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Research article

Postharvest heat treatments to inhibit *Penicillium digitatum* growth and maintain quality of Mandarin (*Citrus reticulata* blanco)Diana B. Queb-González^a, Aurelio Lopez-Malo^{a,*}, María E. Sosa-Morales^b, Rossana Villa-Rojas^{a,c}^a Departamento de Ingeniería Química y Alimentos, Universidad de las Américas Puebla, Cholula, Puebla, Mexico^b Departamento de Alimentos, División de Ciencias de la Vida, Campus Irapuato-Salamanca, Universidad de Guanajuato, Irapuato, Guanajuato, Mexico^c Food Science & Technology, University of Nebraska, Lincoln, NE 68588-6205, USA

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ABSTRACT

Use of fungicides is a common practice as a postharvest treatment to control fruit decay. Nowadays, environment friendly technologies, such as heat treatments, are viable replacements. This study evaluated the effects of post-harvest heat treatments (traditional and microwave-assisted) on mandarins intentionally inoculated with *Penicillium digitatum*. For the studied heat treatments, the target temperature was 50 °C, which was held for 2.5 min. After heating, mandarins were cooled and stored at 25 °C for 13 days. MW treatments effectively prevented mold growth during storage, while HW only delayed it. Control mandarins (without treatment) showed the highest significant weight loss. Neither thermal treatment nor storage affected fruit juice pH ($p > 0.05$). Treated mandarins had a significantly lower vitamin C content than control fruits throughout storage, and all mandarins lost firmness by the 13th day ($p < 0.05$). Control and MW-treated mandarins had lower citric acid content; however, they retained color, total soluble solids (TSS) and had a higher maturity index. While HW mandarins did not have changes in citric acid content, they had higher TSS, and lower maturity index. MW-assisted treatments were effective at inactivating molds and helped retain some nutritional and physical-chemical characteristics of mandarins. However, juice of MW-treated mandarins was not preferred by judges in the sensory tests, the juice was rated lower than that obtained from the other treatment. Postharvest heat treatments may constitute a helpful application to control mandarin fungal decay.

1. Introduction

Mandarins are one of the most important citric crops in Mexico (SAGARPA, 2011), the national production places the country as one of the top ten producers worldwide (FAO, 2016). However, pests and diseases are an important problem for producers. *Penicillium digitatum* and *P. italicum* are two molds that contribute to the decay and loss of citrus fruits during handling and transportation; and due to the airborne nature of their spores, molds can be easily spread among fruits during storage (Ladanyia, 2010). *P. digitatum* and *P. italicum* are the most economically important postharvest pathogens of citrus fruit in all production areas. Both molds are strict wound pathogens that affect all citrus species and cultivars and can infect the fruit in the field, the packinghouse, and during distribution and marketing (Palou, 2014).

The use of postharvest thermal treatments to avoid use of restricted fungicides has proven to be efficient at inactivating pathogen development and can successfully retain quality of citrus fruits. Hot water

immersion and hot air treatments at temperatures above 40 °C and below 60 °C from a few seconds to several hours have shown promising results to control pathogens in diverse fruits, such as apples, pears, citrus, melons, bananas and berries (Sui et al., 2016). Heat treatments have been recently studied in citrus fruits. Gao, Kan, Wan, Chen, Chen & Chen (2018) applied hot air treatments (40 °C, 48 h) and 1% chitosan coating in mandarin fruits, with different effects on citric acid degradation. Soto-Reyes, López-Malo, Rojas-Laguna, Gómez-Salazar & Sosa-Morales, 2018 proposed microwave-assisted hot water treatments (48 °C, 6 min) on grapefruits to control Mexican fruit fly, which preserved the overall quality of the fruits. Microwave heating has also been explored as a successful option for post-harvest treatments of heat sensitive fruits, because its volumetric heating mechanism reduces the time necessary to reach the target temperature, reducing the loss of fruit quality (Villa-Rojas et al., 2011).

Fungal genes related to reactive oxygen species (ROS) are activated when exposed to heat stress over time due to an inadequate system for

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detoxification; these end up accumulating in the organism. The accumulation of ROS leads to an impairment of cellular functions and a loss of viability (Sui et al., 2016). Heat can also induce other alterations that inactivate cellular functionality, such as changes in the cell wall, protein denaturation and destruction of mitochondria and/or outer membranes (Palou & Méndez-Vilas, 2013). However, depending on the temperature and exposure time of the treatment, quality parameters of fruits may be compromised by accelerating ripening and senescence and increasing phytotoxicity (Sui et al., 2016).

The aims of this study were to evaluate a) the effectiveness of thermal treatments to inactivate *Penicillium digitatum* intentionally inoculated in mandarins, and b) the influence of thermal treatment on some quality and sensory attributes in mandarins.

2. Materials and methods

2.1. Materials

Fully matured, and well-developed Murcott Mandarins (*Citrus reticulata* Blanco) were purchased at a farmer's market in Puebla, Mexico; and brought to the laboratory on the same day. Mandarins pre-selected for this study were damage free and had similar external color, size and weight. The target microorganism for this study was *Penicillium digitatum* (MPD-1) obtained from the Food Microbiology Laboratory Culture Collection at Universidad de las Americas Puebla, Mexico. Potato dextrose agar (PDA) was purchased from Becton Dickinson (Mexico).

2.2. Inoculum preparation

P. digitatum inoculum was obtained using a modified method (Gündüz and Pazir, 2013); briefly, the mold was streaked onto a PDA slants and incubated for 7 days at 25 °C. After incubation, conidia were harvested with 5 mL of distilled water and vortexed for 30 s to disperse any conglomerates. Conidia were counted using a Neubauer chamber (Brand, Germany) and an optical microscope (American Optical Co., USA); the suspension was adjusted to a concentration of 10⁶ conidia/mL.

