INTRODUCTION

Kiwifruit (Actinidia delicosa (A. Chev.) C.F. Lianf et. R. Ferguson var. delicosa) is an economically important fruit throughout the world. One of the major causes of postharvest losses of ‘Hayward’ kiwifruit is the fungus Botrytis cinerea Pers.:Fr. After harvest kiwifruit are vulnerable to fungal attack either during cold storage or shelf-life (Eckert and Ogawa 1988). Infections may occur at bloom and continue during fruit maturation and ripening in storage; infection can aslo occur at harvest. Decay occasionally starts on the fruit sepal but mostly develops from the stem end where the stem is snapped off during harvest (Michailides and Grogan 1996). Early infections generally do not cause immediate decay, but the pathogen can resume growth as fruit ripen, usually in cold storage. Growth of B. cinerea is very slow at low temperatures and expression of the disease becomes apparent after 4 to 12 weeks of storage at 0°C. However, fruit that skip infection or do not express disease symptoms during cold storage, once brought to wholesale and retail markets, are exposed to ambient temperatures that favor quick growth of the fungus that may result in extra losses. Therefore inhibition of fungal growth after cold storage would prolong shelf life of kiwifruit significantly. Management of the fungus is obtained usually using a limited number of fungicides that control gray mold of kiwifruit. However, consumer opposition to use of potentially hazardous chemicals on fruit and vegetables and the ability of B. cinerea to rapidly develop resistance to many fungicides has stimulated research on development of alternatives to chemical methods for maintenance of fruit quality (Wisniewski and Wilson 1992).

Several alternatives to chemical control of postharvest decay of fruits and vegetables have been proposed, including heat treatments, controlled or modified atmosphere packaging, biological control, fumigation with natural volatiles, ultraviolet light, calcium salts, and edible coatings (Gennadios and Waller 1990; Klein and Lurie 1992; Biggs et al. 1997; Schirra and D'Hallewin 1997; Marquenie et al. 2002; Zhang et al. 2007). Postharvest heat treatments have been used on fruit and vegetables for disease control and insect disinfestations (Barkai-Golan and Philips 1991). Hot water dipping, vapor heat, or hot dry air treatments have been applied to many horticultural products. If not properly applied, heat treatments may result in fruit damage, which is typically seen as browning of fruit surfaces, pitting, abnormal softening and breakdown of fruit’s flesh (Lurie 1998). Water temperatures of 50°C to 60°C, for various exposure times up to 10 min, can control many postharvest plant pathogens on a number of fruit and vegetables without damaging effects. At lower temperatures duration of heat treatment must be longer to be effective (Barkai-Golan and Philips 1991). Heat treatments have been applied to a great variety of fruit, prior to cool storage in most cases (Lurie 1998). Unfortunately, heat treatments can affect postharvest physiology, ripening processes and fruit quality. Temperatures above 35°C inhibit ripening of many fruits, including kiwifruit (Mitchell 1986; Antunes and Sfakiotakis 2000). However, there are several reports on inhibition of some ripening processes and promotion of others (Lurie 1998).

Studies on hot water dipping of kiwifruit have been reported previously. Irving et al. (1991) reported that pre-storage hot water dipping at 38°C, 42°C, 46°C or 50°C for 8 min did not affect respiration rate, ethylene production or firmness of kiwifruit after 8 weeks at 0°C and an additional 15-day shelf life period. On the contrary, dipping at 54°C increased respiration and ethylene production and softening of fruit. However, Cheah et al. (1992a) showed that hot water dipping at 50°C and 54°C for 8 min effectively controlled B. cinerea rot, with decay reductions of 80% and 92%, respectively, but heat injury developed on treated kiwifruit. Dipping at 46°C resulted in moderate control,
with up to 64% reduction of *B. cinerea* rot. Subsequently Cheah et al. (1992b) showed that hot water dipping at 46°C for 15 min and 48°C for 8 min, resulted in almost complete control of *B. cinerea* rot without causing heat injury or affecting fruit firmness and taste. It was also shown that 50°C was the highest temperature that could be used safely, with 4 min close to the maximum duration of dipping at this temperature (Cheah et al. 1992b). Moreover, these studies demonstrated that hot water dipping treatments applied prior to cold storage changed the physiology and ripening of kiwifruit (Cheah et al. 1992a, 1992b).

