Volatile Constituents of Bing Sweet Cherry Fruit following Controlled Atmosphere Storage

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Volatile constituents of Bing sweet cherry fruit were analyzed by purge and trap sampling from fruit homogenates at harvest and after air or controlled atmosphere storage. Acetic acid and aldehydes were quantitatively the largest volatile compounds present at harvest. Changes during storage in the concentrations of three compounds previously identified as contributors to sweet cherry fruit flavor, benzaldehyde, *E*-2-hexenal, and hexanal, were independent of storage conditions. Ethanol accumulated in fruit stored in 15 or 20% CO_2 with 5% O_2 after 6 weeks of storage. Qualitative and quantitative changes in ester production, particularly ethyl acetate, were coincident with the accumulation of ethanol. 2-Propanol concentrations were consistently highest in fruit stored in 5% O_2 with 0.1% CO_2. Some qualitative changes in volatile production after 4 weeks of storage were unrelated to storage conditions and may reflect metabolic changes occurring during sweet cherry senescence. A number of esters were not detectable after 4 weeks of storage, and several compounds, including butanal, 2-butanone, and pentyl acetate, were only detected after 4 weeks of storage.

Keywords: *Sweet cherries; controlled atmosphere storage; volatiles; esters; alcohols; aldehydes*

INTRODUCTION

Sweet cherry fruits contain many volatile compounds (Mattheis et al., 1992a; Schmid and Grosch, 1986a), and a number of these compounds, including benzaldehyde, *E*-2-hexenal, and hexanal, contribute to fruit flavor and aroma (Schmid and Grosch, 1986b). The majority of sweet cherry volatile compounds are alcohols, aldehydes, esters, and acetic acid, and quantitative and qualitative changes in volatile production occur during fruit development and ripening (Mattheis et al., 1992b).

Sweet cherries are tolerant of low O_2 and/or high CO_2 atmospheres at concentrations that preserve appearance and eating quality as well as reduce decay incidence (Brooks et al., 1932; Chen et al., 1981; Gerhardt and Ryall, 1939; Meheriuk et al., 1995; Patterson, 1982; Porritt and Mason, 1965; Singh et al., 1970). The impact of controlled atmosphere (CA) storage on production of volatile compounds by sweet cherry fruit, however, has not been characterized. Prolonged CA storage of apple fruit can result in reduced ester volatile synthesis and an accompanying loss of flavor (Patterson et al., 1974; Willaert et al., 1983). This residual CA effect increases with storage duration, increased CO_2 and decreased O_2 concentrations (Streif and Bangerth, 1988). Although sweet cherries produce many different esters, the total ester concentration is low relative to total aldehyde concentration (Mattheis et al., 1992b). The reduction in ester production after CA storage of apples has been suggested to result from a lack of substrates for ester synthesis (Patterson et al., 1974; Knee and Hatfield, 1981), reduced fruit respiration following CA storage (Hatfield and Patterson, 1975), and/or reduced fruit sensitivity to ethylene (Bangerth and Streif, 1987). Sweet cherries are a nonclimacteric fruit (Blanpied, 1972) that produce only small amounts of ethylene, and interactions between volatile and ethylene production are less clear.

Recent commercialization of CA container shipments and modified atmosphere packaging technologies for use in the sweet cherry industry prompted this investigation of sweet cherry response to low O_2, high CO_2 storage. Our objective was to characterize sweet cherry volatile composition and fruit quality after storage in low O_2, high CO_2 CA environments.

