High-Temperature Forced-Air Treatment Alters the Quantity of Flavor-Related, Volatile Constituents Present in Navel and Valencia Oranges

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A number of volatile compounds that contribute to orange flavor were quantified following high-temperature forced-air (HTFA) treatment of the fruit to determine if a relationship exists between the flavor loss that is observed following HTFA treatment and the volatile composition of the juice. Following different durations of HTFA treatment, fruit were stored for a period of 4 weeks and juiced and the juice subjected to headspace analysis using either a Tenax/Carbotrap column or a solid-phase microextraction device for trapping of the volatiles. α-Pinene, β-myrcene, and limonene were reduced in amount by 60%, 58%, and 34%, respectively, over the course of the 5-h HTFA treatment. The influence of heat on the amount of decanal was less clear, although in one of the two fruit lots there was little change. The amount of ethanol was reduced by 70% after the initial hour of HTFA treatment and then steadily increased to exceed the initial amount during the remaining 4 h of the treatment. Taste evaluations of the fruit showed a reduction of flavor quality following 4 h or more of treatment. Percent acidity and soluble solids, two other very important determinants of flavor, were nearly unchanged by treatment. Alterations in the volatile constituents of oranges by HTFA treatment may be an important reason behind the negative impact of this treatment on flavor quality.

Keywords: *Volatiles; flavor; quarantine; heat; citrus*

INTRODUCTION

The flavor of fresh oranges is derived from a complex interplay among sugars, acids, and volatile compounds. Volatile compounds are especially important to the flavor of citrus and include alcohols, aldehydes, esters, and hydrocarbons (Nisperos-Carriedo and Shaw, 1990). The effect of a given volatile on flavor can be strongly modified by nonvolatile juice components (Ahmed et al., 1978a) as well as by the synergistic effects of other compounds (Shaw and Wilson, 1980). Those volatiles present at levels below sensory threshold may through additive or synergistic means contribute to overall flavor.

Volatile content in fruit can be modified by the postharvest application of heat. McDonald et al. (1996) noted that heat treatment of tomato fruit reduced the level of volatiles present in the fruit following cold storage. Fallik et al. (1997), working with apples, found that heat acted to reduce the emission of volatiles but that after a period of storage the fruit were able to recover the capability for volatile production. The authors speculated that the change in production was due to a heat-induced alteration in the enzyme systems that catalyze the synthesis of the volatile compounds. Volatile production may also be increased by heat. In an experiment with mandarin oranges, fruit treated at 58 °C for 3 min developed an off-flavor that was attributed to the buildup of ethanol in the juice (Schirra and D'hallewin, 1997).

Increasing legislative pressure on the use of chemicals for postharvest quarantine disinfestation has resulted in a great deal of interest in the development of nonchemical disinfestation treatments. Heat is very effective as an agent to kill insects but can injure the fruit in some instances. In the case of citrus, there have been a number of reports on the negative effects of heat treatment on flavor (Shellie et al., 1993; Shellie and Mangan, 1994 and 1998). The reduction in flavor quality documented in these studies was apparent even when no significant differences in soluble solids or titratable acidity were observed (Shellie et al., 1993; Shellie and Mangan, 1994). No attempt, however, was made to examine the possible effect of the treatments on volatile fruit components. The purpose of this research was to evaluate the possibility that change in flavor quality following heat treatment of navel and Valencia oranges might be due to heat-induced alterations in the quantity of volatile compounds present in the juice.

