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Evaluation of the effect of fruit coating on shelf life extension of lime (*Citrus aurantifolia*) under different storage condition

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ABSTRACT

Purpose: Lime fruit, a non-climacteric, seasonal crop, becomes unmarketable after 1-2 weeks from harvesting. Even though, lime is stored under low temperature conditions, the shelf life of which lasted about 4-6 weeks due to low rate of perishability. Research Method: The study was conducted to evaluate the effect of coating formulation under ambient (30°C, 65% RH), cold (13±2°C and 85% RH), zero energy cooling chamber [ZECC], (16-18°C, 65% RH). Thephysiochemical attributes were evaluated in triplicate and data were analyzed using ANOVA and a probability value of p<0.05 was adopted. Finding: The storage life of citrus fruits was extended up to 15, 30, and 40 days under ambient, ZECC, and cold storage respectively by protecting physicochemical attributes such as low weight loss, TSS, titratable acidity, pH, juice yield, chlorophyll (a, b and total chlorophyll content) and surface colour with respect to L*, a* and b* values. The overall performance of different treatments demonstrated that the wax coating treatment for lime fruits under low temperature storage proved better performance compared with all other treatments. The coated fruits exhibited the retention of physicochemical characteristics significantly (P< 0.05) by lowering the fruit spoilage. Research limitations: Even though mechanical application of wax formulations was effective; it was not practiced in the experiment. Originality/Value: The wax coating treatment can be utilized on commercial scale to enhance the shelf life and to maintain the quality of lime fruits combined with low temperature storage.



INTRODUCTION

Lime (Citrus aurantifolia) is a non-climacteric seasonal fruit and harvesting is confined to a particular time period of a year due to its seasonal behavior. The extent and production of limes in 2014 were 12138 ha and the yield was recorded as 5387 MT (Perera et al., 2015). Lime fruits are widely used in the culinary, medicinal, and food processing industries. The lime harvesting season is usually begins in Sri Lanka in December and goes up to July, while leaving August as the low harvesting season and the September to November period as the off season. During February to March the harvest reaches its peak. The price of lime reduces sharply during January to May due to it's over production (Department of Agriculture, 2006). During the off-season in Sri Lanka, the price is remained around Rs.1000/1kg and drastically dropped down to Rs. 50/1Kg in peak production (Champa et al., 2020). There are few studies that have been conducted to evaluate the different storage, conditions and effective pretreatments on quality improvement of citrus fruits. Therefore, it was important to develop and introduce storage methods combined with different treatments such as fruit coating and modified atmospheric packaging. Tiny injuries and scratches on the surface of fruits can be sealed by coating application (Shahid & Abbasi, 2011). Color of the lime is the main determinant of its acceptability for consumers. After harvesting, lime exhibits rapid green color degradation during storage which reduces the market value of lime. Different methods have been used to avoid chlorophyll degradation and include application of hot water treatment, intermittent warming and surface coating (Khan & Singh, 2017). A number of studies have been performed on the benefits of coating applications on fruits and vegetables. These materials can help to prolong shelf life and color retention, reduce moisture loss (Benítez et al., 2015), inhibit ethylene production (Valero et al., 2013), improve visual appearance, reduce shriveling and wilt while retaining biochemical properties (Ochoa-Reyes et al., 2013), improve texture and color retention, cell membrane stability, and storage life (Deng et al., 2017) inhibit browning (Sanchís et al., 2016). Therefore the study was conducted to find out the effectiveness of wax application under different storage conditions to increase the quality and shelf life of lime fruit.

MATERIALS AND METHODS

Experimental location

The experiment was carried out at the research and development center of National Institute of Postharvest Management (NIPHM), Anuradhapura, Sri Lanka.

Material selection

Lime (*Citrus aurantifolia*) cv. Local fruits in green mature stage (weight $40.2\pm5g$, total soluble solids (TSS) 10.07 ± 0.1 , titratable acidity $7.92\%\pm0.2$); were obtained from a commercial orchard in Anuradhapura, Sri Lanka. Lime fruits free from bruises and diseasesand in mature green colour fruits were selected and transported to the laboratory at NIPHM, Anuradhapura for experimentation. The ready to use wax formulation was used for fruit coating.

