Changes in Some Odor-Active Volatiles in Apples During Storage in Air and in Simulated Low-Ethylene Controlled Atmosphere[1]

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ABSTRACT

The evolution of 13 odor-active volatiles was followed during storage of "McIntosh" apples in air at 0°C and in simulated low-ethylene controlled atmosphere (LCA) at 3.3°C using gas chromato-graphy. Three volatiles were not detected: pentyl acetate, iso pentyl acetate, and butyl propanoate. No differences in the production of hexanal, (E)-2-hexenal, butyl acetate and hexyl acetate in air or in LCA storage were detected. However, LCA severely suppressed the total of the 10 volatiles and butyl pentanoate, and completely suppressed the production of propyl butanoate, butyl butanoate, ethyl 2-methylbutanoate, ethyl butanoate and butyl hexanoate.

COMPENDIO

Se estudió por medio de cromatografía de gases la evolución de 13 compuestos volátiles aromáticos durante el almacenamiento de manzanas "McIntosh" en aire a 0°C y en atmósfera controlada simulada de baja concentración de etileno a 3.3°C. Tres componentes volátiles no fueron detectados: pentilacetato, isopentilacetato y butilpropanoato. Por otra parte no se encontraron diferencias en la producción de hexanol, (E)-2-hexanol, butilacetato y hexilacetato durante el almacenamiento en los dos tipos de atmósferas. Sin embargo, el almacenamiento en atmósfera controlada simulada de bajo etileno redujo severamente la producción total de los 10 volátiles y del butilpentanoato y eliminó completamente la producción del etilbutanoato, propilbutanoato, butilbutanoato, etil 2-metilbutanoato y butilhexanoato.

INTRODUCTION

Controlled atmosphere (CA) storage in which the atmosphere is altered by reducing the oxygen levels and raising the carbon dioxide levels, is a well established method of prolonging the storage life of apples (26). Although the physiological and biochemical basis of the CA effect on apples is not well understood (10), it is already known that CA storage decreases both the rate of ethylene synthesis and action, reduces the rate of respiration, and prevents or delays the appearance of some storage disorders (26). Normal ripening patterns of apples which include the rise in the rate of respiration, degradation of the cell wall, and pigment changes were disrupted by CA storage (12).

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Cold atmosphere storage has also been suggested to suppress apple flavor (14, 29, 30). However, earlier studies (11) concluded that the full characteristic flavor of CA storage apples develops after a short conditioning period in air at room temperature. The severity of CA suppression of flavor components was found to be dependent on both the atmospheric composition and the length of storage (14, 16, 15, 19).

Ripening and senescence in apples is characterized by an array of physical and chemical changes, including changes in color, texture, and flavor. Ethylene which is recognized as the "ripening hormone" is assumed to initiate these changes (1, 3). Ethylene removal during storage of apples was found to be beneficial for further prolonging the storage life of the fruit (17, 18, 19, 20, 21). A program for the commercialization of low-ethylene CA storage (LCA) for apples was started in the state of New York in

1983 (2). Low-ethylene CA storage of "McIntosh" apples, (where fruit is harvested just before the onset of the autocatalytic ethylene production and the where ethylene in the CA room is maintained at ≤ 1 μ l/kg h), has been found to be very effective in prolonging the storage life of the fruit with minimum losses in flesh firmness and acidity (21). However, the fruit was criticized by some consumers for lack of full, ripe flavor and poor flavor when cooked (21).

Sensory studies with "Golden Delicious" apples showed that odor was the first factor to deteriorate during CA storage, preceding the deterioration of the sweet-sour relation and texture (7). Although more than 260 volatiles have been identified to be produced by apples (5), only a few seem to have a significant impact on the sensory quality of the fruit (4).

It is not clear from previous studies how odor-active volatiles are affected by low-ethylene CA storage. In this study the evolution of 13 odor active volatiles in apples was followed during storage of "McIntosh" apples in air and in simulated low-ethylene cold atmosphere.

MATERIALS AND METHODS

Fruit source

"McIntosh" apples (*Malus domestica* Borkh.) were picked on September 24, 1983 from three mature trees grown at the Cornell University Orchard, Ithaca, New York. Trees had been sprayed with 1000 ppm daminozide about 60 - 75 days before harvest. Fruits were sorted and placed at the assigned treatments at the same day. Only fruits free of defects and with uniform medium size were used. Thirty apples per tree were evaluated at harvest for flesh firmness. Another 10 apples per tree were used for the analysis of odor-active volatiles. The remaining apples were stored in air at 0°C or in LCA at 3.3°C. Thirteen odor active volatiles (Table 1) were monitored at different intervals.

