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Changes in quality parameters and bioactive components of seedless lime fruit (*Citrus latifolia*) during cold storage

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Abstract

The postharvest maintenance quality of fruits is significant for industrial applications. The present study was conducted to evaluate of quality parameters and bioactive components of seedless lime (*Citrus latifolia*) fruit during cold storage. Lime fruits were packed in perforated polyethylene bags, which were then put into a perforated expanded polystyrene box, and stored in a cool chamber, at 10°C. Quality parameters including skin color, weight loss, total soluble solids (TSS), juice percentage, technological index, titratable acidity (TTA), reducing and total sugars of the fruits were measured during 56 days of storage. The total phenolic content of the juices was measured using a colorimetric method of Folin-Ciocalteu. Ascorbic acid and major flavonoids of the fruits such as hesperidin and eriocitrin were quantified using HPLC-PDA methods. The results showed that cold storage kept the greenness of the fruit's skin for up to 28 days. A minor weight loss (< 5%) was also recorded in the first 28 days of storage. The technological index of the fruits, reducing total sugars and TSS slightly increased, whereas TA somewhat decreased. Bioactive components such as phenolic and ascorbic acid contents were kept stable (p>0.05) in the first 21 days of storage but considerably reduced after 28 days of storage. A decrease in hesperidin and eriocitrin was also recorded from 21 days of storage but at a lower rate compared to that of phenolic and ascorbic acid contents. Altogether, a simple, convenient cold storage method using a perforated polyethylene bag and expanded polystyrene box can retain the quality and bioactive components of lime fruits for at least 21 days. The findings are significant for the food industrial application of storage lime fruits for fresh utilization or juice processing.

1. Introduction

Lime (*Citrus latifolia*) is an important citrus crop in Mekong Delta, Vietnam, with a total cultivation area of 10,000 hectares and an annual production of 60,000 tons of fruits (Huynh *et al.*, 2021). Lime fruits are very essential for both fresh consumption and the juice processing industry (Rangel *et al.*, 2011). Fresh lime fruit quality is evaluated by its appearance with green skin color and free of dust and pests (Obeed and Harhash, 2006; Kaewsuksaeng *et al.*, 2015; Pongsri *et al.*, 2021). The juice quality is characterized by various parameters such as total soluble solid (TSS), total titratable acidity (TTA), juice percentage, and technological index (TI) (Kluge *et al.*, 2003; Obeed and Harhash, 2006; Stuchi *et al.*, 2009; Sun *et al.*, 2019). In addition, lime juice is also high in bioactive components such as vitamin C, phenolic compounds, and flavonoids (e.g., hesperidin and eriocitrin) (Gattuso *et al.*, 2007; de Moraes Barros *et al.*, 2012; Huang *et al.*, 2018; Dong *et al.*, 2019; Huynh *et al.*, 2021). The attributes for the overall quality of lime fruit are not only common parameters but also the bioactive components of the juice. Lime fruit quality quickly reduces after the fruit is harvested (Kaewsuksaeng *et al.*, 2015). The postharvest deterioration of the lime fruit's quality leads to a significant economic loss (Tavallali and Zareiyan, 2018). Low-temperature storage is commonly applied for lime and lemon. However, choosing the appropriate temperature is very important to avoid chilling injuries. It has been reported that lime fruit has a critical temperature of 7°C. Storage of lime under critical temperature likely causes chilling injury (Pranamornkith *et al.*, 2005; Rivera *et al.*, 2007). Indeed, storage at 10°C is recommended to maintain the quality of the lime fruits (Hardenburg *et al.*, 1990; Kluge *et al.*, 2003).

Various treatments of lime fruits during cold storage

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were recorded such as hot water dipping, intermittent warming, 1-methylcyclopropene, vacuum infiltration with salicylic acid, and electron beam irradiation in combination with shellac coating (Jomori *et al.*, 2003; Kluge *et al.*, 2003; Obeed and Harhash, 2006; Tavallali and Zareiyan, 2018; Pongsri *et al.*, 2021). These treatments usually require more labor, advanced technology and high equipment investment. The appropriate material of the packages used during cold storage is an important factor in retaining the fruit quality. For example, perforated polyethylene bags positively affected the quality of Balady lime (Zagzog and Mohsen, 2012), and kagzi lime (Jadhao *et al.*, 2008) during cold storage. The use of a perforated polyethylene bag could avoid undesirable modified air composition within the package and water condensation lining inside the bag and on the surface of the fruits (Meir *et al.*, 1995). Secondary packaging such as corrugated fiberboard, corrugated plastic, and expanded polystyrene boxes have been commonly used for transport or storage packaging of hard fruit (e.g., citrus fruit) to prevent fruit damage (McGregor, 1989; González-Estrada *et al.*, 2017). Expanded polystyrene boxes have been used for insulation purposes with advantages of high flexibility, economy and safety (Zhao *et al.*, 2020). Recently, polystyrene boxes have been used to maintain the quality of Lisboa lemon and squash (Park, 2000; Obregón-Burgueño *et al.*, 2018). However, the study of using perforated polystyrene boxes during cold storage of seedless lime fruits has been rarely reported. The present study aimed to investigate a simple convenient cold storage of lime fruits using a combination of perforated polyethylene bags and expanded polystyrene boxes for maintaining fruit quality parameters and the bioactive components of the juice.

