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Effect of X-ray Irradiation and Carnauba Wax Coating on Quality of Lime (*Citrus latifolia* Tan.) Fruit

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Abstract. **The quality of Persian (Tahiti) lime (*Citrus latifolia* Tan.) fruit was determined following coating with carnauba wax and X-ray irradiation at doses suitable for disinfestation of quarantine pests. Fruit with or without carnauba wax coating were treated with irradiation doses of 0, 150, 300, or 450 Gy, and stored for 14 days at 13 °C and 6 days at 20 °C to simulate commercial transportation and marketing conditions from Hawaii to the continental United States. The fruit color, weight loss, total soluble solids (TSS) content, and titratable acidity (TA) were analyzed at 7, 14, and 14 + 6 days post irradiation. Wax coating significantly delayed fruit peel discoloration, and reduced fruit weight loss by more than 7% compared with the unwaxed controls. Irradiation did not affect ΔE of the peel for coated fruit at day 14 + 6. Irradiation with or without coating did not affect ΔE of flesh color, weight loss, TSS content, or TA. Wax coating combined with irradiation treatment of limes at doses ≤450 Gy ensured marketable visual quality and chemical composition while providing quarantine security.**

The diversification of Hawaii's agriculture industry depends on exporting new, high-value crops from the islands. Citrus has been grown in Hawaii since before 1800, and during the Gold Rush in the mid-1800s. Hawaii citrus was exported to California, but the citrus industry declined when sugarcane, coffee, and livestock became more profitable (Ebesu 2008). Recently, Mahi Pono LLC (Wailuku, Maui, HI, USA) has planted 3000 acres of Persian (Tahiti) limes (*Citrus latifolia*) on the island of Maui. Once in full production, harvest volumes will exceed local demand and so there is strong interest in potential export options.

Hawaii has several species of tephritid fruit flies, including Mediterranean fruit fly [*Ceratitis capitata* (Wiedemann)], Oriental fruit fly [*Bactrocera dorsalis* (Hendel)], and melon fly [*Zeugodacus cucurbitae* (Hendel)] (Diptera: Tephritidae) that may infest *Citrus* spp., and therefore a quarantine treatment is required before the exportation of lime to overseas markets. X-ray irradiation doses up to 1000 Gy (1 kGy) have been approved by the US Food and Drug Administration for the preservation and disinfestation of fresh fruits and vegetables (FDA 1986). A generic irradiation dose of 150 Gy has been approved by the US Department of Agriculture for all species of fruit flies in the family Tephritidae (Follett 2014; USDA-APHIS 2006). Thus, *Citrus* sp. is approved for export from Hawaii to the continental United States following disinfestation treatment with a minimum dose of 150 Gy, while not exceeding the 1-kGy limit.

Persian limes are typically harvested and marketed while the peel is still green. Under room temperature, the fruit becomes yellow within a few days, which reduces their commercial viability (Rodrigues da Silva et al. 2016). Maintenance of the green color in lime peel postharvest is critical to obtain premium value. Edible coatings are an environmentally friendly technology that has been applied to many products to control moisture transfer, gas exchange, and oxidation processes (Yaashikaa et al. 2023). These coatings can provide an additional protective layer to the produce and can generate the same effect as MA storage by altering the normal internal gas composition of the fruit (Yaashikaa et al. 2023). Carnauba wax, a major edible coating ingredient, has inherent moisture barrier and antifungal properties and is generally recognized as safe by the US Food and Drug Administration (Devi et al. 2023). Carnauba wax coating alone or with incorporated a.i. has been applied extensively to preserve or extend the shelf life of citrus fruit (Devi et al. 2023; Miranda et al. 2021, 2022).

As a crucial pest quarantine technique, irradiation treatments have also been reported to maintain fruit color by inhibiting chlorophyll degradation (Srilaong et al. 2011); however, other reports have revealed negative effects of irradiation on fruit quality. Rodrigues da Silva et al. (2016) found that doses >100 Gy caused accelerated skin yellowing, and doses >150 Gy increased weight loss when subsequently stored at room temperature (24 °C) for 20 d. To alleviate this negative impact, a pre-irradiation wax coating application was proposed. Coatings have been used to delay skin discoloration and weight loss in many fruits and vegetables, including lemons (Bai and Plotto 2012). The objective of this study was to evaluate the effects of wax coatings and X-ray irradiation on the physical and biochemical changes in Persian limes during postharvest storage.