MATERIALS AND METHODS

Experiment 1. Initial Evaluation of the Effect of Quarantine Heat Treatments on Orange Juice Volatile Composition. Unpacked Valencia and navel oranges were obtained in the fall of 1997 from local packing houses. Two lots, each taken from a different grower, were used for both types of oranges. On the following day after the fruit were

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obtained, heat treatments were conducted at the University of California Lindcove Research and Education Center, Lindcove, CA, using a forced-air heat treatment chamber (Aquanomics International, Honolulu, HI). Two different heat treatments were given that were known to be useful for pest disinfestation. The first, effective against Mediterranean fruit fly (Armstrong et al., 1989; U.S. Department of Agriculture, 1998), consisted of increasing the temperature within the chamber to 35 °C and then ramping it up to 48.5 °C over a period of 200 min, at which point the temperature was held constant until the center of the fruit registered a temperature of 47.2 °C for 2 min or more. The total run time is typically around 4 h in length. Humidity during the run was controlled at 60% relative humidity (RH) for the first hour, 80% RH for the second hour, and to 90% RH for the rest of the run. The second, effective against Mexican fruit fly (U.S. Department of Agriculture, 1998), utilized a stepped temperature schedule of 40 °C for 120 min, 50 °C for 90 min, then 52.2 °C until the fruit center reached 47.8 °C. Following treatment, fruit were allowed to cool overnight and were then run over a packing line to be washed and waxed. To simulate commercial marketing conditions, the fruit were stored at 5 °C for 2 weeks, transferred to 12 °C for 1 week, and finally to 20 °C for a final week.

After storage, the fruit were individually juiced and 60 mL of the juice (including pulp) was placed into a 475 mL Teflon sampling jar. Care was taken to ensure the consistency of the extraction procedure throughout the experiment. The jar was sealed and air flowed through the headspace of the jar at 20 mL/min for a period of 1 h at 23 °C through a 100 mm × 4 mm Tenax TA/Carbotrap column. The juice was slowly stirred during the trapping period. Trapped volatiles were desorbed into a gas chromatograph using a short path thermal desorption unit (model TD-2, Scientific Instrument Services, Ringoes, NJ). Desorption occurred at 250 °C for a period of 3 min. The chromatography system used was a Hewlett-Packard G1800A GCD (Hewlett-Packard, Avondale, PA) equipped with a HP-5MS column (30 m × 0.25 mm, Hewlett-Packard). Helium carrier flow was 1 mL/min, and the oven temperature was programmed to increase from 35 °C to 220 °C at 10 °C/min. The temperature of the mass selective detector was maintained at 280 °C and the injector inlet at 250 °C. Identification of volatile components was initially accomplished by matching mass spectra with library values. Quantification and confirmation of the identity of the major volatile, limonene, was performed by placing standards of D-limonene (Sigma Chemical Company, St. Louis, MO) into 60 mL of acetate buffer (pH 3.9) and trapping the volatile headspace for the same amount of time and in the same manner as that used for the orange juice mixture.

Ten individual fruit for each lot were juiced and analyzed for the quantity of limonene in the juice. Prior to statistical analysis, the data were transformed by natural logs in order to stabilize the variances among lots and treatments. The data were then analyzed using PROC GLM (SAS Institute Inc., Cary, NC) and contrast statements were used to test the significance of the difference between control and treatment.

Experiment 2. Heat Treatment Timecourse. Two lots of unpacked navel oranges were obtained in the spring of 1998 from a local packing house and subjected to heat treatment on the following day. The treatment was conducted in the same manner and with the same equipment as the Mediterranean fruit fly treatment described for experiment 1, with the exception that samples of the fruit were taken out of the heat treatment apparatus at 1 h intervals. Also, the total duration of the treatment was lengthened to 5 h. The internal heating profile of the oranges is given in Figure 1. Postheating treatment and storage of the oranges was identical to that in experiment 1.

