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Quality of oranges as influenced by potential radio frequency heat treatments against Mediterranean fruit flies

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Abstract

There has been an increased interest in developing alternative quarantine treatment methods for control of fruit flies under growing international pressure to replace the remaining use of methyl bromide fumigation because of concerns over its role in ozone depletion. The present work explored the possibility of using radio frequency (RF) heating as a means to increase the internal fruit heating rate in water to control pests. Based on the thermal death kinetics of the Mediterranean fruit fly (Medfly), thermal treatments were designed that could provide quarantine security against fruit flies. The main objective of this research was to study the influence of those RF heat treatments on the quality of treated fruit. Treated 'Navel' and 'Valencia' oranges were evaluated for post-harvest quality after 10 days of 4 °C storage. The quality parameters included: weight loss, loss in firmness, color change, total soluble solids, acidity, and change in volatiles. The volatile analysis was done by the SPME-GC/MS technique. The results indicated a significant change in volatile flavor profiles upon RF heat treatments even when there was no significant difference in the other quality parameters. The reduction in process time due to RF heating helped in retention of many volatile compounds in comparison with conventional hot water heating. The treatment that raises fruit temperature from 19 to 48 °C by RF heating in saline water and held then for 15 min in 48 °C hot water would meet the quarantine security without impairing the quality of the treated oranges. However, sensory evaluation for market acceptability of treated oranges should be carried out for complete treatment protocol development.
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1. Introduction

The Mexican fruit fly, *Anastrepha ludens* (Loew), the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the Caribbean fruit fly, *Anastrepha suspensa* (Loew), are quarantine pests of citrus fruit. Due

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to their broad host ranges, presence of these pests in fresh produce can restrict domestic and international commerce. Citrus shipments destined for states such as California, Arizona, and Florida, as well as for export markets including Japan and other Pacific Rim countries require methyl bromide fumigation to meet import quarantine security requirements. Methyl bromide can damage some citrus, e.g. mandarin, oranges (Williams et al., 2000). Moreover, it has been identified as an ozone-depleting chemical. Therefore, its use is being restricted in accordance with an international agreement, the Montreal Protocol (USEPA, 1998). Currently critical use exemptions may make the fumigant available for pre-shipment and quarantine purposes, but future use of methyl bromide is at great risk due to reduced production, increased price and future restrictions imposed on its uses under international agreements. Increasing legislative pressure on the use of chemicals for postharvest quarantine disinfestation has resulted in great interest in the development of non-chemical treatment methods.

Several alternative methods have been explored by many researchers including ionizing radiation, cold storage, and conventional hot air and water heating. All of these methods have drawbacks. For example, a common difficulty with hot air or water heat treatments for large fruit such as citrus and apple is the slow rate of heat transfer resulting in hours of treatment time (Wang et al., 2001b). Shellie and Mangan (1994, 1998), Sharp and MacGuire (1996), Schirra et al. (2005) and Lurie et al. (2004) have extensively studied citrus heat treatments using hot water and moist hot air. A long exposure time requirement and alterations to flavor compounds (Obenland et al., 1999) were the main difficulty in the development of quarantine treatment protocols. Radio frequency (RF) heating has been studied for selected commodities as a rapid disinfestation treatment (Headlee and Burdette, 1929; Frings, 1952; Nelson and Payne, 1982; Wang et al., 2002). RF heating has relative advantages over microwave heating because it provides larger penetration depths, possible differential heating of insects in commodities (Wang et al., 2003), and simple field patterns (Zhao et al., 2000). However, a number of potential problems need to be addressed before RF heat treatments can be successfully used in commercial applications. One potential problem associated with RF heating is possible lack of uniform heating in heterogeneous media (Tang et al., 2000). A large temperature variation among and within fresh fruit reduces the effectiveness of a treatment and may cause severe thermal damage to the fruit. Recently, efforts have been made to overcome non-uniform RF heating of fruit. Birla et al. (2004) have shown improvements in RF heating uniformity of oranges and apples when fruit were submerged in water and kept in motion by water jets during RF heating.

Treatment protocols have been developed using RF energy that can effectively control codling moth (Wang et al., 2001a) and navel orangeworm (Wang et al., 2002) in unshelled walnuts without causing quality losses. These studies focus on dry nuts that have higher heat tolerance than fresh fruit. Exposure to high temperature can alter many fruit ripening processes, such as ethylene production, respiration, fruit softening, and cell wall metabolism, pigment, carbohydrate and volatile metabolism (Lurie, 1998). Many researchers (Shellie et al., 1993; Shellie and Mangan, 1998; Obenland et al., 1999) have reported the negative effects of heat treatment on postharvest quality of citrus fruit. Changes in flavor quality were reported in these studies even with no significant differences in soluble solids or titratable acidity after heat treatment. The instability of fresh orange juice aroma during processing (e.g. heat treatment) and subsequent storage has been studied (Shaw, 1991). Decreased levels of characteristic fresh orange juice aroma compounds on one hand, and off-flavor formation on the other, lead to the distinct aroma differences between fresh and processed juice (Obenland et al., 1999).

Similar to development of any new process or technology, many issues have to be addressed before the RF heating method can be adopted as a commercial process. As RF heating is fast, very short treatment times can be developed. The development of a treatment protocol requires establishment of process parameters and verification and validation of treatment efficacy. The knowledge of thermal death kinetics can be used in selecting time–temperature combinations for RF heat treatments to kill Mediterranean fruit fly (Medfly) in citrus fruit. The objective of this research was to evaluate different treatment conditions that can control Medfly on orange quality. The quality parameters such as weight loss, loss in firmness, color change, total soluble solids, acidity, and the change in volatile compounds from oranges were evaluated after treatments. This study determined suitable treat-

ment conditions to support further efficacy studies with infested fruit.

2. Materials and methods

2.1. Thermal treatment

Freshly harvested 'Navel' and 'Valencia' oranges (*Citrus sinensis* L. *Osbeck*) were procured from Fillmore-Peru Citrus Association, California. The freshly harvested, untreated, unwashed, and unwaxed oranges were delivered overnight to Washington State University, Pullman, WA. The average fruit weight (mean ± S.D.) was 264 ± 21 and 255 ± 18 g for 'Navel' and 'Valencia', respectively. The supplied oranges were stored at 4 °C until used for thermal treatments. The oranges were removed from the cold storage and left overnight at ambient temperature (∼20 °C) to ensure uniform initial fruit temperature. Based on the thermal death kinetics reported by Gazit et al. (2004), 100% mortality of Medfly can be achieved by exposing infested fruit to 48 °C for 15 min, 50 °C for 4 min, or 52 °C for 1 min. Therefore, we chose three temperatures, 48, 50, and 52 °C, and different holding times (Table 1) corresponding to one level above and one level below 100% mortality.

