Effect of Insecticidal Atmospheres at High Temperature Combined with Short Cold-quarantine Treatment on Quality of ‘Valencia’ Oranges

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Additional index words. *Ceratitis capitata*, controlled atmosphere, flavor, vitamin C, phenolics

Abstract. The combination of insecticidal atmosphere (IA) with short cold exposure periods has been effective in controlling the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). In the present work, ‘Valencia’ orange quality was assessed on fruit exposed to IA (95% CO₂) at 23, 28, or 33 °C for 20 h; next stored at 1 °C for 8, 16, or 24 days; and then kept at 20 °C for 7 days to simulate shelf life. Physicochemical, sensory, and nutritional quality parameters were analyzed on treated and control (air-exposed) fruit. No significant negative effects on fruit quality were observed in IA-treated ‘Valencia’ oranges. In addition, the exposure of oranges to 95% CO₂ at 28 °C reduced the weight and firmness loss compared with fruit kept in air. Ethanol content increased in the fruits exposed to 95% CO₂ at 28 or 33 °C, but sensory quality was not adversely affected.

Many countries maintain strict quarantine protocols against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), which is one of the most damaging fruit pests worldwide and may be a major pest of citrus (White and Elson, 2004). The most widely used postharvest disinfection treatment of citrus against this fruit fly involves exposure of the fruit to near freezing temperatures. In the United States, the U.S. Department of Agriculture established a minimum exposure during overseas transit of 14 to 18 d at 1.1 to 2.2 °C (USDA, 2002). Today, extensive research is currently focused on the development of alternative or complementary quarantine treatments, especially for cold-sensitive commodities such as citrus (Jacas et al., 2008).

Controlled atmosphere treatments are known to be effective against fruit flies and

other pests (Follett and Neven, 2006; Mitcham et al., 2003). Different works have investigated the use of insecticidal atmospheres (IA) consisting on high CO₂ shocks previous or after cold exposure of citrus fruit to reduce the duration of the standard cold disinfection quarantine treatment against *C. capitata* and thus alleviate chilling injury (CI) problems (Alonso et al., 2005a, 2005b; Palou et al., 2008a). In general, the effects of insecticidal treatments on treated produce considerably differ depending on species and cultivar (Follett and Neven, 2006). Therefore, the optimum combination of atmosphere gas composition, temperature, and length of application should be pursued for each pest–host system. For example, IA treatments consisting of exposure to 95% CO₂ at 20 °C for 20 h (Alonso et al., 2005a), 98% CO₂ at 22 °C for up to 24 h (Alonso et al., 2005a), and 95% CO₂ at 25 °C for 20 h (Palou et al., 2008a) achieved complete insect mortality on ‘Fortune’ mandarins, ‘Valencia’ oranges, and ‘Clemenules’ mandarins, respectively, without affecting negatively fruit external appearance or sensory properties.

Today, IA at a curing temperature of 33 °C on citrus has also been studied to control postharvest green mold of mandarins (Palou et al., 2008b). These new physical methods

combining heat and gas shocks could be interesting to control established pathogenic infections and/or induce fruit resistance to postharvest diseases. However, little information is available for the effects of high CO₂ citrus exposure at high temperature followed by cold quarantine storage on overall fruit quality.