Following storage, all fruit were juiced and the juice frozen at −20 °C in plastic vials. For volatile analysis, the juice was prepared by thawing in a 25 °C water bath and then centrifuging at 1540*g* for 5 min. The volatile extraction procedure was based upon that of Steffen and Pawliszyn (1996). Fifteen milliliters of supernatant was added to 15 mL of distilled water and placed into a 40 mL vial. The vial was capped with a Teflon-faced septum and placed onto a stir plate to initiate a moderately fast stirring of the juice. At this point, a solid-phase microextraction (SPME) device (Supelco, Bellefonte, PA) with a 100 μm polydimethylsiloxane phase was inserted through the septum into the jar and the analyte collection fiber deployed for 30 min. This time was experimentally determined to be optimum for fiber equilibration for the analytes of interest. The SPME device was then removed from the vial and desorbed for 3 min at 250 °C into a Hewlett-Packard 6890 gas chromatograph. Volatiles were separated using a HP-5MS capillary column (Hewlett-Packard) and a helium flow of 1 mL/min. The Hewlett-Packard 5172 mass-selective detector was maintained at 280 °C. Column temperatures were maintained at 25 °C for 1 min after desorption, increased at 20 °C/min to 40 °C, then increased at 6 °C/min to 130 °C, and finally to 200 °C at 25 °C/min where the temperature was held for 1 min. Identification of volatile components was accomplished by matching spectra to library values and comparing retention times to those of known standards. α-Pinene, β-myrcene, limonene, and decanal were selected for quantification and further analysis based on peak size, reliability of occurrence, and potential importance to orange flavor. These volatiles were quantified by use of a standard mixture (chemicals obtained from Sigma Chemical, St. Louis, MO) diluted in 0.2 M acetate buffer (pH 3.9) and analyzed in the same mannner as the juice. Ethanol was found to be difficult to analyze using this system so was determined directly from the juice supernatant with an ethanol diagnostic kit (Sigma Chemical, Procedure No. 221-UV). Nine to ten individual fruit were juiced and analyzed for volatile content for each lot. Only lot 2 was evaluated for ethanol

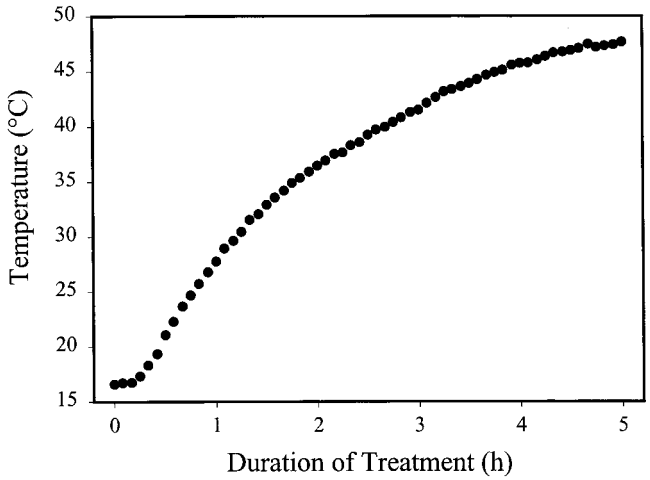

Figure 1. Fruit center temperatures of navel oranges exposed to HTFA treatment. Each value represents the mean from 10 fruit.

Soluble solids were determined from the juice supernatant by means of a temperature-corrected digital refractometer and acidity by use of a pH meter and titration with NaOH. Acidity is expressed as percent citric acid. Determinations from the juice of five separate fruit were made for both soluble solids and acidity. Taste was evaluated by means of an informal taste panel consisting of 15 untrained panelists using a 9-category hedonic scale ranging from dislike extremely to like extremely. Only lot 1 was evaluated for taste.

Prior to statistical analysis, data for the volatiles, with the exception of ethanol, were transformed to stabilize the variance among lots and treatment durations. The least-squares model (PROC GLM, SAS Institute) was utilized for the analysis and included lot as a discrete, fixed effect, treatment duration as a linear continuous effect and also included the interaction between lot and treatment duration. Lot and the interaction were not included in the model for ethanol and taste since they were only measured for one lot. Panelists were fit into the model as a blocking factor for taste.