Experimental design

The fruits were divided into two lots and one was dipped in wax treatment, which contains palm oil 4%, glecerol 22%, sorbitan monooleate (tween 80%) 2%, guar gum 2% mixed with 70% distilled water. The edible wax formulation was used in 1:3 dilutions with water (one part of wax in three parts of water). Then the wax and non-waxed lime samples were kept at



three storage conditions namely ambient (at 30°C and 65% RH), cold (at 13±2°C and 85% RH), and zero energy cooling chamber (ZECC) (at 16-18°C and 65% RH) (Fig. 1).

Storage quality evaluation

Physicochemical parameters such as weight loss%, peel colour, titratable acidity (TA), total soluble solids (TSS), chlorophyll content (a, b and total chlorophyll), juice yield and surface colour were evaluated. Evaluation of physiochemical attributes were conducted at 3 days interval for limes stored at ambient conditionand 6 days under zero energy cooling chamber and 8 days interval under cold storage until the fruits were exhibited the limit of marketability.

Percentage weight loss

Fruit weight was taken after each storage interval and loss in weight during storage was expressed as % of initial weight. Pre-weigh fruit samples weighed on top lording balance (OHAUS, model ARA 520, New Jersey, 07058, USA) after each storage interval. The loss in weight on each sample date was observed.

Fruit juice content (%)

Juice was extracted manually and the juice content (%) was calculated according to the total fruit weight.

Total soluble solids (TSS) °B

TSS is defined as the sugar content expressed in grams for 100g of juice. This parameter has been determined by direct reading on a refractometer {ATAGO, Model: HR-5 (9-90%), Japan}. Reading was reported as °Brix.

Titratable acidity % (TA)

Titratable acidity was determined by the following volumetric method. The juice was neutralized by a NaOH solution (0.1 mol L⁻¹) added by some drops of phenolphthalein as indicator solution. Indeed, under neutral conditions, the NaOH solution turns the juice pink (Horwitz, 1980).

pН

The pH of the sample was measured using a glass electrode pH meter (Model; Thermo Orion 420). The pH was calculated with buffer at pH 4.0 and 7.0 before being used.

Surface colour change

Colour changes during postharvest storage were observed by an increase in the a/b ratio with an increase in yellowness (b) and decrease in greenness (a) orange external colour was evaluated with colour difference meter (Konica Minolta CR 400) which provided L*, a* and b* values. L* is lightness and a* (-greenness to + redness) and b* (- blueness to + yellowness) are the chromaticity coordinates measurements were done in triplicate.

Total chlorophyll content (mg/l)

According to the method explained by Ranganna (1986) the chlorophyll content of the peel of lime treated with coating and packages was determined using equations 1, 2 and 3. Lime peel (2.5 g) was scraped and chlorophyll was extracted into 85% (v/v) acetone solution by placing scraped peel on muslin cloth and extracted the juice using mortar and pestle. The chlorophyll extract was volume up to 50 mL volumetric flask with 85% acetone solution. Chlorophyll



content was measured by using spectrophotometer (DR 6000, USA) at 660 nm and 642 nm wave lengths.

Total chlorophyll (mg/L) =
$$(7.12 \times OD \text{ at } 660 \text{ nm})_{+} (16.8 \times OD \text{ at } 642 \text{ nm})$$
 (1)

Chlorophyll a
$$(mg/L) = (9.93 \times OD \text{ at } 660 \text{ nm}) + (0.777 \times OD \text{ at } 642 \text{ nm})$$
 (2)

Chlorophyll b (mg/L) =
$$(17.6 \times OD \text{ at } 642 \text{ nm}) + (2.81 \times OD \text{ at } 660 \text{ nm})$$
 (3)

Scanning electron micrographs

Scanning electron micrographs of coated and uncoated fruits were examined in the Scanning Reflection Electron Microscope and photographed on Ilford HP3 35 min film.

Statistical analysis

The experiment was conducted as a complete randomized design. The data were analyzed using analysis of variance and means were separated using Duncan's multiple range test at $p \le 0.05$ with SPSS statistical package.

RESULTS AND DISCUSSION

Changes in physicochemical parameters of fresh lime under various storage conditions, including ambient (at 30°C and 65% RH), cold (at 132°C and 85% RH), and zero-energy cooling chamber (at 16-18°C and 65% RH).

SEM images of coated vs. uncoated fruit peel

SEM images clearly showed the existence of wax coating a thin film covering all the natural openings like stomata and lenticels, wounds and stem scars of the treated fruit peels in contrast to the peels of untreated fruit (Fig. 2a, 2b).

