



Optimizing packaging and storage of red mulberry fruits to augment post-harvest life

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ABSTRACT

Mulberry fruits are highly perishable and susceptible to physical damage during post-harvest handling. This study was carried out to assess the shelf life of red mulberry fruits (cv. 'Thar Lohit') under ambient conditions (38-42°C; 55-60% RH) and low temperature storage (5±1°C; 85-90% RH). In addition, effect of packing materials, including plastic punnets, CFB boxes with 0.3% and 0.5% aeration, brown paper bags and muslin cloth bags on storage life was also studied. Plastic punnets and CFB boxes significantly reduced weight loss, minimized spoilage, and delayed biochemical changes during 3 days of storage under ambient conditions. Low-temperature storage combined with plastic punnet box packaging extended storage life up to 8 days while maintaining freshness and market acceptability. Under cold storage, total soluble solids (TSS), sugars, phenols, flavonoids, and antioxidants increased gradually up to 10 days but declined by day 12. The study concluded that red mulberry fruits (cv. Thar Lohit) can be stored in plastic punnets or CFB boxes for 2 days under ambient conditions and 8 days under low temperatures without compromising sensory quality.

Key words: *Morus rubra* L., sugars, anthocyanins, sensory quality, spoilage.

INTRODUCTION

Mulberries have gained popularity as table fruits due to their delicious taste, numerous health benefits, and increasing market demand (Sarolia *et al.*, 15). Red mulberry fruits, in particular, are nutrient-rich and contain essential minerals, vitamins (B₁, B₂, C), carotenes, phenolic compounds like resveratrol, and antioxidants, making them a functional food (Bianco and Mirabella, 4). They are also abundant in anthocyanins, which are known for lowering blood pressure, enhancing vision, and offering various therapeutic benefits (Bao *et al.*, 2). Mulberries are among the most delicate and perishable fruits, characterized by a short shelf life and non-climacteric ripening behaviour (Fan *et al.*, 6). Their high-water content (over 75%), soft texture, and harvesting season, often coinciding with high temperatures and low humidity in arid and semi-arid regions, limit their post-harvest life to just 1-2 days (Han *et al.*, 7).

Given their fragility, mulberries require special care during harvesting, post-harvest handling, and packaging to extend their shelf life and ensure safe transport and marketing. Packaging plays a crucial role in protecting the fruit from mechanical damage, moisture loss, microbial infection, and sunlight exposure while delaying quality degradation (Fan *et al.*, 6). Properly ventilated packaging is essential to minimize damage and preserve the fruit during storage and transportation. By employing proper

packaging, labelling, and marketing techniques under low-temperature conditions, red mulberries can be successfully introduced as a popular table fruit through modern fruit outlets in supermarkets. To this end, a study was conducted to standardize packaging materials and low-temperature storage, as well as to assess the biochemical and sensory changes in red mulberry fruits during storage.

MATERIALS AND METHODS

The experiment was conducted during mulberry harvesting season (April) in two successive years (2023 & 2024) at ICAR-Central Institute for Arid Horticulture, Bikaner. Red mulberry (*Morus rubra*) cv. 'Thar Lohit' fruits, harvested at full maturity (80-100% red surface; TSS 18.30-18.85 °Brix), were procured from 10-year-old clonally propagated experimental orchard. Fruits were handpicked and packed in various materials to minimize bruising during transport to the post-harvest laboratory. Packaging treatments included: plastic punnet boxes with 0.3% (T₁) and 0.5% aeration (T₂); CFB boxes with 0.3% (T₃) and 0.5% aeration (T₄); muslin cloth (T₅), brown paper bags (T₆), and no packaging (control, T₇). Stored under ambient conditions (38-40°C; 55-60% RH) in April month, physical, biochemical, and sensory parameters were analysed daily over three days period.

Another experiment was conducted to evaluate the storage life of mulberry fruits under cold-storage (5±1°C; 85-90% RH) using 250-gram punnet plastic

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boxes with 0.3% aeration for 12 days. Physical, biochemical, and sensory parameters were assessed at two-day intervals. Weight loss (%) was calculated as $[(\text{Initial weight} - \text{Weight at sampling date}) / \text{Initial weight}] \times 100$ and spoilage (%) was determined based on the proportion of decayed fruits showing rotting, lesions, or fungal infection.

