Effect of insecticidal atmosphere and low dose X-ray irradiation in combination with cold quarantine storage on bioactive compounds of clementine mandarins cv. 'Clemenules'

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Abstract

Citrus fruits are a rich source of vitamins and polyphenolic compounds with antioxidant capacity, that need to be maintained during postharvest storage. The aim of this study was to determine the effect of two innovative quarantine treatments, such as insecticidal atmospheres (IA) (95% CO_2 and balance air) applied at 20 or 25 °C for 20 h and low doses X-ray irradiation (0, 30, 54 and 164 Gy), in combination with short periods of cold-quarantine storage on the nutritional quality of 'Clemenules' mandarins. Mandarins were stored at 1.5 ºC for 6, 9, or 12 d before the application of IA treatments or for 0, 6, or 12 d after the X-ray radiation. Nutritional quality of mandarins was determined after the corresponding combination of quarantine treatment (IA or X-ray) with cold quarantine followed by a shelf life period of 7 d at 20 ºC to simulate shelf life conditions. Cold quarantine treatment combined with IA or with X-ray radiation did not affect negatively total antioxidant capacity and total ascorbic acid content of 'Clemenules' mandarins. However, flavanone glycosides (FGs) and total phenolics content were slightly modified. Application of the IA at 20 ºC induced a greater inhibition of the FGs than application at 25 ºC. When X-ray irradiation was applied without a previous quarantine period the synthesis of the FGs increased as irradiation dose increased.

1. Introduction

Spain is the world's largest exporter of fresh citrus fruit. Among the Spanish cultivars, 'Clemenules' (syns.: 'Clementina de Nules', 'Nules') is the leading clementine mandarin (*Citrus reticulata* Blanco) produced around the world. Clementines are characterized by a high sensory quality, seedless, and very easy to peel, which has contributed to an increase in the export shipments to overseas markets such as the USA and Japan (Palou et al 2008).

Many countries maintain strict quarantine measures against the mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). The most widely used postharvest disinfestation treatment of citrus against this fruit fly involves exposure of the fruit to near-freezing temperatures. In the case of the USA, the U.S. Department of Agriculture (USDA) established a minimum exposure during overseas transit of 14 or 18 d below 1.1 or 2.2 ºC, respectively (USDA 2002a). Extensive research is currently focused on the development of alternative or complementary quarantine treatments for reducing cold quarantine storage specially for cold sensitive commodities such as citrus (Alonso et al 2005; Follett & Neven 2006; Palou et al 2008).

Insecticidal atmospheres (IA), with high CO_2 concentrations, and irradiation treatments are known to be effective against fruit flies and other pests (Hallman 1999; Follett & Neven 2006). Different studies have investigated the use of complementary CO_2 treatments previous or after cold exposure of citrus fruit, in order to reduce the duration of the standard cold disinfestation quarantine treatment against *C. capitata* and thus alleviate chilling injury problems (Alonso et al 2005; Palou et al 2008). Complete insect mortality of *C. capitata* with no negative effects on physicochemical and sensory quality of clementine mandarins after 7 d at 20 ºC of shelf life was obtained on fruit first exposed to 1.5 ºC for 3 d and second treated with 95 % CO_2 balanced with air at 25 ºC (Palou et al 2008).

Among the different ionizing radiation sources, the use of X-ray has been approved by the US Food and Drug Administration for food irradiation (US FDA 2004). A generic treatment dose of 100 Gy has been established for quarantine purposes against fruit flies (USDA 2002b). Palou et al (2007) reported complete insect mortality with no negative effects on fruit quality after 7 d at 20°C of shelf life on clementines firstly X-ray irradiated at 30-164 Gy and subsequently exposed to 1°C for 2 d. This combination of treatments considerably reduced quarantine time if compared to standard cold quarantine treatments (1.1-2.2°C for 14-18 d) and therefore showed promise as a potential commercial treatment for Spanish citrus exports.

Traditionally, postharvest quality assessment has been conducted by evaluating physico-chemical quality parameters, such as weight loss, firmness, colour, acidity, and maturity index, among others. Nowadays, nutritional and functional quality has gained great interest, being a component of the overall quality that is very much valued by consumers. Citrus fruits are an important source of vitamin C as well as bioactive compounds such as polyphenolic compounds, mainly flavonoids, with high antioxidant properties (Sánchez-Moreno et al 2003). Postharvest technologies should maintain both nutritional and functional quality of fruits until they reach the consumer. Lee & Kader (2000) remarked the effects of storage temperature and time on vitamin C content of fruits and vegetables. The application of new quarantine treatments might also affect the physiology of the fruit altering their biochemical components. Recent studies show that irradiation of citrus fruit reduced significantly the total ascorbic acid (TAA) content when radiation doses were high (Patil et al 2004; Vanamala et al 2005; Girennavar et al

2008). However, information is still scarce on the effect of new quarantine treatments on nutritional quality of many citrus cultivars. Therefore, the aim of this work was to study the effect of two innovative quarantine treatments, such as IA (95% CO_2 balanced with air) applied at 20 or 25 °C and low doses X-ray irradiation (0, 30, 54 and 164 Gy), in combination with short periods of cold-quarantine storage on the nutritional quality of ‘Clemenules’ mandarins.