It is important to recognize that during shelf life kiwifruit can produce and respond to endogenous ethylene that will increase ripening leading to a ‘ready-to-eat’ firmness (Koukounaras and Sfakiotakis 2007). Therefore, a post-storage heat treatment should control *B. cinerea* rot without negatively affecting fruit physiology.

In recent years consumers have shown a great interest in antioxidants of fruits and vegetables because of their positive effect on human health. Kiwifruit has high concentrations of vitamin C (Mitchell 1994). However, heat treatment may negatively affect the antioxidant content of fruit and vegetables (Jonsson 1991). No information is available on the effect of heat treatment on antioxidant capacity of kiwifruit.

The aim of this study was to investigate the effect of post-storage hot water dips on management of *B. cinerea* rot and quality of ‘Hayward’ kiwifruit during shelf-life.

**MATERIALS AND METHODS**

**Plant material**

‘Hayward’ kiwifruit were harvested, at the stage of usual practises (SSC>6.2%), from the University farm in Thessaloniki, Greece, and kept at 0°C for 8 weeks, in order to reach flesh firmness of at least 20 Newton (N); this represents the minimally accepted firmness levels for wholesale market (Sfakiotakis et al. 2003). Fruit with homogeneity in color and in size (90-110 g), free from any kind of damage and with flesh firmness between 20-35 N were taken from 0°C and put at 20°C for 12 hours before artificial inoculation with the fungus.

**Fungus inoculation of kiwifruit**

The *B. cinerea* pathogenic isolate BPIC1952, provided by the Benaki Phytopathological Institute in Athens/Greece, was used in all experiments. The fungus was routinely grown on potato dextrose agar (PDA; Merk, Germany) and was kept in agar slants at 4°C. The pedicels of kiwifruits that were used for inoculation were gently removed without damaging the fruit flesh. One mycelial disk, 5 mm in diameter, bearing approximately 2x10⁶ conidia, was transferred from the margin of an 8 day old colony, onto each kiwifruit at the stem end, and the inoculum was firmly attached to the fruit in a small cavity formed after removal of pedicel. The concept was to simulate conditions where fruit taken out from cold storage, aparently healthy but carrying inoculum, are brought to the wholesale market. After inoculation and before heat treatment, fruit were put into plastic boxes covered with moist paper, at 24°C for 6 hours to facilitate fungal penetration into the first layer of cells.

**Hot water dip treatment**

Hot water dip treatments were performed in a 150 L, “homemade”, thermostatically controlled water bath. The water bath was filled with fresh tap water for each treatment separately. The required temperature was monitored by a remote squirrel meter (Grant Instruments, Cambridge, UK). Six hours after inoculation each replicate was subjected to one of the following seven hot water dip treatments: 1) 55°C for 1.5 min; 2) 55°C for 3 min; 3) 60°C for 1.5 min; 4) 60°C for 3 min. Following dipping, the fruit were left to dry for 20 to 30 min at room temperature. Fruit were stored at 20°C for 8 days in a modified Mariotte System that consisted of a jar container and an air circulation system that prevented secondary infections and maintained sustainable O₂ concentration (21%) in the container without accumulation of CO₂ (Sfakiotakis 1972). The experiment was performed three times.

The effect of hot water dips on postharvest quality of kiwifruit was evaluated in a separate experiment. Kiwifruit were subjected to hot water dips without prior inoculation with *B. cinerea*. The hot water dip treatments were performed as described above. After treatment fruit were kept at 20°C, in relative humidity ≥90%, for 8 days. There were 3 replicates of each treatment with 12 fruit per treatment. The experiment was performed three times.

**Estimation of Botrytis cinerea rot**

Decay development was evaluated after 8 days at 20°C. Measurements were taken to express the depth of rot into the fruit, indicated by characteristic color change of fruit flesh. Isolations were made from the interior of the fruit, at the border between decayed and healthy tissues to confirm the presence of the fungus. A digital ruler (Mitutoyo Ltd., UK) was used to measure the length of the rotten area after cutting the fruit in equal hemispheres vertically.

