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Varsha Kanojia
Scientist, Processing and Food
Engineering, Punjab
Agricultural University,
Ludhiana, Punjab, India

Preeti Birwal
Scientist, Processing and Food
Engineering, Punjab
Agricultural University,
Ludhiana, Punjab, India

Soumadeep Putatunda
Ph.D. Scholar, Department of
Vegetable Science, College of
Agriculture, O.U.A.T.,
Bhubaneswar, India

Corresponding Author:
Preeti Birwal
Scientist, Processing and Food
Engineering, Punjab
Agricultural University,
Ludhiana, Punjab, India

Effect of storage conditions on physical and biochemical properties of distinct kale (*Brassica oleracea* var. *acephala*) cultivars: A comparative study on Lacinato and KPTL-19

Varsha Kanojia, Preeti Birwal and Soumadeep Putatunda

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Abstract

Kale (*Brassica oleracea* var. *acephala*) has emerged as an exotic superfood of significant interest in India due to its exceptional density of bioactive compounds, vitamins, and dietary minerals. However, its highly perishable green foliage exhibits rapid postharvest senescence, chlorophyll degradation, and physiological weight loss under ambient configurations. This study comprehensively evaluates and compares the postharvest physical and biochemical stability of two distinct kale cultivars i.e. the blistered-leaf 'Lacinato' and the flat, purple-stemmed heirloom 'KPTL-19' under ambient temperature storage and refrigerated storage (5 ± 1 °C). Freshly harvested samples were packed in low-density polyethylene (LDPE) packages and evaluated at 4-day intervals for headspace gas dynamics (O_2 and CO_2), physiological weight loss (PWL), CIELAB color parameters (L^* , a^* , b^*), cutting strength, total soluble solids (TSS), pH, titratable acidity (TA), ascorbic acid (Vitamin C), and total chlorophyll index. Results demonstrated that ambient storage accelerated cellular respiration and rapid moisture depletion, rendering both cultivars completely unmarketable after 4 days, with Lacinato and KPTL-19 exhibiting critical weight losses of 11.2% and 16.9%, respectively, accompanied by severe yellowing and chlorophyll loss (>73%). Conversely, refrigerated storage at 5 °C drastically suppressed metabolic rates and moisture loss, successfully extending the shelf life of both cultivars up to 12-14 days. Refrigeration optimally maintained structural turgidity, preserved greenness, and retained essential nutrients, achieving 84.56% ascorbic acid retention in Lacinato and 46.85% in KPTL-19 at day 12. This comparative investigation definitively concludes that cold-chain integration with proper polymeric packaging is mandatory to mitigate postharvest qualitative and economic losses in commercial kale distribution.

Keywords: *Brassica oleracea* var. *acephala*, Lacinato Kale, KPTL-19, refrigerated storage, postharvest senescence, ascorbic acid, polymeric packaging

1. Introduction

Globalization and shifts in economic standards across developing countries, particularly India, have significantly restructured consumer food perceptions. Modern generations increasingly prioritize nutrient-dense functional foods that contribute directly to physiological health, metabolic regulation, and the prevention of chronic nutritional diseases. Among the cruciferous vegetables (genus *Brassica*), kale (*Brassica oleracea* var. *acephala*) has garnered profound scientific and commercial attention. Characterized by non-heading leafy rosettes, kale is recognized as a premier superfood owing to its high concentration of vitamins (A, C, B6, E, and K), essential dietary minerals (calcium, iron, potassium, magnesium, and phosphorus), and diverse bioactive phytochemicals, including glucosinolates, polyphenols, carotenoids (lutein, zeaxanthin), and anthocyanins. In India, kale is cultivated as an exotic winter crop under cool, dry climates, primarily across Jammu and Kashmir, Himachal Pradesh, Punjab, Uttar Pradesh, and Haryana. Despite its high agricultural potential and ease of cultivation, kale remains an underexploited commodity within the domestic food processing industry due to its high postharvest vulnerability. At harvest, the fresh foliage exhibits an elevated moisture content (approximately 84.5% to 86.0% wet basis) and large surface-to-volume ratios, driving intense physiological respiration and transpiration.

