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Effect of phytosanitary irradiation on the postharvest quality of Seedless Kishu mandarins (Citrus *kinokuni* mukakukishu)

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Running head: Postharvest quality of irradiated mandarins

Abstract

Transnational trade of ‘Seedless kishu’ mandarins (*Citrus kinokuni mukakukishu*) would require them to be subjected to a suitable phytosanitary treatment. Irradiation is used as an effective treatment for many fruit, but the effect on quality of kishu mandarins is unknown. ‘Seedless kishu’ mandarins were treated with gamma irradiation (150, 400, and 1000 Gy) and stored for three weeks at 6 °C and then for one week at 20 °C. Irradiation at 400 and 1000 Gy promoted browning of the calyx end and fungal infection. Irradiation caused immediate reductions in pulp firmness, vitamin E, individual sugars and carotenoids but increased the content of organic acids, except ascorbic acid, and phenolic compounds. The volatile profile of tested fruit was also differentially altered by irradiation. Most of these initial changes were dose dependent. ’Seedless Kishu’ mandarins are significantly sensitive to irradiation and are not suitable for treatment at the studied doses.

Keywords: Phytochemicals; Ionizing energy; Postharvest storage; Citrus; Bioactive compounds; Mandarin

1. Introduction

The cultivation and consumption of mandarins has increased steadily in recent years in the U.S.A. (Baldwin & Jones, 2012). However, the domestic production of mandarins is insufficient and seasonal imports from Spain, Chile, Morocco, and Peru are required to satisfy the domestic demand of this fruit. These seasonal imports provide 30% of the mandarins consumed in the U.S.A (Baldwin et al., 2012). Currently, the importation of

new mandarin varieties from China is under consideration by the United States Animal and Plant Health Inspection Service (APHIS, 2014). The seedless kishu mandarin (*Citrus kinokuni mukakukishu*) is one of the varieties under consideration for import. This variety is also available in California. It is a small, sweet, aromatic mandarin with an easy to peel, thin, tight rind (UCR, 2016), but very little is known about the chemical properties of this variety of mandarin.

International trade of fruit involves the risk of introducing pests if adequate phytosanitary treatments are not applied before fruit shipment. Irradiation represents an alternative method to control insects and possesses advantages over chemical and thermal methods, especially in terms of human safety, fruit quality and environmental impacts (McDonald et al., 2013). The success and advantages of generic phytosanitary irradiation doses for the postharvest control of pests in fruits have clearly been demonstrated (Hallman, 2012). Currently, two generic irradiation doses (150 and 400 Gy) are approved by the APHIS for fruits to be exported to the continental U.S.A., and the maximum irradiation dose allowed in foods by the FDA is 1000 Gy (FDA, 2008; Hallman, 2012). These two generic doses allow the control of the most important quarantine pests of citrus fruits (Hallman, 2012; Zhang, Deng, Fu and Weng, 2014a). However, the success of irradiation as a phytosanitary treatment depends not only on its capacity to kill or neutralize target insects but also on the tolerance of fruits to ionizing energy. Depending upon the fruit, irradiation may result in an increase in ethylene biosynthesis and respiration rate, physiological disorders (rind disorder, loss of glossiness, pitting, and other skin injuries), softening, retardation of color development, deterioration of pulp flavor, accumulation of fermentative metabolites, and the alteration

of levels of some bioactive compounds (Miller, McDonald & Chaparro, 2000; Oufedjikh, Mahrouz, Amiot & Lacroix, 2000; Ladaniya, Singh & Wadhavan, 2003; Alonso, Palou, del Rio & Jacas, 2007; Palou, Marcilla, Rojas, Argudo, Alonso & Jacas, 2007). However, most of these responses in mandarins have mainly been observed at doses that differ from the generic doses, and especially at doses that exceed 1000 Gy. The response of mandarins at irradiation doses of 150 and 400 Gy is virtually not known nor the effect of irradiation on certain chemical attributes of mandarins. Based on literature, it can be hypothesized that a dose of 150 Gy is considerably low for phyto-toxic effects on mandarins, and that at 1000 Gy, some negative impacts may be manifested. Thus, the objectives of this work were to characterize the physical and chemical properties of 'Seedless kishu' mandarins and also to determine the effect of gamma irradiation at generic doses on the physical and chemical attributes of mandarins during simulated sea shipment and subsequent retail distribution.

