AN ABSTRACT OF THE THESIS OF

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Volatile compounds of eight pear varieties were isolated and identified by GC/MS. A total of 112 components were identified from headspace of intact fruits, including 47 compounds reported for the first time in pear. The volatile profiles of these pear varieties were characterized by esters, alcohols, hydrocarbons, aldehydes and ketones. Qualitative and quantitative differences of compounds in the profiles are discussed in terms of flavor differences between pear varieties.

Analysis of volatiles isolated from eating-ripe pears by direct sniffing GC indicated that a group of nine esters including 2-methylpropyl-, butyl-, pentyl- and hexyl acetates, butyl butanoate, ethyl hexanoate, methyl trans-2, cis-4-, ethyl trans-2, cis-4- and ethyl cis-2, cis-4- decadienoates from 'Bartlett', 'Comice' and 'Anjou' pear possessed strong, specific pear-like aroma. A second group of esters was also found to possess very positive aroma and can significantly affect overall sensation by serving as 'contributory to flavor compounds'.

A newly developed, solid-phase microextraction technique was used to follow changes of volatile production of 'Bartlett', 'Packham's Triumph' and 'Anjou' pears during ripening. The major esters were found to increase at different rates and times during fruit ripening. Highly significant correlations between specific groups of odor-

active compounds and pear flavor intensity during ripening indicated the significant contribution of volatiles to overall pear flavor.

The effects of harvest maturity on volatiles differed between fruit varieties. 'Bartlett' harvested at all maturities, upon ripening produced similar amounts of flavor constituents. Higher volatile production was observed in early harvested 'Packham's Triumph'. Delayed harvest significantly reduced volatile production in 'Anjou'. Prolonged storage of fruits resulted in severe reduction of volatile esters in 'Packham's Triumph' and 'Anjou'.

Alcohol acyltransferase, the ester synthesizing enzyme was isolated from 'Bartlett', partially purified, and its properties characterized. The enzyme was estimated to have a molecular weight of 40 kD and was most active at pH 7.5 and 30°C. The enzyme has K_m = 0.6 mM and V_{max} = 0.3 nmol/min for hexanol and K_m = 53 μM and V_{max} = 0.2 nmol/min for acetyl-CoA. Maximum activity of the enzyme was obtained when hexanol and acetyl-CoA were used as substrates.

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APPROVED:

Major professor, representing Horticulture

Chair of Department of Horticulture

Dean of Graduate School

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

Anawat Suwanagul, Author

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This thesis is dedicated to my parents, Dr. Kasem and Pitsamai Suwanagul

RIPENING PEAR FLAVOR VOLATILES: IDENTIFICATION, BIOSYNTHESIS AND SENSORY PERCEPTION

CHAPTER 1

INTRODUCTION

Pears (*Pyrus communis* L.) are one of the most important tree fruit crops grown in the Pacific Northwest region of the U.S. North American pear production is estimated at 824,000 tons with 91% produced by the U.S. California, Washington and Oregon constitute 97% of the U.S. production. Four cultivars 'Bartlett', 'Anjou', 'Bosc' and 'Comice' make up 98% of the production (Wrolstad et al., 1990). In Oregon, pears are the major fruit crop, with wholesale value of $ 155 million in 1993 and a 34 % increase since 1985. Formerly, a large portion of summer pear production was used for processing and only a limited portion was used for fresh market. Recently, the increase in the demand for fresh fruits has shifted the industry to produce fruits for fresh market (Crabtree et al., 1994). Marketing of fresh fruit not only requires fruits with long storage-life but also requires fruits with superior quality.

Appearance is probably the first factor considered in term of quality for consumer purchase, but flavor and texture are usually the most important components when fruit quality is assessed. Good eating pear quality is described by fine melting or buttery texture, juiciness and mild flavor after ripening. Significant changes in pear flavor are usually observed between unripened and ripened fruit. Increased sweetness and flavor complexity and decreased sourness are noticed by consumers. However, there are only slight changes in total soluble solids, titratable acidity, and pH during fruit ripening. Studies of canned pears indicated a close

correlation between aroma and flavor suggesting the importance of volatile aromatics to pear flavor (Quamme and Marriage, 1977).

A wide range of volatile compounds are generated from fruit tissues. Some of which are extremely important components of quality. Consumers exhibit distinct preference patterns in foods that they select to purchase and flavor (aroma) is known to be a primary criterion in this selection. Fruit flavors are comprised of a combination of both taste and aroma. Since taste is generally thought to be limited to only four basic sensations (sweetness, sourness, saltiness and astringency), volatile compounds play a significant role in determining the complexity of flavor. The four basic tastes of the tongue can be contrasted with the potential perception of up to 10,000 distinct odors by human olfactory epithelium (Kendall and Nieson, 1964). Furthermore, human sense of smell is extremely sensitive to low concentrations of substances and the ability to discriminate among them. Therefore, volatile compounds are not only extremely important contributors of taste but they also provide flavor with almost unlimited potential diversity.

Various factors can affect volatile development in fresh fruit. Different varieties or cultivars of fruits have different genetic makeup which results in a different volatile profile that makes them distinct from each other. Preharvest factors such as climate or cultural practices also affect volatile formation during fruit ripening. Several postharvest factors such as harvest maturity, storage temperature, controlled and modified atmosphere storage, ripening stage and postharvest treatments not only have direct effects on storage life of fruits but also have significant effects on volatile flavor compound production. The effects of these factors on volatile development have been extensively studied in various fruits such as apple, banana, peach,

strawberry and tomato (Berger et al., 1986; Drawert and Berger, 1988; Berger et al., 1992;
Albrecht et al., 1992; Perez et al., 1993; Ke et al., 1994). However, only limited information on volatiles has been reported in pears.

Early studies on volatile flavor compounds in pears have been done during 1961 to 1974 by Jennings and co-workers at the University of California, Davis (Jennings, 1961; Jennings and Creveling, 1963; Jennings and Sevenants, 1964; Jennings et al., 1964; Heinz et al., 1966; Creveling and Jennings, 1970; Jennings and Tressl, 1974). With the current techniques and resources, a number of volatile compounds have been isolated and identified, almost entirely from 'Bartlett' pear. Ethyl trans-2, cis-4 decadienoate has been designated as a 'character impact compound' of 'Bartlett' pear and hexyl acetate has been proposed to be a 'contributory flavor compound'. Moreover, several pathways leading to the biosynthesis of those identified compounds have been proposed. However, little further research on pear volatiles has been performed.

The rapid advances in sample preparation and instrumentation technologies has significantly developed flavor research in the past ten to fifteen years. Several new sample extraction techniques provide simple, fast, sensitive and inexpensive means to isolate small amounts of volatile samples without excessive losses, modifications or contaminations. Newly developed high resolution capillary columns significantly improve separation efficiency of complex mixtures of compounds. Direct interfaces between gas chromatographs and mass spectrometers allow rapid and precise identification and quantification of separated compounds. Furthermore, improvements in gas chromatography - olfactometry techniques also provide an opportunity to link human sensitivity with the complex aroma quality of fruits. By

combining the above techniques in a series of experiments, valuable information can be obtained and hopefully used to update and to add to the limited information from the past in order to improve pear flavor quality.

The **main objective** of this study is to provide a basic understanding of volatile compounds responsible for pear flavors and to evaluate internal and external factors which are involved in the development of volatile flavor compounds during storage and ripening of pear varieties.

Specific objectives are as follows:

(1) To quantitatively identify volatile compound profiles of eight pear varieties at eating-ripe stage by using GC-MS.

(2) To identify odor-active compounds from three pear varieties at eating-ripe stage by using direct GC-sniffing technique.

(3) To characterize the changing relationship between volatile compounds and sensory attributes of three pear varieties during ripening.

(4) To determine the effects of harvest maturity and storage durations on volatile flavor compound production in three pear varieties.

(5) To characterize kinetic properties and substrate specificity of alcohol acyltransferase, the enzyme responsible for biosynthesis of the most important volatile flavor compounds in pears.

CHAPTER 2

LITERATURE REVIEW

Flavor

Flavor is a complex sensation perceived when food or beverage is placed in the mouth. The British Standard Institution (1975) previously defined flavor as " the combination of taste and odour". However, flavor may be influenced by sensations of pain, heat and cold, and by tactile sensations. Therefore, the flavor research community has generally defined flavor as "the sum of those characteristics of any material taken in the mouth, perceived principally by senses of taste and smell and also by the general pain and tactile receptors in the mouth, as received and interpreted by the brain" (Hall, 1968) and, more recently, " the combined impression perceived via the chemical senses from a product in the mouth" (Meilgaard et al., 1991).

Besides heat, cold, and irritation sensations which can exist in some foods such as chili peppers or other seasonings, taste sensation and aroma sensation are considered to be the most two important components of flavor. Taste sensation is detected by a group of receptors familiarly known as taste buds which are located on the surface of the tongue. Perhaps as an oversimplification, the sense of taste is commonly categorized as only 4 basic sensations which include; sweetness, sourness, saltiness and astringency. Both non-volatile and volatile compounds in foods are responsible for those sensations.

Unlike taste sensation, aroma or olfaction is detected by receptors called olfactory epithelium which are located on the inner surface of the upper nasal cavity. Since the receptors are not in direct contact with the food itself, organic compounds

in gaseous or vapour state either breathed into the nose or volatilized from the mouth to the nose are responsible for aroma. One striking feature of aroma as compared to taste is the extreme sensitivity to low concentrations of volatile chemical substances and the ability to discriminate among them.

According to Thomson (1986), the sense of smell can detect certain volatile compounds at concentrations as low as 10^{-18} molar. This sensitivity is remarkable even when compared with the performance of sophisticated physical instruments. Kendall and Nielson (1964) found that odor thresholds of humans for some substances were markedly lower than the minimum concentrations detectable by gas chromatography equipped with a flame ionization detector (FID). Furthermore, the ability of human olfactory epithelium to discriminate among many odors is estimated to be over 10,000 distinct odors.

Fruit Aromas

Most fresh fruit aromas are characterized by a specific set of natural volatile compounds that strongly influence buyer appeal and consumer acceptability. These volatile organic compounds are produced under genetic control by individual species and can be influenced by various environmental factors. Typically, fruits produce a wide range of compounds with diverse chemical structures. These include esters, alcohols, aldehydes, ketones, lactones, acids, acetals, phenols, hydrocarbons and heterocyclic compounds. The very first reviews on volatile flavor profiles of various fruits was done by Nursten and Williams (1967) and Nursten (1970). From both reviews, a number of compounds identified from several fruits such as apple, banana, orange and strawberry were tabulated. Since then, the number of identified compounds in many kinds of fruits has increased steadily with improved instrumental

analysis such as gas chromatography with capillary column separation and the combined use of the gas chromatograph with mass spectrometry (GC-MS), as well as improved sample preparation techniques. Recently, a more complete list of volatile compounds isolated and identified in various fruits has been reviewed by Morton and MacLeod (1990).

Volatiles in fruit are generally present in very small amounts, often only in fractions of parts per million. In addition, among the large number of volatiles generated by fruit, typically only a small fraction of those compounds are aroma-active or odor-active and even fewer are aroma impactors.

Gas chromatography-olfactometry (GC-O) is a human bioassay used to determine odor-active compounds in various food products. The technique involves using high-resolution capillary GC to separate odor-active chemicals from the complex mixture of food extracts. An olfactometer combines the effluent compounds from the GC column (in measured concentration) with purified and humidified air and delivers them to a human 'sniffer'. The sniffer smells the air through the nose and records any perceptions both with respect to intensity and, if possible, odor descriptors (Acree, 1993).

Several combinations of gas chromatography and olfactometry (GC-O) techniques have been employed for odor-active compound analysis. The early simplest method involved sniffing individual compounds eluting from the GC column and evaluated odor quality and intensity (Nelson and Acree, 1978; Noble, 1978; Agustyn and Rapp, 1982). Acree et al. (1984) developed a human bioassay called CharmAnalysis for determining odor activity of GC effluent compounds. The name "Charm" has been used which follows its common meaning, " a feature in something or someone that attracts or delights people". The technique measures the dilution

value over the entire time the compounds elute. A similar method called Aroma extraction dilution analysis (AEDA) was developed by Schieberle and Grosch (1984). This method was also based on serial dilution of sample before the GC-O. The most recent method called Osme was developed by McDaniel et al. (1990). Osme is derived from a Greek word meaning, "smell". Unlike CharmAnalysis and AEDA method, the Osme method is not based on odor detection threshold values. The Osme method has been reported to be more sensitive and reproducible to detect the change in odor intensity when physical stimuli change (Piggott, 1990; da Silva et al., 1991). The above technique has been frequently used to analyze odor-active compound in various kinds of foods and beverages including fresh produce such as apple (Cunningham et al., 1986), cucumber and muskmelon (Schieberle et al., 1990), naranjilla fruit (Brunke et al., 1989), bell pepper (Luning et al., 1994; Van Ruth and Roozen, 1994), strawberry (Ulrich et al., 1995) and apple, banana and strawberry (Perez et al., 1993b).

With regard to a fairly large number of aroma compounds in fruits, the question arises as to what is the significance of these compounds. To answer this question, Nursten (1977) classified fruits into four categories on the basis of their flavor volatiles: a) fruits whose aroma is largely given by one compound, i.e. a single character impact compound. b) fruits in which a small number of compounds are involved, c) fruits where a large number of compounds are required to reproduce aroma, and d) fruits whose aroma cannot be reproduced even by a large number of compounds.

Flavor Character of Fruits

Examples of fruits whose aroma is largely given by a single compound or a group of compounds with similar structure such as methyl anthranilate in grapes (Salunkhe and Do, 1976), ethyl ester of trans-2, cis-4 decadienoic acid in 'Bartlett' pear (Heinz and Jennings, 1966) and ethyl 2-methylbutyrate in 'Golden delicious' apple (Flath et al., 1967). In other fruits, aroma character relates to a small group of compounds such as trans-2-hexenal, ethyl 2-methylbutyrate and ethyl 3-methylbutyrate in bilberry (von Sydow et al., 1970) and linalool, trans-hex-2-enal and trans-hex-2-en-1-ol in blueberry (Parliment and Scarpellino, 1977). In others, such as peach, pineapple, apricot and mango, large numbers of components which have been isolated are obviously more important from the point of view of imparting flavor notes, but no claims have been made for any individual or a specific set of compounds which give a recognizable aroma. Those compounds can only be considered as 'contributory to flavor compounds' not as 'character impact components'. However, it is less likely that the character compound(s) do not exist, but rather that the techniques being used for their attempted isolation , separation and determination are inadequate (Williams, 1978). The character impact compounds identified in some fruits are summarized in Table 2.1.

Primary and Secondary Aroma

Drawert et al. (1976) described the flavor formation of fruits into 2 distinct groups, primary and secondary aroma components which are biosynthesized by the whole fruit and the secondary aroma compounds which are enzymatically formed after disruption of the tissue during processing or consumption.

Table 2.1. Character impact compounds responsible for characteristic aroma of selected fruits.

Fruits	*Compounds*	*References*
Apple	ethyl 2-methylbutanoate, hexanal, 2-hexenal	Guadagni (1966), Flath et al. (1967)
	hexyl 2-methylbutanoate	Jacob et al. (1969)
	(Z,Z)-octa-3,5-dien-1-ol and (E,Z)-octa-3,5-dien-1-ol acetate	Iwamoto et al. (1983)
	4-methoxyallylbenzene	Williams et al. (1977)
Banana	amyl esters, isoamyl acetate	McCarthy et al. (1963)
	3-methybutyl acetate, butanoate and 3-methylbutanoate	Berger et al. (1986)
Blueberries	trans-2-hexenal, trans-2-hexenol, linalool	Parliment and Scarpellino (1977)
Cherry	benzaldehyde	Schmid and Grosch (1986)
Grape	methyl anthranilate, ethyl acetate	Salunkhe and Do (1976)
Grapefruit	limonene, terpenes, oxygenated terpenes	Berry et al. (1967)
	nootkatones	Shaw and Wilson (1980)
Lemon	limonene, citral	Salunkhe and Do (1976)
Lime	limonene, citral	Salunkhe and Do (1976)
Papaya	methyl benzoate	MacLeod and Pieris (1983)
Peach	lactones	Do et al. (1969)
Pear	hexyl acetate, decadienoate esters	Jennings and Sevenants (1964), Heinz and Jennings (1966)
Orange	sesquiterpene hydrocarbons, limonene	Salunkhe and Do (1976)
Quince	ethyl 2-methylbut-2-enoate (ethyl tiglate)	Schreyen et al. (1979)
Raspberries	4-(4-hydroxyphenyl)butan-2-one, α- and β-ionones	Winter and Enggist (1977)

The primary aroma or typical aroma of fruits such as banana, peach and pear is generally not present during fruit development but develops entirely during the relatively brief period of fruit ripening (Paillard, 1981). During the brief period of fruit ripening, a minute quantity of lipids, proteins, amino acids and carbohydrates are converted to numerous volatile flavor compounds by endogenous enzyme systems. The primary products of these enzymatic pathways are fatty acids, amino acids, ketones, aldehydes and alcohols. These primary products can be further subjected to oxidation, reduction, decarboxylation, deamination and esterification by specific enzyme systems and converted to various aroma compounds (Drawert, 1974).

Unlike primary aroma components, secondary aroma components are formed during the process of cell disruption. Cellular disruption allows the mixing of enzymes and non-volatile substrates which were separated or compartmentalized within the cell, thereby resulting in the formation of volatile flavor substances (Schreier, 1986). Secondary aroma production generally predominates in various kinds of vegetables such as in *Allium sp.*, Brassicas, cucumbers, tomatoes etc. The fundamental reactions leading to volatiles from non-volatile precursors during tissue disruption have been summarized by Tressl et al. (1975). Cysteine sulfoxide derivatives, thioglucosinolates and unsaturated fatty acids are the major non-volatile precursors widely used in these systems.

Biosynthesis of Volatile Compounds in Fruits

An overall perspective of flavor formation during fruit ripening is shown in Figure 2.1. Basically, flavors are derived from 3 major plant constituents (e.g. carbohydrates, lipids and proteins) under genetic control. Each metabolic pathway is tightly connected to other metabolic pathways. Primary aroma compounds of fruit can

be either generated from direct products of one metabolic pathway or as a result of interaction between pathways.

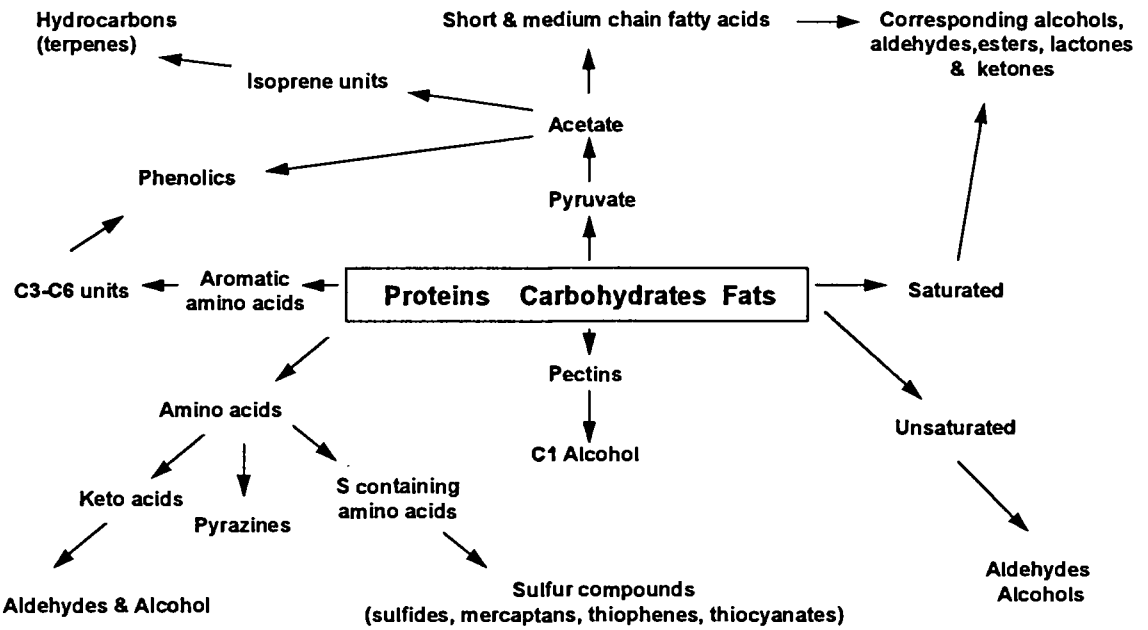

Figure 2.1. Formation of volatile flavor compounds in fruits. Derived from Salunkhe and Do (1976).

Aromas from amino acid metabolism

A summary of aroma formation from proteins through amino acid metabolism is shown in Figure 2.2. Many aroma compounds such as aliphatic and branched chain alcohols, acids, carbonyls and esters are derived from amino acid metabolism.

Studies on ripening banana indicated that L-leucine and L-valine levels of fruit tissue increased three-fold following the climacteric rise of respiration. The level of L-isoleucine, which is only a precursor of minor constituents, remained constant (Tressl and Drawert, 1973). Labeling experiments using postclimacteric banana tissue slices and labeled amino acids showed that L-leucine and L-valine were converted to branched chain compounds such as 3-methyl-1-butanol, 3-methylbutyl

esters, 3-methylbutyrates and 2-ketoisocaproate, which are essential to banana flavor (Myers et al., 1970; Tressl and Drawert, 1973; Tressl and Albrecht, 1986). Yu et al. (1967) also demonstrated that valine, leucine, alanine and aspartic acid could be converted to short chain carbonyls by tomato extracts.

Aromatic amino acids such as tyrosine and phenylalanine may also serve as important precursors to some aromatic flavor compounds which have 'phenolic' or 'spicy' odor characteristics. Cinnamic acid is suggested to be an important

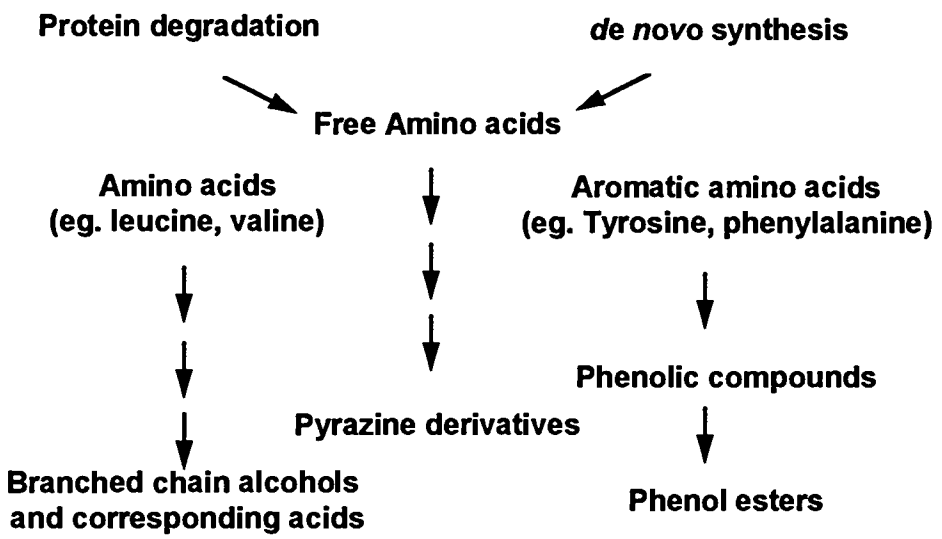

Figure 2.2. Formation of aroma compounds from amino acid metabolism.

intermediate in the pathway (Schreier, 1984). Further esterification of cinnamic acid with methyl or ethyl alcohol to methyl or ethyl cinnamate has been reported in strawberry (Drawert et al., 1973), guava (Torline and Ballschmieter, 1973), cranberry (Anjou and von Sydow, 1967) and passion fruit (Winter and Kloti, 1972). Removal of the acetate moiety from cinnamic acid also yields benzoic acid which can consequently esterify to benzyl esters or be reduced to various benzylaldehydes and benzyl alcohols (Zenk, 1966).

The heterocyclic hydrocarbon compounds, including pyrazines and derivatives, previously known to be formed by heating of many foods such as roasting of nuts or coffee, are believed to be synthesized from condensation mechanisms of α-amino acids, such as valine, isoleucine and leucine (Murray et al., 1975; Nursten and Sheen, 1974). Pyrazine derivatives, especially 3-alkyl-2-methoxypyrazine, have been found in various vegetables such as asparagus, beet root, sweet and red pepper etc. The hypothesis concerning biosynthesis of these pyrazine derivatives was recently reviewed by Leete and Bjorklund (1992).

Aromas from fatty acid metabolism

Figure 2.3 summarized pathways leading to formation of aroma compounds from fatty acid metabolism. Volatile flavors can be derived from lipid via several different pathways included α-oxidation, β-oxidation, hydroxy acid cleavage (lactones) and oxidation through lipoxygenase enzymes, etc. The primary products of these pathways are aldehydes and ketones which can subsequently undergo oxidation, reduction, or esterification to yield various kinds of acids, alcohols, lactones and esters.

β-oxidation is considered to be the most important pathway of ester formation of some fruits such as pears. The decadienoate esters, which are considered as flavor impact compounds of 'Bartlett' pear, are believed to be derived via β-oxidation of linoleic acid (Heinz and Jennings, 1966). Incubation of unsaturated fatty acids with pear puree resulted in the formation of certain flavor compounds (Russell et al., 1981).

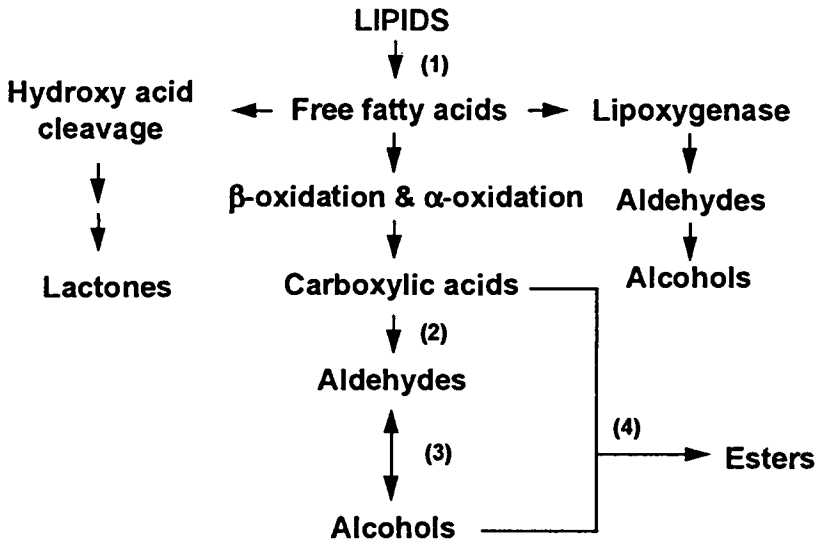

Figure 2.3. Formation of aroma compounds from lipid metabolism. (1) Acyl hydrolase, (2) Pyruvate decarboxylase, (3) Alcohol dehydrogenase and (4) Alcohol acyltransferase.

Jennings and Tressl (1974) proposed a possible pathway leading to the production of mono-, di- and tri-unsaturated esters specific to 'Bartlett' pear (Figure 2.4 and 2.5). Oleic, linoleic and linolenic acid are metabolized, 2 carbons at a time, which results in various chain-length CoA derivatives which then may react with various alcohols to yield esters. During this process, isomerization may occur to yield various cis-trans isomers. It is interesting that the β-oxidation pathway alone can account for all volatile esters which have been identified in pear.

Formation of volatile flavors from fatty acids is also observed in apple. Paillard (1979) demonstrated that straight chain alcohols are formed from aliphatic fatty acids with the same carbon number, or from higher fatty acids after β-oxidation. Even number carbon atom fatty acids give rise to butan-1-ol and hexan-1-ol, and odd carbon atom fatty acids to propan-1-ol and pentan-1-ol. The same pathway was also reported to operate in banana and strawberry (Tressl and Albrecht, 1986).

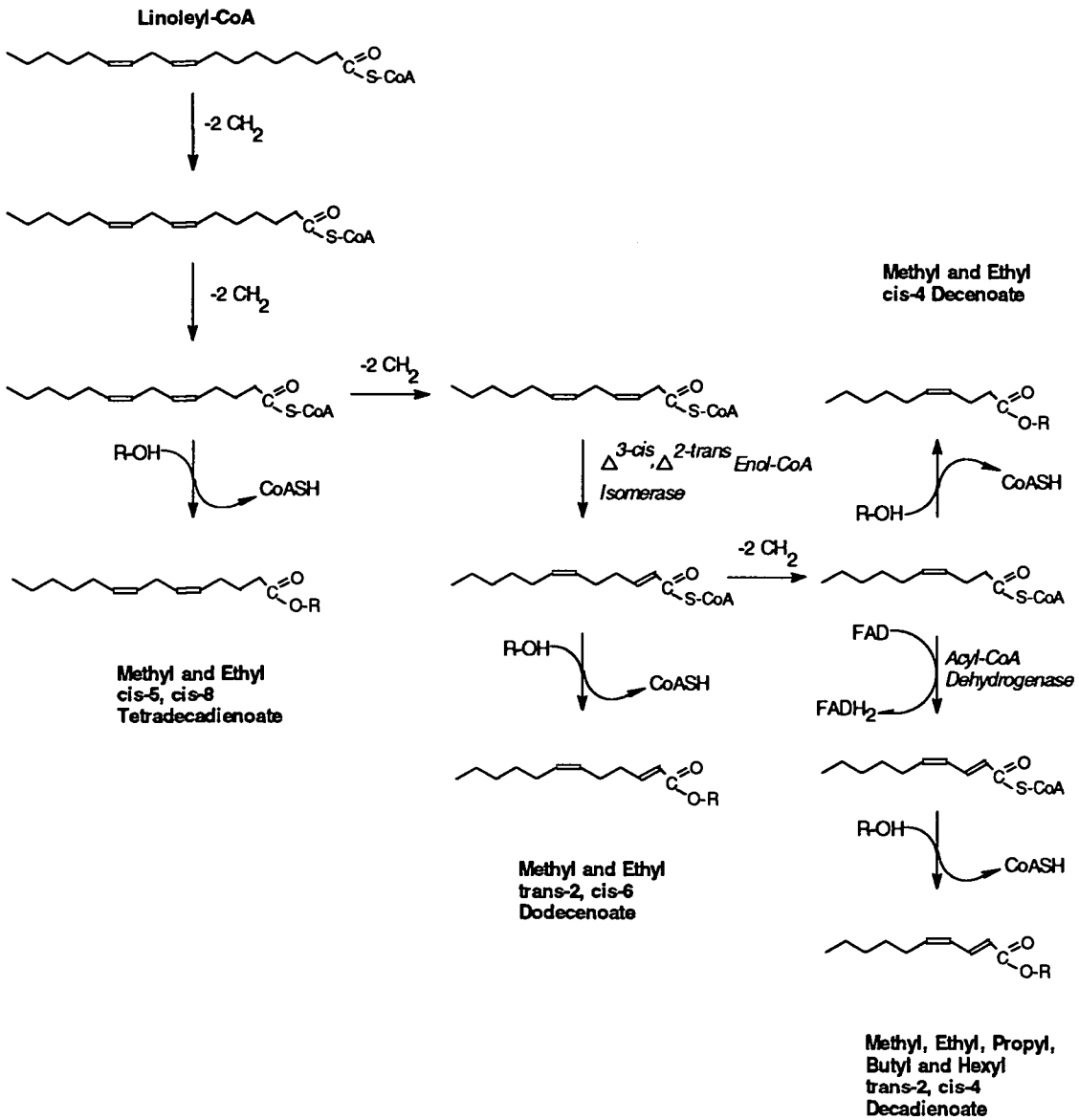

Figure 2.4. Possible pathway leading to the production of mono- and di-unsaturated esters from β-oxidation of unsaturated fatty acids in 'Bartlett' pears (Jennings and Tressl, 1974).

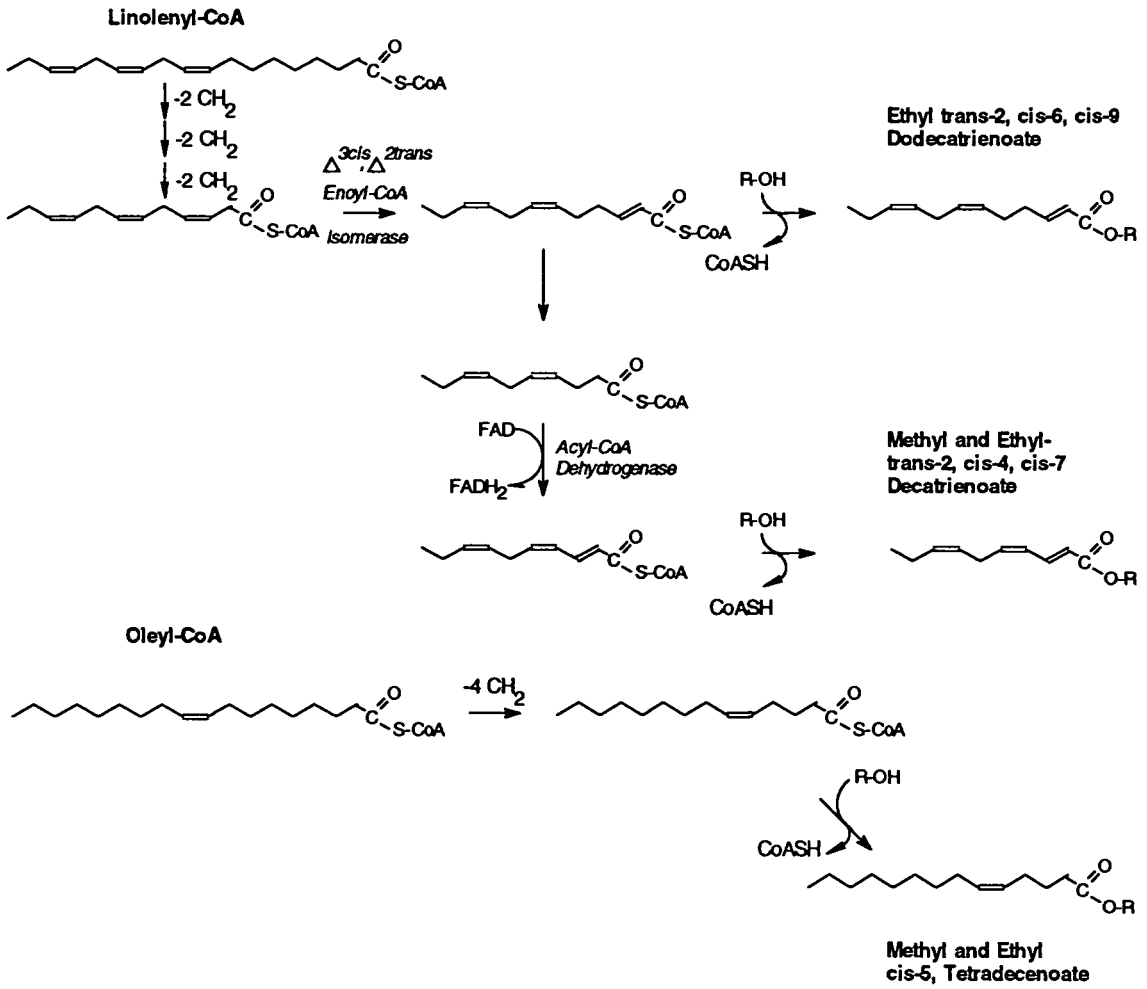

Figure 2.5. Possible pathway leading to the production of mono- and tri-unsaturated esters from β-oxidation of unsaturated fatty acids in 'Bartlett' pears (Jennings and Tressl, 1974).

A large number of volatile flavor compounds may be derived from lipids through lipoxygenase activity. Many of the aliphatic esters, alcohols, acids and carbonyls found in fruits are derived from oxidative degradation of unsaturated fatty acids; mainly linoleic and linolenic acid. Formation of volatiles from oxidative degradation of unsaturated fatty acids have been studied intensively in tomatoes (Kazeniac and Hall, 1970; Jadhav et al., 1972; Stone et al., 1975; Galliard and Matthew, 1977; Schreier and Lorenz, 1981, 1982) and cucumber (Galliard et al., 1977; Sekiya, 1977). The proposed pathway for the formation of some intermediate chain length volatiles from plant lipids through the lipoxygenase pathway is shown in Figure 2.6.

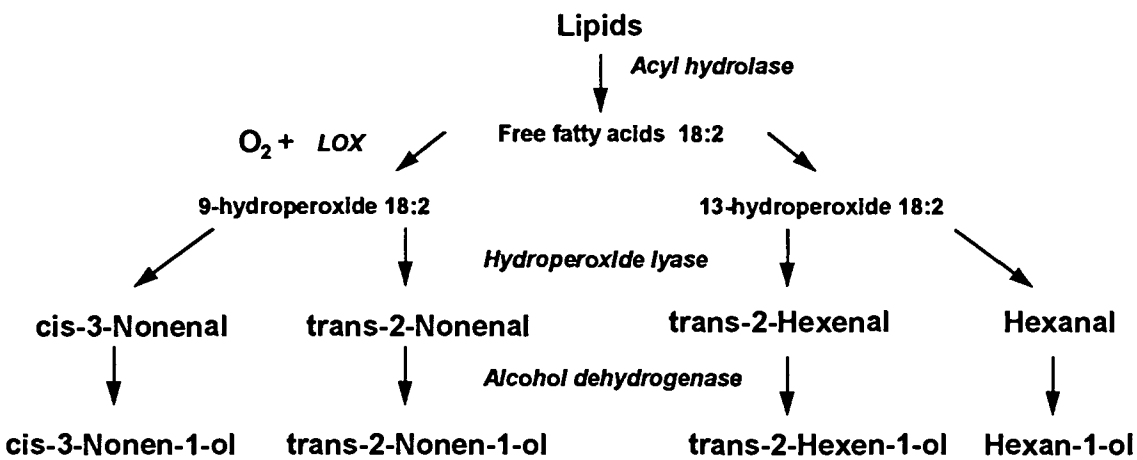

Figure 2.6. Formation of some volatile flavor compounds from linoleic acid through lipoxygenase pathway.

On rupture of plant cells, polyunsaturated fatty acids are released from lipids by the action of acyl hydrolase (lipase) and phospholipases and are further converted by lipoxygenase into 9- and 13- hydroxyperoxides of both C18:2 and C18:3 acids. The fatty acid hydroperoxides were then subjected to cleavage by hydroperoxide

cleaving enzyme. The primary products of the cleavage are C6 and C9 aldehydes with some degree of unsaturation depending on specific origin of fatty acids. This group of aldehydes is reported to be responsible for 'green' or 'grassy' aromas in various fruits or vegetables (Schreier and Lorenz, 1981).

Aldehydes can then be further reduced to alcohols by alcohol dehydrogenase, or isomerized or reduced (double bonds) by isomerase or double bond reducing enzyme to yield other kinds of aldehydes and alcohols. Tomato and cucumber lipoxygenases both favor the formation of 9-hydroperoxide, whilst the hydroperoxide cleaving enzyme was found to act differently. The cleaving enzyme in tomato acts specifically on the 13-hydroperoxide, while in cucumber, it cleaves both 9- and 13-hydroperoxides (Galliard et al, 1976; Galliard et al, 1977).

Beside tomato and cucumber, lipoxygenase has been found to be active in several fruits and vegetables including banana (Tressl and Jennings, 1972; Tressl and Drawert, 1973), apple (Feys et al., 1980; Kim and Grosch,1979), orange (Bruemmer and Roe, 1971) and strawberry (Grost-Allman, 1988).

A further means of formation of flavor compounds from lipids is the formation of lactones. γ- and δ-lactones are known as important flavor components of various fruits such as peaches, apricots, strawberries, mangoes and coconuts. Complete biogenesis of these compounds has not been elucidated. However, many investigators have proposed that an oxidative pathway is involved in the lactone formation (Maga, 1976). Recently, oxidation of linoleic acid by lipoxygenase has been reported to be a major pathway for lactone formation (Albrecht et al., 1992). Tang and Jennings (1968) proposed that lactones could be formed during fatty acid synthesis. The mechanism of the formation involved the double bond shift (from β to

γ position) followed by hydration of γ-δ double bond to yield the corresponding lactone.

Aromas from carbohydrate metabolism

The photosynthetic pathway in plants results in the formation of sugars which are further metabolized into fatty acids and amino acids. Therefore, all plant volatile flavors are indirectly derived from carbohydrate. However, there are some flavor constituents that come directly from carbohydrate metabolism. Terpenes which give characteristic flavors of citrus has been reported to be derived from carbohydrate metabolism. Limonene, a monoterpene hydrocarbon, is a major constituent of most citrus oil. Limonene itself accounts for nearly 90% of citrus oil but possesses very little odor. The oxygenated form of terpene, accounts for not more than 5% of the oil, but generally provides the characteristic of different citrus species (Heath and Reineccius, 1986).

The pathway leading to terpene formation is not fully understood. It has been postulated that terpenes are derived from mevalonic acid through isopentenyl pyrophosphate pathway (Potter and Bruemmer, 1970a). The incubation of ^{14}C labeled mevalonate with orange extract resulted in the formation of linalool, but not limonene (Potter and Bruemmer, 1970c). However, the interconversion between terpene species is proposed to be widespread in all citrus species (Potter and Bruemmer, 1970b).

Factors Affecting the Development of Volatile Compounds in Fruits

Various factors can affect volatile production by fresh produce. Paillard (1981) described factors influencing aroma formation as external and internal factors. The

first factors are associated with pre- and postharvest treatments, the second are involved in the metabolic regulation of the fruits.

External factors

Climatic and cultural practices as well as postharvest handling can significantly affect subsequent aroma production in fruits.

Climatic factors:- Various environmental stresses such as temperature, water availability or nutrient deficiency during plant growth can affect flavor of produce. Simon et al. (1980a,b) reported differences in overall carrot flavor obtained from the same carrot cultivar grown in three different states. The California grown carrot had highest overall flavor with less bitterness and more sweetness than carrots grown in Texas and Florida. The Florida grown carrots had lowest overall flavor and sweetness. However, the authors were unable to determine whether the flavor differences were caused by field temperature, sunlight, water availability or soil nutrition.

Preharvest factors:- Cultural practices, especially fertilization, have influenced volatile formation in various crops. Somogyi et al. (1964) observed an increase in total volatile production in nitrogen fertilized McIntosh apple. Phosphorus and potassium fertilizer applications also showed similar effects and results seemed to be consistent between locations. The effects of plant nutrition on volatile production was far more obvious in vegetable crops. Flavor of onion, garlic, wild onion, cabbage, mustard and watercress have been reported to be strictly dependent on sulphate content in the growing medium. Extreme sulphate deficiency can alter the typical flavor of those crops (Freeman and Mossadeghi 1970, 1971, 1972a,b,c). This is not

a great surprise, since the characteristic flavors of those plants largely derive from S-containing volatiles.

Water in soil during plant growth also influences flavor production. Drought stress generally results in fruits and vegetables with smaller size but more intense flavor. Under limited water availability, plants accumulate more metabolites such as sugars, organic acids and amino acids which serve as precursors for aroma biosynthesis (Freeman, 1979).

Picking date or maturity stage:- Modern postharvest handling tends to harvest many fruit crops just prior to ripening, seeking to obtain maximum storage-life and control of ripening during distribution. However, fruits picked too early can severely affect flavor development patterns. Late picked apples show an immediate increase in total aroma compounds and reach a considerably higher maximum compared to early picked apples. Apples picked too early will not develop their full flavor (Brown et al., 1965; Bachmann, 1983; Dirinck et al., 1989). In peaches, lactones which characterize their flavor, increased as stage of maturity advanced. Fruit harvested too early caused a severe reduction of these compounds (Bayonove, 1974). Artificially ripened immature peaches resulted in only 20% of total lactones content compared to tree-ripened fruits (Do et al., 1969).

Preharvest chemical treatment: Preharvest treatment with an inhibitor of ethylene biosynthesis, aminethoxyvinylglycine (AVG) strongly depressed aroma production of 'Bartlett' pear (Romani et al., 1983) and 'Cox's Orange Pippin' apple (Child et al., 1984). The effect of AVG can be reversed by ethylene treatment. However, the ability of fruit to recover appeared to decline gradually and finally was almost absent after prolonged storage (Bangerth and Streif, 1987).

Postharvest handling or postharvest treatments:-

Storage temperature:

Low temperature storage is currently used to retain postharvest life of most fresh fruits and vegetables. At low temperature, respiration and ethylene production and/or action of fruits were minimized and ripening was delayed. Similar effects were observed on volatile compounds production.

Mattei (1973) and Mattei and Paillard (1973) demonstrated that the production of volatiles in banana is an exponential function of temperature in the range of 5-25°C. Prolonged storage at 10-12°C results in substantially reduced flavor production up to 60%. Higher temperature leads to decreased amounts of volatiles and the formation of fermentation products.

Volatile emissions from apples stored at 4°C has been compared with that from fruit ripening at 15°C. At 4°C, changes in the fruit are greatly retarded, but total volatile emission is still pronounced and reached the peak corresponding to the loss of fruit greenness. Moreover, the chromatograms obtained from both sets of fruits were very similar (Paillard, 1967).

Unlike other fruits, winter pears require a period of time at -1 to 0°C in order to ripen with good quality (Knee, 1987). Fruits stored at this temperature for 60 days and subsequent ripening at 20°C has been reported to result in fruit of maximum flavor (Claypool et al., 1958). The duration of chilling requirement depends upon the variety. Bosc pears required about 15 days, where as 'Comice' and 'Eldorado' needed 45 days and 'Packham's Triumph' and 'Anjou' needed 50 to 60 days (Richardson and Gerasopoulos, 1994; Gerasopoulos, 1988; Hansen, 1966). Low temperature has been reported to encourage the production of unsaturated fatty acids rather than their saturated homologues (Zill and Cheniae, 1962). Important

flavor components of 'Bartlett' pear were postulated to be derived from β-oxidation of unsaturated fatty acids (Jennings and Tressl, 1974).

Accumulation of a volatile compound called α-farnesene by apple and pear fruit during cold storage and its oxidation is postulated to be involved with a physiological disorder, superficial scald (Huelin, 1964; Huelin and Coggiola, 1968). α-Farnesene is subjected to autooxidation (Meigh and Filmer, 1969; Anet, 1972), and the oxidation products may be implicated in superficial scald (Heulin and Coggiola, 1970; Anet, 1974; Anet and Coggiola, 1974; Zoffoli, 1994).

Modified atmosphere (MA) and Controlled atmosphere (CA) storage:

MA and CA-storage technology is commercially used to extend storage life of various fruits and vegetables. Many studies indicate that low O_2 and/or high CO_2 atmosphere generally decrease volatile production during apple ripening (Guadagni et al., 1971a,b; Hatfield and Patterson, 1974; Patterson et al., 1974; Shatat et al., 1978; Paillard, 1981; Streif and Bangerth, 1988; Lidster et al., 1981; Knee and Hatfield, 1981; Willaert et al., 1983; Mattheis et al., 1995). The suppression of volatile formation depends both on the gas composition and storage duration. With increasing CO_2 and decreasing O_2 concentration, aroma volatile production of apple was reduced, especially if both gas treatments were simultaneously applied (Streif and Bangerth, 1988) and partial recovery was observed after returned to air (Yahia et al., 1990). Short-term CA storage (less than 3 months) of apples has almost no effect on volatile biosynthesis. However, long-term storage (320 days) under the same gas conditions results in nearly complete loss of main head space volatiles and there was little formation after subsequent return of fruits to air (Lidster et al., 1983a,

1983b). Dirinck et al. (1984) suggested that short-term CA storage must be used in order to retain fruit quality.

Recently, ultra low oxygen (ULO) storage has been introduced for use on apples in order to obtain maximum shelf-life (Blanpied, 1982). A greater reduction of aroma production was found under this condition. High CO_2 concentration in combination with ULO further suppressed aroma production (Brackmann et al., 1993).

Suppression of aroma production under CA or ULO conditions are not fully understood. Patterson et al. (1974) suggested that the reduction in volatile formation after long-term CA storage of apples may be caused by a loss of substrate or enzyme essential for the formation of esters. According to Knee and Hatfield (1981), the production of esters by 'Cox's Orange' apples in low O_2 atmosphere is limited by the availability of alcohols from which esters are derived. Moreover, the antagonistic effect of high CO_2 atmosphere on aroma production may interfere not only with the enzyme alcohol dehydrogenase but also with carboxylic acid metabolism (DePooter et al., 1987). Recently, Brackmann et al. (1993) indicated that the suppression of aroma production under ULO conditions is related to low fatty acid synthesis and/or degradation and is restricted only to volatiles having straight carbon chains. High CO_2 concentration affects amino acid metabolism resulting in the suppression of volatiles with branched carbon chains. More recently, Ke et al. (1994a, 1994b) proposed modes of O_2 and CO_2 action on ester synthesis in pear and strawberry. According to these two papers, low O_2 and/or high CO_2 directly enhanced activities of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) but decreased the activity of alcohol acyltransferase (AAT). Moreover, CA treatment also caused a decrease in cytoplasmic pH which indirectly enhanced activities of PDC and ADH. The increase

in PDC and ADH activities resulted in the accumulation of ethanol which in turn is used to synthesize ethyl acetate. The lower cytoplasmic pH resulted in severe reduction of AAT activity which in turn resulted in decreased ester synthesis. Hence, fruits stored in CA for long periods will tend to have changes in volatile profiles and in perceived flavor.

High PDC and ADH activities were also observed in apples, pears, berries and plums stored under anaerobic conditions (Kosittrakun, 1989; Richardson and Kosittrakun, 1995). Fruits stored under these conditions developed off-flavor along with high levels of acetaldehyde and ethanol in the tissues. The differences in anaerobic response varied depending on fruit type, variety, temperature and duration of storage. Off-flavor could be partially reversed after subsequent storage in air. However, the level of acetaldehyde and ethanol remained nearly constant and could not account for changes in off-flavors. Similar results were also observed in banana stored under anaerobic conditions but the off-flavor seemed to be closely related to the increase in tissue ethanol (Chantrachit, 1994). Interestingly, banana ester synthesis was blocked under anaerobic conditions, but immediately resumed upon return to air.

Precursor atmosphere (PA) storage:

Supplying intact mature fruits with volatile precursors via the gas phase during short-time storage or so-called PA-(Precursor Atmosphere) can enhance the ester content of various apple cultivars (De Pooter et al., 1981; De Pooter et al., 1983; Bartley et al., 1985; Berger and Drawert, 1984 and Berger et al., 1992), as well as pear, cherry, strawberry (Drawert and Berger, 1988) and banana (Berger et al., 1986a,b). Studies of 'Red Delicious' apple found that ethyl 2-methylbutanoate, which is the flavor impact compound in apples, is increased 20-fold within 48 hr and more

than 50-fold within 8 days in storage atmosphere of 130 mM ethanol (Berger et al., 1992). Hence, PA storage has offered a potential to be used after CA-storage.

Internal factors

Genetic composition determines enzyme systems and their activity in aroma formation. Different fruit species have their own metabolic pathways that make them unique from others. Differences exist even between fruit varieties in the same species. However, it is quite common that varietal differences in fruit aroma are mostly due to quantitative differences in flavor composition rather than qualitative differences. Drawert et al. (1969) reported that the aromatic differences in apple varieties were mostly quantitative in nature and that the aroma composition strongly depended on stage of growth, degree of ripening and storage conditions.

Paillard (1967) reported that the same volatile constituents were present in all nine apple cultivars studied but in variable proportions, and none of those components seem to be a characteristic of a single variety. Similar results were observed in five apple cultivars by Kakiuchi (1986), and Drawert (1978) also reported large proportional differences in C6 aldehydes formed by six apple cultivars.

The yellow varieties of apple have been reported to produce more acetate esters than the red varieties which produced more butanoate esters. Incubation of 'Golden Delicious' apple parenchyma tissue with butyric acid indicated a rapid and almost complete conversion to acetic acid which resulted in the formation of acetate esters rather than butyrate esters (Paillard, 1979). The peels of waxy-coated cultivars converted fatty acid substrates to short chain alcohols and esters while peels of corky-skinned cultivars converted the fatty acids primarily to alcohols and ketones with one less carbon atom.

The ability to produce aroma compounds is also different between different parts of the fruit. The production of volatile esters is much greater in skin tissue than the flesh or the whole fruit (Guadagni et al., 1970a,b).

These differences in metabolism are often responsible for small, yet significant changes in flavor profile and acceptability of the consumers.

Pear Aroma

In the past 40 years, a limited number of studies have been done on pear flavors compared to other fruits such as apples, peaches, strawberries or bananas. Among those articles, papers by Jennings and his co-workers at the University of California, Davis have been the major contributions.

Jennings (1961) isolated and characterized several alcohols and acids from a mixture of the hydrolysis products of 'Bartlett' pear ester by using gas chromatography. The major pear ester acid was reported to be an unsaturated C-10 acid that hydrogenated to give n-capric acid. He also found that the disappearance of ester compounds from the alkaline hydrolysate of pear essence resulted in the disappearance of desirable pear aroma. This indicated that the desirable pear aroma is largely due to volatile esters. The C-10 unsaturated acids that were reported to be a major constituents of pear were later identified as 2,4 decadienoic acid (Jennings and Creveling, 1963).

Jennings and Sevenants (1964) separated 'Bartlett' pear essence by gas chromatography and recovered these essence compounds without losing its desirable aroma. Hexyl acetate and methyl trans-2, cis-4 decadienoate were identified by infrared spectroscopy. The aroma of the former compound was

described as a "contributory flavor compound", and the latter as a "character impact compound" of 'Bartlett' pear.

Later, Jennings et al. (1964) identified ethyl trans-2, cis-4 decadienoate as a flavor component of 'Bartlett' pear. Isolation of trans-2, cis-4 decadienoic acid from a seed oil (*Sapium sebiferum*) provided enough compound to esterify with a series of alcohol moieties. Sensory evaluation of synthesized esters by trained panelists indicated that ethyl-, n-propyl- and n-butyl esters have strong pear-like aroma. The odor of n-amyl and n-hexyl esters appeared to be slightly less desirable, but may have added to the complex response to produce the full, desirable pear flavor.

Heinz et al. (1964) studied the relationship between ultraviolet spectroscopy reading of trapped volatiles and sensory techniques as methods for evaluating the intensity of pear aroma. The absorbance intensities at 263-267 nm was reported to correlate well with pear aroma intensities.

The ester of trans-2, cis-4 decadienoic acid was observed to increase rapidly with the climacteric rise of respiration and reached the maximum stage 1-2 days after the maximum of both the ethylene and CO_2 peak and coincided with optimum eating quality of the fruit. While the production of these esters at one time was proposed to be used as a maturity index. The production of these esters was also severely inhibited by irradiation treatment of fruit which produced flavorless and abnormal ripening (Heinz et al., 1965). However, Romani and Ku (1966) suggested the direct gas sampling method of volatiles as a non-destructive mean to determine pear fruit ripeness stage.

Heinz et al. (1966) compared essence of 'Bartlett' pear prepared by 4 different extraction methods. The major differences between extraction methods was found to be the difference in the relative proportion of high-boiling point compounds to the low-

boiling point compounds. They found that the direct fruit extract and steam-distilled samples tend to result in a lower portion of low boiling-point compounds compared to isopentane extract or charcoal absorption samples. The compounds in the high-boiling point region were similar between all extraction methods. Charcoal absorption followed by Soxhlet extraction of the absorbent seemed to give the best qualitative chromatogram quality. However, the authors failed to give conclusive explanations due to the differences in solvent systems and fruit samples used in the experiments.

A tentative identification of pear essence was done by Heinz and Jennings (1966) and Creveling and Jennings (1970). A long list of volatile compounds from 'Bartlett' pear were reported. The profile was dominated by esters and short-chain alcohols. The assortment of C2 esters, absence of C4 to C6 ester and the abundance of C8 to C18 esters with varying degrees of unsaturation were obvious. This pathway leads to all esters formed in pears.

Jennings and Tressl (1974) studied individual volatile production in ripening 'Bartlett' pear. They observed a cyclic manner among the production of major esters and a newly identified sesquiterpene hydrocarbon compound, α-farnesene. Surprisingly, the production of methyl-, ethyl-, propyl-, butyl-, amyl- and hexyl-acetates exhibited cyclic patterns with maxima and minima out-of-phase with α-farnesene. The decadienoate esters show a similar pattern but the amplitude is very much less than those of acetate esters. The authors suggested that α-farnesene and acetate ester production share the same precursor, acetyl CoA, and there must be some control mechanism alternately regulating the biosynthesis of these groups of compounds. The authors also summarized the possible pathway leading to the production of esters and α-farnesene in 'Bartlett' pear (Figure 2.7).

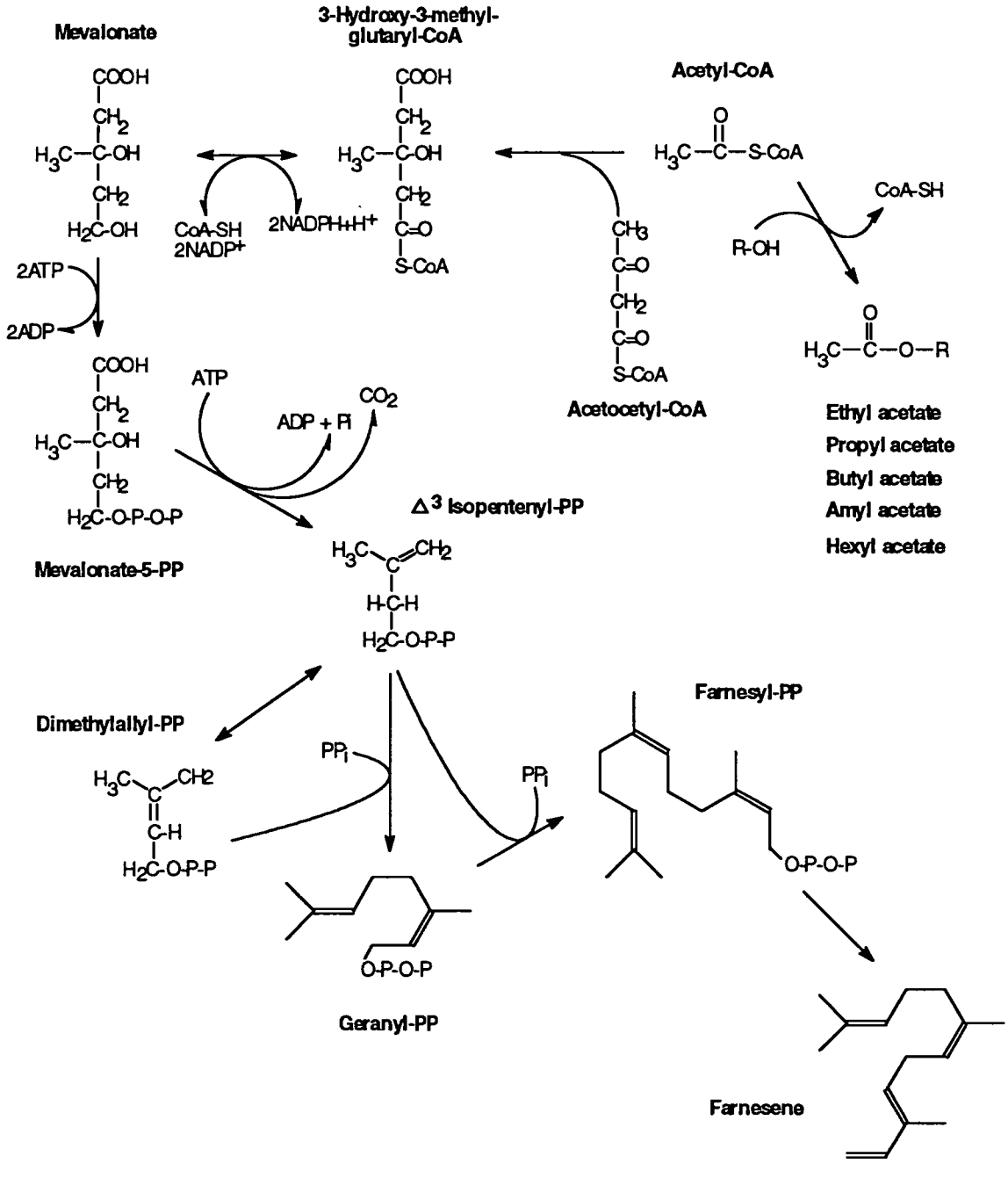

Figure 2.7. Possible pathways leading to the production of esters and α-farnesene in 'Bartlett' pears (Jennings and Tressl, 1974).

Subsequent studies of several pear cultivars by gas chromatography, HPLC, and sensory evaluation have shown that high concentrations of decadienoates are characteristic of the 'Bartlett' pear and the cultivars with 'Bartlett' pear-like flavor (Quamme and Marriage, 1977; Quamme and Spearma, 1983; Quamme, 1984). Aroma profiles from cultivars such as 'Keiffer' and 'Magness' are distinguished from 'Bartlett' by the predominance of compounds in the low boiling point region. Those two pear cultivars have considerably less high boiling point compounds compared to 'Bartlett'. The result from sensory evaluation demonstrated that the 3 cultivars 'Bartlett', 'Magness' and 'Keiffer' can be classified respectively as highly, intermediate and poorly flavored (Russell et al., 1981).

Russell et al. (1981) also demonstrated that incubation of 'Bartlett' pear puree with C18 unsaturated fatty acids resulted in the synthesis of volatile flavors observed by HPLC. A synthesis of 2,4 decadienoate was observed by increased absorbance at 260 nm when a 'Kieffer' pear puree was incubated with 'Bartlett' pear enzymes.

Strandjev (1975) observed a linear increase in volatile production of 'Bartlett' and 'Passe Crassane' pear from unripe, picking, packing and eating-ripe stages. A brief low temperature sterilization (98°C) caused a reduction of low boiling point compounds without loss of aroma quality. The 2,4 decadienoate esters have high boiling points and can be retained in stored pear (Strandjev, 1982).

Shiota (1990) reported a flavor compound profile from 'La France' pear. Aliphatic esters are the principal volatile components which account for more than 70% of the total. Only a small amount of 2,4 decadienoate ester was detected and it appeared to be the trans, trans isomer. Hex-5-enyl acetate is believed to give a 'ketonic estery' characteristic odor of this fruit. The development of volatile compounds during fruit ripening was also observed compared to 'Bartlett' pear. In 'La

France' pear, the concentration of those esters are markedly increased with increasing maturity of fruit and reached the maximum just before the fully ripened stage. Whereas, in 'Bartlett' pear, the aroma components did not linearly increase from an unripened stage to fully ripened stage but reach maximum just before the fully ripe stage.

More recently, Takeoka et al. (1992) has reported the volatile profile from Asian pears (*Pyrus serotina*). A total of 72 compounds were identified including 39 constituents reported for the first time in pear. The studies on odor unit value and threshold data indicated a group of important contributors to pear aroma. Interestingly enough, this group of compounds resembles the flavor impact compounds reported in apples.

Besides volatile compounds from fruits, volatiles from 'Bartlett' and 'Bradford' pear leaves have been investigated by Miller et al. (1989). The hydrocarbon groups were predominant with (E)-β-ocimene, β-caryophyllene and (E,E) α-farnesene present in all samples. α-Copaene was a major component found in 'Bartlett' pear but not in 'Bradford' pear and, linalool was found in considerable amount in 'Bradford' samples but not in 'Bartlett' samples.

Enzymes Involved in Carboxylic Ester Formation in Fruits

Esters are considered to be one of the most important classes of volatiles in fruit aroma (Dirinck et al., 1977; Macku and Jennings, 1987 and Perez et al., 1992). Typical fruit aromas are characterized by those esters such as isoamyl acetate in banana (Berger et al., 1986a), ethyl 2-methylbutanoate in apples (Guadagni et al., 1966 and Flath et al., 1967) and ethyl trans-2, cis-4 decadienoate and hexyl acetate in pears (Jennings et al., 1964).

The pathway leading to esters synthesis in fresh fruits is not completely understood. Incubation of whole fruit or fruit tissues with aldehydes, alcohols or acids resulted in the formation of corresponding esters in various fruits such as apple (Paillard, 1974; Paillard, 1979; Knee and Hatfield, 1981; De Pooter et al., 1981; De Pooter et al., 1983; De Pooter et al., 1987), banana (Tressel and Drawert, 1973; Ueda et al., 1971; Guilliver and Nursten, 1976; Ueda and Ogata, 1978), and strawberry (Yamashita et al., 1975; Yamashita et al., 1976; Yamashita et al., 1977; Yamashita et al., 1979). The esterification of added alcohols and acids was also observed in separated cells of banana, strawberry and melon (Ueda and Ogata, 1976).

Three enzymatic reactions are believed to be involved in esters synthesis in fruits. First, various α-keto acids are converted to corresponding aldehydes by the activity of pyruvate decarboxylase (PDC). Aldehydes were then converted to corresponding alcohols by alcohol dehydrogenase (ADH). The final step is esterification of acids and alcohols to form esters. This step is catalyzed by alcohol acyltransferase (AAT).

Pyruvate decarboxylase and alcohol dehydrogenase are two important enzymes responsible for acetaldehyde and ethanol synthesis during fermentation or anaerobic respiration. Several studies have been done on PDC and ADH due to their important role for fruits stored under controlled atmosphere storage. Their activities were reported to be increased in response to low O_2 and/or high CO_2 stress (Kosittrakun, 1989; Longhurst et al., 1990; Kanellis et al., 1991; Nanos et al., 1992; and Ke et al., 1993). Papers concerning these two enzymes in normal fruit aroma synthesis are very limited. However, these two enzymes play an important role in

ester synthesis by supplying substrates for esterification reactions. The conversion of various α-keto acids to corresponding aldehydes was reported in strawberry. The reaction is assumed to be catalyzed by an enzyme similar to PDC, since the addition of thiamine pyrophosphate and/or Mg^{2+} enhanced the formation of aldehydes. (Yamashita et al., 1978b). Yamashita et al. (1976) reported two forms of ADH activities, NAD-dependent and NADP-dependent which were present in strawberry. Those two ADHs have been isolated, purified and characterized from strawberry seeds (Yamashita et al., 1978a).

Alcohol acyltransferase activity first became of interest to researchers by various microorganisms due to its role in esters synthesis during alcoholic beverage fermentation (Nordstrom, 1961; Howard and Anderson, 1976; Lanza et al., 1976; Armstrong et al., 1984; Sarris and Latrasse, 1985; Malcorps et al., 1991; Malcorps and Dufore, 1993; Kallel-Mhiri et al., 1993; Kallel-Mhiri and Miclo, 1993). The enzyme catalyzes the transfer of the acyl moiety from acetyl-Coenzyme A onto various alcohols, and results in ester formation. The AAT from brewer's yeast has been reported to be localized in both the cytosolic fraction and as a membrane bound enzyme. Only the cytosolic AAT exhibited high enzymatic activity. Furthermore, the enzyme appeared to be tightly related to lipid biosynthesis (Ramos-Jeunehomme et al., 1989). The enzyme has been isolated, purified and characterized in many microorganisms including brewer's yeast (Yoshioka and Hashimoto, 1981), Sake yeast (Akita et al., 1990), *Cladosporium cladosporioides* (Yamakawa et al., 1978) and *Neurospora sp.* (Yamauchi et al., 1989).

Less information concerning this enzyme in higher plants has been reported. Ueda and Ogata (1977) reported the occurrence of AAT enzyme in cell-free extracts

from bananas. Harada et al. (1985) isolated, purified, and characterized AAT from cell-free extracts of banana. More recently, the enzyme has been partially purified and characterized in strawberry, melon and banana (Ueda et al., 1992; Pérez et al., 1993 ; Olias et al., 1995).