2.3. Inoculation of mandarins

Pre-selected mandarins were washed and sanitized in a 200 ppm chlorine solution for 2 min (Food and Drug Administration, 1998) and dried at room temperature in a biosafety cabinet for 2 h before treatment. The exocarp of the fruit was incised four times along the stem area (Figure 1) with a sterile needle. The incisions were 5 mm long and 1 mm deep and served as inoculation spots using 10 µL of conidia suspension (Gündüz and Pazir, 2013).

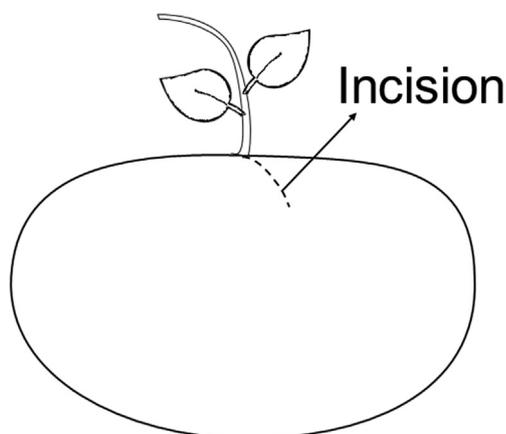


Figure 1. Spot of the incision in mandarins.

2.4. Heat treatments

For traditional (HW) and MW-assisted hydrothermal treatments, mandarins were immersed in tap water using a 1:5 fruit-to-water weight ratio. The objective of all treatments was to maintain the surface temperature of the mandarins at 50 °C for 2.5 min to effectively reduce *P. digitatum* as reported by Palou & Méndez-Vilas (2013).

To determine temperature profiles for the traditional hydrothermal treatment, a separate batch of four washed and sanitized mandarins were immersed in a water bath (Daihan LabTech Co., New Delhi, India), and their temperature was monitored with a type-T thermocouple and data logger (Cole-Parmer, Illinois, USA). For MW-assisted hydrothermal treatment, a separate batch of four washed and sanitized mandarins were immersed in a water-filled container and placed in a microwave oven of 2450 MHz and 1200 W (Panasonic, Guangdong, China), and fiber optic sensors (FISO Technologies, Quebec, Canada) were employed to monitor temperature. Mandarins were heated using either 100% (MW100) or 80% (MW80) of the nominal microwave power. Each temperature profile measurements were done by duplicate.

Three sensors (thermocouples or optical fibers, depending on the treatment) were used for monitoring temperature in the system; below mesocarp, in the center of the fruit and in the surrounding water. The perforation sites for thermocouples or fiber optics were covered with electrical tape to avoid misreading due to water entry. The fruits used to monitor temperature were discarded.

For each of the two treatments, batches of four non-inoculated mandarins were subjected to the process until at least 30 fruits had been treated. The same was repeated with batches of four inoculated mandarins for the sensory analysis. Three types of controls (12 mandarins each) were used for comparison. Mandarins in the first control group (C1) were washed, sanitized, and immersed in water at room temperature for 20 min; those in control group 2 (C2) were washed and sanitized, then wounded and inoculated; those in control group 3 (C3) were washed and sanitized, then wounded and inoculated, followed by an immersion in water at room temperature for 20 min.

After the hydrothermal treatments, the mandarins were cooled down to avoid damage in quality. The cooling was done by immersion in water at 9 °C for 30 min and allowed to dry at room temperature. Dry mandarins were stored in plastic boxes and kept at room temperature (25 °C) for 13 days. Mandarins were stored at 25 °C in order to simulate the conditions of storage in Mexico for this fruit.

2.5. Mold response

Treatment effectiveness was evaluated as the number of wounds with mold growth over the overall number of inoculated wounds (Gündüz and Pazir, 2013); wounds and mold growth were periodically observed during storage.

2.6. Physical, chemical and sensory characteristics

Different characteristics were measured in triplicates for treated and C1 samples. Weight loss of mandarins was determined using an analytical balance (Explorer E12140, Ohaus, USA), the initial weight was compared with the value obtained 24 h after treatment (Sangwanich et al., 2013):

$$\text{Weight loss (\%)} = \left(\frac{A - B}{A} \right) * 100$$

where A and B are the weight (g) of the mandarins before and after treatment, respectively.

To measure other physical-chemical characteristics in accordance with official standards, Mexican (NMX or NOM) or international, juice was extracted from control and treated mandarins. Total soluble solids

(TSS) were measured with a manual refractometer (Atago, Japan), according to NMX-F-103-1982. pH was measured with a pH meter (Conductronic, Hanna, USA), following the standard NMX-F-317-S-1978 (SSA, 1978a). Titratable acidity (TA) of 10 mL of juice diluted in 50 mL of distilled water was determined in accordance with NOM-FF-11-1982 (SSA, 1978b) by titration with sodium hydroxide (0.1 N) and phenolphthalein as an indicator. The maturity index was expressed as the ratio of total soluble solids and acidity (Holland, Ia, Menezes, & Lafuente, 1999).

Firmness was expressed as the maximum force (N) required to compress the equatorial plane of a whole mandarin by 1 cm. Compressions were achieved with a cylindrical probe of 4 cm in diameter attached to a texture meter (Texture Technologist Co., Nueva York, USA) and descending at 0.5 mm/s (Bourne, 1982).

Color of the exocarp or peel was evaluated as coordinates L^* (lightness), a^* (green-red) and b^* (blue-yellow) of the CIELAB system using a portable colorimeter (CR400, Minolta Corp. Tokyo, Japan) (Schirra & D'hallewin, 1997). Net color differences (ΔE) between treated and control samples were calculated with the following equation (Gullett et al., 1972):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where ΔL , Δa , and Δb are the differences between the measurement of the sample after treatment and the control samples (C1).

Ascorbic acid (vitamin C) content was determined as explained in the AOAC method 967.21 by titrating with 2–6 dichlorophenolindophenol (Masamba and Nguyen, 2008).

