ELECTRON BEAM TECHNOLOGY FOR PRESERVING QUALITY ATTRIBUTES OF MANDARINS FOR ENHANCING EXPORT POTENTIAL

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Abstract

There is a need for countries around the world to increase their exports of agricultural products, as the export of these products adds significantly to the economic development of a country. Agricultural exports such as mandarin oranges and other citrus fruits are of high commercial value and are gaining popularity around the world. However, to deal with strict transboundary phytosanitary requirements, these commodities have to be appropriately treated. Ionizing technologies such as gamma, electron beam (eBeam) and X-ray are suitable technologies. The focus of this study was to determine whether the accelerator technology, namely eBeam technology can be combined with cold temperature storage technology to preserve the quality of mandarins. The science question was pursued was whether cold storage before or after eBeam processing was the most beneficial to preserve mandarin quality. The study was performed with mandarins harvested in two different locations: one in California and the other in Chile. There were three different eBeam dose treatments; 0 Gy (un-treated), 50 Gy, and 150 Gy. The cold temperature + eBeam combination treatments consisted of eBeam treatment at a dose of 50 Gy + 3 or 5 days of storage at 1°C either before or after eBeam treatment. After these combination treatments, the fruit were stored for three weeks; 14 days at 7°C and one week at room temperature. The quality attributes from these combination treatments were evaluated based on standard methods normally utilized for evaluating the quality of fruit in commercial trade, namely Citrus Color Index (CCI), maturity index, weight loss, extractable juice volume, pH, vitamin C, and overall appearance. Overall, the results indicate that the observable differences in these quality parameters were attributable to geographical origin of the mandarins and their stage maturity, rather than the eBeam + cold storage combination treatments. The study highlighted that 150 Gy was detrimental to the fruit quality. These results demonstrate the potential for a new phytosanitary treatment of mandarins which would be 50 Gy , followed by refrigerated storage for 3 days at 1°C. These results suggest that eBeam technology can be technologically compatible with citrus fruits. Economic and technical feasibility analyses to build and operate purpose-built accelerator facilities in citrus growing regions of the world still needed.

1. INTRODUCTION

Oranges (*Citrus sinensis*), mandarins (*Citrus reticulata*), and tangelos (*Citrus tangelo*), are economically important crops worldwide and also have large international markets. However, the trade of citrus fruits is limited due to quarantine restrictions put in place to limit the northward spread of tephritid fruit flies and other exotic species from Mexico [1]. To combat this issue, a generic ionizing radiation dose of 150 Gy has been adopted as an international standard for the treatment of fruit flies in any commodity type [2,3]. At present, there are two phytosanitary treatments allowed for control fruit flies. The first is to maintain the fruit for 14 days at 1°C, and the second is irradiation at a minimum absorbed dose of 150 Gy. Irradiation treatment has also been adopted by the International Plant Protection Convention (IPPC) as a generic treatment for all fruit flies in any commodity [3]. Both of these treatments affect the quality of the fruit, as exposure to these high ionizing doses damages the oil glands of the citrus peel [4]. This results in brown spotting or pitting on the surface of the fruit, which can impair its quality and adversely affect its market value. Currently, cobalt-60 is the most commonly used ionizing technology in commercial practice. However this legacy phytosanitary treatment method is facing challenges associated with costs, isotope availability, and the transportation of radioactive materials [5,6]. Additionally, the maximum dose the fruits may experience during commercial cobalt-60 operations can be as high as 450 Gy depending on the ionizing radiation field within the irradiation chamber and the configuration of the pallets being exposed to this field [7]. Therefore, other forms of ionizing technologies available for use as phytosanitary treatments are rapidly evolving.

Electron beam and X-ray technologies are now considered to be environmentally and economically sustainable alternatives to cobalt-60 technology. It has been reported that a lower ionizing dose, combined with a shorter and less harsh cold treatment, may be equally effective in preventing fruit fly reproduction when compared to cobalt-60 processing, without damaging the citrus peel oil glands [8,9]. Additionally, the dose rate of eBeam technology is significantly higher than that of cobalt-60 [10]. Therefore, fruit processed using eBeam technology are exposed to the radiation source for shorter periods of time when compared to cobalt-60 processing. This is important from a visual and nutrient quality perspective, as less time spent being subjected to the radiation field is directly correlated with less quality and physicochemical degradation within the fruit [11]. By applying a low radiation dose and reducing the duration of cold storage, the quality of the mandarins as a function of shelf-life will not be affected.