Consequently, under ambient storage parameters (20-25 °C), kale suffers rapid water depletion, wilting, enzyme-mediated yellowing, and degradation of labile bioactive components within a few days, destroying its marketability.

To optimize domestic commercial logistics and reduce high supply-chain losses, selecting stable cultivars and instituting scientifically validated postharvest handling infrastructure are paramount. Polymeric modified atmosphere packaging, such as low-density polyethylene (LDPE), acts as a partial moisture and gas barrier, establishing an equilibrium gas composition that reduces transpiration and respiration. However, the precise interaction between modern exotic cultivars and temperature treatments remains inadequately characterized in Indian processing research.

The present investigation was therefore executed to evaluate the physiological and biochemical properties of two prominent kale cultivars: 'Lacinato' (Tuscan/Dinosaur kale), celebrated for its narrow, elongated, intensely dark blue-green blistered leaves; and 'KPTL-19', an heirloom variety developed by the ICAR-Indian Agricultural Research Institute (Himachal Pradesh, Kullu Valley) characterized by flat, frilly 'oak leaf' shapes with distinctive purple stems and veins rich in anthocyanins. This research directly establishes the physical and biochemical kinetics of both cultivars under ambient atmospheric storage versus refrigeration (5 ± 1 °C) to provide definitive processing frameworks for shelf-life extension.

2. Materials and Methods

2.1. Plant Material Procurement and Sampling

Freshly harvested, uniform, and disease-free leaves of Lacinato kale (*Brassica oleracea* L. var. *acephala*) were obtained directly from the experimental fields of the Department of Vegetable Science, Punjab Agricultural University (PAU), Ludhiana. Concurrently, leaves of the heirloom kale cultivar KPTL-19 were sourced from the ICAR-Indian Agricultural Research Institute regional center, Kullu Valley, Himachal Pradesh, and transferred under controlled temperature protocols to the laboratory. All primary experimental testing and quantitative analyses were executed at the Department of Processing and Food Engineering (College of Agricultural Engineering and Technology) and the Punjab Horticultural Post-Harvest Technology Centre (PHPTC), PAU, Ludhiana.

2.2. Preliminary Physical parameters Analysis

Prior to storage allotment, initial structural attributes including leaf length, width, and surface area were systematically quantified from randomly selected fresh replicates. Linear measurements (length and width) were obtained using a calibrated metric scale. For the irregularly surfaced leaves, leaf surface area was determined via the standard grid tracing method. Outlines of individual leaves were precisely mapped on 1 mm resolution graph grids, and total leaf area was calculated by quantifying the sum of bounded squares (assuming a base grid resolution of 1x1 cm). Partial boundary squares covering more than 50% of the grid area were registered as integer square units, while stem/petiole surface areas were excluded from the final computational summation.

2.3. Experimental Design and Storage Treatment Packages

Freshly harvested leaves were subjected to a standardized preparatory washing process utilizing tap water to eliminate

field debris, followed by ambient air-drying to completely remove surface moisture. Sample units weighing precisely 50 grams were grouped and enclosed within moisture-retentive Low-Density Polyethylene (LDPE) packaging film pouches. The sample packages were securely enclosed using a manual impulse heat sealing packaging machine. The experimental matrices were distributed into two independent storage environments for a 15-days trial:

1. Ambient Storage: The kale leaves were stored in the month of February and March 2020 at an average ambient temperature of 20 ± 2 °C.
2. Refrigerated Storage: Maintained inside a cold room chamber at temperature of 5 ± 1 °C.

Destructive and non-destructive sample evaluations were conducted at four synchronized intervals: Day 0 (Fresh baseline), Day 4, Day 8, and Day 12.

