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Running head: Postharvest quality of irradiated oranges

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Abstract

The objective of this study was to determine if oranges in the top and bottom layers within a Standard Place Pack were impacted differently by irradiation after long-term storage. ´Barnfield´ Navel oranges were packed in Standard Place Pack cartons and treated with 0, 0.15 or 1 kGy of gamma irradiation. The fruit were stored for three weeks at 5 °C and then for one week at 20 °C. After storage, the fruit from the top and bottom layers were separately evaluated for quality. The development of stem end rind breakdown (SERB) was the main cause of quality loss and was greater in irradiated fruit in the top layer. Fruit in the bottom layer showed more physical damage (flattening) but lower incidence of SERB. The changes in individual sugar content were minimal but significant for layer. The content of individual organic acids was consistently lower in irradiated fruit from the bottom layer. Layer type showed a stronger effect on phenolic compounds than irradiation dose. The tristimulus color, total soluble solids, titratable acidity, and firmness of fruit were not influenced by irradiation dose or layer type. The results show that damage in irradiated Navel oranges depends on dose and layer, with the top layers showing greater physiological damage and bottom layers showing more physical damage.

Keywords: Ionizing energy; Physiological disorders; Chemical composition; Citrus; Phytosanitary treatment; Postharvest quality

Introduction

The US is one of the largest orange producing countries in the world, with California and Florida providing much of the oranges for the fresh market and for processing, respectively (USDA 2017). Fresh oranges from the US are exported to several countries, mainly to South Korea, Canada, and Japan (AMRC 2013). However, the production of oranges in the US has decreased slightly but continuously in recent years, causing an increase in the importation of oranges from countries such as China, Australia, Mexico, Jamaica, and the Philippines (APHIS 2014; USDA 2017). The high volume of international and domestic trade of oranges can infer a high risk for the spread of quarantine pests. Thus, oranges being imported, exported, or even moved within the US must be subjected to phytosanitary treatments before shipment (APHIS 2014). Several postharvest phytosanitary treatments have been approved for citrus fruits but irradiation has advantages over the other treatments in terms of exposure of fruit to unsuitable high or low temperatures for extended periods of time, human safety, and environmental impacts (Hallman 2012).

Generic doses of 0.15 and 0.4 kGy are approved to control many classes of insects except the pupae and adult stages of Lepidoptera (APHIS 2014), while a maximum of 1 kGy is allowed by the FDA for use on fresh fruits and vegetables (Follett and Wall 2013; Hallman 2012). The dose of 0.15 kGy is sufficient to control insects commonly found on oranges and may limit the negative effects on quality, which are manifested in oranges as softening, peel injury, chemical (loss of nutrients, bioactive compounds, volatiles) and sensory changes (Ladaniya et al. 2003; McDonald et al. 2013; Miller et al. 2000; Nagai and Moy 1985). Many of these effects have been related to the

irradiation-mediated increase in ethylene biosynthesis and respiration rate (Ladaniya et al. 2003), but the incidence and severity of these negative effects depend on orange variety, maturity stage, and irradiation dose (Bustos and Mendieta 1988; Miller et al. 2000; Nagai and Moy 1985).

Oranges in the US are generally packed precisely in four layers in 18.1 kg Standard Place Pack cartons. They are typically packed without protective trays between layers, resulting in the compression of fruit, especially in the bottom layer during long-distance shipping (Moresi et al. 2012). This kind of damage can compromise the appearance of the fruit and might cause the rejection of the entire fruit shipment (Mazidi et al. 2016). Compression damage in citrus fruits, as with any other physical damage, triggers a burst of ethylene production (Lu et al. 2014) directly or indirectly impacting the respiration rate, and resulting in softening, peel injury and chemical changes (Porat et al. 2004; Rojas-Argudo et al. 2010, 2012). Compression damage can alter the levels of sugars, organic acids (mainly ascorbic acid), volatiles, and phenols in citrus fruit (Mazidi et al. 2016; Obenland et al., 2018; Rojas-Argudo et al. 2010, 2012). Transportation temperature and distance also influence the severity and incidence of compression damage (Ahmadi 2012; Ahmadi et al. 2010).

Irradiation-induced stress in oranges can elicit a similar physiological response to that of compression damage (Mazidi et al. 2016; Rojas-Argudo et al. 2012). Thus, the combination of compression and irradiation might exacerbate postharvest damage in oranges. In our previous work, we noted that the undesirable effects of irradiation on Navel oranges, especially peel injury, seemed to depend on the position of the fruit within the case, but this phenomenon was not systematically investigated (McDonald

et al. 2013), and the literature does not provide any information in this regard. The objective of this study was to determine if oranges in the top and bottom layers within a Standard Place Pack were impacted differently by irradiation after long-term storage. The 0.15 kGy dose was selected since it is the minimum target dose for oriental fruit fly. A dose of 1 kGy was included to accentuate the impacts of irradiation on the fruit to allow these effects to be detected and measured.

Materials and Methods

Fruit Procurement, Treatment, and Storage

´Barnfield´ Navel Oranges (*Citrus sinensis* (L.) Osbeck) (size 72) were harvested from a commercial orchard in Kern County, CA, USA. The fruit were commercially treated and packed by Paramount Citrus Exchange (Delano, CA, USA). The handling involved three washing steps with chlorine; one at dumping point (150 mg/L), then at high pressure (200 mg/L, 862 kPa) and a final immersion for 3 min in 3% sodium bicarbonate solution containing chlorine (200 mg/L). After rinsing with water, the fruit were treated with Imazalil (300 mg/L) for 30 s in an immersion tank, then rinsed with water. Finally, the fruits were waxed with a carnauba based wax containing Imazalil (1 g/L) and thiabendazole (3.5 g/L). The oranges were bulk packed in 18.14 kg (72-80 fruits) Standard Place Pack cartons (40.6 x 27.9 x 25.4 cm) and refrigerated at 5 °C. In each carton, the fruit were distributed in four layers; each layer containing ~20 fruit. The oranges were transported to Sterigenics, Inc. (Tustin, CA, USA), for treatment, where six cases of oranges were placed two rows high and three across at a precise distance from a ^{60}Co source (~37PBq). Dose mapping was conducted by

