CHANGES OF SOME ODOR-ACTIVE VOLATILES IN CONTROLLED ATMOSPHERE-STORED APPLES

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Accepted for Publication February 21, 1990

ABSTRACT

The effect of controlled atmosphere (CA) storage on the production of 22 odor-active volatiles in 'McIntosh' and 'Cortland' apples was studied. Volatiles were analyzed periodically during ripening in air after harvest, during refrigerated air and CA storage, and during ripening in air after CA storage. Production of most volatiles at a lower rate during ripening after CA storage than during ripening immediately after harvest cannot be attributed entirely to the action of CA. Under the conditions of this study (3% $O_2$ + 3% $CO_2$ + 94% $N_2$ at 0°C for 19 weeks) CA storage caused a "residual suppression" effect on the production of propyl butanoate, butyl hexanoate, and hexyl hexanoate. Results indicate that CA might have altered the normal metabolism of the fruit by blocking the normal production of some volatiles either temporarily or permanently and either partially or completely.

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INTRODUCTION

Controlled atmosphere (CA) storage, in which the storage atmosphere is altered by reducing the O₂ and raising the CO₂ levels, has been used extensively to prolong the storage life of apples (Smock 1979). Apples intended for CA storage should be harvested just before the onset of the climacteric period (Smock 1948).

Although the physiological and biochemical basis of the CA effect are not yet well understood (Kader 1986), CA storage is known to slow down the synthesis and action of ethylene, to reduce fruit respiration, and to prevent or delay the appearance of some storage disorders (Smock 1979). CA storage can disrupt the normal ripening pattern of apples, which includes the climacteric rise in respiration, the degradation of cell wall, and pigment changes (Knee 1971). Although earlier studies by Kidd and West (1936) indicated that CA-stored apples developed their characteristic flavor after a short conditioning period in air at room temperature, recent studies (Knee and Sharples 1981) suggest that CA storage suppresses apple flavor. Sensory evaluation results indicated that odor deterioration preceded the deterioration of the sweet-sour relation and texture in ‘Golden Delicious’ apples stored in 4% O₂ + 8% CO₂ at 4°C (Gorin et al. 1975).

Although taste and texture sensations are essential, it is the presence of trace amounts of volatile components responsible for odor that gives much of the character to the fruit. Odor-causing volatiles in apples are characterized as being of relatively low molecular weight, belonging to different chemical classes, and present in trace amounts (Dimick and Hoskin 1983). More than 300 volatiles have been reported to be produced by apples (Dimick and Hoskin 1983; Van Straten 1977). However, experiments that determined the relative odor activity of volatiles (Cunningham et al. 1986) have shown that only between 20 and 40 volatiles are responsible for apple aroma. These compounds fall into three classes: the ‘apple-peel-smelling’ esters like ethyl 2-methylbutanoate, the lipid oxidation products like (E)-2-hexenal, and the terpenoid B-damascenone. Flath et al. (1967) found that ethyl 2-methylbutanoate, hexanal, and (E)-2-hexenal, with threshold concentrations of 0.1, 5.0 and 17.0 (v/v), respectively, were the main volatile components responsible for the odor in the essence of ‘Delicious’ apples.

Apples which had been stored in CA often failed to synthesize normal quantities of odor volatiles during subsequent ripening (Hatfield and Patterson 1975; Williams and Knee 1977). Patterson et al. (1974) also found a “residual effect of CA” on the production of volatiles in ‘Cox’s Orange Pippin’ and ‘Golden Delicious’ apples. Hatfield and Patterson (1975) found a 75% reduction in the production of some volatile esters in ‘Cox’s Orange Pippin’ apples after a 5-month storage in 2% O₂ with or without 5% CO₂. CA storage also arrested the ability of the peel of ‘Delicious’ apples to develop aroma (Guadagni et al. 1971). The severity of CA suppression of flavor depends on the atmospheric composition and the duration of storage. The lower the O₂ concentration, the higher the CO₂
concentration, and the longer the duration in CA storage, the greater the suppression (Knee and Sharples 1981, Lidster et al. 1983, Patterson et al. 1974). The criteria used in previous studies to determine the CA suppression effect (Halffield and Patterson 1975; Knee and Sharples 1981; Lidster et al. 1983; Patterson et al. 1974) was by comparing the production of volatiles during ripening of the fruit in air after harvest and after CA storage. This comparison ignores the behavior of these volatiles while the fruit is under CA conditions.

