



Received: 14-09-2025
Accepted: 24-10-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Determining Effective Short-Duration Cold-Dip Conditions to Slow Ripening of Banana Cultivar GL3-2 in Storage

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Abstract

This study determined suitable parameters for a short-duration cold-dipping pretreatment designed to suppress physiological and biochemical activities and thereby delay ripening of banana cultivar 'GL3-2' during storage. Immediately after harvest, fruits were dipped in 0.01% azoxystrobin + 0.5% CaCl₂. In Experiment 1, fruit were treated for 3 min at 4 ± 1 °C, 7 ± 1 °C, or 10 ± 1 °C and stored at 15 ± 1 °C. In Experiment 2, fruit were treated at 7 ± 1 °C for 1, 3, 5, or 7 min. Peel color (ΔE^*ab), decay, respiration rate, ethylene production rate, and starch content were monitored every 6 days. The 7 ± 1 °C pretreatment

consistently moderated quality loss relative to 4 °C (risk of chilling-related discoloration) and 10 °C (weaker suppression). For duration at 7 °C, 3–5 min minimized visible deterioration (e.g., lower ΔE^*ab and decay after day 18) and tempered gas-exchange responses without the over-suppression observed at 7 min. Overall, short-duration cold-dipping at 7 ± 1 °C for 3–5 min provided the best balance between ripening control and avoidance of chilling-related disorders, extending shelf life while preserving normal subsequent ripening capacity.

Keywords: Banana Cultivar GL3-2, Short-Duration Cold-Dipping, Ethylene, Respiration, Shelf Life

1. Introduction

Banana cultivar 'GL3-2' (*Musa spp.*, ABB group) ripens rapidly and unevenly after harvest and is prone to mechanical damage during handling, contributing to substantial postharvest losses (approximately 25–50% [1]). For export markets, two major constraints persist: (i) short shelf life that precludes sea shipment and (ii) heterogeneous post-storage quality with common defects such as peel/neck rot, stem-end darkening, and cut-end browning.

Cold treatments can moderate physiological and biochemical changes and thus delay ripening. However, bananas are chilling-sensitive; excessive exposure to low temperatures can induce peel greying/pitting and other physiological disorders. Therefore, identifying an appropriate temperature–time window for a short-duration, cold-dipping pretreatment of this cultivar is essential to stabilize quality and extend shelf life.

2. Materials and Methods

Replicates and statistics. Unless otherwise stated, measurements were performed on three biological replicates (n = 3) per treatment × time point and are reported as mean ± SD.

2.1 Material

Banana cultivar GL3-2 bunches were sourced from Ngoc Thanh Commune, Kim Dong District, Hung Yen Province, Vietnam. Fruit were harvested at 110–120 days after flowering (DAF).

2.2 Experimental Design

Experiment 1 (temperature). Hands were cut from bunches, and uniform hands (13.0 ± 0.5 kg per lot) were selected. Lots were dipped in azoxystrobin 0.01% + CaCl₂ 0.5% at 4 ± 1 °C, 7 ± 1 °C, or 10 ± 1 °C for 3 min. Controls were not dipped. After treatment, cut ends were treated with 1% KAl(SO₄)₂, bananas were drained, packed in 30-μm polyethylene (PE) bags, placed in cartons, and stored at 15 ± 1 °C. At 6-day intervals (days 0, 6, 12, 18, and 24) we measured peel color change (ΔE^*ab),

decay incidence (%), respiration rate ($\text{mL CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), ethylene production rate ($\mu\text{L C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), and starch content (%).

Experiment 2 (duration). Using the optimal temperature identified in Experiment 1, bananas were dipped for 1, 3, 5, or 7 min. Post-treatment handling and storage followed Experiment 1. The same indicators were monitored every 6 days to determine the most suitable short-duration, cold-dipping window.

