AN ABSTRACT OF THE THESIS OF

Theeranuch Chantrachit for the degree of Master of Science in Horticulture
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Title : Banana Quality : Flavor Volatiles under Anaerobic and Aerobic conditions.

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Daryl G. Richardson

Alcoholic off-flavor and accompanying volatile compounds produced by bananas (Cavendishii spp) held at 20°C and subjected to aerobic (air control treatment) or anaerobic conditions (nitrogen treatment) were compared by solid phase micro extraction (SPME), a newly developed method for volatile separation. In general, volatile production was suppressed under anaerobic conditions but resumed, to some extent, after fruits were returned to air. The effects of anaerobic conditions on individual compounds were separated into six groups based on their production trends relative to those of air conditions. It was clear that bananas subjected to anaerobic conditions produced ethanol that could be detected easily by SPME. Anaerobic conditions almost completely blocked the esterification step needed to produced normal volatile profiles of ripening bananas.

The effects of anaerobic conditions on banana flavor and off-flavor development were determined by a taste panel quality scaling method. In order to determine the difference between bananas subjected to both conditions

(aerobic and anaerobic), the "difference from control" method was used in other experiments. Generally, the bananas subjected to anaerobic conditions had better physical appearance than bananas in the air control group but lacked fruit flavor and firmness. Off-flavor developed to a greater extent in bananas under anaerobic conditions than those under the air control which had almost no off-flavor. The correlation between off-flavor and ethanol was very high ($r = 0.87$) while for other volatiles was very low. This finding implies that ethanol is probably the only volatile causing anaerobic off-flavor in ripening bananas. In order to confirm this, headspace ethanol was detected by SPME, and tissue ethanol was extracted and assayed enzymatically. The result showed that tissue ethanol was correlated with headspace ethanol ($r^2 = 0.66$). The threshold for off-flavor development in ripening bananas was 300 mg of tissue ethanol / 100 g FW or 0.5 ppm for headspace ethanol.

Our results indicate that even three days in anaerobic conditions could injure ripening bananas. Thus unlike other fresh fruits, O_2 levels at or below 1% is not suitable for application as postharvest insect control treatment in ripening bananas.

Banana Quality : Flavor Volatiles under Anaerobic and Aerobic Conditions

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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THE LORD BUDDHA NEVER
TEACHES ONE TO RUN AWAY FROM
THE WORLD OF SUFFERING, BUT
ENCOURAGES ONE TO CONFRONT,
UNDERSTAND, AND BE AWARE OF
SUFFERING. ALSO HE TEACHES
ONE TO FACE THE FACTS SO THAT
HE WILL NO LONGER SUFFER.
EVENTUALLY, ONE WILL BE ABLE
TO SURVIVE ON THE EARTH, TO
EAT WITHOUT DESIRE, AND TO
LOVE WITHOUT ATTACHMENT.

Yantra Amaro Bhikkhu.

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BANANA QUALITY : FLAVOR VOLATILES UNDER ANAEROBIC AND AEROBIC CONDITIONS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

After harvest, almost all horticultural products rapidly undergo deterioration mainly due to continuous respiration of plant tissues. Controlled atmosphere (CA) has been applied to many products to reduce metabolic processes. By storing fruits, vegetables, or flowers under atmospheres containing reduced amounts of oxygen and/or increased levels of carbon dioxide concentrations, the quality may be maintained longer. Thus, CA prolongs the postharvest life of horticultural products.

The effect of CA on postharvest quality of perishable products has been assumed to be more on reducing respiration and ethylene production than on other metabolic pathways (Kader, 1985). Beyond the usual purpose, CA has also been applied for quarantine insect control. To succeed in the later objective, several factors also have to be considered: 1) concentration of low O_2 or high CO_2; 2) The time required for lethal exposure to the insect and 3) the tolerance of the plant tissues, which are normally specific for each insect and commodity. The most important element in quarantine control is the characterization of optimum gas concentration and time required to kill the insect without damage to the plant tissues. Therefore, the specific time required for killing the insects must be shorter than the tolerance of those commodities.

Kosittrakun (1989) studied the effects of near anaerobic conditions on mortality of apple maggot (Rhagoletis pomonella). He found the specific time

required for 100 % mortality of insect eggs and larvae at 20° C under nitrogen atmosphere was 7 days. But keeping some varieties of apple (e. g., 'Golden Delicious') and pear ('Bartlett') fruits in these same conditions for that time resulted in off-flavor development. Kosittrakun (1989) also mentioned that the development of off-flavor in apple and pear was at least partially reversible. After return to air for short periods (1 to 4 days) those undesirable flavor characteristics disappeared, if they hadn't accumulated to extremely high levels.

Bananas, for insect quarantine purposes, have not been subjected to CA conditions. The large quantities of ripening volatile compounds produced by banana make them a particularly advantageous system for a basic study of the chemistry of flavor (Wick et al., 1966) and also, therefore, for study of off-flavor development and disappearance. This chapter reviews most aspects involved in fruit flavor including the ripening processes and biochemical changes in flavor during ripening, precursors and biogenesis pathways of banana volatiles, conditions that may lead to off-flavor development, and the compounds suspected to be the causes of off-flavor in ripening banana. Finally, a brief review about methods used for trapping volatile compounds is also included.

The development of off-flavors in stored or packaged fruit and its disappearance is responsible for this study. As previously mentioned, bananas have not been subjected to CA for insect quarantine purposes, but the large quantities of ripening volatiles make bananas an advantageous system for fruit flavor study. Research objectives for these studies are to characterize off-flavor development in banana subjected to anaerobic conditions (nitrogen treatments) and how those conditions affect the production of volatile compounds and eating quality of bananas. The specific time required for off-flavor development and disappearance upon return to air are also to be examined. A final effort seeks to determine which volatile compounds are involved in off-flavors of ripe bananas.

BANANA

Summary of botany

Bananas are monocotyledons and belong to the family Musaceae. The trees are two to nine meters tall, with a pseudostem built up from leaf sheaths. Some seven to nine months after planting of a sucker (a shoot from the rhizome) an inflorescence is formed. Fruits may be suitable for harvest 90-150 days after the inflorescence emergence. The banana fruit is classified as a berry which develops by means of parthenocarpy, i. e. without fertilization. Almost all the edible cultivated parthenocarpic bananas are derived from the wild diploid species *Musa acuminata* (A genome, 2n = 22) or by hybridization between this species with the wild diploid species *M. balbisiana* (B genome, 2n = 22). However the commercially crucial cultivars are normally triploids (2n = 33).

Banana fruit consists of two main parts, peel and pulp. The peel consists mostly of several layers of hypodermal parenchyma imbedded with latex vessels, vascular bundles, chloroplasts containing chlorophyll, and other pigments. The components of banana pulp are not easily revealed due to the large numbers of starch grains in mature but unripened tissues (20-23% of fresh pulp). One characteristic feature of banana fruit is the relatively large proportion of peel which makes up about 80, 40 and 30% of the fresh weight of juvenile, mature, and fully ripe fruits, respectively (Palmer, 1971).

Nutritional and therapeutic values

Nutritional and therapeutic values of bananas have long been studied (Bogert, 1935; Reynold, 1927). Recent work (Stover and Simmonds, 1987) showed that 100 g of banana pulp contains (in gram units) water 75.7, carbohydrate 22.2, protein 1.1, fat 0.2, and ash 0.8. In addition, bananas are a good source of ascorbic acid (20.0% US RDA/100 g in 'Cavendish' variety) and vitamin B6 (25% US RDA/100 g in 'Gros Michel' variety). Bananas also have a low lipid level and high energy value. They have been recommended for obese and geriatric patients and used for persons with peptic ulcers, for treatment of infant diarrhea, in celiac disease, and in colitis (Eddy, 1933; Loesecke, 1950; Stover and Simmonds, 1987).

Production and distribution

Bananas originated in the hot tropical regions of South-East Asia (Loesecke, 1950). Although major producing areas are Asian countries (Abdullah et al., 1990), Central and South America are now the main (70 % of the world volume) producing areas for bananas for international trade (FAO, 1986).

United Nations FAO (1991) estimated world banana production in 1991 at 48 million tons compared to 37 million tons in 1981, a 29 % increase in 10 years. Robert (1987) discussed the distribution of bananas from exporter to consumer. His data did not differ significantly from previous reports (Arthur et al., 1968; Simmonds, 1966) indicating bananas from Central America are shipped mostly to the United States and European Economic Community (EEC). Bananas from

Ecuador go to the United States, Chile, Argentina and Belgium. Bananas consumed in the United Kingdom and other European countries normally come from Africa, Windward Island, and Caribbean countries, while bananas consumed in Asia generally are supplied from Asian countries. The Philippines is the largest banana exporter in Asia, with 900 thousand tons of banana production in 1982, equal to 2.1 % of the world market (FAO, 1986). In Thailand, only three cultivars are of commercial importance, namely, 'Kluai Namwa' (ABB), 'Kluai Hom Thong' (AAA) and 'Kluai Khai' (AA). In 1982, 'Kluai Namwa' accounted for 75% of the total production in thailand. Of the total production, 91% was produced to serve local markets while the rest was exported mainly to Hong Kong (Thailand Centre for Agricultural Statistics, unpublished data). Most export bananas from Thailand are solely produced in limited areas; Ratchaburi, Nakonpathum, Pathumthani, and Nonthaburi provinces, close to Bangkok (Silayoi, 1991).

Maturity stage at harvest

Fruits are normally harvested at the mature green stage. A common indicator of harvest at this stage is the visual fullness of fruit fingers called 'finger angularity'. Some cultivars are harvested at the 'full three-quarters' to 'full' stage of maturity. This maturity index is normally coupled with other visual signs including drying up of leaves and dryness of stylar ends. Days after the emergence of the inflorescence are also used as indicator of harvest. For instance, 'Cavendish' banana is harvested 22-23 weeks from flower emergence (Gorrez et al., 1990).

Bananas are harvested, shipped and stored at the green stage (color stage 1) until they reach their destination where they will be treated with ethylene for ripening and then sold to retail markets. Ripening has been divided into seven stages (Appendix Fig. A 1.1.): 1, green; 2, green with a trace of yellow; 3, more green than yellow; 4, more yellow than green; 5, only a green tip remaining (green tip); 6, all yellow; 7, yellow flecked with brown (Stover and Simmonds, 1987).

Postharvest handling of bananas

Storage temperature

Bananas are native to tropical areas. Like many other tropical fruits, they are sensitive to chilling injury. That is, they are damaged at temperatures below 12°C (Stover and Simmonds, 1987). Bananas for export are normally picked at the green stage (color stage 1), shipped and stored in refrigerated systems at 13-14 °C (56-58 °F) so that they will not soften or sustain much injury during handling (Sommer, 1985). To prolong the preclimacteric period, controlled atmosphere (5-10% CO_2 and/or 1-10% O_2) is applied to the storage rooms and transit vessels (Daun et al., 1973; Liu, 1976; Mapson, 1969; McGlasson and Wills, 1972; Quazi and Freebairn, 1970; USDA, 1986; Wills et al., 1989). Polyethylene bags which create a modified atmosphere (MA) are another means to prolong the preclimacteric period (Stover and Simmonds, 1987). They have to be used with an ethylene absorbent such as potassium permanganate ($KMnO_4$) (Liu, 1970) otherwise other disorders will occur (Fuchs and Temkin-Gorodeiski,

1971). The general recommendation is that bananas stored in controlled atmosphere should be later ventilated with fresh air when ripening is desired (USDA, 1986).

Ripening process

Bananas are ripened commercially in ripening rooms to assure firm pulp texture, good color, and excellent flavor. Banana ripening is most successful at temperatures ranging from 14°C to 20°C with high relative humidity of 90 to 95% (Stover and Simmonds, 1987; USDA, 1986). Ripening is initiated by release of ethylene gas at 100 to 1000 ppm into the ripening room for 24 hours (Sommer, 1985). The ripening rate varies to some extent with ethylene concentration and room temperature. Once the ripening process has initiated, ripening rate can be decreased by lowering the temperature to 13°C or increased by raising the temperature to 18.5°C (Will et al., 1989). Pulp ethylene played a significant role in pulp softening and peel yellowing during ripening (Abeles et al.,1992).

Physiological disorders

Chilling injury

The critical temperature below which chilling symptoms occur is in the region of 12°C to 14°C (Stover and Simmonds, 1987). Both green and ripe bananas are susceptible to chilling injury, but green fruit are slightly more

susceptible (USDA, 1986). Symptoms of chilling injury are a reddish-brown discoloration of the latex vessels in the peel (Stover and Simmonds, 1987), or dark water-soaked areas in the green banana (Snowdon, 1990). These primary symptoms evolve to a poor appearance of the ripe fruits, with flavor and texture slightly affected.

In severely chilled bananas, chilling temperature may delay ripening, cause green or hard ripeness and several changes in biochemical metabolism. Chilling temperature (7 °C) together with low pressure storage (0.14 atm) has been reported to lower the volatile content of butyrates and alcohols, while at the same time leading to an increase in the level of acetate compounds (Murata, 1969). In the most severe cases, chilled bananas turn black and their pulp has an off-taste (Snowdon, 1990).

Browning

Browning of bananas both in pulp when served raw and in peel, from chilling injury, is the result of oxidation of phenolic compounds by polyphenol oxidase (Weaver and Charley, 1974). The main substrate for enzymatic browning in bananas is 3,4-dihydroxyphenylethylamine (dopamine) (Griffiths, 1959). Dopamine in bananas is synthesized from tyrosine but via a different pathway from those of the mammalian systems. In mammalian systems, dopamine is formed by the decarboxylation of DOPA whereas in banana, tyrosine is the intermediate compound (Buckley, 1964). A mechanism of oxidation of dopamine by polyphenol oxidase (PPO) has been proposed by Palmer (1963). The content of dopamine increases during fruit development (Buckley, 1964) and then decreases approximately 20 percent in pulp during

ripening whereas in peel, the concentration of dopamine seems to be constant. As the content of dopamine decreases during ripening, the rate of browning increases (Weaver and Charley, 1974). The pathway of dopamine oxidation is shown below in Fig. 1.1.

Ascorbic acid is a naturally-occurring inhibitor of the oxidation of dopamine. The amount of ascorbic acid increases in green bananas but after the ripening process is initiated, ascorbic acid decreases (Harris and Poland, 1939). This correlates with increasing susceptibility to discoloration of bananas with ripening (Weaver and Charley, 1974). The inhibitory effect of ascorbic acid on browning may be due to its ability to reduce quinones back to the original phenolic compounds, while ascobic acid itself is oxidized to dehydroascorbic acid (Ponting and Joslyn, 1949), alternatively, to direct inhibition of the enzyme (Baruah and Swain, 1953), or to both (Pierpont, 1966).

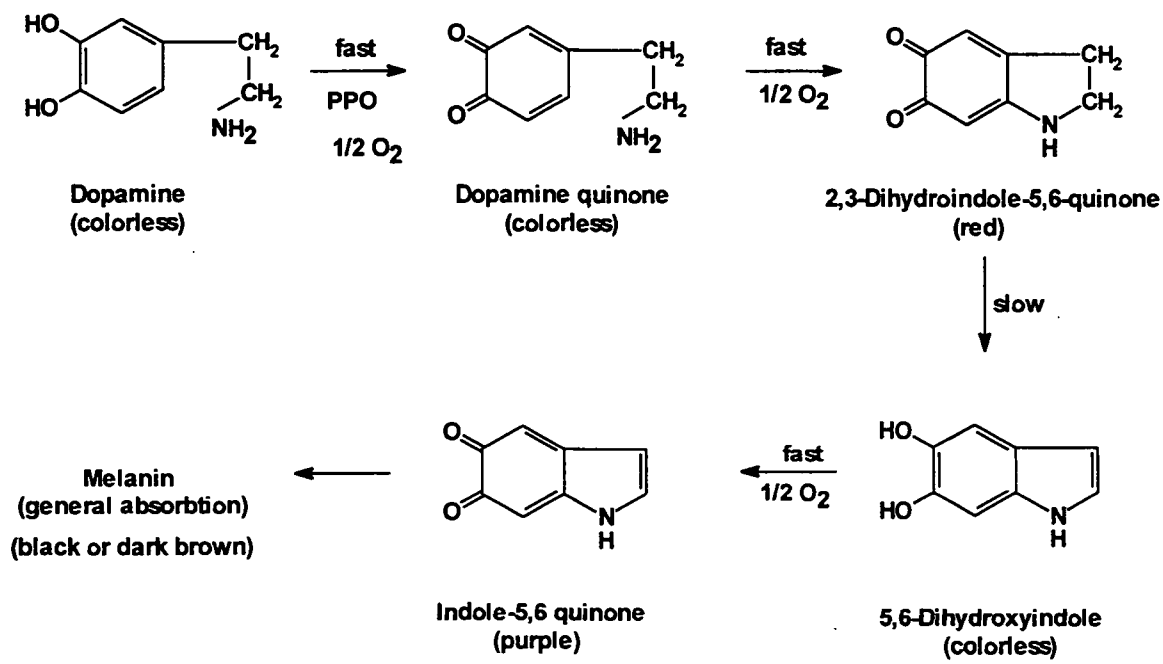

Fig. 1.1 Dopamine oxidation pathway (Palmer. 1963)

PHYSIOLOGY AND BIOCHEMISTRY OF MATURATION

The respiratory climacteric

Fruits can generally be classified as either climacteric or nonclimateric on the basis of their respiration pattern during ripening (Tucker and Grierson, 1987). Climacteric fruits are characterized by an increased rate of respiratory activity (peak of CO_2 production) during ripening while nonclimateric fruit simply exhibit a gradual decline or increase in their respiration (Tucker, 1993). Applying ethylene accelerates the respiratory climacteric in climacteric fruits, but may also result in an increased rate of respiration in nonclimacteric fruit. These phenomena are proportional to the concentration of applied ethylene (Tucker and Grierson, 1987).

Banana is a classic example of a climacteric fruit and was involved in the first discovery of the effect of ethylene on fruit ripening (historical citation as quoted by Abeles et al., 1992). Burg and Burg (1965) reported that unripe bananas show a constant, but low level of ethylene production, around 0.05 μl $kg^{-1} \cdot h^{-1}$ (Seymour, 1993) until the onset of ripening. Ethylene production then increases and this is followed by a rise in the rate of respiration. Peak ethylene production by 'Cavendish' bananas was reported to be around 3 $\mu l \cdot kg^{-1} \cdot h^{-1}$ and then declined (McMurchie et al., 1972). Palmer (1970) noted that as the rate of ethylene production declined, the rate of respiration increased during ripening. The respiratory climacteric in banana was reported to be around three times the rate of CO_2 produced in the preclimacteric stage. This climacteric peak closely corresponds to the optimum stage of eating ripeness in banana (Rhodes, 1970).

Banana ripening and biochemical changes

Generally, ripening of fruit is associated with changes in texture, color and flavor leading to the best eating stage. These changes do not occur in all ripening fruits but do so in bananas. Mostly, banana peel color changes from green to yellow while going through the 7 stages of ripening (Stover and Simmonds, 1987). Texture softens at different rates for each ripening stage partly due to the hydrolysis of starch and pectins in banana pulp (Tucker, 1993). The most important changes which correspond to the best eating quality for ripening banana are the changes in flavor which result from several metabolic reactions.

During ripening, initiated by ethylene, there is a degradation of pectin structure (Loesecke, 1950). However, it is believed that the major cause for softening in ripening banana is degradation of starch in the pulp (Tucker, 1993). Starch is hydrolysed into sugars and CO_2 and some is converted to organic acids and protein. Starch decreases from 20-23% of fresh pulp in green bananas to 1-2% in fully ripe bananas. These changes are concomitant with the increasing sugar content (from 1-2% rising to 20%) in the pulp during ripening (Palmer, 1971). Of the total sugars present in banana pulp, sucrose (13%), glucose (4%) and fructose (3%) are predominant and contribute heavily to the sweet taste of ripe bananas (Seymour, 1993). Color changes during ripening mainly result from degradation of chlorophyll in the peel in the early stages of ripening and the biosynthesis of carotenoids in later stages (Von Loesecke, 1929; Seymour, 1993). Abeles et al. (1992) mentioned that chlorophyll degradation in the peel was inhibited if the peel was removed from the pulp. Browning of peel and pulp after ripening are due to the oxidation of phenolic

compounds in both tissues in which the activity of polyphenoloxidase and concentration of ascorbic acid play a crucial rule (Jayaraman et al., 1982). Tannins, water-soluble phenolics are present and confined to the latex vessels in the peel. They are responsible for reddish-brown color when latex cells are damaged by low temperature (Stover and Simmonds, 1987). Tannins are known to interact with salivary proteins and glycoproteins, causing fruit to taste astringent, hence the loss of astringency in banana pulp during ripening may result from increased polymerization of tannins (Palmer, 1971).

Lipids and total protein content represent only small amounts in banana pulp. They do not change substantially during ripening (Goldstein and Wick, 1969; Loesecke, 1950). Goldstein and Wick (1969) reported that major fatty acids of the pulp and peel are palmitic (16:0), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). However, Wade and Bishop (1978) reported that the fatty acid content of phospholipids was altered during ripening and may result in increased fluidity in cellular membranes. Free amino acids differ from those of total protein content in that during ripening, histidine content increases at the expense of glutamic and aspartic acid and there is a dramatic increase in the content of valine and leucine (Palmer, 1971). Acidity of banana pulp increases during ripening (from pH 5.4 in preclimacteric to pH 4.5 in postclimacteric pulp) (Palmer, 1971). There is a report that the astringent taste of unripe bananas is probably attributable partly to their oxalic acid content, which undergoes significant decarboxylation during ripening (Seymour, 1993).

FRUIT QUALITY AND FRUIT FLAVOR

Fruit quality

Fruit quality may be defined in terms of end use (Wills et al., 1989). In these terms, fruit quality requirements are independently defined for market, storage, transport, eating and processing qualities. The important factors in quality for the consumer are: appearance including size, color, and shape; condition and absence of defects; texture; flavor; and nutritional value (only minor). Appearance and flavor of bananas are the main characteristics which are attractive for customers and responsible for the differences in price (Stover and Simmonds, 1987).

Fruit flavor

Flavor is a combination of taste and aroma. Taste is limited to the tongue's response to salty, sweet, sour and bitter which are experienced when materials producing those sensations are placed in the mouth. The tongue can also react to tactile stimuli and temperature which are part of flavor as well. Aroma is a different sensation detected by the olfactory epithelium in the roof of the nasal cavity. Therefore, all compounds producing aroma are in a vapor stage.

The complexity of the combinations among those sensations makes it virtually impossible to have a simple definition of flavor. Hall (1968) proposed definition for flavor based on the oral contact as: " Flavor is the sensation

produced by a material taken in the mouth, perceived principally by the senses of taste and smell and also by general pain, tactile and temperature receptors in the mouth".

Most flavors are comprised of both taste and odor. Because taste is limited to those four basic sensations: sweet, sour, salty and bitter, odor often plays a very significant role in flavor. An unambiguous example for this phenomenon is when a person catches a cold and can sense flavor characteristics only by taste, tactile and temperature responses, but how flat and uninspired it is without odor (Meyer, 1964).

The aroma of fruit

Odor of fruit can be described as aroma. Because of the essential character of aroma to flavor perception, the vast majority of analytical flavor studies have focused on the volatile constituents. The volatiles of most kinds of fruits are composed of a large number of different chemical compounds. These include esters, lactones, alcohols, acids, aldehydes, ketones, acetals, hydrocarbons and some phenols, ethers, thiol, and heterocyclic oxygen compounds.

Volatiles generally are present in very small amounts, usually less than 100 ppm (Nursten, 1970), such as, 65-338 ppm from 'Valery banana' (Wick et al., 1966); 12-18 ppm in 'Gros Michael' banana (Wick et al., 1969); about 17 ppm in cucumber (Takei and Ono, 1939); and 5-10 ppm in strawberry (Winter et al., 1962). Although a large number of volatiles exist which could contribute to fruit flavor, typically only a very small number of compounds impart the characteristic aroma. Kays (1991) called that group of compounds "character impact

compounds" or CIC. The most important character impact compounds for banana are amyl esters and particularly, isoamyl acetate (Salunke and Do, 1976).

Biogenesis of fruit aroma

Most flavor compounds are produced during the later stages of fruit ripening (Wick et al., 1966). This process involves a number of dynamic changes in both biochemistry and physiology. Produced in small amounts during a short period of ripening, ethylene initially triggers almost all processes needed for fruit ripening: climacteric respiration, softening, hydrolysis of storage material, changes in pigmentation and flavor (Abeles et al., 1992; Moore, 1989). Lyons and Pratt (1964) suggested that increased membrane permeability in response to ethylene may cause higher levels of respiration and correspond to precursors being produced for flavor compounds.

Fruit aroma can be derived normally from a few metabolic pathways which yield all the compounds for flavor. The main pathways involve the conversions of amino acids, fatty acids and carbohydrates. The first two pathways are the main contributors for biogenesis of banana flavor which have already been discussed in the section on banana volatiles. One can state that nearly all plant flavors come indirectly from carbohydrate metabolism. An exception is terpenes which may directly arise from either carbohydrate or lipid metabolism (Heath and Reineccius, 1986). Details about more biogenesis pathways for fruit flavors are proposed elsewhere (Heath and Reineccius, 1986; Tressl and Albrecht, 1985). The interaction among the flavor biogenesis pathways is shown in Fig. 1.2

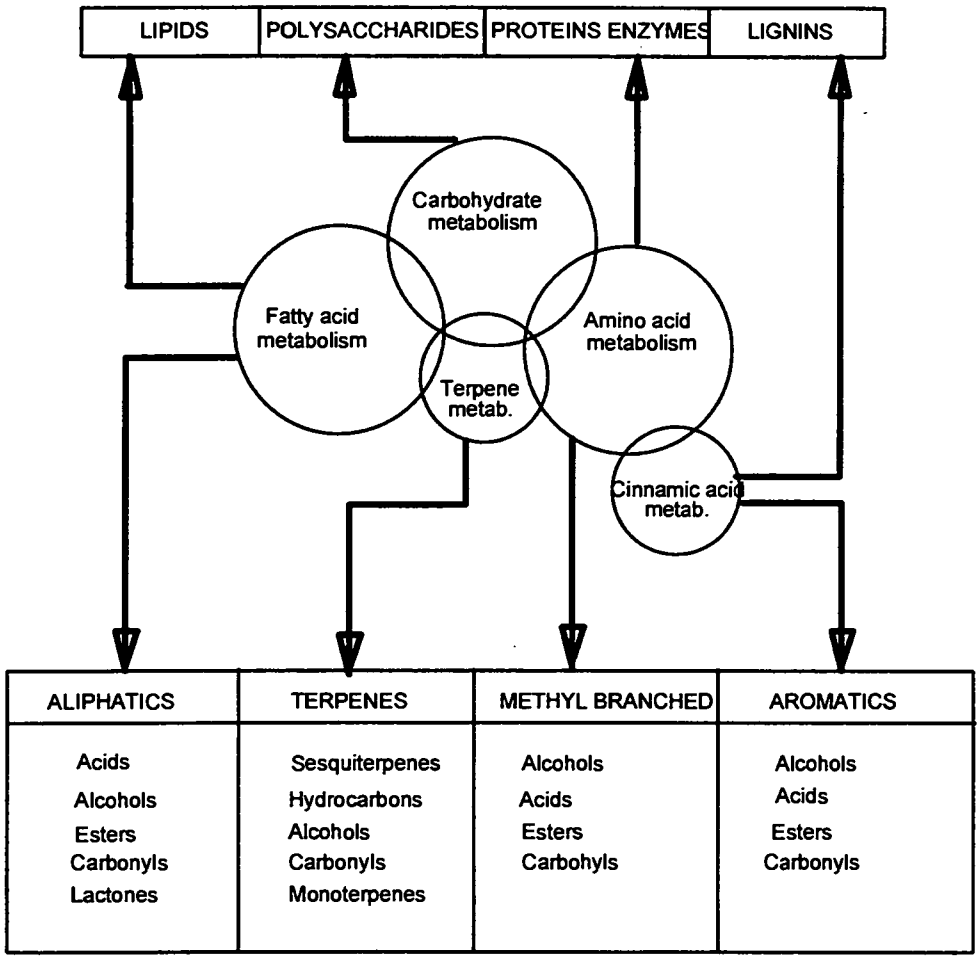

Fig. 1.2 Interactions among metabolic pathways, metabolites and aroma volatile classes (Heath and Reineccius, 1986).

BANANA VOLATILES

Production during ripening

Ripening bananas produce a wide variety of volatile compounds, including esters, alcohols, ketones, aldehydes, and phenol esters. Esters make up to 70% of volatiles and acetates and butyrates predominate within this fraction (Macku

and Jenning, 1987; Tressl and Drawert, 1973). McCarthy et al. (1963) first reported the correlation between volatile compounds identified from GC and flavor profiles. However the exact contribution of each individual volatile to the characteristic flavor and aroma of bananas is not clear (Seymour, 1993). In general, production of volatile compounds increases during ripening until the onset of peel browning, where production reaches either a plateau or decrease (Macku and Jennings, 1987; Tressl and Jennings, 1972). Early reports (Tressl and Jennings, 1972) suggested that acetate and butyrate esters were produced at a rate that varies in a cyclic manner and that the two cycles were out of phase. However, these findings have not been confirmed by more recent investigation (Macku and Jennings, 1987). The number of volatiles which have been reported cover a wide range varying from 200 peaks (Wick et al., 1966) to 350 peaks (Tressl et al., 1970 as quoted by Stover and Simmonds, 1987). The most recent paper (Shiota, 1993) identified eighty six compounds in ripe banana among a total of 152 compounds.

Sensory aspect

It is known that during ripening, the total amount and complexity of volatiles in fruits increase as their flavor and aroma develop (Myers et al., 1969). This implies a fundamental interrelationship between the substances and biochemical processes occurring in the fruits. McCarthy et al. (1963) attributed the characteristic "banana-like" flavor to amyl acetate, amyl butyrate, amyl propionate and isoamyl acetate; distinctive "fruity" or "estery" notesto butyl acetate, butyl butyrate, hexyl acetate and amyl butyrate; "green", "woody" or " musty" notes to methyl acetate, pentanone, butyl alcohol, amyl alcohol and hexyl

alcohol. As early as 1912, Kleber identified amyl acetate as a normal constituent of the ripe banana; and Hultin and Procter (1961) reported that 2-hexenal contributed to green or grassy smell and isoamyl alcohol was significant in the odor of over-ripe banana. Full-bodied or mellow aroma of banana has been attributed to higher boiling point compounds: eugenol, *O*-methyleugenol and elemicin (Wick et al., 1966, 1969). More complicated procedures are required to separate and identify those compounds (Wick et al., 1966).

Biogenesis of banana volatiles

The typical flavor compounds in climacteric fruit are produced during a short ripening period related to the climacteric rise in respiration (Heath and Reineccius, 1986). In early investigations, the selection of potential precursor compounds was arbitrary because little was known about the biosynthesis of volatiles within ripening fruit. An important clue began with a report of accumulation of amino acids during ripening stages (Loesecke, 1950; Palmer, 1971; Wick et al., 1966) and an investigation about the conversion of branched-chain amino acids to branched-chain alcohols by Saccharomyces cerevisiae (Guymon et al., 1961; Ingraham et al., 1961). Thus, a similar process could be hypothesized for the branched-chain alcohols and esters which are major aroma components in banana (Myers et al., 1970). Nowadays, it is well known that the biogenesis of many different individual banana flavors can be accommodated by a few metabolic pathways: from conversions of amino acids, from fatty acid metabolism and from enzymatic oxidative splitting of unsaturated fatty acids.

Conversion of amino acids

Myers et al. (1969, 1970) incubated banana slices with different concentrations of ^{14}C labeled-amino acids, and reported that L-leucine-U-^{14}C was a precursor for isoamyl alcohol and isoamyl acetate.