114 placing 24 alanine pellet dosimeters (FarWest Technology, Inc., Goleta, CA, USA) at
115 various locations in the cases. The dose rate was determined to be 0.637 Gy/s. Six
116 cases of oranges were placed exactly in the same configuration as the dummy cases
117 to receive treatment at a target dose of 0.15 and 1 kGy (4.6-5.5% uncertainty) and
118 Dmax/Dmin ratio of 1.33. Midway through treatment, the boxes were rotated 180° to
119 ensure uniform treatment. After treatment, the oranges were transported to Chapman
120 University, and stored at 5 °C and 95% RH for 3 weeks to simulate sea shipment to
121 Asian markets. After cold storage, the oranges were placed at room temperature (20
122 °C) for one week to simulate retail display. Following this four week storage, twenty
123 fruit from the top and bottom layers of each of the four cases were pooled, to obtain a
124 total of 80 fruit for each layer. Of the 80 fruit per layer, ten were used to measure
125 tristimulus color and ten for firmness. Sixty fruits were distributed in five subsamples,
126 juiced and the juice was used to measure titratable acidity (TA), total soluble solids
127 (TSS), individual sugars, organic acids, and total and individual phenols. Twenty
128 oranges from top and bottom layers from the remaining two cartons were evaluated
129 for stem-end breakdown (SERB), fungal infections, shape deformation and weight
130 loss. All fruits included in the experiment were free of physiological, physical and
131 biological damage (flattening, SERB, fungal infections and insect damage). Their
132 average weight (270.7 ± 3.8 g), peel color (L*= 65.1 ± 0.3, a*= 29.4 ± 0.5, b*= 48.1 ±
133 0.9), internal color (L*= 46.4 ± 0.4, a*= 8.7 ± 0.2, b*= 31.1 ± 0.6), peel firmness, (6.8
134 ± 0.5 N), pulp firmness (3321.8 ± 173.0 N mm), TSS content (12.6 ± 0.03 %) and
135 TA (0.43 ± 0.006%) at the beginning of the experiment were characteristic of ripe
136 Navel oranges.

Peel Damage

Shape deformation by compression was reported as the percentage of fruit showing flattening. These fruit were also grouped according to the severity of compression damage. The severity of flattening was estimated by calculating the percent of fruit surface area showing flattening. These areas were converted into a 5 point scale according to Yue et al. (2007), with 0= no damage, 1= 1-4%, 2= 5-8%, 3= 9-12%, 4=13-15%, and 5= 16% or more of damaged surface. The fruit surface area was determined by measuring their equatorial diameter and assuming a spherical shape while the flattened areas were measured using a Vernier caliper.

The incidence and severity of SERB were determined by digital image analysis, given the irregular shape of SERB lesions and the consequent difficulty to be evaluated using a Vernier caliper. The incidence of SERB was determined by calculating the percent of fruit showing this damage. The severity of SERB was determined by estimating the area and color of SERB lesions by digital image analysis. The peduncle side of 20 fruit per layer was photographed using a digital camera. An algorithm, designed in MATLAB (R2010a, MathWorks, USA), was used to determine the area and tristimulus color of the SERB lesions in the digital images. The digital image is an M×N×P array, where M×N represents the image dimension in pixels, while P is the number of color planes, three in this case, corresponding to the matrices R', G', and B'. In order to convert an R'G'B' digital image to an L*a*b* color space (CIELab), the MatLab object ColorSpaceConverter was used. In the L*a*b* color space, L* indicates lightness, a* is the red (+a)/green coordinate (-a), and b* is the yellow (+b)/blue coordinate (-b) (Chen et al., 2010). For the determination of SERB area, the digital

image was transformed to a gray-scale image. The % of area showing SERB lesions was calculated relative to the area of the fruit in the picture.

The incidence of fungal infections was determined by calculating the percent of fruits per layer showing areas with mycelia. The area of the fungal infection (severity) was not determined since under commercial conditions any fruit showing fungal infection must be discarded, in contrast to fruit showing SERB or flattening.

Tristimulus Color of Fruits

Peel color was measured in areas free of injuries at two equidistant points on the equatorial axis of 10 fruit using a CM-2500d Konica Minolta Spectrophotometer (Ramsey, New Jersey, USA). Then, the oranges were cut at the equatorial axis and two color measurements were taken on the internal surface of each half. The L*, a* and b* values were recorded.

Firmness

Firmness of the peels and intact segments of the fruit was measured. For peel firmness, four peel sections were vertically excised from 10 fruit using a paring knife and evaluated for penetration resistance to a 3 mm puncture probe using a TA-XT2 Texture Analyzer (Texture Technology Corp; Scarsdale, NY, USA), which moved downward through the peel at 3 mm/s until breakpoint. The maximum force (N) required to puncture the peel was recorded. For pulp firmness, the segments from the peeled oranges were carefully separated by hand and distributed in eight subsamples of 150 g each. Each subsample of intact segments was placed into a

Kramer Shear Cell (TA-91) and the five flat-blade press, set at 80 mm from the bottom of the cell platform, was moved downward through the segments at 5 mm/s for 75 mm. The area (N.mm) under the force deformation curve was determined.

Weight Loss, Titratable Acidity (TA) and Total Soluble Solids content (TSS)

Weight loss (%) was determined by measuring the change in weight during storage in the fruit used for peel damage determination. For TA, 5 g of juice were diluted with 50 g of water and titrated to a pH of 8.2 with 0.1N NaOH. TA was calculated using the factor of 0.064 for citric acid, according to McDonald et al. (2013). The content of TSS of the juice was directly determined by placing a few drops of juice on the glass surface of a PAL digital refractometer (Atago Co., LTD, Tokyo, Japan).

Sugars

Glucose, fructose, and sucrose were measured according to Ornelas-Paz et al. (2013), with some modifications. An aliquot of juice (100 µL) was mixed with 2 mL of HPLC water. The mixture was filtered with a 45 µm pore size acrodisk and automatically injected (20 µL) into an Agilent 1100 series HPLC system (Agilent Inc., Santa Clara, CA, USA) equipped with a refractive index detector. The separation was performed in a Sugar SC 1821 (8.0 x 300 mm, 6 µm) column at 80 °C with a Sugar SC-LG (6.0 x 50 mm, 10 µm) precolumn (Showa Denko K.K.; Tokyo, Japan). The mobile phase was 100% HPLC grade water at a flow rate of 0.8 mL/min. The sugars

were quantified using calibration curves constructed with at least three independent sets of dilutions of glucose, sucrose, and fructose.