Esterase, the enzyme which catalyses the reverse reaction of AAT has been found to be implicated in the formation of esters by microorganisms and fruits. Esterase has been isolated, purified and characterized in brewer's yeast (Schermers et al., 1976; Suomalainen, 1981). The enzyme is reported to be located on both sides of the plasma membrane, is relatively labile and has high substrate specificity. Moreover, the enzyme is capable of synthesizing ethyl acetate from ethanol and acetic acid. The activity of esterase was first reported in plants by Schwartz et al. (1964). High carboxylic hydrolase (esterase) activity toward indolphenyl acetate was found among green bean, cabbage, potato tuber, citrus albedo and flavedo and fruits of many cucurbits. However, due to the limited number of esters used in the study and the authors' emphasis on the cucurbitacin esterases, the results may not be relevant to aroma esters in fruits.

Goodenough and Entwist (1982) and Goodenough (1983) isolated, purified and characterized esterase enzyme from *Malus pumila* (apple) fruits. The enzyme has greatest apparent affinity for acetate esters containing 7 or 8 carbon number. The affinity of the enzyme decreased as the carbon number of ester decreased from 8 to 4. The enzyme specificity was also found to increase considerably from immature fruits to large fruit at the climacteric. Goodenough and Riley (1985) observed the esterase activity during fruit maturation of *Malus pumila* compared to two other apple varieties. The specific activity of the enzyme from all varieties increased during the period of fresh weight gain.

Esterase activity has also been implied to interfere with isolation of AAT in banana (Harada et al., 1985) and strawberry (Perez et al., 1993). However, no attempt has been made to verify its actual activity in those fruits.

Recently, the presence of fatty alcohol oxidase which catalyzed the oxidation of fatty alcohols to corresponding fatty aldehydes has been reported in some microorganisms such as *Torulopus candida* (Krauzova et al., 1985, 1986) and *Candida tropicalis* (Kemp et al., 1988). This enzyme has not yet been studied in fruits. However, this enzyme may play a vital role in fruit ester synthesis, since various esters are believed to derived from long chain fatty acids (Paillard, 1979).

Solid-phase Microextraction (SPME)

Solid-phase microextraction (SPME) is a relatively new solvent-free sample extraction technique recently developed by Pawliszyn and co-workers at the University of Waterloo, Canada (Arthur and Pawliszyn, 1990; Arthur et al., 1992a,b; Potter and Pawliszyn, 1992). It basically functions by absorption of organic analytes to the pre-coated fused silica optical fiber followed by thermal desorption of those analytes in the injection port of a gas chromatograph or gas chromatograph-mass spectrometer system with split/splitless or on-column injection (Woolley and Mani, 1992). The SPME technique can be used to concentrate both volatile or non-volatile compounds from either liquid or gaseous (i.e. head-space) samples. Because SPME can attain detection limits of 15 ppt and below for both volatile and non-volatile compounds, the technique was approved by the United States Environmental Protection Agency (EPA) and Ontario Municipal/Industrial Strategy of Abatement (MISA) program to be used for environmental applications (Arthur et al., 1993).

Recently, the hardware and software has been developed to use SPME with the GC autosampler. Hence, the method can now be completely automated (Berg, 1993).

The process of absorption and desorption of analytes by SPME has been mathematically modeled in both liquid (Arthur and Paliszyn, 1990; Louch et al., 1992; Arthur et al., 1993) and head-space samples (Zhang and Pawliszyn, 1993). The results from theoretical modeling are consistent with the experimental data in both types of sampling methods.

The selectivity of analytes can be obtained by altering type and thickness of phase coated fiber. Several fiber coatings have been investigated including poly-dimethylsiloxane (Arthur et al., 1992b; Potter and Pawliszyn, 1992), polyimide (Hawthorne et al., 1992; Arthur et al., 1992a), polyacrylate (Buchholz and Pawliszyn, 1993 and 1994) and Carbowax (Belardi and Pawliszyn, 1989). To date, the most successful phase coated material has been poly-dimethylsiloxane which is available commercially from several optical fiber manufacturers such as Polymicro Technologies (Tucson, AZ) in thicknesses ranging from 15 to 150 µm. Currently, a complete SPME device with various kinds of phase coatings and thicknesses are commercially under patent by Supelco (Bellefonte, PA).

The optimization of the technique for determination of various classes of compounds in different types of samples were reported including: substituted benzene compounds in water (Arthur et al., 1992; Potter and Pawliszyn, 1992), caffeine in beverages (Hawthorne and Miller, 1992), phenolic compounds in ground water (Buchholz and Pawliszyn, 1993), organic compounds in water (Arthur et al., 1992), flavor compounds of ground coffee, fruit juice and vegetable oil (Yang and Peppard, 1994), forensic, pharmaceutical and food samples (Mindrup, 1995) and alcohol and small polar compounds (Shirey et al., 1995).

In comparison with solvent extraction, simultaneous distillation/extraction, conventional solid-phase extraction, purge and trap sampling, which are the most frequently used sample preparation techniques in flavor analysis (Cronin, 1982; Reineccius, 1993), SPME is considered to be a simple, fast, sensitive, inexpensive and versatile method to be used in qualitative and quantitative analysis of selected volatile flavor compounds from food products. Since, there are no solvents involved, the method has a vast advantage over conventional solvent extraction as well as purge and trap sampling in avoiding contaminants from the solvent used or purge air stream.

CHAPTER 3

IDENTIFICATION OF HEADSPACE VOLATILE COMPOUNDS FROM DIFFERENT PEAR (*Pyrus communis* L.) VARIETIES

Anawat Suwanagul and Daryl G. Richardson

Abstract

Eight pear varieties including 'Bartlett', 'Packham's Triumph', 'Anjou', 'Comice', 'Bosc', 'Seckel', 'Vicar of Winkfield' and 'Forelle' were ripened optimally and volatile compounds from dynamic headspace were collected and identified by GC/MS. Among 112 compounds identified from all varieties , 47 compounds are reported the first time in pears. The volatile profile of these eight varieties are characterized by compounds in groups of esters, alcohols, hydrocarbons, aldehydes, and ketones. Esters of short to medium chain alcohols and their corresponding acids with or without some degree of unsaturation are always present as a major group of compounds in all varieties. Esters comprise 60 to 98% of total volatiles among the varieties. Hexyl acetate represents the major ester in all cultivars except 'Bosc' and 'Vicar of Winkfield' which produced butyl acetate as a major ester, and 'Seckel' produced ethyl 2,4 decadienoate as the major ester. Decadienoate esters were detected in all varieties, but varied in amount and isomerization characteristics. Straight-chain alcohols with 2 to 8 carbons are present as the second largest group in the profile. Two isomeric forms (E,E and E,Z) of the sesquiterpene hydrocarbon, α-farnesene, are always present in the profiles but vary considerably in amount. A small number of aldehydes and ketones are also found in some varieties but present in very small amounts. Among the varieties studied, 'Comice' had the highest rate of volatile production at 42 μg/kg/100L of air while 'Seckel' produced the lowest at 0.7 μg/kg/100L.

Introduction

Pears (*Pyrus communis* L.) are one of the most important fruit crops grown in the Pacific Northwest region of the United States. Washington, Oregon and

California contribute about 97% of U.S. production. Four varieties, 'Bartlett', 'Anjou', 'Comice' and 'Bosc' account for 98% of the total production (Wrolstad et al., 1991). In Oregon, pear production is ranked number one in value among all tree fruit and nut crops by farm receipt. The production is shifting from processed products to fresh market with high potential for export (Crabtree et al., 1994). Fresh market fruits not only require superior appearance but also superior flavor and texture.

Complete information involving volatile organic compounds associated with pear fruit aroma is very limited compared to many other fruits. Volatiles were isolated and identified in 'Bartlett' pear (Jennings, 1961; Jennings and Creveling, 1963; Jennings and Sevenants, 1964; Jennings et al., 1964; Heinz et al., 1966; Creveling and Jennings, 1970; Jennings and Tressl, 1974), 'Bartlett' and 'Bradford' pear leaves (Miller, 1989), 'La France' pear (Shiota, 1990) and Asian pear (Takeoke, 1992). According to a recent review by Paillard (1990), a total of 79 pear compounds had been identified. Esters of short to medium chain alcohols and corresponding acids were found to be the most prominent in pear-fruit volatile profiles. Whereas, hydrocarbon compounds such as α-farnesene, β-ocimene and copaene are predominant in pear leaves (Miller, 1989). Among those esters, ethyl trans-2, cis-4 decadienoate is reported as a "character impact compounds" (Jennings et al., 1964) and hexyl acetate as a "contributory flavor compound" (Jennings and Sevenants, 1964) of 'Bartlett' pear.

The aroma intensity of 'Bartlett' pear essence, as determined by sensory evaluation has been shown to correlate well with the magnitude of spectroscopic absorbance at 263-267 nm of the essence. This absorption was identified as being associated with the 2,4 decadienoate esters (Heinz et al., 1964). The decadienoate

esters appeared to be present in pear cultivars with Bartlett-like aroma (Quamme and Marriage, 1977; Russell et al., 1981). Quamme (1984) used high pressure liquid chromatography to compare decadienoate ester levels of 29 canned pear cultivars with data obtained by sensory evaluation. High decadienoate levels appear to be a characteristic of 'Bartlett' and cultivars with similar aroma. However, the information concerning the volatile compound's profile emitted from intact fruits and their contribution to aroma in commercial pear varieties other than 'Bartlett' have not been published.

In the present study, we have quantitatively identified headspace volatile compounds collected from the eating-ripe stage of eight pear varieties grown in Oregon by using GC/MS. The different amounts of compounds evolved and volatile compound profiles may explain differences in susceptibility to certain physiological disorders or the differences in consumer organoleptic perceptions between those pear varieties.

Materials and Methods

Fruit samples

Eight pear varieties including 'Bartlett', 'Packham's Triumph', 'Anjou', 'Comice', 'Seckel' and 'Bosc' were harvested at optimum commercial maturity from the Southern Oregon Experimental Station, Oregon State University, Medford, Oregon. 'Vicar of Winkfield' and 'Forelle' pears were obtained from the National Clonal Germplasm Repository, USDA Agriculture Research Service, Corvallis, Oregon. All fruits were hand harvested at the optimum stage of maturity based on flesh firmness value. Harvesting was done between July and August 1994. Fruits

were stored in 20 kg standard pear cartons with perforated polyethylene film liners at -1°C to 1°C up to 3 months before use.

Preparation of headspace sample

Once the chilling requirement was satisfied (Richardson and Gerasopoulos, 1994), fruits of each variety were removed from cold storage and ripened at 21°C. At eating-ripe stage (4 days for 'Bartlett' pears and 7-8 days for other varieties), 5 to 10 fruits uniform in size and color were weighed (1600-1800 g ea.) and placed in 4 L glass jars. The jar was closed with air-tight Teflon tape sealed lid fitted with inlet and outlet ports. The inlet port was connected to outside air (4th floor of the building) by Tygon tubing which had a series of ORBO-32 activated charcoal tubes (Supelco, Bellefonte, PA) in between. The series of activated charcoal tubes eliminates all possible organic contaminants from outside air. Outside air was pulled through the jar by vacuum, and flow rate was adjusted to 200 ml/min by a needle valve and a flowmeter. A barostat was used to humidify incoming air and to maintain air temperature. After the desired air flow rate was obtained, an ORBO-32 activated charcoal tube was connected to the outlet and volatile compounds evolved from the fruits were trapped. After an 18 hr trapping period, the tubes were capped with plastic caps and stored at 5°C until analyzed by GC/MS. To elute the compounds from the tube, the packed charcoal was carefully transferred into a 1 ml crimp vial and 0.3 ml chromatographic grade carbon disulfide containing 0.226 mg/ml propyl tiglate standard (Aldrich Chemical Company Inc.) was added. The vial was capped, well shaken and allowed to stand at least 30 min before analysis.

GC/MS analysis

A Hewlett-Packard Model 5890 Series II gas chromatograph coupled with Hewlett-Packard Model 5971 quadrupole mass selective detector was used. The GC was equipped with split/splitless injection port and a 60-m, 0.25 mm i.d. and 0.25-μm film thickness Supelcowax 20M (Bellefonte, PA) capillary column. The GC oven temperature was programmed as follows: 60 °C held for 5 min and increased to 220 °C at a rate of 5°C/min. Helium at a linear velocity of 24.8 cm/sec was used as carrier gas. The injection temperature was set at 200°C. Mass spectra ionization potential was set at 70 eV. Transfer line and ion source temperatures were 280 °C and 180 °C, respectively. All injections were made in split mode at 1 to 20 split ratio. Data were acquired with MS Chemstation software (Hewlett-Packard Co., USA).

Identification and quantitation of components

Identification of compounds was based on comparison of spectra and relative abundance with NIST/EPA/MSDC 54K Mass spectral database (Hewlett-Packard Co., USA) by Probability based-matching (PBM) algorithm (McLafferty et al., 1974). Identity of components were confirmed by comparison of Kováts retention index and mass spectra with authentic compounds (if available). In the absence of matched reference spectra, Kováts retention indices were used for tentative compound identification and confirmed by fragmentation pattern of mass spectra.

Kováts retention indices were obtained by comparison to a series C6 to C15 alkanes on the Carbowax 20M column with the same temperature program as for the samples. Retention times of those standard alkane series and unknown samples were used to calculate retention indices (Kovats, 1965).

Estimation of compound concentration was done by comparing integrated peak area of each compound with the internal standard (0.226 mg/ml propyl tiglate), combined with fruit weight and flow rate of air through the sample jars. Individual compound concentrations are expressed as the weight of compound per unit weight of fruit sample per unit volume of headspace air, i.e. ng/kg/100L.

Results and Discussion

'Bartlett' Pears

The headspace volatiles profile from ripened 'Bartlett' pears is shown in Table 3.1. There are 57 esters, 8 alcohols, 2 hydrocarbons and 1 ketone for a total of 68 compounds obtained from the headspace sample. Hexyl acetate and butyl acetate are the only two predominant compounds in the profile, and they account for 39 and 33% of the total, respectively. Hexyl acetate has been reported to be a flavor contributory compound in 'Bartlett' pears (Jennings, 1964). Methyl and ethyl esters of trans-2, cis-4 decadienoate which were reported to be flavor impact compounds are present in much smaller amounts (4-5%). Among those esters identified in the profile, interestingly enough, 21 of them have not previously been reported in 'Bartlett' pears.

Among 8 alcohols found in the profile, 1-hexanol and 1-butanol are predominant and present in very similar amounts (about 1% of the total) and these correspond to acetate esters formed by them. This may reflect fruit ester production dependency on the availability of alcohols found in the tissue. Two branched-chain alcohols (2-methyl 1-propanol and 2-methyl 1-butanol) were also identified. No secondary alcohols were found in the profile.

The only hydrocarbon compounds identified were two isomers (E,E and E,Z) of α-farnesene, which accounted for 0.5% of total volatiles. α-Farnesene is a characteristic compound of pome fruits (Paillard, 1990). The oxidation products of this compound are believed to be implicated in superficial scald (Huelin and Coggiola, 1970; Anet, 1974; Anet and Coggiola, 1974). Shiota (1990) , however, speculated that it could also be responsible for the 'peely fresh green' odor of 'La France' pear fruit.

Table 3.1. Headspace volatile compounds identified from ripened 'Bartlett' pears[a].

Compounds[b]	RT[c]	Kovats[d]	Area%	ng/kg/100L[e]
methyl propanoate[g]	6.69	825	0.02	5.8
ethanol	7.01	851	0.23	57.9
ui. (m/z 43, 55, 72, 101)[h]	7.33	875	0.06	14.2
ethyl propanoate	7.59	895	0.29	72.9
propyl acetate	7.97	921	8.55	2123.7
methyl butanoate	8.20	937	0.33	81.3
2-methylpropyl acetate	8.81	976	0.68	170.1
1-propanol	9.25	1002	0.06	14.5
ethyl butanoate	9.40	1009	1.03	255.8
propyl propanoate	9.61	1019	0.02	5.8
ethyl 2-methylbutanoate	9.85	1030	0.04	10.8
butyl acetate	10.53	1059	33.19	8248.7
2-methyl 1-propanol	10.69	1066	0.15	38.4
isoamyl acetate	11.84	1111	0.83	207.0
1-butanol	12.26	1127	0.96	239.1
butyl propanoate[g]	12.38	1131	0.06	14.1
propyl butanoate	12.95	1151	0.05	13.0
pentyl acetate	13.39	1166	2.49	618.3
methyl hexanoate	13.81	1179	0.29	72.6
2-methyl 1-butanol	14.19	1191	0.14	34.9
1-methylbutyl 2-methylpropanoate[g]	14.60	1205	0.06	14.7
butyl butanoate + 1-methylpropyl butanoate[g']	14.77	1211	0.33	82.6
ethyl hexanoate	15.24	1229	0.68	168.3
1-pentanol	15.53	1240	0.04	10.5
hexyl acetate	16.62	1278	39.48	9811.3
methyl heptanoate	16.93	1288	0.04	9.8
3-methylbutyl 3-methylbutanoate[g]	17.13	1295	0.40	98.5
4-hexen-ol acetate[g]	17.49	1308	0.04	8.9
3-hepten-1-ol acetate[g]	17.62	1313	0.02	4.5
pentyl 2-methylpropanoate[g] + pentyl butanoate[g']	17.76	1318	0.02	5.2

(Continued)

Table 3.1. Continued.

Compounds[b]	RT[c]	Kovats[d]	Area%	ng/kg/100L[e]
1-methylethyl hexanoate[g] + propyl hexanoate[f]	17.82	1321	0.06	15.2
ethyl heptanoate	18.24	1337	0.04	9.1
hexyl propanoate	18.40	1343	0.05	13.3
6-methyl 5-hepten-2-one[g] +hexyl 2-methylpropanoate[g,f]	18.46	1345	0.02	3.9
3-hexen-1-ol acetate (Z)[g]	18.55	1349	0.03	6.6
1-hexanol	18.63	1352	1.06	263.9
heptyl acetate	19.40	1380	0.28	68.6
methyl octanoate	19.88	1397	0.08	19.1
1-methylhexyl butanoate[g]	20.10	1405	0.06	13.7
butyl hexanoate[g]	20.53	1422	0.17	42.0
hexyl butanoate[g]	20.60	1425	0.88	218.3
ethyl octanoate	21.11	1444	0.16	39.2
hexyl 2-methylbutanoate[g]	21.39	1455	0.03	6.4
1-heptanol	21.49	1458	0.03	7.6
2-methylbutyl hexanoate[g]	21.80	1469	0.03	7.0
octyl acetate	22.21	1484	0.08	20.6
4-octen-1-ol acetate[g]	23.61	1532	0.04	10.0
ethyl trans-2 octenoate	24.35	1557	0.11	28.2
methyl 2,4 octadienoate[g]	25.48	1593	0.01	3.2
hexyl hexanoate	25.69	1599	0.07	16.2
methyl 4-decanoate	26.12	1612	0.15	37.9
ethyl decanoate	26.36	1620	0.02	3.9
ethyl 2,4 octadienoate[g]	26.60	1627	0.04	9.4
ethyl 4-decenoate	27.07	1641	0.16	39.0
α-farnesene (E,Z)	28.43	1679	0.04	10.6
α-farnesene (E,E)	29.00	1695	0.45	111.1
ethyl trans-2 decenoate	29.46	1708	0.01	3.7
methyl cis-2, cis-4 decadienoate	29.82	1717	0.02	4.0
methyl trans-2, cis-4 decadienoate	30.20	1727	1.92	478.2
ethyl cis-2, cis-4 decadienoate	30.86	1744	0.05	11.4
ethyl trans-2, cis-4 decadienoate	31.16	1752	2.67	663.2
methyl trans-2, trans-4 decadienoate	31.67	1765	0.24	59.1
ethyl trans-2, trans-4, cis-7 decatrienoate	32.29	1780	0.02	5.8
ethyl trans-2, trans-4 decadienoate	32.78	1792	0.30	74.0
methyl (?) tetradecenoate	34.90	1842	0.01	2.6
Total			100	24851.4

[a] Fruits ripened at 20°C for 6 days, average flesh firmness 23 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

'Packham's Triumph' Pears

Seventy compounds were identified from the headspace sample of ripened 'Packham's Triumph' pears (Table 3.2.). The majority of these compounds were esters (60) and alcohols (7). In addition, two isomers of α-farnesene and one ketone (6-methyl 5-hepten-2-one) were also identified.

Hexyl acetate and butyl acetate were the two major esters which accounted for more than 80% of the profile. Hexyl acetate alone represented more than 50% of the profile. Among esters identified, the aromatic ester, 2-phenylethyl acetate is reported for the first time in pears. 2-Phenylethyl acetate has been reported to be a flavor constituent of various other fruits such as apple, plum, mango, and guava, etc. It was reported to contribute a sweet, rose-, honey-like aroma (Stevens, 1970). Decadienoate esters are also found (2.2%) but less than in 'Bartlett' pear. 'Packham's Triumph' pear is known to have very similar flavor to 'Bartlett' pear. The content of α-farnesene and its isomer are found to be four times higher than 'Bartlett' pear. Not surprisingly, this pear variety has been classified as highly susceptible to scald disorder (Meheriuk et al. 1994; Watkins, 1993).

In general, the volatiles found in this pear are very similar to those of 'Bartlett'. However, one difference is the absence of 1-propanol and the C3 esters in the profile of this pear. Moreover, a high degree of unsaturated acid esters indicated by compounds in the high molecular weight range was noted. Unsaturated fatty acid esters are believed to derive from β-oxidation of unsaturated fatty acids (Jennings and Tressl, 1974).

Table 3.2. Headspace volatile compounds identified from ripened 'Packham's Triumph' pear [a].

Compounds [b]	RT [c]	Kovats [d]	%area	ng/kg/100L [a]
ethanol	7.02	852	0.12	43.5
propyl acetate	7.93	918	0.35	126.6
2-methylpropyl acetate	8.78	974	0.17	60.7
ethyl butanoate	9.37	1008	0.26	94.5
butyl acetate	10.54	1060	25.72	9437.4
isoamyl acetate	11.81	1110	0.78	285.2
1-butanol	12.25	1126	0.20	72.0
pentyl acetate	13.36	1165	1.69	621.6
methyl hexanoate	13.78	1178	0.21	78.1
2-methyl 1-butanol	14.17	1191	0.15	53.8
1-methylpropyl butanoate [g] + butyl butanoate [f]	14.74	1210	0.30	110.0
ethyl hexanoate	15.21	1228	0.83	304.5
1-pentanol	15.51	1239	0.03	12.4
hexyl acetate	16.71	1281	57.14	20962.3
methyl heptanoate	16.95	1289	0.02	8.7
2-methylpentyl acetate [g]	17.09	1293	0.09	34.3
4-hexen-1-ol acetate [g]	17.47	1307	0.02	6.8
3-hepten-1-ol acetate [g]	17.68	1316	0.01	3.8
pentyl 2-methylpropanoate [g]	17.73	1317	0.01	4.5
1-methylethyl hexanoate [g] + propyl hexanoate [f]	17.78	1320	0.06	22.4
ethyl heptanoate	18.21	1336	0.04	14.7
hexyl propanoate	18.36	1342	0.01	5.2
6-methyl 5-hepten-2-one [g] +hexyl 2-methylpropanoate [g,f]	18.43	1344	0.03	12.8
1-hexanol	18.61	1351	0.85	310.6
2-methylpropyl hexanoate	18.78	1357	0.03	9.3
heptyl acetate	19.37	1379	0.47	172.4
methyl octanoate	19.84	1395	0.15	54.1
butyl hexanoate [g]	20.50	1421	0.86	314.9
hexyl butanoate [g]	20.58	1424	1.03	376.9
butyl 2-methylbutanoate [g]	20.87	1435	0.02	7.1
ethyl octanoate	21.07	1443	0.45	164.3
1-heptanol	21.46	1457	0.01	5.0
2-methylbutyl hexanoate [g]	21.74	1467	0.03	11.2
octyl acetate	22.18	1483	0.55	203.1
ethyl 2,4 hexadienoate [g]	22.68	1501	0.01	2.7
butyl 3-hexenoate [g]	22.81	1505	0.01	4.9
3-octen-1-ol acetate [g]	22.90	1508	0.02	9.0
methyl trans-2 octenoate	22.96	1510	0.01	5.4
pentyl hexanoate	23.14	1517	0.02	6.2
4-octen-1-ol acetate [g]	23.56	1531	0.12	43.1
ethyl nonanoate	23.75	1537	0.01	3.1
1-octanol	24.17	1551	0.03	12.5
ethyl trans-2 octenoate	24.29	1555	0.03	10.1
methyl decanoate	25.28	1587	0.07	25.8

(Continued)

Table 3.2. Continued.

Compounds[b]	RT[c]	Kovats[d]	%area	ng/kg/100L[e]
hexyl hexanoate	25.65	1598	0.39	141.6
butyl octanoate [g]	25.73	1600	0.02	8.2
methyl 4-decenoate	26.07	1611	0.31	114.5
ethyl decanoate	26.31	1618	0.23	83.9
ui. (m/z 55, 67, 79, 97, 125, 168) [h]	26.56	1626	0.01	2.8
ui. (m/z 43, 55, 67, 79, 93, 108)[h]	26.72	1630	0.03	10.7
ethyl 4-decenoate	27.03	1639	0.51	186.8
methyl 2,4,6 octatrienoate [g]	27.55	1655	0.02	7.8
alpha farnesene (E,Z)	28.37	1678	0.09	31.4
methyl 2,4,6 octatrienoate [g]	28.45	1680	0.06	23.5
alpha farnesene (E,E)	28.98	1695	1.91	699.4
ui. (m/z 41, 55, 73, 81, 101, 110, 153) [h]	29.28	1703	0.01	4.2
ethyl trans-2 decenoate	29.40	1706	0.02	8.0
methyl cis-2, cis-4 decadienoate	29.77	1716	0.01	3.8
methyl trans-2, cis-4 decadienoate	30.15	1726	0.80	294.0
2-phenylethyl acetate [g]	30.73	1741	0.17	61.7
ethyl cis-2, cis-4 decadienoate	30.80	1743	0.03	10.3
ethyl trans-2, cis-4 decadienoate	31.12	1751	1.38	505.9
ui. (m/z 41, 55, 69, 79, 91, 121) [h]	31.30	1756	0.01	4.1
methyl trans-2, cis-4 decadienoate	31.61	1763	0.02	5.6
ui. (m/z 41,55, 69, 81, 93, 107, 135) [h]	32.15	1777	0.01	3.9
ethyl trans-2, trans-4, cis-7 decadienoate	32.23	1779	0.02	5.8
ethyl trans-2, trans-4 decadienoate	32.71	1791	0.05	17.2
ui. (m/z 41, 93, 107, 135) [h]	32.87	1794	0.01	4.5
ui. (m/z 41, 55, 69, 91, 105, 117, 131, 159, 202) [h]	33.22	1803	0.03	11.1
ui. (m/z 41, 55, 69, 86, 114) [h]	34.30	1828	0.01	5.4
methyl tetradecanoate[g]	34.84	1840	0.04	15.2
ethyl [?] tetradecenoate	35.56	1857	0.02	8.8
methyl cis-5, cis-8 tetradecadienoate	35.78	1861	0.01	4.9
ethyl cis-5, cis-8 tetradecadienoate	36.51	1877	0.06	22.0
methyl 7-hexadecenoate (Z)	39.60	1942	0.01	3.2
ui. (m/z 43,69, 79, 94, 109, 127) [h]	40.24	1954	0.20	74.1
ui. (m/z 43, 55, 69, 93 105, 119, 137,162, 202) [h]	41.19	1973	0.48	176.3
Total			100	36688.2

[a] Fruits ripened at 20°C for 8 days, average flesh firmness 7.8 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

'Anjou' Pears

Table 3.3. shows the headspace volatiles obtained from ripened 'Anjou' pear, a variety whose volatile profile has not been previously studied. There are 111 compounds isolated from the headspace sample. Among 86 compounds identified, 26 compounds were not previously reported in pears. As in other pears, the ester group of compounds are predominant (73) in the profile, followed by alcohols (8), hydrocarbons (2), an aldehyde (1) and a ketone (1).

Table 3.3. Headspace volatile compounds identified from ripened 'Anjou' pears [a].

Compounds[b]	RT[c]	Kovats[d]	area%	ng/kg/100L[e]
ethanol	7.01	851	0.01	2.0
propyl acetate	7.93	918	0.88	142.4
2-methylpropyl acetate	8.78	975	0.17	27.2
ethyl butanoate	9.37	1008	0.10	16.5
butyl acetate	10.58	1062	22.08	3584.4
hexanal	10.69	1066	0.05	7.5
isoamyl acetate	11.81	1110	0.34	54.6
1-butanol	12.25	1127	0.62	101.4
butyl propanoate	12.36	1130	0.12	19.2
pentyl acetate	13.40	1166	1.59	257.6
methyl hexanoate	13.79	1179	0.16	26.4
2-methyl 1-butanol	14.16	1190	0.17	28.4
1-methylpropyl butanoate [g] + butyl butanoate [f]	14.75	1211	0.41	66.3
ethyl hexanoate	15.22	1228	0.49	78.8
1-pentanol	15.51	1239	0.05	8.4
hexyl acetate	16.81	1284	49.18	7982.8
methyl heptanoate	17.01	1291	0.02	2.8
2-methylpentyl acetate [g]	17.08	1293	0.06	9.0
4-hexen-ol acetate [g]	17.49	1308	0.02	3.8
pentyl 2-methylpropanoate [g]	17.74	1318	0.08	13.0
1-methylethyl hexanoate [g] + propyl hexanoate [f]	17.80	1320	0.09	14.9
ethyl heptanoate	18.23	1337	0.06	9.0
hexyl propanoate	18.39	1343	0.27	44.4
6-methyl 5-hepte-2-one [g] + 2-methylhexyl propanoate [g,f]	18.45	1345	0.16	25.3
1-hexanol	18.65	1353	1.03	167.6
2-methylpropyl hexanoate	18.82	1359	0.03	4.7
heptyl acetate	19.38	1379	0.33	53.7
methyl octanoate	19.85	1396	0.21	34.6
1-methylhexyl butanoate [g]	20.03	1403	0.00	0.8

(Continued)

Table 3.3. Continued.

Compounds[b]	RT[c]	Kovats[d]	area%	ng/kg/100L[e]
butyl hexanoate [g]	20.57	1423	1.79	291.0
hexyl butanoate [g]	20.66	1427	1.52	247.2
butyl 4-hexenoate [g]	20.85	1434	0.11	18.0
butyl 2-methylbutanoate [g]	20.90	1436	0.19	31.6
ethyl octanoate	21.10	1444	0.57	92.1
1-heptanol	21.47	1457	0.06	9.0
2-methylbutyl hexanoate [g]	21.75	1467	0.04	6.9
octyl acetate	22.17	1483	0.07	11.7
butyl 2-hexenoate [g]	22.56	1497	0.01	2.2
ethyl cis-3 octenoate	22.72	1502	0.01	1.9
butyl 3-hexenoate [g]	22.87	1507	0.00	0.7
methyl trans-2 octenoate	22.96	1510	0.02	2.7
pentyl hexanoate	23.15	1517	0.21	33.9
heptyl butanoate [g]	23.25	1520	0.01	1.4
propyl octanoate	23.34	1523	0.02	2.5
4-octen-1-ol acetate [g]	23.56	1531	0.05	7.5
ethyl nonanoate	23.75	1537	0.01	1.4
1-octanol	24.17	1551	0.04	7.1
ethyl trans-2 octenoate	24.29	1555	0.03	4.8
hexyl 2-butenoate [g]	24.38	1558	0.01	0.9
2,4 octadien-1-ol acetate [g]	25.18	1583	0.01	1.4
methyl decanoate	25.29	1587	0.19	30.1
hexyl hexanoate	25.74	1601	2.58	418.9
butyl octanoate [g]	25.78	1602	0.16	26.2
methyl 4-decenoate	26.09	1612	0.37	60.2
ethyl decanoate	26.34	1619	0.71	115.7
ui. (m/z 43, 55, 67, 81, 97, 125, 168) [h]	26.56	1626	0.01	1.2
ui. (m/z 43, 55, 67, 79, 93, 108) [h]	26.72	1630	0.01	1.4
nonanol [g]	26.81	1633	0.01	1.6
ethyl 4-decenoate	27.04	1640	0.58	93.8
methyl 2,4,6 octatrienoate [g]	27.56	1655	0.04	5.7
ui. (43, 56, 70, 84, 99, 117, 123) [h]	28.05	1669	0.02	4.0
methyl 2-decenoate	28.14	1671	0.04	5.7
α-farnesene (E,Z)	28.39	1678	0.16	26.4
ui. (m/z 41, 55, 67, 79, 93, 108, 122, 150) [h]	28.46	1680	0.06	10.1
α-farnesene (E,E)	29.05	1697	3.28	532.8
ui. (m/z 41, 43, 55, 73, 84, 101, 110, 123, 153) [h]	29.30	1703	0.11	17.5
methyl cis-2, cis-4 decadienoate	29.78	1716	0.10	16.5
methyl trans-2, cis-4 decadienoate	30.19	1727	1.72	279.8
hexyl octanoate [g]	30.31	1730	0.06	8.9
methyl cis-2, trans-4 decadienoate	30.49	1735	0.01	1.8
ui. (m/z 43, 55, 68, 74, 83, 96, 111, 138) [h]	30.58	1737	0.01	1.7
ethyl cis-2, cis-4 decadienoate	30.81	1743	0.13	20.8
ethyl trans-2, cis-4 decadienoate	31.16	1752	2.13	346.2
ui. (m/z 41, 55, 69, 79, 81, 91, 111, 119, 137, 151) [h]	31.31	1756	0.07	11.6

(Continued)

Table 3.3. Continued.

Compounds[b]	RT[c]	Kovats[d]	area%	ng/kg/100L[e]
ethyl (?) dodecenoate	31.43	1759	0.03	5.3
ethyl cis-2, trans-4 decadienoate	31.50	1761	0.01	2.4
methyl trans-2, trans-4 decatrienoate	31.62	1764	0.05	8.7
ethyl (?) dodecenoate	31.73	1766	0.02	3.6
ethyl (?) dodecenoate	31.94	1772	0.04	6.7
ui. (m/z 41, 55, 69, 88, 97, 117, 125, 138) [h]	32.09	1775	0.01	1.6
farnesene (?,?)	32.16	1777	0.07	10.9
ethyl trans-2, trans-4, cis-7 decatrienoate	32.24	1779	0.04	6.1
ethyl trans-2, trans-4 decadienoate	32.72	1791	0.12	18.8
ui. (m/z 41, 55, 69, 87, 96, 113, 138, 181) [h]	32.79	1792	0.03	4.8
ui. (m/z 41, 55, 67, 79, 93, 107, 119, 135, 204) [h]	32.88	1794	0.05	7.5
ui. (m/z 41, 55, 67, 79, 91, 105, 117, 131, 159, 202)[h]	33.22	1803	0.04	5.8
ui. (m/z 41, 55, 69, 81, 95, 100) [h]	33.36	1806	0.03	5.0
ui. (m/z 41, 55, 73, 81, 96, 101, 115, 127, 138, 181)[h]	33.76	1815	0.07	10.6
ui. (m/z 41, 55, 69, 86, 114, 136, 179)[h]	34.30	1828	0.06	9.7
methyl tetradecanoate [g]	34.50	1833	0.04	5.9
methyl [?] tetradecenoate	34.87	1841	0.70	113.0
ui. (m/z 43, 141, 156) [h]	35.02	1845	0.01	1.2
ethyl tetradecanoate	35.26	1850	0.03	5.1
ethyl [?] tetradecenoate	35.60	1857	0.65	105.2
methyl cis-5, cis-8 tetradecadienoate	35.79	1862	0.21	33.9
ethyl cis-5, cis-8 tetradecadienoate	36.53	1878	0.37	60.6
ui. (m/z 41, 55, 67, 79, 93, 106, 121) [h]	37.21	1892	0.02	3.8
ui. (m/z43, 69, 82, 99, 109)	37.90	1907	0.01	1.7
ui. (m/z 41, 55, 67, 79, 93, 106, 121, 135, 147) [h]	37.98	1909	0.05	8.8
ui. (m/z 43, 74, 81, 143, 185, 199, 227, 239, 270)[h]	39.00	1930	0.04	7.3
ui. (m/z 43, 69, 82, 95, 96, 97, 109, 165) [h]	39.29	1935	0.02	3.1
methyl 7-hexadecenoate (Z)	39.61	1942	0.09	14.9
ui. (m/z 43, 55, 69, 79, 88, 94, 101, 109)[h]	39.94	1949	0.02	4.0
ui. (m/z 43, 55, 69, 79, 94, 109, 127) [h]	40.23	1954	0.21	34.7
methyl hexadecadienoate [g]	40.95	1968	0.06	9.7
ui. (m/z 43, 55, 69, 93, 105, 119, 137, 162, 177, 187, 205) [h]	41.20	1973	0.52	84.2
ui. (m/z 43, 55, 69, 81, 95, 109) [h]	44.65	2037	0.03	5.1
methyl 10-octadecenoate [g]	46.49	2069	0.12	19.8
Total			100	16231.7

[a] Fruits ripened at 20°C for 8 days, average flesh firmness 5.6 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

'Anjou' volatiles are similar to 'Packham's Triumph' pear in that they lack C3 esters and have a high degree of unsaturated acid esters. The major esters are hexyl acetate (49.2%), butyl acetate (22.1%) and hexyl hexanoate (2.6%). Surprisingly, the 2,4 decadienoate esters were found to be as high as observed in 'Bartlett' pear (3.9%) despite the general observation that Anjou pears lack typical 'Bartlett' flavor. Quamme (1984) failed to detect decadienoate esters from 'Anjou' pears by HPLC and therefore did not classify this pear as among the Bartlett-like varieties. A considerably higher content of α-farnesene was also observed (3.4%). Scald incidence has been reported to be higher in this pear compared to 'Packham's Triumph' and 'Bartlett' pear (Zoffoli, 1994). The only aldehyde, hexanal, was found in very small amount. Aldehyde compounds have been previously isolated from pear juice concentrate (Gasco, 1969) but not from the fruits.

'Comice' Pears

Headspace volatile constituents of ripened 'Comice' pears is shown in Table 3.4. There are 56 compounds identified in this pear variety. Esters comprised more than 98% of the total volatiles. The major esters are hexyl acetate and butyl acetate which accounted for more than 92% of the profile. The ester profile is similar to 'Bartlett' pear in having a moderate degree of unsaturated esters. The other two characteristics of 'Comice' pear volatiles are that they lack 2,4 decadienoate esters (0.12%) and both isomers of α-farnesene (0.13%) in the profile. Despite very low decadienoate esters, 'Comice' pears are considered by many to be the best dessert pear variety due to its, juicy, buttery texture and well balanced flavor (Wrolstad et al., 1991). The amount of α-farnesene is only 25 % that of 'Bartlett' pears. Scald incidence was also observed in 'Comice', but started much later during cold storage

(after 6 months) and eventually reached about the same degree of scald as was found in 'Bartlett' after only 3 months in storage (Zoffoli, 1994).

'Comice' is similar to 'Packham's Triumph' pear, in producing an unusual aromatic ester, 2-phenylethyl acetate. Alcohols and ketones found in 'Comice' are almost the same as observed from other varieties. However, the only aldehyde, trans-2-hexenal found in 'Comice' has also been reported in Asian pear volatiles (Takeoka et al., 1992).

Table 3.4. Headspace volatile compounds identified from ripened 'Comice' pears [a].

Compounds [b]	RT [c]	Kovats [d]	area%	ng/kg/100L [e]
ethanol	7.00	850	0.01	4.5
propyl acetate	7.90	916	0.03	14.6
2-methylpropyl acetate	8.76	973	0.01	5.8
ethyl butanoate	9.34	1007	0.08	34.7
ethyl 2-methylbutanoate	9.81	1028	0.01	2.6
butyl acetate	10.54	1060	21.44	8996.6
hex-2-enal	10.74	1068	0.01	3.8
isoamyl acetate	11.75	1108	0.27	113.7
1-butanol	12.21	1125	0.34	142.8
pentyl acetate	13.35	1164	1.32	554.5
methyl hexanoate	13.75	1178	0.07	29.9
2-methyl 1-butanol	14.13	1189	0.12	49.0
1-methylpropyl butanoate [g] + butyl butanoate [f]	14.71	1209	0.23	96.7
ethyl hexanoate	15.18	1227	0.74	308.7
1-pentanol	15.48	1238	0.03	11.9
hexyl acetate	16.81	1284	71.49	29990.6
2-methylpentyl acetate [g]	17.30	1300	0.02	8.2
4-hexen-ol acetate [g]	17.47	1307	0.04	17.3
pentyl 2-methylpropanoate [g]	17.69	1316	0.01	3.4
1-methylethyl hexanoate [g] + propyl hexanoate [f]	17.79	1320	0.06	26.2
ethyl heptanoate	18.20	1336	0.03	12.3
hexyl propanoate	18.36	1342	0.02	8.8
6-methyl 5-hepte-2-one [g] + hexyl 2-methylpropanoate [g,f]	18.43	1344	0.01	2.6
1-hexanol	18.62	1351	0.96	402.1
2-methylpropyl hexanoate	18.78	1357	0.01	4.6
heptyl acetate	19.36	1378	0.29	123.1
ui. (m/z 59, 43, 70, 115, 83) [h]	19.63	1388	0.01	2.3
methyl octanoate	19.82	1395	0.06	25.7
butyl hexanoate [g]	20.47	1420	0.36	149.0
hexyl butanoate [g]	20.55	1423	0.35	145.1
butyl 4-hexenoate [g]	20.79	1432	0.01	4.2

(Continued)

Table 3.4. Continued.

Compounds[b]	RT[c]	Kovats[d]	area%	ng/kg/100L[e]
butyl 2-methylbutanoate [g]	20.85	1434	0.01	4.6
ethyl octanoate	21.06	1442	0.49	207.2
1-heptanol	21.45	1457	0.02	7.0
2-methylbutyl hexanoate [g]	21.71	1466	0.01	2.4
octyl acetate	22.16	1482	0.20	83.3
ui. (m/z 88, 55, 82, 60, 96, 124, 70) [h]	22.23	1485	0.04	15.6
pentyl hexanoate	23.13	1516	0.00	1.5
propyl octanoate	23.32	1523	0.00	1.1
4-octen-1-ol acetate [g]	23.54	1530	0.04	17.8
ui. (m/z 77, 105, 106) [h]	23.89	1542	0.01	2.7
1-octanol	24.15	1550	0.07	30.5
ethyl trans-2 octenoate	24.27	1554	0.00	1.1
hexyl hexanoate	25.62	1597	0.12	50.3
butyl octanoate [g]	25.70	1600	0.06	26.9
methyl 4-decenoate	26.04	1610	0.00	2.0
ethyl decanoate	26.28	1617	0.02	7.8
1-nonanol [g]	26.78	1632	0.00	1.2
ethyl 4-decenoate	26.99	1638	0.03	13.3
α-farnesene (E,Z)	28.34	1677	0.00	2.1
methyl 2,4,6 octatrienoate [g]	28.43	1679	0.01	4.8
α-farnesene (E,E)	28.91	1693	0.13	55.8
methyl trans-2, cis-4 decadienoate	30.11	1725	0.01	2.9
hexyl octanoate [g]	30.27	1729	0.01	4.0
2-phenylethyl acetate [g]	30.70	1740	0.08	31.5
ethyl trans-2, cis-4 decadienoate	31.07	1750	0.11	47.0
ui. (m/z 65, 91, 92, 122) [h]	32.74	1791	0.02	7.0
ui. (m/z 173, 55, 99, 84, 111) [h]	35.97	1866	0.05	22.3
ui. (m/z 43, 45, 69, 81 93, 95, 137, 162) [h]	41.14	1972	0.01	4.1
Total			100	41953.3

[a] Fruits ripened at 20°C for 4 days, average flesh firmness 8.0 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

'Bosc' Pears

Table 3.5. represents headspace volatiles from ripened 'Bosc' pears and is characterized by 17 esters, 4 alcohols, 2 hydrocarbons and 1 aldehyde. Unlike

'Bartlett' or 'Anjou' pear, 'Bosc' pear esters are dominated by butyl acetate, which accounted for more than 55% of the profile followed by hexyl acetate at 24%.

Table 3.5. Headspace volatile compounds identified from ripened 'Bosc' pears [a].

Compounds [b]	RT [c]	Kovats [d]	area%	ng/kg/100L [e]
propyl acetate	7.91	917	1.65	106.6
2-methylpropyl acetate	8.77	974	0.12	7.6
butyl acetate	10.44	1056	55.94	3614.5
hexanal	10.63	1064	0.08	5.3
isoamyl acetate	11.76	1108	0.32	20.7
1-butanol	12.23	1126	6.94	448.1
pentyl acetate	13.32	1163	2.95	190.7
2-methyl 1-butanol	14.14	1190	0.11	7.1
1-methylpropyl butanoate [g] + butyl butanoate [f]	14.70	1209	0.76	48.9
ethyl hexanoate	15.17	1226	0.11	7.0
1-pentanol	15.49	1238	0.29	19.0
hexyl acetate	16.42	1271	24.14	1560.0
1-hexanol	18.57	1350	3.52	227.5
heptyl acetate	19.34	1378	0.07	4.5
butyl hexanoate [g]	20.46	1419	0.36	23.2
hexyl butanoate [g]	20.53	1422	0.47	30.4
ethyl octanoate	22.15	1482	0.04	2.4
4-octen-1-ol acetate [g]	23.54	1530	0.06	3.6
α-farnesene (E,Z)	28.36	1677	0.08	5.1
α-farnesene (E,E)	28.92	1693	0.77	50.0
2-phenylethyl acetate [g]	30.71	1741	0.24	15.4
ethyl trans-2, cis-4 decadienoate	31.07	1750	0.40	25.9
ui. (m/z 41, 57, 89, 117, 151) [h]	32.38	1782	0.30	19.3
ethyl trans-2, trans-4 decadienoate	32.71	1790	0.28	18.4
Total			100	6461.1

[a] Fruits ripened at 20°C for 10 days, average flesh firmness 37 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

Decadienoate esters are present as minor constituents (0.7%). The small amount of decadienoates in 'Bosc' agrees with the earlier reports. Quamme (1984) reported no

decadienoate esters found and classified 'Bosc' pear as lacking Bartlett-like aroma. Among various esters, the unusual aromatic ester, 2-phenylethyl acetate, was also found in this variety.

The amount of butyl acetate corresponds to a high level of 1-butanol (6.9%) observed. All four alcohols identified accounted for 10% of total volatiles. Hexanal is the only aldehyde found in this pear. A very low amount of α-farnesene (0.8%) was observed in this variety.

'Seckel' Pears

Headspace volatiles of ripened 'Seckel' pear are shown in Table 3.6. 'Seckel' pear volatiles are described by 18 esters, 4 alcohols and 2 hydrocarbons. Interestingly, decadienoate esters are the major compounds in the profile, and accounted for more than 41% of total volatiles. Hexyl and butyl acetate, which predominate in 'Bartlett' and 'Anjou' pear, are present in 'Seckel' in much lower amounts, 2.9 and 0.5%, respectively. 'Seckel' pear was previously reported to contain a certain amount of decadienoate esters without carrying Bartlett-like aroma (Quamme, 1984). Wrolstad (1991) reported a cinnamon-like flavor is a characteristic of this pear. However, there is no volatile compound (such as cinamaldehyde) found in this profile which could be linked to that flavor characteristic.

Among alcohols, hexanol and butanol are present in almost the same amount, 5.7 and 5.6%. Total alcohols accounted for more than 13% of the profile which was quite high compared with other pear varieties. α-Farnesene and its isomer are also present in a high amounts (10%), and this pear also has high scald susceptibility.

Table 3.6. Headspace volatile compounds identified from ripened 'Seckel' pears [a].

Compounds [b]	RT [c]	Kovats [d]	area%	ng/kg/100L [e]
ethanol	7.05	854	2.71	18.4
ui. (m/z 43, 55, 72, 101) [h]	7.32	875	0.51	3.4
ethyl butanoate	9.38	1008	1.02	6.9
butyl acetate	10.36	1052	0.55	3.7
1-butanol	12.25	1126	5.55	37.8
2-methyl 1-butanol	14.17	1191	0.34	2.3
1-methylpropyl butanoate [g]	14.73	1210	0.60	4.0
ethyl hexanoate	15.20	1227	1.66	11.3
hexyl acetate	16.38	1270	2.91	19.8
1-hexanol	18.60	1351	5.67	38.5
butyl hexanoate [g]	20.49	1420	0.79	5.4
hexyl butanoate [g]	20.56	1423	8.93	60.8
butyl 4-hexanoate [g]	20.81	1433	0.81	5.5
butyl 2-methylbutanoate [g]	20.88	1435	0.99	6.7
ethyl cis-2 octenoate	21.77	1468	0.42	2.9
hexyl hexanoate	25.66	1598	1.10	7.4
methyl 4-decenoate	26.08	1611	0.43	2.9
ethyl 4-decenoate	27.03	1640	1.93	13.1
α-farnesene (E,Z)	28.39	1678	1.24	8.4
α-farnesene (E,E)	28.96	1694	9.80	66.6
methyl trans-2, cis-4 decadienoate	30.15	1726	9.61	65.3
ethyl cis-2, cis-4 decadienoate	30.82	1743	0.82	5.6
ethyl trans-2, cis-4 decadienoate	31.12	1751	31.51	214.3
methyl trans-2, trans-4 decadienoate	31.63	1764	2.14	14.6
ethyl trans-2, trans-4 decadienoate	32.75	1791	7.75	52.7
ui. (m/z 41, 55, 69, 86, 114) [h]	34.32	1829	0.22	1.5
Total			100	680.1

[a] Fruits ripened at 20°C for 8 days, average flesh firmness 20 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

'Vicar of Winkfield' Pears

There are 35 compounds identified from headspace volatiles of ripened 'Vicar of Winkfield' pears (Table 3.7). Among them, 26 are esters, 6 alcohols, 2 hydrocarbons and 1 ketone compound. Although esters predominate in the profile,

hydrocarbons represent by α-farnesene and its isomers are also major compounds in the profile. Those two farnesene isomers accounted for more than 34% of total volatiles, yet this pear variety is quite insensitive to scald. The second abundant compound in the profile is butyl acetate (23%). Hexyl acetate which normally is present as a major constituent in 'Bartlett' or 'Anjou' pears, is present at only 8.5% of the total. Decadienoate esters are also present but in small amount (2.8%). No compound in the profile has been found to give a typical characteristic of this pear variety.

'Forelle' Pears

Headspace volatiles identified from ripened 'Forelle' pears are presented in Table 3.8. Among 48 compounds identified from the profile, there are 34 esters, 8 alcohols and 6 hydrocarbons. No aldehyde or ketone compounds were found in this variety. Esters comprised more than 85% of the total volatiles. The major esters included hexyl acetate (36%), butyl acetate (28%) and propyl acetate (10%). A very low amount of decadienoate esters were also observed (0.05%). Two aromatics esters, butyl benzoate and hexyl benzoate are reported for the first time in pears and in this variety. Those two compounds have not been reported to be present in any fruits. Alcohols are observed to be present in considerable high ratio compared to Bartlett-like aroma varieties. A total of 8 alcohols comprised more than 10% of total volatiles.

Among 6 hydrocarbon compounds found in the 'Forelle' profile, 1-methyl-4-(1-methylethyl)-1,3 cyclohexadiene, 1-methyl-4-(1-methylethylidene)-cyclohexane, β-phellandrene and 3-carene are the first isolated from pears. The first two have never been reported in any fruit volatiles while the last two terpene hydrocarbons have

Table 3.7. Headspace volatile compounds identified from ripened 'Vicar of Winkfield' pears [a].

Compounds [b]	RT [c]	Kovats [d]	area%	ng/kg/100L [e]
ethanol	7.04	853	0.61	13.4
ethyl propanoate	7.60	895	0.75	16.5
propyl acetate	7.95	920	3.21	70.6
methyl butanoate	8.20	937	0.48	10.6
2-methylpropyl acetate	8.80	976	0.44	9.6
1-propanol	9.26	1004	0.40	8.7
ethyl butanoate	9.40	1012	4.05	89.1
ethyl 2-methylbutanoate	9.84	1030	1.08	23.8
butyl acetate	10.40	1054	23.27	511.5
2-methyl 1-propanol	10.66	1065	0.40	8.8
isoamyl acetate	11.80	1110	1.48	32.6
1-butanol	12.26	1127	2.67	58.7
ui. (m/z 43, 57, 72, 84, 100) [h]	13.10	1156	0.17	3.7
pentyl acetate	13.36	1165	2.01	44.3
methyl hexanoate	13.80	1179	0.58	12.8
2-methyl 1-butanol	14.18	1191	0.90	19.7
butyl butanoate + 1-methylpropyl butanoate [g,f]	14.75	1211	0.70	15.4
ethyl hexanoate	15.22	1228	4.37	96.0
hexyl acetate	16.41	1271	8.55	187.8
1-methylethyl hexanoate [g] + propyl hexanoate [f]	17.79	1320	0.16	3.4
6-methyl 5-hepte-2-one [g]	18.43	1344	0.71	15.6
1-hexanol	18.61	1351	0.34	7.4
methyl octanoate	19.86	1396	0.18	4.0
butyl hexanoate [g]	20.50	1421	1.42	31.2
hexyl butanoate [g]	20.57	1423	0.38	8.4
butyl 2-methylbutanoate [g]	20.89	1436	0.14	3.0
ethyl octanoate	21.09	1443	1.79	39.3
hexyl hexanoate	25.67	1599	0.25	5.5
ethyl decanoate	26.33	1619	0.29	6.4
ethyl 4-decenoate	27.04	1640	0.21	4.6
α-farnesene (E,Z)	28.41	1679	1.17	25.8
α-farnesene (E,E)	29.03	1696	34.00	747.2
methyl trans-2, cis-4 decadienoate	30.16	1726	0.71	15.6
ethyl trans-2, cis-4 decadienoate	31.12	1751	2.13	46.8
Total			100	2197.7

[a] Fruits ripened at 20°C for 8 days, average flesh firmness 27.7 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

been reported in 'Tommy Atkins' mango essence. β-Phellandrene has fatty and oily odor while 3-carene has floral and mango leaf-like odor characteristics (MacLeod and Snyder, 1985). Two isomers of α-farnesene are also found but in a very small amount (1.8%). Lack of information concerning scald incidence in this pear variety make attempts to correlate α-farnesene with physiological scald disorder impossible.

Table 3.8. Headspace volatile compounds identified from ripened 'Forelle' pears [a].

Compounds [b]	RT [c]	Kovats [d]	area%	ng/kg/100L [e]
methyl propanoate [g]	6.95	846	0.06	2.7
ethanol	7.03	852	0.23	11.0
propyl acetate	7.95	920	10.42	497.6
2-methylpropyl acetate	8.80	976	0.58	27.5
1-propanol	9.23	1002	0.43	20.5
ethyl butanoate	9.37	1008	0.09	4.3
butyl acetate	10.48	1057	27.89	1331.3
2-methyl 1-propanol	10.66	1065	0.26	12.6
isoamyl acetate	11.81	1110	1.58	75.6
1-butanol	12.26	1127	3.91	186.5
ui. (m/z 41, 45, 55, 56, 84) [h]	12.65	1140	0.03	1.5
pentyl acetate	13.37	1165	3.26	155.6
1-methyl-4-(1-methylethyl)-1,3-clyclohexadiene [g]	13.68	1175	0.42	20.2
2-methyl 1-butanol	14.17	1191	0.74	35.4
beta phellandrene [g]	14.60	1205	0.05	2.5
butyl butanoate + 1-methylpropyl butanoate [g,i]	14.75	1211	0.47	22.6
ethyl hexanoate	15.21	1228	0.23	11.0
1-pentanol	15.52	1239	0.35	16.9
3-carene [g]	15.70	1246	0.68	32.5
ui. (m/z 51, 78, 104) [h]	16.15	1262	0.03	1.5
3-methylbutyl 2-methylpropanoate [g]	16.26	1266	0.05	2.5
hexyl acetate	16.56	1276	36.31	1733.0
2-methylpentyl acetate [g]	16.70	1280	0.05	2.4
1-methyl-4-(1-methylethylidene)- cyclohexane [g]	16.93	1288	0.08	3.8
4-hexen-1-ol acetate (Z) [g]	17.46	1307	0.03	1.3
1-methylethyl hexanoate [g] + propyl hexanoate [i]	17.79	1320	0.19	9.2
ethyl heptanoate	18.37	1342	0.07	3.5
hexyl propanoate	18.43	1344	0.04	2.0
1-hexanol	18.63	1352	5.20	248.0
2-methylpropyl hexanoate	18.80	1358	0.05	2.2
heptyl acetate	19.38	1379	0.74	35.4
butyl hexanoate [g]	20.50	1421	1.07	50.9
hexyl butanoate [g]	20.57	1423	1.02	48.9
butyl 4-hexenoate [g]	20.82	1433	0.09	4.1

(Continued)

Table 3.8. Continued.

Compounds[b]	RT[c]	Kovats[d]	area%	ng/kg/100L[e]
butyl 2-methylbutanoate [g]	20.88	1435	0.15	7.2
ethyl octanoate	21.08	1443	0.10	4.6
1-heptanol	21.48	1458	0.09	4.3
2-methylbutyl hexanoate [g]	21.75	1468	0.09	4.3
octyl acetate	22.18	1483	0.12	5.8
pentyl hexanoate	23.15	1517	0.03	1.4
4-octen-1-ol acetate [g]	23.57	1531	0.06	2.9
hexyl hexanoate	25.66	1598	0.57	27.4
butyl octanoate [g]	25.74	1601	0.10	4.9
α-farnesene (E,Z)	28.39	1678	0.08	3.7
α-farnesene (E,E)	28.97	1694	1.75	83.4
ethyl trans-2, cis-4 decadienoate	31.11	1751	0.05	2.5
butyl benzoate [g]	31.84	1769	0.04	2.0
hexyl benzoate [g]	36.20	1871	0.04	2.0
Total			100	4772.9

[a] Fruits ripened at 20°C for 8 days, average flesh firmness 27.5 Newtons.
[b] Compounds identified by electron impact mass spectrometer.
[c] Retention time in minute on Carbowax 20M column.
[d] Kovats retention index on Carbowax 20M column.
[e] Relative to propyl tiglate internal standard.
[f] Coeluted compounds.
[g] Identified for the first time in pears.
[h] Unidentified compound and major fragment ions from electron impact MS.

General characteristic of pear volatiles

Volatile flavor profiles in all pear varieties studied are characterized by compounds in groups of esters, alcohols, hydrocarbons, aldehydes and ketones. There is a total of 112 compounds identified among eight pear varieties. Among these compounds, 47 compounds have never been reported in any pear varieties, including Asian pears. The complete list of newly identified compounds and their retention indices are shown in Appendix Table A.3.1.

Esters are always present as a major group of compounds in the profiles ranging from 60% to as high as 98% of total volatiles. Esters of C1 to C8 straight chain alcohols with a minimal degree of unsaturation and their corresponding acids

were widely present in the profile. Methyl and ethyl esters of C10 and higher acids with various degrees of saturation were also observed. A minor group of aromatic esters such as, 2-phenylethyl acetate, butyl and hexyl benzoate were also observed for the first time in some varieties. These unusual esters may play an important role in aroma characteristics which differentiate each variety.

Hexyl acetate, which was reported to be a contributory flavor compound in pear, was observed to be present as a major ester in all pear varieties except 'Bosc', 'Vicar of Winkfield' and 'Seckel' pears. 'Bosc' and 'Vicar of Winkfield' pear produced butyl acetate whereas 'Seckel' produced ethyl 2,4 decadienoate as the major ester in the profile.

Methyl- and ethyl- esters of 2,4 decadienoic acid which have been proposed to be flavor impact compounds of 'Bartlett' type pears were detected from all varieties studied but varied significantly in amount (Tables 3.1-3.8). The highest decadienoate esters was observed in 'Bartlett' pear followed by 'Packham's Triumph' and 'Anjou' pears. 'Forelle' pear produced the least amount of these esters. The level of decadienoate esters content in the fruit has been proposed to be used as a selection criteria for pear breeding purposes (Quamme, 1984). Differences in isomeric forms of 2,4 decadienoate esters were also observed among those varieties. Each isomeric configuration may or may not possess the same aroma characteristic. However, the trans-2, cis-4 isomer of decadienoate esters was the predominant form and was present in all varieties.

Alcohols constitute the second largest category of volatile compounds in pear profiles. They accounted for 1.5 to 14% of total volatile in the eight varieties studied. More free alcohol content was observed in 'Seckel', 'Forelle' and 'Bosc', while 'Packham's Triumph', 'Comice', 'Anjou' and 'Bartlett' pears had lower levels of

alcohols. The varieties with high alcohols have an interesting feature in producing less esters as indicated by the number of esters in the profile. This may reflect low activity of endogenous esterifying enzyme systems in converting alcohols to esters. Among those alcohols identified, the C2 to C9 straight chain alcohols were commonly present in most varieties. Branched-chain alcohols, especially 2-methyl 1- butanol, were also commonly observed in all varieties. There were no secondary or unsaturated alcohols identified in any of the varieties.

Unlike other pome fruits such as apples and quinces, pears produced only very small amounts of aldehydes. Hexanal and hex-2-enal were the only two aldehydes found among these pear profiles. It is possible that the small amount of aldehydes found may be due to the nature of sample preparation in this study (dynamic headspace trapping). While aldehydes have been reported to be present in large amounts in heat processed or solvent extracts, they occur in much smaller quantity in intact fruit (Paillard, 1990).

The two isomeric forms (E,E and E,Z) of α-farnesene were observed in all varieties but varied considerably in the amount. The highest α-farnesene content was found in 'Vicar of Winkfield' followed by 'Packham's Triumph', 'Anjou', 'Bartlett', and 'Comice'. The same order of α-farnesene level was observed from hexane extracts from 'Packham's Triumph', 'Anjou' and 'Bartlett' pear peels (Zoffoli, 1994).

The highest total volatile production during ripening was observed in 'Comice' pears at 42 μg/kg/100L of air followed by 'Packham's Triumph', 'Bartlett' and 'Anjou' at 37, 25 and 16 μg/kg/100L of air, respectively (Tables 3.1-3.8). 'Comice' pears are considered to be the best eating quality pear due to its richness in flavor (Wrolstad

et al., 1991). The lowest volatile production was found in 'Seckel' pear, which was only about 10% of 'Bosc' and only 2% of 'Bartlett' pear.

Varieties can be roughly classified into 3 different groups by using the degree of unsaturated acid which makes up the esters. The group whose esters possessed low degree of unsaturated esters included 'Bosc', 'Vicar of Winkfield', 'Seckel' and 'Forelle'. The intermediate degree of unsaturation esters included 'Bartlett' and 'Comice'. The highly unsaturated esters group included 'Packham's Triumph' and 'Anjou'. The degree of unsaturation may reflect the type of fatty acid substrate used to synthesize esters in each variety. Ester synthesis in pear was reported to be derived from β-oxidation (Heinz and Jennings, 1966; Jennings and Tressl, 1974) and lipoxygenase-catalyzed oxidation (Heinz and Jennings, 1966) of fatty acids. The varieties which contained high amounts of unsaturated fatty acids or those having enzyme systems favoring this type of fatty acid are likely to produce esters with a high degree of unsaturation and yield a large number of esters more than those varieties which contained less unsaturated fatty acids or contained mainly saturated fatty acids.

The present information provides a general view of volatile compounds produced in different pear varieties. The interpretations were done based on differences and similarities of the compounds observed in the profiles compared to limited past research. The gas chromatography-olfactometry procedure is needed to be performed to determine whether a compound or a specific group of compounds have significant contribution to pear aroma. This is done later in Chapter 4.

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CHAPTER 4

ANALYSIS OF ODOR-ACTIVE COMPOUNDS ASSOCIATED WITH PEAR (*Pyrus communis* L.) AROMA

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Abstract

Aroma volatile compounds were isolated and identified from dynamic headspace of 3 pear varieties. Compounds were sensory characterized by direct sniffing compounds eluting from the GC. The results from sniffing GC demonstrated that a group of 31 volatile esters from 'Bartlett', 22 from 'Comice' and 27 from 'Anjou' pears were odor-active. A strong specific pear-like aroma was observed in a group of 9 compounds with slight differences between 3 varieties. Those compounds include 2-methylpropyl acetate, butyl acetate, butyl butanoate, pentyl acetate, ethyl hexanoate, hexyl acetate, methyl trans-2, cis-4 decadienoate, ethyl cis-2, cis-4 decadienoate and ethyl trans-2, cis-4 decadienoate. A second group of volatile esters were found to possess very positive odor characteristics such as floral, sweet, candy or perfume-like aroma and can significantly affect overall sensation by serving as 'contributory to flavor compounds' in pears. The differences between odor-active compound profiles from 3 pear varieties may be used to explain some of the distinct flavors perceived between pear varieties.

Introduction

Pear (*Pyrus communis* L.) is one of the most important tree fruit crops grown in the Pacific Northwest region of the USA. North American pear production is estimated at 824,000 tons. The states of California, Washington and Oregon constitute 97% of the U.S. production. Four cultivars 'Bartlett', 'Anjou', 'Bosc' and 'Comice' make up 98% of the production (Wrostad et al., 1990). A large part of production was formerly used for processing only a limited portion used to supply fresh market. But recently, the increase in the demand for fresh fruits has shifted the industry to produce fruits for fresh market (Crabtree et al., 1994). Marketing of fresh

fruit not only requires fruits superior in appearance but also fruits superior in flavor and texture.

Volatile compounds have been isolated and identified in some pear varieties including 'Bartlett' pear (Jennings,1961; Jennings and Creveling, 1963; Jennings and Sevenants, 1964; Jennings et al., 1964; Heinz et al., 1966; Creveling and Jennings, 1970; Jennings and Tressl, 1974), 'La France' pear (Shiota, 1990) and Asian pear (Takeoke, 1992). More than 79 volatile compounds, predominated by compounds in a group of esters, have been reported as flavor constituents of those pears. Among various esters found in the profile, ethyl trans-2, cis-4 decadienoate has been reported as a 'character impact compound' (Jennings et al., 1964) and hexyl acetate as a 'contributory flavor compound' (Jennings and Sevenants, 1964) of 'Bartlett' pear.

High correlations between aroma and flavor (taste) found among several canned and fresh fruits indicated the significance of aromatic compounds to pear flavor (Quamme and Marriage, 1977). The aroma intensities of extracted 'Bartlett' pear essence exhibited high correlations with the intensity of their absorbance at 263-267 nm. This absorption was identified as associated with 2,4 decadienoic acid esters (Heinz et al., 1964). Subsequent studies of several pear varieties by gas chromatography, HPLC and sensory evaluation have shown that high concentrations of decadienoate esters are characteristic of 'Bartlett' pear and other varieties with Bartlett-like flavor (Quamme and Marriage, 1977; Russel et al., 1981; Quamme and Spearma, 1983; Quamme, 1984). These compounds were proposed for use as criteria for plant breeders to select parent pears in a breeding program (Quamme, 1984).