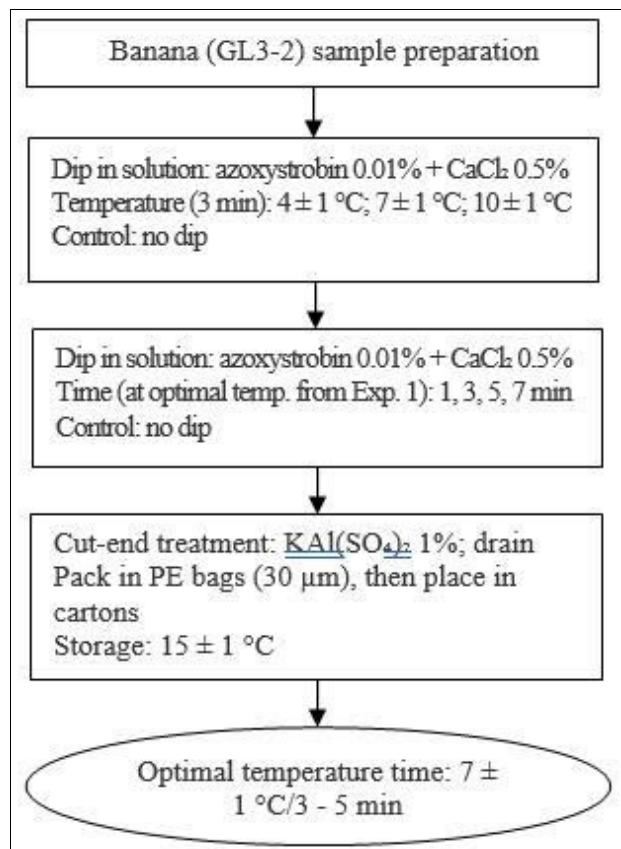


Fig 1: Experimental design

2.3 Analytical Methods

Decay incidence (%). $X = 100 \times A/B$, where A = mass of decayed fruits and B = total mass assessed.

Peel color change (ΔE^*ab). Color (L^* , a^* , b^*) was measured using a Konica Minolta colorimeter in the CIE $L^*a^*b^*$ space. ΔE^*ab was calculated relative to day-0 values as:

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Respiration rate:

$$X = \frac{\% \text{CO}_2 \times (V - v)}{100 \times w \times t} (\text{mL CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1})$$

Where $\% \text{CO}_2$ is the headspace CO_2 concentration, V is container volume, v is displaced fruit volume (so $V - v$ is headspace volume), w is fruit mass (kg), and t is time (h). Gas concentrations were read with a Felix F-960 gas analyzer.

Ethylene production rate:

$$X = \frac{\% \text{C}_2\text{H}_4 \times (V - v) \times 1000}{100 \times w \times t} (\mu\text{L C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1})$$

with variables as above; the factor 1000 converts mL to μL . Starch content (%). Determined according to TCVN 4594:1988 [2].

2.4 Statistical Analysis

Statistical hypotheses were tested by analysis of variance (ANOVA), and treatment means were compared using the least significant difference (LSD) test at $\alpha = 0.05$. Unless otherwise stated, data are presented as mean $\pm$ SD ($n = 3$).

3. Results and Discussion

3.1 Determination of a Suitable Pretreatment Temperature

3.1.1 Effect of Cold Pretreatment Temperature on Decay Incidence and Peel Color Change During Storage

Table 1: Changes in decay incidence (%) and peel color (ΔE^*ab) of Banana cultivar GL3-2 during storage under short-duration cold-dipping pretreatments at different temperatures