2. Materials and methods

2.1 Chemicals

HPLC grade chemicals were purchased from the suppliers as follows: Folin-Ciocalteu's phenol reagent, gallic acid and acetonitrile, ortho-phosphoric acid (Millipore, Merck, Germany), ascorbic acid and glucose (Scharlau, ExpertQ ACS, Barcelona, Spain), hesperidin (Sigma, Saint Louis, MO, USA), eriocitrin (Toronto Research Chemical Inc., Toronto, Canada). Other chemicals were analytically graded.

2.2 Sample procurement

Seedless lime fruits (*Citrus latifolia*) were manually harvested in the early morning from the 6-year-old orchards in Thanh Loi Ward, Long An province, Viet Nam. The fruits were selected for uniformity of size, and color, and without blemishes and other defects. Fruits were selected based on a preliminary color check as (L* = 43.56±1.84, a* = -20.76±0.85, b* =25.89±1.55) using a portable colorimeter (CR-400 Chroma Meter, Konica Minolta, Japan). The fruits were then transported immediately to the laboratory within 4 hours.

2.3 Cold storage conditions

Fruits were washed with clean water, drained and gently wiped with a paper towel before being subjected to storage. Polyethylene bags with the size of 40 cm × 28 cm, a thickness of 0.06 mm were perforated 9 holes (5 mm in diameter) on each side using a domestic perforated tool making a total of 0.16 % of perforation were used for the cold storage. Six lime fruits (around 500 g) were packed in each perforated bag. Expanded polystyrene boxes with a dimension of 31 cm × 22 cm × 24 cm were used as the secondary package for cold storage. Three holes (10 mm in diameter) were made from each side of the boxes (excluding the bottom and the lid), resulting in box perforation of 0.27%. The boxes were divided into two layers, each layer was designed to contain one bag of lime fruit samples. Fruits were divided into eight lots following storage periods of 7, 14, 21, 28, 35, 42 and 56 days. The boxes were kept in a cool chamber, 10°C (± 1°C), relative humidity of 90% (± 2%). At different time intervals of storage (every 7 days), each lot was taken out from the cool chamber and subjected to physicochemical analyses. A total of 12 fruits of each replicate were measured for color, and weight loss. The juice of fruits was mixed to obtain a homogenous sample for measuring total titratable acidity (TTA), total soluble solid (TSS), reducing and total sugars, total phenolic content (TPC), vitamin C, hesperidin and eriocitrin.

2.4 Measurement of the color changes

The color changes were measured using a colorimeter (CR-400 Chroma Meter, Konica Minolta, Japan) according to the method described by Rehman *et al.* (2018). For each lime fruit, the color of two opposite positions around the equatorial plane was recorded. The color was expressed according to the CIELAB system of L*, a* and b*.

2.5 Measurement of the weight loss

Initial weights of lime fruits were recorded before the fruits were subjected to cold storage. After each week of storage, the fruit was taken out from the cool chamber and left at room temperature for 30 mins. The fruits were swiped out of condensed water by using a paper towel and recorded the weight. The weight loss was determined using the following formula:

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$$\text{Weight loss (\%)} = \frac{W_0 - W_s}{W_0} \times 100$$

Where W_0 = initial weight (g) and Ws = weight after storage (g)

2.6 Determination of juice percentage

Around 1000 g of lime fruit was recorded as initial weight (as fruit weight, FW) and the juice was extracted by hand-squeeze described by Topi (2020). The juice was collected and considered as juice weight (JW), and the juice percentage was determined as follows:

$$\text{Juice percentage (\%)} = \frac{JW}{FW} \times 100$$

Where FW = Fruit weight (g) and JW = Juice weight (g)

2.7 Determination of total soluble solids

Total soluble solids (TSS) in extracted lime juices at weekly intervals were measured using an electronic refractometer (Brixacid101, Atago, Japan). The device was calibrated before each lot of measurement using deionized water as a blank. The TSS was measured in triplicates and the results were expressed in degree Brix (Brix).

2.8 Determination of technological index

Technology index (TI) was calculated based on the TSS and juice percentage using the following formula, according to Kluge *et al.* (2003):

$$\text{Technological index (TI)} = \frac{\text{TSS} \times \text{Juice percentage}}{100}$$

2.9 Determination of total titratable acidity

Approximately 5 mL of the extracted lime juices were diluted ten times with distilled water. Then, 10 mL of diluted juices was titrated with NaOH (0.1N) with the color indicator phenolphthalein. The citric acid conversion factor of 0.0064 was used to calculate the total titratable acidity (TTA) as described by Jamil *et al.* (2015)

$$TTA = \frac{V \times K \times 100}{V1}$$

Where V = volume of NaOH added, V_1 = volume of sample, K = 0.0064 (conversion factor for citric acid).