This paper reports the effect of CA storage on the production of 22 odor-active volatiles in ‘McIntosh’ and ‘Cortland’ apples. The criteria used in this study were to follow the changes of each odor-active volatile in the following conditions: (1) during ripening in air at 20°C after harvest, (2) during storage in air at 0°C, (3) during storage in CA at 0°C, and (4) during ripening in air at 20°C after CA storage. Storage in air and CA at 0°C was done to determine the behavior of each volatile under CA storage (CA effect), while the behavior of the volatiles under the four storage conditions determines whether CA has any residual suppression effect (CA residual effect).

MATERIALS AND METHODS

Fruit Source

Several mature, standard-size trees of ‘McIntosh’ and ‘Cortland’ apples (Malus domestica Borkh) grown on the Cornell University orchard, Ithaca, New York were selected. ‘McIntosh’ apples were harvested on September 22, 1984 and ‘Cortland’ apples were harvested on September 29, 1984. The harvesting dates were determined according to the method of optimum maturity evaluation suggested by Liu (1985). Immediately after harvest triplicate samples of 40 apples were tested for flesh firmness and internal ethylene content and the juice was pressed for odor-active volatiles analysis. On the same day comparable fruits were assigned to various storage treatments described below.

Treatments

There were 3 storage treatments: (1) storage in air at 20°C for four weeks with samples taken at 3-days intervals, (2) storage in air at 0°C for four months with samples taken once a week, and (3) storage in CA. The apples for CA storage were held in air at 3.3°C until the CA room was sealed. A semi-commercial CA room (inside dimensions: 8 m × 4.6 m × 4.3 m) was sealed on October 25, 1984 and operated according to the method of Blanpied and Smock (1982). The CA atmosphere was monitored every day using an Orsat gas analyzer and was maintained at 3% O₂ and 3% CO₂ at 0°C. Samples of apples (10 apples/treatment) were taken at different intervals from the CA room.
through a small opening which could be opened and re-sealed quickly. CA storage was terminated on March 12, 1985. CA apples were then ripened in air at 20°C for three weeks and samples were taken at 3-day intervals for analysis. At each sampling time 10 fruits were pressed for odor-active volatiles analysis. Samples removed from 0°C air or CA were held at 20°C overnight in order to allow the fruit temperature to reach 20°C before the analysis.

**Internal Ethylene Measurements**

Internal ethylene content was analysed by withdrawing 1 ml air sample from the central cavity of each fruit using a syringe needle and injecting it into a gas chromatograph (Varian Aerograph Model 3700) equipped with a flame ionization detector and a 0.45 m × 3.2 mm column packed with activated aluminum oxide (Liu and Samelson 1986).

**Odor-Active Volatile Analysis**

The 10 apples were cut into eighths, placed in methanol to inhibit oxidation and pressed using a hydraulic press with a stainless steel basket. The juice was immediately extracted with two thirds of its volume using freon 113 (1, 1, 2-trichloro-1, 2, 2-trifluoroethane) and stirred at 60 rpm for 30 min. The freon layer was separated, dried, by passing through 40 g MgSO₄, and concentrated 30-fold in a rotary evaporator at 35°C and 0.5 Pa (Cunningham et al. 1986; Yahia 1986). The concentrates were stored in amber glass bottles at 0°C until analysis. A Hewlett Packard Model 5840A gas chromatograph equipped with their model 18835B capillary inlet system and a flame ionization detector, and a 25 m × 0.36 mm fused silica column coated with 0.53 micron crosslinked methyl silicone (Hewlett Packard Inc., Palo Alto, CA) was used for odor-active volatile analysis. The column temperature was held at 35°C for 8 min, increased at 3°C/min to 250°C and held for 15 min. The retention indices (Kovats 1965) of 22 authentic standards (Table 1) and an internal standard (Nonanal) were used to identify all volatiles and as a calibration standard for quantitative analysis. Retention indices (RI) were calculated using n-parafin hydrocarbon standards containing n-heptane through n-octadecane. These volatiles have all been shown to be odor-active in apples by Cunningham et al. (1986). Fourteen of the 22 volatiles were verified in a combined sample by mass spectrometry. Mass spectra were obtained with an HP5985 quadrupole mass spectrometer with the same column used for gas chromatographic analysis. The total column effluent was transformed directly to the mass spectrometer source heated at 250°C. The column temperature was maintained for 3 min at 35°C then increased 6°C/min to 225°C. The injector was operated at 250°C in the splitter mode.
<table>
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RESULTS

The ‘McIntosh’ and ‘Cortland’ apples at harvest had an average internal ethylene of 0.244 ppm and 0.061 ppm, respectively. Liu and Samelson (1986) suggested that an internal ethylene of $\geq 0.2$ ppm indicates the onset of the autocatalytic ethylene production in ‘McIntosh’ apples. This onset in turn coincides with the climacteric rise in respiration in the same apples (Liu 1978).