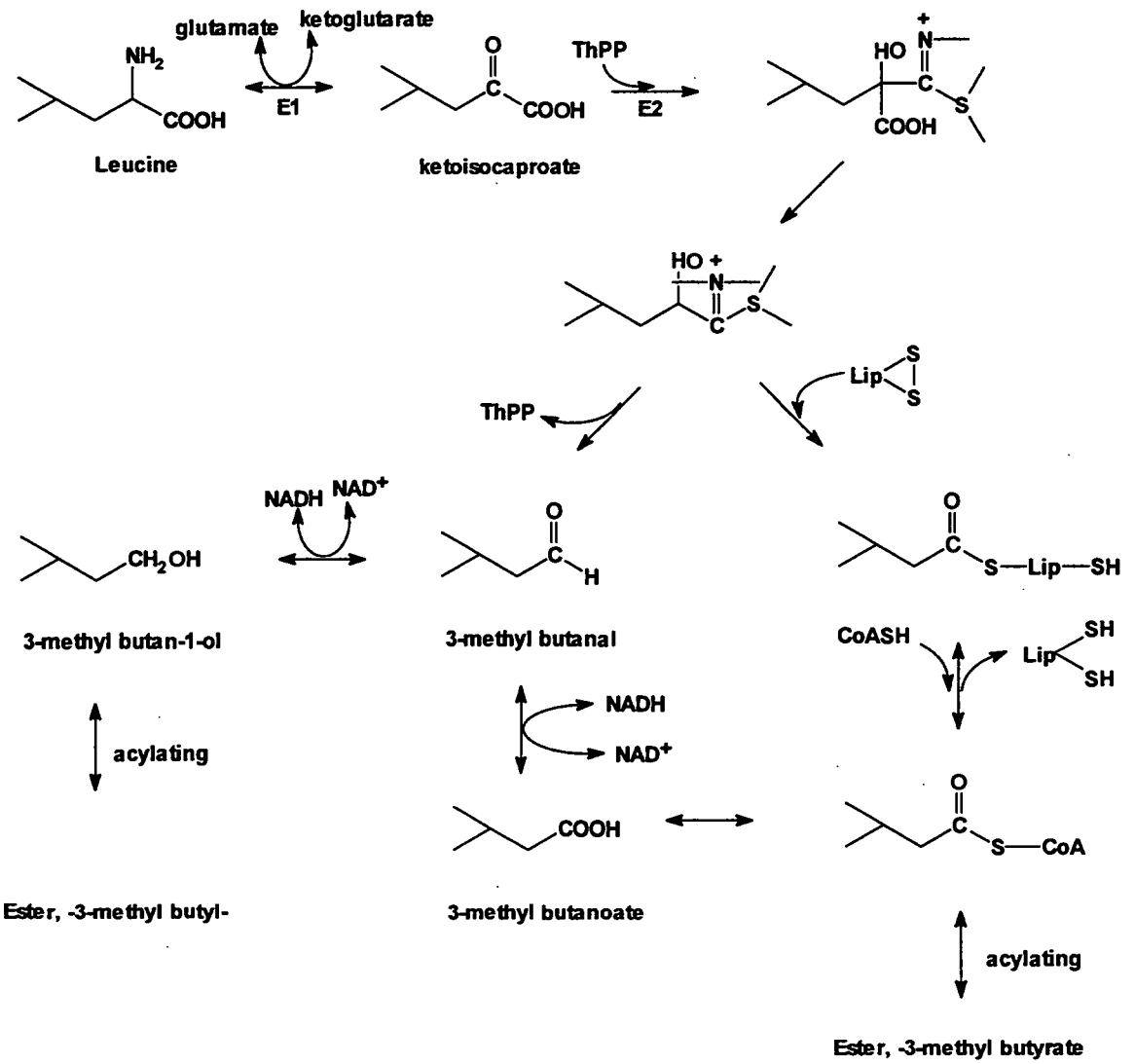

Fig 1.3 Volatile aroma compounds from amino acid precursors as illustrated by leucine. E1=leucine aminotransferase; E2=pyruvate decarboxylase; E3=aldehyde dehydrogenase; ThPP=thiamin pyrophosphate (Heath and Reineccius, 1986).

Tressl and Drawert (1973) supported these results by reporting that the amount of L-leucine and L-valine increased after the climacteric rise in respiration, whereas the level of L-isoleucine, which is a precursor only of some minor constituents, remained constant. Tressl and Drawert (1973) proposed the mechanism that converts those three amino acids into banana volatiles.

Fatty acid metabolism

Fatty acids are another known source for flavor compounds in ripening fruit (review by Forss, 1972). Several different pathways, including β-oxidation, hydroxyacid cleavage and oxidation via lipoxygenase enzyme, metabolism of fatty acids yield as primary products aldehydes and ketones. In addition, various oxidations, reductions, and esterifications also yield substantial quantities of acids, alcohols, lactones and esters which collectively contribute substantially to flavor in various fruits (Heath and Reineccius, 1986). Biogenesis of flavor compounds by climacteric and postclimacteric banana tissues has been prolifically researched by Tressl and coworkers (Tressl and Drawert, 1971; Tressl and Jennings, 1972; Tressl and Drawert, 1973).

Enzymatic oxidative splitting of unsaturated fatty acid

One substantial pathway for biogenesis of banana flavor is the oxidation of linoleic and linolenic acids via lipoxygenase action. Several investigators reported the production of volatile compounds in various fruits via this enzymatic reaction. The enzymatic formation of 2-hexenal and hexanal from linolenic and

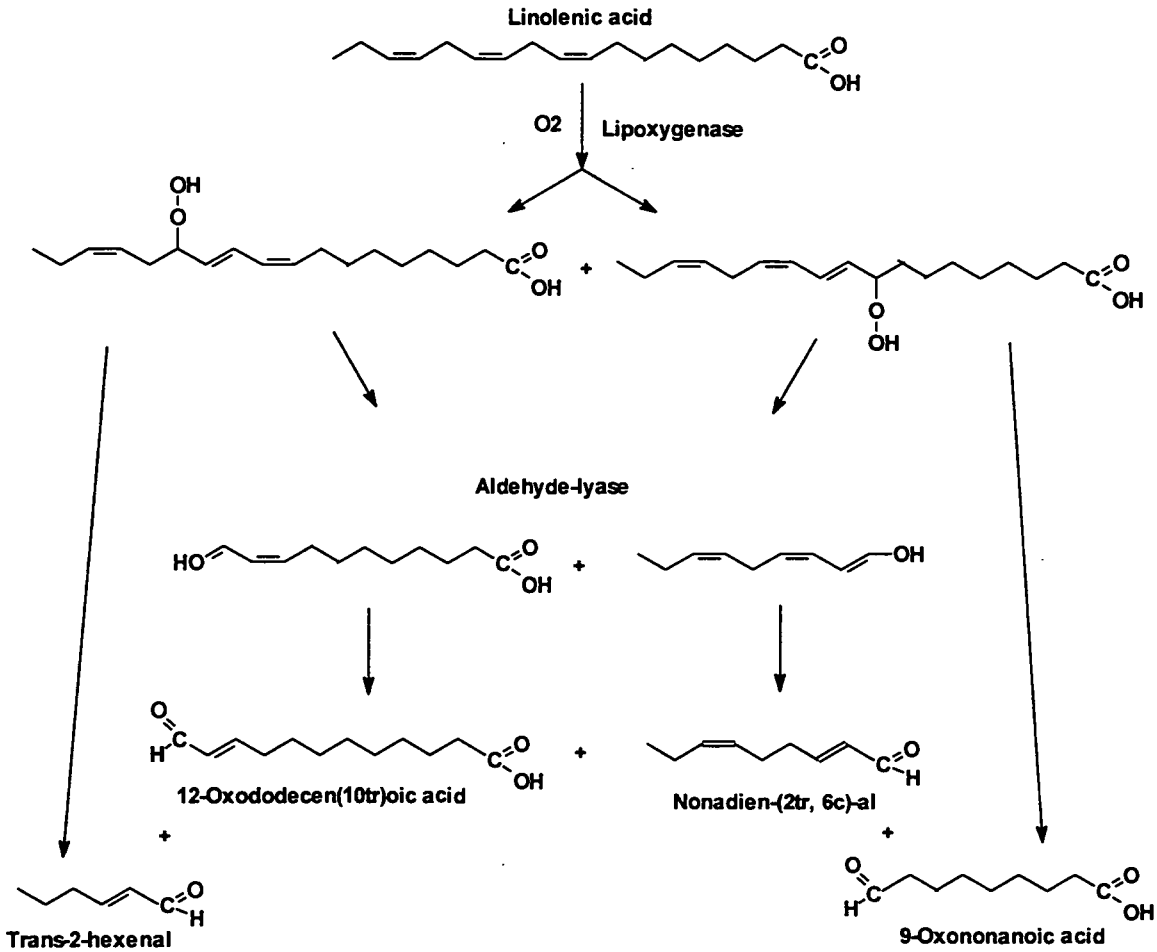

Fig. 1.4 Reaction scheme for enzymatic splitting via lipoxygenase of linolenic acid into flavor volatiles (Tressl and Drawert, 1973)

linoleic acid in apple, pear, plum and grape have been examined by Drawert (1966). Fleming et al. (1968) showed the formation of C9 aldehyde by an enzymatic reaction requiring O_2. The investigation of cis-3-hexenal, trans-3-hexenal, and cis-3-hexenol in tomatoes has been reported by Kazen and Hall (1970) and Stone et al. (1971). Linolenic and linoleic acids are presumed to be precursors of the carbonyl compounds: C6 and C9 aldehydes and C9 and C12 oxo acids in banana homogenates. A possible pathway for enzymatic oxidation via lipoxygenase has been proposed by Tressl and Drawert (1973).

Numerous groups of volatiles derived from fatty acid metabolism via several different pathways have been studied in various fruits. The formation via β-oxidation can be illustrated by the formation in pears whose major flavor impact compounds are decadienoates (Jennings, 1976). Lactones, considerable constituents in peaches, apricots, and coconuts are also derived from oxidative pathways of linolenic acid which involves lipoxygenase action (Maga, 1976).

CONTROLLED ATMOSPHERE (CA) AND INSECT CONTROL

CA and banana

The removal or addition of gas components surrounding the commodity which results in a difference from normal air is called controlled atmosphere (CA) or modified atmosphere (MA) (Kader, 1985). Usually low O_2 concentration reduces respiration and the increase of endogenous ethylene production (Quazi and
Freebairn, 1970) and high CO_2 concentration lowers respiration, ethylene production and can retard coloring of fruit. It has also been reported that low O_2 concentration causes accumulation of ethanol and acetaldehyde within tissues in which alcoholic flavors were determined (Ke and Kader, 1992a).

Preclimacteric bananas can be stored for almost 30 days in low O_2 concentration or high CO_2 concentration. This almost completely stops softening or color change and then the banana can be normally ripened when placed in air (Daun et al., 1973; Liu, 1970,1976; McGlasson and Wills, 1972). Gas components in the chamber need to be varied due to the different varietal

responses, the size of commodity, and particularly the difference in storage temperatures. Low O_2 has been recommended to be between 1-10% (Daun et al., 1973; Mapson, 1969; McGlasson and Wills, 1972; Liu, 1976; Quazi and Freebairn, 1970) and high CO_2 concentration between 5-10% (Daun et al., 1973; McGlasson and Wills, 1972). Low concentration of O_2 delays the production of endogenous ethylene (Quazi and Freebairn, 1970), while high CO_2 concentration directly affects the coloring of banana more than just as a competitive inhibitor of ethylene (Fuchs and Temkin-Gorodeiski, 1971).

Anaerobic conditions and insect control

The environment used for insecticidal disinfestation involves prolonged storage at low or short exposures at a high temperature, low humidity and high carbon dioxide levels. This is often detrimental to fresh fruits and vegetables (Klag, 1985). CA has been reported to be effective for several perishable commodities, such as apple (Chen et al., 1985); cherry (Chen et al., 1981); peach (Ke et al., 1991c); pear (Kosittrakun, 1989); orange (Ke and Kader, 1990) and many other fruits and vegetables. However, CA conditions which are effective against insects without affecting the commodity are rare.

For practical application of insecticidal effect, O_2 concentration below 1% is usually recommended (Ke and Kader, 1992a). The time of treatment by temperature and gas components required for 100% mortality varies with insect species and insect developmental stage (Kader, 1992). However, evaluation of the susceptibility of the insect relative to tolerance of the host commodity offers some potential for CA treatments (Delate and Brecht, 1989). Although these treatments have the advantages of leaving no chemical residues in the fresh

commodities and being safe (once aired out) to workers and the environment, the possible detrimental effects on horticultural crops is still a concern. Mostly, the tolerance of fruits and vegetables to 50-90% CO_2 atmosphere is limited by CO_2 injury, and with anaerobiosis below 1% oxygen, commodities tend to develop off-flavor (Ke et al., 1990; Ke et al., 1991; Ke et al., 1991a). Hence, the period of fruit tolerance to insecticidal CA is limited to the time before the onset of those detrimental effects.

Off-flavor development

Off-flavor related to CA in fresh fruits and vegetables develops normally only when those commodities are exposed to very low O_2 and/or high CO_2 atmosphere. This off-flavor is different from the off-flavor developed in processed products where they are caused by enzymatic or microbial activity (Chan et al., 1973) therefore acidification and heat inactivation of enzymes can prevent development of those detrimental effects.

Off-flavor development in fresh fruits and vegetables is thought to be caused by the accumulation of fermentation products such as ethanol, acetaldehyde, ethyl acetate and probably some other volatiles under the very low-O_2 and/or high CO_2 atmosphere (Ke and Kader, 1992a). Alcoholic off-flavor had a logarithmic relationship to ethanol content of fruits and this off-flavor is the most crucial detrimental effect found to limit fruit tolerance to low O_2 (Ke and Kader, 1992a; Ke et al., 1991a). Fruit ethanol levels have been reported to increase as a result of anaerobic respiration (Lidster et al., 1986). Relative higher storage temperature, higher respiration rate, and greater resistance to gas diffusion enhance off-flavor development while relatively higher oxygen

concentration and higher soluble solids content reduced off-flavor development (Ke and Kader, 1992b). Off-flavor caused by low O_2 and/or high CO_2 atmosphere can be relieved or reversed by exposing some fruits to air. This is partly explained by metabolization of accumulated acetaldehyde and ethanol by plant tissues (Cossins and Beevers, 1963; Fidler, 1968; Knee and Hatfield, 1981). However, more recent research by Kosittrakun (1989) has raised the question about off-flavor caused by compounds other than alcohol or acetaldehyde since reversibility occurred without substantial change in ethanol or acetaldehyde concentration.

ANAEROBIC RESPIRATION AND ITS PRODUCTS

When plants experience anoxic conditions, there is a shift in carbohydrate metabolism from an oxidative to a fermentative pathway. Pyruvate decarboxylase (PDC) is first inactivated (Davies et al., 1974) and lactate dehydrogenase (LDH) is induced initially (Dennis et al., 1992) resulting in accumulation of lactate with a corresponding decrease in pH. At this point, pyruvate decarboxylase and alcohol dehydrogenase (ADH) are induced. While PDC produces acetaldehyde, ADH reduces this to ethanol. Since acetaldehyde is a toxic compound (Smagula and Bramlage, 1977) it must be removed rapidly following its production. That is why ADH plays a significant role to avoid cell intoxification. Once the switch to ethanolic fermentation occurs, the pH of the cell is stabilized and plants then can survive.

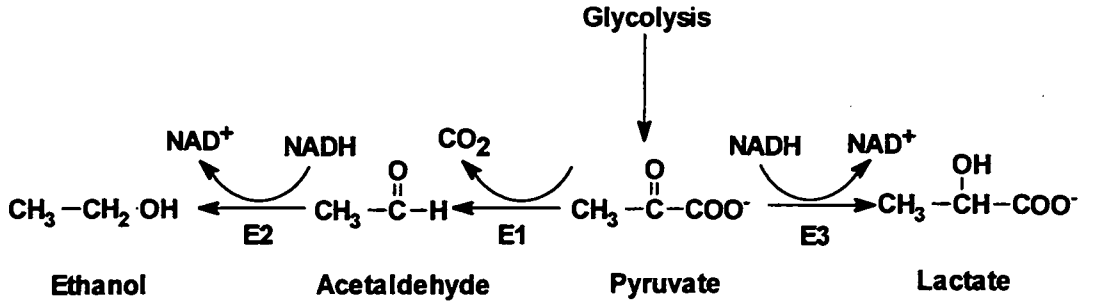

Fig. 1.5 Anaerobic fermentation. E1, pyruvate decarboxylase (PDC), E2, Alcohol dehydrogenase (ADH), E3, lactate dehydrogenase (LDH).

Acetaldehyde

Acetaldehyde occurs naturally as a plant metabolite in relatively small amounts. For example, apples contain 0.5 mg/100 g fresh weight and bananas contain about 5 mg/100 g fresh weight (Fidler, 1968), but whenever anaerobic fermentation or senescence processes play a role, the concentration of acetaldehyde rises considerably. It is believed that acetaldehyde is more cytotoxic than ethanol and its accumulation is a result, rather than cause, of tissue disorganization (Smagula and Bramlage, 1977). However the threshold levels of toxicity have not been established. Whereas the effect of acetaldehyde on fruit deterioration is not clear, other aspects of acetaldehyde on fruit quality have been elucidated. Acetaldehyde accumulation in persimmon fruit in CO_2 enriched atmospheres used to enhance ripening reportedly play a crucial role in reducing fruit astringency (Matsuo et al., 1976; Matsuo and Ito, 1977, 1982). Application of acetaldehyde vapors enhanced the sensory quality of several fruits, such as grapes (Pesis and Frenkel, 1989), pears, tomatoes, and blueberries (Paz, et al., 1982).

Ethanol

Ethanol occurs naturally in either its free form or esterified form everywhere in the plant kingdom, but in only small concentrations in germinating seeds, fruits, and root tips. Under anaerobiosis, ethanol results from reduction of acetaldehyde in the presence of NADH and the production of ethanol is accompanied by an increase in the concentration of acetaldehyde; the ratio of acetaldehyde to ethanol is often of the order of 1:100 (Fidler, 1968). Internal rates of ethanol production varied among plant cultivars and plant parts (Cossins and Beevers, 1963). Threshold levels of toxicity also vary depending upon plant parts and varieties (Jeckson, et al., 1982). Nagodawithana and Steinkraus (1976) reported that internally produced ethanol was much more toxic than when applied exogenously. Application of ethanol vapor inhibited ethylene synthesis and retarded tomato ripening without increasing the rate of ion leakage (Saltveit, 1989; Saltveit and Mencarelli, 1988).

Ethanol has been reported to be correlated with off-flavor developed in fresh fruits subjected to low levels of oxygen (Ke and Kader 1992b; Ke et al., 1991; Ke et al., 1991a; Ke et al., 1991b). Some plant tissues, including storage organs, parts of seedlings, coleoptiles, fruits, roots, stems and leaves can convert parts of added ethanol into CO_2, organic acids, amino acids, lipids, and sugars (Cossins and Beevers, 1963). Knee and Hatfield (1981) reported that apple tissues can convert applied ethanol into ethyl acetate but in small amounts. Ueda et al. (1992) reported that ethanol was predominant among alcohols present in pulp of banana and portions of those alcohols can convert into aroma esters mainly, acetate and butyrate via activity of alcohol acyl CoA transferase (syn. ester synthetase).

SOLID PHASE MICRO EXTRACTION (SPME)

Several methods have been applied for trapping volatile compounds in flavor studies. In early investigations, banana constituents were studied based on classical techniques of organic chemistry. The volatiles had to be isolated and separated by distillation and trapped in different conditions before identification by paper chromatography. By this method, many odor constituents were lost due to, volatility, degradative chemical reactions during distillation and some artifacts produced by damaged tissues. Therefore, methods applied for trapping volatiles without any damage to plant tissues is highly recommended (Tressl and Jennings, 1971).

Solid phase micro extraction (SPME) is an inexpensive, rapid and solvent-free extraction method. It can be modified for trapping plant headspace volatiles. It was first developed for isolation of organic compounds in liquid and aqueous samples by a group of researchers at the University of Waterloo, Ontario, Canada (Supelco, 1993). The SPME unit consists of a length of fused silica fiber coated with a polydimethylsiloxane phase and bound to a stainless steel plunger and a holder which looks like a microliter syringe (see appendix Fig. A 1.2). This method can reduce cost and reduce the long times required for trapping, concentrating, or extracting compounds of interest and may help avoid artifacts from concentration or sample manipulation. The most important factor involved in applying SPME is the time required to reach adsorption equilibrium which is depends mostly on distribution coefficients of the analytes and the thickness of the phase (Shirey, 1994). As mentioned by Yang and Peppard (1994), SPME is a solvent free extraction method which is sensitive to experimental conditions,

therefore any changes of experimental parameters can affect the distribution coefficient and then the adsorption rate.

SPME was used for analysing organic compounds in water (Louch, et al., 1992), determining caffeine in beverages (Hawthorne, et al., 1992) and analysing substituted benzene compounds in ground water (Arthur, et al., 1992). But this is the first time it has been used for analysing volatile headspace compounds of fruits as we are evaluating and applying in the OSU postharvest physiology lab.

CHAPTER 2

EFFECTS OF ANAEROBIC AND AEROBIC CONDITIONS ON PRODUCTION OF BANANA VOLATILE COMPOUNDS

ABSTRACT

Green-tip banana (color stage 5) and all-yellow banana (color stage 6) volatile compounds were studied under aerobic and anaerobic conditions using solid phase micro extraction (SPME). The major groups of banana volatiles consisted of the acetate and butanoate esters of butanol, isobutyl alcohol, pentanol, and isoamyl alcohol. When bananas were held at 20°C under anaerobic condition for five days, production of these volatiles were reduced by approximately 80% compared to normal aerobic conditions. Upon return to air (control conditions) at the same temperature, the volatile recovery was higher in all-yellow bananas (color stage 6) than in green-tip bananas (color stage 5). In general, flavor impact compounds of banana volatiles were suppressed under anaerobic conditions (nitrogen treatments) but production resumed when bananas were returned to air conditions. The effects of nitrogen treatments on individual volatiles were tentatively classified into 6 groups based on relative production trends under anaerobic conditions and the patterns of changes when samples were returned to the normal conditions.

INTRODUCTION

Off-flavor, developed after fruits are subjected to anaerobic conditions, is the most crucial detrimental effect limiting plants tolerance to low O_2 atmospheres required for postharvest insect quarantine treatment. Several investigators reported that this abnormal flavor resulted from accumulation of ethanol and acetaldehyde under anaerobic conditions (Ke and Kader, 1989; Ke and Kader, 1992b; Ke et al., 1991; Ke et al., 1991b; Lidster and et al., 1985). However, recent work (Kosittrakun, 1989) reported that off-flavors developed in fresh commodities (apples and pears), may result from volatile compounds other than ethanol and acetaldehyde both of which persist at high concentration even after off-flavor disappeared when fruits were returned to air.

In order to examine other volatile compounds involved in development of off-flavor, we hypothesized that the accumulation of those volatiles during conditions that cause off-flavor should be determined. However, before monitoring the volatiles accumulated in conditions that produce off-flavor, the production of volatiles under normal conditions must be determined. The objective of these studies therefore is to determine volatile constituent production trends in ripening bananas during aerobic conditions (air conditions) and under anaerobic conditions (nitrogen treatments) and to observe the accumulation of those volatiles which would be suspected to be causes of off-flavor.

Banana volatiles have been studied using different methods of extraction, separation and identification which are responsible for the various patterns of volatile constituents and production (Hultin and Proctor, 1961; Macku and Jennings, 1987; Shiota, 1993; Tressl and Jennings, 1972; Wick et al., 1969). However each of these methods has some drawbacks, thus we carried out

experiments for separation and identification of banana volatiles using our own approach employing solid phase microextraction (SPME). Macku and Jennings (1987) used fused silica capillary needles for sampling volatiles to identify seventeen volatiles from ripening bananas. They reported that the amounts of individual volatiles increased continuously until the onset of peel browning, and then either plateaued or decreased. Tressl and Jennings (1972) reported that the production of two main groups (acetate and butyrate) was in a cyclic manner but that the two cycles were out of phase. In our studies, fused silica needles were used to sample the volatile compounds which were identified by combined gas chromatography (GLC-FID) and mass spectrometry (MS). Total production of volatiles was then reported as the relative peak area based on ripening time. Banana volatiles will be discussed in the categories of 'trends of production' for total volatile, major groups (acetates, butanoates and alcohols) of banana volatiles, flavor impact compounds of bananas and finally for individual volatiles. Production patterns for volatile compounds when treated with nitrogen treatment is emphasized. The responses of bananas in different stages of ripening to anaerobic conditions will also be discussed.

MATERIALS AND METHODS

Fruits and treatments preparation

Mature fresh bananas at different stages of ripeness, green-tip (color stage 5) and all-yellow (color stage 6) were purchased from local markets before starting the experiments. Approximately one kg (six) bananas were placed in sealed 4-liter-jars closed with lids and filled with inlet and outlet tubes. The nitrogen treatments, (dynamic anaerobic conditions) was rapidly established by initially equilibrating the jars with pure nitrogen (99.99%) at a flow rate of 1500 ml/min for 25 min. The flow rate was then reduced to 100 ml/min and kept constant until the experiments ended. Flow boards and capillary tubing were used to control flow. Treatments were all started on the same day and daily measurements were taken. Air control treatments (dynamic aerobic conditions) were applied to the bananas with the same procedures but compressed air was used instead of pure nitrogen. All experiments were conducted at 20°C. After five days, nitrogen treatments (anaerobic conditions) were replaced with compressed air at the same flow rate until experiments terminated (a total of 10 days). Due to the limited time for measurement by SPME (every 12 hr.) for 4 - 6 samples, and the availability of the gas chromatograph (GLC) instrument, it was not possible to complete concurrent replications for the study. In order to confirm the results, the same experiments were repeated sequentially and the results were analyzed.

For identification of volatile compounds, bananas at the same stage and amount were placed in the closed jar and air (100 ml/min) was introduced into the jar. Activated charcoal (ORBO™-32, Supelco, Bellefonte, PA) absorption

disulfide (300 μL) was used to desorb and solubilize the volatiles from the charcoal. Finally, two μL of the sample was injected into GLC/FID and GLC/MS for analysis.

Stages of ripening and changes in color

Color changes during ripening were noted by using the color index chart developed by Stover and Simmonds (1987) (see appendix Fig. A 1.1).

Sampling technique and headspace analysis

Solid phase microextraction (SPME-Supelco, Inc., Bellefonte, PA), consists of a microliter syringe with a fused silica fiber coated with a polydimethylsiloxane phase bound to a stainless steel plunger. To use the unit, the needle including the coated fiber is inserted through the septum that seals the sample vial, then the coated fiber is exposed to the sample. Organic analytes adsorb to the phase on the fused silica. The fiber is then drawn back into the needle and finally, the needle is removed from the sample vial for introduction into the GLC injector port. Duration for headspace sample was formed by preliminary experiment to be optimum at 15 min. 10 μl of methyl hexanoate in carbon disulfide (20 mg/ml) was used as internal standard. Samples were taken every 12 hour for the first five days and then every 24 hour for the last five days, over the period of 10 days.

GLC-FID conditions

Headspace samples were analyzed with a HP5890-Series II gas chromatograph (Hewlett-Packard Co., USA), on 25m x 0.2mm (i.d.) x 0.2μm fused silica capillary column coated with Carbowax 20M (HP™-20M) equipped with flame ionization detector (FID). The signals were processed with a HP-3395 digital integrator (Hewlett-Packard Co., USA) for plotting chromatograms. Injector and detector ports were 200°C and 250°C respectively. The column was held at 60°C for the first five minutes then programmed at 5°C /min to 200°C , then held at 200°C until the end of separation. Helium carrier gas was supplied at a flow rate of 1.0 ml/min.

GLC/MS conditions

Samples were analyzed with a HP5890-Series II gas chromatograph (Hewlett-Packard Co., USA), on 60m x 0.2mm (i.d.) x 0.2μm fused silica capillary column (SUPELCOWAX™-10) equipped with a HP model 5971 Quadrapale mass spectrometer. Injector and detector ports (HP 5971 MS) were held at 200° C and 280°C respectively. The column was held at 60°C for the first five minutes then programmed at 5°C /min to 200°C , then held at 200°C until end of separation. Helium carrier gas was supplied at a flow rate of 1.0 ml/min.

CO_2 and O_2 measurement

A 1.0 ml headspace sample was withdrawn with a hypodermic plastic tuberculin syringe from the sealed jars and injected into a Carle Model 311 gas chromatograph equipped with a Molecular Sieve 5A column (2 m x 3mm O.D., 60/80 mesh) and a HayeSep R column (2m x 3 mm O.D., 80/100 mesh) at 55 °C Separated gases were detected by thermal conductivity detector (TCD). Helium carrier gas flow was 30 ml/min. The sample was injected into the column operated in series position, then switched to bypass at 70 seconds to analyze CO_2 before switching back to series again at 120 seconds for O_2 and N_2. TCD Detector responses were analyzed by a digital integrator model HP 3395 (Hewlett-Packard Co., USA).

RESULTS

Volatile compounds of ripening bananas

Production of banana volatiles was reported as the relative peak area compared to the maximum total volatile compounds present on day 4.5 and was adjusted for the fresh weight of the fruits in each treatment. There were 36 major peaks present on FID-profiles. We were able to identify approximately 24 (65 %) of those peaks by GLC-MS. Some of the identified peaks consisted of more than one compound and these are designated as combined peaks. Several peaks detected by FID were not detected by mass spectrometry; therefore the retention times (Rt.) were used to tentatively assign identities. Fig. 2.1 shows the chromatogram obtained from ripening bananas at color stage 7 and Table 2.1 lists the compounds studied in this work and their order of elution from the chromatographic column.

The total production of volatile compounds for all-yellow bananas increased with time (Fig. 2.2). A maximum level was reached at day 4.5 where the stage of ripening started to change from all-yellow (color stage 6) to yellow flecked with brown (color stage 7) and then, after a few days, volatiles declined. The same pattern was shown in experiments using green-tip bananas (Fig. 2.3) in which total volatile production increased with time. A significant increase in volatile production occurred when bananas changed from color stage 6 to color stage 7, but, differed from that of all-yellow bananas. Total volatile production increased until the last day in the experiments. While the maximum total volatiles produced by green-tip bananas occurred at approximately the same stage of ripening (color stage 6), the level was only 55% of that from all-yellow bananas.

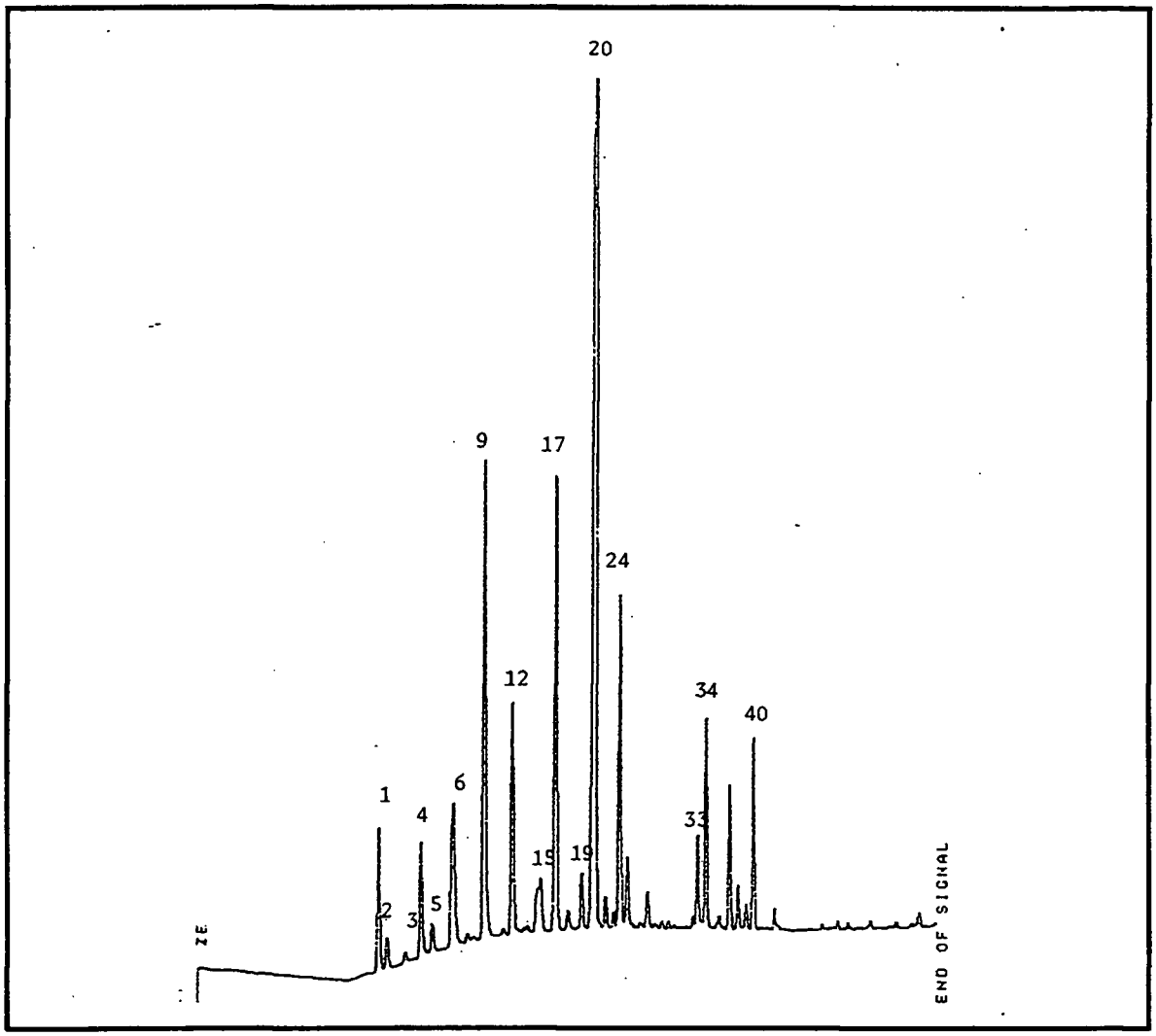

Fig. 2.1 GLC-FID chromatogram of banana volatiles at color stage 7 collected by Solid Phase Micro Extraction (SPME). Absorption time was 15 min with the flow rate of 100 ml/min at 20°C. Chromatography carried out at 60°C for the first 5 min and then programmed at 5°C / min to 200°C as described on page 35.

Table 2.1 Retention time (Rt) of banana volatiles. Identification was based upon GLC/MS matches and relative retention time of references. Volatiles were collected by SPME at 20°C, with flow rate of 100 ml/min. Capillary GLC was carried out at 60°C for the first 5 min and then programmed at 5°C /min to 200°C under condition described on page 35.

