation. Thus, use of coatings (edible films/coatings) which are environment friendly and consumed along with fruit have become popular. Edible films/coatings act as a shield against deteriorating agents and are used for enhancement of fruit shelf life [8].

Edible coatings are used to preserve fruit quality, maintain fruit firmness, and retard microbial damage to fruits and vegetables. They are applied as thin films on the fruit surface, invisible to the naked eye [9]. Lipids, proteins, polysaccharides, and combinations thereof are used to formulate edible coatings [10,11]. Herbal extracts having great medicinal, antioxidant and antimicrobial activities in combination with biopolymer conjugates are used nowadays for treatment of postharvest fruits to enhance the shelf life of fruits. Ginger extract has been used to enhance the shelf life of plantain [12], papaya [13] and mango [14]. Similarly, garlic extract has been used for mango [15] and banana [16].

In the present study extracts of giloy and ajwain were used through synthesis of nanoformulations with combinations of sodium alginate and $CaCl_2$. Giloy (*Tinospora cordifolia*) has many medicinal properties such as immunomodulator, anti-arthritic, anti-osteoporotic and hepatoprotective. As per the ayurvedic system, it is used to treat ailments such as urinary diseases, dyspepsia, and general debility [17]. The silver nanoparticles synthesized using *Tinospora cordifolia* stem showed strong antibacterial properties and a zone of inhibition for antibacterial activity of range from 10 ± 0.58 to 21 ± 0.25 mm was formed [18]. Ajwain (*Trachyspermum ammi* L.) is an herbaceous plant of the *Apiaceae* family. It is mostly cultivated in arid and semi-arid soils having high salt concentrations. Phytochemical studies showed that ajwain is rich in phenolics, glycosides, alkaloids, flavonoids, tannins, and steroids. Thus, these two herbs were used prepare extracts for making edible coatings. Ajwain and giloy have antibacterial properties which help in preventing fruit damage due to bacterial infection. Thus, treatment of guava with edible coatings based on herbal extracts having antibacterial properties will help in maintaining fruit quality for longer periods by preventing bacterial contamination.

Nowadays, nanotechnology provides new avenues for the transfer of active components of plant extracts like polyphenols, vitamins, and antioxidants. Nanotechnology constitutes particles and formulations of size ranging between 100–500 nm [19]. Nanoparticles have a higher surface area to volume ratio compared to large particles, conferring high stability and biological activity.

Temperature has an impact on fruit quality. Low temperatures maintain the fruit quality for longer with improved shelf life. The effect of edible coatings with lower temperature in increasing shelf life has been studied in guava, such as use of carnauba wax and xanthan gum coating at 10 °C which enhanced the shelf life of fruit by up to 30 days [20]. In the present study, ajwain and giloy plant extracts were prepared which were then used for nanoformulations synthesis. Sodium alginate biopolymer was used along with $CaCl_2$ which results in synthesis of a calcium alginate complex. Calcium acts as a scrosslinker and results into herbal extract entrapment inside the alginate and calcium crosslink. Properties of herbal extracts, such as antioxidant, antibacterial and nutritive features, will further improve the efficiency of synthesized nanoformulations. The synthesized nanoformulations were tested for antioxidant and antibacterial properties. Then, nanoformulations treatment on guava was performed at two temperatures, viz. 25 ± 2 °C and 32 ± 2 °C on guava (Hisar Surkha) fruit and analyzed for physiological changes in guava fruit. Temperature impacts the fruit shelf life greatly and thus different temperatures are used for fruit storage.

2. Materials and Methods

2.1. Plant Material

The present investigation was carried out in guava (*Psidium guajava* L.) fruits of variety Hisar Surkha (shelf life 4–5 days) at turning (T) stage procured from the Horticulture Farm, CCS Haryana Agricultural University, Hisar. Measurement of the physiological parameters was performed at the Department of Biochemistry, CCS, Haryana Agricultural University, Hisar, Haryana, India.

2.2. Preparation of Plant Extracts

Ajwain extract: The ajwain seeds were purchased from the local market and air dried. The ajwain extracts were prepared in methanol: acetone (7:3) using hot extraction methods. In the hot extraction method, dried seeds in the ratio 0.1 g/mL of solvent were kept in a water bath for 20 min at 52 °C and then filtered through Whatman filter paper no. 1 and stored at 4 °C.

Giloy extract: The leaves of the giloy plant were collected from the premises of CCS. Leaves and stems were thoroughly washed in the distilled water, cut into pieces, and dried at room temperature. Extraction was carried out in methanol using the Soxhlet extraction method.

Synthesis of herbal nano-formulation(s): Alginate nanoparticles were prepared using cation induced controlled gelification of alginate with a slight modification. Firstly, sodium alginate solution (0.06% in water) was prepared and stirred at 800 rpm for 8 h and then ajwain and giloy extracts were added dropwise to the to 28.5 mL of sodium alginate solution at 1:1 ratio. Then, 1.5 mL of calcium chloride (36 mM) was added to the solution under constant stirring, which forms the crosslinks with alginate and entraps the herbal extracts inside. The mixture is stirred for about an hour. The resultant solution is a herbal nano-formulation (NF) of ajwain and giloy.