Materials and Methods

Fruit. Fresh green Persian limes (*Citrus latifolia* Tanaka) were harvested from Mahi Pono, Kahului, HI (20.8604491°, −156.4420898°). Fruits that were free from damage and uniform in color, shape, and size (100 ± 10 g) were randomly selected and sorted into experimental units of 30 fruits per treatment, with a total of 240 fruit per harvest. All treatments were replicated three times with different batches of fruit. The total average lightness (L^*), chroma (C^*), and hue angle ($h°$) of fruit peel color at day 0 were 49.91, 43.47, and 114.99, respectively. The total average L^*, C^*, and $h°$ of fruit flesh at day 0 were 41.75, 15.84, and 113.91, respectively. The TSS content was 9.01 °Brix, and TA was 1.07% on average at day 0.

Fruit treatment. Lime fruit with or without carnauba wax coating (JBT Corporation, Lakeland, FL, USA; average diameter = 58 nm;

polydispersity index = 0.60; zeta potential = −45 mV) were treated with target absorbed doses of 150, 300, and 450 Gy radiation. The nonirradiated fruit with or without the wax coating were uncoated control and coated control, respectively. There were eight treatment combinations in total: two coatings (coated and uncoated control) × four X-ray irradiations (0, 150, 300, and 450 Gy). For the wax coating treatment, the coating was applied by rubbing 0.5 mL of wax around each fruit with latex gloved hands, until it was fully and evenly covered. The irradiation was carried out at a commercial irradiation facility on the island of Hawaii (RLH Hawaii Pride, Keaau, HI). The facility uses an electron linear accelerator (5 MeV LINAC, model TB-5/15; L-3 Communication Titan Corp., San Diego, CA, USA) and converts the e-beam into X-rays for treatment of produce. Dose mapping to measure variation around each target dose was conducted following our previous method (Sun et al. 2023). The temperature during irradiation was ∼27 °C and treatment time was between 2 and 8 min per box depending on dose. After treatment, the fruit were stored at 13 °C and 90% relative humidity (RH) for 14 d and then another 6 d at ambient conditions (temperature: 20 °C, RH: 40%) to simulate shelf-life conditions.

Color. The fruit peel color measurements were taken at two positions per fruit for nine fruits in each replicate with a chromameter (model CR-300; Minolta Corp., Ramsey, NJ, USA) and recorded as L^*, C^*, and $h°$ under standard illuminant. Internal flesh color measurements were taken at two positions of a flesh cross section per fruit with the same manner as for peel color. The color change from the original color was calculated using the formula: $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta C^*)^2 + (\Delta h°)^2}$.

Weight loss. Weight loss (%) was calculated as the percentage of weight lost per original fruit weight in a sample from each container. In each treatment, there were three containers with 30 fruits in total, and the weight of the same nine fruits was measured.

TSS and TA. For TSS measurement, a 10-g sample of pulp from the same nine fruit in each treatment was homogenized, and the liquid from each individual fruit was measured with a digital refractometer (PAL-3, ATAGO U.S.A., Inc., Bellevue, WA, USA) measuring °Brix. The TA of the 10-time diluted liquid from each individual fruit was determined by an acidity meter (GMK-835F, ATAGO®; ATAGO U.S.A., Inc., Bellevue, WA, USA), and the result was expressed as %.

Statistical analyses. Data were ordered and visualized using Excel (Microsoft Corp., Seattle, WA, USA), and then evaluated using JMP statistical analysis software (version 16; SAS Institute, Cary, NC, USA). Analysis of variance was used to calculate what effect the various treatments had on the quality attributes of lime. For treatments determined to have statistically significant effects, differences in means between different treatments at the same storage time were evaluated using a Tukey's honestly significant difference test at a confidence interval of $\alpha = 0.05$. Measurements of each experimental parameter were performed over at least three replications to determine the response data for lime.

Results and Discussion

Color. The visual appearance of the fruit peel and flesh cross section of lime after coating and irradiation treatment is shown in Fig. 1A and B, respectively, and the chromameter data and statistical analysis are shown in Fig. 2A and B. The coating treatment significantly ($P < 0.05$) delayed fruit peel discoloration compared with controls after 14-d storage at 13 °C (Figs. 1A and 2A). Although there were no significant ($P > 0.05$) differences of exterior L^*, C^*, and ΔE between the irradiation treatments for the coated fruit (C150, C300, and C450), the coated fruit treated with 300 Gy were observed to maintain fruit peel color significantly and substantially compared with the 300 Gy–treated fruit without coating at day 14 + 6 (Figs. 1A and 2A). The combination of coating and irradiation did not have any significant ($P > 0.05$) influence on the internal flesh color compared with the same dosage of irradiation-treated fruit without coating (Figs. 1B and 2B).

