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thesis entitled

PATTERNS IN THE VOLATILE PROFILE FOR 'REDCHIEF DELICIOUS' APPLE FRUIT DURING RIPENING AND SENESCENCE

presented by

MARIA ALEJANDRA FERENCZI GARDINI

has been accepted towards fulfillment
of the requirements for

MASTER ~~OF SCIENCE~~ degree in HORTICULTURE

Major professor

Date 5-1-03

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PATTERNS IN THE VOLATILE PROFILE FOR 'REDCHIEF DELICIOUS' APPLE FRUIT DURING RIPENING AND SENESCENCE

By

María Alejandra Ferenczi Gardini

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

2003

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ABSTRACT

PATTERNS IN THE VOLATILE PROFILE FOR 'REDCHIEF DELICIOUS' APPLE FRUIT DURING RIPENING AND SENESCENCE

By

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The volatile profile of apple fruit was tracked from three weeks prior to eight weeks after the onset of the ethylene climacteric. The peak in ester emanation roughly coincided with the maxima for respiration and ethylene production. The esters were evaluated separately according to the acid and alcohol portion. As ripening progressed, the chain length of the alcohol-derived portion of the predominant ester declined. Prior to the onset of the ethylene climacteric, esters formed with hexyl alcohol predominated. Throughout the early portion of the climacteric, esters with butyl alcohols predominated. Esters formed with propyl alcohols were the predominant esters during the late climacteric and early senescence phase. In late senescence, the esters from ethyl alcohol were the predominant esters. This pattern was not observed in the chain length of the fatty acid portion. Acetate esters predominated prior to the climacteric and also during the latter stages of senescence. In some cases, despite an increase in acid and alcohol substrates availability, the associated esters declined suggesting that there is an enzymatic factor limiting ester formation. The data suggest that the ester precursor production is developmentally regulated throughout ripening and senescence.

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ACKNOWLEDGMENTS

I want to thank my mentor, Dr. Randy M. Beaudry for freely giving me knowledge, wisdom and insight. Randy not only taught me about postharvest physiology, but also life. I sincerely thank Randy for trusting in my work, listening to my ideas and for sharing his ideas. Randy, I really appreciate your endless patience in answering my endless questions. I also want to thank the other members of my committee, Drs. David Dilley and Christopher Benning for taking the time to read this work and for their contributions.

I want to express my special thanks to all the MSU postharvest people: Drs. Robert Herner, Sastry Jayanty, Deirdre Holcroft, Dina Kadyrjanova, Sergei Makhedov, Zhenyong Wang, Nazir Mir, and Ludmila Roze; Najma Mir, Ryan VanAgtmael, Katie Schwallier, Elzette Van Rooyhen, Sukasem Sittipod, Pattra Maneesin, Ann Clements, and particularly to John Golding, Mauricio Canoles, Melissa Whitaker, Melissa Butkiewicz, and Brian Kevany. Thank you for your help, encouragement and especially your friendship. I may forget some details of this thesis, but I will never forget any of you. Many thanks also to all the other MSU graduate students. These are my friends from all over the world. I also very much appreciate the help and support of all the staff and faculty of the MSU Horticultural Department.

Thanks also to the Latino American Community that made us to feel welcome at MSU and not so far away from home. Thanks particularly to the Marquez family, Mari Paz Gonzalez and Luis Flores. But most of all I am

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eternally grateful to the Uruguayans; Ernesto and Daniela Restaino and their adorable children Joaquin and Silvina, and to Jorge Arboleya, who were always ready to help!

I owe a significant debt of gratitude as well to all my work mates and colleagues from the School of Agronomy in Uruguay, including Luis Viega, Tabare Abadie, Mercedes Arias, Natalia Olivo, Giuliana Gambetta, Adriana Telias and the rest of the crew, for their support and encouragement to make this Masters thesis possible.

Very special thanks also to Dr.Albertina Guarinoni, Ing. Agr. Andres Puppo, and Dr. Marius Huysamer, who introduced me to the brave new terrain of postharvest physiology.

Thank you to all my friends, especially to Mariana Cattaneo, Andrea Pastore, Gretel Ruprechter and Fernanda Skowronek for our continuing fresh friendship while being so far away from each other.

I especially want to thank my large extended family, especially my Mum and Dad, Nilda and Roberto, my brother and sister, Arturo and Elisa, and my Mother in law, Cristina, for their patience, continuing support, constant encouragement and love. And a very special thanks to Harry, my Father in law, I am sure you are very happy and proud of my work.

And finally my loving husband Robert. Robert's endless, dedicated love and boundless support has been of constant inspiration in my life and work. Words cannot thank you enough. And finally Harrycito, our little one, he truly helped Mom during the last 40 weeks of this work!. THANK YOU!!

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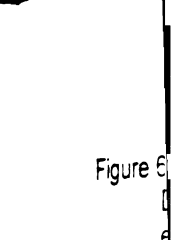

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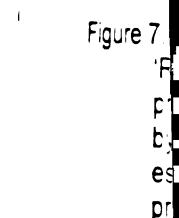

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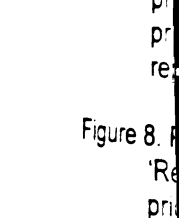

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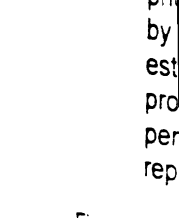

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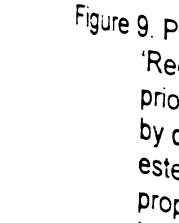

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CHAPTER I: INTRODUCTION

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Consumers consider good quality fruits and vegetables to be those that look good, are firm, and offer good flavor and nutritive value. They buy based on appearance and feel, however their satisfaction and repeat purchases are dependent upon good edible quality. The top three factors ranked by consumers as most influencing their buying decisions, are flavor, appearance and ripeness (Kader, 2002).

Flavor perception is a process that links plant biochemistry with the physiology and psychology of the consumer (Beaudry, 2000). Flavor is composed of taste and aroma. While primarily the sugars, organic acids and phenolics contribute to the fruit taste it is the production of specific organic volatile compounds that determines our sense of aroma. The aroma is the product of the interaction of volatiles molecules retro nasally with the nose olfactory epithelium.

The olfactory system is the most sensitive of the five senses. It can detect odors in parts per trillion, whereas receptors in the tongue can detect taste compounds in parts per hundred (Baldwin, 2000). Aroma compounds contribute heavily to the overall sensory quality of fruit and vegetables. Importantly, the aroma of some fresh horticultural crops including apples has received more attention from both consumers and producers because they perceive insufficient aroma quality (Beaudry, 2000).

The aroma is a complex mixture of different volatile compounds whose composition is specific to species and often to variety. There could be a compound more typical for a specific fruit but in general, the overall aroma quality is the sum of a large number of volatile compounds. In recent years, great

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progress has been made due to advances in physicochemical methods of analysis improving the isolation and identification of a large number of volatile compounds from plant aromas.

Although several of these aroma compounds are complex, large proportions are relatively simple molecules which being volatile at physiological temperatures account for fruit aroma. Paradoxically the most important, both quantitatively and physiologically, volatile compound given off by ripe apples is the olefine, ethylene, which is not directly involved in the aroma or flavor of the fruit (Nursten, 1970). The aroma volatiles are usually present at very low levels, normally in amounts of under a p.p.m. or even p.p.b (v/v). The volatile profile of all fruit is usually very complex. More than 300 volatiles have been isolated from apples (Dimick and Hoskin, 1981). The nature of the volatiles involved is also very diverse and includes esters, alcohols, acids, carbonyl compounds (aldehydes and ketones), and many other chemical groups. The most abundant on a weight basis are esters (78-92%) and alcohols (6-16%) (Dixon and Hewett, 2001).

Studies correlating consumer recognition of the produce with the volatile profile emanating from the produce have shown that only a small number of compounds are responsible for consumer recognition of that commodity (Wills et al., 1998). In most fruit and vegetables, the characteristic aroma is due to the presence of one or two compounds, which are termed "character impact compound". For apples, the key compounds claimed to be responsible for the characteristic green aroma are hexanal and 2-hexenal, and for the ripe aroma ethyl 2-methylbutyrate, 2-methylbutyl acetate, butyl acetate and hexyl acetate

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(Plotto et al.,1999; Fellman, 2000). Ethyl 2-methylbutyrate is a minor component of the aroma fraction but our olfactory senses are extremely sensitive. The threshold concentration, or minimum concentration at which the odor of ethyl 2-methylbutyrate can be detected organoleptically, was found to be 0.001 mL/L. At different stages of maturation, different compounds become the dominant component of flavor.

The biosynthetic pathways for such a wide range of volatiles is also very diverse. However, limited work has been done on elucidating the aroma formation mechanisms. The biosynthesis is further complicated by the fact that while some of these volatiles are synthesized in the intact fruit, others are produced only when the fruit tissue is macerated (Knee, 1993). Volatile precursors include amino acids, membrane lipids and carbohydrates (Figure 1). As a preliminary study on aroma biochemistry the aim of this research was to characterize the patterns in ester biosynthesis during ripening and senescence. This information is hoped improve our understanding of the physiology and biochemistry of ester formation in apples.

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CHAPTER II: LITERATURE REVIEW

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Aroma generation by apple fruit is spontaneous, relying primarily on the developmental stage of the organ. Volatiles are produced by intracellular biogenetic pathways influenced by genetic factors and by ripening and storage conditions (Leahy and Roderick, 1999). Part of apple aroma is that due to tissue disruption by chewing also (Dirinck et al., 1989).

Apple aroma depends upon a complex mixture of organic compounds. Esters are quantitatively and qualitatively the most important compounds and are formed mainly from 2- to 6-carbon alcohols and acids. They are usually saturated and may include branched 4- and 5-carbon units.

Interest in apple volatiles began early in this century. Flath et al. (1967), using commercial essence of Delicious apples as a source of apple volatiles, isolated 56 volatiles compounds. Later Dimick and Hoskin (1981) reported that nearly 300 volatiles have been isolated from apple of which 38% were esters. Of all the volatile compounds identified, only a few esters: butyl acetate, hexyl acetate, 2-methylbutyl acetate and ethyl-2methylbutanoate, are considered major contributors to the characteristic apple-like aroma in most cultivars (Brackmann et al., 1993; Fellman et al., 2000; Song and Bangerth, 1996). Hexyl 2-methylbutanoate is reported also to be important in apple aroma (Rowan et al., 1996).

As an apple ripens naturally, the amount of low-bowling esters tend to build up to a maximum after a period of several weeks (Williams and Knee, 1977). The best volatile composition in 'Starkspur-Golden' apples is comprised of a low content of high boiling-point esters (butanoates) and alcohols and a high content of low boiling-point esters like acetates (Vanoli et al., 1995). Among

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them, pentyl and hexyl acetate were the most abundant esters in quantity. They also identified 3-penten-2-ol considered as a typical compound of ripening 'Golden' apples. Overripeness in 'Golden' apples is correlated with the sum of ethyl butanoate, ethyl propanoate, ethyl-2-methyl propanoate, methyl butanoate, methyl-2-methyl butanoate, ethyl-2-methyl butanoate and ethyl pentanoate (Panasiuk et al., 1980; Patterson et al., 1974; Vanoli et al., 1995; Willaert et al., 1983).

Other volatiles considered key to apple flavor from macerated apple include: trans-2-hexenal, ethyl-2-methylbutanoate, ethyl butanoate, trans-2-hexenol, hexyl acetate, acetate, b-damascenone, ethyl hexanoate and propyl 2-methylbutanoate (Leahy and Roderick, 1999). However, some of them are not present in fresh apples to a significant degree, like trans-2-hexenal (formed upon crushing) and b-damascenone (formed during heat processing) (Dimick and Hoskin, 1981). Schwab and Schreier (1988) identified glycosidically bound volatiles from Jonathan apple fruit.

Cultural and physiological factors affect the production of aroma compounds of apple fruits (Brackmann et al., 1993) but fruit maturity is probably one of the most significant factors (Song and Bangerth, 1996).

APPLE VOLATILES DURING FRUIT DEVELOPMENT AND RIPENING

Apple fruit shows a large increase in CO_2 and ethylene production rates coincident with ripening for what is classified as climacteric fruit (Kader, 1992). Aroma production is closely linked to the onset of the ethylene climacteric and continues to increase as ripening progresses. During ripening, there is a rapid

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increase in metabolites available for biosynthesis of the volatile molecules (Fellman and Mattheis, 1995; Mattheis et al., 1991b; Romani and Ku, 1966; Willaert et al., 1983; Williams and Knee, 1977). An increase in autocatalytic ethylene production and respiratory activity may be essential for a characteristic aroma volatile production (Fan et al., 1998; Song and Bangerth, 1996). However, it is not clear whether the onset of biosynthesis of volatile compounds is concurrent with, or precedes and perhaps plays a role in the initiation of, the climacteric rise in fruit respiration (Fellman et al., 2000).

Early work trying to correlate production of volatiles with the physiological state of the fruit indicates an increase in the production of volatile compounds, which peaked just after the climacteric peak (Brown et al., 1966; Dirinck et al., 1989; Mattheis et al., 1991b; Song and Bangerth, 1996; Tressl and Drawert, 1973).

Yahia et al. (1990), analyzed apples during the maturation and ripening on the tree. Most of the important odor-active volatiles (from apple juice) were formed at or after the onset of ripening and their production followed the autocatalytic evolution of internal ethylene. The ester concentration during ripening of 'Rome' apples increased with advancing harvest date; butyl acetate and 2-methyl-butyl acetate were the main compounds found (Fellman et al., 1993). The acetate concentration of 'Bisbee Delicious' apples also increased during ripening with picking date (Mattheis et al., 1991b). The onset of volatile production was delayed in early picked 'Jonagold' (Hansen et al., 1992), 'Golden Delicious' (Dirinck et al., 1989) and 'Starkspur-Golden' (Vanoli et al., 1995)

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Apples treated with aminoethoxyvinylglycine (AVG), that inhibits ethylene synthesis, and with diazocyclopentadiene (DACP), that inhibits ethylene action, had a reduced production of some volatile esters in both pre and postclimacteric fruit (Fan et al., 1998). The authors suggested that biosynthesis of these esters requires not only continuous ethylene action but also continuous ethylene biosynthesis. On the other hand, acetate ester production was not affected by AVG and DACP in postclimacteric fruit. The authors suggested that sufficient acetate and the enzyme(s) required for ester biosynthesis were present after the initiation of apple ripening regardless of the status of ethylene production or action.

The association of the climacteric with taste and aroma shifts holds for most climacteric crops (Maul et al., 1998; Tressl and Drawert, 1973). This association of the climacteric with maximum rates of ester formation was explained by the fact that ester formation requires acyl-CoA, which is associated with the fundamental metabolism of the cell (Nursten, 1970). Ethylene, recognized as the 'ripening hormone' is responsible for the climacteric rise determining the onset of the ripening of the fruit. Chemical and physical changes occur resulting in changes in color, texture, and flavor. For instance, the chloroplast lamellae break down and the constituents of the membranes, both lipids and proteins, are broken down and may be used as building materials for secondary metabolites. The activity of the enzymes involved in these changes increases during the climacteric producing fatty acids and amino acids, which

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have been shown to act as precursors of apple, banana and strawberry volatiles, all fruits that produce esters as main aroma compounds.

The sequence of aroma volatile production in whole apple fruit changes with advancing of ripeness. The aroma profile in whole and crushed apple fruit changes from aldehydes ("green-notes") to esters ("fruity-notes) during ontogeny (Guadagni et al., 1971; Mattheis et al., 1991b). The concentration of aldehydes declined to no detectable levels by the end of the maturity, when the esters synthesis start as ripening began. Apple fruit reduce aldehydes to alcohols that are subsequently esterified with carboxylic acids (Knee and Hatfield, 1981; Mattheis et al., 1991b).

Preclimacteric 'Golden Delicious' apples are rich in C2-C6 aldehydes, which decrease to trace amounts in climacteric fruits (Fellman et al., 2000). The same progression was observed in 'Bisbee Delicious' apples (Mattheis et al., 1991b). Likewise, there is in 'Starkspur Golden' apples a progressive disappearance of aldehydes (hexanal and (E)-2-hexenal) and a gradual appearance of acetate and butanoate esters during ripening (Vanoli et al., 1995). In 'Gala', 'Delicious', 'Rome' and 'Fuji' apple fruit, acetate ester concentrations increased during ripening as harvest maturity advanced (Fellman et al., 2000).

On the other hand, for some authors aldehydes are important to characteristic apple aroma (Vanoli et al., 1995; Willaert et al., 1983). In McIntosh apples ripe aroma was correlated with C-6 aldehydes (hexanal and 2-hexenal) and overripeness was correlated with esters tentatively identified as ethyl propionate, ethyl 2-methylpropionate, methyl butyrate, methyl-2-methylbutyrate, ethyl butyrate, ethyl 2-methylbutyrate, and ethyl pentanoate (Panasiuk et al.,

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1980). An increasing quantity of (E)-2-hexenal was found in 'Starkspur Golden' apples as the compound responsible for giving the aroma described as 'ripe, aromatic and fruity' (Flath et al., 1967). Other point of view is that aldehydes are produced during chewing (Flath et al., 1967) and could occur in ripen apples (Vanoli et al., 1995), but during ripening they are overwhelmed by the presence of the volatile esters (Guadagni et al., 1971; Mattheis et al., 1991b; Mattheis et al., 1995).

ESTER BIOSYNTHESIS

Dirinck et al. (1989) reported that the generation of aroma esters in apples takes place mainly in the peel, is oxygen-dependent and requires the organization of intact tissue. Peel produced a greater quantity of volatiles than the flesh of intact fruit (Guadagni et al., 1971; Williams and Knee, 1977). This suggested that the primary biochemical system involved in aroma production produces esters and that its activity is located principally in the skin rather than in the flesh of the fruit, apparently because of an abundance of fatty acid substrates resulting from modified metabolic processes and enhanced enzymatic activity (Fellman et al., 2000; Guadagni et al., 1971). Removing the oily, wax coating from the skin did not reduce its ability to produce the esters (Guadagni et al., 1971). Knee and Hatfield (1981), on the other hand showed that the peel has a more active esterifying system than the cortex but the system is qualitatively similar in both tissues.

Amino acids, sugars and lipids all can act as precursors for ester substrates. The final reaction in the pathway for ester formation has been fairly

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well characterized. It is better known in micro-organisms, in the process of fermentation, in which two different enzymes are involved: alcohol acyl-CoA transferase (AAT, EC 2.3.1.84) in the production of esters and esterase in the destruction of esters. AAT catalyzes the transfer of an acyl moiety of an acyl-CoA on to the corresponding alcohol (Dixon and Hewett, 2001), while esterase functions mainly by hydrolyzing esters (Figure 1). Both enzymatic activities have also been described in fruits (Sanz et al., 1997). Experiments using esterase inhibitors have demonstrated that the esterification of alcohols and acids in fruits is a coenzyme A-dependent reaction and that esterase has only hydrolytic activity (Ueda and Ogata, 1977 cited in Perez et al., 1996).

The carbon chain length of the alcohol portion of the esters varies between 1 and 6 carbons. The carbon chain can be both straight and branched. The branched-chains are believed to be derived from amino acids valine (2-methylpropyl-), isoleucine (2-methylbutyl-), and leucine (3-methylbutyl-) (Myers et al., 1970; Perez et al., 1992; Tressl and Drawert, 1973; Wyllie et al., 1995). In the case of isoleucine, it has been proved that there is first a deamination of the amino acid forming 2-methylbutanoic acid, followed by decarboxylation and subsequent reduction to 2-methylbutanol that competes with direct esterification to 2-methylbutanoate esters (Perez et al., 1992; Rowan et al., 1996). The straight-chain alcohols are believed to be reduced forms of short-chain fatty acids (Fellman et al., 2000; Knee and Hatfield, 1981). Alcohols such as butanol and hexanol are produced from fatty acids presumably by b-oxidation followed by reduction in two stages from acetyl-CoA to aldehyde and aldehyde to alcohol (Knee and Hatfield, 1981). The reduction from aldehyde to alcohol is catalyzed

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by the action of alcohol dehydrogenase (ADH, EC 1.1.1.1) on aldehydes. Treatments with aldehydes increased the content of all esters derived from the corresponding alcohols, confirming the activity of alcohol dehydrogenase in apple (Bartley et al., 1985; De Pooter et al., 1983). Vanoli et al. (1995) found that when acetaldehyde was low in concentration, esters reached their maximum production. De Pooter et al. (1983) suggest that apples synthesize esters also through aldehyde reduction.

The ubiquitous nature of ADH may result form the need to eliminate aldehydes, which can be produced by fermentation during low oxigen stress and by lipoxygenase activity following tissue disruption. Actually, ADH is involved in the interconversion of alcohols and aldehydes to supply precursors for ester synthesis and the production of other volatile compounds. Apple tissue has the ability to metabolize added primary alcohols to acetate esters and aldehydes. The formation of aldehydes implies the presence of alcohol dehydrogenase in the tissue. It is also reported the formation of alcohols from acids which implies the presence, in addition to alcohol dehydrogenase, of aldehyde dehydrogenase or an acyl-CoA reductase (Knee and Hatfield, 1981; Tressl and Drawert, 1973). Hexanol could also be derived from hexanal or hexenal, which are fragments resulting from the oxidative cleavage of linoleic or linolenic acids (Knee and Hatfield, 1981).

The acid portion of esters typically has a chain length from 2 to 8 carbons, although there are exceptions. As for the alcohol portion of the molecule, both straight and branched chains are common. The branched-chain compounds are believed to be derived from the same amino acids as the alcohols: valine (-2-

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methyl propanoate), isoleucine (-2-methyl butanoate), and leucine (-3-methyl butanoate). The straight short-chain fatty acids (SSCFAs) are believed to be derived from fatty acid metabolism. Two possibilities seem most likely: short SCFAs may be result from the catabolism of previously formed fatty acids or synthesized *de novo*. Bartley et al. (1985) supports the idea that long chain fatty acids can be precursors to straight chain alcohols, aldehydes and acids, all intermediates for ester formation. They concluded from their results that oxidation of fatty acids is the likely source of precursors for the synthesis of esters with alkyl group (Cn-2, Cn-4) and that the precursors arise because there is a rate limiting step in the b-oxidation pathway. Precursor feeding studies suggest that b-oxidation is responsible for the synthesis of the SCFAs incorporated into esters (Bartley et al., 1985; Brackmann et al., 1993; Rowan et al., 1999). The other possibility is that SCFAs are derived from the pathway of fatty acid synthesis (Tan and Bangerth, 2001). Nursten (1970) implied lipid synthesis as the source of even numbered carbon chains that eventually form esters. He suggested that the intermediates acyl-ACP of fatty acid biosynthesis are very likely to be susceptible to alcoholysis to the corresponding ester as well as to the normal hydrolysis to the free acid. However, the thioesterase B, the enzyme which catalyses the release of SCFAs from the synthetase, has been found in only a few specialized mammalian organs. Fatty acids can be also catabolized through the lipoxygenase pathway. However, this is most active in fruits that produce volatiles by disruption of cells (Bartley et al., 1985; Rowan et al., 1999).

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Knee and Hatfield (1981) suggest that the levels of esters and alcohols in apple tissue result from an equilibrium between synthesis, hydrolysis and diffusion from the tissue. Thus, low concentration of esters could be caused by a lack of precursors, low esterifying activity, high esterase activity or high diffusion rates. Fellman et al. (2000) suggest that the balance between acetate esters and their corresponding alcohols may be regulated by esterase activity.

Applied vapors or solutions of alcohols, organic acids and aldehydes are readily incorporated into esters in intact fruit tissues with low or no ester production (Bartley et al., 1985; Berger and Drawert, 1984; Forney et al., 2000; Knee and Hatfield, 1981; Williams and Knee, 1977; Wyllie and Fellman, 2000). These experiments support the hypothesis that substrate availability is the primary limitation in the production of esters, having qualitative and quantitative effects on the volatile esters profile. Yamashita et al. (1977), working with strawberry, showed that the ester-forming enzyme activity is induced only during the later stages of maturation in strawberry fruit, since no activity was found in unripe fruits. Therefore, they concluded that the lack of most of the volatiles in the immature strawberry fruit is probably due to the absence of volatile precursors and the enzyme forming systems. However, Perez et al. (1993), working with strawberry found that the high level of esterase activity difficult the AAT extraction. This would explain why no ester formation has been detected when homogenized strawberry tissue was incubated with different alcohols by Yamashita et al. (1977). Furthermore, Mattheis et al. (1991b) working with intact 'Bisbee Delicous' apple fruit detected some esters, most notably 2-methylbutyl acetate and butyl acetate, before the onset of ethylene production. Unripe peel

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and cortex tissue were capable of esterifying butanol and 2-methyl propanol to their corresponding acetates (Knee and Hatfield, 1981). These results suggest that the enzymes needed for ester synthesis are functional prior to physiological maturity, indicating that alcohol substrate availability may be the limiting factor in ester formation. Precursor feeding studies in preclimacteric apple fruit (De Pooter et al., 1983; Knee and Hatfield, 1981; Song and Bangerth, 1994) and in preclimacteric banana fruit (Jayanty et al., 2002) demonstrated that the supply of substrates seems to be the limiting factor, rather than the amount of AAT present before the onset of ripening. Rowan et al. (1998) suggested from their feeding experiments with amino acid precursors that there may be competition between substrates, and that enzymatic activity as well as substrate availability may limit aroma biosynthesis.

AAT specificity also plays a key role in this process. Different isoenzymes of AAT present in different fruits have different preferences for the acyl-CoA and the alcohols. This preference is reflected in the volatile profile (Forney et al., 2000; Olias, et al., 1993, 1995; Perez et al., 1993; Ueda et al., 1992). Before AAT was characterized, Knee and Hatfield (1981) suggested that the esterifying system in apple has a relative specificity for longer carbon chain alcohols. Strawberry AAT was found to prefer hexanol when acetyl-CoA was used as an acyl donor although methanol and ethanol were not tested as substrates (Perez et al., 1993). Moreover, the strawberry AAT enzyme seemed to be more active with straight-chain alcohols than against branched-chain alcohols of the same carbon number. Although it had slightly greater activity with acetyl-CoA, AAT acted on various acyl-CoAs (propionate and butanoate). Differences exist among

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acyl-CoA and alcohol specificities between strawberry and banana AAT enzymes (Olias et al., 1995). In both cases, there was a clear correlation between substrate specificity and volatile esters present in the aroma of each fruit (Perez et al., 1992).

Fellman et al. (2000) suggest that apple AAT probably exhibits substrate specificity similar to the final products of the reaction. Treatments of Golden Delicious apples with aldehydes and carboxylic acids suggest that there is a certain selectivity of the apple AAT in the use of the carboxylic acid precursors (De Pooter et al., 1983). They supported the hypothesis that the composition of apple aroma is determined by not only the availability of acids but also by their identity.

The enzyme AAT has been purified and characterized in banana (Harada et al., 1985) and in strawberry (Perez et al., 1993; Perez et al., 1996). The AAT enzyme was localized in the soluble fraction of banana pulp cells (Harada et al., 1985). Strawberry AAT showed to have a pH optimum of 8.0 and optimum temperature of 35°C and an apparent molecular mass of 70 kDa (Perez et al., 1993). It was suggested that AAT could be a membrane-bound enzyme (Perez et al., 1996). Two AAT genes have been cloned from strawberry (Aharoni et al., 2000a) and one gene has been identified in banana and apple (Aharoni et al., 2000b). The size of the AAT gene family in these crops is not known, but several ESTs having high sequence similarity to AAT have been found in the *Arabidopsis* genome (Mekhedov, personal communication). AAT enzymes appear to be a very heterogeneous group with a few common characteristics.