The overall acceptability of mandarin juice was evaluated with a structured hedonic scale test (1–9 points), while the color and firmness of the mandarins was sensory-assessed using a ranking test (Poste et al., 2011). Rankings were converted to scores as explained by Boggs and Hanson (1949) using the table “Score for ordinal (or ranked) data” from Fisher and Yates (1963) to then perform an analysis of variance (ANOVA). All evaluations were done by 20 untrained judges for mandarins stored for 2 and 8 days.

2.7. Data analysis

The study used a randomized block experimental design, with 6 fruits in each of the control groups and at least 30 inoculated and at least 30 non-inoculated fruits for each of the two treatments. Significant differences ($\alpha = 0.05$) of physical-chemical and sensory characteristics, as well as mold inactivation among different treatments and controls were established with ANOVA and Tukey tests, and were calculated with the software Minitab 17 (Minitab Inc., USA).

3. Results and discussion

Fresh mandarins weighed between 145 and 185 g, $42.3 \pm 0.6\%$ of that weight was juice, the exo-mesocarp thickness was 2–3 mm and they had approximately 12–14 seeds. Their firmness was 1101.6 ± 111.8 N, and color parameters of exocarp 61.83 ± 5.02 , 21.08 ± 2.69 and 56.04 ± 4.36 , for L^* , a^* and b^* , respectively. Their juice had 52.2 ± 0.9 mg of ascorbic acid/100 mL of juice, pH 3.8 ± 0.1 , $0.62 \pm 0.1\%$ of citric acid, and 12.8 ± 0.3 °Brix of total soluble solids; corresponding to a maturity index of 20.8 ± 0.5 . Castro et al. (2013) reported similar results for the same mandarin variety, a weight of 136.4–223.9 g, with 36.0–52.2 % of that weight corresponding to juice, 0.73–1.01 % of citric acid and 7.4–12.1 °Brix, corresponding to maturity indexes between 4.4 and 12.0. de Borges and Pio (2003) also found comparable characteristics, with weight between 149.5 and 179.0 g with 50.4–53.5 % of it as juice, 0.9–1.4 % of citric acid and 10.3 to 13.2 °Brix, corresponding to 6.9 to 16.7 of maturity index. Variations in properties are attributed to the fruits being grown in diverse locations under different climates and soil compositions (Kader, 2008).

3.1. Heat penetration curve during thermal treatments

Since different methodologies were applied to heat the fruits, it is important to know the heat distribution and the temperature profiles in each case. Heat penetration curves varied among thermal treatments and the measurement location. Temperature of the mesocarp during HW heating (Figure 2C) showed a rapid increased rate of 0.12 ± 0.005 °C/s (Figure 2C). However, the rate was abruptly reduced by one order of magnitude (0.012 ± 0.005 °C/s) when the mesocarp achieved 41.0 ± 1.0 °C after 150 s of treatment becoming almost asymptotic. The time necessary to reach the target temperature for HW (come up time, CUT) was 997s, the longest among treatments. In contrast, temperature at the mandarin center remained almost constant for approximately 390 s (Figure 2C), afterwards it slowly increased at 0.017 ± 0.005 °C/s. In contrast, both MW treatments (MW100 and MW80) had a linear temperature increase for both mesocarp and center (Figure 2 A and B). As expected, the peel of MW100 treated mandarins heated faster, 0.06 ± 0.009 °C/s and had the shortest CUT 448 s (Figure 2A). While heating rate mandarin peels during MW80 treatments was slower at 0.04 ± 0.012 °C/s and CUT was longer, 660s (Figure 2B).

HW treatments showed an average ΔT of 18.8 ± 1.8 °C, which was relatively constant throughout the process (Figure 2C). The temperature at the center remained below the temperature of the peel and water bath throughout the treatment. On the other hand, for MW treated mandarins, the temperature at the center was quite close to that of the mesocarp throughout the treatment (Figure 2A and B) and their ΔT were relatively small at 2.0 ± 0.9 and 1.0 ± 0.9 °C for MW100 and MW80, respectively.

The variation in heating rates or temperatures at different locations are a result of different heating mechanisms and for MW treatments, power output as well. Conventional heating methods use convection to transfer the thermal energy of the heating medium to the surface of the mandarin peel. Heat transfer by convection (q) in these experiments was a function of the temperature difference between the surface (T_s) of the mandarin mesocarp and heating medium (T_∞), hot water, the contact area (A) between them and convective coefficient (h) of the heat medium: $q = Ah (T_s - T_\infty)$ (Singh and Heldman, 2009). Consequently, the heating rate decreased as the difference in temperatures was reduced with the increase of T_s . On the other hand, MW heating is a consequence of molecular friction caused by a polarization of dipole molecules (e.g. water). This mechanism is volumetric, meaning that, wherever the waves can penetrate, if there are dipoles, heating will occur (Feng et al., 2012). As a result, heating mandarins took a shorter time for MW heating than HW. However, using 80% of power slowed down the heating rate, increasing CUT.

Difference in temperature due to location is also a result of heating mechanisms, once the peel starts heating up, thermal energy transfers through conduction to consecutive layers radially towards center. Heat transfer stops once an equilibrium condition is reached and the fruit has the same temperature as the medium (Ibarz and Barbosa-Cánovas, 2002). As a result, the temperature of the peel is higher than the center as shown in Figure 2 C. In comparison, the volumetric heating of MW reduces the temperature difference between peel and center.

3.2. Mold inactivation

No molds grew on the first three days of storage. However, washing and chlorine sanitizing of mandarins (C1) did not prevent their natural microbiota from growing after 7 days of storage affecting $43.8 \pm 8.8\%$ of the wounds. There was no significant increase in wound colonization during the remainder of the storage time (Figure 3), until reaching 13 days. Inoculated mandarins (C2 and C3) not subjected to heat treatment, almost doubled the percentage of wounds affected by day 7 and increased until all wounds were colonized by day 13 (Figure 3). Water immersion after inoculation (C3) had no significant effect in mold response.