2.10 Determination of the total and reducing sugars

The method developed by Başkan *et al.* (2016) was used with slight modifications. All freshly extracted juices were immediately analyzed for sugars. To determine the reducing sugar, the juice was centrifuged (5000 rpm/min) for 10 mins then the clear supernatant was collected and diluted 50 times with deionized water. Approximately 2 mL of the diluted sample was transferred into a screw cap glass test tube. Then, 2 mL of 3-amino-5-nitrosalicylate (DNS, 100 mL DNS solution contained 1 g DNS, 20 mL NaOH 2 N, 30 g sodium potassium tartrate and deionized water) was added into the test tube. The tube was capped and placed in a boiling water bath for 5 mins. The developed color was measured at the wavelength of 540 nm using a spectrophotometer (Jasco V-730, Japan). Glucose was used as a standard for the quantification of reducing sugar. To determine total sugar, 15 mL of undiluted, clear supernatant was transferred into a screw cap test tube. Then, 0.5 mL of concentrate hydrochloric acid (37%) was added, and the tube was incubated for 10 min at 60°C in a water bath. In the next step, the test tube was immediately chilled in an ice bath. Afterwards, 7.5 mL of 2 N NaOH was added to the test tube. The tube was vortex mixed, and the solution was diluted 50 times with deionized water before being subjected to the DNS reaction. The absorbance was measured similarly to the aforementioned description.

2.11 Determination of total phenolic content

The total phenolic content (TPC) in lime juice was quantified using a modified protocol described by Saikia *et al.* (2015). The phenolic compounds in lime juice were extracted with methanol 80%. The phenolic compounds in the methanolic extract were subjected to a color reaction with Folin-Ciocalteu 10% solution. The developed color complex was measured for absorbance at 765 nm using a spectrophotometer (Jenway 7305, Bibby Scientific, England). Gallic acid standard with a series of dilutions was used to construct the standard curve. The amount of TPC was expressed as mg GAE/100 mL of juice.

2.12 Determination of ascorbic acid

The high-performance liquid chromatography (HPLC) modified protocol of Uckoo *et al.* (2011) and Huynh *et al.* (2021) was used for quantitative analysis of ascorbic acid in lime fruits during cold storage. The lime juice was extracted and immediately diluted six times with 3 mM ortho-phosphoric acid (mobile phase). The diluted sample was then centrifuged, filtered and transferred into HPLC vials for analysis. HPLC system LC-20AD (Shimadzu, Japan) was equipped with a pump (SIL-20A), PDA detector (SPD-20A), degassing unit (DGU-20A) and controller (CBM-20Alite). A reverse phase C18 column (Inertsil, ODS 3, Japan) was used with the isocratic flow of 1 mL/min was employed. The ascorbic acid peak was acquired at a wavelength of 254 nm.

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2.13 Quantification of hesperidin and eriocitrin

Hesperidin and eriocitrin were quantified using the procedure reported by Zhang *et al.* (2014) with modifications. The lime juice was extracted with methanol 80% solution with a ratio of 1:4 (v/v) in the ultrasonic bath (at 30°C, 15 mins). The methanolic extract was then centrifuged (5000 rpm, 10 mins). The supernatant was collected and filtered through a 0.45 µm PTFE syringe filter and transferred into an amber HPLC vial for analysis. The aforementioned Shimadzu LC-20AD similar to ascorbic analysis was used. The mobile phases were deionized water (A) and acetonitrile (B) both acidified with 0.1% formic acid. The stationary phase was the C18 column (ODS 3, Intersil, Japan). A gradient mode of mobile phases was used with the total flow rate of 1 mL/min as follows: 0-20 mins: 20% B, 20-30 min: 30% B, 30-40 mins: 30% B, 40-45 mins: 50% B, 45-52 mins: 50% B, 52-55 min: 70% B, 55-65 mins: 70% B, 65-70 mins: 20% B; 70-80 mins: 20% B. Hesperidin and eriocitrin peaks were acquired and quantified at a wavelength of 280 nm.

2.14 Statistical analysis

GraphPad Prism 8.01 (GraphPad Software Inc.; San Diego, CA, USA) software was used to perform the Analysis of Variance (ANOVA) and Duncan tests at the statistically significant level of $p<0.05$. The presented values are mean±SD of at least three replicates.

3. Results and discussion

3.1 Color change

Color change is an important indicator of physiological alteration and consequently affects the lime fruit quality. In this study, the lime fruits were harvested in the green stage (L*, a*, and b* being 42.46, -20.76 and 25.89, respectively). The color changes of lime fruits during cold storage are presented in Table 1. The cold storage of lime fruits greatly extended the color preservation of the fruits. The lime fruits stored at room temperature showed spoilage only after 14 days of storage (Figure 1B). For cold storage of lime fruits, the results showed that the change of brightness notation (L*), greenness (a*) and yellowness (b*) was significantly different ($p<0.05$). The L* value increased from 42.46 to 75.71, indicating an increase in brightness. It is possible that during the storage the skin color shifted from dark blue to bright yellow as observed in our study (Figure 1A). The a* value changed from -20.76 to -5.84 after 56 days of storage. The shift of a* value toward a higher value represents the loss of greenness. The skin degreening involves in degradation of chlorophyll under the activity of chlorophyllase, chlorophyllase peroxidase, and pheophyntinase (Kaewsuksaeng *et al.*, 2015; Pongsri *et al.*, 2021). Our result agrees with the finding of a previous study where the loss in the greenness of lime fruit during storage was recorded (Kluge *et al.*, 2003; Obeed and Harhash, 2006; Sun *et al.*, 2019). The visual observation from this study demonstrated that the lime fruits considerably reduced in greenness after 35 days of storage (Figure 1). Compared to the control (storage at room temperature, Figure 1B, Table 1), the greenness of cold stored lime fruits was greatly retained. The longer retention of the greenness in lime fruits reflects a longer shelf-life of lime fruits. The cold storage of lime fruits in PE bags thus would be an appropriate method to the extent of the shelf-life of seedless lime fruits. The loss in greenness in lime fruits during cold storage was in accordance with an increase in yellowness. The b* values of lime fruit during cold storage greatly increased (from 25.89 to 63.95). The yellowness of lime fruit was observed (Figure 1A). During the cold storage, the lime fruits shifted into darker, less green and more yellow. The overall color change of lime fruit was remarkably observed after the storage time of 35 days. From the visual observation, lime fruits showed black dots on the surface after 56 days of storage, indicating a sign of quality reduction.