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The relationship between AAT and lipid metabolism in fruits remains unknown (Sanz et al., 1997).

Perez et al. (1996) studied the AAT activity profile during maturation of four strawberry varieties. Only in one variety, AAT activity was detected at the early stages of maturity and all varieties showed an increase in AAT specific activity during maturation. Both absolute and specific AAT activities reached a maximum and then a clear decrease at the overripe stage. Differences among varieties were found not only in relation to maximum AAT values but also in the pattern of AAT activity during fruit maturation. However, the AAT specificity showed similar results as previously reported (Perez et al., 1993). They suggested that high AAT activity should result in higher ester production and subsequently in fruits with enhanced aroma.

No study on apple AAT changes during fruit development has been published; only preliminary studies on the effect of different storage conditions on AAT activity have been carried out (Fellman et al., 1991;Fellman et al., 1993; Fellman and Mattheis, 1995; Ke et al., 1994). Non-treated 'Rome' apple fruit used in Fellman and Mattheis (1995) study showed an increase in AAT activity during the climacteric.

Jayanty et al. (2002) detected AAT gene expression in banana fruit of all stages of ripening. The mRNA for AAT began to accumulate before the onset of aroma production and the maximum level of expression was detected at the onset of natural ester biosynthesis. Similar results were found in white strawberry where AAT expression increased as ripening and color change took place (Aharoni et al., 2000a).

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The enzyme lipoxygenase may play a role in determining the composition of volatile compounds in apple (De Pooter et al., 1983; Fellman et al., 2000). The unsaturated fatty acids linoleic (C18:2 $D^{9,12}$) and linolenic (C18:3 $D^{9,12,15}$) were presumed to be precursors of the carbonyl compounds like the aldehydes hexanal and cis-3 hexenal (Tressl and Drawert, 1973). Unsaturated straight-chain ester volatiles may also be produced by the action of lipoxygenase on unsaturated fatty acids through the intermediacy of the C-6 aldehydes, 3Z-hexenal, 2E-hexenal, and hexanal by the lipoxygenase pathway (Rowan et al., 1999). Fellman et al. (2000) cites a work from Pillard (1986) in 'Golden Delicious' apple where they associated the degreening occurred during ripening with an increase in membrane galactolipids rich in linolenic and linoleic acids from chloroplast degradation. They suggested that these lipids are oxidized by lipoxygenase activity and/or ß-oxidation generating the C_6 aldehydes hexanal and trans 2-hexenal.

Preharvest Factors Affecting Aroma Biosynthesis

Many preharvest factors can affect the development of fruit aroma by impacting ester biosynthesis. Cultivar and rootstock genotype have an important role in determining the flavor quality. Genetic differences between 'Delicious' strains can alter the flavor pattern in apple flesh. However, there appears to be some similarity in the major esters (Fellman et al., 2000). In ripe strawberries, Forney et al. (2000) found both quantitative and qualitative differences in the ester volatiles evolved from different cultivars.

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Miller et al. (1998) conducted studies in 'Delicious' apples to examine whether there is a relationship between red coloration and flavor volatile composition. The effect of canopy position on acetate ester production is opposite to that on anthocyanins, suggesting that the trade-off for high color is a reduction in flavor volatile concentrations. Fellman et al. (2000) detected lower levels of butyl acetate and hexyl acetate in apples with higher proportion of pigmented skin cells. They explained this reduced capacity of acetate ester synthesis by substrate availability limitation. The acetate moieties are used in the synthesis of anthocyanins molecules deposited in peel cell vacuoles. They also found that higher coloring mutations of 'Delicious' had lower levels of the activity of AAT.

Nutrient balance is important for normal production of the compounds responsible for taste and aroma. Esters from freshly-harvest apples from trees high in phosphorus had a higher ester production than those from trees low in phosphorus (Brown et al., 1968). Nitrogenous fertilizers, when used in conjunction with potassium and phosphorus, increased the amount of volatile compounds produced by apples (Somogyi et al., 1964 cited in Brown et al., 1968). However, more recently Fellman et al. (2000) found no statistically significant effect of nitrogen nutrition on the volatiles profile of 'Redspur Delicious' apples. The authors did not find an effect of nitrogen application on the availability of amino acid related precursors. Degradation of chloroplast components and associated macromolecules may create a large pool of amino acids residues needed for synthesis of branched-chain esters.

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Ferrandino et al. (2001) reported the effect of environmental conditions on apple quality and on aroma production. Fruit from higher altitudes (1000 m a.s.l.) and with north exposure had higher quantities of alcohols responsible for fruit aroma. Little is known regarding the impact of other cultural practices on volatile biosynthesis (Fellman et al., 2000).

POSTHARVEST FACTORS AFFECTING AROMA BIOSYNTHESIS

Harvest date and storage regime can positively or negatively effect esters, alcohols, and hydrocarbons (Girard and Lau, 1995). There is an effect of the state of maturity of fruit prior to being placed in store, with a greater volatile emission from fruit from latter harvests (climacteric stages) than from earlier harvests (preclimacteric stages) (Bangerth et al., 1998; Brachmann et al., 1993; Ferrandino et al., 2001; Mattheis et al., 1995; Williams and Knee, 1977).

Controlled atmosphere (CA) storage is commonly used to delay ripening and extend the storage life of apples (Fellman et al., 1993). CA storage utilizes oxygen and carbon dioxide concentrations of about 1 to 5 percent for each gas (Kader, 1992). Many investigations have revealed that CA storage significantly suppresses aroma production (Brackmann et al., 1993; Fellman, et al., 2000; Girard and Lau, 1995; Ke et al., 1994; Mattheis, et al., 1995, 1998; Tough and Hewett, 2001). However the last steps of the ester biosynthesis pathway are active after fruit is removed from CA (Bartley et al., 1985; Brackmann et al., 1993; Knee and Hatfield, 1981). Investigations in the response of apple AAT to regular air and CA storage suggest that inhibition of ripening-related events

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influences subsequent AAT activity after storage (Fellman et al., 1993; Fellman and Mattheis 1995; Fellman et al., 2000).

Other postharvest technique is modified atmosphere packaging (MAP) which objective is to generate an atmosphere similar to CA, with sufficiently low O_2 and/or CO_2 to influence the metabolism of the products being packaged. Song et al. (1997) demonstrated that the impact aroma character volatiles, such as butyl acetate, hexyl acetate, and 2-methylbutyl acetate were significantly reduced under the low O_2 conditions. On the contrary, ethanol and ethyl acetates were identified as the major volatile compounds. The increased AAT activity found in strawberries under passive modified atmosphere (MA) storage conditions (CO_2 > 30%) could be attributed to a detoxifying function of AAT to eliminate the excess of ethanol generated by fermentation (Perez et al., 1996).

Ultralow oxygen (ULO) storage conditions in 'Golden Delicious' apples decreased straight-chain esters such as butyl acetate, while branched-chain esters such as 2-methylbutyl acetate were suppressed by high CO_2 (Brackmann et al., 1993). Suppression of aroma production by ULO conditions seems to be related to low fatty acid synthesis and/or degradation. Suppression of aroma production under high CO_2 concentrations seems to be related to an inhibition of the tricarboxylic acid (TCA) cycle from which most amino acid precursors are derived (Brackmann et al., 1993).

Fermentation induced by anaerobiosis produces large quantities of acetaldehyde and ethanol, which increases the production of ethyl esters (Mattheis et al., 1991a). Brief period of hypoxic conditions (100% CO_2) alters volatile profile of apple fruit (Dixon and Hewett, 2001; Forney et al., 2000). The

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authors suggested that this enhancement of ethyl esters might be due to competitive inhibition by ethanol of biosynthesis of esters from other alcohols and/or to a change in AAT activity and/or substrate specificity of the volatile biosynthetic pathway.

CA treatment in strawberry enhanced activities of fermentation enzymes pyruvate decarboxylase and alcohol dehydrogenase causing ethanol accumulation. As the AAT activity was slightly decreased, the increased ethanol concentration competes with other alcohols for carboxyl groups for esterification reactions and the biosynthesis of ethyl esters increase (Ke et al., 1994). Enhanced apple sensory quality upon application of high amounts of ethanol vapors decreased the concentration of some butyl- and hexyl esters indicating that the esterification of acyl moieties (especially C4 and longer) is likely a competitive reaction (Berger and Drawert, 1984).

Apples treated with the new growth regulator 1-methylcyclopropene (1-MCP), that prevents the action of ethylene have longer storability and are perceived to be less ripe. It decreases the biosynthesis of aroma volatiles by apple fruit to levels similar to those of fruit given CA storage and delay the onset of biosynthesis (Ferenczi and Beaudry not published), yet more acceptable than control apples (Lurie et al., 2002). Similarly, application of 1-MCP on mature green bananas caused a quantitative but not a qualitative change in the composition of the aroma volatiles (Golding et al., 1999).

Dimick and Hoskin (1981) cited works that studied the effect of water loss on flavor volatiles. The measured quantity of esters increased while the alcohols decreased when the rate of weight loss per week increased.

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MEASURING THE VOLATILES COMPONENTS OF APPLE FRUITS

Human olfaction is exceptionally sensitive, capable of detecting very low concentration of volatiles compounds. Humans can discriminate over 10,000 distinct odors. Gas chromatography (GC) detectors vary in sensitivity and they can far exceed the human nose in sensitivity with compounds with little or no olfactory effect, but they can be up to 10,000 times less efficient than the nose, for those compounds which the nose most readily senses (Nursten, 1970).

Certainly when considering apple volatiles, the primary method of component separation is GC and although many identification methods exist, the most useful is GC-MS (Dimick and Hoskin, 1981). Earlier volatiles analyses have been done by the classical flavor isolation procedures of steam distillation and/or solvent extraction. More recently, investigators have employed basically either direct headspace or dynamic headspace purge-and-trap methods (Baldwin et al., 2000). The purge-and-trap method collects the volatile compounds from the air passing over the whole fruit trapping and concentrating them on a solid support such as charcoal or Tenax. The trap is later heated to release volatiles into GC or GC/MS systems. Aroma volatile analysis can also be by extraction of disintegrated tissue or direct measurement of the volatiles in the headspace of fruit tissue discs (Knee and Hatfield, 1976).

However, these methods are expensive and time-consuming processes. The newest method used is solid phase microextraction (SPME), a rapid sampling technique where volatiles interact with a fiber-coated probe that is inserted into the headspace of a sample and then transferred to GC/MS injection

port where the volatiles are desorbed (Matich et al.,1996; Song et al., 1997; Song et al., 1998).

Aside from GC and GC/MS methods, there are sensor arrays called 'electronic noses' (EN) that are useful for discriminating one sample from another based on the volatile profile, rather than for identification/quantification (Baldwin et al., 2000). An EN is comprised of a series of nonspecific gas sensors that are useful for aroma discrimination since their electrical resistance properties are altered by the adsorption of volatile compounds produced by the sample (Maul et al., 1998).

To know which aroma compounds are contributing to flavor, aroma extraction dilution analysis (AEDA) or "Charm" analysis use a sniff port on a GC while diluting the sample. A simpler method is to establish odor thresholds (the level at which a compound can be detected by smell). This is done in the food or in some similar medium since odorants' volatility can change with polarity and viscosity. Log odor units can then be calculated from the ratio of the concentration of a component in a food to its odor threshold. Volatile compounds with positive odor units are assumed to contribute to the flavor of a food, while those with negative units may not (Baldwin et al., 2000).

The concentration of the volatiles in air passing over apples depends on the permeability of the tissue, the concentration of the volatiles in the peel and/or cortex and the extent of enzyme hydrolysis of esters passing through the peel. According to Knee and Hatfield (1976) experiment, the complexity of factors influencing the composition and quantities of volatiles compounds released by whole apples precludes general conclusions about their relation to internal

concentrations, and the evaluation of their role in apple flavor. Low flow rates of air passing over apples would cause an accumulation of esters over several days, while fast flow rates would cause a similarly slow decline. Thus, it is erroneous to calculate rates of production from concentrations of esters found in air streams passing over apples (Knee and Hatfield, 1976).

Each combination of techniques results in a slightly different volatile profile. Methods used to collect and analyze volatiles can cause the loss of certain compounds. In our case, analysis of headspace compounds by SPME is dependent on their individual vapor pressure and their affinity for the fiber. The more volatile compounds are present in higher concentrations in the chamber headspace. This reflects the compound's contribution to the fruit aroma but does not give its true concentration in the tissue. In addition, it has been demonstrated that the less volatile high molecular weight aroma compounds evaporate slowly form the surface of the apples and are depleted from the headspace because of very rapid adsorption by the SPME fiber (Matich et al., 1996). Disruption of the fruit through homogenization removes barriers to diffusion and allows for the determination of true concentrations, but causes enzymatic changes in the volatile profile especially the production of lipoxygenase products such as the aldehydes hexanal, hexanal and their alcohols (Forney et al., 2000).

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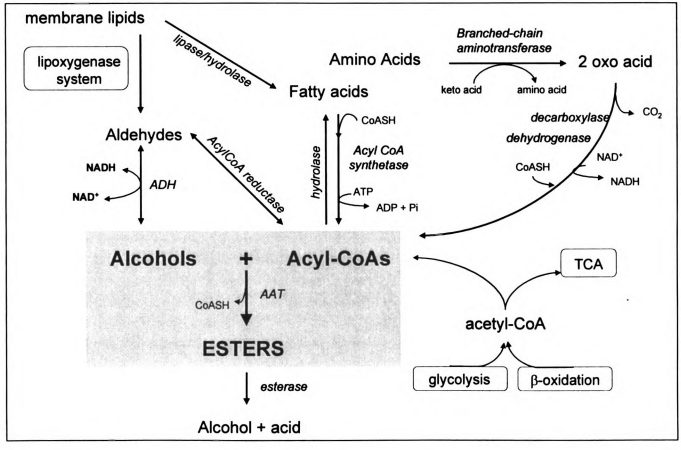

Figure 1. Possible biochemical pathways implicated in volatile ester formation in apple fruit.

CHAPTER III:

PATTERNS IN THE ALCOHOL PORTION OF ESTERS PRODUCED DURING RIPENING AND SENESCENCE OF 'REDCHIEF DELICIOUS' APPLE FRUIT.

INTRODUCTION

Aroma compounds contribute significantly to the flavor of all fresh fruits. However, the aroma of some fresh crops including apples has received more attention from both consumers and producers because they perceive insufficient aroma quality (Beaudry, 2000).

There are several classes of compounds that contribute to aroma. Esters comprise a broadly distributed class of aroma volatiles among various fruit species and contribute significantly to the aroma of apple (*Malus x domestica* Borkh.), pear (*Pyrus communis*), melon (*Cucumis melo*), banana (*Musa sp*), and strawberry (*Fragaria x ananassa* Duch.) fruit. Many factors can affect the development of fruit aroma by impacting the ester biosynthesis: cultivar, growing conditions, fruit maturity, and also storage conditions (Fellman, 2000).

Esters are formed from fatty acids and alcohols (Figure 1 in Chapter 2). The enzyme alcohol acyl-CoA transferase (AAT, EC2.3.1.84) catalyzes the union of an alcohol and the acyl-CoA derivative of a fatty acid. Substrate availability is the primary limitation in the production of esters after storage, having qualitative and quantitative effects on the volatile ester profile (Knee and Hatfield, 1981; Wyllie and Fellman, 2000). Furthermore, AAT has specific preferences for acyl-CoAs and alcohols, which tends to be reflected in the volatile profile (Olias et al., 1995; Perez et al., 1993). It is not known how many different AAT isozymes could be present in apples; only one AAT gene has been identified. No study on apple AAT activity during fruit development has been published; only preliminary studies on the effect of different storage conditions on AAT activity has been carried out (Fellman et al., 1993; Fellman and Mattheis, 1995; Ke et al., 1994).

The carbon chain length of the alcohol portion of the ester varies between 2 and 6 carbons and the acid portion has typically a chain length from 2 to 8 carbons. The carbon chains of the alcohols or fatty acids can be straight or branched. The branched-chain compounds are believed to be derived from amino acids (Nursten, 1970; Perez et al., 1992; Tressl and Drawert, 1973; Wyllie et al., 1995). The straight short-chain fatty acids (SSCFA), between 2 and 8 carbon length, are believed to be derived from fatty acid metabolism, either degradation (Bartley et al., 1985; Brackmann et al., 1993; Fellman et al., 2000; Nursten, 1970; Rowan et al., 1996, 1999) or synthesis (Tan and Bangerth, 2001). The relationship between AAT and lipid metabolism in fruit remains unknown. Although considerable progress has been made in isolating and identifying a large number of volatile compounds from plant aromas, less work has been done on elucidating the aroma formation mechanism.

The aim of this research was to characterize the patterns in ester biosynthesis during ripening and senescence of 'Redchief Delicious' apple to better understand the biochemical origin and fate of these organoleptically significant compounds. Apple fruit were tracked throughout ripening and selected fruit for analysis based on internal ethylene levels. At each stage evaluated, respiration and ester production was measured for five representative fruit. Developmentally dependent patterns in esters were evaluated. An ester matrix was established based on precursor acids and alcohols (Table 1). One axis of the matrix included alcohols (ethanol, propanol, 2-methylpropanol, butanol, 2-methylbutanol, pentanol, and hexanol) and the other axis acids (acetate, propanoate, butanoate, 2-methylbutanoate, pentanoate, hexanoate, heptanoate,

and octanoate). Few alcohol/acid combinations were not detectable. In this paper, we focused on patterns evident in the alcohol portion of esters classed by the acid moiety during ripening and senescence.

MATERIALS AND METHODS

'Redchief Delicious' apples [*Malus sylvestris* (L) Mill. var. *domestica* (Borkh.) Mansf.] were harvested every three to four days at the Michigan State University Horticultural Teaching and Research Center, East Lansing, MI, beginning three weeks prior to the onset of the climacteric and continuing until fruit were considered to have initiated ripening based on internal ethylene content (IEC). The beginning of the climacteric rise was considered to occur when the internal ethylene content was about 0.2 μL/L. The harvest date occurred on October 3rd, which was day 25 of the experiment. Distinct patterns in the ester production were evident.

After the initiation of ripening, the remaining fruit were harvested and held at room temperature for analysis continuing fruit selection for 45 days. Thus, 18 different stages of development of 'Redchief Delicious' apple fruit ranging from unripe through senescent over a period of 70 days were measured. The average IEC of twenty representative fruits at each stage was determined and those five fruit nearest the average were chosen for ester evaluation.

The IEC was determined by withdrawing a 1-mL gas sample from the interior of apples and subjecting the gas sample to gas chromatographic (GC) analysis. The gas chromatograph (Carle Series 400 AGC; Hach Co., Loveland, Colo.) was fitted with a 6-m-long, 2-mm-i.d. stainless-steel column packed with

activated alumina and detection was via a flame ionization detector. The ethylene detection limit was approximately 0.005 $\mu L.L^{-1}$. Ethylene concentrations were calculated relative to a certified standard (Matheson Gas Products, Chicago, Ill.) with an ethylene concentration of 0.979 $\mu L.L^{-1}$.

Volatile analysis procedure was done as described by Song et al. (1997,1998). Ester emissions were sampled by sealing one fruit in each one of five 1-liter Teflon TM chamber. In order to reach a steady-state concentration of apple fruit volatiles in the headspace over the apples, the fruit were maintained in the chambers for approximately three hours at 22°C and the chambers were ventilated with pure air at a rate of approximately 30 mL/min. One chamber with no fruit was used as a blank.

A 1-cm long solid-phase microextraction (SPME) fiber coated with a film thickness of 65 µm of polydimethylsiloxane/divinylbenzene (Supelco Co., Bellefonte, PA) was used to adsorb the volatile sample. The SPME fiber was preconditioned by baking overnight at 260°C.

The fiber was manually inserted through a Teflon-lined half-hole septum into a glass 'tee' located at the outlet of the chambers. Once in the glass 'tee' outlet, the fiber was extended to absorb volatiles for five minutes. The fiber was then retracted prior to removal from the sample container.

Ester analysis was by GC/time-of-flight mass spectrometry (MS). The SPME fiber was inserted in the glass-lined, splitless injection inlet of the GC (230°C) and desorbed for 5 minutes. The volatiles were cryofocussed oncolumn using a liquid nitrogen cryo trap.

The desorbed flavor compounds were separated by a Hewlett-Packard 6890 GC with a capillary column (Supelcowax, 15 m X 0.1 mm i.d., 0.25 μm coating film) (Supelco Co. Bellefonte, PA). The temperature of the GC was programmed from 40 to 240 °C at 50 °C/min. A constant mass flow rate (0.5 mL/min) of the carrier gas (He) in the column throughout the run was maintained. The identification and quantification of the volatiles were by comparison with the National Institute of Standards and Technology database and authenticated standards. Quantification for selected compounds was accomplished using gas standards. Gas standards were created from a mixture of equal volumes of the neat oils of 13 compounds. A sample of 0.5 μL was taken by using a Hamilton 1.0 μL syringe, which was discharged onto a filter paper disk. The filter paper was immediately dropped into a 4.4-L glass volumetric flask fitted with a ground-glass stopper containing a gas-tight Mininert valve (Alltech Assoc., Inc., Deerfield, IL). A new standard was made every month. Volatile aroma compounds were purchased from Sigma Co. and Fluka Chemical Corp. The compounds included in the standard were: 1-butanol, 1-hexanol, cis-3-hexen-1-ol, ethyl alcohol, acetaldehyde, 1-methyl-1-butanol, n-butyl acetate, hexyl acetate, hexyl butyrate, hexyl hexanoate, 3-methylbutyl acetate, 2-methylbutyl acetate, and farnesene mixture. For all compounds identified, not all standards were available.

For each sample, all target compounds were identified. The peak area was determined under the unique ion ID for each specific compound and the total ion count (TIC) was then calculated according to the contribution of the ion to the TIC determined from the NIST library. The quantitative data from the five

replications were averaged and the TIC plotted against time to form curves depicting the production patterns of the volatiles during the 70 days of the experiments runs. It is worth notice that TIC does not reflect quantity, but it can be used for identifying trends over time.

RESULTS

In a typical GC run, ester retention times varied approximately between 55 and 200 seconds (Figure 1). Chromatographic separation was not achieved for many of the volatiles, however determination of the ID unique ions by MS enabled quantification of the responses for many of the target volatiles (Tables 2 and 3).

The initiation of ester biosynthesis followed the climacteric rise in fruit respiration and ethylene content. Total ester production increased rapidly as ethylene biosynthesis increased (Figure 2). Immediately after the peak in ethylene, GC/MS response of total volatiles reached its maximum and then declined. The peak in the TIC for the individual esters classed by alcohol occurred on the analysis date following the maximum in ethylene production (Figures 3a, 4a, 5a, 6a, 7a, 8a, 9a, and 10a). However, some esters were identified at very low levels prior to the onset of the climacteric (e.g. hexyl, butyl and 2methylbutyl acetates; butyl and hexyl butanoates; and butyl and hexyl hexanoates). Typical TICs registered before climacteric were $7x10^5$ for acetates, $9x10^4$ for butanoates and hexanoates. Ester production increased many folds during ripening as evidenced by an increase in the TIC to $1x10^8$, $2x10^7$ and $3x10^7$ respectively.

As ripening progressed, the straight chain length of the alcohol-derived portion of the predominant ester declined. Prior to the onset of the ethylene climacteric, hexanol esters predominated, although their concentration was very low. Then, throughout the early portion of the climacteric, butyl esters tended to predominate. The proportion of propyl esters increased during the late climacteric and early senescence phase. In late senescence, ethyl ester proportion increased (Figure 3c). Butanoate, propanoate and 2-methylbutanoate esters followed similar patterns (Figures 4c, 5c and 7c). The alcohol ester pattern for acetate esters differed slightly, the trends were much less obvious. Despite an increasing incorporation of short-chain alcohols, butanol and hexanol, derived acetate esters maintained their proportions (Figure 6c).

Pentanoate, heptanoate, and octanoate esters were considerably lower than the ester classes previously discussed (Figures 8,9 and 10). Only propyl and ethyl pentanoate esters were identified during the late climacteric and their levels decreased in early senescence (Figure 8). Butyl heptanoate and butyl octanoate were identified early in the climacteric. Propyl and ethyl heptanoate and octanoate esters predominated later in the climacteric (Figures 9 and 10). Free hexanol, butanol, propanol, and 2-methylbutanol were detected after the onset of ethylene climacteric until senescence (Figure 11). Their levels decreased at the peak in the ethylene climacteric (Figures 3b, 4b, 5b and 7b respectively). Only free ethanol was detected throughout the experiment yet no ethyl esters were detected until early senescence (Figures 6b and 11).

Among the compounds present in the standard, 2-methylbutyl acetate had the highest concentration during ripening and senescence. Its concentration was

2800 times higher than its odor threshold (Figure 12a). Only the concentration of butanol was higher than its odor threshold during senescence (Figure 12 b and c).

DISCUSSION

The formation of the aroma compounds is closely correlated with the metabolic changes occurring during fruit ripening. Ester GC/MS response maximized near the peak in ethylene. These finding agree with those of Song and Bangerth (1996) and Fan et al. (1998) who determined that normal ester biosynthesis in apples depends on continuing presence of ethylene. Other authors also found the peak of apple volatile compounds just after the climacteric peak (Brown et al., 1966; Dirinck et al., 1989; Mattheis et al., 1991; Tressl and Drawert, 1973).

The GC/MS response of the esters identified before the onset of the autocatalytic increase was low, however, Mattheis et al. (1991), working with headspace sampling from intact 'Bisbee Delicious' fruit, observed also that 2-methylbutyl acetate preceded the increased ethylene levels associated with the onset of apple ripening. Butyl acetate and hexyl acetate were present in small concentrations during growth or at the time of harvest in 'Golden Delicious' fruit and were only produced in higher amounts during ripening (Willaert et al., 1983). This indicates that the ester biosynthesis system is engaged prior to autocatalytic ethylene formation and that the alcohol and acid precursors are available before the ethylene climacteric. Thus, it appears that either the AAT activity is low or the substrate availability limits ester production in preclimacteric apples. Precursor

feeding studies in preclimacteric apple fruit (De Pooter et al., 1983; Knee and Hatfield, 1981; Song and Bangerth, 1996) demonstrated that the supply of substrates seems to be the limiting factor, rather than the amount of AAT present before the onset of ripening. Similar results were found by Jayanty et al. (2002) in banana fruit. They suggest that the primary limiting factor in ester biosynthesis before natural production is precursor availability, but, as ester biosynthesis is engaged, the activity of AAT exerts a major influence.

The aldehydes (butanal, pentanal, (E)-2-hexenal, and heptanal), and not the esters, have been found to be the main group of volatile compounds detectable from intact immature apples (De Pooter et al., 1987; Fellman et al., 1991, 2000; Flath et al., 1967; Knee and Hatfield, 1981; Mattheis et al., 1991). However, no aldehydes were detected in 'RedChief Delicious' apples in this study.