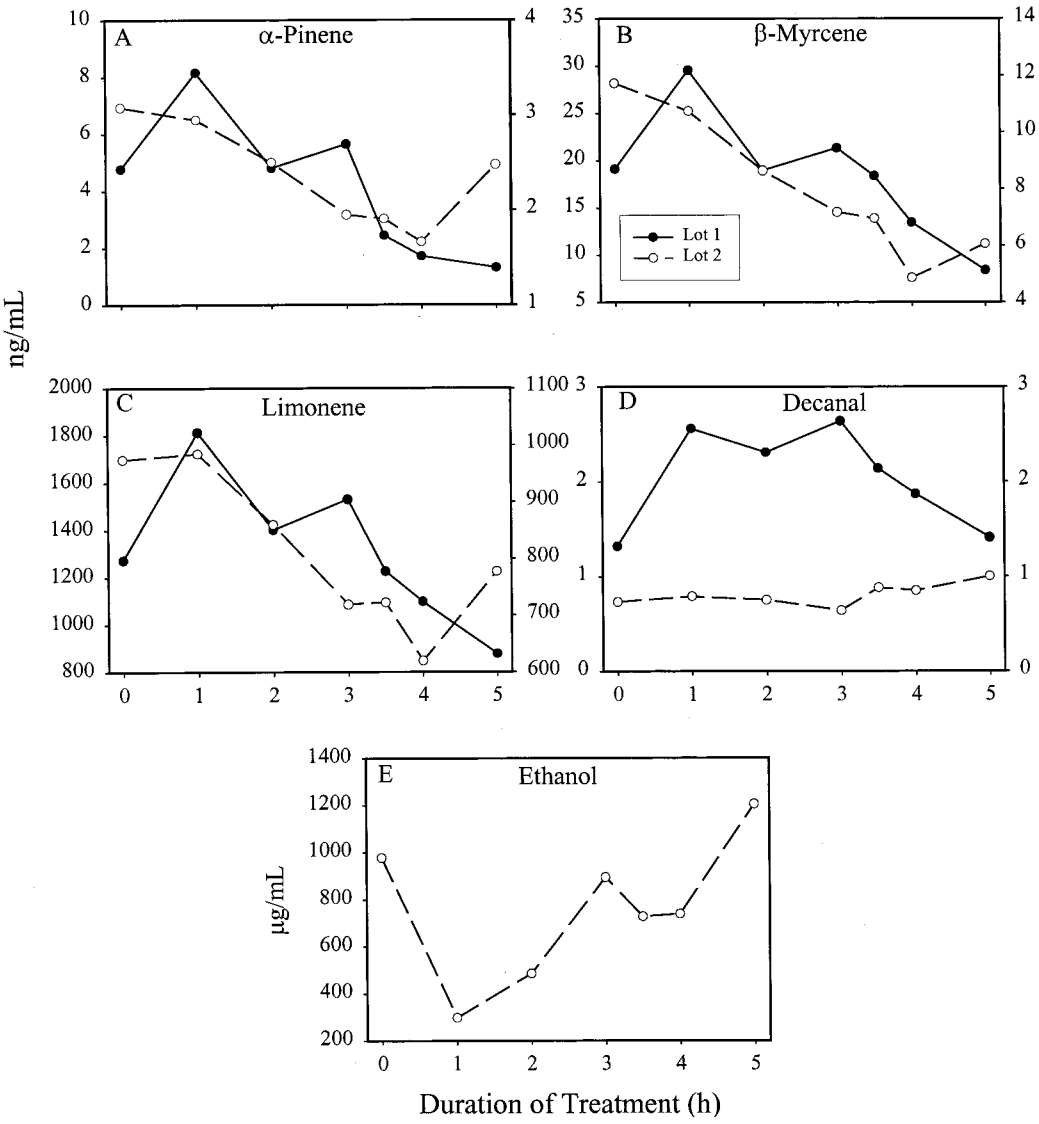

Figure 2. Quantities of selected volatile compounds present in juice from oranges that have been subjected to different durations of HTFA treatment. Each data point represents the mean from 9 to 10 determinations using juice from separate fruit. Lots 1 and 2 were fruit from different grower lots. Labels to the left of each graph correspond to lot 1 with lot 2 being to the right.