MATERIALS AND METHODS

Bing sweet cherries (*Prunus avium* cv. Bing) harvested at commercial maturity (9.5 g fruit weight, 23.6% soluble solids content, 0.81% titratable acidity) were obtained from a commercial orchard on July 12, 1993, and transported to the laboratory within 2 h of harvest. Fruit were surface disinfested by submersion in a solution of 14 mM NaOCl and 150 mg L^{-1} linear ethoxylated alcohol as surfactant for 180 s, then rinsed with tap water. Initial fruit quality and volatile compound analyses were conducted the day of harvest. Cherries for storage were placed in plastic trays (17 cm long × 14 cm wide × 7 cm high) and held in regular cold or CA storage at 1 °C. A tray (17 × 14 × 7 cm) containing 500 mL of water was placed in each storage chamber to minimize fruit desiccation. Concentrations of O_2 (5%) and CO_2 (0.1, 10, 15, or 20%) in the CA chambers (0.14 m^3) were maintained (±0.1%) automatically (TechniSystems, Chelan, WA). Sources of O_2, CO_2, and N_2 were compressed air, compressed CO_2, and a hollow-fiber membrane generator (Permea, St. Louis, MO), respectively. After 1, 2, 4, 8, and 12 weeks, fruit was removed from storage and held at 20 °C inside a covered plastic bin (48 × 35 × 12.5 cm) lined with moist paper towels. Subsequent fruit analyses were performed after 24 and 96 h. The 96 h sampling time was chosen because previous work indicated the profile of volatile compounds produced by sweet cherry fruit changes during this time period (Mattheis et al., 1992b).

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Table 1. Compounds Detected during Storage and Ripening of Bing Sweet Cherries

alcohols	esters	ketones
1-butanol	butyl acetate	2-butanone
2-butoxyethanol	butyl butyrate	2-heptanone
ethanol	butyl hexanoate	3-heptanone
2-ethyl-1-hexanol	butyl 2-methyl-butyrate	3-methyl-2-butanone
1-hexanol	butyl propanoate	6-methyl-5-hepten-2-one
2-methyl-1-butanol	ethyl acetate	4-methyl-2-pentanone
2-methyl-1-propanol	ethyl butyrate	
1-pentanol	ethyl hexanoate	
1-propanol	ethyl 2-methyl-butyrate	acid
2-propanol	ethyl octanoate	acetic acid
aldehydes	ethyl pentanoate	
benzaldehyde	ethyl propanoate	
butanal	hexyl acetate	
decanal	hexyl butyrate	
2-ethylhexanal	hexyl hexanoate	
2-furaldehyde	hexyl 2-methyl-butyrate	
E,*E*-2,4-hexadienal	hexyl propanoate	
hexanal	2-methylbutyl acetate	
E-2-hexenal	methyl butyrate	
heptanal	2-methylbutyl 2-methylbutyrate	
nonanal	methyl 2-methyl-butyrate	
octanal	pentyl acetate	
pentanal	pentyl butyrate	
	propyl acetate	
	propyl hexanoate	
	propyl propanoate	

Fruit quality analyses were conducted as previously described (Mattheis and Roberts, 1993). Soluble solids content (SSC) and titratable acidity (TA) of five replications of six cherries were measured with an Atago refractometer and a Radiometer autotitrator, respectively. Volatile compounds were collected by dynamic headspace sampling of homogenized fruit. Fruit tissue (50 g) with pits removed was added to 50 mL of a saturated $CaCl_2$ solution (Buttery et al., 1987) within 180 s of pit removal and homogenized in a Waring blender for 60 s. A 10 mL subsample was transferred to a 125-mL glass gas washing bottle with a 20-mm fritted disc (40−60 μm pore size). Purified compressed air purged the sample at a flow rate of 100 mL mn^{-1} for 120 s prior to volatile collection. Three replicate volatile samples were prepared for each storage treatment at each sampling time. Volatile compounds in 200 mL of headspace were adsorbed from the exit gas stream onto 50 mg of Tenax GC contained in a glass trap. The traps were subsequently desorbed with a Tekmar 6000 cryofocusing thermal trap desorber. Quantitative and qualitative analyses were performed with a Hewlett Packard 5890A gas chromatograph equipped with a HP 5971A mass selective detector as described previously (Mattheis et al., 1991). Volatile compounds were separated on a capillary column (DB-Wax, 60 m, 0.25 μm film thickness, 0.25 mm internal diameter; J&W Scientific). Quantification of compounds was performed by selected ion monitoring of base peaks with values calculated with response factors generated with standards. Amounts are expressed as nanogram (ηg) of volatile per liter (L) of headspace collected from the 10-mL homogenate/$CaCl_2$ solution. The experiment was analyzed as a completely random design with storage duration and storage treatment as factors by the ANOVA procedure of SAS (SAS Institute). Mean separation for factors with significant F values ($p < 0.05$) was performed with Fisher's least significant difference (LSD).