2. Material and methods

Fruit

Clementine mandarins (*Citrus reticulata* Blanco) cv. ‘Clemenules’ were hand-harvested at commercial maturity (MI=7.45) and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, and dipped in a mixed solution of imazalil (2,500 mg/L) and guazatine (800 mg/L) for 1.5 min. Fruit were allocated into homogeneous groups to apply, subsequently, each one of the combined quarantine treatments.

Materials

Reagents 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), potassium dihydrogen phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-ciocalteu’s phenol reagent, sodium carbonate (Na_2CO_3), gallic acid and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Spain). Methanol was from BDH Prolabo (Poole, UK). 1,4-dithio-DL-threitol (DTT) and hesperidin (hesperitin-7-0-rutinoside, HES) were obtained from Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside, NAT) and didymin (isosakuranetin-7-rutinoside, DID) were purchased from Extrasynthese (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used for the analysis.

Cold and IA quarantine treatments

The mandarins were exposed to the standard cold-quarantine temperature of 1.5±0.5 ºC for 6, 9, or 12 d in a 40 m^3 cold room. Cold-treated fruit were allowed to warm in an air-atmosphere at room temperature (20±2 ºC) for 22–24 h before IA exposure. For each cold quarantine time, three groups of 150 fruit were exposed for 20 h to the following IA treatments: (T1) air-atmosphere at 20±1 ºC (control), (T2) atmosphere containing 95% CO_2 at 20±1 ºC and (T3) atmosphere containing 95% CO_2 at 25±1 ºC. In all cases, RH was 85±5%. IA exposure chambers consisted of hermetic Perspex cabinets (82 cm x 62 cm x 87 cm), fitted with inlet and outlet ports through which CO_2 (Alphagaz, N38, Air Liquide S.A., Madrid, Spain) passed at a rate adjusted to yield a concentration of 95 % (*v/v*) inside the cabinet and balanced with air. Gas was allowed to escape from the outlet port through a bubble tube to maintain the proper gas mixture in the chamber. The desired gas concentrations were regularly reached after 25-30 min of closing the door of the cabinets. Levels of CO_2, O_2, temperature, and RH were continuously monitored by means of the system Control-Tec® (Tecnidex S.A., Paterna, Valencia, Spain). Cabinets were installed inside a 40 m^3 storage room that was also set to each experimental temperature (20 or 25 ºC). Once IA treatments were accomplished, mandarins were coated with a 10% total solids water wax containing polyethylene, shellac, and 0.5% of the fungicide thiabendazole (Brillaqua®, Brillocera S.A., Beniparrell, Valencia, Spain). Coated mandarins were stored 7 d at 20 ºC to simulate commercialization conditions.

X-ray irradiation and cold quarantine treatments

The mandarins were transported in a conditioned truck to the irradiation plant (Beta Gamma Service, BGM, Bruchsal, Germany). During transportation, the fruit were kept at 20±3 ºC. About 36 h later, the fruit were exposed to X-ray irradiation from a source with beam energy of 0.8 MeV and a conveyor speed of 5 m min^{-1}. The following theoretical doses were selected: 0 (control), 25, 50 and 150 Gy. Actual doses were determined by placing 2 cm^2 radiochromatic dosimetry films (Gafchromic® HD-810, International specialty products, Wayne, NJ, USA) at three different heights within three different boxes. Readings (nine per dose) were made with a spectrophotometer at 560 nm and mean and standard error values were 30±1, 54±1, and 164±4 Gy for the respective theoretical doses. Control fruit were not irradiated; they were kept at 20 ºC until the application of the cold quarantine treatments.

Irradiated and non irradiated fruit were exposed to cold-quarantine at 1.5 ºC for 0 (control), 6 and 12 d followed by 7 d of shelf life at 20 ºC.

Determination of bioactive compounds of citrus

Nutritional quality of mandarins was determined at harvest (initial quality) and after the corresponding combination of quarantine treatment (IA or X-ray) with cold quarantine followed by a shelf life period of 7 d at 20 ºC to simulate prompt fruit commercialization. At the end of this period the juice from 3 replicates of 10 fruit each per treatment was obtained, transferred to vials with crimp-top caps and TFE/silicone septum seals and kept at –80 °C until the time of analysis.