Peel discoloration, such as browning, negatively affects the physical appearance of fruits and vegetables, reducing their marketability. Edible coatings have been approved to delay the fruit peel color change (Dhall 2013). Previous research showed that beeswax coating alone, or combined with sodium nitroprusside, effectively maintained chlorophyll by reducing the activity of chlorophyllase and polyphenol oxidase, and the strength of the color green was maintained to a large extent (Hoseini et al. 2023). Similarly, oil emulsion coatings contributed to a longer conservation of the green peel color of lime fruit (Bisen et al. 2012).

Among subtropical fruit, citrus is relatively sensitive to irradiation and the response to treatment is highly variable and dependent on species, hybrid, and cultivar (Wall 2015). Damage to the rind was not observed in our study with limes but other citrus types may

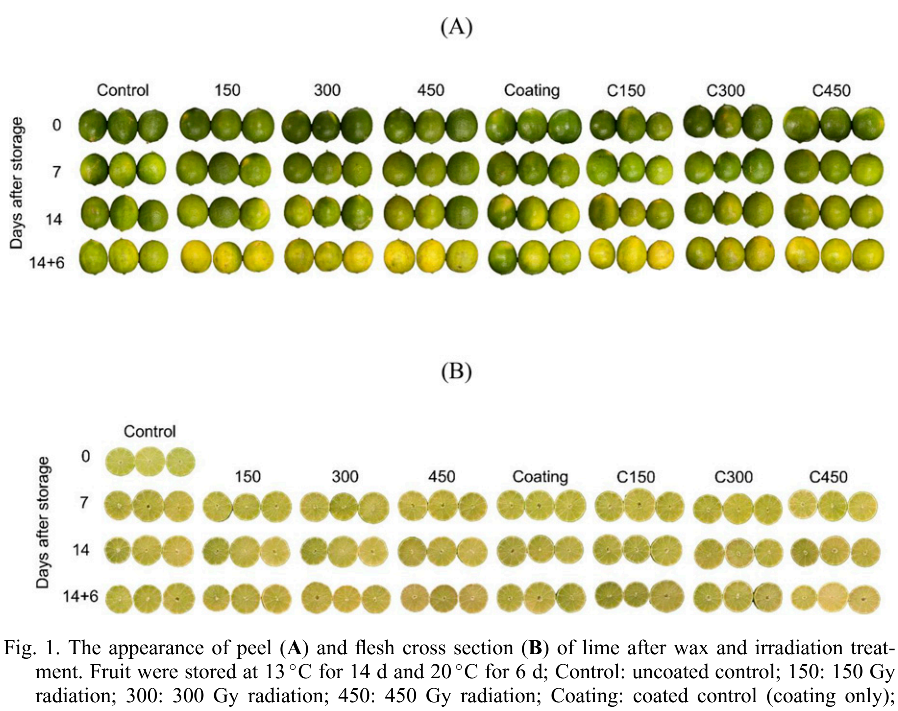

Fig. 1. The appearance of peel (**A**) and flesh cross section (**B**) of lime after wax and irradiation treatment. Fruit were stored at 13 °C for 14 d and 20 °C for 6 d; Control: uncoated control; 150: 150 Gy radiation; 300: 300 Gy radiation; 450: 450 Gy radiation; Coating: coated control (coating only); C150: coating + 150 Gy radiation; C300: coating + 300 Gy radiation; and C450: coating + 450 Gy radiation.