Antibacterial property: The antibacterial activities were determined by modified agar well diffusion assay. Under aseptic conditions and in laminar air flow, 20 mL of nutrient agar medium was dispensed into pre-sterilized petri dishes. Once the media solidified, it was then inoculated with a micro-organism culture of *Escherichia coli* (ATCC no. BAA-2523), *Pseudomonas aeruginosa* (ATCC no. BAA-2796) and *Bacillus cereus* (ATCC no. 7039). The media were then punched with 6mm diameter holes and filled with NFs and herbal extracts at two concentrations, viz., 100 µL and 150 µL. Finally, the petri dishes were incubated for 48 h at 37 °C. The diameter of the zone of inhibition as indicated by a clear area which was devoid of growth of microbes was measured. Each experiment was done in triplicate.

Antioxidant activity: Antioxidant activity was measured using stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as per the method described by Shimada et al. [20]. 3 mL dye (0.5 mM DPPH solution in methanol) (diluted) was mixed with 0.5 mL diluted herbal extracts and NFs solution and incubated in the dark for 20 min. The absorbance was read at 517 nm on a spectrophotometer. Dye mixed with 0.5 mL methanol was used as blank and the scavenging of DPPH was calculated using the following formula:

$$\% \text{ Scavenging capacity of DPPH} = [(Ao - A_1)/Ao] \times 100\%$$

where Ao = Absorbance of blank and A_1 = Absorbance of sample

Treatment of guava fruit with NFs: Guava fruits were procured from the horticulture farm, CCS-HAU, Hisar. They were washed twice with distilled water and then extra water was removed under a fan. Washed guavas were dipped in NFs solution for 3–5 min and then air dried. The treated guava were kept at 25 ± 2 °C and 32 ± 2 °C temperature, maintained in labs. The treated fruits were analyzed till total decay on every alternate day. Uncoated fruits were treated as control and the three treatments were T1, T2 and T3.

2.3. Physiological Characteristics

2.3.1. Physiological Loss in Weight (PLW)

The weight of freshly harvested fruits was recorded at 0 day of storage and termed as initial weight. On each day of observation, the stored fruits were again weighed and termed as final weight on that particular day of observation. The per cent loss in weight on each sampling date was calculated using the following formula:

$$\text{PLW } (\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.3.2. Firmness

Flesh firmness was measured with a handheld fruit pressure tester penetrometer, using a cylindrical plunger of 8 mm diameter and firmness scale of 13 Kg cm^{-2}. The firmness was measured from each side of the equatorial region of the fruit. The firmness of three fruits per treatment was measured and it was expressed in Kg cm^{-2}.

2.3.3. Color Change

Fruit color change was observed according to the biological chart of USDA (1991).

2.4. Statistical Analysis

All data were expressed as Mean ± SD of three replicates. Statistical analysis was performed using SPSS v23.0 software (SPSS for Windows, Chicago, IL, USA). Significant differences among the samples were calculated using one-way ANOVA, followed by post hoc Duncan's multiple comparisons test at 5% level ($p = 0.05$).

3. Results

Guava is a nutrient rich fruit with wide applications, but is limited by its short shelf life. The present investigation was carried out on guava (*Psidium guajava* L.) fruits of variety Hisar Surkha (shelf life 4–5 days) at turning (T) stage. Herbal extracts of ajwain (*Trachyspermum ammi*) seeds and giloy (*Tinosporia cordipholia*) leaves were prepared using hot extraction and Soxhlet extraction methods, respectively. These extracts were used for synthesis of nanoformulations. Three nanoformulations, viz., Alginate: $CaCl_2$ (T1), Alginate: $CaCl_2$: ajwain extract (1:1) (T2), and Alginate: $CaCl_2$: giloy (1:1) (T3) were synthesized using the ionic gelation method. Mean particle size was determined using particle size analyzer (Metrohm instrument). T1 NFs had a mean size of 176.0 nm and polydispersity index (PDI) 0.173 (Figure 1). T2 nanoformulations had a mean size of 42.06 nm and PDI 0.465 (Figure 2) and T3 had a mean size of 216.8 nm and PDI 0.079 (Figure 3).

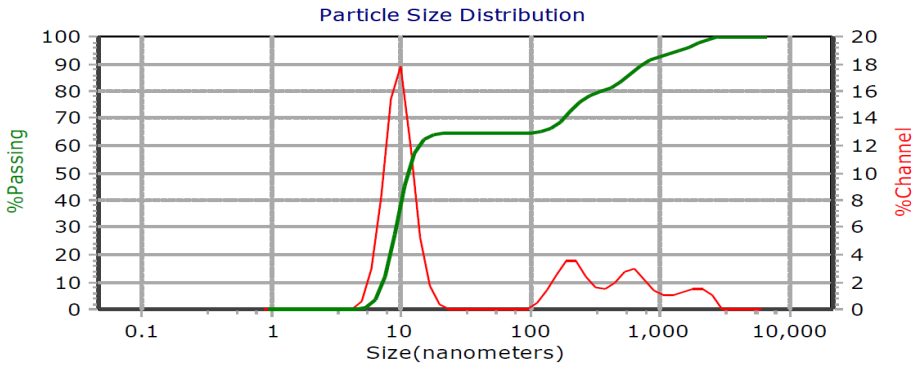

Figure 1. PSA spectrum of T1 NFs.