Peel colour

Harvested, lime fruits were green in colour with L*, a* and b* values of 54.52±2.62, -19.34±0.68 and 38.74±0.87, respectively. The colour degradation of citrus fruits from green to yellow can be seen in all the treatments (p<0.05) and it was significantly lower in wax treated fruits. However, the fruits stored under cold, humid conditions showed a significantly lower rate of color degradation in comparison to the fruits stored under other storage conditions. The non-waxed fruits exhibited a significant increase in a^* value (green to red), and the waxed fruits stored under low temperature conditions exhibited a lower a^* value. Increased b^* values indicate the yellowness of the fruits. Non waxed fruits stored at ambient condition showed peel yellowness by the end of 6 days and then decline drastically compared to waxed fruits which stayed green until 9 days and then gradually exhibited yellowness (Fig. 3). When low temperature stored fruits with wax treatment were compared to untreated fruits, the b* value increased gradually and slowly. This is due to retardation of the senescence process, slowed metabolic as well as enzymatic reaction activities and less degradation in the colour pigment (chlorophyll), which slows the change with coating treatment with low temperature storage. Fruits stored at higher temperatures showed rapid changes in a* value. The color b* value showed a similar pattern, demonstrating significant (p<0.01) increases with temperature and differences between control and coated samples. Similar results have been reported with the application of aloe vera gel to papaya (Marpudi et al., 2011). The effects of controlled atmosphere (5%, 10% O₂ and 1%, 5% CO₂) on the quality and storage life of lime was determined. A low concentration of O₂ (5% O₂) combined with high CO₂ (1% and 5% CO₂) had a storage life of 7 and 4 weeks, respectively. Exposure to 1% CO₂+10% O₂ condition delayed chroma value increasing and delayed hue value decreasing more than



other treatments. Exposure to low O₂ and high CO₂ did not affect titratable acidity (TA) and soluble solid (SS) contents. Vitamin C content of all treatments did not change in 5 weeks during storage (Boonyaritthongchai et al., 2010).

Titratable acidity (TA) %

Variation in acidity in citrus fruits under different storage conditions with wax treatment showed a gradual decrease in acidity during storage, and fruits stored under ambient conditions with wax treatment showed a gradual decrease in acidity during storage. Fruits stored under ambient conditions exhibited a higher reduction in acidity than those stored at ZECC and low temperature storage with an increase in pH. During storage, the increase in respiration may be responsible for the decline in acid content, which is the principal metabolic substance together with sugars. The coating treatment was effective in minimizing the changes in acidity because of restrictions in respiration (Table 1, 2 and 3). The faster rate of decline of acidity in fruits in the control treatment could be due to the faster metabolic reactions occurring within them. The cold-stored wax-coated samples exhibited an increase in their titratable acidity for up to 24 days, which is the same for the non-wax fruits, but the higher titratable acidity was recorded in the wax-coated fruits at the end of storage. In ZECC, the increase was only observed in wax-coated fruits for up to 15 days and in non-waxed fruits for up to 9 days. The fruits in ambient conditions exhibited an increase in their acidity up to the 9th day; thereafter, they decreased. The highest acidity was recorded in coated fruits when compared to uncoated fruits. The higher acidity of coated lime fruit retained in cold storage may be due to less oxygen availability to the fruit in later stages of storage. It appears that the organic acid, which participates in the respiratory process but is not oxidized. The coating treatment preserved significantly (p < 0.05) higher TA for storage periods. This may be due to the fact that the coating treatment resulted in less O₂ being available for the respiratory process, and may therefore delay the utilization of organic acids. In general, the titrable acidity decreased and oBrix increased during storage for both control and coated limes, and the associated change rates were higher at higher temperatures. Similar results were also found in a previous report where the TA of fruits was decreased with extended storage conditions (Bisen et al., 2012). The reduction in acid content of fruits can be attributed to the conversion of organic acids to sugars during the process of respiration (Wills et al., 1998). The increase in acidity and thereafter decreased was observed in all treatments with respect to pH. The decrease was observed at the initial storage and thereafter increased.