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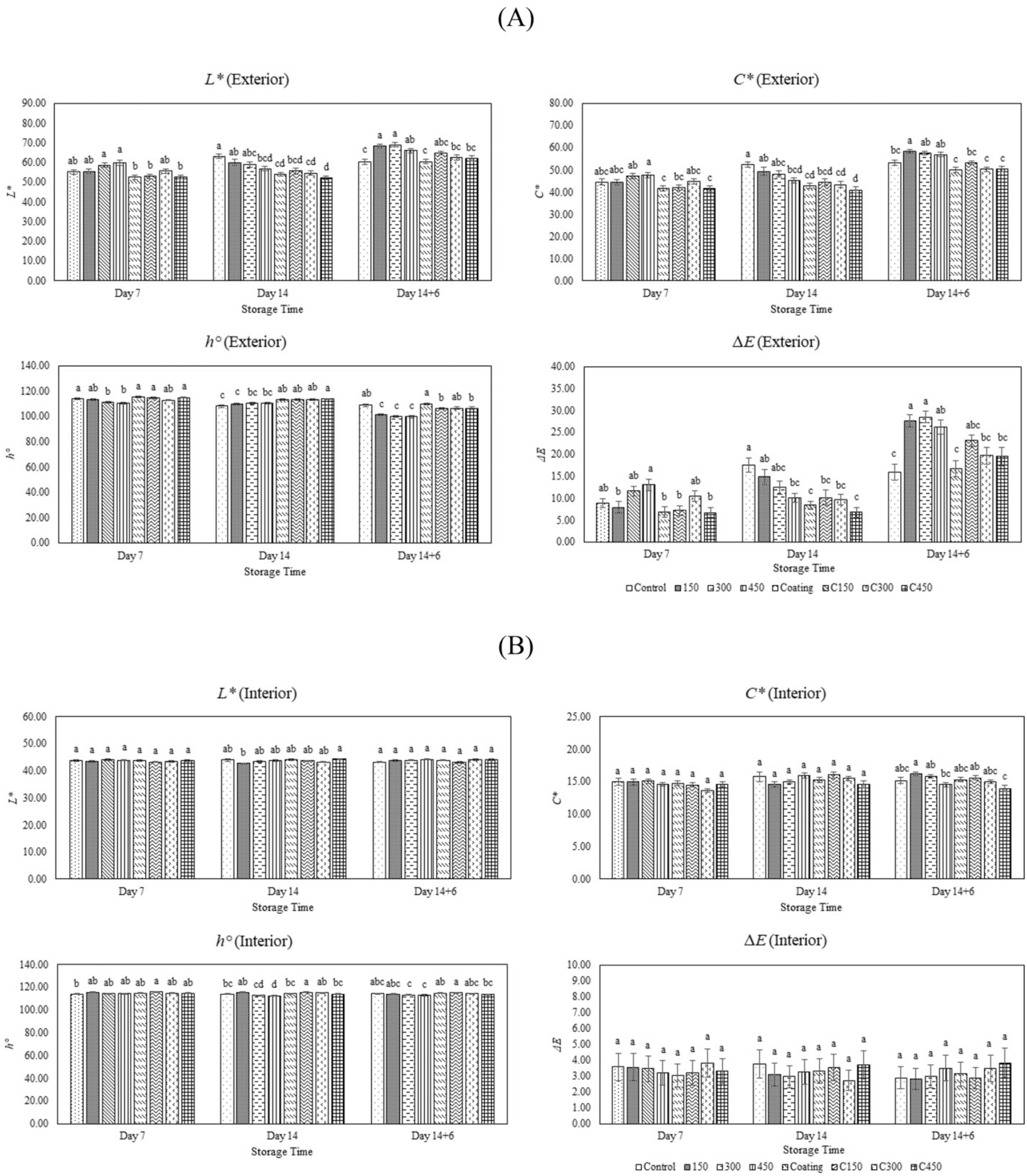

Fig. 2. The effect of wax coating and irradiation treatment and storage on the peel color (**A**) and flesh cross section color (**B**) of lime. Fruit were stored at 13 °C for 14 d and 20 °C for 6 d; Control: uncoated control; 150: 150 Gy radiation; 300: 300 Gy radiation; 450: 450 Gy radiation; Coating: coated control (coating only); C150: coating + 150 Gy radiation; C300: coating + 300 Gy radiation; and C450: coating + 450 Gy radiation. Each value is the mean of three replicates. The vertical bars represent the standard deviations of the means. The different letters indicate significant differences (analysis of variance, $P < 0.05$) between different treatments at the same storage time, according to Tukey's honestly significant difference comparisons test. L^*, average lightness; C^*, chroma; $h°$, hue angle; ΔE, color difference.

be more sensitive to irradiation. A slight to moderate rind browning was observed in X-ray–irradiated 'Clemenules' mandarins at both 195 and 395 Gy after 2 d at 20 °C, and the superficial rind disorder increased deterioration indices for irradiated fruit (Alonso et al. 2007). In contrast, the color of two types of Korean citrus hybrids was not altered after storage for 20 d at 4 °C following irradiation between 400 and 1000 Gy (Jo et al. 2018). Orange cultivars (Ambersweet and Valencia) tolerated 500- to 600-Gy irradiation, but Hamlin, Navel, and Pineapple cultivars were injured at 150 Gy (Miller et al. 2000). Likewise, mandarin hybrids Minneola