Table 1
Experimental design of heat treatments

Treatment name	Heat treatment description	Cultivar
RF48 + 10 RF48 + 15 RF48 + 20	RF heating in saline water to 48 °C and holding at 48 °C for 10, 15, and 20 min	Valencia/Navel
RF50 + 2 RF50 + 4 RF50 + 6	RF heating in saline water to 50 °C and holding at 50 °C for 2, 4, and 6 min	Valencia/Navel
RF52 + 0 RF52 + 1 RF52 + 2	RF heating in saline water to 52 °C and holding for 0, 1, and 2 min	Navel
HW48	Hot water heating at 48 °C for 2.5 h	Valencia
RFA35	Pre-heating in 35 °C hot water for 45 min and followed by RF heating in tap water to 48 °C and holding 15 min	Valencia
Control	No heat treatment	Valencia/Navel

Initial experiments suggested that exposure at 52 °C caused irreversible and undesirable changes in the quality (flavor, and firmness) of treated 'Navel' oranges. Therefore, for 'Valencia' oranges the 52 °C RF treatment was removed and, instead, an experiment was performed to evaluate the effect of pre-heating of fruit (conventional hot water heating) before RF treatment. Pre-heating fruit to a moderate temperature prior to RF treatment could increase throughput and reduce the cost of RF equipment and RF energy on a per unit commodity basis in commercial applications. In principle, RF energy should be used sparingly as a means to overcome the problems associated with conventional heating methods so that it remains economically viable. 'Valencia' oranges were pre-heated in 35 °C hot water for 45 min. The pre-heated fruit were subjected to RF heating in 35 °C tap water to raise the fruit core temperature to 48 °C, and then held at 48 °C in hot water for 15 min.

The RF heating of oranges was conducted in a 12 kW batch type RF heating system (Strayfield Fastran with E-200, Strayfield International Limited, Wokingham, UK). The movement and rotation of oranges in water during RF heating for uniform heating was carried out in a fruit mover. The details of the fruit mover and operating procedure can be found elsewhere (Birla et al., 2004). In preliminary RF heating trials, it was found that 0.006 and 0.004% NaCl salt in tap water were adequate for 'Valencia' oranges and 'Navel' oranges, respectively, in order to minimize differential heating of fruit and water. Prior to starting the RF treatment for oranges, an experiment was conducted to obtain temperature profiles of the fruit subjected to different thermal treatments. At the end of each treatment stage (pre-heating, RF heating, holding, and cooling), two oranges were removed and thermal images were recorded by an infrared imaging camera (ThermaCAM™ Researcher 2001, accuracy ±2 °C, 5 picture recordings per seconds, FLIR Systems, Portland, OR). The temperature of the core and subsurface (5 mm below the surface) was measured by a pre-calibrated thermocouple (Type-T, 0.8-mm diameter and 0.8 s response time, Omega Engineering Ltd., CT) and temperature data during pre-heating and cooling time were recorded every 5 s by a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK).

Eight oranges were placed in the fruit mover and water of preset salt concentration was filled to the top

of the covering plate placed over the oranges. Oranges were kept in motion by means of water jet nozzles mounted on the periphery of the fruit mover. The RF input power (10 kW) was switched off when the water temperature reached the treatment temperature, i.e. 48, 50, or 52 °C. Oranges were then kept on hold at the treatment temperature for 0–20 min depending upon the treatment design, followed by hydro-cooling for 30 min in 3–4 °C chilled water. The core temperature of one randomly selected fruit was measured immediately after RF heating, holding time, and cooling. For a comparative study, conventional hot water heating of 'Valencia' oranges was also carried out in a water bath (Model ZD, Grant, Cambridge, UK) set at 48 °C. Hydro-cooling started after the core temperature reached 47.2 °C. Treated and control oranges were placed in cold storage (∼5 °C and ∼95% RH) for 10 days. Quality analyses of 'Navel' oranges suggested that water loss from both treated and control fruit was considerable and resulted in a loss of firmness. Therefore, treated and control 'Valencia' oranges were waxed (by *Carnauba* natural wax) before placing them in storage to reduce moisture loss. Each treatment combination listed in Table 1 was replicated three times.

2.2. Quality measurement

Weight, firmness, and peel color of each orange were measured before and 10 days after treatment. Each fruit within a treatment group was numbered on its stem end. Color and firmness were measured at three marked spots along the equatorial fruit surface. The firmness was measured by a Texture analyzer (Model TA-XT2, Stable Micro Systems, YL, UK) which was attached with an aluminum disk (50 mm × 20 mm) on cross head. The fruit was kept in position on a concave shaped Nylon disk which was secured in place on the Texture analyzer base during firmness measurement. The firmness was expressed in mm deformation by a 1 kg force on the equatorial fruit surface for 10 s. The change in firmness (difference in post-storage and pre-treatment firmness of individual fruit) was expressed as a percentage of pre-treatment firmness. A positive value suggests that there is a loss of firmness, whereas a negative value suggests that oranges became firmer after the treatments. Peel color was measured at three marked spots on an individual orange by a colorimeter (Model CM-2002, Minolta Corp., Ramsey, NJ) calibrated to a standard white reflective plate. The change in peel color was analyzed as percent change in value of L^*, C^*, and h° color system (L^* = darkness, C^* = chroma, h° = hue angle). The post-storage/treatment color was expressed as a percentage of pre-treatment value of color indices, which were calculated from '*L*', '*a*', and '*b*' values obtained before and after the treatments using the colorimeter. Total soluble solids and percent titratable acidity were measured after 10 days of storage on six oranges for each treatment. Juice was expressed and titratable acidity (TA) was determined by end-point titration of 5 ml juice to pH 8.2 with 0.1N NaOH solution and expressed in terms of the equivalent anhydrous citric acid per 100 ml of juice. Total soluble solids (°Brix) was measured by a hand-held refractrometer (Model N-1α, ATAGO Co. Ltd., Tokyo) and expressed as percent soluble solids in juice. The treated and unteated oranges were visually inspected for external appearance, treatment damage, and decay and assessed organolepticaily for any off-flavor development in the peel.

The measurements of individual quality attributes were subjected to an analysis of variance (ANOVA) and means were separated by L.S.D. ($p < 0.05$) and as multiple pair by Tukey's method (SAS Institute, 1990, Cary, NC).