Table 1. Physiological weight loss (PLW), juice yield (JY), titratable acidity (TA), total soluble solids (TSS) and pH of lime fruits subjected to wax application (W) vs. non-waxed (NW) fruits under cold storage at $13\pm2^{\circ}$ C and 85% RH

		Time in days of	of storage			
Parameter		8	16	24	32	40
PLW [%]	W	1.81±0.43a	9.51±0.48e	13.41±0.76g	17.32±0.52 ^h	20.28±1.28i
	NW	2.72 ± 0.9^{b}	8.16 ± 0.24^{d}	9.56 ± 3.58^{e}	$10.68\pm2.75^{\rm f}$	7.32±2.86°
JY [%]	W	44.51 ± 0.25^{b}	49.96 ± 0.1^{d}	56.32±0.11e	58.17 ± 0.16^{f}	37.66±0.31a
	NW	45.59 ± 0.02^{c}	49.93 ± 0.12^{d}	64.67 ± 0.06^g	75.17 ± 0.96^{h}	37.69±0.1a
TA [%]	W	7.23 ± 0.25^{e}	7.34 ± 1.06^{f}	8.8 ± 0.1^{i}	6.13 ± 0.21^{d}	4.23±0.25°
	NW	7.8 ± 0.1^{g}	7.2 ± 0.15^{e}	8.4 ± 0.1^{h}	3.6 ± 0.06^{b}	2.1±0.15 ^a
TSS [°B]	W	$10.07\pm0.21^{\rm f}$	10.43 ± 0.38^{g}	9.23 ± 0.15^{e}	8.67 ± 0.06^{d}	7.2±0.29 ^b
	NW	10.83 ± 0.81^{i}	11.53±0.25 ^h	12.03 ± 0.06^{j}	7.73 ± 0.18^{c}	7.07 ± 0.29^{a}
pН	W	2.52 ± 2.72^{e}	2.4 ± 0.03^{d}	2.27 ± 0.01^{b}	2.31 ± 0.02^{c}	2.48 ± 0.04^{d}
	NW	2.25 ± 0.02^{b}	2.2 ± 0.02^{a}	2.21±0.01a	2.41 ± 0.01^{d}	2.52±0.02 ^e

Each value represents mean ±SD of three replicates.

Figures with same superscripts are not significantly different (at $p \le 0.05$) along same columns and rows in each parameter.



Table 2. Physiological weight loss (PLW), juice yield (JY), titratable acidity (TA), total soluble solids (TSS) and pH of lime fruits subjected to wax application (W) vs. non-waxed (NW) fruits under zero energy cooling chamber (ZECC) at 16-18°C and 65% RH

	Time in days of storage							
Parameter		3	9	15	21	27	33	
PLW [%]	W	2.55±0.06 ^a	6.24±0.87°	8.03±1.1 ^f	11.32±0.2h	16.42±4.66 ⁱ	18.25±5.39 ^j	
	NW	2.87 ± 0.03^{b}	6.58 ± 0.28^d	6.95 ± 0.44^{e}	9.84 ± 1.3^{g}	20.22 ± 2.22^{k}	22.54 ± 2.21^{1}	
JY [%]	W	40.48 ± 0.23^{a}	46.08 ± 0.14^{k}	47.0 ± 0.1^{1}	45.54 ± 0.75^{j}	45.22 ± 0.27^{i}	45.18±0.07 ^h	
	NW	41.02±0.13°	43.94±0.07g	43.2 ± 0.1^{f}	42.39±1.03e	41.3 ± 0.1^{d}	40.55±0.53b	
TA [%]	W	8.4 ± 0.1^{c}	9.1 ± 0.12^{g}	9.07 ± 0.21^{g}	8.4 ± 0.1^{c}	8.27 ± 0.16^{b}	7.4 ± 0.12^{a}	
	NW	7.37 ± 0.32^{a}	9.17 ± 0.15^{h}	8.97 ± 0.06^{f}	8.9 ± 0.1^{f}	8.67 ± 0.16^{d}	6.8 ± 0.01^{e}	
TSS [°B]	W	10.07±0.21e	10.43 ± 0.38^{e}	11.23 ± 0.15^{h}	11.67 ± 0.06^{j}	7.2 ± 0.29^{c}	6.73 ± 0.21^{b}	
	NW	10.83 ± 0.81^{g}	11.53 ± 0.25^{i}	$12.78\pm0.06k$	10.73 ± 0.18^{f}	8.07 ± 0.29^{d}	6.17±0.15a	
pН	W	2.24 ± 0.03^{a}	2.22 ± 0.04^{a}	2.34 ± 0.02^{b}	2.48 ± 0.01^{c}	2.5 ± 0.01^{d}	3.2 ± 0.01^{e}	
	NW	2.23 ± 0.04^{a}	2.2±0.01a	2.26 ± 0.01^{a}	2.36 ± 0.04^{b}	$2.49\pm0.11c$	2.52 ± 0.12^{d}	

Each value represents mean $\pm SD$ of three replicates.