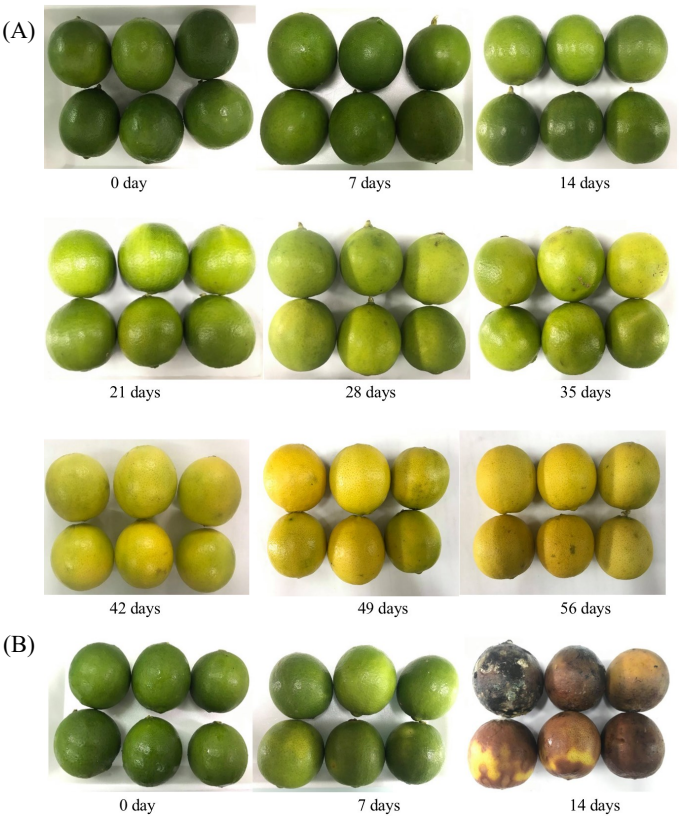

Figure 1. Color changes of seedless lime fruits stored at 10°C for up to 56 days (A) and at room temperature (B) for up to 14 days.

3.2 Weight loss, juice percentage, total soluble solid, and technological index

3.2.1 Weight loss

The weight loss and juice percentage are the important indices of lime fruit during the storage period.

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Table 1. The color changes, weight loss, juice percentage, total soluble solid and technological index of seedless lime stored at 10°C at different storage periods.

Storage time (day)	L* value	a* value	b* value	Weight loss (%)	Juice percentage (%)	TSS (°Brix)	Technological Index
0	42.46±1.84^{f}	-20.76±0.85^{e}	25.89±2.55^{f}	-	40.0±0.2^{f}	7.6±0.2^{f}	3.0±0.2^{e}
7	50.42±1.73^{e}	-20.24±1.61^{e}	38.48±2.96^{e}	4.0±0.7^{d}	40.8±0.1^{e}	7.8±0.1^{f}	3.2±0.1^{de}
14	50.60±3.17^{e}	-19.79±1.18^{e}	39.56±3.93^{e}	4.2±0.1^{d}	41.5±0.1^{d}	8.0±0.1^{e}	3.3±0.1^{d}
21	57.33±3.87^{d}	-19.55±0.77^{de}	45.29±4.14^{d}	4.5±0.3^{cd}	41.6±0.1^{d}	8.1±0.1^{de}	3.4±0.1^{d}
28	60.28±3.31^{c}	-18.72±1.92^{cd}	52.88±3.92^{c}	4.7±0.2^{c}	42.0±0.4^{c}	8.3±0.1^{c}	3.5±0.1^{c}
35	66.69±1.34^{b}	-16.15±1.16^{c}	54.37±3.25^{c}	5.2±0.4^{bc}	42.4±0.6^{c}	8.5±0.1^{b}	3.6±0.1^{b}
42	73.62±2.93^{a}	-10.87±3.16^{b}	61.52±1.74^{b}	5.6±0.6^{b}	42.8±0.3^{bc}	8.6±0.1^{ab}	3.7±0.1^{ab}
49	74.12±3.42^{a}	-10.85±2.34^{b}	61.26±2.71^{ab}	6.4±0.3^{a}	43.7±0.1^{b}	8.7±0.1^{a}	3.8±0.1^{ab}
56	75.71±1.21^{a}	-5.84±1.82^{a}	63.95±1.19^{a}	6.8±0.4^{a}	44.5±0.1^{a}	8.8±0.1^{a}	3.9±0.1^{a}
Control*	50.62±1.73^{e}	-19.76±0.65^{e}	39.58±4.21^{e}	4.6±0.7^{c}	40.8±0.3^{e}	7.7±0.1^{f}	3.1±0.1^{e}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

*Seedless lime fruit stored at room temperature (30°C) at 7 days of storage.