Measured parameters	Treatment	Storage time (days)					
		0	6	12	18	24	30
Decay incidence (%)	Control	0.00 ^a	0.94 ^a	2.67 ^a	5.59 ^a	9.10 ^a	14.57 ^a
	4 $\pm$ 1 °C (CT1)	0.00 ^a	0.20 ^b	1.18 ^c	4.03 ^c	8.67 ^b	13.28 ^b
	7 $\pm$ 1 °C (CT2)	0.00 ^a	0.24 ^b	1.20 ^c	3.62 ^d	6.45 ^d	10.36 ^d
	10 $\pm$ 1 °C (CT3)	0.00 ^a	0.26 ^b	1.87 ^b	4.42 ^b	7.15 ^c	11.52 ^c
Peel color change (ΔE^*ab)	Control	0.00 ^a	0.62 ^b	1.35 ^a	2.87 ^a	4.55 ^a	-
	4 $\pm$ 1 °C (CT1)	0.00 ^a	0.96 ^a	1.26 ^b	2.65 ^b	4.64 ^a	-
	7 $\pm$ 1 °C (CT2)	0.00 ^a	0.28 ^d	0.84 ^d	1.78 ^d	3.55 ^c	-
	10 $\pm$ 1 °C (CT3)	0.00 ^a	0.39 ^c	1.17 ^c	2.48 ^c	4.22 ^b	-

Note: Within a column, identical letters indicate no significant difference (LSD, $\alpha = 0.05$).

Decay incidence (%): Decay increased with storage time for all treatments. At day 6, all cold-pretreated lots (CT1–CT3; letter b) had lower decay than the Control (a). By day 12, CT1 (1.18%) and CT2 (1.20%) were statistically indistinguishable (both c) and both lower than CT3 (1.87%, b) and Control (2.67%, a). At day 18, the ordering by means was CT2 (3.62%, d) < CT1 (4.03%, c) < CT3 (4.42%, b) < Control (5.59%, a). From day 24 onward, the hierarchy stabilized as CT2 < CT3 < CT1 < Control (e.g., day 30: 10.36% < 11.52% < 13.28% < 14.57%), indicating the strongest and most durable suppression of decay at 7 $\pm$ 1 °C. The comparatively higher late-stage decay in CT1 (4 °C) relative to CT2 (7 °C) is consistent with the known chilling sensitivity of bananas, where exposure to 4 °C can predispose fruit to physiological stress and subsequent decay.

Peel color change (ΔE^*ab). ΔE^*ab rose over time across treatments, but the rates diverged. At day 6, CT1 (0.96, a) exhibited the largest early increase, exceeding the Control (0.62, b), whereas CT2 (0.28, d) was minimal and CT3 (0.39, c) intermediate. This pattern persisted through day 24: CT2 consistently minimized ΔE^*ab (0.28 $\rightarrow$ 3.55), CT3 remained intermediate, while Control and CT1 were highest and not different at day 24 (4.55 and 4.64, both a). These data indicate that 7 $\pm$ 1 °C most effectively restrains peel color change, whereas 4 °C heightens the risk of chilling-related discoloration.

Synthesis: Converging evidence from decay and color responses identifies 7 $\pm$ 1 °C as the optimal short-duration pretreatment temperature under our conditions, providing the greatest and most sustained retention of visible quality during storage, while 4 °C carries a chilling-injury risk.

3.1.2 Effect of Cold Pretreatment Temperature on Respiration Rate and Ethylene Production Rate During Storage

Respiration and ethylene production rate are key physiological processes that determine the ripening rate and storability of Banana cultivar GL3-2 after harvest. High respiration rate and elevated ethylene typically accelerate ripening, degrade quality, and shorten storage life. A short-duration cold pretreatment can suppress these processes and thereby extend the storage period of fresh fruit. The changes in respiration rate and ethylene production rate of Banana cultivar GL3-2 subjected to different cold pretreatment temperatures during storage are presented in Table 2.

Table 2: Changes in respiration rate ($\text{mL CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and ethylene production rate ($\mu\text{L C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) of Banana cultivar GL3-2 during storage under different cold pretreatment temperatures