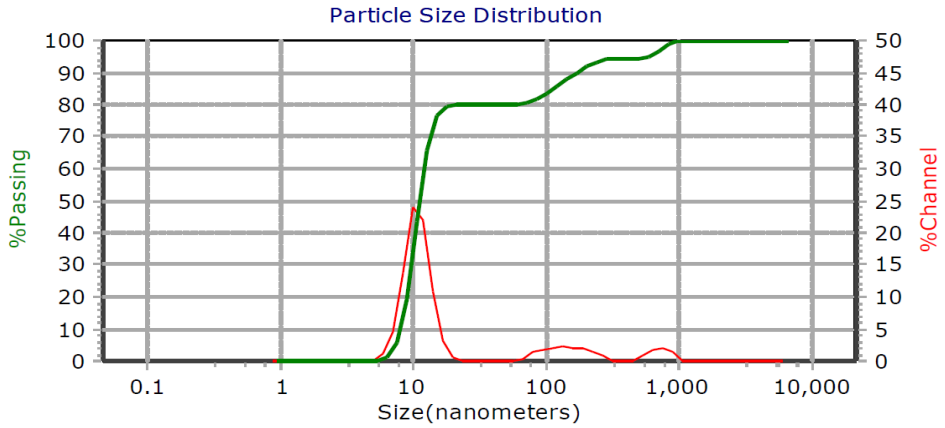

Figure 2. PSA spectrum of T2 NFs.

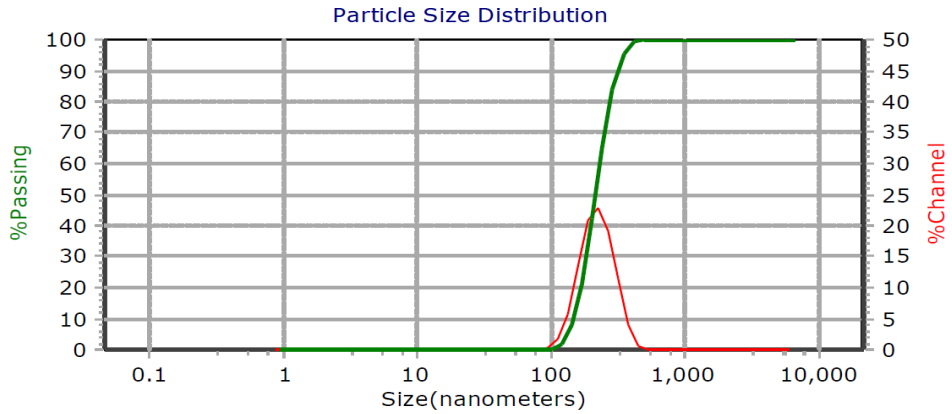

Figure 3. PSA spectrum of T3 NFs.

The efficacy of synthesized and characterized NFs (T1, T2 and T3) was checked by examining their antimicrobial activity and DPPH radical scavenging activity. Antimicrobial activity was determined using the disc agar diffusion method where three bacterial inoculations were used, viz., *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Bacillus cereus* (*B. cereus*). Figure 4 shows the antibacterial activity of different extracts and NFs against three bacterial strains at two concentrations. In each plate, two holes were made marked as L and R and 100 µL and 150 µL concentration of extracts and NFs was added to the respective holes. Then, plates were incubated at 37 °C and formation of inhibition zones was observed. The size of different inhibition zones formed at different concentrations is shown in Table 1. Larger inhibition zone formation was observed at higher concentrations (150 µL) (R hole). The zone of inhibition ranged from 6 ± 0.42 mm to 14 ± 0.92 mm. Ajwain extract, T1, T2 and T3 NFs showed antimicrobial activity against all the three bacterial strains, viz., *E. coli*, *P. aeruginosa* and *B. cereus* whereas giloy extract showed activity against *E. coli* and *B. cereus* but not against *P. aeruginosa*. The zone of inhibition was larger at 150 µL concentration as against 100 µL concentration in all the plates. T1 NFs showed good antimicrobial activity, which was further enhanced in T2, and T3 NFs. T2 showed maximum activity against *E. coli*, *P. aeruginosa* whereas T3 showed maximum activity against *E. coli* and *B. cereus*.

