Heat Treatment Temporarily Inhibits Aroma Volatile Compound Emission from Golden Delicious Apples

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Volatile compounds were collected by porous polymer trapping from Golden Delicious apples (*Malus domestica* Borkh.) that had been heat-treated for 4 days at 38 °C, a treatment developed to reduce physiological and pathological disorders during storage, and then stored at 1 °C. Heat treatment of apple fruits markedly inhibited emission of total volatile esters (compounds commonly associated with apple aroma) and total volatiles (comprised principally of the volatile esters and α-farnesene) of apple within 1 day of treatment. However, after an extended refrigerated storage at 1 °C, the heat-treated fruit recovered and produced more total volatiles, increasing from 4% compared to non-heat-treated fruit directly after heat treatment to 145% of non-heat-treated fruit after 6 weeks of storage. Total volatile production of non-heat-treated fruit declined over 5-fold during the 6 weeks of cold storage, while that of heat-treated fruit increased over 6-fold. Total volatile esters from heat-treated fruit declined after 1 week of storage but had increased 4-fold from the initial sampling date after 6 weeks of storage. The heat treatment effect on emission of volatile compounds was observed immediately following heat treatment. The fruit cuticle and epidermis were not barriers to volatile emission by heat-treated fruit since slicing both heat-treated and non-heat-treated fruit after treatment resulted in total volatile yields similar to intact fruit. Heat treatment apparently temporarily inhibited but did not destroy, or destroyed but allowed resynthesis of, the enzyme systems catalyzing volatile compound synthesis as shown by increasing emission over time by heat-treated apples.

Keywords: *Esters; postharvest storage; flavor; Malus domestica*

INTRODUCTION

Apple fruit quality is comprised of a combination of factors such as texture, sugar and acid content, and aroma compounds. The majority of apple aroma compounds are volatile esters; however, apples also produce a relatively large amount of α-farnesene (Brackmann et al., 1993; Cunningham et al., 1986; Girard and Lau, 1995; Olias et al., 1992). Increases in the concentration of volatile compounds have been detected several weeks before the respiratory climacteric. Following the climacteric, the volatile concentrations decline (Mattheis et al., 1991).

Apples can be stored for several months at low temperature under appropriate controlled atmosphere (CA) conditions. During CA and low-temperature conditions, volatile compound production by apples decreases, although the general appearance of the stored apples can be maintained for a long period (Lidster et al., 1981; Streif and Bangerth, 1988; Willaert et al., 1983). Reduced emission of aroma volatiles has been reported as the factor most likely responsible for diminished flavor (Knee and Hatfield, 1981; Sharples, 1982; Smith, 1984).

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In recent years, prestorage heat treatment for 4 days at 38 °C was found to maintain apple fruit firmness, color, total soluble solids, and organic acids while promoting resistance to physiological disorders such as scald and fungal diseases during storage (Conway et al., 1994; Fallik et al., 1995; Klein and Lurie, 1994). Although various biochemical and physiological alterations have been associated with heat treatment (Klein and Lurie, 1991), the effect of heat treatment on aroma volatile production by apple fruit during short- and long-term cold storage has not been reported. The objective of this study was to determine if heat treatment affects volatile compound emission by Golden Delicious apples.

MATERIALS AND METHODS

Materials. Smoothee Golden Delicious apples were harvested in the preclimacteric stage (ethylene emission was <0.2 mL/g of fresh weight, and the climacteric rise in CO_2 production had not begun) from a research orchard at the University of Kentucky, Lexington, KY. Fruits of uniform color, size, and firmness and without visible defects were stored at 1 °C inside unsealed plastic bags for 5–6 weeks before the heat treatments were applied.

Fruit Treatment. In all experiments heating was performed at 38 °C for 4 days in a thermostatically controlled (±0.5 °C) chamber with the fruit on plastic trays inside unsealed plastic bags to retard water loss. A container of water was placed inside the chamber to maintain relative humidity at approximately 90%. The same number of control fruit was kept in cold storage in unsealed plastic bags while heat treatments were applied to the other fruit.