2. Materials and Methods

2.1 Chemicals and solvents. All reagents and solvents were of analytical or HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A). The standard compounds for sugars (D-(+)-glucose, D-(-)-fructose and sucrose), phenolic compounds (gallic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid, hesperidin, narirutin, rutin and (-)-epicatechin), organic acids (citric, succinic, L-(+)-tartaric, DL-malic, L-ascorbic, oxalic, and fumaric), *all*-rac-α-tocopherol, volatile compounds, and some carotenoids (all-*trans*-β-cryptoxanthin, all-*trans*-lutein, all-*trans*-α and all-*trans*-β-carotene from carrots) were

purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The other carotenoids were obtained from CaroteNature GmbH (Lupsingen, Switzerland).

2.2 Fruit procurement, treatment, and storage

'Seedless kishu' mandarins (*Citrus kinokuni mukakukishu*) were harvested in Exeter, CA, U.S.A. The average weight, diameter, and height of the fruits were 37.7 g, 42.1 mm and 27.9 mm, respectively. They were hand cleaned and packed in 6.8 kg cartons (29.2 cm wide, 43.2 cm long, and 14 cm high) by California Citrus Specialties. Most commercial packing houses do not apply wax or fungicides to these mandarins and none was applied to these fruit. After cleaning, the fruit was ground transported to Chapman University and kept at 20 °C. The next day, the fruit was taken to Sterigenics, Inc. (Tustin, CA, U.S.A.), for treatment. Ten cases of mandarins were located two rows high and five across in front of a ^{60}Co source (~37PBq). Dose mapping was conducted by placing 24 alanine pellet dosimeters (FarWest Technology, In., Goleta, CA, U.S.A.) at various locations in the cases. The dose rate was 0.637 Gy/s. Ten cases of mandarins were placed exactly in the same configuration as the dummy cases to receive treatment at a target dose of 150, 400, and 1000 Gy (4.6-5.5% uncertainty) and Dmax/Dmin ratio of 1.33. Midway through treatment, the boxes were rotated 180° to ensure uniform treatment. After treatment, the mandarins were transported to Chapman University and stored at 6 °C (relative humidity (RH) = 85-90%) for 21 days. Then, the cases were opened and kept at 20 °C (RH = 85-90%) for 7 days. After 2, 21 and 28 days, three cartons of each experimental group were removed from storage. Two cartons were used for physical and chemical analyses. One carton of each treatment was intermittently removed from storage to evaluate the development of disorders and fungal infections. For chemical analyses, at least 150 fruits

were juiced using an Elite Gourmet MaxiMatic Juice Extractor and 20 subsamples of 40 mL each were centrifuged (20000 g/ 20 °C/10 min) to separate the solids and liquid of the juice, according to Stinco et al. (2013). The obtained solids and liquids were distributed into five samples. The solids were evaluated for carotenoid and α-tocopherol content and tristimulus color while the liquid was evaluated for total soluble solids content (TSS), titratable acidity (TA), individual sugars, individual organic acids, individual and total phenolic compounds, and volatile compounds.

2.3 Peel disorders and fungal infections

The incidence of oleocelosis, pitting, rotting and browning was evaluated in one case of fruit per treatment. The cases used for this evaluation were briefly removed from storage and returned to it after evaluation of fruit by five trained judges. The results were expressed as % of total fruit (number of fruit manifesting the disorder/total number of fruit in the case) showing noticeable symptoms of each disorder.

2.4 Evaluation of TSS, TA, tristimulus color, and firmness

TSS was measured in the centrifuged juice using a hand-held refractometer (ATAGO Co. Ltd.; Tokyo, Japan). For TA, the centrifuged juice was diluted with water (1:10, v/v), titrated with 0.1 N NaOH to an end point of pH 8.2 and expressed as citric acid (%). The tristimulus color (L*, a* and b*) of mandarin pulp obtained following centrifugation was evaluated using a CM-2500d Minolta Spectrophotometer (Ramsey, NJ, U.S.A). In

preparation for firmness measurements, ten fruit were scored longitudinally and carefully peeled and segmented. Peel firmness was measured using a TA.XT2 Texture Analyzer (Texture Technology Corp., Scarsdale, N.Y., U.S.A. and Stable Microsystems, Godalming, Surrey, U.K.) at the equatorial axis of the fruit peels with a cylindrical puncture probe (i.d. 3 mm) to penetrate through a distance of 10 mm at a speed of 2 mm/s. The maximum force (N) was recorded. To measure firmness of the flesh, 150 g of mandarin segments were placed in a Kramer Shear Cell (TA-91) and pressed with the five flat-blade attachment at a speed of 4.0 mm/s. The maximum force (N) and area under the curve were recorded.

