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MORALITY BEFORE KNOWLEDGE

Quality improvement of lime fruit (*Citrus aurantifolia*) between packaged and unpackaged conditions combined with different storage temperatures

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ABSTRACT

Background: The use of limes is increasingly widespread worldwide, both as fresh fruit for consumption and for making juice or other drinks. Lime is also used in making jams and candies. The essential oil obtained from the skin is widely used in the pharmaceutical and cosmetic industries for medicines, perfumes, soaps, body lotions, and detergents. However, during fruit storage, the respiration and metabolic activity in lime fruits is directly related to the increase in ambient temperature, which makes the fruit dry out. Improving the shelf life of lime against post-harvest stress while maintaining the sensory and nutritional qualities of fresh produce can be achieved through low-temperature storage and the use of packaging. This study aims to improve the quality of lime by knowing the difference between the provision of packaging and not-given packaging combined with different storage temperatures. **Method:** The experimental design used a single-factor completely randomized design with storage type treatment, which includes room temperature without packaging (control), room temperature with packaging, low temperature without packaging, and low temperature with packaging. Observations of non-destructive characteristics include weight loss, respiration rate, and fruit peel color. Observations of destructive characteristics include juice content, fruit peel softness, Soluble Solid Contents (SSC), Total Acidity (TA), SSC/TA ratio, and ascorbic acid content. **Findings:** Low temperature treatment without packaging provides the best results in delaying weight loss, respiration rate, and SSC/TA ratio. The control treatment provided the best results for peel softness, juice content, and ascorbic acid. **Conclusion:** Low temperature treatment without packaging is recommended for the storage of lime fruit to extend its shelf life. **Novelty/Originality of this Study:** This study examines the combined effects of low temperature and newspaper packaging on extending the shelf life of lime fruits, addressing a significant gap in postharvest storage research for non-climacteric fruits such as lime.

KEYWORDS: low temperature; newspaper packaging; post-harvest.

1. Introduction

Lime is a small citrus fruit, measuring between 4-6 cm in diameter, green in color, and having a sour and fresh taste. In 100 g, lime contains nutritional components including 30 kcal of energy, 0.019 g of fatty acids, 2.8 g of dietary fiber, 11 g of carbohydrates, 29.1 mg of total ascorbic acid, and 1.69 g of total sugar (USDA, 2019). Besides its nutritional content, lime also contains phytochemicals such as flavonoids and alkaloids, as well as essential oils

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(Amorim et al., 2016). Lime belongs to the Rutaceae family and the Citrus genus (Alessandrello, 2021). The use of lime fruit is increasingly widespread worldwide, both as fresh fruit for consumption and for making juice or other drinks. It is also used in making jams and candies. Essential oils obtained from the peel are widely used in the pharmaceutical and cosmetic industries for medicines, perfumes, soaps, body lotions, detergents, and for food and drinks. The peel is also widely used in cooking (Narang & Jiraungkoorskul, 2016). Lime juice has been reported to contain flavonoids and ascorbic acid, which have several bioactivities, including antioxidant properties (Rahmiati et al., 2023). Hesperidin and eriocitrin have been reported as major flavonoids in lime varieties (Huang et al., 2018; Dong et al., 2019). The bioactive components of cold-stored lime fruit juice maintained hesperidin and eriocitrin content for up to 21 days (Huynh et al., 2024).

Experiments on postharvest storage of lime fruit are very important, but information on postharvest storage of lime fruit is still limited. Although lime is a non-climacteric fruit, the senescence process is accelerated by high temperatures and low humidity (Champa et al., 2019). Lime fruit exhibits a high rate of water loss after harvest. It has been proven that in hot, humid tropical areas, the shelf life of lime fruit lasts only about 6-9 days under conditions of around 30-34°C and 70-75% relative humidity (Samaradi-wakara et al., 2018). High temperatures negatively affect the fruit peel, increasing membrane permeability, which causes the peel to shrink and the quality to decrease (Lufu et al., 2020).

To increase the natural resistance of fresh horticultural commodities to post-harvest stress and maintain the sensory and nutritional quality of fresh products, low temperature storage and packaging can be used. A decrease in temperature can slow down the metabolic process of the product, as well as the activity of microorganisms, which have been identified as the main contributors to a decrease in product quality (Makule et al., 2022). Low temperatures can extend shelf life over a long period of time due to a decrease in respiration rate (Jain et al., 2023). For citrus fruits, storage at low temperatures can extend their shelf life compared to storage at room temperature (Arzam & Baba, 2018).