Conventionally, postharvest quality assessment has been conducted to evaluate the physicochemical quality of the fruit through parameters such as weight loss, firmness, maturity index, and acidity, among others. Additionally, the sensory evaluation of fruit is also performed to determine changes during postharvest handling. At present, the nutritional quality is gaining interest, being a component of the overall quality that is very much valued by consumers. In particular, citrus fruits are an important nutritional source of vitamin C and polyphenolic compounds with antioxidant properties such as flavonoids (Sánchez-Moreno et al., 2003). The necessity of preserving the health properties of citrus recommends that postharvest technologies would maintain both functional and nutritional quality until these reach the consumer. Lee and Kader (2000) reviewed the factors affecting postharvest content of vitamin C and concluded that temperature is the most important factor. In general, losses are accelerated by using high temperatures and long storage. However, low-temperature storage can also accelerate the loss of vitamin C in cold-sensitive fruit, even before CI is evident (Miller and Heilman, 1952). Therefore, the exposure of citrus fruit to high CO₂ concentrations at different temperatures and the combination with different periods of cold storage might affect the physiology of the fruit, altering the biochemical components of citrus. Therefore, the aim of this work was to study the effect of IA (95% CO₂) applied at 23, 28, and 33 °C combined with short cold quarantine storage on physicochemical, sensory, and nutritional quality of ‘Valencia’ oranges.

Materials and Methods

Fruit

‘Valencia’ oranges (*Citrus sinensis*) were hand-harvested with an average maturity index of 10.1 from a local grove in Valencia, Spain, and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, and dipped in a mixed solution of imazalil (1000 ppm) for 1 min. Subsequently, fruit were put into 12 homogeneous groups of 80 fruit each, which were placed in separate unlidded commercial cardboard boxes (40 × 29 × 27 cm).

Insecticidal atmosphere treatments

For each period of time, four groups of 80 fruit were exposed for 20 h to the following IA treatments: 1) air atmosphere at 23 ± 1 °C (control); 2) IA containing 95% CO₂ at 23 ± 1 °C; 3) IA containing 95% CO₂ at 28 ± 1 °C; and 4) IA containing 95% CO₂ at 33 ± 1 °C. In all cases, relative humidity (RH) was

Received for publication 16 June 2010. Accepted for publication 24 July 2010.

Adriana Contreras was funded by a scholarship from the Consejo Nacional de Ciencias y Tecnología (CONACyT) from México. Dr. Cristina Rojas-Argudo is supported partially by the European Social Fund.

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85% $\pm$ 5%. IA exposure chambers consisted of hermetic Perspex cabinets (82 $\times$ 62 $\times$ 87 cm) fitted with inlet and outlet ports through which CO₂ (Alphagaz, N38; Air Liquid S.A., Madrid, Spain) passed at a rate adjusted to yield a concentration of 95% (v/v) inside the cabinet and balance air. Gas was allowed to escape from the outlet port through a bubble tube to maintain the proper gas mixture in the chamber. Levels of CO₂, O₂, temperature, and RH were continuously monitored by means of the Control-Tec® system (Tecnidex S.A., Paterna, Valencia, Spain). Cabinets were installed inside a 40-m³ storage room that was also set to each experimental temperature (23, 28, or 33°C). Once IA treatments were accomplished, fruit were coated with a 10% total solids water wax containing polyethylene, shellac, and 0.5% of the fungicide thiabendazole (Brillaqua®; Brilloccera S.A., Beniparrell, Valencia, Spain).

After waxing, the fruits were exposed to the standard cold quarantine temperature of 1 $\pm$ 0.5 °C for 8, 16, or 24 d followed by 7 d of shelf life at 20 °C to simulate prompt fruit commercialization. Approximately 50 additional oranges were used to determine fruit quality at harvest (initial quality). Quality attributes were determined as follows.

Materials

Reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]), potassium dihydrogen phosphate (KH₂PO₄), *meta*-phosphoric acid (MPA), phosphoric acid (H₃PO₄), Folin-Ciocalteu phenol reagents, sodium carbonate (Na₂CO₃), gallic acid, and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinheim, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Spain). 1,4-dithio-DL-threitol (DTT) and hesperidin [hesperitin-7-O-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 were of high-performance liquid chromatography grade and ultrapure water (Milli-Q; Millipore Corporation, Billerica, MA) was used.

Physicochemical quality

Weight loss. Lots of 30 fruits per treatment were used to measure weight loss. The same fruit was weighed at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage of initial weight loss.