The qualitative composition of esters was similar to that found by other investigators (Brackmann et al., 1993; Mattheis et al., 1991; Rowan et al., 1996; Song and Bangerth, 1996; Vanoli et al., 1995). The high concentration of 2-methylbutyl acetate, hexyl acetate and butyl acetate during the early climacteric are the same as those previously reported for other Delicious cultivars (Berger and Drawert, 1984; Brackmann et al., 1993; Dimick and Hoskin, 1983; Fellman et al., 1993, 2000; Kakiguchi et al., 1986; Mattheis et al., 1991, 1995). On the other hand, other compounds such as 3-penten-2-ol associated with the characteristic apple-like aroma of 'Starkspur Golden' fruit (Vanoli et al., 1995) or 4-methoxyally benzene (Kakiguchi et al., 1986), which has been reported as contributing to the spice-like aroma in Jonathan, were not detected in this

experiment. Although there appears to be some similarity in the major esters among different apple cultivars (Fellman et al., 2000), the genotype probably explains the differences found in the quality of the aroma profile of ripe and overripe apples among different studies.

The decrease in total volatile GC/MS response, as ethylene biosynthesis declined, could be attributed to a decrease in activity of the enzymes involved in their biosynthesis, to a lack of substrate availability, or to an enhanced esterase activity (Figure 1 in Chapter 2).

The pattern in the alcohol portion found in hexanoates, butanoates, propanoates, and 2-methylbutanoates has not been previously described in detail for apple fruit. However, some indications of this pattern are evident from studies by Vanoli et al. (1995) who found a high content of low boiling-point esters and alcohol later in ripening and by Panasiuk et al. (1980) and Willaert et al. (1983) who correlated overripeness with more ethyl esters. The change in the alcohol pattern may be related with changing specificity of the AAT enzyme for substrate chain length. But also, this shift in the alcohol portion of the ester with time could indicate a developmentally dependent change in the availability of alcohol precursors from predominantly long to predominantly short chains.

On the other hand, while the chain-length specificity for apple AAT is unknown, that for strawberry has a greater preference for acetyl-CoA to form esters with long-chain alcohols (Perez et al., 1993). This is consistent with ester profile changes during late climacteric in our study when, despite increasing incorporation of short-chain alcohols (ethanol and propanol), butanol- and hexanol-derived acetate esters maintain their proportions. Strawberry AAT has

alcohol substrate specificity in the order hexyl>butyl>amyl>isoamyl using acetyl-CoA as co-substrate, and acyl-CoA substrate specificity in the order acetyl>butyl>>propionyl using butyl alcohol as co-substrate (Perez et al., 1993).

Of all the esters identified, the ester formed with the branched-chain alcohol 2-methylbutanol and acetic acid had the greatest GC/MS response early in climacteric. Interestingly, this branched-chain alcohol was identified forming esters significantly only with acetic acid, which may be related with the specificity of the AAT enzyme.

The fact that free hexanol and propanol were detected from the onset of ethylene climacteric until late in senescence suggests that once the ethylene production starts to increase, the availability of hexanol and propanol is not a limiting factor for ester formation. The decrease in hexanol and propanol at the peak in the ethylene climacteric may have been due to higher AAT activity at that point. Then, as senescence commenced, the activity of AAT declined leaving unreacted hexanol and propanol. The alcohol ethanol is also not a limiting factor for the ester biosynthesis given that it was detected prior and throughout ripening and senescence. The fact that only ethyl esters increased at the end of ripening and in senescence suggests that AAT preference for ethanol may have increased or it could be an AAT isoenzyme present during senescence with higher specificity for ethanol. No free pentanol or 2-methylpropanol was detected and the GC/MS response for these esters were very low suggesting that the availability of the alcohols pentanol and 2methyl-propanol could be a limiting factor for the biosynthesis of these ester classes.

CONCLUSIONS

The ester formation system is present before the onset of the autocatalytic increase but the formation of the characteristic apple aroma compounds is correlated with the metabolic changes occurring during ripening and senescence.

As ripening progressed, there was a change in the alcohol portion of esters from predominantly long to predominantly short straight chains. This change may be related with changing specificity of the AAT enzyme for substrate chain length, or may indicate a developmentally dependent change in the availability of alcohol precursors.

The limiting factor for ester biosynthesis could be substrate availability before onset of the ethylene climacteric, the level of one precursor relative to the other during ripening or a shift from substrate limitation to enzyme limitation later in senescence. Probably there is a different AAT in senescence with higher specificity for short chain alcohols. It could be also possible that acyl-CoA synthetase activity declines and/or there is an increase of esterase activity in senescence.

The data obtained in this experiment will be properly interpreted when more is known about the ester formation system and the family of AAT enzymes. Enzyme specificity in apple for acid and alcohol carbon chain length needs to be more fully characterized.

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Table 1: Matrix of target esters based on acid and alcohol precursors for apple fruit. Detected esters are indicated. Esters in bold type were quantified using gas standards.

Acid/ Alcohol	*Acetate (2C)*	*Propanoate (3C)*	*Butanoate (4C)*	*2-methylbutanoate (branched 5C)*	*Pentanoate (5C)*	*Hexanoate (6C)*	*Heptanoate (7C)*	*Octanoate (8C)*
Ethanol (2C)	Ethyl acetate	Ethyl propanoate	Ethyl butanoate	Ethyl 2-Mbutanoate	Ethyl pentanoate	Ethyl hexanoate	Ethyl heptanoate	Ethyl octanoate
Propanol (3C)	Propyl acetate	Propyl propanoate	Propyl butanoate	Propyl 2-Mbutanoate	Propyl pentanoate	Propyl hexanoate	Propyl heptanoate	Propyl octanoate
2-methylpropanol (branched 4C)	2-Mpropyl acetate	---	---	---	---	2-Mpropyl hexanoate	---	---
Butanol (4C)	**Butyl acetate**	Butyl propanoate	Butyl butanoate	Butyl 2-Mbutanoate	---	Butyl hexanoate	Butyl heptanoate	Butyl octanoate
2-methylbutanol (branched 5C)	**2-Mbutyl acetate**	2-Mbutyl propanoate	2-Mbutyl butanoate	---	---	2-Mbutyl hexanoate	---	---
Pentanol (5C)	Pentyl acetate	---	---	Pentyl 2-Mbutanoate	---	Pentyl hexanoate	---	---
Hexanol (6C)	**Hexyl acetate**	Hexyl propanoate	**Hexyl butanoate**	Hexyl 2-Mbutanoate	---	Hexyl hexanoate	---	---

Table 2: Volatile compounds (esters, alcohols, and acids) identified in 'Redchief Delicious' apple fruit during ripening and senescence as a function of GC retention time (seconds).

COMPOUND	RT (SECONDS)
ethyl acetate	57.4
ethanol	63.3
ethyl propanoate	67.1
propyl acetate	69.9
2-methylpropyl acetate	75.9
propanol	79
ethyl butanoate	79.9
propyl propanoate	81.3
ethyl 2-methylbutanoate	82.7
butyl acetate	86.4
2-methylbutyl acetate	95.2
propyl butanoate	95.5
ethyl pentanoate	97.3
butanol	98
propyl 2-methylbutanoate	98.4
butyl propanoate	98.5
pentyl acetate	104.1
2-methylbutyl propanoate	106.9
2-methylbutanol	108.3
butyl butanoate	112.4
propyl pentanoate	112.8
ethyl hexanoate	115.2
butyl 2-methylbutanoate	115.3
2-methylbutyl butanoate	121.1
Hexyl acetate	122.4
Propyl hexanoate	130.4
Pentyl 2-methylbutanoate	131.8
ethyl heptanoate	132.5
hexyl propanoate	133.6
hexanol	134.4

Table 2 (cont'd).

COMPOUND	RT (SECONDS)
2-methylpropyl hexanoate	135.6
butyl hexanoate	146.6
hexyl butanoate	146.9
propyl heptanoate	147.2
hexyl 2-methylbutanoate	149
ethyl octanoate	149.6
acetic acid	152.6
2-methylbutyl hexanoate	153.8
pentyl hexanoate	162
butyl heptanoate	162.2
propyl octanoate	163.2
propionic acid	166.4
hexyl hexanoate	177.6
butyl octanoate	178
butanoic acid	180.2
2-methylbutanoic acid	186.4
hexanoic acid	212.7

Table 3. Conversion factors for those volatile compounds present in the standard.

COMPOUND	CONVERSION FACTOR
ethanol	$3.06X10^{-5}$
butyl acetate	$2.60X10^{-8}$
2-methylbutyl acetate	$8.92X10^{-8}$
butanol	$1.16X10^{-7}$
2-methylbutanol	$5.67X10^{-8}$
hexyl acetate	$8.93X10^{-9}$
hexanol	$1.47X10^{-8}$
hexyl butanoate	$6.06X10^{-9}$
hexyl hexanoate	$1.65X10^{-8}$

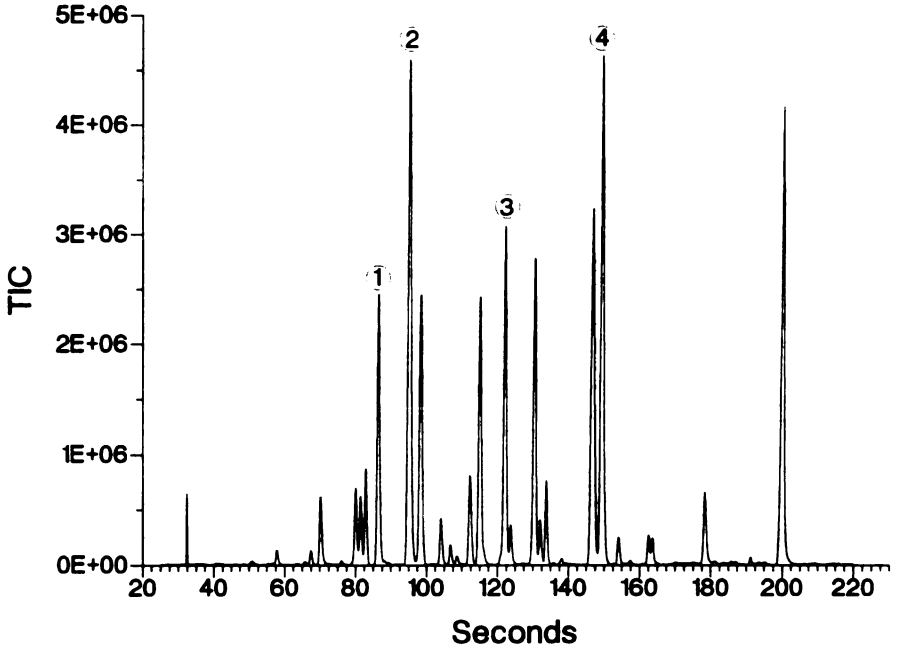

Figure 1. Representative gas chromatograph of the headspace of 'Redchief Delicious' apples at climacteric. Most predominant ester peaks are identified by numbers: 1. butyl acetate; 2. 2-methylbutyl acetate; 3. hexyl acetate; 4. hexyl 2-methylbutanoate.

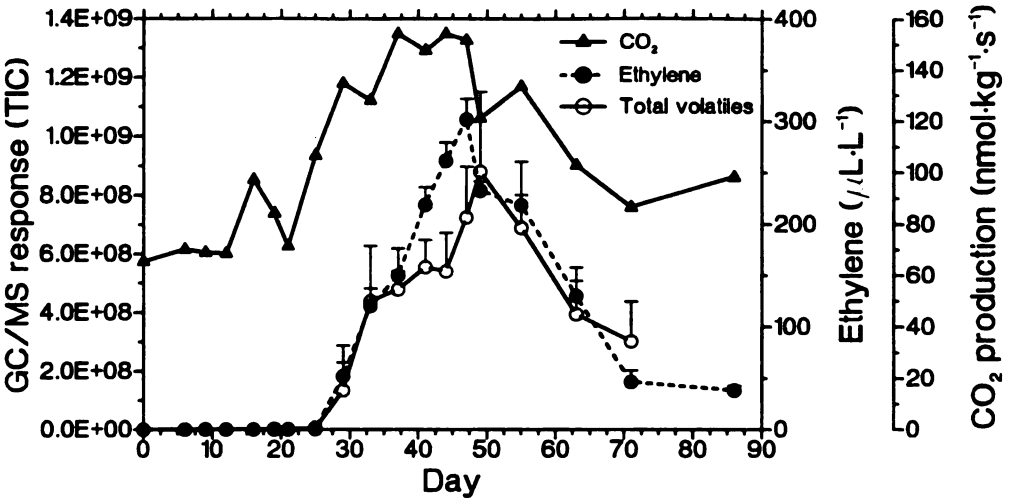

Figure 2. Ontogeny of total esters, ethylene and respiration (CO_2 production) during ripening and senescence of 'Redchief Delicious' apples. The volatile profile of apple fruit was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). Each symbol represents the average of 5 replications.

Figure 3. Pattern of hexanoate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total hexanoate esters and ontogeny of ethylene. (B) GC/MS response (TIC) of ethyl, propyl, 2-methylpropyl, butyl, 2-methylbutyl, pentyl, and hexyl esters of hexanoic acid. (C) Ester proportions (% of total hexanoate esters). Each symbol represents the average of 5 replications.

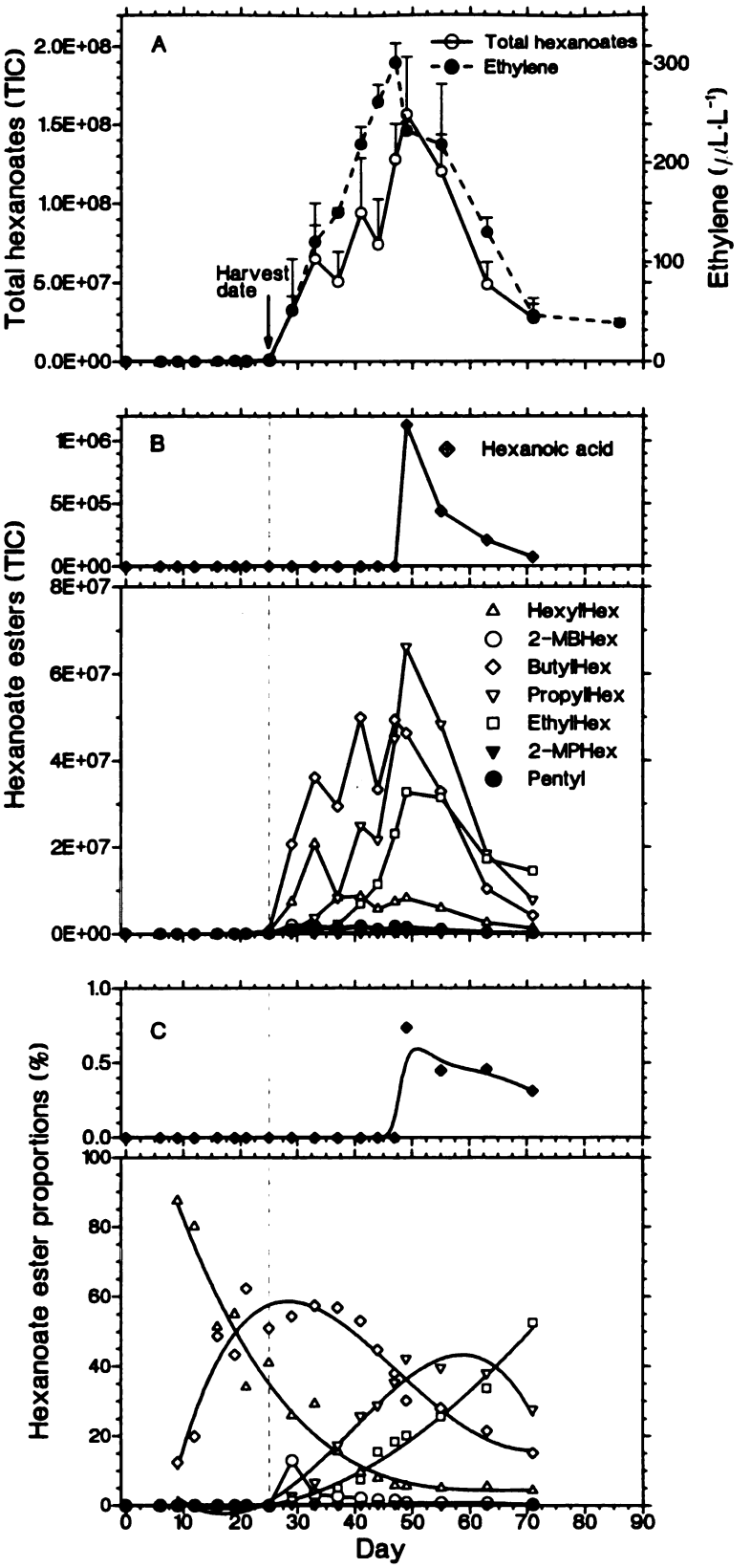
A
Total hexanoates
Ethylene
Harvest date
Total hexanoates (TIC)
Ethylene (μL·L⁻¹)
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
300
200
100
0
B
Hexanoic acid
1E+06
5E+05
0E+00
Hexanoate esters (TIC)
8E+07
6E+07
4E+07
2E+07
0E+00
HexylHex
2-MBHex
ButylHex
PropylHex
EthylHex
2-MPHex
Pentyl
C
Hexanoate ester proportions (%)
1.0
0.5
0.0
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
Day

Figure 3.

Figure 4. Pattern of butanoate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total butanoate esters and ontogeny of ethylene. (B) GC/MS response (TIC) of ethyl, propyl, butyl, 2-methylbutyl, and hexyl esters of butanoic acid. (C) Ester proportions (% of total butanoate esters). Each symbol represents the average of 5 replications.

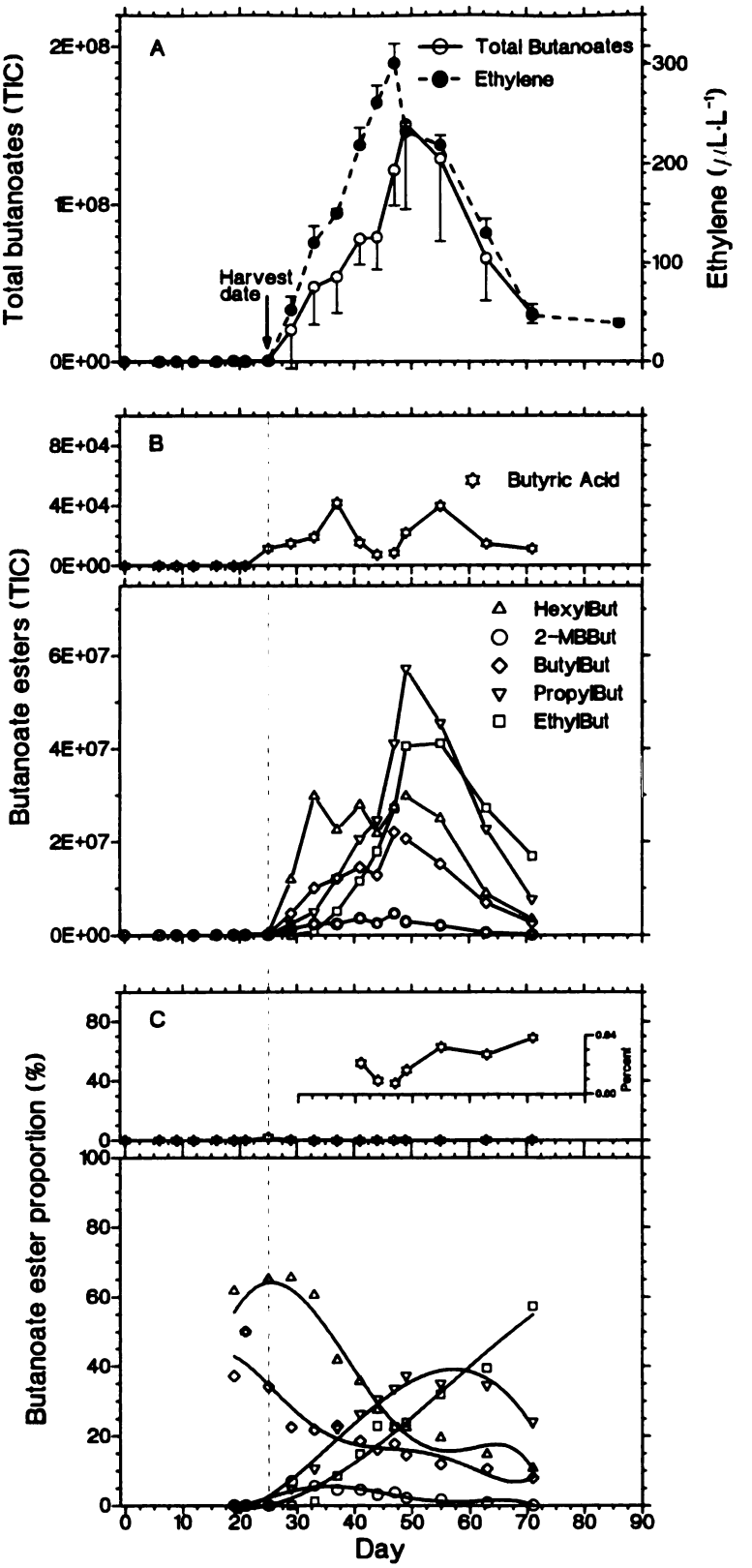
A
Total Butanoates
Ethylene
Total butanoates (TIC)
Ethylene (µL·L⁻¹)
Harvest date
2E+08
1E+08
0E+00
300
200
100
0
B
Butyric Acid
8E+04
4E+04
0E+00
HexylBut
2-MBBut
ButylBut
PropylBut
EthylBut
Butanoate esters (TIC)
6E+07
4E+07
2E+07
0E+00
C
Percent
Butanoate ester proportion (%)
80
40
0
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90
Day

Figure 4.

Figure 5. Pattern of propanoate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total propanoate esters and ontogeny of ethylene. (B) GC/MS response (TIC) of ethyl, propyl, butyl, 2-methylbutyl, and hexyl esters of propanoic acid. (C) Ester proportions (% of total propanoate esters). Each symbol represents the average of 5 replications.

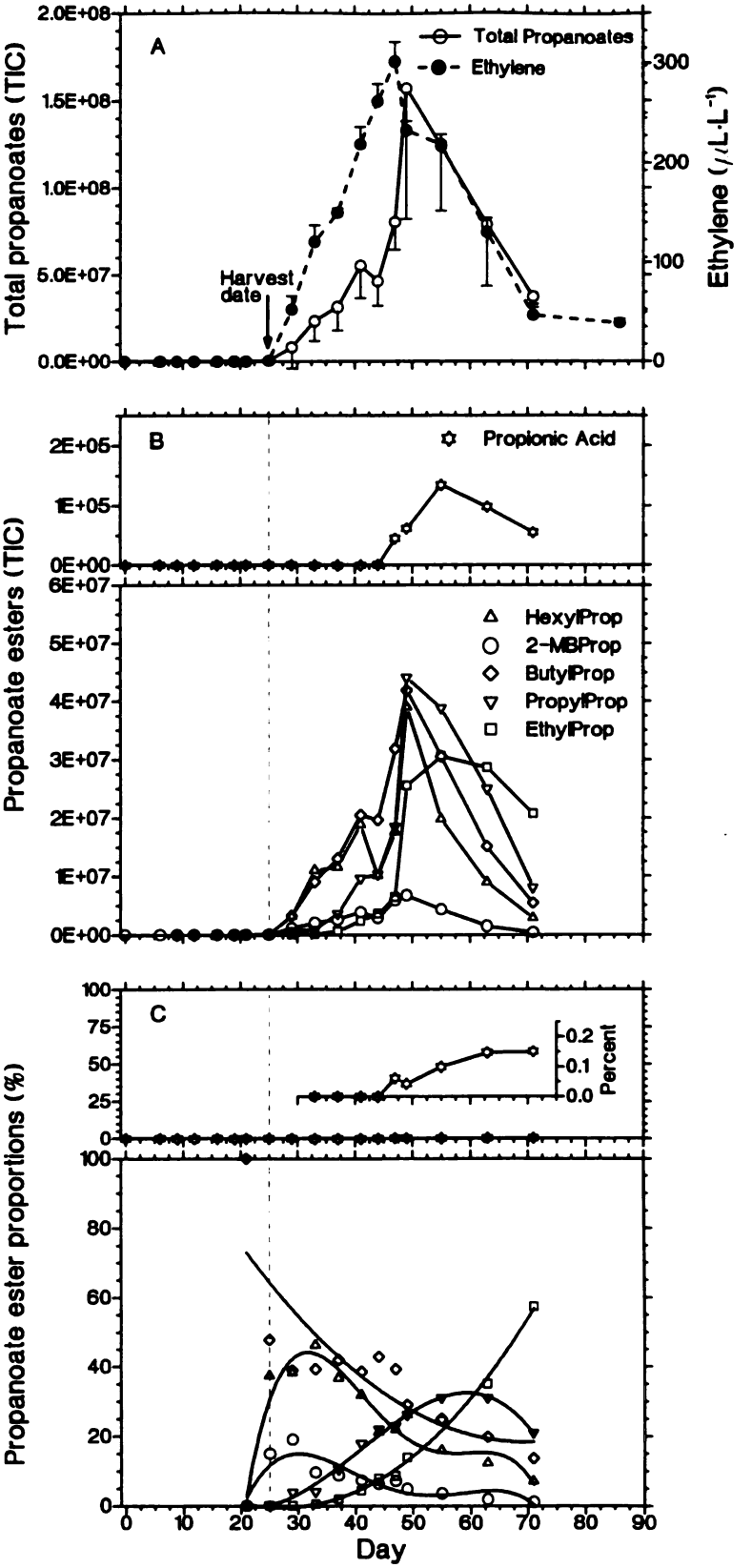
A
Total Propanoates
Ethylene
Total propanoates (TIC)
Ethylene (µL·L⁻¹)
Harvest date
2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
300
200
100
0
B
Propionic Acid
2E+05
1E+05
0E+00
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0E+00
HexylProp
2-MBProp
ButylProp
PropylProp
EthylProp
Propanoate esters (TIC)
C
0.2
0.1
0.0
Percent
100
75
50
25
0
80
60
40
20
0
10
30
40
50
60
70
80
90
Day
Propanoate ester proportions (%)

Figure 5.

Figure 6. Pattern of acetate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total acetate esters (B) GC/MS response (TIC) of ethyl, propyl, 2-methylpropyl, butyl, 2-methylbutyl, pentyl, and hexyl esters of acetic acid. (C) Ester proportions (% of total acetate esters). Each symbol represents the average of 5 replications.