Metabolic and physiological reactions continue to take place and cause unwanted changes in citrus fruits following their harvest, and the rate of these reactions is often influenced by the conditions that the fruits experience during treatment and storage. Citrus fruits are classified as non-climacteric, as their respiration rate does not change once they reach maturity. These fruits are considered to have two independent systems: one involving the exterior of the fruit, or the flavedo because it primarily contains flavonoids, phenolics, carotenoid compounds, and oil glands; and the other involving the interior, or the pulp, that contains pigments, acids and sugars, and bioactive compounds like vitamin C [12]. Since these systems are believed to be independent, they are also believed to have different responses to irradiation treatments. Therefore, the objective of this study was to evaluate these changes as a result of a combination treatment involving low eBeam doses and short cold storage durations. The rationale for this experimental design was to simulate the commercial conditions that citrus fruit experience during transport from the packaging house to the irradiation facility and from the irradiation facility to the retail store.

2. MATERIALS AND METHODS

2.1. Sourcing of Mandarin Oranges and Research Design

This study was conducted on mandarins (*Citrus reticulata*) from two different harvest locations; one in California and the other in Chile. For both studies, the fruits were sourced from a commercial packaging line at Halos – The Wonderful Company in Delano, California. The fruits were shipped via overnight transit to Texas A&M University, with approximately 12 to 15 days elapsing between harvest and arrival. After arrive, the fruits were separated into seven groups (Table 1). Three control groups were selected (0, 50, and 150 Gy) to be compared to four combination treatment groups. There were 50 mandarins per treatment group.

TABLE 1. TREATMENT GROUP DESIGNATIONS

Treatment	Designation
0 Gy Control	0Gy
50 Gy Control	50Gy
150 Gy Control	150Gy
50 Gy + 3 days storage at 1°C	50Gy3D
50 Gy + 5 days storage at 1°C	50Gy5D
3 days storage at 1°C + 50 Gy	3D50Gy
5 days storage at 1°C + 50 Gy	5D50Gy

2.2. Dosimetry and eBeam Treatment

The target eBeam doses were delivered using a 10 MeV, 15 kW linear accelerator. The mandarins were then dose-mapped to determine the dose distribution in the system, the minimum (D_{min}) and maximum (D_{max}) doses, and the Dose Uniformity Ratio (DUR) within the fruits prior to subjecting them to each treatment. The fruits were processed with 11 3/16th high-density polyethylene (HDPE) attenuation sheets, placed above and below the fruit, in order to achieve the minimum and maximum target doses of 50 and 150 Gy, respectively. There were 25 mandarins per tray. The eBeam processing was performed at the National Center for Electron Beam Research at the Texas A&M University, College Station campus. The absorbed eBeam doses within the fruits were measured using L-α-alanine dosimeters (Table 2). Three mandarins were used to measure the absorbed eBeam dose for treatment at 50 and 150 Gy, and dosimeters were inserted into the top, middle, and bottom of each fruit.

TABLE 2. DOSIMETRY DATA

Target Dose (Gy)	Dosimeter Location	Absorbed Dose (Gy)	DUR
	Top	58.33 ± 24.85	
50	Middle	49.00 ± 13.89	1.76
	Bottom	86.33 ± 49.80	
	Top	50.00 ± 2.65	
50	Middle	45.00 ± 1.00	1.11
	Bottom	49.00 ± 2.00	
	Top	208.33 ± 267.32	
150	Middle	218.33 ± 295.89	1.62
	Bottom	135.00 ± 137.71	
	Top	186.33 ± 61.09	
150	Middle	161.33 ± 70.21	1.16
	Bottom	161.00 ± 42.15	

For each control group, 10 fruits were taken for quality assessment at three time points: after treatment, following refrigerated storage (7°C) for 14 days, and following storage at room temperature for one week. For the four treatment groups, the quality was evaluated following the same tine points as the control groups (Figure 1). The quality of the fruits was evaluated through the following physicochemical analyses: juice volume, pH, total soluble solids (TSS), vitamin C, and titratable acidity (TA). The ICM measurements of color and percentage of weight loss were measured in 20 fruits per treatment group, which were monitored throughout storage.

