

3-19-2017

## Effect of Phytosanitary Irradiation on the Postharvest Quality of Seedless Kishu Mandarins (*Citrus kinokuni mukakukishu*)

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### Recommended Citation

Ornelas-Paz, J. d. J., Meza, M. B., Obeland, D., Rodríguez (Frischia), K., Jain, A., Thornton, S., & Prakash, A. (2017). Effect of phytosanitary irradiation on the postharvest quality of Seedless Kishu mandarins (*Citrus kinokuni mukakukishu*). *Food Chemistry*, 230: 712-720. doi: 10.1016/j.foodchem.2017.02.125

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

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23 **Running head:** Postharvest quality of irradiated mandarins

24 **Abstract**

25 Transnational trade of 'Seedless kishu' mandarins (*Citrus kinokuni mukakukishu*) would  
26 require them to be subjected to a suitable phytosanitary treatment. Irradiation is used as  
27 an effective treatment for many fruit, but the effect on quality of kishu mandarins is  
28 unknown. 'Seedless kishu' mandarins were treated with gamma irradiation (150, 400, and  
29 1000 Gy) and stored for three weeks at 6 °C and then for one week at 20 °C. Irradiation  
30 at 400 and 1000 Gy promoted browning of the calyx end and fungal infection. Irradiation  
31 caused immediate reductions in pulp firmness, vitamin E, individual sugars and  
32 carotenoids but increased the content of organic acids, except ascorbic acid, and phenolic  
33 compounds. The volatile profile of tested fruit was also differentially altered by irradiation.  
34 Most of these initial changes were dose dependent. 'Seedless Kishu' mandarins are  
35 significantly sensitive to irradiation and are not suitable for treatment at the studied doses.

36

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

39

40 **1. Introduction**

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

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

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

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

82

## 83 **2. Materials and Methods**

84 **2.1 Chemicals and solvents.** All reagents and solvents were of analytical or HPLC grade  
85 and were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A). The standard  
86 compounds for sugars (D-(+)-glucose, D-(-)-fructose and sucrose), phenolic compounds  
87 (gallic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid, hesperidin, narirutin, rutin and  
88 (-)-epicatechin), organic acids (citric, succinic, L-(+)-tartaric, DL-malic, L-ascorbic, oxalic,  
89 and fumaric), *all-rac*- $\alpha$ -tocopherol, volatile compounds, and some carotenoids (*all-trans*-  
90  $\beta$ -cryptoxanthin, *all-trans*-lutein, *all-trans*- $\alpha$  and *all-trans*- $\beta$ -carotene from carrots) were

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

## 93 2.2 Fruit procurement, treatment, and storage

94 'Seedless kishu' mandarins (*Citrus kinokuni mukakukishu*) were harvested in Exeter, CA,  
95 U.S.A. The average weight, diameter, and height of the fruits were 37.7 g, 42.1 mm and  
96 27.9 mm, respectively. They were hand cleaned and packed in 6.8 kg cartons (29.2 cm  
97 wide, 43.2 cm long, and 14 cm high) by California Citrus Specialties. Most commercial  
98 packing houses do not apply wax or fungicides to these mandarins and none was applied  
99 to these fruit. After cleaning, the fruit was ground transported to Chapman University and  
100 kept at 20 °C. The next day, the fruit was taken to Sterigenics, Inc. (Tustin, CA, U.S.A.),  
101 for treatment. Ten cases of mandarins were located two rows high and five across in front  
102 of a <sup>60</sup>Co source (~37PBq). Dose mapping was conducted by placing 24 alanine pellet  
103 dosimeters (FarWest Technology, In., Goleta, CA, U.S.A.) at various locations in the  
104 cases. The dose rate was 0.637 Gy/s. Ten cases of mandarins were placed exactly in  
105 the same configuration as the dummy cases to receive treatment at a target dose of 150,  
106 400, and 1000 Gy (4.6-5.5% uncertainty) and Dmax/Dmin ratio of 1.33. Midway through  
107 treatment, the boxes were rotated 180° to ensure uniform treatment. After treatment, the  
108 mandarins were transported to Chapman University and stored at 6 °C (relative humidity  
109 (RH) = 85-90%) for 21 days. Then, the cases were opened and kept at 20 °C (RH = 85-  
110 90%) for 7 days. After 2, 21 and 28 days, three cartons of each experimental group were  
111 removed from storage. Two cartons were used for physical and chemical analyses. One  
112 carton of each treatment was intermittently removed from storage to evaluate the  
113 development of disorders and fungal infections. For chemical analyses, at least 150 fruits