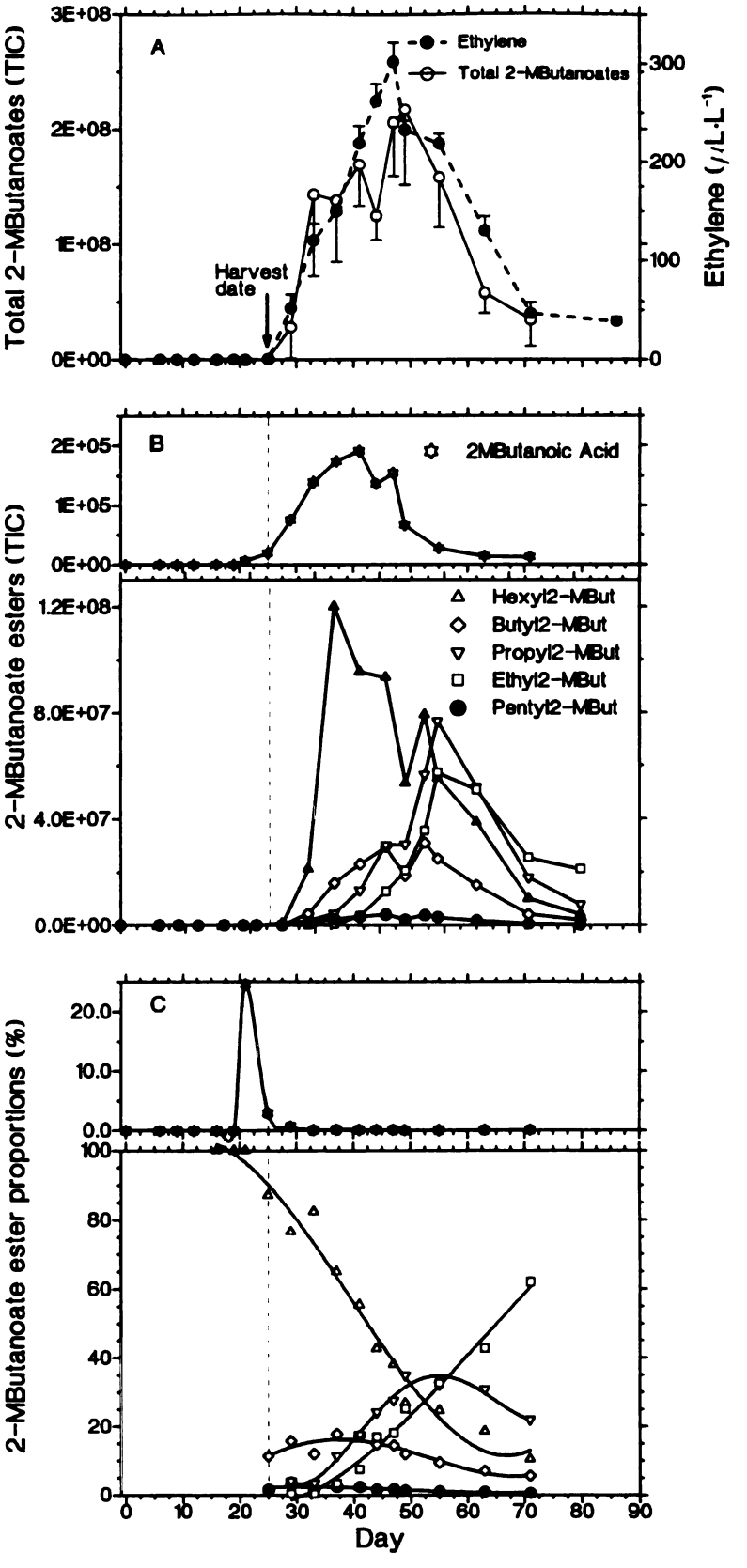
A
Ethylene
Total 2-MButanoates
Harvest date
Total 2-MButanoates (TIC)
Ethylene (μL·L⁻¹)
3E+08
2E+08
1E+08
0E+00
300
200
100
0
B
2MButanoic Acid
2E+05
1E+05
0E+00
2-MButanoate esters (TIC)
Hexyl2-MBut
Butyl2-MBut
Propyl2-MBut
Ethyl2-MBut
Pentyl2-MBut
1.2E+08
8.0E+07
4.0E+07
0.0E+00
C
2-MButanoate ester proportions (%)
20.0
10.0
0.0
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
Day

Figure 6.

Figure 7. Pattern of 2-methylbutanoate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total butanoate esters and ontogeny of ethylene. (B) GC/MS response (TIC) of ethyl, propyl, butyl, pentyl, and hexyl esters of 2-methylbutanoic acid. (C) Ester proportions (% of total 2-methylbutanoate esters). Each symbol represents the average of 5 replications.

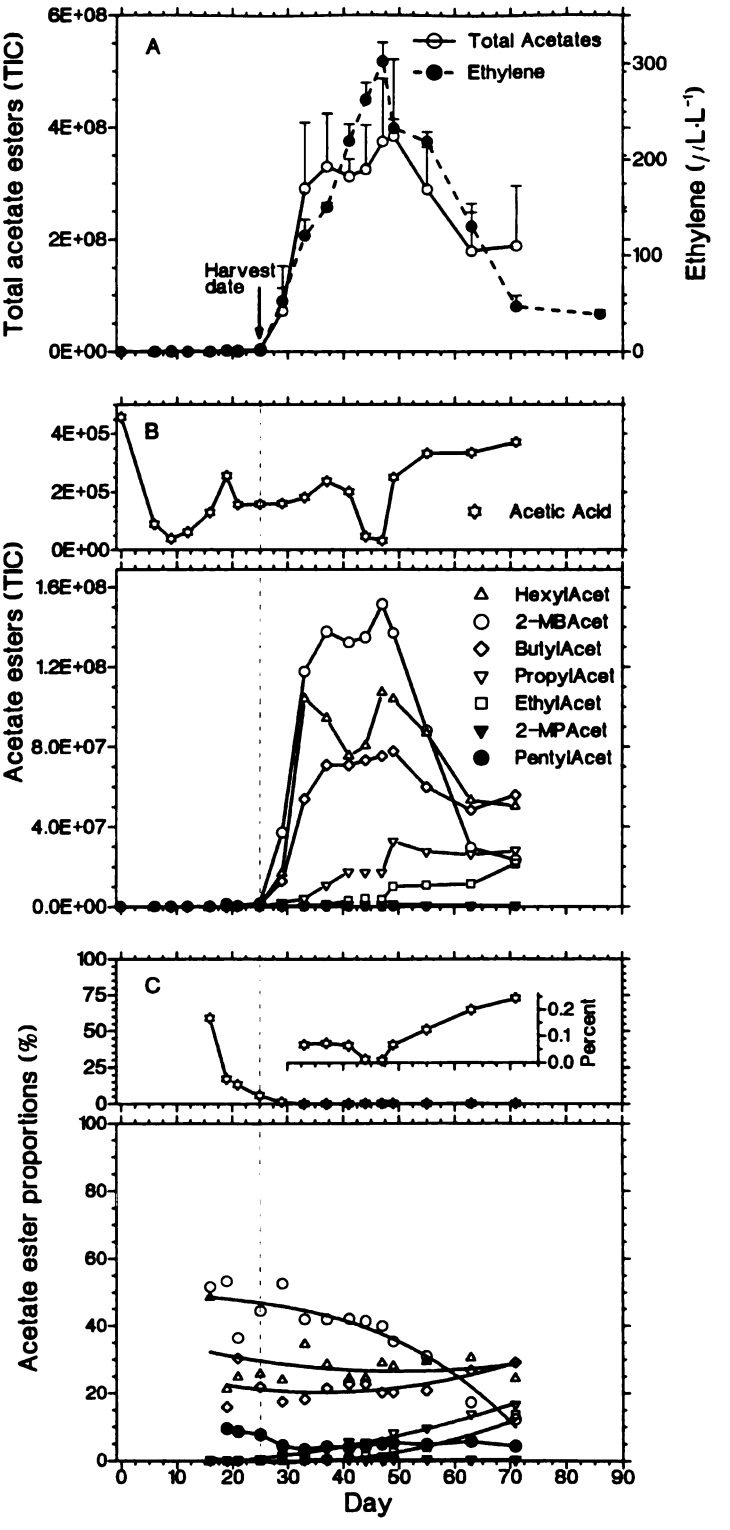
A
Total Acetates
Ethylene
Total acetate esters (TIC)
Ethylene (/L·L⁻¹)
Harvest date
6E+08
4E+08
2E+08
0E+00
300
200
100
0
B
Acetic Acid
4E+05
2E+05
0E+00
Acetate esters (TIC)
HexylAcet
2-MBAcet
ButylAcet
PropylAcet
EthylAcet
2-MPAcet
PentylAcet
1.6E+08
1.2E+08
8.0E+07
4.0E+07
0.0E+00
C
Percent
0.2
0.1
0.0
Acetate ester proportions (%)
100
75
50
25
0
80
60
40
20
0
10
20
30
40
50
60
70
80
90
Day

Figure 7.

Figure 8. Pattern of pentanoate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total pentanoate esters and ontogeny of ethylene. (B) GC/MS response (TIC) of ethyl, and propyl esters of pentanoic acid. (C) Ester proportions (% of total pentanoate esters). Each symbol represents the average of 5 replications.

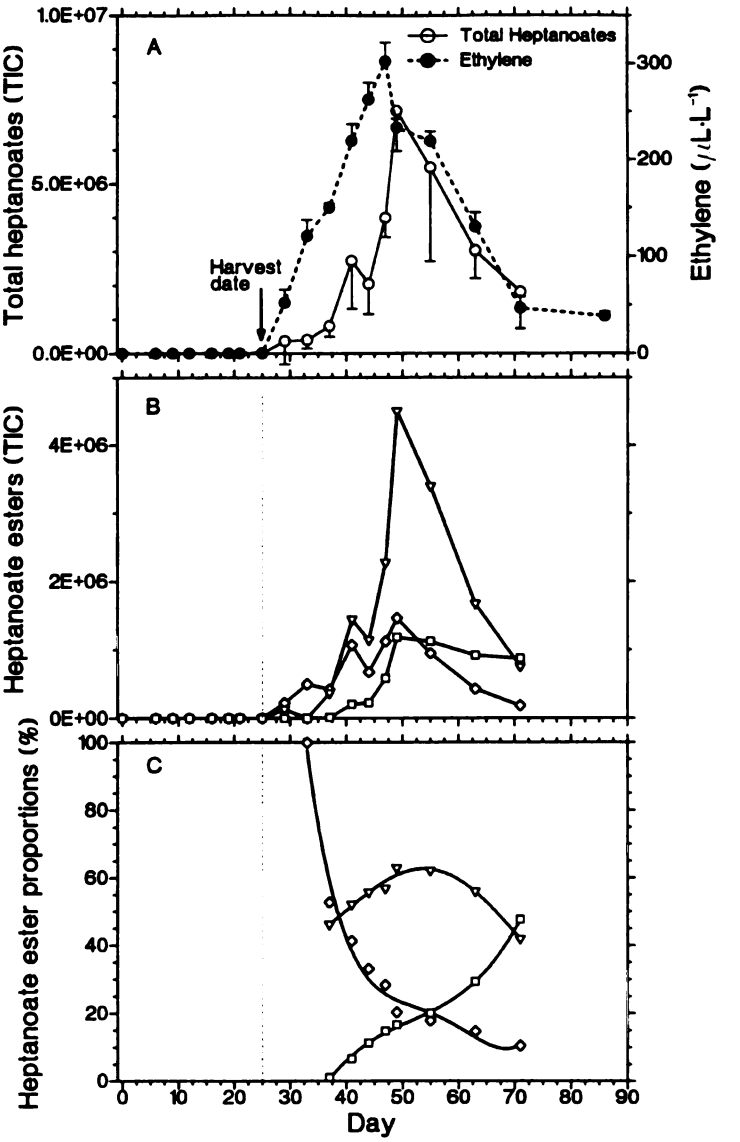
A
Total Heptanoates
Ethylene
Harvest date
Total heptanoates (TIC)
Ethylene (μL·L⁻¹)
1.0E+07
5.0E+06
0.0E+00
300
200
100
0
B
Heptanoate esters (TIC)
4E+06
2E+06
0E+00
C
Heptanoate ester proportions (%)
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90
Day

Figure 8.

Figure 9. Pattern of heptanoate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total heptanoate esters and ontogeny of ethylene. (B) GC/MS response (TIC) of ethyl, propyl, and butyl esters of heptanoic acid. (C) Ester proportions (% of total heptanoate esters). Each symbol represents the average of 5 replications.

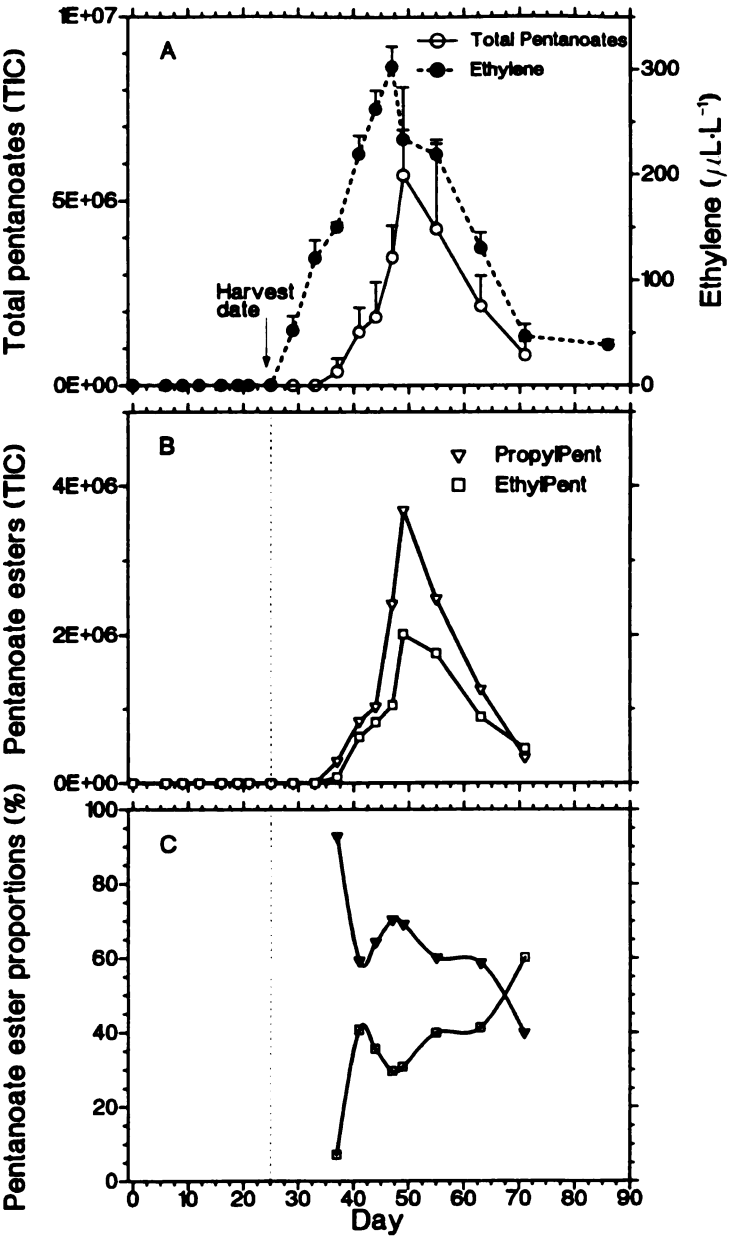
A
Total Pentanoates
Ethylene
Harvest date
Total pentanoates (TIC)
Ethylene (μL·L⁻¹)
1E+07
5E+06
0E+00
300
200
100
0
B
PropylPent
EthylPent
Pentanoate esters (TIC)
4E+06
2E+06
0E+00
C
Pentanoate ester proportions (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
Day

Figure 9.

Figure 10. Pattern of octanoate esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total octanoate esters and ontogeny of ethylene. (B) GC/MS response (TIC) of ethyl, propyl, and butyl esters of octanoic acid. (C) Ester proportions (% of total octanoate esters). Each symbol represents the average of 5 replications.

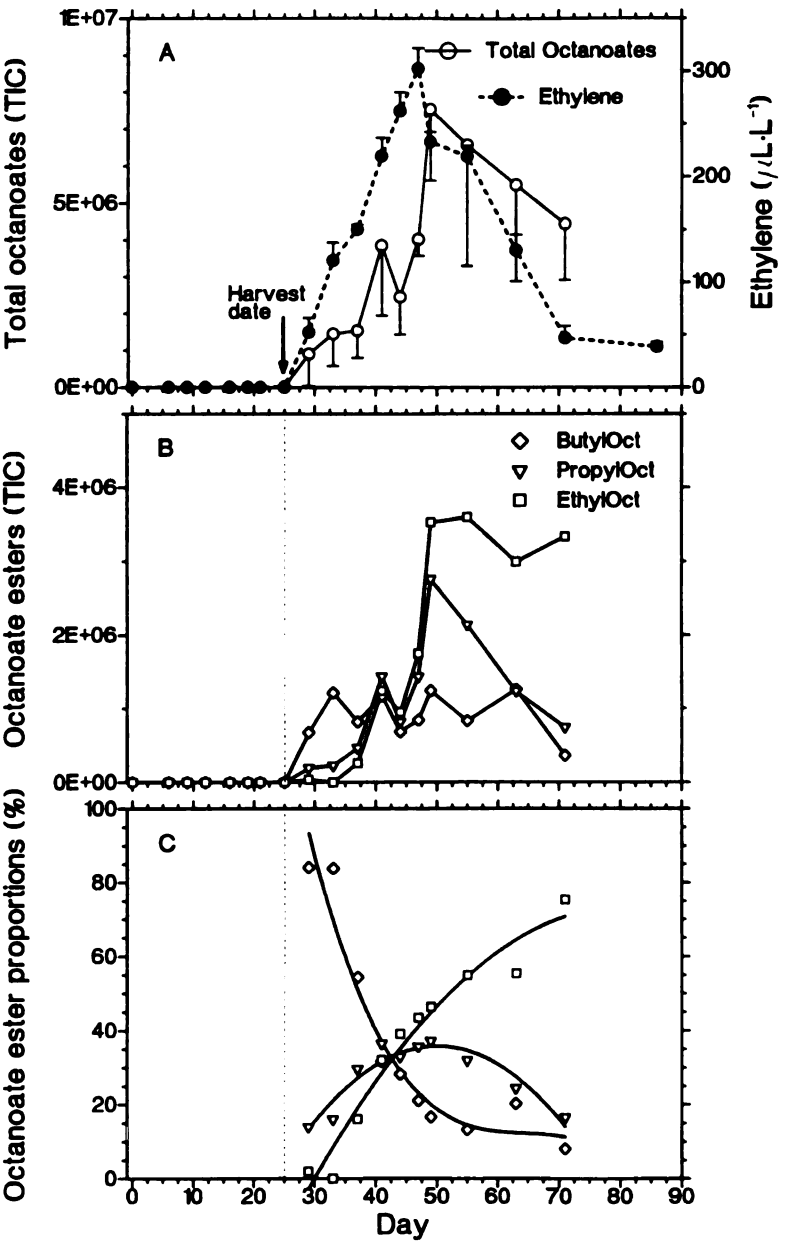
A
Total Octanoates
Ethylene
Harvest date
Total octanoates (TIC)
Ethylene (μL·L⁻¹)
1E+07
5E+06
0E+00
300
200
100
0
B
ButylOct
PropylOct
EthylOct
Octanoate esters (TIC)
4E+06
2E+06
0E+00
C
Octanoate ester proportions (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
Day

Figure 10.

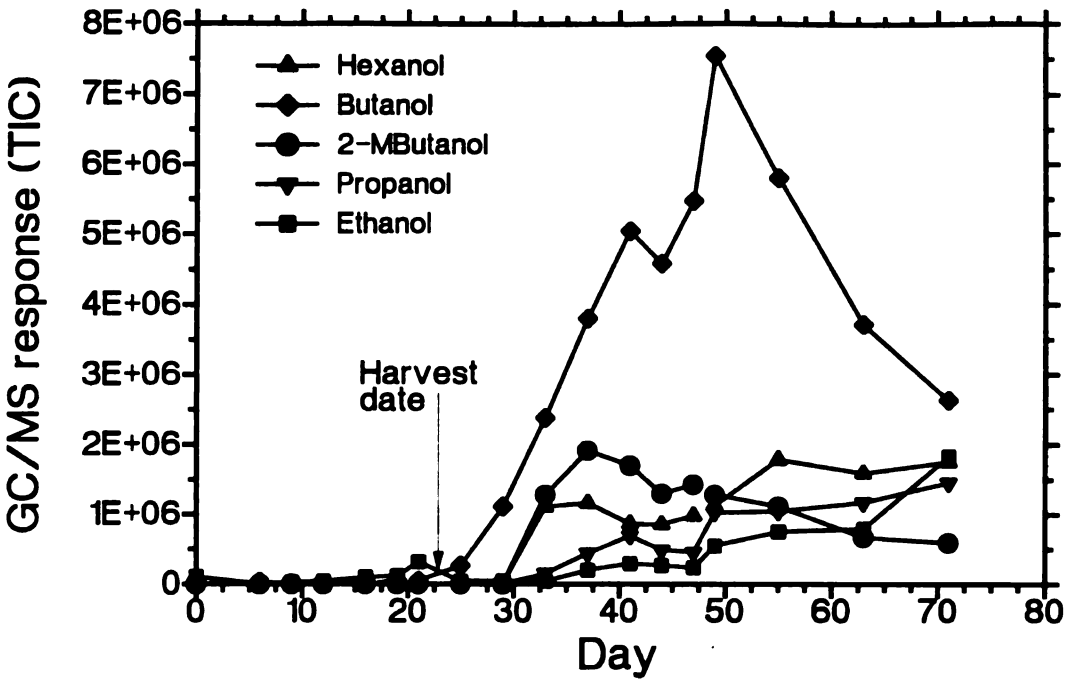

Figure 11. Pattern of alcohols identified during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric which corresponds to the harvest date (indicated by an arrow). Each symbol represents the average of 5 replications.

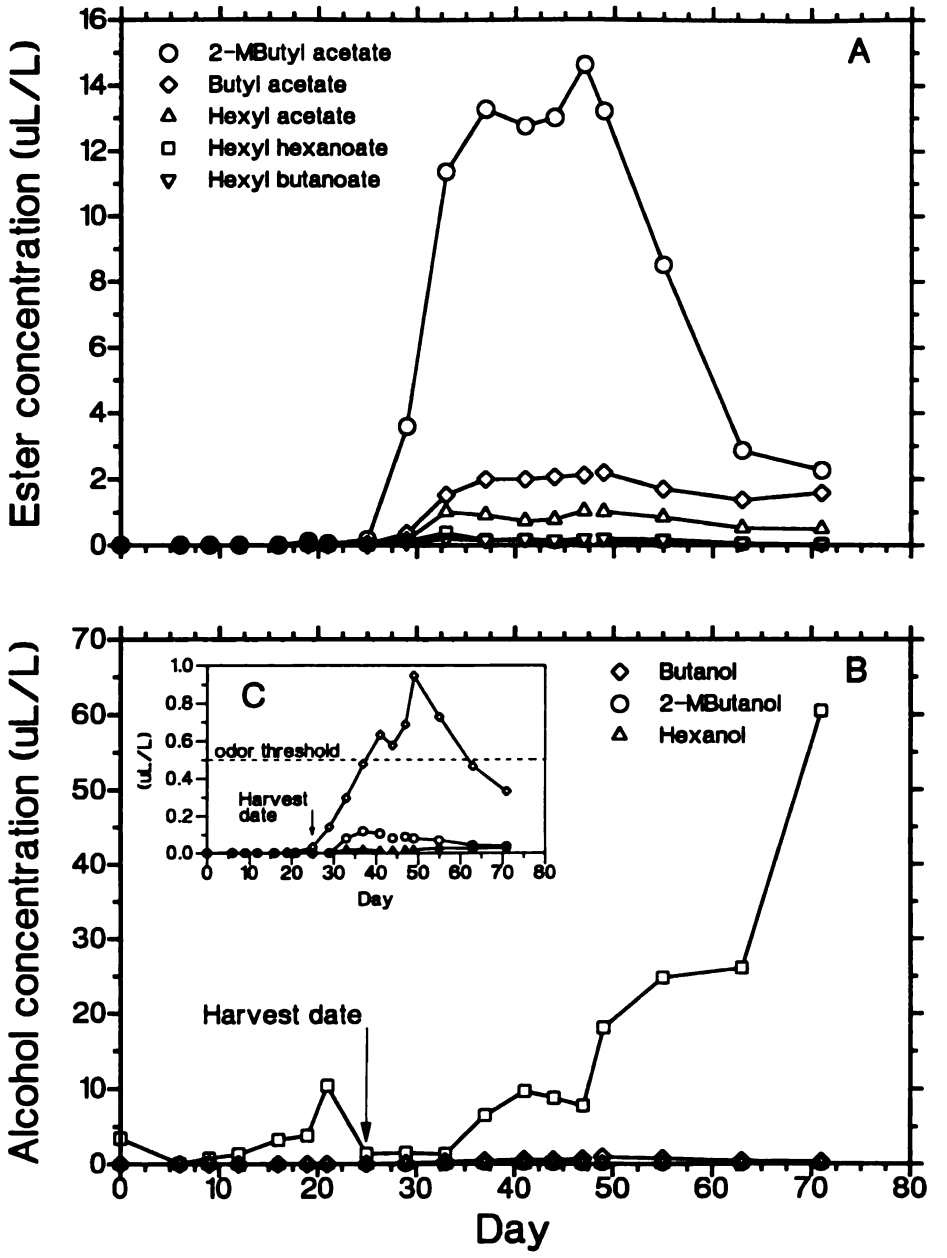

Figure 12. Esters and alcohols content during ripening and senescence of 'Redchief Delicious' apple fruit. (A) Concentration of 2-methylbutyl acetate, butyl acetate, hexyl acetate, hexyl hexanoate and hexyl butanoate esters. (B) Concentration of ethanol, butanol, 2-methylbutanol and hexanol alcohols. (C) Concentration of butanol, 2-methylbutanol and hexanol alcohols. The odor threshold for 2-methylbutyl acetate is 0.005 µL/L, for butyl acetate 0.066 µL/L, for hexyl acetate 0.002 µL/L, for ethanol 100 µL/L, and for butanol and hexanol is 0.5 µL/L (indicated by dashed horizontal line) (Flath et al., 1967). Each symbol represents the average of 5 replications.

CHAPTER IV:

PATTERNS IN THE ACID PORTION OF ESTERS PRODUCED DURING RIPENING AND SENESCENCE OF 'REDCHIEF DELICIOUS' APPLE FRUIT.

INTRODUCTION

Flavor is an important parameter of fruit quality that influence consumer acceptability. Aroma compounds contribute significantly to the flavor of all fresh fruits. However in recent years, the retail industry and consumers recognize apple flavor as needing improvement (Beaudry, 2000). The aroma of apple fruit depends on the concentration of a complex mixture of low molecular weight esters, alcohols, aldehydes, and hydrocarbons. More than 300 volatile compounds have been identified in apple (Dimick and Hoskin, 1981) being the esters the major constituents.

Aroma biosynthesis is affected by many factors that impact the ester biosynthesis: cultivar, growing conditions, fruit maturity, and also storage conditions (Dirinck, et al., 1989; Fellman, 2000; Mattheis et al., 1991). Fruit maturity is probably one of the most significant factors (Song and Bangerth, 1996). It is known that aroma biosynthesis is correlated with ethylene synthesis and action (Brown et al., 1966; Dirinck et al., 1989; Mattheis et al., 1991; Song and Bangerth, 1996; Tressl and Drawert, 1973). However, it is not clear whether the onset of biosynthesis of volatile compounds is concurrent with, or precedes the climacteric rise in fruit respiration (Fellman et al., 2000).

Esters are formed from fatty acids and alcohols (Figure 1 in Chapter 2). The enzyme alcohol acyl-CoA transferase (AAT, EC2.3.1.84) catalyzes the union of an alcohol and the acyl-CoA derivative of a fatty acid. The carbon chain length of the alcohol portion of the ester varies between 2 and 6 carbons and the acid portion has typically a chain length from 2 to 8 carbons. The carbon chains of the alcohols or fatty acids can be straight or branched. The branched-chain

compounds are believed to be derived from amino acids (Nursten, 1970; Perez et al., 1992; Tressl and Drawert, 1973; Wyllie, et al., 1995). The straight short-chain fatty acids (SSCFA), between 2 and 8 carbon length, are believed to be derived from fatty acid metabolism, either degradation (Bartley et al., 1985; Brackmann et al., 1993; Fellman et al., 2000; Nursten, 1970; Rowan et al., 1996, 1999) or synthesis (Tan and Bangerth, 2001).