Total antioxidant capacity (TAC). The TAC was evaluated by the $DPPH^{\bullet}$ assay. Two mL of mandarin juice and 4 mL of methanol HPLC grade were mixed and centrifuged at 12,000 G for 15 min at 5 ºC. Five methanolic dilutions from the supernatant (0.075

mL) were mixed with 2.925 mL of DPPH• (24 mg L^{-1}) and kept in darkness for 40 min at 25±1 ºC. Afterwards, the change in absorbance was determined at 515 nm with a spectrophotometer (Thermo Electron Corporation, Auchtermuchty Fife, UK). The DPPH radical scavenging activity was expressed as effective concentration (EC_{50}), that is the amount of juice necessary to decrease the initial DPPH• concentration by 50% (L juice/kg of DPPH•); thus, lower EC_{50} values mean higher antioxidant capacity (Sánchez-Moreno et al 2003).

Total ascorbic acid (TAA). TAA was determined by the sum of ascorbic acid (AA) plus L-dehydroascorbic acid (DHA), by reducing DHA to AA with DTT. One mL mandarin juice was homogenized with 9 mL of MPA (2.5% w/v). Two mL aliquot was mixed with 0.4 mL of DTT (20 mg mL^{-1}) and allowed to react for 2 h in the dark at room temperature. Afterwards, samples were filtered through a 0.45 µm membrane filter and used for TAA determination by HPLC.

The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200), quaternary pump (Model L-2130), column oven (Model L-2300) and diode array detector (Model L-2450). A reversed-phase C18 LiChrospher®100 column (250 x 4 mm, 5 µm-particle, Merck, Darmstadt, Germany) preceded by a precolumn (4 x 4 mm) was used. Injection volume was 20 µL and oven temperature 25 ºC. The mobile phase was 2% solution of KH_2PO_4, adjusted to pH 2.3 with H_3PO_4. The flow rate was fixed at 1 mL min^{-1} and the wavelength of measurement was 243 nm. AA was identified and quantified by comparison of peak areas with external standard and results were expressed as mg of TAA /100 mL of juice. Analysis were made by triplicate.

Flavanone glycosides (FGs). The main FGs identified in citrus fruit, HES, NAT and DID were determined by HPLC. Two mL of juice were homogenized with 2 mL of DMSO:methanol (1:1 v/v) and centrifuged for 30 min, at 12,000 G and 4 ºC. The supernatant was filtered through one 0.45 µm nylon filter and analyzed by HPLC-DAD using the HPLC equipment described above and the chromatographic system conditions described by Cano et al (2008). The main FGs were identified by matching their respective spectra and retention times with those of commercially obtained standards. NAT, HES and DID contents were calculated by comparing the integrated peak areas of each individual compounds to that of its pure standards. Results were expressed as mg/100 mL.

Total phenolics content (TPC). The TPC was determined using the Folin-Ciocalteu method (Singleton & Rossi 1965). 0.3 mL of mandarin juice was diluted with 1.7 mL of 80% aqueous methanol. Appropriately diluted juice (0.4 mL) was mixed with 2 mL of Folin-Ciocalteu reagent (1:10, v/v diluted with water) and incubated for 1 min before 1.6 mL sodium carbonate (7.5%, w/v) was added. The mixture was incubated for 1 h at room temperature before absorption was measured at 765 nm with a spectrophotometer (Thermo Electron Corporation, Auchtermuchty Fife, UK). TPC was expressed as mg gallic acid equivalents per 100 mL (mg GAE/100 mL). All extracts were analyzed in triplicate.

Statistical Analysis

Data were analyzed using a complete randomized design in a factorial set with 3 repetitions per treatment. Two-way ANOVAs were performed with 3 levels of the factor cold quarantine period and 3 levels of the factor IA in the first experiment and 3 levels of the factor cold quarantine period and 4 levels of the factor X-ray irradiation in the second experiment. Because of significant interactions, individual one-way ANOVAs were also performed for each level of each factor. Specific differences among means were determined by Fisher's protected least significant difference test (LSD; $P \leq 0.5$). Data were analyzed using STATGRAPHICS Plus 2.1 (Manugistics, Inc., Rockville, Maryland, USA).

3. Results and discussion

Two-way ANOVA *P* values for the effect of main factors and interactions on TAC (EC_{50}), TAA, TPC and FGs are shown in Table 1. Because of significant interactions, individual one-way ANOVAs were also performed for each level of each factor for both experiments (means separation in Tables 2 and 3).

3.1. Cold and IA quarantine treatments

Total antioxidant capacity. Table 2 shows the EC_{50} values of treated mandarins. As mentioned earlier, the $DPPH^{\bullet}$ radical decreases by reacting with antioxidants present in the sample; therefore, a higher EC_{50} value indicates a lower TAC of the sample. The TAC of the mandarins were not significantly affected by storage time or by the application of the different IA. Artés-Hernández et al (2007) found that the TAC in fresh-cut 'Lisbon' lemon products stored at different temperatures (0, 2, 5 or 10 ºC) remained constant during 12 d.

Total ascorbic acid. TAA content was not affected by the exposure to CO_2 or the increase in the cold quarantine period, except on mandarins exposed to the IA at 20°C after 9 d of cold storage that had more TAA than the rest of the samples (Table 2). However, this difference although statistically significant was not observed for the rest of the storage periods and could be due to the intrinsic variability among samples.

