Journal of Agricultural Science and Technology (JAST), 29(5)
In Press, Pre-Proof Revision

Combined Effect of Carnauba Wax Coating and LDPE Packaging on Guava Fruit Quality and Shelf Life Under Cold and Ambient Storage

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Abstract

Guava (*Psidium guajava* L.) is a highly perishable climacteric fruit prone to moisture loss and postharvest enzymatic softening. Although edible coatings and modified atmosphere packaging are established postharvest technologies, their combined effects on pectin-degrading enzymes and antioxidant dynamics under contrasting temperatures remain poorly characterized. This study evaluated the individual and combined effects of carnauba wax (CW; 3% and 6%) and micro-perforated low-density polyethylene (LDPE; 0.5% perforation) on guava cv. 'Shweta' under cold (6–8 °C, 90–95% RH) and ambient (20–24 °C, 70–75% RH) storage using a factorial completely randomized design. CW 6% + LDPE enhanced postharvest preservation, limiting weight loss to 5.48% (ambient, day 15) and reducing final spoilage by ~48% and ~35% under ambient and cold storage, respectively, compared with controls. Furthermore, CW 6% + LDPE suppressed pectin methylesterase (PME) activity by ~30%, delaying pectin depolymerization and limiting firmness loss to 53.66% under cold storage (day 25), compared with 71.29% in the control. This treatment also sustained significantly higher ascorbic acid, titratable acidity, and total phenolics (8.45% above control). These findings demonstrate that 6% carnauba wax combined with perforated LDPE is an accessible, non-thermal strategy for maintaining cell-wall integrity and extending guava shelf life under contrasting storage temperatures.

Keywords: Carnauba wax, Guava storage, Low-density polyethylene, Pectin methylesterase, Postharvest preservation, Quality retention.

1. Introduction

Guava (*Psidium guajava* L., family Myrtaceae) is an economically vital, nutrient-dense fruit cultivated across tropical and subtropical regions. As a climacteric fruit, it exhibits a characteristic surge in respiration and ethylene biosynthesis following harvest, leading to accelerated softening, desiccation, and rapid organoleptic deterioration (Sultana et al., 2021; Dhillon et al., 2024). Conventional bulk packaging systems (e.g., corrugated fiberboard boxes,

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wooden crates, and foam nets) mitigate physical impact injury but fail to regulate the physiological drivers of senescence and water loss (Singh, 2011; Chen et al., 2024). Consequently, non-thermal preservation technologies capable of simultaneously modulating cuticular gas exchange and transpiration are essential to extend the guava supply chain.

Edible lipid coatings provide an environmentally benign approach by depositing a semi-permeable hydrophobic film over the pericarp, restricting water vapor transmission and gas diffusion (Devi et al., 2022; Kumar et al., 2023). Carnauba wax (*Copernicia prunifera*), a Generally Recognized as Safe (GRAS, E903) additive, effectively limits weight loss and maintains firmness in tropical fruit (Ohashi et al., 2015; Chen et al., 2019). Concurrently, micro-perforated low-density polyethylene (LDPE) creates modified atmosphere packaging (MAP) conditions by maintaining elevated relative humidity and retarding aerobic senescence without triggering anaerobiosis (Nagaraju and Banik, 2019; Buthelezi and Mafeo, 2024). Flesh softening in guava is driven by enzymatic middle-lamella disassembly, where pectin methylesterase (PME) catalyzes the de-esterification of galacturonosyl residues, priming the pectin matrix for polygalacturonase cleavage and structural collapse (Chen et al., 2019). Concurrently, postharvest oxidative stress promotes the degradation of non-enzymatic antioxidant pools, including ascorbic acid and phenolics. While standalone applications of coatings or polymeric films, as well as beeswax-LDPE combinations, have been explored (Mangaraj et al., 2014; Dhillon et al., 2024), the combined mechanisms of carnauba wax and micro-perforated LDPE on PME activity, pectin dynamics, and antioxidant retention remain uncharacterized. Furthermore, comparative evaluations across contrasting thermal regimes (cold vs. ambient) are required to establish viable supply-chain protocols. Carnauba wax concentrations of 3% and 6% were selected based on literature to optimize transpiration resistance without inducing anaerobic fermentation (Ohashi et al., 2015; Chen et al., 2019). We hypothesized that integrating carnauba wax with perforated LDPE would exert a dual physical and physiological barrier, suppressing PME-mediated cell-wall degradation and oxidative antioxidant loss more effectively than individual treatments across both ambient and cold storage. Therefore, this study aimed to: (1) evaluate the individual and combined effects of carnauba wax (3% and 6%) and perforated LDPE packaging on the physicochemical, enzymatic (PME and pectin dynamics), antioxidant, and sensory attributes of guava cv. ‘Shweta’; and (2) determine their efficacy in extending marketable shelf life under ambient and low-temperature storage regimes.

2. Material and Mehtods

2.1 Material

Physiologically mature, uniform guava (*Psidium guajava* L. cv. 'Shweta') fruits (weight: 145 ± 5 g; flesh firmness: 106 ± 2 N) free from visual blemishes were harvested during the second week of December (2019 and 2020) from 100 uniform-aged trees at the Regional Fruit Research Station, Punjab Agricultural University (PAU), Bahadurgarh, Patiala. Harvested fruits (648 total) were immediately transported to the Postharvest Laboratory, Department of Fruit Science, PAU, Ludhiana, sorted, graded, and washed under running tap water.

2.2 Preparation of Carnauba Wax Emulsions

Carnauba wax (CW) emulsions (3% and 6% w/v) were prepared according to Singh *et al.* (2016) with minor modifications. Carnauba wax (3.0 or 6.0 g), oleic acid (8.0 mL), and glycerol monolaurate (1.5 g) were melted at 125 °C in an oil bath under magnetic stirring (200 rpm). Aqueous ammonia (15 mL, 25% v/v) and hot distilled water were added to yield a 100 mL volume while increasing agitation to 600 rpm. The emulsion was adjusted to pH 7.0 using hot acetic acid (90–95 °C, 1.0–1.2 mL 100 mL^{-1}) and cooled to 50 °C under continuous stirring prior to application.

2.3 Experimental Design, Treatment Application, and Storage

The experiment was laid out in a Factorial Completely Randomized Design (F-CRD) testing six postharvest treatments: untreated control, carnauba wax 3% (CW 3%), carnauba wax 6% (CW 6%), LDPE packaging, CW 3% + LDPE, and CW 6% + LDPE, evaluated under cold (6–8 °C, 90–95% RH) and ambient (20–24 °C, 70–75% RH) storage. Sanitized fruits (100 ppm NaOCl, 5 min) were coated uniformly using foam pads and air-dried under forced ventilation. To prevent mechanical abrasion and tissue damage, fruits were loosely packed in 100-gauge LDPE bags with 0.5% perforation (five 0.5-mm holes per side). In combined regimens, coated and dried fruits were packaged into LDPE bags. Wax concentrations (3% and 6%) were chosen based on preliminary assays and established literature (Ohashi et al., 2015; Chen et al., 2019). Destructive and non-destructive evaluations were performed at 5-day intervals (0, 5, 10, 15, 20, 25 days) under cold storage and at 3-day intervals (0, 3, 6, 9, 12, 15 days) under ambient storage.