Firmness. Firmness of 20 oranges per treatment was determined at the end of each storage time using an Instron Universal Testing Machine (Model 3343; Instron Corp., Canton, MA). Each fruit was compressed between two flat surfaces closing together at a rate of 5 mm·min⁻¹. The instrument gave the deformation after application of a load of 10 N to the equatorial region of the fruit. Results were expressed as percentage of deformation related to initial diameter.

Ethanol and acetaldehyde contents. Ethanol and acetaldehyde contents (EC and AC,

respectively) in juice were determined by head-space gas chromatography. Ten fruits each in three replicates per treatment were analyzed. Five milliliters of orange juice was transferred to 10-mL vials with crimp-top caps and TFE/silicone septum seals and frozen until analysis. EC and AC were analyzed in a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA) equipped with an autosampler, a flame ionization detector, and fitted with a Poropak QS 80/100 column (Waters Corporation, Millord, MA) (1.2 m $\times$ 0.32 cm). Temperatures of the oven, injector, and detector were 150, 175, and 200 °C, respectively. Helium was used as the carrier gas at a flow rate of 28 mL·min⁻¹. One-milliliter sample of the head-space was withdrawn from each vial previously equilibrated in the autosampler incubation chamber for 10 min at 40 °C. EC and AC concentrations were calculated using peak areas of the samples relative to the peak areas of standard solutions. Results were expressed as mg/100 mL juice.

External disorders. Eighty fruit per treatment were inspected for external physiological disorders at the end of each storage period. The different degrees of disorders were rated as 0 = none, 1 = light, 2 = moderate, and 3 = severe. Light was considered when less than 10% of the fruit surface was affected and severe when more than 20% of the fruit surface was affected. Results were converted to an average index.

Internal quality parameters. Soluble solids concentration (SSC) was measured with a digital refractometer (Atago, Model PR1; Atago Co., LTD., Tokyo, Japan) and titratable acidity (TA) was determined by titration with 0.1 N NaOH and phenolphthalein indicator and expressed as grams of citric acid per 100 mL of orange juice. The maturity index (MI) was calculated as SSC/TA ratio. The juice from three replicates of 10 fruit each was used to determine these parameters.

Sensory analysis. Sensory evaluation was conducted by 10 trained judges. Panelists rated flavor on a 9-point scale, in which 1 to 3 represented a range of non-acceptable quality with the presence of off-flavor, 4 to 6 represented a range of acceptable quality, and 7 to 9 represented a range of excellent quality. Off-flavor presence was evaluated using a 6-point scale in which 0 = absence of off-flavor and 5 = high presence of off-flavor.

One sample consisted of whole segments taken from approximately eight individual fruits. Samples were presented to panelist in trays labeled with three-digit random codes and served at room temperature (25 $\pm$ 1 °C). The judges had to taste several segments of each treatment to compensate, as far as possible, for biological variation of material. Mineral spring water was provided for rinsing between samples.

Nutritional quality

DPPH[•] radical-scavenging capacity. The total antioxidant capacity (TAC) was evaluated by the DPPH[•] assay. A total of 0.4 mL of orange juice diluted with 0.8 mL methanol was centrifuged at 12,000 rpm and 4 °C for

20 min. Six methanolic dilutions from the supernatant (0.075 mL) were mixed with 0.2925 mL of DPPH[•] (24 mg·L⁻¹) and kept in darkness for 40 min. Afterward, the change in absorbance at 515 nm was measured in a Multiskan spectrum microplate reader (Thermo Labsystem; Thermo Fisher Scientific Inc., Waltham, MA).

For each dilution, the percentage of remaining DPPH[•] was determined on the basis of the DPPH[•] standard curve. The amount of juice in each dilution was plotted against the amount of DPPH[•] radical remaining. Using the curve obtained, the EC₅₀ value was calculated. This result expressed the amount of orange juice (L) needed to reduce 1 kg of DPPH[•] by 50%; thus, lower values mean higher antioxidant activity.