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

121

### 122 2.3 Peel disorders and fungal infections

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

128

### 129 2.4 Evaluation of TSS, TA, tristimulus color, and firmness

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

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

144

## 145 2.5 Analysis of sugars

146 The content of individual sugars was determined according to Ornelas-Paz et al. (2013),  
147 with some modifications. Aliquots of juice (100  $\mu$ L) were diluted with HPLC grade water  
148 (2 mL), filtered using a nylon membrane with a pore size of 0.45  $\mu$ m (Pall Corp., New  
149 York, U.S.A.), and automatically injected (20  $\mu$ L) into a 1100 series HPLC system (Agilent  
150 Inc., CA, U.S.A.) equipped with a refractive index detector. The sugars (sucrose, glucose  
151 and fructose) were separated in a SUGAR SC 1821 (300 x 8.0 mm I.D., 6  $\mu$ m particle  
152 size) ion-exchange column (Showa Denko K.K.; Tokyo, Japan) at 80 °C. The mobile  
153 phase was HPLC-grade water at a flow rate of 0.8 mL/min. The sugars were identified  
154 by comparing their chromatographic behavior with that of reference compounds.  
155 Quantitative data were obtained by calibration curves constructed with three independent  
156 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<sub>2</sub>SO<sub>4</sub>. 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<sub>2</sub>SO<sub>4</sub> 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

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

181 For total phenols content, 100  $\mu\text{L}$  of filtered juice were mixed with 100  $\mu\text{L}$  of Folin-  
182 Ciocalteu reagent, 3 mL of deionized water and 100  $\mu\text{L}$  of 20%  $\text{Na}_2\text{CO}_3$ . The mixture was  
183 vigorously shaken for 1 min and incubated for 1h in the darkness. The absorbance was  
184 evaluated five times at 765 nm using a FLUOstar Omega microplate reader (BMG  
185 LABTECH Inc.; Cary, NC, U.S.A.). The absorbance values were corrected with those  
186 generated with blank reactions. The quantification was performed using a calibration  
187 curve constructed with several sets of dilutions of gallic acid. The results were expressed  
188 as mg gallic acid equivalents (GAE) per liter of juice.

189

## 190 2.8 Determination of carotenoid and tocopherol content

191 These compounds were analyzed simultaneously according to Ornelas-Paz, Yahia and  
192 Gardea-Bejar (2007). Briefly, 4 g of mandarin pulp was mixed with  $\text{CaCO}_3$  (0.2 g) and 20  
193 mL of methanol. The mixture was filtered through a Whatman paper No. 3, recovering the  
194 methanolic extract. The retained solids were sequentially depigmented with 100 mL of  
195 methanol and 75 mL of a mixture of hexane:acetone (1:1, v/v). The extract was placed  
196 into a separatory funnel and 30 mL of hexane were added. The mixture was shaken  
197 vigorously and 40 mL of 10%  $\text{Na}_2\text{SO}_4$  were added. After phase separation, the upper  
198 phase was recovered and the solvent evaporated at 40  $^\circ\text{C}$  under reduced pressure.  
199 These residues were analyzed without and with saponification in order to identify free and  
200 esterified xanthophylls. For saponification, the residues were dissolved in 30 mL of diethyl  
201 ether and 0.6 mL of 40% KOH in methanol were added. The mixture was vigorously