No.	Retention Time (Rt)	Compound
1	6.07	Ethyl acetate
2	6.33	Ethanol
3*	6.98	Propyl acetate
4	7.53	Isobutyl acetate
5	7.89	Ethyl butanoate
6	8.63	Butyl acetate
7	9.06	Isobutyl alcohol
8	9.28	2-Pentanol
9	9.62	Isoamyl acetate
10	9.78	1-Butanol
11	10.26	Butyl 2-methylpropanoate
12	10.56	Isobutyl butanoate
13	10.77	Pentyl acetate
14	10.94	2-Heptanone
15**	11.34	Isoamyl alcohol
17***	11.94	1-methylpropyl butanoate
18	12.38	Unidentified
19	12.84	Butyl pentanoate
20	13.23	Pentyl butanoate
21	13.43	Hexyl acetate
22	13.61	Unidentified
23	13.89	2-methylbutyl 3-methylbutanoate
24	14.06	Isoamyl isovalerate
25	14.32	Unidentified
26	14.81	Unidentified
27	15.01	Unidentified
28	15.22	Unidentified
29	15.47	Unidentified
30	15.68	Unidentified
32	16.52	Unidentified
33	16.68	1-Methylhexyl butanoate
34	16.96	Hexyl butanoate
36	17.41	Unidentified
37	17.77	Unidentified
38	18.05	Unidentified
40	18.57	4-Hexene-1-ol

* Propyl acetate + 2-Pentanone
** Isoamyl acetate + 2-methylpropyl 3-methylbutanoate
*** 1-methylpropyl butanoate + 1-methylbutyl 2-methylpropanoate

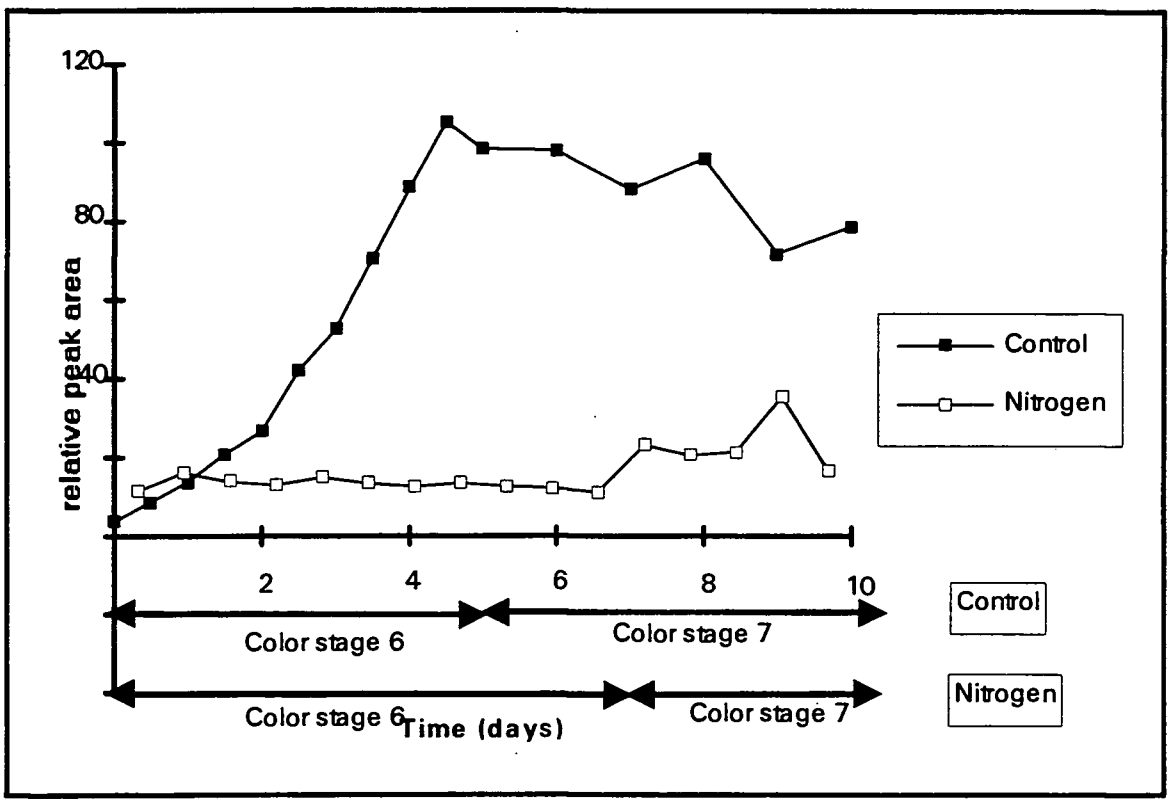

Fig. 2.2 Total volatile relative peak areas produced in air control and nitrogen treatment for bananas started from all yellow stage (color stage 6) relative to color changes during study. Volatiles were measured by SPME at 20°C. Trapping time was 15 min and bananas were sampled every 12 hours over 10 day period.

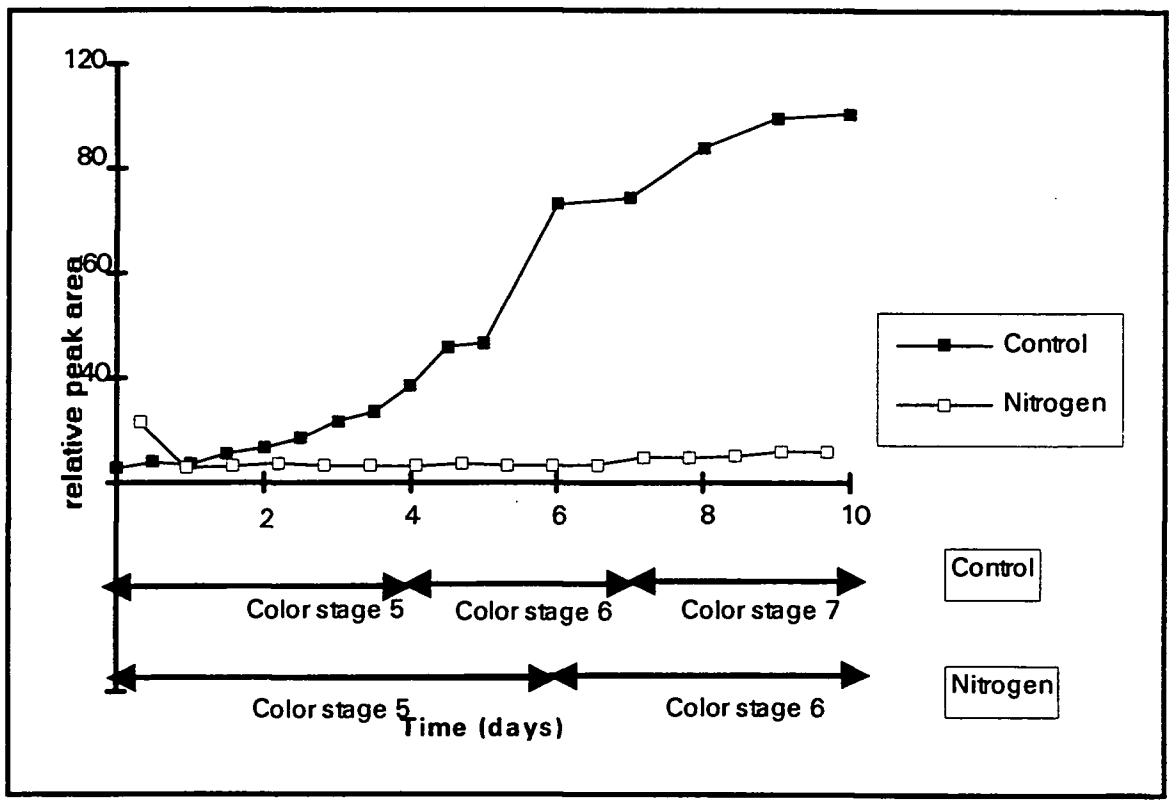

Fig. 2.3 Total volatile relative peak areas produced in air control and nitrogen treatment for bananas started from green tip stage (color stage 5) relative to color changes during study. Volatiles were measured by SPME at 20°C. Trapping time was 15 min and bananas were sampled every 12 hours over 10 day period.

Two main groups of esters are produced by aerobic all-yellow and green-tip bananas, acetates and butanoates. In general, the amounts of individual volatiles increased continuously until the onset of peel browning (color stage 7), after which they either plateaued or decreased. We will focus on the production of compounds which impact banana flavor as designed by McCarthy et al. (1963). In the acetate group, impact volatiles are butyl acetate (fruity flavor), isoamyl acetate (banana-like flavor), pentyl acetate (banana-like flavor) and hexyl acetate (fruity flavor). For unknown reasons, we detected only small amounts of hexyl acetate in our study. For butanoate esters, flavor impact compounds that can be detected by SPME are isobutyl butanoate (fruity flavor) and pentyl butanoate (banana-like flavor). Flavor impact compounds in alcohol groups are mainly responsible for green or woody flavors. In our study, 1-butanol and 2-pentanol were normally detected by SPME. Finally, a high level of ethanol, which was reported as a cause for off-flavor in other fresh fruits (Ke and Kader, 1992b; Ke et al., 1991; Ke et al., 1991b), was also detected by SPME under anaerobic conditions. Relative peak areas for total acetates and flavor impact compounds for all-yellow bananas increased with time as the fruits ripened and after reaching a maximum, production declined; while those for green-tip bananas continously increased throughout the experiments (Fig. 2.4). Trends of production for butanoates and flavor impact compounds (Fig. 2.5) were the same for both all-yellow and green-tip bananas. In this study, butyl acetate and isoamyl acetate were predominant acetate esters, whereas for butanoates butyl butanoate was the major ester. Total alcohols increased linearly with time for all-yellow and green-tip bananas (Fig. 2.6). Production of ethanol showed increasing trends while 2-pentanol showed slightly decreasing trends after 4-5 days.

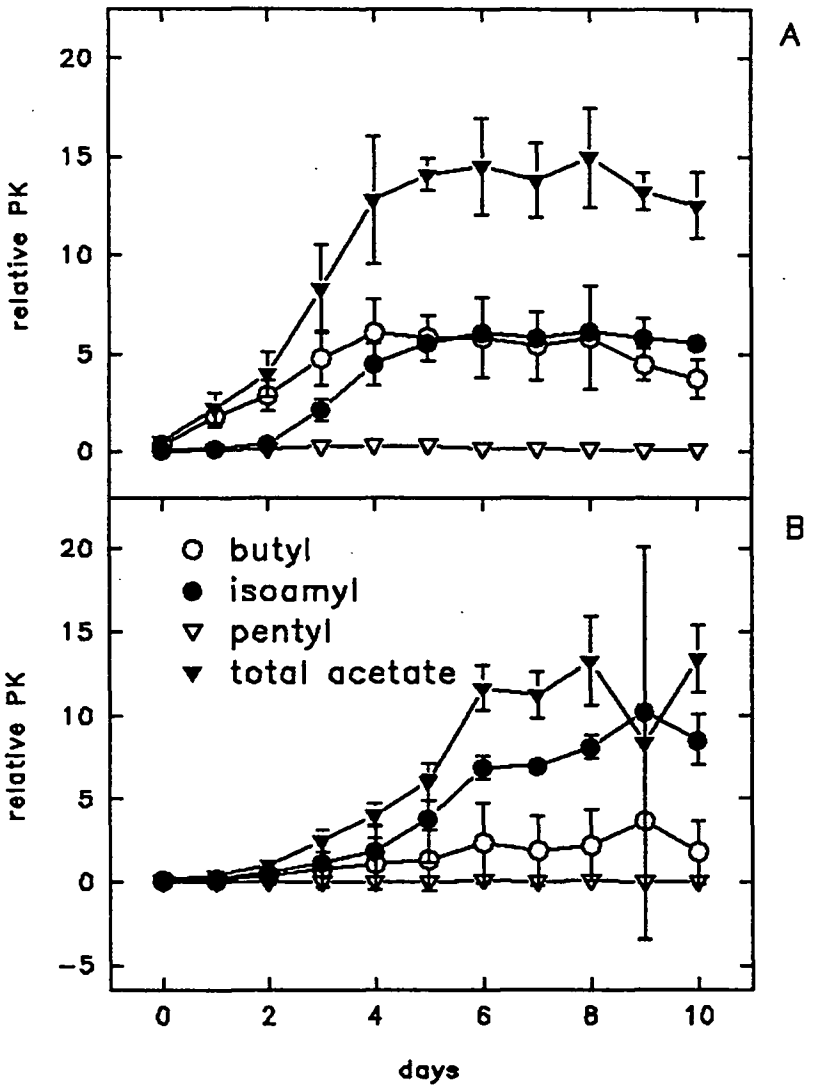

Fig. 2.4 Trends of aerobic production of impact banana volatiles (acetate esters) for all yellow bananas (A) compared to green tip bananas (B). Volatiles were measured by SPME. Trapping time was 15 min at 20°C over the period of 10 days.

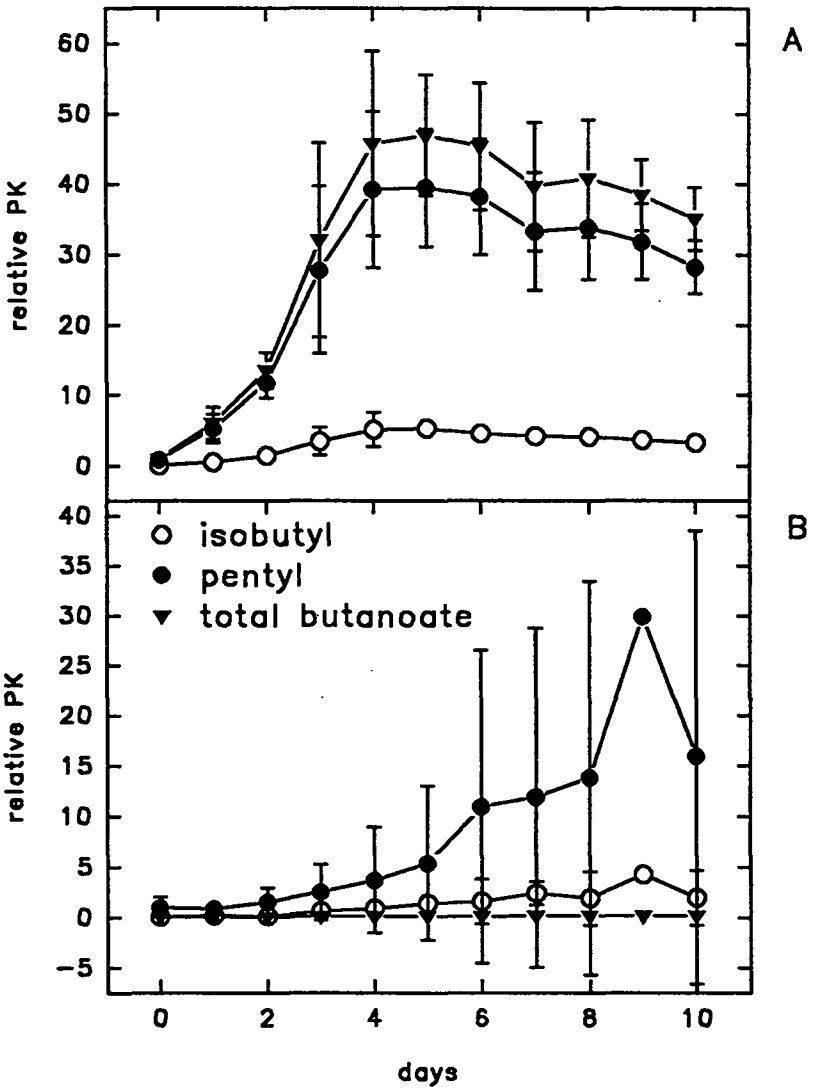

Fig. 2.5 Trends of aerobic production of impact banana volatiles (butanoate esters) for all yellow bananas (A) compared to green tip bananas (B). Volatiles were measured by SPME. Trapping time was 15 min at 20°C over the period of 10 days.

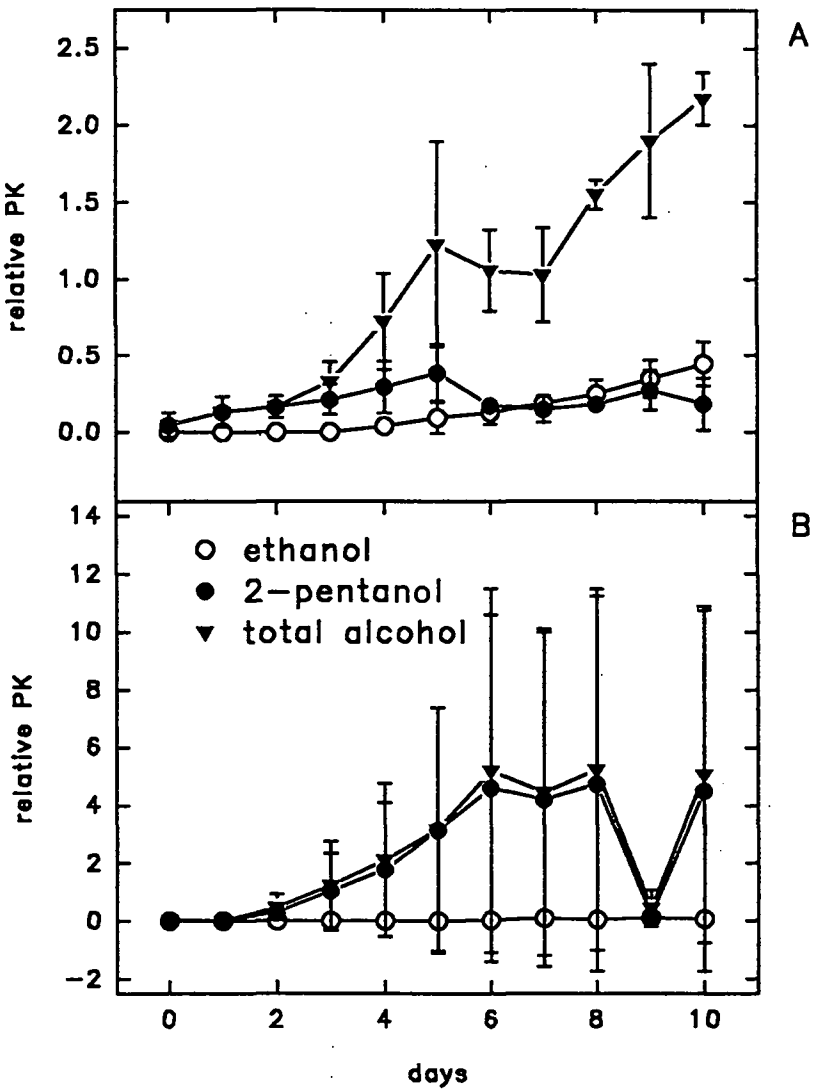

Fig. 2.6 Trends of aerobic production of impact banana volatiles (alcohols) for all yellow bananas (A) compared to green tip bananas (B). Volatiles were measured by SPME. Trapping time was 15 min at 20°C over the period of 10 days.

Effects of nitrogen treatments on banana volatiles

Effects on total volatile production

Generally, nitrogen treatments suppressed the production of most volatile compounds present under normal conditions except for alcohols (ethanol, 1-butanol and 2-pentanol), hexyl acetate and isoamyl isovalerate. Figs. 2.7, 2.8 and 2.9 show chromatograms of volatile compounds on days 0, 5 and 10, respectively for all-yellow bananas ripening in air (A) or with five days of nitrogen treatments (B). Bananas from the five days nitrogen treatments (five days) were then treated with air for an additional five days. Similar responses to nitrogen treatments were found for green-tip bananas, but the volatiles produced were less than volatiles from all-yellow bananas (see appendix Figs A 2.1 to A 2.4 for all volatile chromatograms produced by all-yellow and green-tip bananas).

To examine effects of the anaerobic conditions on volatile production, the relative peak areas of volatiles from bananas under nitrogen treatments were compared to those from bananas kept continuously in air. The volatiles produced under nitrogen treatments are reported as percent of total volatile production under air conditions (% of production). In terms of total volatile production, bananas with color stage 5 (green-tip bananas) and color stage 6 (all-yellow bananas) showed the same trend during the first five days of nitrogen treatment. Total volatile production under nitrogen treatments was approximately 12 percent of that under air. Fig. 2.10 showed results after transferring the N_2-treated bananas to air for another five days, all-yellow bananas showed a higher percent (27%) of production than did the green tip (8%).

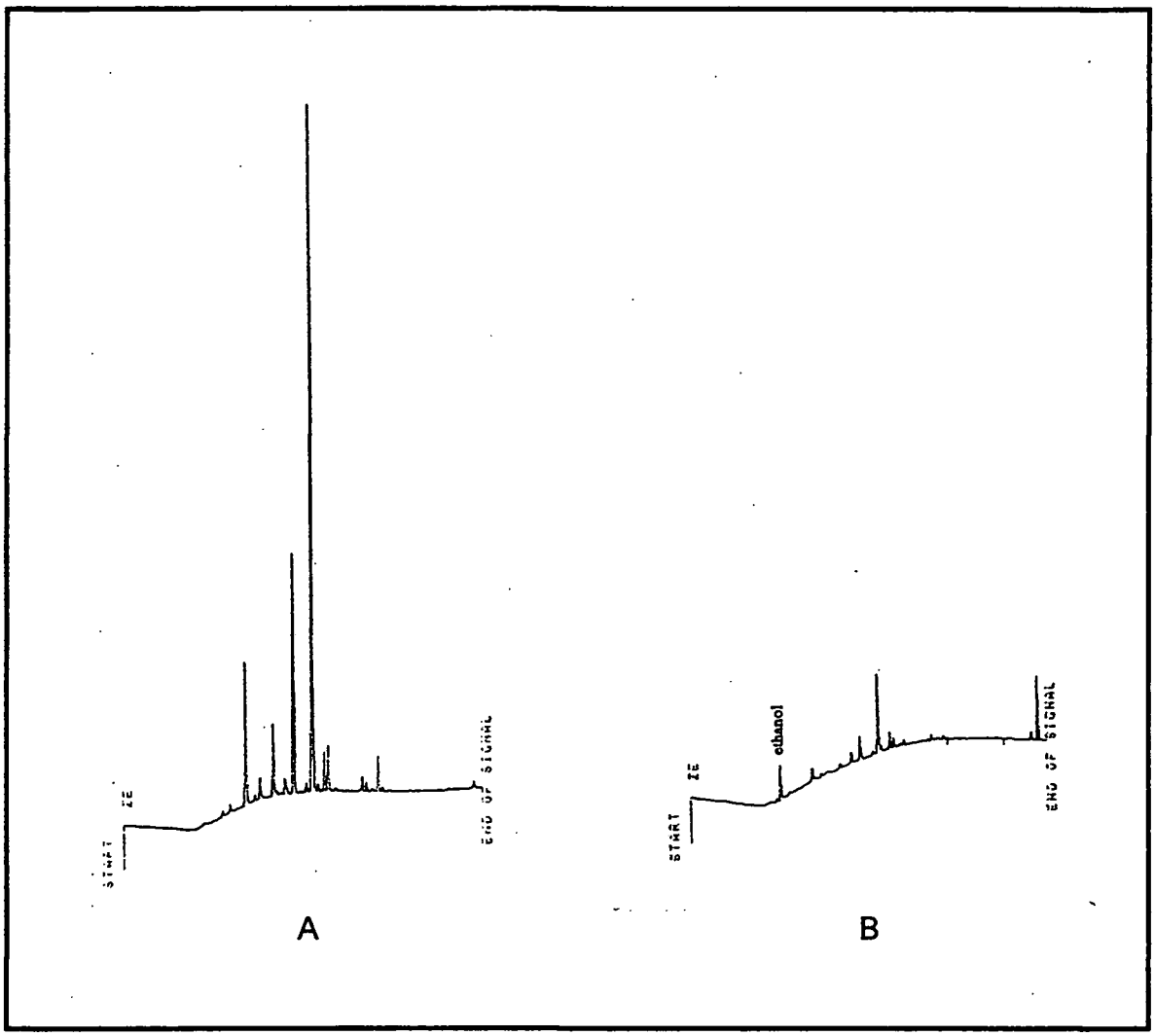

Fig. 2.7 GLC-FID chromatogram for all yellow banana volatiles at day 1 of the experiment. Chromatogram obtained from bananas under air control conditions (A) was compared to that from nitrogen treatment (B). Volatiles were collected by Solid phase microextraction (SPME) at 20°C. Chromatography carried out at 60°C for the first 5 min and then programmed at 5°C / min to 200°C.

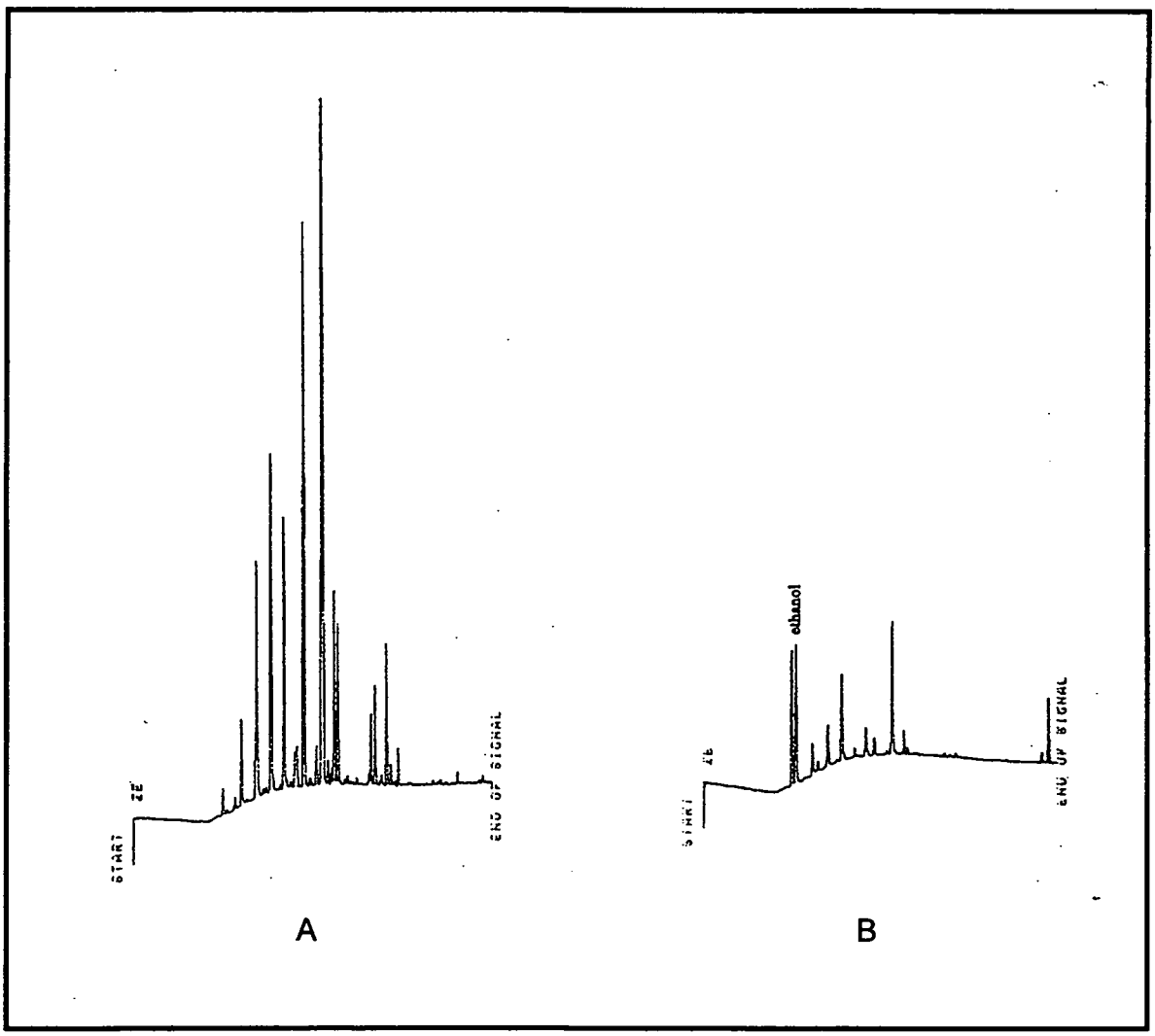

Fig. 2.8 GLC-FID chromatogram for all yellow banana volatiles at day 5 of the experiment. Chromatogram obtained from bananas under air control conditions (A) was compared to that from nitrogen treatment (B). Volatiles were collected by Solid Phase Micro Extraction (SPME) at 20°C. Chromatography carried out at 60°C for the first 5 min and then programmed at 5°C / min to 200°C.

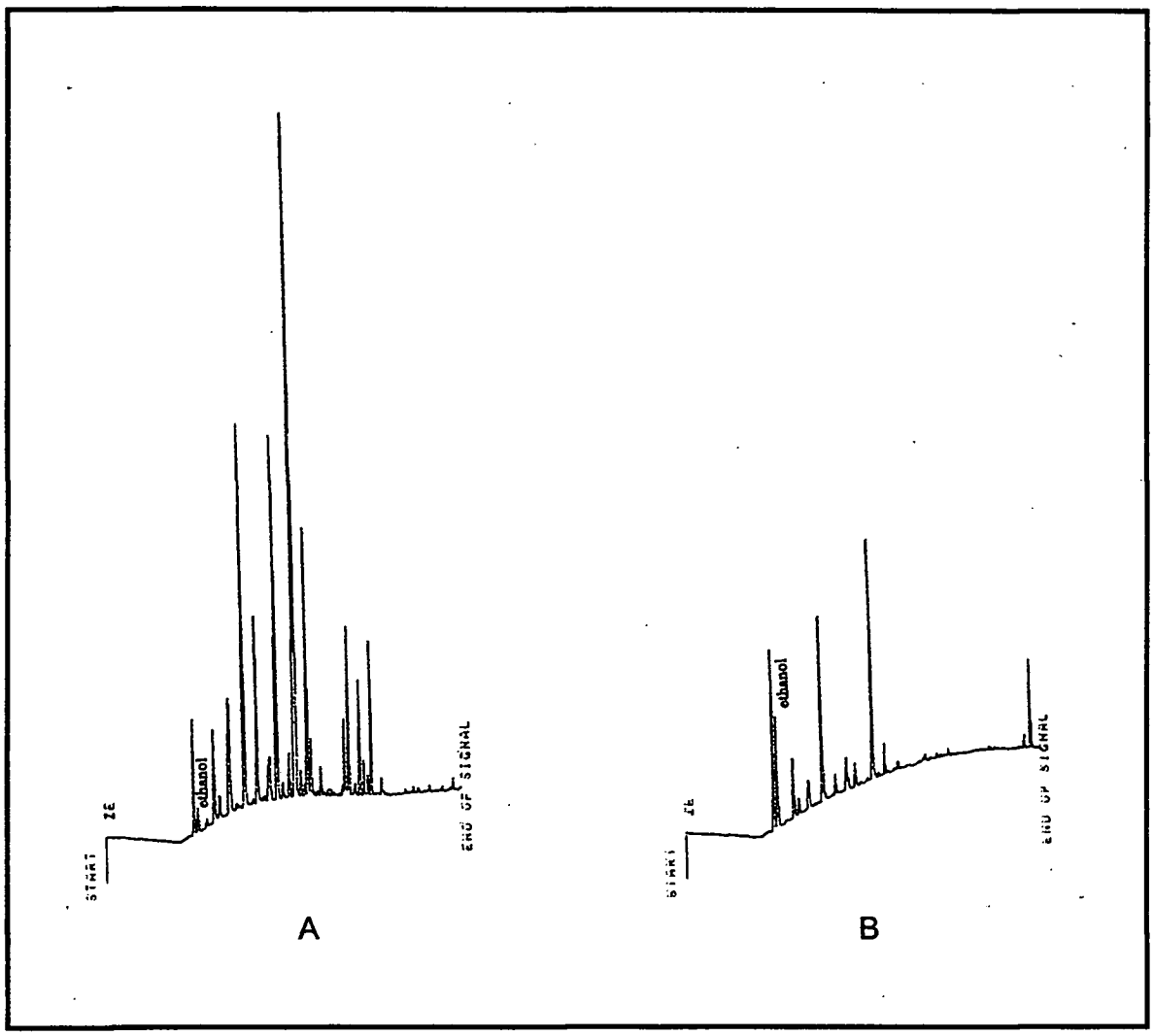

Fig. 2.9 GLC-FID chromatogram for all yellow banana volatiles at day 10 of the experiment. Chromatogram obtained from bananas under air control conditions (A) compared to 5 days under nitrogen treatment plus 5 days in air (B). Volatiles were collected by Solid Phase Micro Extraction (SPME) at 20°C. Chromatography carried out at 60°C for the first 5 min and then programmed at 5°C / min to 200°C.