Table 1 presents the recorded weight loss of lime fruits during cold storage. Overall, weight loss increased during storage and reached 6.8% at 56 days of storage. In the first 28 days of storage, the fruit's weight loss was maintained at a low rate (< 5%). The weight loss of lime fruits stored at room temperature (Table 1) was slightly higher (though not statistically different) than that of lime fruits stored at 10°C. The weight loss value of cold-stored lime fruits in this study was lower than that of the value reported in the literature. Sun *et al.* (2019) studied lemon storage at 10°C and recorded a weight loss of 12% of the green lemon. In another study of cold storage of Persian lime at 4°C, a weight loss of approximately 20% was reported (Tavallali and Zareiyan, 2018). The low weight loss recorded in the present study is possibly due to the use of perforated PE bags in combination with a secondary foam box container. The ability to minimize the weight loss of citrus fruits using perforated bags has been reported earlier. In lime fruit storage, packaging using perforated polyethylene bags greatly reduced the weight loss of kagzi lime (Jadhao *et al.*, 2008) and Balady lime (Zagzog and Mohsen, 2012). In another study, the storage of Baramasi lemon in high-density polyethylene bags also prevented weight loss (Jawandha *et al.*, 2014). The fruits stored in perforated bags possibly result in a high relative humidity inside the package and hence reduce the weight loss. Compared to the control (stored at room temperature), the weight loss of cold-stored lime fruit at 7 days of storage was lower (though not statistically different, $p<0.05$). The expanded polystyrene box (foam box) is commonly used for hard fruit storage (e.g. citrus fruits) (González-Estrada *et al.*, 2017). The utilization of foam boxes was proven to the extent of the shelf-life of squash (Park, 2000), grape (Crisosto *et al.*, 2001), and Lisboa lemon (Obregón-Burgueño *et al.*, 2018). In the present study, the perforated foam box was used as the secondary container to insulate and reduce the moisture loss through high contact surface area that possibly led to a decrease in weight loss as observed.

3.2.2 Juice percentage

Juice content is an important measure of lime fruit quality since it affects the technological value of the fruit. The juice percentage in the present study slightly increased from 40.0 to 44.5% after 56 days of storage (Table 1). Fruit juice content is thought to be correlated to the ripening process, as it increases at the first stage to reach a maximum value and then decreases (Lado *et al.*, 2014; Wijewardane, 2022). However, this trend was not observed in this present study. The juice percentage of lime fruit in this study steadily increased during cold storage. Instead, the results of our study agree with that observed by Kluge *et al.* (2003) when lime (*Citrus latifolia*) was stored at 10°C for 60 days. In the last three weeks of the storage time (from storage day 42 to 56, Table 1) the juice percentage remained unchanged ($p>0.05$). It is possible that the using of perforated PE bags and polystyrene boxes retained more juice weight. As aforementioned, perforated PE bags would diminish the weight loss that is possibly related to the increase in juice percentage. Another possibility causing an increase in lime juice percentage is during ripening the lime's peel becomes more succulent but less tough and fibrous, leading to a more efficient juice extraction (Blankenship, 1987; Ramjan and Ansari, 2018). It is worth noting that though the juice percentage slightly increased, a weight loss was also recorded, which may not preferably contribute to the overall quality of lime fruits.

3.2.3 Total soluble solids

Total soluble solids (TSS) content is an important indicator of citrus fruit quality. The TSS of lime fruits

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slightly increased and reached the highest value of 8.8° Brix after 56 days-storage (Table 1). Compared to the fresh lime, the stored lime fruit at 56 days increased in the TSS by 1.2°Brix. Various factors may be attributed to the increase in the TSS of lime fruits during cold storage. Moisture evaporation, leading to fruit weight loss, is suggested as the main factor causing an increase in TSS (Kaur *et al.*, 2014; Sun *et al.*, 2019). Our study recorded a 6.8% in weight loss which may be a causal correlation to the increase in TSS. Another possible reason is that the ripening of fruit can increase citrus juice quality, reflected by an increase in TSS as proposed by Lado *et al.* (2014). Nonetheless, the findings from this study indicate that perforated polyethylene bags would be effective for maintaining the TSS of lime fruit in the acceptable range during cold storage.

3.2.4 Technological index

The technological index (TI) of lime fruit reflects the industrial juice quality, which is profoundly affected by TSS and juice percentage. Table 1 shows that the TI value significantly increased ($p<0.05$) from 3.0 to 3.9 after 56 days of storage. The increase in TI of the lime juice is more likely contributed by TSS and juice percentage. It was observed that a rise in TI by 0.9 was in accordance with a rise in TSS by 1.2°Brix and a juice percentage of 4.5%. The TI of lime (*Citrus latifolia*) has been reported at a value ranging from 3.2 to 43. An increase in TI value during cold storage of lime fruits has been reported earlier. The TI values during cold storage of Tahiti and Balady limes increased and reached the highest value of 3.76 and 3.75, respectively (Kluge *et al.*, 2003; Zagzog and Mohsen, 2012). A high TI value is desirable for juice manufacture. Thus, the storage methods that retain the high value of TI are preferred. The finding from this study demonstrates that perforated polyethylene bags would be effective for retaining quality parameters such as juice percentage, TSS, and TI values of lime fruit during cold storage.