Measured parameters	Treatment	Storage time (days)				
		0	6	12	18	24
Respiration rate ($\text{mL CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$)	Control	12.45 ^a	9.15 ^a	12.93 ^a	15.68 ^a	18.25 ^a
	4 ± 1 °C (CT1)	2.72 ^d	3.59 ^d	4.61 ^d	5.94 ^d	7.17 ^d
	7 ± 1 °C (CT2)	3.10 ^c	5.50 ^c	9.32 ^c	13.75 ^c	14.25 ^a
	10 ± 1 °C (CT3)	7.98 ^b	8.44 ^b	11.25 ^b	14.73 ^b	17.68 ^b
Ethylene production rate ($\mu\text{L C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$)	Control	0.50 ^a	1.40 ^a	5.80 ^a	7.90 ^a	10.30 ^a
	4 ± 1 °C (CT1)	0.15 ^d	0.22 ^c	0.38 ^d	0.57 ^d	0.74 ^d
	7 ± 1 °C (CT2)	0.21 ^c	0.35 ^b	0.68 ^c	1.40 ^c	1.72 ^c
	10 ± 1 °C (CT3)	0.30 ^b	1.15 ^b	5.05 ^b	7.10 ^b	9.82 ^b

Note: Within a column, identical letters indicate no significant difference (LSD, $\alpha = 0.05$).

Respiration rate: Control fruit showed a steady rise, reaching 18.25 $\text{mL CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ by day 24. CT3 (10 ± 1 °C) followed a similar course (17.68 at day 24), indicating limited inhibition at this temperature. By contrast, CT1 (4 ± 1 °C) and CT2 (7 ± 1 °C) maintained lower rates throughout storage (day 24: 7.17 and 14.25 $\text{mL CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, respectively). Notably, CT2 was significantly lower than Control on days 6–18 (different letters), but approached Control by day 24 (same letter), suggesting a delayed and blunted climacteric rather than its elimination. The smallest numerical values in CT1 reflect strong metabolic suppression near the chilling threshold.

Ethylene production rate. Patterns mirrored respiration. On day 24, Control produced the highest ethylene (10.30 $\mu\text{L C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), followed by CT3 (9.82), whereas CT2 (1.72) and CT1 (0.74) were markedly lower (distinct letters at all time points). Thus, cold pretreatment reduced and delayed ethylene production rate, with stronger inhibition at lower pretreatment temperatures. However, while CT1 achieved the lowest values, the accompanying risk of chilling injury argues against 4 °C as optimal; CT2 (7 °C) instead provided stable physiological regulation (muted ethylene rise, moderated respiration) without abnormal symptoms, consistent with tempered suppression of ACC synthase/oxidase and a delayed climacteric [5, 6].

Synthesis. Gas-exchange responses corroborate that short-duration cold-dipping effectively slows ripening physiology. Among the temperatures tested, 7 ± 1 °C offers the best balance between suppressing respiration/ethylene and avoiding chilling injury.

3.1.3 Effect of Cold Pretreatment Temperature on Starch Content During Storage

In bananas, changes in starch content are key indicators of ripening and senescence. During postharvest ripening, starch is rapidly hydrolyzed to sucrose, glucose, and fructose (with

minor maltose). Therefore, monitoring starch content in the pulp enables assessment of the ripening stage and senescence after harvest.

The evolution of starch content in Banana cultivar GL3-2 subjected to short-duration cold-dipping pretreatments at different temperatures during storage is shown in Fig 1.

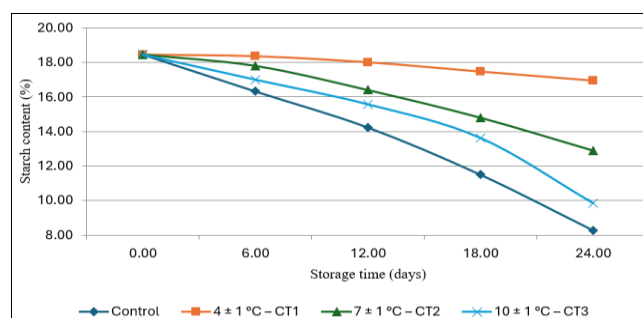


Fig 1: Changes in starch content of Banana cultivar GL3-2 during storage under different cold

Starch content. Starch declined over time in all treatments, but more slowly in cold-pretreated fruit than in the Control; moreover, the rate of decline increased with pretreatment temperature. After 24 days, starch contents were 16.95% (CT1; -8.1%), 12.90% (CT2; -30.0%), 9.84% (CT3; -46.7%), and 8.28% (Control; -55.1%) (changes relative to day 0). These patterns indicate that short-duration cold pretreatment limits starch hydrolysis, with greater hydrolysis at higher pretreatment temperatures - a trend coherent with the respiration and ethylene results.