Many studies in the literature show that AA content of fruits and vegetables decreases as the CO_2 concentration in the storage atmosphere increases and these losses are usually accelerated by using high temperatures and long storage (Lee & Kader 2000; Thompson 2004). Storage at low temperature can also accelerate the loss of vitamin C in cold sensitive fruit, even before chilling injury is evident. For example, Ito et al (1974) reported that in 'Satsuma' mandarins, controlled atmosphere with low-O_2 and high-CO_2 concentrations at 1-4 °C reduced the AA level gradually, while the DHA content increased. In our study, mandarin exposure to 95% CO_2 was performed over a short period of time which could justify that the IA used did not affect TAA content and TAC. Although chilling injury can accelerate the loss of TAA in cold sensitive fruit, Palma et al (2005) did not observe changes in TAA and TAC of 'Fortune' mandarins after 90 d of storage at 5 ºC. Similarly in our work, storage at the cold quarantine temperature of 1.5 ºC did not affect the content of TAA and the TAC of the mandarins (Table 2).

Flavanone glycosides content. Table 2 shows the content of the main flavonoids of 'Clemenules' mandarins after standard cold-quarantine periods and exposed to air or IA. The most abundant flavonoid was HES followed by NAT and DID. In general, HES content increased as cold storage time increased, being this increase less pronounced when the IA was applied at 20 °C. After 12 d of quarantine period, no differences were found in HES content between mandarins exposed to air-atmosphere and IA at 25°C. Samples treated with 95% CO_2 at 20 °C after 9 and 12 d of storage had lower FGs content that control samples, which could indicate a slight inhibition in the synthesis of FGs by this treatment. Palma et al (2005) did not find differences in HES, NAT and DID in 'Fortune' mandarin juice during 90 d of storage at 5 ºC.

Total phenolic content. Table 2 shows the effect of cold quarantine periods and IA treatments on TPC of 'Clemenules' mandarins. TPC of 'Clemenules' mandarins ranged from 49.6 to 59.4 mg GAE/100 mL juice, which was in accordance with those reported in others studies for mandarin fruit (Wang et al 2007). TPC of the mandarins increased as cold quarantine storage increased. This result contrast with that reported by Palma et al (2005) that did not find differences in TPC of 'Fortune' mandarins during 90 d of cold storage at 5 ºC. In strawberry, an increase on the total phenols during storage time was observed although the fruits exposed to air + 20 kPa CO_2 contained lower content of some specific phenolic compounds compared to those exposed to air, indicating that phenolic degradation may increase after exposition to CO_2-enriched atmospheres (Holcroft et al 1998). In our work, total phenols of 'Clemenules' mandarins increased slightly in the fruit kept in high CO_2 and exposed to cold quarantine temperature during 12 d.

3.2. X-ray irradiation and cold quarantine treatments

*Total antioxidant capacity***.** Table 3 shows the changes in the TAC of irradiated and control 'Clemenules' mandarins at harvest and after the different quarantine periods. The EC_{50} values observed during the different storage periods were lower than the initial value measured at harvest, which indicates that the TAC of irradiated and non irradiated clementine mandarins increased after 7 d of storage at 20 ºC. The increase in the TAC might be due to an increase of the compounds of citrus fruit with high antioxidant properties such as TAA and polyphenols. However, this increase was not found in the same samples that were exposed to cold quarantine, followed by the IA treatments, and 7 d storage at 20 ºC (Table 2). In both works, control samples (non-irradiated and air-treated fruit) exposed to similar quarantine conditions and 7 d of storage at 20 ºC behaved differently. Differences in the behavior of the fruit could be due to differences in the handling of the fruit that had to be transported to the irradiation plant in Germany, which implied 4 additional d at 20±3 ºC. However, this should be confirmed with further studies. During storage, however, the TAC expressed as EC_{50} was not significantly affected by storage time at 1°C or by the dose of irradiation (30, 50 and 164 Gy).

Total ascorbic acid content. TAA content of clementine mandarins ranged from 31.67±3.52 to 38.82±1.23 mg AA/100 mL juice (Table 3). These results are within the range of those reported in mandarins and other citrus fruit (Lee & Kader 2000; Cano et al 2008).