Three experiments were conducted as follows: (*Experiment 1*) Sixty fruits were heat-treated as described above; 60 control fruits remained in cold storage. Both groups were subse-

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Table 1. Effect of Heat Treatment and Storage Duration on Volatile Production (Nanograms per Gram of Fresh Weight) by Golden Delicious Apples[a]

| | weeks after heat treatment and cold storage | | | | | | | |
| | 0 | | 1 | | 4 | | 6 | |
volatile	C[b]	H	C	H	C	H	C	H
butyl acetate	31.0[c]	ND[d]	28.2	ND	8.7	1.8	5.9	2.9
2-methylbutyl acetate	8.3	0.7	7.1	0.2	2.2	1.2	1.9	2.3
pentyl acetate	0.8	ND	0.9	ND	0.2	0.1	0.3	0.4
6-methyl-5-hepten-2-one	0.1	0.2	ND	ND	ND	0.2	ND	ND
butyl butanoate	0.8	0.2	1.3	0.1	0.3	0.1	0.1	0.1
hexyl acetate	13.9	0.5	13.5	0.2	6.3	1.3	2.9	3.8
D-limonene	0.2	0.4	4.0	0.8	1.4	0.4	1.2	0.4
butyl 2-methylbutanoate	1.4	0.2	0.7	ND	0.4	0.2	0.4	0.2
hexyl propanoate	1.9	0.7	1.4	ND	0.6	0.3	0.7	1.0
butyl hexanoate + hexyl butanoate[e]	4.9	1.1	3.6	0.7	1.7	1.0	1.2	3.0
hexyl 2-methylbutanoate	4.7	0.3	1.1	0.1	0.8	0.7	1.2	0.6
hexyl hexanoate	3.6	0.5	1.7	0.2	1.6	1.1	1.2	2.8
α-farnesene	94.7	2.4	25.8	4.4	18.5	23.7	12.9	25.8
total volatiles	166.3	7.2	89.3	6.7	42.7	32.1	29.9	43.3
total volatile esters	71.3	4.2	59.5	1.5	22.8	7.8	15.8	17.1

[a] Volatile compounds appear in order of their retention time. Volatiles were collected 24 h after heat treatment or removal from cold storage. [b] C, control; H, heat-treated. [c] Values represent the average of at least two determinations. [d] Not detected. [e] The two compounds yielded the same retention time on the GC.

quently divided into four equal lots before collection of their volatiles. Volatiles from the first lot were collected 24 h following heat treatment. Volatiles from the second, third, and fourth lots were collected following 1, 4, and 6 weeks of storage at 1 °C. All fruits (control and heat-treated) were held for 24 h at room temperature (22 ± 2 °C) before collection of the volatiles. The experiment was repeated twice. (*Experiment 2*) Following heat treatment, 12 control and 12 heat-treated fruits were kept for 24 h at room temperature and then sliced into quarters before collecting volatile compounds. Two replicate experiments were conducted. (*Experiment 3*) Using 12 fruit for controls and for heat treatment, volatiles were collected immediately after heat treatment ended or upon removal from cold storage.

Volatile Compound Collection and Analyses. The emission of volatile compounds from heat-treated and control apples was examined using a slight modification of the procedure of Loughrin et al. (1996). Three fruits were weighed and placed inside a glass sleeve (15 cm diameter, 40 cm length). The air for the apparatus was furnished by an ambient forced air supply and was purified by passage through charcoal and Tenax filters and through multiple layers of compressed charcoal-perfused fabric. Glass tubes containing 50 mg of Super Q absorbent (Alltech Associates, Deerfield, IL) served as volatile compound traps. During operation of the system, a tube was fitted to the outlet side of the apparatus and air was pulled through the collection trap by vacuum. The trap was rinsed prior to volatile collection with 2 mL of CH_2Cl_2. These traps collect about 20% of the volatile compounds in the air flow inside the glass sleeve (J. H. Loughrin, personal communication). During the collections, air was passed through the apparatus at 250 mL/min, and volatile compounds were collected for 2 h. The trap was then removed from the apparatus, and compounds were eluted from it with 250 μL of an 80:20 v/v mixture of hexane/CH_2Cl_2. Cumene was added as an internal standard, and the yield of compounds from apples was expressed on a fruit fresh weight (FW) basis.

Compounds were analyzed by gas chromatography using a Hewlett-Packard 5890 Series II instrument equipped with 60 m × 0.32 mm DB-5 column with a 1.0 μm film thickness (Supelco Inc., Bellefonte, PA). GC operating conditions were as follows: oven temperature at 60 °C for 1 min, programmed to increase at 2 °C/min to 230 °C; injector at 220 °C; flame ionization detector at 240 °C; He carrier linear flow rate at 21 cm/s. Each trapped sample was analyzed at least twice. Gas chromatography/mass spectroscopy was performed on a Hewlett-Packard mass ion detector operated in the electron impact mode and interfaced to a GC equipped with a 30 m × 0.25 mm DB-5 column operated as follows: oven temperature at 40 °C for 1 min, programmed to increase at 2 °C/min to 220

°C; injector at 220 °C. Fourteen compounds with a peak area of 30 000 units and above were further identified by computer library searches and retention time matches of the apple volatile compounds with authentic standards. The authentic compounds were purchased from Aldrich Chemical Co., Milwaukee, WI, or were gifts from Bedoukian Chemicals Co., Danbury, CT. Ylang ylang oil was used to determine the retention time and mass spectral data of α-farnesene due to its high α-farnesene content.