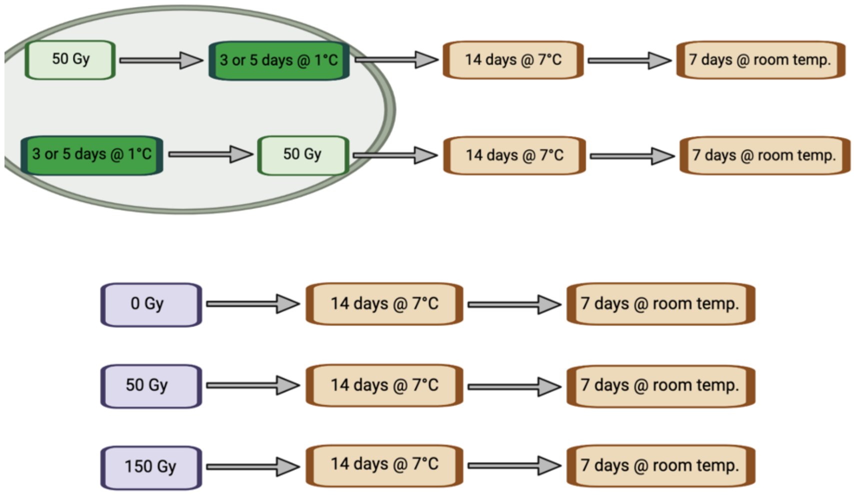

FIG. 1: Image showing the sampling scheme and experimental design of this study.

2.3. Color Determination

The peel color (L^*, a^*, and b^* values) of the 20 mandarins held throughout storage from each treatment group was measured using a calibrated Minolta Colorimeter CR-410 Chroma Meter (Konica Minolta Sensing Americas, Inc., NJ, and USA). A white calibration plate was used to prepare the instrument prior to measurement. Three measurements were taken from each fruit at the equatorial region to obtain an average color reading. These values were then used to determine the CCI of the fruits.

2.4. Determination of Weight Loss

The weight (in grams) of the 20 mandarins held from each treatment group was measured using a digital scale. The results are reported as the average percentage of weight lost by the mandarins.

2.5. Extractable Juice Volume

The mandarins were juiced using a commercially available Hamilton Beach Big Mouth Pro juice extractor and measured using a 100 mL graduated cylinder. A 20 mL aliquot of the juice was used for the pH and TA determination, and the remaining volume was stored under refrigerated conditions in aluminum foil covered, 15 mL polypropylene tubes for approximately one week for the analyses of sugar and vitamin C content.

2.6. Total Soluble Solids (TSS), pH, Titratable Acidity (TA), and Maturity Index

TSS (°Brix) was measured using < 1 mL aliquots of fruit juice and a Rhino Digital Refractometer ATC IP65 (Rhino Technology, Inc., Oakland, CA, USA). The pH of each juice samples was obtained using a MettlerToledo™EasyPlus™Easy Pro Titrator (Mettler-Toledo, LLC, Columbus, OH, USA) and 20 mL of fruit juice. The titrator was calibrated using 1.68 and 4.0 pH buffers prior to measurement. TA (g/100 mL) was determined by titration with 0.1 M NaOH using a MettlerToledo™EasyPlus™Easy Pro Titrator (Mettler-Toledo, LLC, Columbus, OH, USA) and 20 mL of fruit juice. The TSS and TA measurements were used to determine the maturity index of the fruits.