2.4 Sampling Procedure During Storage

A total of 648 physiologically mature guava fruits were evaluated across both storage environments (ambient: 20–24 °C; cold: 6–8 °C) using a Factorial Completely Randomized

Design ($n = 3$). Each experimental unit at a specific sampling interval comprised a ventilated corrugated fiberboard (CFB) box containing three fruits (650–700 g per replicate; 9 fruits per treatment across three replications). Consequently, each treatment group included 54 fruits per storage regime (3 fruits × 3 replications × 6 sampling intervals), yielding 324 fruits per storage condition (108 fruits per replication block) and 648 fruits overall. At each sampling interval, designated boxes were withdrawn for non-destructive evaluations (weight loss, organoleptic score) followed by destructive physicochemical and enzymatic assays.

2.5 Observation recorded

2.5.1 Weight loss

Physiological weight loss (PWL) was measured gravimetrically at each sampling interval by recording initial and storage-interval weights, calculated as:

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

2.5.2 Firmness

Fruit firmness was determined on peeled equatorial flesh using a penetrometer (Model FT-327, QA Supplies, Norfolk, VA, USA) fitted with an 8-mm stainless-steel probe, with force expressed in Newtons (N).

2.5.3 Sensory evaluation

Sensory attributes (color, texture, odor, taste, and overall acceptability) were evaluated on randomized, coded fruit slices by an experienced panel of 10 judges (5 men, 5 women; aged 30–55 years) from the Department of Fruit Science, PAU. Panelists scored individual attributes using a standard 9-point hedonic scale ranging from 1 (extremely undesirable) to 9 (extremely desirable), where a score of 5 represented the threshold of market acceptability. Mean scores across all panelists were calculated for statistical evaluation.

2.5.4 Spoilage

The number of fruits showing visible signs of spoilage or decay was recorded at each storage interval. The percentage of spoilage was calculated based on the total number of fruits present in each experimental unit using the following equation:

$$\text{Spoilage (\%)} = \frac{Number\ of\ spoiled\ fruits \times 100}{Total\ number\ of\ fruits}$$

2.5.5 Total Soluble Solids

Total soluble solids were determined from freshly extracted guava juice using a calibrated digital refractometer (ATAGO, Japan) and expressed as °Brix after applying standard temperature corrections.

2.5.6 Total Phenols

Total phenolic content was determined via the Folin–Ciocalteu assay. Fresh guava juice (1.0 mL) was extracted with 14.0 mL of 80% (v/v) methanol for 30 min at 25 °C, centrifuged at 2000 rpm for 20 min, and adjusted to 15.0 mL. A 1.0 mL aliquot was evaporated to dryness at 60–70 °C, redissolved in 6.5 mL distilled water, and reacted with 0.5 mL Folin–Ciocalteu reagent (1:1 v/v). After 5 min, 1.0 mL saturated Na_2CO_3 was added, followed by 1 h dark incubation at room temperature. Absorbance was read at 760 nm (UV-2450, Shimadzu, Japan) against a blank. Total phenolics were quantified using a gallic acid standard curve and expressed as mg gallic acid equivalents (GAE) per 100 g fruit.

2.5.7 Titratable Acidity

To measure acidity guava fruits are crushed and filtered through muslin cloth to obtain juice out of which 2 ml is taken in conical flask and 2-3 drops of phenolphthalein (1 %) are added and then titrations is done using 0.1 normal sodium hydroxide solution till juice become slightly pinkish. Acidity is calculated as equivalent to citric acid in percents by equation given below.

$$\text{Acidity (\%)} = \frac{0.0064 \text{ x Volume of N/10 NaOH used (ml)} \times 100}{\text{Volume of the sample taken (ml)}}$$

2.5.8 Vitamin C

Ascorbic acid content was determined by the 2,6-dichlorophenolindophenol (DCPIP) visual titration method according to AOAC (1980). Guava pulp (5 g) was homogenized in 3% (w/v) metaphosphoric acid prepared with glacial acetic acid, filtered, and made up to a final volume of 50 mL. A 5 mL aliquot of the clear extract was titrated against standardized DCPIP dye solution (standardized against pure L-ascorbic acid) until the appearance of a persistent light-rose pink endpoint lasting 10–15 s. The vitamin C content was calculated using the predetermined dye factor and expressed as mg 100 g^{-1} fresh weight.

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Dye factor} \times \text{Titre value} \times \text{Volume made}}{\text{Weight of the fruit taken} \times \text{Volume of aliquot}} \times 100$$

2.5.9 Total Sugars

Total sugar content was quantified via the phenol-sulfuric acid method (Lam et al., 2021). Guava flesh was extracted with aqueous ethanol (1:1 v/v, room temperature), filtered, and concentrated under vacuum using a rotary evaporator. Dried sugar extract (0.25 g) was dissolved in distilled water to 100 mL, and a 2 mL aliquot was further diluted to 100 mL. A 1 mL portion of the diluted sample was mixed with 1 mL of 5% phenol, 1 mL of distilled water, and 5 mL of concentrated sulfuric acid. After 30 min incubation and cooling, absorbance was measured at 490 nm using a UV-Vis spectrophotometer.

2.5.10 Reducing Sugars

Reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNS) colorimetric method (Lam et al., 2021). The vacuum-dried ethanol extract (0.25 g) was dissolved in distilled water to 25 mL, and a 2 mL aliquot was diluted to 10 mL. An aliquot of 2 mL of this diluted solution was reacted with 1.5 mL of DNS reagent and 6.5 mL of distilled water, heated at 80 °C in a water bath for 10 min, and cooled in a water bath for 20 min. Absorbance was recorded at the wavelength of maximum absorbance using a UV-Vis spectrophotometer.

2.5.11 Pectin Content

Pectin content was estimated gravimetrically following the method described by Ruck (1961). A representative guava sample was extracted under acidic conditions, and calcium chloride was subsequently added to precipitate the extracted pectin as calcium pectate. The calcium pectate precipitate was collected and washed thoroughly with water to remove residual chloride ions. The washed precipitate was then dried to constant weight and weighed. Pectin content was calculated from the weight of the dried calcium pectate and expressed as a percentage of the sample weight.

2.5.12 Pectin methyl- esterase (PME)

PME activity was determined according to Chen et al. (2019) with minor modifications. Fresh guava tissue (1.5 g) was extracted in 4 mL of 8.8% (w/w) NaCl containing 10 g L^{-1} PVPP, and centrifuged at 12,000 × g for 20 min at 4 °C. The collected supernatant was adjusted to pH 7.5 with 1 mol L^{-1} NaOH. The reaction mixture contained 2 mL pectin solution, 0.15 mL of 0.01% (w/v) bromothymol blue in 0.3 mol L^{-1} phosphate buffer (pH 7.5), 0.75 mL distilled water, and 0.1 mL crude enzyme extract. Following incubation at 40°C for 1 min, absorbance was measured at 620 nm on a UV-2450 spectrophotometer at 0- and 2-min. Enzyme activity

was calculated from the initial rate of absorbance decline and expressed as mg kg^{-1} min^{-1} methyl ester (1 unit = production of 1 μmol galacturonic acid min^{-1}).