Proper packaging is also an effective way to reduce weight loss, such as previous research on Daisy mandarin fruit using film packaging (Dhillon et al., 2016), on mango apple fruit using wax coating combined with plastic wrap and newspaper (Sa'adah et al., 2016), and also on avocado fruit using polyethylene film (Buthelezi & Mafeo, 2024). Packaging can help keep the relative humidity (RH) high, reducing the water vapor pressure deficit (WVPD). However, selecting the right packaging material is crucial to achieving optimal product quality and maintaining a stable RH (Caleb et al., 2013).

In this study, we chose to use newspaper packaging. Newspaper is one packaging material used for storing horticultural products because it is lightweight, clean, and has a smooth surface (Sa'adah et al., 2015). The use of newspaper packaging for agricultural products can inhibit air flow, thereby maintaining water content (Suhartono & Iskandar, 2017). Several studies have evaluated the effect of newspaper packaging on fruit storage, such as in mango apple (Sa'adah et al., 2016), papaya (Sigalingging et al., 2019), crystal guava (Kusmiyati et al., 2019), and tomato (Asjulia & Dyan, 2023).

Most lime fruits are consumed in lime-producing countries. Storage at room temperature is a common practice by retailers in the region (Lerslerwong et al., 2023). This study will be conducted at room or low temperature and will use packaging or not. Thus, improving the shelf life of limes will significantly benefit commercial lime production. This study aims to improve the quality of lime by knowing the difference between the provision of packaging and not-given packaging combined with different storage temperatures.

2. Methods

2.1 Fruit materials and experimental setup

The fruit was harvested at the Greenhouse Cikabayan, IPB University. Harvesting was carried out on April 13, 2023, and the fruit was stored and researched until April 28, 2023. At the time of harvest, the tree was 1.5 years old. The harvested fruit was placed in a cardboard box lined with paper to reduce mechanical damage during transportation to the laboratory. Fruits were harvested in the morning and brought to the laboratory within 10 minutes. The storage and analysis process was conducted at the Postharvest and Biomass Laboratory, Agronomy and Horticulture, IPB University. The fruit was sorted based on peel color, fruit shape, absence of physical defects, and weight (35 ± 5 g) to obtain more uniform samples. The room temperature during the observation ranged from 25.9°C to 27.6°C. The low temperature used in the study was 8°C ± 2°C, and the packaging used was newspaper.

The experimental design employed was a completely randomized design (CRD). The single factors included, Room temperature (RT) without packaging (control), Room temperature with packaging (RTP), Low temperature without packaging (LW), and Low temperature with packaging (LWP). Each treatment had four replications, resulting in a total of 16 experimental units. Within each experimental unit, there were 9 fruits, making a total of 144 fruits used in the experiment. For destructive observations, one fruit was randomly selected every three days from each replication of each treatment. Three fruits were used for non-destructive observations, where the same fruits were individually evaluated every three days.

2.2 Weight loss and respiration rate

Observation of weight loss involves weighing the fruit using analytical balances. Weight loss is calculated as the percentage difference between the previous fruit weight and the weight recorded at each observation, as Gull et al. (2021) stated. The formula used is (Equation 1):

$$Weight\ loss\ (\%) = \frac{(Previous\ weight - Current\ observation\ weight)}{Previous\ weight} \times 100\% \qquad (Eq.\ 1)$$

Respiration rate is determined by measuring the rate of CO_2 gas production from lime fruit after incubation for 3 hours. The citrus fruits are placed in a sealed container with two plastic pipes as outlet channels for CO_2. The amount of CO_2 produced is measured using a Cosmotector, as described by Widodo et al., (2019).

2.4 Peel softness, peel color, and juice content

The softness of the fruit peel was assessed using a method described by Susanto et al., (2018). A Stanhope-SETA penetrometer was used with a 102.5 g cone and an additional load of 50 g applied. The peel was punctured with a needle for 10 seconds to measure its softness. Observation of fruit peel color refers to the method used by Mudyantini et al., (2017) using Munsell Plant Tissue Color Book. This method allows for a standardized evaluation of peel color, ensuring consistent and reliable results across different samples. The assessment is particularly important in this study, which focuses on the quality improvement of lime fruit (*Citrus aurantifolia*) under varying packaging conditions and storage temperatures.

