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Effect of Storage on Some Volatile Aroma Compounds in Fresh-Cut Cantaloupe Melon

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Changes in volatile aroma constituents of fresh-cut cantaloupe melon with storage were determined by headspace solid-phase microextraction gas chromatography−mass spectrometry. The compounds isolated from the fruit immediately after cutting were predominantly aliphatic and aromatic esters. Storage of fruit at 4 °C caused a considerable decrease in concentration of esters and synthesis of the terpenoid compounds β-ionone and geranylacetone over a period of 24 h. This change in the volatile profile with storage is consistent with that of a stress-induced defense response in the cut fruit as an adaptation process to tissue exposure and cell disruption. The same effect occurred in fruit stored at 22 °C and in those treated with sodium azide and ascorbic acid prior to storage. Fruit treated with ascorbic acid and sodium azide had higher concentrations of β-ionone and geranylacetone and retained these compounds better with storage time. The reduction of esters appears to be an important early reaction step in the loss of freshness during storage of fresh-cut cantaloupe.

KEYWORDS: Minimally processed; muskmelon; flavor; *Cucumis melo*; volatiles; phytoalexin; terpenes; fruit

INTRODUCTION

Cut fruit products rapidly lose their typical flavor, even when stored under refrigerated conditions. It is well-known that cut fruit can develop staleness or loss of freshness within a day of refrigerated storage. The rapidly expanding fresh-cut fruit industry has considerably increased interest in the physiological and biochemical changes involved in cut fruit flavor loss (*1*). Fruit flavor is a delicate balance of relative amounts of compounds. Volatile compounds are major determinants of fruit quality as perceived by consumers. Thus, the nature of aroma constituent compounds in cantaloupe melons has been the subject of considerable study (*2*−*5*). Headspace analysis (*4*) and low-temperature headspace solid-phase microextraction (HS-SPME; *6*) analyses of cantaloupe typically yield a mixture of esters. Recovered volatiles in analytical processes such as those that involve solvent extraction, steam distillation, and other SPME conditions indicate the presence of a complex mixture of >250 compounds in the fruit (*2*, *5*). Most studies have focused on the aroma compounds as they relate to the flavor or changes that occur during fruit ripening. The biosynthetic pathways of aroma compounds in cantaloupe melon have also been an area of interest (*7*−*9*).

Biochemical parameters such as pH, titratable acidity, degrees Brix, and organic and amino acids cannot be used as indicators of stored cut cantaloupe quality because they do not change significantly from amounts present in the freshly cut fruit when stored at 4 °C for a period of 2 weeks. At this temperature, the microbial population also remains statistically constant relative to amounts initially present at the time of processing for ∼3−4 days (*10*). Most studies on changes that occur with storage of cantaloupe address maturity effects (*11*); postharvest handling, processing, and packaging treatments as determined from measurements of firmness (*12*, *13*); enzymatic activities (*14*, *15*); microbial growth (*10*, *16*, *17*); and sensory attributes (*16*−*18*). Cantaloupe melon is used more than any other fruit in fresh-cut processing. The objective of this study is to identify changes that occur in some volatile aroma compounds during cold storage of cut cantaloupe melon.

MATERIALS AND METHODS

Fruit Preparation. Cantaloupes (*Cucumis melo* L. var. *reticulatus* Naud.) were purchased from a local supermarket. The fruit, after surface sterilization in a bleach solution (10%), were sliced longitudinally into two halves. After seeds and cavity tissues were removed, half of the fruit was further cut in two along equatorial lines, and several slices (∼1 mm thick) were obtained from the exposed cut end. After ∼2 mm along the slice edges had been cut off, the fruit (3 g) was immediately chopped and the slurry was transferred into a vial (20 mL) containing NaCl (1 g) and into which a magnetic stirring bar was inserted. The vial was fitted with an aluminum septum cap and sealed. Benzothiophene, a compound that has not been identified to be present in cantaloupe melon, was used as an internal standard for gas chromatography−mass spectrometry (GC-MS) analysis and was not detectable in GC-MS analysis of cantaloupe melon to which it was not added. The standard was dissolved in methanol, injected onto the pulverized fruit (1.55 ng/kg), and mixed thoroughly by agitation. Fruits prepared for storage were sliced and placed in glass Petri dishes at 4 and 22 °C,