Ester biosynthesis could be influenced by substrate availability (Knee and Hatfield, 1981; Wyllie and Fellman, 2000), AAT specificity (Olias et al., 1995; Perez et al., 1993), *AAT* expression (Aharoni et al., 2000a; Jayanty et al., 2002), and/or AAT activity levels (Perez et al., 1996). The importance of these factors appears to change as ripening progresses. It is not known how many different AAT isozymes could be present in apples. Two AAT genes have been recently cloned from strawberry, (Aharoni et al., 2000a, 2000b) and one gene has been identified in banana and apple (Aharoni et al., 2000b). No study on apple AAT activity during fruit development has been published; only preliminary studies on the effect of different storage conditions on AAT activity has been carried out (Fellman et al., 1993; Fellman and Mattheis, 1995; Ke et al., 1994).
Although considerable progress has been made in isolating and identifying a large number of volatile compounds from plant aromas, less work has been done on elucidating the aroma formation mechanism.

The aim of this research was to characterize the patterns in ester biosynthesis during ripening and senescence of 'Redchief Delicious' apple to better understand the biochemical origin and fate of these organoleptically significant compounds. Apple fruit were tracked throughout ripening and selected

fruit for analysis based on internal ethylene levels. At each stage evaluated, respiration and ester production was measured for five representative fruit. Developmentally dependent patterns in esters were evaluated. An ester matrix was established based on precursor acids and alcohols (Table 1 in Chapter 3). One axis of the matrix included alcohols (ethanol, propanol, 2-methylpropanol, butanol, 2-methylbutanol, pentanol, and hexanol) and the other axis acids (acetate, propanoate, butanoate, 2-methylbutanoate, pentanoate, hexanoate, heptanoate, and octanoate). Few alcohol/acid combinations were not detectable. In this paper, we focused on patterns evident in the acid portion of esters classed by the alcohol moiety during ripening and senescence.

MATERIALS AND METHODS

'Redchief Delicious' apples [*Malus sylvestris* (L) Mill. var. *domestica* (Borkh.) Mansf.] were harvested every three to four days at the Michigan State University Horticultural Teaching and Research Center, East Lansing, MI, beginning three weeks prior to the onset of the climacteric and continuing until fruit were considered to have initiated ripening based on internal ethylene content (IEC). The beginning of the climacteric rise was considered to occur when the internal ethylene content was about 0.2 µL/L. The harvest date occurred on October 3rd, which was day 25 of the experiment. Distinct patterns in the ester production were evident.

After the initiation of ripening, the remaining fruit were harvested and held at room temperature for analysis continuing fruit selection for 45 days. Thus, 18 different stages of development of 'Redchief Delicious' apple fruit ranging from

unripe through senescent over a period of 70 days were measured. The average IEC of twenty representative fruits at each stage was determined and those five fruit nearest the average were chosen for ester evaluation.

The IEC was determined by withdrawing a 1-mL gas sample from the interior of apples and subjecting the gas sample to gas chromatographic (GC) analysis. The gas chromatograph (Carle Series 400 AGC; Hach Co., Loveland, Colo.) was fitted with a 6-m-long, 2-mm-i.d. stainless-steel column packed with activated alumina and detection was via a flame ionization detector. The ethylene detection limit was approximately 0.005 $\mu L.L^{-1}$. Ethylene concentrations were calculated relative to a certified standard (Matheson Gas Products, Chicago, Ill.) with an ethylene concentration of 0.979 $\mu L.L^{-1}$.

Volatile analysis procedure was done as described by Song et al. (1997,1998). Ester emissions were sampled by sealing one fruit in each one of five 1-liter Teflon TM chamber. In order to reach a steady-state concentration of apple fruit volatiles in the headspace over the apples, the fruit were maintained in the chambers for approximately three hours at 22°C and the chambers were ventilated with pure air at a rate of approximately 30 mL/min. One chamber with no fruit was used as a blank.

A 1-cm long solid-phase microextraction (SPME) fiber coated with a film thickness of 65 µm of polydimethylsiloxane/divinylbenzene (Supelco Co., Bellefonte, PA) was used to adsorb the volatile sample. The SPME fiber was preconditioned by baking overnight at 260°C.

The fiber was manually inserted through a Teflon-lined half-hole septum into a glass 'tee' located at the outlet of the chambers. Once in the glass 'tee'

outlet, the fiber was extended to absorb volatiles for five minutes. The fiber was then retracted prior to removal from the sample container.

Ester analysis was by GC/time-of-flight mass spectrometry (MS). The SPME fiber was inserted in the glass-lined, splitless injection inlet of the GC (230°C) and desorbed for 5 minutes. The volatiles were cryofocussed oncolumn using a liquid nitrogen cryo trap.

The desorbed flavor compounds were separated by a Hewlett-Packard 6890 GC with a capillary column (Supelcowax, 15 m X 0.1 mm i.d., 0.25 μm coating film) (Supelco Co. Bellefonte, PA). The temperature of the GC was programmed from 40 to 240 °C at 50 °C/min. A constant mass flow rate (0.5 mL/min) of the carrier gas (He) in the column throughout the run was maintained.

The identification and quantification of the volatiles were by comparison with the National Institute of Standards and Technology database and authenticated standards. Quantification for selected compounds was accomplished using gas standards. Gas standards were created from a mixture of equal volumes of the neat oils of 13 compounds. A sample of 0.5 μL was taken by using a Hamilton 1.0 μL syringe, which was discharged onto a filter paper disk. The filter paper was immediately dropped into a 4.4-L glass volumetric flask fitted with a ground-glass stopper containing a gas-tight Mininert valve (Alltech Assoc., Inc., Deerfield, IL). A new standard was made every month. Volatile aroma compounds were purchased from Sigma Co. and Fluka Chemical Corp. The compounds included in the standard were: 1-butanol, 1-hexanol, cis-3-hexen-1-ol, ethyl alcohol, acetaldehyde, 1-methyl-1-butanol, n-butyl acetate, hexyl acetate, hexyl butyrate, hexyl hexanoate, 3-methylbutyl

acetate, 2-methylbutyl acetate, and farnesene mixture. For all compounds identified, not all standards were available.

For each sample, all target compounds were identified. The peak area was determined under the unique ion ID for each specific compound and the total ion count (TIC) was then calculated according to the contribution of the ion to the TIC determined from the NIST library. The quantitative data from the five replications were averaged and the TIC plotted against time to form curves depicting the production patterns of the volatiles during the 70 days of the experiments runs. It is worth notice that TIC does not reflect quantity, but it can be used for identifying trends over time.

RESULTS

In a typical GC run, ester retention times varied approximately between 55 and 200 seconds (Figure 1 in Chapter 3). Chromatographic separation was not achieved for many of the volatiles, however determination of the ID unique ions by MS enabled quantification of the responses for many of the target volatiles (Table 2 in Chapter 3). Among the substances found, 38 esters, 5 alcohols and 5 acids were identified and quantified by GC/MS (Table 2 in Chapter 3). According to the GC/MS response, acetate esters were the most abundant compounds. Butyl, hexyl and 2-methylbutyl acetate esters predominated. The branched ester hexyl 2-methylbutanoate was also among the most abundant esters.

Total ester volatiles reached maximum levels at a time, which nearly coincides with the peak in ethylene content and respiratory climacteric (Figure 2

in Chapter 3). Then, as ethylene biosynthesis declined so too did total volatile ester biosynthesis.

The peak in the TIC for the individual esters classed by the acid moiety occurred on the date following the maximum in ethylene production (Figures 1a, 2a, 3a, 4a, 5a, 6a and 7a).

Some esters were identified in small concentration during growth or at the time of harvest and were only produced in higher amounts during ripening (e.g. hexyl, butyl and 2methylbutyl acetates; butyl and hexyl butanoates; and butyl and hexyl hexanoates).

Proportionally, the acetate esters of hexanol, butanol, 2-methylbutanol, propanol, 2-methylpropanol and pentanol predominated prior to the onset of the ethylene climacteric (Figures 1c, 2c, 3c, 5c, 6c and 7c). As ripening progressed, acetate esters decreased while all the other acids increased in their proportions. During senescence, acetate esters predominated again having the highest proportion. In the case of ethyl esters, not only acetate esters of ethanol, but also all acids increased their proportions in the late stages of senescence (Figure 4c).

The TIC for hexyl and butyl esters exhibited a broad peak earlier in climacteric while propyl and ethyl esters all peaked after the ethylene climacteric peak (Figures 1b,2b, 3b and 4b respectively).

The TIC for esters formed with pentanoic acid and longer chain acids like heptanoic and octanoic acids, was considerably low (Figures 1b,2b,3b, 4b and 5b).

Free hexanoic and propanoic acids were detected after the respiration and ethylene climacteric peaks, when the synthesis of total hexanoic and

propanoic esters started to decline (Figure 8). Free butanoic and 2-methylbutanoic acids were detected from the onset of ethylene climacteric and they declined in senescence (Figure 8). Free acetic acid was found throughout the experiment. All free acids declined at the ethylene climacteric peak. No pentanoic, heptanoic or octanoic acids were detected.

Some alcohols increased late in ripening (ethanol, propanol, hexanol), as did acids (hexanoic, propanoic, acetic acid), but the formation of the associated esters did not increase with the exception of propyl and ethyl acetates (Figures 9 and 10).

DISCUSSION

The qualitative composition of esters was similar to that found by other investigators (Brackmann et al., 1993; Fellman et al., 2000; Mattheis et al., 1991; Rowan et al., 1996; Song and Bangerth, 1996; Vanoli et al., 1995). Acetate esters were the most abundant compounds probably because acetyl CoA is the most abundant acyl CoA present in fruit tissue as it is explained by Nursten (1970).

The increase in ester emanation following the onset of the ethylene climacteric is consistent with the findings of Song and Bangerth (1996) and Fan et al. (1998) who determined that normal ester biosynthesis in apples depends on continuing presence of ethylene. The increase in respiration may be related to an increase in substrate availability. Song and Bangerth (1996) suggested that more a general and not a specific increase in metabolic activity is a prerequisite for the stimulation of aroma production. Bangerth et al. (1998) argued that is

rather unlikely that ethylene directly affects the production of so many individual volatile substances. They suggest that ethylene determines an increase of fruit respiration which provides the necessary energy (ATP, NADPH, etc.) for the synthesis of aroma volatile precursors.

The fact that free butanol and ethanol were detected prior the onset of ethylene climacteric but only few esters were identified quite low, suggests that AAT activity may be limiting at this point of time. Other possibilities are that acid availability and/or the conversion of acids to acyl-CoA are limiting. The possibility that acid availability is limiting factor for ester biosynthesis before the onset of ethylene climacteric has been observed previously (Berger and Drawert, 1984; Forney et al., 2000; Knee and Hatfield, 1981; Williams and Knee, 1977). Treatments of Golden Delicious apples with aldehydes and carboxylic acids suggest that there is a certain selectivity of the apple AAT in the use of the carboxylic acid precursors (De Pooter et al., 1983). They supported the hypothesis that the composition of apple aroma is determined by not only the availability of acids but also by their identity. It could be also a different AAT isoenzyme present before the onset of ethylene climacteric with a lower specificity for ethanol since no ethyl esters were detected until late in climacteric.

A pattern in the alcohol portion was found in all but the acetate esters as ripening progressed (discussed in first paper). There was a change in the alcohol moieties in the esters predominantly long to predominantly short chains as fruit ripened. A similar pattern was not observed in the chain length of the fatty acid portion. This fact suggests separate pathways for the substrates for acids and

alcohols or at least no free interconversion by acyl-CoA reductase or other enzyme system.

The decline in free acetic and butyric acids at the ethylene peak suggests that AAT activity achieved its maxima at the ethylene climacteric peak coinciding with the peak in total esters production. At later stages both acids and alcohols are in abundance, yet total ester formation declines. It is possible that the activity of AAT declines during senescence leaving unreacted free acids and alcohols. An increase in the activity of the esterase enzyme during senescence is also a possibility.

The fact that some free alcohols and acids increased late in ripening but the formation of the associated esters did not increase suggests that later in senescence there is a shift from substrate limitation to enzyme limitation. Probably there is a different AAT in senescence with higher specificity for short chain alcohols and/or acyl-CoA synthetase activity declines in senescence. It could be also an increase of esterase activity in senescence. Late in ripening, long- and medium-chain alcohols formed carboxylic esters with acetic acid, and long- and medium-chain fatty acids formed esters with ethanol. This observation suggests that other AAT isoenzyme could be present during senescence with different inherited properties that determines a limit in the number of carbons of the ester molecule.

CONCLUSIONS

Ester formation requires acyl-CoA, which is associated with the fundamental metabolism of the cell. This could explain the association of the climacteric in fruits with maximum levels of esters. However, ester precursors as well as AAT activity are present before the increase in metabolic activity.

Although further work should be done, this study allow us to suggest that different factors are involved in determining volatile ester composition in 'Redchief Delicious' apple fruit: acid and alcohol availability, AAT activity and inherent characteristics; acyl-CoA synthetase activity as well as esterase activity.

Ester formation depends on the availability of alcohols and CoA-derivatives. Thus, both alcohols and acids compete in ester biosynthesis.

The data of this experiment also suggests that there are separate pathways for the substrates for acids and alcohols or at least no free interconversion by acyl-CoA reductase. Whether different AAT isoenzymes predominate at different times during ripening and senescence is not known.

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Figure 1. Pattern of hexanol esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total hexanol esters and ontogeny of ethylene. (B) GC/MS response (TIC) of acetic, propanoic, butanoic, 2-methylbutanoic, and hexanoic esters of hexanol. (C) Ester proportions (% of total hexanol esters). Each symbol represents the average of 5 replications.

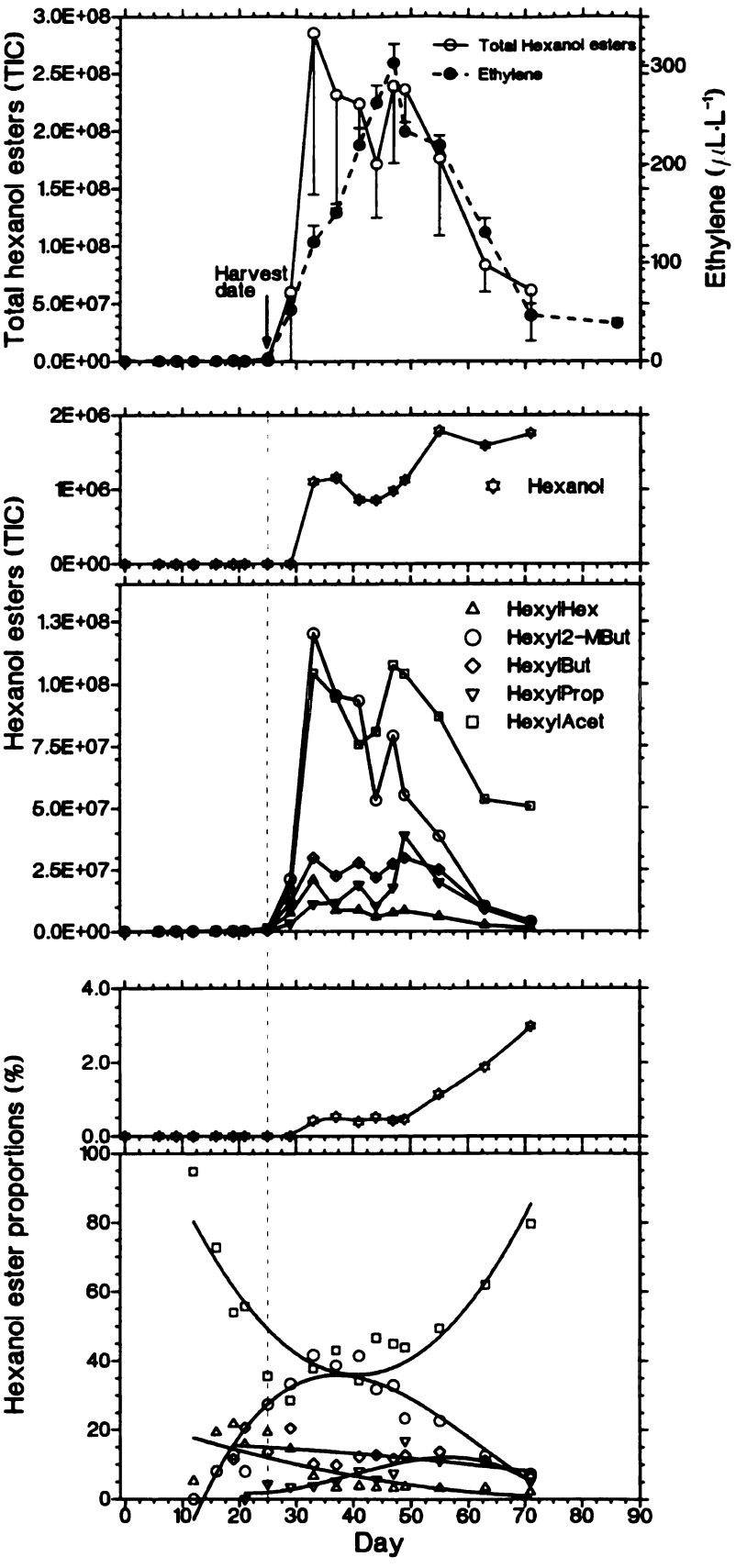
Total hexanol esters (TIC)
Ethylene (µL·L⁻¹)
Total Hexanol esters
Ethylene
Harvest date
Hexanol esters (TIC)
Hexanol
HexylHex
Hexyl2-MBut
HexylBut
HexylProp
HexylAcet
Hexanol ester proportions (%)
Day

Figure 1.

Figure 2. Pattern of butanol esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total butanol esters and ontogeny of ethylene. (B) GC/MS response (TIC) of acetic, propanoic, butanoic, 2-methylbutanoic, hexanoic, heptanoic, and octanoic esters of butanol. (C) Ester proportions (% of total butanol esters). Each symbol represents the average of 5 replications.

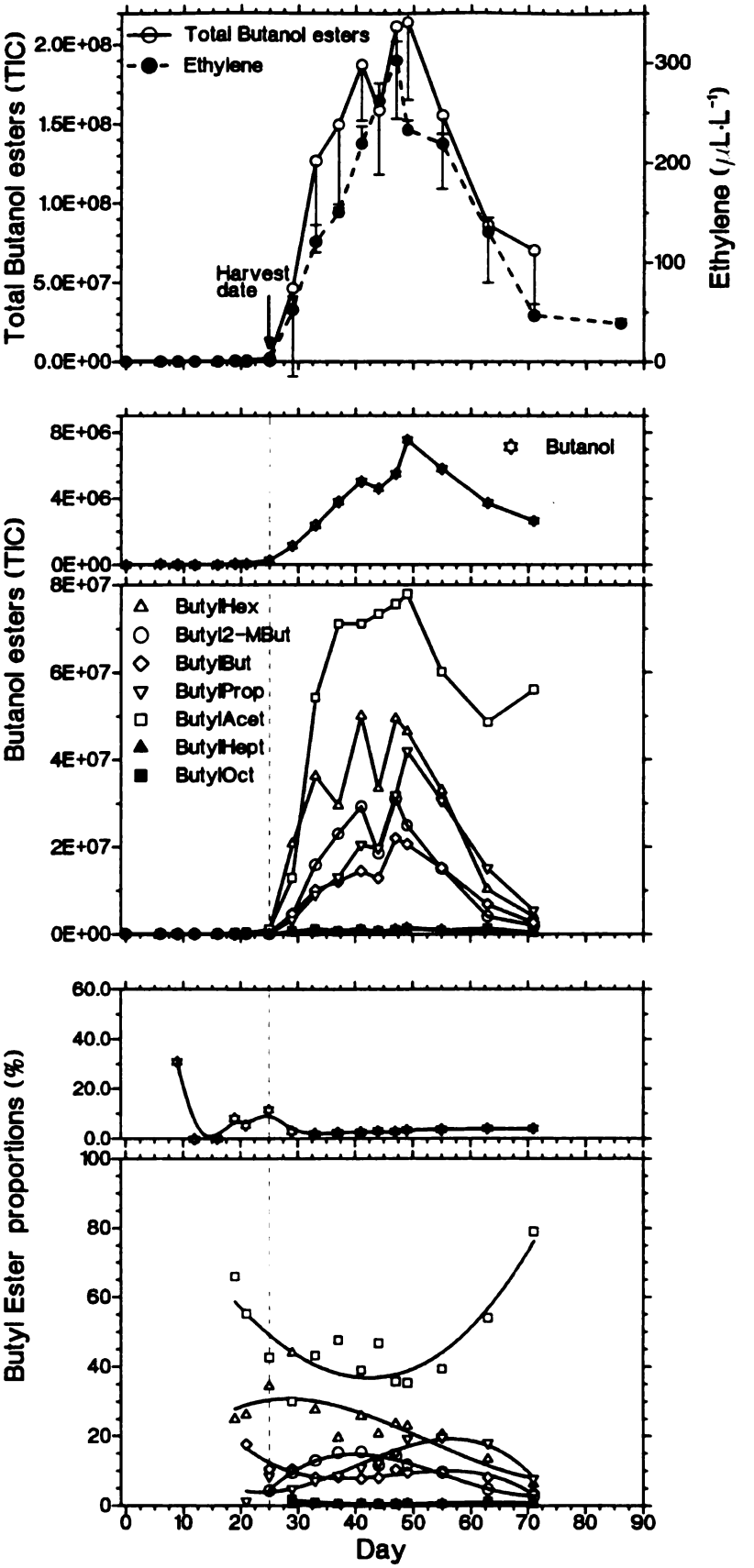
Total Butanol esters
Ethylene
Total Butanol esters (TIC)
Ethylene (μL·L⁻¹)
Harvest date
Butanol
Butanol esters (TIC)
ButylHex
Butyl2-MBut
ButylBut
ButylProp
ButylAcet
ButylHept
ButylOct
Butyl Ester proportions (%)
Day

Figure 2.

Figure 3. Pattern of propanol esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total propanol esters and ontogeny of ethylene. (B) GC/MS response (TIC) of acetic, propanoic, butanoic, 2-methylbutanoic, pentanoic, hexanoic, heptanoic and octanoic esters of hexanol. (C) Ester proportions (% of total propanol esters). Each symbol represents the average of 5 replications.

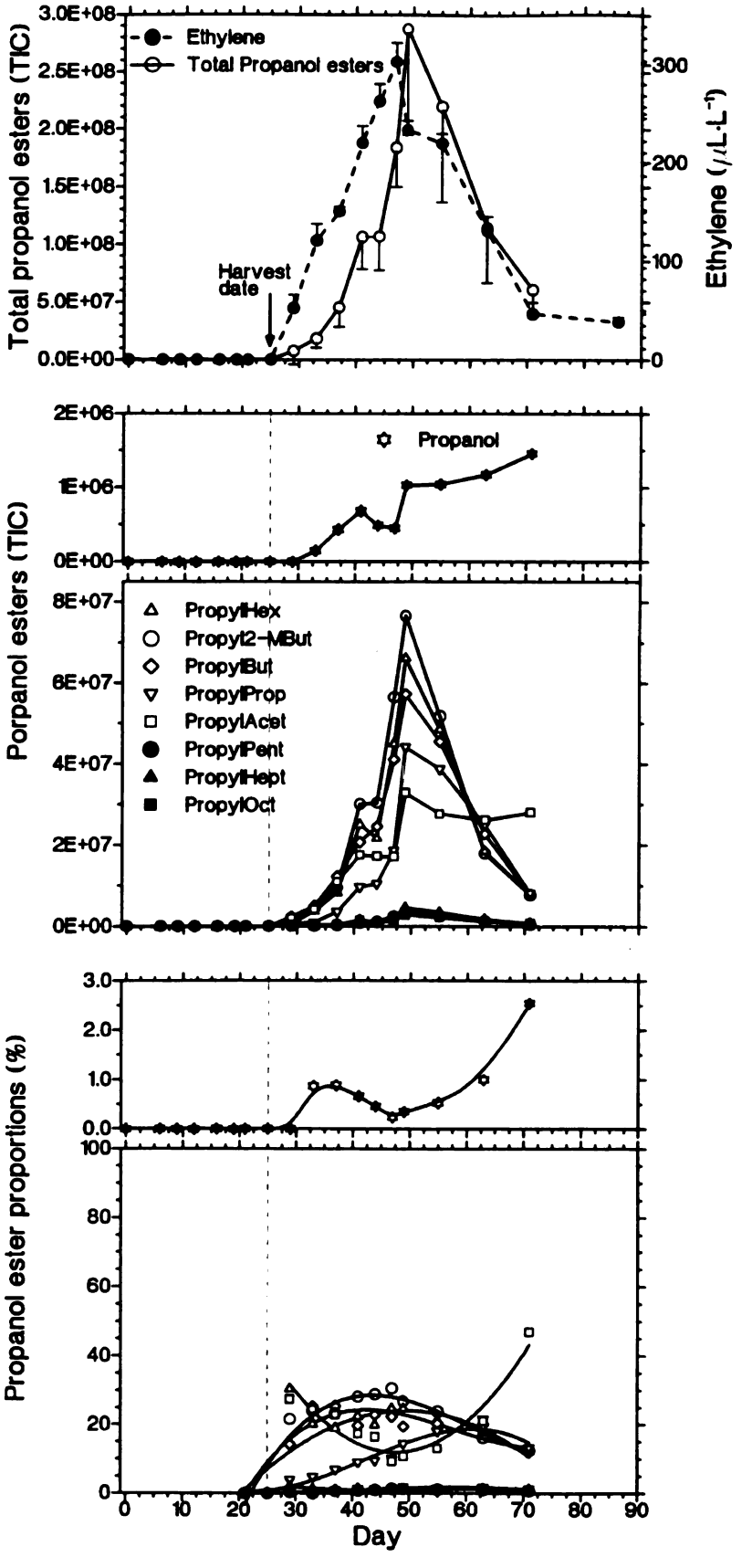
Total propanol esters (TIC)
Ethylene (μL·L⁻¹)
Ethylene
Total Propanol esters
Harvest date
Propanol
Porpanol esters (TIC)
PropylHex
Propyt2-MBut
PropylBut
PropylProp
PropylAcet
PropylPent
PropylHept
PropylOct
Propanol ester proportions (%)
Day

Figure 3.

Figure 4. Pattern of ethyl esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total ethyl esters and ontogeny of ethylene. (B) GC/MS response (TIC) of acetic, propanoic, butanoic, 2-methylbutanoic, pentanoic, hexanoic, heptanoic and octanoic esters of ethanol. (C) Ester proportions (% of total ethyl esters). Each symbol represents the average of 5 replications.