2.3. Volatile compounds analysis

2.3.1. Sample preparation

After 10 days of storage, juice from six oranges (from each treatment group) was hand squeezed, filtered through cheese cloth to remove pulp and filled in a 20 ml plastic (scintillation vial with a cone cap lid). The sample vials were immediately sealed and stored in a freezer until the samples were used for SPME-GC analysis. A solid phase micro-extraction (SPME) technique was used to prepare samples for analysis by gas chromatography (GC) (Steffen and Pawliszyn, 1996). The orange juice samples were removed from the freezer just before volatile component analysis. The sample vial was immersed in tap water for thawing. In a 4 ml SPME vial 1 ml of juice was diluted in 1 ml of de-ionized water containing 0.65 g NaCl, according to Steffen and Pawliszyn (1996), and a 6 mm magnetic stirring bar. The vial was mounted on a SPME stand and a fiber (0.65 μm thick PDMS/DVB stationary

phase, Supelco Inc., Bellefonte, PA) was inserted in the headspace (Yang and Peppard, 1994; Boyd-Bland et al., 1994). The fiber was kept in the headspace for 30 min to absorb the volatile compounds and attain equilibrium (Arthur et al., 1992).

2.3.2. GC/MS analysis

The headspace sample adsorbed on the SPME fiber was injected into a Hewlett-Packard (Agilent, Avondale, PA), 5890II gas chromatograph interfaced with a 5970 mass selective detector system. The volatiles were desorbed into the injection port for 5 min set at 200 °C using a 0.75 mm SPME liner. The injection mode was splitless for 2 min. The MS transfer line was held at 250 °C and the GC programmed according to Mattheis et al. (1991). The carrier gas (Helium) velocity was set at 30.1 cm/s through the fused silica capillary column, a DB-1 (J&W, Folsom, CA) (60 m × 0.32 mm, thickness 0.32 μm). The various flavor compounds present in the orange juice were identified based on comparison of GC retention indices and mass spectra of those contained in the Wiley/NBS library and with those of authentic compounds under the identical experimental conditions. The data were collected and analyzed using the HP Chemstation G 1034C data processing package. The reproducibility of flavor compounds analyzed by the SPME-GC/MS was assessed by analyzing diluted identical samples in replicates and reporting the percent relative standard deviation (% R.S.D.).

2.3.3. Determination of response factors for major flavor compounds

A standard aqueous solution was prepared to determine the response factors for major volatile flavor compounds (ethanol, ethyl acetate, ethyl butanoate, hexenal, α-pinene, β-myrcene, sabinene, limonene, γ-terpinene, l-octanol, decanal, dodecanal, citral, trans-geraniol, L-phellandrene, and valencene). The concentration of these components in the standard solution was compared to the results of Shaw (1991) and our preliminary experiment. The response factors of these components were obtained by dividing GC peak areas by concentrations of each standard component in the standard solution.

3. Results and discussion

3.1. Temperature profiles

Fig. 1 shows the temperature–time history at the core and sub-surface of 'Valencia' oranges (18.9 °C initial temperature) subjected to RF heating in saline water (0.004%), followed by holding at 48 °C for 15 min before hydro-cooling for 30 min. The core temperature of the oranges after 5.5 min of RF heating at 10 kW power input was 46.4 °C whereas surface temperature was 48 °C. After 15 min of holding in hot water at 48 °C, the core temperature was 47.6 °C and it remained above 47 °C for more than 15 min. The addi-

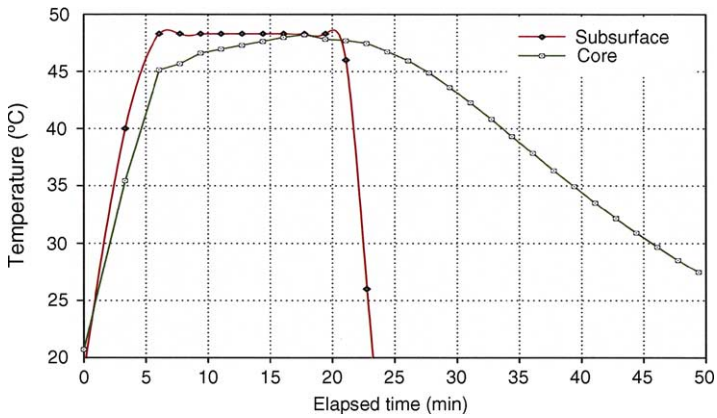

Fig. 1. Temperature–time history of subsurface (10 mm beneath surface) and core of the 'Valencia' oranges recorded during RF48 + 15 heat treatment. The oranges were subjected to RF heating in 0.004% saline water for 5.5 min followed by holding at 48 °C for 15 min before being cooled by 4 °C water for 30 min.

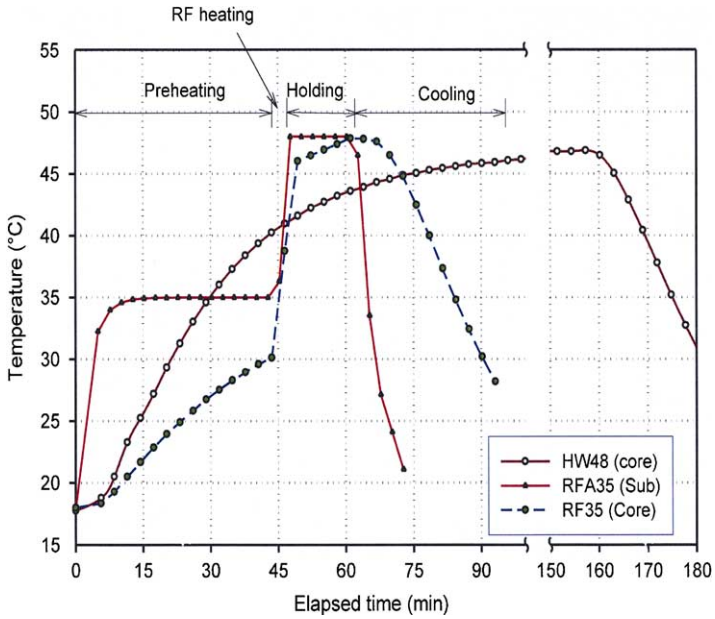

Fig. 2. Temperature–time history of 'Valencia' orange subsurface (10 mm beneath surface) and core subjected to HW48 and RFA35 (pre-heating in 35 °C water for 45 min followed by RF heating in tap water for 2 min and holding at 48 °C for 15 min) treatments.

tion of salt ensured that the core temperature was not more than that of the treatment target temperature during RF heating to avoid prolonged exposure of the core to high temperatures.

Fig. 2 shows the temperature–time profile of the oranges subjected to pre-heating followed by RF heating. After 45 min of pre-heating at 35 °C, core and subsurface (5 mm below the surface) temperatures were 29.6 and 34.6 °C. Upon 2 min of RF heating in 35 °C tap water with 10 kW input RF power, the core and subsurface temperatures were 46.4 and 48 °C. The holding of oranges in hot water for 15 min at 48 °C was carried out to ensure the accumulation of thermal lethality at the core by heat transfer from hot spots to cold spots. Fig. 2 also shows the time–temperature profile of an orange subjected to 48 °C hot water immersion for 2.5 h. Even after such a long exposure time the core temperature of fruit was not higher than 47.2 °C.