Figures with same superscripts are not significantly different (at p≤0.05) along same columns and rows in each parameter.

Table 3. Physiological weight loss (PLW), juice yield (JY), titratable acidity (TA), total soluble solids (TSS) and pH of lime fruits subjected to wax application (W) vs. non-waxed (NW) fruits under ambient condition at 30°C and 65% RH

		Time in days of s	torage			
Parameter		3	6	9	12	15
PLW [%]	W	1.56±0.25a	7.68±0.22 ^b	13.38±0.14 ^d	16.12±0.33 ^f	18.54±0.28 ^h
	NW	8.87 ± 0.02^{c}	14.58±0.23e	17.28 ± 0.25^{g}	19.69 ± 0.04^{i}	22.04 ± 0.51^{j}
JY [%]	W	46.67 0.21 ⁱ	43.11 ± 0.17^{h}	41.84 ± 0.39^{f}	40.37 ± 0.33^{e}	$38.63 \pm 1.54^{\circ}$
	NW	42.93 ± 0.86^{g}	39.13 ± 0.06^{d}	38.73 ± 1.1^{c}	32.89 ± 0.16^{b}	22.40 ± 0.85^{a}
TA [%]	W	8.43 ± 0.15^{d}	8.80 ± 0.17^{f}	9.13 ± 0.06^{i}	9.03 ± 0.21^{h}	8.23 ± 0.25^{c}
	NW	7.7 ± 0.00^{b}	8.83 ± 0.06^{f}	8.9 ± 0.1^{g}	8.6 ± 0.02^{e}	5.23 ± 0.15^{a}
TSS [°B]	W	11.0 ± 0.1^{g}	$10.63 \pm 0.15^{\rm f}$	10.3 ± 0.1^{e}	9.23 ± 0.32^{d}	8.97 ± 0.58^{c}
	NW	12.47 ± 0.29^{h}	13.0 ± 0.20^{i}	13.6 ± 0.0^{j}	6.53 ± 0.06^{b}	4.37 ± 0.15^{a}
pН	W	2.26 ± 0.02^{b}	2.22 ± 0.01^{a}	2.20 ± 0.01^{a}	2.38 ± 0.01^{d}	2.42 ± 0.01^{e}
-	NW	2.26 ± 0.01^{b}	2.22 ± 0.01^{a}	2.18 ± 0.01^{a}	2.24 ± 0.01^{b}	2.3 ± 0.01^{c}

Each value represents mean \pm SD of three replicates.

Figures with same superscripts are not significantly different (at p≤0.05) along same columns and rows in each parameter.

Juice yield (%)

There was a gradual increase in juice yield up to 30 days under cold storage and ZECC storage, and thereafter it declined considerably, where as the fruits stored at ambient conditions showed a continuous decline in juice content (Tables 1, 2, and 3). The wax treatment showed a significant increase in its juice yield compared with non waxed fruits. According to the findings of Wijewardane and Guleria (2009), coated apple fruits exhibited a gradual increase in juice yield up to 120 days of storage at 2±10°C and 85-90% RH; and, thereafter, it declined considerably, whereas, the fruits stored at ambient conditions showed a continuous decline in its juice content. At low temperatures, higher juice yields during the initial storage period may be due to the occurrence and completion of ripening and other associated changes, as a result of certain macro molecules that might have been broken down into smaller molecules during this period. The juice content of fresh lime increases up to 32 days of storage in wax coated and non-waxed fruits, but the increase is less in non-coated fruits when compared to coated fruits. In the samples under ZECC, the increased juice content in coated fruits was only up to 15 days compared to 9 days for non-waxed fruits. The ambient stored samples as a control were increased in their juice content only up to the 3rd day of storage, thereafter, they decreased. The findings of the study were directly related to the findings of Bisen et al. (2012), maximum juice content was recorded by the wax coated lime fruits under cold storage at 32 days of storage. In the present study, the treatments increased



the shelf life and maintained the quality of lime fruits. Similarly, Dashora and Shaffat (1988) also reported similar results in mosambi fruits, Sri Lankan oranges and guava, respectively.