Application of low doses of X-ray irradiation combined with low-temperature quarantine storage did not affect negatively the TAA content of 'Clemenules' mandarins. Rather, an increase in TAA was observed in mandarins stored directly at 20 ºC. Other authors have reported some increases in TAA of 'Clemenules' mandarins after storage at 20 ºC (Rojas-Argudo et al 2007) or gamma irradiation (Abdellaoui et al 1995). However, irradiation effect on TAA seems to depend on irradiation dose, fruit cultivar and maturity stage. Clementine fruits irradiated at 300 and 500 Gy doses along with hot water treatment and stored for 3 weeks at 17 ºC contained higher TAA levels than control samples (Abdellaoui et al 1995). However, in grapefruit a dose of 1,500 Gy decreased TAA content, whereas a dose of 250 Gy did not affect the TAA content (Moshonas & Shaw 1984). Girennavar et al (2008) reported in grapefruit that a dose of 1,000 Gy did not affect the TAA content, whereas a dose of 2,500 Gy significantly reduced the TAA content. Patil et al (2004) reported that early season grapefruit irradiated at up to 700 Gy and stored 35 d did not affect TAA content, whereas in late season fruit an irradiation greater than or equal to 200 Gy caused a marked reduction in TAA content. These authors suggested that in earlier harvest fruit, vitamin C may not be the primary defence mechanism of fruit against the oxidative stress induced by gamma-irradiation, whereas in late season crops the stress induced by irradiation coupled with low temperature stress affecting the TAA content. Therefore, the susceptibility to modify the TAA content on citrus fruit might be avoided through selection of fruit in a optimum maturity stage.

*Flavanone glycosides content***.** In general, FGs content was affected by storage time at 1 ºC and by the irradiation dose applied (Table 3). X-ray irradiated mandarins stored 6 and 12 d at 1 ºC showed a decreased in FGs as the irradiation dose and storage time increased. When mandarins were not exposed to cold quarantine period, the FGs content increased as irradiation dose increased. Vanamala et al (2005) reported in grapefruits that low irradiation dose (300 Gy) increased naringin and NAT contents. Patil et al (2004), in early-season grapefruit, found that the total FGs concentration increased as the fruit was exposed to low doses of irradiation (70 and 200 Gy) followed by storage at 10 ºC for 4 weeks followed by 1 week at 20 ºC, whereas naringin (the more abundant FGs in grapefruit) and NAT levels decreased as the irradiation dose increased (above 200 Gy). The increase in FGs content at low irradiation doses was attributed to an increase in phenylalanine ammonia lyase (PAL) activity during low temperature storage. Whereas, the decline in FGs content of grapefruit at high doses of irradiation was related to their role in counteracting the oxidative stress induced by the gamma irradiation. Therefore, variations in the FGs content at different doses of irradiation may be a result of an equilibrium between gamma irradiation induced oxidative stress and *novo* synthesis of flavonoids by increased PAL activity (Patil et al 2004).

In the group of non-irradiated mandarins (control), HES content increased as quarantine storage increased. Patil et al (2004) also reported higher flavanoid content after cold storage of citrus fruit associated to an increase in the PAL activity during low temperature storage.

*Total phenolics content***.** The TPC of 'Clemenules' mandarin juice is shown in Table 3. The TPC ranged from 50 to 60 mg GAE/100 mL juice, which was in accordance with those reported in others studies for mandarin fruit (Wang et al 2007). In general, our results show that low doses of X-ray irradiation did not significantly affect the TPC of 'Clemenules' mandarins, except for the second cold quarantine period (6 d at 1 ºC) where some differences were found among treatments, being 54 and 164 Gy irradiated mandarins the treatments with the highest TPC. In general, TPC increased as cold quarantine period increased with values from 50 mg GAE/100 mL juice at harvest to 58-60 mg GAE/100 mL juice after 12 d at 1 ºC followed by 1 week at 20 ºC.

Different stresses (irradiation, wounding, nutrient deficiencies, herbicide treatment, and viral, fungi, and insect attacks) have been shown to enhance either PAL synthesis or activity in different plants. PAL has been an indicative of rate-controlling enzyme in phenolic synthesis and wounding of citrus (Patil et al 2004). Many works have shown that irradiation influences phenolic biosynthesis as a response of plant tissue to abiotic stress and irradiation (Dubery 1992). Oufedjikh et al (2000) found that the TPC remained higher in irradiated fruits during 49 d at 3-4 ºC and this content was related to PAL activity, which also reached a maximum at 21 d of storage at 3-4 ºC. However, there were not always evidence of accumulation of phenolic compounds after the peak of PAL activity (Jones 1984; McDonald et al 2000).

4. Conclusion

Results indicate that innovative quarantine treatments, such as IA (95% CO_2, balanced with air) and X-ray irradiation at low doses (30, 54 and 164 Gy) in combination with short periods of cold-quarantine storage (6 to 12 d at 1.5 ºC) did not affect negatively the nutritional quality of ‘Clemenules’ mandarins. The TAC and TAA of mandarins was not affected by these treatments; whereas FGs synthesis was slightly inhibited by application of the IA and increased as X-ray irradiation dose increased.

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References

Abdellaoui, S., Lacroix, M., Jobin, M., Boubekri, C. & Gagnon, M. (1995). Effect of gamma irradiation combined with hot water treatment on phytochemical properties, vitamin C content and organoleptic quality of clementines. *Sciences des Aliments*, **15**, 217-235.

Alonso, M., Jacas, J. & del Río, M.A. (2005). Carbon dioxide diminishes cold tolerance of third instar larvae of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in

'Fortune' mandarins: Implications for citrus quarantine treatments. *Postharvest Biology and Technology*, **36**, 103-111.