Fig. 3 shows the thermal images of oranges taken during the RFA35 experiment (see Table 1), at the end

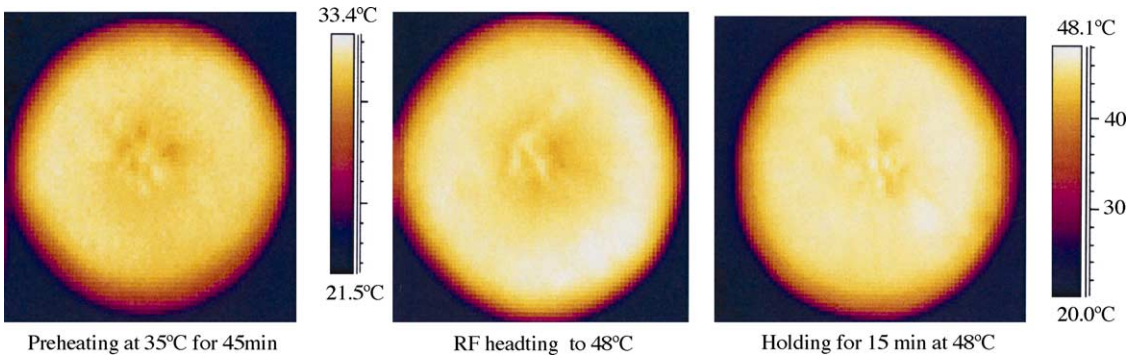

Fig. 3. Thermal images of oranges taken by the infrared imaging camera during RF assisted hot water heat treatment at the end of pre-heating, RF heating, and holding time.

of pre-heating, after RF heating and after holding for 15 min at 48 °C. The pre-heating in hot water established a temperature gradient from surface to core. The pre-heating of the oranges ensured that the core temperature remains below the treatment temperature (48 °C) at the end of RF heating. A thermal image in Fig. 3 showed that 15 min holding at 48 °C eliminated the temperature gradient and ensured a uniform distribution of temperature in the orange. The mean temperature over the orange cross section was 47.4 ± 1.2 and 47.8 ± 0.3 °C before and after holding, respectively.

3.2. Quality analysis

3.2.1. Weight loss

The percent weight loss after 10 days of cold humid storage (5 °C and 95% RH) was significantly higher in 'Navel' oranges (0.6–1.65%) than 'Valencia' oranges (0.2–0.4%) (Table 2). The higher weight loss in 'Navel' oranges was likely caused by no wax coating applied to oranges. Statistical analysis showed a significant effect of temperature on weight loss of the treated 'Navel' oranges. The weight loss was significantly higher in all the treatments in comparison with control oranges (Table 2). Shellie and Mangan (1998) have reported that a hot water treatment (46 °C for 3 h, storage for 4 weeks at 7 °C and 1 week at 23 °C) of oranges caused 10.45% loss of moisture in comparison to 8.46% weight loss from untreated oranges. The weight loss in the 'Navel' oranges subjected to RF treatments corresponding to 48 °C (0.77–0.97%) was significantly less (1.06–1.65%) than that of treatments, to which the oranges were subjected at 50 or 52 °C (Table 2).

The application of wax coating on 'Valencia' oranges before storage improved moisture retention (Table 2). There was no significant weight loss in the 'Valencia' oranges subjected to RF heating in comparison with the control group. A significantly lower weight loss (0.16–0.24%) from 'Valencia' oranges subjected to HW48 or RFA35 treatments in contrast to the control group (0.38%) was likely due to hydration of the orange cells during treatment, and later on retention of absorbed moisture by the wax coating. Therefore, for oranges with a wax coating, weight loss could not be used as a criterion for quality assessment as the negative effect of heat is masked by the wax coating.

3.2.2. Firmness

The loss/gain in firmness was expressed by the percentage change in firmness over the period of 10 days storage. Positive values suggest a loss in firmness and negative values show a gain in firmness upon treatment and storage. In 'Navel' oranges, the effect of heat was pronounced because of excessive weight loss during storage (Table 2). Except treatment RF48 + 10, all the other treatments caused significant loss in firmness

Table 2
Change in the postharvest physical quality traits of 'Navel' and 'Valencia' oranges upon 10 days of storage of oranges subjected to different thermal treatments

Cultivar	Treatment	Weight loss (%)	Firmness change (%)	Peel color change (%)		
				L^*	h°	C^*
Navel	Control	0.60 ± 0.22^{a}	-8.98 ± 9.86^{a}	99.07 ± 1.61^{a}	99.34 ± 2.00^{b}	100.41 ± 2.36^{b}
	48 °C + 10 min	0.77 ± 0.12^{b}	$0.55 \pm 27.93^{a,b}$	98.85 ± 2.04^{a}	$101.09 \pm 3.02^{a,b}$	$1.0014 \pm 3.11^{b,c}$
	48 °C + 15 min	0.93 ± 0.15^{c}	$19.44 \pm 35.44^{b,c,d}$	$98.38 \pm 1.88^{a,b}$	102.02 ± 2.94^{a}	100.68 ± 2.94^{b}
	50 °C + 2 min	1.63 ± 1.53^{e}	$35.46 \pm 41.90^{c,d}$	$98.47 \pm 1.88^{a,b}$	101.98 ± 3.11^{a}	$97.73 \pm 4.22^{c,d}$
	50 °C + 4 min	1.65 ± 0.34^{e}	42.24 ± 49.87^{d}	$97.41 \pm 2.34^{b,c}$	102.09 ± 2.98^{a}	97.15 ± 3.74^{d}
	52 °C + 1 min	$1.27 \pm 0.44^{d,e}$	$23.97 \pm 42.85^{b,c}$	$97.40 \pm 2.36^{b,c}$	$101.1l \pm 2.01^{a}$	$98.44 \pm 4.10^{b,c,d}$
	52 °C + 2 min	1.32 ± 0.34^{e}	16.16 ± 23.32^{b}	$97.41 \pm 2.56^{b,c}$	101.37 ± 3.30^{a}	$99.05 \pm 3.78^{b,c,d}$
Valencia	Control	0.38 ± 0.15^{a}	-10.25 ± 8.60^{a}	97.28 ± 2.97^{a}	98.72 ± 2.89^{a}	$97.99 \pm 5.95^{b,c}$
	48 °C + 10 min	0.36 ± 0.13^{a}	-12.49 ± 14.09^{a}	98.08 ± 2.35^{a}	99.30 ± 2.62^{a}	92.76 ± 4.64^{d}
	48 °C + 15 min	0.39 ± 0.11^{a}	-11.16 ± 14.18^{a}	97.78 ± 3.36^{a}	98.04 ± 7.04^{a}	$93.71 \pm 4.93^{c,d}$
	50 °C + 2 min	$0.32 \pm 0.12^{a,c}$	-13.26 ± 9.99^{a}	97.25 ± 3.81^{a}	$101.37 \pm 6.36^{a,c}$	95.90 ± 3.79^{c}
	50 °C + 4 min	$0.36 \pm 0.10^{a,c}$	-14.56 ± 16.22^{a}	97.71 ± 2.41^{a}	100.27 ± 1.80^{a}	93.77 ± 1.43^{d}
	HW48 °C + 155 min	0.16 ± 0.10^{b}	-6.10 ± 10.82^{a}	98.63 ± 1.30^{a}	99.08 ± 1.84^{a}	$94.08 \pm 3.94^{c,d}$
	RFA35 + 48 °C + 15 min	$0.24 \pm 0.06^{b,c}$	-14.45 ± 8.82^{a}	97.06 ± 2.71^{a}	99.68 ± 3.56^{a}	$100.62 \pm 5.82^{a,b}$