Juice content measurements were conducted using the method described by Navarro-Calderón et al. (2024) with minor adjustments. The volume of juice was measured in milliliters (ml) and then compared to the weight of the fruit. The formula used is (Equation 2):

$$Juice\ content\ (\%) = \frac{Volume\ of\ juice)}{Fruit\ weight} \times 100\% \qquad (Eq.\ 2)$$

2.7 SSC, TA and ratio SSC/TA

The SSC (Soluble Solids Content) and TA (Total Acidity) contents were determined following the method outlined by Ziogas et al. (2022). Measurements were conducted at room temperature using a PAL-BX/ACID 1 Master Kit refractometer (Atago CO. Ltd., Tokyo, Japan), with results expressed in °Brix for SSC and % citric acid for TA. The SSC/TA ratio was calculated by dividing the SSC value by the TA value.

2.8 Ascorbic acid

Ascorbic acid was determined using titration of the filtrate with 0.01 N iodine solution and 1% starch indicator solution, following the method described by Gull et al. (2021). This titration method is widely recognized for its accuracy in quantifying ascorbic acid levels in fruit samples. The precise measurement of ascorbic acid is crucial in this study to evaluate the impact of packaging and storage conditions on the nutritional quality of lime fruit (*Citrus aurantifolia*).

2.9 Data analysis

Data analysis was conducted using Microsoft Excel Office® 2019 and IBM SPSS® 25 software. The data were subjected to analysis of variance at the $\alpha = 0.05$ level. If the analysis of variance yielded significant results, the Duncan multiple range test (DMRT) was performed at the $\alpha = 0.05$ level.

3. Results and Discussion

3.1 Weight loss

The analysis of variance at the $\alpha = 0.05$ level indicated that storage temperature treatments, with or without packaging combinations, significantly affected weight loss on days 3 and 6 (Table 1). Weight loss is attributed to physiological weight reduction due to respiration, water transpiration through peel tissue, and other biological changes occurring in the fruit (Hailu, 2016). Previous research showed that the weight loss of mango fruit (Zhang et al., 2017) and blueberries (Zhou et al., 2014) stored at low temperatures was significantly lower than those stored at room temperature, indicating that lower temperatures can inhibit water transpiration and loss of fruit nutrients, thereby reducing weight loss.

Table 1. Effect of temperature and packaging on weight loss of lime fruit

Treatment	Weight loss (%)				
	Day 3	Day 6	Day 9	Day 12	Day 15
RT	5.68b	5.63c	5.14	2.73	3.53
RTP	5.06b	5.18bc	4.53	2.53	3.18
LW	3.23a	3.30a	4.06	2.07	2.63
LWP	2.76a	4.03ab	5.40	2.56	3.55
Significance	*	*	ns	ns	ns

Details: RT= Room temperature without packaging (control), RTP= Room temperature with packaging, LW= Low temperature without packaging, LWP= Low temperature with packaging. *= Significant effect on statistical test (α<0,05), ns= no significant effect on statistical test (α>0,05)

On day 3, the optimal treatment was low temperature with packaging, as indicated by minimal weight loss, which was not significantly different from low temperature without packaging but significantly different from other treatments. By day 6, the best treatment was low temperature without packaging, not significantly different from low temperature with packaging but significantly different from other treatments. Under low temperature storage conditions, whether using packaging or not, results in reduced cumulative weight loss (Fig. 1). Previous studies on grapes show that low-temperature delays weight loss by reducing the respiration rate and inhibiting various enzyme activities (Luesuwan et al., 2021; Cheng et al., 2023).

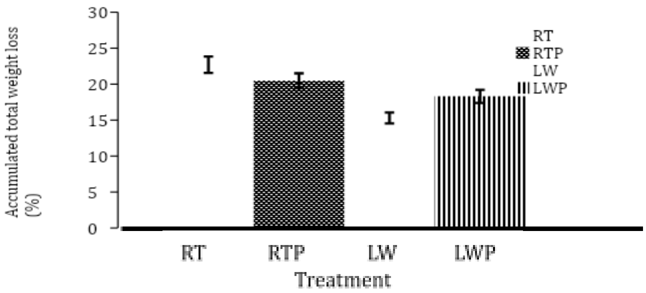

Fig. 1. Accumulated weight loss of lime

The higher the temperature, the greater the respiration, leading to increased weight loss of the product. This research proves that at room temperature, the rate of weight loss is higher compared to lower temperatures (Arzam & Baba, 2018). Elevated temperatures can increase respiration and transpiration rates, resulting in greater water loss, which ultimately contributes to higher weight loss (Silip et al., 2021).