Artés-Hernández, F., Rivera-Cabrera, F. & Kader, A.A. (2007). Quality retention and potential shelf-life of fresh-cut lemons as affected by cut type and temperature. *Postharvest Biology and Technology*, **43**, 245-254.

Cano, A., Medina, A. & Bermejo, A. (2008). Bioactive compounds in different citrus variaties. Discrimination among cultivars. *Journal of Food Composition and Analysis*, **21**, 377-381.

Chun, O.K., Kim, D.O., Smith, N., Schroeder, D., Han, J.T. & Lee, C.Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture*, **85**, 1715-1724.

Dubery, I.A. (1992). Elicitation of enhanced phenylpropanoid metabolism in citrus flavedo by gamma-radiation. *Phytochemistry,* **31**, 2659-2662.

Follett, P.A. & Neven, L.G. (2006). Current trends in quarantine entomology. *Annual Review of Entomology*, **51**, 359-385.

Girennavar, B., Jayaprakasha, G.K., Mclin, S.E., Maxim, J., Yoo, K.S. & Patil, B.S. (2008). Influence of electron-beam irradiation on bioactive compounds in grapefruit (*Citrus Paradisi* Macf.). *Journal of Agricultural and Food Chemistry,* **56**, 10941-10946.

Hallman, G.J. (1999). Ionizing radiation quarantine treatments against tephritid fruit files. *Postharvest Biology and Technology*, **16**, 93-106.

Holcroft, D.M., Gil, M.I. & Kader, A.A. (1998). Effect of carbon dioxide on anthocyanins, phenylalanine ammonia lyase and glucosyltransferase in the arils of stored pomegranates. *Journal of the American Society for Horticultural Science*, **123**, 136-140.

Ito, S., Kakiuchi, N., Izumi, Y. & Iba, Y. (1974). Studies on the controlled atmosphere storage of satsuma mandarin. *Bulletin of the fruit Tree Research Station B Okitsu* **1**, 39-58.

Jones, D.H. (1984). Phenylalanine ammonia-lyase: Regulation of its induction, and its role in plant development. *Phytochemistry*, **23**, 1349-1359.

Lee, K. & Kader, A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, **20**, 207-220.

McDonald, R.E., Miller, W.R. & McCollum, T.G. (2000). Canopy position and heat treatments influence gamma-irradiation-induced changes in phenylpropanoid metabolism in grapefruit. *Journal of the American Society for Horticultural Science*, **125**, 364-369.

Moshonas, M. & Shaw, P.E. (1984). Effects of low dose gamma irradiation on grapefruit products. *Journal of Agricultural and Food Chemistry*, **32**, 1098-1101.

Oufedjikh, H., Mahrouz, M., Amiot, M.J. & Lacroix, M. (2000). Effect of γ-irradiation on phenolic compounds and phenylalanine ammonia-lyase activity during storage in relation to peel injury from peel of *Citrus clementina* Hort. Ex. Tanaka. *Journal of Agricultural and Food Chemistry*, **48**, 559-565.

Palma, A., D´Aquino, S., Agabbio, M. & Schirra, S. (2005). Changes in flavonoids, ascorbic acid, polyphenol content and antioxidant activity in cold-stored ‘Fortune’ Mandarin. *Acta Horticulturae*, **682**, 617-622.

Palou, L., del Río, M.A., Marcilla, A., Alonso, M. & Jacas, J.A. (2007). Combined postharvest X-ray and cold quarantine treatments against the Mediterranean fruit fly in 'Clemenules' mandarins. *Spanish Journal of Agricultural Research,* **5**, 569-578.

Palou, L., Jacas, J.A., Marcilla, A., Alonso, M. & del Río, M.A. (2008). Physico-chemical and sensory quality of 'Clemenules' mandarins and survival of the mediterranean fruit fly as affected by complementary cold and carbon dioxide quarantine treatments. *Postharvest Biology and Technology*, **48**, 443-450.

Patil, B.S., Vanamala, J. & Hallman, G. (2004). Irradiation and storage influence on bioactive components and quality of early and late season 'Rio Red' grapefruit (Citrus paradisi Macf.). *Postharvest Biology and Technology*, **34**, 53-64.

Rojas-Argudo, C., Palou, L., Cano, A., del Río, M.A., Gonzalez-Mas, M.C. & Bermejo, A. (2007). Efecto de la aplicación de Rayos X a dosis moderadas sobre los componentes bioactivos de mandarinas 'Clemenules'. *Revista Iberoamericana de Tecnología Postcosecha*, **8**, 74-81.

Sánchez-Moreno, C., Plaza, L., de Ancos, B. & Cano, M.P. (2003). Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juice. *Journal of the Science of Food and Agriculture*, **83**, 430-439.

Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of total phenolics with phospomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144-158.

Thompson, A.K. (2004). *Controlled atmosphere storage of fruits and vegetables*. Pp 56-70. Wallingford, UK: CAB International.