Total ascorbic acid. Total AA was determined by the sum of AA plus L-dehydroascorbic acid by using the reducing agent DTT (Sánchez-Mata et al., 2000). One milliliter of orange juice was diluted to 10 mL with 2.5% (w/v) MPA. Two milliliters of this solution was mixed with 0.4 mL of DTT (20 mg·mL⁻¹) for 2 h in darkness. Afterward, the extracts were filtered through a 0.45- μ m Millipore filter before being analyzed by high-performance liquid chromatography (HPLC).

The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi, Darmstadt, Germany) equipped with an L-2200 autosampler, L-2130 quaternary pump, L-2300 column oven, and L-2450 diode array detector. System conditions were: injection volume 20 μ L, oven 25 °C, detector wavelength 243 nm, flow rate 1 mL·min⁻¹, column Lichospher 100 RP-18 of 25 $\times$ 0.4 cm preceded by a precolumn (4 $\times$ 4 mm) with a 5- μ m particle size (Merck, Darmstadt, Germany). The mobile phase was 2% KH₂PO₄ adjusted to pH 2.3 with H₃PO₄. Results were expressed as mg AA/100 mL of juice.

Flavanone glycosides. HES, NAT, and DID (mg/100 mL) were determined by the method described by Cano et al. (2008) slightly modified. Two milliliters of orange juice was homogenized with 2 mL DMSO: methanol (1:1 v/v) and centrifuged for 30 min at 12,000 rpm and 4 °C. The supernatant was filtered through one 0.45- μ m nylon filter and analyzed by HPLC-DAD using the HPLC equipment described previously. System conditions were: injection volume 10 μ L, oven 25 °C, detector wavelength 280 nm, flow rate 1 mL·min⁻¹, column Lichospher 100 RP-18 of 25 $\times$ 0.4 cm preceded by a precolumn (4 $\times$ 4 mm) of a 5- μ m particle size (Merck). The mobile phase was acetonitrile (A): 0.6% acetic acid (B) with initial condition of 10% A for 2 min, reaching 75% A in the next 28 min, then back to the initial condition in 1 min, and held for 5 min before the next sample injection. The main flavanone glycosides (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 with that of its pure standards. Results were expressed as mg/100 mL.

Total phenolic content. The orange juices were analyzed for total phenolics by the Folin-Ciocalteu colorimetric method. A total of 0.3 mL of orange juice was diluted with 1.7 mL of 80% aqueous methanol. Appropriately diluted extract (0.4 mL) was mixed with 2 mL of Folin Ciocalteu commercial reagent (previously diluted with water 1:10, v/v) 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. The absorbance of the resulting blue solution was measured spectrophotometrically at 765 nm (Thermo UV1; Thermo Electron Corporation, Auchtermuchty Fife, U.K.) and the concentration of total phenolics was expressed as gallic acid equivalents per 100 mL (mg GAE/100 mL). All extracts were analyzed in triplicate.

Statistical analysis. Statistical analysis was performed using STATGRAPHICS Plus 4.1 (Manugistics, Inc., Rockville, MD). Significance between means was determined by least significant difference at $P \leq 0.05$.

Results and Discussion

Physicochemical quality. The use of high temperatures is known to enhance the insecticidal activity of quarantine treatments (Vincent et al., 2003), but also it might increase fruit weight loss in the same way that conventional curing of citrus increases fruit weight loss (Plaza et al., 2003; Porat et al., 2000). Figure 1 shows the weight loss of 'Valencia' oranges exposed to the IA treatments (95% CO₂) at 23, 28, or 33 °C followed by cold quarantine storage at 1 °C for 8, 16, or 24 d and a shelf life period at 20 °C for 7 d. The exposure of 'Valencia' oranges to the IA at different temperatures did not increase the weight loss compared with the control. Interestingly, oranges exposed to IA at 28 °C had the lowest weight loss. It is known that exposure to moderate temperatures and high RH induces wound healing by biosynthesis of lignin and other phenolic compounds (Mulas and Schirra, 2007; Nunes et al., 2007). Therefore, this mild heat treatment probably induced positive changes in the rind of the mandarins that helped reduce orange weight loss.