Several combinations of gas chromatography and olfactometry (GC-O) techniques have been employed for odor-active compounds analysis. The

techniques included the simple sensory sniffing-port panel method (Nelson and Acree, 1978; Noble, 1978; Agustyn and Rapp, 1982; Linssen et al., 1993), CharmAnalysis (Acree et al., 1984; Acree, 1993), Aroma Extraction Dilution Analysis or AEDA (Schieberle and Grosch, 1984) and the Osme method (McDaniel et al., 1990). The above techniques have been widely used to analyze odor-active compound in various foods and beverages such as cheese (Arora et al., 1995), and wine (Miranda-Lopez et al., 1992). The techniques have also been used successfully for fresh produce such as naranjilla fruit (Brunke et al., 1989), cucumbers and muskmelon (Schieberle et al., 1990), bell pepper (Luning et al., 1994; Van Ruth and Roozen, 1994), strawberry (Ulrich et al., 1995) and apple, banana and strawberry (Perez et al., 1993b).

The objective of this study was to apply the gas chromatography-olfactometry method to analyze odor-active compounds obtained from the headspace of 3 ripening pear varieties including 'Bartlett', 'Comice' and 'Anjou' pears. The results of this study can be used to identify a compound or a group of compounds that significantly contribute to pear aroma.

Materials and Methods

Fruit samples

'Bartlett', 'Anjou' and 'Comice' pears were harvested from the Southern Oregon Experimental Station, Oregon State University, Medford, Oregon. All fruits were hand harvested at optimum stage of maturity based on commercially recognized flesh firmness values. Harvesting was done between July and August

1994. Fruits were stored at -1°C to 1°C until chilling requirements (2 to 4 months for 'Anjou' and 'Comice' pears) were satisfied.

Preparation of headspace sample

Fruits of each variety after chilling requirements were satisfied, were removed from cold storage and ripened at 21°C. At the optimum eating-ripe stage (4 days for 'Bartlett', 6 days for 'Comice' and 8 days for 'Anjou'), 5 to 10 fruits uniform in size and color were weighed (1600-1800 g total) and placed in 4 L glass jars with air-tight Teflon seal lids and inlet and outlet ports. Outside air (4th floor of the building) was pulled to pass through the jar by vacuum at a flow rate of 200 ml/min. Volatile compounds emitted from the fruits were trapped on an ORBO-32 activated charcoal tube (Supelco, Bellefonte, PA) for a period of 18 hr. The tubes were capped with plastic caps and stored at 5°C. To elute the compounds from the tube, packed charcoal was carefully transferred into a 1 ml crimp vial and 0.3 ml of 0.226 mg/ml propyl tiglate standard in chromatographic grade carbon disulfide was added. The vial was capped, well shaken and allowed to stand at least 30 min before GC/MS or gas chromatography-olfactometry analysis.

Gas chromatography-olfactometry (GC-O)

Head-space volatile compounds of ripened 'Bartlett', 'Anjou' and 'Comice' pears obtained by carbon disulfide extraction from ORBO-32 activated charcoal tubes were separated by a Hewlett-Packard Model 5890A gas chromatograph equipped with a 30-m, 0.32-mm i.d. and 0.5-μm film thickness Supelcowax10 capillary column (Supelco, Bellefonte, PA). The GC oven temperature was programmed as follows: 60°C held for 5 min and increased to 220°C at a rate of

5°C/min. Helium at linear velocity of 23.5 cm/sec was used as a carrier gas. The injection port and the detector temperature were set at 200°C and 250°C, respectively. All injections were performed splitless mode with 0.5 min purge time. At the end of the capillary column, the GC effluent can be connected to either a flame ionization detector (FID) for chromatograms or to a sniffing port for a human nose to determine odor characteristics.

A modified Osme method (McDaniel et al., 1990) was used for odor-active compounds analysis. At the sniffing port, the GC effluent was mixed with humidified (65 to 85% R.H.) air at 10.2 L/min. The supplied air carries the effluent compound through the delivery system to the human subject who was instructed to breathe normally. Panelists were asked to estimate odor intensity based on an ordinal scale 1 to 5 (1= just detectable, 2= slight, 3= moderate, 4= strong and 5= very strong) and to verbally describe odor characteristics (Acree et al., 1984b). Each sample was sniffed 3 times by each of three panelists which were familiarized with the GC-O technique. Each sniffing session lasted approximately 35 min and was conducted on separate days to avoid olfactory fatigue. Description and mean intensity were presented for odors that were agreeably perceived at least two out of three runs for each panelist and two out of three runs between panelists, regardless of the difference in odor descriptions. To determine the identity of compounds in the profile, a chromatographic run with FID was made just before each sniffing session. The co-chromatogram was used to compare with the result from GC/MS analysis.

Estimation of compound concentration at the end of sniffing port was done by comparing the integrated peak area of each compound obtained from FID with the internal standard (0.226 mg/ml propyl tiglate) by assuming all response factors of 1. Compounds as eluted from the GC column were subjected to further dilution by

humidified air supplied at the sniffing port. Dilution was calculated based on linear velocity of GC effluent and velocity of humidified air supplied.

GC/MS analysis

A Hewlett-Packard Model 5890 Series II gas chromatograph coupled with Hewlett-Packard Model 5971 quadrupole mass selective detector was used. The GC was equipped with split/splitless injection port and a 60-m, 0.25 mm i.d. and 0.25-µm film thickness Supelcowax 20M (Supelco, Bellefonte, PA) capillary column. The GC oven temperature was programmed as follows: 60 °C hold for 5 min, and then increased to 220 °C at a rate of 5 °C/min. Helium at a linear velocity of 24.8 cm/sec was used as carrier gas. The injection temperature was set at 200°C. Mass spectra ionization potential was set at 70 eV. Transfer line and ion source temperatures were 280 °C and 180 °C, respectively. All injections were made in split mode at 1 to 20 split ratio. Data were acquired with MS Chemstation software (Hewlett-Packard Co., USA).

Identification of compounds was assigned based on comparison of spectra and relative abundance with NIST/EPA/MSDC 54K Mass spectral database (Hewlett-Packard Co., USA) by a Probability based-matching (PBM) algorithm (McLafferty et al., 1974). We accepted as resonable matches PBM > 75%, most were > 95%. In the absence of matched reference spectra, Kováts retention index were used for tentative compound identity and fragmentation patterns were deduced from the spectra obtained.

Results

'Bartlett' pears

The aroma description and intensity of the odor active compounds isolated from headspace of 'Bartlett' pears are listed in Figure 4.1 and Table 4.1. Among the complex mixture of approximately 64 compounds separated on the GC column, 31 compounds were odor-active. Eleven compounds clearly possessed fruity or estery aroma. Specific pear aroma was obtained from 7 different compounds including 2-methylpropyl acetate, butyl butanoate, pentyl acetate, ethyl hexanoate, hexyl acetate, ethyl cis-2, cis-4 decadienoate and ethyl trans-2, cis-4 decadienoate. The highest intensity of pear-like aroma was observed from pentyl acetate and hexyl acetate. Those two compounds were rated to have a strong to very strong pear and fruity odor. Three isomers of ethyl decadienoates are found to possess different odor characteristics. The cis-2, cis-4 isomer provided pear-fruity scent and the trans-2, cis-4 provided pear-peel-green while the trans-2, trans-4 isomer provided alcoholic or fermented-like characteristic. Note that all of these three compounds would give similar responses if not resolved by HPLC, such as used by Quamme's group.

Besides the eleven compounds which possessed pear or fruity aroma, another 7 compounds were found to have positive odor characteristics by providing floral, sweet, candy or perfume aroma. Those compounds were in a group of esters such as propyl acetate, isoamyl acetate, 1-methylpropyl butanoate and 3-methylbutyl 3- methylbutanoate. The odor intensity of those compounds ranged from just detectable to slight on an ordinal score. Another 3 compounds in the group of esters were found to possess mushroom-like odor characteristics. These compounds include 4-hexen-1-ol acetate, 2-methylpropyl hexanoate and ethyl -2,4-octadienoate.

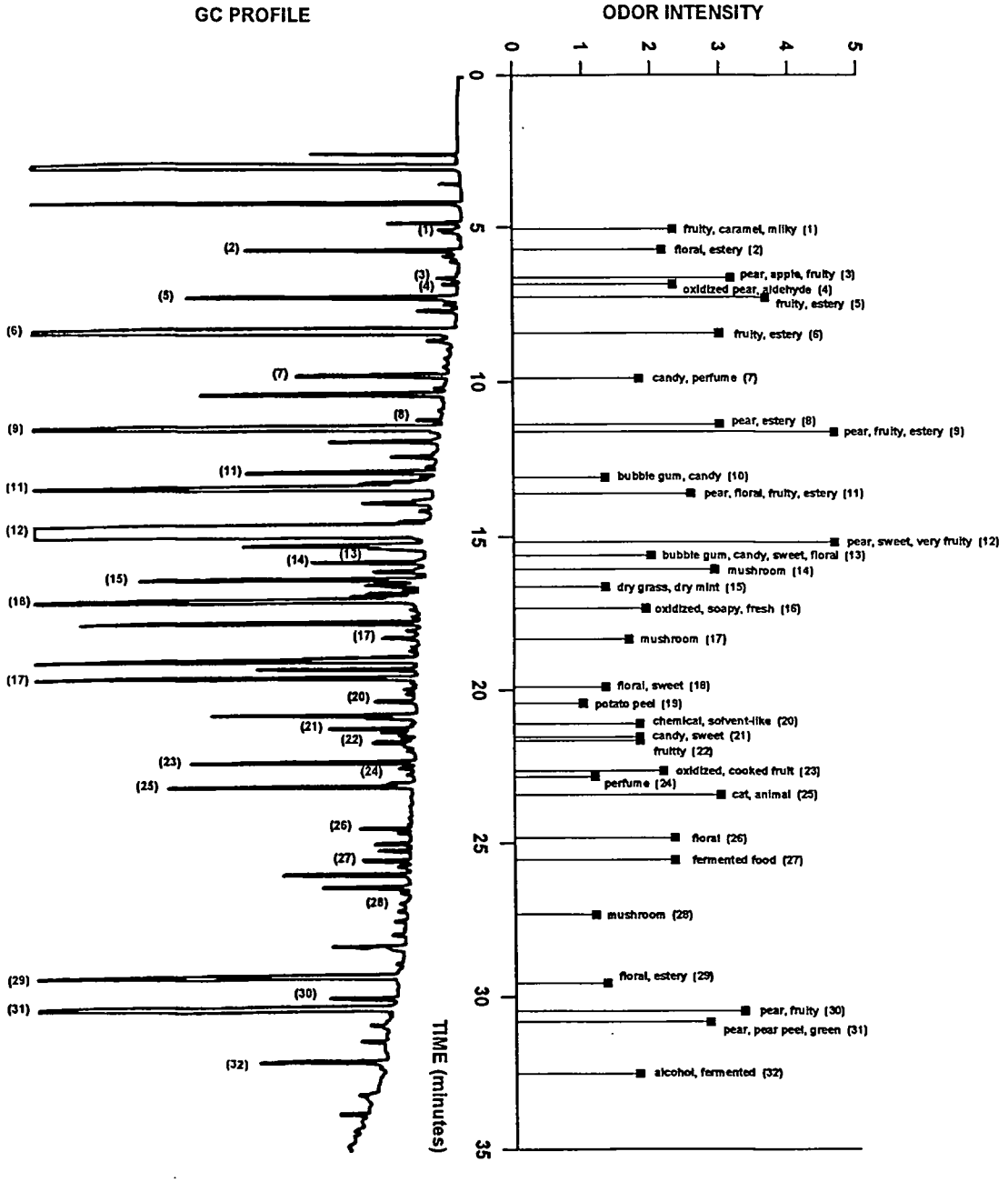

Figure 4.1. Gas chromatogram (FID) and odor description and mean value of intensity at the sniffing port of headspace volatiles from 'Bartlett' pear.

Table 4.1. List of odor-active compounds identified from 'Bartlett' pears.

Peak Number[a]	Retention time (min)	Compounds	Odor Quality	Conc. (ppb)[b]	Odor Intensity[c]
1	5.05	Ethyl propanoate	fruity, caramel, milky	15	2.3
2	5.72	Propyl acetate	floral, estery	125	2.2
3	6.62	2-Methylpropyl acetate	pear, apple, fruity	18	3.2
4	6.83	1-Propanol	oxidized pear, aldehyde	11	2.3
5	7.26	Ethyl butanoate	fruity, estery	181	3.7
6	8.42	Butyl acetate	fruity, estery	12448	3.0
7	9.89	Isoamyl acetate	candy, perfume	129	1.8
8	11.36	Butyl butanoate	pear, estery	19	3.0
9	11.61	Pentyl acetate	pear, fruity, estery	1830	4.7
10	13.07	1-Methylpropyl butanoate	bubble gum, candy	137	1.3
11	13.59	Ethyl hexanoate	pear, floral, fruity, estery	1056	2.6
12	15.17	Hexyl acetate	pear, floral, sweet, very fruity	73860	4.7
13	15.61	3-Methylbutyl 3-methyl butanoate	bubble gum, candy, sweet, floral	39	2.0
14	16.06	4-Hexen-1-ol acetate	mushroom	80	2.9
*15	16.63	Propyl tiglate	dry grass, dry minty	222	1.3
16	17.34	1-Hexanol	oxidized, soapy, fresh	978	1.9
17	18.34	2-Methylpropyl hexanoate	mushroom	12	1.7
18	19.89	Ethyl octanoate	floral, sweet	252	1.3
19	20.42	1-Heptanol	potato peel	11	1.0
20	21.09	Octyl acetate	chemical, solvent-like	134	1.8
21	21.52	Ethyl 2,4 hexadienoate	candy, sweet	64	1.8
22	21.64	Ethyl 3-octenoate (Z)	fruity	23	1.8
23	22.63	Methyl trans-2 octenoate	oxidized, cooked fruit	148	2.2
24	22.82	4-Octen-1-ol acetate	perfume	12	1.2
25	23.41	Unidentified	cat, animal	164	3.0
26	24.81	Hexyl hexanoate	floral	33	2.3
27	25.54	Ethyl decanoate	fermented food	21	2.3
28	27.32	Ethyl 2,4 octadienoate	mushroom	10	1.2
29	29.55	Methyl trans-2, cis-4 decadienoate	floral, estery	353	1.3
30	30.47	Ethyl cis-2, cis-4 decadienoate	pear, fruity	48	3.3
31	30.82	Ethyl trans-2, cis-4 decadienoate	pear, pear peel, green	2614	2.8
32	32.58	Ethyl trans-2, trans-4 decadienoate	alcohol, fermented	97	1.8

* Compound used as an internal standard.

[a] Peak number corresponds to Figure 4.1.

[b] Estimate concentrations at the end of sniffing port in ppb. (Calculation based on dilution of the GC effluent by humidified air supplied at the sniffing port.)

[c] Odor intensity ranked from 1= just detectable, 3= moderate to 5= very strong.

'Comice' pears

Among 72 compounds separated from capillary GC, at least 22 compounds from headspace volatiles of 'Comice' pear were found to be odor active compounds (Figure 4.2 and Table 4.2). Among 9 compounds which possessed fruity aroma, six were found to have pear-like aroma characteristics. These esters included butyl acetate, pentyl acetate, ethyl hexanoate, hexyl acetate, methyl trans-2, cis-4 decadienoate and ethyl trans-2, cis-4 decadienoate. High odor intensity ranging from moderate to strong pear aroma were again observed from hexyl acetate and butyl acetate while ethyl trans-2, cis-4 decadienoate was rated lower as slight to moderate in intensity. The highest odor intensity was obtained from isoamyl acetate which was characterized as candy or perfume-like aroma. Along with isoamyl acetate, six other compounds were found to contribute the same positive characteristic of odor as floral, candy, sweet or perfume aroma. Those compounds were 2-methylpropyl acetate, pentyl octanoate, 4-octen-1-ol acetate, hexyl hexanoate, methyl 2,4,6 octatrienoate and 2-phenylethyl acetate. The last compound is an aromatic ester which represents the first time this compound was identified in pears. It has been reported to contribute sweet, rose-, honey-like aroma in several fruits such as apple, plum, mango and guava (Stevens, 1970).

'Anjou' pears

The aroma of 'Anjou' pear was characterized by a group of 27 odor active compounds (Figure 4.3 and Table 4.3). Nine esters possessed fruity or estery odor. Seven of them included butyl acetate, pentyl acetate, hexyl acetate, butyl 4-hexenoate plus butyl 2-methylbutanoate (coeluted), methyl trans-2, cis-4 decadienoate, ethyl cis-2, cis-4 decadienoate and ethyl trans-2, cis-4 decadienoate

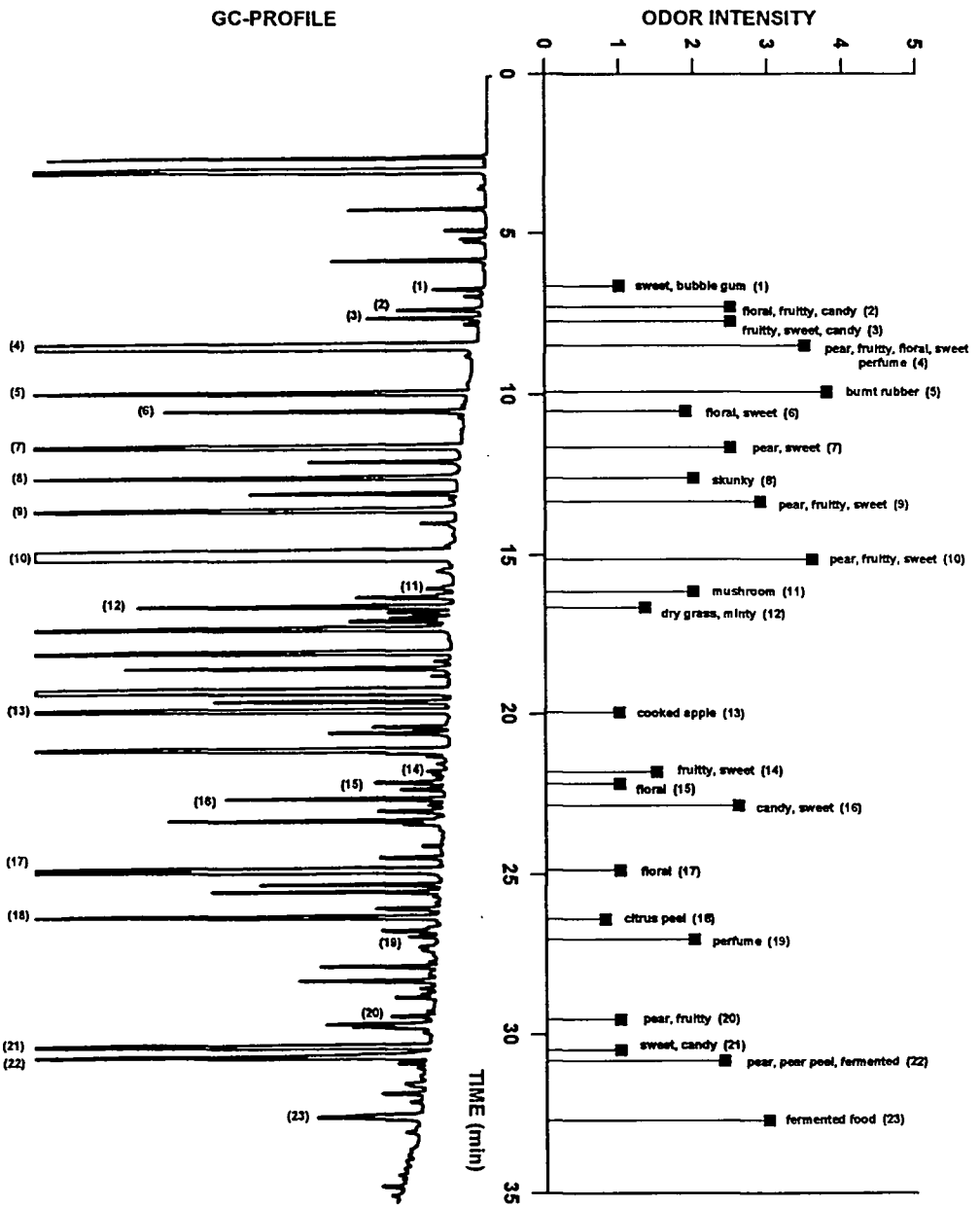

Figure 4.2. Gas chromatogram (FID) and odor description and mean value of intensity at the sniffing port of headspace volatiles from 'Comice' pear.

Table 4.2. List of odor-active compounds identified from 'Comice' pears.

Peak Number[a]	Retention time (min)	Compounds	Odor Quality	Conc. (ppb)[b]	Odor Intensity[c]
1	6.65	2-Methylpropyl acetate	bubble gum, sweet	10	1.0
2	7.30	Ethyl butanoate	floral, fruity candy	18	2.5
3	7.75	Ethyl 2-methylbutanoate	fruity, sweet, candy	3	2.5
4	8.50	Butyl acetate	pear, fruity, floral, sweet, bubble gum, very perfume	7459	3.5
5	9.93	Isoamyl acetate	candy, perfume	162	3.8
6	10.52	1-Butanol	medicinal	68	1.9
7	11.65	Pentyl acetate	pear, sweet	495	2.5
8	12.60	2-Methyl 1-butanol	skunky	97	2.0
9	13.34	Ethyl hexanoate	pear, sweet, fruity	222	2.9
10	15.15	Hexyl acetate	pear, sweet	15501	3.6
11	16.16	4-Hexen-1-ol acetate (Z)	mushroom	6	2.0
*12	16.67	Propyl tiglate	dry grass, minty	76	1.4
13	19.95	Ethyl octanoate	cooked apple	412	1.0
14	21.81	Ethyl 3-octenoate (Z)	fruity, sweet	5	1.5
15	22.17	Pentyl octanoate	floral	19	1.0
16	22.86	4-Octen-1-ol acetate	candy, sweet	5	2.6
17	24.87	Hexyl hexanoate	floral	196	1.0
18	26.39	Ethyl 4-decenoate	citrus	109	0.8
19	27.01	Methyl 2,4,6 octatrienoate	perfume	7	2.0
20	29.55	Methyl trans-2, cis-4 decadienoate	pear, fruity	11	1.0
21	30.50	2-Phenylethyl acetate	sweet, candy	263	3.6
22	30.84	Ethyl trans-2, cis-4 decadienoate	cooked pear fruit, green	157	2.4
23	32.69	Ethyl trans-2, trans-4 decadienoate	fermented food, sour	45	3.0

* Compound used as an internal standard.

[a] Peak number corresponds to Figure 4.2.

[b] Estimate concentrations at the end of sniffing port in ppb. (Calculation based on dilution of the GC effluent by humidified air supplied at the sniffing port.)

[c] Odor intensity ranked from 1= just detectable, 3= moderate to 5= very strong.

were characterized as pear-like aroma. Highest pear aroma intensity was observed from hexyl acetate followed by butyl acetate. Both compounds were rated between strong to very strong odor. Three isomeric forms of 2,4 decadienoate esters were also found to have different odor characteristics as those observed in 'Bartlett' pears. Ethyl cis-2, cis-4 decadienoate was described as pear or fruity while ethyl trans-2, cis-4 decadienoate was characterized by ripened pear, pear blend or fruity aromas.

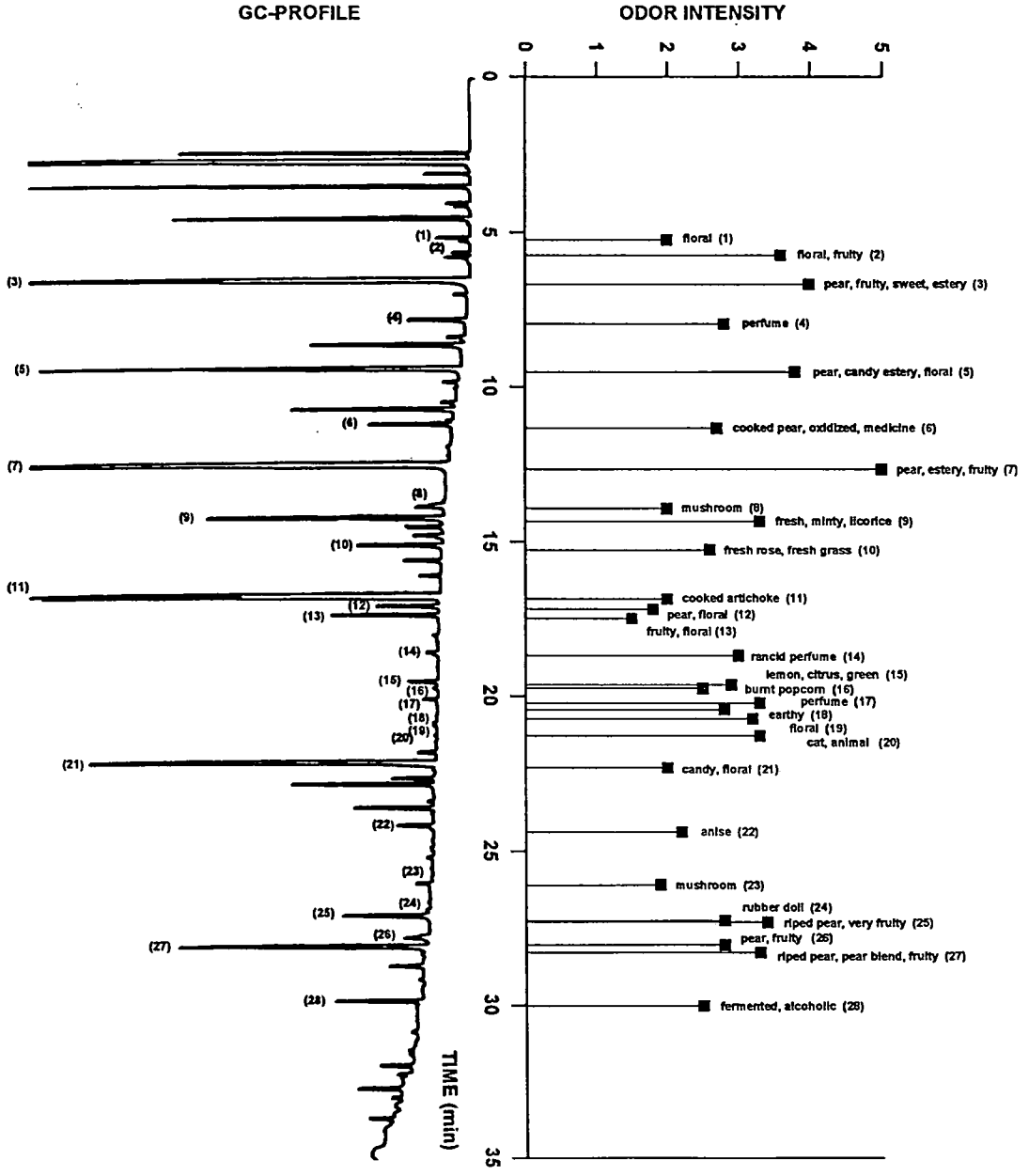

Figure 4.3. Gas chromatogram (FID) and odor description and mean value of intensity at the sniffing port of headspace volatiles from 'Anjou' pear.

Table 4.3. List of odor-active identified compounds from 'Anjou' pears.

Peak Number[a]	Retention time (min)	Compounds	Odor Quality	Conc. (ppb)[b]	Odor Intensity[c]
1	5.25	2-Methylpropyl acetate	floral	28	2.0
2	5.74	Ethyl butanoate	floral, fruity	15	3.6
3	6.67	Butyl acetate	pear, estery, sweet, fruity	4578	4.0
4	7.96	Isoamyl acetate	perfume	59	2.8
5	9.53	Pentyl acetate	pear, candy, estery, floral	375	3.8
6	11.35	Ethyl hexanoate	cooked pear, oxidized, medicine	75	2.7
7	12.66	Hexyl acetate	pear, estery, fruity	4276	5.0
8	13.93	4-Hexen-1-ol acetate (Z)	mushroom	16	2.0
*9	14.34	Propyl tiglate	fresh, minty, licorice	222	3.3
10	15.25	1-Hexanol	fresh rose, fresh grass	83	2.6
11	16.84	Butyl hexanoate	cooked artichoke	552	2.0
12	17.18	Butyl 4-hexenoate + Butyl 2-methylbutanoate	pear, floral	58	1.8
13	17.49	Ethyl octanoate	fruity, floral	93	1.5
14	18.69	Octyl acetate	rancid perfume	18	3.0
15	19.64	Pentyl hexanoate	lemon, citrus, green (citrus-leaf)	30	2.9
16	19.75	Heptyl butanoate + Propyl octanoate	burnt popcorn	24	2.5
17	20.22	4-Octen-1-ol acetate	perfume	12	3.3
18	20.43	1-Octanol	earthy	40	2.8
19	20.72	Ethyl trans-2 octenoate	floral	43	3.2
20	21.28	unidentified	cat, animal, skunky	22	3.3
21	22.3	Hexyl hexanoate	candy, floral	311	2.0
22	24.38	Methyl 4-decenoate	anise	41	2.2
23	26.09	Octyl hexanoate + Methyl 2-decenoate	mushroom	8	1.9
24	27.25	unidentified	rubber doll	15	2.8
25	27.3	Methyl trans-2, cis-4 decadienoate	ripe pear, very fruity	89	3.4
26	28.04	Ethyl cis-2, cis-4 decadienoate	pear, fruity	27	2.8
27	28.28	Ethyl trans-2, cis-4 decadienoate	ripe pear, pear blend, fruity	235	3.3
28	30.01	Ethyl trans-2, trans-4 decadienoate	fermented, alcoholic	85	2.5

* Compound used as an internal standard.

[a] Peak number corresponds to Figure 4.3.

[b] Estimate concentrations at the end of sniffing port in ppb. (Calculation based on dilution of the GC effluent by humidified air supplied at the sniffing port.)

[c] Odor intensity ranked from 1= just detectable, 3= moderate to 5= very strong.

Ethyl trans-2, trans-4 decadienoate possessed fermented or alcoholic odor characteristic.

Like the findings with 'Bartlett' and 'Comice' pears, six other esters also possessed positive odor characteristics as floral, sweet, candy or perfume-like

aromas. These compounds included 2-methylpropyl acetate, isoamyl acetate, octyl acetate, 4-octen-1-ol acetate, ethyl trans-2 octenoate and hexyl hexanoate and were rated from slight to moderate in odor intensity.

4-Hexen-1-ol acetate, octyl hexanoate and methyl 2-decenoate were described as mushroom-like aroma while pentyl hexanoate was described as green lemon or citrus-like odor.

Discussion

In general, all odor-active compounds in 'Bartlett', 'Comice' and 'Anjou' pears were found to be esters. Depending on variety, nine to eleven compounds generally possessed fruity aroma. The results from sniffing GC clearly indicate that pear aroma is not solely dependent on the few compounds which have been reported earlier. Jennings and Sevenants (1964) reported hexyl acetate as a 'contributory flavor compound' while Jennings et al. (1964) described ethyl trans-2, cis-4 decadienoate as a 'character impact compound' for 'Bartlett' pear. However, those authors did not provide any details on whatever sensory evaluation techniques were used to reach their conclusions. In our study, strong, specific pear-like aroma was detected in a group of 9 compounds. These compounds included 2-methylpropyl acetate, butyl acetate, butyl butanoate, pentyl acetate, ethyl hexanoate, hexyl acetate, methyl trans-2, cis-4 decadienoate, ethyl cis-2, cis-4 decadienoate and ethyl trans-2, cis-4 decadienoate. Hexyl acetate and some of the acetate esters of lower carbon number alcohols possessed a strong pear-like aroma while two isomers of ethyl decadienoate ester possessed similar pear odor quality, but somewhat lower in intensity. This group of compounds were found in all three pear varieties and were consistently described by panelists as very estery, fruity and having pear-like aroma. Thus, those

9 compounds collectively are likely to be described as 'flavor impact compounds' in pear.

Besides compounds which possessed pear-like aroma, other compounds possessed very positive odor characteristics such as floral, sweet, candy or perfume-like aroma. Those compounds were observed to be slightly different between three pear varieties and can significantly affect overall sensation by serving as 'contributory to flavor compounds' in pears.

The comparison between odor active compound profiles obtained from these three pear varieties indicated the possibility that some compounds might have a significant effect to provide a typical odor for each pear variety. For instance, 2-phenylethyl acetate in 'Comice' pear and pentyl hexanoate in 'Anjou' pear, were compounds observed to be fairly potent aroma active compounds and were found specific to the variety. However, we ought not make a conclusion based only on this observation, since flavor is a complex sensation of a combination of taste and aroma. Moreover, in fresh fruit, texture can have a significant effect on overall flavor, because the compounds have to be released by crushing of cells in chewing. In this case, the difference in flavor between pear varieties can best be explained by the proportional blending between all odor active compounds in the profile, the specific volatile compound(s) in the profile, the difference in taste such as sweetness or other non-volatile descriptors or the combination of above factors. Therefore, more research effort concerning sensory analysis is warranted to refine those questions.

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CHAPTER 5

DEVELOPMENT OF VOLATILE COMPOUNDS DURING RIPENING OF PEAR (*Pyrus communis* L.) VARIETIES IN RELATION TO SENSORY PERCEPTION

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Abstract

Headspace volatile flavor compounds, flesh firmness and sensory attributes were measured in 'Bartlett', 'Packham's Triumph' and 'Anjou' pears during ripening at 20°C. The production of volatile compounds during ripening of pears are largely dominated by the increase of various esters and alcohols concomitant with the rapid decrease of hydrocarbons, α-farnesenes. The highest rate of ester production was observed in fruits at the optimum eating-ripe stage. The production of major esters among three varieties were found to increase at different rates and times during ripening. Furthermore, various esters were found to increase at rates that vary in a cyclic manner. The maximum pear flavor intensity and general acceptability as judged by an experienced taste panel were obtained from fruits after ripening for 4-6 days for 'Bartlett', 10 days for 'Packham's Triumph' and 6-8 days for 'Anjou'. Several odor active compounds were found to correlate well with pear flavor intensity and general acceptability. Those compounds included ethyl acetate, propyl acetate, methyl and ethyl trans-2, cis-4- decadienoate, ethyl cis-2, cis-4 decadienoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl trans-2 octenoate. The above compounds have been previously reported to possess pear-, fruity- or floral-like odor characteristics. The difference in significant levels of these compounds along with other odor active compounds may be responsible for differences in overall aroma complexity between these pear varieties.

Introduction

Generally, both summer and winter pears are harvested in a mature but unripe- stage of development. The fruits are then stored until marketed fresh or processed. In both cases, the fruit must be ripened in order to achieve optimum

eating quality. Fruits upon removal from cold stored are best ripened at 20°C (Claypool et al., 1958; Mellenthin and Wang, 1976; Chen et al., 1983). The duration of ripening to obtain good dessert quality varies between pear varieties and length of time in storage, normally 4 days for 'Bartlett', 4-5 days for 'Bosc' and 'Comice' and 7-10 days for 'Anjou' and 'Packham's Triumph' (Wrolstad et al., 1991).

Volatile flavor components of 'Bartlett' pear have been extensively investigated (Jennings,1961; Jennings and Creveling, 1963; Jennings and Sevenants, 1964; Jennings et al., 1964; Heinz et al., 1966; Creveling and Jennings, 1970; Jennings and Tressl, 1974). Ethyl trans-2, cis-4 decadienoate has been reported to be a character impact compound (Jennings et al., 1964) and hexyl acetate as a contributory flavor compound (Jennings and Sevenants, 1964). The decadienoate esters appeared to be present in pear cultivars with Bartlett-like aroma (Quamme and Marriage, 1977; Russell et al., 1981). Quamme (1984) used high pressure liquid chromatography and UV absorbance at 267nm to correlate decadienoate ester levels of 29 canned pear cultivars with data obtained by sensory evaluation. The results indicated that high decadienoate levels appear to be a characteristic of 'Bartlett' and other cultivars with similar aroma.

The production of decadienoate esters of 'Bartlett' as observed by ultraviolet spectrophotometry increased rapidly in fruit during the climacteric rise of respiration and reached the maximum 1-2 days after the maximum production of ethylene and CO_2, and coincided with optimum eating quality of the fruit (Heinz et al., 1965). Similar results were observed for other major esters including ethyl, propyl, butyl, and hexyl acetate production from dynamic headspace of 'Bartlett' pears (Jennings and Tressl, 1974). Recently, the development of volatile compounds during fruit ripening has been reported in 'La France' pear compared to those of 'Bartlett' (Shiota, 1990).

The concentration of major esters in 'La France' increased markedly as fruit increased in maturity and reached maximum just before the fully ripened stage. However, a non-linear production of major esters were observed in 'Bartlett' with the maximum observed just before the fully ripe stage.

Solid-phase microextraction (SPME) is a new solvent-free sample extraction technique developed at the University of Waterloo, Canada (Arthur and Pawliszyn, 1990; Arthur et al., 1992a,b; Potter and Pawliszyn, 1992). It basically consists of an absorption of analytes on a GLC liquid phase pre-coated on a fused silica fiber followed by thermal desorption of those analytes in the GC injection port. The SPME technique can be used to concentrate both volatile or non-volatile compounds in both liquid or gaseous (headspace) samples. The selectivity of analytes has recently been improved by altering type and thickness of the phase coated fiber (Mindrup, 1995). The method has been successfully applied for determination of various classes of compounds in different types of samples such as substituted benzene and phenolic compounds in water (Arthur et al, 1992; Potter and Pawliszyn, 1992; Bucholz and Pawliszyn, 1993), caffeine in beverages (Hawthorne and Miller, 1992) and halogenated compounds in foods (Page and Lacroix, 1993). Recently, the technique has been applied to determine flavor compounds from various food samples (Woolley and Mani, 1994; Yang and Peppard, 1994; Mindrup, 1995).

In the previous studies (Chapters 3 and 4), we tentatively identified volatile compound profiles from various pear varieties at the eating ripe stage using GC/MS and determined aroma active compounds in some pear varieties by using the gas chromatography-olfactometry technique. In this study, the newly developed solid-phase microextraction (SPME), was applied to detect and quantitate various volatile aroma components from static headspace of three pear varieties including 'Bartlett',

'Packham's Triumph', and 'Anjou' during ripening. The evolution of volatile compounds during fruit ripening were used to correlate with data obtained from sensory evaluation. The results from the study not only may provide a broader picture of development of volatile flavor compounds in ripening various pear varieties, which was not the focus of the previous research, but may also explain the direct relationship between a specific compound or group of compounds and consumer sensory perception.

Materials and Methods

Fruit samples

'Bartlett', 'Packham's Triumph' and 'Anjou' fruit were hand harvested at the Southern Oregon Experimental Station, Oregon State University, Medford, Oregon during 1993. Fruits were harvested at optimum maturity stages based on commercial flesh firmness value ('Bartlett 89N; 'Packham's Triumph' 69N; 'Anjou' 62N). All fruits were dipped in 500 ppm Benomyl (fungicide) and 2,300 ppm Ethoxyquin solution (anti-scald), air dried, then packed in 20 kg standard pear boxes with perforated polyethylene liners and stored at -1 to 1 °C for 2 months before use.

Volatiles sample preparation

After the chilling requirement was satisfied, fruits of each variety were removed from cold storage and ripened at 20°C in perforated polyethylene bags. To obtain volatile compound samples from fruits, a set of 5 to 6 fruits weighing between 1,200 to 1,400g were placed in 4L glass jars and sealed with air-tight Teflon seal lids. The jars were allowed to stand at ripening temperature (20°C) for 1 hr before collecting samples. Headspace volatile compounds emitted from fruits were collected

by the solid-phase microextraction (SPME) technique. A pre-heated 100 µm film thickness of poly(dimethylsiloxane) coated fused silica fiber SPME (Cat. No. 5-7300, Supelco, Bellefonte, PA) was inserted into the jar through a rubber stopper. The fiber was allowed to equilibrate with head-space volatile compounds for 30 min before direct thermal desorption in the gas chromatograph injection port. A Hewlett Packard model 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and a 25 m, 0.2 mm i.d. and 0.2 µm film thickness Carbowax 20M (HP-20M, Hewlett Packard) capillary column was used. The GC oven temperature was programmed with an initial hold at 60°C for 5 min and increased to 220°C at a rate of 5°C/min. High purity (99.9995 %) helium carrier gas was regulated at a linear velocity of 17.0 cm/sec. The injector and detector temperatures were set at 200°C and 250°C, respectively. All injections were made in split mode at 1:10 split ratio. Data were collected and analyzed by a HP 3395 Integrator (Hewlett Packard, USA). The same set of fruits were used to sample head-space volatile compounds throughout the course of ripening.

Identification and quantitation of compounds

Identification of head-space volatile compounds was done by comparing retention time of peaks obtained from GC-FID with retention times of identified compounds obtained from GC/MS using the same Carbowax 20M column. Ethyl ester retention index (Van den Dool and Kratz, 1963) was constructed by using retention times of C2 to C10 ethyl esters as external standards. A Hewlett-Packard Model 5890 Series II gas chromatograph coupled with Hewlett-Packard Model 5971 quadrupole mass selective detector was used for GC/MS analysis. The GC was equipped with split/splitless injection port and a 60-m, 0.25 mm i.d. and 0.25-µm film

thickness Supelcowax 20M (Bellefonte, PA) capillary column. The GC oven temperature was programmed as follows: hold at 60 °C for 5 min and then increased at 5 °C/min to 220 °C and hold at 220°C for 30 min. Helium at a linear velocity of 24.8 cm/sec was used as carrier gas. The injection temperature was set at 200°C. Mass spectral ionization potential was set at 70 eV. Transfer line and ion source temperatures were 280 °C and 180 °C, respectively. All injections were made in 1:20 split mode ratio. Data were acquired and integration performed with MS Chemstation software (Hewlett-Packard Co., USA). Identification of compounds was based on retention time and comparison of mass spectra and relative abundance with NIST/EPA/MSDC 54K Mass spectral database (Hewlett-Packard Co., USA) and our own pear volatiles spectra library constructed in the Postharvest Physiology Lab., Department of Horticulture, Oregon State University by a Probability based-matching (PBM) algorithm (McLafferty et al., 1974).

Estimation of compound concentrations was done by comparing integrated peak area of each compound with a peak of an internal standard (5 mg/ml propyl tiglate) spiked through the jar at the beginning of incubation period and assuming all response factors of 1. The propyl tiglate standard was found to elute in the middle of the GC chromatogram. Concentrations of compound were expressed as the weight of compound per unit weight of fruit per unit time, i.e. µg/kg/hr.

Sensory evaluation

Sensory evaluations were performed on fruits at 2 day intervals after removal from cold storage. Five fruits were randomly selected at specific ripening periods for the test. Fruits ready for the test were assigned three digit random numbers, longitudinally cut into 8-12 pieces and presented to the panelists within 5 min before

the session. Preliminary observations indicated only small variations between fruits in texture (as indicated by flesh firmness values) and certain taste indicators (total soluble solids, titratable acidity and pH).

A group of 8 panelists were selected from a group of colleagues in the Department of Horticulture, Oregon State University based both on taste panel experience with pears and availability. Panelists were not specially trained but all were familiar with pear quality evaluation, descriptive analysis and consumer tests. The same group of panelists was used throughout the experiment. An isolated and comfortable area in the Postharvest physiology lab was prepared to facilitate sensory evaluation. All tests were performed under fluorescent lights at room temperature (20°C). Panelists were asked to rate the fruit they tasted for pear flavor intensity and texture based on a 16-point category scale (0=none to 15=extreme) and general acceptability based on a 9-point hedonic scale (see sample ballot in Appendix 5.1). Panelists were requested to wash their mouths with cool tap water after tasting each sample. Each session contained not more than six samples and not more than two sessions were performed on the same day. Data were analyzed using the SAS general linear model procedure (SAS Institute, Inc., Cary, NC) based on a completely randomized design. Least significant differences tests (LSD) at the 0.05 level of significance were used to separate means. Data presented are the averages of scores and standard error of means (n= 8) for each ripening period. The Pearson correlation coefficients (r-values) between the production of compounds and the sensory data were performed with the same software using compound concentrations as independent variables and sensory scores as dependent variables.

A separate set of five fruits from each treatment were used to measure flesh firmness during the course of ripening. Flesh firmness was measured on two peeled

sides using a UC-Davis firmness penetrometer (Western Ind. Supply, CA) equipped with an 8 mm diameter tip.

Results and Discussion

In comparison with solvent extraction, simultaneous/distillation extraction, and conventional solid-phase extraction or purge and trap sampling, which are the most frequently used sample preparation techniques in flavor research (Cronin, 1982; Reineccius, 1993), the SPME technique has proven to be a fast, simple, sensitive, repeatable, and inexpensive method to detect and quantify volatile compounds during fruit ripening. The technique has the advantage over various solvent extractions or purge and trap methods by reducing a possibility of losses of compounds during concentration or by contamination from solvents used for extraction or purged air streams. Furthermore, a static headspace trapping of compounds by SPME over a fixed time allowed us to accurately follow the changes of particular volatile compounds such as α-farnesene or various decadienoates esters without excessive losses due to oxidation.

Sample GC chromatograms obtained from SPME of static headspace of 'Bartlett' pear at 0, 4 and 8 days of ripening at 20°C are shown in Figure 5.1.

'Bartlett' pears

The highest general acceptability taste panel score was observed for 'Bartlett' fruits after 4 days of ripening when fruits had flesh firmness values of 11N (Figure 5.2). Fruits at this firmness have been reported to have juicy, melting, buttery texture and have maximum dessert quality (Chen et al., 1981). The highest pear flavor intensity score was observed 6 days after ripening at 20°C which agreed with the

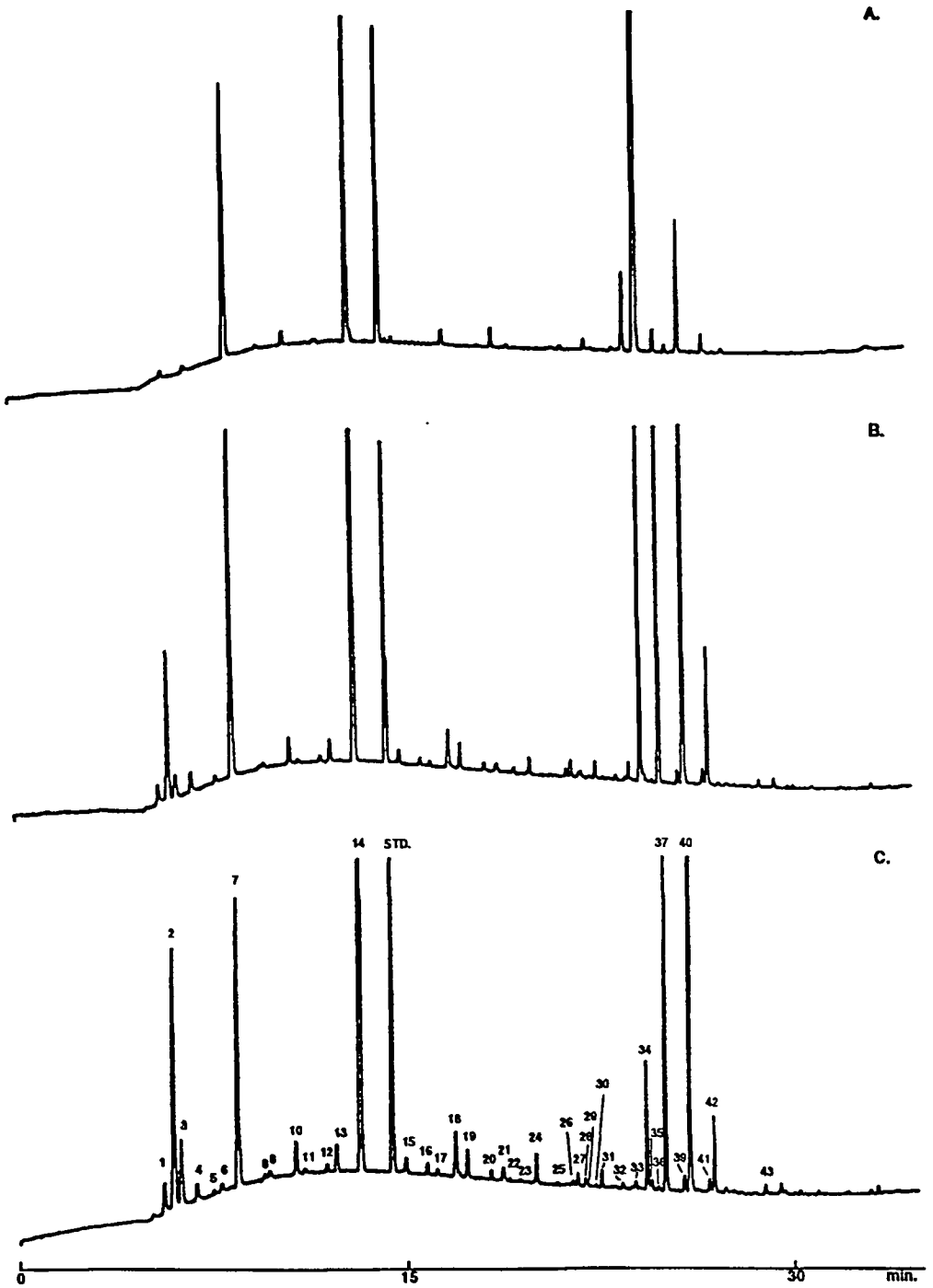

Figure 5.1. Gas chromatogram of headspace volatile compounds from 'Bartlett' pear during ripening for 0 (A), 4 (B) and 8 (C) days at 20°C. Compounds were extracted from headspace sample using SPME technique, directly desorbed in the GC injection port and separated by Carbowax 20M, 25 m x 0.2 mm I.D. capillary column with FID detection. Identification of peaks are listed in Table 5.1.

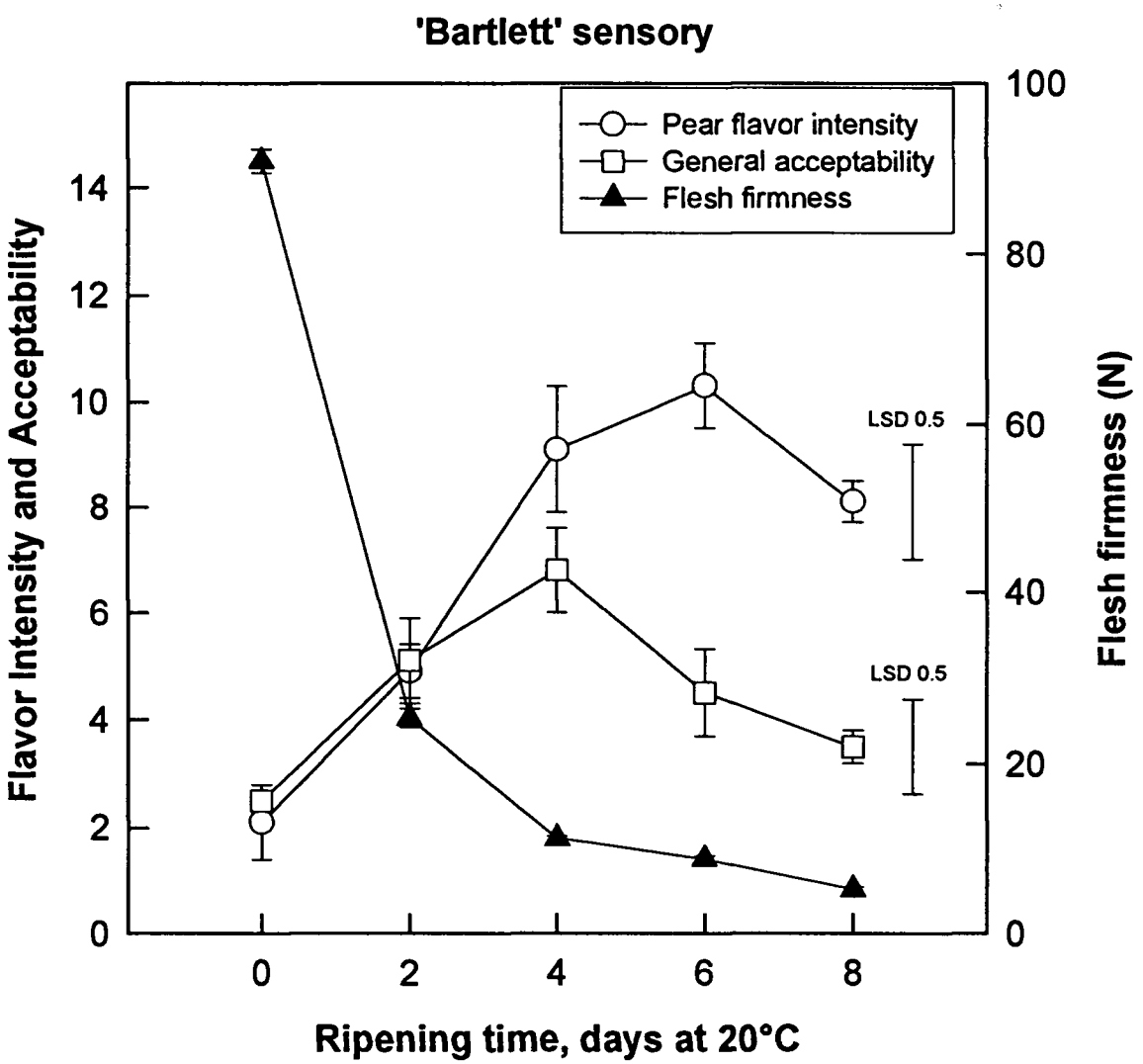

Figure 5.2. Changes in sensory attributes and flesh firmness of 'Bartlett' pear during ripening at 20°C.

timing of the maximum production of esters. The higher general acceptability score which was obtained 2 days before fruits reached the maximum in flavor intensity indicated that consumer acceptance is not solely dependent upon fruit aroma but also on fruit texture.

The changes of volatile compound profiles detected from static headspace of 'Bartlett' pear during ripening at 20°C are shown in Table 5.1. There are 43 compounds detected by the SPME technique. Compounds can be classified into three groups, esters, alcohols and hydrocarbons (represented by two isomers of α-farnesene). A non-linear pattern of the total volatile production was observed throughout the ripening period (Figure 5.3). A rapid decline in the production of both forms of α-farnesene was accompanied by an increase in the total esters at the beginning of fruit ripening. The production of α-farnesene continued to decline as fruits advanced in ripeness and may reflect oxidation of farnesenes. The oxidation of these sesquiterpene hydrocarbons (α-farnesenes) to conjugated trienes is reportedly implicated in superficial scald, a physiological disorder commonly found in apple and pear (Huelin, 1964; Huelin and Coggiola, 1968; Huelin and Coggiola, 1970; Anet, 1974; Anet and Coggiola, 1974; Zoffoli, 1994). However, no scald symptoms were observed in fruits used in this experiment. The decrease of α-farnesene, in this case, may be caused by the direct diffusion or volatilization of compounds from the fruit tissues.

A cyclic pattern of total ester production was observed by two maxima at days 2 and 6 of ripening. Similar results were observed in ripening 'Bartlett' pear by both Jennings and Tressel (1974) and Shiota (1990), but not in 'La France' pear where the esters linealy increased as fruit advanced in ripening. Jennings and Tressel (1974)

Table 5.1. Volatile compounds identified from static headspace of 'Bartlett' pear during ripening at 20°C[a].

Pk. No.	Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
				0	1	2	3	4	6	8
1	5.70	1.60	methyl acetate	nd	nd	5.7	7.3	9.8	11.6	5.0
2	6.07	2.07	ethyl acetate	5.8	8.8	29.3	40.9	62.0	79.8	82.9
3	6.36	2.41	ethanol	nd	1.1	9.1	7.7	11.1	15.9	23.9
4	6.97	3.09	propyl acetate	3.2	2.9	3.8	5.7	7.3	9.2	5.6
5	7.52	3.66	2-methylpropyl acetate	nd	nd	nd	nd	0.6	nd	nd
6	7.89	4.02	ethyl butanoate	nd	nd	1.7	2.0	3.1	3.5	4.2
7	8.56	4.37	butyl acetate	107.5	163.3	169.3	144.4	145.1	134.3	93.8
8	9.61	4.89	isoamyl acetate	nd	nd	nd	1.5	0.9	2.5	2.2
9	9.79	4.97	1-butanol	1.7	2.0	3.2	2.7	3.1	1.2	3.2
10	10.79	5.40	pentyl acetate	5.4	8.8	10.9	9.5	nd	10.3	9.6
11	11.12	5.54	methyl hexanoate	nd	nd	nd	1.1	2.3	1.8	1.4
12	12.00	5.88	butyl butanoate + 1-methylpropyl butanoate	1.4	3.4	3.0	3.0	4.0	2.7	2.5
13	12.33	6.00	ethyl hexanoate	nd	2.1	nd	6.4	10.2	8.3	8.2
14	13.29	6.44	hexyl acetate	140.2	360.1	423.8	372.1	433.2	453.8	374.2
-	14.52	6.95	propyl tiglate (internal standard)	-	-	-	-	-	-	-
15	15.02	7.15	1-hexanol	2.3	4.3	6.3	5.0	5.1	6.2	4.9
16	15.85	7.46	heptyl acetate	nd	1.9	2.5	2.3	2.8	3.1	3.1
17	16.25	7.60	methyl octanoate	nd	nd	nd	1.7	1.9	2.3	1.6
18	16.96	7.85	hexyl butanoate + butyl hexanoate	6.4	20.8	22.3	13.6	15.5	16.3	13.5
19	17.39	8.00	ethyl octanoate	nd	1.7	5.6	5.4	8.4	9.3	7.5
20	18.32	8.45	octyl acetate	nd	2.1	2.9	0.9	2.9	3.0	2.9
21	18.77	8.67	unknown	nd	nd	0.8	1.5	4.1	2.5	4.6
22	18.86	8.71	methyl trans-2 octenoate	6.6	5.6	3.5	nd	nd	nd	nd
23	19.47	8.99	4-octen-1-ol acetate	1.6	4.1	3.6	1.6	1.7	1.7	0.9
24	20.08	9.26	ethyl trans-2 octenoate	nd	nd	3.1	3.3	5.4	6.7	8.0
25	20.89	9.61	methyl 2,4 octadienoate	nd	nd	nd	nd	nd	nd	1.0
26	21.49	9.86	hexyl hexanoate	1.1	3.5	3.4	2.5	3.2	2.7	1.7
27	21.68	9.93	methyl 4-decanoate	nd	1.8	2.6	3.5	6.1	5.5	3.2
28	21.98	10.05	ethyl decanoate	nd	nd	nd	1.7	1.4	1.5	2.0
29	22.07	10.09	ethyl 2,4 octadienoate	nd	0.8	1.0	1.7	1.6	1.5	nd

(Continued)

Table 5.1. Continued.

Pk. No.	Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
				0	1	2	3	4	5	7
30	22.45	10.24	ethyl 4-decenoate	4.2	1.0	nd	nd	nd	nd	nd
31	22.61	10.30	ethyl 2,4 octadienoate	nd	2.6	2.2	3.9	4.5	1.4	4.5
32	23.48	10.63	methyl 2-decenoate	0.5	1.4	nd	0.9	2.2	2.6	1.6
33	23.94	10.80	α-farnesene (E,Z)	22.0	12.5	11.3	7.1	6.3	4.8	2.0
34	24.42	10.98	α-farnesene (E,E)	1016.0	634.5	389.6	165.7	109.6	75.0	28.4
35	24.54	11.02	ethyl trans-2 decenoate	nd	nd	nd	nd	4.7	4.9	3.3
36	24.81	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	1.5	1.6	1.7
37	25.13	11.23	methyl trans-2, cis-4 decadienoate	6.7	33.7	78.6	86.1	119.7	119.9	80.9
38	25.58	11.39	unknown	2.8	1.3	nd	nd	nd	nd	nd
39	25.84	11.48	ethyl cis-2, cis-4 decadienoate	nd	1.1	1.4	2.6	4.3	4.8	3.9
40	26.08	11.56	ethyl trans-2, cis-4 decadienoate	38.1	138.6	222.1	180.6	270.8	260.0	186.5
41	26.84	11.81	methyl trans-2, trans-4 decadienoate	nd	2.6	3.0	3.5	4.8	7.2	3.3
42	27.05	11.88	ethyl trans-2, trans-4 decadienoate	5.4	39.5	46.3	30.0	39.5	32.3	17.0
43	29.06	12.51	methyl tetradecenoate	nd	nd	1.3	1.6	2.7	37.7	2.3
			Total	1378.7	1468.0	1473.1	1131.0	1323.1	1349.4	1006.7

[a] Fruits were harvested at mature unripe stage (flesh firmness 89N) and stored for 2 months at 0°C before use. Compounds were trapped and analysed using SPME technique. Units are calculated based on 180.8 μg propyl tiglate used as internal standard (response factor = 1) and expressed in μg compound kg^{-1} hr^{-1}. nd refers to not detected or detected below 0.05 μg kg^{-1} hr^{-1}.

[b] Retention times on Carbowax 20M column (See Materials and methods for details).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

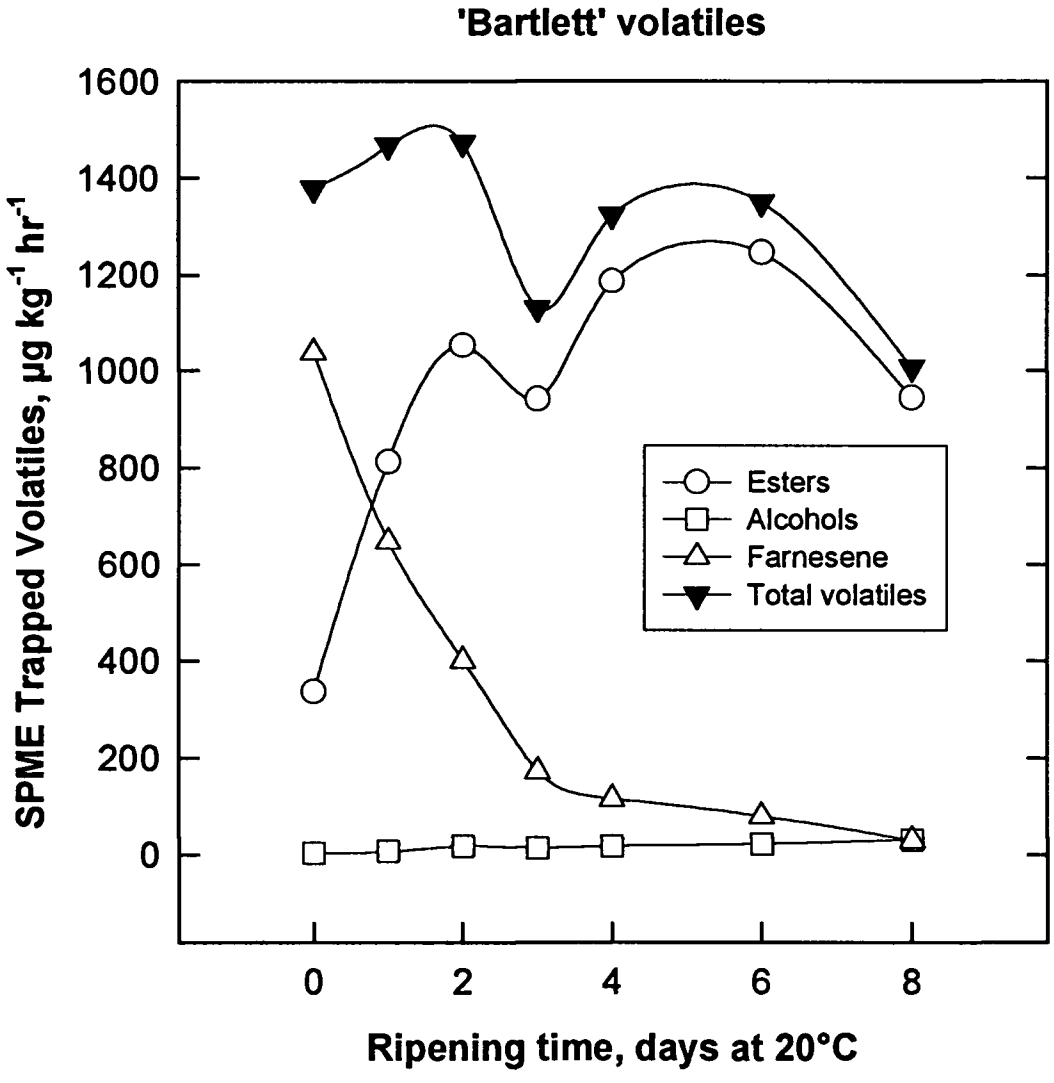

Figure 5.3. Changes in total volatiles, esters, alcohols and farnesenes from static headspace of 'Bartlett' pear during ripening at 20°C.

also reported an increase in α-farnesene production in a cyclic manner out-of-phase with the major acetate esters. The authors proposed that α-farnesene and acetate esters may share a common precursor, acetyl CoA, or some unknown mechanism regulates the production of these compounds during fruit ripening.

The production of alcohol compounds were found to gradually increase throughout the ripening period (Figure 5.3). Three major alcohols were found to be ethanol, 1-butanol and 1-hexanol (Table 5.1). Non-significant changes in alcohol levels during ripening suggests that alcohol availability probably is not a limiting factor in ester synthesis.

Butyl acetate, hexyl acetate, methyl and ethyl trans-2, cis-4 decadienoate were found to be major esters in 'Bartlett' volatile profiles. Hexyl acetate has been reported to be a 'flavor contributory compound' in 'Bartlett' pear (Jennings and Sevenants, 1964). Ethyl trans-2, cis-4 decadienoate was reported as a 'character impact compound' in 'Bartlett' pear (Jennings et al., 1964). However, our previous study indicated all the above compounds possess moderate to strong characteristic pear or fruity odors except methyl trans-2, cis-4 decadienoate which possesses a mild floral odor (Chapter 4). The production of hexyl acetate and ethyl trans-2, cis-4 decadienoate during fruit ripening exhibited a parallel pattern as observed for the total volatiles (Figure 5.4). The production of hexyl acetate and ethyl trans-2, cis-4 decadienoate increased rapidly after fruit began to ripen and reached the first peak at day 2. The production of these two esters then slightly declined on day 3, followed by an increase and reached the second maximum on day 6 of ripening, then gradually declined as fruit advanced in ripening.