2.7. Vitamin C Content

Vitamin C content (µg/mL) was performed by the Integrated Metabolomics Analysis Core (IMAC) at the Texas A&M University, College Station campus. The vitamin C extraction was done by passing 500 µL of fruit juice through a 0.2 µm nylon filter (Merck Millipore, Burlington, MA) and diluting the filtrate 1:1000 in water. Targeted liquid chromatography (LC-QQQ) analysis was performed on a TSQ Altis mass spectrometer (Thermo Scientific, Waltham, MA) coupled to a binary pump UHPLC (Vanquish, Thermo Scientific), with an injection volume of 10 µL. Chromatographic separation was achieved on a Syneri Fusion 4 µm, 150 mm x 2 mm reverse phase column (Phenomenex, Torrance, CA) maintained at 30°C using a solvent gradient method, with a flow rate of 0.4 mL min^{-1}. Sample acquisition and data analysis was performed using Trace Finder 4.1 (Thermo Scientific).

2.8. Statistical Analysis

For the statistical analysis, JMP Software (Version 14.0, SAS Institute Inc., Cary, NY, USA) was used. One-way analysis of variance (ANOVA) was then utilized to analyze and identify differences between the 7 treatment groups at each time point and within each treatment group across the three time points. A student's t-test was then used to examine statistically significant differences (α=0.05). The results are reported as the mean ± standard deviation.

3. RESULTS

3.1. CCI

The effect of the combination treatment on the CCI of the mandarins is shown in Figure 2. For the California harvested mandarins, all treatment groups experienced decreases in CCI except for the 150Gy and both of the five-day treatment groups. For the Chile harvested mandarins, all treatment groups experienced decreases in CCI. However, any changes in CCI for both harvest locations were not statistically significant (α=0.01).

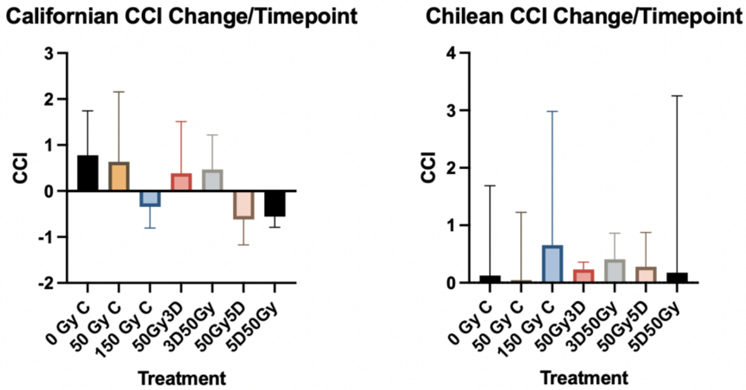

FIG. 2. Graph detailing the average change in CCI value for each of the treatment groups (α=0.01).

3.2. Overall Appearance

The effect of the combination treatments on the overall appearance of the mandarins is shown in Figure 3. For the California harvested mandarins, visual damage such as brown spotting, pitting, and bruising are clearly visible by the third time point in the 150Gy and 50Gy5D samples. Similar brown spotting and dehydration developed in the 0GyC group. Minimal damage is seen in the mandarins that underwent the other three combination treatments. For the Chile harvested mandarins, visual damage and blemishes are clearly visible on the surface of the 150Gy and 5D50Gy samples. It is also shown that already existing brown spots on the fruit peel of the 0GyC, 50GyC, 50Gy5D samples became more pronounced over time. Minimal visual damage can be seen in the mandarins that underwent the combination treatments with a three-day cold storage duration (50Gy3D and 3D50Gy).

FIG. 3. Digital images taken of 20 mandarins held throughout storage.

3.3. Percentage of Weight Loss

The effect of the combination treatments on the percentage of weight loss experienced by the mandarins is shown in Figure 4. For the California harvested mandarins, all treatment groups lost weight during storage except for the 150Gy group. For the Chile harvested mandarins, all of the treatment groups lost weight during storage. However, any changes in the percentage of weight lost by the mandarins in both harvest locations were not statistically significant (α=0.01).