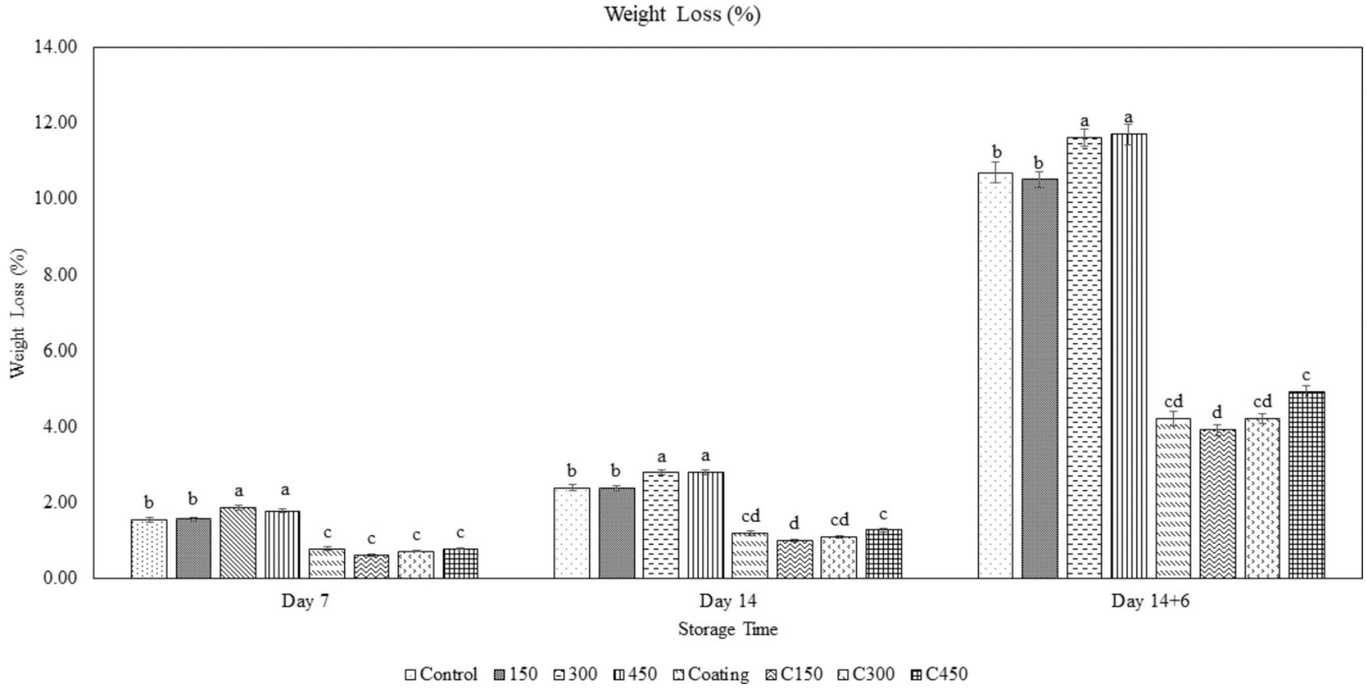

Fig. 3. The effect of wax coating and irradiation treatments and storage on the weight loss of lime. Fruit were stored at 13 °C for 14 d and 20 °C for 6 d; Control: uncoated control; 150: 150 Gy radiation; 300: 300 Gy radiation; 450: 450 Gy radiation; Coating: coated control (coating only); C150: coating + 150 Gy radiation; C300: coating + 300 Gy radiation; and C450: coating + 450 Gy radiation. Each value is the mean of three replicates. The vertical bars represent the standard deviations of the means. The different letters indicate significant differences (analysis of variance, $P < 0.05$) between different treatments at the same storage time, according to Tukey's honestly significant difference comparisons test.

and Murcott showed tolerance at 500 to 600 Gy, but peel pitting occurred for Fallglo, Sunburst, and Temple cultivars at 150 Gy (Miller et al. 2000). Because green color is important to lime marketability, a combination of treatments may be useful to extend shelf life. For example, ultraviolet-B at 8.8 $kJ \cdot m^{-2}$ delayed chlorophyll breakdown in lime peel, thereby maintaining the green color of lime fruit peel (Srilaong et al. 2011).

Weight loss. The weight loss of lime fruit increased progressively in all treatments during storage (Fig. 3). The weight loss of fruits was impacted by both coating and irradiation. The coating treatment significantly ($P < 0.05$) reduced the fruit weight loss at 7-, 14-, and 14 + 6-d storage. The weight loss of all the uncoated fruit with or without irradiation was between 10.5% and 11.7% after storage at 13 °C for 14 d and 20 °C for 6 d, whereas the weight loss for all the coated fruit treatments (coating alone and coating + irradiation) was between 3.9% and 4.9% (Fig. 3). The coating treatment reduced the fruit weight loss by 58% to 64% compared with the treatments without coating at day 14 + 6. Higher doses of irradiation (300 and 450 Gy) without coating significantly ($P < 0.05$) increased the weight loss of lime compared with control and the dose of 150 Gy (Fig. 3). For the combination of coating and irradiation treatments, the 150 Gy–treated fruit showed significantly ($P < 0.05$) lower weight loss than the other irradiation treatments at both day 14 and day 14 + 6 compared with the coated control (Fig. 3).