Six of the 22 volatiles were not detected in any of the apples at the threshold of detection (Ca 1 ng/g). They were butyl- and pentyl acetate, propyl-, butyl- and pentyl propanoate, and pentyl 2-methylbutanoate. Propyl 2-methylbutanoate and ethyl pentanoate were only produced by apples held at 20°C in air. The other 14 volatiles detected followed different trends. Table 2 summarizes the behavior of the different volatiles detected during the experiment.

Figure 1 shows the changes in the total of the odor-active volatiles measured during the different storage regimes. The production of odor-active volatiles increased rapidly during ripening in air at 20°C, reached a maximum and then decreased rapidly. CA storage had a tendency to suppress the production of these volatiles compared to storage in air at 0°C but the effect was small and gradual. The amount of volatiles produced during ripening in air at 20°C after CA was considerably lower than that before CA.

Apples stored in air and in CA at 0°C produced similar amounts of hexanal, (E)-2-hexenal, hexyl acetate, methyl butanoate, methyl 2-methylbutanoate, and butyl 2-methylbutanoate. The changes in hexanal and methyl 2-methylbutanoate are shown in Fig. 2 and 3. Hexyl acetate concentrations were highest at harvest time, declined rapidly in all storage regimes, and reached a very low level in less than two months after harvest (data not shown). The production of ethyl- and propyl butanoate, hexyl 2-methylbutanoate, and methyl-, butyl- and hexyl hexanoate were either severely or completely suppressed by CA compared to air storage at 0°C. The changes in ethyl butanoate and methyl hexanoate are shown in Fig. 4 and 5. Ethyl 2-methylbutanoate was only produced by apples kept in air at 20°C (Fig. 6).

Apples ripened in air at 20°C after CA storage produced similar amounts of ethyl butanoate (Fig. 4) and propyl 2-methylbutanoate (Table 2) as apples ripened before storage (immediately after harvest). However, apples ripened after CA storage produced less total volatiles (Fig. 1), and less hexanal (Fig. 2), (E)-2-hexenal (data not shown), methyl butanoate (data not shown), propyl butanoate (Fig. 7), butyl 2-methylbutanoate (data not shown), and hexyl 2-methylbutanoate (data not shown), than apples before storage. Hexyl acetate, butyl hexanoate (Fig. 8) and hexyl hexanoate were produced in very low amounts during ripening after CA storage than before storage. Methyl 2-methylbutanoate (Fig. 3), ethyl 2-methylbutanoate (Fig. 6), and ethyl- and butyl pentanoate were produced in larger amounts during ripening after CA storage than before storage.
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FIG. 1. CHANGES IN THE CONTENTS OF THE TOTAL ODOR-ACTIVE VOLATILES OF 'MCINTOSH' AND 'CORTLAND' APPLES AT DIFFERENT STORAGE CONDITIONS

'McIntosh' apples had higher ethyl butanoate, propyl butanoate, ethyl 2-methylbutanoate, propyl 2-methylbutanoate, and ethyl pentanoate and lower (E)-2-hexenal concentration than 'Cortland' apples (Table 2).

DISCUSSION

The results in this study indicate that the "CA effect" can be quite independent of the "CA residual effect". "CA residual effect" is defined as "the suppression
FIG. 2. CHANGES IN THE CONCENTRATION OF HEXANAL IN 'MCINTOSH' AND 'CORTLAND' APPLES AT DIFFERENT STORAGE CONDITIONS

...effect which lasts during ripening of the fruit after CA storage and is clearly due to the action of CA". Most volatiles behaved differently under CA storage than when the fruit was ripened after storage (Table 2). For example, some volatiles were completely suppressed during CA storage, but during ripening after storage, some were produced normally, some were slightly suppressed, while others were produced in higher amounts. In most cases the volatile production during ripening after CA storage was lower than that before CA storage. The difference cannot be attributable entirely to a "CA residual effect". For instance, the loss of production in certain volatiles after CA compared to before CA is probably due...
to a gradual loss of these volatiles and/or their precursors during long term CA storage as a result of natural senescence rather than to the inhibitory action of CA. However, there are some cases where the loss after CA storage might involve a real "CA residual effect". For example, there was a complete suppression in the production of propyl butanoate during CA storage and more than 50% suppression in its production during ripening after CA storage (Fig. 7). The severe suppression of butyl hexanoate during CA storage as well as during
FIG. 4. CHANGES IN THE CONCENTRATION OF ETHYL BUTANOATE IN 'MCINTOSH' AND 'CORTLAND' APPLES AT DIFFERENT STORAGE CONDITIONS

Ripening after CA storage (Fig. 8) is another example that clearly involves a suppression due to the CA action. Hexyl hexanoate was also severely suppressed in CA storage as well as after CA storage (data not shown).