RESULTS

Experiment 1 was conducted to determine if quarantine heat treatments that were developed for fruit flies cause any alteration in volatile compound composition or quantity. Dynamic headspace analysis of the juice from heated and nonheated oranges indicated the presence of 30−40 compounds of which 80% or more on a peak area basis was limonene. Due to its abundance and status as a compound important to orange flavor (Nisperos-Carriedo and Shaw, 1990), it was decided to focus on the quantification of limonene in experiment 1. Results indicated that HTFA treatment for Mexican fruit fly resulted in a 49% loss in limonene from the fresh juice as compared to untreated controls (Table 1). Nearly an identical result was obtained with navel oranges using the same treatment as well as from that developed for the Mediterranean fruit fly.

Evidence of a substantial loss in volatile content due to heat treatment led us to conduct further research (experiment 2) in which the effect of HTFA treatment duration was examined and a greater number of volatiles quantified. Volatiles were chosen for quantification on a basis of abundance, ease of identification, and potential impact on flavor. The results of the volatiles work is presented in Figure 2 and the statistical analysis of the data in Table 2. With the exception of decanal, the overall changes in volatile amount due to heat treatment were statistically significant (Table 2). α-Pinene, *β*-myrcene, and limonene all showed a similar pattern of decrease in amount due to treatment. Lots 1 and 2 differed significantly in the amount of these three volatiles present, but both lots responded in nearly the same manner to heat. Decanal amount was not affected by heat in lot 2 but was found to increase and then

Table 1. Limonene Present in Orange Juice Following Quarantine Heat Treatment of Intact Valencia or Navel Oranges

	limonene (*µ*g/mL)[a]	
heat treatment[b]	valencia	navel
control	6.54	6.52
Mediterranean fruit fly		3.60 (0.0001)
Mexican fruit fly	3.33 (0.0020)	3.41 (0.0001)

[a] Mean values from two fruit lots and 10 fruit/lot. Numbers in parentheses indicate level of significance (Pr > F) of difference between control and heat treatments. [b] Quarantine insect heat disinfestation treatments. See Materials and Methods for details.

Table 2. Mean Squares for Volatiles, Soluble Solids, Acidity, and Taste[a]

		volatiles[b]					
source	df	α-pinene	β-myrcene	limonene	decanal	df	ethanol[c]
lot	1	14.43*	18.62*	6.79*	25.64*		
duration	1	4.45*	9.30*	2.25*	0.09	1	3.62*
duration*lot	1	0.07	0.00	0.01	0.23		
error	126	0.28	0.28	0.12	0.23[d]	52	0.21

	quality factors			
source	df	soluble solids	acidity	df	taste[e]
lot	1	0.35	0.00		
duration	1	0.05	0.04*	1	11.84*
duration*lot	1	0.05	0.00		
error	56	1.04	0.01	74	1.18

[a] (*) indictes statistical significance at $P \leq 0.05$. [b] With the exception of ethanol, values are calculated from natural log transformations of the data. [c] Actual mean squares = values $\times 10^6$. Ethanol was quantified only in one lot. Analysis performed with control value deleted. [d] Error degrees of freedom = 122. [e] Taste determination was performed on only one lot.

decrease in amount in lot 1 (Figure 2D). Ethanol initially declined following 1 h of treatment but then increased in amount from 1 to 5 h (Figure 2E).

Quality evaluation of the fruit from experiment 2 is presented in Figure 3. Acidity, although determined to be significantly decreased by HTFA treatment (Table 2), was decreased by only 0.1% in the most extreme case. Soluble solids content was not altered by treatment in either lot. No significant differences were determined between lots for acidity or soluble solids (Table 2). Taste worsened due to heat treatment, but the decrease only became definitive following 4 h or more of heat.