3.3 Total titratable acid, reducing sugar and total sugar

3.3.1 Total titratable acidity

The high acidity of lime fruits is dedicated to the fruit contributing to the taste of juice. In this experiment, the total titratable acidity (TTA) of lime fruits was measured, and the results are presented in Table 2. During storage, the TTA reduced from 6.75 % to 5.87% during 56-day cold storage, making a decrease in TTA by 13%. The TTA values of lime fruits kept constant ($p>0.05$) in the first 14 days of storage, then slightly decreased afterwards. The pattern of change in TTA in this study is in agreement with what was observed by Rapisarda *et al.* (2008) in the orange cold storage study. In general, a decrease in the TTA of lime fruits during storage has been reported (Kluge *et al.*, 2003; Tavallali and Zareiyan, 2018; Sun *et al.*, 2019; Wijewardane, 2022). The decrease in TTA is probably due to the oxidation of organic acids (Abu-Goukh and Elshiekh, 2008) or the conversion of organic acids to sugars (Guo *et al.*, 2020). The sugar changes during cold storage of lime fruits should be analyzed to clarify the decrease in TTA. Nevertheless, a minor reduction in TTA after 56 days of storage implies the retention of sourness and thus the sensory quality of lime fruits.

3.3.2Reducing and total sugars

The sugar content in lime fruits is likely much lower than other tropical fruits. The reducing and total sugars of fresh fruit were 0.53 and 1.04 g/100 mL (Table 2). The results agree with those obtained by (Ziena, 2000) in which the reducing and total sugars were reported as 0.58 and 0.78 g/100 mL, respectively. The results in Table 2 show that reducing sugar and total sugar significantly increased in accordance with the extension

Table 2. The total titratable acidity, reducing sugar and total sugar of seedless lime stored at 10°C at different storage periods.

Storage periods (days)	Total titratable acidity (g/100 mL)	Reducing sugar (g/100 mL)	Total sugar (g/100 mL)
0	6.75±0.06^{a}	0.53±0.03^{h}	1.04±0.02^{i}
7	6.67±0.04^{ab}	0.58±0.03^{h}	1.20±0.02^{h}
14	6.56±0.06^{b}	0.73±0.02^{g}	1.42±0.01^{g}
21	6.43±0.06^{c}	0.83±0.03^{f}	1.61±0.02^{f}
28	6.37±0.06^{c}	0.96±0.03^{e}	1.88±0.02^{e}
35	6.24±0.04^{d}	1.08±0.01^{d}	2.14±0.02^{d}
42	6.14±0.05^{de}	1.17±0.02^{c}	2.26±0.03^{c}
49	6.03±0.06^{e}	1.24±0.03^{b}	2.40±0.02^{b}
56	5.87±0.06^{f}	1.33±0.04^{a}	2.58±0.03^{a}
Control*	6.15±0.03^{de}	0.60±0.02^{h}	1.22±0.01^{h}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

*Seedless lime fruit stored at room temperature (30°C) at 7 days of storage.

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in the storage time. The highest values of reducing sugar and total sugars were 1.33 and 2.58/100 mL, respectively at 56 days storage period. The increase in reducing sugar appears to involve fruit ripening during storage. The metabolism of citrus fruits during ripening can lead to an increase in soluble sugar. The mechanism may involve the production of sugar from citric acid during the tricarboxylic cycle (Guo *et al.*, 2020). Our results support this concept since a decrease in TTA was recorded. Limes and lemons contain greater proportions of acids than sugars and during ripening the acid content is still predominant (Selvaraj and Edward Raja, 2000; Bisen *et al.*, 2012). In the present study, regardless of a reduction in TTA, the acidity of the fruits was still predominantly found at a level more than two-fold higher than the sugar content after 56 days of storage. Though there was a minor increase in reducing and total sugar contents, it is unlikely that this change may affect the sensory quality of cold storage lime fruits.

3.4 Changes in ascorbic acid, total phenolic, hesperidin, and eriocitrin of lime fruit during cold storage

Ascorbic acid is an important quality indicator of lime juice contributing to the juice's nutritional value. The chromatography method used gave a resolution of ascorbic acid since the target compound was reproducibly eluted at 5.87 min (Figure 2), suggesting the appropriate method for the quantification of ascorbic acid. The quantitative changes in ascorbic acid of lime fruits during storage were measured and the results are illustrated in Figure 3A. The results showed that the ascorbic acid significantly decreased from 29.7 to 19.3 mg/100 mL during cold storage. In the first two weeks of the storage, the ascorbic acid remained unchanged (p>0.05). However, a gradual reduction in ascorbic acid was recorded from 21 to 35 days of storage. After 35 days of storage, total ascorbic acid was significantly reduced, resulting in a total 35% reduction in total ascorbic acid at 56 days. The reduction in vitamin C during the storage of citrus fruits has been recorded in previous studies (Choi *et al.*, 2002; Zagzog and Mohsen, 2012; Sun *et al.*, 2019). It is proposed that vitamin C could be degraded by ascorbic acid oxidase (Van den Broeck *et al.*, 1998; El-Ishaq and Obirinakem, 2015). Furthermore, the reduction in vitamin C found in the present study is probably involved in physiological changes in fruits during storage. The ripening of fruits during cold storage can accelerate changes in physiological properties and cause a rapid reduction in vitamin C. This phenomenon could be confirmed by a visual observation in which the color changed from green to yellow as aforementioned (Figure 1B) indicating the ripening of lime fruits and in turn, led to a significant decrease in vitamin C after 35 days of storage. It has been reported that the vitamin C content was retained for 30 days of storage when the lemon was in the green stage whereas that of the yellow stage significantly degraded (Sun *et al.*, 2019).