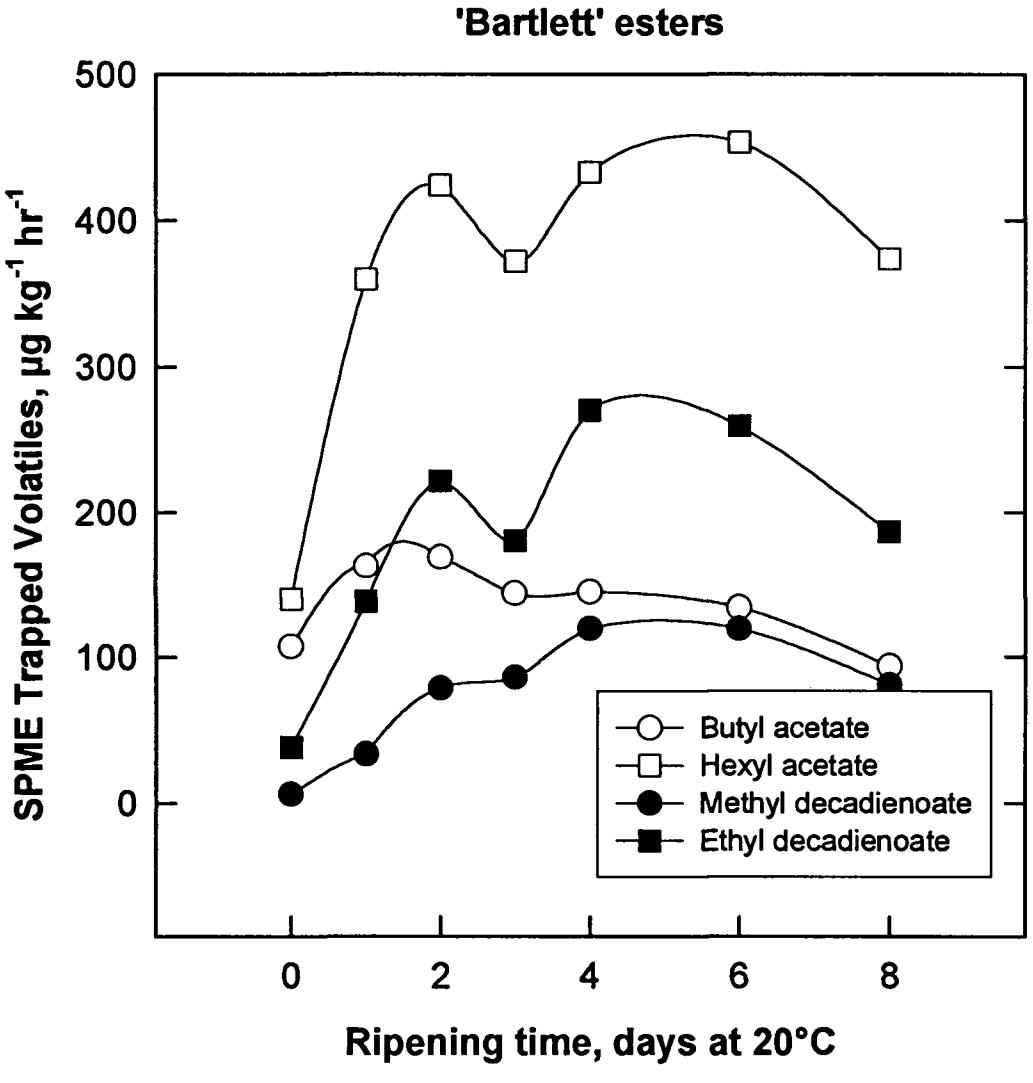

Figure 5.4. Changes in selected esters from static headspace of 'Bartlett' pear during ripening at 20°C.

The changes in these two esters during fruit ripening may account for the cyclic changes found in the total volatiles. Since the acyl moieties are needed for esterification, two phases of ester production may reflect different sources of substrates used for ester synthesis. Degradation of fatty acids by β-oxidation is believed to be the main source of the acyl moiety used for ester synthesis in various fruits such as apple (Paillard, 1979), banana and strawberry (Tressel and Albrecht, 1986) and pear (Heinz and Jennings, 1966; Jennings and Tressel, 1974). Incubation of unsaturated fatty acids with pear puree also resulted in the formation of certain esters (Russell et al., 1981). As fruit ripening starts, chloroplast breakdown is readily observed by the change in fruit color from green to yellow. In this case, a breakdown of lipid membranes of the chloroplasts is likely to be the source of acyl moieties used for the first phase of ester synthesis. The second phase of ester production started just before fruits reached the fully ripe stage. At this stage, a significant decrease in mitochondrial yield with a concomitant increase in free oleic acid from extracts of 'Bartlett' pear fruit particulates was reported (Romani et al., 1961). Free fatty acids released from this stage of fruit ripening can significantly account for the second upsurge of ester production as observed in this study. However, more research is needed to clarify this observation, but this could possibly explain the cyclic nature of ester production.

The production of butyl acetate and methyl trans-2, cis-4 decadienoate exhibited different patterns than were found for hexyl acetate and ethyl trans-2, cis-4 decadienoate. Butyl acetate and methyl trans-2, cis-4 decadienoate gradually increased and reached maxima at different times (2 days and 4-6 days, respectively) and then declined as fruit advanced further in ripening (Figure 5.4).

Nineteen of the compounds produced during fruit ripening were found to have significant (p-value < 0.05) correlations to the pear flavor intensity score obtained from the taste panel (Table 5.2). Sixteen compounds were found to have positive correlations to the pear flavor intensity. Among these compounds, nine compounds

Table 5.2. Correlation coefficient (r) between A) pear flavor intensity and B) general acceptability and selected volatile compounds during ripening of 'Bartlett' pear at 20°C.[a]

Pk. No.[b]	Compounds	r[c]	p-value	Aroma quality[d]
A. Pear Flavor Intensity				
Positive correlation				
39	ethyl cis-2, cis-4 decadienoate	0.99	0.001	pear, fruity
19	ethyl octanoate	0.96	0.009	cooked apple
17	methyl octanoate	0.96	0.011	-
35	ethyl trans-2 decenoate	0.95	0.013	-
2	ethyl acetate	0.94	0.018	-
27	methyl 4-decenoate	0.94	0.019	anise
37	methyl trans-2, cis-4 decadienoate	0.93	0.020	floral, estery
4	propyl acetate	0.93	0.021	floral, estery
41	methyl trans-2, trans-4 decadienoate	0.93	0.022	-
11	methyl hexanoate	0.92	0.026	-
36	methyl cis-2, cis-4 decadienoate	0.92	0.028	-
13	ethyl hexanoate	0.92	0.028	pear, fruity, floral
1	methyl acetate	0.91	0.031	-
6	ethyl butanoate	0.91	0.031	fruity, estery
24	ethyl trans-2 octenoate	0.90	0.036	floral
32	methyl 2-decenoate	0.88	0.046	-
Negative correlation				
22	methyl trans-2 octenoate	-0.97	0.006	oxidized,cooked fruit
34	α-farnesene (E,E)	-0.93	0.022	-
33	α-farnesene (E,Z)	-0.91	0.033	-
B. General Acceptability				
12	butyl butanoate + 1-methylpropyl butanoate	0.98	0.004	pear, estery
26	hexyl hexanoate	0.90	0.040	floral

[a] Only compounds significant at p-value < 0.05 are shown.
[b] Peak number corresponds to Table 5.1.
[c] Pearson correlation coefficient (n = 6).
[d] Odor-active compounds identified from Chapter 4.

were previously identified as odor active compounds in 'Bartlett' pear (Chapter 4). Furthermore, six compounds including ethyl cis-2, cis-4 decadienoate, ethyl octanoate, methyl trans-2, cis-4 decadienoate, propyl acetate, ethyl hexanoate and ethyl butanoate were among the compounds which were found to possess pear or fruity aroma. High correlations with flavor were also observed for hexyl acetate and ethyl trans-2, cis-4 decadienoate, the two potential flavor impact compounds reported in 'Bartlett' pear (Jennings et al., 1964; Jennings and Sevenants, 1964 and Chapter 4). However, their significance levels were higher than 0.05% (0.09 % and 0.07 % , respectively).

Highly significant negative correlations with pear flavor were found among three compounds including methyl trans-2 octenoate and the 2 isomers of α-farnesene (E,E and E,Z). The first compound has been reported to possess a mild oxidized or cooked fruit aroma in 'Bartlett' pear (Chapter 4). Although the latter two compounds were not found to possess any odor characteristics in the GC-O tests, the significance of these compounds should not be omitted. Since compounds such as α-farnesene are suspected to relate to peely fresh green odor of 'La France' pear (Shiota, 1990) their disappearance may permit positive detection of the specific pear odors from the aroma pool also α-farnesenes are easily oxidized, and little information is known about the odor activity of oxidized farnesenes.

Fewer compounds were found to significantly correlate to the general acceptability score during fruit ripening (Table 5.2). These compounds included butyl butanoate + 1-methyl propyl butanoate and hexyl hexanoate. Butyl butanoate was reported to possess a moderate pear like-odor characteristic while hexyl hexanoate possessed moderate floral aroma. The smaller number of compounds as well as

differences of compounds between pear flavor intensity and general acceptability indicated that aroma is not the only factor which relates to overall acceptability by consumers.

'Packham's Triumph' pears

A significant increase in pear flavor intensity was observed in fruit ripened for 6 days at 20°C. The score for flavor intensity remained high until the end of the 12 day ripening period (Figure 5.5). A similar pattern was also observed in the general acceptability score which was highest between day 6 to day 12 of ripening. Flesh firmness decreased from 67 N initially, to 10 N on day 6 when the significant increase in general acceptability was observed.

The production of volatile compounds from static headspace of 'Packham's Triumph' pear during ripening at 20°C are shown in Table 5.3. A total of 52 compounds were detected above the rate of 0.05 µg/kg/hr by the SPME technique. Similar to 'Bartlett', the esters, alcohols and hydrocarbons were found to be major groups of compounds found in the profiles.

A rapid decline in the total volatiles was observed during the first 4 days of ripening due to the rapid decrease of the two α-farnesenes (Figure 5.6). While total volatiles decreased, there was a non-linear increase in the production of esters. A cyclic pattern of ester production and a gradual increase in total alcohol production during fruit ripening were observed to be similar to those found in 'Bartlett'.

The production of four major esters in 'Packham's Triumph' during ripening is shown in Figure 5.7. Hexyl acetate exhibited the most obvious cyclic pattern of ester production. The production of hexyl acetate appeared to increase, reaching maxima on days 1, 6 and 8 and then declined as ripening advanced. The production of butyl

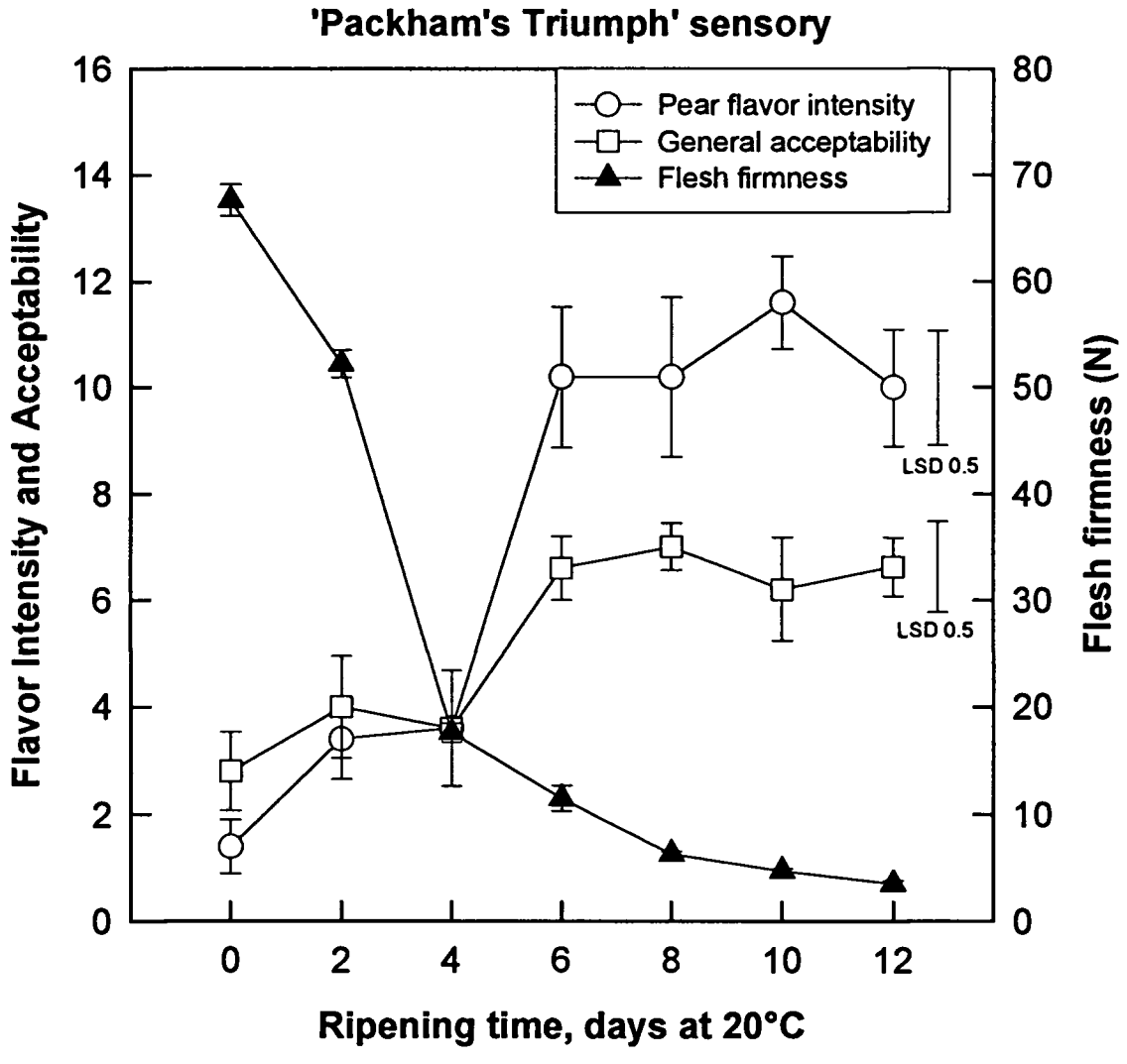

Figure 5.5. Changes in sensory attributes and flesh firmness of 'Packham's Triumph' pear during ripening at 20°C.

Table 5.3. Volatile compounds identified from static headspace of 'Packham's Triumph' pear during ripening at 20°C[a].

Pk. No.	Rt[b] (min)	I_E[c]	Compounds	Days at 20°C											
				0	1	2	3	4	5	6	7	8	9	10	12
1	5.69	1.59	methyl acetate	nd	nd	nd	nd	nd	nd	2.6	0.5	5.0	nd	0.3	nd
2	5.88	1.83	unknown	nd	nd	nd	nd	nd	nd	2.1	nd	nd	nd	4.2	7.1
3	6.02	2.01	ethyl acetate	nd	nd	nd	nd	0.8	8.1	18.4	18.9	34.2	34.8	47.9	52.7
4	6.32	2.37	ethanol	nd	nd	nd	nd	nd	nd	1.1	1.9	5.1	7.6	11.2	13.0
5	6.97	3.10	propyl acetate	1.2	nd	nd	nd	0.9	3.1	4.1	3.1	5.5	5.4	3.4	5.1
6	7.49	3.63	2-methylpropyl acetate	nd	nd	nd	1.0	nd	nd	nd	nd	nd	nd	nd	0.5
7	8.55	4.37	butyl acetate	79.9	136.4	183.1	151.0	150.3	158.0	160.1	123.9	133.4	103.6	95.0	76.7
8	9.57	4.87	isoamyl acetate	nd	nd	nd	nd	0.7	nd	0.3	0.5	nd	nd	nd	nd
9	9.73	4.95	1-butanol	nd	0.7	nd	0.4	0.8	0.7	nd	nd	nd	0.9	1.8	2.0
10	10.78	5.40	pentyl acetate	4.1	8.2	9.8	9.2	9.5	10.4	5.9	8.9	10.1	7.5	7.6	4.2
11	11.97	5.87	1-methylpropyl butanoate + butyl butanoate	8.0	1nd	9.3	8.3	6.9	5.7	4.4	2.0	2.7	0.4	nd	nd
12	12.39	6.02	ethyl hexanoate	nd	nd	nd	1.6	nd	3.4	3.8	3.1	1.7	1.2	4.5	0.8
13	12.51	6.09	1-pentanol	2.8	nd	nd	nd	nd	nd	nd	0.9	nd	nd	nd	nd
14	13.29	6.44	hexyl acetate	132.4	427.4	370.6	289.3	327.4	362.6	389.3	323.7	388.8	290.8	300.2	230.9
-	14.52	6.95	propyl tiglate (internal standard)	-	-	-	-	-	-	-	-	-	-	-	-
15	14.78	7.05	unknown	4.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	15.02	7.15	1-hexanol	3.0	4.9	3.7	0.9	2.5	1.2	1.8	nd	nd	1.0	1.3	1.1
17	15.85	7.46	heptyl acetate	nd	2.1	2.4	1.9	1.6	4.0	3.0	3.5	4.9	4.0	2.2	4.0
18	16.23	7.60	methyl octanoate	nd	nd	nd	nd	nd	2.4	1.1	1.9	1.3	nd	2.0	nd
19	16.96	7.85	butyl hexanoate	41.9	8nd	98.8	67.9	64.0	61.8	49.4	30.2	29.8	18.0	15.1	7.9
20	17.40	8.00	ethyl octanoate	nd	nd	nd	0.9	3.8	10.1	12.1	11.1	15.2	10.7	9.9	6.0
21	18.35	8.46	octyl acetate	2.1	5.6	12.0	9.7	9.0	13.3	11.6	7.7	10.7	6.1	5.6	3.8
22	18.86	8.71	butyl 3-hexenoate	10.4	8.0	7.9	4.6	2.4	1.2	nd	nd	nd	nd	nd	nd
23	19.25	8.89	pentyl hexanoate	nd	nd	0.8	0.8	nd	1.8	nd	nd	nd	nd	nd	nd
24	19.46	8.98	4-octen-1-ol acetate	nd	4.5	6.1	4.1	2.8	4.0	1.6	1.3	1.2	1.7	1.7	nd
25	19.93	9.19	1-octanol	nd	0.9	0.6	0.5	1.4	1.5	nd	0.7	nd	nd	nd	nd
26	21.03	9.67	methyl decanoate	1.1	nd	nd	nd	nd	5.6	4.1	2.8	4.2	1.6	1.3	nd

(Continued)

Table 5.3. Continued.

Pk. No.	Rt[b] (min)	I_E[c]	Compounds	Days at 20°C 0	1	2	3	4	5	6	7	8	9	10	12
27	21.49	9.86	hexyl hexanoate	18.0	27.7	40.5	26.5	23.9	33.8	25.5	14.9	18.2	10.4	10.2	4.9
28	21.68	9.93	methyl 4-decenoate	nd	nd	nd	nd	3.9	8.7	9.0	7.1	14.2	7.8	2.7	5.2
29	22.06	10.09	ethyl 2,4 (?) octadienoate	nd	1.0	1.3	0.8	4.4	12.4	11.6	10.6	16.0	8.3	7.4	4.7
30	22.44	10.24	ethyl 4-decenoate	31.0	35.0	1.9	nd	nd	nd	nd	nd	nd	nd	nd	nd
31	22.58	10.29	ethyl 2,4 (?) octadienoate	nd	0.5	40.1	21.9	13.3	6.9	5.8	4.0	3.6	1.0	2.5	nd
32	22.63	10.31	unknown	12.2	3.2	2.7	3.9	5.7	12.3	15.4	16.5	31.9	15.9	17.1	12.0
33	22.91	10.42	methyl 2,4,6 octatrienoate	nd	nd	nd	nd	0.7	2.6	3.0	1.8	3.3	nd	1.1	nd
34	23.79	10.75	unknown	nd	0.7	1.7	1.5	0.8	5.5	6.9	6.2	9.8	3.7	4.9	0.7
35	23.94	10.80	α-farnesene (E,Z)	28.1	15.8	17.1	7.5	4.7	4.8	6.0	4.5	5.8	3.3	2.1	2.7
36	24.43	10.98	α-farnesene (E,E)	2433.6	1698.1	1230.0	733.3	418.9	427.6	442.2	296.0	369.9	242.3	232.0	151.7
37	25.13	11.23	methyl trans-2, cis-4-decadienoate	1.6	3.0	8.6	9.9	18.7	38.0	39.2	28.2	45.5	26.4	28.7	19.6
38	25.36	11.31	unknown	17.9	20.6	16.7	11.8	9.2	8.6	8.5	2.7	7.1	1.9	6.5	3.6
39	25.58	11.39	2-phenylethyl acetate	12.6	6.4	18.9	13.7	9.1	7.8	6.9	2.4	6.4	2.6	4.6	nd
40	25.83	11.47	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	nd	nd	nd	nd	0.7	1.3	1.8	nd
41	26.08	11.56	ethyl trans-2, cis-4-decadienoate	10.4	20.9	38.9	24.4	29.0	45.8	59.7	48.8	86.0	53.1	70.4	48.4
42	27.03	11.87	ethyl trans-2, trans-4 decadienoate	nd	12.5	6.8	4.1	4.3	9.5	11.5	10.5	16.8	10.4	11.2	7.3
43	27.77	12.11	unknown	nd	0.9	0.6	nd	nd	nd	nd	nd	nd	nd	nd	nd
44	27.81	12.12	unknown	3.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
45	29.55	12.66	unknown	nd	0.6	1.2	0.5	nd	nd	nd	nd	nd	nd	nd	nd
46	29.62	12.68	ethyl tetradecenoate	nd	nd	nd	nd	nd	2.7	5.1	2.7	5.3	3.1	3.0	1.6
47	32.24	13.42	unknown	nd	nd	nd	0.7	nd	1.8	1.5	2.4	5.6	2.8	3.8	3.6
48	31.07	13.10	unknown	nd	nd	nd	nd	nd	nd	nd	0.8	4.7	0.4	4.0	3.0
49	32.16	13.40	unknown	nd	nd	nd	nd	nd	nd	nd	nd	1.6	0.8	nd	nd
50	33.12	13.66	methyl cis-7 hexadecenoate	nd	nd	1.2	nd	0.4	2.5	1.1	2.3	2.8	nd	nd	nd
51	33.42	13.74	unknown	nd	nd	nd	nd	nd	2.0	nd	3.6	2.5	0.7	0.6	nd
	Total			2860.7	2535.7	2133.4	1412.6	1131.9	1292.5	1336.1	1012.6	1311.5	891.5	929.9	684.8

[a] Fruits were harvested at mature unripe stage (flesh firmness 69N) and stored for 2 months at 0°C before use. Compounds were trapped and analysed using SPME technique. Units are calculated based on 180.8 μg propyl tiglate used as internal standard (response factor = 1) and expressed in μg compound kg^{-1} hr^{-1}. nd refers to not detected or detected below 0.05 μg kg^{-1} hr^{-1}.

[b] Retention times on Carbowax 20M column (See Materials and methods for details).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

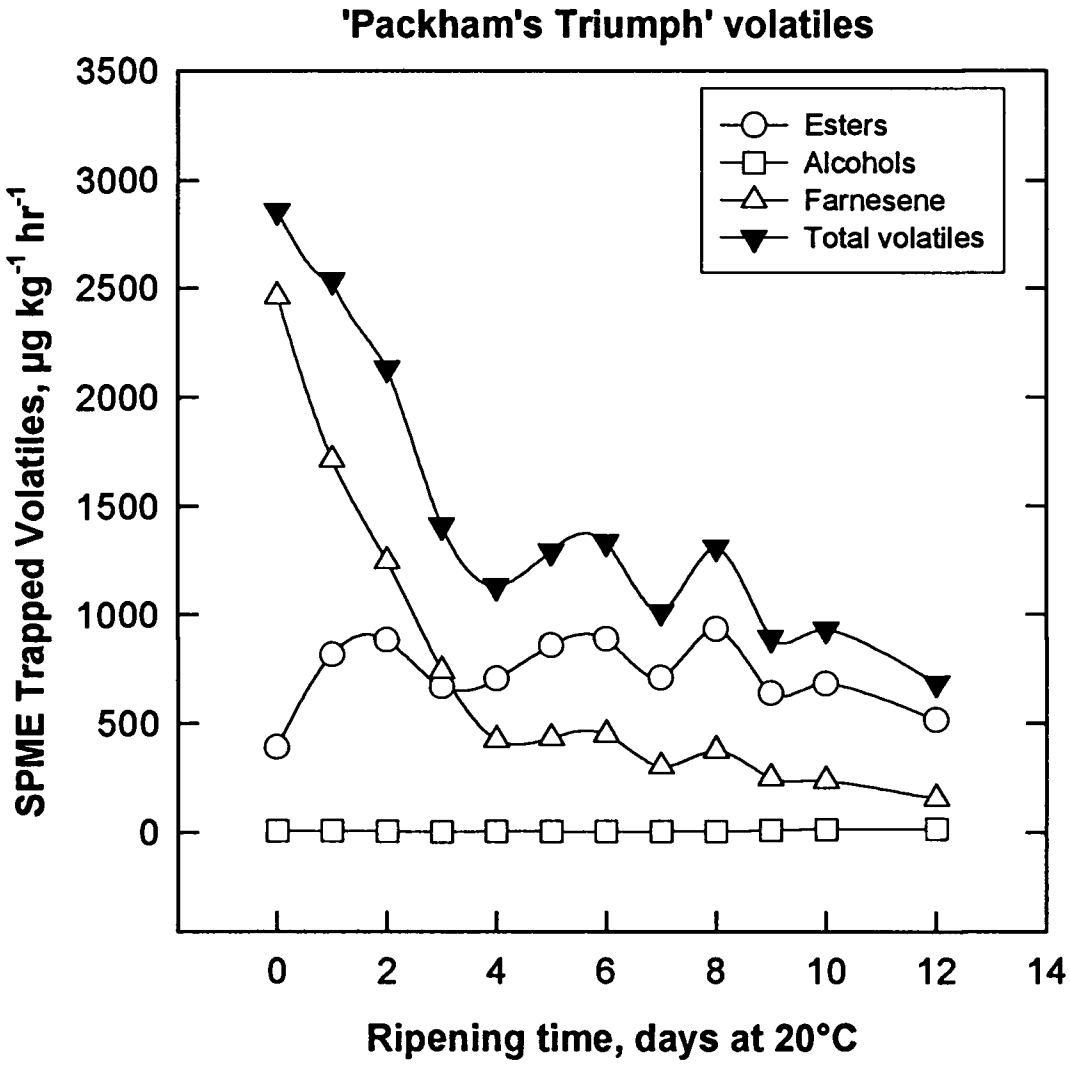

Figure 5.6. Changes in total volatiles, esters, alcohols and farnesenes from static headspace of 'Packham's Triumph' pear during ripening at 20°C.

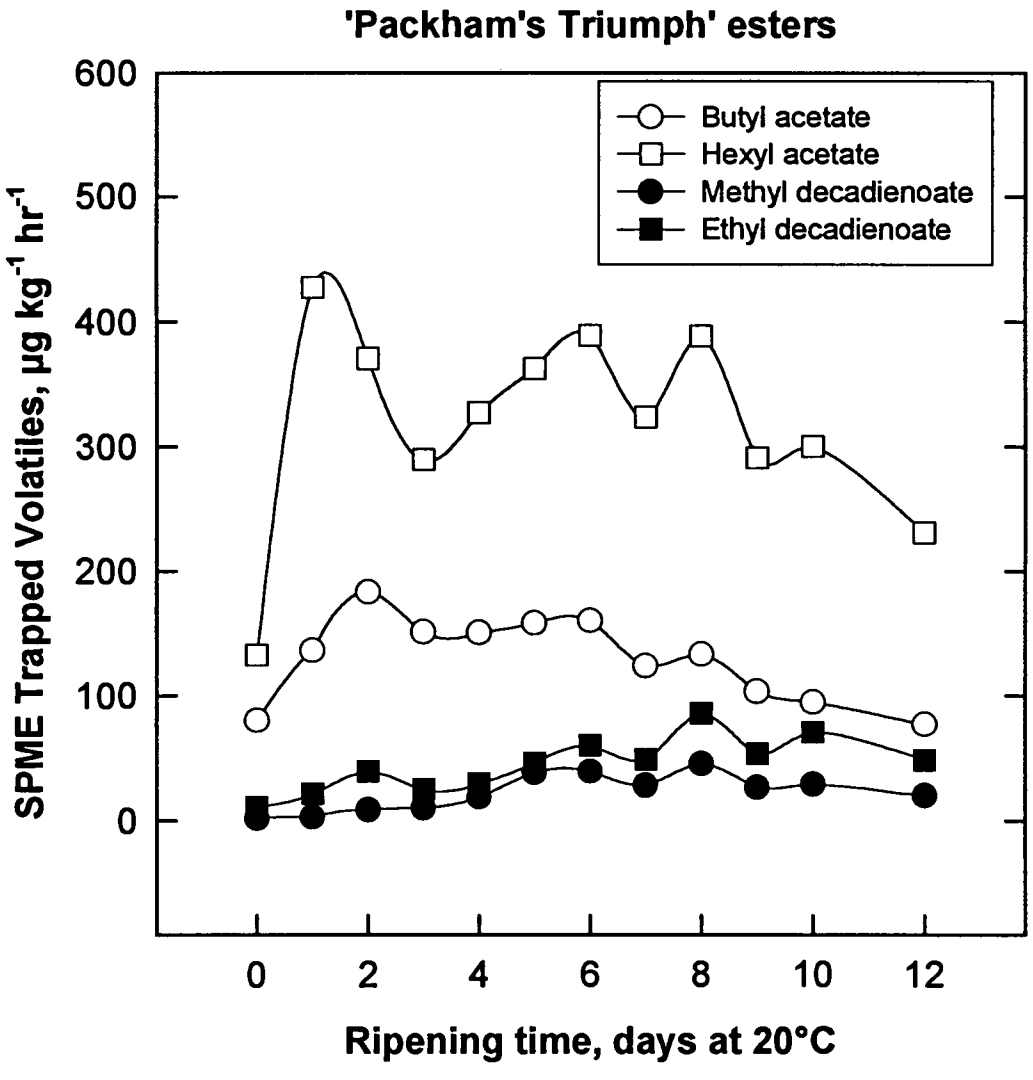

Figure 5.7. Changes in selected esters from static headspace of 'Packham's Triumph' pear during ripening at 20°C.

acetate rapidly increased during the first 2 days of the ripening period and then gradually declined throughout 12 days of ripening. Ethyl and methyl trans-2, cis-4 decadienoate gradually increased and reached a peak on day 8 of ripening. Ethyl trans-2, cis-4 decadienoate of 'Packham's Triumph' pear was considerably lower than 'Bartlett' even though this pear variety is known to have flavor similar to 'Bartlett' pear.

Seventeen compounds produced during fruit ripening were found to have a significant correlation (p-value < 0.05) with pear flavor intensity scores obtained from the taste panel (Table 5.4). Among ten compounds which were found to have positive correlations, seven compounds were odor active compounds. These compounds included ethyl trans-2, cis-4 decadienoate, methyl trans-2, cis-4 decadienoate, propyl acetate, ethyl octanoate and ethyl hexanoate which possessed pear, fruity or floral-like odor characteristics and also were found in 'Bartlett' pear. Interestingly, these two pear varieties are known to have a very similar flavor.

Unlike 'Bartlett' pear, a larger number of compounds were found to have a negative correlation to 'Packham's Triumph' pear flavor intensity. These compounds included the two isomers of α-farnesene, 1-methylpropyl butanoate, 2-phenylethyl acetate and 1-hexanol. 1-Methylpropyl butanoate and 2-phenylethyl acetate were both reported to possess a sweet or candy-like aroma in 'Bartlett' and 'Comice' pear, respectively (Chapter 4). The 2-phenylethyl acetate has also been reported to contribute a sweet, rose-, honey-like aroma in various fruits such as apple, plum, mango and guava (Stevens, 1970).

A number of similar compounds were found to highly correlate with the general acceptability scores and with pear flavor intensity scores. High positive

correlations between compounds such as ethyl and propyl acetate, ethyl and methyl trans-2, cis-4 decadienoate or ethyl octanoate with general acceptability of fruit during ripening may be used to emphasize the significance of these compounds to overall fruit aroma.

Table 5.4. Correlation coefficient (r) between A) pear flavor intensity and B) general acceptability and selected volatile compounds during ripening of 'Packham's Triumph' pear at 20°C.[a]

Pk. No.[b]	Compounds	r[c]	p-value	Aroma quality[d]
A. Pear Flavor Intensity				
Positive correlation				
41	ethyl trans-2, cis-4-decadienoate	0.88	0.009	pear, pear peel, green
3	ethyl acetate	0.88	0.009	-
5	propyl acetate	0.87	0.011	floral, estery
20	ethyl octanoate	0.86	0.013	cooked apple
47	unknown	0.84	0.018	-
42	ethyl trans-2, trans-4 decadienoate	0.82	0.024	alcohol, fermented
46	ethyl tetradecenoate	0.82	0.024	-
37	methyl trans-2, cis-4-decadienoate	0.82	0.025	floral, estery
12	ethyl hexanoate	0.81	0.029	pear, fruity, floral estery
18	methyl octanoate	0.76	0.046	-
Negative correlation				
11	1-methylpropyl butanoate + butyl butanoate	-0.90	0.006	pear, estery
22	butyl 3-hexenoate	-0.90	0.007	-
38	unknown	-0.85	0.015	-
16	1-hexanol	-0.83	0.021	fresh grass, oxidized
36	α-farnesene (E,E)	-0.82	0.025	-
35	α-farnesene (E,Z)	-0.80	0.033	peelyfresh green
39	2-phenylethyl acetate	-0.77	0.042	sweet, rose, honey
B. General Acceptability				
Positive correlation				
41	ethyl trans-2, cis-4-decadienoate	0.92	0.003	pear, pear peel, green
20	ethyl octanoate	0.90	0.005	cooked apple
5	propyl acetate	0.90	0.005	floral, estery
42	ethyl trans-2, trans-4 decadienoate	0.90	0.006	alcohol, fermented
46	ethyl tetradecenoate	0.89	0.008	-
37	methyl trans-2, cis-4-decadienoate	0.88	0.009	floral, estery

(Continued.)

Table 5.4. Continued.

Pk. No.[b]	Compounds	r[c]	p-value	Aroma quality[d]
17	heptyl acetate	0.86	0.014	-
47	unknown	0.86	0.014	-
29	ethyl 2,4 (?) octadienoate	0.84	0.017	-
3	ethyl acetate	0.81	0.029	-
34	unknown	0.78	0.039	-
28	methyl 4-decenoate	0.77	0.043	anise
Negative correlation				
22	butyl 3-hexenoate	-0.87	0.012	-
16	1-hexanol	-0.85	0.016	fresh grass, oxidized
11	1-methylpropyl butanoate + butyl butanoate	-0.81	0.029	pear, estery
38	unknown	-0.80	0.031	-
36	α-farnesene (E,E)	-0.77	0.042	-
35	α-farnesene (E,Z)	-0.74	0.056	peelyfresh green
39	2-phenylethyl acetate	-0.69	0.085	sweet, rose, honey

[a] Only compounds significant at p-value < 0.05 are shown.
[b] Peak number corresponds to Table 5.3.
[c] Pearson correlation coefficient (n = 7).
[d] Odor-active compounds identified from Chapter 4.

'Anjou' pears

The highest score for pear flavor intensity and general acceptability was observed in 'Anjou' fruit after 6 days of ripening (Figure 5.8). Interestingly, there are no corresponding increases in any of major ester compounds found during this period (Figure 5.10). Flesh firmness of fruits decreased from an initial value of 67 N to 11 N at day 6 , when the maximum eating quality was obtained.

Volatile compounds identified from static headspace of 'Anjou' pear during ripening at 20°C are shown in Table 5.5. A total of 46 compounds were detected above 0.05 μg/kg/hr. Similar to volatiles observed in 'Bartlett' and 'Packham's Triumph' pear, esters, alcohols and hydrocarbons were among the compounds detected by the SPME method.

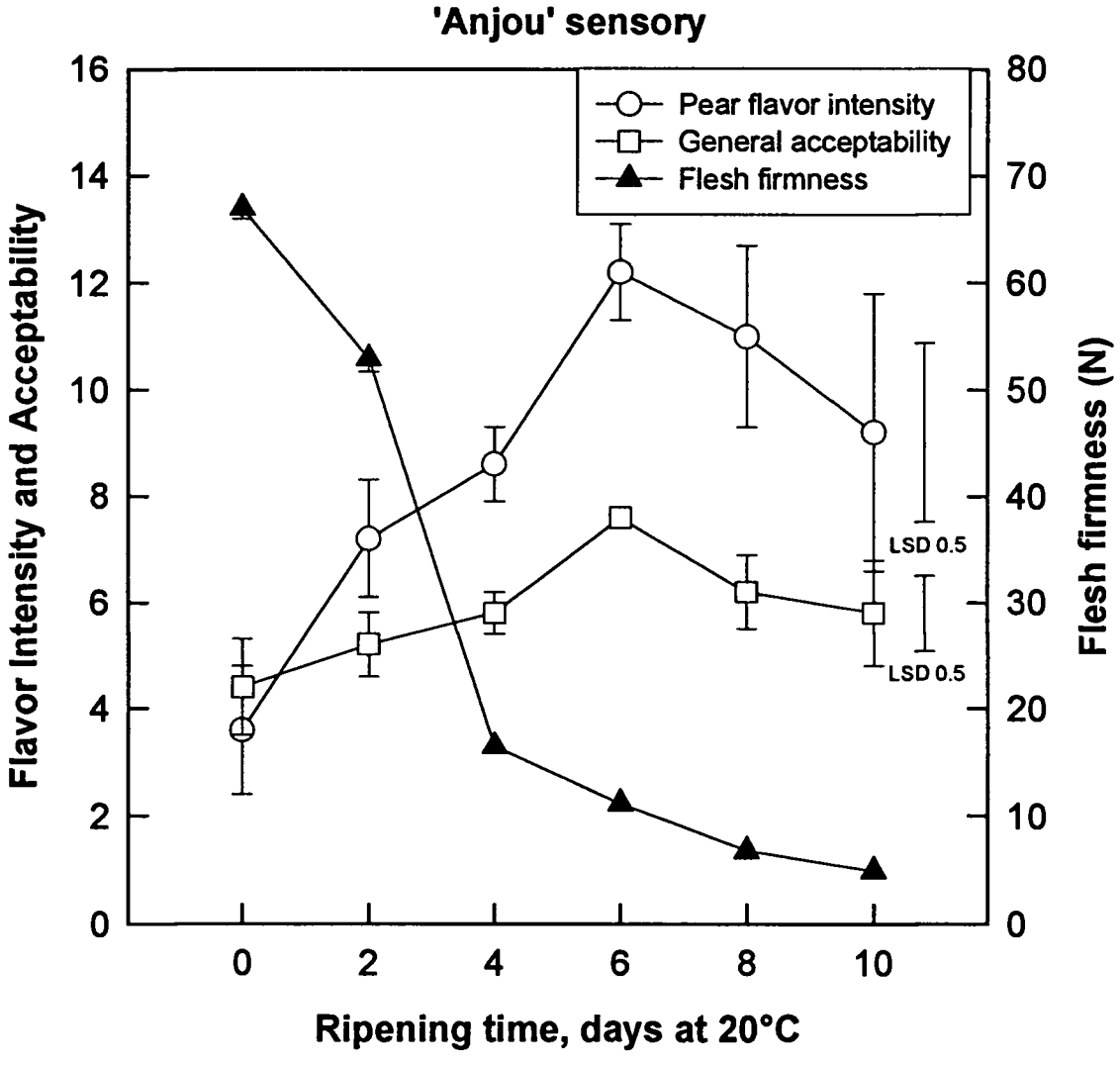

Figure 5.8. Changes in sensory attributes and flesh firmness of 'Anjou' pear during ripening at 20°C.

Table 5.5. Volatile compounds identified from static headspace of 'Anjou' pear during ripening at 20°C[a].

Pk. No.	Rt[b] (min)	I_E^c	Compounds	Days at 20°C											
				0	1	2	3	4	5	6	7	8	9	10	12
1	6.32	2.37	ethanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.5	4.8	2.0
2	6.95	3.06	propyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.2
3	7.19	3.32	2-methylpropyl acetate	nd	nd	6.4	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	8.47	4.32	ethyl butanoate	nd	nd	nd	nd	nd	nd	nd	0.4	nd	nd	nd	nd
5	8.52	4.35	butyl acetate	0.6	1.5	7.0	10.5	9.3	12.7	14.0	8.0	27.9	30.7	35.0	41.9
6	9.59	4.88	isoamyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.6
7	9.77	4.96	1-butanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.9
8	10.24	5.17	butyl propanoate	nd	nd	nd	nd	nd	0.9	nd	nd	nd	nd	nd	nd
9	10.76	5.39	pentyl acetate	nd	nd	nd	nd	nd	1.6	3.6	5.1	17.1	3.1	8.4	8.4
10	11.96	5.86	1-methylpropyl butanoate + butyl butanoate	3.4	7.6	14.7	14.3	11.3	9.1	7.2	5.8	4.2	0.7	0.9	nd
11	12.41	6.03	ethyl hexanoate	nd	nd	1.6	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	13.28	6.43	hexyl acetate	6.2	24.7	18.8	18.4	18.4	30.4	32.1	55.4	89.5	134.1	151.9	205.6
-	14.52	6.95	propyl tiglate (internal standard)	-	-	-	-	-	-	-	-	-	-	-	-
13	15.01	7.14	1-hexanol	0.7	4.1	3.1	0.6	1.6	0.6	nd	0.3	nd	nd	nd	nd
14	15.45	7.31	2-methylpropyl hexanoate	nd	nd	nd	nd	0.6	nd	nd	2.3	4.6	5.3	6.3	6.5
15	15.85	7.46	heptyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	1.5	0.9	3.4	3.6
16	16.20	7.59	methyl octanoate	nd	nd	nd	nd	nd	0.3	1.7	1.7	3.4	2.2	0.5	0.9
17	16.96	7.85	butyl hexanoate+hexyl butanoate	89.6	106.7	177.1	182.7	154.5	155.9	108.3	101.9	86.0	52.7	39.4	11.5
18	17.33	7.98	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.1	0.5	1.4
19	17.40	8.00	ethyl octanoate	nd	nd	nd	nd	nd	1.8	4.6	6.8	11.2	9.5	7.3	5.6
20	18.04	8.32	2-methylbutyl hexanoate	nd	nd	nd	nd	1.8	2.1	1.2	0.5	nd	nd	nd	nd
21	19.25	8.89	pentyl hexanoate	3.8	5.0	10.4	12.1	1nd	12.1	9.1	8.5	9.2	1.7	3.0	nd
22	21.03	9.67	ethyl trans-2 octenoate	nd	nd	nd	0.5	2.8	6.8	6.4	8.5	9.8	4.5	5.2	1.9
23	21.49	9.86	hexyl hexanoate	57.5	70.8	100.1	112.6	89.6	112.3	77.5	89.6	110.2	54.5	40.9	21.2
24	21.68	9.93	methyl 4-decenoate	nd	nd	nd	nd	nd	4.8	3.6	5.8	7.2	5.4	5.6	4.1
25	22.07	10.09	unknown	nd	nd	nd	1.0	1.8	8.6	11.5	13.9	30.1	22.4	17.2	8.4
26	22.44	10.24	ethyl 4-decenoate	11.0	8.0	8.3	2.7	0.6	2.6	0.6	nd	nd	nd	nd	nd
27	22.60	10.30	methyl 2,4,6 octatrienoate	nd	nd	nd	nd	nd	2.1	3.0	3.7	7.2	9.5	12.4	4.9

(Continued)

Table 5.5. Continued.

Pk. No.	Rt[b] (min)	I_E[c]	Compounds	Days at 20°C											
				0	1	2	3	4	5	6	7	8	9	10	12
28	22.84	10.39	unknown	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.5	4.1
29	23.82	10.76	methyl 2-decenoate	nd	nd	nd	nd	nd	nd	nd	0.9	nd	1.4	nd	nd
30	23.94	10.80	α-farnesene (E,Z)	28.6	13.9	13.3	11.8	6.4	6.3	1.3	5.2	5.4	6.8	4.7	nd
31	24.43	10.98	α-farnesene (E,E)	2780.5	1359.8	1187.0	935.2	556.5	460.2	347.9	348.5	436.3	449.8	275.4	276.4
32	25.13	11.23	methyl trans-2, cis-4 decadienoate	nd	2.6	4.1	9.6	13.1	23.1	19.6	29.1	40.8	32.9	31.5	24.2
33	25.58	11.39	unknown	7.0	1.1	1.4	1.4	nd	nd	nd	0.4	1.6	nd	nd	nd
34	25.67	11.42	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	1.5	1.9	1.9	2.0	2.2	4.3	0.9	nd	nd
35	26.08	11.56	ethyl trans-2, cis-4 decadienoate	2.6	4.6	6.1	6.5	7.6	13.5	12.9	17.5	38.2	44.7	35.4	30.7
36	26.48	11.69	methyl trans-2, trans-4 decadienoate	nd	nd	nd	1.1	nd	1.1	2.5	2.8	7.1	6.1	4.4	4.0
37	27.04	11.87	ethyl trans-2, trans-4, cis-7 decadienoate	nd	nd	nd	nd	nd	1.9	2.6	4.3	5.5	1nd	8.1	6.6
38	29.09	12.52	methyl tetradecenoate	1.0	nd	2.0	1.7	2.0	1.4	2.5	2.7	4.2	2.5	2.9	0.9
39	29.57	12.66	ethyl tetradecanoate	1.1	1.5	1.3	nd	nd	nd	nd	nd	nd	nd	nd	nd
40	29.64	12.68	unknown	nd	nd	nd	nd	2.3	5.2	6.7	9.7	24.1	15.9	18.0	17.2
41	30.36	12.89	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	nd	nd	nd	1.0	3.4	4.8	17.5	17.1	17.4	14.7
42	31.09	13.10	unknown	nd	nd	nd	nd	nd	nd	nd	nd	4.7	6.1	6.8	5.0
43	31.49	13.22	unknown	nd	nd	nd	nd	nd	nd	nd	nd	1.7	nd	0.8	0.5
44	32.02	13.36	unknown	1.6	2.1	3.0	4.9	5.5	2.2	4.0	1.0	3.8	4.1	2.1	1.3
45	33.13	13.66	methyl 7-hexadecenoate	nd	nd	nd	nd	0.8	1.2	nd	1.4	1.6	nd	nd	nd
46	37.05	14.64	unknown	nd	nd	nd	1.5	nd	1.2	0.4	3.2	4.9	2.1	3.3	2.5
				2995.2	1614.1	1565.7	1330.6	898.8	884.9	690.6	752.0	1020.8	942.2	754.8	718.9

[a] Fruits were harvested at mature unripe stage (flesh firmness 62N) and stored for 2 months at 0°C before use. Compounds were trapped and analysed using SPME technique. Units are calculated based on 180.8 μg propyl tiglate used as internal standard (response factor = 1) and expressed in μg compound kg^{-1} hr^{-1}. nd refers to not detected or detected below 0.05 μg kg^{-1} hr^{-1}.

[b] Retention times on Carbowax 20M column (See Materials and methods for details).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

The changes in esters, alcohols, α-farnesenes and total volatiles in 'Anjou' during ripening resemble that observed in 'Packham's Triumph'. Total volatiles decreased at the early stage of fruit ripening as a result of a rapid decline in α-farnesenes (Figure 5.9). Total volatiles then slightly increased following an increase in total ester production, reaching the peak at day 8 and then gradually declined as fruit further ripened. 'Anjou' total ester production throughout the ripening period appeared to be about half that of 'Bartlett' and 'Packham's Triumph'. A slight increase in total alcohols was also observed as 'Anjou' ripening progressed.

The production of selected esters from headspace of 'Anjou' pear during ripening at 20°C are shown in Figure 5.10. Unlike 'Bartlett' and 'Packham's Triumph', 'Anjou' produced relatively high amounts of butyl hexanoate + hexyl butanoate (coeluted peak) and hexyl hexanoate esters. The production of these two (or three) esters increased rapidly during early stages of fruit ripening, reached a peak on day 3 and then declined during the remainder of the ripening period. The production of hexyl acetate increased gradually during the first 6 days of ripening and then began to increase rapidly as fruit reached the eating-ripe stage. The increase in hexyl acetate continued toward the end of the ripening period. A slight increase in ethyl trans-2, cis-4 decadienoate production was also observed in fruit at the eating-ripe stage and continued to remain at a high level until the end of ripening.

Compared to 'Bartlett' and 'Packham's Triumph' pears, only five compounds were found to significantly correlate (p-value < 0.05) to pear flavor intensity during fruit ripening of 'Anjou' (Table 5.6). Ethyl trans-2 octenoate was the only compound found to positively correlate to pear flavor intensity. GC-O analysis of 'Anjou' volatiles revealed that this compound possessed a moderate to strong floral odor

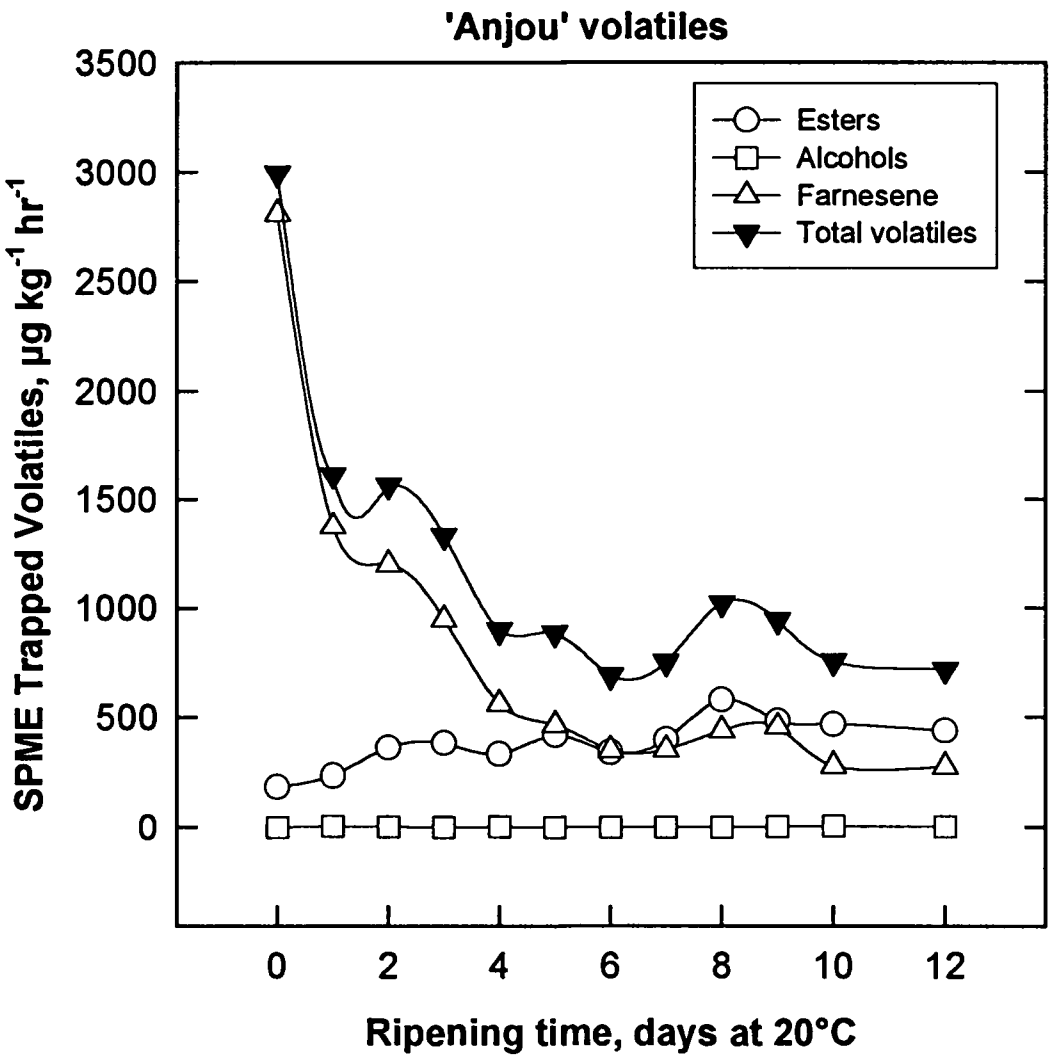

Figure 5.9. Changes in total volatiles, esters, alcohols and farnesenes from static headspace of 'Anjou' pear during ripening at 20°C.

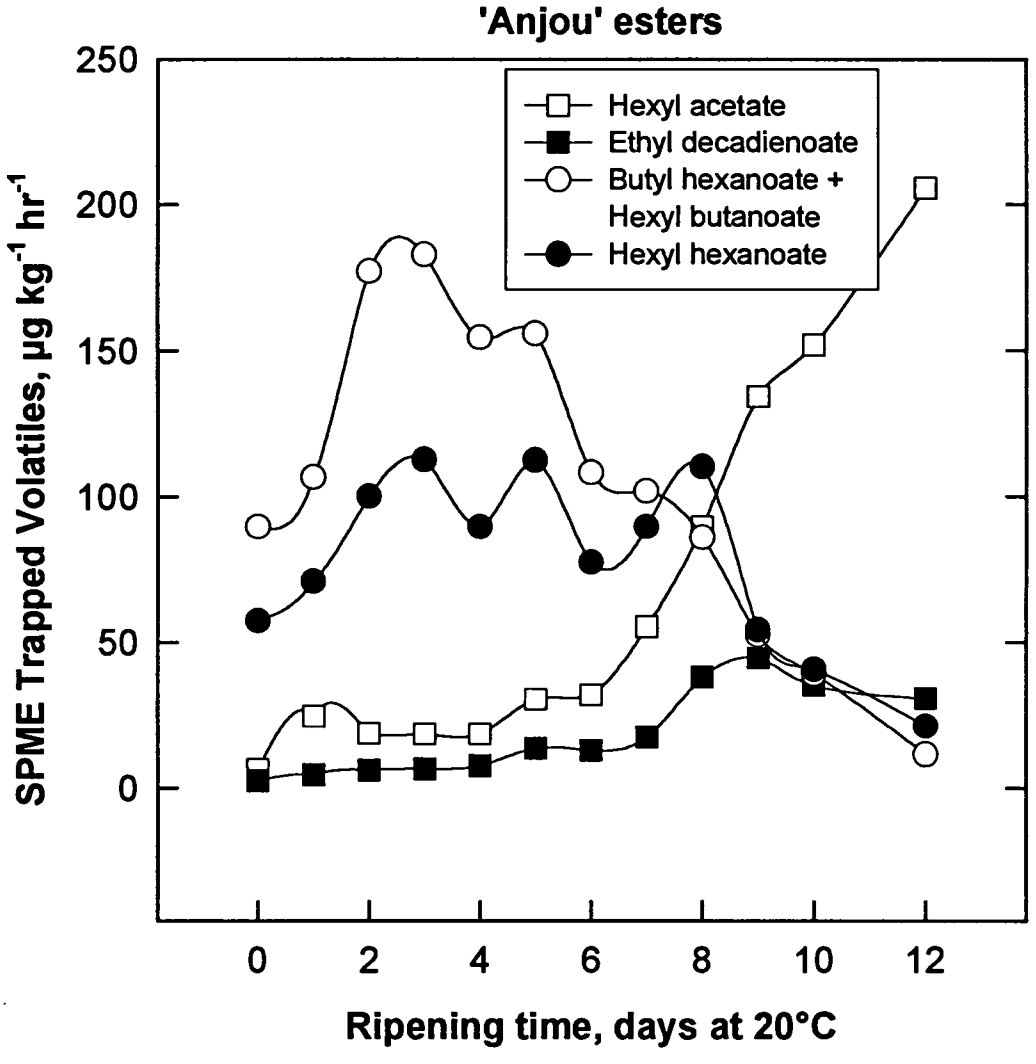

Figure 5.10. Changes in selected esters from static headspace of 'Anjou' pear during ripening at 20°C.

characteristic (Chapter 4). Similar to 'Bartlett' and 'Packham's Triumph' pears, the two isomers of α-farnesene were found to negatively correlate to 'Anjou' pear flavor intensity during ripening. High negative correlation was also observed for ethyl 4-decenoate. This compound was found to possess mild citrus-like aroma in 'Comice' pear.

None of the compounds were found to significantly correlate to general acceptability during fruit ripening. The lack of flavor compounds correlated to pear flavor intensity and general acceptability during ripening of 'Anjou' pear was not a surprise, since this pear variety is known to have milder flavor than other pear varieties.

Table 5.6. Correlation coefficient (r) between pear flavor intensity and selected volatile compounds during ripening of 'Anjou' pear at 20°C.[a]

		Pear Flavor Intensity		
Pk. No.[b]	Compounds	r[c]	p-value	Aroma quality[d]
Positive correlation				
22	ethyl trans-2 octenoate	0.83	0.022	floral
32	methyl trans-2, cis-4 decadienoate	0.73	0.065	floral, estery
37	ethyl trans-2, cis-4 decadienoate	0.52	0.237	pear, pear peel, green
Negative correlation				
31	α-farnesene (E,E)	-0.86	0.025	peelyfresh green
30	α-farnesene (E,Z)	-0.86	0.033	-
26	ethyl 4-decenoate	-0.83	0.023	citrus
33	unknown	-0.77	0.041	-

[a] Only compounds significant at p-value < 0.05 are shown.
[b] Peak number corresponds to Table 5.5.
[c] Pearson correlation coefficient (n = 7).
[d] Odor-active compounds identified from Chapter 4.

Conclusion

In conclusion, the production of volatile compounds during ripening of pear can be explained by the increase of the production of various esters and alcohols compounds concomitant with the rapid decrease of both isomers of the sesquiterpene hydrocarbon, α-farnesene.

A non-linear increase in total esters was observed in all varieties due to a cyclic pattern of increasing characteristics of particular esters as well as differences in times of increase among the esters (Figure 5.4, 5.7 and 5.10). However, the highest rate of ester production was observed in fruits at the optimum eating-ripe stage (4 to 8 days depending on the variety). No significant changes in the production of headspace alcohols were found during fruit ripening.

Some slight differences in the pattern of development of the major esters during fruit ripening were found among the three pear varieties studied. In 'Bartlett' and 'Packham's Triumph', hexyl acetate was found to rapidly increase as fruit started to ripen, reaching the peak at the fully ripe stage followed by a decline at later stages of fruit ripening. While in 'Anjou', hexyl acetate was found to increase rapidly in fruit at eating-ripe stage and continued to increase toward the end of ripening. Ethyl trans-2, cis-4 decadienoate, in all varieties, continued to increase from the beginning of fruit ripening. The production of this compound was found to reach a peak slightly before fruits reached optimum eating quality (4 days for 'Bartlett' and 8 days for 'Packham's Triumph'). A slight delay in the production of ethyl trans-2, cis-4 decadienoate was observed in 'Anjou' pear where the maximum production was reached 3 days after fruit attained the optimum eating quality.

Maximum pear flavor intensity and general acceptability were obtained from fruits after ripening for 4-6 days for 'Bartlett', 10 days for 'Packham's Triumph' and 6 days for 'Anjou'.

The production of various esters which possessed pear, fruity or floral odor characteristics were found to correlate positively with pear flavor intensity scores obtained from sensory evaluation during fruit ripening. These esters included propyl acetate, methyl and ethyl trans-2, cis-4 decadienoate, ethyl cis-2, cis-4 decadienoate, ethyl hexanoate, ethyl octanoate and ethyl trans-2 octenoate. However, the levels of those esters varied between pear varieties and may be responsible for subtle differences in overall aroma between pear varieties.

Highly significant negative correlations were consistently observed in the two isomers of α-farnesene. These compounds have been described as responsible for peely or green-like odor characteristics and the disappearance of them may contribute as other volatiles increase in ripe fruit aroma.

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CHAPTER 6

EFFECTS OF HARVEST MATURITY AND STORAGE DURATION ON VOLATILE PRODUCTION IN RIPENING PEAR VARIETIES

Anawat Suwanagul and Daryl G. Richardson

Abstract

Changes in headspace volatile profiles and sensory attributes during fruit ripening were investigated in 'Bartlett', 'Packham's Triumph' and 'Anjou' pears harvested at early, optimum and late maturity stages at specific time intervals after storage at 0°C. The effects of harvest maturity on volatile flavor synthesis vary among fruit varieties. 'Bartlett' harvested at all maturity stages produced relatively the same amount of flavor constituents during ripening. Higher volatile production was observed in 'Packham's Triumph' harvested at earlier maturity stages. Delayed harvest resulted in substantial reduction of volatiles in 'Anjou'. Harvest maturity had minor effects on overall fruit quality after storage. Fruits harvested at different maturity stages ripened to about the same maximum eating quality and pear flavor intensity after storage. Late harvest fruits, however, tend to ripen to optimum eating quality quicker and have shorter shelf-life than fruit harvested at earlier maturity stages.

Prolonged storage resulted in increased ester and alcohol production in 'Bartlett'. However, significant decreases in various volatile flavor constituents were observed in 'Packham's Triumph' after storage for 4 months and 'Anjou' after 6 months. Sensory evaluation data indicated minor differences in general acceptability and pear flavor intensity of fruits after prolonged storage. Fruits harvested at different maturity stages, after prolonged storage, were subject to various degrees of scald disorder. Late harvest 'Bartlett' were the most susceptible to scald, while late harvest 'Packham's Triumph' and 'Anjou' were the most resistant.

Introduction

The developmental stage of maturity at the time of harvest is one of the most important factors affecting fruit quality. Apples picked too early will not develop their full flavor. By contrast, apples picked after optimum harvest maturity show an immediate increase in aroma development and reach considerably higher maxima compared to early picked fruits (Brown et al., 1965; Bachmann, 1983; Dirinck et al., 1989).

Harvest maturity indices of pear has been reviewed by Kingston (1992). Flesh firmness has been reported to be the most reliable parameter to determine pear maturity (Wang, 1982). The optimum harvest firmness for pear varieties was summarized by Wrolstad et al. (1991). Numerous volatile compounds have been isolated and identified in pears (Jennings, 1961; Jennings and Creveling, 1963; Jennings and Sevenants, 1964; Jennings et al., 1964; Heinz et al., 1966; Creveling and Jennings, 1970; Jennings and Tressl, 1974; Russell et al., 1981 and Shiota, 1990). The effect of harvest maturity on some volatiles and sensory characteristics has been studied in 'Comice' and 'Conference' pears (Zerbini and Spada, 1993). In both varieties, early harvest fruits resulted in less production of low boiling point esters after ripening. However, no significant differences in sensory attributes were found among fruits harvested at different maturities. Less mature fruits tend to store very well and remained capable of producing relatively high amounts of high boiling point esters.

Besides the volatile esters which are important to aroma of the fruit, pears produce high amounts of the sesquiterpene hydrocarbon, α-farnesene (Jennings and Trssel, 1974). The oxidation of α-farnesene to conjugated trienes has been to be

implicated in superficial scald which is a physiological disorder commonly found in apples and pears (Huelin, 1964; Huelin and Coggiola, 1968; Huelin and Coggiola, 1970; Anet, 1974; Anet and Coggiola, 1974). The effect of harvest maturity on susceptibility of fruit to scald has been reported in apples (Anet, 1972; Meir and Bramlage, 1988; Blanpied et al., 1991) and pears (Zoffoli, 1994).

Earlier in this thesis (Chapter 5), the volatile profiles of fruits during ripening at 20°C was investigated in 'Bartlett', 'Packham's Triumph' and 'Anjou' pear. The changes in amounts of certain volatiles during fruit ripening was found to correlate very well with pear flavor intensity scores obtained by sensory evaluation. Those compounds were identified by direct sniffing method as odor-active compounds which possessed moderate to strong pear-or fruity-like odor characteristics (Chapter 4). The present study was conducted to determine whether production of those compounds changes during fruit ripening due to harvest maturity and storage duration.

Materials and Methods

Fruit samples

Fruits of 'Bartlett', 'Packham's Triumph' and 'Anjou' were hand harvested from the Southern Oregon Experimental Station, Oregon State University, Medford, Oregon during 1993. They were harvested at three maturity stages based on commercial flesh firmness value. Early, optimum and late harvest fruits were picked at 98, 89 and 80 Newtons (N) for 'Bartlett' ; 73, 69 and 63 N for 'Packham's Triumph' and 68, 62 and 58 N for 'Anjou', respectively. All fruits were harvested from the same block of uniform mature trees in the same orchard. Flesh firmness was measured on

two peeled sides of 10 fruits using a UC-Davis Firmness Penetrometer (Western Ind. Supply, CA) with 8 mm diameter tip. All fruits were dipped in 500 ppm Benomyl and 2,700 ppm Ethoxyquin solution, then air dried and packed in 20 kg standard pear boxes with perforated polyethylene liners and stored at -1 to 1 °C.

Volatile sample preparation

After 2 and 4 months storage for 'Bartlett', and 1 month intervals from 2 to 6 months for 'Packham's Triumph' and 'Anjou', fruits were removed from cold storage and ripened at 20°C in perforated polyethylene bags. To obtained volatile samples from fruit, a set of 5 to 6 fruits weighing between 1,200 and 1,400g were placed in 4L glass jars and sealed with air-tight Teflon seal lids. The jar was allowed to stand at ripening temperature for 1 hr before collecting samples. Head-space volatile compounds evolved from fruits were collected by solid-phase microextraction (SPME) technique. A 0.1 μM film thickness dimethyl polysiloxane coated SPME fiber (Supelco, Bellefonte, PA), previously heat-cleaned at 250°C was inserted into the jar through a rubber stopper. The fiber was allowed to equilibrate with head-space volatile compounds at 20°C for 30 min before direct thermal desorption in the gas chromatograph injection port. Separation of volatiles was accomplished using a Hewlett Packard model 5890 Series II gas chromatograph equipped with flame ionization detector (FID) and a 25 m, 0.2 mm i.d. and 0.2 μm film thickness Carbowax 20M column (HP-20M, Hewlett Packard). The GC oven temperature was programmed with an initial hold at 60°C for 5 min and then increased to 220°C at a rate of 5°C/min. Helium at a linear velocity of 17.0 cm/sec was used as a carrier gas. The injector and detector temperatures were set at 200°C and 250°C, respectively. All injections were made in split mode at 1:10 split ratio. Data were collected and

analyzed by a HP 3395 Integrator (Hewlett Packard, USA). The same set of fruits were used to sample head-space volatile compounds throughout the course of ripening. Identification of compounds was performed by GC/MS as described earlier in Chapter 5. Quantitative estimation of compounds was done by comparing integrated peak areas of each compound with a peak of an internal standard (5 mg/ml propyl tiglate) applied through the sample jar at the beginning of the incubation period. The concentration of compounds was calculated based on response factor of FID equal to 1 and expressed as the unit weight of compound per unit weight of fruit per unit time, i.e. µg/kg/hr.

Sensory evaluation

Sensory evaluations were performed on ripening fruits at 2 day intervals from samples held in storage for specific time intervals. Five fruits were randomly selected at each sampling time. Fruits ready for the test were assigned three digit random numbers, longitudinally cut into 8-12 pieces, cored, and presented to the panelists within 5 min before the session. A group of 8 panelists were selected from a group of colleagues in the Department of Horticulture, Oregon State University based both on taste panel experience with pears and availability. Panelists were not specially trained but all were familiar with pear quality, descriptive analysis, and consumer tests. The same group of panelists was used throughout the experiment. An isolated and comfortable area in the Postharvest physiology lab was prepared to facilitate sensory evaluation. All tests were performed under fluorescent lights at room temperature (20±2°C). Panelists were asked to rate the fruit they tasted for pear flavor intensity and texture based on 16-point category scales (0=none to 15=extreme) and general acceptability was based on a 9-point hedonic scale. Cool

tap water was provided for panelists to wash their mouths after tasting each sample. To avoid sensory fatigue, each session contained not more than seven samples and no more than two sessions were performed on the same day.

Results and Discussion

'Bartlett' pears

Effects of harvest maturity and storage duration on pear flavor intensity and overall eating quality during ripening of 'Bartlett' pear are shown in Figure 6.1. Fruits harvested at different maturity stages ripened to optimum eating quality at different rates. Late harvest fruits ripened rapidly after removal from cold storage. The highest eating quality score was observed after 6, 4 and 2 days for fruits harvested at early, optimum and late maturity stage, respectively. A slightly higher eating quality score was also observed in late harvested 'Bartlett' fruit after storage for 2 months, but this score decreased thereafter.