Simulated LCA storage

Apples were put into 19 l glass jars with about 50 fruits per jar. The jars filled with apples were left unstoppered in air at 3.3°C for 24 h to promote quick cooling and then stoppered. Premixed CA gases containing 3% O_2, 3% CO_2, and 94% N_2 were humidified and metered through each jar at 200 ml/minute. The atmosphere inside the jars was monitored three times a week. Gas samples were collected from the outlet of the jar, and O_2 and CO_2 contents were measured using an Orsat gas analyzer.

Table 1. Odor-active volatiles analyzed.

butyl acetate	ethyl butanoate
pentyl acetate	propyl butanoate
iso pentyl acetate	butyl butanoate
hexyl acetate	
butyl propanoate	ethyl 2-methylbutanoate
hexanal	butyl pentanoate
(E) - 2-hexenal	butyl hexanoate

Ethylene production and flesh firmness measurements

The rate of ethylene production of LCA-stored apples was monitored at intervals by withdrawing 1 ml air sample from the effluent gas of each jar and injecting it into a gas chromatograph. The gas chromatograph was a Varian Aerograph Model 3700 equipped with a flame ionization detector and a 0.46 m x 3.2 mm column packed with activated aluminum oxide.

Flesh firmness was measured on two opposite paired surfaces of each fruit using an "Effigi" fruit penetrometer with an 11 mm plunger.

Odor-active volatile analysis

The 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 collected in a graduated cylinder containing 10 per cent (v/v) of the expected juice volume of methanol, and extracted with two thirds of its volume Freon 113 (1, 1, 2-trichloro-1, 2, 2-triflouroethane) by stirring at 60 rpm for 30 minutes. The Freon layer was separated, dried using $MgSO_4$, and concentrated in a rotary evaporator at 35°C and 0.5 Pa to a 30-fold concentrate (30). The concentrates were stored in amber glass bottles at 0°C until analysis. A gas chromatograph equipped with a flame ionization detector, and a 12.5 m x 0.36 mm fused silica column coated with 0.53 microns crosslinker methyl silicone was used for the analysis of the 13 volatiles. These volatiles have all been shown to be odor active by Cunningham *et al.* (4). The column temperature was held at 35°C for 3 min, increased 4°C/min to 250°C and held for 15 minutes. The retention indices (15) of 13 authentic standards (Table 1) were used to identify all volatiles and as a calibration standard for quantitative analysis. Retention indices were calculated using n-paraphin hydrocarbon standards containing n-heptane through n-octadecane.

RESULTS AND DISCUSSION

The ethylene production by fruits stored in LCA was lower than 0.4 ppm, which met the requirement of LCA storage of less than 1 ppm. This low concentration of ethylene was maintained by a continuous flow of ethylene-free CA gas mixture. The changes in flesh firmness during air and LCA storage are shown in Figure 1. Firmness losses of LCA-stored fruits were minimal while those apples stored in air had a significant loss. These results indicate that LCA is very effective in reducing the loss in flesh firmness over an extended storage period. Similar results have been shown by others (2, 20, 21).

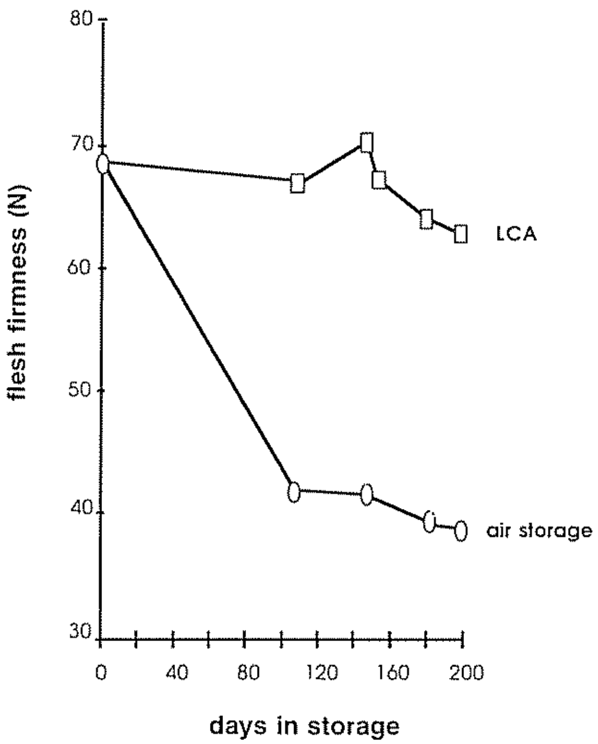

Fig. 1. Effects of LCA and air storage on the flesh firmness of "McIntosh" apples.