Organic Acids

One mL of juice was mixed with 3 mL of 5 mM H_2SO_4. The mixture was filtered using a 45 μm pore acrodisk and automatically injected (20 μL) into the HPLC system described above, which is also equipped with a diode array detector. The separation was performed using an Aminex HPX-87H ion exchange column (7.8 x 300 mm; Bio-Rad Laboratories, Hercules, CA, USA) at 60 °C. The mobile phase was 5 mM H_2SO_4 and acetonitrile (90:10, v/v) at flow rate of 0.4 mL/min. Oxalic, citric, tartaric, malic, quinnic, succinic, and fumaric acids were monitored at λ=210 nm while ascorbic acid was monitored at λ=260 nm. The quantification was based on calibration curves constructed with at least three independent sets of dilutions of standard compounds.

Phenolic Compounds

The analysis of individual and total phenols was performed simultaneously. The juice was filtered with a membrane of 0.45 μm pore size and directly injected (100 μL) into the HPLC described previously. The separation of phenolic compounds was performed using a Kinetex C18 column (4.6 x 100 mm) (Phenomenex; Torrance, CA, USA) at 30 °C. The phenolic compounds were monitored at λ= 280, 320, 350 and 520 nm. The mobile phase consisted of 2% acetic acid (A), and acetonitrile (B), according to the following gradient: 100% A at 0 min, 93% A at 12 min, 89% A at 20 min, 86%A at 35 min, 84% A at 36 min, 82% A at 41 min, 79% A at 44, 0% A from min 55 to

60. The flow rate was 1 mL/min. The phenolic compounds were identified and quantified by using reference compounds. The UV-Vis spectrum of each phenol was also used for identification purposes.

For total phenolic 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 1 h in the dark. The absorbance was evaluated five times at 765 nm using a FLUOstar Omega microplate reader (BMG LABTECH Inc.; Cary, NC, USA). The absorbance values were corrected with those generated with blank reactions. Quantification was based on a calibration curve constructed with several sets of dilutions of gallic acid. The results were expressed as mg GAE per liter of juice.

Statistical Analysis

The effects of irradiation dose and layer were determined using a linear mixed effects model and pairwise Tukey Kramer Test, using a level of significance of 0.05. Analysis was conducted using R 3.2.3 software with lme4, multcomp, and car packages (R Core Team, 2015, Vienna, Austria).

Results and Discussion

Peel Damage

SERB, flattening and fungal infections were observed in control and irradiated fruit from both layers (Table 1). SERB was characterized by collapsed, darkened, and

248 sunken rind tissue around the calyx, as described by Ritenour et al. (2004). It was
249 observed after cold storage in all treatments, but was more evident after storage at
250 room temperature. This disorder has been observed in other studies for non-irradiated
251 oranges (Alférez et al., 2003). The incidence of SERB lesions increased with
252 irradiation dose (Table 1) ($P<0.05$). Image analysis showed that the area of the
253 lesions was similar for fruit in the top and bottom layers ($P>0.05$) (Fig. 1A). Irradiation
254 dose and layer also affected the L* and b* values in the SERB lesions (Figs. 1B and
255 1C). Fruit treated with 1 kGy showed darker lesions as compared to 0.15 kGy and
256 control fruit ($P<0.05$) and, the fruit from top layer showed darker lesions in all
257 experimental groups as compared with fruit from the bottom layer ($P<0.05$).
258 Differences in gas composition between the top and bottom layers might explain the
259 differences in SERB incidence. Fruit from the bottom layer might produce more CO_2
260 and ethylene because that fruit was subjected to higher stress by compression
261 (flattening). Also, given the higher density of CO_2, fruit in the bottom layer was
262 probably exposed to higher CO_2 levels which might avoid the oxidation of phenolic
263 compounds. This hypothesis might explain the less darkening (higher L* values) of
264 SERB lesions for control and irradiated fruit in the bottom layer (Fig. 1). Porat et al.
265 (2004) demonstrated that modified atmosphere packaging (reduced levels of O_2 and
266 increased levels of CO_2) reduced SERB in oranges. On the other hand, most of the
267 C_2H_4 would diffuse from bottom to the top and accumulate there because of its lower
268 density ($CO_2 > O_2 > C_2H_4$). Higher levels of C_2H_4 could lead to increased phenylalanine
269 ammonia lyase (PAL) activity resulting in the production of phenols which are then
270 oxidized by polyphenol oxidase and peroxidase to *o*-quinones that further polymerize

271 to the brown pigments characteristic of SERB lesions (Banerjee et al., 2015).
272 Unfortunately, the low irradiation doses do not inactivate the polyphenol oxidase and
273 peroxidase responsible for phenol oxidation and formation of brown pigments. Alférez
274 et al. (2003) associated the ethylene production of oranges with their susceptibility to
275 develop postharvest browning. Increased ethylene production has also been
276 associated with the development of peel injury in irradiated and wounded citrus fruits,
277 probably due to its involvement in the activation of enzymes such as peroxidase and
278 PAL which are resposible for citrus browning (Ladaniya 2008; Lu et al. 2014;
279 McDonald et al. 2000; Porat et al. 2004). PAL was observed to increase immediately
280 after irradiation treatment in Clementine mandarins (Oufedjikh et al., 2000) and
281 grapefruit (Riov et al., 1975) and correlated with an increase in phenolic compounds
282 in damaged peel cells. Guerrero et al. (1967) attributed rapid rind breakdown to higher
283 respiratory rates in ´Washington Navel´ oranges irradiated with 0.5 to 6 kGy.
284 Flattening was clearly observed in all tested fruit. As expected, fruit in the bottom layer
285 always presented larger flat areas than fruit in top layer for all treatments ($P<0.05$)
286 (Table 2). However, although the incidence of flattening in both layers decreased as
287 the irradiation dose increased, the severity of flattening increased with irradiation dose
288 (Tables 1 and 2). Thus, fruit treated with 0.15 and 1 kGy showed a lower incidence of
289 flattening as compared to control fruit, but the severity of the damage increased with
290 irradiation dose (Tables 1 and 2). Nevertheless, the overall flattened area was no
291 more than 12% of total surface in irradiated fruit and was not the primary contributing
292 factor to a decrease in quality. These findings demonstrate a differential effect of
293 irradiation in non-damaged and compressed (damaged) areas of the oranges. Some