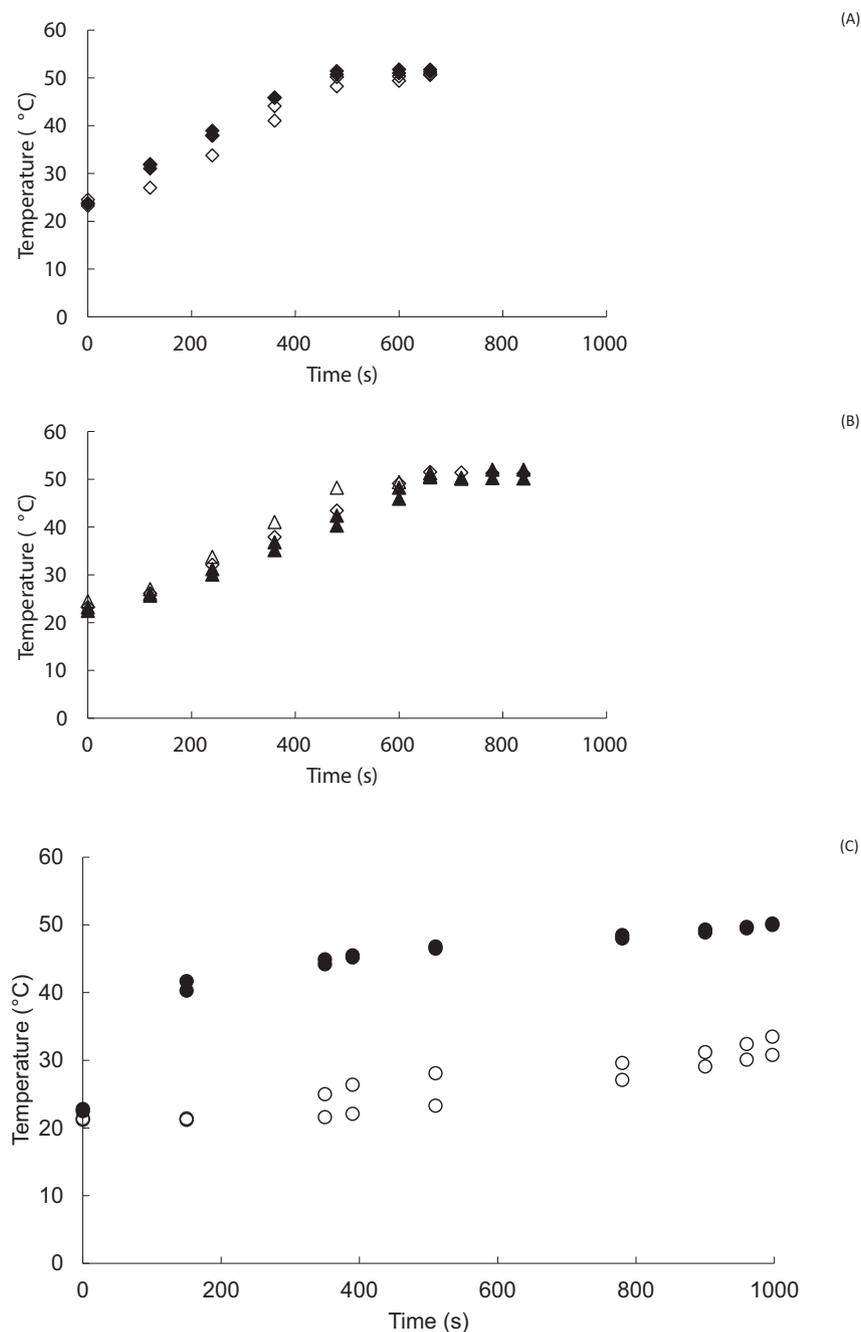


Figure 2. Heat penetration curves at the mesocarp (◆▲●) and center (◇△○) of Murcott mandarins, during different thermal treatments: Microwave assisted at 100 (A◆◇) and 80 (B▲△) % of nominal power and hot water bath (C●○). Target temperature at the mesocarp was 50 °C.

Heat treatments were more efficient to prevent mold growth than chlorine sanitizing (mandarins from controls C1 C2 and C3). Thermal treatments presented colonization of the wounds at a later storage time than all controls (Figure 3). HW-treated mandarins showed mold growth after 10 days of storage with 25 % of the wounds colonized, less than the amount affected in non-inoculated mandarins (C1). As storage time advanced, mold colonization increased as it did for the controls. After 13 days of storage mold growth on HW-treated mandarins was not significantly different from C1, but showed significant differences from C2 and C3 (Figure 3). Interestingly, MW-treated mandarins did not show mold growth after 13 days of storage.

Other studies have reported similar or greater inactivation of molds with thermal treatments. Hong et al. (2014) inoculated with *P. digitatum*,

P. italicum and *Geotrichum citri-aurantii* in wounds inflicted on mandarins, treated with hot water at 40 °C for 2 min and stored them for 4 weeks at 25 °C. Infection of the wounds of heat treated mandarins only reached 20%, while 90% of wounds in control mandarins were infected.

As reported by this study, MW power influenced mold inactivation treatments and has also been reported. Sisquella, Viñas, Teixidó, Picouet and Usall (2013) reported brown rot of MW-treated nectarines for 50 s at 10, 15, 17.5 and 20 kW was reduced by 0, 1, 91 and 91%, respectively. The increase in power allowed the nectarine surface to reach higher temperature, 34, 39, 44 and 46 °C, respectively, leading to greater brown rot inactivation.