RESULTS AND DISCUSSION

Fifty-four volatile compounds were detected during the course of this study (Table 1). Volatiles with the largest concentrations detected at harvest and after storage were acetic acid, alcohols, aldehydes, esters, and ketones (Figure 1). Acetic acid and aldehydes were

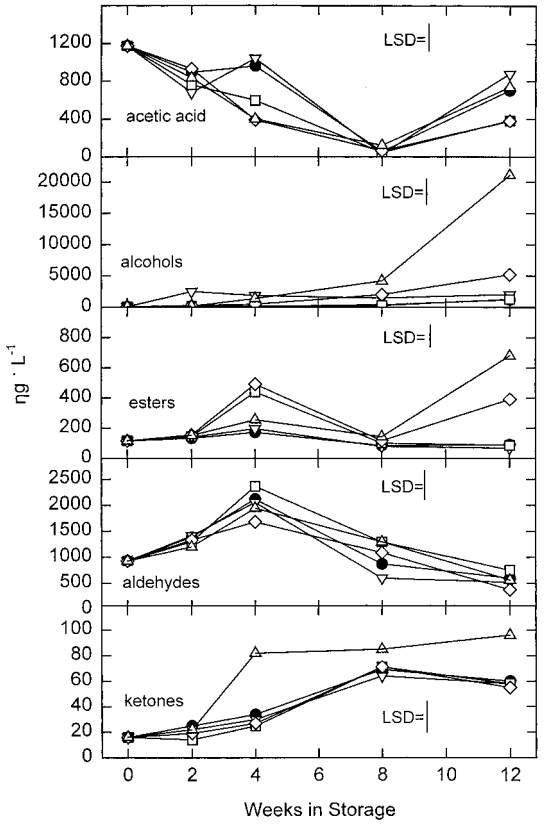

Figure 1. Concentrations of acetic acid, alcohols, aldehydes, esters, and ketones released from sweet cherry homogenates. Fruit samples were analyzed at harvest and after 24 h at 21 °C after removal from storage. Least significant difference values (LSD) are indicated. Treatments: (●) air; (▽) 5/0.1%, O_2/CO_2; (□) 5/10%, O_2/CO_2; (◇) 5/15%, O_2/CO_2; (△) 5/20%, O_2/CO_2.

quantitatively the largest portion of volatile compounds detected from harvest through 4 weeks of storage. At 8 weeks, acetic acid concentration decreased in fruit from all storage treatments including air. The amount of acetic acid increased during the 4-day post-storage period at 21 °C to values of previous weeks (data not shown). No consistent treatment differences in acetic acid or aldehyde concentration were observed. The total ketone concentration remained low relative to the other volatiles throughout the experiment. Fruit stored in 5% O_2 with 20% CO_2 had the largest total ketone concentration after 4 and 12 weeks of storage; however, no treatment differences were evident after 2 or 8 weeks of storage.

Total aldehyde concentration increased between harvest and 4 weeks of storage for all treatments. Aldehyde concentration then decreased to harvest levels by the end of the storage period. Three aldehydes identified by Schmid and Grosch (1986b) as important contributors to sweet cherry juice aroma were benzaldehyde, hexanal, and *E*-2-hexenal. Benzaldehyde concentrations increased for all treatments except 5% O_2/0.1% CO_2 after 2 weeks, at which time concentrations for all treatments were not significantly different from harvest values (Figure 2). Hexanal and *E*-2-hexenal concentrations peaked for most treatments after 4 weeks of storage (hexanal concentration in cherries stored in 5% O_2/15% CO_2 was highest after 8 weeks), then amounts decreased approaching values at harvest for all treatments by the end of the storage period. Hexanal and *E*-2-hexenal are products of lipoxygenase (LOX) activity (Galliard et al., 1977) and benzaldehyde is produced