294 studies have demonstrated that irradiation causes different biochemical responses in
295 wounded citrus fruits as compared to fruit that are not wounded (Rojas-Argudo et al,
296 2012); however, there is no information regarding biochemical responses of oranges
297 subjected to compression stress and irradiation as compared with oranges subjected
298 to only irradiation. Besides the mechanical weakening of fruit by compression,
299 ethylene biosynthesis in compressed areas of irradiated oranges might be higher than
300 in not compressed areas, causing a higher enzymatic softening around flattened areas
301 and increasing the severity of this kind of damage. The individual effects of
302 compression and irradiation on ethylene biosynthesis in citrus fruit have been
303 demonstrated previously (Ladaniya et al. 2003; Lu et al. 2014).
304 The incidence of fungal infections was similar for fruit in both layers for all experimental
305 groups ($P>0.05$) (Table 1). Vilanova et al. (2014) observed that the susceptibility of
306 oranges to postharvest infections was increased by fruit wounding. In our study, the
307 0.15 kGy dose did not increase mold growth as compared to the control. However,
308 fruit treated with a dose of 1 kGy showed higher levels of fungal infection most likely
309 due to damage caused to fruit cell walls and release of nutrients that encourage fungal
310 growth (Ladaniya et al. 2003; Zhang et al. 2014). While flattening and decay incidence
311 are both higher in bottom layer fruit and fruit treated at 1 kGy, the high occurrence of
312 decay in the top layer of the 1 kGy fruit, suggests that irradiation by itself at this dose
313 level enhances decay. Rojas-Argudo et al. (2012) demonstrated that low irradiation
314 doses (0.51 kGy) stimulated the biosynthesis of antifungal compounds in citrus fruits
315 and that higher irradiation doses (0.875 kGy) inhibited such biosynthesis, favoring
316 postharvest infections.

Tristimulus Color, Firmness and Weight Loss

Neither irradiation dose nor fruit layer affected tristimulus color and firmness of peel or pulp ($P>0.05$) (data not shown). Similarly, McDonald et al. (2013) demonstrated that the color of Navel oranges was not affected by irradiation doses of up to 0.6 kGy. Weight loss after storage ranged from 6.4 to 9% and was not impacted by irradiation treatment and layer type ($P>0.05$) (data not shown). Miller et al. (2000) demonstrated that irradiation at 0.15, 0.3 and 0.45 kGy did not significantly alter the color, firmness and weight loss in five orange cultivars, including Navel oranges.

Total Soluble Solids (TSS) and Sugars

TSS values, ranging from 12 to12.7% (data not shown), were similar to those reported previously for oranges (McDonald et al. 2013; Miller et al. 2000), and were not affected by irradiation dose or layer type ($P>0.05$). Similarly, Miller et al. (2000) evaluated the effect of irradiation (0.15-0.45 kGy) on TSS content in fruit from five orange cultivars, including Navel oranges, and found that irradiation did not affect TSS.

Sucrose was the most abundant of measured sugars in the oranges ($P<0.05$), followed by glucose and fructose, which showed similar content ($P>0.05$) (Fig. 2). Similar sugar composition has been reported previously for oranges (Kelebek et al. 2009; Roussos 2011). The changes in sugar content as a function of irradiation dose and layer type were very small but significant in some cases. In fruit from top layer, sucrose content tended to decrease with the irradiation dose while glucose and fructose increased ($P<0.05$). Similar results were reported for mandarins treated with

0.15, 0.4 and 1 kGy (Ornelas-Paz et al. 2017). However, irradiation caused a different alteration of sugar content in fruit in the bottom layer, where a slight decreasing trend was observed for glucose and fructose while sucrose content was not altered significantly ($P>0.05$) (Fig. 2). These opposite trends in sugars for fruit in top and bottom layers suggest sugar conversion in the top layer fruit and increased usage of glucose and fructose in the bottom layer fruit. Some studies have demonstrated that irradiation can increase the activity or biosynthesis of enzymes involved in sugar conversion (invertases, sucrose synthases, fructokinase, hexokinase and sucrose phosphate synthases) (Shi et al. 2016; Yativ et al. 2010). Other studies have demonstrated that, depending on severity, physical damage can induce biological stress in fruits and ethylene biosynthesis, causing the expression of gene coding enzymes involved in the sugar composition of citrus fruits (Ladaniya et al. 2003; Lu et al. 2014; Rojas-Argudo et al. 2012; Shi et al. 2016). The conversion of sucrose to glucose and fructose is a genetic response of fruit to satisfy the demand for hexoses due to the increased respiration rate mediated by ethylene exposure and/or wounding, but generated hexoses are also used for signaling and as precursors for sucrose biosynthesis in highly damaged fruit because sucrose confers tolerance to fruit against damage (Cao et al., 2013; Lin et al., 2015). Thus, bottom fruit showed lower levels of glucose and fructose without alteration of the sucrose content probably because the hexoses were used for respiration and also to maintain normal levels of the protective sucrose.

Titratable Acidity (TA) and Organic Acids

363 TA values (0.44-0.49%) showed no effect of layer or irradiation dose (P>0.05) (data
364 not shown). Other studies have also shown that irradiation doses of up to 3 kGy do
365 not significantly alter the TA of orange juice (Miller et al. 2000). Our TA values were
366 similar to those previously reported for oranges (Flores et al. 2012; Kelebek et al.
367 2009; Roussos et al. 2011). The most abundant organic acids found in 'Barnfield'
368 Navel oranges were citric and quinnic acids while fumaric and oxalic were the least
369 abundant, as reported by Flores et al. (2012).
370 With few exceptions, the content of tested individual organic acids was consistently
371 lower in irradiated fruit in the bottom layer as compared with that of top layer (P<0.05)
372 (Fig. 3), suggesting that the metabolic activity was exacerbated in irradiated fruit by
373 irradiation and physical damage, leading to a higher respiration rate and consequently
374 to an increased utilization of organic acids. Different types of stress accelerate the
375 glycolysis and tricarboxylic acid cycle in citrus fruits, enhancing the transition from
376 sucrose metabolism to organic acid metabolism and leading to extensive citrate
377 degradation mainly through the gamma-aminobutyric acid and acetyl-CoA pathways
378 (Lin et al., 2015). These changes are genetically regulated, with the gene cascade
379 Aco3-IDH2/3-GAD4 serving as the major contributor to acid degradation (Chen et al.,
380 2012). Thus, irradiation and physical damage might induce a higher reduction of the
381 organic acid content in the bottom fruit as compared with the top fruit. This behavior
382 was clearly observed for ascorbic acid, which has been related to oxidation of ascorbic
383 acid by irradiation-generated reactive oxygen species (Wong and Kitts 2001).
384 Recently, Ramírez-Cahero and Valdivia-López (2018) demonstrated that irradiation
385 (0.5, 0.7 and 1 kGy) of ascorbic acid model solutions led to the formation of several