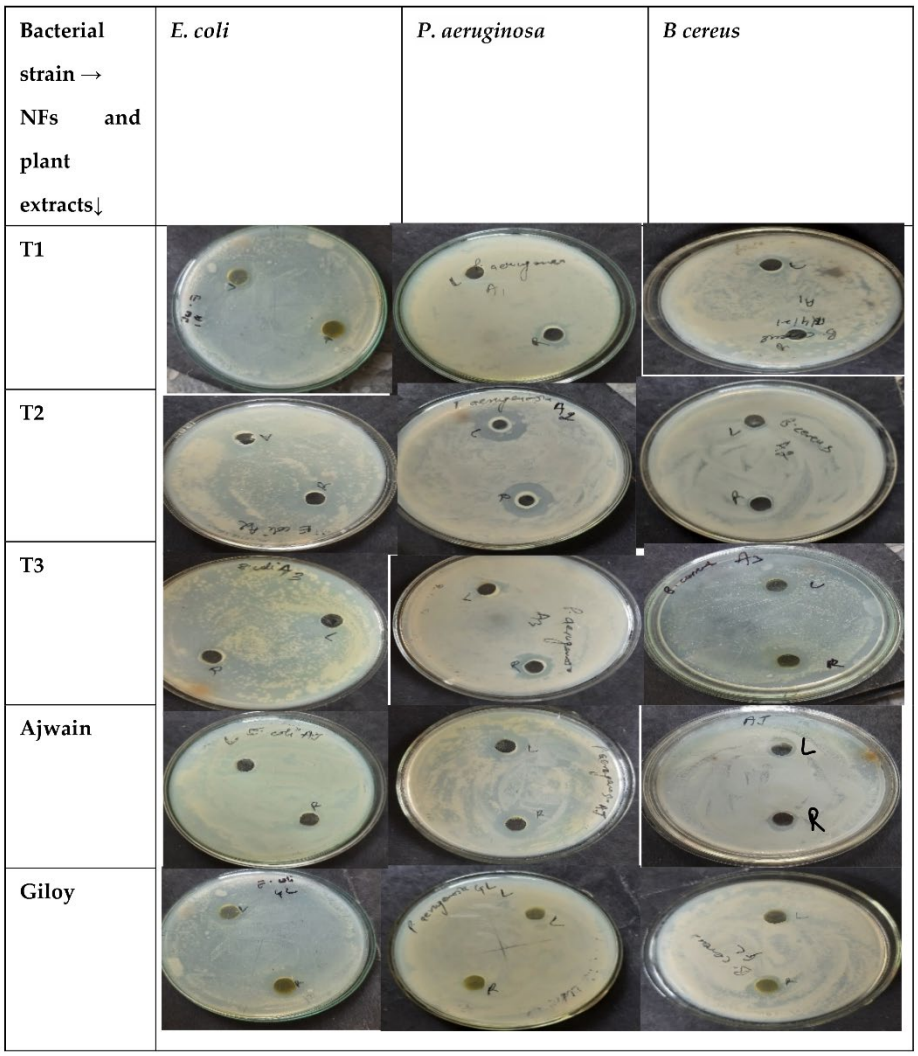

Figure 4. Antibacterial activity of NFs and extracts with the disc agar diffusion method.

Table 1. Zone of inhibition (in mm) at different concentration of herbal extracts and their NFs.

Bacterial Strains→ Antibacterial Solutions ↓	Zone of Inhibition (in mm) at Different Concentration of Herbal Extracts and Their NFs					
	E. coli		*P. aeruginosa*		*B. cereus*	
	100 µL	**150 µL**	**100 µL**	**150 µL**	**100 µL**	**150 µL**
T1	8 ± 0.12	12 ± 0.34	8 ± 0.42	10 ± 0.23	10 ± 0.50	12 ± 0.41
T2	**12 ± 0.45**	**14 ± 0.17**	**13 ± 0.51**	**14 ± 0.62**	8 ± 0.26	11 ± 0.34
T3	**13 ± 0.46**	**14 ± 0.92**	8 ± 0.28	10 ± 0.41	**11 ± 0.23**	**14 ± 0.33**
Ajwain	8 ± 0.26	10 ± 0.27	13 ± 0.43	14 ± 0.38	6 ± 0.42	8 ± 0.36
Giloy	10 ± 0.15	12 ± 0.43	-	-	7 ± 0.54	10 ± 0.53

3.1. DPPH Radical Scavenging Activity

All extracts and NFs showed DPPH radical scavenging activity. Highest activity was observed for T2 (93.27%) followed by T3 (71.67%), T1(67.04%), ajwain extract (89.90%) and giloy extract (67.05%) as shown in Table 2. Thus, the synthesized NFs have higher efficacy than their extracts.

Table 2. Antioxidant activity of different herbal extracts and NFs.