USDA (2002a). Importation of clementines from Spain: Final rule. United States Department of Agriculture. *Federal Register*, 67, 64701-64739.

USDA (2002b). Irradiation phytosanitary treatments of imported fruits and vegetables. Final rule. United states Department of Agriculture. *Federal Register*, 67, 65016-65029.

US FDA (2004). Irradiation in the production, processing and handling of food. Final rule. United States Food and Drug Administration. *Federal Register*, 69, 76844-76847.

Vanamala, J., Cobb, G., Turner, N.D., Lupton, J.R., Yoo, K.S., Pike, L.M. & Patil, B.S. (2005). Bioactive compounds of grapefruit (Citrus paradise cv. Rio Red) respond differently to postharvest irradiation, storage, and freeze drying. *Journal of Agricultural and Food Chemistry*, **53**, 3980-3985.

Wang, Y. C., Chuang, Y. C. & Ku, Y.H. (2007). Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. *Food Chemistry*, **102**, 1163-1171.

Table 1. ANOVA *P* values (α=0.05) for the effect of cold quarantine storage (CQ), insecticidal atmosphere (IA), X-ray treatment (X-ray) and interactions on total antioxidant capacity and bioactive compounds of 'Clemenules' mandarins.

	TAC (EC_{50})	TAA	TPC	FGs		
				NAT	HES	DID
CQ	0.147	0.202	0.003	0.544	<0.001	0.021
IA	0.299	0.117	<0.001	0.036	0.081	<0.001
CQ x AI	0.258	0.093	0.001	0.075	0.026	0.064
CQ	0.057	<0.001	<0.001	0.132	<0.001	0.166
X-ray	0.463	0.478	0.163	0.446	0.197	0.150
CQ x X-ray	0.258	0.093	<0.001	0.075	0.026	0.065

P≤ 0.05 indicates a significant effect at the 5% level.
TAC=total antioxidant capacity, TAA=total ascorbic acid, TPC=total phenolic content, FGs=flavanone glycosides, NAT=narirutin, HES=hesperidin, DID=didymin.

Table 2. Total antioxidant capacity and bioactive compounds of ‘Clemenules’ mandarins exposed to cold quarantine at 1.5 ºC for 6, 9, or 12 d followed by 20-h exposure to air-atmosphere at 20 ºC (control) or insecticidal atmospheres (IA, 95 % CO_2) at 20 or 25 ºC.

Cold quarantine period (days)	**IA treatment**	**TAC (EC_{50}) L juice/kg DPPH**	**TAA mg/100 mL juice**	**TPC ng GAE/100 mL juice**	**FGs (mg / 100 mL juice)**		
					NAT	**HES**	**DID**
Initial (at harvest)		391.5 ± 41.1	32.73 ± 3.00	49.58 ± 1.37	2.52 ± 0.19	20.15 ± 0.76	0.33 ± 0.02
6	Control (air-20 ºC)	331.0 ± 26.5 a A	29.03 ± 2.70 a A	54.01 ± 1.27 a A	2.48 ± 0.19 a A	20.31 ± 1.16 ab A	0.30 ± 0.01 a A
	95% CO_2-20 ºC	355.8 ± 40.1 a A	29.74 ± 4.09 a A	55.45 ± 1.56 a A	2.53 ± 0.27 a A	19.68 ± 1.06 a A	0.29 ± 0.03 a A
	95% CO_2-25 ºC	395.5 ± 59.9 a A	29.75 ± 2.53 a A	59.06 ± 0.86 b B	2.89 ± 0.18 b B	21.09 ± 0.65 b AB	0.30 ± 0.01 a B
9	Control (air-20 ºC)	388.9 ± 18.0 a A	29.35 ± 2.59 a A	56.42 ± 0.14 a B	2.72 ± 0.15 b A	22.19 ± 0.41 b B	0.31 ± 0.01 b A
	95% CO_2-20 ºC	376.8 ± 66.5 a A	35.98 ± 1.79 b A	56.98 ± 1.90 a AB	2.38 ± 0.16 a A	21.19 ± 0.99 b B	0.25 ± 0.02 a A
	95% CO_2-25 ºC	408.5 ± 28.6 a A	30.04 ± 0.58 a A	54.75 ± 1.25 a A	2.52 ± 0.10 a A	19.97 ± 0.91 a A	0.26 ± 0.01 a A
12	Control (air-20 ºC)	377.8 ± 25.0 a A	28.72 ± 1.60 a A	56.68 ± 0.27 a B	2.65 ± 0.12 b A	22.77 ± 1.05 b B	0.31 ± 0.01 c A
	95% CO_2-20 ºC	433.9 ± 22.9 a A	29.60 ± 4.05 a A	59.31 ± 0.69 b B	2.47 ± 0.08 a A	21.56 ± 0.49 a B	0.27 ± 0.00 a A
	95% CO_2-25 ºC	381.3 ± 46.8 a A	32.22 ± 2.00 a A	59.35 ± 0.57 b B	2.64 ± 0.03 b AB	22.74 ± 1.14 b B	0.30 ± 0.01 b B

TAC=total antioxidant capacity, TAA=total ascorbic acid, TPC=total phenolic content, FGs=flavanone glycosides, NAT=narirutin, HES=hesperidin, DID=didymin

Previous to TAC, TAA, TPC and FGs determinations, treated fruit was kept at 20 ºC for 7 d to simulate shelf life conditions.