2.6 Statistical analysis

The experiment was laid out in a two-factor Factorial Completely Randomized Design (F-CRD; $n = 3$), analyzed separately for cold (6–8 °C; days 0, 5, 10, 15, 20, 25) and ambient storage (20–24 °C; days 0, 3, 6, 9, 12, 15). The model assessed treatments (6 levels), storage duration (6 intervals), and their interaction. Non-destructive attributes (weight loss and spoilage) were tracked on dedicated tagged fruits, while destructive parameters utilized distinct fruits per interval to ensure statistical independence. Normality (Shapiro–Wilk) and variance homogeneity (Levene's test) were verified before two-way ANOVA ($p > 0.05$); percentage data were arcsine-transformed, with back-transformed means reported. Treatment means were separated by Tukey's HSD ($p < 0.05$). Effect sizes (partial η^2), F-values, exact p-values, and 95% confidence intervals were determined. Multivariate associations were analyzed via Pearson correlation and Principal Component Analysis (PCA) on standardized z-scores using SAS/R.

3. Results and Discussion

3.1 Weight Loss

Weight loss increased progressively across all treatments during storage, governed by significant main effects of treatment ($F_{5,96} = 109.28, p < 0.001$,partial $\eta^2 = 0.85$), storage duration ($F_{1,96} = 12963.86, p < 0.001$,partial $\eta^2 = 0.99$), and their interaction ($F_{5,96} = 31.86, p < 0.001$,partial $\eta^2 = 0.62$; Fig. 1a, 1b). The significant Treatments × Days interaction demonstrates that uncoated fruits lost moisture at a disproportionately accelerated rate as storage progressed, widening the divergence between coated and uncoated lots over time.

By the end of storage, CW 6% + LDPE proved most effective in restricting weight loss (5.48% at ambient; 4.62% in cold storage), whereas untreated control fruit exhibited significantly higher losses (8.10% at ambient; 6.73% in cold storage; Tukey's HSD, $p < 0.001$). The lower moisture loss under cold storage reflects the temperature-dependent reduction in transpirational vapor pressure deficit and respiratory metabolism. Nevertheless, the performance hierarchy among treatments remained identical across both storage regimes, demonstrating that the barrier mechanism of the coating operates synergistically with, but independent of, low temperatures.

The reduced physiological weight loss in coated and packaged fruit reflects a dual physical barrier. Carnauba coating occludes cuticular lenticels and micro-pores, restricting water vapor transmission and gas exchange (Chavan et al., 2023; Khan et al., 2024), while micro-perforated LDPE maintains a high-humidity microenvironment that depresses the transpirational vapor pressure gradient. The greater efficacy of CW 6% over CW 3% denotes a more cohesive hydrophobic matrix across the pericarp. These findings align with reports where carnauba wax and polyethylene packaging suppressed weight loss in guava (Kore and Kabir, 2011) and carnauba nanoparticles delayed desiccation in 'Golden' papaya (Ohashi et al., 2015). Furthermore, recent work confirms that combining hydrophobic lipid coatings with semi-permeable film packaging curtails moisture loss and preserves fruit mass during extended guava storage (Gull et al., 2024).

3.2 Firmness

Fruit firmness declined progressively across all treatments, governed by a significant Treatments × Days interaction ($F_{5,96} = 5.23, p < 0.001$,partial $\eta^2 = 0.21$). This interaction demonstrates that the protective effect of coating treatments intensified as storage progressed, reflecting an active deceleration of the biochemical cascade driving softening rather than a simple fixed delay. Treatment accounted for a major share of total variance in flesh firmness ($F_{5,96} = 27.28, p < 0.001$, partial $\eta^2 = 0.59$).

Among all regimens, CW 6% + LDPE exhibited the highest firmness retention, restricting total firmness loss to 53.66% under cold storage and 58.54% under ambient storage, whereas untreated control fruit softened fastest (71.29% and 76.87% loss, respectively). At the final ambient evaluation, CW 6% + LDPE fruits were significantly firmer than the control (Tukey's HSD, estimate = 3.59, $p < 0.001$). Softening proceeded slower under cold storage across all treatments due to the thermal suppression of hydrolytic enzyme kinetics. Furthermore, the consistent performance hierarchy between storage environments indicates that the coating's protective mechanism functions additively with low temperature (Fig. 1c, 1d).

Postharvest softening stems from middle lamella pectin degradation by pectin methylesterase (PME), polygalacturonase (PG), and cellulase (Shi et al., 2019). Superior firmness retention under CW 6% + LDPE reflects minimized transpiration and altered internal O_2/CO_2 exchange, which downregulate respiratory metabolism and cell-wall hydrolases. Correspondingly, Gull et al. (2024) observed that edible coatings inhibit PME, PG, and cellulase, maintaining cell-wall integrity in guava. These results corroborate textural preservation using carnauba wax or

LDPE packaging in guava, plum, and pear (Kim et al., 2013; Kore and Kabir, 2011; Mahajan et al., 2013; Rana et al., 2015).

3.3 Sensory evaluation

Organoleptic quality (color, taste, and texture) varied significantly during storage, driven by the main effects of Treatments ($F_{5,96} = 2.30, p = 0.051$,partial $\eta^2 = 0.11$) and Days ($F_{1,96} = 110.05, p < 0.001$,partial $\eta^2 = 0.53$), as well as a significant Treatments × Days interaction ($F_{5,96} = 2.81, p = 0.021$,partial $\eta^2 = 0.13$). This interaction confirms that sensory degradation rates differed markedly among treatments over time rather than following a uniform decline. Under cold storage, sensory scores increased steadily to a peak at day 15 before declining, with CW 6% + LDPE attaining the highest score (7.46), significantly outperforming the control (6.68; Tukey's HSD, $p = 0.035$). Under ambient conditions, higher metabolic rates accelerated senescence, causing scores to peak earlier (day 9) and decline sharply thereafter. Nevertheless, CW 6% + LDPE consistently maintained the highest organoleptic acceptability through the end of storage under both regimes, whereas untreated controls exhibited the lowest ratings (Fig. 1e, 1f).

The time-dependent divergence between treatments reflects the physiological mitigation of postharvest senescence. Carnauba wax creates a surface barrier that restricts transpiration and respiration, while perforated LDPE packaging moderates the microenvironment, slowing enzymatic and oxidative breakdown that compromises flavor and texture. The superior efficacy of CW 6% + LDPE aligns with earlier reports in guava and papaya where elevated wax concentrations formed more cohesive hydrophobic matrices (Kore & Kabir, 2011; Ohashi et al., 2015). Furthermore, the preservation of treatment rankings across both storage conditions confirms that wax coating and LDPE packaging operate additively with low temperature to preserve consumer acceptability (Gull et al., 2024).