3.2 Respiration rate

Analysis of variance at α = 0.05 level indicated that storage temperature treatments, with or without packaging combinations, significantly affected the respiration rate on days 3, 9, 12, and 15 (Table 2). The respiration rate pattern (CO_2 production) in lime fruits exhibits a non-climacteric pattern. Non-climacteric fruits do not show a significant increase in respiration rate during the ripening process (Kim et al., 2015). The control treatment and the room temperature treatment combined produced higher respiration rates. This can be attributed to temperature influencing the respiration process. Temperature is a critical factor, as fruits stored at room temperature have a shorter shelf life compared to refrigerated fruits (Minas et al., 2013). On day 15, the lowest respiration rate was observed in the unpackaged low-temperature treatment (Table 2), significantly different from other treatments, suggesting that fruit quality lasts longer when respiration and transpiration rates are minimized by maintaining low storage temperatures (Fatchurrahman et al., 2022).

Table 2. Effect of temperature and packaging on respiration rate of lime fruit

Treatment	Respiration rate (mg CO_2/kg jam)					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RT	618.77	531.41b	626.49	452.59b	558.47b	601.25b
RTP	549.98	527.09b	539.67	270.38a	274.63a	573.02b
LW	625.19	241.44a	561.72	355.40a	323.52a	307.84a
LWP	618.68	343.19a	705.10	514.99b	480.24b	519.95b
Significance	ns	*	ns	*	*	*

Details: RT= Room temperature without packaging (control), RTP= Room temperature with packaging, LW= Low temperature without packaging, LWP= Low temperature with packaging. *= Significant effect on statistical test (α<0,05), ns= no significant effect on statistical test (α>0,05)

3.3 Peel softness

Analysis of variance at α = 0.05 level indicated that storage temperature treatments, with or without packaging combinations, significantly affected peel softness on days 3, 12, and 15 (Table 3). On day 15, the highest peel softness was observed in the control and low temperature with packaging treatments, both recording a value of 4.33 mm kg^{-1}. These values were not significantly different from those of room temperature without packaging but were significantly different from room temperature with packaging (Table 3).

Table 3. Effect of temperature and packaging on peel softness of lime fruit

Treatment	Fruit peel softeness (mm kg^{-1})					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RT	4.46	5.18b	4.46	3.61	4.46b	4.33b
RTP	4.07	4.26b	4.00	3.34	5.25c	4.20b
LW	4.07	4.66b	3.67	2.69	5.31c	2.82a
LWP	4.72	3.41a	3.80	3.21	3.54a	4.33b
Significance	ns	*	ns	ns	*	*

Details: RT= Room temperature without packaging (control), RTP= Room temperature with packaging, LW= Low temperature without packaging, LWP= Low temperature with packaging. *= Significant effect on statistical test (α<0,05), ns= no significant effect on statistical test (α>0,05)

The higher the measurement result, the softer the fruit, which is crucial for limes as it indicates better quality. Hard lime peels tend to have a shorter shelf life and higher weight loss due to reduced fruit juice volume. The quality of fruit firmness is primarily determined by the cell wall structure, strength, and intercellular adhesion, regulated by enzymes like β-galactosidase (β-GAL) and pectin lyase (PL), which can be influenced by postharvest conditions (Dobón-Suárez et al., 2021; Mi et al., 2023). Newspaper packaging helps inhibit airflow, thereby maintaining optimal moisture levels (Suhartono and Iskandar, 2017). Consistent with this research, newspaper packaging helps preserve fruit softness, preventing it from becoming hard and dry.

3.4 Peel color

Fruit color is one of the most critical parameters influencing consumer preferences. It is determined by pigmentation in the pulp and peel during ripening (Tilahun et al., 2019). Low-temperature storage, with or without packaging, results in a more uniform green-yellow hue. The peel color of the fruit begins to transition to bright yellow by days 3 and 6, indicating the onset of senescence.