2.5 Analysis of sugars

The content of individual sugars was determined according to Ornelas-Paz et al. (2013), with some modifications. Aliquots of juice (100 µL) were diluted with HPLC grade water (2 mL), filtered using a nylon membrane with a pore size of 0.45 µm (Pall Corp., New York, U.S.A.), and automatically injected (20 µL) into a 1100 series HPLC system (Agilent Inc., CA, U.S.A.) equipped with a refractive index detector. The sugars (sucrose, glucose and fructose) were separated in a SUGAR SC 1821 (300 x 8.0 mm I.D., 6 µm particle size) ion-exchange column (Showa Denko K.K.; Tokyo, Japan) at 80 °C. The mobile phase was HPLC-grade water at a flow rate of 0.8 mL/min. The sugars were identified by comparing their chromatographic behavior with that of reference compounds. Quantitative data were obtained by calibration curves constructed with three independent sets of dilutions of reference compounds (six concentration points for each set).

157

158 2.6. Analysis of organic acids

159 This analysis was based on the methodology described by Ornelas-Paz et al. (2013). One
160 milliliter of centrifuged juice was diluted with 3 mL of 5 mM H_2SO_4. The mixture was filtered
161 and injected into the HPLC system described above but connected to a diode array
162 detector. The separation of the organic acids was performed in an Aminex HPX-87H (300
163 x 7.8 mm I.D., 9 µm particle size) ion-exchange column (Bio-Rad Laboratories., CA,
164 U.S.A.) at 60 °C. The mobile phase was composed of 5mM H_2SO_4 and acetonitrile (90:10,
165 v/v) at a flow rate of 0.4 mL/min. The ascorbic acid was monitored at λ= 260 nm while the
166 other organic acids were monitored at λ=210 nm. The identification and quantification of
167 the acids were performed using standard compounds.

168

169 2.7. Analysis of phenolic compounds

170 The analysis of individual and total phenols was performed simultaneously. The juice was
171 filtered with a membrane of 0.45 µm pore size and directly injected (100 µL) into the HPLC
172 described previously. The separation of phenolic compounds was performed in a Kinetex
173 C18 (100 x 4.6 mm I.D., 5 µm particle size) (Phenomenex; Torrance, CA, U.S.A.) at 30
174 °C. The phenolic compounds were monitored at λ= 280, 320, 350 and 520 nm. The mobile
175 phase consisted of 2% acetic acid (A), and acetonitrile (B), according to the following
176 gradient: 100% A at 0 min, 93% A at 12 min, 89% A at 20 min, 86% A at 35 min, 84% A
177 at 36 min, 82% A at 41 min, 79% A at 44, 0% A from min 55 to 60. The flow rate was 1
178 mL/min. The phenolic compounds were identified and quantified by using reference

compounds. The UV-Vis spectrum of individual phenols was also used for identification purposes.

For total phenols content, 100 µL of filtered juice were mixed with 100 µL of Folin-Ciocalteu reagent, 3 mL of deionized water and 100 µL of 20% Na_2CO_3. The mixture was vigorously shaken for 1 min and incubated for 1h in the darkness. The absorbance was evaluated five times at 765 nm using a FLUOstar Omega microplate reader (BMG LABTECH Inc.; Cary, NC, U.S.A.). The absorbance values were corrected with those generated with blank reactions. The quantification was performed using a calibration curve constructed with several sets of dilutions of gallic acid. The results were expressed as mg gallic acid equivalents (GAE) per liter of juice.

2.8 Determination of carotenoid and tocopherol content

These compounds were analyzed simultaneously according to Ornelas-Paz, Yahia and Gardea-Bejar (2007). Briefly, 4 g of mandarin pulp was mixed with $CaCO_3$ (0.2 g) and 20 mL of methanol. The mixture was filtered through a Whatman paper No. 3, recovering the methanolic extract. The retained solids were sequentially depigmented with 100 mL of methanol and 75 mL of a mixture of hexane:acetone (1:1, v/v). The extract was placed into a separatory funnel and 30 mL of hexane were added. The mixture was shaken vigorously and 40 mL of 10% Na_2SO_4 were added. After phase separation, the upper phase was recovered and the solvent evaporated at 40 ºC under reduced pressure. These residues were analyzed without and with saponification in order to identify free and esterified xanthophylls. For saponification, the residues were dissolved in 30 mL of diethyl ether and 0.6 mL of 40% KOH in methanol were added. The mixture was vigorously