Fruit firmness significantly decreased after 24 d of storage at 1 °C (Table 1). Although no relevant differences were observed between IA-treated fruit and control fruit, fruit exposed to the IA at 28 or 23 °C maintained higher firmness values than control fruit after 8 or 24 d of cold storage, respectively, which is in accordance with the lower weight loss of these treatments under those storage conditions (Fig. 1). In general, the treatments had no harmful effect on fruit firmness and the maximal percentage of deformation remained below the 5% threshold established for firmness in citrus fruit (Martínez-Jávega et al., 1998). Similar results have been reported on mandarins and oranges exposed to combined cold and IA quarantine treatments (Alonso et al., 2005a, 2005b; Palou et al., 2008a).

Figure 2 shows the EC and AC of 'Valencia' oranges exposed to combined IA and cold quarantine treatments. The EC and AC of CO₂-treated oranges remained fairly constant as cold quarantine storage time

increased. 'Valencia' oranges exposed to high CO₂ had more EC and AC than those exposed to air. EC and AC were lower in fruit exposed to the IA at 23 °C than in those exposed at 28 and 33 °C. Under these high-temperature

Fig. 1. Weight loss of 'Valencia' oranges exposed to air (control) or an insecticidal atmosphere (IA, 95% CO₂) at 23, 28, or 33 °C for 20 h followed by a cold quarantine storage at 1 °C for 8, 16, or 24 d and a shelf life of 7 d at 20 °C. Bars indicate SD at $P \leq 0.05$.

Table 1. Firmness of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO₂) at 23, 28, or 33 °C for 20 h followed by a cold quarantine storage at 1 °C for 8, 16, or 24 d and a shelf life of 7 d at 20 °C.

Cold quarantine period (days)	IA treatment	Firmness (% deformation)
Initial (at harvest)		2.24 ± 0.41
8	Control (air at 23 °C)	2.28 ± 0.36 b
	IA 23 °C	2.24 ± 0.30 ab
	IA 28 °C	2.08 ± 0.20 a
	IA 33 °C	2.36 ± 0.27 b
16	Control (air at 23 °C)	2.27 ± 0.36 a
	IA 23 °C	2.05 ± 0.41 a
	IA 28 °C	2.21 ± 0.38 a
	IA 33 °C	2.20 ± 0.36 a
24	Control (air at 23 °C)	3.13 ± 0.38 b
	IA 23 °C	2.75 ± 0.44 a
	IA 28 °C	2.93 ± 0.49 ab
	IA 33 °C	3.14 ± 0.43 b

Previous to firmness measurement, fruit was kept at 20 °C for 7 d to simulate shelf life conditions. Values give means ± SD (n = 3). For each cold quarantine period, means with the same letter are not different at $P \leq 0.05$.

Fig. 2. Ethanol and acetaldehyde contents of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO₂) at 23, 28, or 33 °C for 20 h followed by a cold quarantine storage at 1 °C for 8, 16, or 24 d and a shelf life of 7 d at 20 °C. Bars indicate SD (n = 3).

conditions, EC exceeded the limit slightly, set up at 200 mg/100 mL juice, considered by some authors as the level of off-flavor build-up risk (Hagenmaier, 2002; Ke and Kader, 1990).

An increase in the concentration of these fermentative volatile compounds resulting from exposure to high CO₂ and/or low O₂ atmospheres has also been described in other citrus cultivars (Alonso et al., 2005a; Ke and Kader, 1990; Pesis and Avissar, 1989). Although the levels of volatile built up as a consequence of controlled atmosphere exposure depends on fruit cultivar, treatment conditions, and duration, these works report similar volatile built up to the results found in our work when IA treatments were applied at temperatures below 25 °C. In general, the level of ethanol, acetaldehyde, and other internal volatiles associated with anaerobic conditions increased as the temperature of the application of the IA treatment increased.