compounds (2-furaldehyde, 2(*5H*)-furanone, 2-furoic acid, furfuryl alcohol, glycolaldehyde, and formic, oxalic, succinic and L-tartaric acids) with the formation of these compounds dependent on irradiation dose. This direct degradation of certain organic acids by irradiation, and consequent increase in others could occur in our study, but the bottom layer almost always showed a reduction in organic acids at 1 kGy, due most likely to accelerated glycolysis and respiration rate of the bottom layer fruit. The negative effects of irradiation and mechanical damage on ascorbic acid content have been separately reported for oranges (Ladaniya 2008; Lee and Kader 2000). In contrast to irradiated fruit, the acid content in control group was generally higher in fruit from bottom layer as compared with that of the top layer ($P<0.05$). Recently, Ornelas-Paz et al. (2017) also observed a generally higher content of organic acids in non-irradiated mandarins after simulated sea shipment as compared to irradiated fruit. Our findings demonstrated that irradiation and compression and their combination affected differentially the metabolism of oranges.

Total and Individual Phenols

The total phenolic content in fruit of the same layer was unchanged with irradiation ($P>0.05$), but showed minor differences between layers. Fruit in the bottom layer exhibited a lower total phenolic content, as compared to fruit in the top layer ($P<0.05$) (Fig. 4). This suggested a wounding-mediated deterioration of phenolic compounds. The concentration of phenolic substances following irradiation can increase with low doses but higher doses can lead to reduced synthesis or destruction (Oufedjikh et al., 2000). Some studies have demonstrated that the combination of wounding and

409 irradiation at some doses reduced the biosynthesis of phenolic compounds, as
410 compared with the individual effects of wounding and irradiation, promoting a higher
411 incidence of fungal infections (Rojas-Argudo et al., 2012). This might explain the
412 higher incidence of fungal infections observed in this study for fruit in the bottom layer
413 (Table 1). Some studies have demonstrated that wounding causes an immediate
414 increase in the concentration of antifungal compounds, i.e. phytoalexins, which
415 prevent spore germination and mycelium growth but do not damage the fungal
416 structures or their viability (Kim et al., 1991; Ben Yehoshua et al., 1992). This
417 wounding-mediated increase of antifungal compounds is transient (Rojas-Argudo et
418 al., 2012), favoring the initiation of the disease in wounds as the levels of antifungal
419 compounds decrease during storage (Kim et al., 1991; Ben Yehoshua et al., 1992).
420 Thus, the combination of this transient effect of wounding and the well-known
421 phytotoxic effect of irradiation can, in combination, exacerbate the incidence of fungal
422 infections.
423 Some phenolic acids (chlorogenic, *p*-coumaric, and ferulic acids) and flavonoids (rutin,
424 narirutin, hesperidin and naringenin) were identified and quantified in the juice of the
425 tested fruit (Fig. 4). The content of narirutin (93.3-100.2 mg/L) did not change
426 significantly as a function of irradiation or layer type ($P>0.05$) (data not shown).
427 Hesperidin, narirutin, and naringenin were the most abundant phenolic compounds in
428 tested oranges. Similar concentrations of these compounds have been reported
429 previously for oranges (Agcam et al. 2014; Rocco et al. 2014). In general, oranges in
430 the bottom layer had a lower ($P<0.05$) concentration of hesperidin, *p*-coumaric acid,
431 rutin and naringenin compared to oranges in the top layer, although this trend was

less evident for naringenin. Wounding and other types of mechanical injury increase PAL activity and the content of phenolic compounds. However, irradiation is able to inhibit the wounding-mediated activation of PAL, favoring the reduction of phenolic content by injury (Banerjee et al., 2015). This might be the reason why fruit from bottom layer showed a lower content of individual phenols. In contrast, the content of chlorogenic acid was lower in bottom layer of control fruit, while the opposite was observed for irradiated oranges ($P<0.05$). This phenomenon might be a consequence of irradiation-mediated transformation of phenols. As indicated above, the content of total phenols showed minor changes among experimental groups, suggesting the transformation of phenolic compounds by irradiation or compression damage, as reported by Breitfellner et al. (2003) in irradiated strawberries. In our study, the effect of irradiation dose on individual phenolic compounds was lower than that of the layer type ($P<0.05$). Only the content of hesperidin and chlorogenic acid was clearly affected by irradiation dose ($P<0.05$). McDonald et al. (2013) did not observe changes in the phenolic content of Navel oranges treated with several irradiation doses (0.2-0.6 kGy). In our study, the phenolic content in tested fruit depended on layer type, showing the negative effect of compression damage on this quality attribute. Several studies have already demonstrated that physical damage of citrus fruits alters the content of some individual phenols (Mazidi et al. 2016; Rojas-Argudo et al. 2010, 2012). Our study demonstrated that the combination of physical damage and irradiation affected differently the content of phenolic compounds of citrus fruits.

Conclusions

This work demonstrated that the position of the fruit within a case plays a role in the postharvest quality of irradiated oranges. The observed chemical changes seemed to be a response to stress caused by irradiation as well as location in the case, as evidenced by small alterations in sugars, acids and phenol compounds. Irradiation exacerbated SERB but unexpectedly this disorder was more severe in the top than in the bottom fruit, probably due to differences in the gas composition and/or relative humidity inside the case or phenolic compounds. Flattening and fungal decay depended on irradiation dose and layer type, once again highlighting the combined effect of irradiation and fruit placement in the case. This study shows that for large and heavy fruit such as oranges, which are often packed in multiple layers, packaging type should be considered when evaluating the effect of irradiation on quality. Fruit treated at 0.15 kGy showed minimal alterations in quality independent of fruit position inside the case, demonstrating that Navel oranges tolerate phytosanitary irradiation at this low dose.

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652
653 **Table captions**

Table 1. Incidence of stem end rind breakdown (SERB), flattening and fungal infections in oranges taken from top and bottom layers of Standard Place Pack cartons after storage (3 weeks at 5 °C + 1 week at 20 °C).

Table 2. Percentage of fruits from top and bottom layers of Standard Place Pack cartons showing different levels of flattening after storage (3 weeks at 5 °C + 1 week at 20 °C).

Figure captions

Figure 1. Percentage of fruit surface area affected by SERB (A) and L* (B) and b* (C) values on SERB lesions in oranges taken from top (▬) and bottom (▬) layers of Standard Place Pack cartons after storage (3 weeks at 5 °C + 1 week at 20 °C)**.** Data represent the mean value of twenty fruits ± the standard error.