shaken and kept in the darkness for 16 h. Then, the sample was washed with water and the organic solvent evaporated at reduced pressure. Crude and saponified residues were dissolved in methanol (4 mL), filtered and injected in the HPLC system (20 µL) described above. The separation was performed in a YMC C30 column (150 x 4.6 mm I.D., 3 µm particle size) (YMC America Inc., Allentown, PA, U.S.A.) at 15 °C. The UV-Vis spectra of carotenoids was recorded from 300 to 750 nm in steps of 1 nm. The α-tocopherol was monitored with a fluorescence detector (λex= 294 nm, λem = 326 nm). The mobile phase was composed of water (solvent A), methanol (solvent (B) and methyl *tert*-butyl ether (MTBE, solvent C) according to the following gradient: 4%A/ 94.5%B/ 1.5%C at 0 min, 4%A/ 68%B/ 28%C at 31 min, 4%A/ 30%B/ 66%C at 83 min. The identification of carotenoid esters was performed by comparing the chromatographic behavior of crude and saponified extracts. The chromatographic behavior and UV-Vis of peaks of the sample were also compared with those of reference compounds. The quantification was performed using calibration curves constructed with reference compounds. The *cis* isomers were quantified as all-*E* carotenoids.

2.9 Volatile analysis

Five samples per treatment (5 mL each) were placed into headspace vials (12 x 32 mm) and 5 mL of saturated sodium chloride was added. 1-pentanol (1.1 mL, final concentration of 490 µg/L) was added to each sample as internal standard. The headspace vials were capped with a Teflon-coated septum. The analysis of volatile components was completed using solid phase microextraction (SPME) with a 75-µm carboxen/polydimethylsiloxane fiber (Supelco, St. Louis, MO, USA). A Gerstel MPS-2 system (Gerstel, Linthicum, MD, USA) was utilized to automate the analytical procedure.

225 Initially, the sample was equilibrated at 40 °C for 15 min followed by deployment of the
226 SPME fiber into the headspace vial and the trapping of headspace volatiles at 40 °C for
227 30 min, all processes with agitation at 4.2 s^{-1}. This was followed by desorption of the
228 volatile compounds from the SPME fiber at 280 °C in the splitless inlet of an Agilent 7980
229 GC (Agilent, Palo Alto, CA) equipped with an FID detector at 280 °C. The flow of hydrogen
230 and air were 0.67 mL/s and 7.5 mL/s, respectively. The separation was performed with
231 an Agilent HP-5MS ultra-inert column (30m x 0.25 mm I.D., 0.25 µm film thickness) using
232 He as carrier gas (0.02 mL/s). Oven temperature was held at 32 °C for 3 min and then
233 ramped up to 200 °C at a rate of 0.1 °C/s. Identification of compounds was performed by
234 using standards, retention indices for *n*-alkanes, and comparing the MS spectra of volatile
235 compounds with those of Wiley/NBS library. The MS spectra were obtained by switching
236 the GC from the FID detector to the mass spectrometer (Agilent 5975) and analyzing
237 identical samples. Semi-quantification of volatile concentrations from the FID data was
238 obtained by utilizing calibration curves generated from standards placed into deodorized
239 mandarin juice. The quantification of ethanol, esters, alcohols aldehydes, terpenes,
240 terpene alcohols and ketones was performed using calibration curves made with ethanol,
241 ethyl acetate, 3-methyl butanol, *E*-2-hexenal, α-pinene, 4-terpineol, and carvone,
242 respectively.

243

244 2.10 Statistics

245 The data were analyzed using a completely randomized design. The statistical
246 significance of the differences between treatments was determined using ANOVA

followed by the Tukey–Kramer post hoc test; 0.05 was the significance limit. Data analysis was performed using JMP statistical software (SAS Institute Inc., Cary, NC, U.S.A.).