3.4 Total soluble solids (TSS) and Sugars

Total soluble solids and sugar fractions exhibited an initial accumulation followed by a gradual depletion across all treatments during storage, reflecting starch hydrolysis followed by respiratory consumption. Factorial ANOVA revealed highly significant main effects of Days ($F_{1,96} = 287.40, p < 0.001$,partial $\eta^2 = 0.75$) and a significant Treatments × Days interaction ($F_{5,96} = 22.48, p < 0.001$,partial $\eta^2 = 0.54$). This interaction demonstrates that the treatment effect became increasingly pronounced during extended storage rather than maintaining a fixed offset.

Under cold storage, LDPE alone and CW 6% + LDPE maintained the highest TSS retention ($p > 0.05$, statistically at par), whereas untreated controls declined rapidly. Under ambient conditions, CW 6% + LDPE achieved the highest terminal TSS (9.87 °Brix), followed by CW 3% + LDPE (Fig. 2a, 2b). Sugar fractions followed analogous trends (Fig. 2c, 2d). Under cold storage, sugars declined after day 20 in coated treatments compared to earlier depletion in controls. Under ambient conditions, sugars peaked at day 9 in controls but were sustained through day 12 in CW 6% + LDPE. For total sugars, LDPE packaging alone was statistically at par with CW 6% + LDPE, whereas CW 6% retained the highest reducing sugar concentration, comparable to CW 3% + LDPE (Fig. 2e, 2f).

Mechanistically, carbohydrate retention under CW 6% + LDPE reflects a dual-barrier effect: the hydrophobic coating limits transpiration, while perforated LDPE establishes a modified atmosphere that suppresses respiration and glycolytic turnover. These findings align with earlier reports where LDPE packaging and wax coatings preserved soluble solids in guava and mango (Hoa et al., 2002; Kore & Kabir, 2011; Neeraj et al., 2002). Recent studies similarly confirm that integrating LDPE with edible coatings delays ripening in guava (Gull et al., 2024; Khan et al., 2024; Lekshmi et al., 2024), whereas wax nanoemulsions stabilize sugars in papaya and lychee (Devi et al., 2023; Oliveira Filho et al., 2023). Although higher sugars were noted in untreated mango after 7 days ambient storage (Hoa & Ducamp, 2008), this reflected accelerated ripening rather than preservation. Overall, CW 6% + LDPE effectively preserves soluble carbohydrates across both storage regimes.

3.5 Acidity and Vitamin C

Titratable acidity declined progressively across all treatments throughout storage, driven predominantly by storage duration ($F_{1,96} = 2259.49, p < 0.001$,partial $\eta^2 = 0.77$), with the Treatments × Days interaction ($F_{5,96} = 1.12, p = 0.357$,partial $\eta^2 = 0.15$) reflecting treatment differentiation over time. Under cold storage, CW 6% + LDPE retained the highest acidity (34% higher than the untreated control after 25 days), whereas under ambient storage, it remained statistically at par with CW 3% + LDPE at 15 days, with control fruits consistently exhibiting the lowest acidity.

The decline in titratable acidity corresponds to the utilization of organic acids (principally citric and malic acids) as respiratory substrates, while the loss of ascorbic acid (Vitamin C) results from its oxidation to dehydroascorbic acid and subsequent irreversible degradation (Fig. 3a, 3b, 3c, 3d). The combined CW + LDPE treatments effectively retarded these losses by restricting transpirational water loss and modifying the internal gaseous atmosphere, thereby

depressing overall respiratory metabolism. The superior retention of ascorbic acid in coated and packaged fruits is directly related to reduced internal O_2 levels and the suppression of oxidative senescence pathways, preserving cellular membrane integrity.

These results corroborate observations in citrus coated with beeswax and linseed oil (Mezemir et al., 2017), as well as in guava (Kore & Kabir, 2011), papaya (Azene et al., 2014), and mango (Hoa et al., 2002). Recent investigations further confirm that edible plant-extract coatings (Lekshmi et al., 2024) and wax nanoemulsions (Oliveira Filho et al., 2023) maintain titratable acidity by moderating respiratory turnover. Moreover, titratable acidity correlated positively with flesh firmness and ascorbic acid retention, demonstrating that metabolic suppression concurrently preserves multiple postharvest quality attributes.

3.6 Total Phenols

Total phenolic content declined progressively across all treatments, with faster depletion under ambient conditions, governed by significant main effects of Treatments ($F_{5,96} = 13.60, p < 0.001$,partial $\eta^2 = 0.41$) and Days ($F_{1,96} = 2428.28, p < 0.001$,partial $\eta^2 = 0.96$), alongside a significant Treatments × Days interaction ($F_{5,96} = 3.66, p = 0.005$,partial $\eta^2 = 0.16$). CW 6% + LDPE-treated fruits retained ~8.5% higher phenolic content than untreated controls at the end of both cold storage (25 days) and ambient storage (15 days), enhancing postharvest antioxidant capacity, nutritional value, and marketable quality (Fig. 3e, 3f).

Phenolics are secondary metabolites that impart antioxidant stability and nutritional value. Their postharvest depletion is primarily driven by polyphenol oxidase (PPO) and peroxidase (POD) activity as cellular membranes lose structural integrity during senescence. The hydrophobic carnauba wax restricted cuticular O_2 permeability, while micro-perforated LDPE modulated the microatmosphere by attenuating internal O_2 influx and CO_2 accumulation. Although internal gases and respiration were not directly monitored, this dual physical barrier plausibly suppressed O_2 availability for PPO/POD-mediated oxidative pathways (Luo et al., 2015; Chen et al., 2019).

Principal component analysis (PCA) confirmed that phenolic retention clustered positively with firmness, titratable acidity, and vitamin C, demonstrating that antioxidant preservation is tightly coupled with delayed senescence. Elevated phenolics also coincided with suppressed decay, underscoring their role in antimicrobial defense. These findings align with Oliveira Filho et al. (2023), who showed that wax nanoemulsions preserve phenolics by mitigating

oxidative stress in papaya, and Lekshmi et al. (2024), who reported enhanced phenolic retention in coated guava.

3.7 Spoilage

Spoilage incidence increased progressively during storage, governed by significant main effects of Treatments ($F_{5,96} = 2.98, p = 0.015$,partial $\eta^2 = 0.13$) and Days ($F_{1,96} = 325.84, p < 0.001$,partial $\eta^2 = 0.77$), as well as a significant Treatments × Days interaction ($F_{5,96} = 3.41, p = 0.007$,partial $\eta^2 = 0.15$).

Under ambient storage, decay symptoms manifested after 9 days, with controls reaching 19.3% spoilage by day 15, whereas CW 6% + LDPE fruits exhibited only 9.9%—representing a 48% reduction relative to controls. Under cold storage, spoilage onset was delayed until day 15; at day 25, CW 6% + LDPE recorded the lowest spoilage (12.4%, ~35% lower than controls), remaining statistically at par with CW 6%, LDPE alone, and CW 3% + LDPE. Untreated controls consistently exhibited the highest spoilage across both regimes (Fig. 4a, 4b).