Interim conclusion. Across visual quality (decay, ΔE^*ab), gas exchange (respiration, ethylene), and starch dynamics, 7 ± 1 °C emerges as the most suitable pretreatment temperature to extend shelf life without inducing physiological disorders. Accordingly, this temperature was used in the subsequent experiment to determine the optimal treatment duration.

3.2 Determination of an Appropriate Duration for the Cold Pretreatment

Fruits were fully immersed in the same 0.01% azoxystrobin + 0.5% CaCl_2 solution at 7 ± 1 °C for 1, 3, 5, or 7 min. After pretreatment, samples were drained, packed in 30- μm PE bags, placed in cartons, and stored at 15 ± 1 °C. Changes in indicators relevant to the postharvest storability of banana cultivar GL3-2 are summarized below.

3.2.1 Effect of Cold Pretreatment Duration on Decay Incidence and Peel Color Change During Storage

Table 3: Changes in decay incidence (%) and peel color (ΔE^*ab) of Banana cultivar GL3-2 during storage under different cold pretreatment durations

Measured parameters	Treatment	Storage time (days)						
		0	6	12	18	24	30	
Decay incidence (%)	0 - Control	0.00 ^a	0.94 ^a	2.67 ^a	5.59 ^a	9.10 ^a	14.57 ^a	
	1 min - CT4	0.00 ^a	0.67 ^a	2.03 ^a	5.16 ^a	8.95 ^a	14.10 ^a	
	3 min - CT5	0.00 ^a	0.23 ^b	1.18 ^c	3.65 ^b	6.43 ^b	10.35 ^c	
	5 min - CT6	0.00 ^a	0.20 ^b	1.09 ^{bc}	3.60 ^b	6.40 ^b	10.27 ^c	
	7 min - CT7	0.00 ^a	0.18 ^b	1.03 ^c	3.58 ^b	7.52 ^b	11.43 ^b	
	0 - Control	0.00 ^a	0.60 ^a	1.31 ^a	2.86 ^a	4.52 ^a	-	
Changes in peel color (ΔE^*ab)	1 min - CT4	0.00 ^a	0.48 ^b	1.05 ^b	2.15 ^c	4.14 ^b	-	
	3 min - CT5	0.00 ^a	0.28 ^c	0.84 ^c	1.78 ^d	3.55 ^c	-	
	5 min - CT6	0.00 ^a	0.16 ^c	0.78 ^c	1.37 ^c	3.22 ^c	-	
	7 min - CT7	0.00 ^a	0.64 ^a	1.18 ^{ab}	3.15 ^a	7.27 ^a	-	
	0 - Control	0.00 ^a	0.60 ^a	1.31 ^a	2.86 ^a	4.52 ^a	-	
	1 min - CT4	0.00 ^a	0.48 ^b	1.05 ^b	2.15 ^c	4.14 ^b	-	

Note: Within a column, identical letters indicate no significant difference (LSD, $\alpha = 0.05$). “-” denotes not determined

Decay incidence: Decay increased with storage time across all durations. On days 6–18, the 3- and 5-min treatments consistently produced lower decay than 1 min and Control; 7 min was numerically as low as, or lower than, 3–5 min up to day 18 (e.g., day 12: 1 min = 2.03%, 3 min = 1.18%, 5 min = 1.09%, 7 min = 1.03%, Control = 2.67%). However, by day 24–30, the ranking stabilized as 3–5 min < 7 min < 1 min < Control (day 30: 10.35% $\approx$ 10.27% < 11.43% < 14.10% < 14.57%), indicating that 7-min fruits ultimately decayed more than 3–5 min during prolonged storage.