The exposure of 'Valencia' oranges to the IA did not cause any rind damage. These results are in agreement with Alonso et al. (2005b) and Palou et al. (2008a) in 'Fortune' and 'Clemenules' mandarins, respectively. However, Ke and Kader (1990) described that longer exposures to high CO₂ atmospheres (9 to 14 d) induced severe rind injuries in 'Valencia' oranges.

Application of IA treatments did not affect TA, SSC, or MI (data not shown). Similarly, Alonso et al. (2005a) and Palou et al. (2008a) showed no effect of high CO₂ exposure for short storage periods on internal fruit quality parameters of 'Fortune' and 'Clemenules' mandarins, respectively. However, Pesis and Avissar (1989) showed that the exposure of oranges to high CO₂ atmospheres for 20 to 40 h decreased TA and increased MI.

Sensory analysis. Overall flavor and off-flavors were unaffected by the IA treatment or the length of the cold quarantine period. Judges evaluated the oranges as acceptable at the end of storage and having a very slight

off-flavor (Table 2). Similarly, no negative influence on fruit sensory quality was found on 'Valencia' oranges or 'Fortune' mandarins exposed to IA for short periods (Alonso et al., 2005a, 2005b). However, the flavor of 'Clemenules' mandarins decreased after short-term exposure to high CO₂ atmospheres (Palou et al., 2008a). These differences are because several factors such as electrical conductivity, acidity, SSC, and cultivar can influence the perception of off-flavors (Ke and Kader, 1990; Ke et al., 1991).

Nutritional quality. Table 3 shows the DPPH• radical-scavenging capacity (RSC) of 'Valencia' oranges treated with IA at high temperature combined with short cold quarantine periods. The DPPH• RSC of the oranges was unaffected by the exposure to 95% CO₂ or when the cold quarantine period increased, except IA 33 °C-treated oranges. In this treatment, EC₅₀ values increased (i.e., DPPH• RSC decreased) at the end of the cold quarantine storage ($P \leq 0.05$). A high correlation between

phenolic compounds and TAC in different fruits and vegetables has been widely reported (Kevers et al., 2007). In citrus fruit, AA in addition to phenolic content might contribute from 33% to 95% of the TAC (Gil-Izquierdo et al., 2002; Palma et al., 2005; Sánchez-Moreno et al., 2003). In our work, neither TAA nor total phenolics decreased in IA 33 °C-treated oranges; therefore, other phytochemicals such as carotenoids would be involved in the observed DPPH• RSC decrease.

TAA content of 'Valencia' oranges ranged from 34 to 42 mg/100 mL (Table 3). The TAA content of 'Valencia' oranges was unaffected by 95% CO₂ exposure in fruits stored at cold quarantine temperature for 8 or 16 d. However, after 24 d cold quarantine storage, TAA increased in control fruit but no increase was observed in the rest of the treatments. In accordance with our results, Rapisarda et al. (2008) reported an increase in the TAA content of 'Valencia' oranges during storage at 6 °C. Therefore, exposure to 95% CO₂ could

Table 2. Sensory analysis of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO₂) at 23, 28, or 33 °C for 20 h followed by a cold quarantine storage at 1 °C for 8, 16, or 24 d and a shelf life of 7 d at 20 °C.