DISCUSSION

In this study we have utilized HTFA treatments that have been developed for quarantined insects to discover if the amount of heat needed for these treatments affects fruit volatile constituents and thus determine whether the volatiles have relevance to the flavor quality problem that has been previously observed with HTFA-treated oranges. In the case of the Mediterranean fruit fly treatment, a center temperature of 47.2 °C is required for disinfestation with the treatment protocol that we were using. To reach this temperature required between 4 and 5 h (Figure 1), by which time the amounts of α-pinene, β-myrcene, and limonene were all substantially decreased. All three of these compounds are believed to contribute positively to orange flavor (Ahmed et al., 1978a,b), with limonene, due to its high abundance in orange juice, being especially important. Reductions in the amounts of these volatiles could potentially have a negative effect on flavor. Other important flavor-related volatiles may also show such trends with regard to heat and should be investigated. Ethanol, although not elevated over the control value at 4 h, nevertheless showed a trend toward increasing amounts with increased heat and could be a problem if more extreme heat treatments than the one utilized in this study were used. Ethanol is normally present in orange juice and may contribute to the flavor at moderate levels by providing an enhancement to other flavors (Nisperos-Carriedo and Shaw, 1990). However, an excessive amount, as may be observed following storage under controlled atmospheres (Ke and Kader, 1990) or improper waxing (Cohen et al., 1990), is thought to

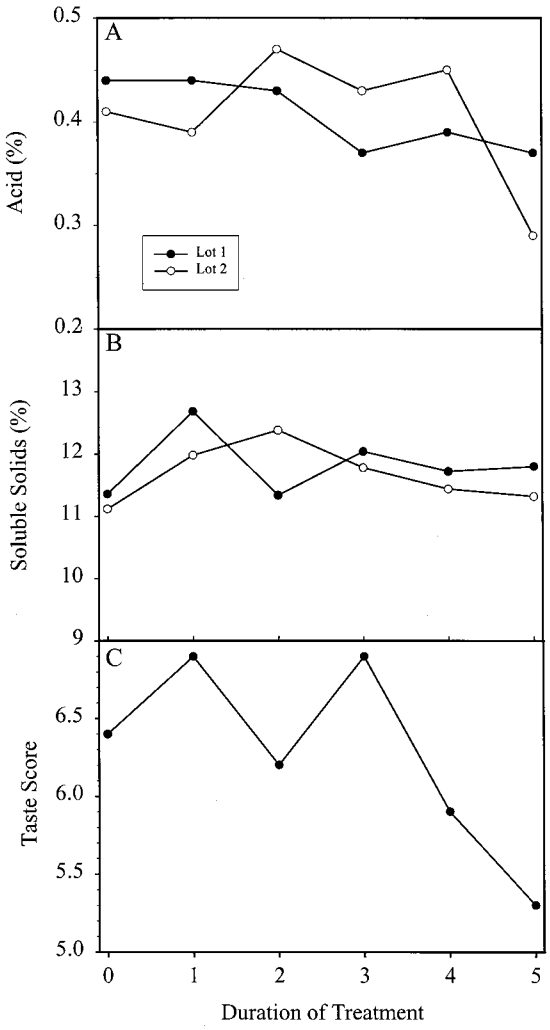

Figure 3. Percent acid, soluble solids, and taste as affected by different durations of HTFA treatment. Six fruit were measured for each time point to determine percent acid and soluble solids. Fifteen panelists were used to gather taste data. The taste ratings were as follows: 7, liked moderately; 6, liked slightly; 5, neither liked nor disliked. Lots 1 and 2 were fruit from different grower lots.

cause off-flavor. The increase in ethanol in heated citrus fruit and its potential impact on flavor has been previously reported (Schirra and D'hallewin, 1997).

In contrast to the volatiles, we found HTFA to have little or no effect on soluble solids or acidity, two other important determinants of flavor. This result has been reported previously (Shellie et al., 1993; Shellie and Mangan, 1994) and gives additional significance to our findings concerning the effect of HTFA on volatiles.

It is important that HTFA treatments for orange insect disinfestation be developed that are both efficacious and do not alter the flavor of the fruit. Flavor, however, is a subjective measurement and is difficult to assess accurately. It is possible that measurements of individual volatiles or numerous volatiles in combination with multivariate analysis techniques (Moshonas and Shaw, 1997) could be used as a more sensitive means with which to monitor the impact of a given HTFA treatment on flavor. In this manner it may be possible to fine-tune HTFA treatments to lessen heat damage. Further investigations regarding volatile constituent changes and heat could also be done to uncover the biochemical basis for these changes, possibly en-

abling the discovery of means to prevent heat-induced flavor quality loss.

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