Results present means ± standard deviation (n=3). For each cold quarantine period, mean values followed by different lower case letter indicate statistical differences among IA treatments according to Fisher’s protected LSD test ($P \leq 0.05$). For each IA treatment, means with different capital letter indicate statistical differences among different quarantine periods according to Fisher’s protected LSD test ($P \leq 0.05$).

Table 3. Total antioxidant capacity and bioactive compounds of 'Clemenules' mandarins irradiated with X-rays at 0, 30, 54, or 164 Gy and exposed to cold quarantine at 1.5 ºC for 0, 6, or 12 d.

Cold quarantine period (days)	**X-ray treatment**	**TAC (EC_{50}) (L juice/kg DPPH)**	**TAA (mg/100 mL juice)**	**TPC (mg GAE/100 mL juice)**	**FGs (mg / 100 mL juice)**		
					NAT	**HES**	**DID**
Initial (at harvest)		391.5 ± 41.1	32.73 ± 3.00	49.58 ± 1.37	2.52 ± 0.19	20.15 ± 0.76	0.33 ± 0.02
0	Control	233.6 ± 16.2 a A	34.41 ± 1.88 a A	53.48 ± 0.33 a A	2.46 ± 0.19 ab A	20.84 ± 0.92 a A	0.32 ± 0.01 ab A
	30 Gy	227.2 ± 20.3 a A	37.60 ± 1.37 a A	52.73 ± 0.75 a A	2.42 ± 0.02 a A	20.71 ± 0.63 a A	0.31 ± 0.01 a A
	54 Gy	240.2 ± 51.1 a A	38.82 ± 1.23 a B	53.87 ± 1.12 a A	2.73 ± 0.07 bc A	22.33 ± 0.54 b A	0.34 ± 0.01 bc A
	164 Gy	272.9 ± 33.3 a A	35.92 ± 3.15 a A	54.84 ± 2.19 a A	3.01 ± 0.47 c B	24.58 ± 1.27 c A	0.36 ± 0.03 c B
6	Control	259.5 ± 16.8 a A	33.40 ± 1.72 a A	54.37 ± 1.00 a A	2.72 ± 0.17 a A	22.87 ± 1.69 a B	0.31 ± 0.03 a A
	30 Gy	244.5 ± 15.9 a A	31.67 ± 3.52 a A	56.42 ± 0.74 ab B	3.13 ± 0.36 b C	26.67 ± 2.76 b B	0.38 ± 0.06 b B
	54 Gy	275.2 ± 19.6 a A	32.67 ± 2.03 a A	58.00 ± 0.59 bc B	2.72 ± 0.19 a A	24.46 ± 0.94 a B	0.35 ± 0.01 ab B
	164 Gy	273.2 ± 53.9 a A	35.64 ± 1.96 a A	58.98 ± 1.73 c A	2.84 ± 0.06 a B	24.35 ± 0.64 a A	0.34 ± 0.01 a B
12	Control	271.2 ± 7.8 a A	32.55 ± 1.55 a A	57.43 ± 0.37 a B	2.65 ± 0.26 ab A	24.92 ± 0.40 b C	0.33 ± 0.03 b A
	30 Gy	278.5 ± 35.8 a A	35.23 ± 3.12 a A	59.89 ± 1.42 a C	2.81 ± 0.20 b B	24.83 ± 0.53 b B	0.35 ± 0.02 c B
	54 Gy	288.7 ± 12.3 a A	32.20 ± 0.98 a A	57.60 ± 1.32 a B	2.81 ± 0.09 b A	24.26 ± 0.93 b B	0.34 ± 0.01 bc A
	164 Gy	258.4 ± 31.0 a A	33.44 ± 2.40 a A	56.71 ± 4.27 a A	2.43 ± 0.23 a A	23.15± 1.25 a A	0.29 ± 0.02 a A

TAC=total antioxidant capacity, TAA=total ascorbic acid, TPC=total phenolic content, FGs=flavanone glycosides, NAT=narirutin, HES=hesperidin, DID=didymin

Previous to TAC, TAA, TPC and FGs determinations, fruit was kept at 20 ºC for 7 d to simulate shelf life conditions.

Results present means ± standard deviation (n=3). For each cold quarantine period, mean values followed by different lower case letter indicate statistical differences among X-ray treatments according to Fisher's protected LSD test ($P \leq 0.05$). For each X-ray treatment, means with different capital letter indicate statistical differences among different quarantine periods according to Fisher's protected LSD test ($P \leq 0.05$).