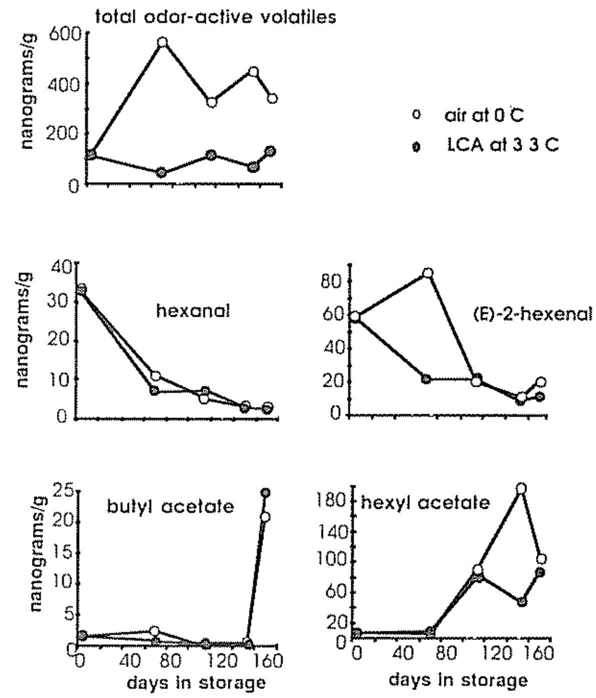

Fig. 2. The production of the total and some odor-active volatiles in "McIntosh" apples during storage in air and simulated low-ethylene CA (LCA).

Three of the 13 volatiles monitored were not detected. These were pentyl acetate, iso pentyl acetate, and butyl propanoate. Figures 2 and 3 show the production of the total and 10 odor-active volatiles during air and LCA storage. There were no large differences in the production of hexanal, (E)-2-hexenal, butyl acetate, and hexyl acetate in air or in LCA storage. However, LCA severely suppressed the production of total volatiles and butyl pentanoate, and completely suppressed the production of ethyl butanoate, propyl butanoate, butyl butanoate, ethyl 2-methylbutanoate, and butyl hexanoate. The six volatiles suppressed contribute significantly to apple flavor (4, 5).

These results clearly indicate that different odor-active volatiles are affected differently by LCA storage. This selective effect is helpful to allow future work to concentrate only on those volatiles affected by a low-ethylene cold atmosphere. The volatiles not affected include two aldehydes and two acetates (Fig. 2), while the affected volatiles include members of butanoates, 2-methylbutanoates, pentanoates and hexanoates (Fig. 3). This raises the question of whether the "CA effect" in general might be related to chemical structure. In case this proved to be a valid assumption, future work would be able to concentrate only on a few chemical groups rather than on many individual volatiles.

Storage of apples in CA (0.5% — 1% CO_2, 3°C, and no control over O_2) decreased the production of alcohols, aldehydes, ketones and esters compared to apple in air (22). Head space volatile analysis indicated that "Cox's Orange Pippin" apples transferred from CA storage into air at 20°C produced lower rates of butanol, butyl-and hexyl acetate compared to similar apples ripened after harvest (2). The atmosphere of 5% CO_2 + 2% O_2 for 5.5 months almost completely inhibited the production of these volatiles. Lidster *et al.* (16) found that CA storage (3% O_2 + 5% CO_2 at 2.8°C) suppressed the head space ethanol and acetaldehyde in "McIntosh" apples