RESULTS

In experiment 1, a considerable decrease over 6 weeks of cold storage in total volatile compounds (comprising esters, α-farnesene, D-limonene, and 6-methyl-5-hepten-2-one) from non-heat-treated (control) fruit stored at 1 °C was observed, declining from 166 ng/g of FW at the first sampling date to 30 ng/g of FW (Table 1). This trend was also observed in total volatile esters, which as a group are considered important contributors to apple aroma (Cunningham et al., 1986). In contrast, the total volatile production increased with time in heat-treated fruit from 7 to 43 ng/g of FW over 6 weeks. Volatile esters from heat-treated fruit declined after 1 week of storage but increased 4-fold from the initial sampling date by 6 weeks of storage. The percentages of total volatile production by heat-treated compared to non-heat-treated fruits were 4, 8, 75, and 145% at 0, 1, 4, and 6 weeks of storage, respectively.

Most of the volatile compounds identified from the Golden Delicious fruit were aliphatic esters. At the first sampling date, the most abundant volatiles collected and measured from the non-heat-treated fruit were α-farnesene, butyl acetate, hexyl acetate, and 2-methylbutyl acetate at 94.7, 31.0, 13.9, and 8.3 ng/g of FW, respectively (Table 1). These comprised 88% of the total volatile production; all other compounds were below 5 ng/g of FW. After 1 week of cold storage, a sharp decrease in α-farnesene was observed from non-heat-treated fruit. A marked decrease in the three major esters, butyl acetate, hexyl acetate, and 2-methylbutyl acetate, was observed in non-heat-treated apples after 4 and 6 weeks of cold storage.

The major volatiles produced in the heat-treated apples at the first sampling date were α-farnesene and butyl hexanoate/hexyl butanoate (2.4 and 1.1 ng/g of

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Table 2. Volatile Compound Production (Nanograms per Gram of Fresh Weight) by Golden Delicious Fruit as Affected by Slicing Heat-Treated and Non-Heat-Treated Apples in Quarters[a]

	sliced fruit	
volatile	control	heat-treated
butyl acetate	31.0[b]	1.4
2-methylbutyl acetate	20.3	5.3
pentyl acetate	1.5	0.3
6-methyl-5-hepten-2-one	ND[c]	ND
butyl butanoate	0.9	ND
hexyl acetate	8.0	0.7
D-limonene	8.4	1.0
butyl 2-methylbutanoate	0.8	0.3
hexyl propanoate	1.1	5.3
butyl hexanoate + hexyl butanoate[d]	2.5	0.6
hexyl 2-methylbutanoate	1.1	0.4
hexyl hexanoate	1.0	0.3
α-farnesene	19.9	3.9
total volatiles	96.5	19.5
total volatile esters	68.2	14.6

[a] Fruit were sliced and volatiles collected 24 h after heat treatment and cold storage. [b] Values represent the average of at least two determinations. [c] Not detected. [d] The two compounds yielded the same retention time on the GC.

Table 3. Volatile Compounds Emission (Nanograms per Gram of Fresh Weight) by Golden Delicious Apples Measured Immediately following Heat Treatment or Cold Storage

volatile	control	heat-treated
butyl acetate	14.7	0.3
2-methylbutyl acetate	4.4	0.6
pentyl acetate	0.3	0.1
6-methyl-5-hepten-2-one	ND[a]	ND
butyl butanoate	0.7	0.1
hexyl acetate	7.3	0.2
D-limonene	3.0	0.2
butyl 2-methylbutanoate	0.2	0.1
hexyl propanoate	0.8	ND
butyl hexanoate + hexyl butanoate[b]	2.4	1.1
hexyl 2-methylbutanoate	1.6	0.1
hexyl hexanoate	1.4	0.1
α-farnesene	20.7	0.6
total volatiles	57.5	3.5
total volatile esters	33.8	2.7

[a] Not detected. [b] The two compounds yielded the same retention time on the GC.

FW, respectively) (Table 1). The total volatile productions by heat-treated fruit were similar after 0 and 1 week of cold storage (Table 1). However, butyl and pentyl acetate were not detected on the first two sampling dates of heat-treated fruit. A sharp increase in α-farnesene emission, from 4 to 24 ng/g of FW, occurred with heat-treated fruit from 1 to 4 weeks of cold storage. The amount of most other volatiles produced by heat-treated fruit increased slightly after 4 weeks of cold storage, and all were detected. By 6 weeks of cold storage of heat-treated fruit, the production of half of the volatiles had increased (butyl acetate, 2-methylbutyl acetate, hexyl acetate, butyl hexanoate/hexyl butanoate, and hexyl hexanoate), while others remained nearly the same as at 4 weeks.