Inactivation of molds by conventional treatments was not significantly different at any storage day (Figure 3). However, MW-treated

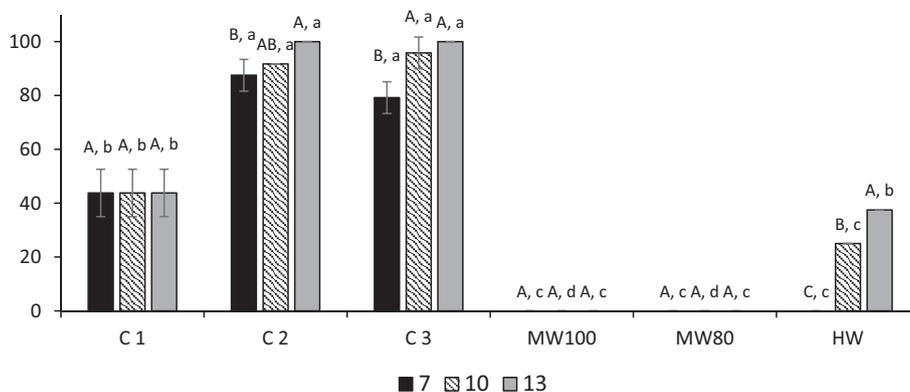


Figure 3. Wounds infected with *P. digitatum* during storage at 25 °C of control and treated mandarins. Different small cap letters indicate significant difference ($p < 0.05$) among treatments on the same storage day, different capital letters indicate significant difference ($p < 0.05$) during storage time for the same treatment.

mandarins show no mold growth. MW inactivation mechanisms of spores are not completely understood and remain controversial; some authors have either not found non-thermal effects or have found them negligible (Jeng et al., 1987; Welt et al., 1994) while others suggest there is evidence of electric field effects (Celandroni et al., 2004). Regardless, what has been clear is the inactivation mechanism of MW is different from that of conventional methods, causing more cellular disruption and loss of genetic material than other heating methods making it more efficient for spore inactivation (Kim et al., 2009).

3.5 Effect of treatments on physical, chemical and sensory characteristics.

3.3. Weight loss

Control and treated mandarins significantly loss weight during storage at room temperature ($p < 0.05$). Weight loss of treated (0.9–1.8%) and control ($2.3 \pm 0.1\%$) mandarins after the first day of storage was not significantly different ($p \geq 0.05$). At day 8, the weight loss of MW100 mandarins ($7.1 \pm 0.6\%$) was significantly ($p < 0.05$) lower compared to other heat-treated and control mandarins (12.3–15.8%). By the end of storage. Control mandarins showed a significantly ($p < 0.05$) higher weight loss ($34.6 \pm 2.3\%$) than all other mandarins (18.3–22.5%).

The results obtained in this study present an advantage when using MW and HW compared to untreated mandarins, and agree with reports by Shen et al. (2013), showing control and hot water (50, 52 and 54 °C for 3 min) mandarins lost weight during storage at 10 °C for 15–60 days. Only mandarins treated at 50 °C lost significantly less weight than untreated fruit. In contrast, MW treatment results reported by Zhang et al. (2004) and Karabulut and Baykal (2002) had no significant differences compared to untreated peaches after 30 and 40 days in cold storage (0–2 °C). A reduction in water loss due to thermal treatment may be linked to a change in the distribution and topology of the natural wax coat of fruits. Heat treated fruits present a smoother coating that covers the pores of the peel, while the cracked and roughed natural distribution of the coating in non-treated fruits allows the exposure of the pores to the environment. However, severe treatments can damage or even remove this natural coating (Schirra & D'hallewin, 1997).

3.4. pH, acidity, soluble solids and maturity index

pH of control and treated mandarin juices varied between 3.81 – 4.56 without significant difference among treatments at the same storage days (1, 8, 13) or among storage time for the same treatment ($p > 0.05$). Shen et al. (2013) also reported pH of heat-treated mandarin juices did not vary significantly with that of controls after 30 days of storage.

Citric acid content did not change significantly after HW treatment compared to C3 (Figure 4) or after 8 days of storage compared to their initial value and C3. In contrast, for C3 and MW-treated mandarins, the concentration of citric acid was significantly reduced by the 13th storage

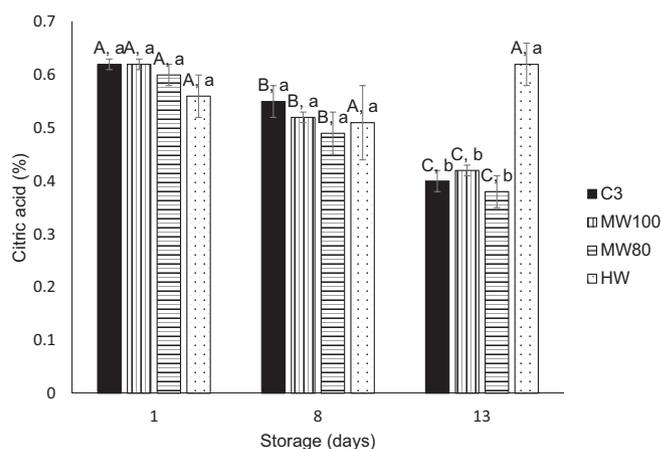


Figure 4. Changes in citric acid during storage at 25 °C of control and treated mandarins. Different small cap letters indicate significant difference ($p < 0.05$) among treatments on the same storage day, different capital letters indicate significant difference ($p < 0.05$) during storage time for the same treatment.

day (Figure 4). Other studies have reported little or insignificant differences in citric acid content during storage of citrus fruits (Hong et al., 2014). TA of citrus fruits has been shown to fluctuate during storage (Sun et al., 2013); therefore, it is not possible to say with certainty if the differences between treatments are due to the natural metabolic cycle or a disruption in the acid-metabolism of the fruit.