Total phenolic content (TPC) which reflects the reducing capacity was measured by using Folin-Ciocalteu method and the results are presented in Figure 3B. The TPC of the juice significantly (p<0.05) decreased from 345.5 to 200.4 mg GAE/100 mL during 56 days of storage. During the first 28 days of storage, the TPC was quite stable, remaining at the level of over 300 mg GAE/100 mL. The extent of storage time beyond 28 days caused a considerable reduction in TPC. Specifically, the 56-day storage sample exhibited a TPC

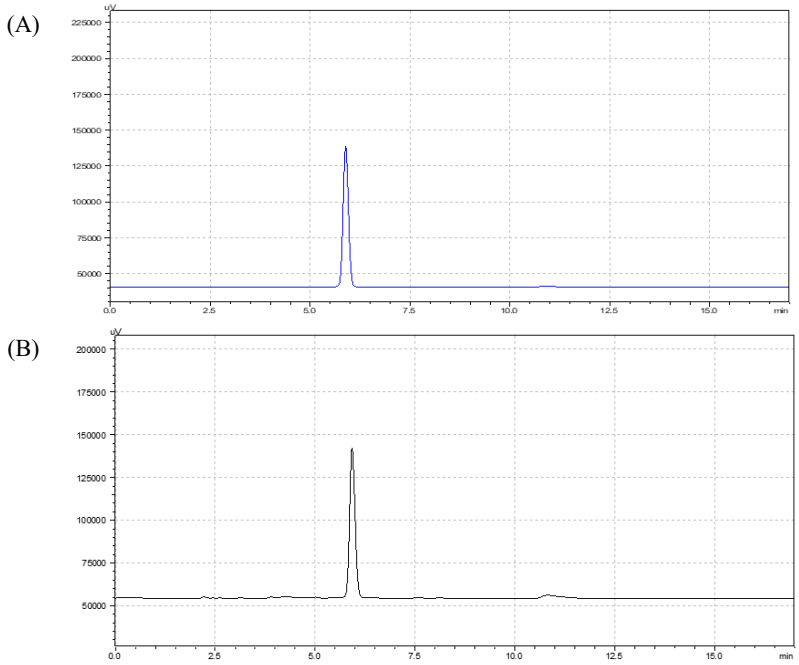

Figure 2. Ascorbic acid standard (A) and in lime (*Citrus latifolia*) juice (B). Chromatograms were acquired by HPLC-PDA analysis at 254 nm.

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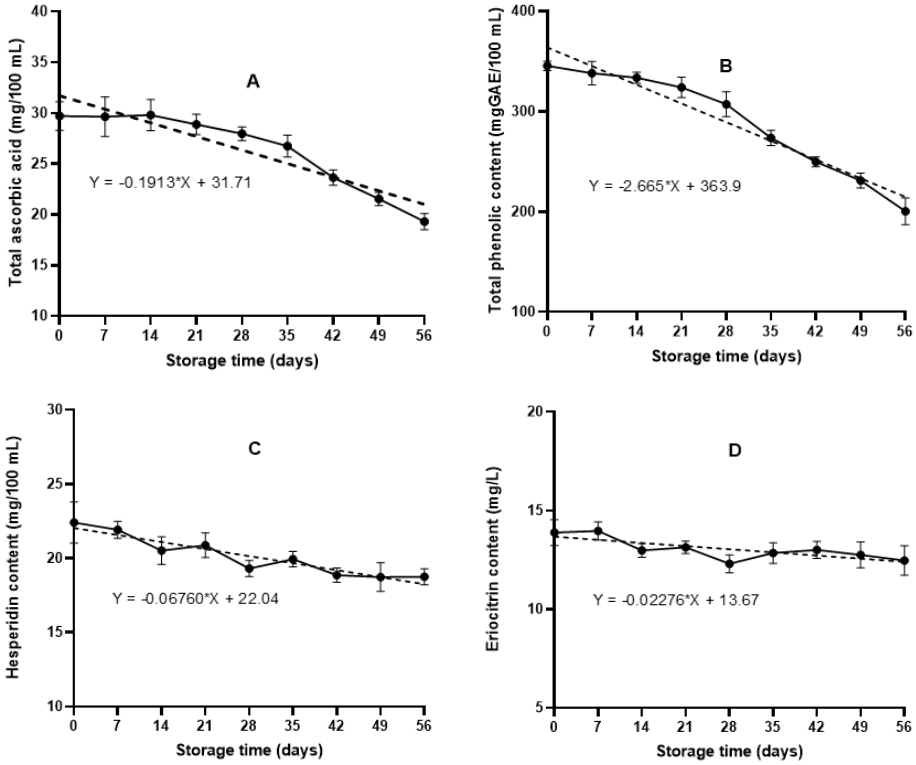

Figure 3. Ascorbic acid (A), total phenolic content (B), hesperidin (C) and eriocitrin (D) contents of lime juice during 56 days of storage.