Total soluble solids (TSS) were determined using digital refractometer (MA 871, Milwaukee, Japan) and expressed as °Brix. Titratable acidity was estimated by titration against 0.1N NaOH using phenolphthalein as indicator (AOAC, 1). Total monomeric anthocyanins were determined by pH differential method (Lee *et al.*, 10). Total phenols were measured by Folin-Ceoucaltu method. Total flavonoids and total antioxidants were analyzed through method suggested by Medini *et al.* (12) and Berwal *et al.* (3), respectively. The total soluble sugars were analyzed by following the phenol sulphuric acid method (Dubois *et al.*, 5). Reducing sugar concentration was estimated by following the method of Somogyi and Nelson (17). Sensory analysis was carried based on the basis of fruit taste, color, flavour and overall acceptability according to 9-point hedonic scale score. Data were recorded in triplicate and analysed at 5% significance level using Web Based Agricultural Statistics Software Package 2.0, developed by ICAR-Central Coastal Agricultural Research Institute, Goa and expressed as mean \pm standard error.

RESULTS AND DISCUSSION

Mulberry fruits are highly perishable due to their thin skin and rapid post-harvest water losses. Packaging significantly impacts physiological weight loss (PLW) during storage. After one day, the lowest PLW was observed in T₁ (3.53 \pm 0.02%), T₃ (3.62 \pm 0.01%), and T₆ (3.88 \pm 0.1%) with no significant differences, while the highest PLW occurred in T₇ (7.57 \pm 0.21%). Over time, PLW increased across all treatments due to evaporation and transpiration (Fig. 1). Plastic punnet and CFB boxes with 0.3% aeration (T₁ and T₃) effectively minimized PLW compared to other treatments. By the third day, T₃ (8.27 \pm 0.17%) and T₁ (8.59 \pm 0.19%) had the lowest PLW, while T₇ (control) recorded the highest (15.80 \pm 0.53%), followed by T₅ (12.65 \pm 0.12%) and T₄ (10.42 \pm 0.42%). Reduced PLW in treatments like brown paper bags, plastic, and CFB boxes was attributed to maintaining higher humidity in the headspace, which delayed water loss and inhibited cell wall-degrading enzymes such as polygalacturonase and pectin esterase activity. In contrast, rapid water loss was observed in control due to unrestrained transpiration and evaporation losses. These findings align with previous

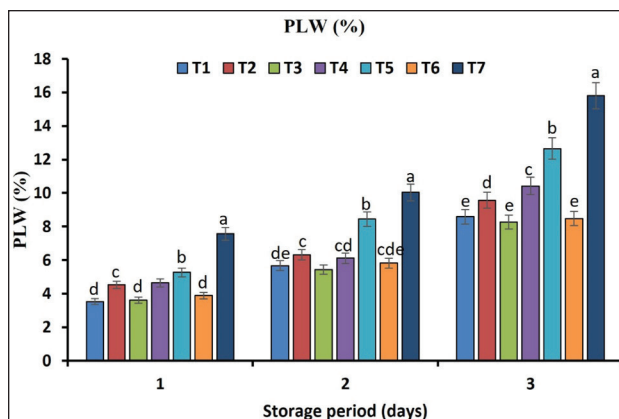


Fig. 1. Effect of packaging on physiological weight loss (%) during 3 days storage at ambient conditions.

studies on packaging and storage of mulberry fruits (Tinebra *et al.*, 18).

Fruits undergoes rapid physiological and biochemical changes during post-harvest storage which influenced by packaging material and headspace environment. This study revealed a significant effect of packaging on total soluble solids (TSS), titratable acidity, and the TSS/acid ratio (Table 1). Fruits stored in plastic punnets and CFB boxes with 0.3 and 0.5% aeration (T₁, T₂, T₃ and T₄) exhibited minimal increases in TSS (21.60–21.83 °Brix) and TSS/acid ratio (34.10–34.75) after 2 days, compared to significant increases in control (T₇), T₆, and T₅. By the third day, TSS ranged from 22.69–23.06 °Brix in T₁ to T₄, with no significant differences among treatments. Fruits in T₅, T₆, and T₇ spoiled completely by the second day. The slower increase in TSS in fruits packed in plastic and CFB boxes was attributed to the humid microclimate inside the packaging, which delayed ripening and polysaccharide hydrolysis. Higher TSS in control and other treatments was likely due to moisture loss results in concentrating soluble solids. Titratable acidity declined gradually in plastic punnets and CFB boxes but dropped sharply in control due to organic acid utilization as a respiratory substrate. Slower acidity loss in superior packaging was associated with reduced respiration and ethylene production. The TSS/acid ratio increased across all treatments due to rising TSS and declining acidity, with the most pronounced increase in control, T₆, and T₅. These findings align with previous studies on mulberry and pear storage (Tinebra *et al.*, 18; Kaur *et al.*, 8).