TSS did not vary significantly among treatments after being stored for 1 and 8 days ($p > 0.05$), with values between 12.0 – 15.0 °Brix. TSS of HW-treated mandarins significantly increased after 13 days of storage from 12.3 ± 0.4 to 15.3 ± 0.4 °Brix and from 13.8 ± 1.1 to 17.3 ± 0.4 °Brix, in MW-treated fruits. TSS content is an important factor in fruit quality and it's related with juice yield. During the ripening period TSS values increase in a fluctuating sigmoidal trend (Berk, 2016). Other authors reported no significant changes in TSS after storage of citrus fruits (Hong et al., 2014; Karabulut and Baykal, 2002; Zhang et al., 2004). However, an increase in TSS content has also been reported and would be expected (Mbogo et al., 2010; Sun et al., 2013).

The sugar-to-acid ratio in citrus fruits may be used a maturity index which can be related to changes during storage and define acceptable maturity to compare, as in this case, the effect of applied treatments. This index (19.3–22.1) was not significantly different among control and treated mandarins after a day of storage (Figure 5). Only MW80 treated mandarins had a significantly higher index (Figure 5) at day 8 (25.4 ± 1.9) compared to day one (20.1 ± 0.5); however, there was no difference among treated mandarins (22.5–25.4) and control on that storage day. By

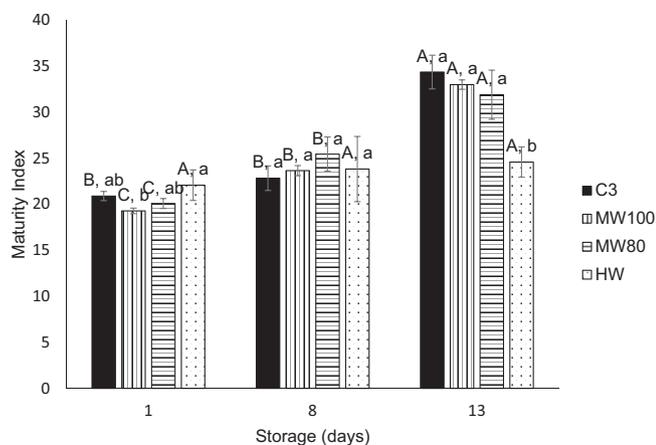


Figure 5. Changes in maturity index during storage at 25 °C of control and treated mandarins. Different small cap letters indicate significant difference ($p < 0.05$) among treatments on the same storage day, different capital letters indicate significant difference ($p < 0.05$) during storage time for the same treatment.

the 13th storage day, control, MW-treated mandarins had reached a significantly higher maturity index compared to the beginning of the storage period (Figure 5). Only HW-treated mandarins had a significantly lower index than control and MW-treated mandarins by the end of storage (Figure 5). Maturity index is a great indicator of sensory quality and consumer acceptability of citric juices. As the fruits mature and the acidity tends to increase while the sugars decrease, the index would generally increase (Berk, 2016) as illustrated by the results shown in Figure 5. The results agree with previous reports by Hong et al. (2007) on the increase of maturity index of heat-treated mandarins during storage.

3.5. Vitamin C

Ascorbic acid content of mandarins was significantly reduced by half or less of its original concentration after all heat treatments as shown in Figure 6. On subsequent storage days controls and MW100 had a significant reduction in vitamin C concentration (Figure 6) in comparison with the first storage day. Even with the reduction in concentration, control mandarins had a significantly higher concentration of vitamin C than treated mandarins throughout the storage period (Figure 5). By the end of the storage, MW80 and HW-treated mandarins had significantly

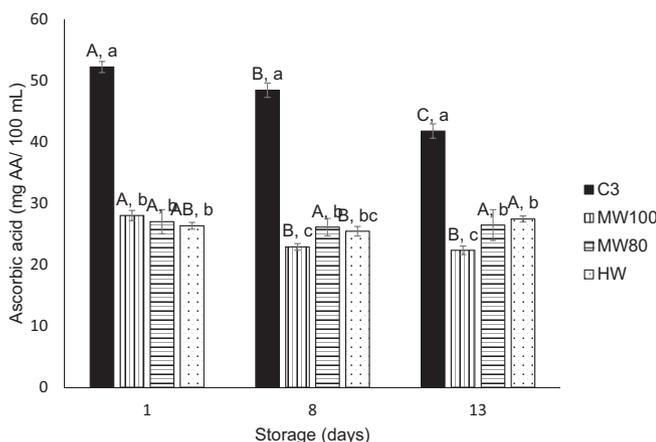


Figure 6. Changes in ascorbic acid (vitamin C) during storage at 25 °C of control and treated mandarins. Different small cap letters indicate significant difference ($p < 0.05$) among treatments on the same storage day, different capital letters indicate significant difference ($p < 0.05$) during storage time for the same treatment.

higher concentrations of ascorbic acid than MW100 mandarins ($p < 0.05$), as it is shown in Figure 6.

The results are in contrast with some studies where no changes in ascorbic acid were reported during storage or between control and heat-treated fruits (Hong et al., 2014; Shen et al., 2013; Zhang et al., 2004). However, the studies by Hong et al. (2014) and Zhang et al. (2004) do not state whether they observed significant differences immediately after treatment, but rather report values after prolonged storage times (15–60 days) at cold temperatures (2–10 °C). Hence, it is not possible to say if vitamin C concentration differed at the beginning and became similar after sufficient storage time had elapsed. Shen et al. (2013) on the other hand, did not find any significant difference in vitamin C after treatment or during storage of mandarins. Loss of ascorbic acid is expected during storage due to its sensitivity to oxygen, heat and enzyme degradation, especially at storage temperatures of 25 °C and above (Berk, 2016). This study showed heat treatments and storage negatively affect the vitamin C content of mandarins.