Total soluble solids (TSS) °B

The TSS in general was increased up to 25 days during storage and a subsequent decrease was observed. The TSS was gradually increased up to 15 days of storage at ambient conditions, although, the marketability was decreased. Wijewardane and Guleria (2013) a continuous increase in TSS was observed in the fruits treated with the polysaccharides based coating treatment for guava fruits. All treatments showed an increase in TSS at the initial storage period and decreased thereafter. This increase in TSS is due to the hydrolysis of starch into simple (soluble) sugars, which is higher during fruit ripening. The fruits under ambient conditions as a control exhibited the increase up to the 6th day and the 3rd day in waxed and non-waxed fruits. Higher retention of TSS was observed in wax coated fruits under cold storage conditions. The increase in TSS of up to 16 days may be attributed to the hydrolysis of acid and deposition of polysaccharide with the advancement of the storage period, as reported by Omayma et al. (2010) in guava. In general, the TSS demonstrated an increasing trend, which was slower with coated fruits as compared to the control. Increased TSS in control may be due to accumulation of different solutes in vacuoles of cells as a result of the normal respiratory and physiological process of fruit ripening, which hydrolyses starch into sugars. The reason for the slower rate of TSS increase in coated samples is obviously due to the slowing down of the respiratory and physiological ripening activities (Maftoonazad & Ramaswamy, 2019). The fruits stored in ambient conditions always obtained a higher TSS value due to the high ambient temperature and low relative humidity, which may lead to high water loss (Champa & Gamage, 2020). The results are in line with the findings of Yimenu et al., (2017). The increase in TSS content of fruits is directly correlated with the hydrolytic activities of starch that indicates fruits are undergoing the ripening process (Hassan et al., 2014). Moreover, as the water loss from untreated fruits was higher compared with other treatments, thus the concentration of sugars was increased leading towards improved TSS content.

Chlorophyll content (mg/l)

Chlorophyll degradation was identified throughout the storage in all storage treatments, and the degradation was less when samples were kept under cold storage, followed by the ZECC and ambient conditions. In early storage, the presence of a high concentration of chlorophyll in the fresh lime is significantly related to the higher intensity of green colour observed. Thus, the progression of storage, chlorophyll degradation (Hernandez-Munoz et al., 2008), and the predominance of xanthophylls and other catetonoids pigments are the reasons for the color change from green to yellow (Tables 4, 5, 6, and Fig. 3). When the storage period has enhanced the degradation in chlorophyll a, b, and total chlorophyll, it was observed in all storage conditions. The fruits under cold storage might be delayed ripening due to slow degradation of chlorophyll and decreased enzymatic acidity, which are responsible for slow degradation of chlorophyll. It may also be due to reducing the rate of water loss and less availability of oxygen within the fruit, which slows down the rate of ripening (Bisen et al., 2012). Treatment of fruits with bio-wax significantly reduced physiological weight loss (PWL) in contrast to untreated control samples, and this reduction was nearly 50% when fruits were at ambient conditions and 30% when the fruits were under cold room conditions (Champa et al., 2020). Application of pre-storage UV-C irradiation (from 3.4 to 10.5 kJ m⁻² of UV-C) maintained the quality of lime fruit during storage in air at 10 °C by



delaying peel yellowing, reduced ethylene production, lowered respiration rate, reduced calyx detachment and increased overall acceptability (Pristijono, et al., 2019).

Table 4. Chlorophyll a (Chl.a), chlorophyll b (Chl.b),total chlorophyll (T.Chl.) of lime fruits subjected to wax application (W) vs. non-waxed (NW) fruits under cold storage at 13 ±2°C and 85% RH

		Time in days of	of storage			
Parameter		8	16	24	32	40
Chlorophyll a [mg/l]	W	2.36 ± 0.02^{i}	1.94±0.05 ^h	1.84 ± 0.43^{g}	1.63±0.04 ^f	0.47±0.11 ^b
	NW	2.36 ± 0.01^{i}	1.52 ± 0.01^{e}	0.53 ± 0.01^{c}	0.2 ± 0.01^{a}	0.83 ± 0.09^{d}
Chlorophyll b [mg/l]	W	2.21 ± 0.02^{i}	2.15 ± 0.1^{h}	2.10 ± 0.5^{g}	1.18 ± 0.06^{e}	0.57 ± 0.18^{c}
	NW	1.24 ± 0.02^{f}	1.19±0.01e	0.68 ± 0.01^{d}	0.26 ± 0.03^{b}	0.11±0.23a
Total Chlorophyll [mg/l]	W	3.57 ± 0.02^{j}	3.49 ± 0.05^{i}	3.31 ± 0.49^{g}	2.81 ± 0.04^{f}	0.04 ± 0.29^{a}
	NW	3.42 ± 0.04^{h}	2.71 ± 1.01^{e}	1.21 ± 0.12^{d}	0.47 ± 0.03^{c}	0.18 ± 0.59^{b}

Each value represents mean ±SD of three replicates.