2.4. Analytical Procedures for Quality and Biochemical Parameters

2.4.1. Headspace Gas Composition Analysis

To track internal packaging atmosphere dynamics driven by respiration, the volumetric concentration (%) of gaseous Oxygen (O₂) and Carbon Dioxide (CO₂) inside the sealed pouches was measured non-destructively using a GS6600 O₂ & CO₂ Headspace Analyzer (Systech Illinois/Gaspac 6000). The dual detector infrared and zirconia sensor unit was calibrated using ambient atmospheric baseline standards. An airtight hypodermic sampling needle probe was driven through an adhesive rubber septum fixed to the external pouch wall, automatically aspirating fixed gas volumes to display stabilized concentration data on screen.

2.4.2. Physiological Weight Loss (PWL)

Gravimetric moisture loss kinetics were tracked using a precise digital weighing balance. The cumulative physiological weight loss percentage (PWL) relative to the fundamental baseline mass was calculated for individual sampling dates via the following standard equation:

$$\text{PWL (\%)} = [(W_0 - W_t) / W_0] \times 100$$

where W₀ represents the baseline fresh packaging weight (50 g) at Day 0, and W_t denotes the absolute package weight recorded at specific testing intervals (t = 4, 8, 12 days).

2.4.3. Surface Color Evaluation (CIELAB)

Surface color changes were tracked utilizing a Konica Minolta CR-400 Handheld Portable Chroma-Meter, calibrated against a standard white tile configuration. The CIELAB tristimulus color space values were captured across three primary coordinates: L* (lightness ranging from 0 = black to 100 = white), a* (chromaticity defining red when positive and green when negative), and b* (chromaticity defining yellow when positive and blue when negative). Multiple surface point readings were executed at designated leaf blade and midrib coordinates to construct accurate averages. The Total Color Difference (ΔE) representing cumulative deviation relative to fresh baseline tissue was determined mathematically via standard Euclidean distance algorithm tracking over time.

2.4.4. Cutting Strength

Cutting strength of kale leaves were analyzed utilizing a TA.XT Plus Digital Texture Analyzer (Stable Micro

Systems). The mechanical configuration relied on a standardized razor-sharp cutting blade probe geometry to execute a defined shear test across the sample structures. The technical program was set to the following operation parameters: contact force threshold = 15 g; pre-test travel velocity = 2.0 mm/s; cutting test velocity = 1.0 mm/s; return post-test velocity = 15.0 mm/s; total target travel clearance distance = 40 mm. Samples were secured flat over a slotted platform, and the peak cutting shear force required to cleave the central tissue zone was registered in Newtons (N).

2.4.2. Biochemical Analysis (TSS, pH, Acidity, Vitamin C, and Chlorophyll)

Total Soluble Solids (TSS) concentration was observed via a hand-held digital refractometer, with data reported in degrees Brix (°Bx). Active solution acidity (pH) was tracked concurrently by immersing the automated glass combination electrode probe of a digital pH meter directly into 10 mL of extracted sap. Titratable acidity (TA) was quantified utilizing the standard acid-base titration method against a standard 0.1 N Sodium Hydroxide (NaOH) solution with phenolphthalein indicator. Ascorbic acid was quantified via the classic 2,6-dichlorophenol indophenol (DCPIP) visual

titration method using a 0.4% oxalic acid stabilizing extract phase. Total chlorophyll content was determined spectrophotometrically at absorbance wavelengths of 660 nm and 642.5 nm following exhaustive tissue tissue extraction inside an 85% acetone extracting medium matrix.