In general, progressive increases in pear flavor intensity were observed during fruit ripening. A significant delay in the increase of pear flavor intensity score was observed in early harvest fruit compared to fruit harvested at later stages. The highest pear flavor intensity of fruit harvested at early, optimum and late maturity was obtained after ripening for 8, 6 and 2 days, respectively. The intensity scores for early harvest fruits at the maximum were slightly lower than those observed in optimum and late harvest fruits (Figure 6.1).

After storage for 4 months, 'Bartlett' fruits harvested at all three maturity stages were subject to various degrees of senescent scald, a physiological disorder. Fruits harvested at the late stage of maturity (M3) were over-ripe and developed

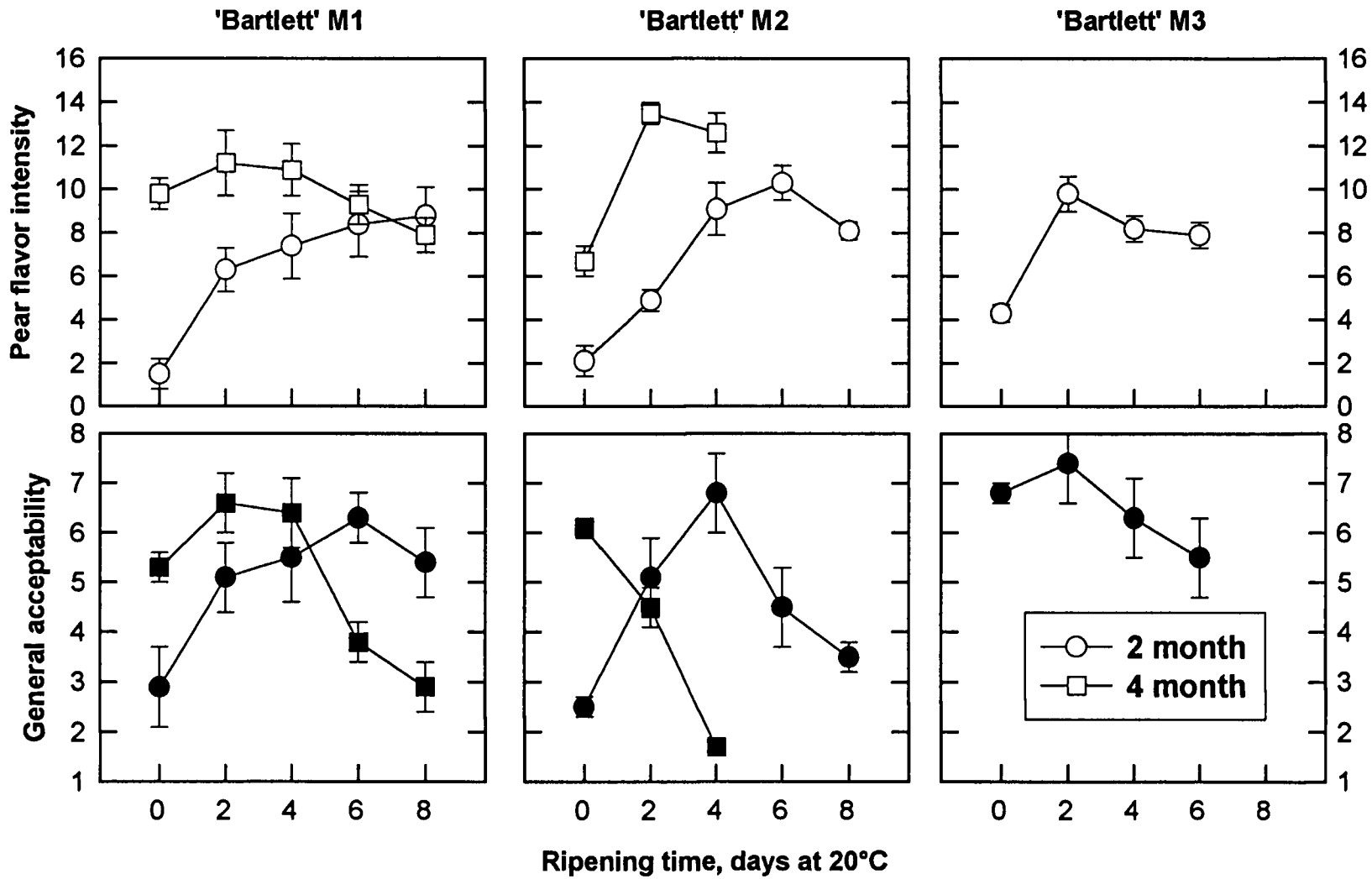

Figure 6.1. Changes in sensory attributes of 'Bartlett' pear harvested at early (M1), optimum (M2) and late (M3) maturity stages after storage for 2 to 4 months at 0°C. Vertical bars represent standard error of means (n= 6 to 8).

severe scald symptoms upon removal from cold storage. Therefore, no sensory evaluation was performed on fruits at this maturity stage. Fruits harvested at optimum maturity stage, even though no scald symptoms were found, turned yellow upon removal from cold storage. A relatively high eating quality score was observed in this fruit on day 0 of ripening but declined rapidly as fruit started to develop scald symptoms (day2). Early harvest fruits were relatively green and no scald was found after 4 months in storage. Fruits harvested at this early maturity stage ripened normally, reaching optimum eating quality within 2 days which coincided with the time when fruits obtained the highest flavor intensity score. No scald incidence was found in this fruit during 8 days of ripening.

Changes in static headspace volatile compounds during ripening of 'Bartlett' pears harvested at three different harvest maturities after 0°C storage for 2 and 4 months are shown in Appendix Tables A.6.1 to A.6.5. Fruits harvested at optimum maturity (M2), after 4 months in storage, were ripened and exhibited senescent scald after 3 days of ripening at 20°C. No data were obtained from late harvested fruits at 4 months storage due to over-ripeness and severe scald symptoms.

Changes in total esters, total α-farnesenes (E,Z and E,E isomers) and total alcohols during ripening of 'Bartlett' pear harvested at early, optimum and late maturity stages after storage for 2 and 4 months are shown in Figure 6.2.

After 2 months in cold storage, a similar pattern of total ester production during ripening was observed in fruits harvested at early and optimum maturity stages. Total esters gradually increased as fruits started to ripen, reaching the peak at about day 5 and declined as fruit ripening advanced. A relatively high rate of total ester production was observed in late harvest fruits at the very beginning of ripening,

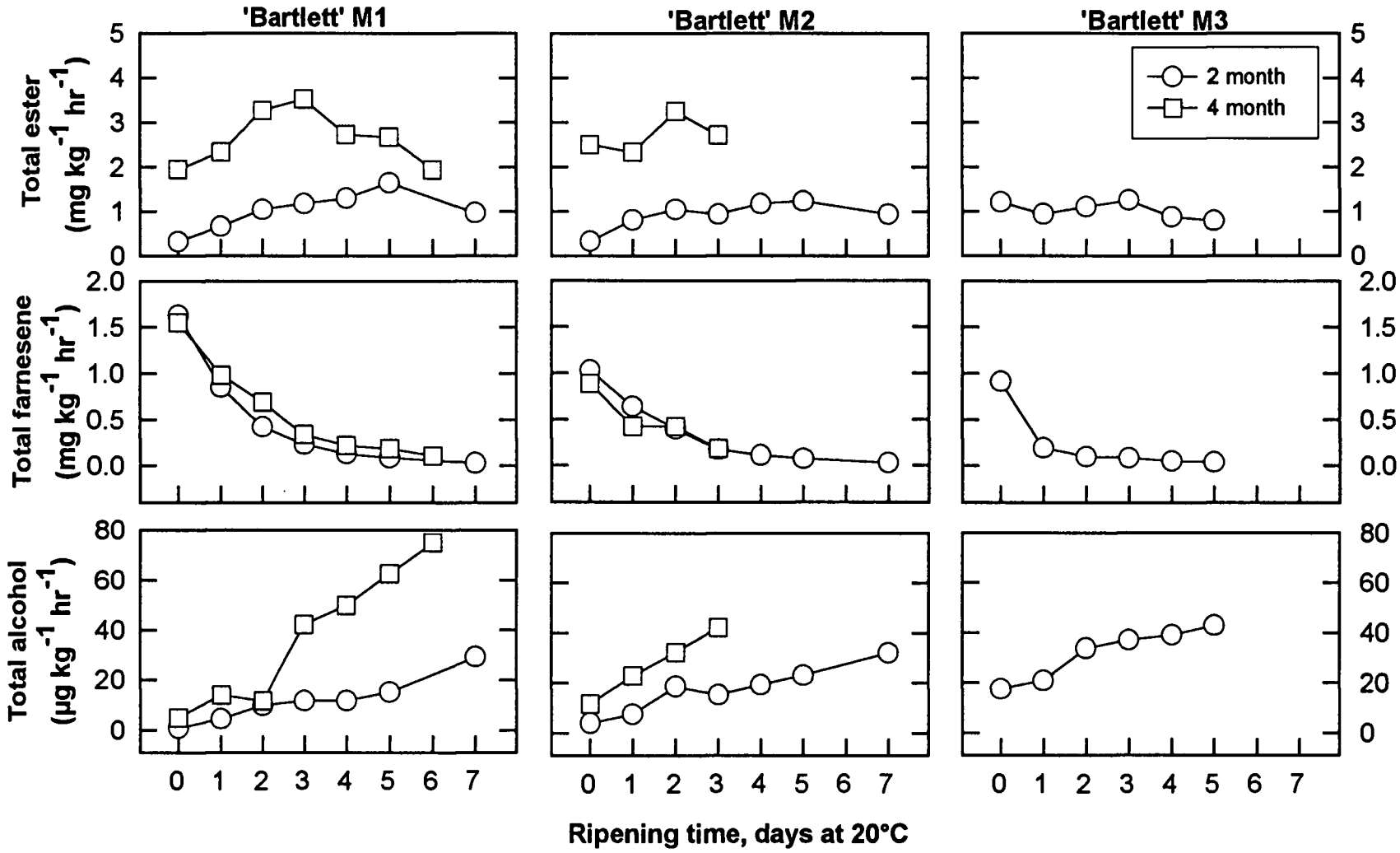

Figure 6.2. Changes in total ester, farnesene and alcohol production during ripening of 'Bartlett' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage at 0°C for 2 to 4 months.

but then gradually declined as ripening advanced. The maximum rate of ester production of late harvest fruit was similar to fruit harvested at optimum maturity, it just occurred much earlier.

A considerable increase in ester production rate during fruit ripening was observed in fruits after storage for 4 months. High rates of ester production were observed in both early and optimum harvest fruits after removal from cold storage. The ester production continued to increase and reached a peak within 3 and 2 days in early and optimum harvest fruits, respectively, then gradually declined as fruit reached the over-ripe stage. However, ester production rate of optimum harvest fruit still remained at a high level up until 4 days of ripening when fruit developed scald symptoms.

A slight difference in α-farnesene level was observed among fruits harvested at different maturity stages. Higher α-farnesene was observed in earlier harvest fruit upon removal from cold storage. However, a progressive decline of α-farnesene was observed in fruits from all harvest maturities as they advanced in ripening. Similar patterns were observed for fruits after storage for 2 and 4 months. After 4 months storage, as mentioned earlier, fruits harvested at optimum maturity exhibited severe senescent scald symptoms after 3 days of ripening. However, only slight differences in α-farnesene levels were found between fruits after 2 and 4 months in storage.

Higher alcohol production during fruit ripening was noted in late mature fruit (Figure 6.2). A linear increase in alcohol production was observed in fruits harvested at all maturity stages throughout the course of ripening. A significant increase in alcohol production was observed in fruits after storage for 4 months. Higher alcohol

production may reflect the higher rate of ester production in this pear variety after prolonged storage.

The effects of harvest maturity and storage duration on selected flavor impact compounds of 'Bartlett' pear during ripening are presented in Figures 6.3 and 6.4.

Ethyl hexanoate, ethyl butanoate and hexyl acetate are among saturated esters whose production during fruit ripening highly correlated to the pear flavor intensity score obtained from the taste panel (Chapter 5). Furthermore, these three compounds also possess a strong pear or fruity-like aroma by the GC-O technique (Chapter 4). Only minor differences in the production of these compounds were observed from fruit harvested at different maturity stages (Figure 6.3). In fruits stored for 2 months, the production of these compounds gradually increased during fruit ripening, reached the maximum at different times depending on harvest maturity and then declined as fruit advanced in ripening. Only slight differences in the magnitude of these compounds were found among fruits harvested at different maturity stages. Only a slight increase in the production of ethyl butanoate was observed in early harvested fruit compared to fruits harvested at later maturity stages.

Relatively higher production rates of all saturated esters were observed in fruits harvested at all maturity stages after storage for 4 months (Figure 6.3). Slightly more ester production was observed in early harvest fruit compared to optimum harvest fruit.

Very similar to what was observed for saturated esters, only minor differences in the production rate of ethyl trans-2, cis-4 and ethyl cis-2, cis-4 decadienoates were observed from fruit harvested at different maturity stages (Figure 6.4). These unsaturated esters, especially with ethyl trans-2, cis-4 decadienoate have been reported to be a character impact compounds of 'Bartlett' pear (Jennings and

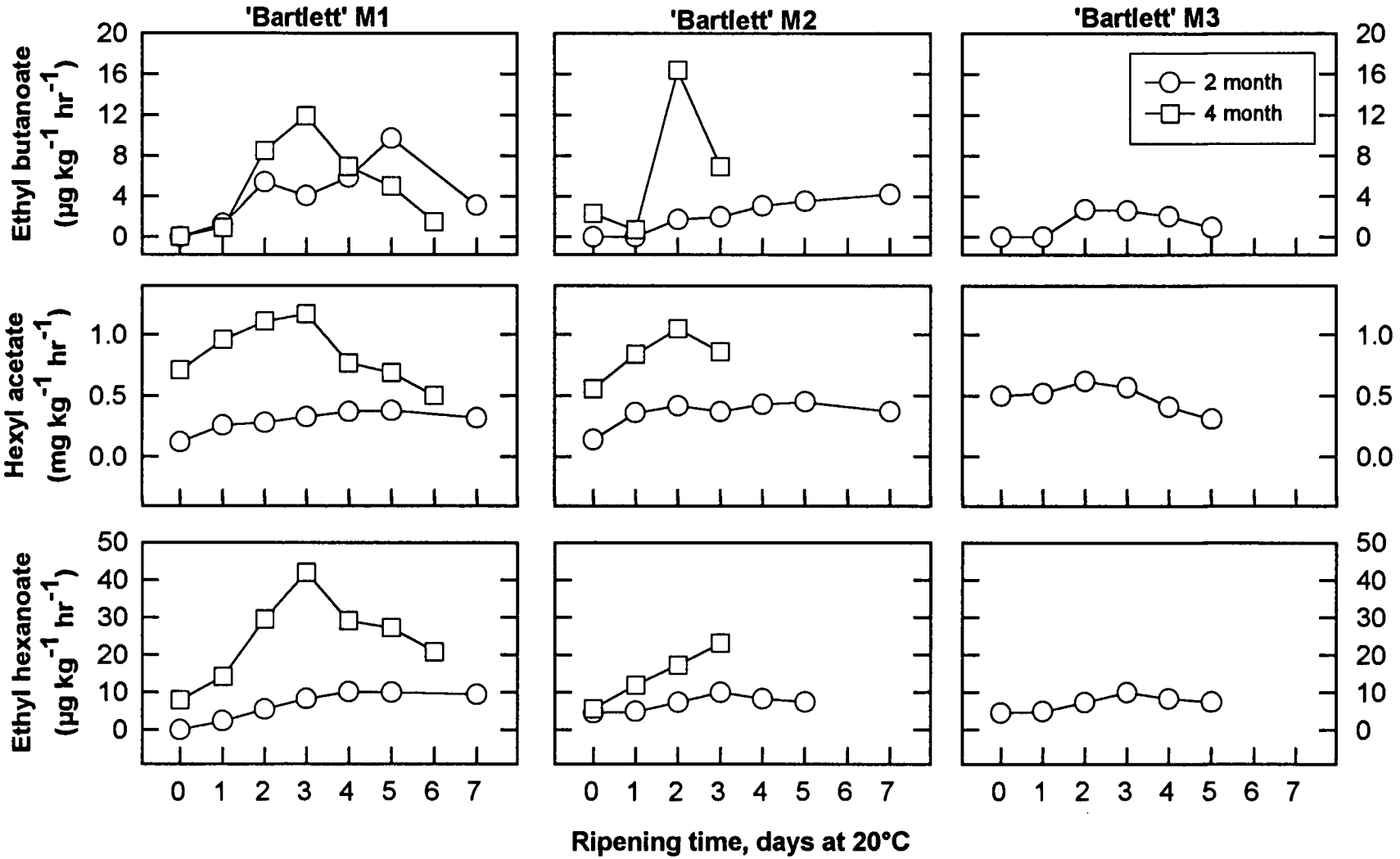

Figure 6.3. Changes in selected flavor impact compounds during ripening of 'Bartlett' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage at 0°C for 2 to 4 months.

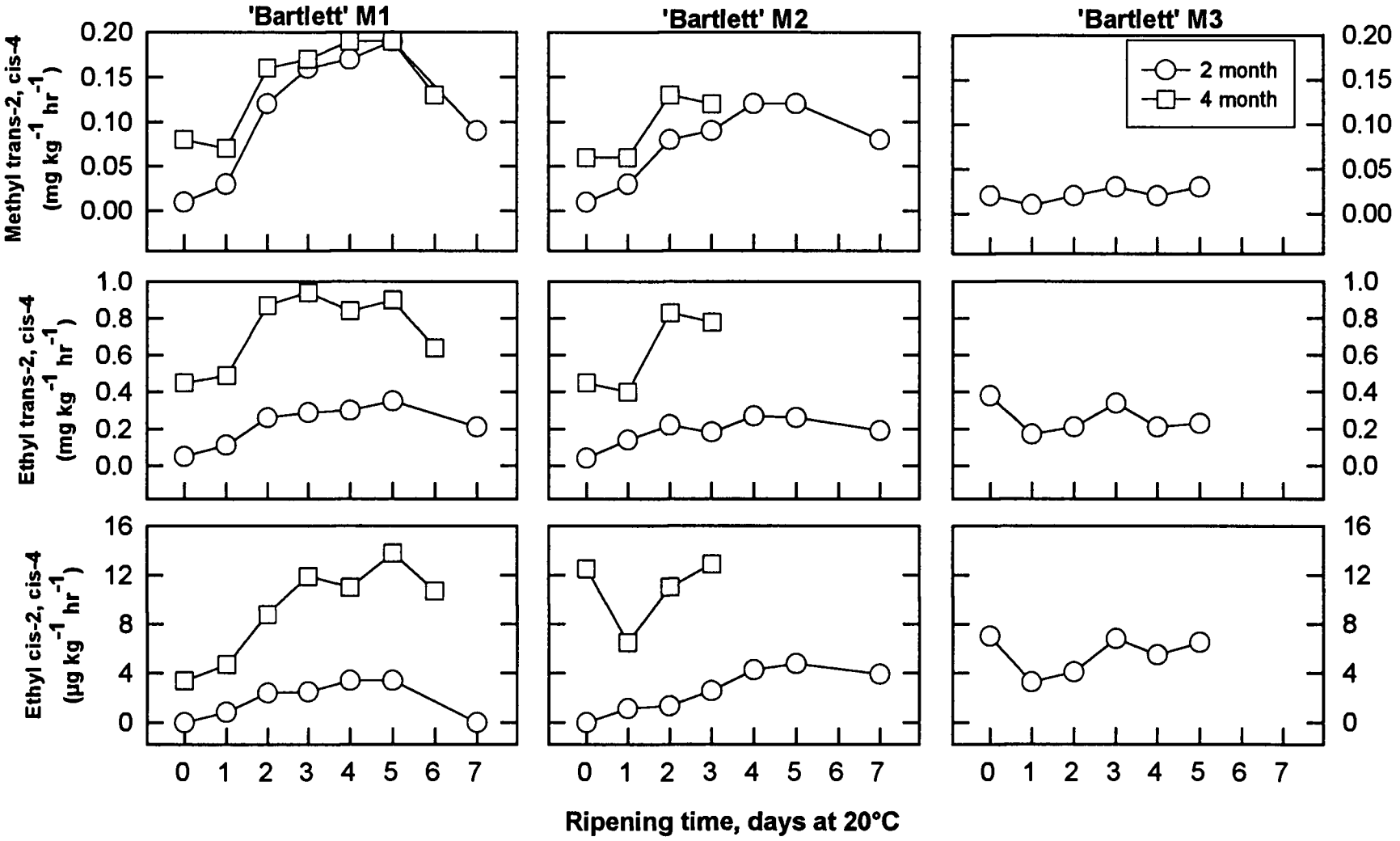

Figure 6.4. Changes in decadienoate ester production during ripening of 'Bartlett' pear harvested at early (M1), optimum (M2) and late (M3) maturity stages after storage at 0°C for 2 to 4 months.

Sevenants, 1964). However, a significant increase in methyl trans-2, cis-4 decadienoate production was observed in fruits harvested at the early stage of maturity. This unsaturated ester has been found to possess a mild estery or floral aroma in 'Bartlett' pear (Chapter 4).

In general, harvest maturity has minor effects on overall 'Bartlett' pear aroma as indicated by sensory evaluations as well as evolution of headspace volatile compounds during fruit ripening. Fruits were ripened to about the same maximum pear flavor intensity regardless of harvest maturity. Early harvest fruits not only are able to ripen to the same acceptable eating quality as fruits harvested at later maturities but also have longer shelf-life than fruits harvested at later maturity stages. Similarly, late harvest fruits tend to ripen to optimum eating quality faster and have shorter shelf-life than early harvest pears.

Relatively minor differences in the magnitude of major flavor impact volatile production during ripening were also observed among fruits harvested at different maturity stages. Interestingly, the production of total esters in early harvest fruit appeared to be at about the same level or slightly higher than that of optimum harvest fruit or fruit harvested later. More volatile esters have been reported in 'Comice' and 'Conference' pears harvested at later maturity stages (Zerbini and Spada, 1993). Since the volatile measurements were performed on all fruits on the same day (in this case, after 6 days of ripening), the differences in volatiles observed in their research may caused by differences in fruit ripeness rather than differences in harvest maturity.

Prolonged cold storage of fruits resulted in a rapid increase in pear flavor intensity during fruit ripening. The increase in flavor intensity was accompanied by increases in total volatile ester and alcohol production. A significant increase in major

flavor impact compounds was also observed in fruits after storage. Similar results were reported in 'Golden Delicious' apple after storage at 3°C for 2 to 4 months. Fruits upon removal from cold storage produced greater amounts of volatiles than fruits before storage or from storage at higher temperature (Grevers and Doesburg, 1965). Pear fruits, especially winter pears have been known to ripen to maximum flavor after specific time in cold storage (Claypool et al., 1958; Knee, 1987). Low temperature also was reported to encourage the production of unsaturated fatty acids (Zill and Cheniae, 1962). Important flavor components of 'Bartlett' pear were postulated to be derived from β-oxidation of several unsaturated fatty acids (Jennings and Tressl, 1974).

Fruits after storage for 4 months, upon removal from cold storage ripened to the optimum rapidly and exhibited various degrees of scald symptoms depending on harvest maturity. Higher scald incidence was observed in fruits with more advanced harvest maturity. The oxidation of α-farnesene is believed to be involved in scald formation (Huelin, 1964; Huelin and Coggiola, 1968; Huelin and Coggiola, 1970; Anet, 1974; Anet and Coggiola, 1974). However, in this study, only slight differences in α-farnesene evolution were observed in 'Bartlett' pears stored for 2 to 4 months. The results suggested that α-farnesene level may not be a good indication for scald incidence prediction. The results of this study were also somewhat different from results obtained from peel hexane extraction of 'Bartlett' pear harvested at the same three maturity stages (Zoffoli, 1994). In that study, the highest α-farnesene level, as measured by spectrophotometry, was reported in late harvest fruit after storage for 2 months. As the storage duration increased to 3.5 months, a rapid decline in α-farnesene was observed in fruits harvested at all maturity stages. However, after 3.5

months storage, the lowest α-farnesene was detected in late harvest fruits, and this coincided with the highest scald incidence. Contrary to the present study, the highest level of α-farnesene was observed in early fruit compared to fruits harvested later. Besides, there was no decrease in α- farnesene in fruits stored for 4 months. However, higher scald incidence was observed in fruit with more advanced harvest maturity.

'Packham's Triumph' pears

The effects of harvest maturity and storage duration on sensory attributes during ripening of 'Packham's Triumph' pear are shown in Figure 6.5. Similar to 'Bartlett' pear, fruits harvested at different maturity stages ripened to optimum eating quality at different rates. After 2 months in storage, late harvest fruit ripened to maximum acceptability after 6 days at 20°C, while slight longer times were required for optimum and early harvest fruits (7 and 8 days, respectively). Furthermore, the magnitude of the maximum acceptability score seemed to increase in fruits harvested at more advanced maturity. A slightly longer time was required for early harvest fruits to ripen to the maximum pear flavor intensity. The maximum pear flavor intensity score was observed in late harvested fruit after 6 days of ripening, while 10 days were required for both early and optimum harvest fruits. A slightly higher intensity score was observed in optimum harvested fruit compared to fruit harvested at early and late maturity stages.

Prolonged storage of fruits for 4 to 6 months, resulted in shorter time required for fruit to reach optimum eating quality and pear flavor intensity. After 4 months storage, late, optimum and early harvest fruits ripened to optimum eating quality

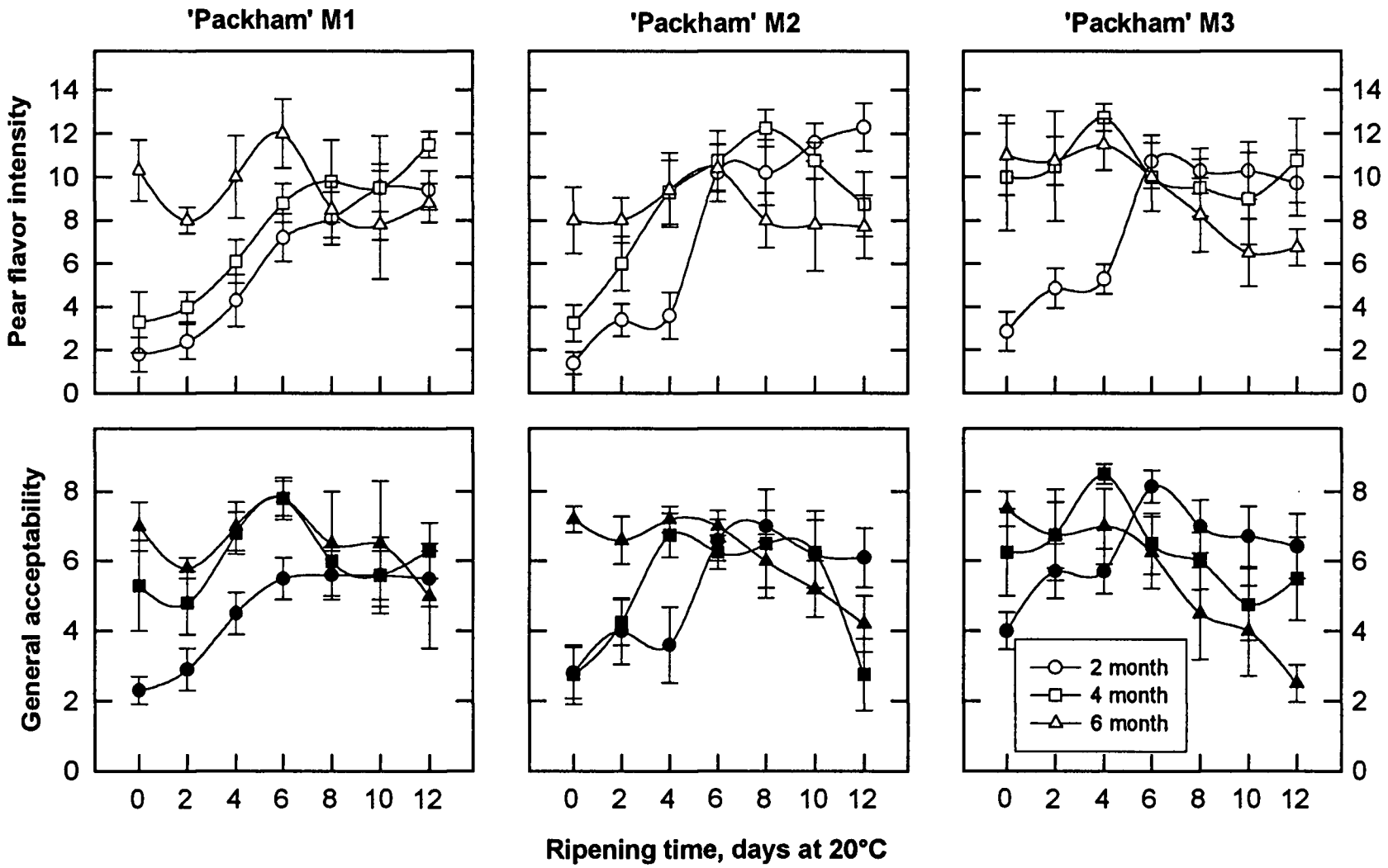

Figure 6.5. Changes in sensory attributes of 'Packham's Triumph' pear harvested at early (M1), optimum (M2) and late (M3) maturity stages after storage for 2,4 or 6 months at 0°C. Vertical bars represent standard error of means (n = 6 to 8).

within 4, 4 and 6 days at 20°C, respectively. The highest pear flavor intensity scores of these fruits were observed after 4, 8 and 8 days of ripening, respectively.

Late and optimum harvest fruits were ripened to optimum after removal from cold storage for 6 months. However, they required 4 and 6 more days to produce the maximum flavor intensity. Fruits harvested at early stages ripened slower. It required 6 days at 20°C to reach the maximum eating quality and flavor intensity score.

Changes in volatile production during ripening of 'Packham's Triumph' pear harvested at early, optimum and late maturity stages after storage at 0°C for 2 to 6 months are tabulated in Appendix Tables A.6.6 to A.6.11.

The effects of harvest maturity and storage duration on total esters, α-farnesenes, and alcohol production during ripening of 'Packham's Triumph' pear are shown in Figures 6.6, 6.7 and 6.8, respectively. Total ester production rate of fruits harvested at all maturities increased rapidly after 2 months of cold storage (Figure 6.6). Production rates were irregular due to various ester increases at different times during fruit ripening. However, a significant reduction in total ester production can be observed in late harvest fruit after storage for 2 months. Prolonged storage of fruits for 4 to 6 months resulted in about 3-fold reduction in total ester production in all fruits. Fruits after storage for 6 months showed a tendency to produce less esters than fruits after storage for 4 months. However, the differences in ester production of fruits after storage for 4 and 6 months were not obvious.

Effects of harvest maturity and storage duration on total α-farnesene production of 'Packham's Triumph' pear are shown in Figure 6.7. Slightly less α-farnesene was observed in late harvest fruit upon removal from cold storage after 2 months. Prolonged storage of fruits for 4 to 6 months resulted in significantly less

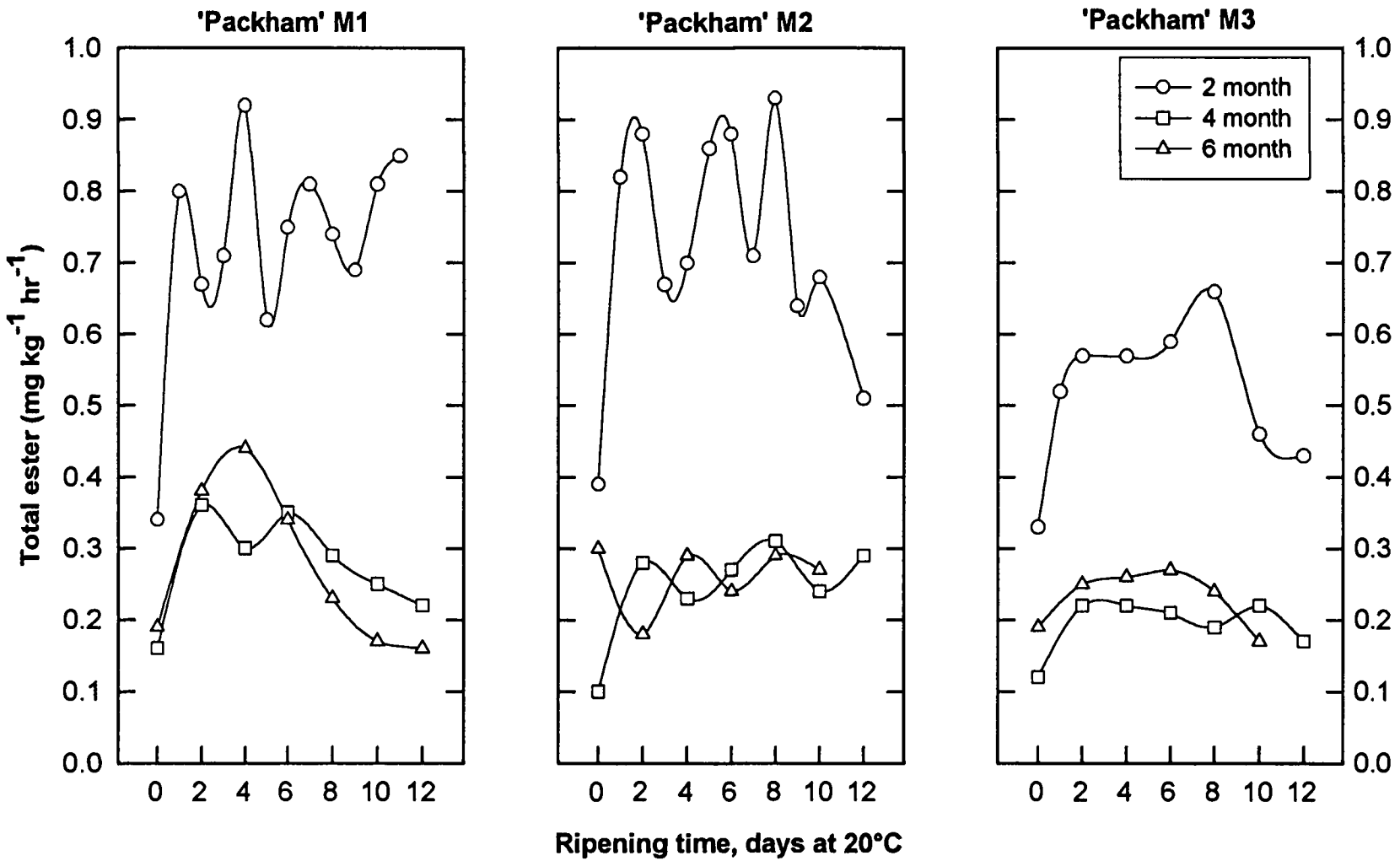

Figure 6.6. Changes in total ester production during ripening of 'Packham's Triumph' pear harvested at early (M1), optimum (M2) and late (M3) maturity stages after storage for 2, 4 to 6 month at 0°C.

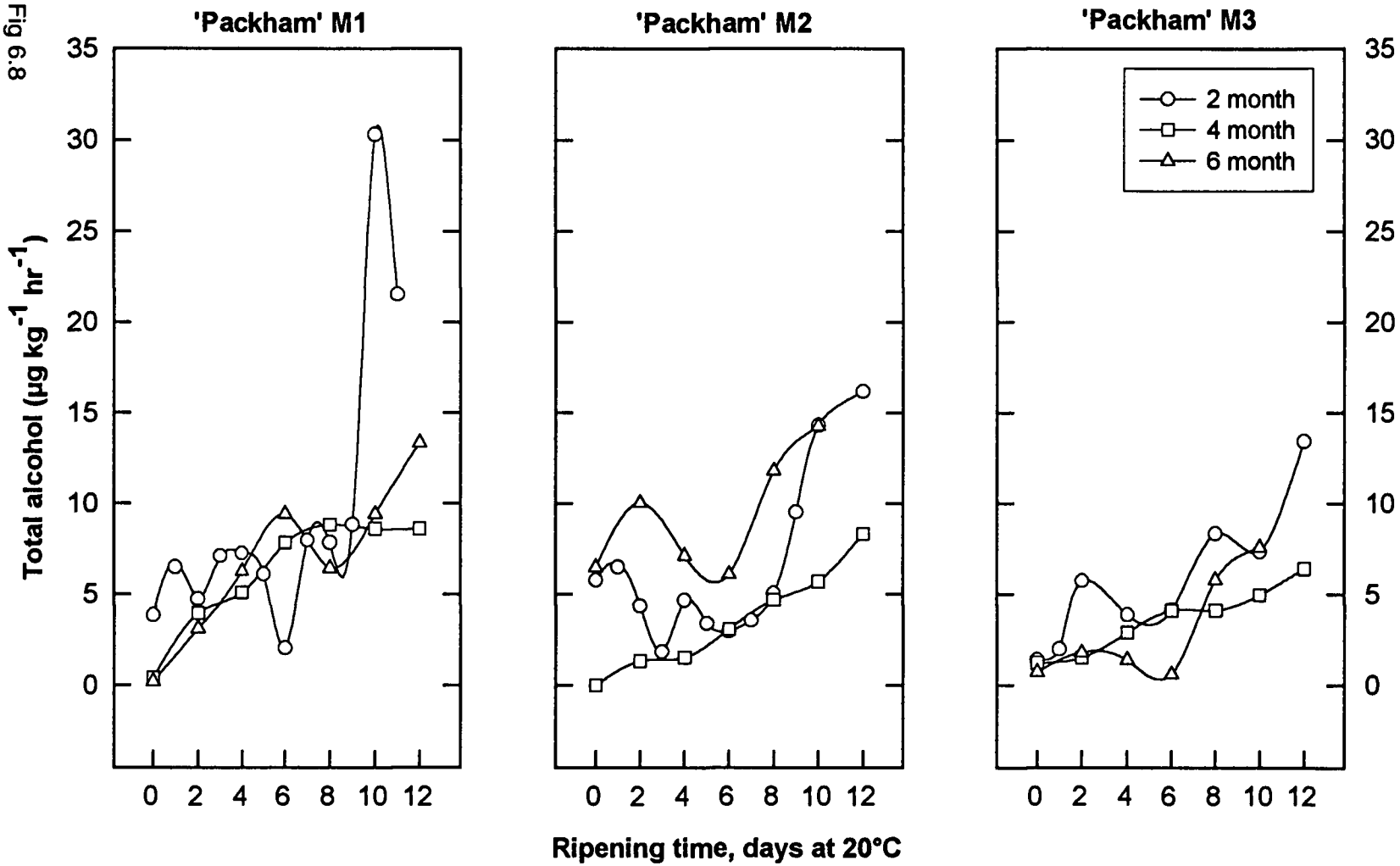

Figure 6.7. Changes in total alcohol production during ripening of 'Packham's Triumph' pear harvested at early (M1), optimum (M2) and late (M3) maturity stages after storage for 2, 4 to 6 month at 0°C.

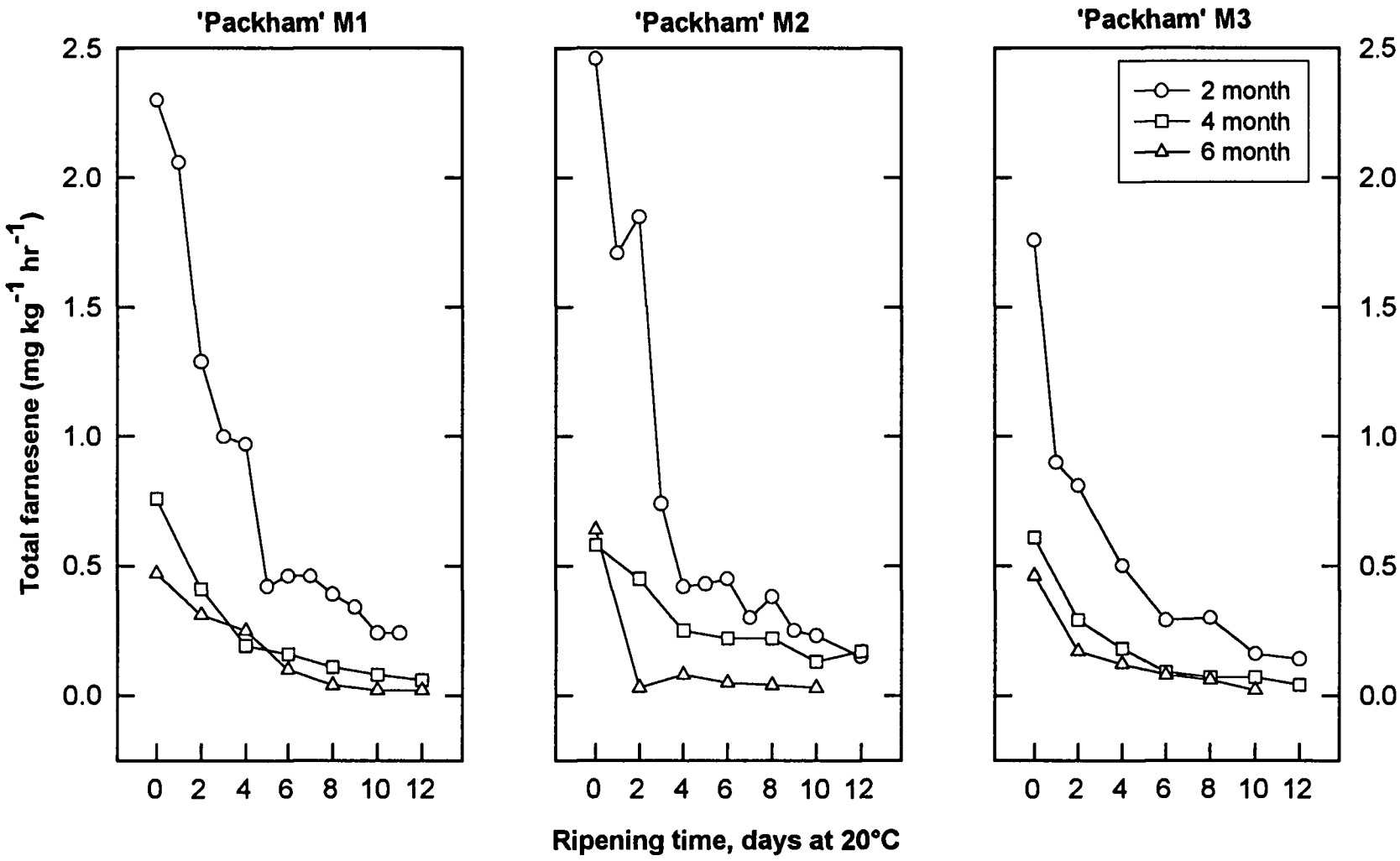

Figure 6.8. Changes in total farnesene production during ripening of 'Packham's Triumph' pear harvested at early (M1), optimum (M2) and late (M3) maturity stages after storage for 2, 4 to 6 month at 0°C.

α-farnesene in fruits harvested at all maturities. No significant differences in α-farnesene levels were found among fruits harvested at different maturities after storage for 4 to 6 months. α-Farnesene levels in fruits at all storage durations tend to decrease to low levels as fruits advanced in ripening. No scald symptom was observed in any of these fruits after 4 months storage. However, fruits harvested at different maturity stages exhibited differences in scald susceptibility after storage for 6 months. Early harvest fruits started to develop scald symptoms after ripening for 8 days. The scald intensity in this fruit appeared to develop slowly and remained at low levels toward the end of ripening period (day 12). In optimum harvest fruits, the disorder was observed after day 6 of ripening and spread very rapidly, finally with complete darkening of fruit after 10 days. No scald incidence was observed in late harvest fruit throughout the ripening period.

Irregular total alcohol production was observed in fruits harvested at all maturities during ripening (Figure 6.8). Similar to what was observed in total ester production, fruits after storage for 2 months exhibited the most severe fluctuation in alcohol production during ripening. This fluctuation may reflect the irregular production of particular alcohols and the dependency of ester synthesis in using those alcohols as precursors. However, no severe reduction in alcohols production during fruit ripening was observed in fruit after storage for 4 to 6 months.

Effects of harvest maturity and storage duration on production of selected flavor impact compounds in 'Packham's Triumph' pear are shown in Figures 6.9, 6.10 and 6.11. Ethyl acetate, propyl acetate, ethyl hexanoate, methyl and ethyl trans-2, cis-4 decadienoate production were found to highly correlate to both pear flavor intensity and general acceptability of 'Packham's Triumph' fruits during ripening at

20°C (Chapter 5). For fruit stored for 2 months, a substantial increase in the production of ethyl and propyl acetate were observed in early harvest fruit after ripening for 6 days (Figure 6.9). Production of these two esters in optimum and late harvest fruits gradually increased toward the end of ripening period. Prolonged storage of fruits for 4 to 6 month resulted in a severe reduction of those two esters in fruits regardless of harvest maturity.

A similar increase in ester production as a result of early harvest was also observed in ethyl hexanoate production during ripening of 'Packham's Triumph' pear after storage for 2 months (Figure 6.10). Early harvest fruit showed a substantial increase in the production of ester after 3 days of ripening. Less effects of harvest maturity were found in fruits after prolonged storage for 4 to 6 months. Fruits harvested at all maturity stages ripened after 6 months gradually produced ethyl hexanoate reaching the peak but much lower level compared to the fruits after storage for 2 months. Maximum ester production appeared to be the same between fruits stored for 4 and 6 months.

A rapid increase in hexyl acetate production was observed in all fruits after storage for 2 months (Figure 6.10). Higher production was observed in fruits harvested at optimum maturity stage. Hexyl acetate production of fruits during ripening declined substantially after storage for 4 to 6 months. Again, no differences in ester production rate were found between fruit stored for 4 and 6 months.

Similar to what was observed in ethyl hexanoate production, methyl trans-2, cis-4 decadienoate production rapidly increased after 2 to 3 days of ripening (Figure 6.11). Fruits harvested at early or optimum maturity stages produced higher amounts of this ester than fruit harvested at late stage of development. Prolonged storage of fruits for 4 to 6 months also resulted in less production of this ester.

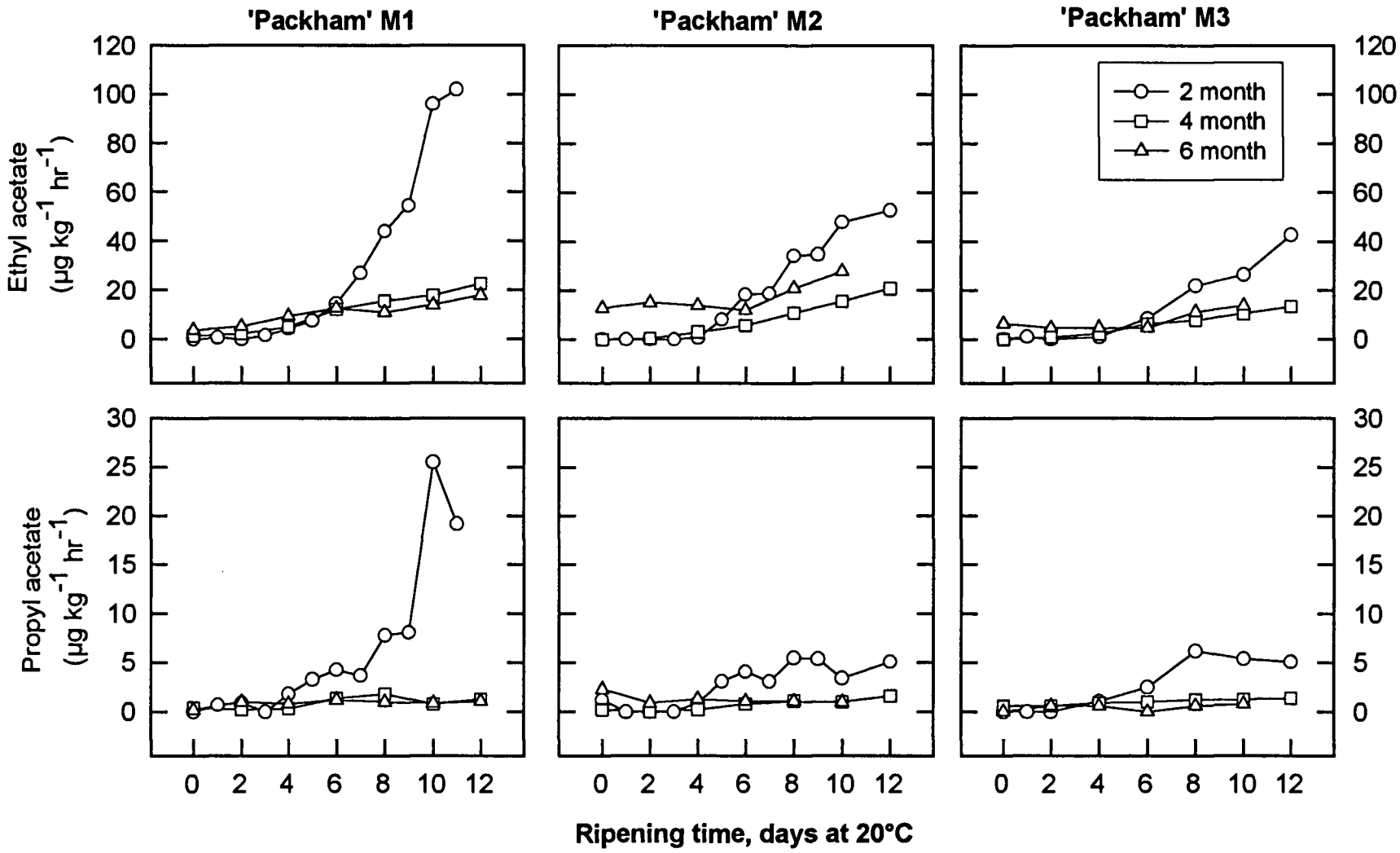

Figure 6.9. Changes in ethyl acetate and propyl acetate production during ripening of 'Packham's Triumph' pear harvested at early(M1), optimum (M2) and late(M3) maturity stages after storage at 0°C for 2 to 6 months.

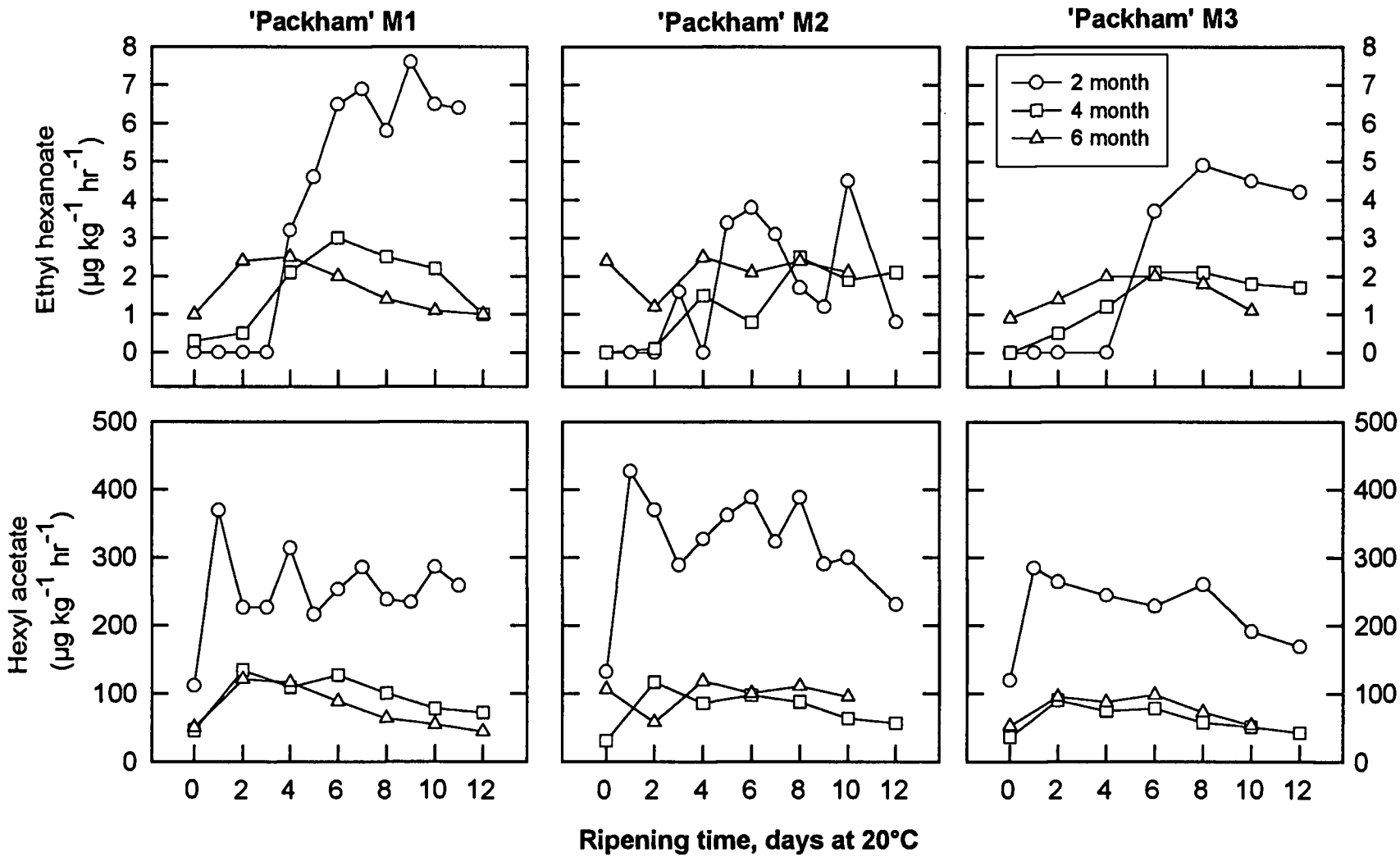

Figure 6.10. Changes in ethyl hexanoate and hexyl acetate production during ripening of 'Packham's Triumph' pear harvested at early(M1), optimum (M2) and late(M3) maturity stages after storage at 0°C for 2 to 6 months.

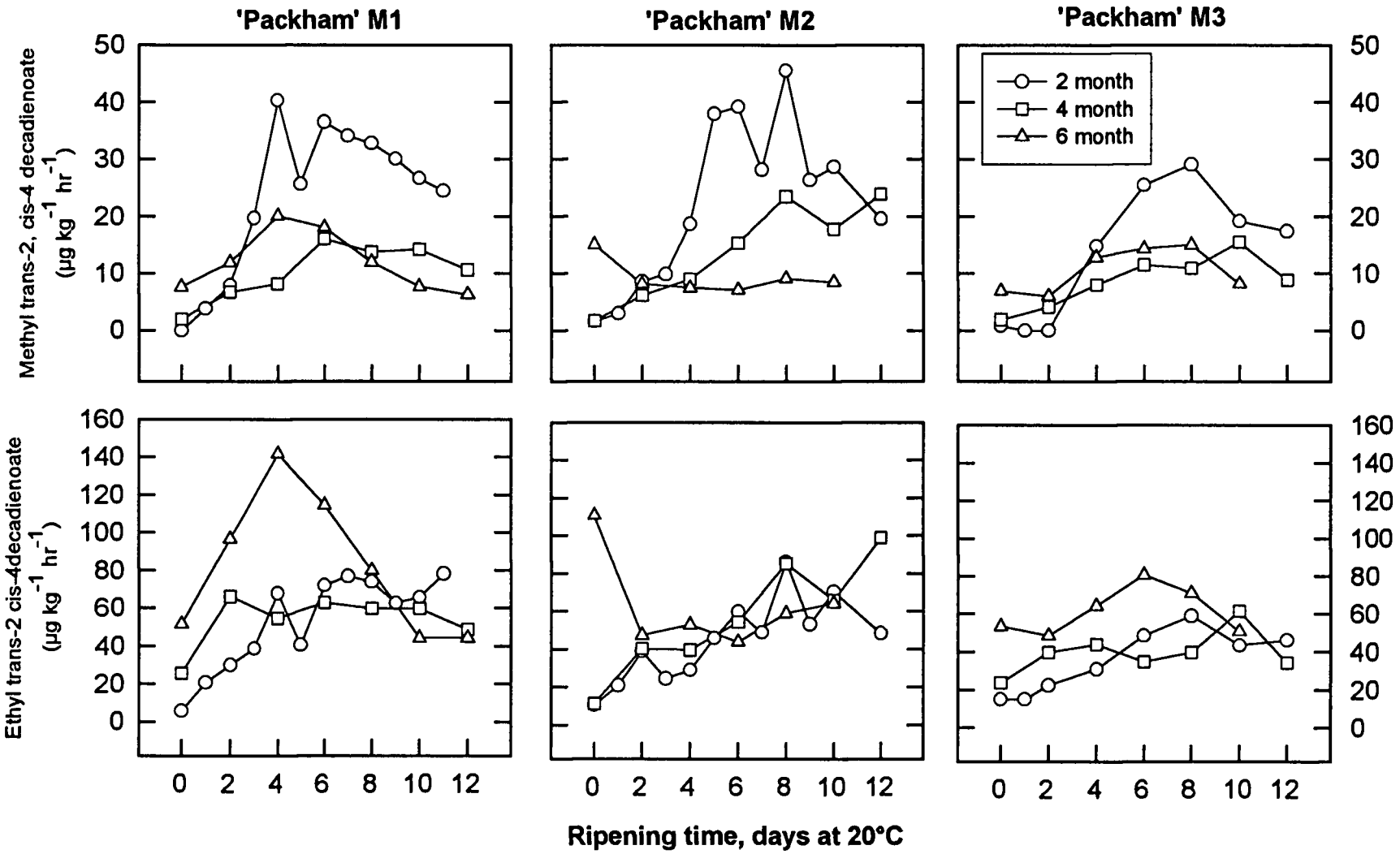

Figure 6.11. Changes in decadienoate ester production during ripening of 'Packham's Triumph' pear harvested at early(M1), optimum (M2) and late(M3) maturity stages after storage at 0°C for 2 to 6 months.

Only a slight increase in ethyl trans-2, cis-4 decadienoate was observed in early harvested fruits after storage for 2 months (Figure 6.11). Fruits harvested at all maturity stages were still capable to produce higher amounts of this ester after storage for 4 to 6 months. However, an unusual increase in this decadienoate ester production during ripening was observed in early harvested fruit after storage for 6 months.

Similar to what was observed in 'Bartlett' pear, harvest maturity had a minor affect on overall 'Packham's Triumph' pear aroma. Results from sensory evaluation indicated that fruits harvested at all harvest maturity stages could be ripened to almost the same maximal pear flavor intensity score after storage for 2 to 6 months. However, late harvest resulted in less time for fruits to reach maximum flavor and acceptability during ripening at 20°C. Early harvested fruits were ripened at slower rate and retained high eating quality longer than late harvest fruits. In contrast, late harvest fruits ripened to slight higher eating quality but had shorter marketable life than early harvest fruits at the same storage duration. No differences in sensory attributes were reported in 'Comice' pear harvested at different maturity stages (Zerbini and Spada, 1993). Early harvested fruit tend to store very well and have longer storage life than fruits harvested at later stages of maturity.

Unlike 'Bartlett' pear, large differences in ester production during ripening were observed in 'Packham's Triumph' fruits harvested at different maturity stages. As indicated by the total esters and selected flavor impact esters production during fruit ripening, fruits harvested at earlier maturity seemed to produce more esters than fruits harvested at later maturity. This observation has not been reported in other fruits since it has been long known that apples harvested before optimum stage of maturity will not produce the same amount of volatile flavor compounds and result in

low eating quality fruits after ripening (Brown et al., 1965; Bachmann, 1983; Dirinck et al., 1989). Similar results were reported in peaches (Do et al., 1969) and tomatoes (Shah et al., 1969).

Prolonged storage of 'Packham's Triumph' pears for 4 to 6 months resulted in less volatile flavor compounds in fruit of all harvest maturities. The acceptability and pear flavor intensity of ripened fruits were still rated as high as fruits ripened after storage for 2 months. The results suggested that sensory perceptions may not depend solely on volatiles esters which contributed mainly to fruit aromas. Other non-volatile compounds which contribute to taste sensations as well as texture attributes may also play a significant role in pear flavor and overall acceptability of fruits during ripening.

However, the variation among judges or panelists used in the test can not be ruled out, since it is not unusual for panelists to be a major source of variation in sensory evaluation of products (Hall and Lingnert, 1984). The inconsistent use of different levels of the rating scale among judges or within the same judges frequently causes lack agreement of the results (Heymann and Noble, 1987).

'Anjou' pears

The effect of harvest maturity and storage duration on sensory attributes during ripening of 'Anjou' pear are shown in Figure 6.12. Similar to 'Bartlett' and 'Packham's Triumph' pear, fruits harvested at different maturity stages ripened to optimum eating quality at different rates. After 3 months in cold storage, early harvest and late harvest fruit gradually ripened to optimum acceptability after 10 days at 20°C. Fruits harvest at optimum or commercial maturity ripened rapidly and reached optimum acceptability within 2 days. General acceptability of fruits harvested at this

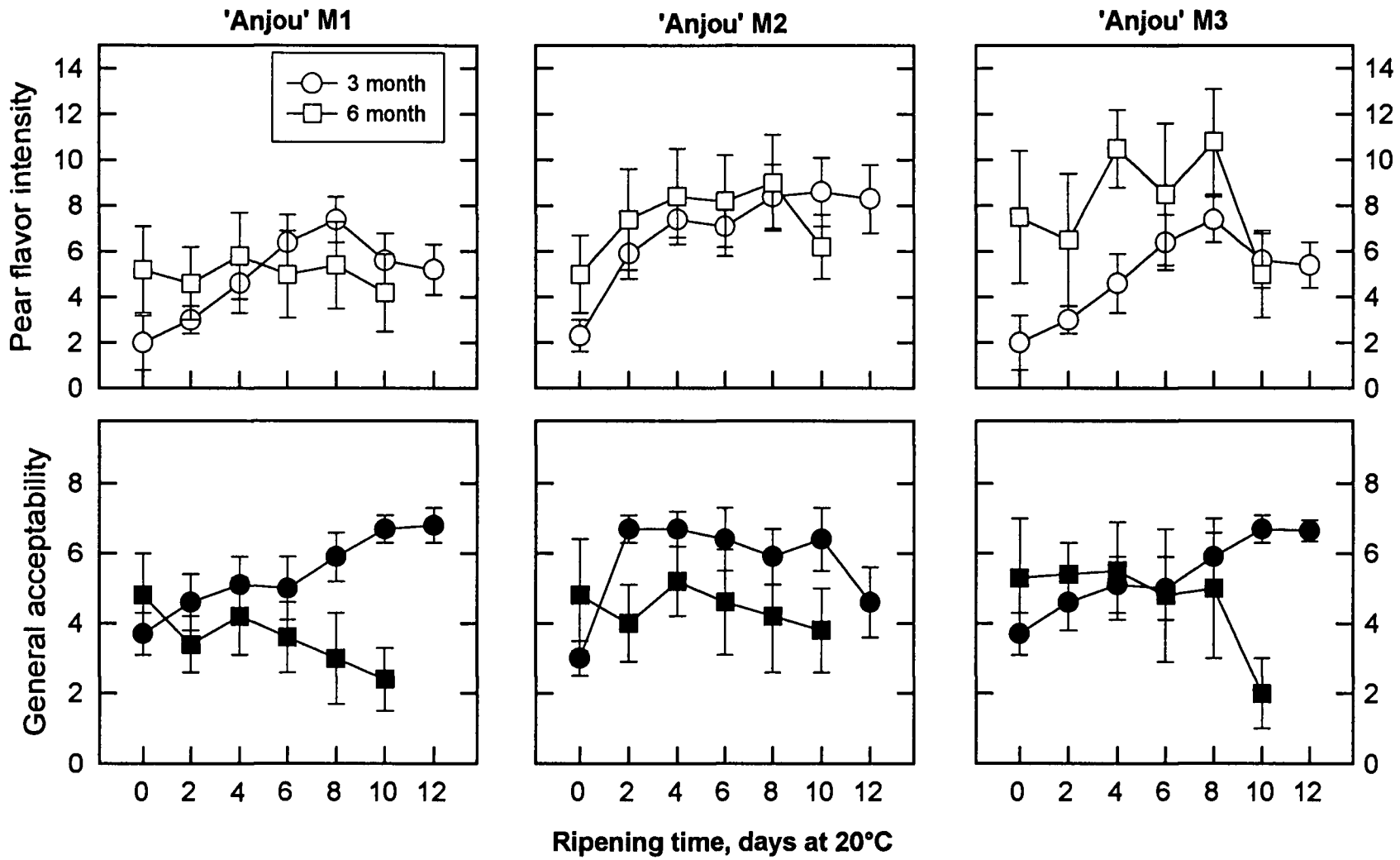

Figure 6.12. Changes in sensory attributes during ripening of 'Anjou' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage for 3 to 6 months at 0°C. Vertical bars represent standard error of means (n = 6 to 8).

maturity still remained at high level up to 10 days, then started to decline. A gradual increase in pear flavor intensity score was also observed in early and late harvested fruits after storage for 3 months. The highest pear flavor intensity scores were observed after 8 days of ripening then started to decline as fruit advanced in ripening. By contrast, a rapid increase in pear flavor intensity score was observed in fruit harvested at optimum maturity. Maximum pear flavor intensity was observed about the same time as early and late harvest fruits (day 8). However, the intensity score in this fruits remained at high level even toward the end of ripening (day 12).

Prolonged storage of fruits for 6 months significantly shortens the time for fruits to reach optimum eating quality. Fruits harvested at all maturity stages were rated at maximal acceptability the day of removal from cold storage (day 0). Thereafter, rapid decrease in acceptability scores were observed in early harvest fruits as ripening advanced. Meanwhile, acceptability scores of optimum and late harvest fruits still remained relatively high until day 4 of ripening before decreasing. Relative high pear flavor intensity scores were observed in optimum and late harvest fruits compared to early harvest fruits after storage for 6 months. Pear flavor intensity scores for optimum and late harvested fruits also increased during fruit ripening with higher magnitude than what was observed in fruit stored for 3 months. No increase in flavor intensity score was observed in early harvest fruit during ripening.

Changes in static headspace volatile profiles during ripening of 'Anjou' pear harvested at early, optimum and late maturity stages after storage for 2 to 6 months at 0°C are presented in Appendix Tables A.6.12 to A.6.25.

The effects of harvest maturity and storage duration on total ester, α-farnesenes and alcohols production during ripening of 'Anjou' pear after storage for 3

to 6 months are shown in Figures 6.13, 6.14 and 6.15, respectively. A significant difference in ester production rate during ripening was observed between fruits harvested at different maturity stages after storage for 3 months (Figure 6.13). A rapid increase in ester production was observed in both early and optimum harvest fruit at early stage of ripening. Ester production in both fruit reached maxima at the same magnitude and ripening time (day 8). In contrast, the ester production of late harvest fruit increased gradually and reached the maximum on day 6 with one-third the magnitude of fruits harvested at earlier maturities. Prolonged storage for 6 months resulted in severe reduction of ester production in early and optimum harvest fruit to the same level as observed in late harvest fruit after storage for 3 months.

Effects of harvest maturity and storage duration on total α-farnesene production of 'Anjou' pear during fruit ripening are shown in Figure 6.14. Less α-farnesene content at the time of removal from cold storage (day 0) was observed in late harvest after storage for 3 months. No scald symptoms were detected at this storage duration. After storage for 6 months, a significant reduction in α-farnesene content was observed in early and optimum harvest fruits, while late harvest fruit the α-farnesene remained the same as was observed after 3 months. At this storage duration, early and late harvest fruits started to develop scald symptoms during ripening. The symptoms observed in these fruits were small dark discoloration spots scattered throughout the fruit after ripening for 6 to 8 days. However, the incidence of scald remained at low level until the end of ripening period. Similar to 'Packham's Triumph' pear, no scald symptom was observed in late harvest fruit at this storage duration.