Figure 2. Content of sucrose, glucose, and fructose in juice of Navel oranges taken from top (▬) and bottom (▬) layers of Standard Place Pack cartons after storage (3 weeks at 5 °C + 1 week at 20 °C)**.** Data represent the mean value of five measurements ± the standard error.

Figure 3. Content of organic acids in juice of Navel oranges taken from top (▬) and bottom (▬) layers of Standard Place Pack cartons after storage (3 weeks at 5 °C + 1 week at 20 °C)**.** Data represent the mean value of five measurements ± the standard error.

Figure 4. Content of total and individual phenols in juice of Navel oranges taken from top (▬) and bottom (▬) layers of Standard Place Pack cartons after storage (3 weeks at 5 °C + 1 week at 20 °C). Data represent the mean value of five measurements ± the standard error.

Table 1.

Irradiation dose (kGy)	Layer	Damaged fruit (%)		
		SERB	Flattening	Fungal infections
0	Top	10.0±10.0a	58.8±21.2b	8.8±8.8a
	Bottom	6.3±6.3a	100±0a	19.4±2.3a
0.15	Top	48.2±23.2a	35.7±35.7b	12.1±0.4a
	Bottom	16.3±3.8b	95±5.0a	13.9±2.8a
1	Top	100±0a	6.3±6.3b	22.2±0.7a
	Bottom	100±0a	81.3±6.3a	27.8±5.6a

Data represent the mean values ± the standard error. Mean values in the same column for every irradiation dose connected by the same letter are not significantly different.

Table 2

Irradiation dose (kGy)	Layer	Damage level					
		0	1	2	3	4	5
0	Top	41.3±21.3a	58.8±21.3a	0.0±0.0b	0.0±0.0a	0.0±0.0a	0.0±0.0a
	Bottom	0.0±0.0b	87.5±12.5a	12.5±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
0.15	Top	64.3±35.7a	35.7±35.7a	0.0±0.0b	0.0±0.0b	0.0±0.0a	0.0±0.0a
	Bottom	5.0±5.0b	55.0±5.0a	22.5±2.5a	17.5±7.5a	0.0±0.0a	0.0±0.0a
1	Top	93.8±6.3a	6.3±6.3b	0.0±0.0b	0.0±0.0b	0.0±0.0a	0.0±0.0a
	Bottom	18.8±6.3b	50.0±0a	18.8±6.3a	12.5±0.0a	0.0±0.0a	0.0±0.0a

Severity values were based on % of fruit surface showing flat areas: 0 (no damage), 1 (1-4%), 2 (5-8%), 3 (9-12%), 4 (13-15%), and 5 (>16%). Data represent the mean values ± the standard error. Mean values in the same column for every irradiation dose connected by the same letter are not significantly different.

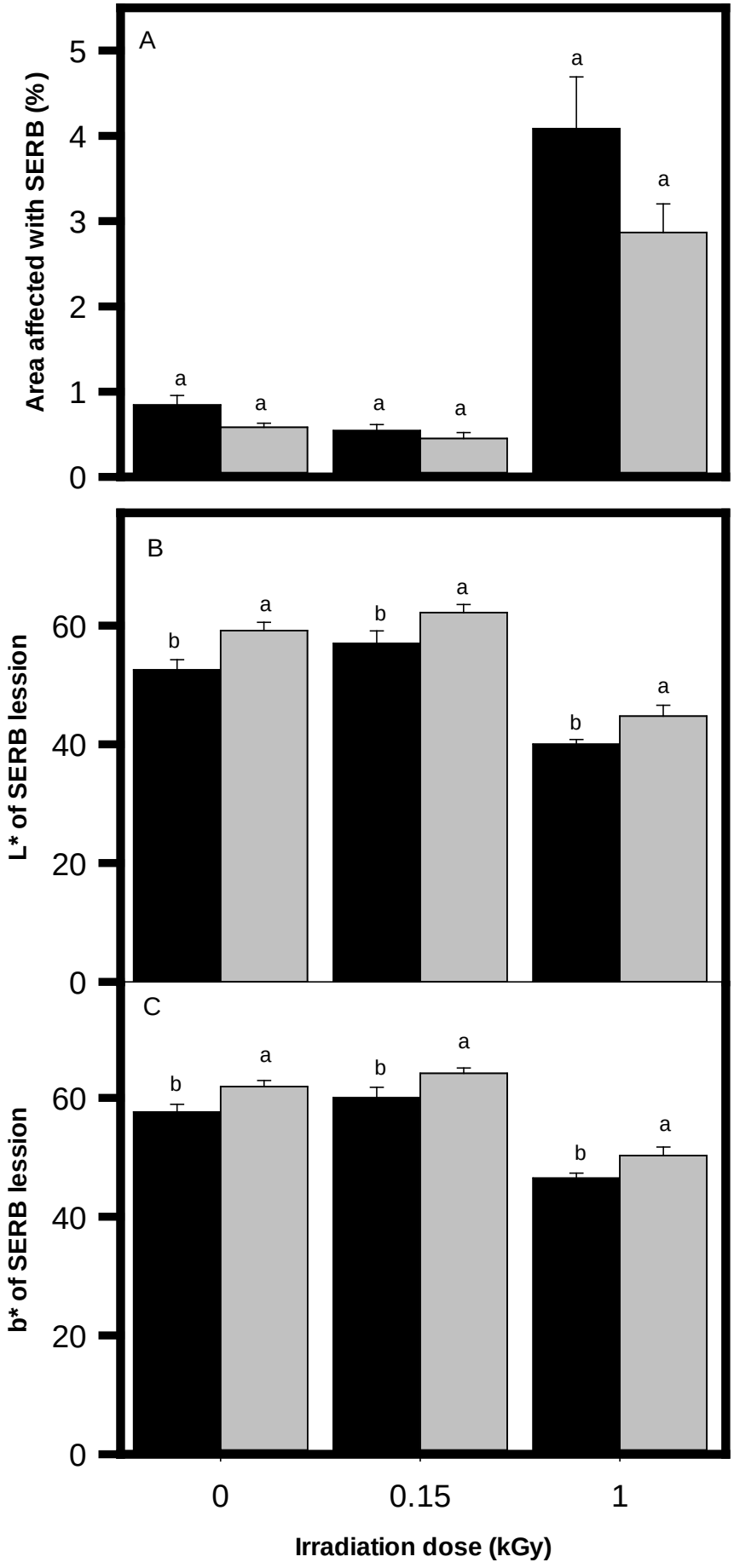
A
Area affected with SERB (%)
0
1
2
3
4
5
a
a
a
a
a
a
B
L* of SERB lession
0
20
40
60
b
a
b
a
b
a
C
b* of SERB lession
0
20
40
60
b
a
b
a
b
a
0
0.15
1
Irradiation dose (kGy)

Fig 1.