Figures with same superscripts are not significantly different (at p≤0.05) along same columns and rows in each parameter.

Table 5. Chlorophyll a (Chl.a), chlorophyll b (Chl.b), total hlorophyll (T. Chl.) of lime fruits subjected to wax application (W) vs. non-waxed (NW) fruits under zero energy cooling chamber (ZECC) at 16-18°C and 65% RH

		Time in days	of storage				
Parameter		3	9	15	21	27	33
Chl.a [mg/l]	W	0.88±0.01e	0.51±0.23e	0.47 ± 0.04^{d}	0.41±0.04°	0.1±0.11a	0.1±0.01a
	NW	0.87 ± 0.02^{f}	0.28 ± 0.09^{b}	0.27 ± 0.02^{b}	0.27 ± 0.02^{b}	0.1 ± 0.02^{a}	0.1 ± 0.01^{a}
Chl.b [mg/l]	W	5.57 ± 0.04^{h}	2.67 ± 0.06^{g}	$1.89\pm0.05^{\rm f}$	$0.89\pm0.05^{\rm f}$	0.27 ± 0.05^{b}	0.15 ± 0.05^{a}
- 0 -	NW	2.34 ± 0.03^{g}	0.68 ± 0.25^{d}	0.49 ± 0.04^{d}	0.32 ± 0.05^{c}	0.17 ± 0.06^{a}	0.13±0.01a
T.Chl.[mg/l]	W	3.45 ± 0.04^{g}	$1.48\pm0.17^{\rm f}$	1.3±0.09e	1.3±0.09e	1.27 ± 0.64^{e}	0.8 ± 1.31^{d}
- 0 -	NW	3.21 ± 0.05^{g}	0.86 ± 0.36^{d}	0.77 ± 0.06^{c}	0.77 ± 0.07^{c}	0.67 ± 0.58^{b}	0.23 ± 0.02^{a}

Each value represents mean ±SD of three replicates.

Figures with same superscripts are not significantly different (at p≤0.05) along same columns and rows in each parameter.

Table 6. Chlorophyll a (Chl.a),cChlorophyll b (Chl.b), total chlorophyll (T. Chl.) of lime fruits subjected to wax application (W) vs. non-waxed (NW) fruits under ambient condition at 30°C and 65% RH

		Time in days of	f storage			
Parameter		3	6	9	12	15
Chlorophyll a [mg/l]	W	0.79 ± 0.07^{g}	0.74 ± 0.28^{f}	0.71 ± 0.02^{e}	0.68 ± 0.01^{d}	0.57 ± 0.01^{c}
	NW	0.89 ± 0.01^{h}	$0.53 \pm 0.05^{\circ}$	0.43 ± 0.02^{b}	0.40 ± 0.01^{b}	0.32 ± 0.01^{a}
Chlorophyll b [mg/l]	W	2.78 ± 0.2^{h}	2.72 ± 0.61^{g}	2.66 ± 0.1^{f}	2.53 ± 0.11^{e}	2.43 ± 0.11^{d}
	NW	$2.66 \pm 0.03^{\rm f}$	1.19 ± 0.07^{c}	0.69 ± 0.02^{b}	0.66 ± 0.10^{b}	0.56 ± 0.1^{a}
Total Chlorophyll	W	3.12 ± 0.27^{h}	2.87 ± 1.01^{g}	2.56 ± 0.1^{f}	2.19 ± 0.01^{e}	2.07 ± 0.09^{d}
[mg/l]	NW	3.55 ± 0.03^{i}	1.71 ± 0.12^{c}	1.12 ± 0.01^{b}	$1.05\pm1.17^{\rm a}$	1.02 ± 0.03^{a}

Each value represents mean ±SD of three replicates.

Figures with same superscripts are not significantly different (at p≤0.05) along same columns and rows in each parameter.