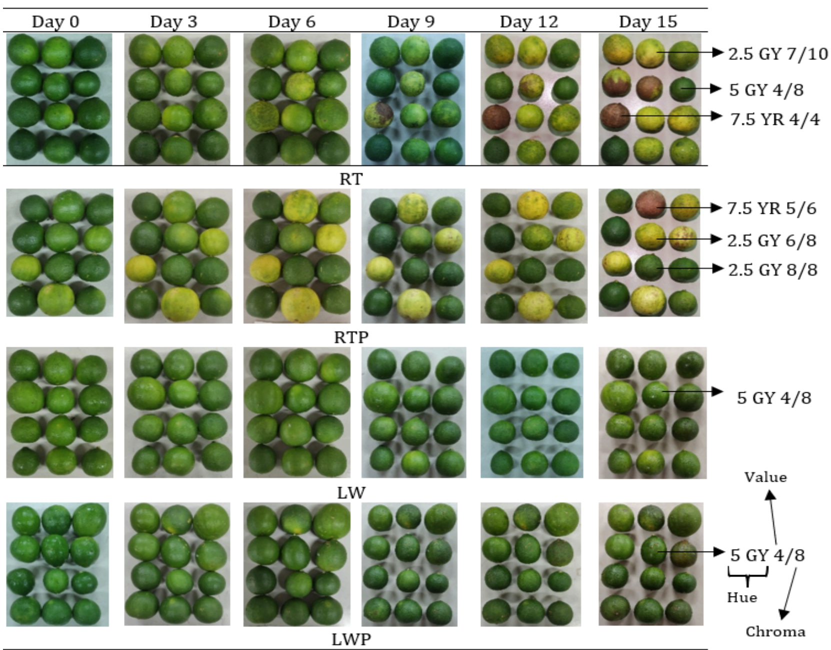

Fig. 2. Discoloration of lime peel during ripening

Post-harvest decline in lime fruit quality is marked by the loss of green color due to chlorophyll breakdown (Opio, 2017). This color change in the fruit peel occurs as chlorophyll degrades, giving way to carotenoids and resulting in the shift from green to yellow (Chen et al., 2021). Ethylene plays a role in regulating these color changes by facilitating chlorophyll degradation and promoting the accumulation of carotenoids or anthocyanins (Iqbal et al., 2017).

Hue indicates the predominant color of an object, whether red (R), green (G), or yellow (Y). Value indicates the brightness of the color, ranging from 0 to 10, with higher values indicating brighter colors. Chroma indicates the intensity of a color, ranging from 0 to 20, with higher Chroma values indicating stronger color intensity (Fig. 2).

3.5 Juice content

Analysis of variance at α = 0.05 level showed that storage temperature treatments, with or without packaging combinations, significantly influenced juice content on days 3, 6, 9, and 15 (Table 4). Lime fruit typically contains a water content ranging from 37.25% to 43.96% (Manuha et al., 2019), which aligns with findings from this research. In Hex Chlamys edulis fruit, water content is significantly higher at the ripe stage compared to stages of unripe, medium ripeness, and overripeness (Arena et al., 2021). Consistent with these findings, ripe limes contain more juice compared to unripe limes. The control treatment ripens faster than cold storage, resulting in higher juice content initially. However, prolonged storage accelerates senescence. Low temperatures, with or without packaging, lead to reduced juice content but are advantageous for transportation and prolonged shelf life.

Table 4. Effect of temperature and packaging on juice content of lime fruit

Treatment	Juice content (%)					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RT	41.50	18.87a	52.25c	39.84b	43.05a	42.80b
RTP	37.31	40.57b	29.24a	17.32a	43.46a	31.06a
LW	30.78	47.69b	27.32a	14.35a	50.68a	26.28a
LWP	27.33	24.01a	41.19b	23.64a	41.72a	25.73a
Significance	Ns	*	*	*	ns	*

Details: RT= Room temperature without packaging (control), RTP= Room temperature with packaging, LW= Low temperature without packaging, LWP= Low temperature with packaging. *= Significant effect on statistical test (α<0,05), ns= no significant effect on statistical test (α>0,05)