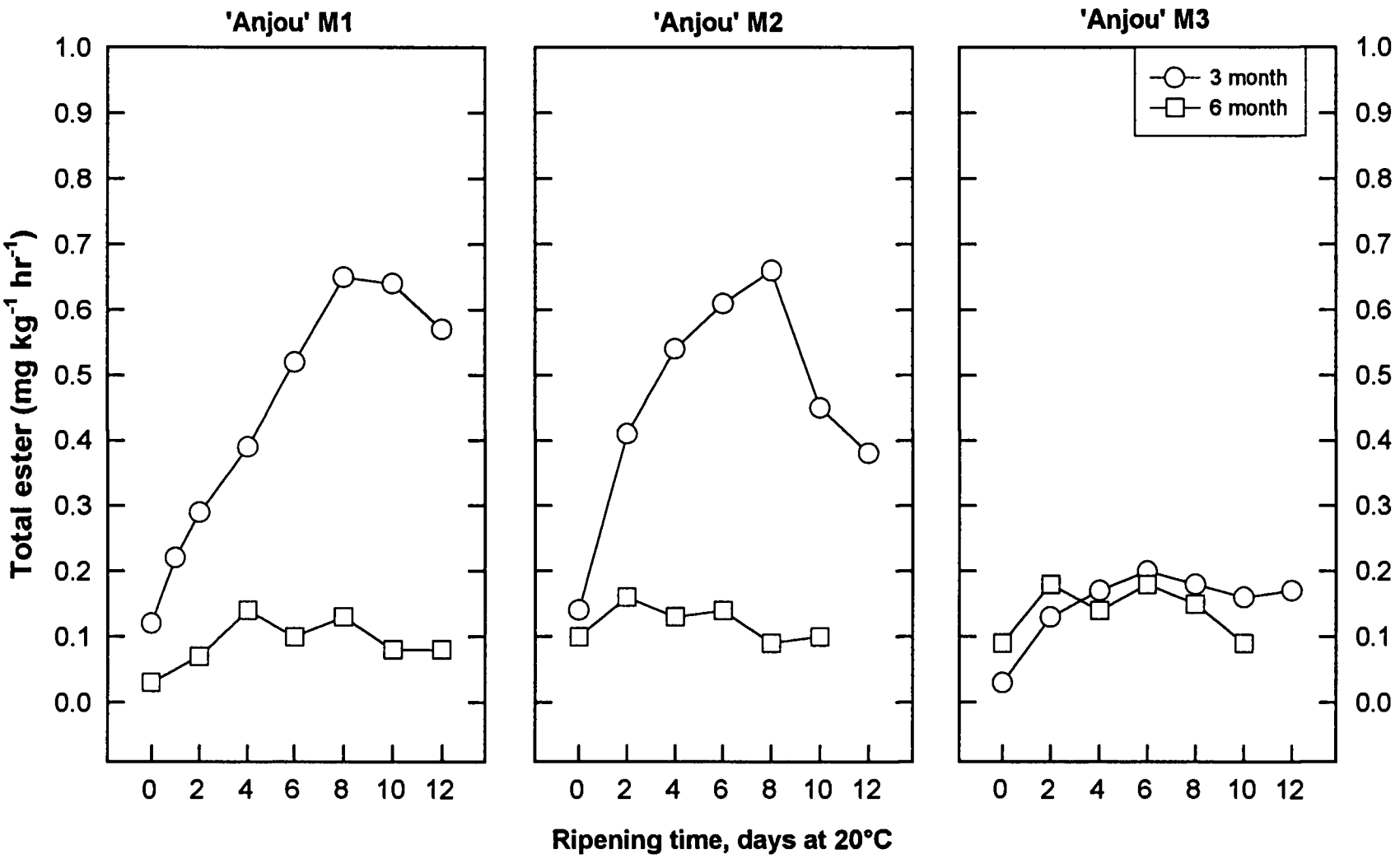

Figure 6.13. Changes in total ester production during ripening of 'Anjou' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage for 3 to 6 months at 0°C.

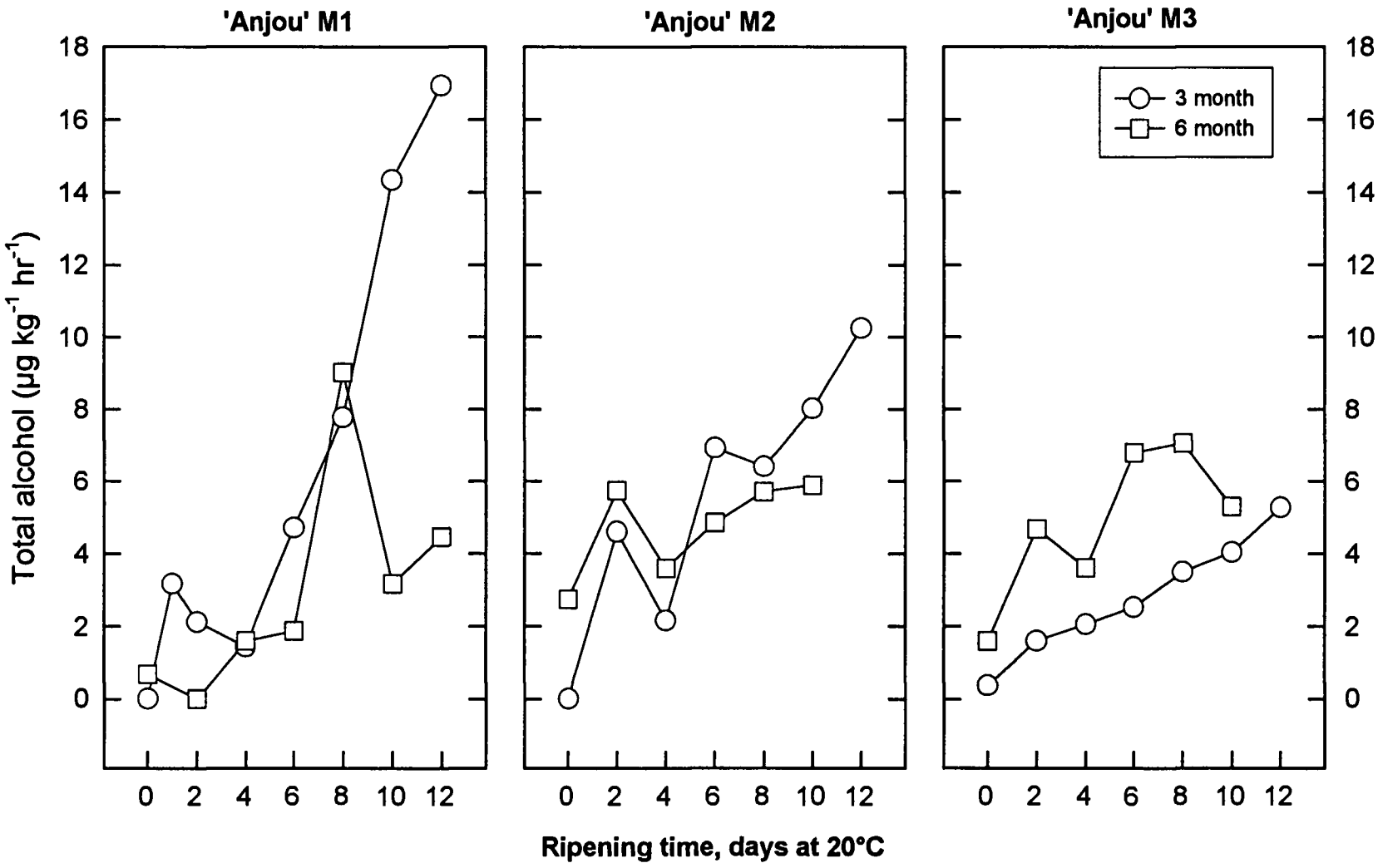

Figure 6.14. Changes in total alcohol production during ripening of 'Anjou' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage for 3 to 6 months at 0°C.

As with ester production, delay in harvest also resulted in less alcohol production during fruits ripening (Figure 6.15). The lowest alcohol production rate was observed in late harvest fruit after storage for 3 months. Prolonged storage for 6 months also resulted in less alcohol production in early and optimum harvest fruits but not for late harvest fruit. Alcohol production in late harvest fruit after storage for 6 months was higher than that observed after 3 months.

The effects of harvest maturity and storage duration on the production of important flavor impact compound production of 'Anjou' pear during ripening are shown in Figures 6.16, 6.17 and 6.18. Butyl hexanoate and hexyl hexanoate are two main components in 'Anjou' pear volatile compounds. The first compound has a cooked artichoke aroma which may be characterized as typical 'Anjou' aroma. The latter compound possessed a candy or floral-like aroma which was categorized as contributory to pear aroma (Chapter 4). The production of both compounds increased at the early stage of fruit ripening and declined at the end of ripening when maximum acceptability and pear flavor intensity of fruits were obtained. A severe reduction of both compounds was observed in fruit harvested at later stage of maturity after storage for 3 months (Figure 6.16). Prolonged storage for 6 months resulted in a severe decrease of both compounds during fruit ripening, regardless of harvest maturity.

Pentyl acetate, ethyl octanoate, methyl 4-decenoate and ethyl trans-2, cis-4 decadienoate were among the compounds found to have high positive correlation to pear flavor intensity during fruit ripening (Chapter 5). These compounds (except methyl 4-decenoate) were found to possess pear or fruity-like odor by sniffing GC. Methyl 4-decenoate possessed an anise-like odor which can contribute to typical 'Anjou' aroma (Chapter 4). Again, a severe reduction of both compounds was

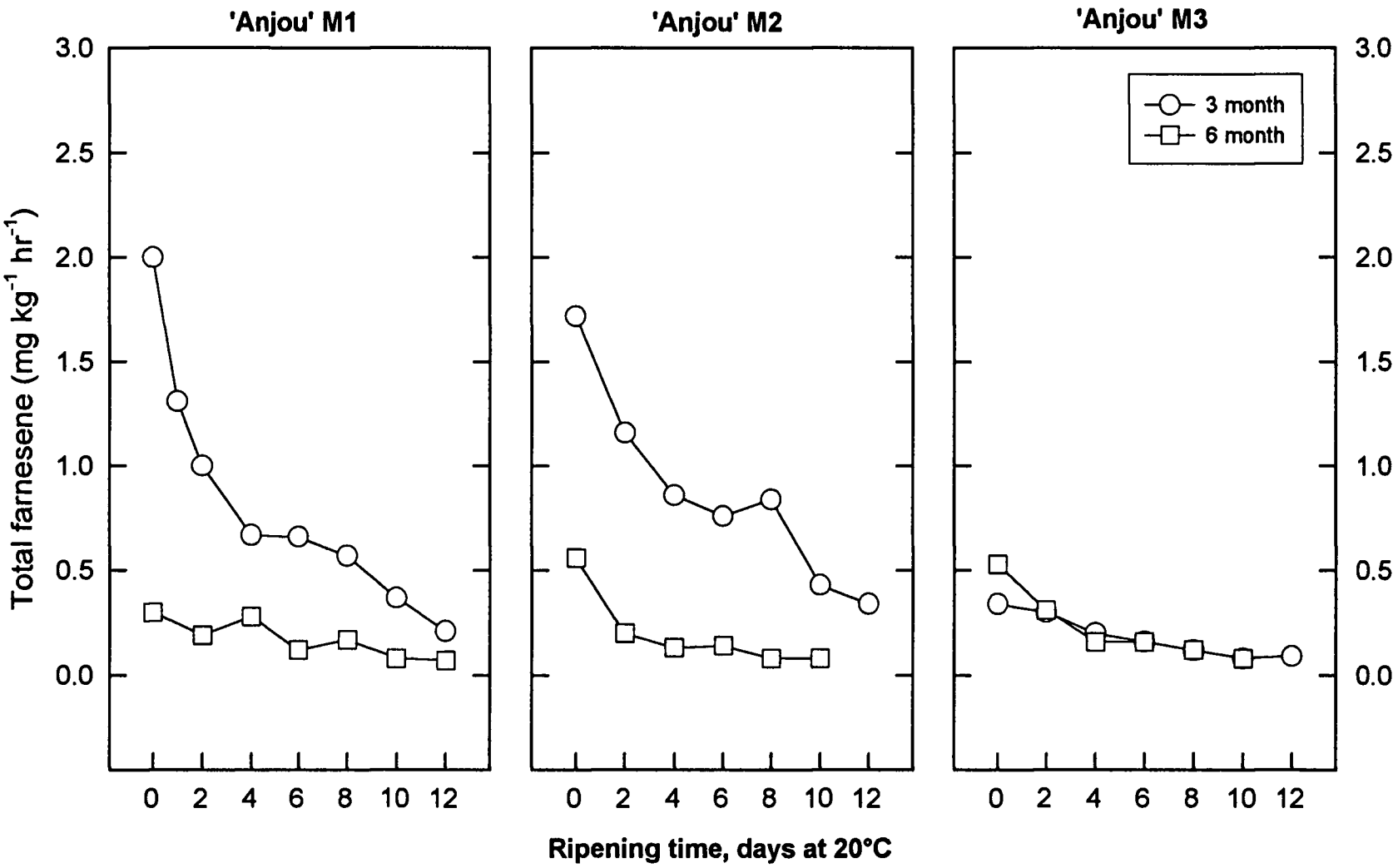

Figure 6.15. Changes in total farnesene production during ripening of 'Anjou' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage for 3 to 6 months at 0°C.

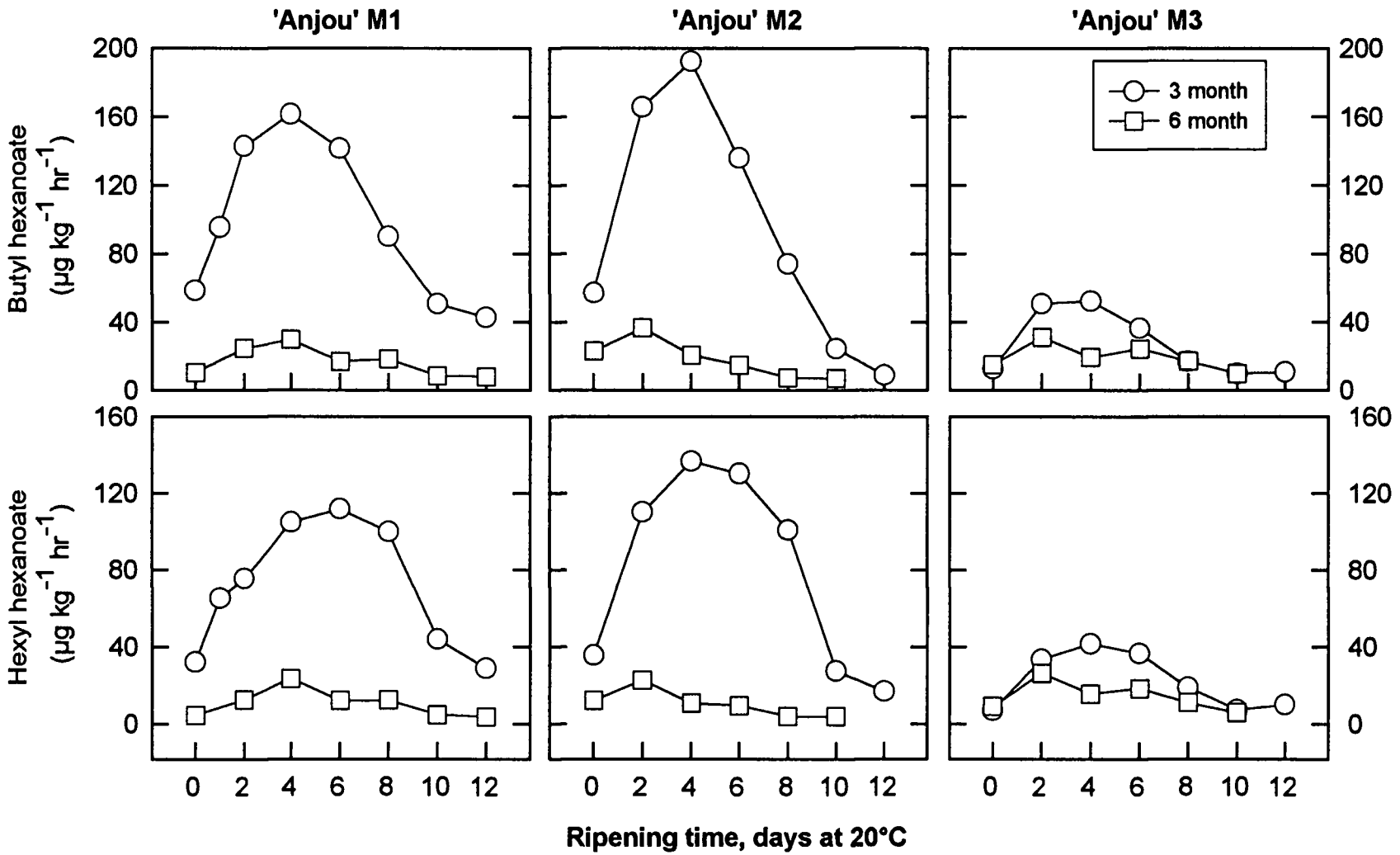

Figure 6.16. Changes in butyl hexanoate and hexyl hexanoate productions during ripening of 'Anjou' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage for 3 to 6 month at 0°C.

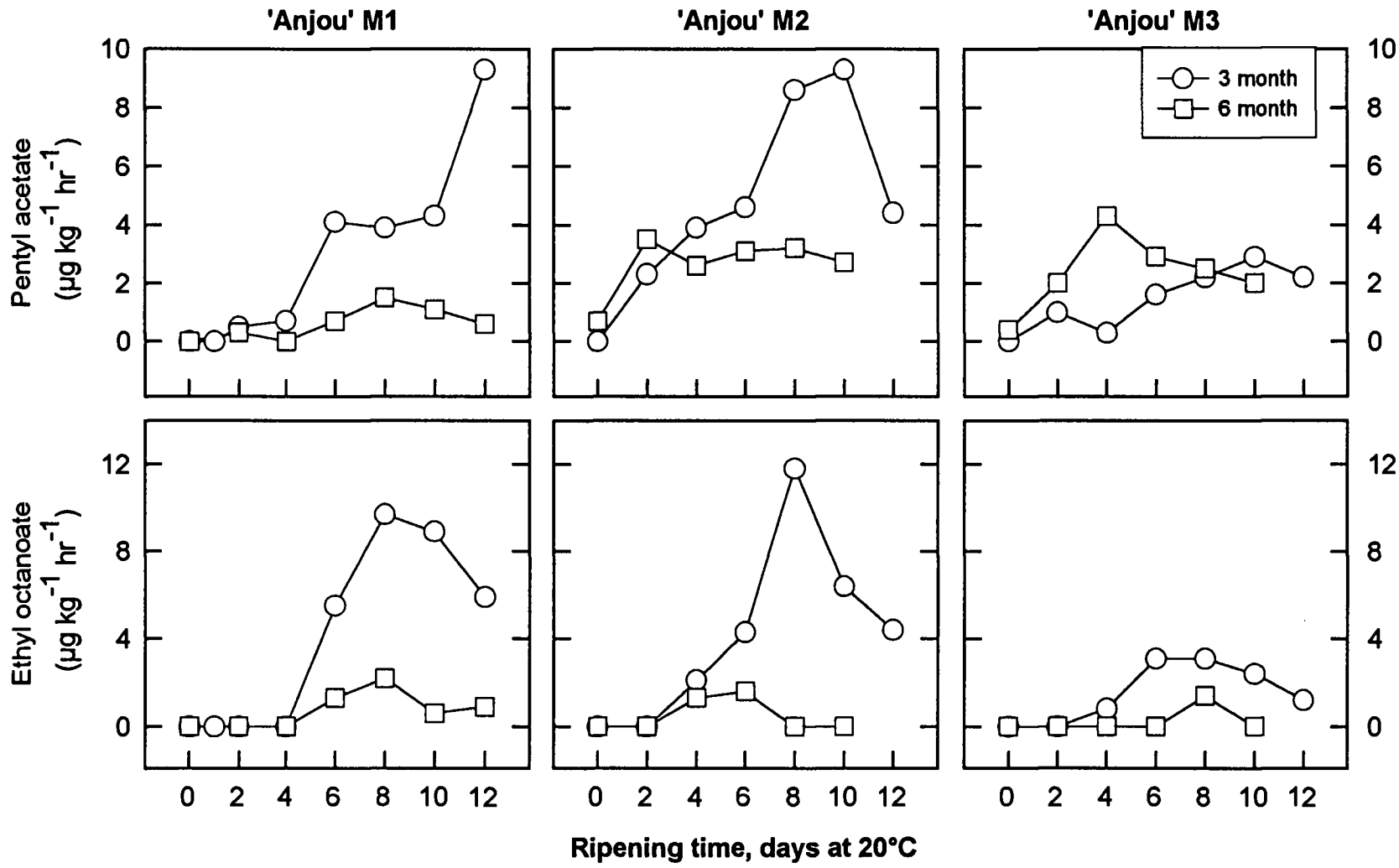

Figure 6.17. Changes in pentyl acetate and ethyl octanoate production during ripening of 'Anjou' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage for 3 to 6 month at 0°C.

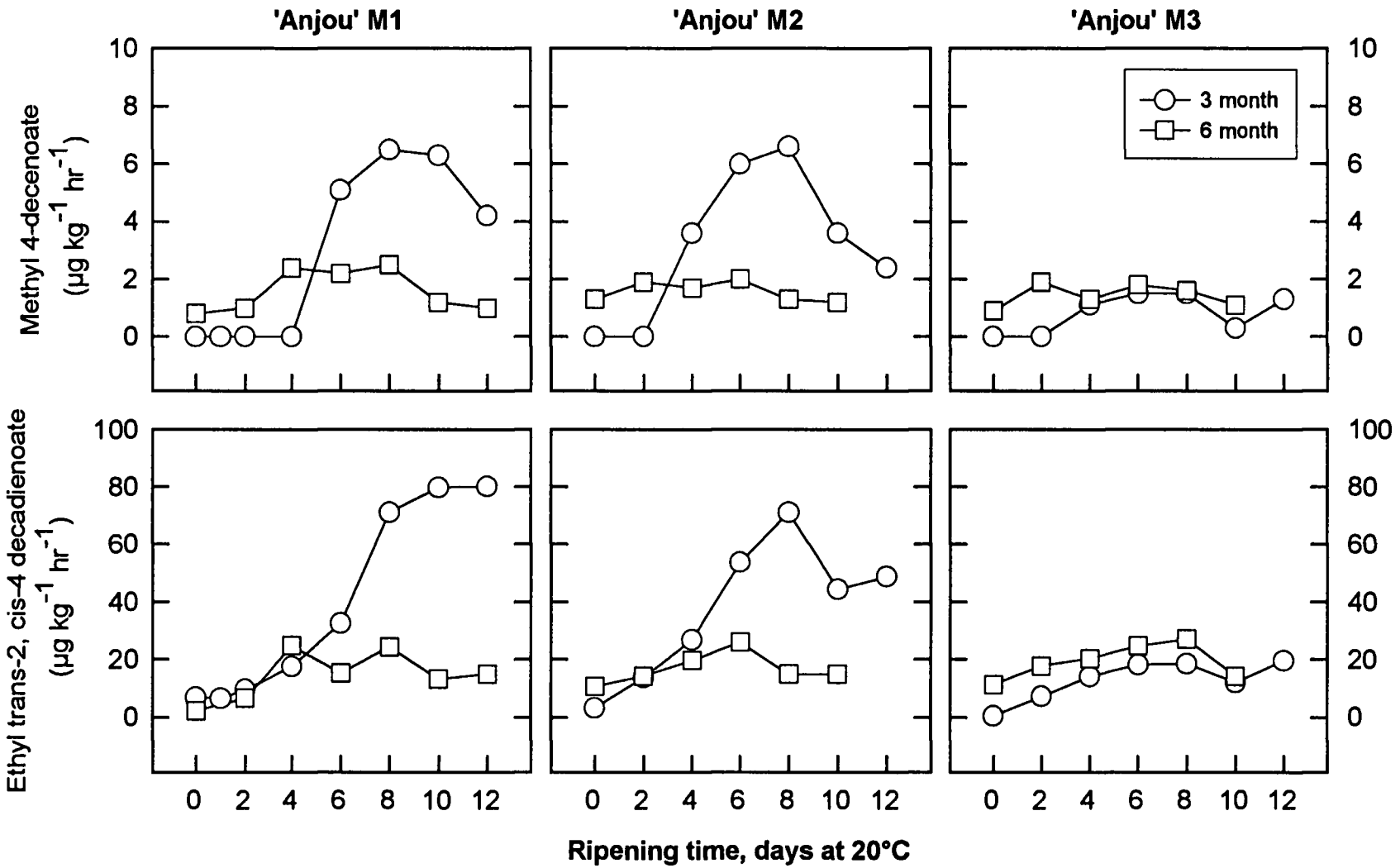

Figure 6.18. Changes in methyl 4-decenoate and ethyl trans-2, cis-4 decadienoate production during ripening of 'Anjou' pear harvested at early(M1), optimum(M2) and late(M3) maturity stages after storage for 3 to 6 month at 0°C.

observed in late harvest fruit after storage for 3 months. Prolonged storage for 6 months also resulted in less of both compounds during fruit ripening, regardless of harvest maturity (Figures 6.17 and 6.18).

In general, effects of harvest maturity and storage duration on volatile compounds and sensory attributes of 'Anjou' pear during ripening were similar to those found in 'Packham's Triumph' pear. But even less of the important volatiles esters were observed in late harvest fruit and fruit harvested at all maturity stages after storage for 6 months. A severe reduction in volatiles has been reported in apple after storage for 8 months at 4°C (Paillard, 1967). The author suggested that during long term storage apples may exhaust their substrates or lose their capacity to synthesize aroma compounds. Alcohols are important substrates used in ester synthesis. In this study, total alcohols production of fruit after storage for 6 months appeared to be the same or even higher than in fruits after storage for 2 months. Therefore, the reduction of esters formation observed in pear after prolonged storage is not likely limited by substrate alcohol, but may be directly affected by the decrease in the ability of enzyme(s) used in ester biosynthesis. More research needs to be done to elucidate this observation.

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CHAPTER 7

PARTIAL PURIFICATION AND SOME PROPERTIES OF ALCOHOL ACYLTRANSFERASE FROM RIPENING 'BARTLETT' PEARS

Anawat Suwanagul and Daryl G. Richardson

Abstract

Alcohol acyltransferase, the enzyme involved in volatile esters formation in many fruits was studied in 'Bartlett' pears. The activity of enzyme increased during fruit ripening and reached the maximum after 4 days at 20°C. The soluble protein from ripened pear was purified about 9 fold by ammonium sulfate fractionation, gel filtration and anion-exchange chromatography. The molecular weight estimated by SDS-PAGE was 40 kD. The purified enzyme was most active at pH 7.5 and temperature 30°C. The enzyme has a K_m and V_{max} value for hexyl alcohol at 0.6 mM and 0.3 nmol/ min and for acetyl-CoA at 53 μM and 0.2 nmol/ min, respectively. The partially purified enzyme was used to test for substrate specificity using various alcohols and acyl-CoAs . The maximum activity of the enzyme was obtained when hexyl alcohol and acetyl- CoA were used as substrates.

Introduction

Esters generally occupy a large portion of volatile compounds evolved from fruit tissues and they typically are among the most important odor active substances synthesized by ripening fruits. Typical fruit aromas are characterized by those esters such as isoamyl acetate in banana (Berger et al., 1986), ethyl 2-methylbutanoate in apples (Guadagni et al., 1966 and Flath et al., 1967) and ethyl trans-2, cis-4 decadienoate and hexyl acetate in pears (Jennings et al., 1964). The pathway leading to ester synthesis in fresh fruit is not completely understood. During the brief period of fruit ripening, minute quantity of lipids, carbohydrates and proteins are enzymatically converted to highly distinct volatile flavor compounds. Various straight-chain alcohols and carboxylic acids are formed from the degradation of fatty acids, presumably by β-oxidation (Palliard, 1979). Oxidation of unsaturated fatty acids via

lipoxygenase have also been shown to yield unsaturated alcohols and aldehydes (Stone et al., 1975). Branched chained alcohols tend to be derived from branched amino acids (Myers et al., 1970). These primary volatiles are then converted to various esters by endogenous enzyme systems.

Incubation of whole fruits or tissue slices with aldehydes, alcohols or acids results in the formation of various esters in diverse fruits such as apple (Paillard, 1979; Knee and Hatfield, 1981; De Pooter et al., 1981; De Pooter et al., 1983; De Pooter et al., 1987), banana (Tressel and Drawert, 1973; Ueda et al., 1971; Ueda and Ogata, 1978), and strawberry (Yamashita et al., 1975; Yamashita et al., 1976; Yamashita et al., 1977; Yamashita et al., 1979). The esterification of added alcohols and acids was also observed in isolated cells of banana, strawberry and melon (Ueda and Ogata, 1976). The esterification of alcohols and acids to esters has been reported to be a coenzyme A dependent reaction (Ueda and Ogata, 1977). The reaction is catalyzed by the activity of the enzyme alcohol acyltransferase (AAT).

Alcohol acyltransferase activity first became of interest to researchers of various microorganisms due to its role in ester synthesis during alcoholic beverage fermentation (Nordstrom, 1961; Howard and Anderson, 1976; Lanza et al., 1976 and Sarris and Latrasse, 1985). The enzyme catalyzes the transfer of the acyl moiety basically from acetyl-Coenzyme A (sic) onto various alcohols, and results in ester formation. The enzyme has been isolated, purified and characterized in many microorganisms including brewer's yeast (Yamawaka et al., 1978; Yoshioka and Hashimoto, 1981), Sake yeast (Akita et al., 1990), *Cladosporium cladosporioides* (Yamakawa et al., 1978) and *Neurospora sp.* (Yamauchi et al., 1989). Only limited information concerning this enzyme has been generated for higher plants. Ueda and Ogata (1977) and Yoshioka et al. (1982) reported the occurrence of AAT enzyme in

cell-free extracts from bananas. Harada et al. (1985) isolated, purified and characterized AAT from cell-free extracts of banana. More recently, the enzyme has been partially purified and characterized in strawberry and banana (Pérez et al., 1993 ; Olias et al., 1995). However, information regarding whether this enzyme is implicated in other fruit volatile ester biosynthesis is still very limited.

In this paper, the activity of AAT isolated from 'Bartlett' pear at various ripening stages was observed. The enzyme was partially purified and some properties of the enzyme are reported. The partially purified enzyme was tested for substrate specificity using different alcohols and acyl CoAs as substrates.

Materials and Methods

Fruit samples

Mature 'Bartlett' pear fruits were obtained from the Southern Oregon Experimental Station, Oregon State University, Medford, Oregon. Fruits were hand harvested based on optimum commercial flesh firmness value (89 Newtons). Fruits were stored at -1°C and ripened at 20°C at specific time intervals before extraction.

Preparation of crude enzyme extract

Pulp tissue (30 g) from four fruits were peeled, cut into slices and homogenized in 30 ml cold 100 mM potassium phosphate, pH 8.0 buffer containing 50 mM mercaptoethanol (ME), 5 mM EDTA and 1 g of insoluble polyvinylpolypyrrolidone (PVPP). The homogenate was filtered through 2 layers of muslin sheet before centrifugation at 18,000 x g for 30 min at 4°C. The supernatant was used as a crude enzyme extract. To determine the effects of detergent on crude

enzyme extract, Triton X-100 at concentration of 1% was added to the extraction buffer before homogenization.

Enzyme purification

All procedures were performed at 0 to 4°C or in an ice bath. Crude protein extracts were purified as follows:

Step 1. $(NH_4)_2SO_4$ at 20% saturation was slowly added to crude extract with constant stirring. After 1 hr incubation, the extract was centrifuged at 12,000x g for 30 min. The supernatant was transferred to a fresh prechilled centrifuge tube. The pellet containing cell debris and pectic materials was discarded.

Step 2. To the supernatant was then slowly added $(NH4)_2SO_4$ with constant stirring until 90% of saturation. After 1 hr of incubation, crude protein was collected by centrifugation at 12,000 x g for 30 min. The protein pellet was redissolved in a small volume of 10 mM K-phosphate buffer, pH 8.0 containing 0.1 mM dithiothreitol (DTT) and 12 mM mercaptoethanol and desalted on Econo-Pac 10 DG desalting column (Bio-Rad) equilibrated with the same buffer.

Step 3. The desalted protein was applied to a Bio-gel P100-120 (Bio-Rad) column (1 x 50 cm) equilibrated with 10 mM K-P buffer, pH 8.0, containing 0.1 mM DTT. The column was eluted with the same buffer at a flow rate of 10 ml/hr and 10 ml fractions were collected.

Step 4. The active fractions from gel filtration were pooled and concentrated by semipermeable membrane (Immersible CX-100, Millipore Corp.). The concentrated protein was applied to DEAE-cellulose (Cellex D, Bio-Rad) column (1.5 x 30 cm) equilibrated with 10 mM K-P buffer pH 8.0 containing 0.1 mM DTT. A stepwise elution was run with the same buffer containing 0.0 (100 ml), 0.15M (100

ml) and 0.3 M NaCl (100 ml), respectively. The column flow rate was maintained at 5 ml/hr and 10 ml fractions were collected.

The fractions containing AAT activity were pooled and concentrated with the same CX-100 membrane. The protein was considered as the most purified enzyme and used for characterization of AAT properties.

Assay of AAT activity

Assay of AAT activities were performed in 11 ml vials sealed with rubber cap. The standard assay reaction consisted of 10 mM K-P buffer, pH 8.0, 0.25 mM acetyl-CoA, 0.05 mM DTT, 20 mM hexanol, 50 µg bovine serum albumin (BSA) and the appropriate volume of enzyme extract (50-100 µl) in a total volume of 500 µl. The reaction mixtures were incubated at 28°C for 30 min before transfer to 20°C. Solid-phase microextraction (SPME) with 100 µm polydimethylsiloxane coating fiber (Supelco, Cat. No. 5-7300) was inserted into the vial through a rubber cap. The fiber was allowed to equilibrate in the headspace of the reaction vial for 5 min before removal and was thermally desorbed in the 200°C injection port of the gas chromatograph. The reaction product, hexyl acetate, was determined by a Hewlett-Packard Model 5890 series II gas chromatograph equipped with flame ionization detector (FID) and 60-m, 0.25 mm i.d. and 0.25-µm film thickness Supelcowax 20M (Bellefonte, PA) capillary column. The GC oven temperature was programmed as follows: 60 °C held for 5 min and increased to 220 °C at a rate of 5 °C/min. Helium at a linear velocity of 24.8 cm/sec was used as carrier gas. The injections and detector temperature were set at 200 °C and 250 °C, respectively. All injections were performed splitless mode with 0.5 min purge time.

To quantitate the ester formation from the assay reaction, the exact amounts of hexyl acetate ranging from 12.5 to 225 nmol were introduced into the enzyme-free reaction mixture. The mixture was allowed to incubate under the same conditions as did the samples. SPME analyses were performed on the standard as with the samples. Gas chromatographic peak area and hexyl acetate concentration were used to develop a standard curve (Appendix Figure A.7.1). The amount of hexyl acetate formation of samples was calculated from the standard curve of standard hexyl acetate against the peak area of the gas chromatograph. Standard curves for other esters were similarly constructed and used to quantify esters produced in the substrate specificity experiment (Appendix Figures A.7.2, A.7.3, A.7.4).

One unit of enzyme activity was defined as the amount of enzyme which yielded 1 μmol of hexyl acetate in 1 min.

Protein determination

Protein was determined according to the method described by Bradford (1976), using Bio-Rad dye binding protein assay (Bio-Rad, Richmond, CA). Bovine serum albumin (BSA) ranging from 20 to 100 μg/ml was used as a standard protein.

Effect of pH and temperature

The effects of pH on pear AAT activity were performed in a standard assay reaction containing 10 mM K-P buffer, pH ranging from 2.1 to 11.2. The pH between 4.2 and 8.4 were obtained by adjusting the ratio of K_2HPO_4 and KH_2PO_4, while 1 N phosphoric acid and KOH were used to adjust pH below 4.2 and above 8.4, respectively. The reactions were incubated at 28°C for 30 min before evolved hexyl acetate was measured. The effects of temperature on the enzyme were performed in

standard assay reaction containing 10 mM K-P, pH 8.0. The reactions were incubated in the temperature range from 4 to 60°C for 30 min before measuring the amount of hexyl acetate produced. The most purified enzyme obtained from the DEAE-cellulose column was used in both experiments.

Enzyme kinetics

The determination of AAT K_m and V_{max} of hexyl alcohol were carried out at 28°C using standard assay reaction containing hexyl alcohol ranging from 0.3 to 20 mM, while concentration of acetyl-CoA was maintained at constant saturation at 250 µM. Standard assay reactions containing acetyl-CoA ranging from 2 to 250 µM and constant saturating concentration of hexyl alcohol (20 mM) were used for the determination of K_m and V_{max} of acetyl-CoA. Double reciprocal or Lineweaver-Burk plots were constructed and used for apparent K_m and V_{max} determinations.

Molecular weight determination

SDS-PAGE was performed according to the method of Laemmli (1970). Approximately 200 µg of most purified protein eluted from DEAE-cellulose was loaded on 12% SDS-PAGE minigel (10.0 X 7.0 cm) with 4% stacking gel. The electrophoresis was performed at constant 200 V at 20°C for 40 min. Proteins were visualized by Coomassie Brilliant Blue R-250 staining. The SDS-PAGE low molecular weight protein standard (Bio-Rad) was used for AAT molecular weight determination.

Results and Discussion

Effect of detergent

Detergents have been reported to be necessary for the extraction of strawberry AAT which is suggested to be a membrane-bound enzyme (Pérez et al., 1993). High AAT activity was also observed from banana extracted with buffer containing Triton X-100 (Harada et al., 1985). The effect of Triton X-100 in banana extract was suggested to be indirect by protecting enzyme from inactivation or to prevent the enzyme from coagulation. In the case of the pear extract, the recovery of total protein was higher from the extract with the buffer containing 1% Triton X-100 compared to the extract without Triton X-100. However, crude protein extract from buffer containing Triton X-100 showed a reduction in AAT activity (Table 7.1). The specific activity of protein from the extract containing Triton X-100 was observed to be 50% that of the extract without Triton X-100. Less AAT activity was observed as fruits advanced in ripeness. The results suggested that pear AAT may not be a membrane-bound enzyme or that Triton X-100 has some unknown negative effect on the assay.

Table 7.1. Effect of Triton X-100 on the extraction of Alcohol acyltransferase from unripened and ripened 'Bartlett' pear fruit tissue[a].

Tissue	Extraction Buffer	Protein (mg)	Protein (%)	Total Activity (units)[b]	Specific activity (units/mg protein)
unripened	+Triton	64.8	0.26	0.434	0.007
	-Triton	37.0	0.15	0.517	0.014
ripened	+Triton	74.1	0.30	0.414	0.006
	-Triton	30.0	0.12	0.729	0.024

[a] Crude protein extract obtained from 30 g of unripened pear (flesh firmness 40 Newtons) and ripened pear (flesh firmness 18 Newtons) were used for the assay.

[b] one unit of alcohol acyltransferase is defined as the amount of enzyme which produced 1 µmol of hexyl acetate /min.

Activity of AAT from fruit extracts has been reported to be related to the activity of esterase enzyme which catalyzes the reverse reaction of AAT. Esterase is believed to be associated with pectic materials of the fruit (Ueda and Ogata, 1976). Macerozyme has been reported to be essential for obtaining AAT activity in cell-free extracts of banana (Ueda and Ogata, 1977) and strawberry (Perez et al., 1993), since esterase was eliminated during Macerozyme treatment and subsequent washing process. In the case of pears which contain high levels of pectin, the reduction of AAT activity may be related to the changing pectin content of the fruit, since pectin appeared to be more dissolved in the extract containing Triton X-100. However, there was no esterase activity detected in any of those pear extracts.

Determination of optimum enzyme quantity and assay reaction time

The production of hexyl acetate from crude enzyme preparation in relation to quantity of enzyme and time in incubation at 28°C is shown in Figure 7.1. The production was a linear function of incubation time from 5 min to 2 hr when 50 µl (55 µg protein) was used. With higher concentration of protein, 100 µl (110 µg protein) and 200 µl (220 µg protein), the linear relationship was found within 60 and 40 min of incubation, respectively. The 30 min incubation period was selected to be used throughout this experiment.

Change in alcohol acyltransferase activity during fruit ripening

The specific activity of AAT obtained from 20-90% $(NH_4)_2SO_4$ fractionation is shown in Figure 7.2. The activity increased rapidly from unripened fruits to ripened fruits, reaching the maximum after 4 days at 20°C and then gradually declined as fruits ripened further. Interestingly, the maximum in AAT activity corresponds to the

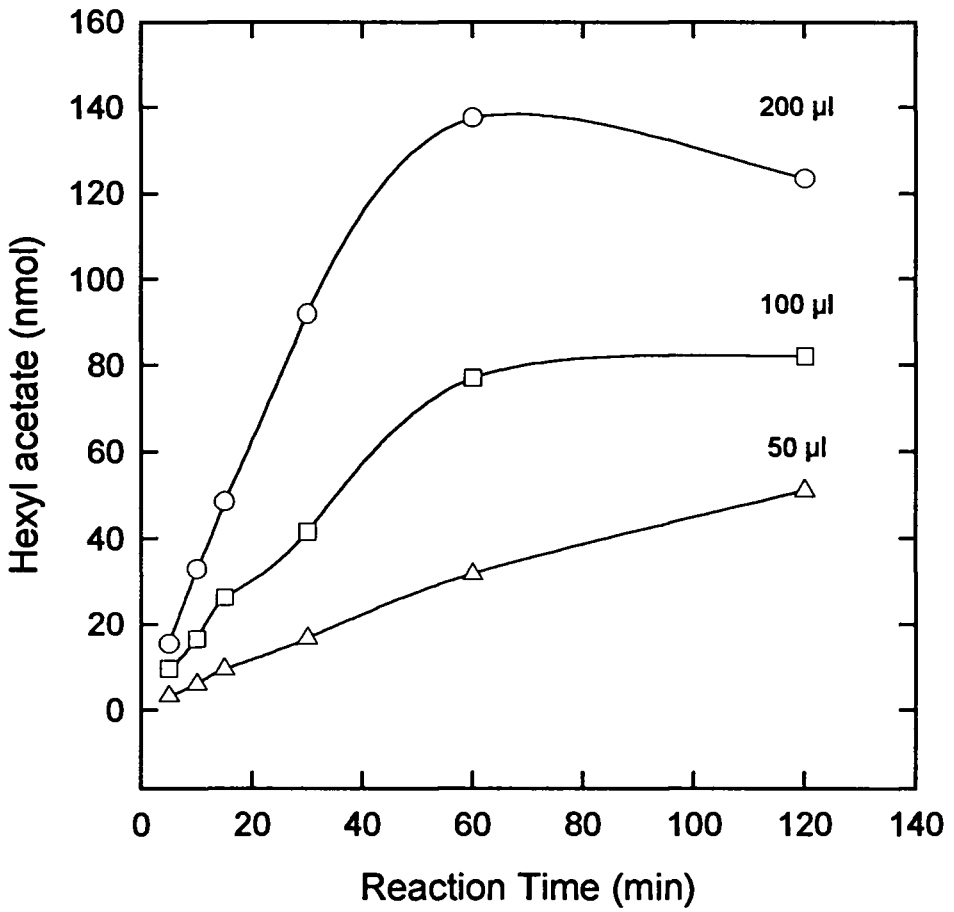

Figure 7.1. Time course of hexyl acetate production by alcohol acyltransferase preparation from 'Bartlett' pears. Crude protein extract from eating-ripe fruit (4 days of ripening) was used. Protein concentration was approximatly 1µg/µl.

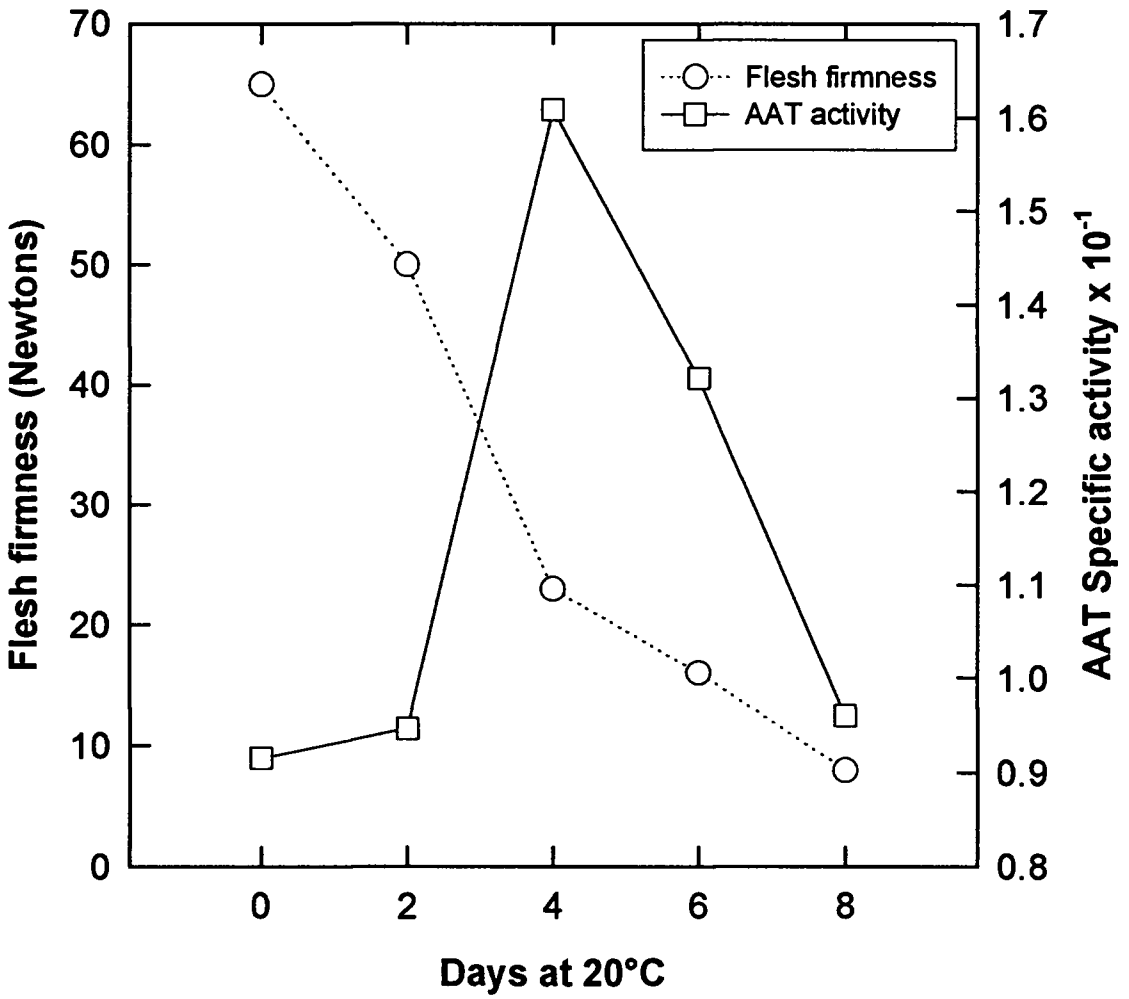

Figure 7.2. Specific activity of alcohol acyltransferase and flesh firmness of 'Bartlett' pear during ripening at 20°C.

time when fruit is reaching the optimum eating-stage (25 Newtons flesh firmness). Similar changes were reported from tissue slice assay and cell-free extracts of AAT from banana (Harada et al., 1985).

AAT Purification

The purification of ripened 'Bartlett' pear AAT is summarized in Table 7.2. A total of 37 mg of soluble protein, equivalent to 0.12% of fresh tissue, was obtained from crude extract of ripened 'Bartlett' pear in buffer without Triton X-100. Ammonium sulphate fractionation between 20 to 90% saturation recovered 70% of the soluble protein. A single protein peak corresponding to the peak that contained AAT activity was obtained from Bio-gel P100-120 gel filtration of desalted precipitate (Figure 7.3). Only 2.6 fold purification was achieved through these steps. Active fractions (2-5) were pooled and concentrated before subjected to DEAE-cellulose anion-exchange chromatography. The active fractions containing AAT activity were eluted at 0.15 M NaCl (Figure 7.4). The protein obtained from pooled active fractions (13-16) accounted for only 4% of total soluble protein, but 34% of total activity. The highest increase in specific activity (approximately 9 fold) was obtained from the active fractions eluted from this column. The purification efficiency was considered low compared to those reported for cell-free banana extract AAT (Harada et al., 1985) and strawberry (Perez et al., 1993). Moreover, 'Bartlett' pear AAT specific activity value at the end of the purification process was 25% that reported for banana and 10% that reported for strawberry.

Table 7.2. Purification of Alcohol acyltransferase from ripened 'Bartlett' pear[a].

Purification step	Total protein (mg)	Total activity (units)[b]	Specific activity (units/mg protein)	Purification (fold)	Yield (%)
crude extract	37.0	0.748	0.019	1	100
$(NH_4)_2SO_4$ 20-90% ppt.	25.5	0.723	0.028	1.5	96.7
Bio-gel P100-120	14.2	0.700	0.049	2.6	93.5
DEAE-cellulose	1.5	0.258	0.175	9.1	34.5

[a] 30 g of eating-ripe (4 days of ripened at 20°C, average flesh firmness 23 Newtons) fruit was used.

[b] one unit of alcohol acyltransferase is defined as the amount of enzyme which produced 1 µmol of hexyl acetate /min.

Molecular weight determination

SDS-PAGE of partially purified AAT from 'Bartlett' pear is shown in Figure 7.5A. The molecular weight of this enzyme estimated by a plot between log of molecular weight of standard protein versus their relative mobility was 40 kD (Figure 7.5B). The estimate of molecular weight appeared to be the same as reported from banana (Harada et al., 1985) but somewhat smaller than strawberry AAT, 70 kD (Perez et al., 1993). A difference in molecular weight was also observed between enzyme from pear and various microorganisms. The molecular weight of AAT has been reported from *Cladosporium sp.* (22 kD ; Yamakawa et al., 1978), Neurospora sp. (30 kD; Yamauchi et al., 1989) and Sake yeast (50 kD; Akita et al., 1990).

Effect of pH and temperature

The enzyme was most active at pH 7.5 (Figure 7.6). The relatively high activity (above 80%) was also observed between pH 7.0 to 8.4. Assay with the pH below 5.5 and above 8.4 resulted in significant loss in enzyme activity. The optimum

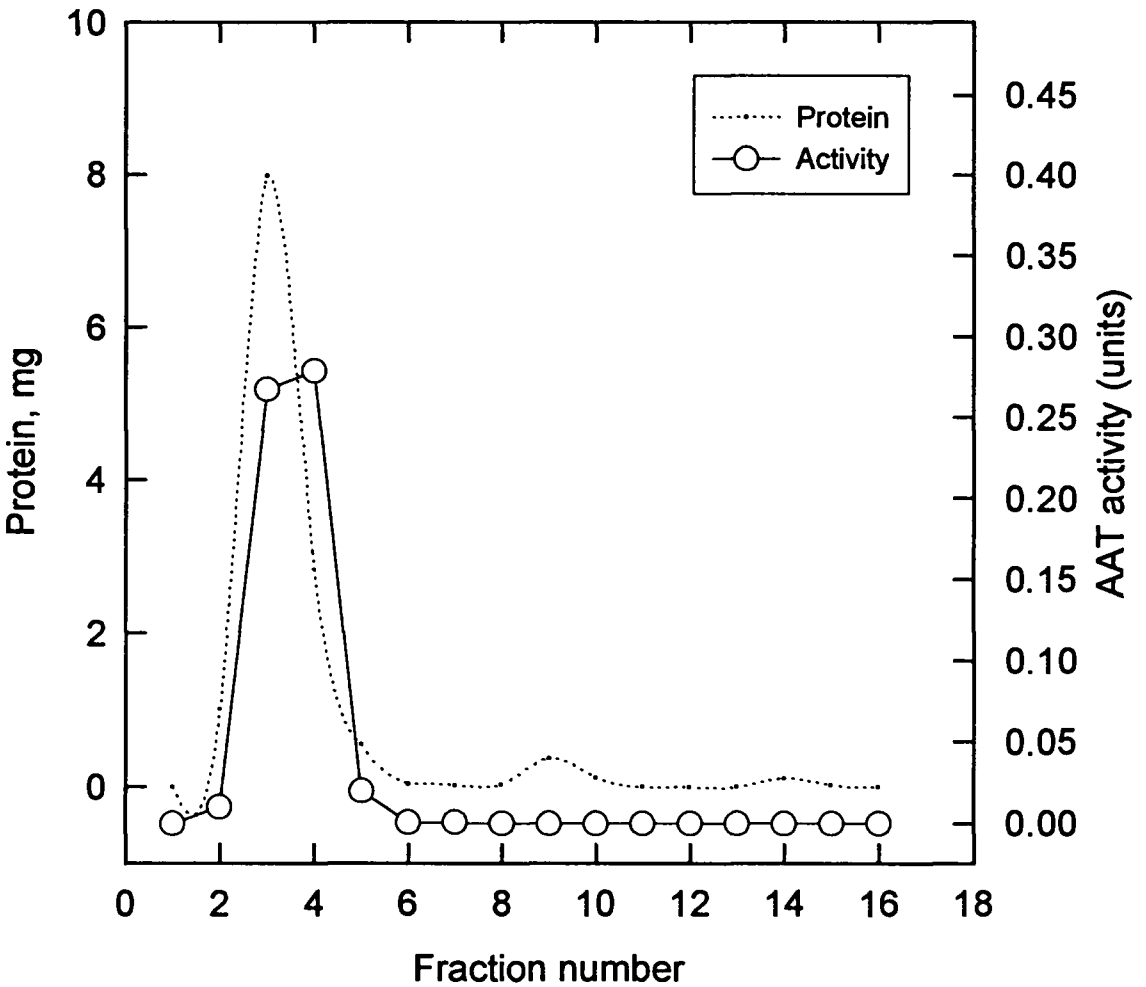

Figure 7.3. Elution profile of 'Bartlett' pear Alcohol acyltransferase crude fraction on Bio-gel P100-120 column. Fractions of 10 ml were collected at a flow rate of 10 ml/min.

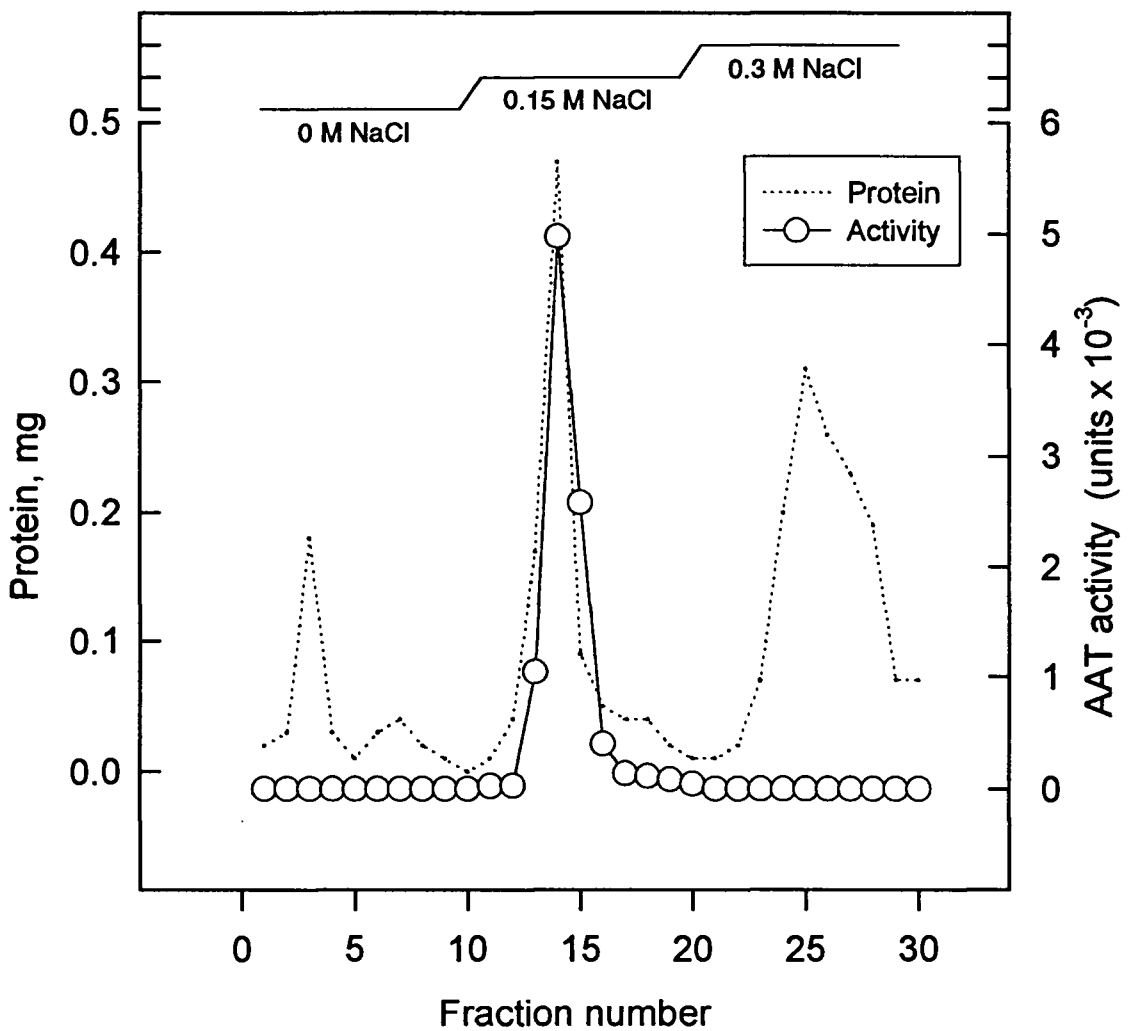

Figure 7.4. Elution profile of crude 'Bartlett' pear Alcohol acyltransferase on DEAE anion exchange column. Protein was eluted with 0-0.3 M NaCl and fractions of 10 ml were collected at a flow rate of 5 ml/min.

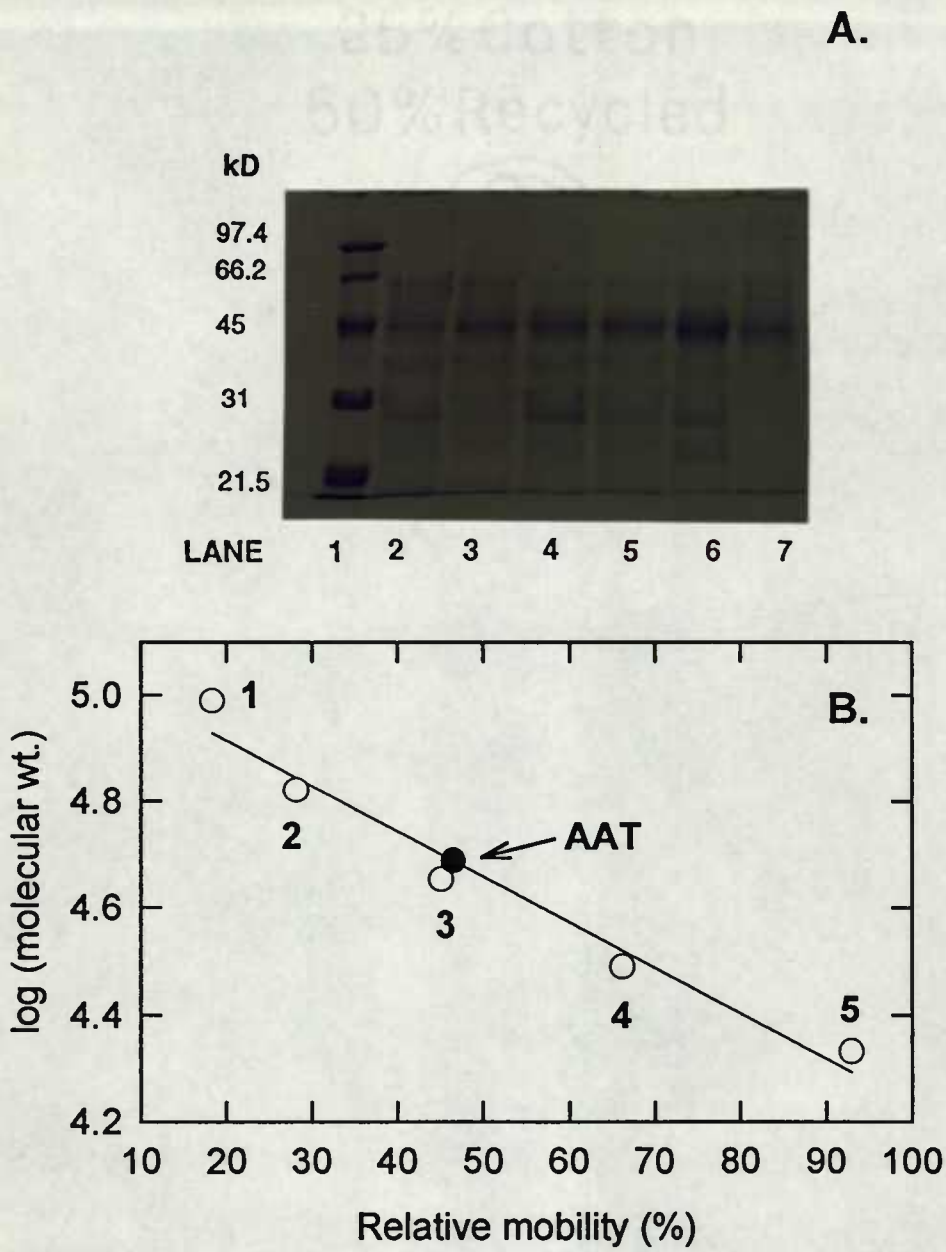

Figure 7.5. A. SDS-PAGE of partially purified alcohol acyltransferase from 'Bartlett' pear. (lane 1, molecular weight marker; lane 2-6, native protein of 'Barlett' pear after 0,2,4,6 and 8 days ripening at 20°C; lane 7, partial purified 'Bartlett' pear AAT)

B. Estimation of 'Bartlett' pear AAT molecular weight. 1. phosphorylase b (97.4 kD), 2. BSA (66.2 kD), 3. ovalbumin (45 kD), 4. carbonic anhydrase (31 kD) and 5. trypsin inhibitor (21.5 kD).

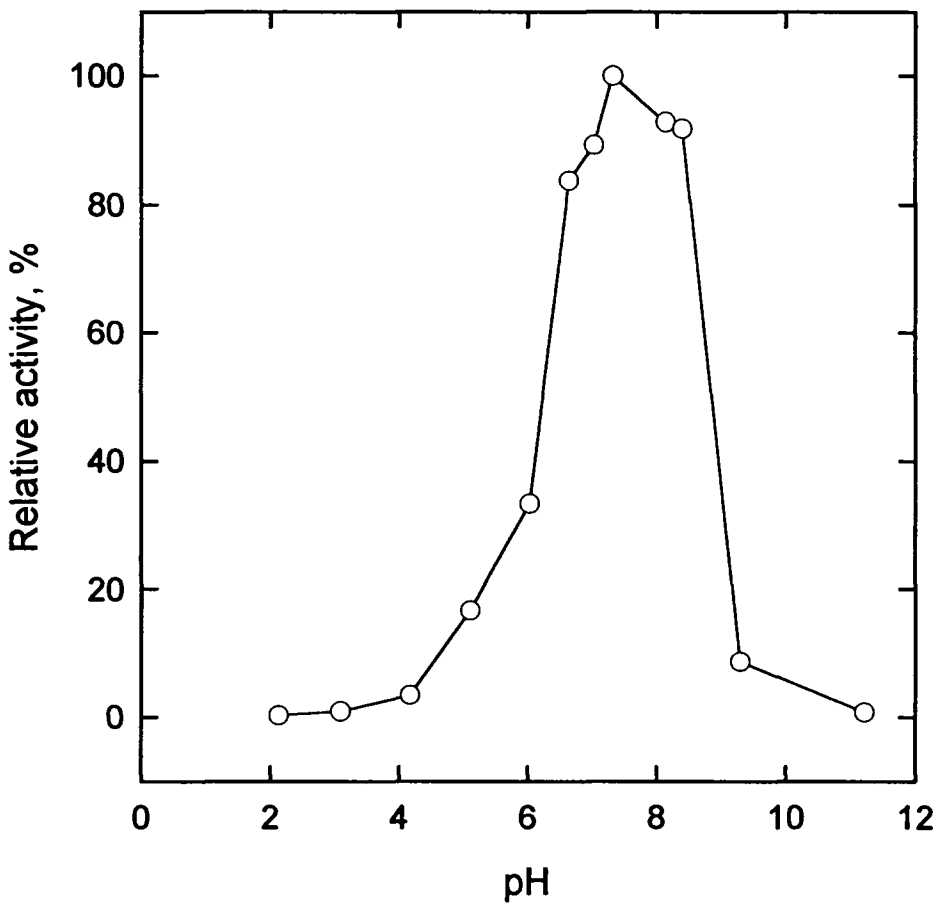

Figure 7.6. Effect of pH on partially purified 'Bartlett' pear alcohol acyltransferase activity.

pH of pear AAT is slightly lower than those reported in strawberry (Perez et al., 1993) and banana (Harada et al., 1985), which had optimum pH between 8 and 8.5.

The optimum temperature for AAT activity was observed to be at 30°C (Figure 7.7). The activity of enzyme remained relatively high (above 60%) in the temperature range from 13 to 45°C. The pear AAT enzyme exhibited a broader range of temperature activity compared to those reported from various AAT from microorganisms (Yamawaka et al., 1978; Yoshioka and Hashimoto, 1981; Yamauchi et al., 1989) and strawberry (Perez et al., 1993).

Kinetic parameters

The apparent K_m and V_{max} value of 'Bartlett' pear AAT for hexyl alcohol as a substrate at standard conditions (pH 8.0 and 28°C) was estimated from Lineweaver-Burk plot as 0.63 mM and 0.3 nmol/ min, respectively (Figure 7.8). The K_m value obtained for pear AAT is only slightly higher than that reported for AAT from banana (Harada et al., 1985) but significantly lower (approximately 5 times) than that of strawberry (Perez et al., 1993). The V_{max} value is also observed to be about 6 times lower than that reported in strawberry.

With acetyl CoA as a substrate, the apparent K_m and V_{max} value of 'Bartlett' pear AAT estimated in the same way was found to be 52.6 μM and 0.21 nmol/min, respectively (Figure 7.9). This K_m value corresponds to the value reported in banana (Harada et al., 1985) and strawberry (Perez et al., 1993). However, the V_{max} value is about 11 times lower than that in strawberry.