Anthocyanin levels increased in all packaging treatments during ambient storage. By day 2, the highest anthocyanin content was in the control (288.56 mg/L), followed by T₅ (262.96 mg/L) and T₆ (260.98 mg/L), while T₁–T₄ showed lower levels

Table 1: Effect of packaging material on biochemical changes and spoilage of mulberry fruits during 3 days storage at ambient conditions.

Storage period (days)	Packaging treatment						
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
TSS (°Brix)							
0	18.48±0.03	18.48±0.03	18.48±0.03	18.48±0.03	18.48±0.03	18.48±0.03	18.48±0.03
1	20.29±0.10 ^d	20.64±0.17 ^c	20.27±0.25 ^d	20.57±0.06 ^{cd}	21.39±0.14 ^b	21.69±0.22 ^b	22.00±0.19 ^a
2	21.60±0.15 ^c	21.71±0.14 ^c	21.73±0.17 ^c	21.83±0.23 ^c	22.56±0.15 ^b	22.60±0.16 ^b	23.01±0.18 ^a
3	22.69±0.36 ^a	23.00±0.25 ^a	23.01±0.08 ^a	23.06±0.50 ^a	-	-	-
Titratable acidity (%)							
0	0.738±0.02	0.738±0.02	0.738±0.02	0.738±0.02	0.738±0.02	0.738±0.02	0.738±0.02
1	0.719±0.01 ^a	0.708±0.02 ^{ab}	0.700±0.01 ^{bc}	0.693±0.09 ^{bc}	0.693±0.01 ^{bc}	0.686±0.07 ^{cd}	0.675±0.01 ^d
2	0.634±0.01 ^a	0.629±0.02 ^a	0.625±0.05 ^a	0.628±0.02 ^a	0.590±0.04 ^b	0.562±0.01 ^c	0.554±0.01 ^c
3	0.504±0.01 ^a	0.501±0.02 ^a	0.498±0.00 ^a	0.490±0.01 ^a	-	-	-
TSS/acid ratio							
0	25.04±0.18	25.04±0.18	25.04±0.18	25.04±0.18	25.04±0.18	25.04±0.18	25.04±0.18
1	28.22±0.14 ^c	29.17±0.15 ^c	28.96±0.21 ^c	29.67±0.31 ^c	30.87±0.27 ^b	31.63±0.14 ^a	32.59±0.58 ^a
2	34.10±0.71 ^c	34.54±0.53 ^c	34.78±1.04 ^c	34.75±0.51 ^c	38.24±0.84 ^b	40.27±1.21 ^a	41.59±1.04 ^a
3	45.09±1.95 ^a	45.93±1.11 ^a	46.31±0.60 ^a	47.02±2.33 ^a	-	-	-
Total anthocyanins (mg/L)							
0	128.60±1.50	128.60±1.50	128.60±1.50	128.60±1.50	128.60±1.50	128.60±1.50	128.60±1.50
1	152.00±1.45 ^d	154.83±1.50 ^d	151.96±1.36 ^d	154.10±1.10 ^d	173.96±1.01 ^b	166.10±1.47 ^c	185.40±3.04 ^a
2	210.30±0.45 ^d	227.45±1.62 ^c	217.95±2.42 ^{cd}	224.04±1.32 ^c	262.96±2.43 ^b	260.98±2.06 ^b	288.56±2.89 ^a
3	285.30±2.92 ^c	302.35±3.37 ^b	309.51±2.13 ^{ab}	316.40±3.11 ^a	-	-	-
Spoilage (%)							
1	00±00	00±00	00±00	00±00	28.08±1.58 ^b	20.60±0.84 ^c	33.59±2.61 ^a
2	14.32±1.01 ^d	13.49±0.93 ^d	13.92±1.90 ^d	14.00±1.35 ^d	59.31±2.51 ^c	66.06±2.36 ^b	74.38±2.13 ^a
3	55.78±3.58 ^d	56.82±2.36 ^{cd}	59.67±2.15 ^c	67.93±2.86 ^b	100±00 ^a	100±00 ^a	100±00 ^a

Data are presented in mean± standard error of three replications. Different small letters in the same line demonstrate significant difference ($p < 0.05$) within the same storage period.