3.6 SSC, TA, and ratio SSC/TA

Analysis of variance at α = 0.05 level indicated that storage temperature treatments, with or without packaging combinations, significantly influenced Soluble Solids Content (SSC) from day 3 to day 15 (Table 5). SSC is a crucial parameter in assessing fruit quality, affecting taste and directly impacting consumer preferences (Sun et al., 2019). SSC values for fruits stored at room temperature were higher than those stored at low temperatures, either with packaging or without packaging. The highest SSC value at 15 days of age was the room temperature treatment with packaging, resulting in a value of 8.4^{o}Brix, while the lowest value was the low temperature treatment without packaging, resulting in a value of 7.30^{o}Brix. The increase in SSC occurred due to starch hydrolysis, sucrose synthesis, and gluconeogenesis (Farcuh et al., 2018), which happened at a higher rate in room temperature treatment than at low temperature. Previous research has shown that lower storage temperatures for 'Qingcuili' plums led to slower accumulation of soluble sugars and reduced titratable acids (Feng et al., 2024).

Table 5. Effect of temperature and packaging on SSC of lime fruit

Treatment	SSC (⁰Brix)					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RT	7.65a	8.35b	7.67b	8.37c	8.25b	8.20b
RTP	8.20b	8.22b	7.92b	7.55b	8.05b	8.40b
LW	7.42a	8.22b	6.65a	6.40a	8.07b	7.30a
LWP	7.10a	70.4a	6.80a	6.82a	7.02a	7.45a
Significance	*	*	*	*	*	*

Details: RT= Room temperature without packaging (control), RTP= Room temperature with packaging, LW= Low temperature without packaging, LWP= Low temperature with packaging. *= Significant effect on statistical test (α<0,05), ns= no significant effect on statistical test (α>0,05)

Analysis of variance at α = 0.05 level indicated that storage temperature treatments, with or without packaging combinations, significantly affected Total Acidity (TA) on days 3 and 12 (Table 6). The lowest TA was observed on day 0, followed by an increase on day 3, and then a decrease as the fruit storage period increased. The reduction in TA may result from citric acid's susceptibility to oxidative damage influenced by environmental factors during ripening, as well as starch hydrolysis, which leads to an increase in total sugars and a decrease in acidity (Hailu, 2016).

Table 6. Effect of temperature and packaging on TA of lime fruit

Treatment	TA (%)					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RT	5.17	8.43b	7.50	7.31	7.57b	7.27
RTP	5.66	7.98b	7.46	6.95	6.55a	7.06
LW	5.92	8.15b	7.92	8.15	7.82b	7.85
LWP	5.52	7.14a	7.67	7.65	7.74b	7.50
Significance	ns	*	Ns	ns	*	ns

Details: RT= Room temperature without packaging (control), RTP= Room temperature with packaging, LW= Low temperature without packaging, LWP= Low temperature with packaging. *= Significant effect on statistical test (α<0,05), ns= no significant effect on statistical test (α>0,05)

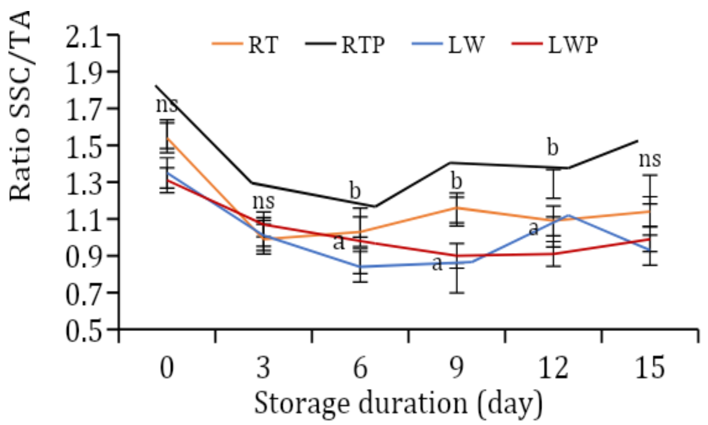

Fig. 3. Storage temperature treatment pattern with packaging or packaging tanpan to SSC/TA ratio.

Analysis of variance at α = 0.05 level showed that storage temperature treatments, with or without packaging combinations, significantly influenced the SSC/TA ratio on days 6, 9, and 12 (Fig. 3). The higher the SSC/TA ratio, the more mature the fruit (Susanto et al., 2018). Therefore, for limes, a lower SSC/TA ratio indicates better quality because it indicates a longer shelf life (Fig. 4).