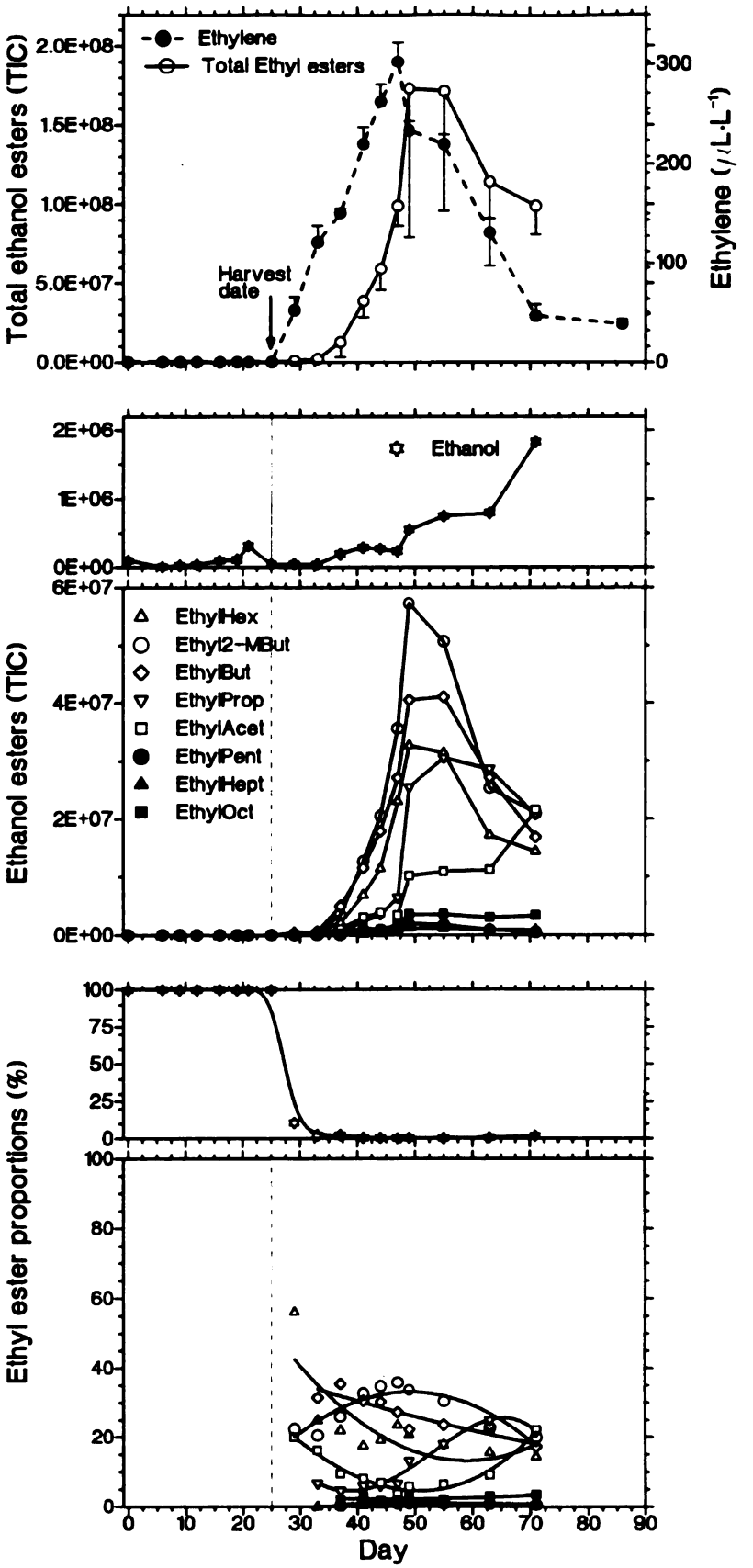
Ethylene
Total Ethyl esters
Total ethanol esters (TIC)
Ethylene (µL·L⁻¹)
Harvest date
Ethanol
Ethanol esters (TIC)
EthylHex
Ethyl2-MBut
EthylBut
EthylProp
EthylAcet
EthylPent
EthylHept
EthylOct
Ethyl ester proportions (%)
Day

Figure 4.

Figure 5. Pattern of 2-methylbutanol esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total 2-methylbutanol esters and ontogeny of ethylene. (B) GC/MS response (TIC) of acetic, propanoic, butanoic, and hexanoic esters of 2-methylbutanol. (C) Ester proportions (% of total 2-methylbutanol esters). Each symbol represents the average of 5 replications.

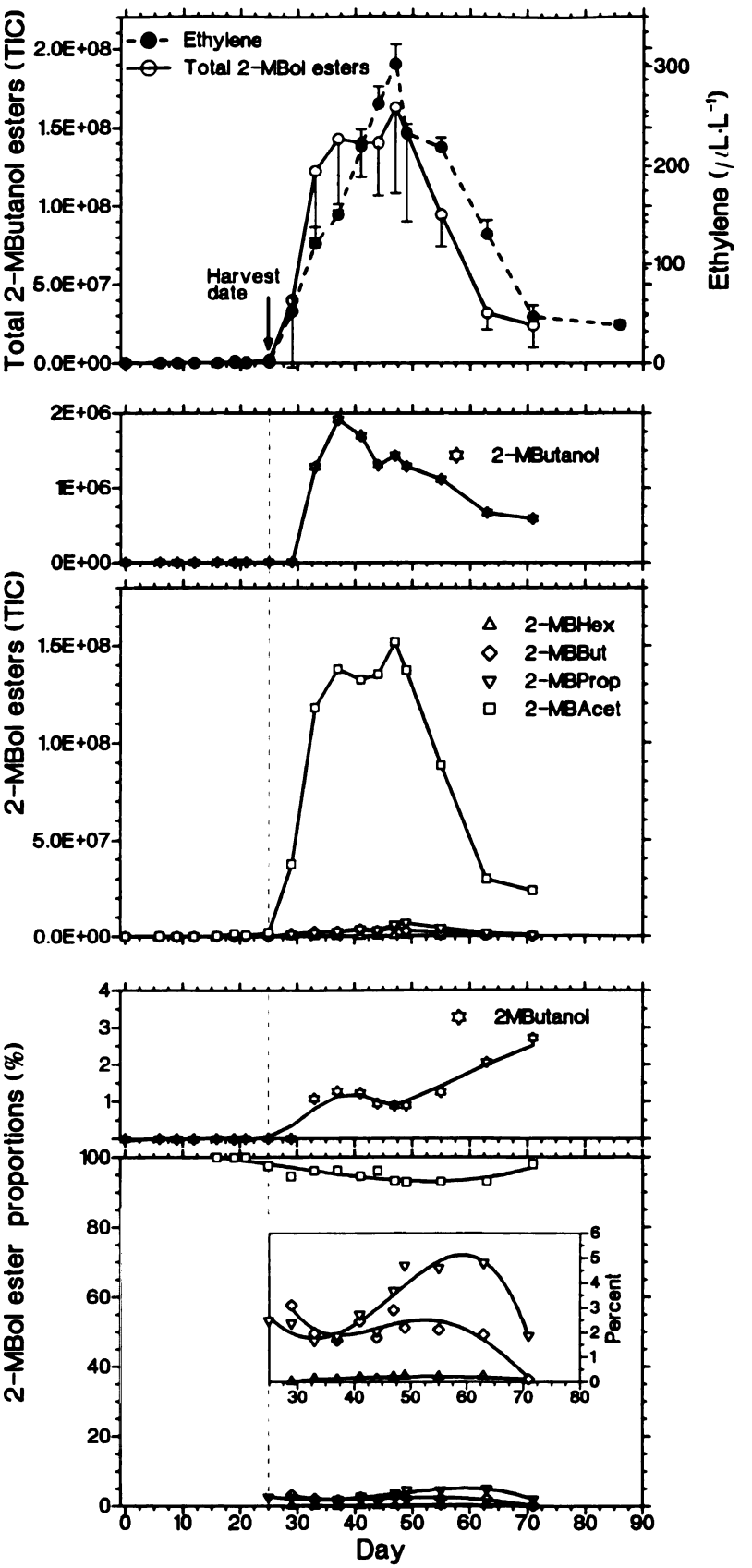
Ethylene
Total 2-MBol esters
Harvest date
Total 2-MButanol esters (TIC)
Ethylene (µL·L⁻¹)
2-MButanol
2-MBHex
2-MBBut
2-MBProp
2-MBAcet
2-MBol esters (TIC)
2MButanol
2-MBol ester proportions (%)
Percent
Day

Figure 5.

Figure 6. Pattern of 2-methylpropanol esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total 2-methylpropanol esters and ontogeny of ethylene. (B) GC/MS response (TIC) of acetic, and hexanoic esters of 2-methylpropanol. (C) Ester proportions (% of total 2-methylpropanol esters). Each symbol represents the average of 5 replications.

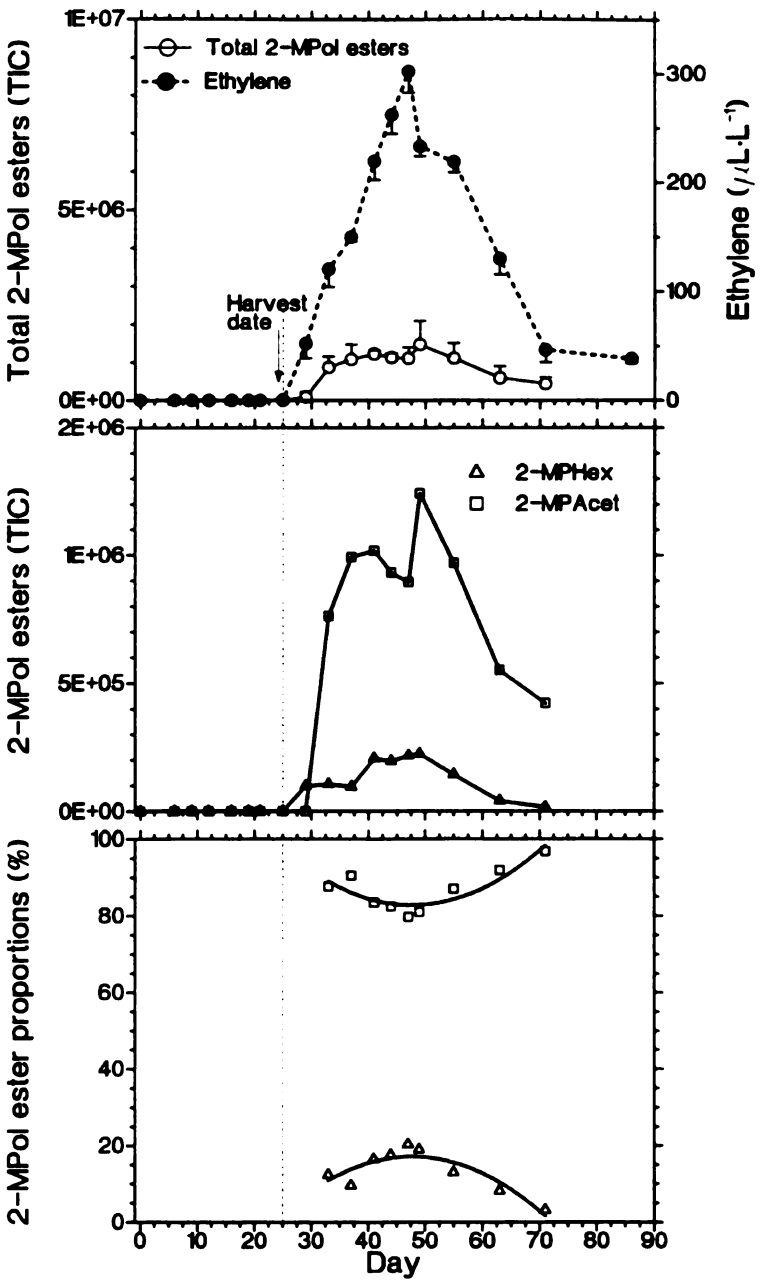
Total 2-MPol esters
Ethylene
Harvest date
Total 2-MPol esters (TIC)
Ethylene (µL·L⁻¹)
2-MPHex
2-MPAcet
2-MPol esters (TIC)
2-MPol ester proportions (%)
Day

Figure 6.

Figure 7. Pattern of pentanol esters during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric (indicated by dashed vertical line). (A) GC/MS response (TIC) of total pentanol esters and ontogeny of ethylene. (B) GC/MS response (TIC) of acetic, 2-methylbutanoic, and hexanoic esters of pentanol. (C) Ester proportions (% of total pentanol esters). Each symbol represents the average of 5 replications.

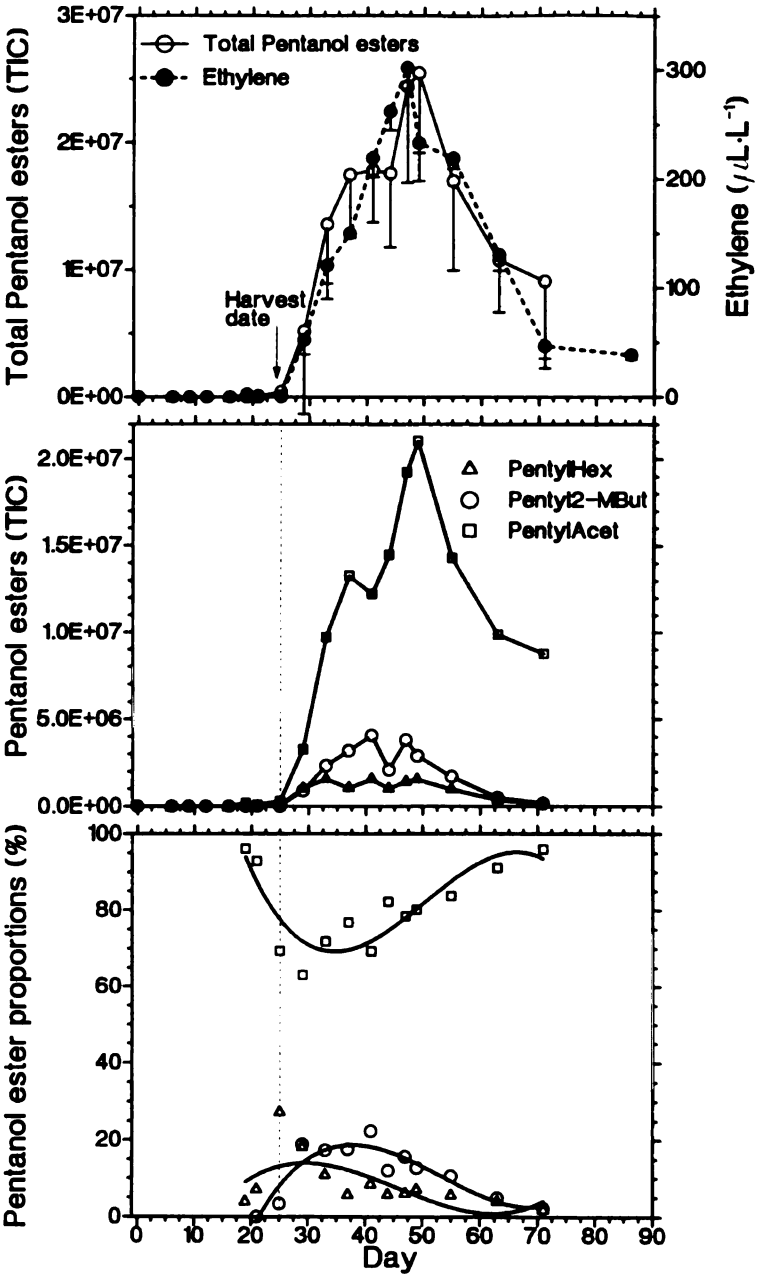
Total Pentanol esters
Ethylene
Harvest date
Total Pentanol esters (TIC)
Ethylene (µL·L⁻¹)
PentylHex
Pentyt2-MBut
PentylAcet
Pentanol esters (TIC)
Pentanol ester proportions (%)
Day

Figure 7

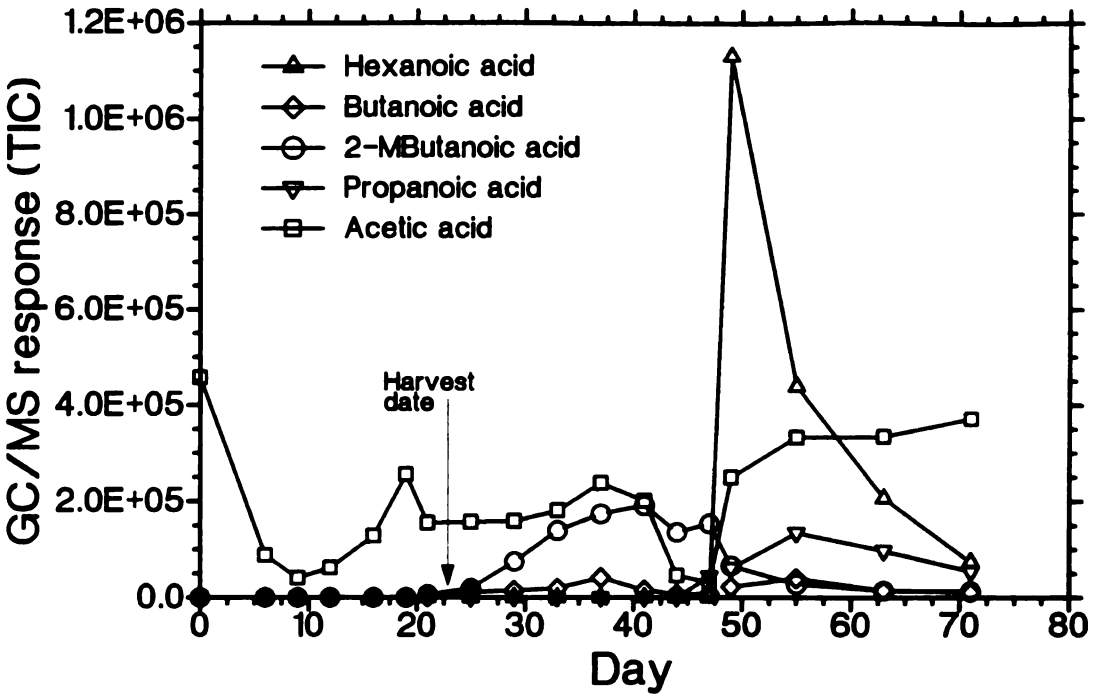

Figure 8. Pattern of acids identified during ripening and senescence of 'Redchief Delicious' apple. The volatile profile was tracked from 3 weeks prior to eight weeks after the onset of the ethylene climacteric which corresponds to the harvest date (indicated by an arrow). Each symbol represents the average of 5 replications.

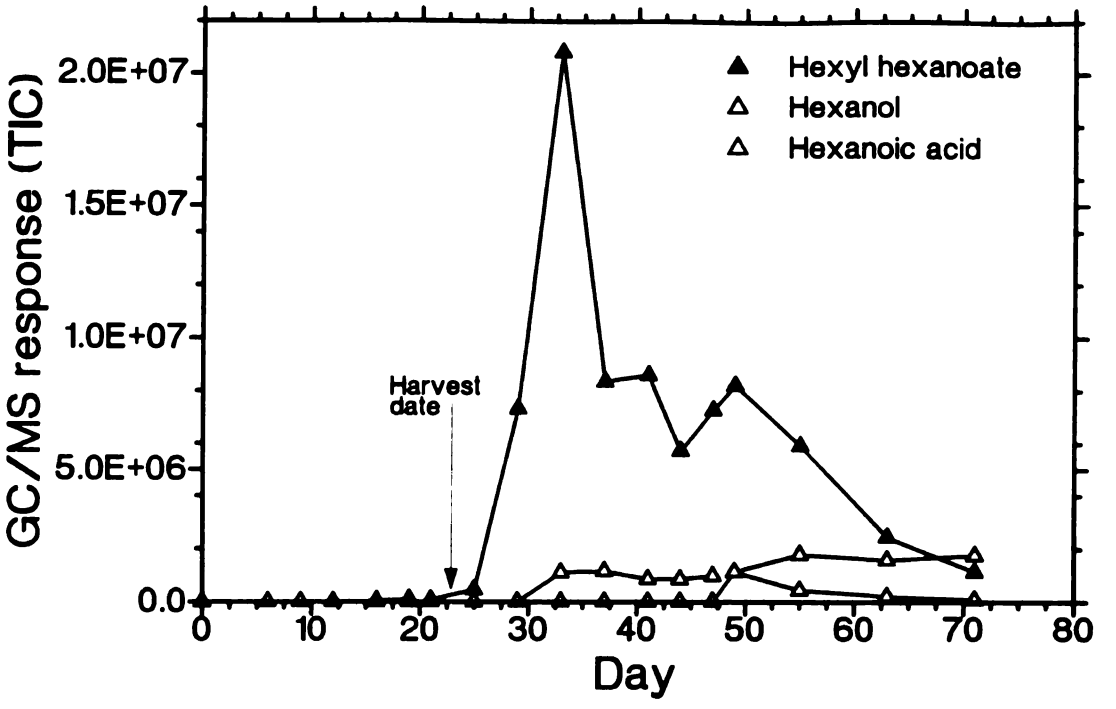

Figure 9. Pattern of hexanol, hexanoic acid and the associated ester (hexyl hexanoate) during ripening and senescence for 'Redchief Delicious' apple fruit. Each symbol represents the average of 5 replications.

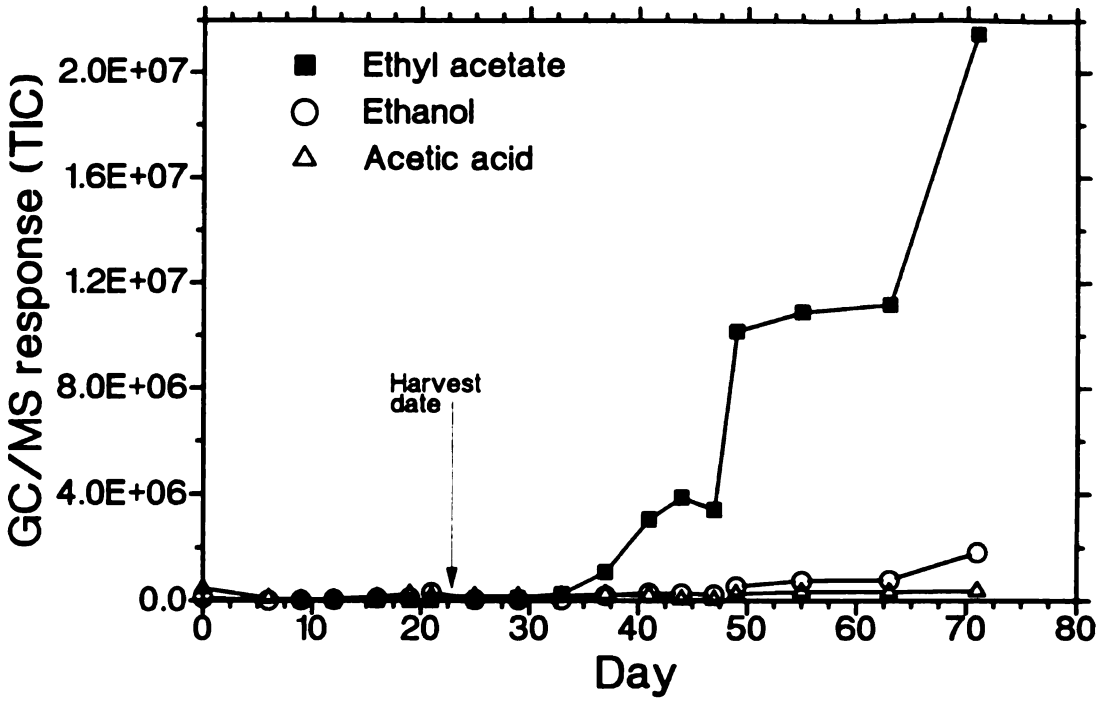

Figure 10.Pattern of ethanol, acetic acid and the associated ester (ethyl acetate) during ripening and senescence for 'Redchief Delicious' apple fruit. Each symbol represents the average of 5 replications.

APPENDIX

Table 1. Original data of Figure 2 (Chapter 3). Ethylene content and CO_2 production. Each value is the average of 5 replications.

Date	Ethylene (uL/L)	STD	CO2 (nmol/kg.s)	STD
0	0.0384	0.00368	65.5	36.5
6	0.108	0.0518	70.3	4.92
9	0.0530	0.00580	69.1	6.77
12	0.0455	0.0155	68.6	5.82
16	0.0843	0.0156	97.4	8.57
19	0.122	0.143	84.5	5.91
21	0.0404	0.0205	71.6	6.81
25	0.499	0.337	106.8	10.9
29	52.3	13.6	134.8	19.3
33	120.6	16.8	128.2	9.31
37	150.1	4.27	154.2	11.8
41	219.2	17.2	147.7	12.8
44	262.0	17.6	154.2	12.0
47	302.0	19.7	151.7	5.33
49	232.8	8.98	121.3	15.6
55	218.8	9.78	133.6	6.85
63	130.3	14.5	102.9	33.4
71	46.6	11.4	86.7	6.72
86	38.4	4.32	98.4	14.7

Table 2. Original data of Figure 3a and 3b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each hexanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthylHex	STD	PropylHex	STD	2MBHex	STD	ButylHex	STD	PentylHex	STD
0	0	0	0	0	0	0	29271	28870	0	0
6	0	0	0	0	0	0	10977	10984	0	0
9	0	0	0	0	0	0	9443	9535	0	0
12	0	0	0	0	0	0	2301	2430	0	0
16	0	0	0	0	0	0	32716	17728	0	0
19	0	0	0	0	0	0	99549	84395	8024	16049
21	0	0	0	0	0	0	137742	39657	10134	12625
25	0	0	0	0	0	0	646325	351455	93416	32550
29	421437	487935	654102	552094	21042	42084	20616358	24040158	1020584	1345066
33	577627	516782	3691264	1640575	141842	65390	36097986	18539768	1575490	828949
37	2137145	970004	8272978	2966730	159236	85051	29405778	12023640	1050047	431866
41	6847946	2629705	24965865	10750818	270027	90106	49981416	18113135	1556691	608011
44	11338885	4713892	21548327	8960472	162350	36077	33261137	12804755	1017022	407698
47	22967379	1837889	44939244	6885093	305646	56694	49362289	13232105	1412054	321262
49	32648885	15270685	66110142	16235952	303353	77343	46340896	10879940	1541751	400994
55	31367495	16920827	48410198	23244686	189345	121552	32831460	13727708	976807	494428
63	17123204	7408752	18384857	4616789	66683	21621	10240947	2378727	363505	72529
71	14318458	6624179	7721103	4118061	18233	14990	4083233	1755348	135212	59666

Table 2 (cont'd).

