Hot Water Combined with Calcium Treatment Improves Physical and Physicochemical Attributes of Kiwifruit (Actinidia delicosa cv. Hayward) during Storage

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Abstract. Kiwifruit has a short storage life and encounters severe disorders during maintenance mainly as a result of its climacteric behavior. The role of calcium and heat treatment in delaying degenerative processes during storage has been revealed. This study investigated the effects of hot water combined with calcium (Ca) dips on the quality of kiwifruit (Actinidia delicosa cv. Hayward). Whole fruits were treated with hot water for 5, 10, and 15 minutes at 47 °C, therefore dipped in CaCl₂ solution (2% w/v) and stored at 0 °C for up to 120 days. During storage, fruit were sampled at 0, 30, 60, 90, and 120 days for postharvest quality evaluation. Postharvest evaluations included chromatic parameters (L*, a*, b*, hue, and chroma), firmness, and physiological parameters [phenolic content and polyphenol oxidase (PPO)]. Heating combined with Ca dips significantly reduced PPO activity. The results showed that mild heat treatments in combination with CaCl₂ maintained chromatic parameters for kiwifruits compared with control and hot water or CaCl₂ treatments solely. In addition, the levels of total phenolic compounds remained significantly higher for hot water combined with CaCl₂-treated fruit as compared with control or untreated fruits. According to the results, hot water treatments had a significant firming effect, whereas CaCl₂ dips solely had less effect on chromatic parameters. However, the influence of hot water treatments was dependent on application time with (treatment that showed significant results) showing significant improved kiwifruit postharvest qualities. Overall, with this simple and non-contaminant technology, after long-term storage, quality of kiwifruit could be even greater than in recently harvested fruits.

The kiwifruit was introduced to the world market from New Zealand in 1950s. The export of fresh fruit led to rapid expansion (Barboni et al., 2010). Production is almost exclusively of the cultivar Hayward because of its longer storage life and its larger fruit size (Franco et al., 2006). The increasing market demand for this fruit has challenged postharvest and food technologists to develop procedures to lengthen storage life (Piga et al., 2003).

With attention to the risk of improper use of chemicals in postharvest technology and consumer’s demand for healthy products, study on application of postharvest treatments such as heat treatment is necessary (Shafiee et al., 2010). Heat treatments have already been used to control postharvest decay and to improve the storage quality in intact fruits as a result of changes it induces in physiological and physicochemical characteristics and post-processing quality (Beirão-da-Costa et al., 2006). Heat treatments also inhibited ripening, softening, and improved postharvest quality. This was observed in the case of whole fruit with apples (Klein and Lurie, 1990), strawberries (Civello et al., 1997), citrus fruit (Porat et al., 2000), and mangoes (Benitez et al., 2006). The mechanism by which a heat treatment causes changes in fruit ripening such as inhibition of ethylene synthesis and cell wall degrading enzymes may be the result of changes in gene expression and protein synthesis. During a high-temperature treatment, the mRNA of fruit-ripening genes decreases and those of heat shock proteins accumulates (Lurie, 1998).

Ca plays a significant role in maintaining quality in a number of different fruits (Hopkirk et al., 1990). Pre- and postharvest Ca application has been demonstrated to produce beneficial effects on whole fruit quality, decreasing the incidence of physiological disorders (Serrano et al., 2004) and delaying softening (Antunes et al., 2004). Ca is directly involved in strengthening plant cell walls through its ability to crosslink with carboxyl groups of polyuronide chains of pectins found in the middle lamella (Lara et al., 2004). Furthermore, Ca ions help in the stabilization of cell membranes (Picchioni et al., 1995) and affecting cellular turgor pressure (Mignani et al., 1995).

Temperature can have an effect on Ca uptake. A combination of heat treatment followed by Ca dip has also been applied for the primary purpose of controlling postharvest pests and/or diseases and has been found to have satisfactory results in maintaining or improving the texture of several products. In this sense, Ca application, combined or not with heat treatments, maintained firmness in a wide variety of fruit and vegetables including lettuce (Roura et al., 2008).

According to our review, there is no report on postharvest application of Ca and hot water on qualitative parameters of kiwifruit during cold storage. The overall aim of this research was to evaluate the effect of hot water combined with Ca solution treatments to maintain qualitative characteristics of kiwifruit during cold storage.

Materials and Methods

Plant material

Mature, unripe kiwifruit (Actinidia delicosa cv. Hayward) of medium-sized (80 to 120 g) fruits, free from visible defects or decay, were harvested from a commercial orchard in Gorgan, Iran, with average firmness of 9.8 kg·cm⁻² and 7% °Brix. Fruits were immediately transferred to the postharvest laboratory at Shiraz University.