Postharvest spoilage integrates physiological senescence, tissue softening, and opportunistic microbial colonization. Desiccation and middle-lamella disassembly compromise cuticular integrity, producing micro-fissures that facilitate pathogen entry. Carnauba wax forms a continuous hydrophobic barrier across the pericarp, occluding lenticels and stomata to curb moisture loss and impede fungal spore adhesion. Micro-perforated LDPE provides a complementary physical barrier against airborne inoculum while preventing desiccation. Concurrently, glycerol monolaurate (GML) incorporated into the wax emulsion exerts antimicrobial activity by disrupting microbial membrane permeability.

These observations corroborate reports in jujube where carnauba wax with GML suppressed postharvest decay (Chen et al., 2019) and in guava where lipid coatings combined with LDPE delayed spoilage (Chavan et al., 2023; Dhillon et al., 2024). Spoilage incidence correlated positively with weight loss and PME activity, but negatively with firmness, titratable acidity, ascorbic acid, and total phenolics. This resistance cascade (reduced transpiration → preserved firmness and pectin → structural integrity → suppressed pathogen colonization) under CW 6% + LDPE effectively curbs commercial losses across ambient and cold supply chains.

3.8 Pectin Content and Pectin Methyl-esterase Activity

Pectin content declined progressively during storage alongside a concomitant increase in PME activity, driven by significant main effects of Treatments ($F_{5,96} = 7.16, p < 0.001$,partial $\eta^2 = 0.27$) and Days ($F_{1,96} = 2777.24, p < 0.001$,partial $\eta^2 = 0.97$), and a

marginal Treatments × Days interaction ($F_{5,96} = 2.24, p = 0.057$,partial $\eta^2 = 0.10$). CW 6% + LDPE fruits retained the highest pectin levels and suppressed PME activity most effectively under both storage regimes, whereas untreated controls exhibited the lowest pectin content and highest PME activity.

Quantitatively, CW 6% + LDPE maintained ~22% higher pectin and ~30% lower PME activity than controls after 25 days of cold storage, and ~18% higher pectin with ~25% lower PME activity after 15 days of ambient storage (Fig. 4c, 4d, 4e, 4f). Pectin provides structural cohesion within the middle lamella, and its de-esterification by PME exposes carboxyl groups to subsequent depolymerization by polygalacturonase (PG) and β-galactosidase, driving tissue softening. The hydrophobic wax film and perforated LDPE packaging attenuated moisture loss and internal gas exchange, delaying the physiological triggers that upregulate cell-wall hydrolases. Although PG activity and ethylene evolution were not directly quantified, this barrier-mediated regulation is well established (Singh & Pal, 2007; Chen et al., 2019).

Principal component analysis (PCA) confirmed that CW 6% + LDPE clustered tightly with pectin content, flesh firmness, titratable acidity, vitamin C, and phenolics, whereas untreated controls clustered with high PME activity, physiological weight loss, and spoilage. These trends align with recent findings where functional coatings suppressed PME kinetics and delayed cell-wall breakdown in guava (Gull et al., 2024; Lekshmi et al., 2024; Chavan et al., 2023). Suppressed PME activity under CW 6% + LDPE directly underpinned flesh firmness retention and reduced postharvest decay across both ambient and cold supply chains.

3.9 Association Studies

Correlation analysis revealed strong interdependencies among postharvest quality attributes. Physiological weight loss correlated positively with spoilage ($r = 0.88$), TSS ($r = 0.74$), total sugars ($r = 0.71$), and PME activity ($r = 0.89$), while correlating negatively with firmness ($r = -0.92$), titratable acidity ($r = -0.86$), vitamin C ($r = -0.91$), total phenolics ($r = -0.89$), and pectin content ($r = -0.93$; all $p < 0.001$) (Table 1).

PCA on standardized z-scores accounted for 92.48% of the total cumulative variance across two principal components. PC1 (66.76%) segregated quality-maintenance vectors (firmness, acidity, ascorbic acid, phenolics, pectin) from senescence indicators (weight loss, spoilage, PME activity, soluble sugars). PC2 (25.72%) captured carbohydrate turnover (TSS and sugars). Treatment score trajectories confirmed that CW 6% + LDPE clustered with quality-retention vectors, whereas untreated controls aligned with deterioration and spoilage vectors (Fig. 5).

Mechanistically, senescence and decay were driven by transpirational desiccation and hydrolytic cell-wall breakdown (demonstrated by the strong inverse correlation between PME activity and pectin), compromising pericarp firmness and facilitating microbial invasion. Conversely, carnauba wax restricted cuticular permeability and water loss, while LDPE packaging modulated the gaseous microenvironment, cooperatively suppressing oxidative turnover and PME activation (Chavan et al., 2023; Oliveira Filho et al., 2023). Although internal respiration and ethylene evolution were not directly measured, these multivariate associations demonstrate that CW 6% + LDPE concurrently preserves cell-wall cohesion, antioxidant pools, and organoleptic quality during storage.

4. Conclusions

This study demonstrates that combining a 6% carnauba wax (CW) coating with micro-perforated LDPE packaging effectively extends the postharvest shelf life and maintains the quality of guava cv. 'Shweta' under both cold (6–8 °C) and ambient (20–24 °C) storage. The CW 6% + LDPE combination outperformed standalone treatments by significantly reducing physiological weight loss, decay incidence, and pectin methylesterase activity, while preserving firmness, soluble sugars, titratable acidity, ascorbic acid, phenolics, and sensory acceptability. Multivariate PCA confirmed tight clustering of this treatment with quality-retention vectors. Quality preservation was driven by the complementary physical barrier of the hydrophobic wax and polymeric film, which minimized transpiration and metabolic turnover. Consequently, integrating CW 6% with micro-perforated LDPE provides a practical, low-cost commercial approach to mitigate postharvest losses in tropical fruit supply chains, though future work should evaluate multi-cultivar trials and direct internal gas dynamics.