Date	HexylHex	STD	HexAcid	STD	TOTAL	T-STD	2MPHex	STD
0	20501	15597	0	0	49772	40598	0	0
6	34619	39636	0	0	36477	48789	0	0
9	53075	54628	0	0	62517	64139	0	0
12	7051	3067	0	0	7482	5456	0	0
16	38352	23466	0	0	42641	47095	0	0
19	101594	39677	0	0	209167	135251	0	0
21	78843	33743	0	0	226719	79639	0	0
25	460018	154743	0	0	1199759	502495	0	0
29	7318974	5760272	0	0	32246622	32448276	97700	122506
33	20813162	13151454	0	0	64924620	35324814	104915	39221
37	8334268	4404864	0	0	50790858	18690099	94629	32021
41	8579113	2721448	0	0	94314595	34814888	204752	75792
44	5711116	1861748	0	0	74353097	28482080	194758	119678
47	7256644	1699872	0	0	128232201	22471421	216025	39820
49	8199351	1647541	1130795	361354	156589928	36656078	222811	53001
55	5912301	3069608	439078	57381	120669796	55201941	142036	56531
63	2443574	788055	206422	31670	48965913	13877318	40128	13058
71	1129604	479566	73361	10464	27468719	12751529	15438	12929

Table 3. Original data of Figure 3c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each hexanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthylHex	STD	PropylHex	STD	2MBHex	STD	ButylHex	STD	PentylHex	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	45.9	25.3	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	27.9	6.8	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	12.5	6.4	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	19.9	20.1	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	48.7	7.5	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	43.4	12.5	1.7	3.4
21	0.0	0.0	0.0	0.0	0.0	0.0	62.2	6.4	3.7	5.1
25	0.0	0.0	0.0	0.0	0.0	0.0	50.9	10.9	8.1	1.1
29	1.4	1.5	2.6	1.5	0.0	0.0	54.4	11.5	2.7	1.3
33	0.7	0.5	6.7	2.9	0.3	0.2	57.5	5.0	2.4	0.4
37	5.0	3.3	17.4	5.5	0.3	0.1	56.9	3.1	2.0	0.5
41	7.4	0.8	25.7	2.8	0.3	0.0	53.1	2.0	1.6	0.1
44	15.3	2.7	28.6	2.6	0.2	0.0	44.8	2.6	1.4	0.0
47	18.3	2.5	35.2	2.2	0.2	0.0	37.9	3.8	1.1	0.1
49	20.1	7.6	42.2	1.7	0.2	0.1	30.1	6.4	1.0	0.3
55	25.5	3.6	39.6	2.0	0.2	0.1	28.0	3.0	0.8	0.1
63	33.5	6.3	37.9	1.6	0.1	0.0	21.5	3.1	0.8	0.3
71	52.4	5.6	27.5	3.1	0.1	0.1	15.1	2.7	0.5	0.2

Table 3 (cont'd).

Date	HexylHex	STD	HexAcid	STD	2MPHex	STD
0	54.1	25.3	0.0	0.0	0.0	0.0
6	72.1	6.8	0.0	0.0	0.0	0.0
9	87.5	6.4	0.0	0.0	0.0	0.0
12	80.1	20.1	0.0	0.0	0.0	0.0
16	51.3	7.5	0.0	0.0	0.0	0.0
19	55.0	14.5	0.0	0.0	0.0	0.0
21	34.1	5.2	0.0	0.0	0.0	0.0
25	41.0	10.0	0.0	0.0	0.0	0.0
29	25.8	5.5	0.0	0.0	0.2	0.2
33	29.2	7.4	0.0	0.0	0.2	0.1
37	15.6	6.1	0.0	0.0	0.2	0.0
41	9.6	2.0	0.0	0.0	0.2	0.0
44	7.8	0.6	0.0	0.0	0.3	0.2
47	5.7	0.7	0.0	0.0	0.2	0.0
49	5.5	1.5	0.7	0.2	0.1	0.0
55	5.0	1.1	0.4	0.2	0.1	0.0
63	5.3	2.0	0.5	0.1	0.1	0.0
71	4.2	1.2	0.3	0.1	0.1	0.0

Table 4. Original data of Figure 4a and 4b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each butanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyBut	STD	PropylBut	STD	ButylBut	STD	2MBBut	STD
0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	0	0	0	0	64141	56504	0	0
21	0	0	0	0	98565	31860	0	0
25	0	0	0	0	210901	172931	0	0
29	0	0	2347656	4053182	4592362	6231025	1284570	1346404
33	624006	445313	4925625	2902871	10109972	4920176	2353173	783887
37	4985463	4712371	12205288	7282436	12115969	4476873	2355238	1278181
41	11553408	2657331	20681929	4734229	14537831	2917477	3542875	1110539
44	17821390	4645574	24472199	7251317	12824617	4099373	2469688	606945
47	27034520	5595307	41084204	8408003	21950694	6896731	4588010	1368395
49	40499172	29577609	57183333	23544422	20576979	5140195	2835945	1112742
55	41101317	21073235	45550838	19989043	15210561	5972483	2014705	646160
63	27129520	14709235	22656584	9517372	6832704	2403132	541888	208712
71	16818676	2282781	7618930	3363527	2581723	1629826	38378	76755

Table 4 (cont'd).

Date	HexylBut	STD	Butiric Acid	STD	Total	STD
0	0	0	0	0	0	0
6	0	0	0	0	0	0
9	0	0	0	0	0	0
12	0	0	0	0	0	0
16	0	0	0	0	0	0
19	106137	98249	0	0	170277	153934
21	95277	21122	0	0	193842	29314
25	371397	218513	11411	7790	582298	383706
29	11841891	12780833	14723	12269	20066480	24300839
33	29752798	15936174	19164	8809	47765573	24330672
37	22479664	9052356	41776	34879	54141621	23129131
41	27844560	6102573	15521	801	78160603	16500967
44	21733541	7216808	7072	2123	79321434	20440705
47	27377250	5767197	8340	1652	122034678	22780150
49	29700085	4145533	21828	1183	150795514	53823139
55	24958839	11620169	39654	18281	128836260	52332963
63	8841211	1793243	14553	3668	66001906	27234793
71	3363309	1582112	11094	3325	30421016	6436478

Table 5. Original data of Figure 4c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each butanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyBut	STD	PropylBut	STD	ButylBut	STD	2MBBut	STD	HexylBut	STD	Butiric Acid	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	38.2	3.1	0.0	0.0	61.8	3.1	0.0	0.0
21	0.0	0.0	0.0	0.0	50.0	10.9	0.0	0.0	50.0	10.9	0.0	0.0
25	0.0	0.0	0.0	0.0	34.8	5.6	0.0	0.0	65.2	5.6	2.0	1.0
29	0.0	0.0	4.9	5.7	22.6	5.7	7.0	5.1	65.5	6.5	0.2	0.1
33	1.2	0.5	10.6	3.9	21.9	4.2	5.6	1.5	60.6	9.1	0.0	0.0
37	8.4	4.7	22.3	5.7	23.0	2.2	4.4	1.6	41.9	10.4	0.1	0.0
41	14.8	1.2	26.5	1.9	18.7	0.4	4.5	0.8	35.7	2.7	0.0	0.0
44	22.8	4.5	30.6	3.0	16.0	2.1	3.1	0.5	27.4	5.5	0.0	0.0
47	22.5	4.3	33.6	1.9	17.7	3.0	3.7	0.7	22.5	2.0	0.0	0.0
49	23.7	9.7	37.2	3.0	14.4	2.8	2.2	1.1	22.4	8.2	0.0	0.0
55	31.9	6.6	35.0	2.3	12.0	1.9	1.7	0.7	19.5	4.4	0.0	0.0
63	39.3	4.5	34.4	2.2	10.6	0.6	0.9	0.4	14.8	3.7	0.0	0.0
71	57.2	11.4	24.1	5.5	7.9	3.2	0.1	0.2	10.7	3.4	0.0	0.0

Table 6. Original data of Figure 5a and 5b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each propanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyProp	STD	PropylProp	STD	ButylProp	STD	2MBProp	STD
0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
21	0	0	0	0	8581	7897	0	0
25	0	0	0	0	139442	86556	42570	24661
29	0	0	644314	1110801	3174711	4865423	1158803	1646371
33	105384	43376	941441	481036	9096621	4564120	2033500	794939
37	629833	583330	3590217	2782503	13072961	5737589	2576796	716256
41	2307403	611144	9651243	2564022	20487639	4399861	3853517	888264
44	3529672	1453971	10285910	3936606	19584661	5474476	2858585	797032
47	6425338	1412944	18486532	5489040	31797027	7915894	5840342	1676660
49	25460544	24268191	44055985	28708395	41805099	13963796	6762870	2534115
55	30553428	13082888	38787968	14062208	30543370	9041307	4287982	1044700
63	28556819	14972286	24896236	12395221	15085637	5763118	1470564	512391
71	20691470	2718228	8036932	2791292	5375799	3008448	436195	266185

Table 6 (cont'd).

Date	HexylProp	STD	Propanoic Acid	STD	Total	STD
0	0	0	0	0	0	0
6	0	0	0	0	0	0
9	0	0	0	0	0	0
12	0	0	0	0	0	0
16	0	0	0	0	0	0
19	0	0	0	0	0	0
21	0	0	0	0	8581	7897
25	99947	32830	0	0	281958	131040
29	3098954	4681272	0	0	8076782	12302094
33	11007748	5925726	0	0	23184694	11577150
37	11547921	4847828	0	0	31417728	13586303
41	18859010	12121339	0	0	55158812	18715783
44	10066958	4421056	0	0	46325786	14384486
47	17605724	3957220	43943	20058	80154964	15827974
49	38929830	18083994	60582	21408	157014329	74957882
55	19819200	9720505	134736	93064	123991948	37217114
63	8970346	2974729	97184	18436	78979603	35479600
71	2762459	1601056	54124	26737	37302855	6142811

Table 7. Original data of Figure 5c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each propanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyProp	STD	PropylProp	STD	ButylProp	STD	2MBProp	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	47.7	7.4	14.9	7.2
29	0.0	0.0	3.7	3.4	38.9	2.7	19.1	4.7
33	0.6	0.3	4.1	1.0	39.4	4.0	9.7	2.2
37	1.8	1.0	10.6	3.9	42.0	4.0	8.8	1.6
41	4.3	0.6	17.9	2.3	38.6	5.1	7.3	1.3
44	7.8	2.0	21.9	3.7	42.8	2.3	6.3	1.0
47	8.5	3.2	23.0	4.4	39.3	3.5	7.2	0.9
49	13.8	6.4	26.1	4.5	29.0	7.3	4.9	2.0
55	24.4	6.8	31.1	3.0	25.0	4.3	3.6	0.7
63	35.0	2.8	31.0	2.3	19.8	2.2	1.9	0.3
71	57.3	12.8	21.0	4.1	13.6	5.6	1.1	0.5

Table 7 (cont'd).

Date	HexylProp	STD	Propanoic Acid	STD
0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0
25	37.3	5.0	0.0	0.0
29	38.2	2.1	0.0	0.0
33	46.3	4.8	0.0	0.0
37	36.8	7.5	0.0	0.0
41	31.9	8.5	0.0	0.0
44	21.2	3.8	0.0	0.0
47	22.0	2.2	0.1	0.0
49	26.2	11.1	0.0	0.0
55	15.9	5.4	0.1	0.0
63	12.2	2.6	0.1	0.1
71	7.0	3.1	0.1	0.1

Table 8. Original data of Figure 6a and 6b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each acetate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyAcet	STD	PropylAcet	STD	2MPAcet	STD	ButylAcet	STD
0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	315079	256356
21	0	0	0	0	0	0	337764	176161
25	0	0	17577	16559	0	0	1050342	963652
29	94745	143468	1882391	2810212	0	0	12818637	14942446
33	249219	87520	4179384	1696435	762528	254806	54146614	23147058
37	1077315	629146	10886393	5280206	992087	362716	71037720	23704322
41	3064187	644551	17406687	2258505	1017543	59477	71012405	7149925
44	3875462	530726	17125120	4931143	932427	174103	73272213	15992980
47	3418096	1993177	17026138	4267359	893603	255908	75478679	21189544
49	10150084	6478902	32769527	20598573	1244184	669805	77847884	31438710
55	10877748	4411685	27613313	10297076	969778	366924	60086307	16703655
63	11174483	7625333	26074213	17912964	550712	304741	48494788	26388419
71	21460458	3577260	27975216	10315463	421810	156716	55956359	34143263

Table 8 (cont'd).

Date	2MBAcet	STD	PentylAcet	STD	HexylAcet	STD	Acetic Acid	STD	TOTAL	T-STD
0	0	0	0	0	0	0	457664	193691	0	0
6	0	0	0	0	0	0	87778	64875	0	0
9	0	0	0	0	484541	362475	40183	23245	484541	362475
12	0	0	0	0	159930	212918	61773	34743	159930	212918
16	58499	31890	0	0	45143	38255	128231	23217	103643	63645
19	1254389	1511859	192848	172124	365244	242627	255713	89422	2127560	2079319
21	462426	300946	90935	40523	278214	96445	155089	27091	1169340	462239
25	1767945	965201	294510	241510	993031	642259	157147	38493	4123404	2696118
29	37286455	39491682	3255680	4256479	16647937	19120059	159182	25169	71985845	80618633
33	117813571	41546774	9701514	4169348	104124341	48569120	181115	43154	291235739	117034876
37	137695013	40390054	13269900	3105414	94390994	33589032	237785	68076	329641991	94280513
41	132275573	19558097	12221866	2446700	75537871	7213295	201049	32584	312821998	30864852
44	134965494	32693994	14477114	4908086	80581347	27034660	45634	33289	325507976	78966728
47	151625723	51431223	19247366	6165300	107277585	30919595	31345	13963	375255869	111518957
49	136999822	54401137	21039946	8861422	103918970	28730673	248964	92033	384386465	136953469
55	88103319	19442297	14306434	6245273	86814194	32860097	332634	66362	289058364	76057784
63	29654771	10311451	9864723	4067426	53246779	20676363	333463	146020	179212863	84055285
71	23401251	13929582	8758086	6730896	50381435	39893329	370883	67405	188487732	106611683

Table 9. Original data of Figure 6c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each acetate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyAcet	STD	PropylAcet	STD	2MPAcet	STD	ButylAcet	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	16.0	3.8
21	0.0	0.0	0.0	0.0	0.0	0.0	30.3	10.1
25	0.0	0.0	0.4	0.4	0.0	0.0	21.9	6.4
29	0.1	0.1	1.5	1.1	0.0	0.0	17.5	1.7
33	0.1	0.0	1.5	0.3	0.3	0.1	18.3	1.9
37	0.3	0.2	3.2	1.2	0.3	0.1	21.5	3.7
41	1.0	0.1	5.6	0.5	0.3	0.0	22.8	1.7
44	1.3	0.3	5.3	1.3	0.3	0.1	22.6	1.5
47	1.0	0.5	4.6	0.8	0.2	0.1	20.2	2.1
49	2.4	1.0	8.1	2.9	0.3	0.1	20.2	3.0
55	3.9	1.3	9.7	2.3	0.3	0.1	20.9	1.7
63	5.9	1.1	13.7	2.9	0.3	0.0	26.6	2.3
71	13.5	4.0	16.4	4.0	0.2	0.0	29.1	1.1

Table 9 (cont'd).

Date	2MBAcet	STD	PentylAcet	STD	HexylAcet	STD	Acetic Acid	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
9	0.0	0.0	0.0	0.0	100.0	0.0	11.3	0.0
12	0.0	0.0	0.0	0.0	100.0	0.0	42.6	16.9
16	51.6	30.6	0.0	0.0	48.4	30.6	58.9	17.7
19	53.3	14.2	9.5	0.7	21.2	10.7	17.3	9.2
21	36.4	13.0	8.6	3.2	24.7	3.6	13.2	5.3
25	44.5	7.1	7.7	2.7	25.6	4.3	5.6	3.9
29	52.6	5.0	4.4	1.0	23.9	3.6	1.2	1.5
33	42.0	5.2	3.3	0.2	34.5	3.4	0.1	0.0
37	41.9	2.9	4.1	0.4	28.5	4.7	0.1	0.0
41	42.1	3.1	3.9	0.6	24.2	2.0	0.1	0.0
44	41.5	2.3	4.4	0.6	24.5	2.9	0.0	0.0
47	40.0	1.7	5.1	0.4	28.9	2.5	0.0	0.0
49	35.4	4.9	5.4	0.6	28.0	3.2	0.1	0.0
55	31.0	4.7	4.8	0.9	29.5	5.2	0.1	0.0
63	17.3	2.1	5.7	0.7	30.5	4.4	0.2	0.1
71	12.2	3.4	4.2	0.8	24.3	5.0	0.2	0.1

Table 10. Original data of Figure 7a and 7b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each 2-methylbutanoate ester during ripening and senescence. Each value is the average of five replications.

Date	Ethyl 2MB	STD	Propyl 2MB	STD	Butyl 2MB	STD	Pentyl 2MB	STD
0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
25	0	0	0	0	104327	75767	20340	27715
29	152679	233676	1740252	2825496	4384347	4975584	904956	944413
33	353482	263169	4482135	2143970	15901600	6617092	2322501	976100
37	3501731	2724209	13128531	6042727	22964648	8310149	3177797	1090933
41	12640809	3458374	30054950	8299517	29233181	7172209	4059718	1322052
44	20467110	4639623	30309579	7163857	18536013	4583335	2097059	618619
47	35571674	6803576	56496777	11808892	31064140	12894338	3793095	1319126
49	57268147	29424149	76615014	29047530	24953727	7704638	2890612	759639
55	50752873	19685744	51784686	17514047	15044368	5032103	1708131	596900
63	25364528	10548858	17888372	6150454	4068701	1326856	501501	166228
71	20950729	13053165	7663060	5070864	1899878	1181909	180549	136544

Table 10 (cont'd).

Date	Hexyl 2MB	STD	2MBut.acid	STD	Total	STD
0	31453	18945	0	0	31453	18945
6	82061	157701	0	0	82061	157701
9	76386	149879	0	0	76386	149879
12	0	0	0	0	0	0
16	20243	40485	0	0	20243	40485
19	73248	62451	0	0	73248	62451
21	44784	26885	5951	7319	44784	26885
25	686325	286813	19061	2984	810993	375573
29	21075123	18444550	75312	90436	28257357	27067524
33	120162130	61458139	139916	73727	143221848	70584034
37	95360764	46824076	174175	85430	138133470	53746663
41	93228085	20183863	191335	54050	169216742	36095328
44	53150051	9684543	135962	42659	124559812	21304459
47	79111397	26359436	153673	70397	206037083	46684293
49	55309068	22035896	66349	55231	217036569	65614263
55	38685752	14088269	27633	5150	157975810	43763170
63	10075762	3001475	13965	2387	57898864	18007316
71	3998692	3556797	12850	11378	34692908	22745650

Table 11. Original data of Figure 7c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each 2-methylbutanoate ester during ripening and senescence. Each value is the average of five replications.

Date	Ethyl 2MB	STD	Propyl 2MB	STD	Butyl 2MB	STD	Pentyl 2MB	STD	Hexyl 2MB	STD	2MBut. acid	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	24.6	38.7
25	0.0	0.0	0.0	0.0	11.2	6.7	1.6	2.1	87.2	7.4	2.9	1.3
29	0.3	0.3	3.9	3.1	15.7	4.1	3.6	0.9	76.5	7.0	0.7	0.8
33	0.3	0.2	3.5	1.5	12.0	2.7	1.7	0.2	82.5	4.4	0.1	0.0
37	3.3	2.5	11.4	5.8	17.9	4.2	2.4	0.3	65.0	12.3	0.1	0.0
41	7.4	1.2	17.6	2.6	17.2	1.9	2.3	0.3	55.4	4.9	0.1	0.0
44	16.8	4.2	24.1	2.1	14.7	1.6	1.7	0.3	42.7	3.7	0.1	0.0
47	18.1	4.8	27.6	3.0	14.5	2.7	1.8	0.2	38.0	5.6	0.1	0.0
49	25.1	10.4	34.9	4.1	11.9	3.0	1.4	0.3	26.8	10.8	0.0	0.0
55	32.5	7.9	32.2	2.8	9.4	2.1	1.1	0.2	24.8	6.1	0.0	0.0
63	42.8	4.6	30.7	2.3	7.0	0.6	0.9	0.3	18.6	5.9	0.0	0.0
71	61.9	6.3	21.8	3.5	5.5	1.2	0.5	0.1	10.3	2.7	0.0	0.0

Table 12. Original data of Figure 8a and 8b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each pentanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthylPent	STD	PropylPent	STD	TOTAL	T-STD
0	0	0	0	0	0	0
6	0	0	0	0	0	0
9	0	0	0	0	0	0
12	0	0	0	0	0	0
16	0	0	0	0	0	0
19	0	0	0	0	0	0
21	0	0	0	0	0	0
25	0	0	0	0	0	0
29	0	0	0	0	0	0
33	0	0	0	0	0	0
37	76937	153874	294275	208037	371212	357848
41	618090	363340	831813	355214	1449903	659494
44	816981	625746	1033382	360683	1850363	946785
47	1048185	494059	2416754	647512	3464939	854659
49	2007939	1166898	3672964	1262302	5680903	2397611
55	1748907	1129521	2485505	1310986	4234412	2404482
63	890759	345002	1265480	486509	2156239	816871
71	469181	166723	346320	240053	815501	386189

Table 13. Original data of Figure 8c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each pentanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthylPent	STD	PropylPent	STD
0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0
37	7.2	14.3	92.8	14.3
41	40.7	9.2	59.3	9.2
44	35.6	21.3	64.4	21.3
47	29.6	13.0	70.4	13.0
49	30.7	13.3	69.3	13.3
55	39.8	6.2	60.2	6.2
63	41.3	4.0	58.7	4.0
71	60.2	11.3	39.8	11.3

Table 14. Original data of Figure 9a and 9b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each heptanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthylHept	STD	PropylHept	STD	ButylHept	STD	TOTAL	T-STD
0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
29	0	0	131080	262161	226811	421849	357891	683721
33	0	0	0	0	497950	257046	398360	257046
37	10602	8865	366954	151891	423174	161779	800731	311218
41	203052	117891	1444632	839818	1069769	468755	2717453	1407853
44	227216	96767	1143075	505538	674823	317734	2045115	892376
47	580500	76468	2277162	416553	1124889	225169	3982551	583943
49	1183431	422467	4503977	753603	1463033	354965	7150442	1194078
55	1120688	649957	3395604	1737152	947340	463638	5463633	2767475
63	918074	401342	1672974	414697	427366	109209	3018414	818061
71	869548	555178	755740	436322	183256	94265	1808544	1080327

Table 15. Original data of Figure 9c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each heptanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthylHept	STD	PropylHept	STD	ButylHept	STD
0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0
33	0.0	0.0	0.0	0.0	100.0	0.0
37	1.1	0.9	46.2	7.4	52.7	7.1
41	6.6	3.4	52.1	4.0	41.3	4.6
44	11.2	2.1	55.7	4.4	33.1	4.5
47	14.8	2.0	56.9	3.6	28.4	4.3
49	16.6	5.5	63.0	2.6	20.4	3.5
55	20.0	2.6	62.1	2.4	17.9	2.8
63	29.3	6.3	55.9	3.2	14.7	3.3
71	47.6	4.0	41.9	3.3	10.5	1.3

Table 16. Original data of Figure 10a and 10b (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each octanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyOct	STD	PropylOct	STD	ButylOct	STD	TOTAL	T-STD
0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
29	37047	74094	192307	267718	672331	542960	901685	862824
33	0	0	228164	146707	1216621	754579	1444785	871841
37	254074	248631	464398	235182	818692	366376	1537165	745398
41	1238569	647637	1436260	785404	1171636	485382	3846465	1909283
44	948782	403244	816250	374705	683590	263724	2448622	1021089
47	1741731	265230	1431517	170326	840421	125850	4013668	449619
49	3526770	1396520	2752961	676157	1241261	311977	7520992	1928921
55	3598015	1835550	2134222	1112249	830617	365442	6562854	3269594
63	2990646	1508280	1232519	537749	1255879	1321248	5479043	2611457
71	3334404	1133654	740812	295478	360290	135253	4435506	1528365

Table 17. Original data of Figure 10c (chapter 3). Fractions, corrected GC/MS response (total ion counts) of each octanoate ester during ripening and senescence. Each value is the average of five replications.

Date	EthyOct	STD	PropylOct	STD	ButylOct	STD
0	0	0	0	0	0	0
6	0	0	0	0	0	0
9	0	0	0	0	0	0
12	0	0	0	0	0	0
16	0	0	0	0	0	0
19	0	0	0	0	0	0
21	0	0	0	0	0	0
25	0	0	0	0	0	0
29	2	3	14	10	84	13
33	0	0	16	5	84	5
37	16	14	30	3	54	12
41	32	2	36	3	32	3
44	39	5	33	4	28	1
47	43	3	36	1	21	3
49	46	9	37	7	17	3
55	55	3	32	2	13	2
63	55	9	24	6	20	14
71	75	4	17	3	8	1

Table 18. Original data of Figure 11 (chapter 3). Absolute, corrected GC/MS response (total ion counts) of each alcohol during ripening and senescence. Each value is the average of five replications.

Date	ethanol	propanol	butanol	2MButanol	hexanol	TOTAL
0	102490	0	0	0	0	102490
6	2074	0	29076	0	0	31151
9	24093	0	5678	0	0	29771
12	40091	0	0	0	0	40091
16	96202	0	0	0	0	96202
19	114523	0	48471	0	0	162994
21	313734	0	45205	0	0	358938
25	41024	0	258310	0	0	299334
29	44436	0	1108201	0	0	1152637
33	40313	148509	2375003	1274840	1105836	4944501
37	194638	432912	3793554	1910647	1158022	7489773
41	291800	686108	5036189	1697844	861260	8573202
44	264820	486290	4576437	1294249	851308	7473104
47	233425	449579	5468774	1427208	978473	8557458
49	545401	1026204	7540814	1281669	1123745	11517832
55	747978	1043687	5800859	1112980	1787472	10492976
63	788108	1167695	3708011	665438	1588272	7917525
71	1827758	1454652	2628005	588482	1751484	8250381

Table 19. Original data of Figure 12 (chapter 3). Concentration (ppm) of esters and alcohols during ripening and senescence. Each value is the average of five replications.

Date	ButylAcet	2MBAcet	HexylAcet	HexylBut	HexylHex	Ethanol	Butanol	2-MButanol	Hexanol
0	0.000	0.000	0.000	0.000	0.000	3.390	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.001	0.069	0.004	0.000	0.000
9	0.000	0.000	0.005	0.000	0.001	0.797	0.001	0.000	0.000
12	0.000	0.000	0.002	0.000	0.000	1.326	0.000	0.000	0.000
16	0.000	0.006	0.000	0.000	0.001	3.182	0.000	0.000	0.000
19	0.009	0.121	0.004	0.001	0.002	3.789	0.006	0.000	0.000
21	0.009	0.045	0.003	0.001	0.001	10.379	0.006	0.000	0.000
25	0.029	0.170	0.010	0.002	0.008	1.357	0.032	0.000	0.000
29	0.360	3.595	0.161	0.078	0.131	1.470	0.139	0.000	0.000
33	1.521	11.358	1.005	0.195	0.371	1.334	0.298	0.078	0.018
37	1.995	13.274	0.911	0.147	0.149	6.439	0.476	0.117	0.018
41	1.994	12.752	0.729	0.182	0.153	9.653	0.632	0.104	0.014
44	2.058	13.011	0.778	0.142	0.102	8.760	0.574	0.079	0.014
47	2.120	14.617	1.035	0.179	0.129	7.722	0.686	0.087	0.016
49	2.186	13.207	1.003	0.194	0.146	18.042	0.946	0.079	0.018
55	1.688	8.494	0.838	0.163	0.105	24.744	0.728	0.068	0.028
63	1.362	2.859	0.514	0.058	0.044	26.071	0.465	0.041	0.025
71	1.572	2.256	0.486	0.022	0.020	60.464	0.330	0.036	0.028

Table 20. Original data of Figure 1a and 1b (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each hexyl ester during ripening and senescence. Each value is the average of five replications.