Nfs and Herbal Extracts	DPPH Radical Scavenging Activity (%)
T1	67.04 ± 0.39
T2	93.27 ± 1.43
T3	71.67 ± 1.09
Ajwain	89.90 ± 1.17
Giloy	67.05 ± 1.04
C.D. at 5%	0.31

3.2. Physiological Loss in Weight (PLW)

Figure 5 shows the physiological loss in weight (PLW) of guava fruits during storage and the effect of T1, T2 and T3 nanoformulations (NFs) coatings on fruit ripening. PLW ranges from 3.16% to 17.21% and 3.23% to 15.57% during storage at 25 ± 2 °C and 32 ± 2 °C, respectively. PLW increased during storage from the 2nd day after treatment (DAT) to the 10th and 12th DAT, where fruits totally decayed at 32 ± 2 °C and 25 ± 2 °C, respectively. Maximum increase in PLW was observed in control fruits (uncoated) followed by T1, T3 and T2 coated fruits at both temperature storage conditions. The maximum PLW was 17.21% and 15.57% in control fruits at 12 DAT and 10 DAT during storage at 25 ± 2 °C and 32 ± 2 °C, respectively. Minimum PLW was observed in T2 coated fruits with values of 12.28% and 9.97% at incubation and room temperature, respectively. Storage at 25 ± 2 °C maintained the fruit quality 2 days longer than storage at 32 ± 2 °C.

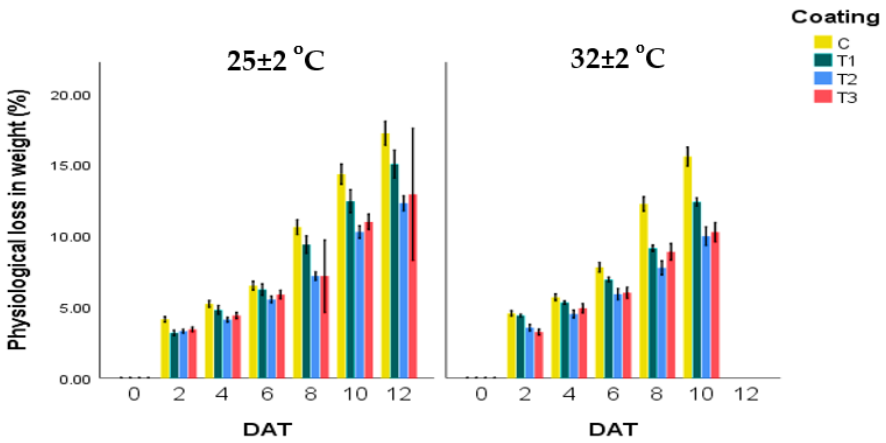

Figure 5. Effect of nanoformulation coatings on physiological loss in weight (%) of Hisar Surkha at (**a**) 25 ± 2 °C and (**b**) 32 ± 2 °C during storage.

C.D. at 5% Level of Significance	
Storage temperature	0.12
DAT	0.20
Storage temperature × DAT	0.28
Treatment	0.16
Storage temperature × Treatment	N/A
DAT × Treatment	0.40
Storage temperature × DAT × Treatment	0.57

3.3. Fruit Firmness

Figure 6 shows the change in firmness of guava fruits after the application of T1, T2 and T3 nanoformulations (NFs) coatings during storage, which ranges from 4.47 to

8.41 kg/cm^2 and 4.84 to 8.37 kg/cm^2 during storage at 25 ± 2 °C and 32 ± 2 °C, respectively. Fruit firmness decreased during storage. Maximum decrease in firmness was observed in control fruits (uncoated) followed by T1, T3 and T2 coated guava fruits at both temperature storage conditions. In control fruits, it decreased from 8.41 kg/cm^2 to 4.47 kg/cm^2 and 8.37 kg/cm^2 to 4.84 kg/cm^2 at 25 ± 2 °C and 32 ± 2 °C storage, respectively. In coated fruits at 25 ± 2 °C, firmness decreased from 8.41 kg/cm^2 to 5.08 kg/cm^2, 5.78 kg/cm^2 and 5.52 kg/cm^2 kg/cm^2 in T1, T2 and T3, respectively. In coated fruits at 32 ± 2 °C, firmness decreased from 8.37 kg/cm^2 to 5.62 kg/cm^2, 6.01 kg/cm^2 and 5.58 kg/cm^2 in T1, T2 and T3 treated fruits, respectively. Minimum decrease in firmness was observed in T2 coated fruit at both storage temperatures.