3.6. Firmness

Heat-treated mandarins were significantly softer than controls, except for MW100, after a day of storage (Figure 7). By the 8th day of storage, firmness of MW80 treated mandarins were significantly lower than controls, and no significant difference in firmness was registered compared to day one (Figure 7). At the end of storage, control and treated mandarins, except MW80, were significantly softer compared to day 1 (Figure 7). Degradation of protopectins into their soluble versions, pectin and pectic acid, softens fruits; this process is slower for citrus compared to climacteric fruits (Ladanya, 2010). In contrast to other studies (Hong et al., 2014; Karabulut and Baykal, 2002), heating did not prevent loss of firmness. However, other authors (Hong et al., 2007) have also recorded softening of fruits during storage, which can be attributed to degradation of protopectins, coupled with the loss of water suggesting there was a loss of turgor and a consequent loss of firmness.

3.7. Color

There was no significant change in ΔE among control and treated mandarins at the first (4.76–6.21) and eight (4.39–8.30) day of storage. By the end of storage, ΔE of control and MW-treated mandarins (5.54–8.98) was not significantly different among them or compared to day 1. However, ΔE of HW (15.7 ± 8.8) mandarins was higher compared to day one and other treatments on day 13.

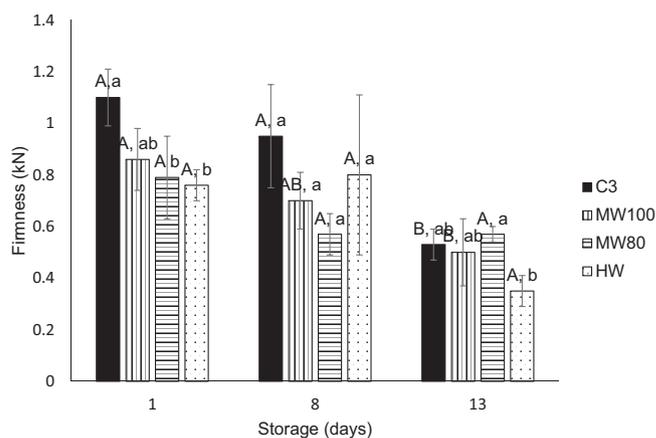


Figure 7. Changes in firmness during storage at 25 °C of control and treated mandarins. Different small cap letters indicate significant difference ($p < 0.05$) among treatments on the same storage day, different capital letters indicate significant difference ($p < 0.05$) during storage time for the same treatment.

Table 1. Changes in color parameters L^* , a^* , b^* , and net color difference (ΔE) during storage at 25 °C of mandarins washed, sanitized, and immersed in water for 20 min (C1), treated with hot water (HW) or microwaved at 100 and 80% of nominal power (MW100 and MW80).

Storage time (day)	Control	MW100	MW80	HW
Net Color difference (ΔE)				
1	6.21 ± 2.25 ^{A,a}	5.11 ± 3.1 ^{A,a}	5.50 ± 1.86 ^{A,a}	4.76 ± 3.09 ^{A,a}
8	4.39 ± 1.58 ^{A,a}	8.30 ± 2.29 ^{A,a}	5.94 ± 4.17 ^{A,a}	7.26 ± 3.73 ^{A,a}
13	6.10 ± 1.59 ^{A,a}	5.54 ± 3.36 ^{A,a}	8.98 ± 1.68 ^{A,a}	15.66 ± 8.84 ^{A,a}
L^*				
1	61.83 ± 5.02 ^{A,a}	64.37 ± 1.52 ^{A,a}	65.18 ± 1.23 ^{A,a}	63.59 ± 1.64 ^{A,a}
8	63.22 ± 2.20 ^{A,a}	68.03 ± 1.35 ^{A,a}	64.19 ± 2.71 ^{A,a}	64.30 ± 2.15 ^{A,a}
13	61.56 ± 1.92 ^{A,a}	63.53 ± 3.95 ^{A,a}	64.74 ± 0.92 ^{A,a}	56.16 ± 9.15 ^{A,a}
a^*				
1	21.08 ± 2.69 ^{A,a}	21.34 ± 2.24 ^{A,a}	20.59 ± 1.55 ^{A,a}	23.39 ± 2.63 ^{A,a}
8	22.60 ± 1.02 ^{A,a}	18.80 ± 2.73 ^{A,a}	23.69 ± 3.25 ^{A,a}	23.45 ± 5.44 ^{A,a}
13	23.31 ± 2.76 ^{A,a}	21.75 ± 1.28 ^{A,a}	25.64 ± 0.96 ^{A,a}	24.00 ± 1.09 ^{A,a}
b^*				
1	56.04 ± 4.36 ^{A,a}	59.77 ± 3.06 ^{A,a}	59.86 ± 2.15 ^{A,a}	58.43 ± 3.18 ^{A,a}
8	56.07 ± 3.83 ^{A,a}	64.61 ± 2.96 ^{A,a}	58.69 ± 4.31 ^{A,a}	57.85 ± 5.00 ^{A,a}
13	58.24 ± 2.91 ^{A,a}	58.34 ± 4.54 ^{A,a}	63.10 ± 1.74 ^{A,a}	49.60 ± 9.75 ^{A,a}

For each parameter, different small cap letters indicate significant difference ($p < 0.05$) among treatments on the same storage day, different capital letters indicate significant difference ($p < 0.05$) during storage time for the same treatment.

Lightness (L^*) did not change significantly among treated and control mandarins (61.8–68.0) during storage (Table 1). By the end of storage (day 13) HW mandarins (56.2 ± 9.2) were darker compared to day one and other treatments (61.6–64.7) (Table 1). a^* and b^* presented positive values for all mandarins throughout storage, placed them in the yellow-red zone of the CIELab color space. No significant differences for a^* parameter were registered for control or treated mandarins (18.8–25.6) among treatments throughout storage or among different storage days for the same treatments (Table 1). b^* color parameter showed a similar trend to L^* , with no significant difference among control and treated mandarins (56.0–64.6) during storage (Table 1). However, HW (49.6 ± 9.7) mandarins had a lower b^* at the end of storage compared to the first day (Table 1).