3. Results and discussion

3.1 Damage and decay

Fungal infection and browning of the calyx end were the main causes of damage and decay in irradiated fruit (Table 1). Control fruit did not develop fungal infections or browning during the experiment. The incidence of fungal infections was minimal (0.6-1.3%) and independent of irradiation dose during cold storage. Similar rates of spoilage were observed in mandarins of several genotypes irradiated up to 300 Gy during refrigerated storage (Miller et al., 2000). In the fourth week of storage at 20 °C, fruit treated at 150 Gy maintained low incidence of rotting (1.3%). However, 400 and 1000 Gy treated samples were heavily infected and incidence of fungal infection in these fruit in their cases was so high, that it was difficult to evaluate the fruit, thus no tests could be performed at the last test day. Similarly, Zhang et al. (2014a) demonstrated that the postharvest decay of Shatang mandarins increased at irradiation dose at and above 400 Gy. Ladaniya et al. (2003) also demonstrated that mandarins irradiated with high doses (1000-1500 Gy) were more susceptible to fungal infections than those treated with low irradiation doses (250-500 Gy). Rojas-Argudo et al. (2012) showed that high levels of phytoalexins provide resistance to fungal rot in mandarins. However, they observed that physical damage in irradiated mandarins, especially when stored at ambient temperatures, can lead to a decrease in phytoalexins and the resultant development of

fungal infection. This is in line with our observation of fungal rot developing in irradiated mandarins but only when the fruit was stored at room temperature.

Browning of the calyx (Fig.1), was observed exclusively in irradiated fruit, with 150 Gy fruit showing the lower incidence of this disorder (76.6 %) after the third week of storage, as compared with fruit treated at the higher doses (91.9-99.3%). The severity of browning seemed to increase with irradiation dose, especially for fruit located on the top layer of the cases but browning severity was not objectively evaluated in this study and further studies are needed in this regard. Irradiation-induced peel injury has been observed in oranges (Macfarlane and Roberts, 1968, Guerrero et al., 1967) and grapefruit and mandarins (Ladaniya, 2008) and attributed to higher respiratory rates, and increased activity of enzymes such as peroxidase and phenyl alananine lyase leading to an increase in phenolic compounds. Pitting, a disorder observed in irradiated citrus fruit (McDonald et al., 2013) was not manifested in the irradiated mandarins in this study.

Irradiation at 400 and 1000 Gy decreased pulp firmness (Supplementary Table 1). Similar results have been reported for mandarins of several genotypes (Miller et al., 2000; Ladaniya et al., 2003). This effect might be a consequence of irradiation-mediated modification of cell wall components. Bustos and Mendieta (1988) demonstrated that irradiation doses below 1000 Gy were able to modify protopectin and pectin, two polysaccharides involved in fruit firmness, of Valencia oranges. On the other hand, there was no significant changes in peel firmness due to irradiation or storage (data not shown).

Irradiation did not cause an immediate effect on tristimulus color of pulp; however, yellow and red components (a* and b* values) were reduced in the 400 and 1000 Gy irradiated

samples after 21 days of cold storage (Supplementary Table 1). This decrease in a* and b* values of the pulp coincided with darkening of the peel, but did not correlate with carotenoid values. The color values for control and 150 Gy fruit were similar during storage. Similarly, Mitchell, McLauchlan, Isaacs, Williams, and Nottingham (1992) observed moderate effects of irradiation (75 and 300 Gy) on tristimulus color of 'Ellendale' and 'Imperial' mandarins, especially of a* and b* values, after storage. The tristimulus color of clementines was minimally affected by irradiation (300 Gy) during cold storage (Mahrouz et al., 2002).

3.2 TSS and sugars

The contents of TSS and individual sugars are shown in Table 2. Our values for TSS are within the range typically reported (8.3-15.5%) for mandarins (Mahrouz et al., 2002; Mahmoud, Mohamed, Botros & Sabri, 2011). Sucrose was the most abundant of the individual sugars, followed by fructose and glucose, within the concentration ranges previously reported for this fruit and particularly similar to those reported for 'Garbí', 'Fortune' and 'Kara' mandarins (Matsumoto & Ikoma, 2012; Sdiri, Bermejo, Aleza, Navarro & Salvador, 2012).