Peel color (ΔE^*ab). Color change increased over time in all treatments, but 7 min showed the largest increases at every time point (e.g., day 6: 0.64; day 24: 7.27), with visible dark-green/greyish tones indicative of chilling injury. By contrast, 3–5 min consistently minimized ΔE^*ab (day 24: 3 min = 3.55; 5 min = 3.22), while 1 min was intermediate (4.14) and Control remained high (4.52). Thus, 3–5 min best restrained peel color transition, whereas 7 min promoted discoloration consistent with chilling symptoms.

Synthesis. Integrating decay and color responses, 3–5 min represents the most favorable duration window: it reduces decay (particularly after day 18) while avoiding the chilling-related discoloration observed at 7 min, and it suppresses ripening more effectively than 1 min.

3.2.2 Effect of Cold Pretreatment Duration on Respiration Rate and Ethylene Production Rate During Storage

The effects of pretreatment duration on respiration rate and ethylene production rate of Banana cultivar GL3-2 during storage are presented in Table 4.

Table 4: Changes in respiration rate and ethylene production rate of Banana cultivar GL3-2 during storage under different cold pretreatment durations

Measured parameters	Treatment	Storage time (days)				
		0	6	12	18	24
Respiration rate (mL CO ₂ ·kg ⁻¹ ·h ⁻¹)	0 - Control	12.45 ^a	9.65 ^a	12.93 ^a	15.68 ^a	18.25 ^a
	1min - CT4	5.72 ^b	8.25 ^b	11.84 ^b	15.00 ^b	17.82 ^b
	3 min - CT5	3.10 ^c	5.48 ^c	9.31 ^c	13.76 ^c	14.23 ^c
	5 min - CT6	3.05 ^c	5.44 ^c	9.25 ^c	13.73 ^c	14.20 ^c
	7 min - CT7	2.95 ^c	4.80 ^d	5.94 ^d	7.05 ^d	9.95 ^d
Ethylene production (μL C ₂ H ₄ ·kg ⁻¹ ·h ⁻¹)	0 - Control	0.50 ^a	1.40 ^a	5.80 ^a	7.90 ^a	10.30 ^a
	1min - CT4	0.45 ^a	1.15 ^b	5.05 ^b	7.10 ^b	9.82 ^b
	3 min - CT5	0.22 ^c	0.35 ^c	0.68 ^c	1.38 ^c	1.72 ^c
	5 min - CT6	0.20 ^c	0.32 ^c	0.66 ^c	1.40 ^c	1.70 ^c
	7 min - CT7	0.15 ^d	0.25 ^d	0.43 ^d	0.81 ^d	1.15 ^d

Note: Within a column, identical letters indicate no significant difference (LSD, $\alpha = 0.05$).

Respiration rate: in all cold-pretreated samples increased with storage time, and the rate of increase was inversely related to pretreatment duration. The 7-min treatment showed the smallest rise in respiration, whereas the 1-min treatment showed the largest. At a given time point, respiration followed the order CT7 < CT6 $\approx$ CT5 < CT4 < Control (note: lower values indicate stronger suppression). The low respiration in CT7 (7 min) suggests over-suppression of physiological and biochemical activity, consistent with incipient chilling injury; conversely, CT4 (1 min) provided insufficient suppression. Treatments of 3–5 min yielded similar, moderate respiration—indicating that cold had permeated the fruits sufficiently to temper metabolism without causing injury.

The data also indicate that short-duration cold pretreatment delayed the occurrence of the climacteric peak. The Control

rose rapidly and reached a peak of 18.25 mL CO₂·kg⁻¹·h⁻¹ on day 24; the 1-min treatment also peaked at day 24 but at a lower value (17.82 mL CO₂·kg⁻¹·h⁻¹). In contrast, the 3-, 5-, and 7-min treatments had not yet reached a respiration peak within the 24-day observation period. These findings are consistent with climacteric fruit physiology and prior reports [3, 5], which show that brief cold treatments can modulate respiration-related enzymes (e.g., cytochrome oxidase), thereby slowing ripening.