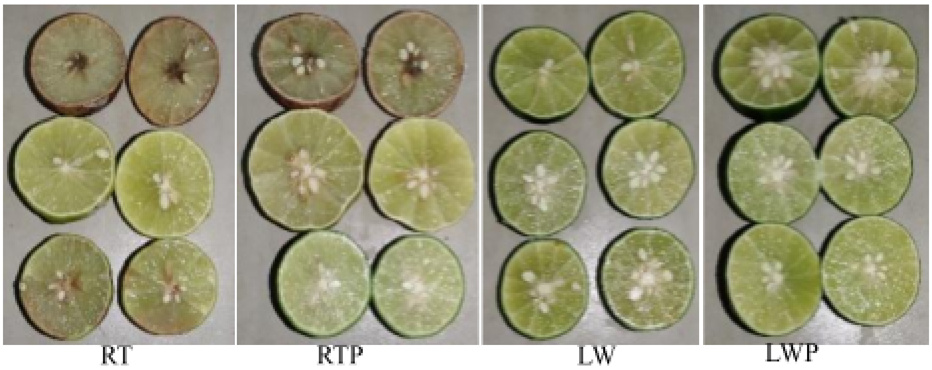

Fig. 4. Correlation ratio of PTT / TA to the maturity level of lime fruit

3.7 Ascorbic acid

Ascorbic acid is often used as an indicator of overall reduction in product quality during processing, storage, and postharvest handling (Giannakourou & Taoukis, 2021). Analysis of variance at α = 0.05 level indicated that storage temperature treatments, with or without packaging combinations, significantly affected ascorbic acid levels on days 6, 9, and 15 (Table 6). The best treatment was the control, which was not significantly different from room temperature with packaging but significantly different from the other treatments (Table 8). On day 15, storage at low temperature without packaging or with packaging resulted in lower levels of ascorbic acid. Previous research stated that from several citrus genotypes such as ordinary Citrus, Hybrid, Mandarin, and Orange, there was a decrease in ascorbic acid by increasing storage temperature, but this did not occur in the Grapefruit variety and lime, which increased by increasing storage temperature (Budiarto et al., 2024). Therefore, room temperature can maintain the ascorbic acid content of lime than low temperature.

Table 6. Effect of temperature and packaging on ascorbic acid of lime fruit

Treatment	TA (%)					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RT	74.50	49.25	52.8b	77.00b	68.15	88.00b
RTP	73.26	29.70	52.8b	48.40a	55.00	57.20a
LW	71.79	44.75	37.4a	50.60a	37.40	45.20a
LWP	74.65	38.45	53.9b	74.80b	40.70	44.20a
Significance	tn	tn	*	*	tn	*

Details: RT= Room temperature without packaging (control), RTP= Room temperature with packaging, LW= Low temperature without packaging, LWP= Low temperature with packaging. *= Significant effect on statistical test (α<0,05), ns= no significant effect on statistical test (α>0,05)

4. Conclusions

Low temperature treatment without packaging yields the best results in delaying weight loss, respiration rate, and SSC/TA ratio. The control treatment showed superior results in peel softness, juice content, and ascorbic acid levels. Therefore, low temperature treatment without packaging is recommended for storing lime fruit to prolong shelf life. Suggestions for future research include the need for treatments related to increasing lime juice content by accelerating fruit ripening, such as using ethylene. Still, they must be combined with low temperature storage so that the shelf life of the fruit remains long even though it is given a treatment to accelerate ripening.

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Author Contribution
Conceptualization, I.H.; Methodology, I.H.; Software, I.H.; Validation, I.H., A.D.S; Formal Analysis, I.H., A.D.S; Investigation, I.H.; Resources, I.H., A.D.S; Data Curation, I.H.; Writing – Original Draft Preparation, I.H.; Writing – Review & Editing, I.H., A.D.S; Visualization, I.H.; Supervision, I.H.; Project Administration, I.H., A.D.S; and Funding Acquisition, I.H.

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Not applicable

Informed Consent Statement
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Data Availability Statement
Primary data is requested based on confidentiality or ethical constraints.

Conflicts of Interest
The authors declare no conflict of interest.

Open Access

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