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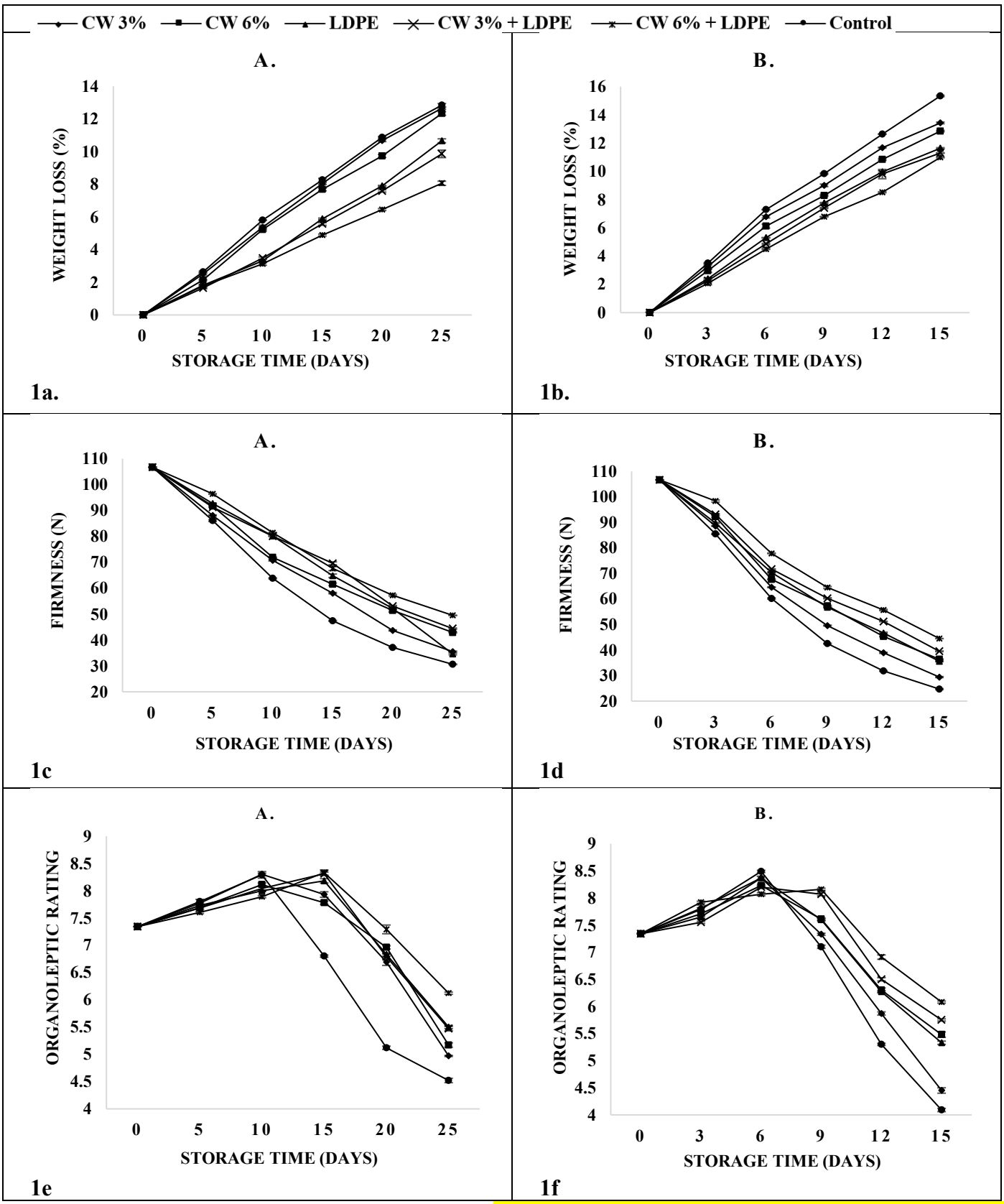
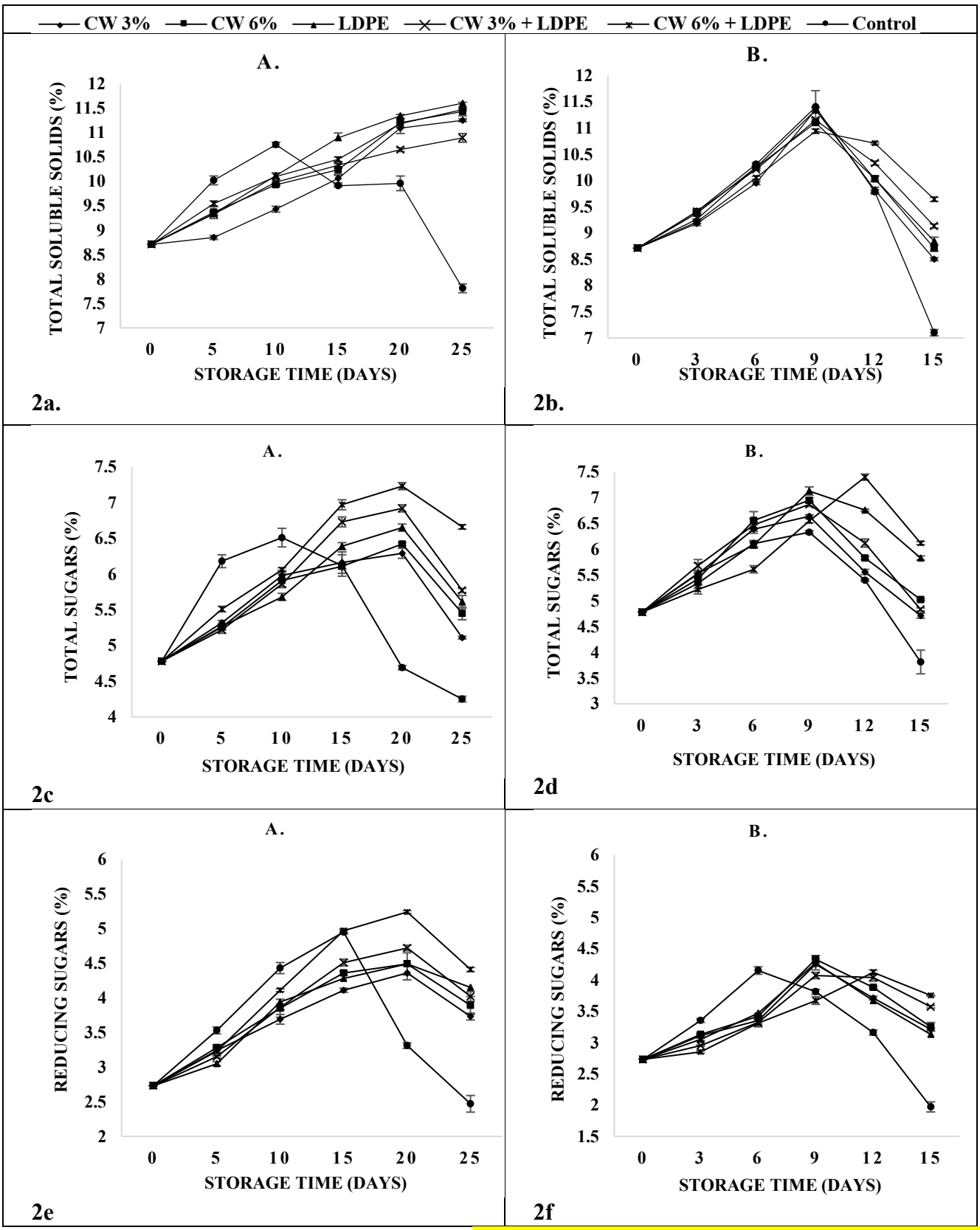
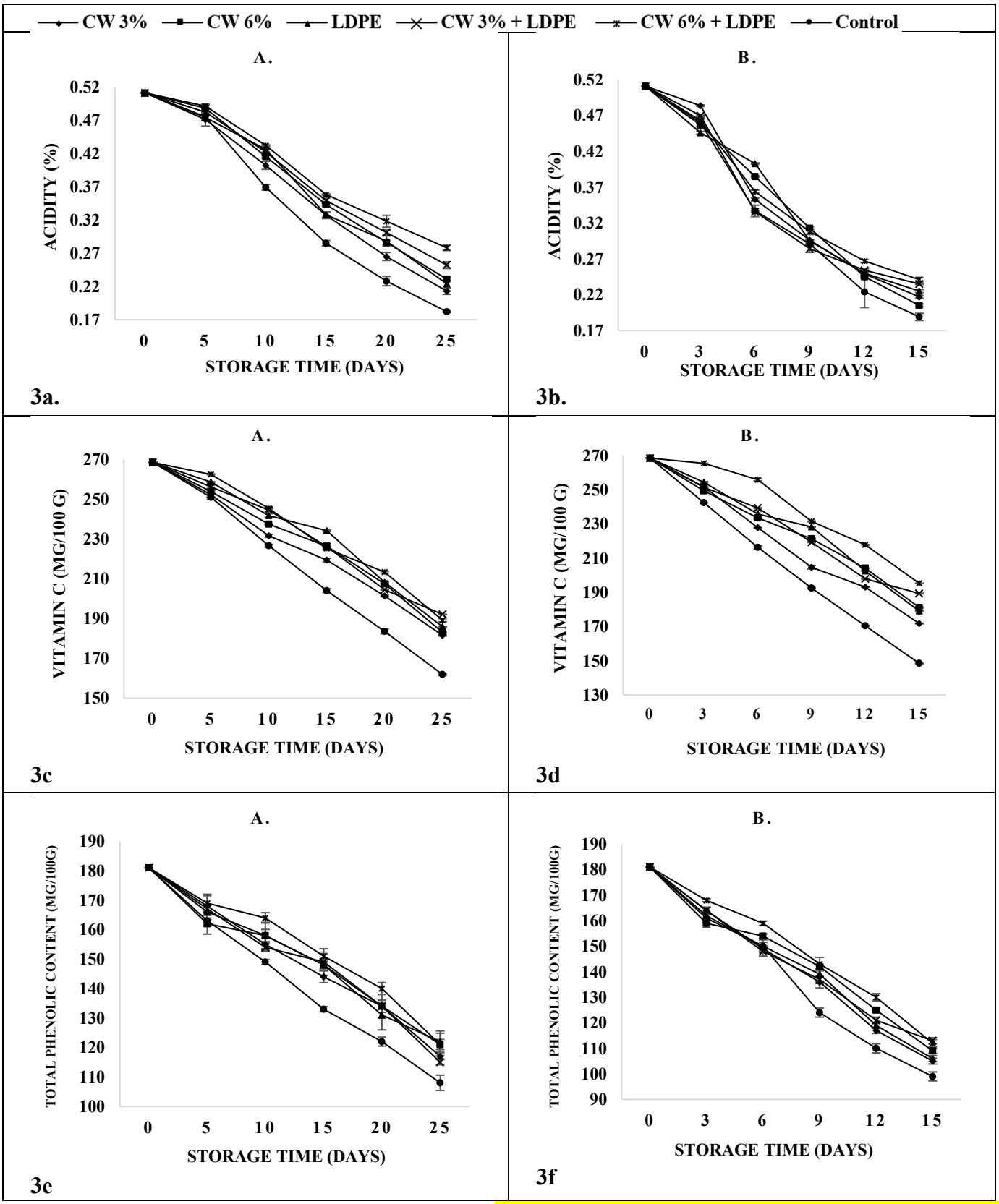