202 shaken and kept in the darkness for 16 h. Then, the sample was washed with water and  
203 the organic solvent evaporated at reduced pressure. Crude and saponified residues were  
204 dissolved in methanol (4 mL), filtered and injected in the HPLC system (20  $\mu$ L) described  
205 above. The separation was performed in a YMC C30 column (150 x 4.6 mm I.D., 3  $\mu$ m  
206 particle size) (YMC America Inc., Allentown, PA, U.S.A.) at 15 °C. The UV-Vis spectra of  
207 carotenoids was recorded from 300 to 750 nm in steps of 1 nm. The  $\alpha$ -tocopherol was  
208 monitored with a fluorescence detector ( $\lambda_{ex}$ = 294 nm,  $\lambda_{em}$  = 326 nm). The mobile phase  
209 was composed of water (solvent A), methanol (solvent B) and methyl *tert*-butyl ether  
210 (MTBE, solvent C) according to the following gradient: 4%A/ 94.5%B/ 1.5%C at 0 min,  
211 4%A/ 68%B/ 28%C at 31 min, 4%A/ 30%B/ 66%C at 83 min. The identification of  
212 carotenoid esters was performed by comparing the chromatographic behavior of crude  
213 and saponified extracts. The chromatographic behavior and UV-Vis of peaks of the  
214 sample were also compared with those of reference compounds. The quantification was  
215 performed using calibration curves constructed with reference compounds. The *cis*  
216 isomers were quantified as all-*E* carotenoids.

## 217 2.9 Volatile analysis

218 Five samples per treatment (5 mL each) were placed into headspace vials (12 x 32 mm)  
219 and 5 mL of saturated sodium chloride was added. 1-pentanol (1.1 mL, final  
220 concentration of 490  $\mu$ g/L) was added to each sample as internal standard. The  
221 headspace vials were capped with a Teflon-coated septum. The analysis of volatile  
222 components was completed using solid phase microextraction (SPME) with a 75- $\mu$ m  
223 carboxen/polydimethylsiloxane fiber (Supelco, St. Louis, MO, USA). A Gerstel MPS-2  
224 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<sup>-1</sup>. 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

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

249

### 250 **3. Results and discussion**

#### 251 3.1 Damage and decay

252 Fungal infection and browning of the calyx end were the main causes of damage and  
253 decay in irradiated fruit (Table 1). Control fruit did not develop fungal infections or  
254 browning during the experiment. The incidence of fungal infections was minimal (0.6-  
255 1.3%) and independent of irradiation dose during cold storage. Similar rates of spoilage  
256 were observed in mandarins of several genotypes irradiated up to 300 Gy during  
257 refrigerated storage (Miller et al., 2000). In the fourth week of storage at 20 °C, fruit  
258 treated at 150 Gy maintained low incidence of rotting (1.3%). However, 400 and 1000 Gy  
259 treated samples were heavily infected and incidence of fungal infection in these fruit in  
260 their cases was so high, that it was difficult to evaluate the fruit, thus no tests could be  
261 performed at the last test day. Similarly, Zhang et al. (2014a) demonstrated that the  
262 postharvest decay of Shatang mandarins increased at irradiation dose at and above 400  
263 Gy. Ladaniya et al. (2003) also demonstrated that mandarins irradiated with high doses  
264 (1000-1500 Gy) were more susceptible to fungal infections than those treated with low  
265 irradiation doses (250-500 Gy). Rojas-Argudo et al. (2012) showed that high levels of  
266 phytoalexins provide resistance to fungal rot in mandarins. However, they observed that  
267 physical damage in irradiated mandarins, especially when stored at ambient  
268 temperatures, can lead to a decrease in phytoalexins and the resultant development of

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

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

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

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

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

299

### 300 3.2 TSS and sugars

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

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

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

324

### 325 3.3 Organic acids

326 The TA and concentration of organic acids in tested fruit are shown in Table 2. Our values  
327 of TA are within the range typically reported (0.2-1.9%) for irradiated and non-irradiated  
328 mandarins from several cultivars (Miller et al., 2000; Palou et al., 2007). The organic acids  
329 evaluated in this study have also been reported previously for mandarins and other citrus  
330 fruits, although the content of oxalic, fumaric, and tartaric acids are often not quantified in  
331 mandarins (Matsumoto et al., 2012; Sdiri et al., 2012). As expected, citric acid was the  
332 most abundant among tested acids. Our values for this acid were similar to those reported  
333 (4.9-16.9 g/L) for several mandarin genotypes (Matsumoto et al., 2012; Sdiri et al., 2012).  
334 Citrus fruits are regarded as an important source of ascorbic acid. The initial content of