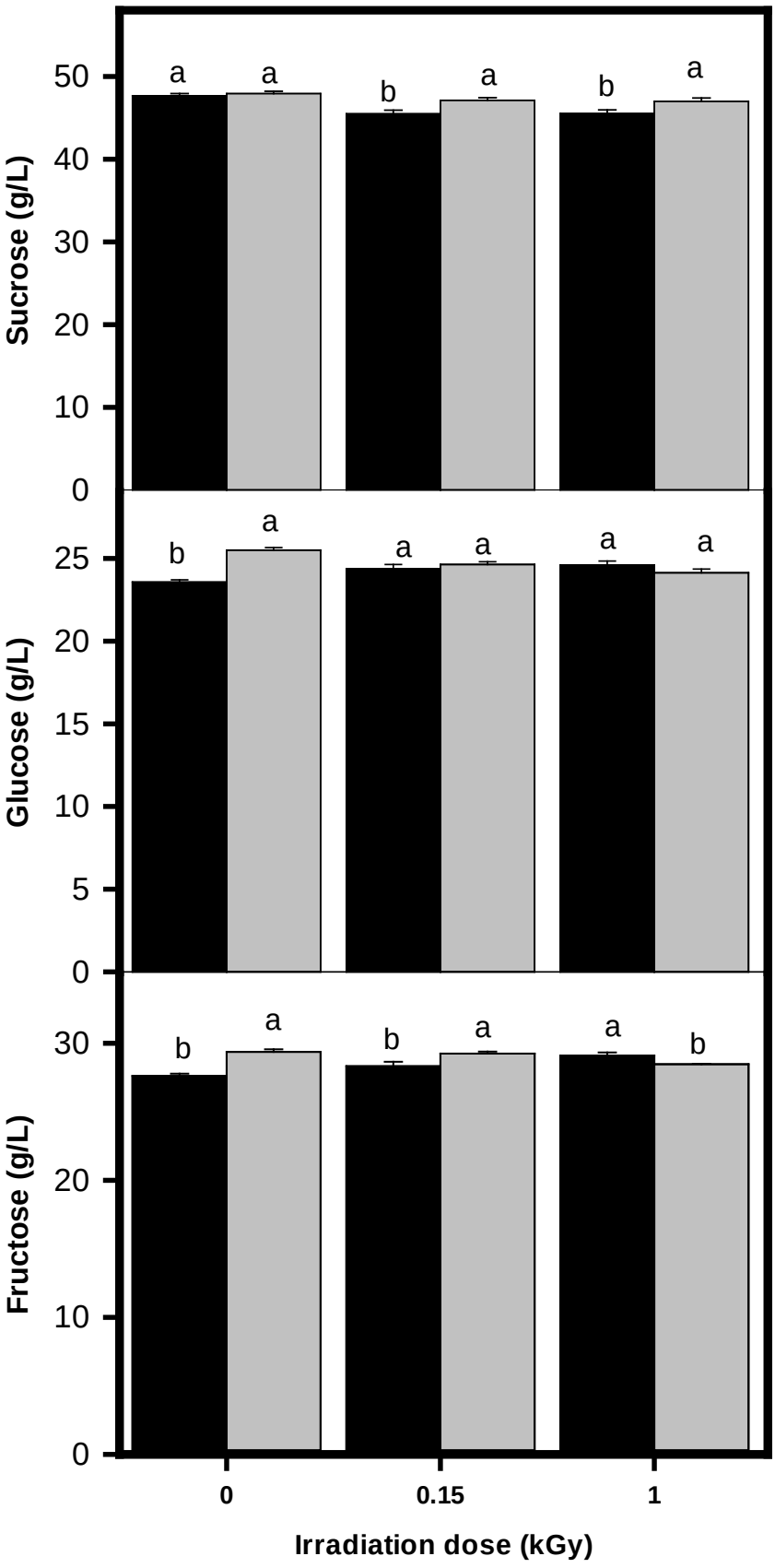
Sucrose (g/L)
50
40
30
20
10
0
a a b a b a
Glucose (g/L)
25
20
15
10
5
0
b a a a a a
Fructose (g/L)
30
20
10
0
b a b a a b
0
0.15
1
Irradiation dose (kGy)