Entries with different superscripts letters (a–c) in the same column of each cultivar are significantly different ($p < 0.05$).

in comparison to the control oranges. In the case of 'Valencia' oranges, control as well as treated oranges became firmer after 10 days of storage. There was no significant difference in firmness between control and treated oranges. This gain in firmness might be attributed to the wax coating that prevented moisture loss during storage. Even the oranges subjected to hot water treatment (HW48) did not lose firmness. Therefore, we conclude that prevention of moisture loss by the wax coating can maintain firmness.

3.2.3. Skin color

Change in skin color was calculated from the color measurement before and after 10 days of treatment. In 'Navel' oranges, L^* value of the peel was significantly lower in the oranges subjected to a temperature of 50 °C for more than 4 min or 52 °C for more than 1 min in comparison to controls (Table 2). The lower value of L^* indicates a darker shade of peel color. Physical observation also suggested that 'Navel' oranges subjected to RF heat treatment at 50 °C and 4 min or more holding time or exposure to 52 °C lost the luster of peel surface. The loss of luster might be due to diffusion of peel essential oil from peel to hot water. The change in hue angle that signifies the shift toward yellow or red within the yellow to red quadrant was significantly higher in all treatments except for the heat treatment RF48 + 10 (Table 2). The trend of chroma value or color intensity change was not clear but in most of the treatments the color intensity did not change significantly in comparison with the control oranges.

In 'Valencia' oranges, peel color in terms of L^* did not change significantly for all treatments. The values of hue for all treatments were not statistically different from control oranges except for the treatment RF50 + 6 min. The color intensity of oranges was again found to vary from treatment to treatment, but in comparison with control oranges most of the treatments were not significantly different. The lowest color intensity was recorded for the treatment RF48 + 10 (Table 2).

3.2.4. Total soluble solids/titratable acidity

There was no significant difference in the value of total soluble solids (TSS) for all treatments (data not shown). The mean value of TSS was 10.78 ± 0.5 and 10.24 ± 0.5% for 'Navel' and 'Valencia' oranges, respectively. A sharp decrease in TA in heat-treated fruit may be an indication of heat damage (Schirra et al., 2005). But in the present study we did not find a significant difference in values of TA for all heat treatments in comparison with the control (data not shown). The mean value of acidity in treated oranges were 0.98 ± 0.04 and 1.04 ± 0.05 g/100 ml for 'Navel' and 'Valencia' oranges, respectively.

3.2.5. Visual observations

In the present study, we observed incidences of decay and off-flavor development in the peel of oranges subjected to HW48 treatment. Shellie and Mangan (1998) reported that hot water heating (46 °C for 4 h) of oranges inflicted deleterious effects on fruit flavor, and decay incidence. McGuire (1991) also reported a higher incidence of decay and off-flavor development in grapefruit that were subjected to hot water (48 °C for 3 h) than in grapefruit treated by forced hot air at 48 °C. The RF heat treatments did not cause any visible peel damage except for treatments at 52 °C. Control oranges showed the onset of stem rot after 10 days of cold-humid storage. Upon 10 days of storage 12% of control oranges, 22% of HW48 and 8% of RF52 treated oranges were found with some decay. The onset of stem rot in untreated control oranges was likely due to a large load of active pathogens, whereas an increased incidence of decay in heat-treated oranges, HW48 and RF52 was likely due to pathogens invading areas on the fruit injured by heat treatment. Mulas et al. (2001) studied the response of 'Tarocco', 'Moro', 'Sanguinello' and 'Doppio sanguigno' blood oranges to hot water heat treatment. They observed that a fruit core temperature of 44 °C for 100 min or 46 °C for 50 min did not induce visible damage to the fruit, but inflicted deleterious effects on quality attributes such as the development of off-flavors and off-taste, decreased fruit firmness and reduced fruit resistance to decay.

A distinct oily odor from the peel of 'Valencia' oranges was detectable by the nose in all heat treatments, however, such odor was not detected from 'Navel' oranges. The impairment of water-gas exchange by the wax coating has been studied by Cohen et al. (1990) and Baldwin et al. (1995), who reported the notable increase in the synthesis of volatiles associated with anaerobic conditions, such as ethanol, methanol, and acetaldehyde which might be the reason behind off-flavor development. Secondly, the movement and rotation of oranges during RF heating in the fruit mover might have caused peel bruising that could lead to

the phenomenon called Oleocellosis, or oil spotting on orange peel. This is a common peel injury of citrus fruit that is usually caused by mechanical damage that forces the toxic oil out of the oil glands. This oil kills nearby parenchyma, epidermal and subepidermal cells of the flavedo. Cells killed by oil are readily invaded by fungi resulting in increased decay (Wardowski et al., 2004). In the present study, the distinct flavor development in 'Valencia' orange peel might be attributed to the combined effect of the wax coating and bruised peel oil glands. This speculation was based on the observation that no distinct off flavors from the peel of untreated, waxed 'Valencia' oranges were detected. A consideration of mechanical damage is very important in designing a system for the handling and movement of citrus fruit.