Weight loss is a consequence of fruit dehydration due to changes in respiration rate and surface transfer resistance to water vapor due to the occurrence of small fissures connecting the internal and external atmospheres (Baldwin et al. 1995). Persian lime fruit have little or no natural surface cuticle, and therefore are subject to excessive water loss. One of the most efficient ways to mitigate fruit weight loss is by applying an edible coating to create an additional thin layer of protection over a fruit's peel capable of improving its natural water semi-impermeable properties (Sun et al. 2022). The carnauba wax coating in our study significantly reduced weight loss in limes during storage, and has been shown to reduce postharvest weight loss for many other fruits, including strawberries (Oliveira Filho et al. 2022), papayas (Miranda et al. 2022), and pomegranates (Meighani et al. 2015). The mode of action of a wax coating is probably primarily due to covering and sealing the stomata and cracks on the fruit surface (Lufu et al. 2020). This sealing of cracks or natural openings reduces water loss. Moreover, fruits treated with wax coatings showed a reduction of respiration rate and ethylene production (Li et al. 2018), and therefore had reduced weight loss. Wax coating treatments have also been shown to be effective in terms of maintenance of membrane functionality and integrity, with lower losses of phospholipids and proteins and reduced ion leakage (Firdous et al. 2023).

Irradiation, especially at high doses (e.g., >300 Gy), could cause damage to the fruit peel cell membranes, thereby increasing weight loss. For example, irradiation treatments have been shown to increase membrane changes normally associated with ripening, such as microviscosity, and a decrease in fatty acid saturation, with the trend of higher weight loss that typically occurs during ripening (Arvanitoyannis et al. 2009). At even higher doses (>1000 Gy), irradiation can significantly modify the microstructure of fruit tissues and consequently their barrier properties and susceptibility to postharvest dehydration, which can therefore lead to reduced postharvest weight loss of the fruit. The most common effect of very high doses is the exacerbation of postharvest dehydration, particularly for citrus fruit, including navel oranges and grapefruit (Prakash and Ornelas-Paz 2019). Likewise, other tropical fruit treated with a high dose of irradiation showed increased fruit weight loss in a manner similar to our results (Wall and Khan 2008). This might be due to damage to the cuticle and epicuticular wax layer of the fruit surface by irradiation (Jaramillo Sánchez et al. 2021).

Total soluble solids content (SSC) and TA. The total soluble solids content (°Brix; SSC) of both treated and control fruits was between 9% and 9.5% after 7- and 14-d storage at 13 °C, and they were not significantly ($P > 0.05$) affected by coating or irradiation treatments (Fig. 4). However, at 13 °C for 14 d and 20 °C for 6 d, the SSC of the coating + 450 Gy irradiation-treated fruit (C450) was significantly lower than uncoated control, 150 Gy, and coating + 150 Gy (C150)-treated fruit (Fig. 4).

The average SSC of Persian lime fruit was reported as 7.6% to 10% (Akaber et al. 2024). Others have reported an average SSC of 8.8% to 10.8% for Persian lime depending on the root stock (Garcia-Munoz et al. 2021). Paraffin wax–treated limes showed lower SSC than unwaxed controls, and this was