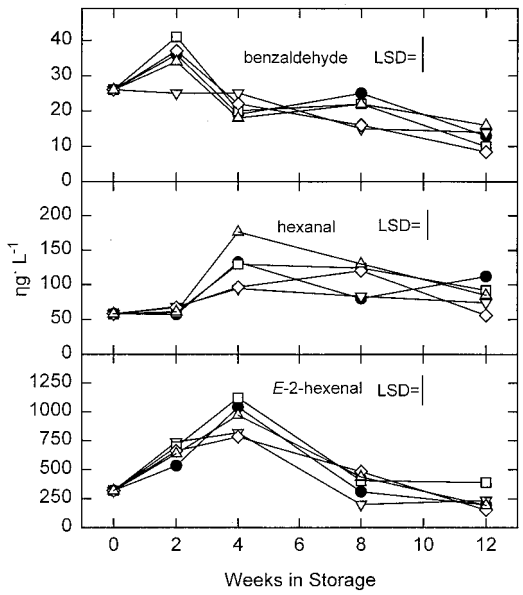

Figure 2. Concentrations of benzaldehyde, hexanal, and *E*-2-hexenal released from sweet cherry homogenates. Fruit samples were analyzed at harvest and after 24 h at 21 °C after removal from storage. Least significant difference values (LSD) are indicated. Treatments: (●) air; (▽) 5/0.1%, O_2/CO_2; (□) 5/10%, O_2/CO_2; (◇) 5/15%, O_2/CO_2; (△) 5/20%, O_2/CO_2.

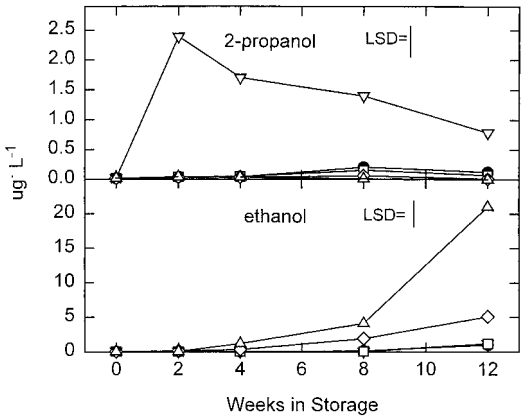

Figure 3. Concentrations of 2-propanol and ethanol released from sweet cherry homogenates. Fruit samples were analyzed at harvest and after 24 h at 21 °C after removal from storage. Least significant difference values (LSD) are indicated. Treatments: (●) air; (▽) 5/0.1%, O_2/CO_2; (□) 5/10%, O_2/CO_2; (◇) 5/15%, O_2/CO_2; (△) 5/20%, O_2/CO_2.

from hydrolysis of amygdalin contained in cherry pits (Nahrstedt, 1972). Enzyme activity that results in production of these compounds appears to be largely insensitive to the CA conditions used in this study because no consistent treatment differences in the amounts of these compounds were observed. Storage duration rather than atmosphere composition appears to be the determining factor for production of volatile aldehydes by sweet cherries within this range of O_2 and CO_2 concentrations.

Total alcohol concentration was low through 4 weeks after harvest, then ethanol concentration increased in fruit held in 5% O_2 with 15 or 20% CO_2 (Figure 3). The increase in ethanol concentration indicates fermentative metabolism occurred under the high CO_2 concentrations as ethanol did not accumulate in fruit held in air or 5% O_2 with 0.1 or 10% CO_2. Fermentative metabolism and ethanol accumulation during exposure to high CO_2 concentrations has been reported in apples (Thomas, 1925), pears (Ke et al., 1990), oranges (Ke and Kader, 1990), strawberries (Ke et al., 1991), blueberries (Saltveit and Ballinger, 1983a), and grapes (Saltveit and Ballinger, 1983b). Sweet cherry ethanol concentration increased with CO_2 concentration, and the critical CO_2 concentration was >10% in this experiment. Because ethanol accumulation did not begin until after 4 weeks in storage, sweet cherry tolerance to low O_2, high CO_2 atmospheres appears to be dependent on fruit developmental stage as has been observed with pear fruit (Ke et al., 1994). Fermentative metabolism in fruit exposed to high CO_2 concentrations may occur due to inhibition of tricarboxylic acid cycle enzymes, particularly succinate dehydrogenase (Frenkel and Patterson, 1973; Knee, 1973), and subsequent increase in pyruvate decarboxylase and alcohol dehydrogenase activity (Ke et al., 1994). Cherries stored in 5% O_2 with 0.1% CO_2 had a higher 2-propanol concentration compared with fruit from the other storage treatments. This treatment effect was also observed after 4 days at 21 °C (data not shown).