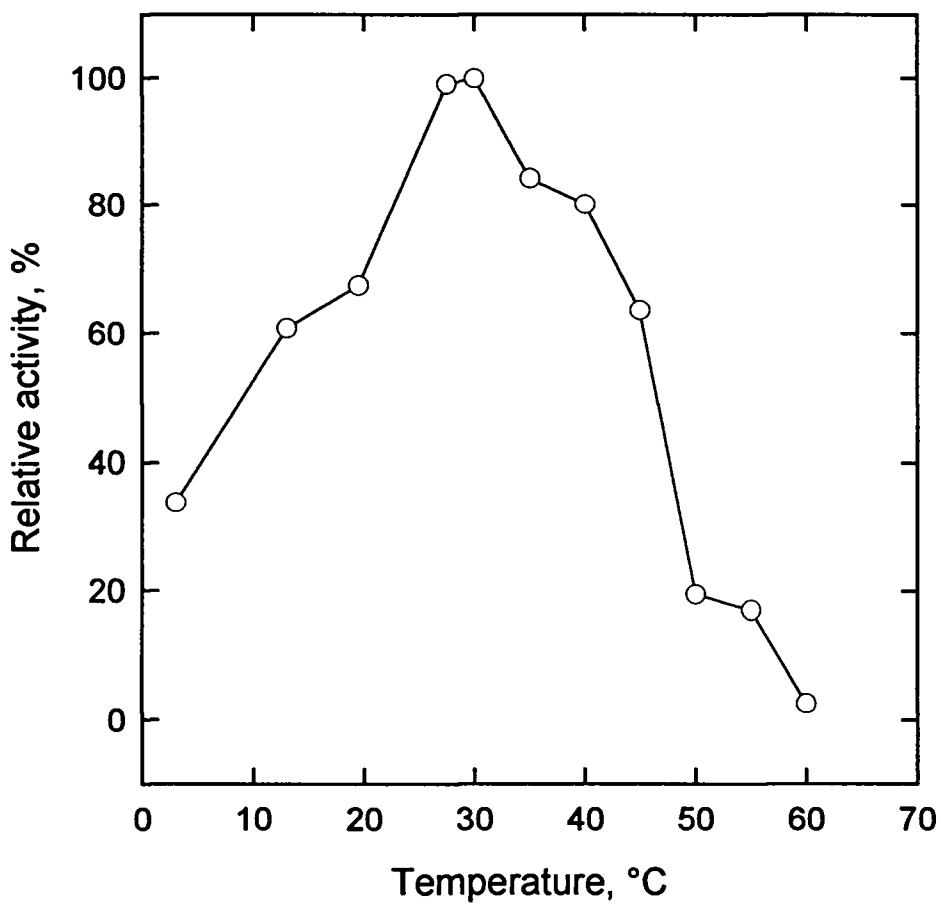

Figure 7.7. Effect of temperature on partially purified 'Bartlett' pear alcohol acyltransferase activity.

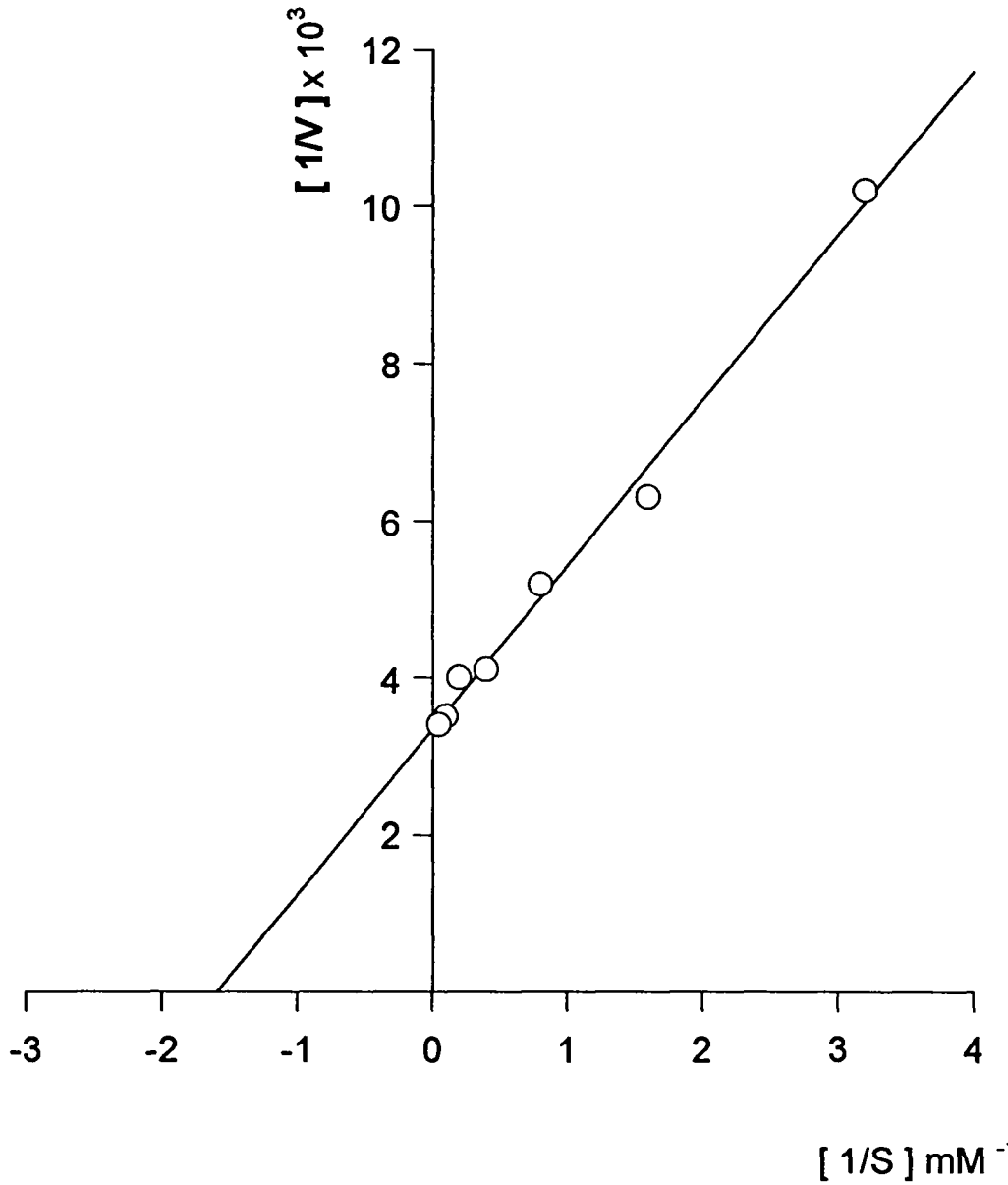

Figure 7.8. Lineweaver-Burk Plot of the concentration of hexanol versus the rate of hexyl acetate formation from the most purified alcohol acyltransferase of 'Bartlett' pear.

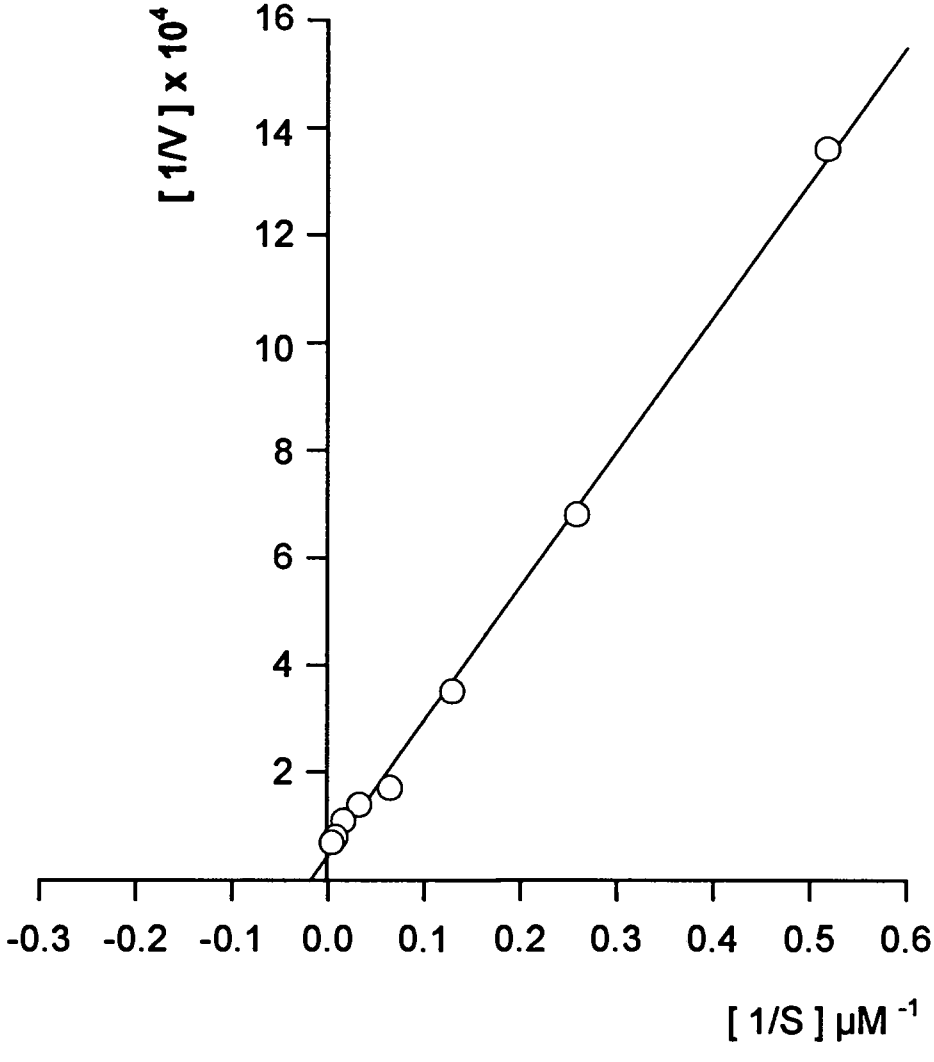

Figure 7.9. Lineweaver-Burk Plot of the concentration of acetyl CoA versus the rate of hexyl acetate formation from the most purified alcohol acyltransferase of 'Bartlett' pear.

Enzyme stability

Sulfhydryl group integrity of the enzyme has been reported to be important for the activity of many acyl transferases, both from microorganisms and higher plants (Yoshioka and Hashimoto, 1981; Harada et al., 1985; Perez et al., 1993). Without SH-group protection the enzyme can lose its activity up to 80% within 24 hr. A combination of 1 mM DTT and 12 mM ME has been reported to be the best reagent to preserve the AAT activity from strawberry (Perez et al., 1993). In this experiment, the partially purified AAT from pear stored under those conditions appeared to be slightly more labile than those reported from strawberry. The enzyme lost approximately 50% of its activity within 8 days after storage at 0-4°C (Figure 7.10).

Substrate specificity

A difference in substrate specificity was observed for AAT from 'Bartlett' pear when different alcohols and acyl Coenzyme A's (Table 7.3) as substrates. The enzyme activity increased gradually as the number of carbons of alcohol increased from 1 to 6 carbons and declined sharply when more than 6 carbon alcohols were used. The maximum activity was obtained when hexyl alcohol was used as a substrate. No enzyme activity was detected when secondary alcohols were tested. Moreover, the enzyme seemed to be more active with straight-chain alcohols than branched-chain alcohols with the same carbon number. The high specificity of pear AAT enzyme for hexyl alcohol is expected since hexyl acetate was found to be the most prominent ester in the pear volatile profile. Hexyl alcohol was also found to be a preferred substrate for AAT extracted from strawberry (Perez et al., 1993). Interestingly, butyl, isobutyl and isoamyl alcohols were reported to be the preferred

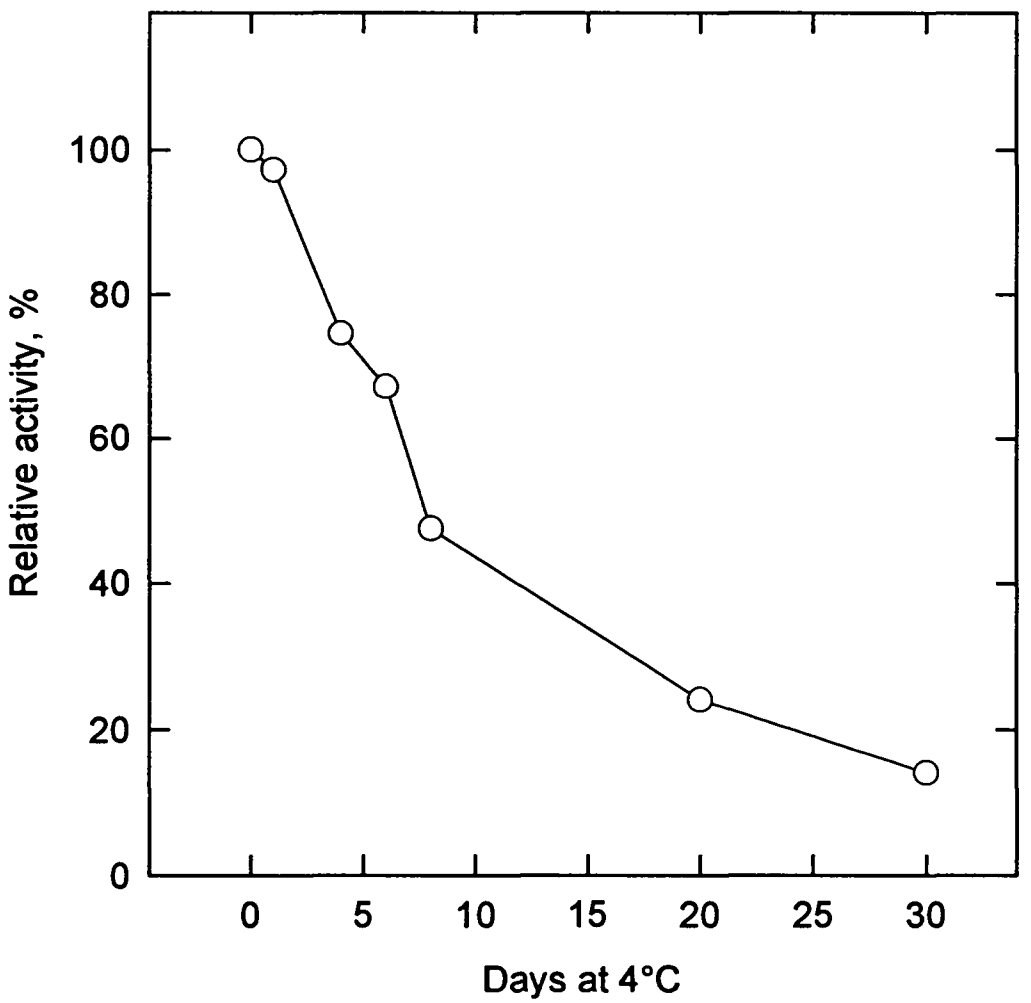

Figure 7.10. Stability of partially purified alcohol acyltransferase from 'Bartlett' pear held at 4°C.

substrates for banana AAT (Ueda et al., 1992; Olias et al., 1995). Inactivity of pear AAT enzyme for secondary alcohol was expected since ester of secondary alcohol has not been detected in 'Bartlett' pear volatile profiles.

The highest enzyme activity was obtained from 2 carbon acyl coenzyme A, acetyl CoA (Table 7.3). The increase in carbon number of the acyl group of coenzyme A from 4 to 12 carbons resulted in significant decreases of enzyme activity. Acetate esters were found to be the major esters in pear aroma profiles. Similar results have been reported for AAT from *Neurospora sp.* (Yamauchi et al., 1989) and also banana and melon (Ueda et al., 1992). The specificity of fruit AAT for lower carbon number of acyl moiety indicates the distinction between AAT from plants and microorganisms. In brewer's yeast, the enzyme was most active when C6 to C10 acyl moieties were used, while no activity was detected from C2 and C3 acyl moieties. Moreover, a low enzyme activity for high carbon number of the acyl moiety can be considered as an undesirable feature of pear AAT, since the 2,4 decadienoate esters which have been reported as the flavor impact compounds in this pear (Jennings, 1964) is likely to be derived from the unsaturated 10 carbon acyl moiety. However, attempts to test this unsaturated acyl will have to wait until later due to the current unavailability of this type of acyl CoA.

Table 7.3. Substrate specificity of 'Bartlett' pear alcohol acyltransferase[a].

Substrate[b]	Carbon no.[c]	Ester Products	Amount, nmol
A. *Alcohols*			
methanol	1	methyl acetate	1.3
ethanol	2	ethyl acetate	2.2
1-propanol	3	propyl acetate	3.8
2-propanol	3	propan-2-ol acetate	0
2-methyl-1-propanol	4	2-methylpropyl acetate	0.5
1-butanol	4	butyl acetate	4.3
2-butanol	4	butan-2-ol acetate	0
1-pentanol	5	pentyl acetate	7.4

(Continued)

Table 7.3. Continued.

Substrate[b]	Carbon no.[c]	Ester Products	Amount, nmol
2-methyl-1-butanol	5	2-methylbutyl acetate	1.5
3-methyl-1-butanol	5	isoamyl acetate	5.4
1-hexanol	6	hexyl acetate	7.9
1-heptanol	7	heptyl acetate	2.9
1-octanol	8	octyl acetate	0.5
2-ethyl-1-hexanol	8	2-ethylhexyl acetate	0.3
B. *Acyl Coenzyme A*			
acetyl CoA	2	hexyl acetate	11.2
n-butyryl CoA	4	hexyl butanoate	3.0
n-hexanoyl CoA	6	hexyl hexanoate	0.5
n-octanoyl CoA	8	hexyl octanoate	0.03
n-decanoyl CoA	10	hexyl decanoate	0.02
n-lauroyl CoA	12	hexyl lauryate	0

[a] Protein eluted from DEAE-cellulose column was used.

[b] A. Alcohols at 20 mM were added to the standard assay reaction contained 0.25 mM acetyl CoA incubated at 28°C for 30 min.
B. Acyl Coenzyme A's at 0.25 mM were added to the standard assay reaction contained 20 mM hexyl alcohol and incubated at 28°C for 1 hr.

[c] Carbon number of each acyl group and alcohol which used for a substrate.

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CHAPTER 8

GENERAL CONCLUSIONS

Volatile profiles of eight pear varieties including 'Bartlett', 'Packham's Triumph', 'Anjou', 'Comice', 'Bosc', 'Seckel, 'Vicar of Winkfield' and 'Forelle' at eating-ripe stage are characterized by esters, alcohols, hydrocarbons, aldehydes and ketones. Among a total of 122 compounds identified by GC/MS, 47 compounds are reported for the first time in pears. Esters are always present as a major group of compounds in the profiles ranging from 60 to 98% of the total. Hexyl acetate and ethyl trans-2 cis-4 decadienoate (previously reported to be important flavor components) and α-farnesene, a hydrocarbon associated with scald disorder in pears, are found in all varieties studies but varied in the significant amounts. Qualitative and quantitative differences of volatile esters in the profiles likely reflect the subtle flavor differences among pear varieties.

Analysis of volatile compounds isolated from eating-ripe pears by direct sniffing GC indicated that a group of 9 volatile esters from 'Bartlett', 'Comice' and 'Anjou' pear possessed strong pear or fruity-like odor characteristics. These compounds included 2-methylpropyl acetate, butyl acetate, butyl butanoate, pentyl acetate, ethyl hexanoate, hexyl acetate, methyl trans-2, cis-4 decadienoate, ethyl cis-2, cis-4 decadienoate and ethyl trans-2, cis-4 decadienoate. A second group of esters such as propyl acetate, isoamyl acetate or hexyl hexanoate etc. were also found to possess very positive aroma and can significantly affect overall sensation by serving as 'contributory to flavor compounds' in pear varieties.

The production of volatile compounds during ripening of 'Bartlett', 'Packham's Triumph' and 'Anjou' pears are characterized by the increase of various esters and

alcohols concomitant with decreases of the hydrocarbon, α-farnesenes. The highest rate of ester production is observed in fruit at the optimum eating-ripe stage. The major esters were found to increase at different rates and times during fruit ripening. Several odor-active compounds correlate well with pear flavor intensity during fruit ripening. Those compounds reported to possess pear or fruity-like or other positive odor characteristics significantly contribute to pear flavor during fruit ripening.

Effects of harvest maturity on volatile flavor compound production vary among pear varieties. Maturity had only slight effects on 'Bartlett' volatiles during ripening. Early harvest has a tendency to increase volatile production in 'Packham's Triumph' and 'Anjou' pear and late harvest resulted in severe reduction of volatile production in 'Anjou'. Harvest maturity was observed to have minor effects on fruit quality after ripening. Fruits harvested at different maturity stages can be ripened to about the same eating quality and pear flavor intensity after ripening. However, late harvest fruits tend to ripen to optimum eating quality faster and have shorter shelf-life than fruits harvest at earlier maturities. Prolonged storage resulted in an increase in ester and alcohol production in 'Bartlett' , but a severe reduction of various flavor compounds in 'Packham's Triumph' and 'Anjou' after storage for 4 and 6 months, respectively. Fruits after prolonged storage for 4 to 6 months, were still able to ripen to about the same eating quality and flavor intensity as fruit stored for 2 months. However, susceptibility to scald disorder increased after prolong storage.

The highest activity of alcohol acyltransferase (AAT), the enzyme involved in volatile ester synthesis in 'Bartlett' pear coincided with optimum ripening at 20°C for 4 days. The partially purified enzyme had a molecular weight of 40 kD and was most active at pH 7.5 and 30°C. The enzyme has a K_m = 0.6 mM and V_{max} = 0.3 nmol/min

for hexyl alcohol and for acetyl-CoA K_m= 53 μM and V_{max} = 0.2 μM/min. Tests for substrate specificity indicated that the AAT was most active when hexyl alcohol and acetyl-CoA were used as substrates.

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APPENDICES

PEAR FLAVOR TESTING BALLOT

Name:_____ Date:_____

BALLOT 1
16-POINT CATEGORY SCALE

Instruction:
- Please fill in sample code numbers from left to right as they appear.
- Start from the left, evaluate **Pear Flavor Intensity** and **Mealiness** by using the following scale:

0 = None	8 =
1 = Just detectable	9 = Moderate to large
2 =	10 =
3 = Slight	11 = Large
4 =	12 =
5 = Slight to moderate	13 = Large to extreme
6 =	14 =
7 = Moderate	15 = Extreme

Sample Code #	____	____	____	____	____	____	____
Pear Flavor Intensity	____	____	____	____	____	____	____
Mealiness	____	____	____	____	____	____	____

BALLOT 2
9-POINT HEDONIC SCALE

Instruction:
- Please fill in sample code numbers from left to right as they appear.
- Evaluate **how well** you like the sample in term of **Overall Eating Quality** by place **X** on the line that best describe the sample.

Sample Code #	____	____	____	____	____	____	____
Like extremely	____	____	____	____	____	____	____
Like very much	____	____	____	____	____	____	____
Like moderately	____	____	____	____	____	____	____
Like slightly	____	____	____	____	____	____	____
Neither like nor dislike	____	____	____	____	____	____	____
Dislike slightly	____	____	____	____	____	____	____
Dislike moderately	____	____	____	____	____	____	____
Dislike very much	____	____	____	____	____	____	____
Dislike extremely	____	____	____	____	____	____	____

Figure A.5.1. Sample ballot sheet used for sensory evaluation of pear varieties during ripening and storage.

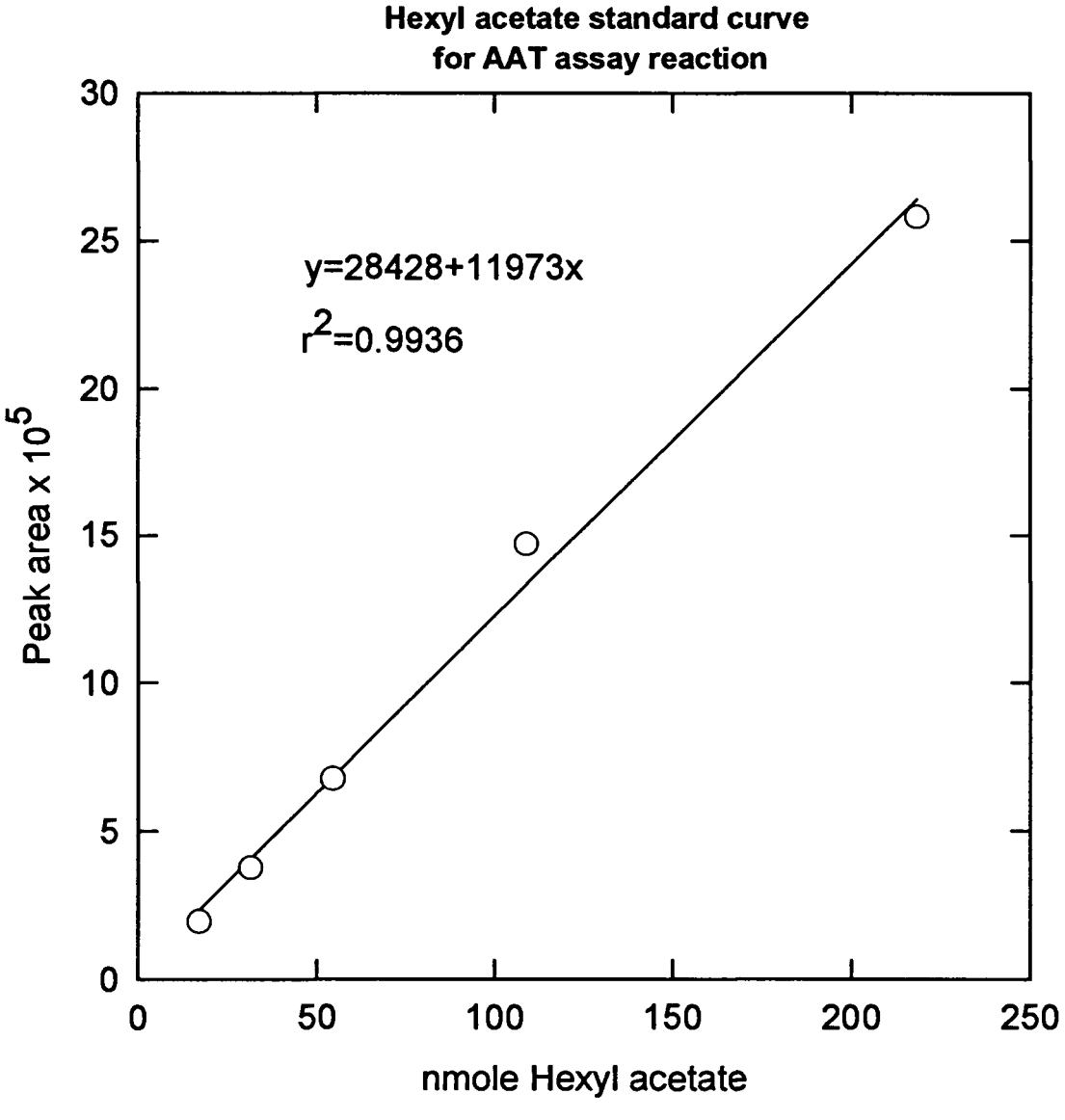

Figure A.7.1 Hexyl acetate standard curve used for alcohol acyltransferase enzyme assay.

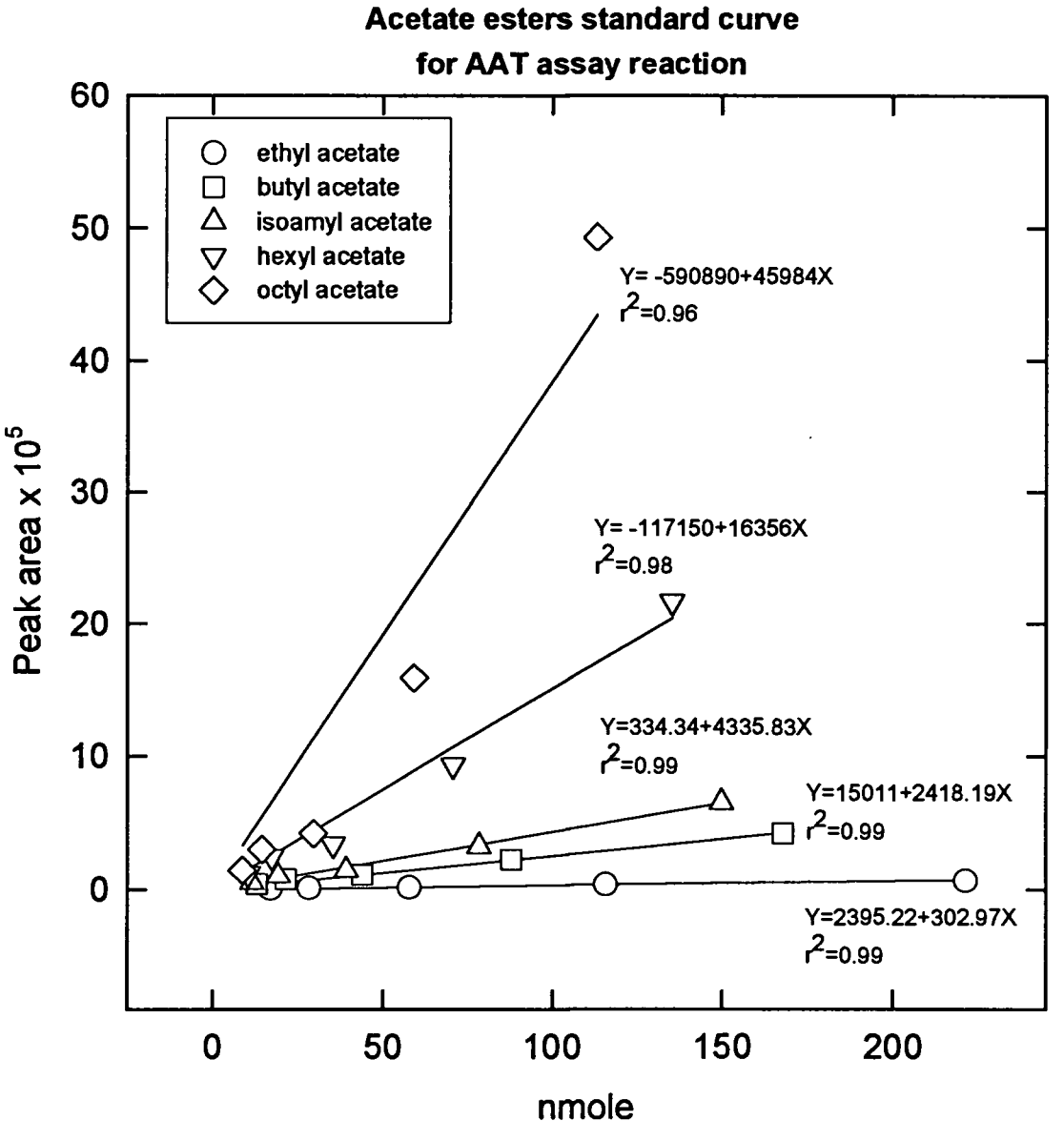

Figure A.7.2. Acetate esters standard curves used for alcohol acytransferase substrate specificity assay.

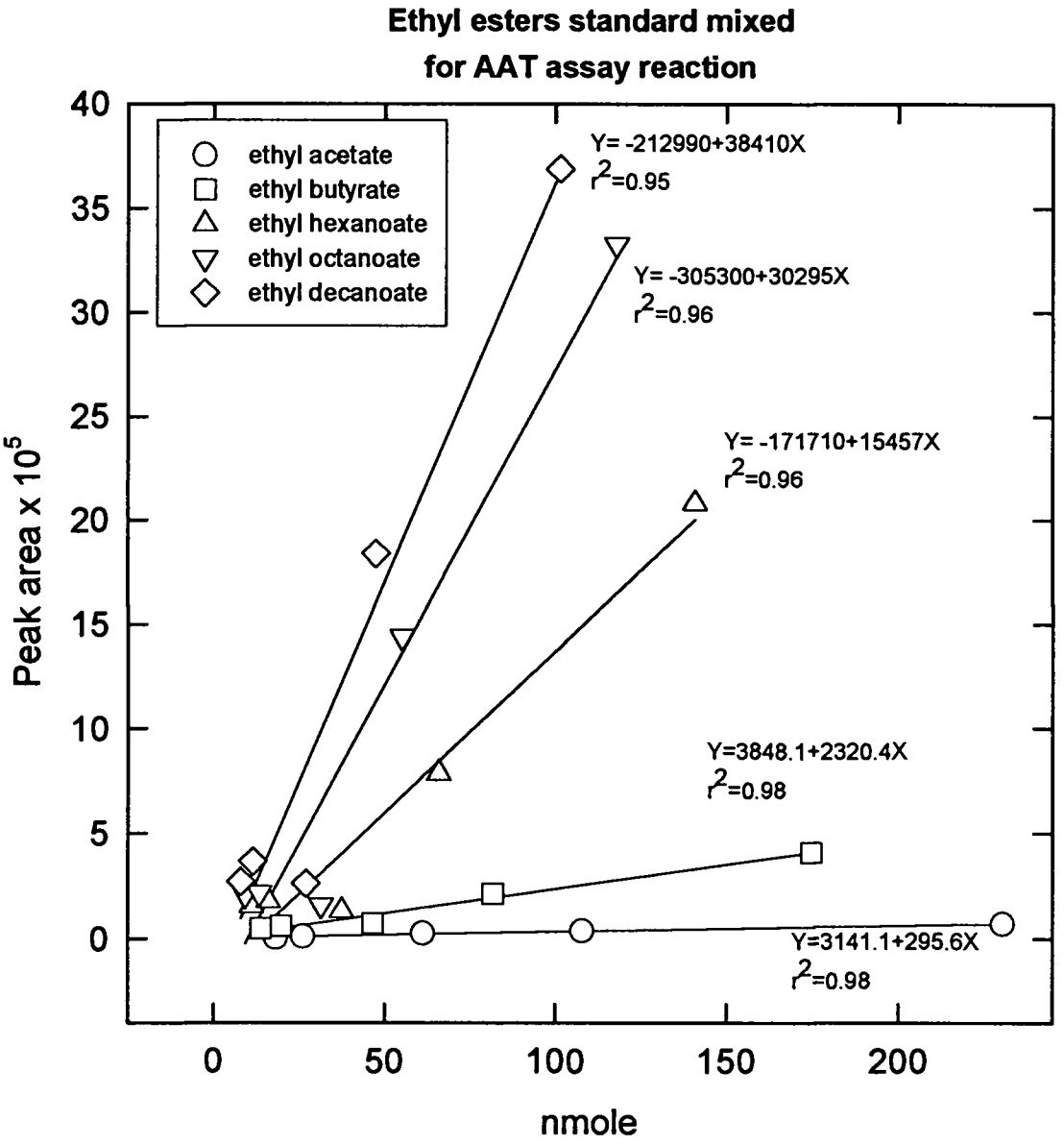

Figure A.7.3. Ethyl esters standard curves used for alcohol acytransferase substrate specificity assay.

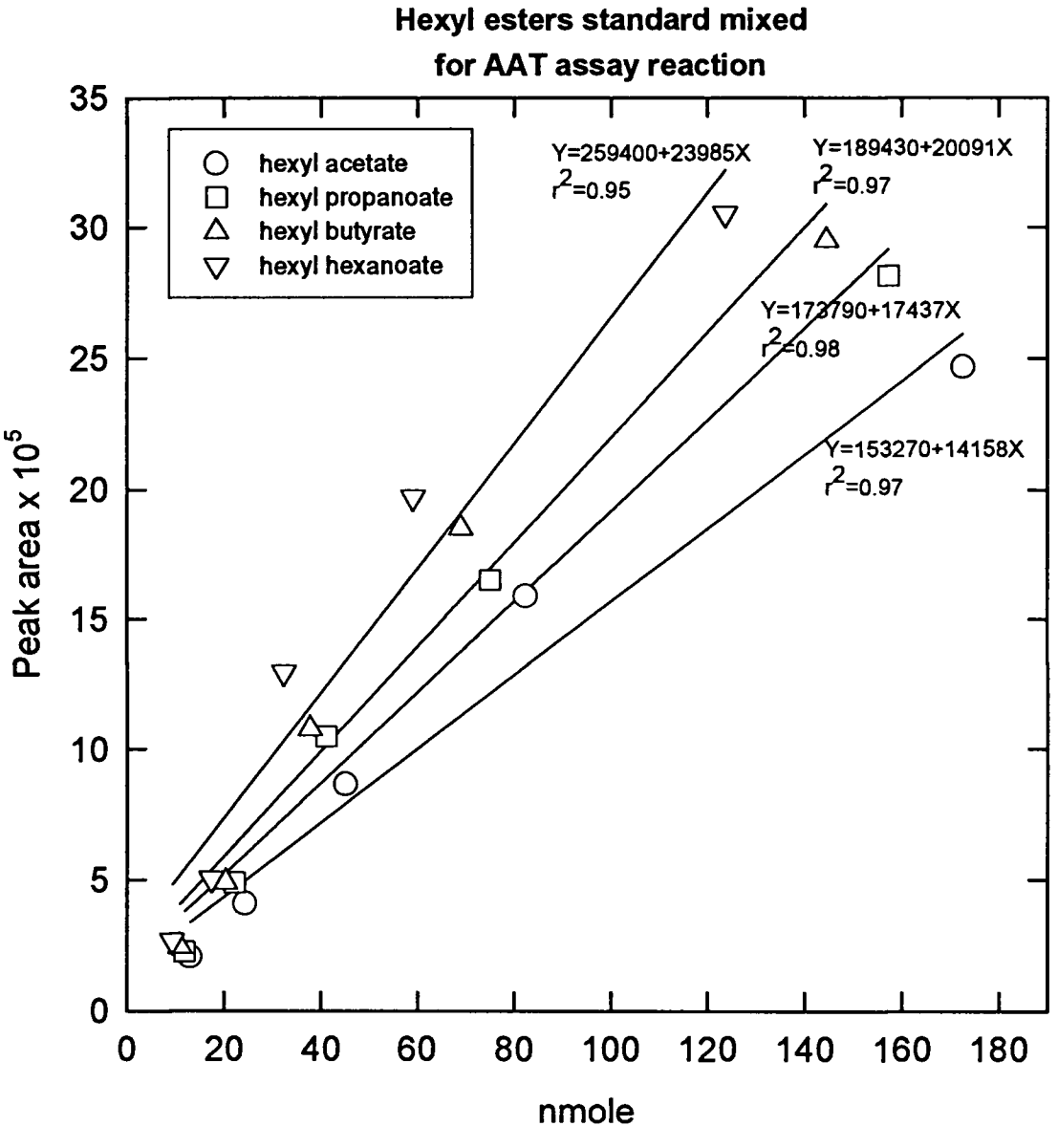

Figure A.7.4. Hexyl esters standard curves used for alcohol acyltransferase substrate specificity assay.

Table A.3.1. Newly identified volatile compounds among 8 pear varieties.[a]

No.	Rt[b]	Kovats[c]	Compounds	Variety[d]
1	6.69	825	methyl propanoate	B,F
2	12.38	1131	butyl propanoate	B
3	13.68	1175	1-methyl-4-(1-methylethyl)-1,3-clyclohexadiene	F
4	14.60	1205	β-phellandrene	F
5	14.60	1205	1-methylbutyl 2-methylpropanoate	B
6	14.77	1211	1-methylpropyl butanoate	B,P,A,C,Bo,S,V,F
7	15.70	1246	3-carene	F
8	16.26	1266	3-methylbutyl 3-methylbutanoate	F
9	16.93	1288	1-methyl-4-(1-methylethylidene)-cyclohexane	F
10	17.09	1293	2-methylpentyl acetate	P,A,C,F
11	17.13	1295	3-methylbutyl 2-methylpropanoate	B
12	17.49	1308	4-hexen-ol acetate	B,P,A,C,F
13	17.62	1313	3-hepten-1-ol acetate	B,P
14	17.73	1317	pentyl 2-methylpropanoate	B,P,A,C
15	17.76	1318	pentyl butanoate	B
16	17.82	1321	1-methylethyl hexanoate	B,P,A,C,V,F
17	18.43	1344	hexyl 2-methylpropanoate	P,C
18	18.46	1345	6-methyl hepte-2-one	B,P,A,C,V
19	18.46	1345	2-methylhexyl propanoate	B,A
20	18.55	1349	3-hexen-1-ol acetate (Z)	B
21	20.10	14.05	1-methylhexyl butanoate	B,A
22	20.53	1422	butyl hexanoate	B,P,A,C,Bo,S,V,F
23	20.60	1425	hexyl butanoate	B,P,A,C,Bo,S,V,F
24	20.85	1434	butyl 4-hexenoate	A,C,S,F
25	20.87	1435	butyl 2-methylbutanoate	P,A,C,S,V,F
26	21.39	1455	hexyl 2-methylbutanoate	B
27	21.80	1469	2-methylbutyl hexanoate	B,P,A,C,F
28	22.56	1497	butyl 2-hexenoate	A
29	22.68	1501	ethyl 2,4 hexadienoate	P
30	22.81	1505	butyl 3-hexenoate	P, A
31	22.90	1508	3-octen-1-ol acetate	P
32	23.25	1520	heptyl butanoate	A
33	23.61	1532	4-octen-1-ol acetate	B,P,A,C,Bo,F
34	24.38	1558	hexyl 2-butenoate	A
35	25.18	1583	2,4 octadien-1-ol acetate	A
36	25.48	1593	methyl 2,4 octadienoate	B
37	25.73	1600	butyl octanoate	P,A,C,F
38	26.60	1627	ethyl 2,4 octadienoate	B
39	26.81	1633	1-nonanol	A,C
40	27.55	1655	methyl 2,4,6 octatrienoate[e]	P,A,C
	(28.45)	(1680)		
41	30.31	1730	hexyl octanoate	A,C
42	30.73	1741	2-phenylethyl acetate	P,C,Bo
43	31.84	1769	butyl benzoate	F
44	34.84	1840	methyl tetradecanoate	P,A

(Continued)

Table A3.1. Continued.

No.	Rt[b]	Kovats[c]	Compounds	Variety[d]
45	36.20	1871	hexyl benzoate	F
46	40.95	1968	methyl hexadecadienoate	A
47	46.49	2069	methyl 10-octadecenoate	A

[a] Compounds were optained from fruits at eating-ripe stage. Separation was performed on 60 m x 0.25 mm. I.D. x 0.25 µm Carbowax 20M capillary column. Temperature was programmed as 60°C held for 5 min and increased to 220°C at the rate of 5°C/min. Identification was performed by electron impact mass spectrometer equipped quadrupole mass filter.

[b] Retention time in minute on Carbowax 20M column.

[c] Kovats retention index on Carbowax 20M column.

[d] Varieties: B= Bartlett, P= Packham's Triumph, A= Anjou, C= Comice, Bo= Bosc, S= Seckel, V= Vicar of Winkfield and F= Forelle.

[e] Two unidentified isomers obtained at different retention time.

Table A.6.1. Changes in volatile compounds during ripening of 'Bartlett' pear harvested at early stage of maturity (M1) after storage at 0°C for 2 months[a].

Rt[b] (min.)	I_F[c]	Compounds	Days at 20°C 0	1	2	3	4	5	7
5.67	1.56	methyl acetate	nd	0.8	6.9	7.4	14.3	19.1	15.3
6.07	2.07	ethyl acetate	nd	10.4	27.2	49.8	87.7	110.1	115.5
6.38	2.44	ethanol	nd	nd	3.4	5.5	10.8	15.2	26.6
6.95	3.08	propyl acetate	3.8	3.2	5.7	6.9	9.8	31.3	7.1
7.52	3.66	2-methylpropyl acetate	nd	nd	4.1	nd	2.5	54.9	0.4
7.94	4.04	ethyl butanoate	nd	1.3	5.4	4.0	5.8	9.7	3.1
8.55	4.37	butyl acetate	105.6	166.7	160.7	149.1	134.0	157.8	76.1
9.61	4.89	isoamyl acetate	1.6	nd	nd	2.8	2.1	2.6	1.9
9.77	4.96	1-butanol	nd	2.0	4.7	3.3	nd	nd	nd
10.77	5.40	pentyl acetate	5.9	9.5	13.6	10.6	11.3	10.4	8.5
11.11	5.54	methyl hexanoate	nd	nd	nd	1.4	2.6	2.0	nd
11.96	5.87	butyl butanoate + 1-methylpropyl butanoate	nd	4.1	3.5	2.1	0.8	2.0	1.6
12.32	6.00	ethyl hexanoate	nd	2.4	5.5	8.3	10.1	1nd	9.5
13.28	6.43	hexyl acetate	116.6	256.7	280.9	327.8	368.3	377.2	319.6
15.01	7.14	1-hexanol	0.9	2.6	1.9	2.9	0.9	nd	2.7
15.86	7.46	heptyl acetate	nd	1.2	1.8	1.4	3.1	nd	3.2
16.25	7.60	methyl octanoate	nd	nd	0.9	2.2	3.1	3.9	1.1
16.96	7.85	hexyl butanoate + butyl hexanoate	9.3	23.9	19.3	18.5	15.5	14.3	10.1
17.40	8.00	ethyl octanoate	nd	0.9	6.2	nd	11.7	12.9	9.3
18.32	8.45	octyl acetate	1.1	1.8	1.9	3.2	3.1	4.3	nd
18.78	8.67	unknown	nd	nd	1.3	2.8	5.2	6.5	3.9
18.86	8.71	methyl trans-2 octenoate	7.7	5.9	4.1	nd	nd	nd	nd
19.46	8.98	4-octen-1-ol acetate	1.2	3.7	3.0	2.2	nd	1.9	1.0
20.08	9.26	ethyl trans-2 octenoate	nd	nd	2.8	4.0	4.0	8.3	8.4
20.87	9.60	methyl 2,4 octadienoate	nd	nd	nd	nd	nd	1.4	1.2
21.06	9.68	methyl decanoate	nd	nd	1.9	1.7	1.7	1.4	nd
21.50	9.86	hexyl hexanoate	2.4	3.0	5.4	5.2	3.8	2.9	1.8
21.69	9.94	methyl 4-decanoate	nd	1.2	8.4	12.3	15.7	17.9	8.3
21.97	10.05	ethyl decanoate	nd	nd	1.2	2.0	1.8	2.6	2.5
22.07	10.09	ethyl 2,4 octadienoate	nd	nd	4.8	5.2	5.0	4.1	2.0
22.44	10.24	ethyl 4-decenoate	4.0	nd	nd	nd	nd	nd	nd
22.61	10.30	ethyl 2,4 octadienoate	0.5	1.2	10.3	15.0	17.3	26.0	16.4
22.91	10.42	methyl 2,4,6 octatrienoate	nd	nd	2.2	2.6	2.5	2.9	0.8
23.49	10.64	methyl 2-decenoate	nd	1.8	2.4	2.5	3.0	4.2	1.4
23.80	10.75	unknown	1.1	0.5	3.7	4.4	3.9	5.5	2.1
23.94	10.81	alpha farnesene (E,Z)	34.6	17.9	12.1	9.0	6.0	5.8	1.9
24.43	10.98	alpha farnesene (E,E)	1597.8	833.5	408.4	222.4	121.6	76.4	28.1
24.81	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	0.9	1.7	2.6	1.4
25.13	11.23	methyl trans-2, cis-4 decadienoate	11.0	34.0	121.3	157.1	166.7	187.9	92.2
25.58	11.39	unknown	1.0	nd	nd	nd	nd	nd	nd
25.84	11.48	ethyl cis-2, cis-4 decadienoate	nd	0.8	2.4	2.5	3.4	3.4	nd
26.09	11.56	ethyl trans-2, cis-4 decadienoate	45.1	109.5	263.6	293.2	303.7	351.4	211.9
26.84	11.81	methyl trans-2, trans-4 decadienoate	nd	nd	1.9	2.7	3.8	7.0	2.9
27.05	11.88	ethyl trans-2, trans-4 decadienoate	10.8	28.3	66.9	61.2	51.7	54.6	20.3

(Continued)

Table A.6.1. Continued.

Rt[b] (min.)	I_F[c]	Compounds	Days at 20°C 0	1	2	3	4	5	7
27.48	12.02	unknown	nd	nd	nd	1.7	2.0	5.3	1.6
27.77	12.11	unknown	nd	0.7	2.0	1.8	nd	2.1	nd
28.63	12.38	unknown	nd	nd	nd	0.9	1.6	nd	nd
29.08	12.52	methyl tetradecenoate	1.4	nd	2.7	nd	3.9	12.2	2.9
29.57	12.66	unknown	1.1	nd	nd	3.1	4.7	13.0	3.6
30.39	12.90	unknown	nd	nd	1.0	1.9	2.7	11.7	2.3
Total			1964.8	1529.5	1487.3	1425.6	1435.0	1656.7	1030.6

[a] Fruits were harvested at mature unriped stage (flesh firmness 98 N) and stored for 2 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.2. Changes in volatile compounds during ripening of 'Bartlett' pear harvested at early stage of maturity (M1) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C 0	1	2	3	4	5	6
5.21	0.93	acetaldehyde	nd	nd	1.2	12.3	12.9	12.3	4.4
5.65	1.53	methyl acetate	nd	1.2	2.0	20.4	19.9	19.8	16.5
6.05	2.04	ethyl acetate	64.2	81.4	151.0	303.1	273.3	283.6	247.0
6.32	2.37	ethanol	1.6	6.8	6.8	38.8	48.4	59.9	71.7
6.95	3.08	propyl acetate	8.8	8.3	11.5	15.4	4.2	8.2	4.4
7.86	3.99	ethyl butanoate	nd	0.9	8.5	11.9	6.9	5.0	1.5
8.54	4.36	butyl acetate	452.6	447.5	444.8	402.9	216.6	174.3	114.4
9.54	4.86	isoamyl acetate	1.4	nd	nd	nd	nd	nd	nd
9.76	4.96	1-butanol	1.1	1.6	1.5	3.5	nd	1.1	nd
10.77	5.40	pentyl acetate	7.6	19.6	25.4	30.1	23.7	28.9	31.4
11.11	5.54	methyl hexanoate	nd	nd	nd	3.3	1.0	nd	nd
11.95	5.86	butyl butanoate + 1-methylpropyl butanoate	2.1	4.2	5.0	0.7	nd	nd	nd
12.32	6.00	ethyl hexanoate	7.9	14.2	29.5	42.0	29.0	27.2	20.8
13.28	6.43	hexyl acetate	706.2	956.4	1112.5	1173.1	765.7	686.0	502.5
15.01	7.14	1-hexanol	2.4	5.8	3.5	nd	1.5	1.7	3.3
15.84	7.45	heptyl acetate	nd	2.9	7.0	8.8	9.0	9.1	10.4
16.24	7.60	methyl octanoate	nd	nd	nd	3.6	3.0	1.4	nd
16.87	7.82	unknown	nd	nd	nd	nd	0.8	1.4	1.1
16.95	7.85	hexyl butanoate + butyl hexanoate	20.2	38.2	46.7	16.3	10.1	8.2	2.9
17.08	7.89	unknown	nd	nd	nd	nd	3.2	2.6	1.6
17.41	8.00	ethyl octanoate	4.8	12.2	28.0	37.9	27.7	27.9	19.2
18.33	8.46	octyl acetate	3.8	6.7	11.6	16.1	16.8	23.2	15.1
18.77	8.67	unknown	nd	nd	4.6	8.1	10.6	10.2	6.0
19.45	8.98	4-octen-1-ol acetate	4.2	8.4	9.2	8.4	1.2	1.9	3.5
20.06	9.25	ethyl trans-2 octenoate	2.6	3.8	12.0	16.8	19.7	22.0	15.7
21.49	9.86	hexyl hexanoate	10.9	8.6	11.7	10.0	4.8	2.2	nd
21.67	9.93	methyl 4-decenoate	nd	3.4	12.3	14.1	11.9	10.7	5.0
21.98	10.05	methyl 4-decanoate	nd	nd	2.4	3.6	4.8	1.7	2.9
22.07	10.09	ethyl 2,4 octadienoate	1.8	7.5	10.5	12.8	7.2	6.1	3.4
22.35	10.20	unknown	nd	nd	1.3	nd	1.1	nd	nd
22.43	10.23	ethyl 4-decenoate	17.3	13.7	10.1	nd	5.9	3.1	nd
22.61	10.30	ethyl 2,4 octadienoate	10.6	17.1	26.1	31.7	27.7	30.7	19.2
23.15	10.51	unknown	nd	1.3	2.2	nd	nd	2.2	0.5
23.43	10.62	methyl 2-decenoate	nd	nd	1.2	0.8	3.9	2.7	1.8
23.80	10.75	unknown	5.3	6.1	8.3	7.7	5.5	6.3	3.6
23.93	10.80	α-farnesene (E,Z)	41.3	23.6	19.7	13.2	10.4	9.6	6.6
24.40	10.97	α-farnesene (E,E)	1510.6	959.1	671.8	325.3	207.7	174.5	98.5
24.54	11.02	ethyl trans2-decenoate	nd	nd	17.7	15.6	14.5	16.0	11.5
24.82	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	3.6	nd	nd	nd	1.7
25.13	11.23	methyl trans-2, cis-4 decadienoate	76.0	73.2	157.7	172.1	191.9	188.6	134.8
25.56	11.38	unknown	1.8	nd	nd	nd	nd	nd	nd
25.84	11.48	ethyl cis-2, cis-4 decadienoate	3.4	4.7	8.8	11.9	11.0	13.8	10.7
26.08	11.56	ethyl trans-2, cis-4 decadienoate	451.7	485.6	873.5	937.3	841.9	896.6	642.2

(Continued)

Table A.6.2. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C 0	1	2	3	4	5	6
26.84	11.81	methyl trans-2, trans-4 decadienoate	4.8	6.2	7.9	10.0	5.6	4.9	2.5
27.04	11.88	ethyl trans-2, trans-4 decadienoate	70.3	104.2	187.5	141.7	113.0	102.9	63.8
27.47	12.01	unknown	nd	1.7	1.6	nd	0.5	4.0	3.2
27.74	12.10	unknown	0.8	2.7	0.5	nd	1.3	nd	nd
28.64	12.38	unknown	nd	nd	nd	2.3	1.7	2.6	nd
29.03	12.50	methyl tetradecenoate	0.9	nd	nd	nd	nd	nd	nd
29.08	12.51	unknown	5.4	8.0	11.5	16.3	10.2	15.1	10.9
29.64	12.68	unknown	nd	nd	nd	4.8	1.5	2.9	1.1
30.18	12.84	unknown	nd	nd	1.5	1.6	3.3	1.9	1.1
30.38	12.90	unknown	nd	nd	2.9	2.1	3.6	4.1	2.2
31.09	13.10	unknown	nd	2.2	5.8	7.9	5.2	5.7	2.6
Total			3504.3	3349.0	3980.5	3915.8	3001.3	2925.3	2123.2

[a] Fruits were harvested at mature unriped stage (flesh firmness 98 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.3. Changes in volatile compounds during ripening of 'Bartlett' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 2 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	1	2	3	4	5	6
5.70	1.60	methyl acetate	nd	nd	5.7	7.3	9.8	11.6	5.0
6.07	2.07	ethyl acetate	5.8	8.8	29.3	40.9	62.0	79.8	82.9
6.36	2.41	ethanol	nd	1.1	9.1	7.7	11.1	15.9	23.9
6.97	3.09	propyl acetate	3.2	2.9	3.8	5.7	7.3	9.2	5.6
7.52	3.66	2-methylpropyl acetate	nd	nd	nd	nd	0.6	nd	nd
7.89	4.02	ethyl butanoate	nd	nd	1.7	2.0	3.1	3.5	4.2
8.56	4.37	butyl acetate	107.5	163.3	169.3	144.4	145.1	134.3	93.8
9.61	4.89	isoamyl acetate	nd	nd	nd	1.5	0.9	2.5	2.2
9.79	4.97	1-butanol	1.7	2.0	3.2	2.7	3.1	1.2	3.2
10.79	5.40	pentyl acetate	5.4	8.8	10.9	9.5	nd	10.3	9.6
11.12	5.54	methyl hexanoate	nd	nd	nd	1.1	2.3	1.8	1.4
12.00	5.88	butyl butanoate + 1-methylpropyl butanoate	1.4	3.4	3.0	3.0	4.0	2.7	2.5
12.33	6.00	ethyl hexanoate	nd	2.1	nd	6.4	10.2	8.3	8.2
13.29	6.44	hexyl acetate	140.2	360.1	423.8	372.1	433.2	453.8	374.2
15.02	7.15	1-hexanol	2.3	4.3	6.3	5.0	5.1	6.2	4.9
15.85	7.46	heptyl acetate	nd	1.9	2.5	2.3	2.8	3.1	3.1
16.25	7.60	methyl octanoate	nd	nd	nd	1.7	1.9	2.3	1.6
16.96	7.85	hexyl butanoate + butyl hexanoate	6.4	20.8	22.3	13.6	15.5	16.3	13.5
17.39	8.00	ethyl octanoate	nd	1.7	5.6	5.4	8.4	9.3	7.5
18.32	8.45	octyl acetate	nd	2.1	2.9	0.9	2.9	3.0	2.9
18.77	8.67	unknown	nd	nd	0.8	1.5	4.1	2.5	4.6
18.86	8.71	methyl trans-2 octenoate	6.6	5.6	3.5	nd	nd	nd	nd
19.47	8.99	4-octen-1-ol acetate	1.6	4.1	3.6	1.6	1.7	1.7	0.9
20.08	9.26	ethyl trans-2 octenoate	nd	nd	3.1	3.3	5.4	6.7	8.0
20.89	9.61	methyl 2,4 octadienoate	nd	nd	nd	nd	nd	nd	1.0
21.49	9.86	hexyl hexanoate	1.1	3.5	3.4	2.5	3.2	2.7	1.7
21.68	9.93	methyl 4-decanoate	nd	1.8	2.6	3.5	6.1	5.5	3.2
21.98	10.05	ethyl decanoate	nd	nd	nd	1.7	1.4	1.5	2.0
22.07	10.09	ethyl 2,4 octadienoate	nd	0.8	1.0	1.7	1.6	1.5	nd
22.45	10.24	ethyl 4-decenoate	4.2	1.0	nd	nd	nd	nd	nd
22.61	10.30	ethyl 2,4 octadienoate	nd	2.6	2.2	3.9	4.5	1.4	4.5
23.48	10.63	methyl 2-decenoate	0.5	1.4	nd	0.9	2.2	2.6	1.6
23.94	10.80	α-239 farnesene (E,Z)	22.0	12.5	11.3	7.1	6.3	4.8	2.0
24.42	10.98	α-farnesene (E,E)	1016.0	634.5	389.6	165.7	109.6	75.0	28.4
24.54	11.02	ethyl trans-2 decenoate	nd	nd	nd	nd	4.7	4.9	3.3
24.81	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	1.5	1.6	1.7
25.13	11.23	methyl trans-2, cis-4 decadienoate	6.7	33.7	78.6	86.1	119.7	119.9	80.9
25.58	11.39	unknown	2.8	1.3	nd	nd	nd	nd	nd
25.84	11.48	ethyl cis-2, cis-4 decadienoate	nd	1.1	1.4	2.6	4.3	4.8	3.9
26.08	11.56	ethyl trans-2, cis-4 decadienoate	38.1	138.6	222.1	180.6	270.8	260.0	186.5
26.84	11.81	methyl trans-2, trans-4 decadienoate	nd	2.6	3.0	3.5	4.8	7.2	3.3

(Continued)

Table A.6.3. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	1	2	3	4	5	6
27.05	11.88	ethyl trans-2, trans-4 decadienoate	5.4	39.5	46.3	30.0	39.5	32.3	17.0
29.06	12.51	methyl tetradecenoate	nd	nd	1.3	1.6	2.7	37.7	2.3
Total			1378.7	1468.0	1473.1	1131.0	1323.1	1349.4	1006.7

[a] Fruits were harvested at mature unriped stage (flesh firmness 89 N) and stored for 2 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.4. Changes in volatile compounds during ripening of 'Bartlett' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C			
			0	1	2	3
5.23	0.96	acetaldehyde	nd	1.2	1.9	2.0
5.62	1.49	unknown	nd	nd	0.4	3.4
5.68	1.57	methyl acetate	1.5	2.6	2.1	nd
6.05	2.04	ethyl acetate	55.2	67.8	102.1	155.8
6.33	2.38	ethanol	5.6	12.4	21.0	33.6
6.90	3.02	unknown	nd	0.6	nd	nd
6.95	3.07	propyl acetate	4.2	3.0	6.2	4.4
7.69	3.82	2-methylpropyl acetate	nd	nd	1.7	nd
7.89	4.01	ethyl butanoate	2.3	0.7	16.4	6.9
8.54	4.36	butyl acetate	322.0	322.5	331.1	261.2
9.76	4.96	1-butanol	1.4	1.8	3.4	1.4
10.24	5.17	butyl propanoate	nd	1.4	nd	nd
10.64	5.34	unknown	nd	2.1	nd	nd
10.77	5.40	pentyl acetate	10.7	30.3	23.7	20.9
11.95	5.86	butyl butanoate + 1-methylpropyl butanoate	1.9	2.5	4.6	0.8
12.33	6.00	ethyl hexanoate	5.6	11.9	17.3	23.2
13.28	6.43	hexyl acetate	558.7	840.2	1052.7	859.3
14.76	7.05	unknown	1.8	nd	nd	nd
15.02	7.15	1-hexanol	4.8	8.7	7.9	7.1
15.84	7.45	heptyl acetate	0.9	1.5	6.3	8.0
16.96	7.85	hexyl butanoate + butyl hexanoate	13.0	28.9	41.9	25.8
17.41	8.00	ethyl octanoate	4.7	8.4	17.9	22.7
18.31	8.45	octyl acetate	1.3	4.7	9.2	11.0
18.78	8.67	unknown	0.9	0.9	1.8	4.1
19.45	8.98	4-octen-1-ol acetate	2.2	4.4	7.0	4.6
20.06	9.25	ethyl trans-2 octenoate	2.7	4.9	10.5	14.8
21.49	9.85	hexyl hexanoate	5.8	6.3	7.9	4.7
21.86	10.01	methyl 4-decanoate	4.9	1.8	4.1	3.7
21.97	10.05	ethyl decanoate	nd	nd	3.5	5.6
22.05	10.08	ethyl 2,4 octadienoate	nd	1.1	4.5	2.8
22.44	10.23	ethyl 4-decenoate	7.6	1.7	4.3	nd
22.61	10.30	ethyl 2,4 octadienoate	3.5	5.1	6.1	7.4
23.07	10.48	unknown	2.8	nd	nd	nd
23.41	10.61	methyl 2-decenoate	nd	nd	nd	1.0
23.78	10.75	unknown	nd	nd	nd	0.7
23.93	10.80	α-farnesene (E,Z)	33.8	15.9	15.8	8.8
24.40	10.97	α-farnesene (E,E)	862.5	413.2	410.6	172.8
24.53	11.02	ethyl trans-2 decenoate	nd	nd	13.3	12.9
25.12	11.23	methyl trans-2, cis-4 decadienoate	63.1	55.9	126.4	123.0
25.55	11.38	unknown	2.6	nd	nd	nd
25.77	11.45	unknown	3.4	nd	nd	nd
25.84	11.48	ethyl cis-2, cis-4 decadienoate	12.5	6.5	11.0	12.9
26.08	11.56	ethyl trans-2, cis-4 decadienoate	447.2	398.2	833.0	777.4

(Continued)

Table A.6.4. Continued

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C 0	1	2	3
26.33	11.64	unknown	2.6	nd	nd	nd
26.53	11.71	unknown	5.2	nd	nd	nd
26.67	11.75	unknown	1.1	nd	nd	nd
26.82	11.81	methyl trans-2, trans-4 decadienoate	1.2	1.4	4.6	9.1
27.04	11.87	ethyl trans-2, trans-4 decadienoate	51.3	67.1	123.7	97.1
27.45	12.01	unknown	nd	nd	1.2	4.4
27.77	12.11	unknown	1.0	nd	nd	nd
28.63	12.38	unknown	nd	nd	nd	0.9
29.04	12.50	methyl tetradecenoate	1.0	1.7	7.2	9.9
Total			2514.5	2339.6	3264.1	2726.0

[a] Fruits were harvested at mature unriped stage (flesh firmness 89 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.5. Changes in volatile compounds during ripening of 'Bartlett' pear harvested at late stage of maturity (M3) after storage at 0°C for 2 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	1	2	3	4	5
5.26	1.00	acetaldehyde	nd	nd	1.2	2.6	3.1	nd
5.67	1.56	methyl acetate	nd	nd	2.9	2.3	2.6	3.1
6.07	2.06	ethyl acetate	31.2	26.2	42.9	54.9	62.4	66.8
6.36	2.42	ethanol	5.1	6.9	16.0	21.4	26.3	31.2
6.97	3.10	propyl acetate	1.9	2.0	4.0	2.0	1.1	0.9
7.40	3.54	2-methylpropyl acetate	1.0	nd	nd	nd	nd	nd
7.90	4.02	ethyl butanoate	nd	nd	2.7	2.6	2.0	0.9
8.55	4.37	butyl acetate	177.2	132.8	129.0	121.3	88.5	71.6
9.77	4.96	isoamyl acetate	4.0	4.1	4.8	4.3	3.0	3.5
10.79	5.40	1-butanol	5.5	6.2	7.5	7.0	5.5	5.8
11.93	5.85	pentyl acetate	nd	0.7	1.0	nd	nd	nd
12.33	6.00	ethyl hexanoate	4.5	4.9	7.3	1nd	8.3	7.5
13.29	6.44	hexyl acetate	503.7	523.5	615.4	573.3	407.1	313.9
14.79	7.06	unknown	3.6	nd	nd	nd	nd	nd
15.02	7.15	1-hexanol	7.0	7.9	10.3	8.9	7.4	6.1
15.86	7.46	heptyl acetate	nd	nd	2.7	3.4	2.4	nd
16.96	7.85	butyl hexanoate	7.3	8.2	8.7	7.5	1.5	2.1
17.07	7.89	hexylbutanoate	nd	nd	nd	4.3	5.1	7.2
17.41	8.00	ethyl octanoate	3.8	5.2	7.4	11.1	7.5	6.3
17.93	8.27	unknown	1.6	1.0	nd	1.3	1.2	nd
18.32	8.45	octyl acetate	3.7	3.7	4.6	6.2	5.0	4.2
18.76	8.66	unknown	0.9	nd	nd	nd	1.6	1.5
19.46	8.98	4-octen-1-ol acetate	2.6	4.4	5.0	3.6	3.2	1.6
19.94	9.20	1-octanol	nd	1.2	1.9	1.3	1.3	1.2
20.07	9.26	ethyl trans-2 octenoate	2.7	3.6	4.7	8.2	6.0	6.3
21.49	9.86	hexyl hexanoate	3.1	2.4	2.2	1.8	0.9	nd
21.95	10.04	ethyl decanoate	nd	0.9	nd	2.2	1.2	1.2
22.07	10.09	ethyl 2,4 octadienoate	nd	1.3	nd	2.1	1.2	nd
22.44	10.24	ethyl 4-decenoate	2.6	nd	nd	nd	nd	nd
22.62	10.31	ethyl 2,4 octadienoate	5.3	2.5	1.3	2.0	nd	nd
23.17	10.52	methyl 2,4,6 octatrienoate	nd	2.2	nd	nd	nd	nd
23.63	10.69	methyl 2-decenoate	nd	0.8	nd	nd	nd	nd
23.80	10.75	unknown	1.5	3.7	nd	nd	nd	0.4
23.94	10.80	α-farnesene (E,Z)	21.9	7.0	3.5	3.6	2.5	2.3
24.41	10.97	α-farnesene (E,E)	890.2	187.5	91.0	82.9	45.2	37.7
24.53	11.02	ethyl trans-2 decenoate	nd	2.2	2.4	4.4	2.6	2.4
25.12	11.23	methyl trans-2, cis-4 decadienoate	20.9	9.4	17.0	31.2	15.7	31.0
25.58	11.39	unknown	2.6	nd	nd	nd	nd	nd
25.84	11.48	ethyl cis-2, cis-4 decadienoate	7.0	3.3	4.1	6.8	5.5	6.5
26.09	11.56	ethyl trans-2, cis-4 decadienoate	378.6	166.5	210.2	341.3	214.8	232.0
26.84	11.81	methyl trans-2, trans-4 decadienoate	21.7	11.3	8.4	13.3	8.3	7.9
27.04	11.88	ethyl trans-2, trans-4 decadienoate	23.0	18.8	20.7	26.2	13.8	11.5
27.48	12.02	unknown	nd	nd	1.1	1.9	1.6	1.8