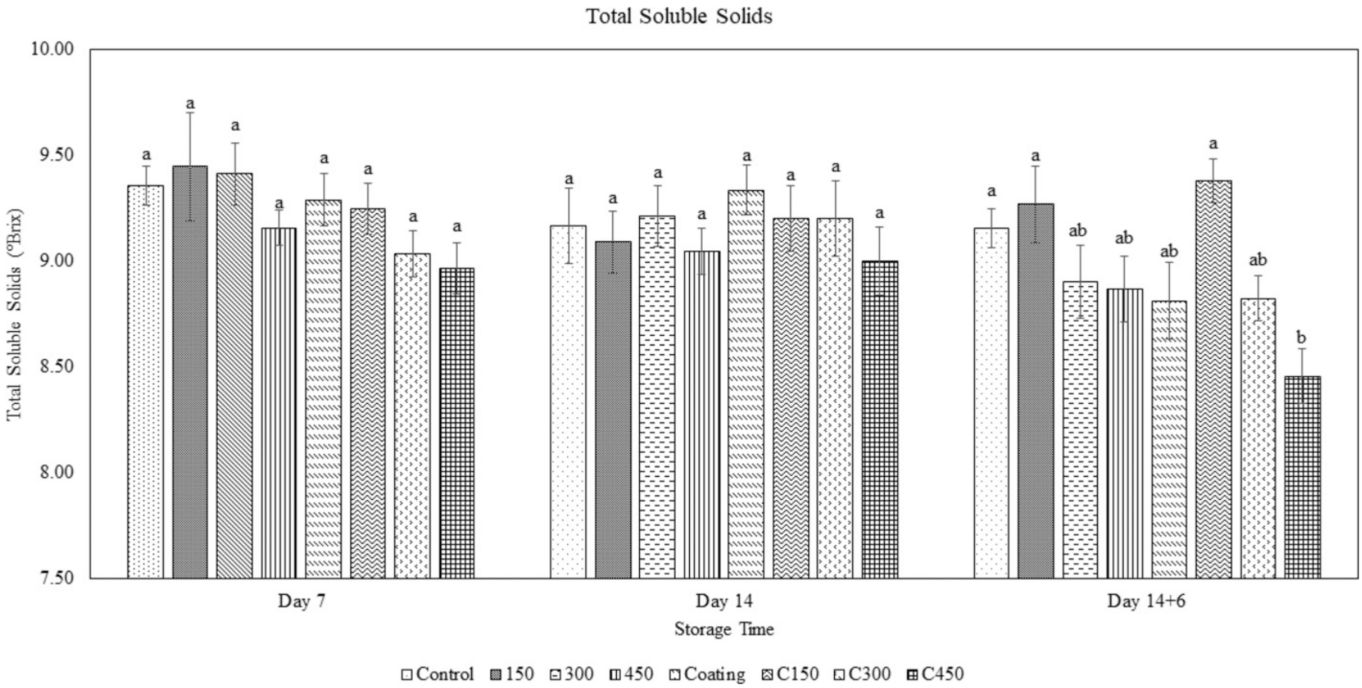

Fig. 4. The effect of wax coating and irradiation treatment and storage on the total soluble solids content (SSC) of lime. Fruit were stored at 13 °C for 14 d and 20 °C for 6 d; Control: uncoated control; 150: 150 Gy radiation; 300: 300 Gy radiation; 450: 450 Gy radiation; Coating: coated control (coating only); C150: coating + 150 Gy radiation; C300: coating + 300 Gy radiation; and C450: coating + 450 Gy radiation. Each value is the mean of three replicates. The vertical bars represent the standard deviations of the means. The different letters indicate significant differences (analysis of variance, $P < 0.05$) between different treatments at the same storage time, according to Tukey's honestly significant difference comparisons test.

probably because of a lower concentration of juice as a result of dehydration in unwaxed fruit (Bisen et al. 2012). Previous research showed that the postharvest irradiation treatment did not affect the SSC of Korean citrus fruits (Jo et al. 2018). The 75- and 300-Gy irradiation treatments did not alter the individual sugars content in lemons and mandarins, but a slight decrease of sucrose and increase of glucose and fructose have been observed in 150- and 400-Gy irradiated mandarins (Ornelas-Paz et al. 2017). The changes in individual sugars have been attributed to the irradiation-mediated activation or biosynthesis of enzymes involved in the metabolism of