(210–227 mg/L). The control exhibited a 124.38% increase in anthocyanins, compared to 63.55% in plastic punnet packaging. Lower anthocyanin accumulation in plastic punnets and CFB boxes was attributed to a humid micro-environment reducing phenylalanine-lyase (PAL) activity, consistent with findings from pomegranate aril studies (Lashkari, 9).

Packaging significantly influenced fruit spoilage during storage under ambient conditions (Table 1). On the first day, no spoilage was observed in plastic punnets and CFB boxes (T₁–T₄), while 33.59% of fruits spoiled in the control. By the second day, minimal spoilage was recorded in T₂ (13.49%), T₃ (13.92%), T₄ (14.00%), and T₁ (14.32%), with no significant differences, compared to high spoilage in control (74.38%), muslin cloth (66.05%), and brown paper bags (59.31%) packing. By the third

day, spoilage reached 55.78% and 59.67% in plastic punnets and CFB boxes, respectively, while all fruits in control, T₅, and T₆ were spoiled. Reduced spoilage in T₁, T₂, T₃ and T₄ was attributed to protection from bruising and reduced pathogen activity. In contrast, higher spoilage in control, muslin cloth, and paper bags was due to juice oozing creating favourable conditions for fungal growth and fruit shrivelling.

It is concluded from the experiment that the plastic punnet and CFB boxes packaging with 0.3% aeration extended shelf life for 2 days with minimum PLW % losses at ambient conditions of arid region.

During 12 days of low-temperature storage, mulberry fruits showed gradual increase in physiological weight loss (PLW), spoilage (%) and reduction in juice (%) (Fig. 2, Table 2). PLW increased from 2.21% on day 2 to 8.31% by day 12,

Table 2. Effect of low temperature storage on physical and biochemical changes in mulberry fruits during 12 days storage.

Storage days	TSS (°Brix)	Titrateable acidity (%)	TSS/acid ratio	Total anthocyanins (mg/L)	Total phenols (mg GAE/g)	Total flavonoids (mg CatE/g)	Total sugars	Reducing sugars	Juice %	Spoilage %
0	20.42 ± 0.10 ^d	0.704 ± 0.06 ^a	29.13 ± 2.52 ^d	15.55 ± 0.27 ^g	0.554 ± 0.10 ^d	0.025 ± 0.01 ^d	127.81 ± 8.00 ^d	31.51 ± 2.63 ^f	69.76 ± 1.32 ^a	0 ± 00
2	22.11 ± 0.11 ^b	0.597 ± 0.03 ^b	37.08 ± 2.22 ^c	19.97 ± 0.15 ^f	0.966 ± 0.07 ^c	0.066 ± 0.03 ^c	136.24 ± 4.09 ^a	65.11 ± 5.59 ^e	67.24 ± 0.93 ^b	0 ± 00
4	23.30 ± 0.00 ^a	0.528 ± 0.01 ^c	44.13 ± 1.25 ^b	20.68 ± 0.49 ^e	1.098 ± 0.04 ^c	0.068 ± 0.00 ^c	148.27 ± 5.95 ^c	109.17 ± 5.34 ^{cd}	64.11 ± 0.87 ^c	0 ± 00
6	23.37 ± 0.37 ^a	0.476 ± 0.03 ^{cd}	49.21 ± 2.65 ^a	23.32 ± 0.20 ^d	1.298 ± 0.16 ^b	0.070 ± 0.02 ^c	155.43 ± 6.80 ^{bc}	111.27 ± 3.86 ^{bc}	60.45 ± 0.70 ^d	0 ± 00
8	21.91 ± 0.10 ^{bc}	0.450 ± 0.00 ^d	48.70 ± 0.35 ^a	29.27 ± 0.22 ^c	1.047 ± 0.05 ^c	0.068 ± 0.01 ^c	155.07 ± 8.58 ^{bc}	103.28 ± 4.95 ^d	59.27 ± 0.94 ^d	2.28 ± 0.58 ^a
10	21.87 ± 0.15 ^{bc}	0.451 ± 0.03 ^d	48.73 ± 2.79 ^a	37.26 ± 0.22 ^b	1.474 ± 0.05 ^b	0.103 ± 0.04 ^b	164.87 ± 6.40 ^b	126.86 ± 3.90 ^a	56.57 ± 0.74 ^e	6.43 ± 1.21 ^b
12	21.77 ± 0.20 ^c	0.425 ± 0.02 ^d	51.09 ± 1.51 ^a	39.21 ± 0.17 ^a	1.327 ± 0.09 ^a	0.082 ± 0.02 ^a	158.07 ± 5.16 ^b	117.50 ± 4.10 ^a	53.11 ± 1.63 ^f	9.11 ± 1.83 ^c