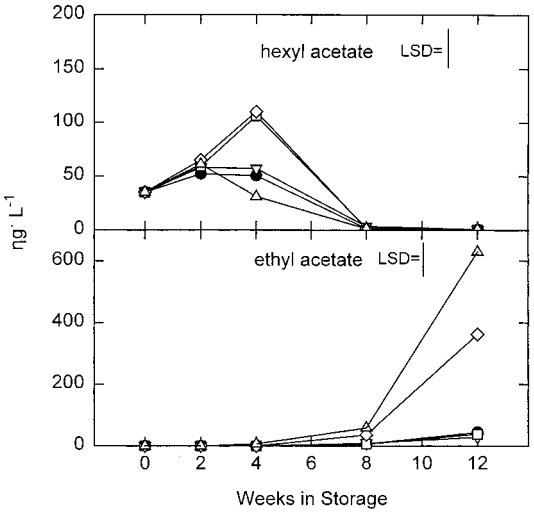

Figure 4. Concentrations of hexyl acetate and ethyl acetate released from sweet cherry homogenates. Fruit samples were analyzed at harvest and after 24 h at 21 °C after removal from storage. Least significant difference values (LSD) are indicated. Treatments: (●) air; (▽) 5/0.1%, O_2/CO_2; (□) 5/10%, O_2/CO_2; (◇) 5/15%, O_2/CO_2; (△) 5/20%, O_2/CO_2.

The peak in ester concentration after 4 weeks of storage was mainly due to increased concentrations of hexyl acetate (Figure 4) and other hexyl esters. The highest hexyl acetate concentrations were in fruit stored in 5% O_2 with 10 or 15% CO_2. This peak in hexyl-ester concentration likely resulted from increased amounts of C6 aldehydes present in the fruit (Figure 2). Concentrations of hexanal, *E*-2-hexenal, and other C6 aldehydes also were relatively high after 4 weeks of storage, although hexanal concentrations were highest for fruit stored in 5% O_2 with 20% CO_2 or air. Activities of alcohol dehydrogenase (ADH) and/or acylalcohol transferase (AAT), the enzyme catalyzing ester synthesis (Harada et al., 1985), may have been higher in the 10 and 15% CO_2 treatments, resulting in enhanced conversion of aldehydes to alcohols (by ADH) to esters (by AAT) and leaving a lower residual aldehyde concentration. The increase in ethanol after 8 weeks of storage led to accumulations of ethyl acetate in cherries held in 15 and 20% CO_2.

Qualitative changes in volatile compounds occurred in the latter stages of senescence regardless of storage treatment (Table 2). The amounts of ethyl propanoate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl hexanoate,

Table 2. Changes in Selected Sweet Cherry Volatile Compounds after Removal from Storage[a]

compound	storage period					
	0 weeks	1 week	2 weeks	4 weeks	8 weeks	12 weeks
butanal	nd[b,c]	nd/nd	nd/nd	nd/nd	nd/22	25[d]
2-butanone	nd	nd/nd	nd/nd	2/37	18/24	64
pentyl acetate	nd	nd/nd	nd/nd	nd/29	14/10	6
ethyl acetate	nd	nd/nd	nd/nd	8/41	116/69	1102
ethyl propanoate	nd	9/2	3/5	4/nd	nd/nd	nd
ethyl butyrate	2	25/14	14/19	11/nd	nd/nd	nd
ethyl 2-methylbutyrate	1	25/12	10/24	5/nd	nd/nd	nd
ethyl hexanoate	2	42/20	17/27	25/nd	nd/nd	nd
ethyl octanoate	nd	4/1	1/6	16/nd	nd/nd	nd
butyl 2-methylbutyrate	1	20/9	5/13	27/nd	nd/nd	nd
propyl hexanoate	1	13/7	2/10	25/nd	nd/nd	nd
hexyl propanoate	1	15/10	6/15	26/nd	nd/nd	nd

[a] After removal from storage, cherries were held in air at 21 °C, and analyses were conducted after 1 or 4 days; values are sums of amounts from all treatments. [b] Not detected. [c] ng L^{-1} headspace. [d] All day-4 fruit decayed.