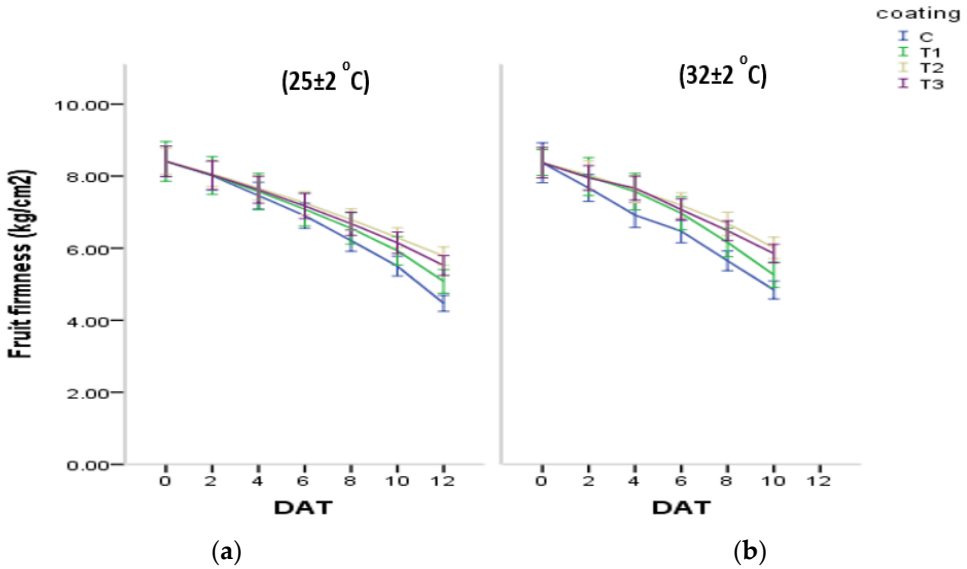

Figure 6. Effect of nanoformulation coatings on fruit firmness (kg/cm^2) of Hisar Surkha at (**a**) 25 ± 2 °C and (**b**) 32 ± 2 °C during storage.

C.D. at 5% Level of Significance	
Storage temperature	0.04
DAT	0.08
Storage temperature X DAT	0.12
Treatment	0.06
Storage temperature X Treatment	0.09
DAT X Treatment	0.17
Storage temperature X DAT X Treatment	0.23

3.4. Color Change

The change in color of guava fruits during the storage period was observed according to the biological chart of USDA (1991). The results showed that the color of guava fruits varied turning to yellow in all the treated as well as control fruits. In control fruits, the color changed to the yellow stage at 4th DAT and at 6th DAT at 32 ± 2 °C and 25 ± 2 °C, respectively. The prepared NFs delayed the change in color in all the treatments for both storage conditions. T2 showed most prominent results for delay of color change, and the yellow stage appeared on the 6th and 8th DAT at 32 ± 2 °C and 25 ± 2 °C, respectively, as compared to control fruits which attained yellow colored stage on 4th and 6th DAT (Figures 7 and 8).

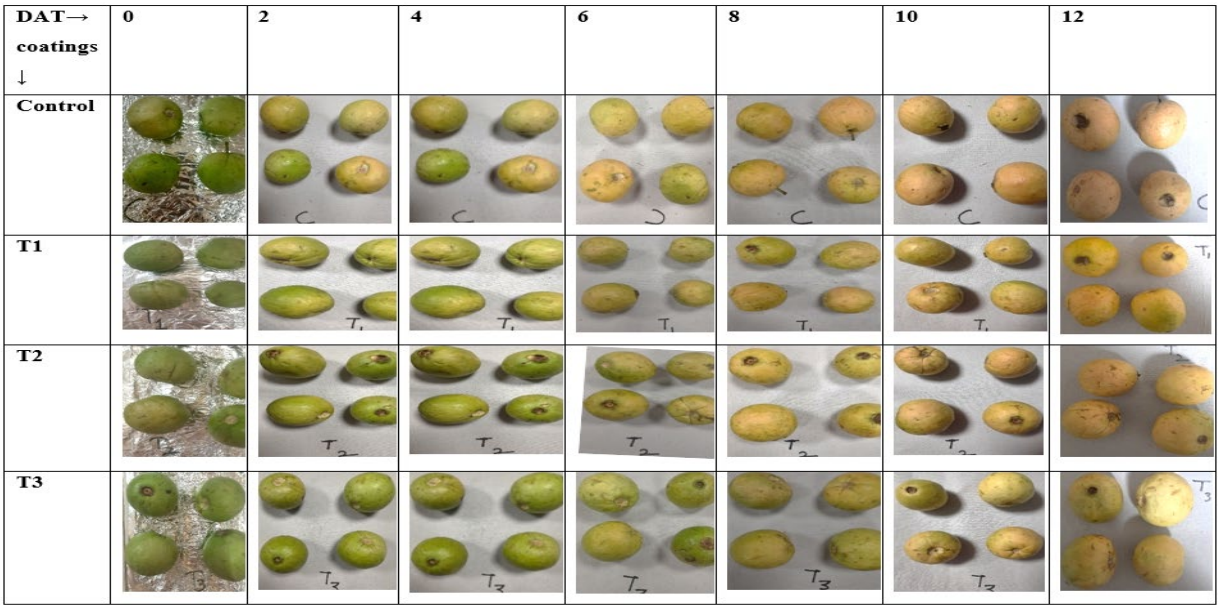

Figure 7. Effect of nanoformulations on color change of guava fruits (Hisar Surkha) during storage at 25 ± 2 °C.