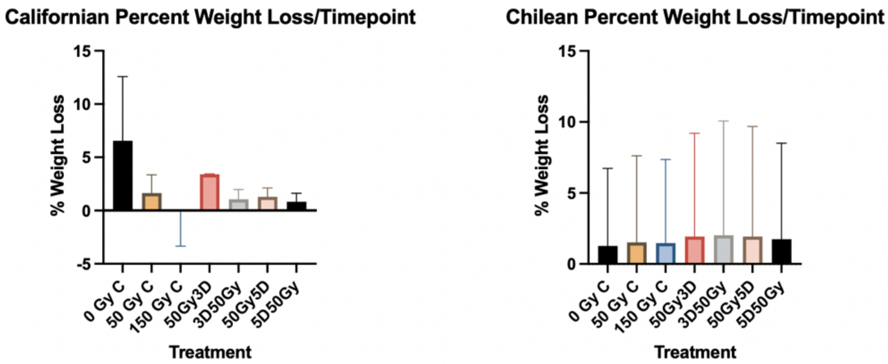

FIG. 4. Graph detailing the average change in percentage of weight loss for each of the treatment groups (α=0.01).

3.4. Extractable Juice Volume

The effect of the combination treatments on the extractable juice volume (mL) of the mandarin fruits is shown in Figure 5. For the California harvested mandarins, losses in extractable juice volume were seen in all of the treatment groups except in the 50GyC, 150GyC, and 5D50Gy samples. For the Chile harvested mandarins, losses in extractable juice volume were seen except in the 50Gy3D and 3D50Gy samples. However, any changes in extractable juice volume for both harvest locations were not statistically significant (α=0.01).

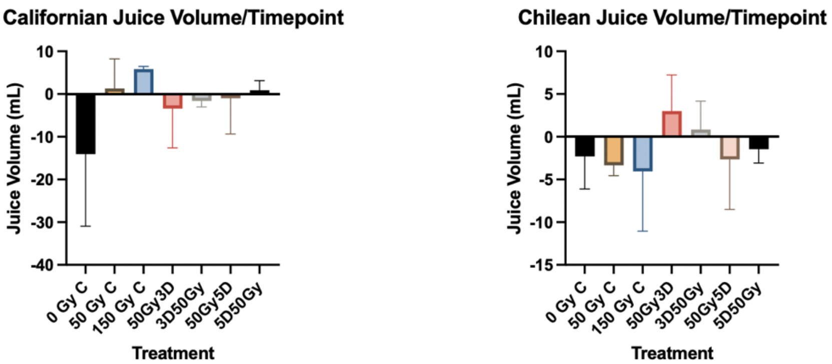

FIG. 5. Graph detailing the average change in juice volume for each of the treatment groups (α=0.01).

3.5. Maturity Index

The effect of the combination treatments on the maturity index (ºBrix/TA) of the mandarin fruits is shown in Figure 6. For the California harvested mandarins, all of the treatments experienced decreases in their maturity index except for the 0Gy and 50Gy5D treatment groups. For the Chile harvested mandarins, all of the treatments experienced decreases in their maturity index during storage except for the 50Gy3D, 3D50Gy, and 5D50Gy treatment groups. However, any changes in the maturity index of the mandarins from both harvest locations were not statistically significant (α=0.01).

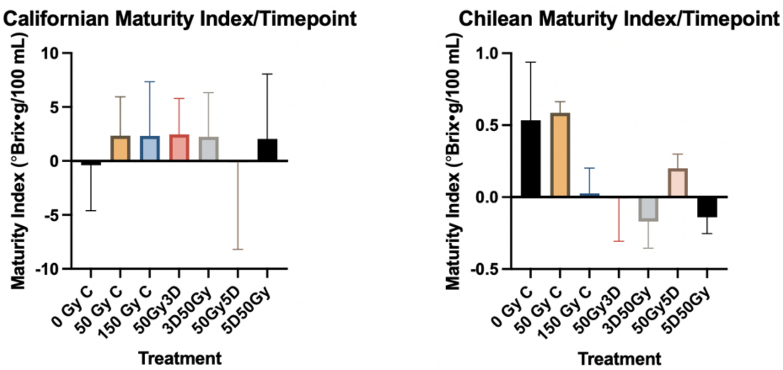

FIG. 6. Graph detailing the average change in maturity index for each of the treatment groups (α=0.01).