3. Results and Discussion

3.1. Morphological and Baseline Characteristics of Fresh Cultivars

The two cultivars exhibited striking morphological distinctions at harvest. Fresh 'Lacinato' leaves exhibited an elongated, narrow, blade-like architecture with an average baseline length of 34.41 cm and width of 9.06 cm, resulting in a mean individual leaf surface area of 220.71 cm² (Table 1). Visually, Lacinato displayed an intensely dark, blue-green color with a profoundly crinkled, embossed surface texture. In contrast, 'KPTL-19' leaves presented an 'oak leaf' morphology with deeply frilly, toothed margins and distinctive purple coloration along the stem and primary veins, indicating a high concentration of vacuolar anthocyanin pigments. KPTL-19 leaves possessed a baseline length of 22.5 cm and width of 14.0 cm, providing a mean surface area of 235.0 cm² per leaf.

Table 1: Initial Baseline Morphological and Dimensional Profiles of Fresh Lacinato and KPTL-19 Kale Leaves.

Cultivar Variety	Mean Leaf Length (cm)	Mean Leaf Width (cm)	Mean Surface Area (cm ²)	Characteristic Surface Structural Morphology
Lacinato	34.41 ± 8.12	9.06 ± 1.05	220.71 ± 66.8	Elongated, narrow blade; highly blistered, embossed bumpy texture
KPTL-19	22.50 ± 1.20	14.00 ± 0.85	235.00 ± 12.4	Loose rosette; flat, frilly oak-leaf shape with prominent purple stems

3.2. Headspace Gas Concentration

The respiratory exchange of kale tissue inside sealed LDPE bags altered the headspace gas composition over time. At room temperature (20.7 °C), both cultivars exhibited a rapid decline in O₂ and a corresponding surge in CO₂ concentrations by Day 4 (Table 2). Inside Lacinato packages, O₂ dropped from 21.0% to 15.6%, while CO₂ rose

from 0.03% to 4.2%. Similarly, KPTL-19 packages ambient-stored for 4 days experienced a drop in O₂ from 19.7% to 17.4% and an increase in CO₂ to 2.3%. This rapid atmospheric shift highlights the high metabolic rate of green leafy vegetables under ambient conditions, which accelerates tissue senescence.

Table 2: Headspace Gas Concentrations (% v/v) in Sealed Polymeric Packages of Lacinato and KPTL-19 Kale.

Storage Period	Lac. Atm O ₂	Lac. Atm CO ₂	Lac. Ref O ₂	Lac. Ref CO ₂	KPTL Atm O ₂	KPTL Atm CO ₂	KPTL Ref O ₂	KPTL Ref CO ₂
Day 0	21.00	0.03	21.00	0.03	19.70	1.40	19.70	1.40
Day 4	15.60	4.20	19.20	1.20	17.40	2.30	18.50	1.90
Day 8	—	—	17.80	2.30	—	—	17.60	2.50
Day 12	—	—	16.70	3.50	—	—	17.10	2.80

3.3. Physiological Weight Loss (PWL)

Weight loss in harvested leafy vegetables is driven primarily by transpirational water loss, which directly degrades visual quality, mass, and freshness. Under ambient storage conditions, both cultivars suffered rapid and severe moisture

loss. By Day 4, Lacinato leaves lost 11.2% of their initial weight, while KPTL-19 lost 16.9% (Table 3). Since weight loss exceeding 5% to 10% causes visible wilting and shriveling, both cultivars became entirely unmarketable and were discarded after Day 4 under ambient conditions.

Table 3: Cumulative Physiological Weight Loss (PWL%) Kinetics of Kale Cultivars.

Storage Period	Lacinato Ambient	Lacinato Refrigerated	KPTL-19 Ambient	KPTL-19 Refrigerated
Day 0	0.00	0.00	0.00	0.00
Day 4	11.20	7.00	16.90	7.00
Day 8	—	13.00	—	11.20
Day 12	—	19.80	—	15.00

3.4. Color Change in Surface

Postharvest leaf yellowing, driven by enzyme-mediated chlorophyll degradation that unmasks underlying carotenoids, is a primary indicator of senescence. Surface color analysis showed a rapid increase in lightness (L*) and yellowness (b*) under ambient conditions (Table 4). By Day 4, Lacinato's L* increased from 35.6 to 41.6, and its b* rose from 7.3 to 24.1, shifting the greenness parameter (a*) from-

7.2 to a positive 1.9. This resulted in a high Total Color Difference (TCD or ΔE) of 20.02, reflecting severe yellowing. KPTL-19 experienced a similar shift under ambient conditions: its L* rose from 29.8 to 49.5 and its b* increased from 10.75 to 45.8, while its a* values crossed into positive coordinates (2.2), indicating a rapid loss of greenness.