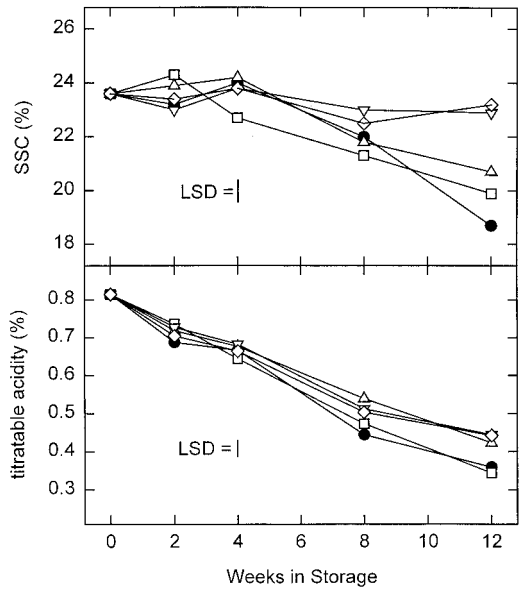

Figure 5. Changes in sweet cherry soluble solids content and titratable acidity during storage. Fruit samples were analyzed at harvest and after 24 h at 21 °C after removal from storage. Least significant difference values (LSD) are indicated. Treatments: (●) air; (▽) 5/0.1%, O_2/CO_2; (□) 5/10%, O_2/CO_2; (◇) 5/15%, O_2/CO_2; (△) 5/20%, O_2/CO_2.

ethyl octanoate, butyl 2-methylbutyrate, propyl hexanoate, and hexyl propanoate increased between 1 and 4 weeks of storage compared with amounts detected at harvest. Emission of these esters after an additional 4-day ripening period at 21 °C was dependent on storage duration. Amounts of these esters decreased after 1 week, increased after 2 weeks, and were not detectable after 4 weeks of storage plus the 4-day ripening period. Butanal, 2−butanone, and ethyl- and pentyl acetates were detected initially 4 or 8 weeks after harvest. The changes in ethyl acetate may have been in response to increased amounts of ethanol, although apples (Mattheis et al., 1991) and strawberries (Ke and Kader, 1994) produce more ethyl esters in general after ethanol accumulated due to anaerobic storage conditions. The qualitative change in volatile production occurred in air-stored as well as CA-stored fruit, suggesting a general consequence of fruit ripening rather than a low O_2 and/or high CO_2 effect.

Reduction of sweet cherry SSC was slowed by the CA treatments; fruit stored in the highest CO_2 concentrations retained SSC at the harvest concentration (Figure 5). Titratable acidity loss was greater in comparison to SSC, and treatment effects were observed only after 8 and 12 weeks of storage. Highest TA concentrations after 8 and 12 weeks were in fruit stored in the highest CO_2 concentrations; however, TA concentrations for these treatments were still nearly 50% less than harvest values.

These results indicate storage duration and atmosphere impact on the volatile composition of Bing sweet cherry fruit. Concentrations of benzaldehyde, hexanal, and *E*-2-hexenal changed during the storage period but were near harvest concentrations at the end of 12 weeks of storage. The contribution of these compounds to fruit flavor presumably is similar at harvest and after 12 weeks of storage. Fruit appearance was good through 8 weeks storage, but the fruit lacked luster after 12 weeks. Informal sensory evaluations of these fruit indicated the highest CO_2 treatments developed off-flavors after 12 weeks of storage, whereas other treatments lacked flavor after 8 and 12 weeks. Though informal, the sensory evaluations indicate factors other than benzaldehyde, hexanal, and *E*-2-hexenal concentrations are important for sweet cherry flavor after extended storage times. The change in the SSC/TA ratio may be one factor having an impact on sweet cherry flavor and consumer acceptance. Reducing the rate of acidity loss may be a critical determinant in extending the marketing period for sweet cherry fruit, although this remains to be determined by controlled sensory studies.

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