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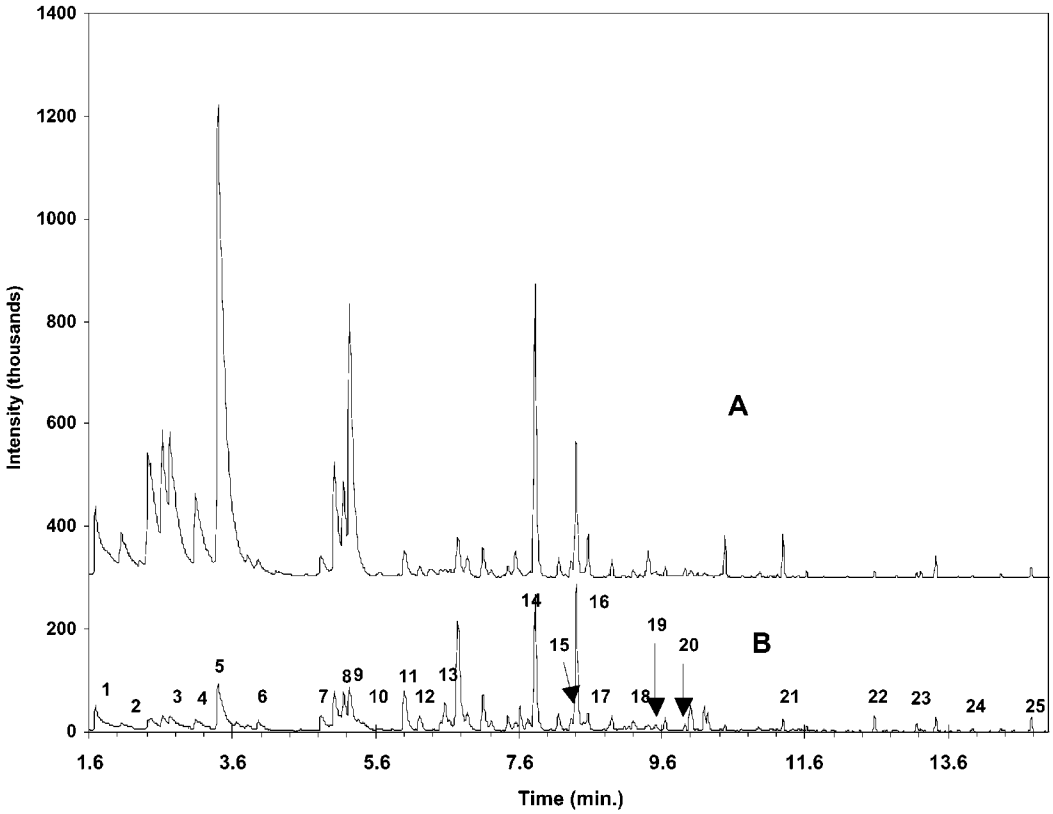

Figure 1. Total ion chromatogram of aroma compounds in fresh-cut cantaloupe melon (A) and in the cut fruit stored at 4 °C for 24 h (B).

respectively. Replicate samples from three separate melons were removed and prepared for analysis at various times as described. Treatment of fruit with ascorbic acid and sodium azide was carried out by transferring cut fruit pieces into polypropylene baskets and dipping in cold water (4 °C), containing ascorbic acid (2.5 mM) or sodium azide (70 mM), for 1 min. After the fruits were dipped in the solutions, they were allowed to drain for ~1 min before being transferred into glass Petri dishes for storage. Control experiments in which cut cantaloupe was dipped in water without the added ascorbic acid or sodium azide had a volatile recovery profile similar to that of the undipped fruit.

GC-MS Analysis. Volatile components of the fruit were extracted by headspace solid-phase microextraction (HS-SPME) using a fused silica fiber coated with a 100 μm layer of dimethylpolysiloxane. The fiber was conditioned by inserting it into the GC inlet for 2 h prior to use for volatile compound adsorption. The fruit and NaCl mixture was initially stirred in a water bath maintained at 30 °C for 30 min. The SPME fiber was then inserted into the sample headspace for 15 min while stirring continued at the same temperature. Desorption of the fiber took place in the GC inlet at 250 °C for 4 min.

GC-MS analysis was performed on a Hewlett-Packard HP-6890 series system utilizing an HP-5 MS cross-link 5% phenyl methyl siloxane (30 m × 0.25 mm × 0.25 μm) column. The injection port was operated in a splitless mode with helium as the carrier gas. The oven was programmed at an initial temperature of 60 °C, ramped to 215 °C at the rate of 8 °C/min and then to 260 °C at °C/min, and held for 15 min. The mass spectrometer was operated in a scan mode from 40 to 400 amu, using 70 eV electrons for ionization. Compounds were identified from their retention times using a commercially available library, authentic reference compounds, and MS fragmentation patterns. Relative amounts of the compounds recovered are expressed as peak areas relative to the peak area of the internal standard.