Data are presented in mean ± standard error of three replications. Different small letters in the same line demonstrate significant difference ($p < 0.05$) within the same storage period.

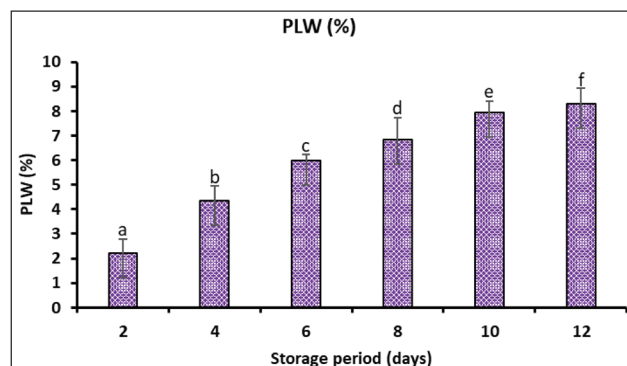


Fig. 2. Effect of low-temperature storage on physiological weight loss during storage of 12 days.

attributed to reduced evaporation and physiological processes under cold storage. Juice content declined significantly from 69.76% initially to 53.11% by day 12, primarily due to moisture loss. Spoilage symptoms appeared on day 8 (2.28%) and reached 9.11% by day 12, driven by ongoing metabolic activities that caused cellular disintegration and senescence (Kaur *et al.*, 8).

During storage, a significant increase in TSS was observed over 8 days, followed by a decline on the 10th and 12th days (Table 2). Titrateable acidity decreased to 0.425% by the end of storage, leading to an enhanced TSS/acid ratio due to starch hydrolysis, sugar synthesis, and organic acid degradation (Park *et al.*, 14). The subsequent decline in TSS was attributed to sugar utilization for respiration and metabolic activities.

Anthocyanin content in mulberry fruits increased significantly from 15.55 mg/L to 39.21 mg/L by the 12th day, attributed to the breakdown of chlorophylls and carotenoids during ripening. Total phenolic content (TPC) and flavonoid content (TFC) rose until the 10th day but declined on the 12th day, likely due to ROS-induced degradation. This aligns with findings by Singh *et al.* (16) in cold-stored strawberry fruits. Total antioxidant activity (TAA) peaked on the 10th day, with CUPRAC reaching 2.10 mg AAE/g and DPPH inhibition at 47.79%, before declining by the 12th day (Fig. 3). The increase in TAA corresponded to higher TPC, TFC, and anthocyanin levels, while the decline mirrored their reductions, consistent with Li *et al.* (11). Total and reducing sugars increased significantly until the 10th day due to starch hydrolysis and juice concentration from dehydration. A decline on the 12th day was linked to complete starch hydrolysis, metabolic breakdown, and fruit senescence, consistent with Yavic (19) findings in white mulberry storage.

No significant variation was observed in the sensory parameters of fresh mulberry fruits during the first 6 days. By the 8th day, slight changes in taste and overall acceptability were noted (Fig. 4). However, significant reductions in sensory parameters were observed on the 10th and 12th days due to spoilage, colour changes, dehydration, and imbalances in the TSS/acid ratio. Post-harvest fruit acceptability during storage depends on maintaining an appropriate balance between TSS and acidity. Biochemical and physiological changes during storage disrupted this