Treatments

Kiwifruits were divided into eight groups (each group considered for one treatment). The first three groups were treated with hot water for 5, 10, and 15 min (HW5, HW10, and HW15). One group was treated with CaCl₂ (2% w/v) solution for 10 min. Three other groups were treated with hot water for 5, 10, and 15 min and then dipped in 2% CaCl₂ solution for 10 min. One group without any treatment was considered a control. The temperature of hot water was 47 °C. Four replicates and eight fruits per replicate were considered for each treatment. The treated fruits were air-dried at 24 ± 1 °C, labeled and packaged into ventilated bags, then stored at 0 ± 1 °C and 90% ± 5% relative humidity for 4 months. Samples, each one containing two fruits, were taken every month during storage for quality evaluation and after analyses.

Physical and physicochemical assays

Firmness. Firmness was measured on two opposite sides of the equatorial zone of the peeled fruit using a texture analyzer (Stevens-Lira, U.K.) fitted with an 8-mm diameter cylindrical probe. A piece of skin ≈2 mm was removed. The penetration depth was 9 mm and the cross-head speed was 2 mm·s⁻¹. Firmness was expressed as kg·cm⁻².

Extraction and assay of PPO activities. The PPO activity was performed using the method described by Murr and Morris (1974)
with slight modification. One gram of frozen tissue was homogenized in 0.2 mol·L\(^{-1}\) sodium phosphate buffer (pH 6.5) containing 1% polyvinylpyrrolidone centrifuged at 15,000 rpm for 20 min at 4°C in a refrigerated centrifuge. PPO activity was determined in a 2-mL total reaction mixture containing 1 mL of aliquot of the supernatant, 200 mL of pyrocatechol solution (0.2 M), and 800 mL sodium phosphate buffer pH 6.2. One unit of PPO activity was defined as the amount of enzyme that caused the increase in absorbance of 0.01 at 410 nm in 1 min under the specified conditions.

**Total phenolic contents.** Total phenolics were extracted and determined according to the method of Gutfinger (1981). Briefly, 5 mg of the dried fruit sample was placed directly into a solution of 5 mL of 80% methanol and was homogenized. Thereafter, samples were filtered and centrifuged at 14,000 rpm for 20 min and the supernatant was reserved. Each extract (1 mL) at concentration of 1 mg·mL\(^{-1}\) was mixed with 1 mL of 2% Na\(_2\)CO\(_3\). After standing for 3 min, 0.2 mL of 50% Folin-Ciocalteu reagent was added to the mixture and allowed to stand for 30 min. The mixture was centrifuged at 13,400 \(\text{g}\) for 5 min. The absorbance was measured at 750 nm and total phenolic content (TPC) was expressed as gallic acid equivalents (GAE).

**Color.** Color was determined using digital imaging (Afshari-Jouybari and Farahnaky, 2011). Fruits were cut equatorially with a sharp knife and the cut surface was photographed in a chamber. Angle light with the horizontal surface of the images was 45°. After transferring the images to a computer, Photoshop image processing software (Adobe Photoshop.CS, Middle Eastern version) was performed after the recording of individual \(L^*\), \(a^*\), and \(b^*\) parameters. \(L^*\) is lightness and \(a^*\) (−greenness to +redness) and \(b^*\) (−blueness to + yellowness) are chromaticity coordinates. The \(a^*\) and \(b^*\) values were converted to chroma \([C^* = (a^* ^2 + b^* ^2)^{1/2}]\) and hue angle \([h^* = \tan^{-1}(b^*/a^*)]\).

**Statistical analysis.**

Data were subjected to analysis of variance. Sources of variation were time of storage and treatments and the interaction of treatment \(\times\) storage time. Mean comparisons were performed using the least significant difference test to examine if differences between treatments and storage time were significant at \(P < 0.05\). All analyses were performed with SAS software package Version 9.1 for Windows (SAS Institute Inc., Cary, NC).

**Results and Discussion**

**Firmness.** Fruit firmness decreased during cold storage and the rate of decrease was significantly higher in control fruits compared with those treated with HW + Ca treatments (Fig. 1). The control fruits showed a decrease in firmness reaching up to 1.05 (kg·cm\(^{-2}\)) after 120 d, whereas the firmness of samples treated with HW + Ca for 5, 10, and 15 min were 4.62, 3.58, and 5.05 (kg·cm\(^{-2}\)), respectively. It has been noted that the positive effect of hot water treatment on kiwifruit firmness is the result of higher levels of Ca linked to pectate within the cell wall. In general, the effect of Ca on tissue firmness is explained by its complexing to the cell wall and middle lamella polygalacturonic acid residues imparting improvement of structural integrity (Morris, 1980). The beneficial effect of Ca treatments on firmness of fresh-cut products has been studied in some researches (Diouma et al., 2009; Luna-Guzmán et al., 1999; Rico et al., 2007).

Heat treatments had a significant firming effect on kiwifruits stored at 0°C for up to 120 d; however, there were not significant differences between 5 and 10 min (Fig. 1). The effect of heat treatment observed in this
study was probably the result of the temporary suspension of enzyme synthesis involved in cell wall degradation (Obenland and Neipp, 2005).