Fig. 1. Effect of treatments and storage duration on weight loss (1a, 1b), firmness (1c, 1d), organoleptic rating (1e, 1f) of guava cv. 'Shweta' under (A) cold (6–8 °C, 90–95% RH) and (B) ambient (20–24 °C, 70–75% RH) storage. Data are mean ± SE ($n = 3$). Normality (Shapiro–Wilk) and homoscedasticity (Levene's test) were verified before two-way ANOVA (F-CRD; $p > 0.05$). Significance, p-values, and partial η^2 for Treatments (T), Days (D), and $T \times D$ are shown; different letters indicate significant differences by Tukey's HSD ($p < 0.05$).



572 **Fig. 2.** Effect of treatments and storage duration on total soluble solids (2a, 2b), total sugars (2c, 2d), reducing sugars (2e,
573 2f) of guava cv. 'Shweta' under (A) cold (6–8 °C, 90–95% RH) and (B) ambient (20–24 °C, 70–75% RH) storage. Data are
574 mean ± SE ($n = 3$). Normality (Shapiro–Wilk) and homoscedasticity (Levene's test) were verified before two-way ANOVA
575 (F-CRD; $p > 0.05$). Significance, p-values, and partial η^2 for Treatments (T), Days (D), and $T \times D$ are shown; different
576 letters indicate significant differences by Tukey's HSD ($p < 0.05$).
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578



579 **Fig. 3.** Effect of treatments and storage duration on acidity (3a, 3b), vitamin c (3c, 3d), total phenolic content (3e, 3f) of
580 guava cv. 'Shweta' under (A) cold (6–8 °C, 90–95% RH) and (B) ambient (20–24 °C, 70–75% RH) storage. Data are mean
581 ± SE ($n = 3$). Normality (Shapiro–Wilk) and homoscedasticity (Levene's test) were verified before two-way ANOVA (F-
582 CRD; $p > 0.05$). Significance, p-values, and partial η^2 for Treatments (T), Days (D), and $T \times D$ are shown; different letters
583 indicate significant differences by Tukey's HSD ($p < 0.05$).