3.3. Flavor analysis

Tables 3 and 4 show the concentrations of the major volatile compounds identified in the 'Navel' and 'Valencia' orange juice samples using SPME and GC/MS techniques after treatment and 10 days of cold storage. The last row in the tables shows the percent relative standard deviation (% R.S.D.) in each treatment for all the volatile components. In calculation of the % R.S.D., acetaldehyde and ethanol were excluded because we could not accurately quantify these components in every sample since the peaks representing these components were often too small and too poorly resolved for accurate determinations. Therefore, the concentrations of acetaldehyde and ethanol may not be accurate. However, the areas were averaged for the replicates whose peaks had large enough values so we might view the effect of different heat treatments on these compounds. The values of R.S.D. (<10%) indicated excellent reproducibility of SPME-GC/MS analysis under the analytical conditions used. This level of reproducibility was adequate to separate the effect of different heat treatment regimes on the flavor compounds. The quantitative values determined for the terpenes, esters, alcohols, and aldehydes listed in Table 4 are in agreement with reported literature values for most of the compounds found in hand squeezed unheated orange juice (Shaw, 1986; Nisperos-Carriedo and Shaw, 1990). The data in Table 4 also lists the odor threshold (OT, ppm, in water) of the major compounds as compiled by Rychlik et al. (1998).

The GC/MS analysis of untreated orange juice showed that typical citrus-like flavor was contributed by more than 31 volatile components present in varying quantities. Among those 31 flavor compounds, 16

Table 3
Concentration (μg/ml) of the major volatiles compounds quantified using the SPME-GC/MS technique in 'Navel' oranges subjected to different RF heat treatment regimes and 10 days of cold storage

Volatile compound	LV (μg/ml)	Control	52 °C		50 °C		48 °C	
			1 min	2 min	2 min	4 min	10 min	15 min
Acetaldehyde	3–8.5 a	38.6 ± 3.8	39.8 ± 36.7	41.7 ± 12.6	40.5 ± 41.6	37.5 ± 20.3	35.3 ± 5.4	45.8 ± 8.3
Ethanol	64–900 a	57.0 ± 19.2	67.9 ± 13.6	79.9 ± 43.8	81.9 ± 48.5	102.8 ± 15.6	107.3 ± 12.0	79.0 ± 24.1
Ethyl acetate	0.01–0.58 b	0.11 ± 0.03	0.05 ± 0.01	0.02 ± 0.02	ND	0.05 ± 0.00	0.01 ± 0.01	0.04 ± 0.00
Hexanal	0.02–0.65 a	0.06 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.00
Ethyl butanoate	0.26–1.02 b	0.66 ± 0.14	0.02 ± 0.01	0.01 ± 0.02	0.13 ± 0.18	0.10 ± 0.06	0.12 ± 0.04	0.09 ± 0.05
α-Pinene	0–0.22 b	0.75 ± 0.10	0.26 ± 0.06	0.24 ± 0.15	0.27 ± 0.05	0.21 ± 0.06	0.61 ± 0.01	0.70 ± 0.02
Sabinene	0–0.15 b	0.64 ± 0.04	0.93 ± 0.15	0.84 ± 0.21	1.17 ± 0.31	0.88 ± 0.41	0.69 ± 0.20	0.62 ± 0.21
β-Myrcene	1.54 c	17.5 ± 1.10	10.5 ± 1.70	11.4 ± 0.50	15.5 ± 2.60	12.2 ± 1.70	12.8 ± 0.80	12.8 ± 1.8
Limonene	1–278 a	61.2 ± 4.10	39.6 ± 3.90	38.0 ± 3.00	54.4 ± 2.90	42.8 ± 2.10	58.0 ± 9.40	53.2 ± 3.2
γ-Terpinene	0.04–0.46 b	0.01 ± 0.02	0.02 ± 0.03	0.05 ± 0.01	0.12 ± 0.04	0.08 ± 0.10	0.05 ± 0.02	0.04 ± 0.03
Linalool	0.15–4.6 b	0.58 ± 0.18	0.95 ± 0.19	1.23 ± 0.42	1.11 ± 0.15	1.14 ± 0.25	0.95 ± 0.05	1.23 ± 0.09
L-α-Terpineol	0.09–1.1 a	0.30 ± 0.05	0.69 ± 0.07	0.70 ± 0.19	0.73 ± 0.12	0.66 ± 0.14	0.64 ± 0.08	0.67 ± 0.05
Decanal	0.01–0.15 a	0.83 ± 0.26	1.50 ± 0.95	1.83 ± 0.88	l.25 ± 0.27	1.78 ± 0.71	1.11 ± 0.39	2.09 ± 0.62
Dodecanal	NA	0.11 ± 0.05	0.29 ± 0.07	0.27 ± 0.20	0.68 ± 0.75	0.20 ± 0.14	0.22 ± 0.01	0.35 ± 0.02
Valencene	0.8–15 b	1.57 ± 0.39	1.16 ± 0.45	1.32 ± 0.76	1.73 ± 0.10	1.30 ± 0.52	1.26 ± 0.29	1.16 ± 0.18
R.S.D. (%)		11.5	13.5	11.5	9.7	9.9	14.8	8.5

LV: literature values cited from (a) Shaw (1986), (b) Nisperos-Carriedo and Shaw (1990), (c) Steffen and Pawliszyn (1996); linear range (0.03–0.00013); ND: not detected; NA: not available.

Table 4
Concentration (μg/ml) of the major volatile compounds quantified using the SPME-GC/MS technique in 'Valencia' oranges subjected to different heat treatment regimes and 10 days of cold storage