Cold quarantine period (days)	IA treatment	Off-flavor (0–5)	Flavor (1–9)
Initial (at harvest)		0.00 ± 0.00	7.12 ± 0.71
8	Control (air at 23 °C)	0.67 ± 1.30 a	6.33 ± 1.44 a
	IA 23 °C	1.25 ± 1.42 a	5.58 ± 1.56 a
	IA 28 °C	1.08 ± 1.24 a	5.33 ± 1.61 a
	IA 33 °C	1.08 ± 1.44 a	5.00 ± 1.86 a
16	Control (air at 23 °C)	0.82 ± 0.98 a	5.36 ± 1.80 a
	IA 23 °C	1.00 ± 1.26 a	5.09 ± 1.64 a
	IA 28 °C	1.36 ± 1.50 a	4.91 ± 1.76 a
	IA 33 °C	1.55 ± 1.21 a	5.00 ± 2.05 a
24	Control (air at 23 °C)	0.46 ± 0.78 a	6.62 ± 1.19 a
	IA 23 °C	1.38 ± 1.45 a	5.54 ± 1.51 a
	IA 28 °C	1.23 ± 1.36 a	5.46 ± 1.66 a
	IA 33 °C	0.92 ± 1.26 a	5.92 ± 1.61 a

Previous to sensory evaluation, fruit was kept at 20 °C for 7 d to simulate shelf life conditions. Values give means ± SD. For each cold quarantine period, means with the same letter are not different at $P \leq 0.05$.

Table 3. DPPH• radical-scavenging capacity (DPPH• RSC) and bioactive compounds of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO₂) at 23, 28, or 33 °C for 20 h followed by a cold quarantine storage at 1 °C for 8, 16, or 24 d and a shelf life of 7 d at 20 °C.

Cold quarantine period (days)	IA treatment	DPPH• RSC (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)		233.0 ± 14.2	34.71 ± 1.40	93.20 ± 4.82	2.86 ± 0.24	21.75 ± 1.21	0.97 ± 0.08
8	Control (air at 23 °C)	224.3 ± 10.8 a A	39.15 ± 1.47 a A	91.23 ± 4.47 a A	3.26 ± 0.13 a A	27.83 ± 0.31 a A	1.02 ± 0.08 a A
	IA 23 °C	229.5 ± 7.1 a A	37.57 ± 1.23 a AB	90.21 ± 2.02 a A	2.85 ± 0.05 a A	27.47 ± 0.86 a A	0.96 ± 0.00 a A
	IA 28 °C	238.6 ± 19.5 a A	39.30 ± 1.18 a A	93.54 ± 1.64 a A	3.02 ± 0.53 a A	26.36 ± 1.36 a A	0.90 ± 0.11 a A
	IA 33 °C	206.3 ± 3.6 a A	36.21 ± 1.17 a A	98.70 ± 3.85 a A	2.95 ± 0.28 a A	26.88 ± 1.61 a A	0.87 ± 0.05 a A
16	Control (air at 23 °C)	220.8 ± 7.6 a A	38.72 ± 1.10 a A	94.10 ± 0.38 a A	3.05 ± 0.20 a A	24.97 ± 1.09 a A	0.94 ± 0.08 a A
	IA 23 °C	228.7 ± 14.8 a A	35.50 ± 1.53 a A	99.74 ± 7.21 a A	2.84 ± 0.21 a A	25.08 ± 1.23 a A	0.84 ± 0.07 a A
	IA 28 °C	219.5 ± 35.6 a A	35.85 ± 1.29 a A	99.10 ± 5.28 a A	2.86 ± 0.22 a A	25.27 ± 0.28 a A	0.79 ± 0.07 a A
	IA 33 °C	194.9 ± 11.7 a A	36.21 ± 2.60 a A	107.19 ± 11.20 a A	2.97 ± 0.23 a A	27.51 ± 1.01 b A	0.80 ± 0.03 a A
24	Control (air at 23 °C)	216.1 ± 2.1 a A	42.31 ± 1.65 b B	105.86 ± 4.99 a B	2.88 ± 0.18 a A	27.67 ± 0.61 a A	0.85 ± 0.07 a A
	IA 23 °C	227.0 ± 5.7 a A	38.84 ± 0.49 a B	100.66 ± 4.19 a A	2.94 ± 0.11 a A	26.66 ± 1.27 a A	0.88 ± 0.01 a A
	IA 28 °C	241.6 ± 17.2 a A	36.94 ± 2.22 a A	103.55 ± 9.38 a A	3.20 ± 0.11 a A	25.88 ± 0.64 a A	0.94 ± 0.06 a A
	IA 33 °C	234.8 ± 2.8 a B	38.01 ± 0.36 a A	107.80 ± 2.46 a A	3.16 ± 0.17 a A	26.11 ± 0.56 a A	0.90 ± 0.09 a A