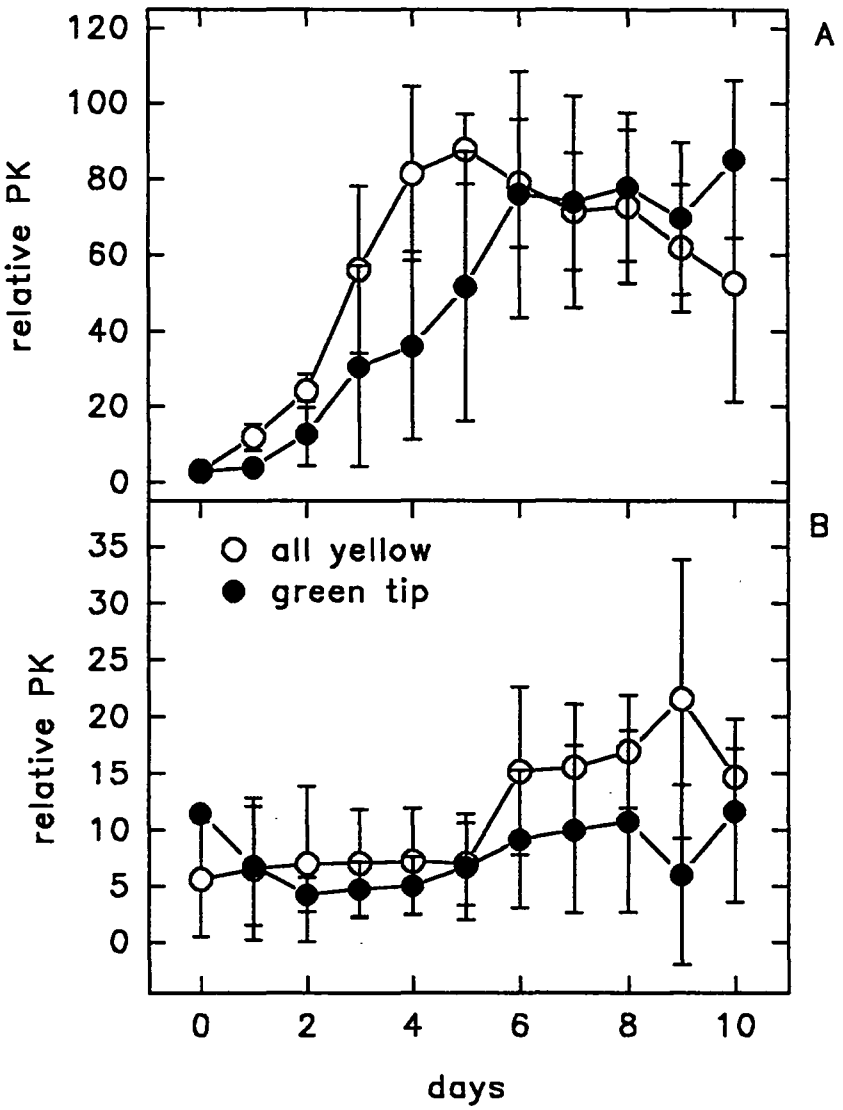

Fig. 2.10 Trends of total volatile production for all yellow bananas and green tip bananas in air control (A) compared to nitrogen treatment (B) . Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

Effects on flavor impact compounds

The effects of nitrogen atmosphere on the specific flavor impact volatiles are shown in Figs. 2.11, 2.12 and 2.13 for all-yellow bananas and Figs. 2.14, 2.15 and 2.16 for green-tip bananas. In general, after nitrogen treatments, total acetates and total butanoate esters decreased while production of alcohols increased. Production of total acetate esters and total butanoate esters was suppressed during nitrogen treatments. Production of total acetates increased dramatically the first day after return to air (Fig. 2.11 and 2.14 for all-yellow and green-tip bananas, respectively). Production of total butanoates increased after N_2 treated all-yellow bananas were returned to air (Fig. 2.12), but continuously decreased for green-tip bananas (Fig. 2.15). While acetates and butanoates were suppressed during nitrogen treatments, production of total alcohols increased (Fig. 2.13 and 2.16 for all-yellow and green-tip bananas, respectively). Production of total alcohols under air conditions was very low compared to nitrogen treatments. During the five days that bananas were kept under nitrogen, production of total alcohols for all-yellow bananas steadily increased with time and then decreased slightly when the bananas were transferred to air. The patterns of ethanol production for green-tip bananas was the same as for all-yellow bananas as production increased with time but then slightly decreased on the first day after fruits were returned to air.

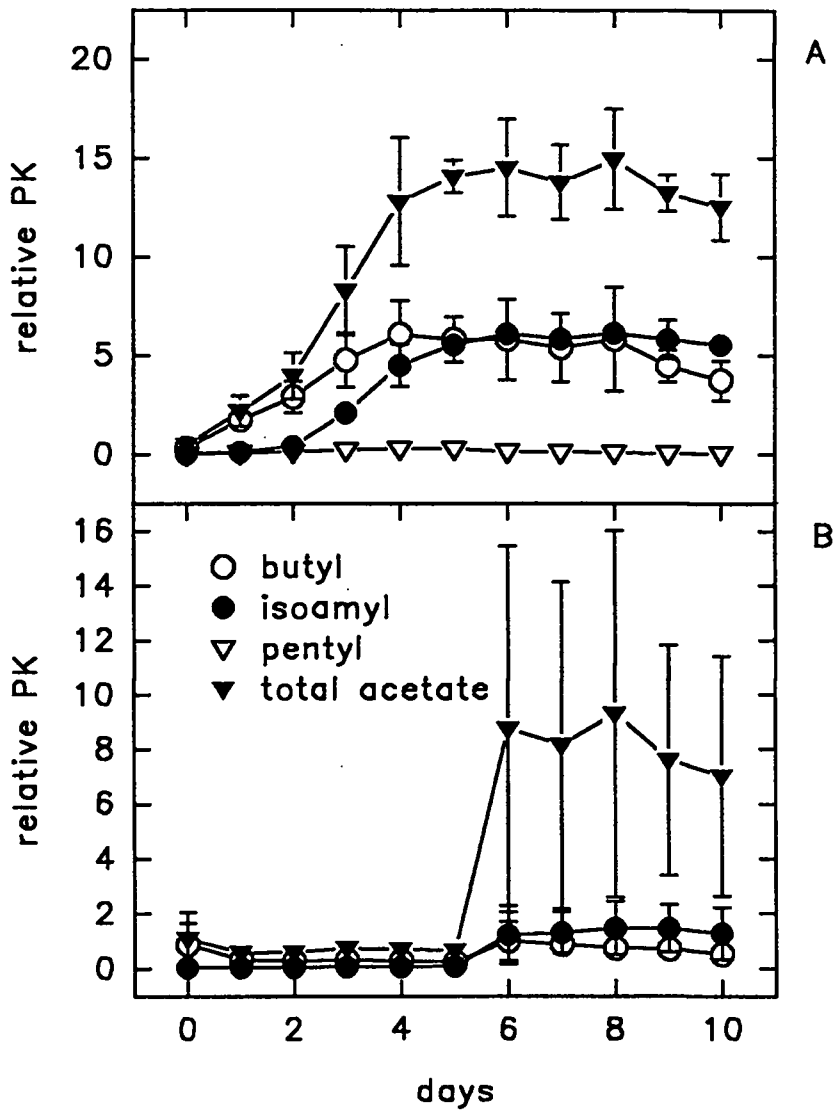

Fig. 2.11 Trends of production for total acetate esters and impact volatiles produced by all yellow bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

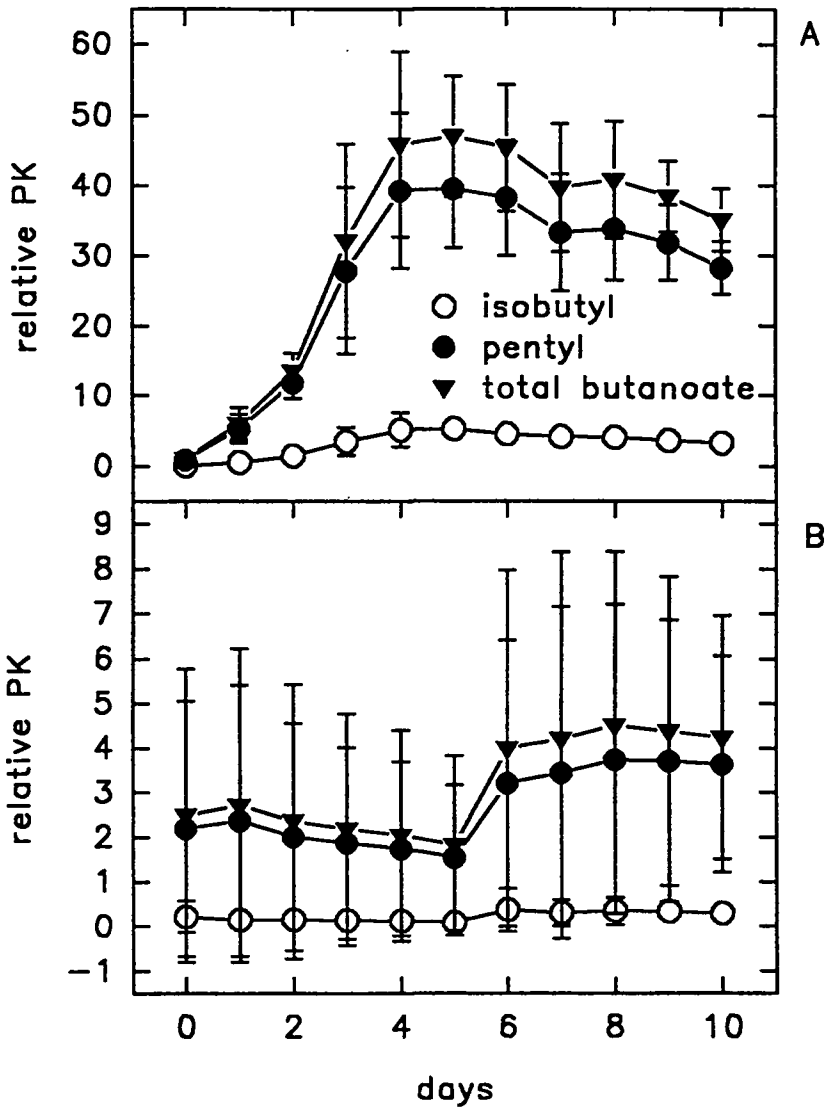

Fig. 2.12 Trends of production for total butanoate esters and impact volatiles produced by all yellow bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

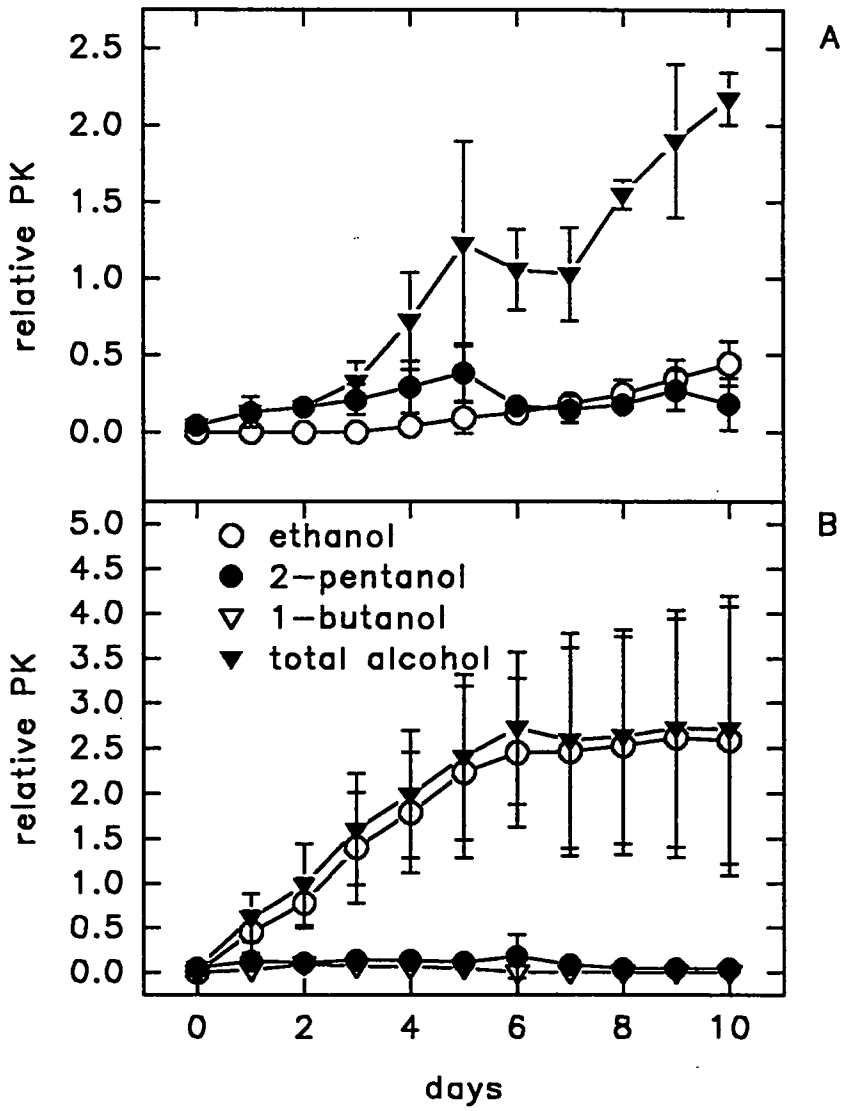

Fig. 2.13 Trends of production for total alcohols and impact volatiles produced by all yellow bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

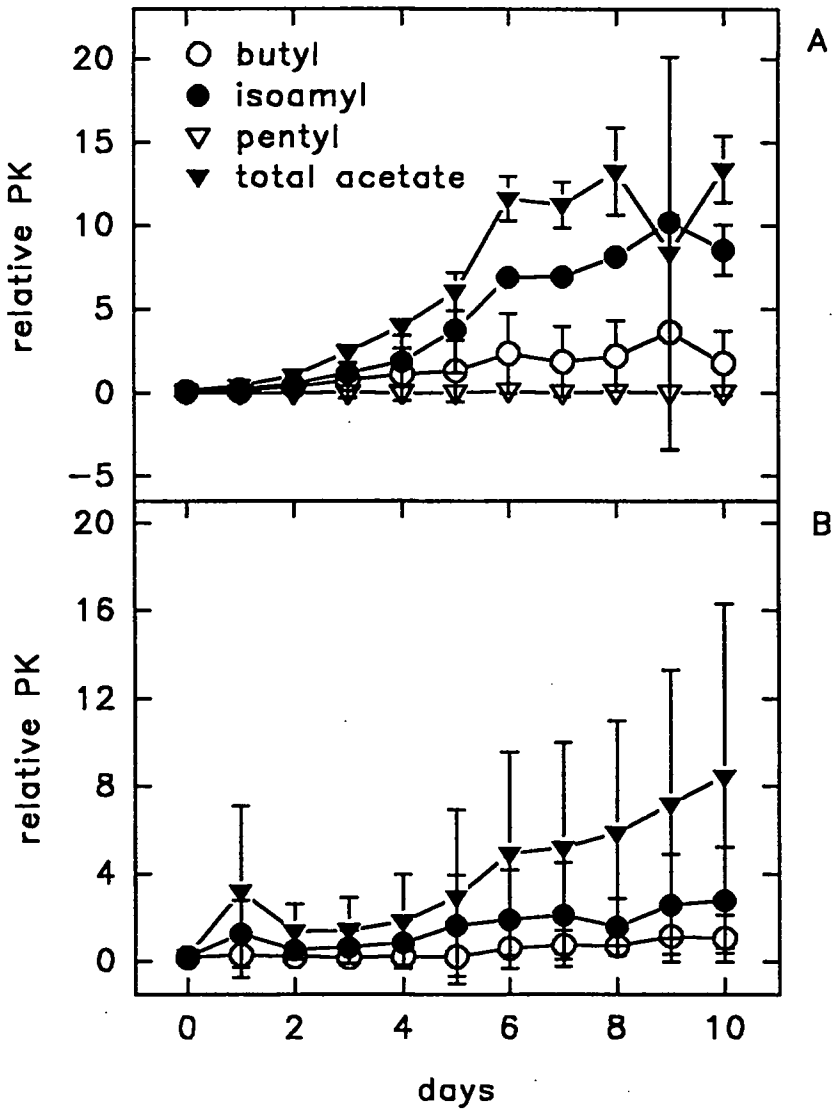

Fig. 2.14 Trends of production for total acetate esters and impact volatiles produced by green tip bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

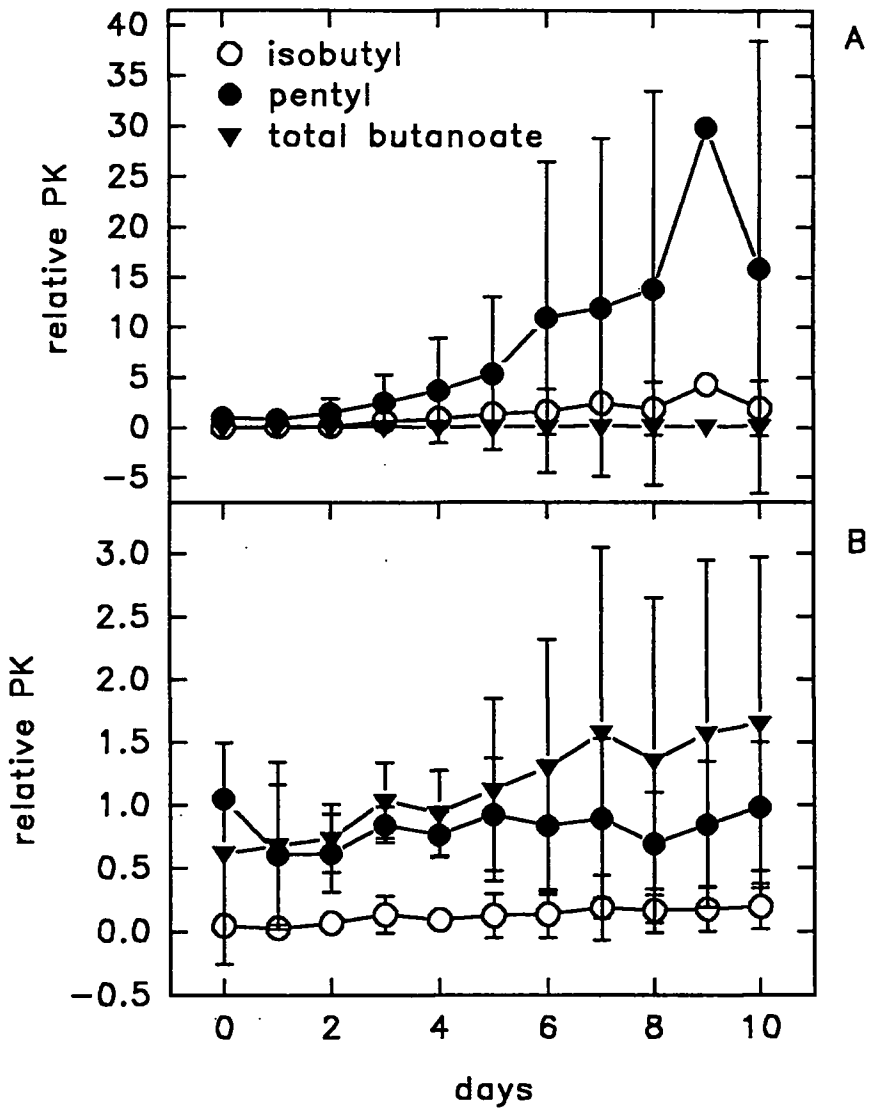

Fig. 2.15 Trends of production for total butanoate esters and impact volatiles produced by green tip bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

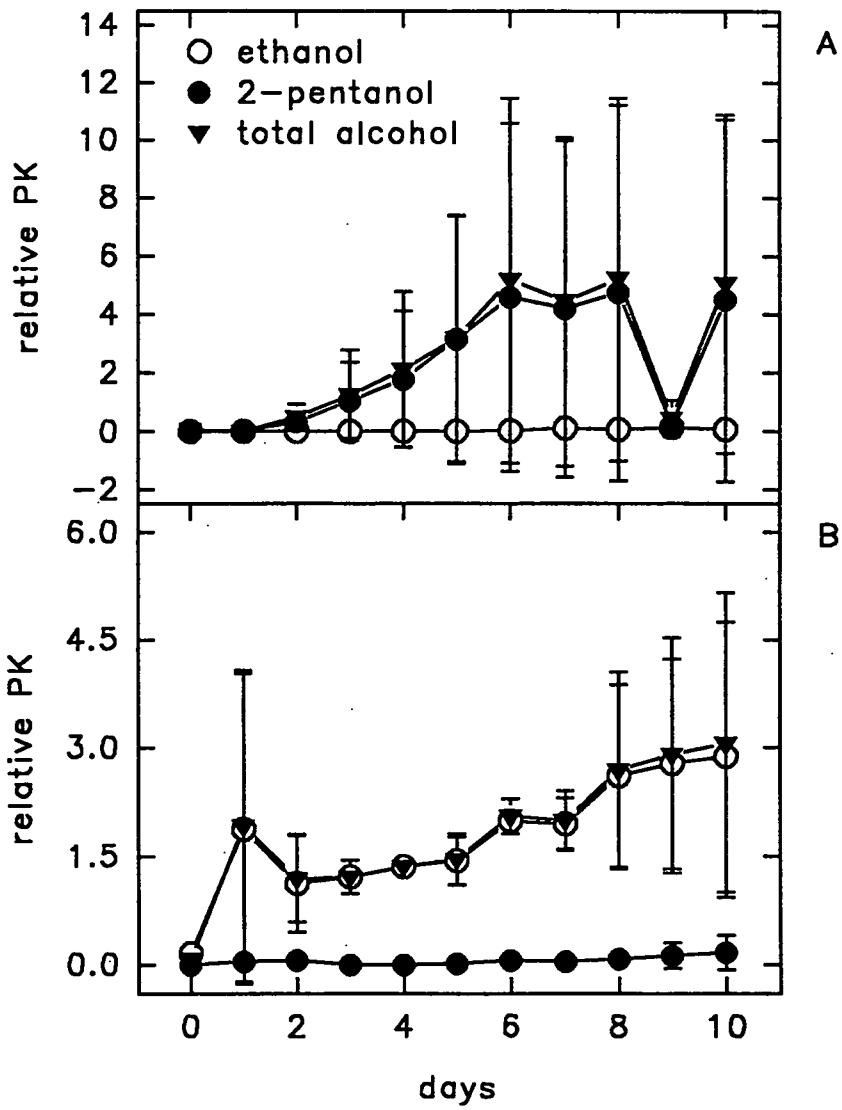

Fig. 2.16 Trends of production for total alcohols and impact volatiles produced by green tip bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

The accumulation of total alcohols under nitrogen treatments was due mainly to the accumulation of ethanol for both all-yellow and green-tip bananas. Figs. 2.17 and 2.18 show an overview of the effects of anaerobic conditions on total volatiles produced by all-yellow and green-tip bananas, respectively.

Effects on individual volatile production

The effects of anaerobic conditions on individual volatile compounds were tentatively classified into six groups (table 2.2) based on relative production trends under anaerobic conditions and after fruits were transferred to air conditions. The trends of production under anaerobic conditions were essentially the same for all-yellow and green-tip bananas. However, green-tip bananas produced less volatiles than all-yellow bananas.

Fig. 2.19 represents compounds in trend group 1, which produced no volatile compounds during nitrogen treatments, but resumed production of these compounds at trace levels after bananas were returned to air. This group consisted of isobutyl alcohol, butyl 2-methylpropanoate, pentyl acetate, 2-heptanone, 2-methylbutyl, 3-methylbutanoate, 4-hexene-1-ol and unidentified compounds at Rt. = 12.38 min, 15.22 min, and 16.52 min.

Trend group 2 (Fig. 2.20) is represented by volatiles which were produced at very low levels during nitrogen treatments but increased in production after bananas were returned to air. Some compounds increased to levels greater than those under air conditions during the first few days after fruits were returned to air and then declined. Included in this group were isobutyl acetate, butyl acetate,

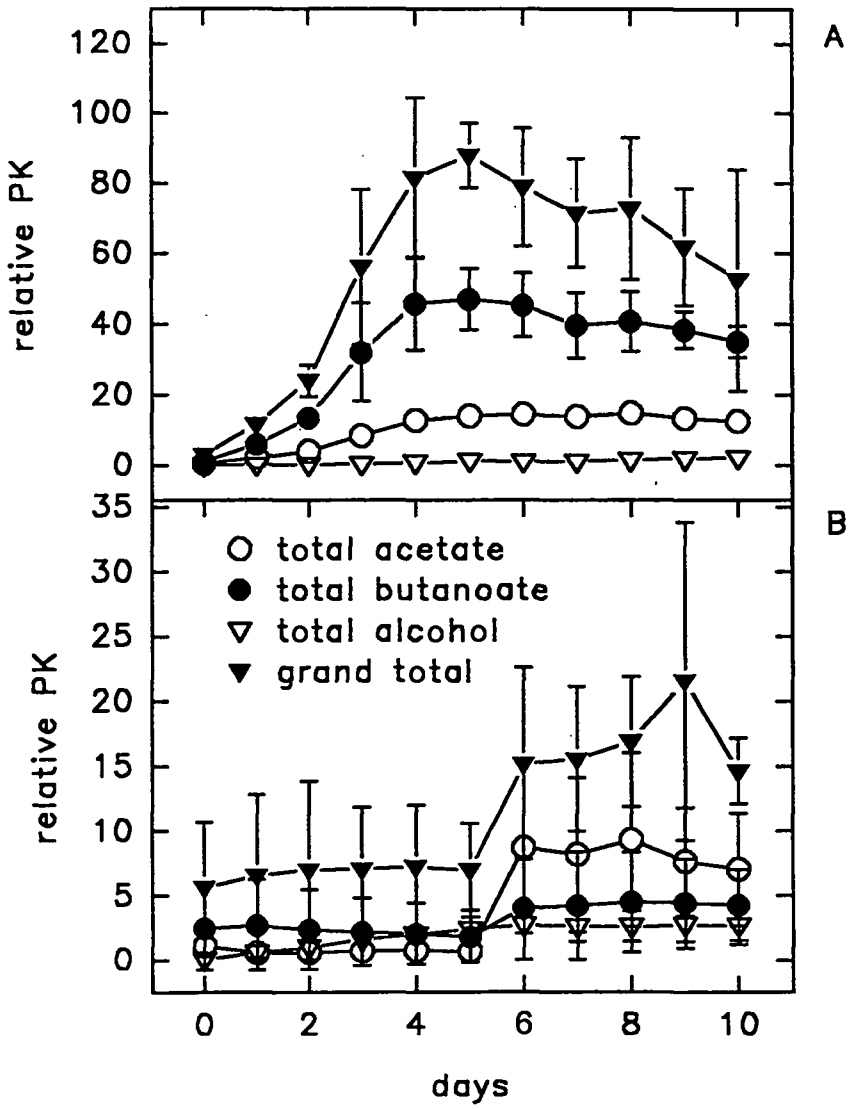

Fig. 2.17 Trends of total volatile production produced by all yellow bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured further for other 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

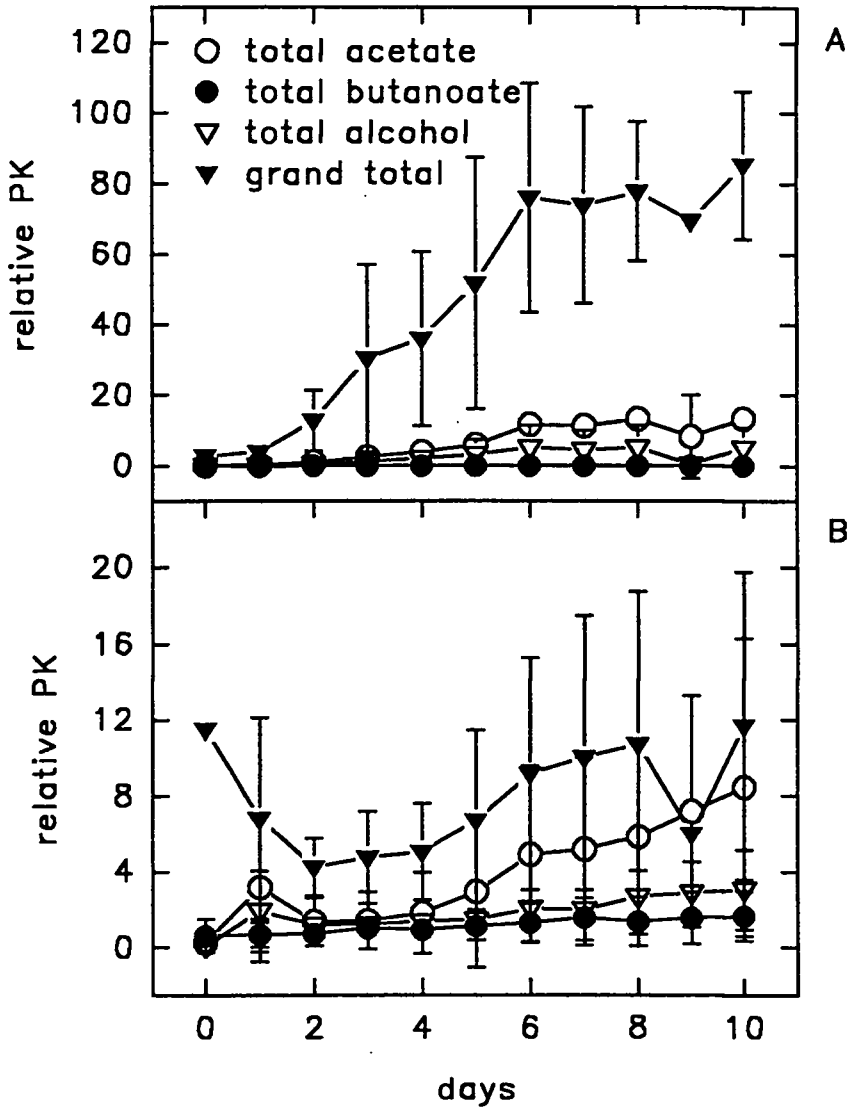

Fig. 2.18 Trends of total volatile production produced by green tip bananas under air control (A) compared to nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured further for other 5 days. All fruits were held at 20°C and measurements were taken for total of 10 days.

Table 2.2 Banana volatile compounds classified into 6 trend groups based on percent of production and patterns of changes during and after nitrogen atmosphere.

Group	Compound (Rt)
1	Isobutyl alcohol (9.06)
	Butyl 2-methylpropanoate (10.26)
	2-Heptanone (10.94)
	Pentyl acetate (10.77)
	2-methylbutyl 3-methylbutanoate (13.89)
	4-Hexene-1-ol (18.57)
	Unidentified peaks (12.38, 15.22, 15.47, 16.52)
2	Ethyl acetate (6.07)
	Isobutyl acetate (7.53)
	Ethyl butanoate (7.89)
	Butyl acetate (8.63)
	Isoamyl acetate (9.62)
	Pentyl butanoate (13.23)
3	Propyl acetate (6.98)
	Isoamyl alcohol (11.34)
	1-methylpropyl butanoate (11.94)
	Butyl pentanoate (12.84)
	Isoamyl isovalerate (14.06)
	1-Methylhexyl butanoate (16.68)
	Unidentified peak (15.01)
4	Hexyl butanoate (16.96)
	Unidentified peaks (14.32, 17.77, 18.05)
5	Ethanol (6.33)
	1-Butanol (9.78)
	Hexyl acetate (13.43)
6	2-Pentanol (9.28)
	Unidentified peaks (13.61, 14.81, 15.68)

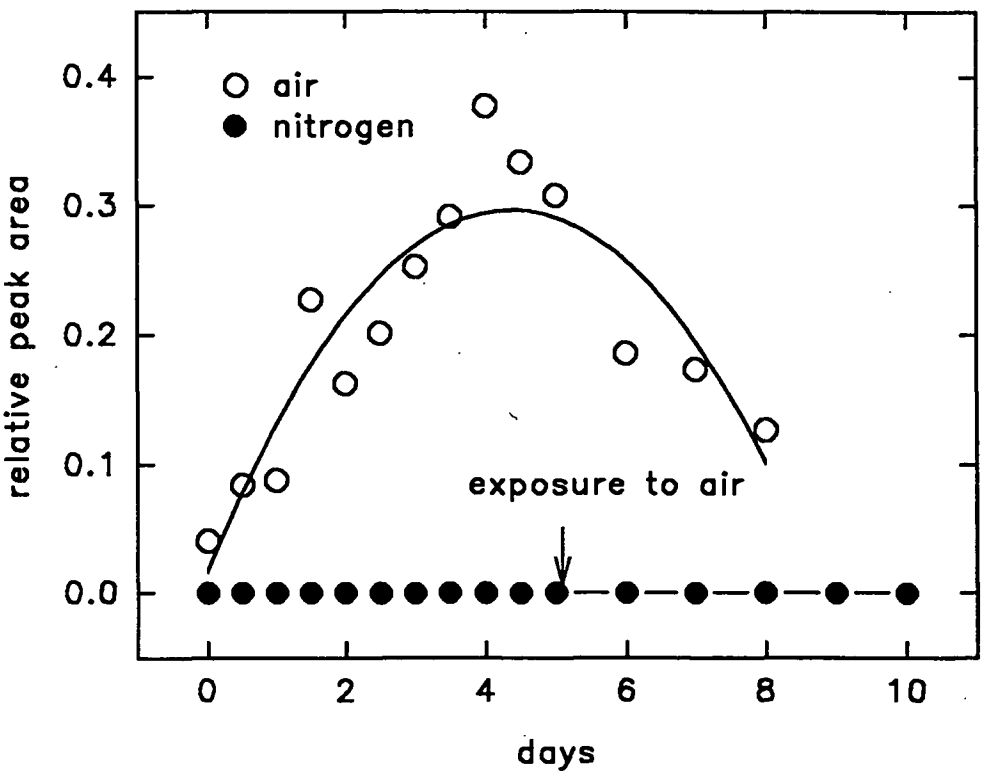

Fig. 2.19 Trend group 1: total volatile relative peak area of pentyl acetate. There was no production of this compound during nitrogen atmosphere (day 0 - day 5), with only a small amount produced after fruits were returned to air (day 6 - day 10).