The effect of irradiation on TSS has extensively been studied, finding that this variable is slightly or not affected by wide range of irradiation doses (75-2400 Gy) in several mandarin genotypes (Mitchell et al., 1992; Mahmoud et al., 2011). In our study, we did not observe a consistent effect of irradiation on TSS or glucose and fructose. Sucrose, however, was reduced by irradiation at 400 and 1000 Gy. After three weeks, the 1000 Gy

fruit showed higher glucose and fructose concentrations suggesting that these changes were a consequence of a sugar interconversion process whereby the higher dose affected the activity or the biosynthesis of enzymes such as invertases, sucrose synthases, and sucrose phosphate synthases (Yativ, Harary & Wolf, 2010). The *novo* biosynthesis of these enzymes could also be involved in these changes since some studies in other nonclimateric fruits such as Chinese bayberry fruit have demonstrated that irradiation promoted the expression of genes coding these enzymes (Shi, Cao, Shao, Chen, Yang & Zheng, 2016). An increase in sugar content immediately after irradiation has been observed in mangoes (Naresh, Varakumar, Variyar, Sharma & Reddy, 2015) and Chinese bayberry fruit (Shi et al., 2016). In our study, the effect of storage on sugar content was not apparent.

3.3 Organic acids

The TA and concentration of organic acids in tested fruit are shown in Table 2. Our values of TA are within the range typically reported (0.2-1.9%) for irradiated and non-irradiated mandarins from several cultivars (Miller et al., 2000; Palou et al., 2007). The organic acids evaluated in this study have also been reported previously for mandarins and other citrus fruits, although the content of oxalic, fumaric, and tartaric acids are often not quantified in mandarins (Matsumoto et al., 2012; Sdiri et al., 2012). As expected, citric acid was the most abundant among tested acids. Our values for this acid were similar to those reported (4.9-16.9 g/L) for several mandarin genotypes (Matsumoto et al., 2012; Sdiri et al., 2012). Citrus fruits are regarded as an important source of ascorbic acid. The initial content of

this acid in tested mandarins was in the range typically reported (21-600 mg/L) previously for this fruit (Mitchell et al., 1992; Mahrouz et al., 2002).

Irradiation did not affect TA (Table 2). Miller et al. (2000) observed that the TA values increased the irradiation dose in Murcott mandarins; however, in other mandarin genotypes, irradiation either did not alter or decreased TA. Interestingly, the content of individual organic acids increased immediately after irradiation application (Table 2) as compared to the control. The increase in organic acids suggests alterations of the normal function of the tricarboxylic acid cycle. Similarly, Surendranathan and Nair (1980) observed the accumulation of organic acids in irradiated preclimateric bananas as compared with non-irradiated fruit. They demonstrated that gamma irradiation shifted the glycolytic pathway to the pentose phosphate pathway, causing a reduction in the production of energy and an increased usage of proteins to enhance the gluconeogenic flux. The increase of organic acids and reducing sugars by irradiation and the probable involvement of amino acids on such increases of sugars and acids was also hypothesized in carrots (Ismail, Afifi & Fahmy, 1977). Few studies are available in this regard, with limitations in the range of irradiation doses, storage conditions, and fruit type. Thus, our data suggest that irradiation modified the activity of several enzymes (i.e. phosphoenolpyruvate carboxykinase, isocitratelyase, fructose diphosphatase, Asp-α-KG, Ala-α-KG, and those of glyoxylate cycle) that initially caused an increase in organic acid content at the beginning of the experiment. After storage for three weeks however, the impact of irradiation was diminished. In regards to ascorbic acid, there was an initial increase in this acid but after three weeks of storage, ascorbic acid showed a clear dose dependent decrease (Table 2). Similar dose dependent reductions in ascorbic acid have

been observed in Nagpur mandarins (Ladaniya et al., 2003) and Imperial mandarins (Mitchell et al., 1992). The extent of decrease in mandarins has been shown to depend on genotype (Mitchell et al., 1992). The irradiation-mediated loss of vitamin C in vegetable foods has been attributed to the direct oxidation of ascorbic through the action of free radicals generated by the water radiolysis as well as by the involvement of ascorbic acid in the protection of other compounds against the oxidative damage (Wong & Kitts, 2001).