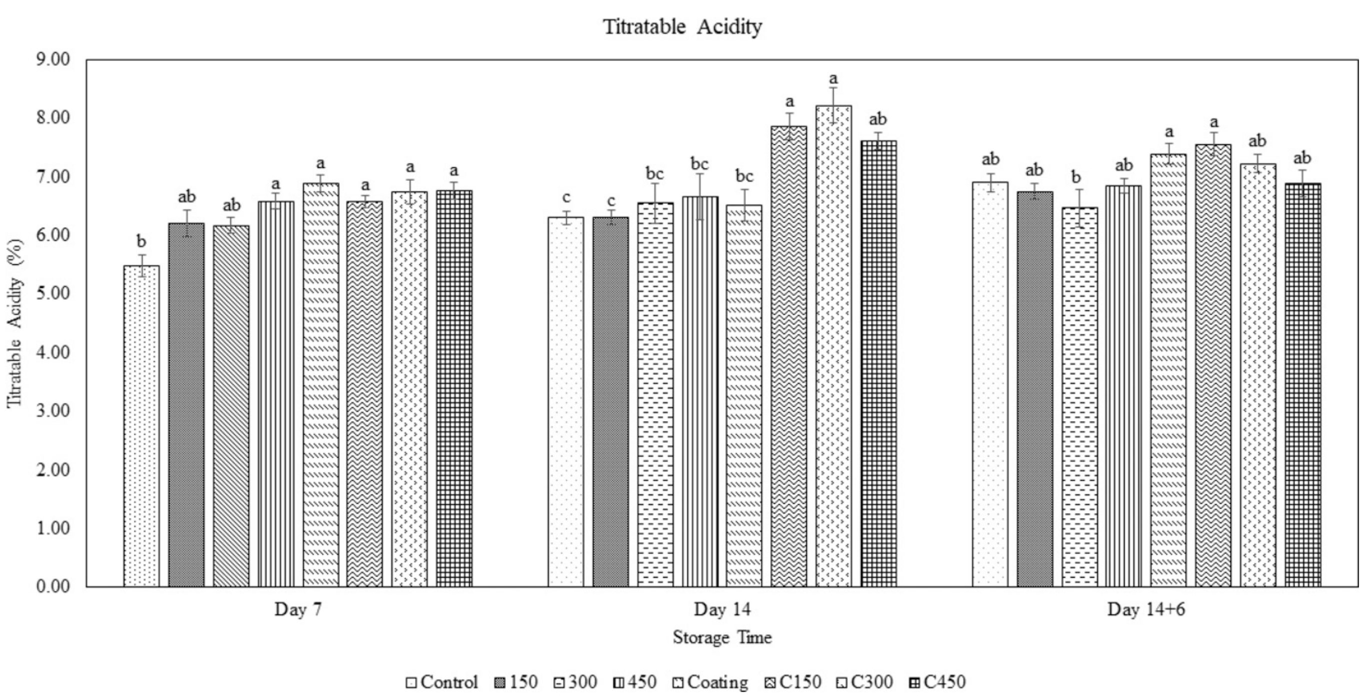

Fig. 5. The effect of wax coating and irradiation treatment and storage on the titratable acidity of lime. Fruit were stored at 13 °C for 14 d and 20 °C for 6 d; Control: uncoated control; 150: 150 Gy radiation; 300: 300 Gy radiation; 450: 450 Gy radiation; Coating: coated control (coating only); C150: coating + 150 Gy radiation; C300: coating + 300 Gy radiation; and C450: coating + 450 Gy radiation. Each value is the mean of three replicates. The vertical bars represent the standard deviations of the means. The different letters indicate significant differences (analysis of variance, $P < 0.05$) between different treatments at the same storage time, according to Tukey's honestly significant difference comparisons test.

these chemicals. It has also been demonstrated that irradiation promotes the expression of genes encoding these enzymes (Prakash and Ornelas-Paz 2019).

The TA of both treated and control fruits were between 5% and 8%, and the combination of irradiation and coated fruit had significantly ($P < 0.05$) higher TA (> 6.5%) than uncoated control (< 6.0%) at day 7 and day 14 (Fig. 5). At day 7, the 450 Gy–treated fruit had significantly higher TA than uncoated control. At day 14 + 6, the TA of irradiated and coated treated fruit was higher than uncoated control, but not significantly (Fig. 5).

As one of the main variables of fruit quality, TA represents fruit organic acids, such as citric, malic, and quinic acid. It normally decreases because organic acids are enzymatically broken down over time during storage (Dhall 2013). A decline in TA in wax-coated fruits has been observed (Hassan et al. 2014); however, postharvest irradiation did not affect the TA of Korean citrus fruits (Jo et al. 2018). Low-dose irradiation (75 and 300 Gy) had little effect on TA and individual organic acids of lemons and mandarins (Ornelas-Paz et al. 2017). Clementine mandarin cv. Clemenules retained fruit quality when treated with 195- and 395-Gy radiation (Alonso et al. 2007). Dosages from 400 to 700 Gy reduced the quality of early-season stored ‘Rio Red’ grapefruit, but not late-season fruit (Patil et al. 2004). The irradiation-mediated respiration rate of fruits might be involved in the changes observed in sugars and organic acids (Prakash and Ornelas-Paz 2019).

Conclusion

The carnauba wax coating appeared to mitigate color changes of lime (*Citrus latifolia* Tan.) fruit across all irradiation doses and storage times. Irradiation generally increased color changes, but the relationship was not linear and varied with time. After 14 d at 13 °C + 6 d at 20 °C, all samples experienced color changes, but coated fruit had a more consistent and often lower change across all irradiation doses, which suggests that coating could be an effective method for preserving the surface color of produce during cold storage and subsequent room temperature exposure. The results also suggested that the use of a coating was effective in reducing weight loss in cold stored fruit, and this effect persisted even after exposure to higher temperatures. The increase in irradiation dose generally positively correlated with increased weight loss, but the impact was less pronounced in coated fruit. Low-dose irradiation (150–450 Gy) after application of a carnauba wax coating could be used to maintain quality and improve quarantine safety of fresh lime fruit. This information could be valuable for postharvest management strategies to extend the shelf life of Persian limes and other fresh produce.

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