**CO₂ and ethylene production**

Carbon dioxide (CO₂) and ethylene production were measured every two days starting at ‘day 0’, the day of treatment. Two ml air samples were taken after closing the fruits in 3 L glass jars for 40 min. CO₂ concentration was measured by injecting the gas sample into a stream of N₂ carrier gas flowing through an CO₂/O₂ analyzer (model Combo 280, David Bishop Instruments, UK), whereas ethylene concentration was measured by injecting the gas sample into a Varian 3300 gas chromatograph (Varian Instruments, Walnut Creek, CA) equipped with a stainless steel column filled with Porapak, length 100 cm, diameter 0.32 cm, at 50°C and a flame-ionization detector at 120°C.

**Color**

Fruit color was measured at two opposite sides after skin removal, using a chromameter (Minolta CR-200, Minolta, Osaka, Japan), equipped with an 8-mm measuring head and a C illuminant (6774 K) calibrated using the manufacturer’s standard white plate. Color changes were quantified in the L*, a* and b* color space. L* refers to lightness of the kiwifruit flesh, ranging from black (0) to white = 100. Hue angle ([h°=tan⁻¹(b*/a*)-180°]) when a*<0 and b*<0 and chroma values [C=(a*²+b*²)¹/²] were calculated from a* and b* values (McCuen 1992). At day 0, color was measured using 3 replications of 12 fruit each, with the same characteristics as those that received treatments.

**Flesh firmness**

Whole fruit flesh firmness was measured using a Chatillon penetrometer fitted with a 7.9-mm tip, on opposite sides after skin removal. At day 0, flesh firmness was measured using 3 replications of 6 fruit each, with the same characteristics as those that received treatments.

**Compositional analysis**

After storage, all fruit of each replicate were macerated in a blender for compositional analysis. Soluble solids content (SSC) of juice from the blended material was measured using an Atago PR-1 refractometer (Atago Co Ltd, Tokyo, Japan). pH was also measured in the juice of the blended material and the titratable acidity (TA) was determined by adding 50 ml distilled water into 5 g of clear juice and the sample titrated to pH 8.2 with 0.1 N NaOH with the result expressed as percentage (%/citric acid.

Ascorbic acid was extracted in 1% oxalic acid and measured by using Reflectoquant ascorbic acid test strips in an RQflex reflectometer (Merck, Darmstadt, Germany).

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ac-
tivity was determined using a modified method of Brand-Williams et al. (1995): samples were homogenized in 25 ml methanol and centrifuged at 5000 \( \times g \) for 10 min. The supernatant was diluted with methanol up to 25 ml, and 50 \( \mu l \) of the extract were added to 2950 \( \mu l \) of 100 \( \mu M \) DPPH methanolic solution in a test tube. Tubes were covered with parafilm, vortexed thoroughly and kept in the dark at 24°C. The reduction in the absorbance of the resulting solution was measured at 517 nm after 30 min. Control solution consisted of 50 \( \mu l \) methanol and 2950 \( \mu l \) DPPH. A standard curve was developed using ascorbic acid. DPPH radical scavenging activity was expressed as milligrams of ascorbic acid equivalents antioxidant capacity (AEAC) per kg of fresh weight.

At day 0, SSC, pH, TA ascorbic acid content and DPPH radical scavenging activity were measured using 3 replications of 12 fruit each, with the same characteristics as those that received the treatments.

**Statistical analysis**

Data analysis and ANOVA was performed according to the completely randomized design and mean separation by LSD at the 0.05 level. For \( CO_2 \) and ethylene production and the depth of the fungal growth into the fruit, means \( \pm S.E. \) are presented.

**RESULTS**

**Effect of water dipping on Botrytis cinerea decay of kiwifruit**

A high incidence of typical gray mold by \( B. \ cinerea \) developed in the control (Fig. 1). The fungal rot progressed 8.79 mm into fruit and infected tissue turned dark oily-green and was water-soaked. The fungus was isolated from the interior of all control fruit. All hot water-treated fruit showed complete inhibition of \( B. \ cinerea \) decay. No fungus was isolated from the interior of any hot-water treated fruit. Even heat treatment at the lowest temperature and for the shortest time (50°C for 1.5 min) was able to inhibit fungal decay completely at the stem end of kiwifruit (Fig. 1).