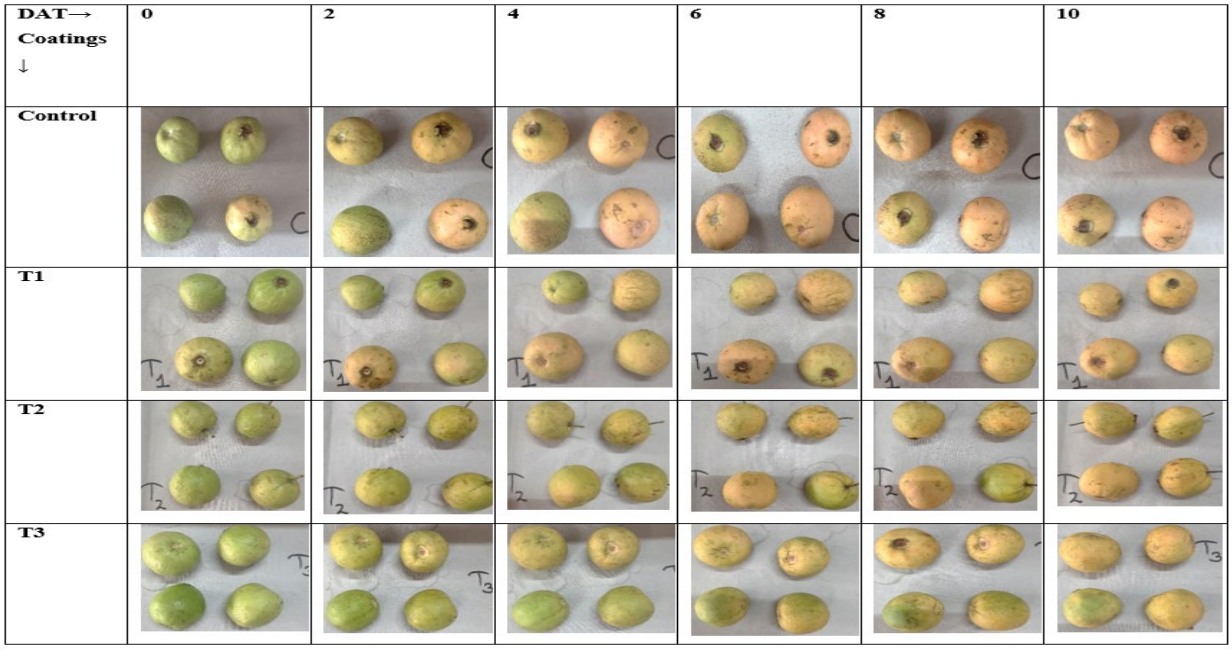

Figure 8. Effect of nanoformulations on color change of guava fruits (Hisar Surkha) during storage at 32 ± 2 °C.

4. Discussion

The guava (*Psidium guajava* L.) with chromosome number 2*n* = 22 is a tree within the the *Myrtaceae* family. Commonly known as "Apple of the Tropics", it is grown in tropical and subtropical climatic regions. It is an evergreen, easily adaptable and resilient tree. Loss occurs due to improper storage of fruit and lack of packaging amenities. Fruits are imperative for good health as they provide macro and micronutrients to the body. Edible coatings are an effective method in reducing post-harvest losses. In guava, use of edible coatings such as xanthan gum, carnauba wax, 2% chitosan, carboxymethyl cellulose and cashew gum, cassava starch and cinnamon essential oil has been reported [21–24]. In the

present study, herbal extracts of ajwain (*Trachyspermum ammi*) seeds and giloy (*Tinosporia cordipholia*) leaves and stems were prepared. Addition of these extracts improves the coatings' efficiency. Similar results have been reported by Murmu and Mishra [25], where gum arabic: sodium caseinate: tulsi extract improved the shelf life of guava fruit to 7 days against control which had 4 days shelf life at 28 °C. Table 3 shows the effect of different edible coatings on shelf life of fruits.

Table 3. Effect on edible coatings on shelf life of guava.

Coating	Effect of Coatings	Deposition	References
Gum Arabic and Moringa extract	Sustained fruit firmness, TSS, antioxidant activity and total sugars	Dipping	[26]
Arabic Gum, Sodium Caseinate and Tulsi Extract	Reduced evaporation, transpiration and oxygen transmission	Dipping	[25]
Gum Arabic and Cinnamon Essential Oil	Reduced PLW, maintenance of firmness, caretonenoid content, and chlorophyll content, as compared to control fruit	Dipping	[11]
Chitosan and Ruta Graveolens Essential Oil	Antimicrobial activity against *Colletotrichum gloesporioides* and maintenance of fruit firmness; lesser PLW	Dipping	[27]
Agar (4%) + Pomegranate Seed oil (0.4 mL/L)	Reduction in PLW; fruit color and TSS maintained by coating and no change in carotenoid content	Dipping	[28]

Synthesis of herbal nanoformulations (NFs) was carried out using alginate: $CaCl_2$ in combination with ajwain and giloy extracts using the ionotropic gelation method. Ion or ionotropic gelation, also known as polyelectrolyte gelation or complexation, is the most widely used method of nanoencapsulation synthesis using biocompatible and non-toxic polymers [29]. This gelation is the result of mixing of oppositely charged biopolymers at low concentration in solutions, which should be less than their gelling points. Alginate is produced by marine brown algae and is used in the synthesis of edible coatings and nanocapsules. The different sized insoluble nanoparticles of calcium alginate can be formed using soluble sodium alginate and calcium chloride at different concentrations. Particles of 100 nm to 2 mm have been synthesized using sodium alginate and $CaCl_2$ for drugs delivery [30].