In experiment 2, the total amounts of volatile compounds collected from sliced heat-treated and sliced non-heat-treated fruit were 19.5 and 96.5 ng/g of FW, respectively (Table 2). Total volatile esters were also greater from sliced control fruit. Slicing non-heat-treated fruit sharply increased the amount of 2-methylbutyl acetate, hexyl propanoate, and D-limonene trapped compared to intact heat-treated fruit, but reduced several others (Tables 1 and 2). Relatively large amounts of 2-methylbutyl acetate and hexyl propanoate (5.3 ng/g of FW) were produced by sliced heat-treated fruit compared to intact fruit, while α-farnesene production was reduced in sliced control compared to intact control fruit.

Measuring the volatiles immediately following heat treatment or cold storage, as compared to waiting 24 h as in experiments 1 and 2, showed a relatively low total compound production, 3.5 and 57.5 ng/g of FW in heat-treated and non-heat-treated fruit, respectively (Table 3). These totals are lower than the amounts measured in experiments 1 (Table 1) and 2 (Table 2). The maximum individual volatile compounds produced by heat-treated fruit right after treatment were butyl hexanoate and hexyl butanoate, measured at 1.1 ng/g or less (Table 3).

DISCUSSION

Storage conditions and the length of storage result in volatile compound loss in many apple cultivars

(Brackman et al., 1993; Girard and Lau, 1995; Knee and Hatfield, 1981; Lidster et al., 1981). In our work, the total amount of volatile compounds collected from non-heat-treated (control) fruit after 6 weeks of cold storage was <20% of that from the initial sampling date (Table 1). Patterson et al. (1974) attribute the decline in aroma volatiles of apples to a loss of substrates or enzymes essential for the formation of esters. In addition, evaporation of aroma compounds obviously contributes to their decline. Prominent aliphatic esters detected in non-heat-treated fruit were butyl acetate, 2-methylbutyl acetate, hexyl acetate, butyl hexanoate/hexyl butanoate, and hexyl 2-methylbutanoate as previously reported for Golden Delicious (Brackmann et al., 1993; De Pooter et al., 1987; Olias et al., 1992).

There is little information about the effect of heat treatment on aroma volatile production of apples or other fresh produce. Recently, it was reported that prestorage heat treatment of tomato fruits reduced the levels of some volatiles during storage (McDonald et al., 1996). To the best of our knowledge, the current study is the first to show that a 4 day heat treatment of apple fruits temporarily but markedly reduces total volatile and volatile ester emission (Table 1). This was evident immediately after heat treatment (Table 3). Volatile emission from both control and heat-treated fruit increased in the 24 h after treatment or removal from cold storage. Preliminary analyses of volatile emission at 48 h after treatment indicated similar levels by control and heat-treated fruit as that collected at 24 h (data not shown). While cold storage may suppress volatile compound production, within 24 h after removal from cold storage apple volatile production capacity increases rapidly, in contrast to longer term inhibition of volatile production after removal from CA storage (Guadagni et al., 1971; Hatfield and Patterson, 1975).

Slicing both heat-treated and non-heat-treated fruit did not alter the treatment differences observed; heat treatment also reduced volatile emission from slices (Table 2). Thus, heat treatment did not alter physical barriers to volatile emission at the fruit surface.

The amount of α-farnesene emitted from the heat-treated apples was very low following heat treatment compared to non-heat-treated fruit (Table 1). Although Golden Delicious fruit do not get scald in storage, a similar response to heat treatment by other cultivars might explain, in part, the reduction in scald in heat-

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treated fruit (Lurie et al., 1990), as α-farnesene has been linked to the development of storage scald (Morozova et al., 1974).

The marked and temporary reduction in volatile compound emission by heat-treated apples could be a result of the inhibition of the ripening processes as described by Lurie and Klein (1990). Suppression of volatile emission is particularly marked under conditions that delay ripening (Hatfield and Patterson, 1975). Our data suggest that heat treatment temporarily inhibited but did not destroy, or destroyed but allowed resynthesis of, the enzyme systems catalyzing volatile compound synthesis. After a progressively extended storage period, heat-treated fruit recovered the ability to produce most compounds, emitting 6-fold more total volatiles and 4-fold more volatile esters than observed at the initial sampling date (Table 1). The levels of volatile esters were comparable to those of controls after 6 weeks. This suggests that the enzymes regained function, or increased in amount, during storage as shown by the increasing emission. This trend by heat-treated apples contrasts to the decline in volatile production observed with non-heat-treated control fruit. The results suggest that heat treatment to control postharvest pathogens and maintain fruit quality would not adversely affect apple aroma when used in conjunction with current commercial storage conditions and periods of several weeks or more.

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