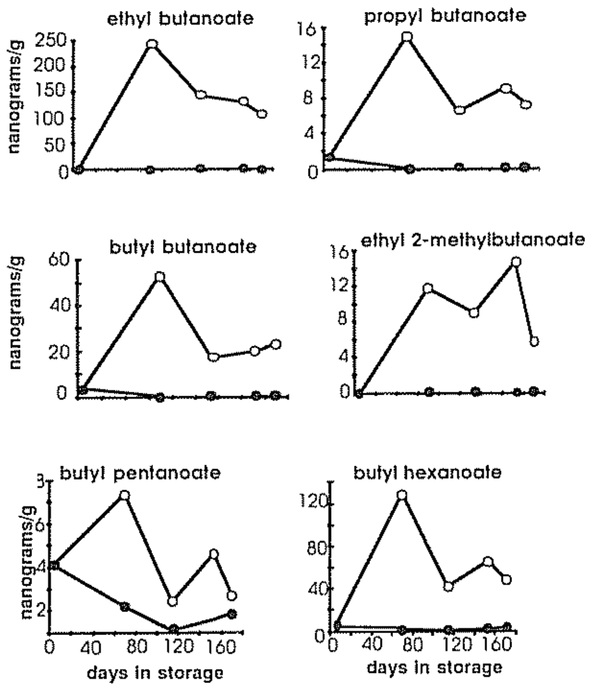

Fig. 3. The production of some odor-active volatiles in "McIntosh" during storage in air and simulated low ethylene CA (LCA).

compared to similar apples stored in air at 0°C. Acetaldehyde, ethanol, ethyl butyrate and hexanal were further suppressed when the apples were stored in a more strict CA atmosphere (1.5% CO_2 + 1.5% O_2 or 1.0% O^2 + 1.5% CO_2 at 2.8°C). Low-oxygen storage over a long period (1.0% O_2 +1.5% CO_2 for 320 days) completely suppressed the formation of these volatiles even after the apples were moved to air. Apples stored in lower-ethylene CA conditions (3% O_2 + 5% CO_2 and < 5 ppm ethylene) produced less acetaldehyde, ethyl alcohol, ethyl acetate, and ethyl butanoate than apples stored in higher ethylene CA (> 500 ppm), which in turn produced a smaller amount of these volatiles than apples held in air (6).

The suppression of odor volatiles by CA was suggested to be due to the limited supply and further metabolism of the volatiles precursors rather than to the limited enzyme activity (14). The esterifying enzymes were reported to be operating at similar rates in apple fruits stored in air or in 2% O_2 (28). Since whole apples in low oxygen atmospheres were able to esterify added alcohols as rapidly as apples in air, it was concluded that the low levels of esters in apples from low O_2 atmospheres were a consequence of low rates of alcohol synthesis (13). Alcohols were found to be produced from fatty acids supplied to excised apple tissue (24), and that oxygen was required in the synthesis of these fatty acids at the desaturation steps (8). The assumption that low levels of some esters are due to low rates in alcohol synthesis might be possible, however there are several situations that cannot be fully explained by this hypothesis. The results described in this paper show that some esters were not suppressed by a cold atmosphere. In addition, not all esters are thought to be derived through fatty acid and alcohol metabolism. For example, some amino acids were found to be the precursors of some banana esters (23, 27). The difference in the effect of CA on the different volatiles might be due to the diverse origins and pathways that lead to the production of these odor volatiles.

However, LCA maintained minimum losses in flesh firmness of "McIntosh" apples stored for about seven months, yet it caused a suppression of six of the most important flavor volatiles in the fruit. The suppression of volatiles was selective and might be related to chemical structure. Further work is needed to evaluate this hypothesis and to determine the behavior of these volatiles during the ripening of the fruit after storage.

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RESEÑA DE LIBROS

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En este libro se reúne, en forma altamente práctica y técnica, un conjunto de recomendaciones para el establecimiento, producción y cosecha de la alfalfa. Este atributo hace que la guía sea de gran utilidad a productores, técnicos y particularmente extensionistas.

Los valores de los parámetros edáficos y agronómicos se refieren a las condiciones reinantes en los Estados Unidos de América. Sin embargo, las exigencias fisiológicas de la alfalfa son típicas (salvo variaciones entre ecótipos, como es de esperar) y muchas de las recomendaciones agronómicas son extrapolables a otros ambientes. Estas dos razones hacen que la obra sea de interés para otros países, particularmente para aquellos con condiciones agroecológicas análogas a las regiones estadounidenses productoras de alfalfa.

Una tercera razón para recomendar esta obra es su presentación. Es muy fácil de leer, y hay en ella un balance óptimo entre lo técnico y lo práctico y una combinación magistral de artes gráficas. Esta publicación constituye un buen ejemplo de cómo divulgar en forma efectiva recomendaciones técnicas a extensionistas, productores y profesores.

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