Table 1. Volatile Compounds Recovered in Fresh-Cut Cantaloupe Melon[a]

peak	compound	relative amount[b]
1	ethyl acetate	1.95
2	ethyl propionate	1.21
3	ethyl isobutyrate	2.06
4	ethyl 2-methylbutyrate	2.05
5	2-methylbutyl acetate	9.92
6	methyl hexanoate	0.39
7	ethyl (methylthio) acetate	0.32
8	ethyl hexanoate	1.37
9	hexyl acetate	3.6
10	1,8-cineole	0.08
11	1,3-butanediol diacetate	0.34
12	2,3-butanediol diacetate	0.13
13	ethyl heptanoate	0.08
14	benzyl acetate	1.73
15	ethyl octanoate	0.09
16	benzothiopene (standard)	1
17	octyl acetate	0.27
18	ethyl phenylacetate	0.06
19	phenylethyl acetate	0.16
20	phenyl hexanoate	0.06
21	3-phenylpropyl acetate	0.16
22	geranylacetone	0.01
23	β-ionone	0
24	dihydroactinidiolide	0.01
25	diethyl phthalate	0.05

[a] Peaks correspond to those identified in **Figure 1**. [b] Expressed as peak areas relative to that of the internal standard.

RESULTS AND DISCUSSION

Figure 1 shows chromatograms of changes in volatile aroma compounds of fresh-cut cantaloupe with storage at 4 °C. The volatiles recovered in cantaloupe analyzed immediately after cutting are essentially aliphatic and aromatic esters (**Table 1**). Methylbutyl acetate and hexyl acetate were the most prominent compounds. These two compounds contribute to the fruity character in a number of fruits (*19*, *20*) and are typically found in relatively large quantities in cantaloupe (*4*, *21*). The low-temperature isolation procedure used minimizes formation of

Table 2. Relative Amounts of Aliphatic and Aromatic Esters and Terpenoids Present in Fresh-Cut Fruit and Fruit Treated with Ascorbic Acid and Sodium Azide during Storage at 4 and 22 °C

		4 °C			4 °C and ascorbic acid			4 °C and sodium azide	22 °C	
compound	fresh-cut	1[a]	3	7	1	3	7	1	1	2
aliphatic esters	23.43a	4.21b	2.6c	1.79c	3.76b	2.24c	2.94bc	4.13b	4.68b	6.71d
aromatic esters	2.11a	1.04b	0.88bc	0.47c	1.74ab	2.1a	2.21a	2.17a	1.45ab	5.08d
terpenoids	0.01a	0.1b	0.06bc	0.05c	0.17d	0.1b	0.1b	0.16d	0.03c	0.04c

[a] Number of days in storage. The letters a–d are used to compare mean values within each row. Mean values without the same letters are significantly different by Duncan's multiple-range test ($p < 0.1$). Values are expressed as peak areas relative to that of the internal standard.

heat-induced secondary reaction products and avoids solvent interactions with constituent compounds that could occur when solvent extraction methods are used. The compounds identified, which are consistent with previously reported volatile aroma components of cantaloupe melon (*2*, *4*), are thus expected to maintain relative amounts that are more representative of the balance maintained in the fruit. Storage of fruit pieces over a period of 24 h at 4 °C resulted in a sharp decrease in the total volatiles (**Figure 1**). The most drastic drop occurred in the relative amount of aliphatic esters in the fruit. After the initial decrease in the aliphatic and aromatic esters during the first 24 h, the loss of these volatile aroma compounds with storage time was minimal over a period of 7 days (**Table 2**). Concurrent with the decrease in the concentration of esters was the formation of the terpenoid compounds β-ionone and geranylacetone. The amount of the terpenoid compounds, unlike the esters, decreased after the first day. Terpenoid contents in fruits stored for 3 and 7 days were the same.

The reduction of esters appears to be an important early reaction step in the loss of freshness during storage of fresh-cut cantaloupe; these esters could potentially serve as precursor compounds for synthesis of secondary volatile aroma compounds. We recently reported a similar decrease in aliphatic esters and synthesis of terpenoid compounds as a result of UV light induced stress in fresh-cut cantaloupe (*6*). Fruit exposure to UV light for 15 min decreased the concentration of aliphatic esters by >60%. Cyclic and acyclic terpenoids, including phytoalexin compounds β-ionone, geranylacetone, and terpinyl acetate, were also produced. These compounds, particularly β-ionone, were effective in inhibiting microbial growth in the fruit. It seems obvious from the similar patterns of changes in volatile compounds as a result of the UV light induced biological stress and those observed in this study as a consequence of storage that the defense response of the fruit tissue plays a critical role in altering the flavor of the cut fruit. An intense biological stress is placed on the tissue by UV light, causing the rapid and simultaneous loss of esters and phytoalexin production, whereas during storage the process is slower. These changes in amounts of esters and terpenoid compounds are expected to affect fruit flavor. Esters are important flavor compounds in cantaloupe, whereas terpenoid compounds such as β-ionone and geranylacetone have characteristic aroma properties (*22*, *23*).