**Effect of hot water dipping on \( CO_2 \) and ethylene production**

During storage at 20°C control fruit showed a continuous increase of \( CO_2 \) production (Fig. 2). All hot water-treated fruit exhibited significantly lower \( CO_2 \) production than control for the whole storage period. The only exception was fruit treated at 55°C, for 3 min; on the sixth day of storage these fruit had lower, but not significantly different \( CO_2 \) production compared to the control. Ethylene production in control fruit started to increase on the day 4 of storage and continued to increase until day 8 (Fig. 3). Fruit treated with 50°C for 1.5 min showed similar or higher values than the control. All the other treatments in general showed significantly lower ethylene production than control from the fourth to eighth day of storage.

**Effect of hot water dips on flesh firmness**

Flesh firmness of fruit before treatment and storage at 20°C about 25 N (dashed line in Fig. 4). After 8 days of storage, flesh firmness decreased significantly in all treatments (Fig. 4). Fruit treated at 55°C or 60°C for 1.5 or 3 min exhibited significantly higher firmness than the control, and they did not differ from one another.

**Effect of hot water dipping on fruit color**

In control fruit, \( L^* \) and \( C^* \) values were significantly lower after storage at 20°C than on day 0 (Table 1). In contrast, hue angle showed small changes. Hot water dips did not affect fruit color compared to the control, apart from significantly higher \( L^* \) and \( C^* \) value in fruit treated at 55°C for 1.5 min. No differences were observed in \( H^\circ \) values.

**Effect of hot water dips on composition**

After 8 days of storage, no significant differences in SSC were observed among the treatments (Table 2). After 8 days of storage, pH increased and titratable acidity decreased in control (Table 2). Fruit treated with 55°C for 1.5, or 3 min.
and 60°C for 1.5 or 3 min, showed significantly lower pH and higher titratable acidity than the control, without significant differences among them. Ascorbic acid content of control fruit decreased after storage (Table 3). In all heat treatments ascorbic acid content was similar to control, and after 8 days only fruit treated with 50°C for 1.5 min had significantly higher ascorbic acid content. DPPH radical scavenging activity of the control slightly decreased after 8 days storage at 20°C. The results showed that there were no significant differences among control and hot water treated fruit (Table 3).

DISCUSSION

In a previous study it was shown that hot water dips at temperatures of 50°C and 54°C for 8 min applied prior to cold storage, gave high level of control of B. cinerea rot, but caused heat injury to kiwifruit (Cheah et al. 1992a). In contrast, lower temperatures like 46°C, for 15 min and 48°C for 8 min gave a high, although not complete, level of control of B. cinerea rot without causing heat injury (Cheah et al. 1992b). The present investigation deals with another approach of hot water dip treatments after storage to protect fruit during marketing. Post cold storage, hot water dips of kiwifruit at 50°C, 55°C or 60°C, for 1.5 or 3 min, provided an effective treatment against B. cinerea and prolonged shelf life of fruit at conditions that normally favor rapid decay development. Complete inhibition of decay development on artificially and heavily inoculated kiwifruit was achieved by the treatment. These results are similar to those
of Fallik et al. (1991) where hot water rinsing and brushing of artificially inoculated, or naturally infected apples at 55°C for 15 sec, reduced decay development significantly, while maintaining quality after prolonged storage and marketing simulation. Also, heat treatment has been reported to be effective in reducing decay development and maintenance of postharvest quality in bell peppers (Fallik et al. 1999), melons (Fallik et al. 2000) and grapefruits (Porat et al. 2000).