3.6. pH

The effect of the combination treatments on the pH of the mandarin fruits is shown in Figure 7. For the California harvested mandarins, all treatment groups experienced increases in pH except for the 0GyC group. For the Chile harvested mandarins, all of the treatment groups experienced increases in pH during storage except for the 50Gy3D, 3D50Gy, and 5D50Gy samples. However, any changes in pH for both harvest locations were not statistically significant (α=0.01).

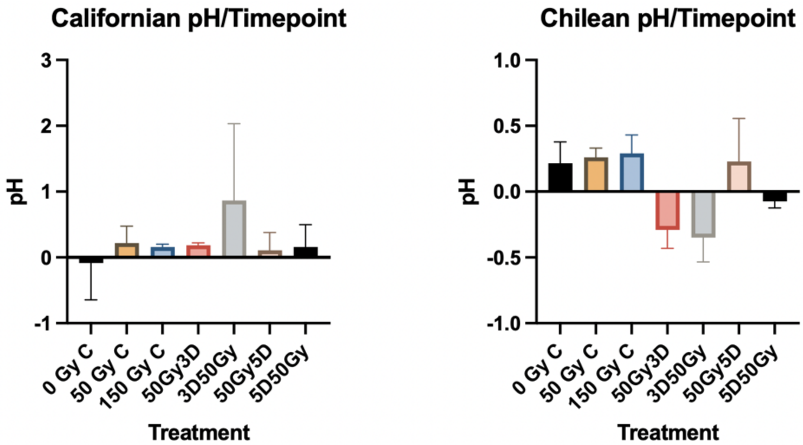

FIG. 7. Graph detailing the average change in pH for each of the treatment groups (α=0.01).

3.7. Vitamin C Content

The effect of the combination treatments on the vitamin C content (µg/mL) of the mandarin fruits is shown in Figure 8. For the California harvested mandarins, all of the treatment groups experienced decreases in vitamin C content except for the 0GyC, 150GyC, and 3D50Gy samples. For the Chile harvested mandarins, all of the treatment groups experienced decreases in vitamin C content. However, any changes in vitamin C content for both harvest locations were not statistically significant (α=0.01).

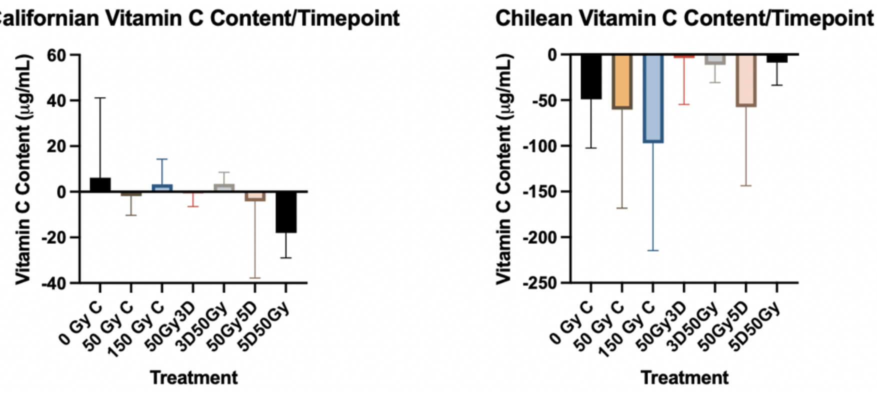

FIG. 8. Graph detailing the average change in vitamin C content for each of the treatment groups (α=0.01).

4. DISCUSSION

The most important attributes for the visual indication of citrus fruit quality are peel color (*L**, *a**, *b**, and CCI values) and overall appearance, and they are also key metrics that influence consumer acceptance [13]. In this study, it was found that the combination treatments had no negative effect on the peel color characteristics in the mandarin fruits. Marked visual damage was seen in the 150GyC mandarins from both harvest locations, which is consistent with previous reports [14,15]. These studies illustrate that this physical damage is understood to be due to the activation of the phenylalanine ammonia-lysate enzyme by irradiation doses, which may enhance the synthesis of phenolic compounds. These compounds are accumulated in the flavedo cell, resulting in cell death and subsequent peel necrosis and pitting. This deterioration could also be the result of an increase in the transpiration rate of the mandarins in the early stages of storage, which has been reported to induce postharvest senescence of citrus fruits [16,17].