Date	HexylAcet	STD	HexylProp	STD	HexylBut	STD	Hexyl2MBut	STD
0	0	0	0	0	0	0	31453	18945
6	0	0	0	0	0	0	82061	157701
9	369335	364737	0	0	0	0	76386	149879
12	159930	212918	0	0	0	0	0	0
16	45143	38255	0	0	0	0	20243	40485
19	365244	242627	0	0	106137	98249	73248	62451
21	278214	96445	0	0	95277	21122	44784	26885
25	993031	642259	99947	32830	371397	218513	686325	286813
29	16647937	19120059	3098954	4681272	11841891	12780833	21075123	18444550
33	104124341	48569120	11007748	5925726	29752798	15936174	120162130	61458139
37	94390994	33589032	11547921	4847828	22479664	9052356	95360764	46824076
41	75537871	7213295	18859010	12121339	27844560	6102573	93228085	20183863
44	80581347	27034660	10066958	4421056	21733541	7216808	53150051	9684543
47	107277585	30919595	17605724	3957220	27377250	5767197	79111397	26359436
49	103918970	28730673	38929830	18083994	29700085	4145533	55309068	22035896
55	86814194	32860097	19819200	9720505	24958839	11620169	38685752	14088269
63	53246779	20676363	8970346	2974729	8841211	1793243	10075762	3001475
71	50381435	39893329	2762459	1601056	3363309	1582112	3998692	3556797

Table 20 (cont'd).

Date	HexylHex	STD	HEXANOL	STD	Total	STD
0	20501	15597	0	0	51955	32578
6	27696	38060	0	0	109756	195486
9	53075	54628	0	0	498795	516089
12	5641	3935	0	0	165571	215649
16	23011	26142	0	0	88397	93180
19	101594	39677	0	0	646222	335578
21	78843	33743	0	0	497118	131660
25	460018	154743	0	0	2610718	1260974
29	7318974	5760272	0	0	59982879	59937577
33	20813162	13151454	1105836	437582	285860178	141015756
37	8334268	4404864	1158022	453842	232113610	95543683
41	8579113	2721448	861260	122495	224048640	35805950
44	5711116	1861748	851308	324432	171243013	46725081
47	7256644	1699872	978473	230814	238628600	66561822
49	8199351	1647541	1123745	827278	236057305	28683634
55	5912301	3069608	1787472	508488	176190285	67362663
63	2443574	788055	1588272	660768	83577672	23441505
71	1129604	479566	1751484	1072620	61635499	44018830

Table 21. Original data of Figure 1c (chapter 4). Fractions, corrected GC/MS response (total ion counts) of each hexyl ester during ripening and senescence. Each value is the average of five replications.

Date	HexylAcet	STD	HexylProp	STD	HexylBut	STD	Hexyl2MBut	STD	HexylHex	STD	HEXANOL	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	51.6	28.3	48.4	28.3	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	32.1	33.9	67.9	33.9	0.0	0.0
9	61.4	32.9	0.0	0.0	0.0	0.0	5.7	11.1	32.9	34.7	0.0	0.0
12	94.8	3.7	0.0	0.0	0.0	0.0	0.0	0.0	5.2	3.7	0.0	0.0
16	72.6	25.9	0.0	0.0	0.0	0.0	8.1	16.2	19.2	16.7	0.0	0.0
19	53.8	21.8	0.0	0.0	11.5	10.7	12.9	12.9	21.8	13.4	0.0	0.0
21	55.6	9.5	0.0	0.0	20.6	7.0	8.0	4.6	15.8	4.7	0.0	0.0
25	35.5	7.5	4.3	1.6	13.5	3.0	27.4	5.3	19.2	4.6	0.0	0.0
29	28.4	7.0	3.4	2.0	20.4	3.4	33.4	9.8	14.5	2.7	0.0	0.0
33	37.6	3.7	3.8	0.5	10.3	1.6	41.6	3.9	6.6	2.2	0.4	0.1
37	42.9	6.5	5.2	1.4	9.9	1.9	38.7	7.0	3.3	0.6	0.5	0.2
41	34.2	3.6	8.3	4.8	12.3	1.0	41.4	4.4	3.8	0.8	0.4	0.1
44	46.5	2.8	5.7	1.3	12.7	2.4	31.8	4.2	3.3	0.5	0.5	0.2
47	44.8	2.7	7.5	0.8	11.6	0.8	33.0	3.6	3.1	0.2	0.4	0.1
49	43.7	9.8	16.8	8.4	12.7	1.6	23.4	8.6	3.5	0.7	0.5	0.3
55	49.3	3.7	11.1	2.1	13.8	1.6	22.6	5.7	3.2	0.4	1.1	0.5
63	61.8	8.2	11.1	3.1	11.1	2.2	12.7	3.7	3.3	1.6	1.9	0.3
71	79.4	6.3	4.9	1.3	6.3	1.8	7.3	5.2	2.1	0.6	3.0	0.3

Table 22. Original data of Figure 2a and 2b (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each butyl ester during ripening and senescence. Each value is the average of five replications.

Date	ButylAcet	STD	ButylProp	STD	ButylBut	STD	Butyl2MBut	STD	ButylHex	STD
0	0	0	0	0	0	0	0	0	29271	28870
6	0	0	0	0	0	0	0	0	8781	10760
9	0	0	0	0	0	0	0	0	9443	9535
12	0	0	0	0	0	0	0	0	1841	2361
16	0	0	0	0	0	0	0	0	19630	21106
19	315079	256356	0	0	64141	56504	0	0	99549	84395
21	337764	176161	8581	7897	98565	31860	0	0	137742	39657
25	1050342	963652	139442	86556	210901	172931	104327	75767	646325	351455
29	12818637	14942446	3174711	4865423	4592362	6231025	4384347	4975584	20616358	24040158
33	54146614	23147058	9096621	4564120	10109972	4920176	15901600	6617092	36097986	18539768
37	71037720	23704322	13072961	5737589	12115969	4476873	22964648	8310149	29405778	12023640
41	71012405	7149925	20487639	4399861	14537831	2917477	29233181	7172209	49981416	18113135
44	73272213	15992980	19584661	5474476	12824617	4099373	18536013	4583335	33261137	12804755
47	75478679	21189544	31797027	7915894	21950694	6896731	31064140	12894338	49362289	13232105
49	77847884	31438710	41805099	13963796	20576979	5140195	24953727	7704638	46340896	10879940
55	60086307	16703655	30543370	9041307	15210561	5972483	15044368	5032103	32831460	13727708
63	48494788	26388419	15085637	5763118	6832704	2403132	4068701	1326856	10240947	2378727
71	55956359	34143263	5375799	3008448	2581723	1629826	1899878	1181909	4083233	1755348

Table 22 (cont'd).

Date	ButylHept	STD	ButylOct	STD	Butanol	STD	Total	STD
0	0	0	0	0	0	0	29271	28870
6	0	0	0	0	29076	33533	8781	10760
9	0	0	0	0	5678	8078	9443	9535
12	0	0	0	0	0	0	1841	2361
16	0	0	0	0	0	0	19630	21106
19	0	0	0	0	48471	41789	478769	338906
21	0	0	0	0	45205	45258	582652	209840
25	0	0	0	0	258310	164619	2151337	1610046
29	226811	421849	672331	542960	1108201	1142479	46485557	55877382
33	398360	257046	1216621	754579	2375003	969616	126967774	57835855
37	423174	161779	818692	366376	3793554	1716166	149838941	50495945
41	1069769	468755	1171636	485382	5036189	960729	187493876	35182167
44	674823	317734	683590	263724	4576437	940541	158837054	40490625
47	1124889	225169	840421	125850	5468774	1069410	211618139	58401594
49	1463033	354965	1241261	311977	7540814	2179821	214228880	49094359
55	947340	463638	830617	365442	5800859	924304	155494024	46351236
63	427366	109209	1255879	1321248	3708011	1805253	86406022	36519509
71	183256	94265	360290	135253	2628005	940289	70440537	41092665

Table 23. Original data of Figure 2c (chapter 4). Fractions, corrected GC/MS response (total ion counts) of each butyl ester during ripening and senescence. Each value is the average of five replications.

Date	ButylAcet	STD	ButylProp	STD	ButylBut	STD	Butyl2MBut	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	66.0	12.5	0.0	0.0	9.2	7.8	0.0	0.0
21	55.1	10.2	1.2	1.2	17.6	3.1	0.0	0.0
25	42.5	10.5	8.4	4.3	10.5	2.8	4.4	2.3
29	29.9	3.2	4.7	2.1	10.4	3.5	9.3	1.7
33	43.2	2.6	7.2	1.4	8.0	1.3	13.0	2.1
37	47.5	5.0	8.7	1.6	8.2	1.1	15.3	1.4
41	38.9	5.8	10.9	1.2	7.7	0.4	15.5	1.7
44	46.7	4.0	12.3	1.2	8.0	0.7	11.7	0.8
47	35.7	4.6	15.2	1.3	10.3	0.9	14.3	2.3
49	35.3	9.0	19.1	2.2	9.6	0.4	11.8	2.9
55	39.4	4.8	19.7	1.4	9.6	1.2	9.7	2.6
63	53.9	5.4	17.9	2.9	8.1	1.0	4.9	0.9
71	78.9	3.0	7.6	1.6	3.6	0.4	2.8	1.3

Table 23 (cont'd).

Date	ButylHex	STD	ButylHept	STD	ButylOct	STD	Butanol	STD
0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	100.0	0.0	0.0	0.0	0.0	0.0	58.2	0.0
9	100.0	0.0	0.0	0.0	0.0	0.0	31.1	0.0
12	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	24.8	11.1	0.0	0.0	0.0	0.0	8.2	4.6
21	26.1	8.5	0.0	0.0	0.0	0.0	5.3	5.0
25	34.2	6.9	0.0	0.0	0.0	0.0	11.5	2.0
29	43.8	4.5	0.2	0.3	1.7	1.0	2.9	0.6
33	27.5	3.1	0.3	0.1	0.9	0.5	1.9	0.3
37	19.5	3.8	0.3	0.0	0.5	0.2	2.4	0.8
41	25.8	5.6	0.5	0.2	0.6	0.2	2.6	0.3
44	20.6	3.2	0.4	0.1	0.4	0.1	2.8	0.3
47	23.6	2.5	0.5	0.1	0.4	0.1	2.7	0.7
49	22.8	7.5	0.7	0.3	0.6	0.2	3.4	0.3
55	20.5	3.5	0.6	0.2	0.5	0.1	3.7	0.5
63	13.3	4.2	0.6	0.2	1.2	0.7	4.0	0.3
71	6.2	1.0	0.3	0.0	0.6	0.1	4.0	0.9

Table 24. Original data of Figure 3a and 3b (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each propyl ester during ripening and senescence. Each value is the average of five replications.

Date	PropylAcet	STD	PropylProp	STD	PropylBut	STD	Propyl2MBut	STD	PropylPent	STD
0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0
25	17577	16559	0	0	0	0	0	0	0	0
29	1882391	2810212	644314	1110801	2347656	4053182	1740252	2825496	98849	197698
33	4179384	1696435	941441	481036	4925625	2902871	4482135	2143970	0	0
37	10886393	5280206	3590217	2782503	12205288	7282436	9479610	3094560	294275	208037
41	17406687	2258505	9651243	2564022	20681929	4734229	30054950	8299517	831813	355214
44	17125120	4931143	10285910	3936606	24472199	7251317	30309579	7163857	1033382	360683
47	17026138	4267359	18486532	5489040	41084204	8408003	56496777	11808892	2416754	647512
49	32769527	20598573	44055985	28708395	57183333	23544422	76615014	29047530	3672964	1262302
55	27613313	10297076	38787968	14062208	45550838	19989043	51784686	17514047	2485505	1310986
63	26074213	17912964	24896236	12395221	22656584	9517372	17888372	6150454	1265480	486509
71	27975216	10315463	8036932	2791292	7618930	3363527	7663060	5070864	346320	240053

Table 24 (cont'd).

Date	PropylHex	STD	PropylHept	STD	PropylOct	STD	Propanol	STD	Total	STD
0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	17577	16559
29	654102	552094	131080	262161	192307	267718	0	0	7690952	11896007
33	3691264	1640575	0	0	228164	146707	148509	78337	18448012	8163012
37	8272978	2966730	366954	151891	464398	235182	432912	255693	45560115	16774656
41	24965865	10750818	1444632	839818	1436260	785404	686108	137637	106473379	27968883
44	21548327	8960472	1143075	505538	816250	374705	486290	178807	106733843	29096384
47	44939244	6885093	2277162	416553	1431517	170326	449579	153416	184158328	34202929
49	66110142	16235952	4503977	753603	2752961	676157	1026204	460266	287663904	92755352
55	48410198	23244686	3395604	1737152	2134222	1112249	1043687	261775	220162335	83843478
63	18384857	4616789	1672974	414697	1232519	537749	1167695	610471	114071235	47410264
71	7721103	4118061	755740	436322	740812	295478	1454652	311270	60858114	23148619

Table 25. Original data of Figure 3c (chapter 4). Fractions, corrected GC/MS response (total ion counts) of each propyl ester during ripening and senescence. Each value is the average of five replications.

Date	PropylAcet	STD	PropylProp	STD	PropylBut	STD	Propyl2MBut	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	27.3	7.1	3.9	3.5	14.2	12.8	21.5	3.2
33	24.4	8.5	4.9	0.7	25.1	5.3	24.0	4.2
37	22.9	6.1	7.0	2.7	25.4	5.5	23.2	8.0
41	17.3	3.8	9.1	1.1	19.7	1.8	28.1	1.0
44	16.4	4.0	9.4	2.0	22.8	1.3	28.8	1.7
47	9.2	1.6	10.0	1.8	22.3	1.4	30.6	1.7
49	10.7	3.9	14.3	5.5	19.4	2.1	26.8	4.7
55	13.0	3.0	18.1	3.6	20.3	2.2	23.9	3.8
63	21.0	4.8	21.5	4.4	19.9	1.5	16.0	2.0
71	46.8	5.8	13.3	1.2	12.3	0.7	12.4	6.7

Table 25 (cont'd).

Date	PropylPent	STD	PropylHex	STD	PropylHept	STD	PropylOct	STD	Propanol	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.3	0.6	30.3	16.7	0.4	0.8	2.0	1.8	0.0	0.0
33	0.0	0.0	20.2	1.9	0.0	0.0	1.2	0.8	0.9	0.6
37	0.6	0.3	19.0	4.8	0.8	0.2	1.0	0.5	0.9	0.4
41	0.8	0.2	22.4	5.3	1.3	0.4	1.3	0.4	0.7	0.1
44	1.0	0.2	19.8	4.0	1.1	0.3	0.7	0.2	0.5	0.1
47	1.3	0.2	24.6	1.7	1.3	0.2	0.8	0.1	0.2	0.1
49	1.3	0.1	24.6	6.8	1.8	0.6	1.1	0.6	0.4	0.1
55	1.1	0.2	21.1	3.7	1.5	0.3	0.9	0.3	0.5	0.2
63	1.1	0.1	17.6	5.1	1.6	0.5	1.3	0.7	1.0	0.1
71	0.5	0.2	12.3	1.9	1.2	0.2	1.2	0.1	2.5	0.5

Table 26. Original data of Figure 4a and 4b (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each ethyl ester during ripening and senescence. Each value is the average of five replications.

Date	EthylAcet	STD	EthylProp	STD	EthylBut	STD	Ethyl2MBut	STD	EthylPent	STD
0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0
29	94745	143468	0	0	0	0	152679	233676	0	0
33	249219	87520	105384	43376	624006	445313	353482	263169	0	0
37	1077315	629146	629833	583330	4985463	4712371	3501731	2724209	76937	153874
41	3064187	644551	2307403	611144	11553408	2657331	12640809	3458374	618090	363340
44	3875462	530726	3529672	1453971	17821390	4645574	20467110	4639623	816981	625746
47	3418096	1993177	6425338	1412944	27034520	5595307	35571674	6803576	1048185	494059
49	10150084	6478902	25460544	24268191	40499172	29577609	57268147	29424149	2007939	1166898
55	10877748	4411685	30553428	13082888	41101317	21073235	50752873	19685744	1748907	1129521
63	11174483	7625333	28556819	14972286	27129520	14709235	25364528	10548858	890759	345002
71	21460458	3577260	20691470	2718228	16818676	2282781	20950729	13053165	469181	166723

Table 26 (cont'd).

Date	EthylHex	STD	EthylHept	STD	EthylOct	STD	Ethanol	STD	Total	STD
0	0	0	0	0	0	0	102490	107336	0	0
6	0	0	0	0	0	0	2074	1409	0	0
9	0	0	0	0	0	0	24093	34177	0	0
12	0	0	0	0	0	0	40091	60141	0	0
16	0	0	0	0	0	0	96202	101288	0	0
19	0	0	0	0	0	0	114523	156116	0	0
21	0	0	0	0	0	0	313734	421148	0	0
25	0	0	0	0	0	0	41024	19642	0	0
29	421437	487935	0	0	37047	74094	44436	29232	705909	916384
33	577627	516782	0	0	0	0	40313	13511	1909719	1140602
37	2137145	970004	10602	8865	254074	248631	194638	71288	12673100	9615526
41	6847946	2629705	203052	117891	1238569	647637	291800	63331	38473463	10215912
44	11338885	4713892	227216	96767	948782	403244	264820	34811	59025496	13476103
47	22967379	1837889	580500	76468	1741731	265230	233425	129246	98787423	12725312
49	32648885	15270685	1183431	422467	3526770	1396520	545401	277009	172744972	93689124
55	31367495	16920827	1120688	649957	3598015	1835550	747978	248459	171120472	75357639
63	17123204	7408752	918074	401342	2990646	1508280	788108	366059	114148033	53031520
71	14318458	6624179	869548	555178	3334404	1133654	1827758	436381	98912925	18311252

Table 27. Original data of Figure 4c (chapter 4). Fractions, corrected GC/MS response (total ion counts) of each ethyl ester during ripening and senescence. Each value is the average of five replications.

Date	EthylAcet	STD	EthylProp	STD	EthylBut	STD	Ethyl2MBut	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	19.9	22.8	0.0	0.0	0.0	0.0	22.3	14.8
33	16.2	8.4	6.8	3.2	31.5	12.4	20.6	14.3
37	9.5	2.5	4.7	1.3	35.4	7.0	25.9	5.1
41	8.1	0.8	6.0	0.6	30.5	2.9	32.7	1.2
44	6.8	1.2	6.0	1.8	30.2	3.2	34.8	3.3
47	3.7	2.1	6.6	1.5	27.2	2.4	35.8	2.3
49	5.8	0.8	13.1	5.3	22.2	4.6	33.6	4.8
55	6.5	1.2	18.0	3.9	23.6	2.3	30.4	3.0
63	9.2	1.6	24.9	5.5	23.2	2.2	22.6	1.7
71	22.0	2.7	21.7	4.9	17.3	2.2	20.0	8.5

Table 27 (cont'd).

EthylPent	STD	EthylHex	STD	EthylHept	STD	EthylOct	STD	Ethanol	STD
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0
0.0	0.0	55.9	36.4	0.0	0.0	1.9	3.2	10.6	7.3
0.0	0.0	24.9	15.6	0.0	0.0	0.0	0.0	2.5	0.9
0.2	0.5	22.0	8.8	0.2	0.2	2.0	2.4	2.4	1.8
1.6	0.8	17.5	2.8	0.5	0.2	3.1	0.9	0.8	0.1
1.2	0.8	19.1	5.4	0.4	0.1	1.6	0.5	0.5	0.1
1.0	0.5	23.4	1.7	0.6	0.1	1.8	0.3	0.2	0.1
1.0	0.4	20.6	6.5	0.8	0.3	2.7	1.4	0.3	0.1
1.0	0.3	17.8	4.3	0.6	0.2	2.1	0.6	0.5	0.1
0.8	0.1	15.6	4.6	0.8	0.2	2.9	1.5	0.7	0.1
0.5	0.1	14.3	5.2	0.9	0.5	3.4	0.9	1.8	0.3

Table 28. Original data of Figure 5a and 5b (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each 2-methylbutyl ester during ripening and senescence. Each value is the average of five replications.

Date	2MButyl Acet	STD	2MButyl Prop	STD	2MButyl But	STD	2MButyl Hex	STD	2MButanol	STD	Total	STD
0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
16	58499	31890	0	0	0	0	0	0	0	0	58499	31890
19	1254389	1511859	0	0	0	0	0	0	0	0	1254389	1511859
21	462426	300946	0	0	0	0	0	0	0	0	462426	300946
25	1767945	965201	42570	24661	0	0	0	0	0	0	1810515	984542
29	37286455	39491682	1158803	1646371	1284570	1346404	21042	42084	0	0	39750870	42483217
33	117813571	41546774	2033500	794939	2353173	783887	141842	65390	1274840	368229	122342085	43069732
37	137695013	40390054	2576796	716256	2355238	1278181	159236	85051	1910647	852651	142786282	41673233
41	132275573	19558097	3853517	888264	3542875	1110539	270027	90106	1697844	230755	139941993	21383115
44	134965494	32693994	2858585	797032	2469688	606945	162350	36077	1294249	405080	140456116	33714045
47	151625723	51431223	5840342	1676660	4588010	1368395	305646	56694	1427208	458902	162359721	54307719
49	136999822	54401137	6762870	2534115	2835945	1112742	303353	77343	1281669	493533	146901990	56906992
55	88103319	19442297	4287982	1044700	2014705	646160	189345	121552	1112980	221007	94595351	20538000
63	29654771	10311451	1470564	512391	541888	208712	66683	21621	665438	302615	31733906	10595750
71	23401251	13929582	436195	266185	38378	76755	18233	14990	588482	265428	23894057	14221772

Table 29. Original data of Figure 5c (chapter 4). Fractions, corrected GC/MS response (total ion counts) of each 2-methylbutyl ester during ripening and senescence. Each value is the average of five replications.

Date	2MButylAcet	STD	2MButylProp	STD	2MButylBut	STD	2MButylHex	STD	2MButanol	STD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	97.5	0.9	2.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0
29	94.5	2.0	2.4	0.9	3.1	2.1	0.0	0.0	0.0	0.0
33	96.2	0.5	1.7	0.2	2.0	0.3	0.1	0.1	1.1	0.2
37	96.4	1.0	1.8	0.3	1.7	0.7	0.1	0.0	1.3	0.4
41	94.6	0.9	2.7	0.4	2.5	0.5	0.2	0.1	1.2	0.1
44	96.0	0.7	2.0	0.3	1.8	0.4	0.1	0.0	0.9	0.2
47	93.2	0.9	3.7	0.4	2.9	0.5	0.2	0.0	0.9	0.1
49	92.9	1.8	4.7	0.8	2.2	1.1	0.3	0.1	0.9	0.1
55	93.1	1.2	4.6	0.8	2.1	0.5	0.2	0.1	1.3	0.4
63	93.0	2.0	4.8	1.3	1.9	0.8	0.2	0.1	2.0	0.3
71	98.0	0.5	1.8	0.4	0.1	0.2	0.1	0.1	2.7	0.5

Table 30. Original data of Figure 6a and 6b (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each 2-methylpropyl ester during ripening and senescence. Each value is the average of five replications.

Date	2MPAcet	STD	2MPHex	STD
0	0	0	0	0
6	0	0	0	0
9	0	0	0	0
12	0	0	0	0
16	0	0	0	0
19	0	0	0	0
21	0	0	0	0
25	0	0	0	0
29	0	0	97700	122506
33	762528	254806	104915	39221
37	992087	362716	94629	32021
41	1017543	59477	204752	75792
44	932427	174103	194758	119678
47	893603	255908	216025	39820
49	1244184	669805	222811	53001
55	969778	366924	142036	56531
63	550712	304741	40128	13058
71	421810	156716	15438	12929

Table 31. Original data of Figure 6c (chapter 4). Fractions, corrected GC/MS response (total ion counts) of each 2-methylpropyl ester during ripening and senescence. Each value is the average of five replications.

Date	2MPAcet	STD	2MPHex	STD
0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0
29	0.0	0.0	100.0	0.0
33	87.6	2.8	12.4	2.8
37	90.5	3.1	9.5	3.1
41	83.5	5.4	16.5	5.4
44	82.4	10.7	17.6	10.7
47	79.7	4.3	20.3	4.3
49	81.0	9.7	19.0	9.7
55	87.0	4.4	13.0	4.4
63	91.9	3.4	8.1	3.4
71	96.8	2.8	3.2	2.8

Table 32. Original data of Figure 7a and 7b (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each pentyl ester during ripening and senescence. Each value is the average of five replications.

Date	PentylAcet	STD	Pentyl2MBut	STD	PentylHex	STD	Total	STD
0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
19	192848	172124	0	0	8024	16049	200872	171569
21	90935	40523	0	0	10134	12625	101070	50437
25	294510	241510	20340	27715	93416	32550	408266	295316
29	3255680	4256479	904956	944413	1020584	1345066	5181220	6510386
33	9701514	4169348	2322501	976100	1575490	828949	13599506	5901330
37	13269900	3105414	3177797	1090933	1050047	431866	17497744	4546287
41	12221866	2446700	4059718	1322052	1556691	608011	17838275	4092385
44	14477114	4908086	2097059	618619	1017022	407698	17591195	5828823
47	19247366	6165300	3793095	1319126	1412054	321262	24452514	7600287
49	21039946	8861422	2890612	759639	1541751	400994	25472309	8471139
55	14306434	6245273	1708131	596900	976807	494428	16991372	7087416
63	9864723	4067426	501501	166228	363505	72529	10729729	4091248
71	8758086	6730896	180549	136544	135212	59666	9073846	6820829

Table 33. Original data of Figure 7c (chapter 4). Fractions, corrected GC/MS response (total ion counts) of each pentyl ester during ripening and senescence. Each value is the average of five replications.

Date	PentylAcet	STD	Pentyl2MBut	STD	PentylHex	STD
0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0
19	96.1	7.8	0.0	0.0	3.9	7.8
21	92.8	8.9	0.0	0.0	7.2	8.9
25	69.4	6.8	3.4	4.3	27.2	6.4
29	63.0	11.5	18.9	5.2	18.1	9.8
33	71.8	2.1	17.3	1.7	10.9	3.0
37	76.7	3.8	17.6	2.5	5.7	1.4
41	69.2	5.2	22.4	3.6	8.4	1.9
44	82.2	1.6	12.1	2.0	5.7	0.8
47	78.3	3.3	15.7	2.3	6.0	1.1
49	80.1	8.7	12.8	5.3	7.1	3.5
55	83.7	3.4	10.7	3.0	5.6	1.0
63	91.1	2.7	4.9	1.5	4.0	1.6
71	95.9	1.6	2.3	1.2	1.8	0.6

Table 34. Original data of Figure 8 (chapter 4). Absolute, corrected GC/MS response (total ion counts) of each acid during ripening and senescence. Each value is the average of five replications.

Date	Acetic Acid	Propanoic Acid	Butiric Acid	2MBut.acid	HexAcid	TOTAL
0	457664	0	0	0	0	457664
6	87778	0	0	0	0	87778
9	40183	0	0	0	0	40183
12	61773	0	0	0	0	61773
16	128231	0	0	0	0	128231
19	255713	0	0	0	0	255713
21	155089	0	0	5951	0	161039
25	157147	0	11411	19061	0	187619
29	159182	0	14723	75312	0	249217
33	181115	0	19164	139916	0	340195
37	237785	0	41776	174175	0	453735
41	201049	0	15521	191335	0	407906
44	45634	0	7072	135962	0	188668
47	31345	43943	8340	153673	0	237302
49	248964	60582	21828	66349	1130795	1528517
55	332634	134736	39654	27633	439078	973735
63	333463	97184	14553	13965	206422	665588
71	370883	54124	11094	12850	73361	522312