Table 4: Evolution of CIELAB Tristimulus Color Coordinates and Total Color Difference (TCD) in Kale Leaves.

Storage Period	Lac L*	Lac a*	Lac b*	Lac TCD	KPTL L*	KPTL a*	KPTL b*	KPTL TCD
Day 0 (Fresh)	35.60	-7.20	7.30	0.00	29.80	-8.30	10.75	0.00
Day 4 Ambient	41.60	1.90	24.10	20.02	49.50	2.20	45.80	41.51
Day 4 Refrig.	38.10	-10.30	9.40	4.48	34.40	-5.90	21.80	12.24
Day 8 Refrig.	40.50	-9.60	10.80	6.50	38.70	1.60	32.00	25.43
Day 12 Refrig.	40.20	-9.70	11.20	6.50	41.80	2.90	43.80	36.70

3.5. Cutting Strength

The peak mechanical cutting force required to shear kale leaves increased across all experimental groups, driven by water loss and cell wall lignification. Under ambient storage, Lacinato's cutting strength increased from 2.70 N to 3.39 N by Day 4 (Table 5). This change was even more

pronounced in KPTL-19, which surged from an initial 4.26 N to 7.74 N by Day 4. This rapid increase in cutting force under ambient conditions stems from severe moisture depletion, which leaves the vascular ribs tough, fibrous, and leathery, thereby reducing consumer acceptability.

Table 5: Peak Mechanical Cutting Strength/Shear Force (Newtons) of Lacinato and KPTL-19 Kale.

Storage Period	Lacinato Ambient	Lacinato Refrigerated	KPTL-19 Ambient	KPTL-19 Refrigerated
Day 0	2.70	2.70	4.26	4.26
Day 4	3.39	2.93	7.74	5.82
Day 8	—	3.10	—	6.56
Day 12	—	3.22	—	7.44

3.6. Total Soluble Solids, pH, and Titratable Acidity

Total Soluble Solids (TSS) increased in both cultivars over the storage period, primarily driven by moisture loss, which concentrates the solutes in the cell sap, alongside the enzymatic breakdown of starch reserves into soluble sugars. Under ambient storage, Lacinato's TSS rose from 1.0 to 2.5

°Bx by Day 4, while KPTL-19 increased from 4.5 to 5.8% (Table 6). Under refrigeration at 5 °C, this concentration effect was much more gradual: Lacinato reached 2.5 °Bx at Day 12, while KPTL-19 increased slowly to 5.2% at Day 12.

Table 6: Physicochemical Profiles (TSS, pH, and Titratable Acidity) of Stored Kale Cultivars.

Storage Period	Lac TSS	Lac pH	Lac TA	KPTL TSS	KPTL pH	KPTL TA
Day 0 (Fresh)	1.00	5.87	0.48	4.50	6.14	0.32
Day 4 Ambient	2.50	5.80	0.56	5.80	5.54	0.50
Day 4 Refrig.	1.70	5.85	0.51	4.60	6.05	0.39
Day 8 Refrig.	2.00	5.82	0.54	4.90	6.01	0.43
Day 12 Refrig.	2.50	5.80	0.58	5.20	5.89	0.48

3.7. Changes in Ascorbic Acid (Vitamin C)

Ascorbic acid is a highly labile antioxidant compound that is highly susceptible to postharvest thermal and oxidative degradation, making it an excellent indicator of overall nutritional quality retention in vegetables. Ambient storage conditions dramatically accelerated the loss of Vitamin C (Table 7). By Day 4, Lacinato's ascorbic acid content fell from 324 mg/100 g to 184.6 mg/100 g—a 43.0% loss.