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

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

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

364

### 365 3.4 Phenolic compounds

366 The phenolic content in tested fruit is shown in Table 3. Our values of total phenols (TPC)  
367 are within the range reported (263.1-557.3 mg/L) for eleven mandarin cultivars cultivated  
368 in Spain (Simón-Grao et al., 2014). Similar values of TPC were also reported for Chinese  
369 mandarins (Zhang et al., 2014b). Three phenolic acids and four flavonoids were identified  
370 in the juice of tested mandarins (Table 3). Ferulic acid was the most abundant phenolic  
371 acid in tested juice, as observed in fruit from other genotypes (Shen, Sun, Qiao, Chen,  
372 Liu & Ye, 2013). Hesperidin and narirutin were the most abundant flavonoids in tested  
373 juice, as reported for many other mandarin genotypes (Zhang et al., 2014b; Shen et al.,  
374 2013; Oufedjikh, Mahrouz, Lacroix, Amiot & Taccini, 1998). The levels of hesperidin in  
375 tested fruit were similar to those reported (229-287 mg/L) for irradiated and non-irradiated  
376 mandarins (Rojas-Argudo et al., 2012). Interestingly, other flavonoids characteristic of  
377 mandarins, like naringin, naringenin and neohesperedin, were not detected. Recently,  
378 Zhang et al. (2014b) demonstrated that the flavonoid composition in mandarins strongly  
379 depended on genotype, with some genotypes showing quite similar qualitative

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

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

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

403

### 404 3.5 Carotenoids and $\alpha$ -tocopherol

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

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

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

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

446

### 447 3.6 Volatiles

448 Fifty volatile compounds were identified in control and irradiated mandarin samples  
449 consisting of aldehydes, alcohols, esters, sesquiterpenes, monoterpenes and ketones  
450 (data not shown). These components have been commonly found in other varieties of  
451 mandarins (Tietel, Plotto, Fallik, Lewinsohn, Porat, 2010; Ummarat, Arpaia, Obenland,  
452 2015). Thirty-nine of these volatiles were significantly changed due to irradiation  
453 treatment at one or more of the three time points (Table 5). At the beginning of the  
454 experiment, irradiated fruit had higher volatile concentrations following both 150 and 400  
455 Gy treatments but had reduced concentrations in 13 volatiles, relative to the control, after  
456 1000 Gy treatment. This loss in concentration was particularly prevalent in the aldehydes  
457 (4), ketones (2) and terpene alcohols (4). Alcohols were increased by irradiation treatment  
458 the greatest degree and, with the exception of octanol, concentrations were greater than  
459 those in untreated fruit, even at 1000 Gy. Storage for 3 weeks at 6 °C after irradiation  
460 treatment magnified the effect of the 400 Gy and 1000 Gy treatments and resulted in  
461 greater increases in most of the volatile compounds relative to that seen in fruit at the  
462 beginning of the experiment. In this case, 1000 Gy caused significant increases in nearly  
463 every volatile compound. The large impact of cold storage was most dramatically  
464 illustrated in the response of ethanol to irradiation at 6 and 20 °C. In contrast, for many  
465 of the volatiles the effect of 150 Gy treatment was lessened and only six of the 39  
466 compounds remained significantly different from the untreated control following cold  
467 storage. Holding the mandarins an additional week at 20 °C following cold storage also  
468 had a large impact on the response to irradiation at 150 Gy. In this case, ethanol and 2-  
469 methyl-3-buten-2-ol and most of the esters were further increased in amount while many

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

482

#### 483 **4. Conclusions**

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

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

494

#### 495 **Acknowledgements**

496 J.J. Ornelas-Paz thanks CONACYT (Mexico) for providing support for his sabbatical leave  
497 at Chapman University. This research was funded by a TASC grant from USDA-FAS.  
498 The authors wish to thank David Karp for his suggestions and Lance Walheim (California  
499 Citrus Specialties) for providing the fruit needed for this study.

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651

## 652 **Figure Captions**

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