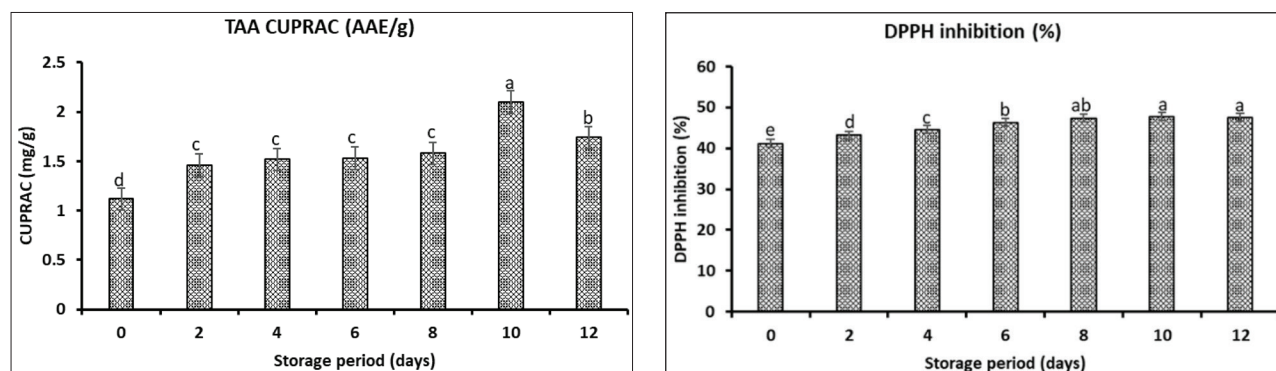


Fig. 3. Effect of low temperature on changes in total antioxidants (CUPRAC & DPPH) during 12 days storage. The values presented represent the means of three replicates \pm standard error, with varying lowercase letters denotes significant differences between treatments.

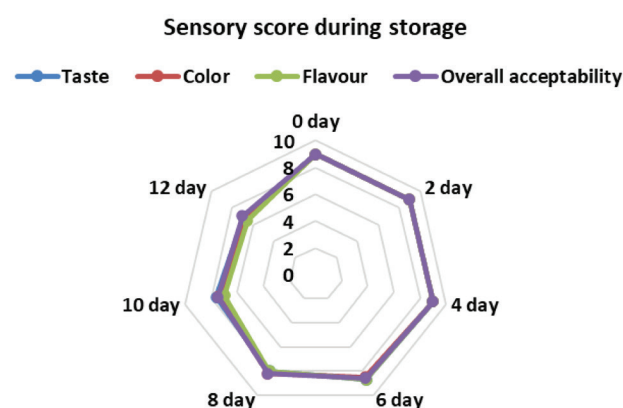


Fig. 4. Changes sensory parameters of mulberry fruits during 12 days storage under low temperature conditions.

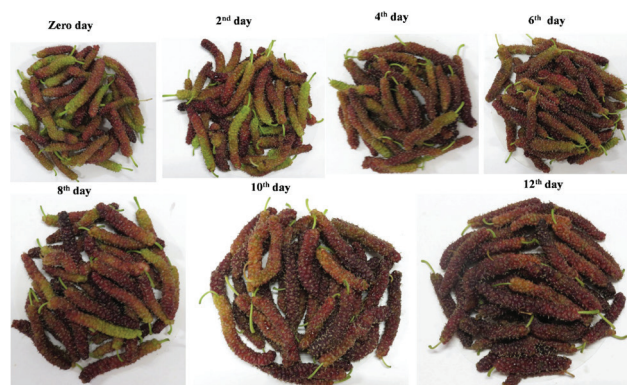


Fig. 4. Sequential colour changes during low-temperature storage of mulberry fruits for 12 days.

balance, with an increase in TSS and a sharp decline in acidity by the 10th and 12th days. Additionally, moisture loss, spoilage, and colour changes further contributed to the decline in sensory quality, rendering the fruits unsuitable for marketing or fresh consumption.

The study concludes that red mulberry fruits (cv. 'Thar Lohit'), manually harvested at full maturity and packed in plastic punnets or CFB boxes with 0.3% aeration, can be successfully stored for 2 days under ambient conditions (38-42°C; 55-60% RH). Packaging in plastic or CFB boxes effectively protected the fruits from bruising, minimized weight loss, and prevented dehydration by maintaining a humid microclimate inside the box. Cold storage (5 \pm 1°C; 85-90% RH) combined with plastic punnet packaging extended the shelf life to 8 days while preserving bioactive compounds, sensory quality and market acceptability.

AUTHORS' CONTRIBUTION

Conceptualization (PSG); Methodology(PSG); Writing original draft (PSG); Biochemical analysis (MKB); Experimentation (KLK); Statistical analysis(KLK); Manuscript editing(DKS); Resources (DKS).

DECLARATION

The authors confirm no conflicts of interest with respect to the concerned manuscript.

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