Physiological and mechanical damage as a consequence of storage and treatment conditions has also been reported to impact the amount of weight lost by citrus fruits [18]. Previous studies on mangoes have shown that treatment with ionizing energy may cause similar physiological damage to fruits, possibly due to structural damage to the cells and the breakdown of these cells and their compounds [19]. This damage may contribute to the insignificant amount of weight lost by most of the mandarins during storage, most likely due to water loss. It has been reported that weight loss in tomatoes exposed to ionizing doses in refrigerated storage conditions (10ºC) was significantly less than the weight loss observed in untreated fruits [20]. However, this study concluded that an eBeam treatment consisting of low doses and short cold storage periods are not sufficient in imparting significant effects to the weight of the mandarins.

Citrus fruits are well known sources of ascorbic acid (vitamin C), and it has been reported that this nutrient is particularly sensitive to degradation by ionizing energy and cold storage conditions [21]. Moreover, the degradation products of vitamin C contribute to the formation of brown pigments and subsequent quality losses in citrus juices during storage [22]. However, the results of this study indicate that the combination treatment had no effect on the vitamin C content of the mandarins. It has been reported in a previous study that only doses exceeding 1 kGy significantly reduce the vitamin C content in citrus fruit [23]. Therefore, it can be deduced that vitamin C remained unaffected due to the conditions of the combination treatment, with its low radiation dose and short refrigerated storage period.

The analyses to evaluate the quality of the mandarin pulp following combination treatment included extractable juice volume, TSS, pH, TA, and Maturity Index. Independent of the harvest location, and in spite of the insignificant differences found, there were more changes in extractable juice volume associated with variations of the fruit rather than those associated with irradiation treatment or storage condition. Similar to the weight loss possibly being linked to the transpiration rate in the mandarins, the insignificant decrease in TA in these fruits has also been linked to an increase in transpiration rates [24]. More specifically, organic acids, like citric and malic acid, that normally accumulate in the fruit during development are used in the TCA cycle during ripening which leads to their depletion [25]. Additionally, there were no changes seen in the TSS of the pulp as a result of the combination treatments. This is consistent with other studies which show that, like vitamin C, doses at or below 1 kGy do not alter TSS [25,26].

The criteria for determining the maturity of citrus fruits involves two factors: internal changes to the fruit flesh, or pulp, and external changes in the color of the fruit peel, or flavedo [27]. This study focused on both of these changes to directly measure fruit maturity. Therefore, for the scope of this project, citrus fruits like mandarins are considered to be at peak maturity when their total soluble solids: acidity ratio, or maturity index, has reached a minimal level of palatability and when they showed optimal flavedo colors, which has already been discussed. This study illustrated that the proposed combination treatments did not impact the maturity of the mandarins during storage.

5. CONCLUSION

This study of citrus fruit quality, as a result of the combination treatment consisting of eBeam and cold storage, complements the efficacy studies of the treatment when used for phytosanitary treatment. Therefore, these studies support the adoption of the combination treatment for use in phytosanitary applications. The evaluation of the four different combination treatments in this study on mandarins (*Citrus reticulata*) demonstrated the feasibility of utilizing a phytosanitary treatment involving a 50 Gy eBeam dose and cold storage for 3 days at 1°C. When this treatment is applied to the mature, commercially acceptable fruit, the physiological, chemical, and nutritional qualities are not impacted. Furthermore, this combination treatment allows for the fruit to remain suitable for commercial purposes, without any pitting or discoloration. Ultimately, this study provides strong evidence that the combination treatment can be applied to citrus fruits as an alternative to damaging alternatives. Overall, there is evidence that Chile-harvested mandarins may be more susceptible to physicochemical degradation by eBeam treatment doses as compared to the Californian mandarins. However, whether this difference is due to the time lag differences between the harvests of the mandarins from the two locations or the combination treatment is unknown.

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