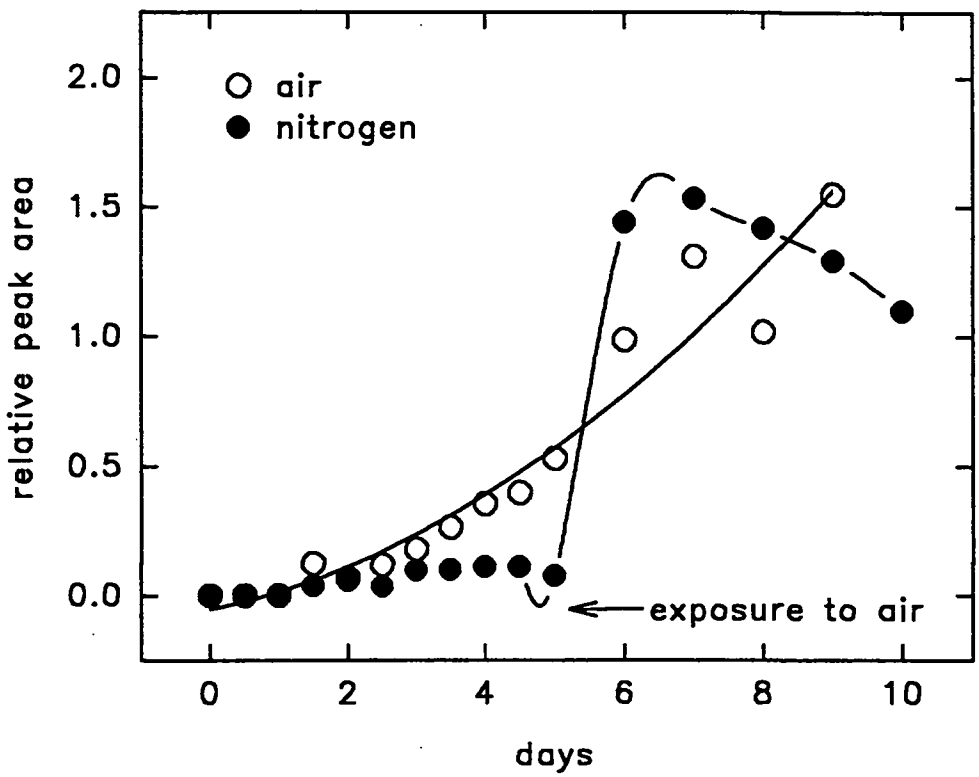

Fig. 2.20 Trend group 2: total volatile relative peak area of ethyl acetate. Ethyl acetate was produced in a small amount during nitrogen atmosphere (day 0 - day 5), but higher production occurred as bananas were returned to air (day 6 - day 10). For ethyl acetate, production increased higher than that of air control after fruits were returned to air in the first few days.

isoamyl acetate, isobutyl butanoate, ethyl acetate, ethyl butanoate, and pentyl butanoate which are mainly flavor impact compounds for banana.

Trend group 3 (Fig. 2.21.) includes those volatiles that responded to nitrogen treatments by producing levels greater than air controls for the first few days, but this level then decreased when fruits were transferred to air conditions. The following compounds were in this group: propyl acetate, isoamyl acetate, 1-methylpropyl butanoate, butyl pentanoate, isoamyl isovalerate. 1-methylhexyl butanoate and an unidentified compound at Rt. = 15.01 min.

Compounds whose production was unchanged during nitrogen treatments and remained unchanged even after fruits were transferred to air conditions were in trend group 4 (Fig. 2.22). In the constant air conditions (controls), an un-identified compound (Rt. = 14.32 min) increased to a maximum about day 4, then decreased to about 1/3 of the peak values. Compounds in this group were hexyl butanoate, and unidentified compounds at Rt. = 14.32 min, 17.41 min, 17.77 min. and 18.05 min.

The expected effect of nitrogen treatment appeared in banana volatile compounds classified as trend group 5 (Fig. 2.23). Ethanol represents a typical example in this group. The accumulation of ethanol was obvious in bananas treated with a nitrogen treatments for five days and was strongly linear with time (R^2 = 0.99). After fruits were returned to air, a slight decrease in ethanol production occurred within the first few days but high levels of production continued until the experiment was terminated. Other compounds in this group were 1-butanol and hexyl acetate. Both of these were present at very low levels only when samples were kept in nitrogen, but were not detected by SPME after bananas were returned to air.

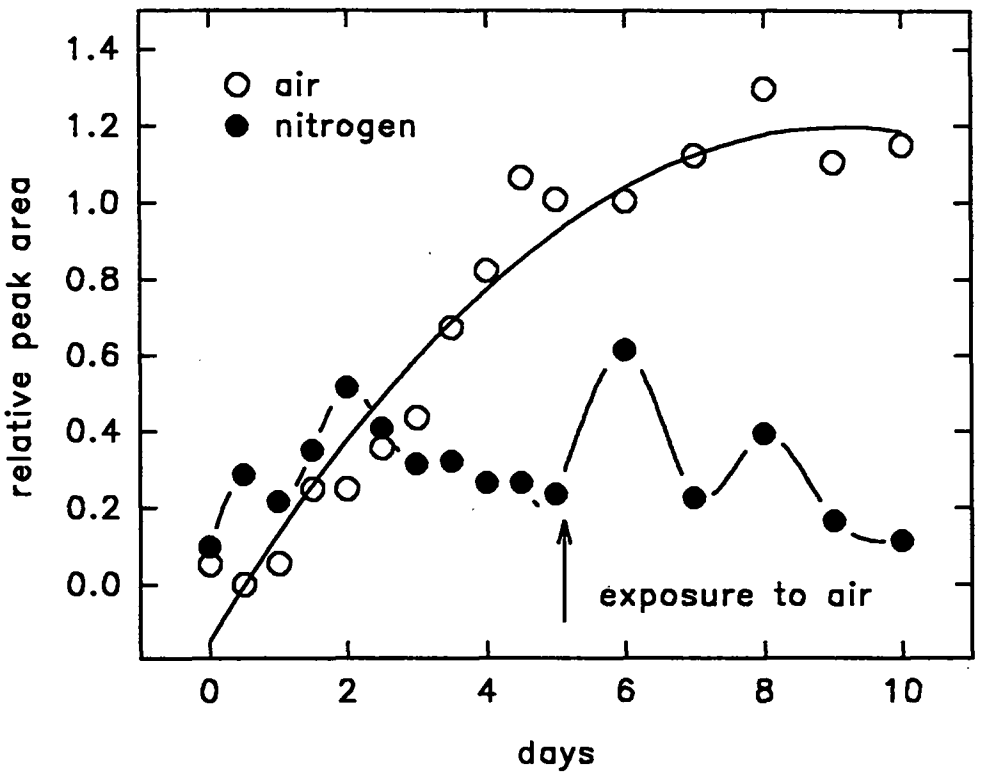

Fig. 2.21 Trend group 3: total volatile relative peak area of butyl pentanoate. The production of this compound during nitrogen atmosphere (day 0 - day 5), exceed that in air control for the first few days, then decreased even after fruits were returned to air (day 6 - day 10)

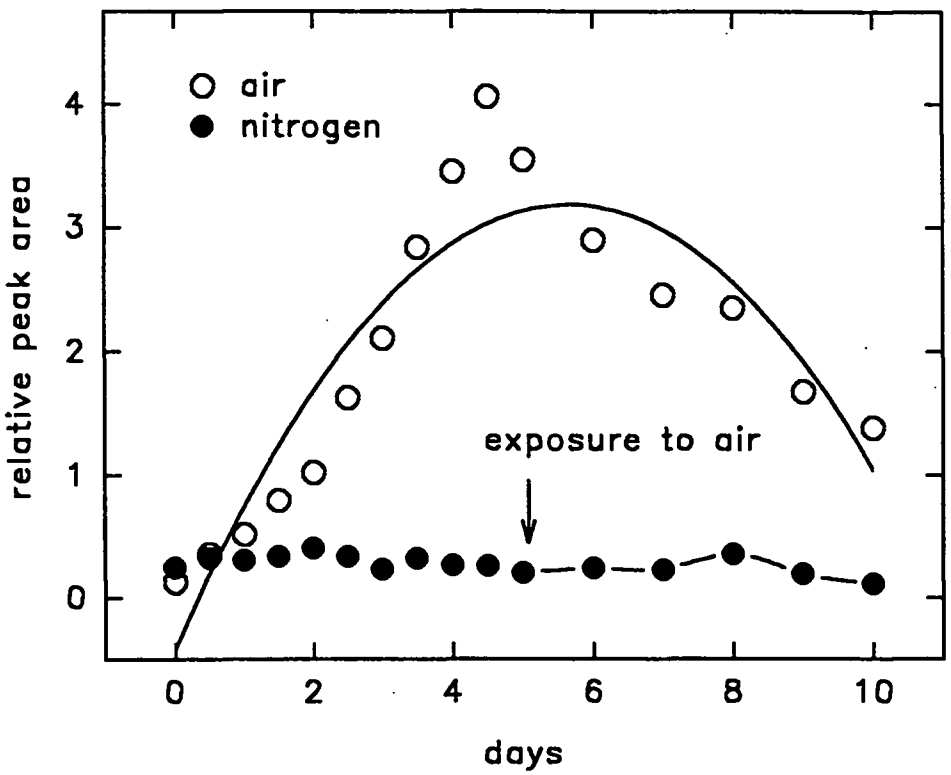

Fig. 2.22 Trend group 4: total volatile relative peak area of unidentified peak at Rt = 14.32 min. The production was constant during nitrogen atmosphere (day 0 - day 5), and upon return to air (day 6 - day 10). Air control showed an interesting rise to a maximum, then steady declined after day 5.

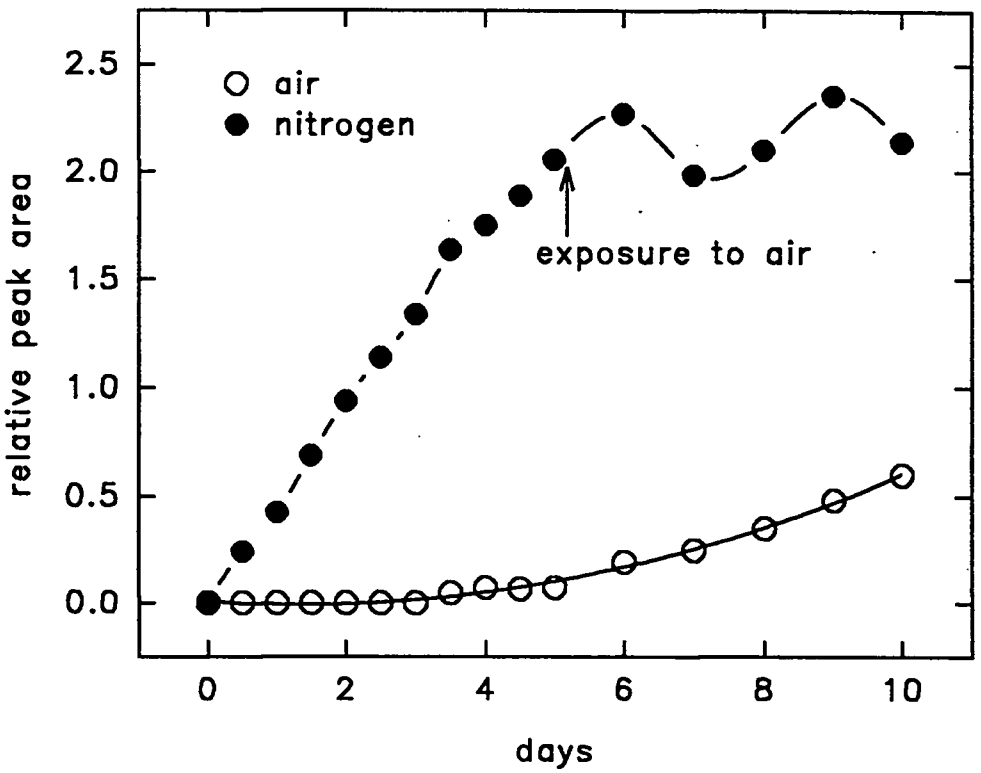

Fig. 2.23 Trend group 5: total volatile relative peak area of ethanol. The accumulation of this compound dramatically increased during nitrogen atmosphere (day 0 - day 5), and still maintained production after return to air (day 6 - day 10). Air control showed little production for the first 5 days, then slightly increased to day 10.

Trend group 6 (Fig. 2.24) is represented by compounds which were produced at the same level under nitrogen as under air controls. A consistent pattern of volatile production continued until the last day of nitrogen treatments. When fruits were returned to air, volatile production decreased and in some cases ceased. 2-pentanol and unidentified compounds at Rt. = 13.61 min, 14.81 min and 15.68 min were in this last group.

We were most interested in treatment, which resulted in increased levels of volatiles which were expected to correlate with off-flavor characteristics in later studies. Very few volatiles increased their production under nitrogen treatments. These included three alcohols (ethanol, 2-pentanol, and 1-butanol), one acetate ester (hexyl acetate), and one isovalerate ester (isoamyl isovalerate). Compounds such as 1-butanol and hexyl acetate were present only under nitrogen treatments and were absent later when bananas were returned to air. Another alcohol, isobutyl alcohol was present only in the air conditions and was never present under nitrogen treatments.

CO_2 and O_2 concentration

Table 2.3 shows means and standard deviations of carbon dioxide, oxygen and nitrogen data (from 4 replications) in this study. Under anaerobic conditions (nitrogen treatment) oxygen was controlled at or below 0.2%. The accumulation of carbon dioxide, as a result of anaerobic respiration, was flushed out by nitrogen and maintained at or below 0.017%. After returning fruits to air conditions, percent of oxygen was re-equilibrated at the normal conditions (21.35%).

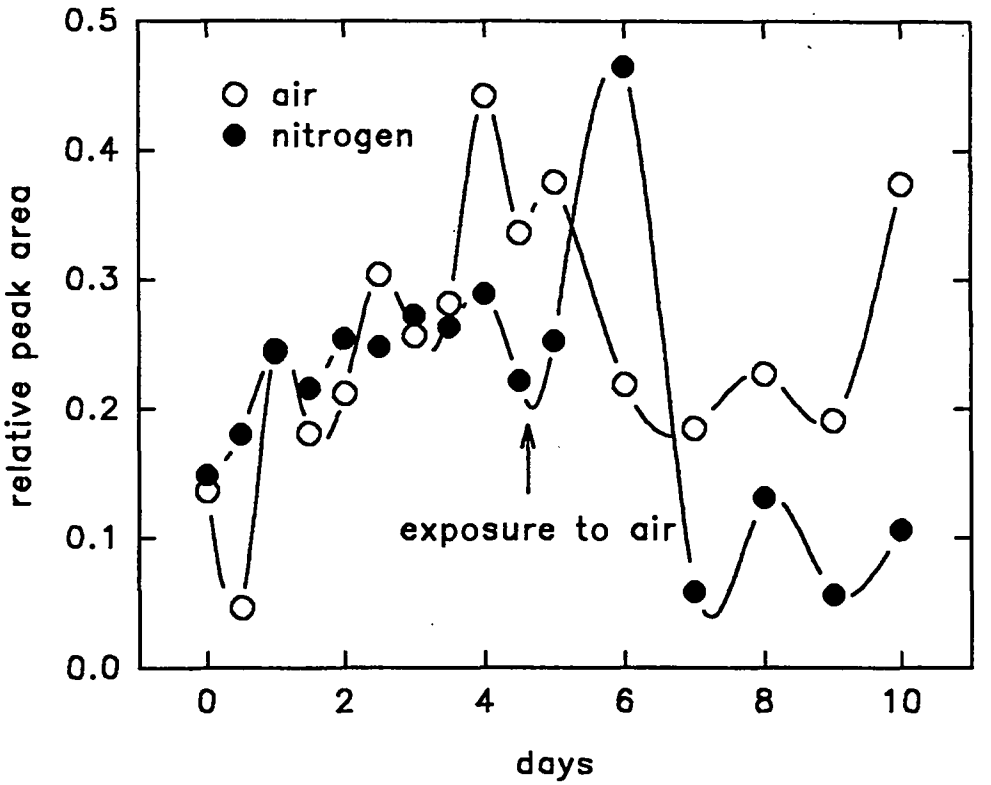

Fig. 2.24 Trend group 6: total volatile relative peak area of 2-pentanol. Production of these volatile compounds were almost the same as those from the control during nitrogen atmosphere (day 0 - day 5), and only slightly declined after fruits were returned to air (day 6 - day 10).

Table 2.3 Means and standard deviations of percent of CO_2, O_2, and N_2 present during bananas treated with nitrogen atmosphere for 5 days (day 0 - day 5) and after returned fruits back to air for other 5 days (day 6 - 10).

Day	% CO_2 (STD)	% O_2 (STD)	% N_2 (STD)
0	0.023 (0.003)	0.124 (0.073)	99.451 (1.387)
1	0.018 (0.002)	0.151 (0.156)	99.373 (1.804)
2	0.018 (0.003)	0.065 (0.020)	98.668 (2.808)
3	0.017 (0.002)	0.150 (0.081)	97.164 (0.812)
4	0.015 (0.002)	0.069 (0.042)	97.215 (1.633)
5	0.013 (0.001)	0.169 (0.042)	99.456 (2.754)
6	0.020 (0.001)	20.860 (0.397)	77.178 (1.498)
7	0.018 (0.002)	21.498 (0.083)	79.382 (0.181)
8	0.018 (0.002)	21.430 (0.157)	79.359 (0.937)
9	0.017 (0.004)	21.096 (0.047)	76.932 (1.096)
10	0.018 (0.003)	21.904 (0.728)	80.743 (3.443)
Mean in N2 treatment	0.017 (0.002)	0.121 (0.069)	98.55 (1.86)
Mean upon return to air	0.018 (0.002)	21.35 (0.282)	78.72 (1.431)

DISCUSSION

Banana volatiles

As previously mentioned because of time and instrument limitations, the replicated experiments for volatile production were conducted sequentially. There were two main groups of esters produced by the bananas in this study, acetates and butanoats. Due to the anaerobic conditions (nitrogen treatments) that we set for our experiments, alcohols were the other predominant volatiles formed in this study.

Banana volatiles have been reported to include up to 350 compounds (Tressl et al., as quoted by Macku and Jennings, 1987), but the number varies due to sampling and separation methods (Hultin and Proctor, 1961; Macku and Jennings, 1987; McCarthy et al., 1963; Shiota, 1993; Tressl and Jennings, 1972; Wick et al., 1969). Differences in methodology yield different results in the volatile composition of ripening bananas. Tressl and Jennings (1972) used Porapak Q, and obtained 13 identified compounds while Macku and Jennings (1987) reported 17 compounds separated by fused silica capillary needles. Recently, Shiota (1993) extracted banana volatiles with a mixture of distilled pentane and dichloromethane and identified eighty six compounds out of 152 separated components. Our study used a method similar to that of Macku and Jennings (1987), but the adsorbent material coated on the needle was the different.We obtained separation of 36 major components of banana volatiles. However, with the limitation of the quality of separation, and the fact that some peaks consist of more than one compound, 24 of those components were unambiquously identified.

Results from all experiments which were conducted at different times shows total volatile production under air was significantly differrnt from that in the nitrogen treatment. After fruits were returned to air for 3 days, anaerobic production of total volatile compounds was still greater than that from nitrogen treatments. However, by day 9 and day 10, production of volatiles under anaerobic conditions had caught up with volatiles produced under constant aerobic conditions.

Because they started from different stages of ripening, all-yellow bananas (color stage 6) and green-tip bananas (color stage 5), produced greater quantities of volatiles at the end of the experiments. Total volatile production eventually decreased for all-yellow bananas, but continuously increased for green-tip bananas. These results are likely due to senescence in all-yellow bananas at the end of experiment where the peel became dark brown in color and the pulp had a musty flavor. This is normally considered to be after the edible stage (Macku and Jennings, 1987). The main groups of banana volatiles (e.g., acetates, butanoates and alcohols) were similar to those reported by Macku and Jennings (1987). Generally, individual compounds continuously increased until the onset of peel brown spots, then either leveled off or decreased, but quantitative results for individual compounds were different. For instance, other authors reported acetate esters as predominant among volatiles, but our study showed that butanoate esters were the major banana volatiles detected by SPME. This finding may be due to the sensitivity of SPME based on the thickness of the coating materials (Louch et al., 1992; Shirey, 1994). The cyclic manner of production reported for acetate esters and butanoate esters (Tressl and Jennings, 1972), was not evident in our study. These findings support the fact that separation method is absolutely critical as was mentioned

support the fact that separation method is absolutely critical as was mentioned in several papers (Hultin and Proctors, 1961; Macku and Jennings, 1987; Tressl and Jennings, 1972; Wick et al., 1969).

Effects of anaerobic conditions on volatile production

As several investigators reported, off-flavor is an important detrimental effect of ethanol accumulation under anaerobic conditions (Ke and Kader, 1990; Ke et al., 1991; Ke et al., 1991; Ke et al., 1991a). However, none of the above authors studied total volatile compounds produced under specific anaerobic conditions.

In this study, total volatiles decreased for bananas held under anaerobic conditions (nitrogen treatments). Nitrogen treatments had a greater effect on green-tip bananas than on all-yellow bananas (the lower percentage of volatile production, upon return to air, from green-tip bananas than from all-yellow bananas). Production of flavor impact compounds for both acetate and butanoate groups were suppressed by nitrogen treatment, but production resumed when bananas were transferred to air. The recovery of volatile production when bananas were returned to air seemed to be greater for acetate esters than butanoate esters. This implied that the anaerobic conditions may have longer lasting residual effects on butanoate esters than acetate esters. Different effects were shown for alcohol production. Ethanol, accounting for about 90% of total alcohols, accumulated during the nitrogen treatments. Under nitrogen treatments, isobutyl alcohol and 4-hexene-1-ol were not produced, while 1-butanol was present only in small amounts.

group 1 (e.g., 2-pentanol) where this compound was not produced even after fruits were transferred to air. Almost all flavor impact compounds, i. e. isoamyl acetate, pentyl butanoate, butyl acetate, butyl butanoate, were suppressed only in the nitrogen treatments, but resumed production when fruits were returned to air, even though the production upon return to air was less than those for bananas in continuous air. This may have residual effects on loss in fruit flavor. Production of hexyl acetate, with a characteristic "fruity" attribute (McCarthy et al., 1963) was detected only in small amounts during the nitrogen treatment. Among the six groups of nitrogen effects, ethanol was the most interesting compound. Not only did it produce off-flavor (Ke and Kader, 1990; Ke et al., 1991; Ke et al., 1991a; Ke et al., 1991b; Ke and Kader, 1992b) but it was easily observed to accumulate as detected by SPME. Several papers reported that both ethanol and acetaldehyde normally accumulated during anaerobic conditions and were responsible for off-flavor in fresh fruits (Fidler, 1968; Ke and Kader, 1990; Ke et al., 1991a; Smagula and Bramlage, 1977), but with SPME, acetaldehyde could not be detected. A possible explanation is that its production was below the level of detection for either SPME or GLC. Ke et al. (1991b) reported that the range of ethanol levels are typically 100 times higher than that of acetaldehyde.

Several external factors are involved in fruit tolerance to insecticidal CA. For instance, O_2 and CO_2 concentration, temperature during storage and temperature after returning the fruits to air. Ke and Kader (1990) reported effects of temperature on ethanol content in oranges. He mentioned that ethanol accumulation increased after transferring fruits from 0.02% O_2, 5°C to air, 10°C where oranges had higher respiration rates and probably maintained a relatively high anaerobic respiration that resulted in increased ethanol content. These findings may explain the high level of ethanol accumulation in this study after

transferring the bananas to air conditions at 20°C. Although low temperature has been reported to prolong fruit tolerance to low oxygen atmospheres (Ke and Kader, 1990; Ke and Kader, 1992b; Ke et al., 1991a; Ke et al., 1991b) temperatures below about 12°C (USDA, 1986) can cause chilling injury in bananas. In this study, at low temperatures, such as 13 - 15 °C, there was less volatile production (data not shown). Therefore, keeping bananas at 20°C may be a more suitable condition for studying banana volatiles. Mattei (1979) reported that production of banana volatiles was exponential with temperature between 20-35°C and the maximum production occurred at about 25°C.

In conclusion, while this study may lack some concurrent replications due to the scheduling limitations of the equipment, the results can be discussed in terms of volatile production trends. Bananas from two subsequent experiments showed the same trends for volatile production. Production of flavor impact volatile compounds by bananas in nitrogen ceased or decreased but resumed when bananas were returned to air conditions. The effects of nitrogen treatments on individual compounds were tentatively classified into six groups based on relative production and the patterns of those compounds produced after bananas were transferred to air. Compounds accumulated during the nitrogen treatments were mainly ethanol, 1-butanol, and hexyl acetate. Among those compounds, ethanol was the dominant volatile. Hexyl acetate and 1-butanol was accumulated in small amounts when compared to production of ethanol. In our study, ethanol was the only compound that accumulated during nitrogen treatments and appears to correlate with off-flavor in ripening bananas.

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CHAPTER 3

EFFECTS OF ANAEROBIC CONDITIONS ON ETHANOL PRODUCTION CORRELATED TO OFF-FLAVOR DEVELOPED IN RIPENING BANANAS

ABSTRACT

This study involves the effects of anaerobic conditions on banana volatile production and fruit quality. Production of flavor impact compounds under anaerobic conditions was determined by using SPME. However, when bananas were returned to air these volatiles resumed production, to some extent. The effects of anaerobic conditions (nitrogen treatments) on fruit quality were determined by using different methods of evaluation by taste panel, scaling method and 'difference from control'. Bananas placed under nitrogen treatments had better appearance when compared to bananas under air conditions but they lacked fruit flavor and had soft pulp if kept under nitrogen longer than three days. Ethanol production was significantly increased with time under nitrogen treatments, and this was highly correlated with off-flavor developed in the ripening bananas ($r = 0.87$). The detection threshold of tissue ethanol related to off-flavor developed in ripening bananas was approximately 300 mg / 100 g FW, or 0.5 ppm determined in headspace analysis. Apparently ripening bananas cannot tolerate anaerobic conditions for longer than three days, without adversely affecting fruit quality.

INTRODUCTION

In the last decade, the potential of short-term exposure to controlled atmosphere (CA), O_2 at or below 1%, for postharvest insect disinfestation has generated increasing interest. Although several benefits have been proposed, this treatment must also consider residual effects on the quality of fruits for human consumption. The most important detrimental effect of the low O_2 in controlled atmosphere is development of alcoholic off-flavors in several fruits, such as reported in mango and papaya (Yahia, 1985); strawberry (Aharoni et al., 1981; Ke et al., 1991a; 1991b); peach (Ke et al., 1991a; 1991b); sweet potato (Delate and Brecht, 1989); oranges (Ke and Kader, 1990) and in apples and pears (Kosittrakun, 1989). Therefore, CA treatment can be used as a postharvest quarantine procedure only when it kills the insect of interest without detrimentally affecting the quality of the commodity. In other words, plant tolerance to such a condition is an equally important characteristic for the successful application of this treatment. Under anaerobic conditions (O_2 levels at or below 1%), some plants accumulate γ-aminobutyrate, succinate, or malate (Mazelis and Vennesland, 1957; Streeter and Thompson, 1972), but the majority, particularly fresh fruits, produce ethanol and acetaldehyde (Cossins and Beevers, 1963; Nichols and Patterson, 1987; Norman and Craft, 1971; Smagula and Bramlage, 1977). This results in alcoholic off-flavor in those commodities (Lidster et al., 1987; Ke and Kader, 1990; Ke et al., 1991; Ke et al., 1991b; Ke and Kader, 1992b).

Bananas, as do other climacteric fruits, produce volatile compounds during fruit ripening and as the volatiles are produced, fruit flavor develops. But when subjected to low O_2 levels at or below 1% (anaerobic conditions), the

patterns of volatile production changed. The main impact volatiles decreased in anaerobic conditions, while ethanol dramatically increased. It has not been reported that ethanol accumulates in anaerobic conditions correlated with off-flavor developed in ripening bananas, nor has the tolerance of ripening bananas to such CA conditions for insect control been studied. Therefore, a major purpose of these studies is to study off-flavor development in ripening bananas under anaerobic conditions (nitrogen treatments) and to determine the volatile compounds that correspond to off-flavor in ripening bananas. In order to determine fruit tolerance to such conditions, fruit qualities are evaluated by trained panelists. In our study, banana qualities were evaluated by two different methods. In order to examine the development of off-flavor and other fruit attributes, a 0-15 scaling method was applied (Larmond, 1991). Differences in fruit attributes between bananas subjected to nitrogen treatment and air control were evaluated by 'difference from control' methods (Meilgaard, 1987; Meilgaard et al., 1991).

Gilliver and Nursten (1976) concluded that increases in esters, produced by banana slices, were much more marked when the appropriate alcohol was added. Recently, there were reports about correlation between short-chain aliphatic alcohols and their corresponding aldehydes and esters in fruits volatiles. For example, short-chain aliphatic alcohols were converted into corresponding aldehydes and acetates in apple tissues (Knee and Hatfield, 1981). In ripening banana, Ueda et al. (1992) studied the conversion of alcohols into corresponding aldehydes and esters and reported that the esterification of alcohols into the corresponding esters by banana, melon and strawberry was successful via the activity of alcohol acyl CoA transferase (ester synthetase). Therefore, an additional purpose of these studies is to characterize the correlation between alcohols and their corresponding esters accumulated due to anaerobic condition

(nitrogen treatments). Since we know that subjecting bananas to anaerobic conditions results in accumulation of ethanol more than other volatile compounds, we will focus on this production. We also hypothesize that high production of ethanol in nitrogen treatments might result in the production of corresponding ethyl and acetate esters after fruits are transferred to air; via the activity of enzyme alcohol acyl CoA transferase as previously mentioned by Ueda et al. (1992). Due to time constraints however, we did not attempt purification or direct measurement of the activity of alcohol acyl CoA transferase nor did we attempt to measure the activity of the conversion of alcohols into corresponding esters. This investigation had measured headspace ethanol accumulation under anaerobic conditions as determined by a new method of solid phase microextraction (SPME). Therefore, in order to corroborate the presence and the quantity of ethanol, tissue ethanol was extracted and quantified by the NADH coupled spectrophotometric according to the enzymatic method of Beutler (1983). Correlation between tissue ethanol and headspace ethanol was also established.

MATERIALS AND METHODS

Fruits and treatments preparation

Samples for headspace analysis

All-yellow bananas, color stage 6 (Stover and Simmonds, 1987), were purchased from local markets and kept at 12°C in cardboard cartons lined with perforated polyethylene film until used in the experiments. Approximately one kg of whole bananas was placed in each of three 4-liter-jars with lids attached to inlet and outlet tubes. The three jars were connected together and each jar was used as one replication. Dynamic nitrogen conditions were rapidly established by equilibrating the jars with pure nitrogen (99.99%) for 25 min (1500 ml/min) before the flow rate was reduced to 100 ml/min and this rate was maintained until the experiment ended. Air conditions (controls) were conducted in the same manner by using compressed air instead of pure nitrogen. All fruits were held at 20°C throughout the experiments. Flow boards and capillary tubing were used for flow control.

Samples for evaluation using scaling method and tissue ethanol

Bananas, at the same stage of ripening (color stage 6), were purchased from the same local market in order to reduce variation between fruits and were then prepared for the two conditions, air conditions and nitrogen treatments, as

described above. To evaluate fruit quality, bananas were taken daily from the jars, peeled and then presented to panelists who evaluated their physical appearance and qualities including off-flavor characteristics by a (0 to 15 cm) scaling method. Each day, fruits from the same jars were separated for tissue ethanol extraction. All samples were held at 20°C until the end of the experiments.

Samples for evaluation using 'difference from control' method

Bananas, selected at the same stage of ripening (color stage 6), were purchased from the same local market and prepared as for the method described in parts A and B. Bananas in each jar were subjected to nitrogen treatments beginning on different days to give different periods of exposure at the end of experiments, so that taste panelists could taste all stages of treatment at the same time.

Sampling techniques and GLC conditions

Solid phase microextraction (SPME-Supelco, Inc., Bellefonte, PA) was used for sampling headspace samples for a period of 15 min before taking the sample to the GLC for analysis. Samples were taken every 24 hr over a period of 10 days. Recovery of volatile production after treatment with nitrogen for five days were also examined, but without fruit quality evaluation.

Headspace samples were separated and analyzed using a HP5890-Series II gas chromatograph (Hewlett-Packard Co., USA), equipped with 25m x

0.2mm (i.d.) x 0.2μm fused silica capillary column coated with Carbowax 20M (HP™-20M). Separated volatiles were detected by flame ionization detector (FID). Injection and detection ports were 200°C and 250°C, respectively. The column was held at 60°C for the first 5 minutes then programmed at 5°C /min to 200°C , then held at 200°C until end of the separation. Helium carrier gas was supplied at a flow rate of 1.0 ml/min To plot chromatograms, the signals were processed with a HP-3395 digital integrator (Hewlett-Packard Co., USA).

Standard curves for relative peak area of ethanol and its esters

Anhydrous ethanol was obtained from Quantum Chemical Co. USA. Ethyl acetate and ethyl butanoate were from Sigma Chemical Co. (St. Louis, MO). Standard curves for headspace concentration were established by injecting different concentrations of compound on Whatman no. 1 filter paper (Whatman Int. Ltd., Maidstone, UK) and placed in 4-liter-jars with lids that had been sealed with parafilm against leaks. The jars were then incubated for 15 min using a magnetic stirring bar before allowing SPME absorption for another period of 15 min. Samples, with three replications, were analyzed by GLC/FID using the same column and temperature program for volatiles analysis.