During fruit ripening there is a reduction of flesh firmness, and in climacteric fruit, increase in respiration, ethylene production rate and in sugar:acid ratio. Inhibition or acceleration of ripening process has been observed in fruit following heat treatments. In kiwifruit the ripening was incomplete or inhibited at temperatures of 38°C or higher (Antunes and Sfakiotakis 2000). Apples that had received a pre-storage treatment at 38°C for 3 or 4 days were firmer than non-heated fruit after 6 months of storage at 0°C and subsequent shelf life for 7 days at 20°C (Porrit et al. 1978; Fallik et al. 2000). Positive results on maintenance of quality during shelf life in ‘Kensington’ mango fruit were shown following hot water hot water treatment at 53°C for 15 min, or combination of exposure to hot air at 22°C to 42°C for 4 to 6 h, prior to dipping in hot water at 45°C for 30 min, or 47°C for 15 min (Jacobi and Gils 1997). On the contrary, forced-air heat treatment of mangoes and papayas for 4 h at 50°C led to faster softening after treatment (Shellie and Mangan 1994). Irving et al. (1991) reported that pre-storage dipping of kiwifruit in water at 54°C for 8 min, accelerated softening, respiration and ethylene production rates when fruit were transferred to bench conditions after a period of cool storage. Cheah et al. (1992b) showed that 4 min was the maximum duration for dipping at 50°C in order to preserve kiwifruit quality. The present study showed that when kiwifruit were heated after cold storage, at temperatures from 50°C to 60°C, for shorter times like 1.5 or 3 min and kept at bench conditions for 8 days, ethylene production, respiration and fruit softening rates decreased, indicating inhibition of the ripening process and extension of fruit shelf life. However, regardless of the time of application of hot water dips (pre- or post storage), fruit response to heat depends on many factors, including species or cultivar, condition of fruit prior to treatment, temperature and duration of treatment, as well as the mode of heat application e.g. hot air vs. hot water and others (Lurie 1998). Further research is necessary to clarify the mechanism responsible for reduction of ethylene production after hot water dipping at 55°C and 60°C for 1.5 and 3 min. In such a short time treatment, only the peel and outer layers of fruit flesh are heated and not the interior of the fruit. In case that the non-heated inner part of kiwifruit continues to produce ethylene, it is possible that the hot water dip triggers a mechanism that blocks ethylene production in the outer cells and then a signal is transferred to the interior cells causing a reduction of ethylene production in the whole fruit.

Flavor of kiwifruit is determined by soluble solids concentration and acidity. A minimum of SSC of about 8% and 4% total citric acid levels in the flesh as good flavor (Biggs et al. 2004). Hot water dips, at all temperatures and exposure times tested, did not affect SSC that was maintained within the consumer-accepted values. In addition, citric acid content decreased in control and in both 50°C treatments during storage. In the 55°C and 60°C treatments citric acid was significantly higher than the control. Flavor characteristics of various fruit, specifically SSC and TA, have been reported to be either improved or suppressed by heat treatments. In apples, nectarines and strawberries titratable acidity was reduced while soluble solids concentration was not affected after heat treatments (Klein and Lurie 1990; Lay-Yee and Rose 1994; Garcia et al. 1995). In grapefruit hot air treatment at 43.5°C for 4.5 hours, did not affect titratable acidity or soluble solid content (Miller and McDonald 1997). On the other hand, sugar content increased or acidity decreased in some fruit by heat treatment as in apples that were sweeter after a heat treatment compared to non-heated fruit (Klein et al. 1997).

Hot water dips had minor affects on kiwifruit color. Earlier studies with fresh-cut kiwifruit have shown that the effect of heat treatment, at 25-50°C, for 10-90 min, on colour was negligible at both, firm ripe and soft ripe fruit maturity stages (Beirão da Costa et al. 2006). Color was not affected by heat treatment in papaya (Paul and Chen 1990), grapefruit (Porat et al. 2000), blood oranges (Schirra et al. 2004) and mandarins (Schirra and D’Halluin 1997; Hong et al. 2007).

Hot water dips at temperatures of 50-60°C had no effect on antioxidant content and vitamin C, providing treated fruit of good quality. Heat treatments have been reported to affect these constituents either negatively or positively in other commodities. Highest content of total ascorbic acid was observed in non-heated tomato (Soto-Zamora et al. 2005), but in strawberry higher level of ascorbic acid and antioxidant capacity was found in heat-treated fruit than in control (Vicente et al. 2006).

In conclusion, hot water dipping of kiwifruit at temperatures 50-60°C for 1.5 or 3 min, after cold storage, prevents B. cinerea decay for at least 8 days. Also dipping at 55°C or 60 for 1.5 or 3 min delayed softening, by reducing ethylene production and respiration, without affecting quality attributes such as SSC, citric acid and total antioxidants. It is suggested that hot water dips at 55°C for 1.5 min, provide an effective, non-chemical method to extend storability by protecting from B. cinerea and preserving quality of ‘Hayward’ kiwifruit under conditions that favor decay. Thus such treatments should minimize losses that occur during short-distance market circulation in inland wholesale markets and in retail market displays. However a large scale trial should be conducted in order to evaluate the economic and operational perspective prior to commercial implementation.

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