Ethylene production rate. in all treatments increased over storage time, but the rate of increase depended strongly on pretreatment duration. The control (no pretreatment) rose fastest and reached 10.30 μL C₂H₄·kg⁻¹·h⁻¹ by day 24. The 1-min treatment (CT4) also remained high—9.82 μL C₂H₄·kg⁻¹·h⁻¹ at day 24—indicating that such a short exposure was insufficient to meaningfully moderate ethylene biosynthesis.

By contrast, the 3-min (CT5), 5-min (CT6), and 7-min (CT7) treatments exhibited substantially lower ethylene production rate, with CT7 reaching only 1.15 μL C₂H₄·kg⁻¹·h⁻¹ at day 24. This pattern is consistent with a temperature-driven effect on the ethylene biosynthetic pathway—specifically suppression of ACC synthase (ACS) and ACC oxidase (ACO), the key enzymes governing ethylene formation. As reported by [3, 5], brief cold exposures may not penetrate sufficiently to curb physiology, whereas longer exposure can strongly inhibit ACS/ACO activity. In our study, the 7-min treatment produced near-minimal ethylene at most time points during storage, suggesting physiological over-suppression (not tissue death, but markedly reduced metabolic activity), which in practice corresponded to poor sensory quality at ripeness.

Synthesis. Gas-exchange responses corroborate that 3–5 min strikes the best balance, adequately dampening respiration and ethylene without the over-suppression seen at 7 min or the insufficiency at 1 min.

3.2.3 Effect of Cold Pretreatment Duration on Starch Content During Storage

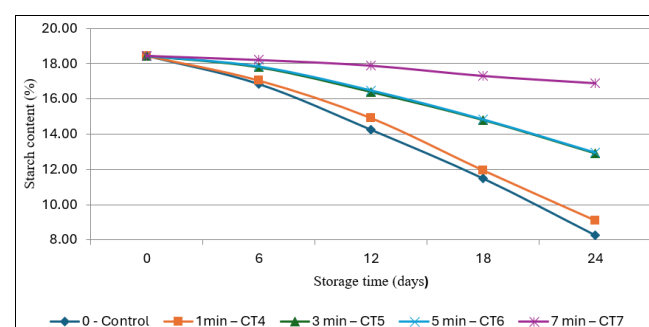


Fig 2: Changes in starch content of Banana cultivar GL3-2 during storage under different cold pretreatment durations

Starch content: Starch declined in all treatments but more slowly in cold-treated fruit than in the Control. For the cold-treated samples, at a given time point the starch content was inversely related to the degree of ripening and thus increased with pretreatment duration—i.e., longer exposures preserved higher residual starch. This reflects stronger suppression (or near suspension) of physiological and biochemical activity, thereby limiting starch hydrolysis to soluble sugars.

After 24 days, starch contents were lowest in the Control (8.25%), followed by 1 min (9.10%), 3 min (12.90%), 5 min (12.95%), and 7 min (16.91%). These results indicate that

the short-duration cold-dipping pretreatment limited starch-to-sugar conversion during storage, with the extent of limitation increasing with treatment duration.

Interim conclusion. Considering visible quality (decay, ΔE^*ab), gas exchange (respiration, ethylene), and starch dynamics, a pretreatment duration of 3–5 min at 7 ± 1 °C is most appropriate to extend shelf life while maintaining the fruit's capacity to ripen normally.

4. Conclusion

Based on the present results, the short-duration, cold-dipping pretreatment effectively suppressed key physiological and biochemical processes, thereby extending the shelf life of banana cultivar GL3-2. Given the cultivar's pronounced chilling sensitivity, the pretreatment must temper ripening without compromising subsequent ripening capacity. Accordingly, the appropriate condition for the pretreatment stage is 7 ± 1 °C for 3–5 min (in the azoxystrobin 0.01% + CaCl_2 0.5% dip used in this study).

5. Conflicts of Interest

The authors declare no conflicts of interest.

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