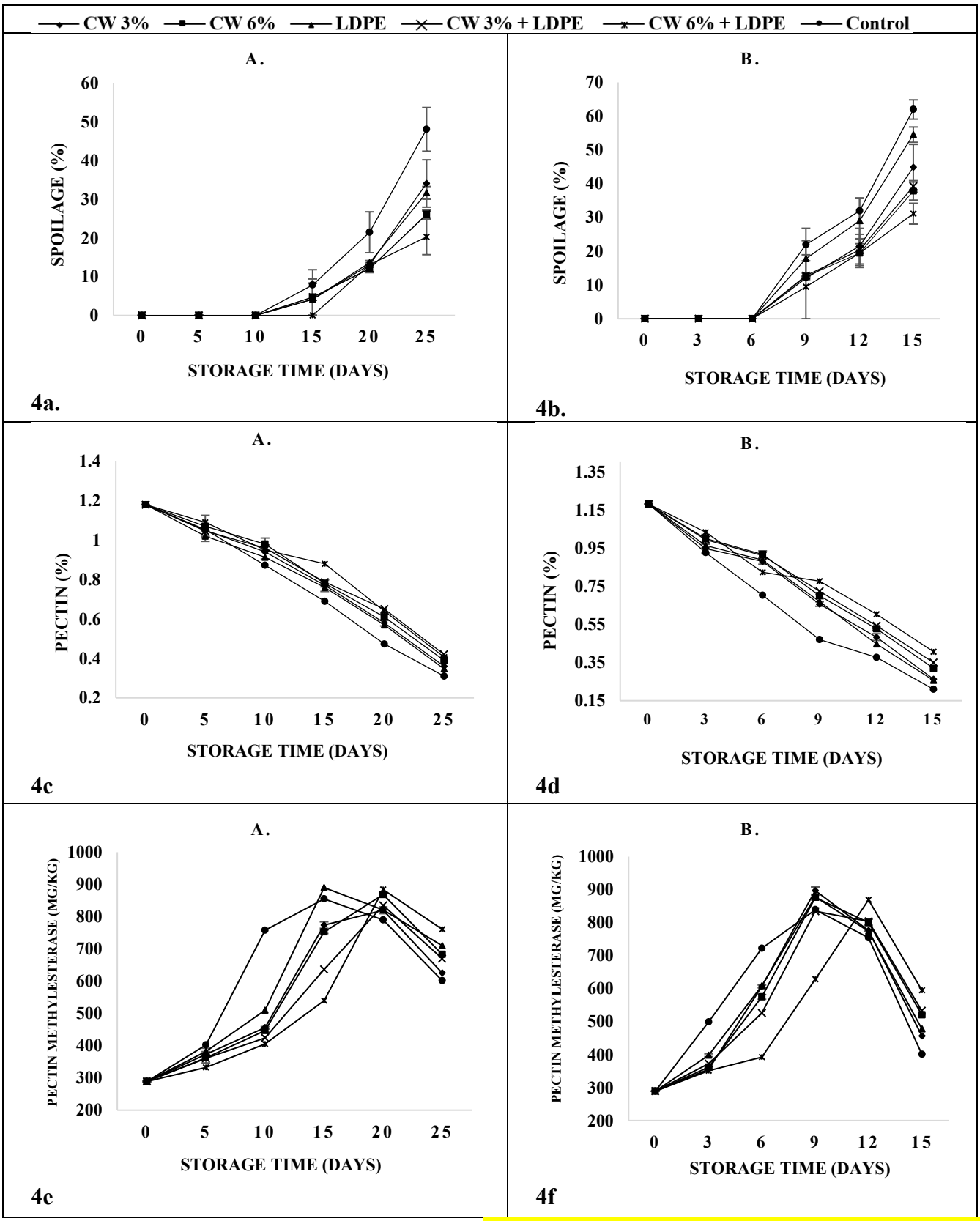
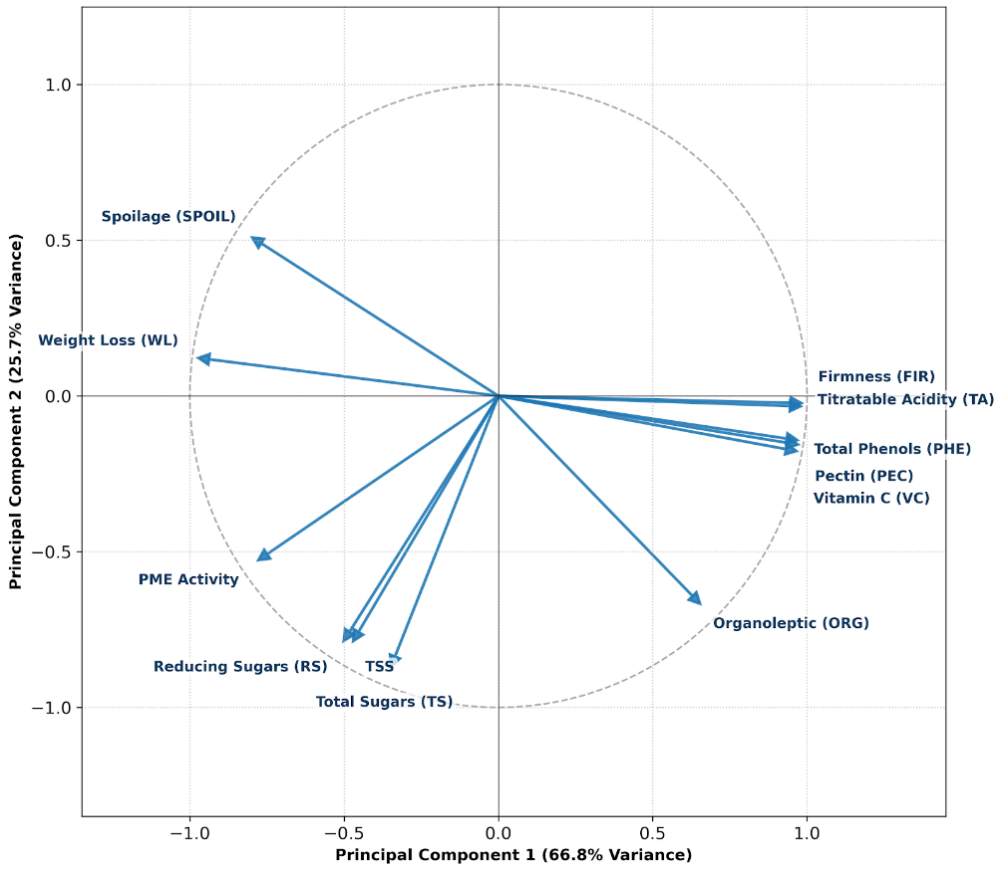


Fig. 4. Effect of treatments and storage duration on spoilage (4a, 4b), pectin (4c, 4d), pectinmethylesterase (4e, 4f) of guava cv. 'Shweta' under (A) cold (6–8 °C, 90–95% RH) and (B) ambient (20–24 °C, 70–75% RH) storage. Data are mean ± SE ($n = 3$). Normality (Shapiro–Wilk) and homoscedasticity (Levene's test) were verified before two-way ANOVA (F-CRD; $p > 0.05$). Significance, p-values, and partial η^2 for Treatments (T), Days (D), and $T \times D$ are shown; different letters indicate significant differences by Tukey's HSD ($p < 0.05$).

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591 **Fig. 5.** Principal component analysis of different studied variables of guava.

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602 **Table 1. Correlation of different variables of guava.**

	WL	FIR	ORG	SPOIL	TSS	TS	RS	TA	VC	PHE	PEC	PME
WL	1											
FIR	-0.984**	1										
ORG	-0.687**	0.629**	1									
SPOIL	0.820**	-0.779**	-0.861**	1								
TSS	0.350**	-0.427**	0.152*	-0.021	1							
TS	0.229**	-0.318**	0.384**	-0.111	0.798**	1						
RS	0.377**	-0.474**	0.158*	-0.003	0.829**	0.821**	1					
TA	-0.974**	0.977**	0.645**	-0.794**	-0.426**	-0.316**	-0.453**	1				
VC	-0.975**	0.973**	0.744**	-0.846**	-0.304**	-0.175*	-0.358**	0.955**	1			
PHE	-0.970**	0.966**	0.708**	-0.847**	-0.329**	-0.237**	-0.368**	0.961**	0.969**	1		
PEC	-0.968**	0.963**	0.737**	-0.860**	-0.344**	-0.209**	-0.364**	0.963**	0.970**	0.976**	1	
PME	0.696**	-0.751**	-0.173*	0.337**	0.769**	0.700**	0.782**	-0.752**	-0.658**	-0.663**	-0.660**	1

603 **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed). WL- Weight Loss, FIR-Firmness, ORG -
604 Organoleptic, SPOIL- Spoilage, TSS-Total Soluble Soilds, TS- Total Sugars, RS- Reducing Sugars, TA- Titratable Acidity, VC- Vitamin C, PHE- Total
605 Phenols, PEC-Pectin, PME- Pectin Methyl Esterases.