There are a few reports of the breakdown of esters as a plant tissue defense response. In *Lactarius* species, esters that are otherwise inactive are enzymatically converted into the aldehydes and alcohols with antimicrobial properties when injured (*24*). The compounds formed are responsible for the bitter and astringent tastes that are common when the fruiting bodies are broken. In many fruits, the natural defense mechanism against microbial infections provided by tannins appear to be associated with the hydrolysis of ester linkages between gallic acid and polyols (*25*). Infection of Japanese pear by *Venturia nashicola* is accompanied by de-esterification (*26*). The stress-induced hydrolysis of polygalacturonide esters by pectin methylesterase that takes place during solubilization of the cell wall is well-known (*27*, *28*). The reduction of ester concentration observed in this study is unrelated to microbial stress. Changes in volatile aroma compounds in cantaloupe treated with sodium azide were similar to the control that was not treated with the biocide. The treated fruit, however, produced higher amounts of terpenoid compounds during storage.

Plant tissue stress-induced enzymatic hydrolysis of esters involves their esterase-mediated conversions to acids and alcohol (*24*). Subsequent reactions involve fatty acid degrading enzymes and/or alcohol dehydrogenase. Most primary aroma compounds in cut fruits are believed to be products of β-oxidation of fatty acids and secondary compounds the result of fatty acid oxidation via the lipoxygenase (LOX) pathway (*9*). Many of the natural volatile compounds that control microbial growth in fruits are also typical products of the LOX reaction pathway (*25*). Our attempts to correlate the reduction in aliphatic and aromatic esters during storage with synthesis of fatty acids, aldehydes, and alcohols by extracting the volatile compounds at higher temperatures (45 and 60 °C) were unsuccessful. Although the extraction of volatiles at higher temperatures facilitated detection of many heavier compounds, including fatty acids, aldehydes, and alcohols, the extraction procedure significantly decreased the amount of esters and increased terpenoid concentrations in the freshly processed fruit. The new compounds emitted from the fresh fruit and stored fruit when higher SPME extraction temperatures were used could not be correlated with the de-esterification that also occurred with storage. This observation appears to be due to the fact that the end products can serve as precursors in the formation of other fatty acids and their esters, alcohols, and aldehydes, and such reactions increase with increased extraction temperature (*29*, *30*). The volatile compounds extracted from cantaloupe melon using an SPME procedure and a higher extraction temperature (40 °C) than the temperature we used for the extraction of volatile compounds were recently reported (*5*).

Our results indicate that the loss of esters is an important early reaction step related to the loss of freshness during storage of cut cantaloupe melon. This reaction could potentially provide precursors for synthesis of other compounds that may further alter the typical cantaloupe melon flavor. The initial degradation of esters is unaffected by fruit storage temperature. When the cut fruit was kept at 22 °C, a similar rapid reduction in ester compounds occurred during the first day of storage (**Table 2**). At this temperature, however, an additional day of storage caused an increase in the amount of esters apparently through the involvement of secondary reaction products of ester degradation in β-oxidation reactions (*31*). An indication that lower temperatures favor synthesis of terpenoid compounds in the fruit tissue is the lower content of these compounds at 22 °C than at 4 °C after 24 h of storage.

Ascorbic acid reduces oxidative stress and enhances muskmelon anionic peroxidase antioxidative action apparently by way of ascorbate−POD complex and metal ion cofactors (*15*). Dipping cut cantaloupe in ascorbic acid solutions had no effect on the loss of aliphatic ester compounds during cold storage (**Table 2**). Aromatic esters were, however, better retained with storage. The treatment also increased the concentration of phytoalexin terpenoids produced during the first day of storage. In a similar pattern with fruits that were untreated with ascorbic acid, terpenoid concentration decreased after the first day of storage, but higher amounts were subsequently retained than in the untreated fruit. Phytoalexin production typically extends the shelf life of plant tissues (*32*). The better retention of the phytoalexin compounds in ascorbic acid treated cut cantaloupe might be responsible in part for the fruit's extended shelf life, as determined by color retention, when the cut fruit is dipped in ascorbic acid solution (*14*). Lower temperatures and reduced oxidative and microbial stress during storage thus appear to favor production of phytoalexin terpenoid compounds as evidenced by their reduced quantities in fruit stored at 22 °C and those that were untreated with either sodium azide or ascorbic acid.

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