Previous to DPPH• RSC and bioactive compounds determination, fruit was kept at 20 °C for 7 d to simulate shelf life conditions. Values give means ± SD ($n = 3$). For each cold quarantine period, different treatments with the same lower case letter are not different at $P \leq 0.05$. For each treatment and different quarantine periods, means with the same capital letter are not different at $P \leq 0.05$.

DPPH• RSC = DPPH• radical-scavenging capacity; TAA = total ascorbic acid; TPC = total phenolic content; FGs = flavanone glycosides; NAT = narirutin; HES = hesperidin; DID = didymin.

diminish the capacity for the AA synthesis during fruit storage.

The FG contents of 'Valencia' oranges exposed to IA and cold quarantine were in the range of those reported for citrus fruit (Table 3) (Dhuique-Mayer et al., 2005; Nogata et al., 2006). The HES, NAT, and DID contents of 'Valencia' oranges were unaffected by the increase in the cold quarantine period ($P \leq 0.05$). Palma et al. (2005) did not find significant differences in HES, NAT, or DID in 'Fortune' mandarin during 3 months of storage at 5 °C. In general, the FG contents were unaffected by the exposure to 95% CO₂, except on oranges exposed to the IA at 33 °C after 16 d of cold quarantine storage that had more HES than the rest of the samples.

Total phenolic content (TPC) of 'Valencia' oranges ranged from 90.21 ± 2.02 to 107.80 ± 2.46 mg/100 mL (GAE) (Table 3). Gil-Izquierdo et al. (2002) found that TPC of orange juice and pulp after domestic and commercial squeezing was 87.8 and 71.7 mg/100 mL, respectively. Gardner et al. (2000) also found that total polyphenols ranged from 50.4 ± 1.0 to 75.5 ± 1.8 mg/100 mL (GAE) in three commercial orange juices.

In general, there was an increase in the TPC of 'Valencia' oranges when the cold quarantine period increased. This result is in accordance with Patil et al. (2004), which reported higher flavanoid content after cold storage of citrus fruit. This was associated with an increase in the phenylalanine ammonia lyase (PAL) activity during low-temperature storage. On the other hand, other works have shown that cold storage either did not influence or decreased the citrus TPC. For example, Palma et al. (2005) did not find differences in the TPC of 'Fortune' mandarins after 90 d of storage at 5 °C, whereas Rapisarda et al. (2008) found a decrease of total phenolics in 'Valencia' oranges after 40 d of storage at 6 °C, which was attributed to senescence during storage.

It can be concluded from these results that the exposure of 'Valencia' oranges to 95% CO₂ at 23, 28, or 33 °C combined with short cold quarantine periods did not induce any harmful effect on physicochemical, sensory, or nutritional citrus quality. Exposure of 'Valencia' oranges to 95% of CO₂ at 28 °C for 20 h was beneficial to maintain the fruit quality, reducing weight and firmness loss. Combination of the IA and cold quarantine periods of 8 or 16 d did not affect the TAA content of the fruits; whereas when the cold quarantine period increased to 24 d, fruit exposed to high CO₂ atmospheres had lower TAA content than control fruit. Therefore, this high CO₂ atmosphere, alone or combined with curing temperatures, could be applied as insecticidal treatment or for the control of citrus moulds without negatively affecting the quality of 'Valencia' orange.

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