Tissue ethanol extraction

Approximately 10 g of banana pulp was homogenized in a Sorval Omni-mixer with 15 ml tris-maleate buffer pH 9, plus 2% PVP, for 20 seconds then permitted to stand for five seconds before homogenizing again for another 20

seconds. Approximately two g of celite was added, before the third homogenization. The mixture then was vacuum filtered through Whatman No. 1 filter paper (Whatman Int., Ltd., Maidstone, UK), and the residue washed two times with five ml of tris-maleate buffer. The extracts then were sealed and kept at 4°C until used.

Tissue ethanol concentrations were determined enzymatically according to Beutler (1960) using an enzyme assay kit (Boehringer and Mannheim™). Original extracts were diluted 1:100 before being used as substrate for enzymatic assays. This assay is based on the principle that ethanol is quantitatively oxidized in the presence of the enzyme alcohol dehydrogenase (ADH) by nicotinamide-adenine dinucleotide (NAD^+) to acetaldehyde. The alkaline condition, tris-maleate pH 9, shifts the equilibrium reaction to the side of acetaldehyde and NADH. Acetaldehyde is also oxidized by nicotinamide-adenine dinucleotide (NAD^+) to acetic acid in the presence of Aldehyde dehydrogenase (AlDH). The amount of NADH formed is equal with the amount of acetaldehyde, but with half the amount of ethanol. NADH was measured by its absorbance at 340 nm using a Bausch & Lomb 2000 spectrophotometer. Tissue ethanol concentration values were averaged from three replications.

Scaling method for quality evaluation

Fruit appearance, fruit flavor, off-flavor, texture, and overall quality were evaluated daily by seven panelists who detected the difference between normal flavor and off-flavor in the preliminary experiment (data not shown). For this method of evaluation, unstructured scaling or visual analog scales consisted of 15 cm-horizontal lines. Each anchor point was labeled with a word or expression.

expression. A separate line was used for each sensory attribute to be evaluated (see Panelist ballot appendix Fig. A 3.1). The distances measured from the left point of the line were recorded and then data were analyzed by StatGraphics computer software.

'Difference from control' method for quality evaluation

Bananas from each day (days 1 - 5) and both treatments (air and nitrogen treatments) were evaluated within one day for their eating quality by 47 panelists using 'difference from control' method. To determine the differences, each subject was presented a control sample plus two test samples and then rated the magnitude of difference by using 9-point-category scale (see Panelist ballot appendix Fig. A 3.2). The data then were analyzed for the difference in means and variance by using StatGraphics computer software.

RESULTS

Total production of volatiles under anaerobic conditions

In general, after five days of nitrogen treatments, the production of total volatiles was only 13% of the volatiles produced by bananas under air conditions. Fig. 3.1. shows trend of total production for the main groups of volatile compounds produced in air (A) and nitrogen (B). Production of acetates and butanoates decreased during nitrogen treatments but increased after fruits were returned to air (Figs. 3.2 and 3.3 for acetates and butanoates, respectively). Nitrogen treatments apparently had greater effects on production of butanoates than acetates. Under air conditions, maximum relative peak area for total butanoate was higher than acetate (around 40 and 15 for total butanoate and total acetate, respectively), but after bananas were returned to air, relative peak area of total acetate was higher than butanoate (approximately 6 and 3 for total acetate and total butanoate, respectively). While production of acetates and butanoates decreased under nitrogen treatments, production of alcohols increased, but then slightly decreased after fruits were returned to air (Fig. 3.4). Table 3.1. shows means of total volatile production for all main groups and evaluated scores for quality attributes. To determine effects of nitrogen treatments on volatile production, percent of production in air was calculated by comparing relative peak area obtained under nitrogen treatment to that obtained under air conditions. While production of acetates and butanoates decreased under nitrogen treatments, alcohols dramatically increased. However, the trend for percent ethanol production decreased with time in the nitrogen treatments.

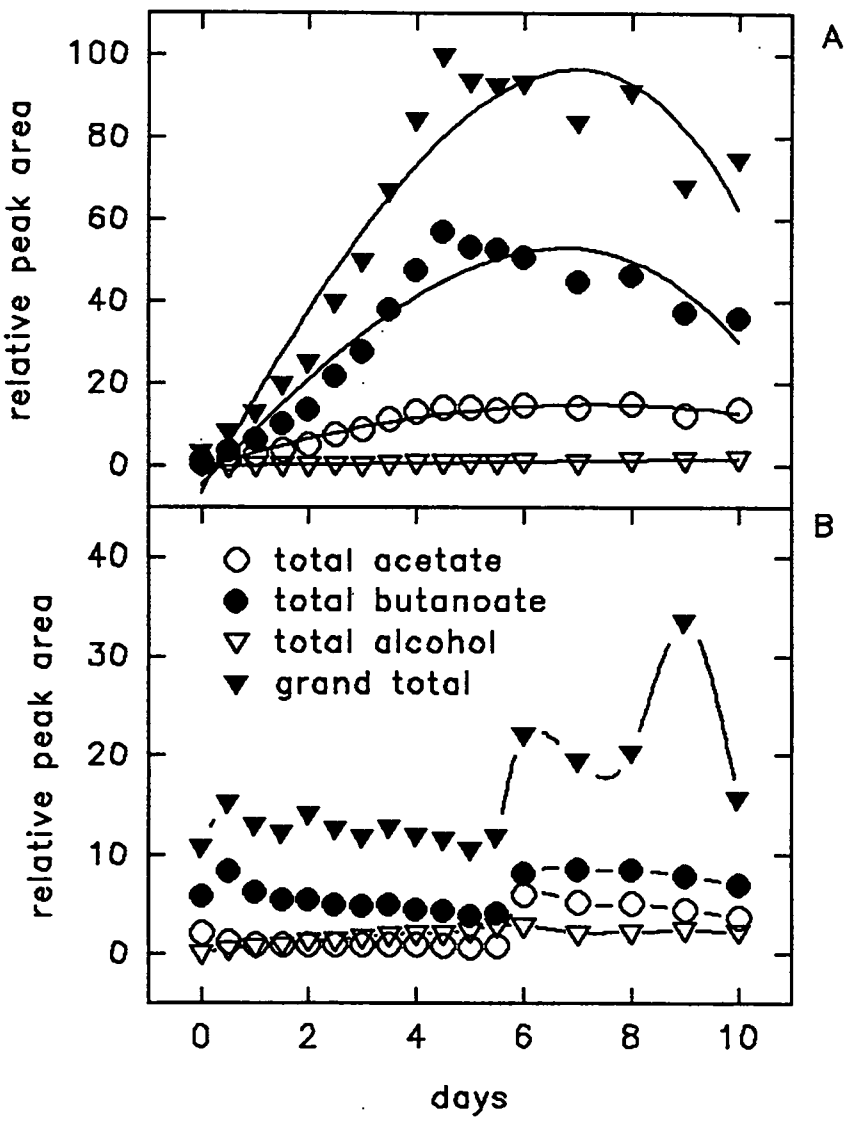

Fig. 3.1 Trends of total volatile production for all yellow bananas (color stage 6) under air control (A) compared nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days.

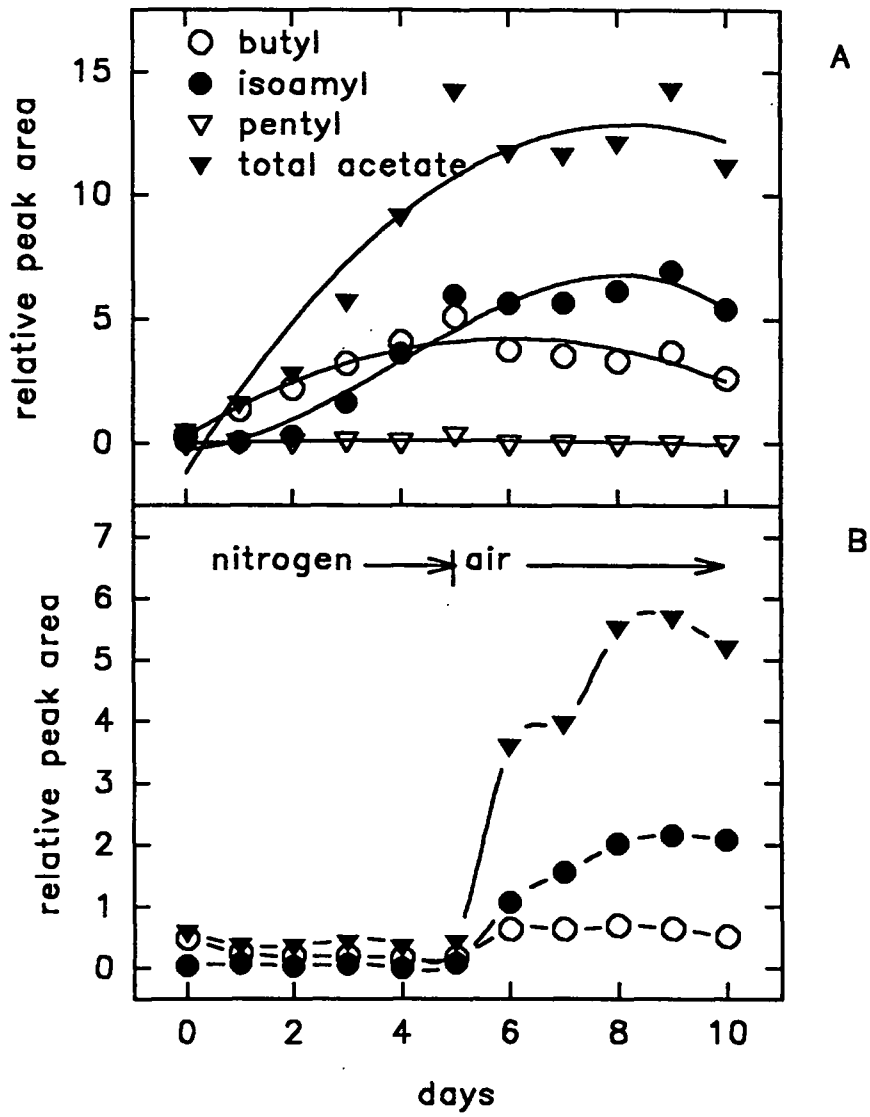

Fig. 3.2 Trends of production for total acetate and flavor impact compounds produced by all yellow bananas (color stage 6) under air control (A) compared nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. Relative peak area is calculated as a percent of maximal total volatiles produced in air at 4.5 days.

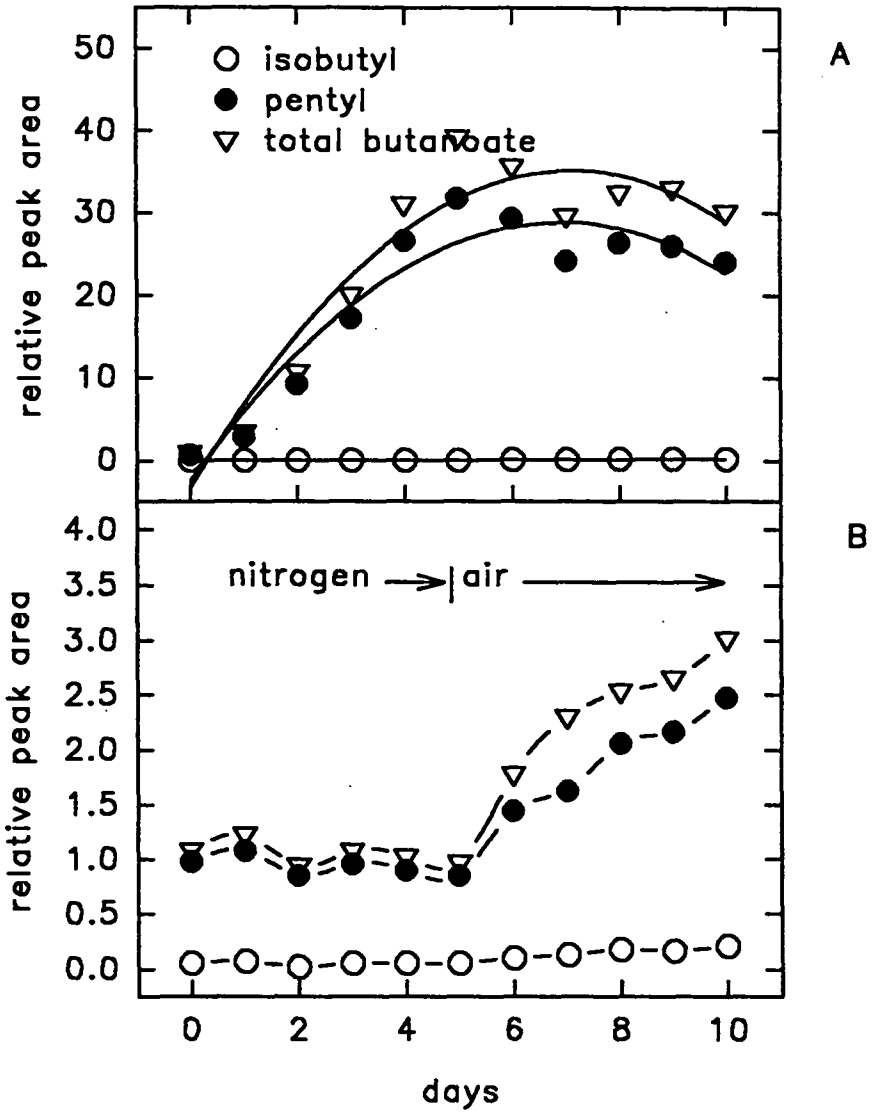

Fig. 3.3 Trends of production for total butanoate and flavor impact compounds produced by all yellow bananas (color stage 6) under air control (A) compared nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. Relative peak area is calculated as a percent of maximal total volatiles produced in air at 4.5 days.

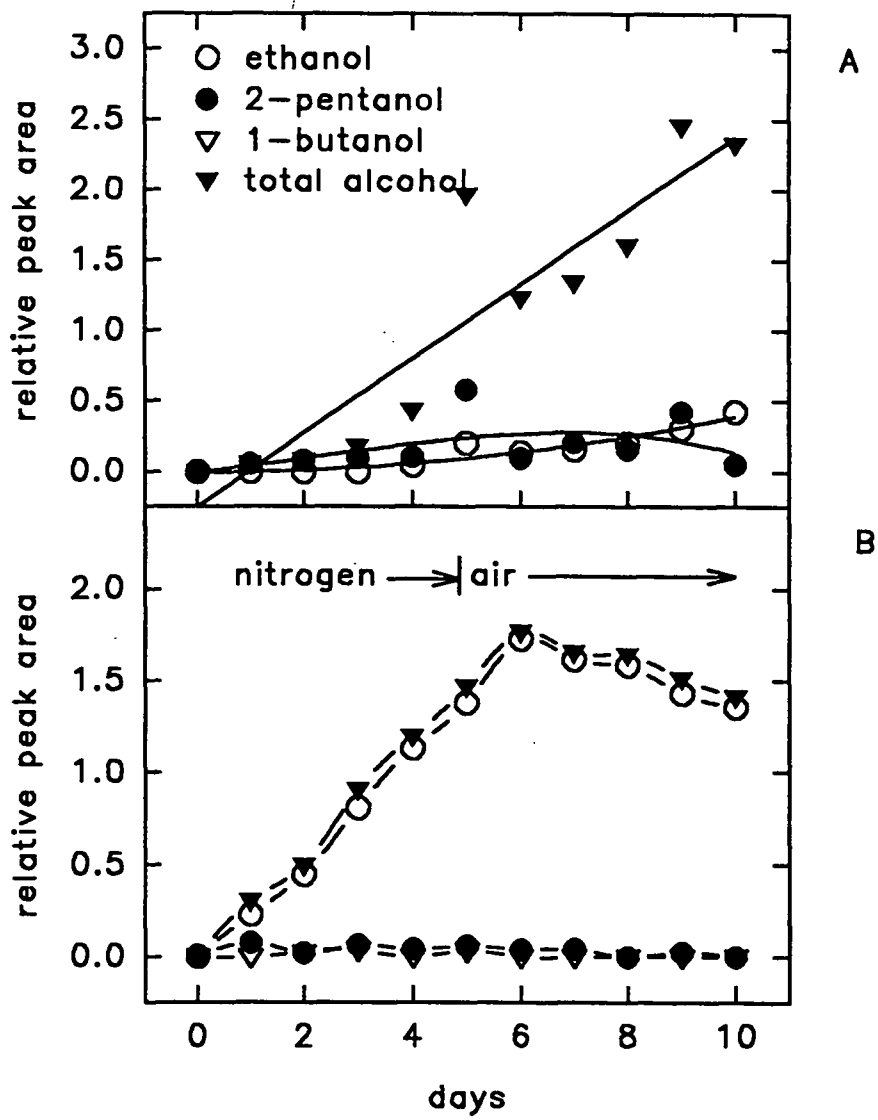

Fig. 3.4 Trends of production for total alcohols and flavor impact compounds produced by all yellow bananas (color stage 6) under air control (A) compared nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. Relative peak area is calculated as a percent of maximal total volatiles produced in air at 4.5 days.

Table 3.1 Means for main volatile compounds (calculated as percent of maximal volatile production) and evaluated scores for test panel. Volatile compounds were calculated from 3 replications and test panel scores were evaluated by 7 panelists (n = 7).

	Compounds			Fruit attributes				
Treatment	acetate	butanoate	alcohol	appearance	fruit-flavor	off-flavor	texture	overall qualit
Air								
0	0.492	0.422	0.000	11.4	8.9	2.4	8.1	9.5
1	1.350	2.923	0.035	12.6	9.0	3.0	8.8	9.4
2	2.500	8.405	0.062	9.6	9.9	1.5	5.3	7.8
3	5.140	16.505	0.235	9.7	10.8	0.9	6.9	11.8
4	7.331	23.764	0.368	7.0	11.9	0.4	9.3	12.6
5	9.983	26.780	1.191	6.2	11.9	0.8	9.3	12.6
$LSD_{0.05}$	2.862	7.456	0.494	2.47	3.51	2.66	4.34	3.78
Nitrogen								
0	0.760	1.195	0.067	8.5	7.3	2.6	9.4	9.4
1	0.579	1.655	0.481	11.0	6.4	5.9	8.0	7.3
2	0.523	1.689	0.885	11.7	8.9	6.2	6.4	6.3
3	0.642	1.709	1.461	11.9	6.9	6.1	4.3	4.6
4	0.590	1.622	2.072	9.0	6.9	10.9	3.3	1.9
5	0.673	1.554	2.420	10.1	2.9	13.3	4.6	1.5
$LSD_{0.05}$	0.508	1.442	1.164	3.83	4.67	4.19	4.04	3.78

Production of ethanol and its corresponding esters

The off-flavor which developed in ripening bananas under anaerobic condition was primarily characterized as "alcoholic off-flavor". This was the same as the off-flavor reported by Ke and Kader (1992b). Therefore, production of alcohols and their corresponding esters was primarily emphasized in our study. Five alcohols were detected by SPME. They are: ethanol, 1-butanol, 2-pentanol, 4-hexen-1-ol and isobutyl alcohol. Among those alcohols, only ethanol showed significant accumulation under nitrogen treatmenta (Table 3.2). This production accounts for more than 90% of total alcohols in the fourth and fifth days under the nitrogen treatmenta and showed strong linear-relationships with time (R^2 = 0.72). While ethanol accumulated during nitrogen treatmenta, other alcohols showed various production patterns. Isobutyl alcohol was not detected at all under nitrogen treatments and 4-hexen-1-ol although present, was considerably suppressed by the nitrogen. Production of 1-butanol was detected at low levels during nitrogen treatments, but was not detected after the bananas were returned to air. Production of 2-pentanol was high under nitrogen treatments, but decreased when bananas were returned to air.

From graphic results, most alcohols did not show a correlation with their corresponding esters (data not shown). The exception was ethanol and its corresponding esters, ethyl acetate and ethyl butanoate (Fig. 3.5). While ethanol accumulated during nitrogen treatments, ethyl acetate and ethyl butanoate decreased. After bananas were transferred to air, production of ethanol decreased slightly while production of ethyl acetate and ethyl butanoate were dramatically increased. This finding shows that ethanol may be converted into

Table 3.2 Means and standard deviations for production of five alcohols found in both air control and nitrogen treatment. (as reported by area) and percent of maximal volatile production when compared to air control. Data were calculated from total of 3 replications.

Treatments	Days in the treatments				
	1	2	3	4	5
Air control					
Ethanol	0.000 (0.00)	0.000 (0.00)	0.011 (0.02)	0.034 (0.03)	0.183 (0.13)
Isobutyl alcohol	0.000 (0.00)	0.000 (0.00)	0.061 (0.08)	0.065 (0.01)	0.433 (0.25)
2-pentanol	0.041 (0.03)	0.073 (0.04)	0.138 (0.10)	0.124 (0.03)	0.403 (0.21)
1-butanol	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)
4-hexen-1-ol	0.000 (0.00)	0.000 (0.00)	0.065 (0.05)	0.191 (0.06)	0.378 (0.17)
Total alcohol	0.041 (0.03)	0.073 (0.04)	0.276 (0.25)	0.415 (0.11)	1.398 (0.74)
N_2 treatment					
Ethanol	0.420[1] (0.17)[2]	0.929 (0.43)	1.538 (0.63)	2.271 (0.98)	2.682 (1.13)
Isobutyl alcohol	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)
2-pentanol	0.108 (0.02)	0.063 (0.09)	0.117 (0.06)	0.097 (0.05)	0.121(0.08)
1-butanol	0.036 (0.03)	0.047 (0.04)	0.061 (0.03)	0.056 (0.05)	0.072 (0.06)
4-hexen-1-ol	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.007 (0.01)	0.000 (0.00)
Total alcohol	0.065 (0.23)	1.039 (0.53)	1.716 (0.70)	2.432 (1.07)	2.876 (1.24)
% of ethanol to total alcohol	74	89	90	93	93

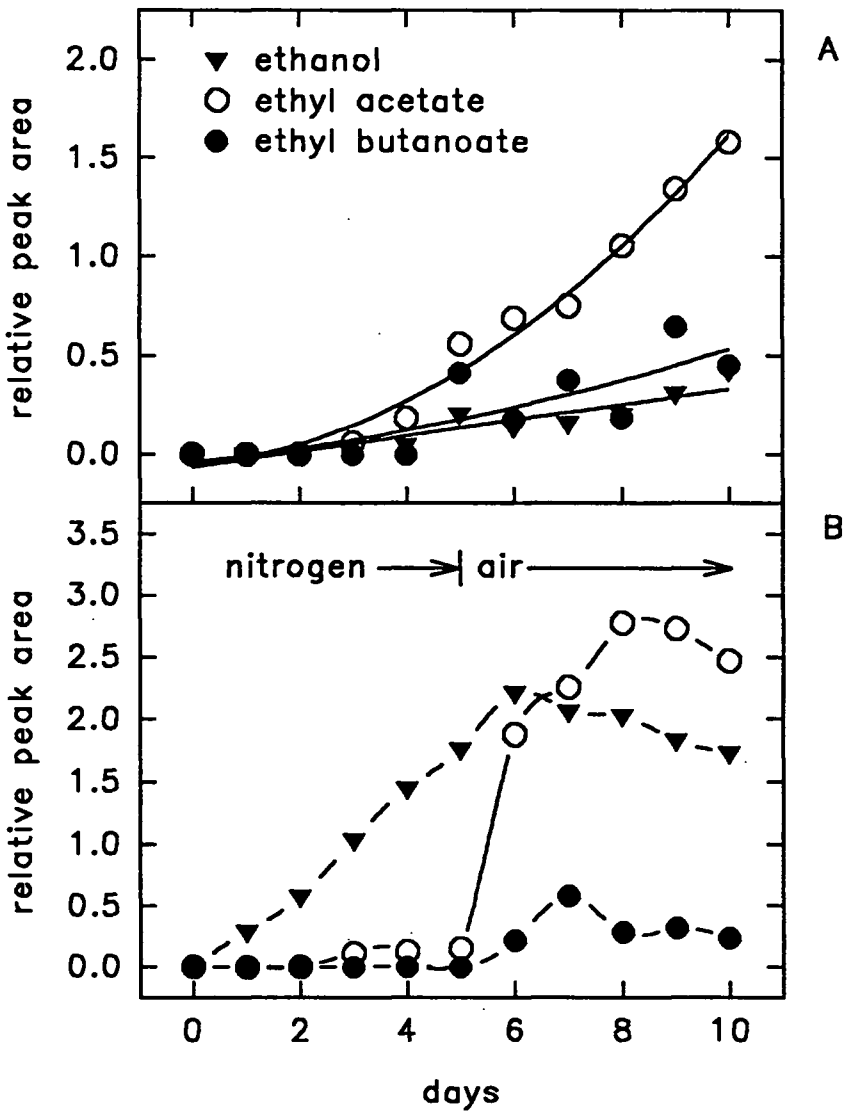

Fig. 3.5 Trends of production for ethanol and its esters (based on relative peak area) produced by all yellow bananas (color stage 6) under air control (A) compared nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. Relative peak area is calculated as a percent of maximal total volatiles produced in air at 4.5 days.

ethyl acetate and ethyl butanoate by esterification via activity of enzyme alcohol acyl CoA transferase as mentioned by Ueda et al. (1992).

As previously mentioned, this phenomenon has not been observed for other alcohols and their esters. In order to clearly graph this relationship, the concentrations of those volatiles of interest were calculated using standard curves (Figs. 3.6, 3.7 and 3.8 for ethanol, ethyl acetate, and ethyl butanoate, respectively). The production for ethanol, ethyl acetate and ethyl butanoate were then replotted again as shown in Fig. 3.9.

Tissue ethanol measurement

The methodology for calculating tissue ethanol concentration is based on the difference between absorbance of endogenous NADH (before adding the enzymes) and the reduced NADH from tissue ethanol (after adding the enzymes). Tissue ethanol from bananas under air conditions varied from 0 mg/100 g FW (day 1-2) to 120 mg/100 g FW (day 5) (Table 3.3) whereas banana tissue subjected to nitrogen treatments varied from 53 mg/100 g FW to 506 mg/100 g FW (day 5) (Table 3.4). These findings demonstrated a strong linear relationship between tissue ethanol and time under the nitrogen treatment (R^2 = 0.94). The relative peak area of headspace SPME ethanol was correlated with enzymatically-determined ethanol in the pulp for bananas kept in nitrogen (R^2 = 0.66). Fig. 3.10 shows the correlation between headspace ethanol and tissue ethanol during five days in the nitrogen treatments.

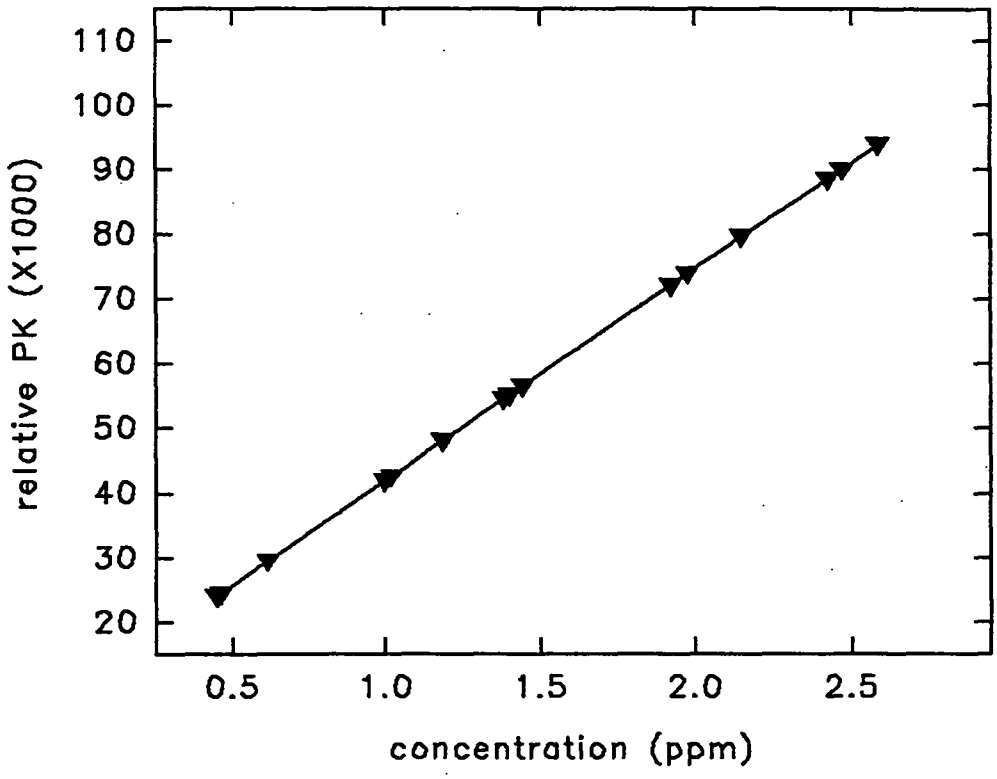

Fig. 3.6 Standard curve for headspace ethanol concentration in 4-liter-jars quantified by SPME. Equilibrating time was 15 min. and trapping time was 15 min. at 25°C.

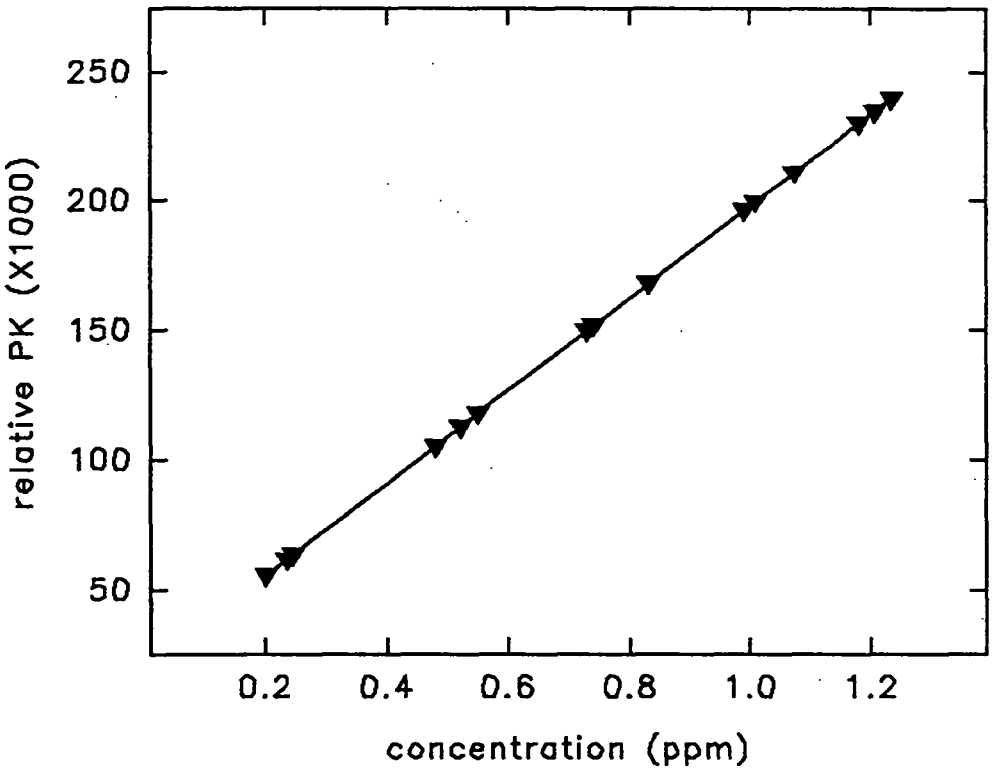

Fig. 3.7 Standard curve for headspace ethyl acetate concentration in 4-liter-jars quantified by SPME. Equilibrating time was 15 min. and trapping time was 15 min. at 25°C.

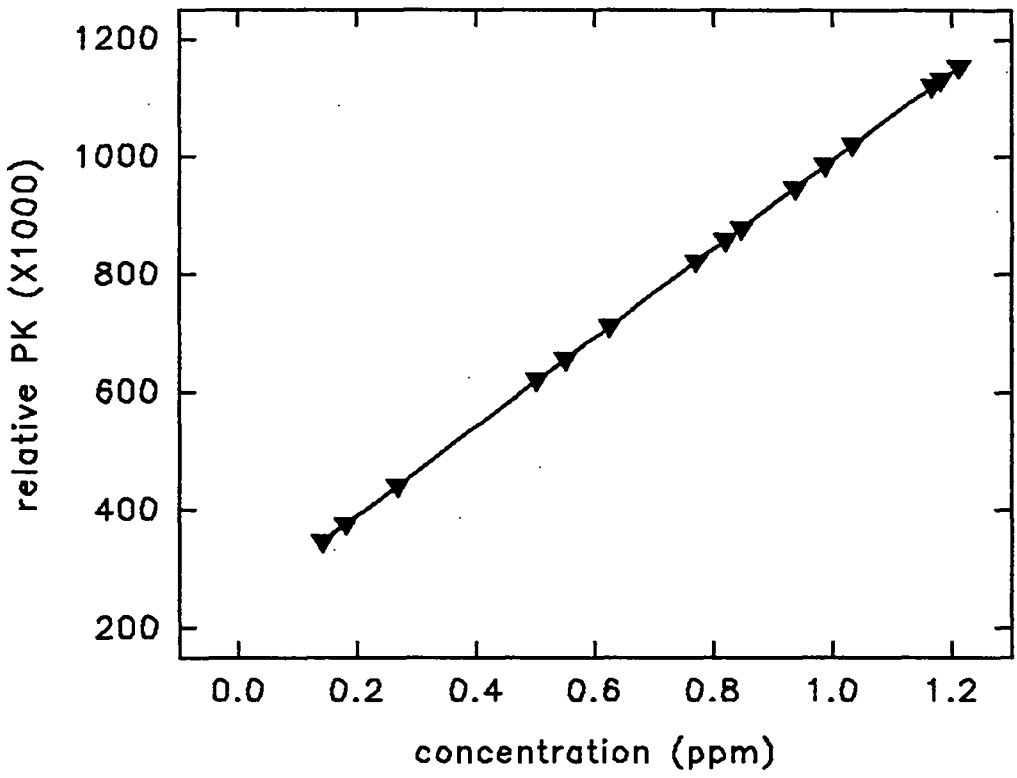

Fig. 3.8 Standard curve for headspace ethyl butanoate concentration in 4-liter-jars quantified by SPME. Equilibrating time was 15 min and trapping time was 15 min at 25°C.