Color. Color parameters were significantly different between HW treatments and control. Hue angle was lowest in control fruits after 120 d storage (62.34) (Fig. 2). In general, chroma value decreased during storage as well as ripening, and it was lower in control (31.87) compared with HW + Ca-treated fruits (46.26, 45.87, and 45.52 for 5, 10, and 15 min, respectively) (Fig. 3). The application of heat treatment interrupts normal protein and chlorophyllase synthesis probably temporarily in heat-treated fruits (Brodl, 1989). According to other research, chroma values decrease significantly in control fruits during cold storage and shelf life, indicating less color intensity (Koukounaras and Sfakiotakis, 2007). Therefore, the results obtained in this work could be explained through the combined inhibitory effect of HW + Ca. Overall results indicated that HW + Ca treatments significantly suppressed color development of kiwifruit stored at 0 °C for 120 d compared with sole Ca treatments. Such finds suggested that HW + Ca treatments have a potential to act as an alternative color loss prevention method for long-term storage.

Total phenolic compounds and PPO. Control fruit exhibited a significant reduction in TPC during cold storage from the initial values (45.45 mg GAE/100 g) (Table 1). A sharp increase occurred during the first 30 d of storage (59.15 mg GAE/100 g) and decreased thereafter. After 120 d of storage, the TPC of control fruit was less than 30 mg GAE/100 g, whereas it was more than 48 mg GAE/100 g in the CaCl2 (2%) + HW (15 min)-treated fruit. Fruits dipped in HW + Ca showed a significant difference with respect to total phenolics, which was associated with lower PPO activity. PPO is involved in the oxidation of polyphenols into quinones (Liu et al., 2005). The combination of Ca and HW treatments had additive effects, but no significant difference was found between them. The PPO degradative activity was then partially inhibited by HW + Ca treatment, as will be later discussed. The authors suggested that the maintenance of cellular integrity conferred by CaCl2 diminished the possible contact of the enzyme with its substrate, resulting in a lower rate of TPC.

PPO activity increased sharply during first 2 months. There was no significant difference at Days 0 and 30 between different treatments, but HW treatment significantly prevented the increase in PPO activity compared with controls during storage (Fig. 4). PPO content of control samples declined significantly compared with treated samples. The fruits treated with Ca + HW had higher PPO activity than the other treatments or the control. The initial PPO activity of untreated kiwifruits was 0.52 (U-min⁻¹·g⁻¹ fresh weight). After 120 d, PPO in samples control was 1.48 (U-min⁻¹·g⁻¹ fresh weight), whereas those in treated samples with Ca + HW 5, 10, and 15 were 1.24, 1.11, and 1.05, respectively. Higher duration of HW + Ca treatment showed significantly least levels of PPO activity compared with all other treatments during 4-month storage period. Similar observations were reported by Jin et al. (2009) and Torres et al. (2010). PPO is not a very heat-stable enzyme at temperatures higher than 40 °C (Garcia and Barrett, 2002). Also,
Whole fruits were non-treated (control), dipped in 2% CaCl2 solution, heat-treated at 47 °C for 5 min (H5), 10 min (H10), 15 min (H15) alone, or in combination with CaCl2 (2%) solution. TPC = total phenolic content; GAE = gallic acid equivalents; HW = hot water; LSD = least significant difference.

| Treatment                      | Storage time (day) | Time (1.33) | Treatment (1.68) | Time × treatment (3.77) |
|--------------------------------|--------------------|-------------|------------------|-------------------------|
| 0                              | 30                 | 60          | 90               | 120                     |
| Control                        | 45.45              | 59.15       | 42.15            | 41.05                   | 29.45                  |
| HW (5 min)                     | 45.25              | 57.92       | 53.55            | 48.32                   | 34.62                  |
| HW (10 min)                    | 44.5               | 61.85       | 48.12            | 46.82                   | 36.4                   |
| HW (15 min)                    | 45                 | 53.97       | 57.37            | 48.65                   | 43.7                   |
| CaCl2 (2%)                     | 45.5               | 54.52       | 51.07            | 44.5                    | 38.5                   |
| CaCl2 (2%) + HW (5 min)        | 45.75              | 52.92       | 58               | 54.25                   | 45.45                  |
| CaCl2 (2%) + HW (10 min)       | 44                 | 49.2        | 52.92            | 52.22                   | 41.57                  |
| CaCl2 (2%) + HW (15 min)       | 43.75              | 52.82       | 58.87            | 56.25                   | 48.52                  |

Time values are in brackets

TPC = total phenolic content; GAE = gallic acid equivalents; HW = hot water; LSD = least significant difference.

Table 1. Changes in TPC (milligrams GAE/100 g) of kiwifruit during 120 d storage at 0 °C.

The effect observed in this study was probably the result of the temporary suspension of enzyme synthesis involved in cell wall degradation (Obenland and Neipp, 2005). Also, HW treatment solely inhibited the activity of PPO; however, the effect was not as good as HW + Ca treatment.

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