Volatile compounds	Retention time (min)	LV (μg/ml)	OT (ppm)	Control (μg/ml)	HW 48 °C	RFA35	RF 50 °C		RF 48 °C	
					2.5 h	48 °C + 15 min	2 min	4 min	10 min	15 min
Acetaldehyde	3.83	3–8.5 a	0.01 a	29.1 ± 13.6	53.1 ± 13.7	32.1 ± 14.2	30.7 ± 12.8	22.9 ± 17.3	11.0 ± 6.9	24.2 ± 7.0
Ethanol	4.30	64–900 a	NA	19.7 ± 8.8	64.9 ± 13.5	54.8 ± 7.2	53.1 ± 2.5	82.2 ± 77.0	26.3 ± 5.0	35.7 ± 4.9
Ethyl acetate	6.03	0.01–0.58 b	0.5	0.05 ± 0.01	ND	ND	ND	ND	0.04 ± 0.00	0.02 ± 0.00
Hexanal	13.4	0.02–0.65 b	0.015	0.13 ± 0.08	0.01 ± 0.02	0.02 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Ethyl butanoate	14.0	0.26–1.02 b	0.001	0.52 ± 0.06	0.12 ± 0.08	0.21 ± 0.02	0.03 ± 0.00	0.02 ± 0.02	0.27 ± 0.12	0.18 ± 0.00
α-Pinene	22.1	0–0.22 b	0.030	0.19 ± 0.05	0.04 ± 0.03	0.22 ± 0.04	0.12 ± 0.00	0.16 ± 0.09	0.12 ± 0.04	0.14 ± 0.03
Sabinene	23.8	0–0.15 b	NA	0.25 ± 0.10	0.16 ± 0.06	0.45 ± 0.07	0.43 ± 0.04	0.48 ± 0.18	0.18 ± 0.05	0.52 ± 0.01
β-Myrcene	24.7	1.54 c	0.016	6.51 ± 0.80	1.56 ± 0.84	6.50 ± 0.08	3.36 ± 0.20	5.18 ± 1.35	4.33 ± 0.80	4.74 ± 0.25
Limonene	26.7	1–278 a	0.034	29.5 ± 3.1	13.7 ± 1.1	25.3 ± 0.1	26.3 ± 0.6	25.2 ± 3.3	21.5 ± 2.9	22.8 ± 2.5
1-Octanol	27.5	NA	NA	0.06 ± 0.00	0.08 ± 0.09	0.05 ± 0.01	0.11 ± 0.02	0.06 ± 0.04	0.06 ± 0.00	0.09 ± 0.00
γ-Terpinene	27.7	0.04–0.46 b	NA	0.03 ± 0.02	0.02 ± 0.00	0.04 ± 0.00	0.03 ± 0.04	0.02 ± 0.02	0.01 ± 0.02	0.05 ± 0.01
Linalool	28.9	0.15–4.6 a	0.001	0.61 ± 0.04	1.12 ± 0.60	0.64 ± 0.04	0.86 ± 0.10	0.98 ± 0.24	0.51 ± 0.02	0.66 ± 0.04
L-α-Terpineol	32.0	0.09–1.1 a	NA	0.17 ± 0.01	0.57 ± 0.36	0.26 ± 0.01	0.35 ± 0.00	0.39 ± 0.08	0.26 ± 0.03	0.38 ± 0.02
Decanal	32.33	0.01–0.15 a	0.007	0.33 ± 0.07	0.13 ± 0.07	0.35 ± 0.10	0.49 ± 0.10	0.27 ± 0.09	0.21 ± 0.07	0.44 ± 0.10
Dodecanal	38.57	NA	NA	0.12 ± 0.02	0.01 ± 0.00	0.06 ± 0.00	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.06 ± 0.01
Valencene	41.51	0.8–15 b	NA	2.11 ± 0.89	0.20 ± 0.06	0.76 ± 0.22	0.70 ± 0.03	1.03 ± 0.41	1.19 ± 0.31	0.67 ± 0.11
R.S.D. (%)	–	–		13.9	21.6	2.8	20.8	4.0	15.7	9.8

OT, odor threshold; data complied by Rychlik et al. (1998), ND, not detected; NA, not available; LV, literature values cited from: (a) Shaw (1986), (b) Nisperos-Carriedo and Shaw (1990), (c) Steffen and Pawliszyn (1996); linear range (0.03–0.00013).

major volatiles were selected and quantified based on their abundance in the juice and contribution in overall citrus flavor. Sizer et al. (1988) broadly categorized volatile orange flavor compounds and reported that 75–98% of flavor compounds are hydrocarbons, 0.6–1.7% aldehydes, 1% esters, 1% ketones, and 1–5% alcohols. In the present study, the volatile compounds identified in the juice of both 'Navel' and 'Valencia' orange varieties were similar, but abundance of many volatiles was higher in 'Navel' oranges (Tables 3 and 4).

The volatile compounds responsible for the delicate, fruity flavor of orange, including ethyl butanoate, ethyl hexanoanate, octanal (Ahmed et al., 1978), were present in relatively low quantities in untreated oranges. These compounds have very low odor thresholds (ppb range) thus making them indispensable for the fresh orange flavor (Table 4). Upon heat treatments all of these components were diminished to a level at which detection and quantitation by the present method was not reproducible (data not shown). Ethyl butanoate is the major volatile ester in orange juice and it is an important contributor to desirable top-notes in orange flavor (Ahmed et al., 1978). A general decrease in the amount of this ester is associated with decreased fresh orange flavor quality (Nisperos-Carriedo and Shaw, 1990). In both varieties, ethyl butanoate levels were somewhat reduced by the heat treatments (Tables 3 and 4). Heat is known to inactivate enzyme systems responsible for the synthesis of esters, but a study by Fallik et al. (1997) on apple suggested that heat treatment only temporarily inhibits aroma volatile emission, mainly esters. Therefore, we would expect to see renewed biosynthesis of ethyl butanoate upon long term storage of treated oranges.

Heat treatments led to significant changes in acetaldehyde and ethanol concentrations. In the most severe heat treatment, i.e. HW48 ethanol and acetaldehyde increased two to three-fold (Table 4). An increase in the concentrations of the ethanol and acetaldehyde were least in the oranges subjected to heat treatment RF48 + 10 and RF48 + 15 in comparison with other heat treatments. Ethanol build-up after heat treatment is a well-documented trend shown in the literature. A study by Schirra and D'hallewin (1997) showed a two-fold increase in ethanol levels in oranges after a heat treatment at 58 °C for 3 min. In the present study, we also observed the same trend of increased ethanol with increasing holding time and temperature particularly in 'Navel' oranges (Table 4). Ethanol build-up might be attributed to long exposure at high temperature, which increases the respiration rate leading to onset of the anaerobic pathway. Ethanol concentration was also considerably higher in the oranges subjected to RFA35 and HW48 heat treatments (Table 4). Obenland et al. (1999) reported that oranges exposed to 48.5 °C forced air heating for more than 200 min (47.2 °C core temperature) caused a large increase in ethanol build-up in the range of 1200 μg/ml. Though ethanol enhances other flavors, its build-up, along with acetaldehyde will lead to an off-flavor in oranges (Cohen et al., 1990).

Limonene was the most abundant volatile component in orange juice. 'Navel' oranges contained two times more limonene than 'Valencia' oranges (Tables 3 and 4). The loss of limonene in thermal treatments was observed, but losses were very high (30–50%) for heat treatments HW48, RF52, and RF48 + 20 min (Tables 3 and 4 and Fig. 4).

The compound α-terpineol is a thermal degradation product of limonene and it is a known contributor to the off-flavor in orange juice at levels of 2 ppm or higher (Tatum et al., 1975). It is evident from a trend shown in Fig. 4 that decrease in limonene is associated with spiking in volatiles such as linalool and L-α-terpineol. The quantity of these components dramatically increased by more than two times in the oranges subjected to either HW48 or RF heating at 52 °C (Fig. 4 and Table 3). A reduction in important flavor components and increases of linalool and L-α-terpineol concentrations upon heat treatment could be a possible explanation for poor orange juice flavor quality (Nisperos-Carriedeo and Shaw, 1990). The oranges subjected to RFA35 and RF48 + 10 and15 heat treatments showed the minimum increase in these two components (Fig. 4).