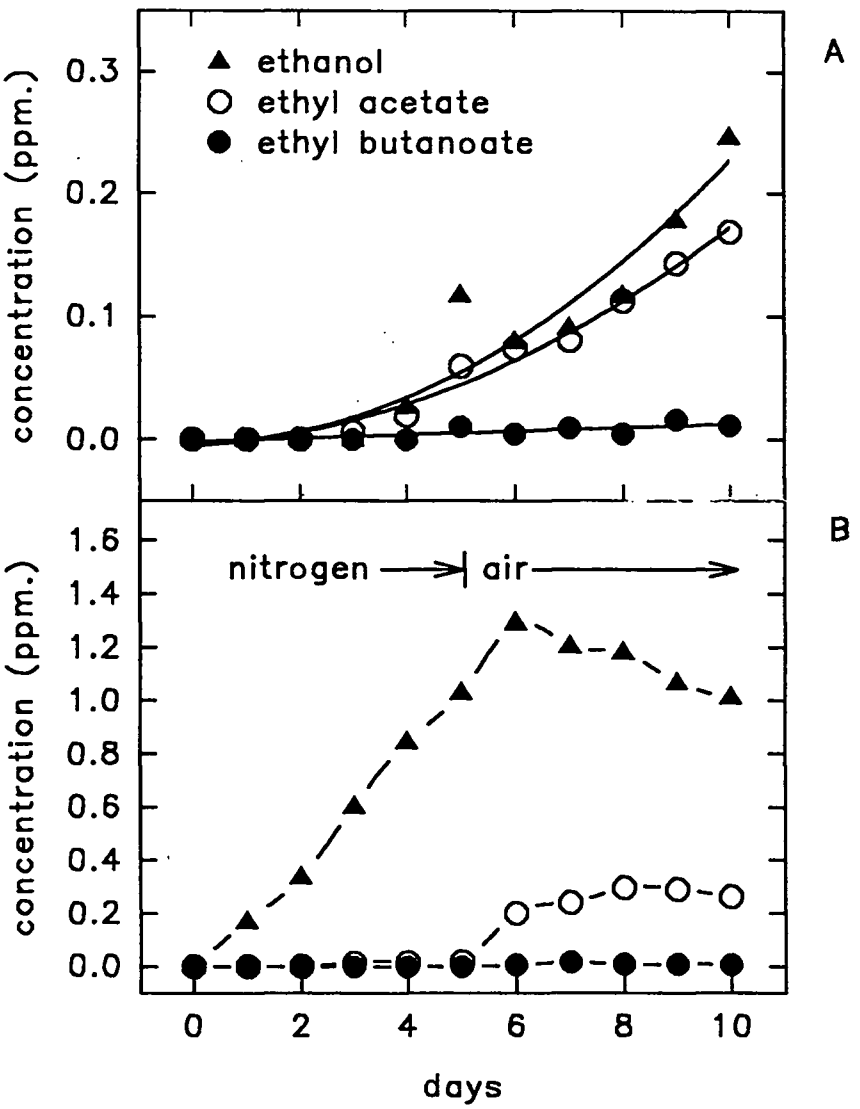

Fig. 3.9 Trends of production for ethanol and its esters (based on concentration, ppm.) produced by all yellow bananas (color stage 6) under air control (A) compared nitrogen treatment (B). Bananas from 5-day nitrogen treatment were returned to air on day 6 and volatiles were then measured for another 5 days. Relative peak area is calculated as a percent of maximal total volatiles produced in air at 4.5 days.

Table 3.3 Means and standard deviations of headspace ethanol (ppm) and tissue ethanol (mg/100 g FW) for Bananas under air treatment.

Day	Headspace ethanol (ppm)		Tissue ethanol (mg/100g FW)	
	Means	STD	Means	STD
0	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	0.01	0.01	25.29	25.29
4	0.02	0.02	63.23	34.61
5	0.11	0.08	119.96	55.19
$LSD_{0.05}$	0.058		87.93	

Significance level p = 0.05. Means were average from total of 3 replications (n =3).

Table 3.4 Means and standard deviations of headspace ethanol (ppm) and tissue ethanol (mg/100 g FW) for Bananas under nitrogen treatment.

Day	Headspace ethanol		Tissue ethanol	
	Means	STD	Means	STD
0	0.175	0.162	52.78	3.45
1	0.245	0.058	134.36	7.54
2	0.541	0.146	161.71	30.54
3	0.896	0.213	299.93	7.54
4	1.324	0.332	369.99	5.44
5	1.564	0.383	506.28	37.45
$LSD_{0.05}$	0.749		53.60	

Significance level p = 0.05. Means were average from total of 3 replications (n =3).

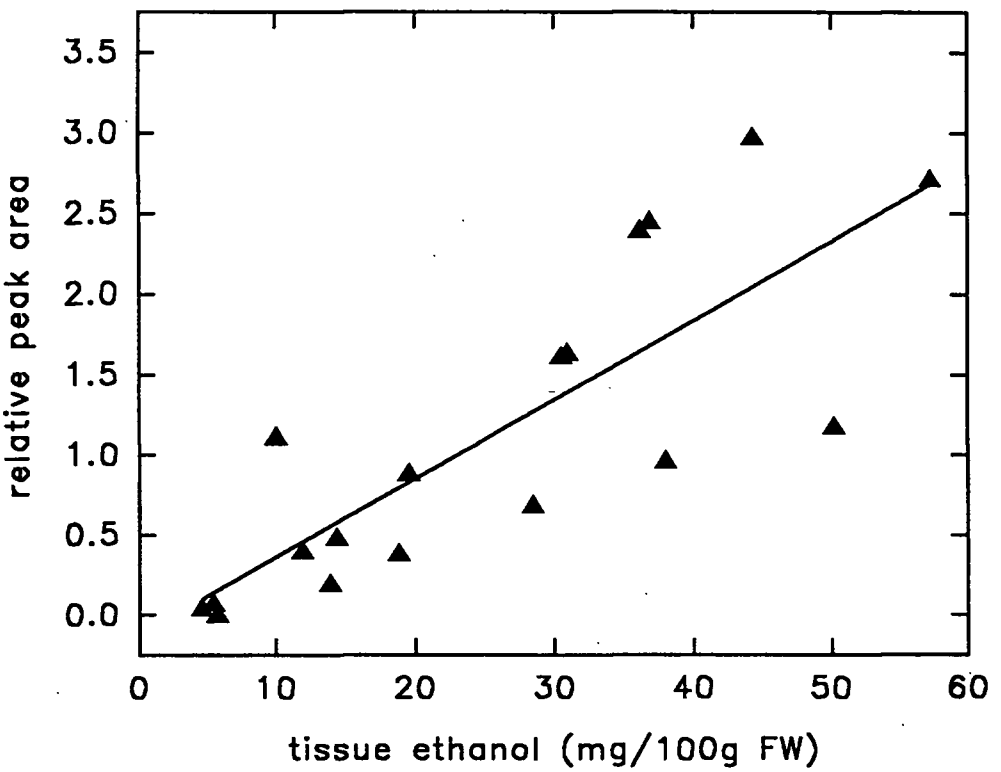

Fig. 3.10 Correlation between relative peak area and tissue ethanol for all yellow bananas (color stage 6) when subjected to nitrogen conditions for total of 5 days.

Fruit quality and flavor evaluation

Scaling method

Five attributes (fruit appearance, fruit flavor, off-flavor, texture and overall quality) were evaluated by partially trained panelists. Bananas under nitrogen treatments and air conditions were removed from the treatments every day and evaluated by the test panelists consecutively for five days.

From test panel, only slight changes in color were discerned by panelists in bananas from nitrogen treatments, while bananas placed in air had a much less pleasing appearance. This was reflected in lower scores for fruit appearance in air (Fig. 3.11). Despite better appearance, bananas subjected to nitrogen treatments had lower scores for fruit flavor (Fig. 3.12) and higher scores for off-flavor development (Fig. 3.13). The texture of bananas in air did not show significant changes in firmness (Fig. 3.14). Overall quality scores showed the same trend as those for fruit-flavor and texture in which bananas in the air control were more desirable to all panelists than bananas from the nitrogen treatments (Fig. 3.15).

Correlations were examined between off-flavor evaluation scores and certain volatiles produced by bananas under nitrogen treatments. The majority of volatiles had very low correlation coefficients (data not shown), except for ethanol whose correlation coefficient was very high ($r = 0.87$). Fig. 3.16 shows the correlation between headspace ethanol (ppm) and off-flavor scores and between headspace ethanol (ppm) and tissue ethanol (mg/ 100 g FW).

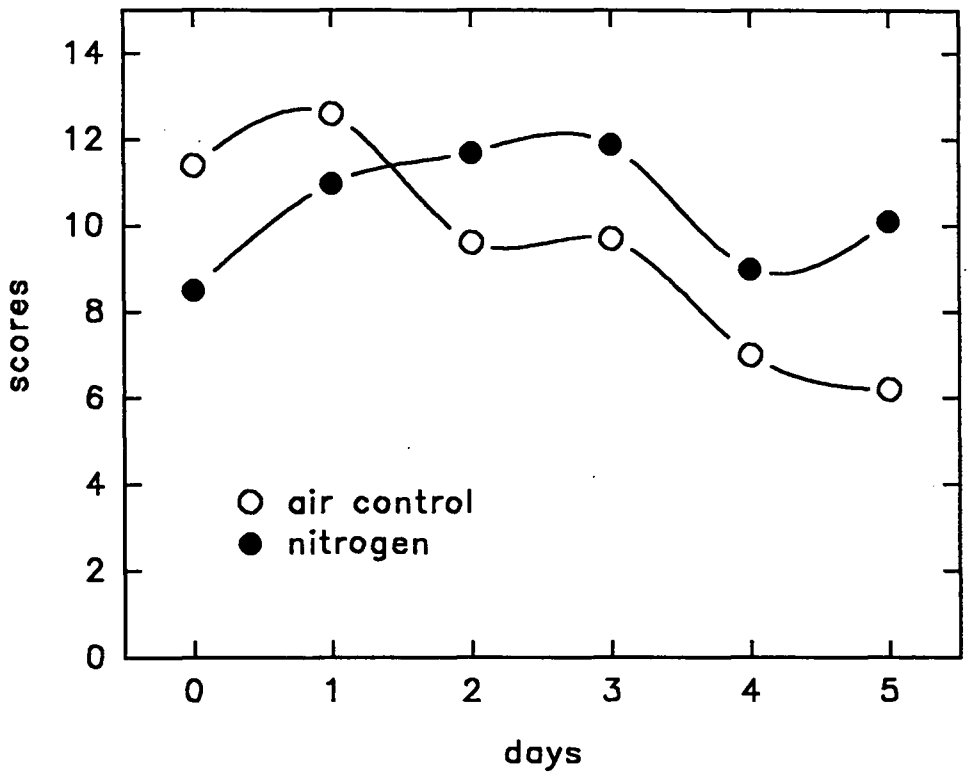

Fig. 3.11 Average score for fruit appearance for bananas from both air treatment and nitrogen treatment. Response range was from 0 - 15. High score means more desirable characteristic. This attribute was evaluated by using scaling method of evaluation (n = 7).

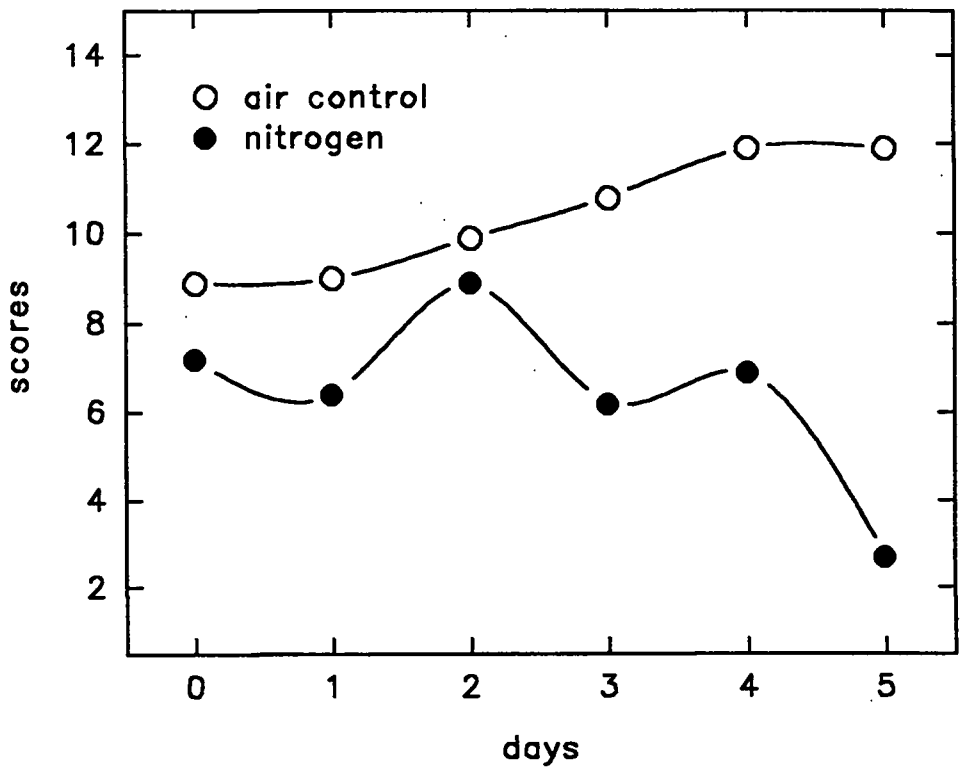

Fig. 3.12 Average score for fruit flavor for bananas from both air treatment and nitrogen treatment. Response range was from 0 - 15. High score means more desirable characteristic. This attribute was evaluated by using scaling method of evaluation (n = 7).

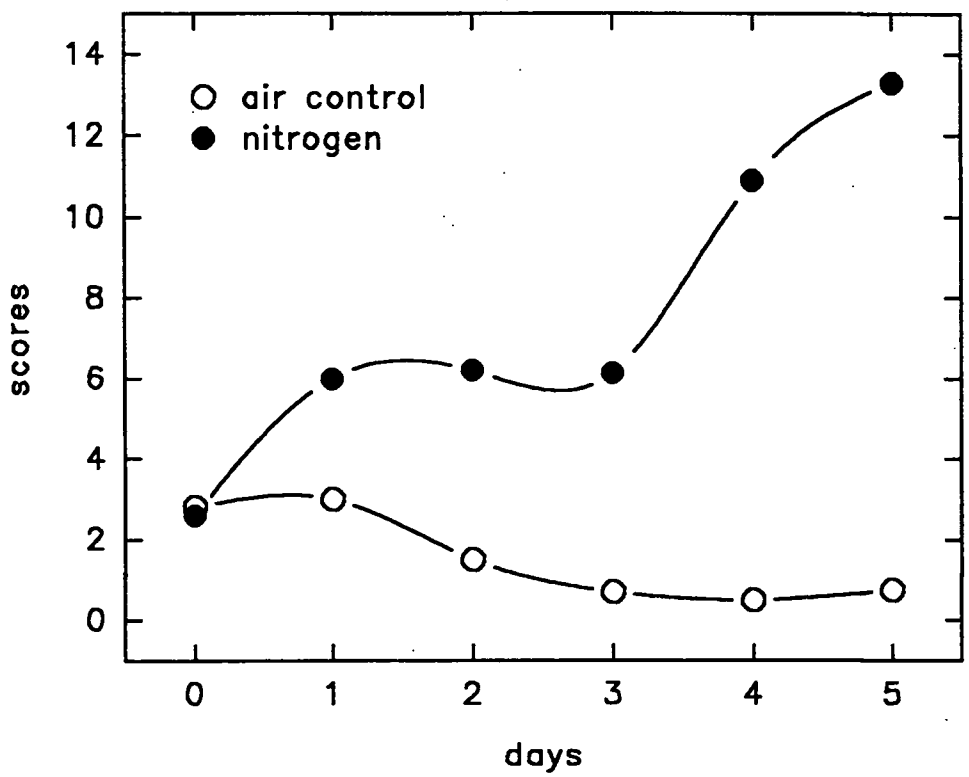

Fig. 3.13 Average score for off-flavor for bananas from both air treatment and nitrogen treatment. Response range was from 0 - 15. High score means less desirable characteristic. This attribute was evaluated by using scaling method of evaluation (n = 7).

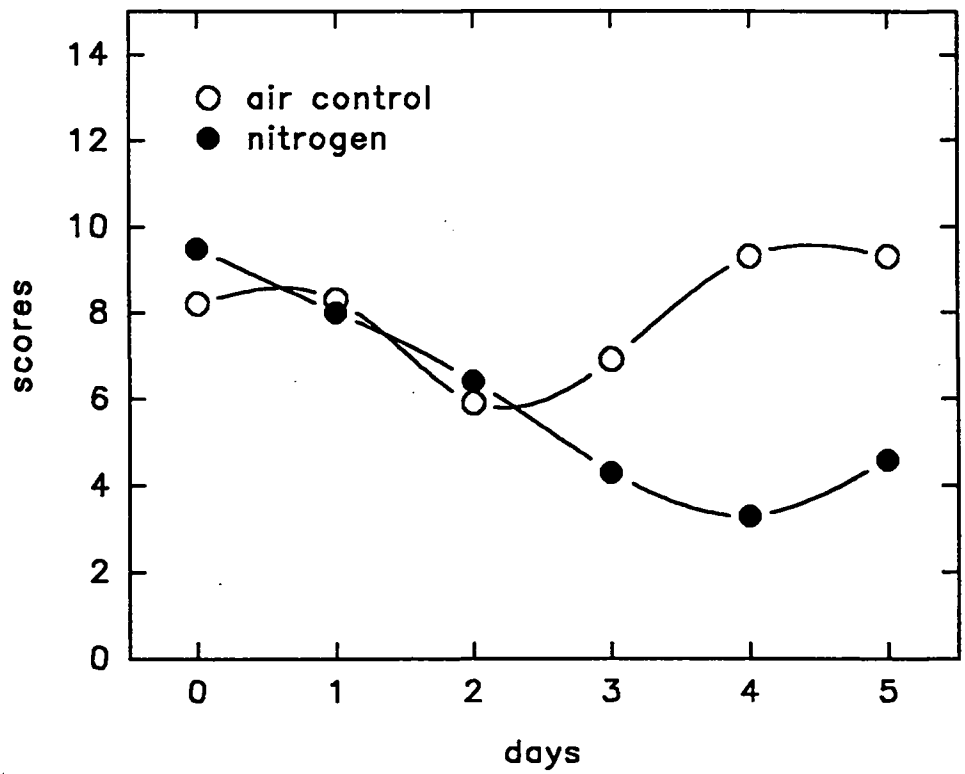

Fig. 3.14 Average score for texture for bananas from both air treatment and nitrogen treatment. Response range was from 0 - 15. High score means firmer texture characteristic. This attribute was evaluated by using scaling method of evaluation (n = 7).

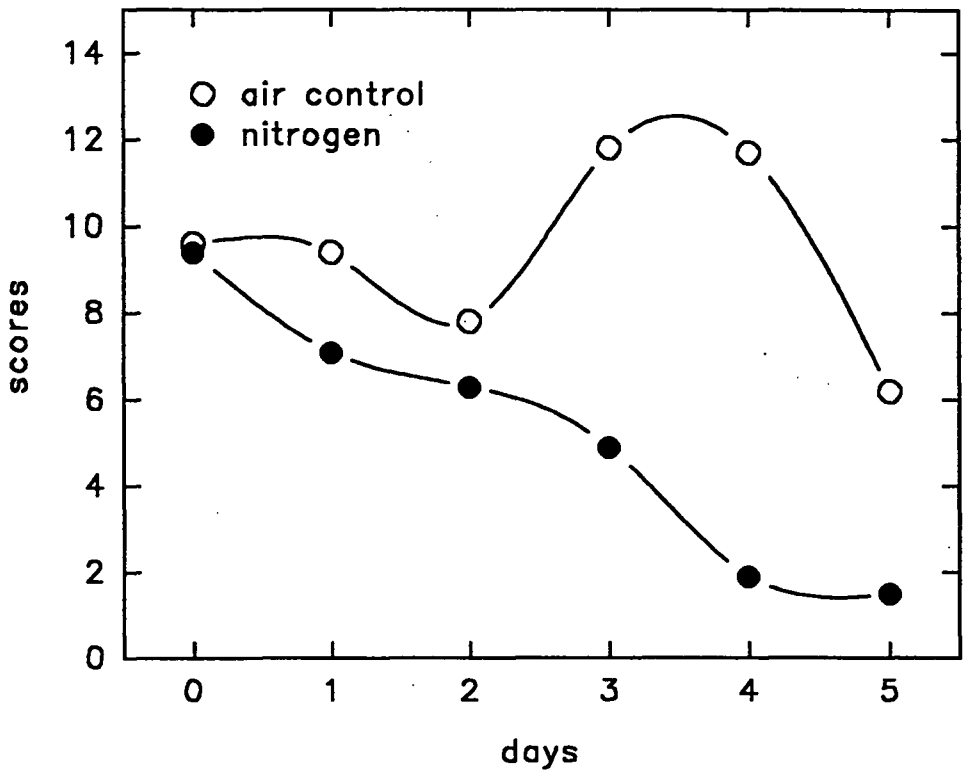

Fig. 3.15 Average score for overall quality for bananas from both air treatment and nitrogen treatment. Response range was from 0 - 15. High score means more desirable characteristic. This attribute was evaluated by using scaling method of evaluation (n = 7).

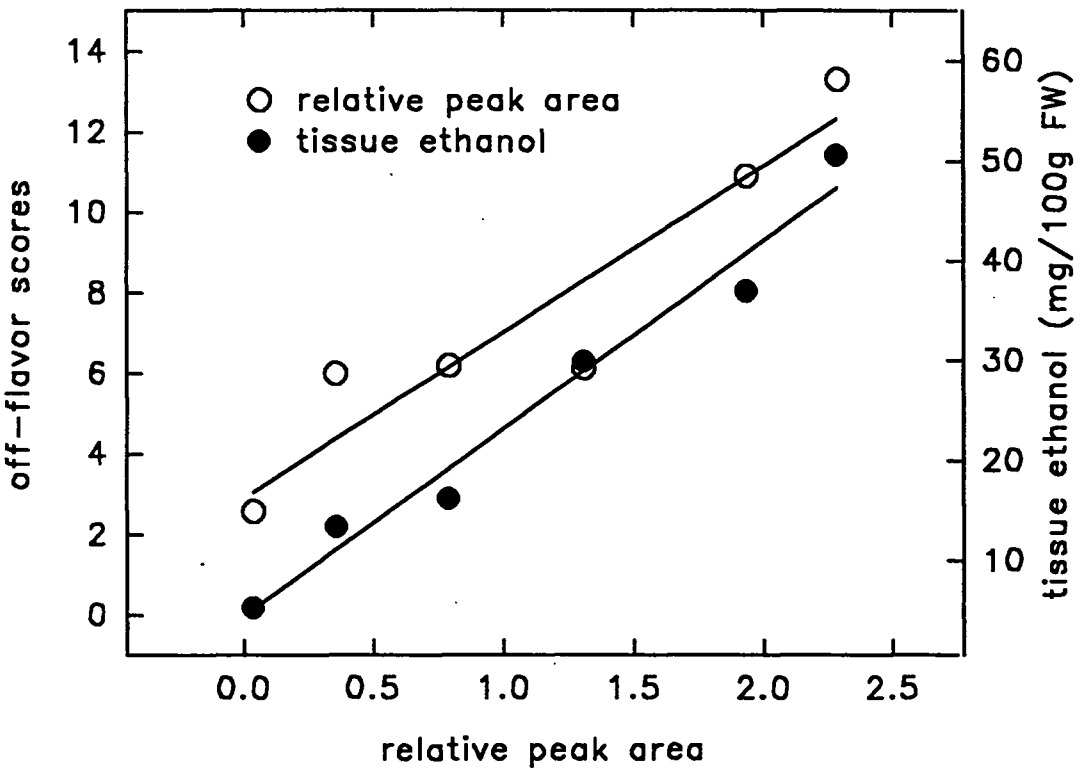

Fig. 3.16 Correlation between means of off-flavor, mean of relative peak area of headspace ethanol and mean of tissue ethanol for all yellow bananas when subjected to nitrogen treatment for total of 5 days.

'Difference from control' method

Fruits kept at 20°C under air conditions or nitrogen treatments for 5, 4, 3, 2, and 1 days were evaluated by 47 panelists on a single day for the differences in their appearance, aroma, flavor and overall liking attributes compared to the control. The magnitude of the differences was recorded without indicators of direction for those differences except for overall like or dislike attribute. Therefore, the results were very difficult to interpret (see appendix Table 3.1) and we decided not to use this information.

DISCUSSION

Effects of anaerobic conditions on ethanol production

Various methods have been reported for separating and detecting ethanol and acetaldehyde accumulated during anaerobic conditions using the GC. For instance, gas analysis has been conducted from direct headspace samples of plant tissues or fruits (Delate and Brecht, 1989; Norman and Craft, 1971), or headspace samples from fruit extracts or juice (Ke and Kader, 1990; Ke et al., 1991b; Ke et al., 1994). Another method that has been reported was directly injecting juice or fruit extraction into the GC (Ke and Kader, 1992b; Ke et al., 1991; Ke et al., 1991a). The discrepancy in these methods of analysis may result in differences in the threshold concentrations of ethanol and acetaldehyde for off-flavor found in treated fruits. However, SPME has not been previously used for detecting ethanol and acetaldehyde in headspace plant volatiles. Therefore, in our study we also extracted tissue ethanol for the enzymatic assay according to Beutler (1960) as another means to confirm and quantify the concentration of ethanol in ripening bananas. Preliminary experiments showed that SPME has a high potential for separating fruit volatiles when compared to the traditional methods used for flavor study such as the charcoal adsorption method. It has slightly differences in sensitivity to volatile compounds (see appendix Fig. A 3.3 for comparison of chromatograms of volatiles trapped using the two methods, activated charcoal and SPME). SPME also has different sensitivity for different volatile compounds produced by ripening bananas. To confirm the degree of difference in sensitivity for SPME, the same

concentrations of ethanol, ethyl acetate and ethyl butanoate were used as headspace samples for SPME detection. It seems to us that SPME has greater sensitivity for ethyl butanoate than ethyl acetate and has the lowest sensitivity for ethanol (see appendix Fig. A 3.4).

After bananas were placed under nitrogen treatments, headspace ethanol increased steadily from the first day in nitrogen, whereas under continuous air, it was first detected on day 3 at very low levels. The increase of headspace ethanol was strongly correlated with tissue ethanol extracted from bananas in the same jars. However, headspace concentration of ethanol as determined by SPME method did not represent the rate of ethanol produced by those bananas due to the flow through system (100 ml/min of gases) and the SPME method itself. As mentioned by Yang and Peppard (1994), it is very difficult to determine fully quantitative recovery by SPME. Because SPME is a single-batch process, the amount of an analytes adsorbed on the SPME fiber and the resulting sensitivity are determined both by adsorption kinetics and distribution coefficient. Standard curves for ethanol, ethyl acetate, ethyl butanoate were conducted by modifying the method according to Norman and Craft (1971). By using standard curves, we can directly convert the peak areas to concentrations (ppm) for compounds of interest, but these concentration are only specific to this experimental environment.

Effects of anaerobic conditions on fruit quality

Results from the scaling method of evaluation imply that bananas kept up to three days under nitrogen treatments have highly desirable scores for their appearance, based on fruit color that did not change, but they have lower quality

bananas kept more than three days in nitrogen treatments would develop black color on the peel within 12 hours after bananas were returned to air. The intensity of black color development was directly related with time under the nitrogen. This event has a plausible explanation related to the activity of polyphenol oxidase (PPO) enzymes, because color changes in banana peel mainly result from the oxidation of phenolic compounds (dopamine) by polyphenol oxidase (Palmer, 1963; Weaver and Charley, 1974). This enzyme requires O_2 to oxidize dopamine into melanin, a black or dark brown end product. Therefore, PPO may be suppressed when bananas were subjected to anaerobic conditions resulting in an accumulation of substrate which can be quickly oxidized by that enzyme after fruits were returned to air. However, the activity of PPO and the accumulation of dopamine were not measured in our study.

Because flavor impact compounds were suppressed under nitrogen treatments, this was reflected in lower scores for fruit flavor. After four days in nitrogen treatments, some bananas showed bruises on the pulp, lacked fruit flavor, and a musty flavor was mentioned by some panelists. The result is that bananas under nitrogen treatments had lower average scores for overall quality reflecting less desirable conditions than those under air conditions. We did conduct banana quality evaluation after those bananas were returned to air, but there were high variations in evaluated scores for all fruit attributes and the evaluation scores were not consistent for consecutive days (data not shown). This result may due to the replacement of some trained panelists and the fact that the bananas may have changed very slightly after they were returned to air, and panelists could not detect the differences. Too many samples presented for evaluation at the same time may have fatigued panelists and was another confounding factor adding to wide variation.

Off-flavor development was an expected result of nitrogen treatments. Off-flavor scores increased with time in nitrogen particularly after three days. However, some panelists recorded off-flavor in both air samples and nitrogen treatments samples in the first few days of the experiment. These factors may result from the difference in desired taste of certain panelists. Bananas in the first few days of the experiment were still perceived as unripe and therefore with atypical flavor that might have been judged as "off-flavor". Therefore, the 'off-flavor' developed in those days was not the same as the 'alcoholic off-flavor' which was our concern. Due to the variation in quality evaluation after bananas had been returned to air, we cannot discuss about the reversibility of off-flavor after five days of nitrogen treatments. One interesting observation was that the development of black color was faster in bananas kept under nitrogen for five days. Therefore, we assume that after five days in nitrogen, no reversal of banana attributes was occurring.

Correlation between off-flavor and volatile compounds

From the correlation between off-flavor and volatile compounds produced under nitrogen treatments, it was clear that ethanol is the most probable cause of off-flavor in ripening banana. Our study confirms similar results found by Ke and Kader (1990) in orange; Aharoni et al.(1981) and Ke et al.(1991a) in strawberry; Ke et al.(1991b) in peach; Delate and Brecht (1989) in sweet potato. Moreover, Kosittrakun (1989) suggested that there may be other compounds involved in off-flavor development in apple and pear. To confirm the result that ethanol is the cause for off-flavor in ripening bananas, we tried to carry out one experiment for testing the effects of applied ethanol on banana fruit flavor. This

experiment was conducted by treating normally air-ripened bananas with a dynamic system containing certain amount of ethanol, according to the method of Berger et al. (1992). However, after five days, headspace ethanol did not show a good correlation with tissue ethanol concentration nor had off-flavor developed in those bananas (data not shown). The threshold of ethanol concentration for off-flavor development was not clear in this study, perhaps due to the panelists misunderstanding about the meaning of "off-flavor" in the first few days. Thus, results showed that off-flavor developed from the first day in the experiment where no tissue ethanol was detected. However, later there was strong evidence (off-flavor evaluated scores and tissue ethanol measurement) which showed that off-flavor was highly developed after three days of nitrogen treatments where tissue ethanol was 300 mg/100 g FW and headspace ethanol was 0.54 ppm. The threshold for headspace ethanol found in our study was quite low (0.5 ppm) when compared to that in other fruits, such as 100 μL /L in strawberry and up to 1000-4000 μL /L in orange (Ke and Kader, 1990). This confirms the problem that the actual concentration of headspace volatiles in the flow-through system could not be readily determine by SPME.

Correlation between headspace ethanol and its corresponding esters

Production of ethanol slightly decreased while production of ethyl acetate and ethyl butanoate dramatically increased after bananas were returned to air. Therefore the ethanol which accumulated under nitrogen treatments was correlated, to some extent, to production of its corresponding esters, ethyl acetate and ethyl butanoate after fruits were returned to air. This finding can be explained by esterification of ethanol to esters via activity of the enzyme alcohol

acyl CoA transferase as proposed by Ueda et al. (1992). Therefore, esterification is another possible means for ethanol metabolism under return to aerobic conditions. However, the mechanism for regulation of ethanolic fermentation in fruit is still unclear (Ke et al., 1994).

In conclusion, anaerobic conditions (nitrogen treatments) cause reduction of flavor impact compounds of banana flavor which corresponds to lack fruit flavor. However, there were slight changes to banana peel color under nitrogen. As a typical result of anaerobic conditions, ethanol accumulation is the most important detrimental effect. This is the major cause of off-flavor in ripening bananas as well as in some other fresh fruits. The off-flavor threshold for tissue ethanol appears to be about 300 mg/100 g FW or at 0.5 ppm for headspace ethanol. Banana tolerance to anaerobic conditions had not been determined by physiological measurement e. g., soluble solid content (SSC) or resistance to gas diffusion, but was based on the quality evaluation. In order to determine fruit tolerance, reversibility of all fruit attributes should be determined, particularly for off-flavors, the most important detrimental effects that limit fruit tolerance to low O_2. Our data shows that ripening bananas can be kept in anaerobic conditions at 20°C for no longer than three days because other detrimental effects will occur: lack of fruit flavor, softening of pulp, or changes in the peel color after transfer to air. Although accumulation of ethanol under anaerobic conditions caused off-flavor in fresh fruits as previously mentioned, postharvest application of ethanol and acetaldehyde enhanced some fruit sensory quality for example in pears, tomatoes and blueberries (Paz et al., 1981), strawberries (Morris et al., 1979).