Fig. 2

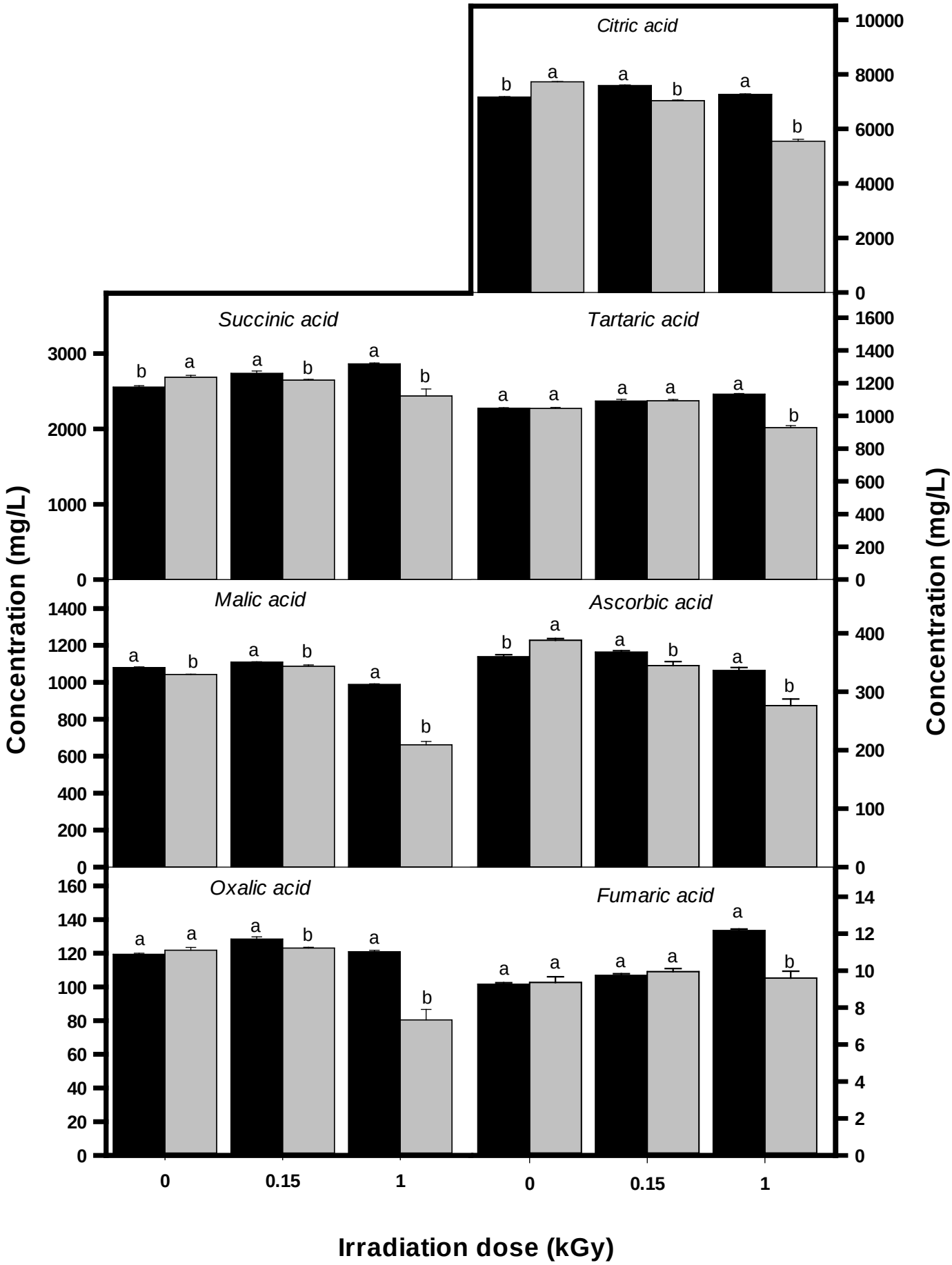
Citric acid
Succinic acid
Tartaric acid
Malic acid
Ascorbic acid
Oxalic acid
Fumaric acid
Concentration (mg/L)
Concentration (mg/L)
Irradiation dose (kGy)
0
0.15
1
0
0.15
1

Fig. 3

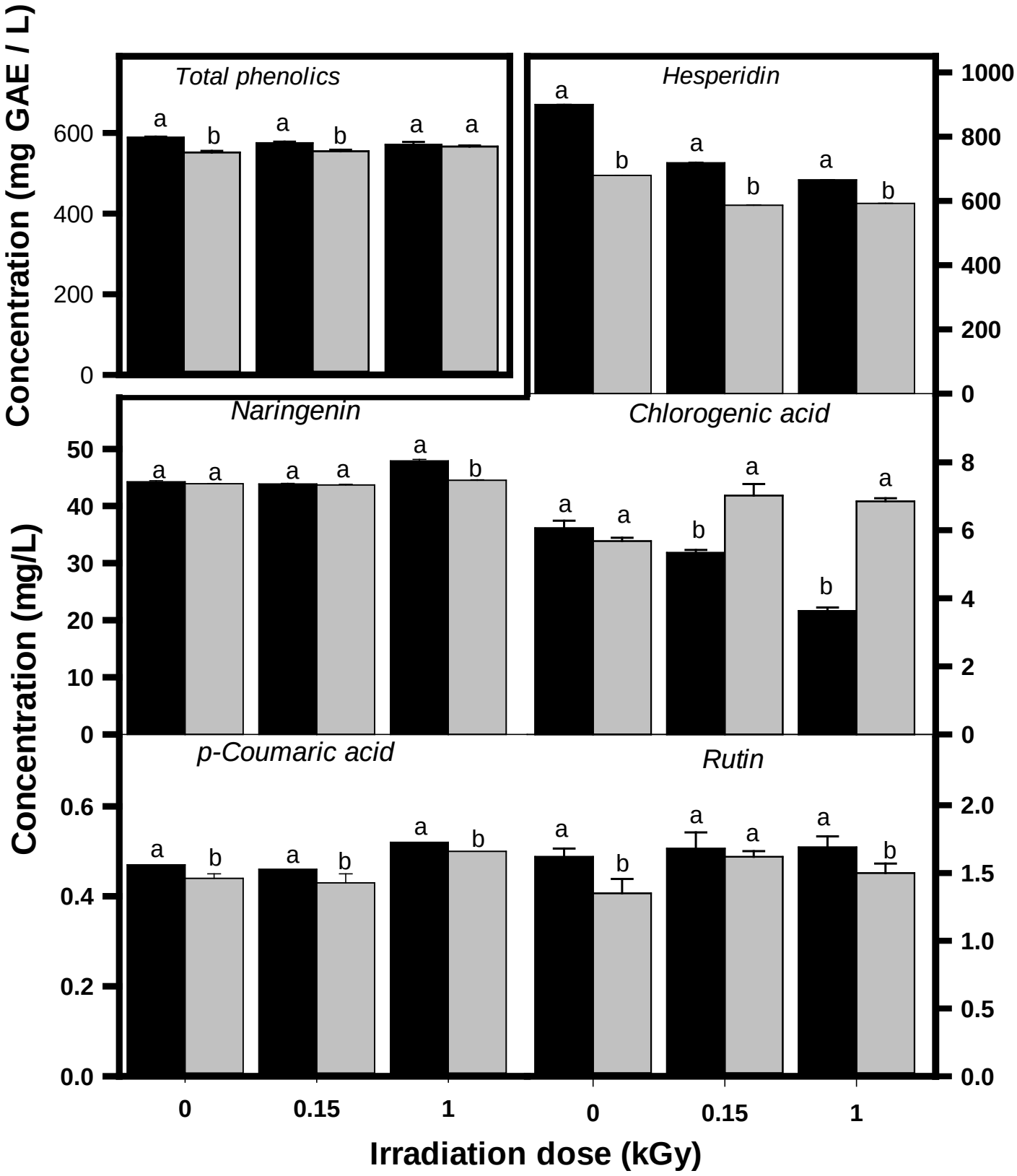
Total phenolics
Hesperidin
Naringenin
Chlorogenic acid
p-Coumaric acid
Rutin
Concentration (mg GAE / L)
Concentration (mg/L)
Irradiation dose (kGy)
0
0.15
1

Fig. 4