The color of mandarins, as for many fruits, is intrinsically associated with its level of maturity and therefore acceptability in the market. Green fruits (derived from chlorophyll) are perceived as immature while mature fruits are the ones with yellow, orange or reddish tones (given by carotenoids) (Ladanyia, 2010). Color of control and MW-treated mandarins remained constant throughout storage as reported by Sisquella et al. (2013) for MW-treated peaches. In contrast with reports by Hong et al. (2007), HW-treated mandarins lost lightness and yellowness (b^*) by the end of storage.

3.8. Sensory evaluation

Texture of MW80 and HW-treated mandarins ranked top among judges while control and MW100 were at the bottom of the rank without significant difference among them after 2 days of storage (Table 2). By the 8th day, texture of control mandarins was the lowest ranked, while no difference among treated mandarins was detected (Table 2).

Color of HW mandarins was the highest ranked after two days of storage, while all other treated mandarins were second best without significant difference among them, and controls were the lowest ranked (Table 2). After being stored for 8 days, color of treated mandarins was ranked higher than color of controls (Table 2).

Neither subjective parameter, color nor texture, had a significant ($p \geq 0.05$) linear correlation (Pearson coefficients not shown) with their objective counterpart, ΔE and firmness, at either storage day. Therefore,

Table 2. Ranking score of mandarins washed, sanitized, and immersed in water for 20 min (C1), treated with hot water (HW) or microwaved at 100 and 80% of nominal power (MW100 and MW80) and stored for 2 and 8 days at 25 °C. Different letters indicate significant difference ($p < 0.05$) among treatments on the same storage day.

Storage (Day)	Sample	Texture	Color
2	Control	0.9 ± 0.3 ^a	0.9 ± 0.2 ^a
	MW100	1.2 ± 0.3 ^{ac}	1.2 ± 0.3 ^b
	MW80	1.5 ± 0.4 ^{bc}	1.3 ± 0.3 ^b
	HW	1.5 ± 0.4 ^b	1.6 ± 0.3 ^c
8	Control	1.0 ± 0.1 ^a	0.9 ± 0.1 ^a
	MW100	1.4 ± 0.4 ^b	1.4 ± 0.3 ^b
	MW80	1.6 ± 0.3 ^b	1.5 ± 0.3 ^b
	HW	1.4 ± 0.4 ^b	1.5 ± 0.3 ^b

in this study the perceived rank of the color and texture of mandarins cannot be explained or related with any measurable physical changes. Nonetheless, there was a consistent low ranking for control mandarins, while MW and HW-treated fruits were given a higher preference and therefore considered more acceptable to the public.

Color of mandarin juice was acceptable for all mandarins during either storage day (Table 3); most were “Liked moderately” (score of 7) while MW100 held the lowest average score of 6.4 that corresponds to “Like slightly”.

Juice taste was acceptable for all mandarins except MW100, which was “Slightly disliked” after two storage days (Table 3). And juice of MW mandarins was “Slightly” to “Moderately” disliked by day 8 of storage. Following a similar trend, general acceptance of mandarin juice of MW-treated mandarins was below acceptance levels (Table 3).

Neither juice taste nor general acceptability had a significant linear correlation (Pearson coefficient not shown) with either TSS, citric acid content or maturity index. However, even though juice color was acceptable for all mandarins, juice from MW-treated mandarins was disliked in general due to its taste. Judges referred to MW-treated mandarin juice as “bitter”, “cooked” or “overripe”. And even though smell was not evaluated, comments of a “fermented” smell for MW-treated mandarins were commonly mentioned by judges.

4. Conclusion

MW-treated mandarins effectively prevented mold growth on stored mandarins, and also retained some quality attributes like pH, color and TSS; while others were not favorably affected, such as citric acid content, higher maturity and unacceptable juice taste and overall acidity. On the other hand, HW-treated mandarins had a delay of mold growth while retaining qualities such as citric acid content, pH and acceptable juice taste and overall acceptability. But, they lost some important attributes such as color and showed lower maturity index. All treated mandarins lost almost half their vitamin C, an important nutritional attribute for citrus fruits.

Table 3. Acceptability score of mandarin juice washed, sanitized, and immersed in water for 20 min (C1), treated with hot water (HW) or microwaved at 100 and 80% of nominal power (MW100 and MW80) and stored for 2 and 8 days at 25 °C.

Storage (Day)	Sample	Color	Taste	General acceptance
2	Control	7.8 ± 1.5	7.9 ± 1.5	7.9 ± 1.3
	MW100	6.4 ± 2.1	4.1 ± 1.9	4.3 ± 1.9
	MW80	7.7 ± 0.9	6.9 ± 1.9	7.0 ± 1.6
	HW	7.7 ± 1.7	7.2 ± 1.9	7.2 ± 1.9
8	Control	7.5 ± 1.4	7.5 ± 1.3	7.6 ± 1.4
	MW100	7.3 ± 1.3	3.0 ± 1.6	3.6 ± 1.8
	MW80	7.6 ± 1.1	4.1 ± 1.8	4.7 ± 1.8
	HW	7.2 ± 1.7	6.0 ± 1.7	6.0 ± 1.7

In general, the applied heat treatments (HD or assisted with MW) retarded or inactivated mold growth; however, depending on the treatment, they also negatively affected some quality attributes such as vitamin C content, and had an effect on the flavor of the juice. Derived from these findings, more studies are needed to determine the correct combination of time and temperature of treatment to achieve mold inactivation without detrimental the quality of the fruit.

Declarations

Author contribution statement

Diana B. Queb-González: Performed the experiments; Analyzed and interpreted the data.

Aurelio Lopez-Malo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

María E. Sosa-Morales: Analyzed and interpreted the data; Wrote the paper. Rossana Villa-Rojas Performed the experiments; Contributed reagents, materials, analysis tools or data.

Competing interest statement

The authors declare no conflict of interest.

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Additional information

No additional information is available for this paper.

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