The NFs exhibited good antioxidant and antimicrobial activity due to addition of herbal extracts. Ajwain and giloy extracts were reported as having antibacterial properties but at nanoscale these activities were further enhanced. This is due to nanoparticles' smaller size to volume ratio which provides a larger surface area for herbal extracts' bioactive compounds' interaction with the fruit surface. Herbal extracts are full of antioxidant content and thus, enhance fruit resistance against reactive oxygen species. All the synthesized nanoformulations are of nano size. Figure 5 shows the physiological loss in weight (PLW) of guava fruits during storage and the effect of T1, T2 and T3 nano-formulations (NFs) on fruit ripening. The maximum PLW was 17.21% and 15.57% in control fruits at 12 DAT and 10 DAT during storage at 25 ± 2 °C and 32 ± 2 °C, respectively. Minimum PLW was observed in T2 coated fruits, with 12.28% and 9.97% weight loss at 25 ± 2 °C and 32 ± 2 °C, respectively. PLW is the result of respiration and moisture evaporation between the fruit tissue and surrounding environment, and is affected by post-harvest treatments [31]. The loss in PLW of guava is due to loss of bound and free moisture through evapotranspiration and respiration. Higher moisture loss also impacts parameters such as firmness and color change [32]. Sharma and Saini [33] reported improved shelf life till 12th day for guava coated with flaxseed protein: guar gum: glycerol as compared to uncoated control guava

which shriveled on the 7th day (approx. 70%). Uncoated fruits' premature ripening leads to tissue damage which makes them more prone to microbial attack. Similar results were observed for tomato fruit treated with starch coating [34].

Figure 6 shows the change in firmness of guava fruits after the application of T1, T2 and T3 NFs during storage, which ranges from 4.47 to 8.41 kg/cm^2 and 4.84 to 8.37 kg/cm^2 during storage at 25 ± 2 °C and 32 ± 2 °C, respectively. Maximum decrease in firmness was observed in control fruits (uncoated) followed by T1, T3 and T2 coated guava fruits at both temperature storage conditions. One reason behind firmness loss is the action of hydrolytic enzymes such as β-D-gluctosidase hydrolysis of pectin in guava [35]. Uncoated fruits lose moisture and become inflexible in sustaining firmness compared to NFs treated fruits. NFs have a binding capacity and regulate moisture and gaseous exchange; this maintains fruit firmness. Santos et al. [36] observed similar results in guava fruits coated with zein protein and tannic acid. Coating of 'Rocha' pears with nano-emulsions of sodium alginate with citral and lemongrass essential oil preserved the firmness of fruit for a longer time compared to control fruit [37].

In the present study, the results showed that the color of guava fruits varied, turning yellow in all the treated as well as control fruits. In control fruits, the color changed to the yellow stage at the 4th DAT and at the 6th DAT at 32 ± 2 °C and 25 ± 2 °C, respectively. The prepared NFs delayed the change in color in all the treatments under both storage conditions. T2 showed most prominent results where color change was delayed, and the yellow stage appeared on the 6th and 8th DAT at 32 ± 2 °C and 25 ± 2 °C, respectively, as compared to control fruits which attained the yellow colored stage on the 4th and 6th DAT (Figures 7 and 8). The color change in fruits from green to yellow is ascribed to pigment content changes such as decrease in chlorophyll and increase in carotenoid content during storage. A 400% increase in carotenoids and 72% decrease in chlorophyll content was observed in plume guava stored at 27 °C and resulted in color change from green to yellow [38]. Moreover, the color change is affected by the action of polyphenol oxidase in the presence of oxygen which causes a browning reaction in fruits [39]. Coating of 'Rocha' pears with nano-emulsions of sodium alginate with citral and lemongrass essential oil reduced color change development as compared to uncoated control fruits [37]. Application of edible coatings replaces the O_2 and releases CO_2 which slows down the oxidation of fruits which in turn cause lesser ethylene production. Water loss from the fruit surface is also minimized which results into higher firmness and shelf life of guava. Freshness and quality are also preserved for longer time [40].

5. Conclusions

Treatment of guava with NFs significantly reduced the oxidative damage to fruits and enhanced the fruit shelf life by 6–8 days at incubator and room temperature storage. Damage was less at a lower temperature (25 ± 2 °C) compared to a higher temperature (32 ± 2 °C). Physiological loss in fruits was minimum in T2 NFs treated fruits compared to T3 and T1. Control fruit faced the maximum oxidative damage. Ajwain is an effective substrate for herbal NFs synthesis and its role in increasing the shelf life of guava could thus be explored further on a larger scale.

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