Other significant volatile hydrocarbons influenced by heat treatment include α-pinene, sabinene, β-myrcene and valencene. The flavor compound α-pinene has a positive contribution to flavor, whereas valencene has a citrus-like aroma and sabinene contributes a warm, spicy aroma and flavor (Arctander, 1969). The flavor of β-myrcene has a musty geranium odor (Högnadòttir and Rouselff, 2003). In the present study, the heat treatments RF50, RF52, and HW48 reduced α-pinene amount by half and β-myrcene by more than 25% (Tables 3 and 4). The effect of heat treatment on sabinene was not consistent; therefore, a

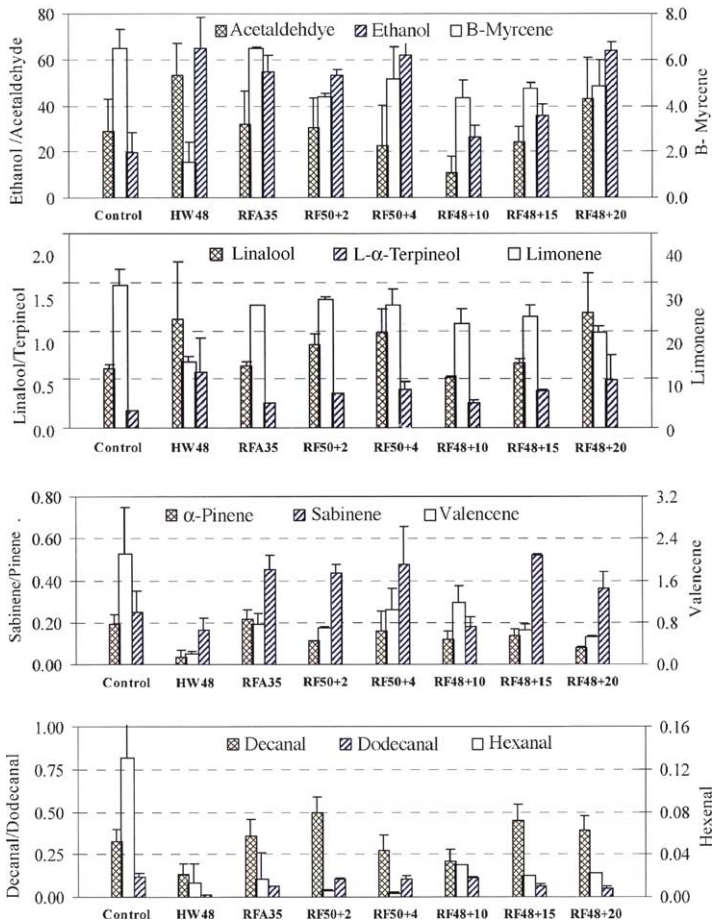

Fig. 4. Volatile compounds concentration (μg/ml, ppm) in 'Valencia' oranges subjected to different heat treatments (temperature + holding, min) and 10 days of cold storage.

definite conclusion could not be drawn. The amount of valencene was found to slightly decrease with severity of heat treatments and maximum loss was observed in oranges treated by HW48 (Table 4). Obenland et al. (1999) reported a substantial loss of α-pinene, β-myrcene and limonene in oranges subjected to 48.5 °C high-temperature forced-air over 200 min.

The aldehyde hexenal, believed to contribute green, grassy orange notes, was found to decrease substantially upon heat treatments (Tables 3 and 4 and Fig. 4). Another aldehyde, decanal, contributes to the green soapy flavors in oranges (Buettner and Schieberle, 2001) was found more in 'Navel' orange (0.8 ppm) than that of 'Valencia' orange (0.33 ppm). However, Ahmed et al. (1978) found that 0.72 ppm of decanal made a negative contribution to orange juice flavor. In the present study, we observed a large increase in decanal after heat treatments of 'Navel' oranges whereas heat treatments of 'Valencia' oranges did not show a consistent trend (Tables 3 and 4). Obenland et al. (1999) reported an abrupt increase in the amount of decanal in oranges exposed to humid hot air of 48.5 °C up to 3 h, but this started decreasing with further increase in exposure time.

The effect of heat treatments on some volatiles that are abundant in peel oil such as decanal, myrcene, sabinene, linalool, and limonene, should be interpreted with caution because during sample preparation some portion of the peel oil might have mixed with juice. This is due to the fact that heat-treated oranges required more

hand pressure to squeeze juice from the vesicles and so peel oil might be squeezed out too. The heat might change cell wall structure and results in less extractability of juice from the sacs.

The flavor analysis enabled us to choose RF48 + 15 and RFA35 as potential RF heat treatments that merit further investigations for complete treatment protocol development. In the present study, the advanced SPME GC/MS technique was used for detection and quantification of the volatile components in orange juice. However, due to the extremely low concentrations of potent orange juice odorants such as methyl and ethyl esters, hexenal, octanal, etc., direct identification and quantitation in the headspace by means of instrumental methods such as GC/MS is sometimes difficult (Buettner and Schieberle, 2001). Therefore, the high sensitivity of the human nose and taste buds should be employed in a confirmatory test using sensory evaluation techniques to judge consumer acceptability of RF heat-treated oranges.

With the reported thermal death kinetic information for Medfly (Gazit et al., 2004) and the treatment conditions determined in this study to minimize quality changes in oranges, our next logical step will be validation by conducting in-situ efficacy studies. We plan to conduct experiments with a 12 kW 27 MHz radio frequency system at Hilo (HI), USA on infested orange fruit with the treatment conditions determined in this study.

4. Summary

RF heating was explored as a tool to expedite the internal heating rate of oranges in the hot water treatment in order to decrease exposure time. The selection of time and temperature combinations for different RF heat treatments was based on the thermal death kinetics study of the Medfly. We hypothesized that a reduction in exposure time at elevated temperature would retain the postharvest quality of the treated oranges. But slow cooling after RF heating resulted in more quality damage at 52 °C even for very short time exposures than at 48 °C for 20 min. Considering the overall analysis of quality attributes such as color, firmness, weight loss and change in flavor components, RF treatment corresponding to a target temperature of 48 °C and holding for 15 min seems to be the best case scenario. If we consider the overall prospect for developing an RF heating process, hot water pre-heating followed by RF heating treatment seems to be the best option for practical implementation. To validate optimal RF heat treatments, further confirmative studies are required on sensory evaluation, simulated marketing period storage, and consumer acceptability. Based on results of our previous and present studies, a next logical step will be an efficacy test. This test is an essential step for validation of the RF heat treatment protocol.

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