Since the low levels of O_2 at or below 1% is required for effective insect control in fresh fruits (Ke et al., 1991a; Ke et al., 1991b; Ke and Kader, 1992b), our results indicate that even three days in that condition could injure ripening

bananas. As a result, this treatment can not be applied for postharvest insect quarantine treatment in ripening bananas if insects of interest require lethal exposure time longer than three days. Other possible means to treat bananas may involve using green bananas (preclimacteric bananas) instead of ripening bananas (climacteric or postclimacteric bananas). Because green bananas are normally less sensitive to such stress conditions due to the difference in both physiological and biochemical components.

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CONCLUSIONS

Solid phase microextraction (SPME) coupled with capillary GLC/FID or GLC/MS separated 36 major volatile compounds produced by ripening bananas under normal conditions at 20°C. Major banana volatiles were acetate and butanoate esters which accounted for more than 70% of total volatiles. Flavor impact compounds for banana increased with time, reached a maximum, then decreased. In general, volatile production decreased after bananas reached color stage 7 (yellow flecked with brown) for a few days.

Anaerobic conditions (nitrogen treatments) markedly decreased production (by more than 80%) of volatile compounds but most flavor impact compounds resumed production after fruits were returned to air (normal conditions). Anaerobic conditions suppressed production of butanoate compounds more than acetate compounds and also had greater effects on green tip banana (color stage 5) compared to all yellow bananas (color stage 6). In our study, we categorized effects of anaerobic conditions into 6 groups based on trends in volatile production during anaerobic conditions and after fruits were returned to air (normal conditions). While production of flavor impact compounds were suppressed during anaerobic conditions, alcohol production increased, particularly ethanol which accounted for more than 90% of total alcohols produced by anaerobically ripening bananas. While SPME was used to quantify concentration of headspace volatiles, tissue ethanol was also measured enzymatically in ripening bananas. Production of tissue ethanol increased with time ($r^2 = 0.99$) (approximately 10 fold in 5 days) during anaerobic conditions. After fruits were returned to air, production of ethanol slightly decreased but still was high compared to production of other volatiles.

Smaller quantities of flavor impact compounds produced during nitrogen treatments were related to low taste panel scores for fruit flavor (banana flavor). A significant increase in off-flavor scores occurred after fruits were kept in nitrogen for 3 days when tissue ethanol rose to around 300 mg/ 100 g FW. Bananas in nitrogen treatments had better appearance when compared to air control. However, after returning fruits to air for 12 hours, black color developed on the peel. The longer the bananas were kept under nitrogen treatment, the faster the black color developed on the peel.

Correlations between off-flavor development and specific volatile production under nitrogen treatment were determined but it was only ethanol that showed a high positive correlation ($r = 0.94$). Therefore, ethanol is probably the cause of off-flavor in ripening bananas under anaerobic conditions.

Ethanol accumulated under nitrogen treatments was expected to be a probable precursor for esterification after fruits were returned to air via enzymatic activity of alcohol acyl CoA transferase. Production of ethyl acetate and ethyl butanoate were suppressed during nitrogen treatments but both dramatically increased after fruits were returned to air. However, proof of the conversion of ethanol into its corresponding esters and the activity of enzyme alcohol acyl CoA transferase were not measured in our study.

One advantage of anaerobic conditions might be for potential use as a postharvest insect control treatment. From our results, anaerobic conditions longer than 3 days (nitrogen treatments) can potentially damage the ripening bananas. Therefore, this treatment might not be suitable for application as a banana postharvest insect control. However, this treatment might be safer if green bananas (preclimacteric bananas, or color stage 1) are used instead of ripening bananas. Preclimacteric fruits tend to tolerate stress environments better than climacteric or ripening fruits.

One factor to be considered if this treatment should be applied as a postharvest insect control is which insects are of interest. The different insects, their stages of growth and development likely will require different times and stress intensities for lethal treatment. Therefore this treatment will be effective only when the time required for killing the insects of interest is less than the time before fruit tolerance is lost under the same conditions.

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APPENDIX

Table A 3.1 Means[1] and standard deviations[2] for large taste panel (n = 47) from difference from control method of evaulation. This method is used to detect the differences for bananas quality attributes between air and nitrogen treatments by using 9-point-category scale.

Day	Attributes			
	Appearance	Aroma	Flavor	Overall liking
	[1]Means [2](STD)	Means (STD)	Means (STD)	Means (STD)
1	3.34**(2.54)	1.79**(2.11)	2.93**(2.58)	-0.74ns(3.09)
2	2.19**(1.99)	1.04**(2.30)	1.63**(2.57)	-0.64ns(3.10)
3	2.38**(2.34)	2.04**(2.13)	2.40**(2.65)	-0.70ns(3.17)
4	4.32**(2.25)	3.41**(2.25)	4.28**(2.42)	-1.63**(3.36)
5	2.44**(2.09)	1.98**(2.64)	2.20**(2.42)	-2.04**(2.83)

Means were averaged from total of 47 judges. Means of the difference were obtained by subtracting different scores from bananas in nitrogen by those from air control, ns indicates non significant level and ** indicates significant level at alpha = 0.01

Fig. A 1.1 The seven stages of banana ripening :1, green; 2, green with a trace of yellow; 3, more green than yellow; 4, more yellow than green; 5, only a green tip remaining (green tip); 6, all yellow; 7, yellow flecked with brown (Stover and Simmonds, 1987).

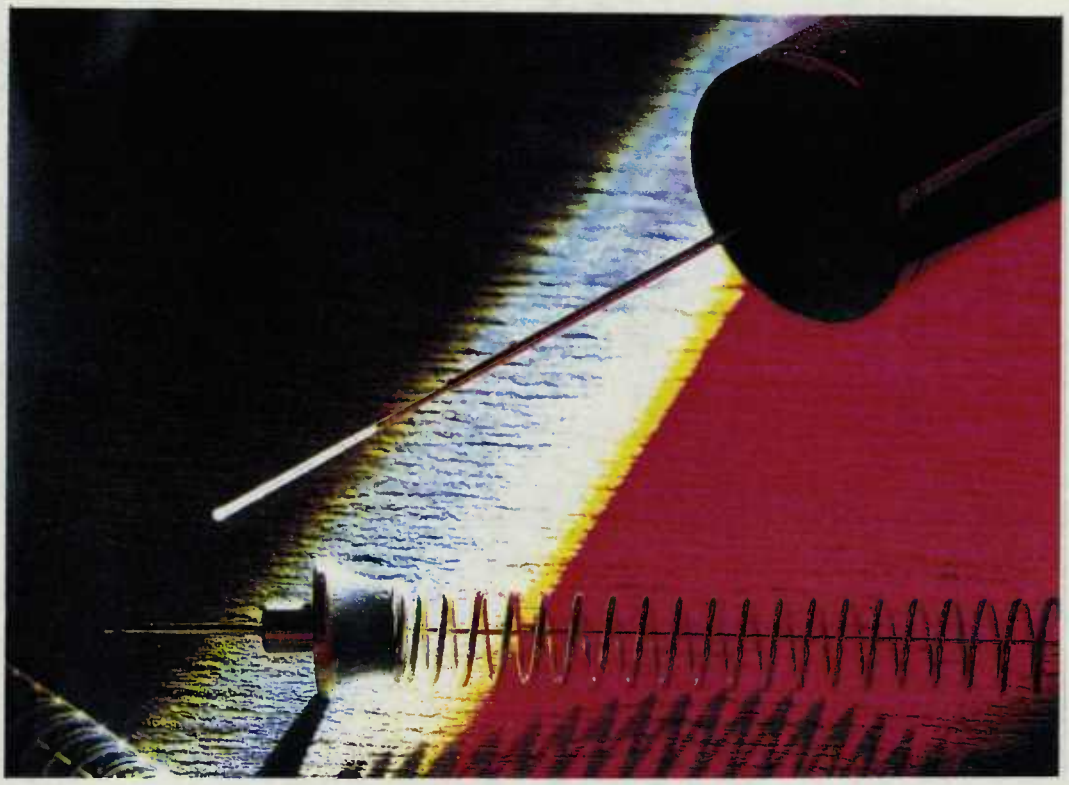

Fig. A 1.2 Solid phase micro extraction (SPME-Supelco, Inc., Bellefonte, PA), a microliter syringe with a fused silica fiber coated with polydimethylsiloxane.

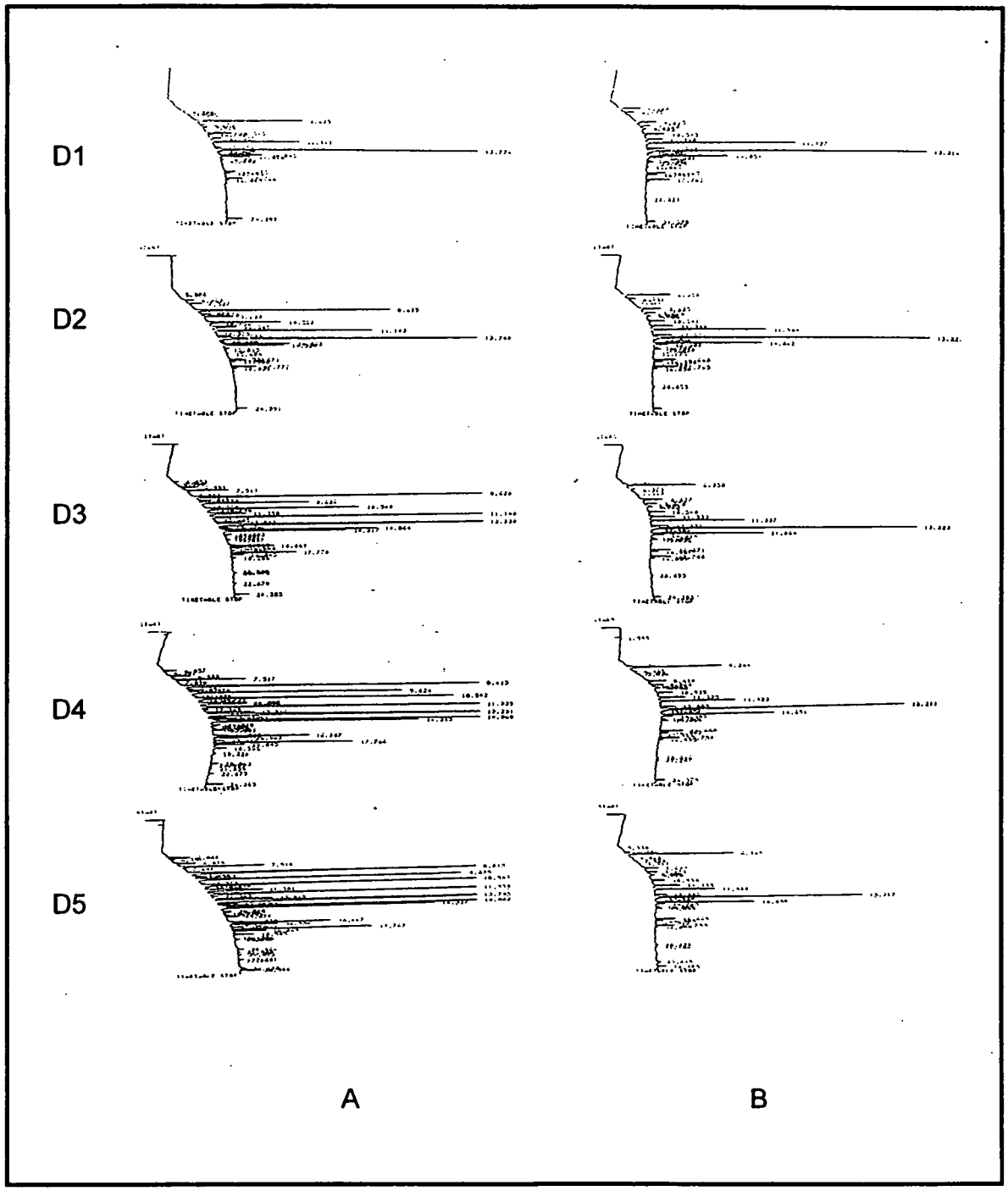

Fig. A 2.1 FID-Chromatograms for the first five days (day 1- day 5) in experiments for all yellow bananas. Volatiles produced under air conditions (A) and under nitrogen treatment (B) were collected by SPME at 20°C with the flow rate of 100 ml/min Chromatography carried out at 60°C for the first 5°C min and then programmed at 5 °C / min to 200°C .

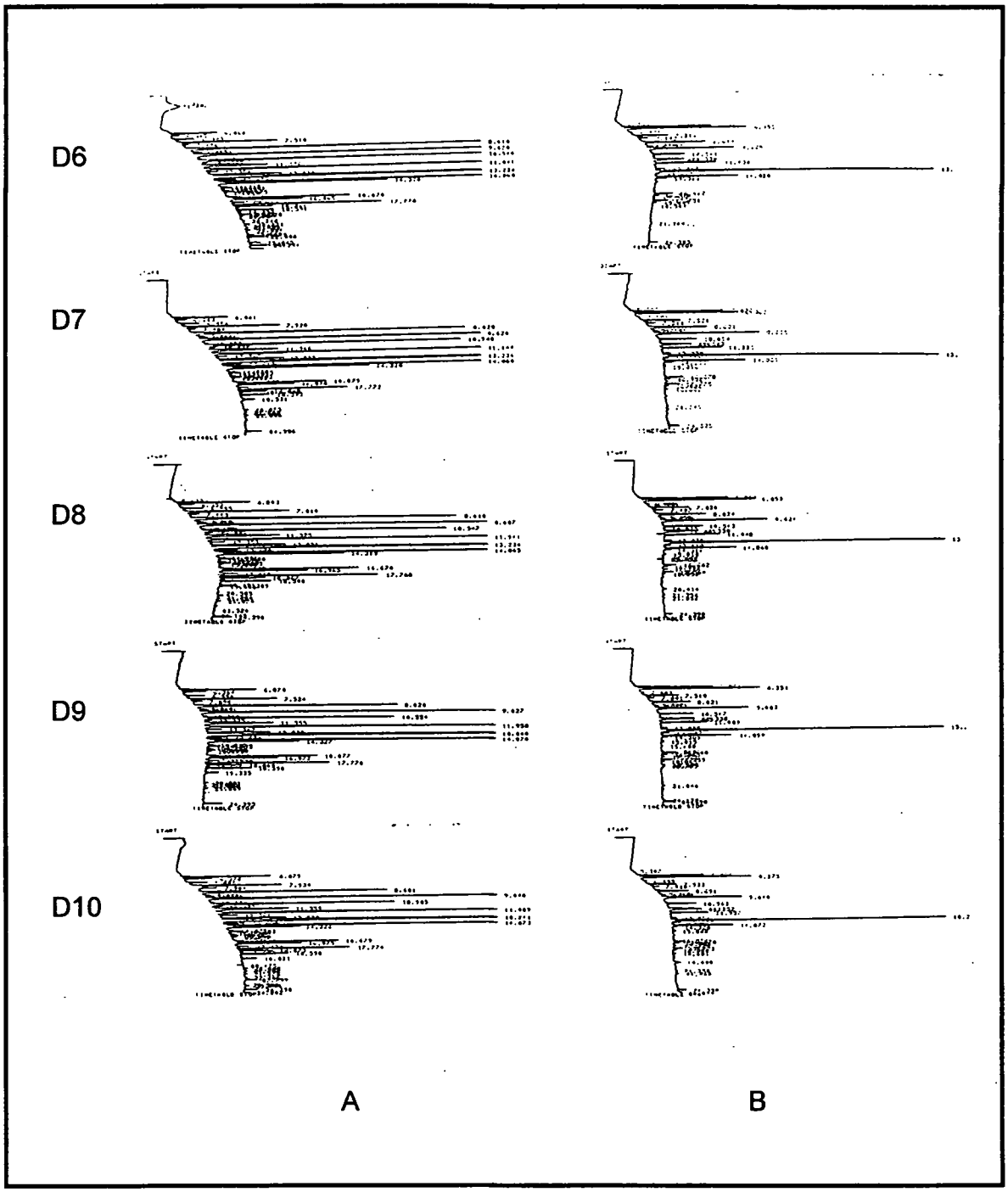

Fig. A 2.2 FID-Chromatograms for the last five days in experiments (day 6 - day 10) in experiments for all yellow bananas. Volatiles produced under air conditions (A) and upon return to air for bananas under nitrogen treatment (B) were collected by SPME at 20°C with the flow rate of 100 ml/min Chromatography carried out at 60°C for the first 5°C min and then programmed at 5 °C / min to 200°C .

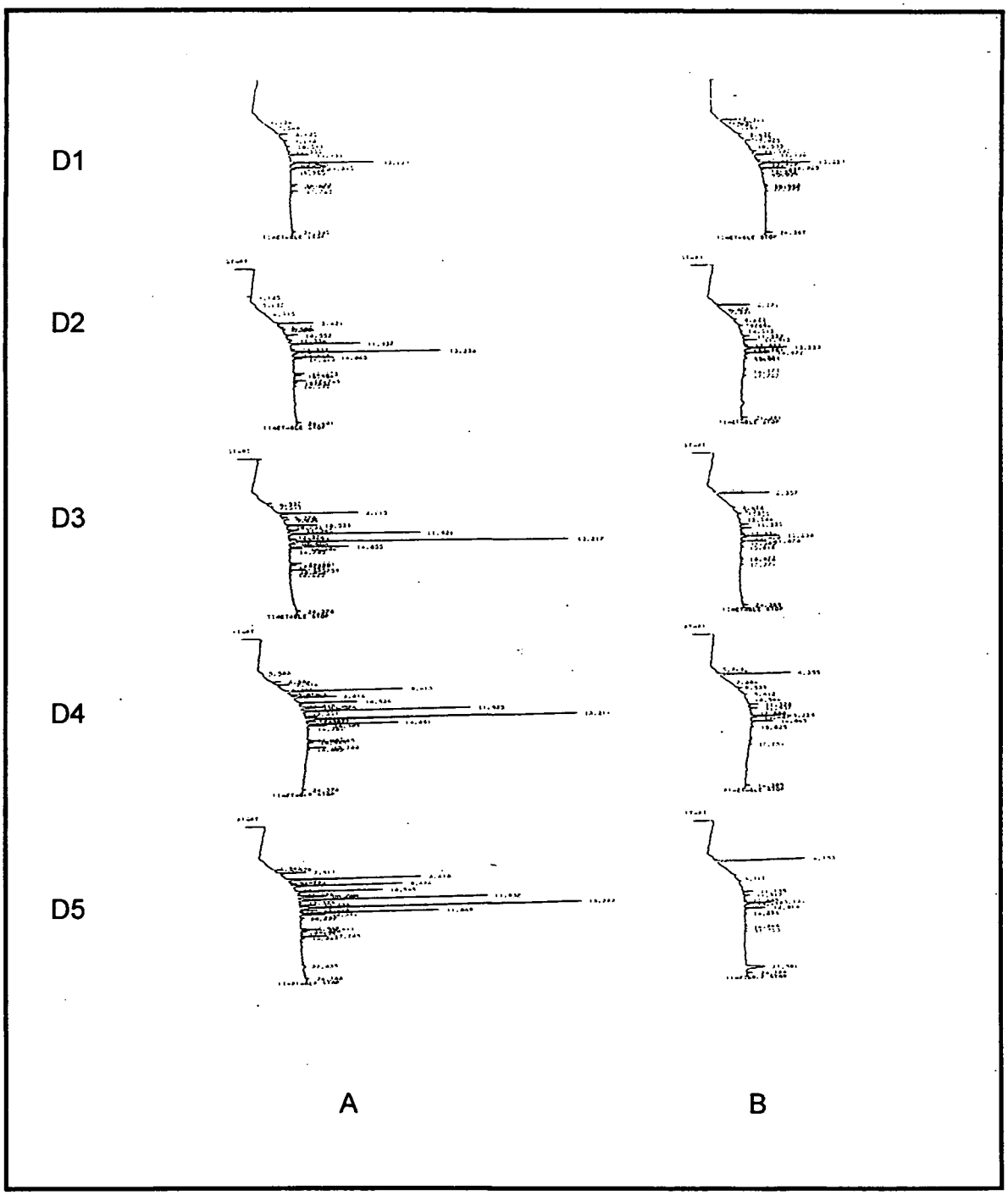

Fig. A 2.3 FID-Chromatograms for the first five days (day 1- day 5) in experiments for green tip bananas. Volatiles produced under air conditions (A) and under nitrogen treatment (B) were collected by SPME at 20°C with the flow rate of 100 ml/min Chromatography carried out at 60°C for the first 5°C min and then programmed at 5 °C / min to 200°C .

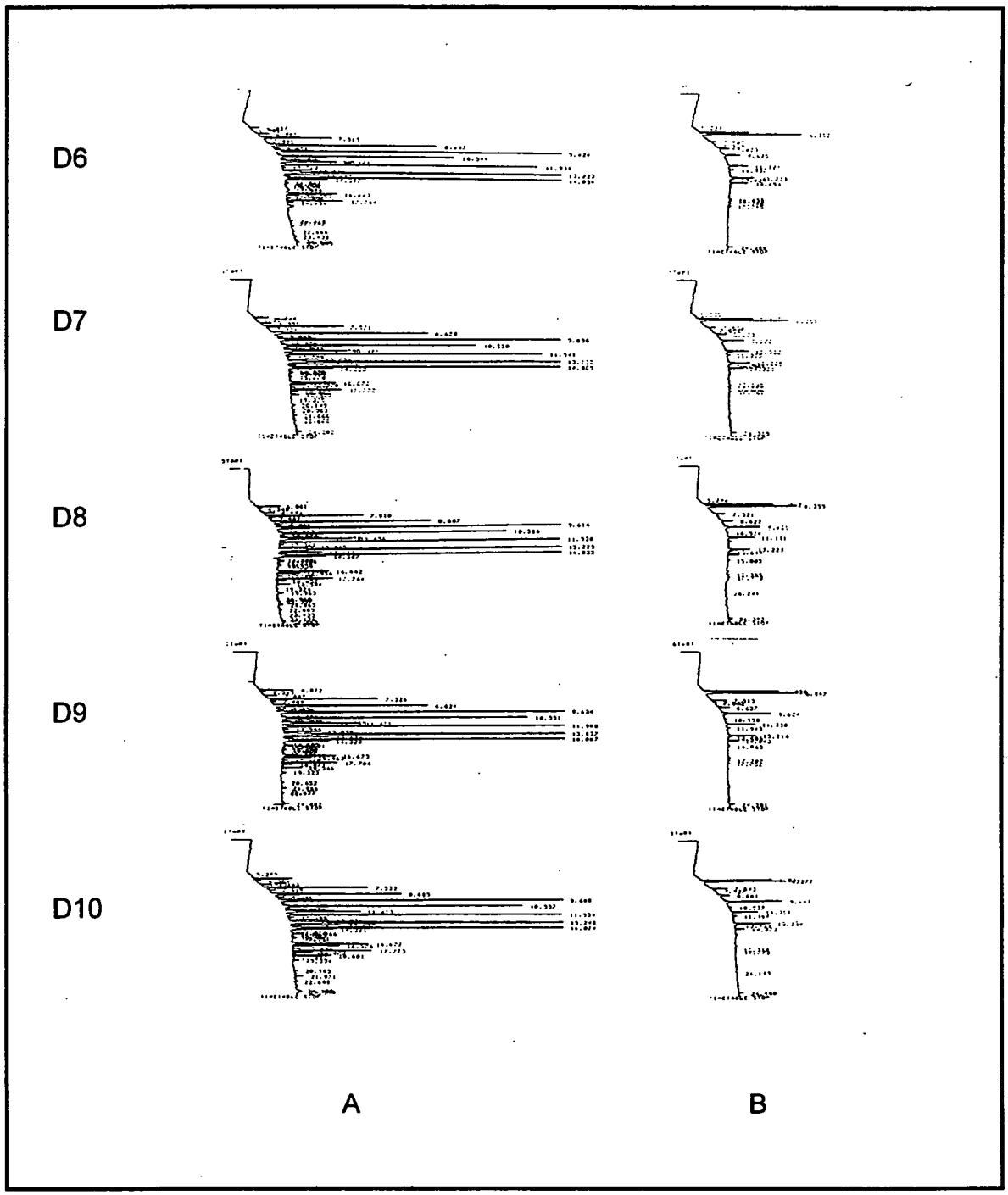

Fig. A 2.4 FID-Chromatograms for the last five days in experiments (day 6 - day 10) in experiments for green tip bananas. Volatiles produced under air conditions (A) and upon return to air for bananas under nitrogen treatment (B) were collected by SPME at 20°C with the flow rate of 100 ml/min Chromatography carried out at 60°C for the first 5°C min and then programmed at 5 °C / min to 200°C .

Fruit Flavor and Eating Quality Ballot

for Banana

Name----------Date----------

Direction : Please evaluate the fruit for appearance,aroma, fruit flavor, off-flavor, texture and overall quality by placing a mark in each line below. Be sure to put the number of each sample on the top of your marked.

I. Fruit Appearance.

I----------I
Poor Good

II. Banana Aroma.

I----------I
Low High

III. Off-flavour Aroma.

I----------I
None Strong

IV. Banana Flavor.

I----------I
Low High

V. Off-Flavor.

I----------I
None Strong

VI. Texture.

I----------I
Soft Firm

VII.Overall Quality.

I----------I
Dislike Like

Fig. A 3.1 Ballot for quality evaluation using scaling method.

Name :_____ Date_____Time_____

Banana Testing Ballot

1. Difference from control :

Instruction : You have been presented two coded samples and a control. Test the control sample, then each coded sample, and rate each coded sample for three attributes as to how different they are from the control.

	Appearance (Looking) Codes#		Aroma (Smelling) Codes#		Flavor (Tasting) Codes#	
	____	____	____	____	____	____
No difference	____	____	____	____	____	____
Just detectable	____	____	____	____	____	____
Slightly Different	____	____	____	____	____	____
Slight to Moderate	____	____	____	____	____	____
Moderately Different	____	____	____	____	____	____
Moderately to Largely	____	____	____	____	____	____
Largely Different	____	____	____	____	____	____
Largely to Extremely	____	____	____	____	____	____
Extremely Different	____	____	____	____	____	____

2. Please check overall qualities for each sample code :

Codes #	Like extremely	Neither like nor dislike	Dislike extremely
--------	I----------I----------I		
--------	I----------I----------I		

Fig. A 3.2 Ballot for quality evaluation using difference from control method

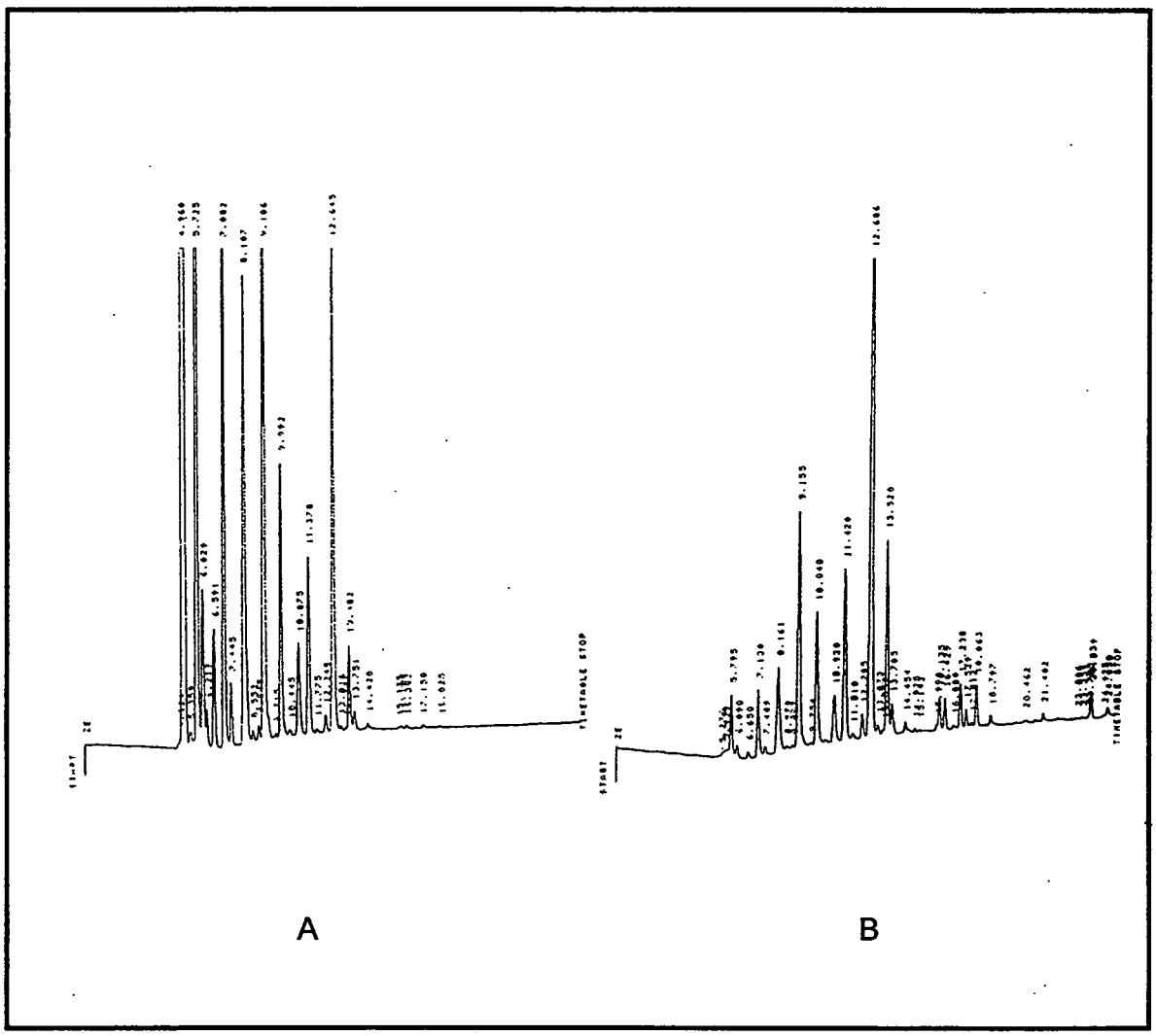

Fig. A 3.3 A typical FID chromatogram of banana volatiles (color stage 7) trapped by activated charcoal (A) compared to SPME (B). Chromatography carried out at 60°C for the first 5 min and then programmed at 5°C / min to 200°C.

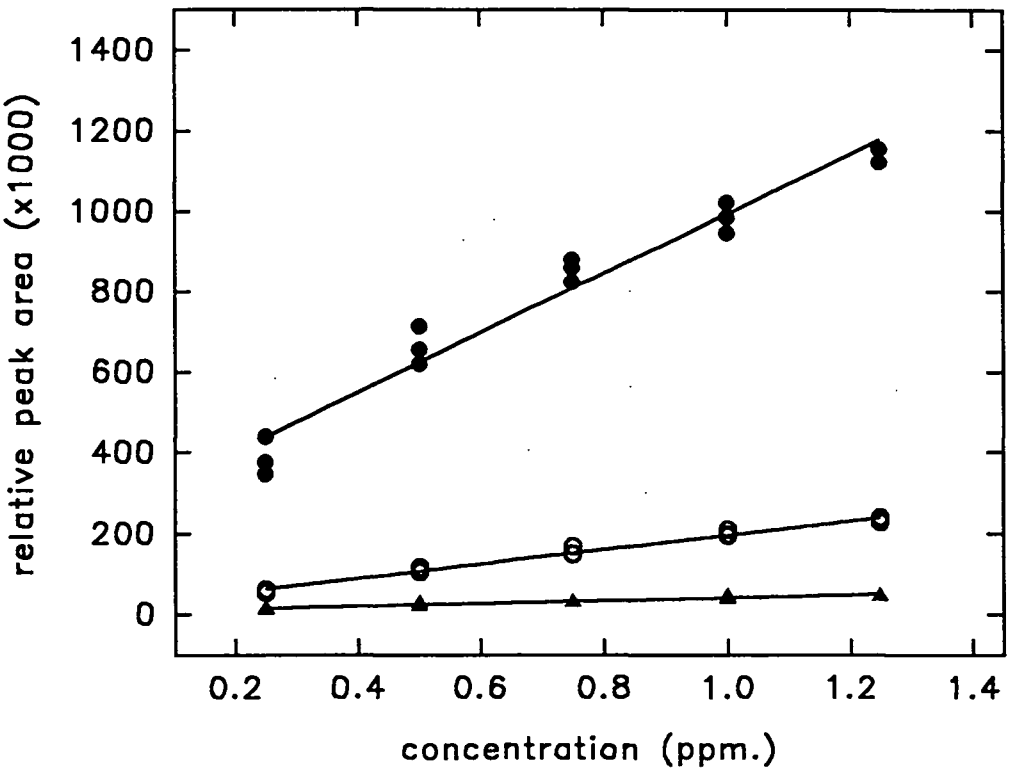

Fig. A 3.4 Graph depicts sensitivity of SPME to the same concentration of ethanol and its corresponding esters, ethyl acetate and ethyl butanoate. Samples were equilibrated for 15 min and SPME was then allowed to absorb for a period of 15 min All samples were conducted at room temperature (25°C)