34.7% lower than the 28-day storage sample (204.1 vs. 307.2 mg GAE/100 mL). The changing pattern of TPC in this study was different from that observed in a study of cold storage Tahiti and Persian lime in which the TPC remarkably decreased at least 60% after 60 days of storage at the temperature of 4°C (Tavallali and Zareiyan, 2018). The finding suggests that storage of lime fruits in perforated PE bags and foam boxes at 10°C exhibited a lower degradation of the phenolic content of the lime juice. It has been documented that the activity of polyphenol oxidase in accordance with the ripening of lemon fruits results in a decrease in the total phenolic concentration (Dong *et al.*, 2019; Sun *et al.*, 2019). The rate of TPC degradation was higher than that of ascorbic acid degradation, reflected by the fitted slopes of degradation curves (-2.665 vs. -0.1913, Figure 3A and Figure 3B). The higher rate of reduction in TPC led to a shorter storage time (28 vs. 35 days). The findings suggest that to maintain an acceptable level of TPC, lime fruits would not be extended in storage time after 28 days.

Flavonoids are important bioactive compounds contributing to the health benefits and are thus considered important quality parameters of lime fruit's quality. Hesperidin and eriocitrin have been reported as the main flavonoids in lime varieties (Gattuso *et al.*, 2007; Huang *et al.*, 2018; Dong *et al.*, 2019). The chromatograms of hesperidin, eriocitrin standards, and lime fruits are demonstrated in Figure 4. Though some other compounds were detected, eriocitrin (eluted at 9.76 min) and hesperidin (eluted at 20.9 min) were found as major peaks of the lime fruits. The result confirms the abundance of hesperidin and eriocitrin in the lime juice. During the cold storage, the hesperidin of lime slightly decreased from 22.4 to 18.7 mg/100 mL, accounting for a 17% reduction in hesperidin content. The change in hesperidin during cold storage depends on the citrus variety. Rapisarda *et al.* (2008) studied cold storage of orange varieties at 6°C and found the hesperidin content of two varieties decreased during 60 days of storage. In another study, hesperidin of lemon stored at 4°C was reduced by at least 40% after 35 days (Serna-Escolano *et al.*, 2021). The findings in the present study reveal that during cold storage the minor degradation in the hesperidin of lime fruits. The eriocitrin content of lime fruits in our present study was 16 times lower than hesperidin content and measured at the level of 13.8 mg/L. A minor decrease in eriocitrin was detected in lime fruit during storage (though not statistically different, $p>0.05$) after 56 days of storage. The result was in good agreement with the result obtained by Serna-Escolano *et al.* (2021). Overall, the change in flavanones of lime fruits during cold storage was negligible. However, a considerable decrease in TPC was detected in this study implying for degradation of other antioxidants in the lime fruit during storage. In citrus varieties, besides flavonoids, other phenolic acids (e.g. chlorogenic, caffeic, ferulic acids and more) also contribute to the total phenolic content of the juice (Xi *et al.*, 2017). Thus, the decrease in TPC of the lime fruit during cold storage is more likely involved in a reduction in phenolic acids

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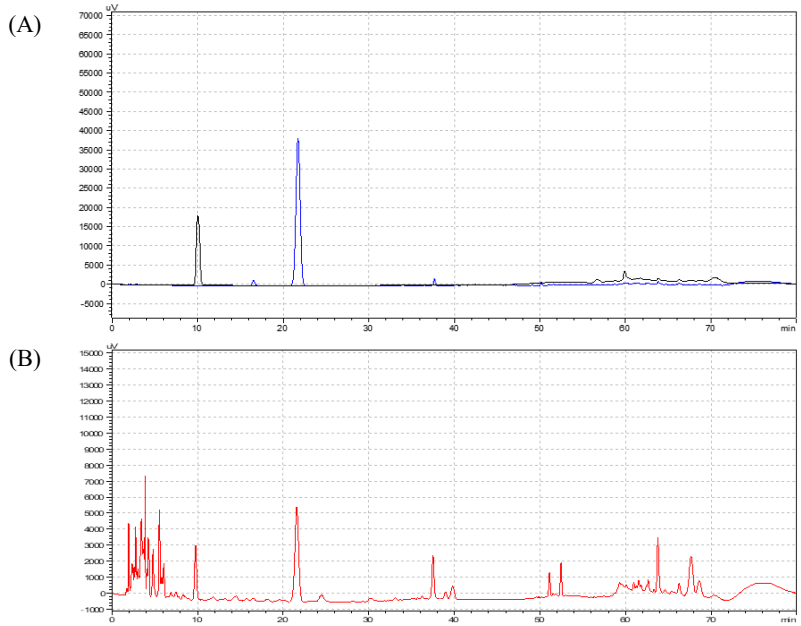

Figure 4. Hesperidin and etriocitrin standards (A) and in lime (*Citrus latifolia*) juice (B). Chromatograms were acquired by HPLC-PDA analysis at 280 nm.

rather than flavonoids (e.g., hesperidin and eriocitrin).

4. Conclusion

Postharvest cold storage of lime fruit in perforated PE bag and box was conducted to maintain the storability by tracking the quality of lime fruits during 56 days of storage. Overall, the storage condition retained the greenness for up to 28 days while other quality parameters such as weight loss, TSS, juice percentage, TI, TTA, and sugar value were in an acceptable range, indicating the satisfactory quality of stored lime fruits. Regarding juice's bioactive components, cold-stored lime fruits maintained the TPC, vitamin C, hesperidin, and eriocitrin contents for up to 21 days of storage. It can be concluded that lime cold-stored at 10°C in perforated PE bags and expanded polystyrene boxes can maintain the fruit's quality for up to 3 weeks. The findings, thus, would be significantly applied in the food industry such as choosing appropriate storage conditions while waiting for further processing.

Conflict of interest

The authors declare no conflict of interest.

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