3.4 Phenolic compounds

The phenolic content in tested fruit is shown in Table 3. Our values of total phenols (TPC) are within the range reported (263.1-557.3 mg/L) for eleven mandarin cultivars cultivated in Spain (Simón-Grao et al., 2014). Similar values of TPC were also reported for Chinese mandarins (Zhang et al., 2014b). Three phenolic acids and four flavonoids were identified in the juice of tested mandarins (Table 3). Ferulic acid was the most abundant phenolic acid in tested juice, as observed in fruit from other genotypes (Shen, Sun, Qiao, Chen, Liu & Ye, 2013). Hesperidin and narirutin were the most abundant flavonoids in tested juice, as reported for many other mandarin genotypes (Zhang et al., 2014b; Shen et al., 2013; Oufedjikh, Mahrouz, Lacroix, Amiot & Taccini, 1998). The levels of hesperidin in tested fruit were similar to those reported (229-287 mg/L) for irradiated and non-irradiated mandarins (Rojas-Argudo et al., 2012). Interestingly, other flavonoids characteristic of mandarins, like naringin, naringenin and neohesperedin, were not detected. Recently, Zhang et al. (2014b) demonstrated that the flavonoid composition in mandarins strongly depended on genotype, with some genotypes showing quite similar qualitative

composition of phenolic compounds (phenolic acids and flavonoids) to that observed in the present study.

Irradiation immediately increased the content of total and individual phenols, except hesperidin (Table 3). Similar increases of phenolic compounds, including that of hesperidin, after application of low (510 and 875 Gy) and high irradiation (37900 Gy) doses has been observed in Clemenules and mandarin pomaces (Kim, Lee, Lee, Nam & Lee, 2008; Rojas-Argudo et al., 2012). In contrast, Oufedjikh et al. (1998) observed small decreases in hesperidin and other phenolic compounds immediately after irradiation application (300 Gy) in Clementines. The causes for this immediate increase in phenols by irradiation in mandarins and other fruits has been attributed to the stimulation of phenylalanine ammonia-lyase (PAL) activity as a response of the fruit to the stress caused by ionizing energy, as occurs with other stressing postharvest treatments (Rojas-Argudo et al., 2012; Shen et al., 2013). Undoubtedly, the irradiation-mediated generation of free radicals from water splitting could also reduce the levels of phenolic compounds; however, our data suggest that this negative effect was low or masked by the positive effect of PAL stimulation by irradiation.

The TPC and tested individual phenols significantly increased in control fruit during storage (Table 3) although the effect of storage was not clear for irradiated fruit. After cold storage, the total phenolics was highest in the 1000 Gy mandarins but individual phenol content was mostly lower in irradiated than in control fruit, demonstrating that irradiation exert a negative effect on the content of some phenolic during long storage of mandarins. Oufedjikh et al. (1998) also observed that the phenolic content was consistently lower in irradiated than in control mandarins during cold storage for 49 days.

3.5 Carotenoids and α-tocopherol

The crude extract contained 20 different carotenoid species according to their UV-Vis spectra, including *Z* and all-*E* isomers of free and esterified of carotenoids. Excepting data of β-cryptoxanthin, all-*E* and *Z* isomers of free xanthophylls were observed in small amounts. The content of the different isomers of each carotenoid were grouped in their free and esterified forms and shown in Table 4. β-cryptoxanthin and violaxanthin, in free and esterified form, were the most abundant xanthophylls in tested fruit. Similar concentrations and relative abundances for these xanthophylls have been reported in Ponkan and Satsuma mandarins (Lin & Chen, 1995; Matsumoto, Ikoma, Kato, Nakajima & Hasegawa, 2009). Luteoxanthin, mutatoxanthin and zeinoxanthin were not observed in tested fruit. β-carotene was more abundant than α-carotene; both of which were observed at very small amounts (Table 6), agreeing with previous studies on mandarin carotenoids (Lin et al., 1995; Matsumoto et al., 2009). The carotene content was significantly lower than that of total xanthophylls, as observed in fruit from several mandarin genotypes (Matsumoto et al., 2009).

The effect of irradiation on carotenoid composition of mandarins has not been previously documented. In our study, irradiation did not promote all-*E* to *Z* isomerization, as evidenced by the absence of increases in the content of *Z* isomers as irradiation dose increased. As expected, irradiation immediately reduced the content of tested carotenoids in a dose dependent trend (Table 4). This detrimental effect of irradiation on carotenoid content might be attributed to the irradiation-mediated generation of hydroxyl radicals

from water splitting and the subsequent production of other highly reactive free radicals, like peroxyl and alkoxyl radicals, which show strong reactivity with carotenoids and vitamin E and concomitant depletion of these antioxidants (Bramley et al., 2000; van den Berg et al., 2000). Such radicals are characteristic of membrane lipid oxidation (Bramley et al., 2000), allowing the hypothesis that irradiation caused lipid oxidation in tested fruit. This inference is supported by the immediate and sustained reduction of α-tocopherol levels in tested fruit as the irradiation dose increased (Table 4) since tocopherols are the most potent antioxidants of lipids in the cellular membranes (Bramley et al., 2000). The initial reduction of the carotenoid content by irradiation was statistically similar for free and esterified versions of the same xanthophyll. Similarly, Pérez-Gálvez and Mínguez-Mosquera (2002) demonstrated that antioxidant capacity and degradation susceptibility of some carotenoids by free radicals were not dependent on esterification.