It has been reported (Williams and Knee 1977) that esterifying enzymes operate at similar rates in fruits stored in air or in 2% O_2. Therefore the suppression of odor volatiles by CA was suggested to be due to the limited supply and further metabolism of the volatile precursors (Knee and Sharples 1981). Since whole apples in low O_2 atmospheres were able to esterify added alcohols as rapidly as apples in air, it was concluded by Knee and Hatfield (1981) that the low levels
of esters in apples stored in low O₂ were a consequence of low rates of alcohol synthesis. Alcohols were found to be produced from fatty acids supplied to excised apple tissue (Paillard 1979), and oxygen is required in the synthesis of these fatty acids at the desaturation steps (Harris and James 1969). Although the suggestion that low levels of some esters are due to low rates of alcohol synthesis
FIG. 6. CHANGES IN THE CONCENTRATION OF ETHYL 2-METHYLBUTANOATE IN 'MCINTOSH' AND 'CORTLAND' APPLES AT DIFFERENT STORAGE CONDITIONS

might be true there are situations which cannot be fully explained by this hypothesis. Several volatiles were not suppressed by CA as shown in Table 2. Furthermore, the CA effect on different members of the same acid or alcohol group was not the same (Table 2). On the other hand, different pathways may be involved in ester formation in apples. Some methyl branched esters in bananas have been found to be derived from amino acids (Myers et al. 1970, Tressl and Drawert 1973). Nevertheless, reduced $O_2$ levels in CA is most likely to be an important factor behind the suppression of volatiles production.

The difference in the effect of CA on various volatiles in apples might be due to the diverse origins and pathways which lead to the production of these odor components. Since some volatiles are produced normally in air after CA storage while others are either partially or completely suppressed, or stimulated, may
FIG. 7. CHANGES IN THE CONCENTRATION OF PROPYL BUTANOATE IN 'MCINTOSH' AND 'CORTLAND' APPLES AT DIFFERENT STORAGE CONDITIONS

indicate that CA storage might have altered the normal metabolism of the fruit. Some pathways might not be affected, while others are blocked either temporarily or permanently and either partially or completely. It may also be likely that some
FIG. 8. CHANGES IN THE CONCENTRATION OF BUTYL HEXANOATE IN ‘MCINTOSH’ AND ‘CORTLAND’ APPLES AT DIFFERENT STORAGE CONDITIONS

pathways or mechanisms are diverted to different directions, or that new synthetic pathways become operative. The stimulation in the production of methyl- and ethyl 2-methylbutanoate and ethyl- and butyl pentanoate might be due to the operation of new pathways. The increase in these volatiles, especially ethyl 2-methylbutanoate, needs to be closely examined because of their importance. Ethyl 2-methylbutanoate has a very low odor threshold (0.0001 ppm), and has
been described as the character-impact odor of ‘Delicious’ apples (Flath et al. 1967). Ethyl 2-methylbutanoate is probably a postharvest-induced volatile, because it was present in a very high concentration in apples ripened off the tree or after long term storage (Fig. 6). On the other hand, a study of 40 apple cultivars ripened on the tree showed that ethyl 2-methylbutanoate contributed less than 1% of their odor (Cunningham et al. 1986). It seems likely that this compound is not a major component of the flavor of fruit ripened on the tree as it is to the flavor of postharvest-ripened and postharvest-processed apples. Furthermore, its postharvest formation may be induced by low temperature.

Any valid conclusion about CA, especially in relation to flavor, must take into consideration the exact gas composition, temperature, and the storage duration. Differences in these three factors influence the effects of CA on the flavor components of apples (Lidster et al. 1983; Yahia et al. 1985). Under the condition of this study (3% O₂ + 3% CO₂ + 94% N₂ at 0°C for 19 weeks) we found that CA caused a residual suppression on the production of three volatiles; propyl butanoate, butyl hexanoate, and hexyl hexanoate. Cunningham et al. (1986) found that these volatiles are important to apple flavor. For example, hexyl hexanoate was among the five most intense odor compounds in ‘McIntosh’ and ‘Cortland’ apples. More severe suppression would be expected if the CA room was sealed sooner, and if the storage duration was longer. Therefore, relatively short periods of CA storage can be used without the risk of severe loss in the volatiles components of apple odor.

REFERENCES