Under the same conditions, KPTL-19's Vitamin C plummeted from 111 mg/100 g to 32 mg/100 g, representing a severe 71.17% loss within just 4 days. Refrigerated storage at 5 °C drastically suppressed enzymatic oxidation, preserving ascorbic acid content across both cultivars, maintaining an exceptional retention rate of 84.56% in Lacinato and 46.85% in KPTL-19 at Day 12.

Table 7: Quantitative Loss and Retention Percentages of Ascorbic Acid (Vitamin C) in Stored Kale.

Cultivar and Environment Variety	Initial Fresh Conc. (mg/100 g)	Terminal Content (mg/100 g)	Total Vit C Loss (%)	Total Retention (%)
Lacinato Ambient (Day 4)	324.00	184.60	43.00	57.00
Lacinato Refrigerated (Day 12)	324.00	274.00	15.44	84.56
KPTL-19 Ambient (Day 4)	111.00	32.00	71.17	28.83
KPTL-19 Refrigerated (Day 12)	111.00	52.00	53.15	46.85

3.8. Total Chlorophyll Retention

Postharvest chlorophyll degradation is driven by specific enzymes, such as chlorophyllase and pheophytinase, whose activity is highly dependent on ambient temperature and cellular stress. Under ambient storage conditions, both cultivars lost green pigments rapidly. By Day 4, Lacinato's total chlorophyll fell from 2.84 mg/gm to 0.73 mg/gm—a

severe 74.30% loss (Table 8). Similarly, KPTL-19's Chlorophyll Index fell from 95% to 38% by Day 4, representing a 60.0% reduction. Refrigerated storage at 5 °C effectively slowed down chlorophyll degradation, maintaining significantly higher pigment stability at Day 12 across both varieties.

Table 8: Total Chlorophyll Content Degradation and Retention Dynamics in Stored Kale.

Cultivar and Environment Configuration	Initial Baseline Chlorophyll	Terminal Bounded Chlorophyll	Total Chlorophyll Loss (%)	Total Pigment Retention (%)
Lacinato Ambient (Day 4)	2.84 mg/gm	0.73 mg/gm	74.30	25.70
Lacinato Refrigerated (Day 12)	2.84 mg/gm	1.05 mg/gm	63.00	37.00
KPTL-19 Ambient (Day 4)	95.00% Index	38.00% Index	60.00	40.00
KPTL-19 Refrigerated (Day 12)	95.00% Index	51.00% Index	46.32	53.68

4. Conclusions

This comparative investigation demonstrates that storage temperature is the key factor determining postharvest physical and biochemical quality retention in exotic kale cultivars. Under ambient room conditions, both 'Lacinato' and 'KPTL-19' cultivars undergo rapid quality decline driven by intense respiration, transpirational water loss, and enzymatic breakdown. This leads to critical physiological weight loss (>11%), rapid chlorophyll degradation (>60%), and a significant loss of essential Vitamin C within 4 days, rendering the fresh produce completely unmarketable. Enclosing the fresh kale in moisture-retentive polymeric LDPE packaging coupled with immediate refrigeration at 5 °C successfully delays senescence by suppressing respiration and water loss. This cold-chain configuration effectively extended the commercial shelf life of both cultivars from 2 days to 12-14 days. Refrigeration optimally preserved the green color, structural texture, and nutritional value of the leaves, achieving high nutrient retention, including 84.56% of ascorbic acid in Lacinato at Day 12. This research confirms that combining polymeric packaging with mechanical refrigeration is essential to maintain the quality, maximize shelf life, and reduce postharvest losses of kale within the commercial supply chain.

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