In general, cold storage reduced the levels of carotenoids and α-tocopherol (Table 4), with this effect being more evident for control than for irradiated fruit. However, the levels of tested compounds increased in irradiated and control fruit upon removal from cold storage, suggesting that carotenoids are being generated. The effect of temperature storage on carotenoid biosynthesis has previously been studied. Matsumoto et al. (2009) demonstrated that the content of many carotenoids was higher in fruit stored at 20 °C than 5 °C, explaining the increased levels of some carotenoids observed in the present study by the end of the experiment. They observed that cold storage (5 °C) reduced the carotenoid content, as occurred in our study.

3.6 Volatiles

Fifty volatile compounds were identified in control and irradiated mandarin samples consisting of aldehydes, alcohols, esters, sesquiterpenes, monoterpenes and ketones (data not shown). These components have been commonly found in other varieties of mandarins (Tietel, Plotto, Fallik, Lewinsohn, Porat, 2010; Ummarat, Arpaia, Obenland, 2015). Thirty-nine of these volatiles were significantly changed due to irradiation treatment at one or more of the three time points (Table 5). At the beginning of the experiment, irradiated fruit had higher volatile concentrations following both 150 and 400 Gy treatments but had reduced concentrations in 13 volatiles, relative to the control, after 1000 Gy treatment. This loss in concentration was particularly prevalent in the aldehydes (4), ketones (2) and terpene alcohols (4). Alcohols were increased by irradiation treatment the greatest degree and, with the exception of octanol, concentrations were greater than those in untreated fruit, even at 1000 Gy. Storage for 3 weeks at 6 ºC after irradiation treatment magnified the effect of the 400 Gy and 1000 Gy treatments and resulted in greater increases in most of the volatile compounds relative to that seen in fruit at the beginning of the experiment. In this case, 1000 Gy caused significant increases in nearly every volatile compound. The large impact of cold storage was most dramatically illustrated in the response of ethanol to irradiation at 6 and 20 °C. In contrast, for many of the volatiles the effect of 150 Gy treatment was lessened and only six of the 39 compounds remained significantly different from the untreated control following cold storage. Holding the mandarins an additional week at 20 ºC following cold storage also had a large impact on the response to irradiation at 150 Gy. In this case, ethanol and 2-methyl-3-buten-2-ol and most of the esters were further increased in amount while many

of the other volatiles lost in concentration relative to that measured following cold storage. Ethanol has often been noted to be a marker of stress in fruit tissues and has been previously reported to accumulate in enhanced amounts in citrus as a result of irradiation (McDonald et al., 2013), indicating an impact of the irradiation treatment on the metabolism of the fruit. In comparison to the prior research, however, ethanol concentrations were considerably lower in this study, even following irradiation. This was likely in a large part due to the fact that the 'Seedless Kishu' were unwaxed and probably had higher internal oxygen levels. Ethyl esters, compounds that can increase in response to high ethanol levels and contribute to off-flavor (Tietel, Fallik, Lewinsohn & Porat, 2011), were also present in much smaller amounts than observed in the prior study. This may indicate that it might be safer to irradiate unwaxed fruit, as was done in this test, in terms of considering the potential effects on flavor.

4. Conclusions

Seedless Kishu mandarins have physical and chemical attributes similar to other mandarins, although there were some differences such as the lack of phenolic compounds- naringin, naringenin and neohesperedin. The kishu mandarins were highly sensitive to gamma irradiation even at 150 Gy, the lowest generic doses approved by USDA-APHIS for postharvest phytosanitary treatment. Irradiation negatively affected the appearance, firmness, and promoted fungal infections during storage. Irradiation also significantly modified the composition of many compounds involved in the sensory, nutrient and health-promoting attributes of mandarins. These effects were in many cases

dose-dependent. We conclude that the Kishu mandarin is not a good candidate to be treated with irradiation for phytosanitary purposes.

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651

652 **Figure Captions**

653 **Fig. 1.** Damage evident in irradiated kishu mandarins after three weeks of storage.