Fig. 1. Zero Energy Cooling Chamber (ZECC)



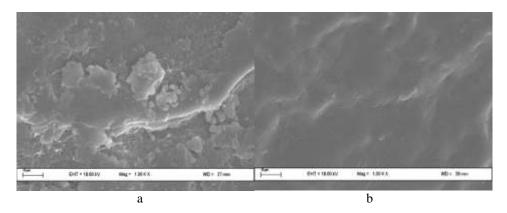
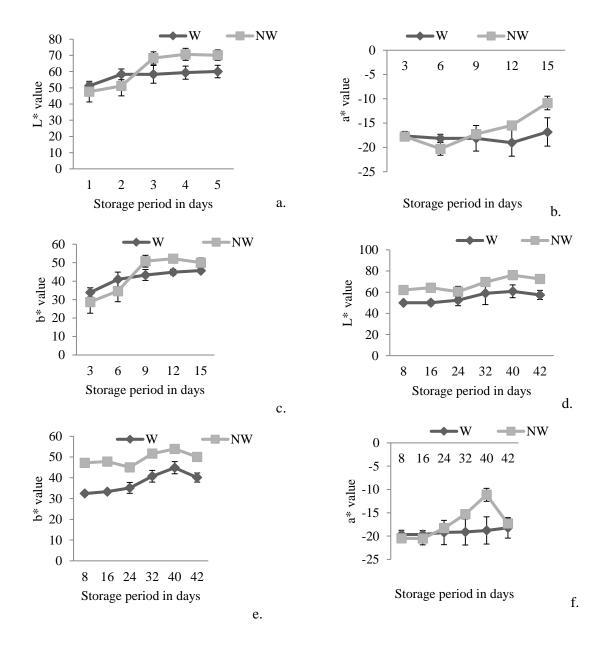


Fig. 2. SEM images of the uncoated (a) and coated (b) lime fruit peel- after 07 DOS under ambient conditions (at $32\pm2^{\circ}$ C and relative humidity 70%) are shown in figure 2(a) and 2(b).





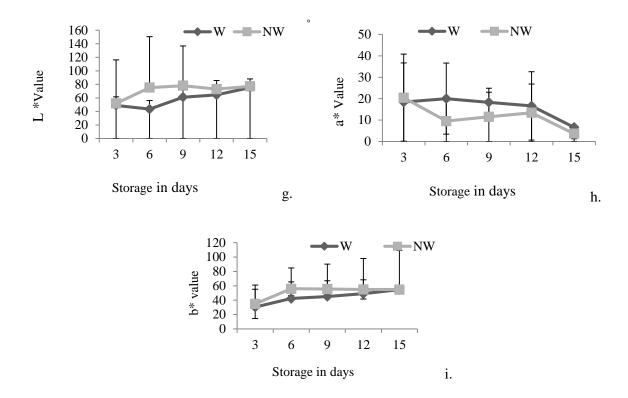


Fig. 3. Variation in peel colour (L^* , a^* , b^*) of lime fruits with wax coating treatment (W) vs. non-waxed (NW) fruits during storage under ambient at 30 °C and 65% RH, (a, b, c); cold at 13 ± 2 °C and 85% RH, (d, e, f) and under zero energy cooling chamber at 16-18 °C and 65% RH (g, h, i). L^* 0: Black, 100: white $a^*=$ (-) greenness, (+) redness; $b^*=$ (-) blueness, (=): yellowness. Vertical bars represent mean \pm S.E. of three replicates.

CONCLUSION

The overall performance of different treatments can be concluded that the wax coating treatment for lime fruits under low temperature storage proved to have better performance compared with all other treatments. The coated fruits exhibited the retention of physicochemical characteristics significantly (P< 0.05) by lowering the fruit spoilage. The storage life of citrus fruits was extended by up to 15, 30, and 40 days under ambient, ZECC, and cold storage conditions, respectively. Storage of fruits under low temperatures was most effective when compared with the other two storage conditions, and ZECC was the most suitable alternative as a low cost storage technique to be used to reduce physiological weight loss in fresh lime. Most of the quality parameters, such as physiological weight loss, total soluble solids, juice percentage, titratable acidity, chlorophyll a, b, and total chlorophyl content, external colour, and TSS, remained relatively stable in the lime fruits treated with wax coating. According to the results of the present study, the thewax coating treatment can be utilized in a commercial scale to enhance the shelf life and to maintain the quality of fresh lime.

Conflict of Interest

The author declares that there is no conflict of interest to report.



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