(Continued)

Table A.6.5. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C 0	1	2	3	4	5
27.79	12.12	unknown	3.1	2.2	1.8	1.8	nd	nd
29.04	12.50	methyl tetradecenoate	nd	nd	1.5	3.4	2.9	3.3
29.79	12.73	unknown	1.3	1.4	nd	2.8	1.7	2.9
30.39	12.90	unknown	nd	nd	nd	1.2	0.3	0.9
Total			2150.0	1166.1	1245.1	1386.3	970.3	883.4

[a] Fruits were harvested at mature unriped stage (flesh firmness 80 N) and stored for 2 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.6. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at early stage of maturity (M1) after storage at 0°C for 2 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C											
			0	1	2	3	4	5	6	7	8	9	10	11
5.62	1.49	methyl acetate	nd	nd	1.6	nd	nd	2.9	0.5	5.6	7.0	11.1	2nd	20.3
6.03	2.02	ethyl acetate	nd	0.8	nd	1.7	4.5	7.7	14.7	27.2	44.3	54.8	96.3	102.2
6.33	2.38	ethanol	nd	nd	nd	nd	1.7	2.1	0.9	5.0	7.8	8.8	27.7	21.5
6.91	3.03	propyl acetate	nd	0.7	0.9	nd	1.8	3.3	4.3	3.7	7.8	8.1	25.5	19.2
7.51	3.65	2-methylpropyl acetate	nd	nd	nd	nd	nd	nd	nd	1.9	1.4	0.9	1.3	8.4
7.88	4.01	ethyl butanoate	nd	nd	nd	nd	nd	nd	nd	1.5	3.3	1.9	nd	12.2
8.52	4.36	butyl acetate	67.1	129.7	145.1	148.8	151.2	122.9	133.3	135.6	111.8	96.8	105.6	119.1
9.57	4.87	Isoamyl acetate	nd	nd	1.6	nd	nd	nd	nd	1.5	3.1	nd	2.6	8.3
9.75	4.95	1-butanol	nd	2.5	2.1	2.5	3.8	2.2	nd	1.5	nd	nd	nd	nd
10.76	5.39	pentyl acetate	2.9	7.3	7.8	7.5	nd	8.1	8.3	9.5	7.7	11.1	10.3	14.6
11.94	5.86	1-methylpropyl butanoate + butyl butanoate	8.9	17.6	9.3	14.6	14.2	6.9	5.9	4.8	nd	3.8	2.3	nd
12.33	6.00	ethyl hexanoate	nd	nd	nd	nd	3.2	4.6	6.5	6.9	5.8	7.6	6.5	6.4
12.49	6.08	1-pentanol	1.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13.27	6.43	hexyl acetate	112.0	369.7	226.3	226.5	313.9	216.4	253.2	285.8	238.0	234.4	286.4	258.9
14.76	7.04	ethyl heptanoate	2.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15.02	7.15	1-hexanol	2.1	4.0	2.6	4.6	1.7	1.8	1.2	1.5	nd	nd	2.6	nd
15.83	7.45	heptyl acetate	nd	2.1	2.1	1.9	3.2	2.4	2.4	3.4	3.3	3.8	3.3	5.5
16.23	7.60	methyl octanoate	nd	nd	nd	nd	nd	1.1	nd	3.0	3.1	3.2	3.4	3.2
16.95	7.85	butyl hexanoate	57.7	119.1	108.5	99.6	114.3	53.9	49.7	39.1	23.8	19.4	14.4	12.3
17.38	7.99	ethyl octanoate	nd	nd	nd	3.8	8.7	7.8	14.9	14.9	15.7	13.0	12.3	14.5
18.31	8.45	octyl acetate	nd	5.2	7.7	9.6	11.2	7.7	8.6	9.1	7.6	7.9	7.1	6.6
18.85	8.70	butyl 3-hexenoate	11.5	11.6	11.7	8.7	9.0	5.0	2.4	2.8	nd	nd	nd	nd
19.24	8.88	pentyl hexanoate	nd	nd	1.7	nd	2.7	1.2	nd	nd	nd	nd	nd	nd
19.42	8.97	4-octen-1-ol acetate	nd	4.1	2.8	4.1	3.5	1.8	1.9	1.8	1.7	1.7	nd	1.5
21.01	9.66	methyl decanoate	1.0	nd	nd	4.2	5.5	4.8	3.0	4.1	4.1	2.8	2.7	2.9
21.14	9.71	unknown	2.2	2.3	2.3	3.1	nd	nd	nd	nd	nd	nd	nd	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	14.8	30.1	32.7	36.9	53.8	23.8	21.2	17.5	12.2	9.3	7.5	8.0
21.66	9.93	methyl 4-decenoate	nd	nd	nd	4.5	7.6	5.7	10.5	11.6	12.4	11.2	11.6	12.0
22.04	10.08	ethyl 2,4 (?) octadienoate	nd	0.9	1.8	6.0	15.6	7.9	16.7	15.2	17.8	12.5	10.6	10.6

(Continued)

Table A.6.6. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C											
			0	1	2	3	4	5	6	7	8	9	10	11
22.43	10.23	ethyl 4-decenoate	17.8	26.1	21.9	16.1	12.5	4.4	4.9	2.9	3.4	2.9	nd	nd
22.68	10.33	ethyl 2,4 (?) octadienoate	2.0	4.3	2.9	8.8	14.7	9.7	24.2	25.7	27.0	25.1	27.8	33.1
22.90	10.41	methyl 2,4,6 octatrienoate	nd	nd	nd	nd	2.3	1.7	3.1	3.2	3.3	2.7	2.1	3.1
23.79	10.75	methyl 2,4,6 octatrienoate	1.3	1.4	nd	3.7	6.1	4.7	9.3	9.4	9.8	4.5	8.6	5.2
23.93	10.80	α-farnesene (E,Z)	19.6	17.2	11.3	10.2	10.3	5.0	5.0	5.2	4.6	3.9	3.9	3.6
24.42	10.98	α-farnesene (E,E)	2276.9	2045.7	1280.7	986.0	960.7	413.7	456.6	455.7	388.0	333.7	240.2	239.1
25.11	11.22	methyl trans-2, cis-4-decadienoate	nd	3.8	7.9	19.7	40.3	25.7	36.5	34.1	32.8	30.1	26.7	24.5
25.35	11.31	methyl cis-2, trans-4-decadienoate	17.0	22.4	15.8	14.0	14.2	9.4	11.2	1nd	8.2	7.7	7.3	7.8
25.56	11.38	2-phenylethyl acetate	11.0	12.1	12.8	11.1	10.2	5.1	6.6	4.8	3.6	3.1	2.3	3.4
25.85	11.48	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.2	nd	3.0
26.07	11.55	ethyl trans-2, cis-4-decadienoate	5.8	20.7	3nd	38.6	67.7	40.6	72.0	76.8	73.9	62.7	65.7	78.2
27.00	11.86	ethyl trans-2, trans-4 decadienoate	3.0	5.4	4.8	7.0	15.9	9.2	14.8	17.8	17.7	14.3	13.9	15.2
27.79	12.11	unknown	5.6	5.0	4.6	4.7	3.7	1.2	nd	nd	0.9	nd	nd	nd
29.54	12.65	unknown	nd	1.2	2.1	nd	nd	1.3	nd	0.9	2.4	2.0	2.8	1.4
29.63	12.68	ethyl tetradecenoate	nd	nd	nd	nd	2.4	1.9	1.9	4.9	5.9	5.4	4.1	5.3
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	nd	nd	1.5	1.4	3.0	3.9	6.6	5.1	6.5	9.4
30.92	13.05	unknown	nd	1.5	nd	nd	3.6	2.4	1.7	2.7	4.9	3.7	5.2	11.3
33.13	13.66	methyl 7-hexadecenoate	nd	nd	nd	nd	nd	nd	1.7	0.9	2.1	1.7	2.1	2.4
33.41	13.74	unknown	nd	nd	nd	nd	2.4	1.9	1.5	1.1	1.8	1.8	2.4	1.3
37.04	14.64	unknown	nd	nd	nd	nd	nd	4.2	3.6	3.5	5.1	nd	4.0	nd
Total			2645.0	2874.2	1963.3	1708.6	1899.6	1045.6	1217.7	1278.1	1141.6	1035.6	1085.5	1115.5

[a] Fruits were harvested at mature unriped stage (flesh firmness 73 N) and stored for 2 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min)

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.7. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at early stage of maturity (M1) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E°	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.63	1.51	methyl acetate	nd	nd	0.1	0.9	0.9	0.7	1.2
6.06	2.05	ethyl acetate	1.5	2.4	5.0	12.4	15.7	18.2	22.8
6.32	2.37	ethanol	nd	1.3	2.2	4.6	5.4	6.3	7.6
6.95	3.07	propyl acetate	0.4	0.2	0.3	1.4	1.8	0.8	1.3
8.53	4.36	butyl acetate	35.6	56.3	47.8	52.9	39.1	29.0	24.4
9.68	4.92	isoamyl acetate	nd	1.2	0.2	nd	0.2	nd	0.4
9.74	4.95	1-butanol	nd	1.7	0.2	nd	0.3	nd	nd
10.76	5.40	pentyl acetate	1.1	3.3	3.1	3.4	1.0	2.3	4.6
11.95	5.86	1-methylpropyl butanoate + butyl butanoate	0.7	1.2	0.6	1.2	0.5	0.3	nd
12.48	6.07	1-pentanol	0.3	0.5	2.1	3.0	2.5	2.2	1.0
13.27	6.43	hexyl acetate	46.1	134.6	109.1	127.1	101.1	78.1	71.9
14.76	7.05	ethyl heptanoate	0.6	nd	nd	nd	nd	nd	nd
15.03	7.15	1-hexanol	0.1	0.4	0.7	0.2	0.7	0.1	nd
15.83	7.45	heptyl acetate	nd	1.1	1.1	1.6	1.6	1.3	0.6
16.23	7.60	methyl octanoate	nd	nd	nd	0.6	0.2	0.2	nd
16.73	7.77	pentyl 2-methylpropanoate	nd	nd	nd	nd	0.3	nd	nd
16.95	7.85	butyl hexanoate	6.2	18.5	14.3	12.4	7.1	6.3	4.5
17.38	7.99	ethyl octanoate	0.4	2.7	4.0	4.8	3.7	1.8	2.8
18.30	8.44	octyl acetate	nd	0.9	1.4	1.9	1.5	1.2	1.0
18.85	8.70	butyl 3-hexenoate	2.1	1.3	0.4	nd	nd	nd	nd
19.41	8.96	4-octen-1-ol acetate	nd	0.6	0.1	0.3	nd	nd	nd
20.06	9.25	ethyl trans-2 octenoate	nd	0.4	0.3	0.6	0.6	0.3	0.5
21.04	9.67	methyl decanoate	nd	nd	nd	0.8	nd	nd	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	2.8	5.8	4.3	6.1	4.6	3.6	2.4
21.64	9.92	methyl 4-decenoate	nd	0.4	1.5	3.0	2.2	1.9	1.5
22.05	10.08	ethyl 2,4 (?) octadienoate	0.5	2.9	3.3	4.1	2.6	2.3	1.9
22.43	10.23	ethyl 4-decenoate	16.6	15.0	7.3	4.3	2.9	2.3	1.3
22.61	10.30	ethyl 2,4 (?) octadienoate	2.5	11.1	8.4	10.4	8.4	8.4	5.4
22.88	10.41	methyl 2,4,6 (?) octatrienoate	nd	nd	0.1	0.5	nd	0.5	nd
23.79	10.75	methyl 2,4,6 (?) octatrienoate	0.6	3.4	3.0	3.5	2.3	2.0	1.0
23.93	10.80	α-farnesene (E,Z)	10.0	5.6	2.8	2.4	1.7	1.6	1.2
24.42	10.98	α-farnesene (E,E)	753.7	400.4	192.1	161.6	104.5	82.3	56.9
25.12	11.23	methyl trans-2, cis-4-decadienoate	2.0	6.7	8.2	16.0	13.8	14.2	10.6
25.35	11.31	methyl cis-2, trans-4-decadienoate	3.2	3.6	2.4	1.8	1.9	1.6	1.4
25.56	11.38	2-phenylethyl acetate	4.1	5.2	3.9	2.4	1.0	1.9	0.8
25.82	11.47	ethyl cis-2, cis-4 decadienoate	nd	0.7	0.2	1.0	0.6	1.6	1.7
26.07	11.56	ethyl trans-2, cis-4-decadienoate	25.6	66.0	54.6	62.8	59.7	59.7	48.8
27.04	11.88	ethyl trans-2, trans-4 decadienoate	4.8	12.9	10.2	11.4	8.0	7.3	5.6
27.73	12.10	unknown	nd	0.1	0.1	0.3	0.3	0.2	nd
29.03	12.50	methyl tetradecenoate	nd	0.6	0.2	0.3	0.9	0.5	1.2
29.64	12.68	ethyl tetradecenoate	nd	nd	nd	nd	0.9	0.5	nd
30.38	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	0.4	1.5	1.3	1.4	1.4
31.05	13.09	unknown	nd	0.3	0.2	1.7	1.6	2.2	1.8

(Continued)

Table A.6.7. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
32.26	13.43	unknown	0.3	nd	0.2	nd	0.2	0.1	0.3
33.10	13.65	methyl cis-7 hexadecadecenoate	nd	nd	nd	0.5	nd	nd	nd
Total			921.8	769.4	496.3	525.6	403.7	345.2	289.8

[a] Fruits were harvested at mature unriped stage (flesh firmness 73 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min)

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.8. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at early stage of maturity (M1) after storage at 0°C for 5 months[a].

Rt[b] (min.)	I_E^c	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.68	1.57	methyl acetate	nd	0.1	0.5	1.5	nd	1.6	0.2
6.07	2.07	ethyl acetate	2.8	3.3	7.3	12.6	12.6	17.0	15.2
6.34	2.39	ethanol	0.8	1.7	3.3	5.1	5.1	6.5	5.9
6.98	3.10	propyl acetate	0.9	0.6	1.2	1.5	1.3	1.7	1.3
7.51	3.65	2-methylpropyl acetate	nd	0.7	nd	0.5	0.2	0.2	nd
7.90	4.03	ethyl butanoate	nd	0.7	1.1	0.1	0.6	0.4	0.3
8.55	4.37	butyl acetate	36.2	37.6	48.9	40.6	32.3	30.6	21.2
9.61	4.89	isoamyl acetate	nd	0.4	nd	0.7	0.6	1.0	0.4
9.76	4.96	1-butanol	0.3	0.9	0.7	1.1	0.9	1.0	0.6
10.78	5.40	pentyl acetate	1.7	3.0	3.8	4.2	2.5	3.3	2.5
11.95	5.86	1-methylpropyl butanoate + butyl butanoate	1.3	2.5	1.0	1.3	0.8	0.6	0.4
12.34	6.00	ethyl hexanoate	nd	2.5	3.3	3.0	2.2	2.1	1.5
13.28	6.43	hexyl acetate	43.0	84.4	128.4	120.3	97.0	90.2	57.5
14.77	7.05	ethyl heptanoate	0.8	nd	nd	nd	nd	nd	nd
15.04	7.15	1-hexanol	0.5	1.0	1.3	1.0	0.8	nd	0.9
15.86	7.46	heptyl acetate	nd	1.0	1.7	1.8	1.8	0.9	1.2
16.25	7.60	methyl octanoate	nd	0.6	0.5	0.5	0.2	0.1	nd
16.93	7.84	butyl hexanoate	6.8	17.3	17.0	11.3	7.8	6.2	3.6
17.40	8.00	ethyl octanoate	1.1	3.1	4.3	3.2	2.2	1.9	1.4
18.33	8.46	octyl acetate	0.4	1.2	1.4	nd	1.1	1.2	0.5
18.85	8.70	butyl 3-hexenoate	1.9	0.4	1.2	nd	0.2	nd	nd
19.27	8.90	pentyl hexanoate	nd	0.4	0.6	0.4	0.2	nd	nd
19.46	8.98	4-octen-1-ol acetate	nd	0.6	0.7	0.4	nd	nd	nd
20.06	9.25	ethyl trans-2 octenoate	nd	0.3	1.0	1.0	0.7	0.6	0.4
21.08	9.68	methyl decanoate	nd	0.7	0.3	0.6	nd	nd	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	2.6	5.8	6.0	4.5	2.4	2.3	1.2
21.67	9.93	methyl 4-decenoate	0.7	2.2	2.9	3.0	1.9	1.7	0.8
21.98	10.05	ethyl decanoate	nd	0.3	0.5	0.4	nd	nd	nd
22.07	10.09	ethyl 2,4 (?) octadienoate	1.0	4.3	4.1	3.2	2.1	1.7	1.1
22.44	10.23	ethyl 4-decenoate	7.4	7.1	6.4	3.3	2.1	1.7	0.7
22.61	10.30	ethyl 2,4 (?) octadienoate	3.7	11.8	14.5	9.9	8.3	7.8	4.5
22.91	10.42	methyl 2,4,6 (?) octatrienoate	nd	0.6	0.9	0.6	0.3	0.5	nd
23.80	10.75	methyl 2,4,6 (?) octatrienoate	2.0	3.3	3.7	nd	1.9	1.5	0.9
23.93	10.80	α-farnesene (E,Z)	7.5	3.7	3.6	1.0	1.3	1.1	0.8
24.41	10.98	α-farnesene (E,E)	426.6	206.9	168.7	76.9	47.6	38.6	21.8
24.81	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	0.2	0.5	nd
25.12	11.23	methyl trans-2, cis-4-decadienoate	5.5	11.2	19.1	17.4	13.9	14.2	6.6
25.36	11.31	methyl cis-2, trans-4-decadienoate	1.4	0.8	1.3	1.0	0.8	0.9	0.7
25.56	11.38	2-phenylethyl acetate	1.4	1.8	1.9	1.0	0.7	nd	0.3
25.84	11.47	ethyl cis-2, cis-4 decadienoate	0.8	1.1	1.6	nd	nd	2.2	1.8
26.08	11.56	ethyl trans-2, cis-4-decadienoate	35.5	83.2	106.7	68.8	68.0	74.8	50.2
27.04	11.88	ethyl trans-2, trans-4 decadienoate	6.3	16.7	18.2	11.4	9.3	8.4	4.6
27.31	11.96	unknown	0.4	0.4	0.6	nd	0.3	0.6	nd
27.76	12.11	unknown	0.4	0.5	nd	0.7	0.2	nd	0.6
29.04	12.50	methyl tetradecenoate	0.3	1.2	1.9	1.6	1.4	1.5	1.1
29.63	12.68	ethyl tetradecenoate	nd	0.6	1.0	1.0	0.8	nd	0.3
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	0.5	0.9	1.4	1.7	0.8	1.1	0.9

(Continued)

Table A.6.8. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
31.07	13.10	unknown	0.6	1.1	1.5	1.9	1.4	1.9	1.3
32.16	13.40	unknown	nd	0.5	0.4	nd	0.5	0.5	0.3
33.14	13.66	methyl cis-7 hexadecadecenoate	nd	nd	0.3	0.5	nd	nd	0.3
33.42	13.74	unknown	nd	nd	0.3	0.6	0.4	0.4	0.2
34.11	13.92	unknown	nd	0.2	0.3	0.7	0.4	0.3	0.2
35.83	14.35	unknown	nd	nd	nd	0.7	nd	nd	nd
37.07	14.65	unknown	0.4	0.5	nd	1.0	0.3	nd	nd
Total			603.6	531.3	597.2	425.7	338.4	331.2	216.3

[a] Fruits were harvested at mature unriped stage (flesh firmness 73 N) and stored for 5 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on Carbowax 20M column (See Materials and methods for details).

[c] Ethyl ester retention indices.

Table A.6.9. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at early stage of maturity (M1) after storage at 0°C for 6 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.07	2.07	ethyl acetate	3.8	5.3	9.6	12.8	11.1	14.2	18.1
6.37	2.42	ethanol	nd	1.7	4.3	7.2	5.9	8.0	11.9
6.95	3.08	propyl acetate	0.2	1.0	0.8	1.2	1.0	0.9	1.1
7.54	3.68	2-methylpropyl acetate	nd	nd	nd	0.2	nd	0.2	nd
7.90	4.03	ethyl butanoate	nd	0.8	0.8	0.5	0.6	0.6	nd
8.55	4.37	butyl acetate	36.5	42.9	39.9	32.6	21.2	19.7	17.7
9.56	4.87	isoamyl acetate	1.3	nd	0.4	nd	nd	0.4	0.4
9.78	4.97	1-butanol	nd	0.9	1.1	1.0	0.5	0.6	0.6
10.76	5.40	pentyl acetate	1.6	3.1	2.1	nd	2.7	2.3	2.4
11.99	5.88	1-methylpropyl butanoate + butyl butanoate	0.8	1.8	1.3	0.6	0.5	nd	0.4
12.33	6.00	ethyl hexanoate	1.0	2.4	2.5	2.0	1.4	1.1	1.0
12.46	6.06	1-pentanol	0.2	nd	nd	nd	nd	nd	nd
13.28	6.43	hexyl acetate	50.9	121.3	116.3	89.3	63.9	54.7	44.5
14.78	7.05	ethyl heptanoate	1.3	nd	nd	nd	nd	nd	nd
15.02	7.14	1-hexanol	nd	0.5	0.8	1.2	nd	0.8	0.8
15.86	7.46	heptyl acetate	nd	1.6	1.9	1.5	0.7	1.2	1.0
16.95	7.85	butyl hexanoate	4.7	14.4	9.9	5.4	3.6	2.8	2.2
17.40	8.00	ethyl octanoate	nd	3.1	3.5	2.4	1.5	nd	nd
18.33	8.46	octyl acetate	nd	1.6	1.9	1.4	0.7	0.6	0.5
18.86	8.71	butyl 3-hexenoate	0.8	nd	nd	nd	nd	nd	nd
19.45	8.98	4-octen-1-ol acetate	nd	1.2	0.8	0.5	nd	nd	nd
20.07	9.25	ethyl trans-2 octenoate	nd	1.0	1.4	nd	0.8	0.3	nd
21.18	9.73	unknown	0.6	nd	nd	nd	nd	nd	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	2.1	3.6	4.7	2.5	1.4	nd	0.6
21.68	9.93	methyl 4-decenoate	nd	2.4	2.9	1.5	0.9	0.5	0.5
21.96	10.05	ethyl decanoate	nd	0.5	0.5	0.4	nd	nd	nd
22.07	10.09	ethyl 2,4 (?) octadienoate	nd	1.9	3.3	1.1	1.4	0.5	nd
22.43	10.23	ethyl 4-decenoate	11.5	11.8	9.2	4.8	2.6	1.9	1.2
22.61	10.30	ethyl 2,4 (?) octadienoate	2.1	16.0	17.8	8.7	4.9	2.6	2.4
22.89	10.41	methyl 2,4,6 (?) octatrienoate	nd	nd	0.7	nd	nd	nd	nd
23.79	10.75	methyl 2,4,6 (?) octatrienoate	1.1	4.4	4.5	1.9	1.2	0.5	1.0
23.93	10.80	α-farnesene (E,Z)	9.2	6.9	5.7	2.6	1.3	0.6	0.6
24.40	10.97	α-farnesene (E,E)	462.6	306.7	242.9	95.4	42.4	19.4	20.6
25.11	11.23	methyl trans-2, cis-4-decadienoate	7.6	11.9	20.1	18.1	12.0	7.7	6.3
25.34	11.30	methyl cis-2, trans-4-decadienoate	0.7	1.3	1.7	1.3	0.6	0.1	0.7
25.55	11.38	2-phenylethyl acetate	2.8	3.7	4.3	2.5	1.1	0.7	0.6
25.83	11.47	ethyl cis-2, cis-4 decadienoate	1.7	2.2	3.2	2.9	2.1	1.6	1.9
26.07	11.55	ethyl trans-2, cis-4-decadienoate	51.6	96.5	141.8	114.7	80.0	44.3	44.3
27.03	11.87	ethyl trans-2, trans-4 decadienoate	6.8	15.8	23.3	13.8	8.9	5.0	3.9
27.32	11.96	unknown	0.8	0.6	nd	0.5	nd	nd	nd
27.48	12.02	unknown	nd	nd	nd	0.8	0.6	nd	nd
27.76	12.11	unknown	nd	0.3	0.5	nd	0.2	1.0	1.1
27.83	12.13	unknown	1.2	nd	nd	1.7	nd	nd	nd
29.04	12.50	methyl tetradecenoate	nd	1.6	3.0	3.3	1.4	1.1	1.3
29.62	12.68	ethyl tetradecenoate	nd	nd	0.6	0.7	0.4	0.2	0.5
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	1.1	1.3	0.9	0.9	0.7	0.4
31.07	13.10	unknown	0.5	1.1	2.1	1.4	1.6	0.8	1.1

(Continued)

Table A.6.9. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
32.15	13.40	unknown	nd	0.4	1.0	0.5	nd	0.3	0.3
33.10	13.65	methyl cis-7 hexadecadecenoate	nd	0.6	0.4	nd	nd	nd	nd
34.13	13.92	unknown	nd	0.7	0.9	0.7	0.4	nd	nd
Total			666.1	696.5	695.4	442.4	282.3	197.7	191.9

[a] Fruits were harvested at mature unriped stage (flesh firmness 73 N) and stored for 6 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min)

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.10. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 2 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C											
			0	1	2	3	4	5	6	7	8	9	10	12
5.69	1.59	methyl acetate	nd	nd	nd	nd	nd	nd	2.6	0.5	5.0	nd	0.3	nd
5.88	1.83	unknown	nd	nd	nd	nd	nd	nd	2.1	nd	nd	nd	4.2	7.1
6.02	2.01	ethyl acetate	nd	nd	nd	nd	0.8	8.1	18.4	18.9	34.2	34.8	47.9	52.7
6.32	2.37	ethanol	nd	nd	nd	nd	nd	nd	1.1	1.9	5.1	7.6	11.2	13.0
6.97	3.10	propyl acetate	1.2	nd	nd	nd	0.9	3.1	4.1	3.1	5.5	5.4	3.4	5.1
7.49	3.63	2-methylpropyl acetate	nd	nd	nd	1.0	nd	nd	nd	nd	nd	nd	nd	0.5
8.55	4.37	butyl acetate	79.9	136.4	183.1	151.0	150.3	158.0	160.1	123.9	133.4	103.6	95.0	76.7
9.57	4.87	isoamyl acetate	nd	nd	nd	nd	0.7	nd	0.3	0.5	nd	nd	nd	nd
9.73	4.95	1-butanol	nd	0.7	nd	0.4	0.8	0.7	nd	nd	nd	0.9	1.8	2.0
10.78	5.40	pentyl acetate	4.1	8.2	9.8	9.2	9.5	10.4	5.9	8.9	10.1	7.5	7.6	4.2
11.97	5.87	1-methylpropyl butanoate + butyl butanoate	8.0	10.0	9.3	8.3	6.9	5.7	4.4	2.0	2.7	0.4	nd	nd
12.39	6.02	ethyl hexanoate	nd	nd	nd	1.6	nd	3.4	3.8	3.1	1.7	1.2	4.5	0.8
12.51	6.09	1-pentanol	2.8	nd	nd	nd	nd	nd	nd	0.9	nd	nd	nd	nd
13.29	6.44	hexyl acetate	132.4	427.4	370.6	289.3	327.4	362.6	389.3	323.7	388.8	290.8	300.2	230.9
14.78	7.05	unknown	4.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15.02	7.15	1-hexanol	3.0	4.9	3.7	0.9	2.5	1.2	1.8	nd	nd	1.0	1.3	1.1
15.85	7.46	heptyl acetate	nd	2.1	2.4	1.9	1.6	4.0	3.0	3.5	4.9	4.0	2.2	4.0
16.23	7.60	methyl octanoate	nd	nd	nd	nd	nd	2.4	1.1	1.9	1.3	nd	2.0	nd
16.96	7.85	butyl hexanoate	41.9	8nd	98.8	67.9	64.0	61.8	49.4	30.2	29.8	18.0	15.1	7.9
17.40	8.00	ethyl octanoate	nd	nd	nd	0.9	3.8	10.1	12.1	11.1	15.2	10.7	9.9	6.0
18.35	8.46	octyl acetate	2.1	5.6	12.0	9.7	9.0	13.3	11.6	7.7	10.7	6.1	5.6	3.8
18.86	8.71	butyl 3-hexenoate	10.4	8.0	7.9	4.6	2.4	1.2	nd	nd	nd	nd	nd	nd
19.25	8.89	pentyl hexanoate	nd	nd	0.8	0.8	nd	1.8	nd	nd	nd	nd	nd	nd
19.46	8.98	4-octen-1-ol acetate	nd	4.5	6.1	4.1	2.8	4.0	1.6	1.3	1.2	1.7	1.7	nd
19.93	9.19	1-octanol	nd	0.9	0.6	0.5	1.4	1.5	nd	0.7	nd	nd	nd	nd
21.03	9.67	methyl decanoate	1.1	nd	nd	nd	nd	5.6	4.1	2.8	4.2	1.6	1.3	nd
21.17	9.72	unknown	2.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
21.49	9.86	hexyl hexanoate + butyl octanoate	18.0	27.7	40.5	26.5	23.9	33.8	25.5	14.9	18.2	10.4	10.2	4.9

(Continued)

Table A.6.10. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C											
			0	1	2	3	4	5	6	7	8	9	10	12
21.68	9.93	methyl 4-decenoate	nd	nd	nd	nd	3.9	8.7	9.0	7.1	14.2	7.8	2.7	5.2
22.06	10.09	ethyl 2,4 (?) octadienoate	nd	1.0	1.3	0.8	4.4	12.4	11.6	10.6	16.0	8.3	7.4	4.7
22.44	10.24	ethyl 4-decenoate	31.0	35.0	1.9	nd	nd	nd	nd	nd	nd	nd	nd	nd
22.58	10.29	ethyl 2,4 (?) octadienoate	nd	0.5	40.1	21.9	13.3	6.9	5.8	4.0	3.6	1.0	2.5	nd
22.63	10.31	unknown	12.2	3.2	2.7	3.9	5.7	12.3	15.4	16.5	31.9	15.9	17.1	12.0
22.91	10.42	methyl 2,4,6 (?) ctatrienoate	nd	nd	nd	nd	0.7	2.6	3.0	1.8	3.3	nd	1.1	nd
23.79	10.75	unknown	nd	0.7	1.7	1.5	0.8	5.5	6.9	6.2	9.8	3.7	4.9	0.7
23.94	10.80	α-farnesene (E,Z)	28.1	15.8	17.1	7.5	4.7	4.8	6.0	4.5	5.8	3.3	2.1	2.7
24.43	10.98	α-farnesene (E,E)	2433.6	1698.1	1830.0	733.3	418.9	427.6	442.2	296.0	369.9	242.3	232.0	151.7
25.13	11.23	methyl trans-2, cis-4-decadienoate	1.6	3.0	8.6	9.9	18.7	38.0	39.2	28.2	45.5	26.4	28.7	19.6
25.36	11.31	unknown	17.9	20.6	16.7	11.8	9.2	8.6	8.5	2.7	7.1	1.9	6.5	3.6
25.58	11.39	2-phenylethyl acetate	12.6	6.4	18.9	13.7	9.1	7.8	6.9	2.4	6.4	2.6	4.6	nd
25.83	11.47	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	nd	nd	nd	nd	0.7	1.3	1.8	nd
26.08	11.56	ethyl trans-2, cis-4-decadienoate	10.4	20.9	38.9	24.4	29.0	45.8	59.7	48.8	86.0	53.1	70.4	48.4
27.03	11.87	ethyl trans-2, trans-4 decadienoate	nd	12.5	6.8	4.1	4.3	9.5	11.5	10.5	16.8	10.4	11.2	7.3
27.77	12.11	unknown	nd	0.9	0.6	nd	nd	nd	nd	nd	nd	nd	nd	nd
27.81	12.12	unknown	3.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
29.55	12.66	unknown	nd	0.6	1.2	0.5	nd	nd	nd	nd	nd	nd	nd	nd
29.62	12.68	ethyl tetradecenoate	nd	nd	nd	nd	nd	2.7	5.1	2.7	5.3	3.1	3.0	1.6
32.24	13.42	unknown	nd	nd	nd	0.7	nd	1.8	1.5	2.4	5.6	2.8	3.8	3.6
31.07	13.10	unknown	nd	nd	nd	nd	nd	nd	nd	0.8	4.7	0.4	4.0	3.0
32.16	13.40	unknown	nd	nd	nd	nd	nd	nd	nd	nd	1.6	0.8	nd	nd
33.12	13.66	methyl cis-7 hexadecadecenoate	nd	nd	1.2	nd	0.4	2.5	1.1	2.3	2.8	nd	nd	nd
33.42	13.74	unknown	nd	nd	nd	nd	nd	2.0	nd	3.6	2.5	0.7	0.6	nd
Total			2862.0	2535.7	2733.4	1412.6	1131.9	1292.5	1336.1	1012.6	1311.5	891.5	929.9	684.8

Table A.6.10. Continued.

[a] Fruits were harvested at mature unriped stage (flesh firmness 69 N) and stored for 2 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min)

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.11. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 3 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.56	1.41	unknown	nd	nd	nd	nd	nd	0.4	2.3
5.62	1.49	methyl acetate	nd	nd	0.3	1.0	3.3	8.8	20.6
6.06	2.06	ethyl acetate	nd	nd	7.4	21.3	47.3	59.0	96.2
6.35	2.40	ethanol	nd	nd	1.3	3.8	3.8	6.7	25.4
6.96	3.09	propyl acetate	1.1	nd	2.6	1.8	5.7	6.5	7.4
8.55	4.37	butyl acetate	98.5	165.9	175.8	161.4	126.4	82.8	70.3
9.60	4.89	isoamyl acetate	nd	nd	nd	nd	1.2	1.6	0.8
9.74	4.95	1-butanol	nd	0.9	nd	nd	nd	nd	nd
10.77	5.40	pentyl acetate	2.8	8.0	10.3	9.8	7.4	6.4	5.4
11.96	5.86	1-methylpropyl butanoate + butyl butanoate	1.3	6.5	5.6	3.2	nd	nd	nd
12.34	6.00	ethyl hexanoate	nd	nd	2.6	3.7	5.0	6.1	5.8
12.47	6.07	1-pentanol	0.5	nd	nd	nd	nd	nd	nd
12.93	6.28	unknown	1.3	nd	nd	nd	nd	nd	nd
13.28	6.43	hexyl acetate	121.3	304.6	325.4	359.5	272.8	205.0	182.3
13.41	6.49	methyl heptanoate	8.4	nd	nd	nd	nd	nd	nd
14.77	7.05	ethyl heptanoate	3.9	nd	nd	nd	nd	nd	nd
15.03	7.15	1-hexanol	1.9	3.5	2.9	1.7	0.3	1.4	2.2
15.84	7.45	heptyl acetate	nd	1.7	2.5	4.0	3.0	3.0	1.1
16.21	7.59	methyl octanoate	nd	nd	nd	2.6	0.4	2.2	0.6
16.77	7.79	pentyl 2-methylpropanoate	nd	nd	nd	4.2	nd	nd	nd
16.95	7.85	butyl hexanoate	32.7	53.3	53.1	51.6	19.2	12.7	5.9
17.40	8.00	ethyl octanoate	nd	2.4	7.5	12.4	13.3	11.2	9.9
18.33	8.46	octyl acetate	nd	7.0	10.6	9.4	6.9	4.5	3.2
18.86	8.71	butyl 3-hexenoate	6.7	0.9	nd	nd	nd	nd	nd
19.45	8.98	4-octen-1-ol acetate	nd	3.2	1.6	2.3	0.5	0.5	nd
20.07	9.25	ethyl trans-2 octenoate	nd	nd	nd	nd	0.8	0.3	1.4
21.02	9.66	methyl decanoate	nd	nd	0.6	nd	nd	nd	nd
21.17	9.72	unknown	nd	nd	2.4	3.8	4.3	0.7	2.3
21.48	9.85	hexyl hexanoate + butyl octanoate	13.7	18.7	26.5	21.1	19.5	7.9	6.4
21.66	9.93	methyl 4-decenoate	nd	1.1	8.5	11.7	22.9	12.1	10.5
22.05	10.08	ethyl 2,4 (?) octadienoate	nd	1.7	11.9	15.7	25.8	10.1	8.9
22.24	10.16	unknown	nd	nd	nd	nd	3.2	nd	0.8
22.43	10.23	ethyl 4-decenoate	32.4	47.9	24.6	13.5	17.8	7.6	3.5
22.59	10.30	ethyl 2,4 (?) octadienoate	1.0	10.0	18.0	23.2	36.0	30.9	33.0
22.91	10.42	methyl 2,4,6 (?) octatrienoate	nd	nd	1.8	2.8	1.9	2.1	1.2
23.03	10.46	unknown	3.8	nd	nd	nd	nd	nd	nd
23.80	10.75	methyl 2,4,6 (?) octatrienoate	1.2	2.3	7.1	9.7	10.2	6.8	7.9
23.93	10.80	α-farnesene (E,Z)	25.2	13.9	8.4	6.9	7.5	6.2	6.4
24.42	10.98	α-farnesene (E,E)	2314.3	1220.5	649.9	509.3	427.4	305.1	226.5
25.12	11.23	methyl trans-2, cis-4-decadienoate	1.5	11.6	40.4	49.4	57.4	49.6	40.1
25.35	11.31	methyl cis-2, trans-4-decadienoate	8.2	11.2	10.3	7.3	7.5	6.1	2.1
25.57	11.38	2-phenylethyl acetate	7.7	17.1	19.6	13.5	12.5	10.1	7.8
25.84	11.48	ethyl cis-2, cis-4 decadienoate	nd	nd	1.3	nd	1.2	2.7	3.4
26.08	11.56	ethyl trans-2, cis-4-decadienoate	18.1	94.6	101.1	102.0	133.5	127.1	116.5
27.05	11.88	ethyl trans-2, trans-4 decadienoate	2.9	14.2	16.6	19.8	24.2	20.5	19.4

(Continued)

Table A.6.11. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
29.03	12.50	methyl tetradecenoate	nd	nd	nd	nd	nd	1.4	1.7
29.55	12.66	ethyl tetradecenoate	nd	0.4	0.6	0.9	4.4	1.7	5.1
30.36	12.89	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	nd	3.3	5.5	4.8	5.7
31.09	13.10	unknown	nd	0.8	nd	nd	4.9	5.1	5.0
33.12	13.66	methyl cis-7 hexadecadecenoate	nd	nd	nd	1.9	nd	nd	nd
37.04	14.64	unknown	nd	nd	2.9	3.4	0.8	2.0	1.4
37.98	14.86	unknown	nd	3.0	1.0	nd	nd	nd	nd
	Total		2710.3	2027.1	1563.1	1472.8	1345.6	1039.5	956.1

[a] Fruits were harvested at mature unriped stage (flesh firmness 69 N) and stored for 3 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min)

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.12. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.66	1.55	methyl acetate	nd	nd	0.2	0.2	0.8	2.0	2.0
6.04	2.03	ethyl acetate	nd	0.3	3.0	5.7	10.8	15.6	20.8
6.32	2.37	ethanol	nd	0.3	1.2	2.2	4.3	5.7	7.7
6.94	3.06	propyl acetate	0.2	nd	0.2	0.8	1.1	1.0	1.6
8.55	4.37	butyl acetate	25.5	43.8	37.9	36.2	30.0	22.4	19.0
9.74	4.95	1-butanol	nd	0.7	0.1	0.2	0.4	nd	0.4
10.77	5.40	pentyl acetate	0.8	2.5	2.3	1.5	2.0	1.2	0.9
11.94	5.86	1-methylpropyl butanoate + butyl butanoate	nd	1.7	0.8	0.9	nd	nd	nd
12.28	5.98	ethyl hexanoate	nd	0.1	1.5	0.8	2.5	1.9	2.1
13.28	6.43	hexyl acetate	31.3	117.2	86.1	98.1	88.1	63.5	56.8
15.00	7.14	1-hexanol	nd	0.4	0.3	0.7	nd	nd	0.2
15.83	7.45	heptyl acetate	nd	0.6	0.4	1.4	0.7	1.0	1.1
16.24	7.60	methyl octanoate	nd	nd	nd	0.3	0.2	nd	0.2
16.95	7.85	butyl hexanoate	3.9	17.5	13.5	6.3	3.7	3.5	6.9
17.39	8.00	ethyl octanoate	nd	1.3	2.0	3.7	4.9	3.3	3.1
18.32	8.45	octyl acetate	nd	1.7	1.4	1.9	2.0	0.6	0.9
18.85	8.70	butyl 3-hexenoate	1.2	nd	nd	nd	nd	nd	nd
19.44	8.97	4-octen-1-ol acetate	nd	0.8	0.5	0.8	nd	nd	nd
20.06	9.25	ethyl trans-2 octenoate	nd	nd	nd	0.3	0.9	0.2	0.4
21.01	9.66	methyl decanoate	nd	nd	nd	nd	0.1	nd	nd
21.04	9.67	unknown	nd	nd	nd	nd	0.3	0.3	0.2
21.48	9.85	hexyl hexanoate + butyl octanoate	2.5	7.1	5.0	5.8	4.0	2.4	3.1
21.65	9.92	methyl 4-decenoate	nd	0.3	1.3	2.4	3.3	2.5	2.6
22.06	10.09	ethyl 2,4 (?) octadienoate	nd	1.5	2.1	3.3	4.1	2.7	1.4
22.43	10.23	ethyl 4-decenoate	13.8	17.0	8.6	7.8	6.5	1.5	3.0
22.61	10.30	ethyl 2,4 (?) octadienoate	0.9	4.1	4.0	7.3	11.4	8.7	9.7
22.89	10.41	methyl 2,4,6 (?) octatrienoate	nd	nd	nd	0.2	0.2	0.1	nd
23.78	10.75	methyl 2,4,6 (?) octatrienoate	0.3	1.3	1.4	2.0	2.8	1.7	1.5
23.93	10.80	α-farnesene (E,Z)	7.9	6.1	3.7	3.3	4.0	2.8	3.9
24.41	10.98	α-farnesene (E,E)	574.5	448.8	246.5	218.7	215.0	127.7	167.2
25.11	11.23	methyl trans-2, cis-4-decadienoate	1.7	6.2	9.0	15.3	23.5	17.8	23.9
25.34	11.30	methyl cis-2, trans-4-decadienoate	0.8	2.0	1.4	1.0	0.9	0.5	1.2
25.57	11.38	2-phenylethyl acetate	3.3	5.3	4.7	5.5	6.1	3.9	3.5
25.83	11.47	ethyl cis-2, cis-4 decadienoate	nd	0.4	0.4	0.4	1.6	1.7	3.0
26.07	11.56	ethyl trans-2, cis-4-decadienoate	11.3	40.2	39.5	54.3	85.1	65.8	98.9
27.03	11.87	ethyl trans-2, trans-4 decadienoate	1.6	7.1	6.4	8.8	11.6	9.1	10.9
29.05	12.51	methyl tetradecenoate	nd	nd	0.4	0.2	0.2	0.3	1.6
29.54	12.65	ethyl tetradecenoate	nd	0.4	nd	0.1	0.5	0.9	0.8
30.35	12.89	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	0.1	0.7	1.4	1.5	2.5
31.08	13.10	unknown	nd	nd	nd	0.3	1.8	1.9	3.1
32.14	13.39	unknown	nd	nd	nd	nd	0.4	0.4	0.5
33.09	13.65	methyl cis-7 hexadecadecenoate	nd	nd	nd	0.1	0.3	nd	0.5
Total			681.5	736.6	485.7	499.3	537.4	376.0	466.9

(Continued)

Table A.6.12. Continued.

[a] Fruits were harvested at mature unriped stage (flesh firmness 69 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min)

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.13. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 5 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.72	1.63	methyl acetate	nd	nd	0.9	0.5	1.2	2.4	1.4
6.08	2.08	ethyl acetate	2.6	4.0	5.2	7.8	12.5	17.4	17.0
6.38	2.44	ethanol	0.6	2.0	2.1	3.3	5.0	6.2	6.0
6.98	3.11	propyl acetate	0.7	0.3	0.6	1.0	1.3	0.9	1.4
8.56	4.37	butyl acetate	31.1	44.5	32.9	30.6	25.2	19.2	15.0
9.61	4.89	isoamyl acetate	0.8	0.5	0.3	0.3	0.7	nd	0.5
9.78	4.97	1-butanol	0.8	0.6	0.9	0.3	0.7	nd	nd
10.78	5.40	pentyl acetate	0.7	3.0	2.4	2.2	1.9	1.9	1.8
11.99	5.88	1-methylpropyl butanoate + butyl butanoate	0.8	2.0	1.0	nd	0.3	nd	0.2
12.35	6.01	ethyl hexanoate	nd	1.3	1.5	1.5	2.2	1.8	1.5
13.29	6.44	hexyl acetate	35.2	137.8	81.5	75.9	63.6	52.6	43.0
14.77	7.05	ethyl heptanoate	0.9	nd	nd	nd	nd	nd	nd
15.02	7.15	1-hexanol	0.8	1.9	nd	nd	0.7	0.9	1.2
15.85	7.46	heptyl acetate	nd	1.3	1.1	1.0	1.2	1.3	0.9
16.27	7.61	methyl octanoate	nd	nd	0.3	0.5	0.5	nd	nd
16.96	7.85	butyl hexanoate	3.6	15.3	9.6	6.5	4.3	3.2	2.9
17.41	8.00	ethyl octanoate	nd	1.7	2.3	2.5	2.5	1.7	1.6
18.34	8.46	octyl acetate	nd	nd	1.4	1.4	nd	1.0	0.7
18.87	8.71	butyl 3-hexenoate	0.9	1.3	0.4	nd	nd	nd	nd
19.47	8.99	4-octen-1-ol acetate	nd	1.2	nd	0.7	0.5	nd	0.4
2nd7	9.25	ethyl trans-2 octenoate	nd	0.6	0.6	0.7	0.5	0.8	nd
21.08	9.69	methyl decanoate	nd	nd	nd	nd	0.4	nd	nd
21.49	9.85	hexyl hexanoate + butyl octanoate	1.3	4.2	2.8	2.7	1.7	1.4	1.1
21.70	9.94	methyl 4-decenoate	nd	1.4	1.5	2.1	2.0	1.8	1.3
22.08	10.10	ethyl 2,4 (?) octadienoate	nd	1.6	1.7	2.6	1.6	1.5	1.0
22.44	10.24	ethyl 4-decenoate	7.4	15.4	8.1	6.1	2.9	2.2	1.4
22.61	10.30	ethyl 2,4 (?) octadienoate	1.4	10.2	7.8	8.0	6.1	6.8	5.1
22.92	10.42	methyl 2,4,6 (?) octatrienoate	nd	nd	nd	0.7	0.4	0.3	0.1
23.80	10.75	methyl 2,4,6 (?) octatrienoate	0.8	2.8	2.0	2.3	1.5	1.7	1.2
23.94	10.80	α-farnesene (E,Z)	5.1	6.1	2.5	2.6	1.7	1.8	1.5
24.41	10.98	α-farnesene (E,E)	305.6	339.1	134.4	125.1	93.3	74.1	64.8
25.13	11.23	methyl trans-2, cis-4-decadienoate	2.3	7.8	9.0	13.2	10.9	13.0	8.1
25.36	11.31	methyl cis-2, trans-4-decadienoate	0.9	2.0	1.2	1.3	0.9	0.5	0.6
25.58	11.39	2-phenylethyl acetate	1.5	5.5	4.9	4.0	1.9	1.6	1.0
25.82	11.47	ethyl cis-2, cis-4 decadienoate	0.5	1.1	1.1	1.4	1.2	1.8	1.3
26.08	11.56	ethyl trans-2, cis-4-decadienoate	14.5	55.3	54.6	62.9	44.3	56.3	50.0
27.05	11.88	ethyl trans-2, trans-4 decadienoate	2.2	9.9	8.6	9.5	7.2	7.2	7.2
27.78	12.11	unknown	nd	0.3	0.5	0.3	0.3	0.7	1.3
27.83	12.13	unknown	1.6	1.0	0.9	1.0	0.8	1.4	1.2
29.65	12.68	ethyl tetradecenoate	nd	nd	nd	0.5	0.8	0.7	0.3
30.18	12.84	unknown	nd	nd	nd	nd	nd	0.5	0.6
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	0.5	0.5	0.8	1.2	1.3
31.14	13.12	unknown	1.8	nd	0.7	0.7	0.9	1.5	1.7
32.16	13.40	unknown	nd	nd	nd	nd	0.3	0.4	0.3
33.12	13.66	methyl cis-7 hexadecadecenoate	nd	nd	nd	nd	0.3	nd	0.2

(Continued)

Table A.6.13. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
33.42	13.74	unknown	nd	nd	nd	0.3	0.3	nd	nd
34.83	14.10	unknown	0.4	nd	nd	nd	nd	nd	1.7
	Total		426.7	682.8	387.7	384.7	307.1	289.6	249.7

[a] Fruits were harvested at mature unriped stage (flesh firmness 69 N) and stored for 5 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min)

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.14. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 6 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	2	4	6	8	10
5.25	0.99	acetaldehyde	nd	nd	nd	nd	0.5	0.9
5.69	1.58	methyl acetate	nd	0.8	0.6	nd	1.6	2.7
6.09	2.09	ethyl acetate	12.8	15.1	13.9	12.0	20.9	27.8
6.37	2.43	ethanol	0.7	8.5	4.9	4.3	8.6	11.9
6.99	3.11	propyl acetate	2.3	0.9	1.3	1.1	1.1	1.0
7.54	3.68	2-methylpropyl acetate	1.1	0.2	0.2	nd	nd	nd
7.88	4.01	ethyl butanoate	1.5	0.6	0.7	0.5	0.6	0.4
8.57	4.38	butyl acetate	71.3	21.0	43.2	33.4	36.8	33.2
9.57	4.87	isoamyl acetate	2.4	0.4	0.7	nd	0.6	nd
9.81	4.98	1-butanol	4.8	0.6	1.1	0.6	1.8	0.8
10.79	5.41	pentyl acetate	6.1	2.5	3.5	2.7	6.3	3.5
11.99	5.88	1-methylpropyl butanoate + butyl butanoate	3.5	nd	0.8	0.7	0.4	nd
12.35	6.01	ethyl hexanoate	2.4	1.2	2.5	2.1	2.4	2.1
13.30	6.44	hexyl acetate	106.8	58.3	118.4	100.6	111.0	95.8
14.77	7.05	ethyl heptanoate	0.6	nd	nd	nd	nd	nd
15.05	7.16	1-hexanol	0.9	0.9	1.1	1.2	1.3	1.6
15.86	7.46	heptyl acetate	0.5	1.3	nd	1.7	1.9	nd
16.96	7.85	butyl hexanoate	6.0	3.0	6.8	4.8	4.0	3.8
17.40	8.00	ethyl octanoate	1.0	nd	1.9	1.6	1.7	1.4
18.33	8.46	octyl acetate	1.1	0.7	1.2	1.1	1.4	1.3
18.86	8.71	butyl 3-hexenoate	1.5	nd	nd	nd	0.6	nd
19.46	8.98	4-octen-1-ol acetate	nd	nd	0.5	nd	0.7	nd
20.06	9.25	ethyl trans-2 octenoate	0.7	0.4	nd	0.6	0.6	0.6
21.19	9.73	unknown	0.8	nd	nd	nd	nd	nd
21.49	9.86	hexyl hexanoate + butyl octanoate	2.9	nd	2.8	2.1	1.8	1.6
21.67	9.93	methyl 4-decenoate	0.3	0.5	0.9	0.9	1.1	0.8
21.95	10.04	ethyl decanoate	nd	nd	nd	0.2	0.3	0.5
22.06	10.09	ethyl 2,4 (?) octadienoate	nd	0.5	1.1	0.8	1.3	0.9
22.44	10.24	ethyl 4-decenoate	11.5	2.0	4.5	3.5	2.9	2.3
22.61	10.30	ethyl 2,4 (?) octadienoate	4.3	2.8	4.6	4.2	5.2	4.8
22.92	10.42	methyl 2,4,6 (?) octatrienoate	nd	nd	nd	nd	0.3	nd
23.81	10.76	methyl 2,4,6 (?) octatrienoate	1.5	0.5	1.2	1.1	1.2	1.1
23.94	10.80	α-farnesene (E,Z)	17.4	0.6	2.3	1.5	1.4	nd
24.42	10.98	α-farnesene (E,E)	620.8	24.8	78.1	45.5	42.5	29.1
25.13	11.23	methyl trans-2, cis-4-decadienoate	15.1	8.2	7.5	7.1	9.1	8.5
25.36	11.31	methyl cis-2, trans-4-decadienoate	2.6	0.1	1.9	1.7	1.5	1.2
25.57	11.38	2-phenylethyl acetate	3.8	0.8	2.2	2.0	1.6	1.2
25.85	11.48	ethyl cis-2, cis-4 decadienoate	3.7	1.7	1.3	1.3	2.4	2.5
26.08	11.56	ethyl trans-2, cis-4-decadienoate	110.9	47.3	52.8	43.9	58.9	64.2
27.04	11.88	ethyl trans-2, trans-4 decadienoate	12.0	5.4	7.5	5.6	8.0	5.2
27.33	11.97	unknown	0.9	nd	0.3	nd	0.4	nd
27.83	12.13	unknown	3.8	1.0	1.0	nd	0.7	0.9
29.07	12.51	methyl tetradecenoate	1.4	1.2	1.8	1.7	1.8	1.6
29.58	12.66	ethyl tetradecenoate	nd	0.2	0.3	0.5	0.3	nd
30.36	12.89	ethyl cis-5, cis-8 tetradecadienoate	0.5	0.7	nd	0.5	1.1	0.7

(Continued)

Table A.6.14. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	2	4	6	8	10
31.09	13.10	unknown	0.7	0.8	0.7	0.8	1.6	0.7
33.58	13.78	unknown	nd	nd	nd	2.0	nd	nd
33.88	13.86	unknown	nd	nd	nd	1.2	nd	nd
Total			1043.0	215.6	375.8	297.1	350.2	316.3

[a] Fruits were harvested at mature unriped stage (flesh firmness 69 N) and stored for 6 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.15. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at late stage of maturity (M3) after storage at 0°C for 2 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C							
			0	1	2	4	6	8	10	12
5.64	1.52	methyl acetate	nd	nd	nd	nd	nd	1.0	1.7	4.2
6.05	2.04	ethyl acetate	nd	1.0	nd	0.9	8.6	21.9	26.4	42.7
6.33	2.38	ethanol	nd	nd	nd	nd	2.7	6.5	6.6	11.8
6.95	3.08	propyl acetate	nd	nd	nd	1.1	2.5	6.2	5.4	5.1
8.54	4.36	butyl acetate	76.4	94.2	120.1	128.7	115.2	112.9	79.2	68.8
9.75	4.95	1-butanol	nd	0.6	2.2	1.1	nd	nd	nd	nd
10.76	5.39	pentyl acetate	2.9	7.5	7.7	4.3	6.6	7.0	5.2	4.9
11.90	5.84	1-methylpropyl butanoate	nd	nd	nd	nd	0.3	1.5	0.3	nd
11.96	5.86	butyl butanoate	7.0	9.6	10.4	6.3	2.4	nd	nd	nd
12.32	6.00	ethyl hexanoate	nd	nd	nd	nd	3.7	4.9	4.5	4.2
13.28	6.43	hexyl acetate	119.9	284.9	264.4	244.6	228.7	260.3	191.3	169.3
14.78	7.05	ethyl heptanoate	2.4	nd	nd	nd	nd	nd	nd	nd
15.02	7.15	1-hexanol	1.4	1.4	3.6	1.1	nd	1.8	0.5	1.6
15.83	7.45	heptyl acetate	nd	nd	nd	1.4	0.6	2.5	2.4	0.6
16.23	7.60	methyl octanoate	nd	nd	nd	nd	1.4	2.2	1.7	nd
16.95	7.85	butyl hexanoate	43.0	59.1	77.7	65.7	30.6	20.9	3.4	2.0
17.37	7.99	ethyl octanoate	nd	nd	nd	2.0	13.9	14.9	9.8	7.1
18.33	8.46	octyl acetate	0.9	3.4	4.2	7.1	8.0	7.1	4.0	2.7
18.86	8.71	butyl 3-hexenoate	8.1	4.1	3.9	1.5	0.9	1.5	nd	nd
19.46	8.98	4-octen-1-ol acetate	nd	1.8	2.6	1.5	1.0	1.6	1.2	nd
19.93	9.19	1-octanol	nd	nd	nd	1.7	1.4	nd	0.2	nd
21.04	9.67	methyl decanoate	nd	nd	nd	2.0	4.0	3.6	2.1	nd
21.50	9.86	hexyl hexanoate + butyl octanoate	15.3	13.5	21.7	18.7	16.9	14.1	5.2	3.5
21.66	9.93	methyl 4-decenoate	nd	nd	nd	0.3	5.3	8.6	6.7	4.3
22.07	10.09	ethyl 2,4 octadienoate (?)	nd	nd	1.4	6.5	18.5	18.3	10.3	6.5
22.44	10.24	ethyl 4-decenoate	19.3	15.4	16.5	9.8	4.1	3.2	0.4	0.8
22.58	10.29	ethyl 2,4 octadienoate (?)	nd	0.3	2.0	2.1	9.4	15.3	13.8	10.4

(Continued)

Table A.6.15. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C							
			0	1	2	4	6	8	10	12
22.90	10.41	methyl 2,4,6 octatrienoate	nd	nd	nd	nd	2.4	2.0	1.0	nd
23.80	10.75	methyl 2,4,6 octatrienoate	1.0	nd	0.7	2.2	4.2	6.2	4.8	2.9
23.94	10.81	α-farnesene (E,Z)	16.5	6.9	7.5	4.4	3.1	4.5	3.0	1.6
24.43	10.98	α-farnesene (E,E)	1741.4	895.8	804.5	496.9	291.9	294.1	156.5	142.3
25.13	11.23	methyl trans-2, cis-4-decadienoate	0.8	nd	nd	14.8	25.5	29.1	19.2	17.4
25.36	11.31	methyl cis-2, trans-4-decadienoate	8.5	5.2	2.5	4.8	2.9	1.3	nd	2.2
25.58	11.39	2-phenylethyl acetate	9.1	4.7	5.8	5.0	3.6	3.5	nd	nd
25.82	11.47	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	nd	1.3	nd	nd
26.08	11.56	ethyl trans-2, cis-4-decadienoate	15.0	15.0	22.4	30.8	48.7	59.0	43.5	46.2
26.30	11.63	unknown	nd	nd	nd	nd	nd	nd	0.6	1.6
27.04	11.88	ethyl trans-2, trans-4 decadienoate	1.8	1.8	3.7	6.2	11.7	12.3	7.9	7.5
29.03	12.50	methyl tetradecenoate	nd	nd	nd	nd	nd	0.8	nd	1.2
29.63	12.68	ethyl tetradecenoate	nd	nd	nd	nd	2.7	3.4	2.3	2.5
30.38	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	nd	nd	3.3	1.9	3.6	3.5
31.07	13.10	unknown	nd	nd	nd	nd	0.8	3.8	1.6	4.9
32.16	13.40	unknown	nd	nd	nd	nd	nd	0.8	0.7	1.1
33.10	13.65	methyl cis-7 hexadecadecenoate	nd	nd	nd	nd	0.6	0.6	nd	1.2
33.41	13.74	unknown	nd	nd	nd	nd	1.9	1.8	1.1	0.7
37.04	14.64	unknown	nd	nd	nd	nd	3.2	1.3	1.1	nd
Total			2090.7	1426.4	1385.5	1073.6	893.2	965.7	629.1	587.3

[a] Fruits were harvested at mature unriped stage (flesh firmness 63 N) and stored for 2 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 μg propyl tiglate used as internal standard (response factor = 1) and expressed in μg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 μg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2μm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.16. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at late stage of maturity (M3) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E°	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.60	1.47	methyl acetate	nd	nd	nd	0.1	nd	nd	1.5
6.05	2.05	ethyl acetate	nd	0.8	2.2	6.4	7.9	10.5	13.5
6.37	2.43	ethanol	nd	0.4	1.3	3.0	3.4	4.4	5.9
6.95	3.07	propyl acetate	0.6	0.6	0.9	1.0	1.2	1.3	1.4
7.53	3.67	2-methyl propyl acetate	nd	nd	0.5	nd	0.2	0.1	0.1
7.86	3.99	ethyl butanoate	nd	nd	0.4	nd	0.4	nd	0.3
8.53	4.36	butyl acetate	26.3	33.0	32.5	29.5	23.2	18.6	16.5
9.57	4.87	isoamyl acetate	nd	nd	0.2	0.4	0.4	0.4	0.7
9.76	4.96	1-butanol	0.5	nd	0.6	0.4	nd	nd	nd
10.76	5.39	pentyl acetate	1.2	2.5	2.3	2.7	1.9	1.7	1.5
11.95	5.86	1-methylpropyl butanoate + butyl butanoate	1.4	1.6	1.2	1.1	0.7	0.5	0.4
12.34	6.00	ethyl hexanoate	nd	0.5	1.2	2.1	2.1	1.8	1.7
13.27	6.43	hexyl acetate	36.4	90.3	74.5	78.5	57.7	51.1	43.0
14.76	7.05	ethyl heptanoate	1.0	nd	0.3	nd	nd	nd	nd
15.01	7.14	1-hexanol	0.8	1.1	1.0	0.6	0.7	0.6	0.5
15.87	7.46	heptyl acetate	nd	0.7	0.8	1.0	1.1	1.0	0.8
16.19	7.58	methyl octanoate	nd	nd	nd	0.6	0.3	0.4	nd
16.95	7.85	butyl hexanoate	4.7	12.9	10.5	5.0	4.0	4.3	3.4
17.40	8.00	ethyl octanoate	nd	nd	2.7	3.7	2.8	2.9	2.5
18.34	8.46	octyl acetate	nd	1.2	1.4	1.1	0.9	0.9	0.7
18.84	8.70	butyl 3-hexenoate	1.2	0.7	0.8	nd	nd	0.6	nd
19.46	8.98	4-octen-1-ol acetate	nd	0.8	0.9	0.5	0.3	nd	0.3
2nd8	9.26	ethyl trans-2 octenoate	nd	nd	0.5	0.5	0.3	0.4	0.4
21.06	9.68	methyl decanoate	nd	nd	0.5	0.5	0.4	0.3	0.4
21.48	9.85	hexyl hexanoate + butyl octanoate	2.0	4.3	3.7	2.1	1.4	1.3	1.2
21.68	9.93	methyl 4-decenoate	nd	0.7	1.7	2.4	1.9	1.9	1.2
22.07	10.09	ethyl 2,4 (?) octadienoate	nd	2.1	3.1	3.2	2.3	2.4	2.1
22.43	10.23	ethyl 4-decenoate	8.3	8.3	6.0	2.9	2.1	1.8	1.0
22.60	10.30	ethyl 2,4 (?) octadienoate	1.1	4.7	6.5	6.5	5.3	7.0	4.5
22.91	10.42	methyl 2,4,6 octatrienoate	nd	nd	0.5	0.8	0.5	0.4	0.4
23.80	10.75	methyl 2,4,6 octatrienoate	0.5	1.4	2.3	1.9	1.8	1.9	1.1
23.92	10.80	α-farnesene (E,Z)	8.7	4.3	3.1	1.4	1.5	1.9	1.0
24.42	10.98	α-farnesene (E,E)	602.8	285.8	179.2	87.3	68.8	71.5	40.6
25.12	11.23	methyl trans-2, cis-4-decadienoate	1.9	4.1	8.0	11.5	10.9	15.5	8.8
25.35	11.31	methyl cis-2, trans-4-decadienoate	0.9	1.0	1.0	0.4	0.6	1.0	0.9
25.56	11.38	2-phenylethyl acetate	3.6	3.1	3.4	1.6	3.0	1.7	0.9
25.83	11.47	ethyl cis-2, cis-4 decadienoate	0.4	0.6	0.7	1.1	1.3	1.9	1.5
26.07	11.55	ethyl trans-2, cis-4-decadienoate	23.8	39.7	44.0	34.7	39.7	61.6	34.3
26.86	11.82	ethyl trans-2, trans-4, cis-7 decatrienoate	nd	nd	0.2	nd	nd	nd	1.5
27.04	11.87	ethyl trans-2, trans-4 decadienoate	3.1	6.7	7.9	6.2	6.6	8.8	5.6
27.30	11.96	unknown	nd	nd	nd	nd	nd	7.3	0.4

(Continued)

Table A.6.16. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
27.82	12.13	unknown	1.7	0.5	nd	0.3	nd	0.7	3.3
29.08	12.52	methyl tetradecenoate	0.2	0.5	0.5	nd	nd	1.1	0.9
29.77	12.72	unknown	0.3	0.3	nd	nd	0.8	0.9	0.7
30.18	12.84	unknown	nd	nd	nd	nd	0.3	0.4	nd
30.38	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	0.3	0.4	1.1	1.0	1.5	8.2
31.09	13.10	unknown	nd	0.5	0.4	1.5	1.2	2.3	1.3
33.14	13.66	methyl cis-7 hexadecadecenoate	nd	0.2	0.2	0.6	0.5	0.3	0.5
Total			733.2	516.4	409.9	306.0	266.2	297.0	217.8

[a] Fruits were harvested at mature unriped stage (flesh firmness 63 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.17. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at late stage of maturity (M3) after storage at 0°C for 5 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.69	1.58	methyl acetate	nd	nd	0.5	0.4	nd	nd	0.3
6.08	2.08	ethyl acetate	7.9	4.8	11.2	13.9	13.8	11.9	15.6
6.37	2.42	ethanol	1.0	2.0	5.6	5.6	6.5	5.4	9.3
6.97	3.10	propyl acetate	1.5	1.2	1.3	1.4	0.9	nd	1.0
7.49	3.63	2-methylpropyl acetate	nd	0.4	0.3	nd	nd	nd	nd
7.89	4.01	ethyl butanoate	nd	0.8	nd	1.2	nd	nd	0.7
8.56	4.37	butyl acetate	44.2	33.3	36.6	32.0	24.3	19.2	15.9
9.60	4.88	isoamyl acetate	1.0	0.5	nd	0.6	0.6	0.2	0.3
9.76	4.96	1-butanol	nd	1.0	1.1	0.7	nd	nd	0.7
10.78	5.40	pentyl acetate	1.6	2.8	2.5	2.4	2.5	2.3	2.5
11.99	5.88	1-methylpropyl butanoate + butyl butanoate	1.1	nd	nd	nd	nd	nd	nd
12.32	6.00	ethyl hexanoate	nd	0.7	1.4	1.5	1.2	1.2	1.1
12.50	6.08	1-pentanol	0.6	nd	nd	nd	nd	nd	nd
13.28	6.43	hexyl acetate	65.4	124.3	109.3	90.1	73.9	60.8	51.0
14.76	7.04	ethyl heptanoate	0.9	nd	nd	nd	nd	nd	nd
15.02	7.15	1-hexanol	0.8	0.9	1.1	1.1	0.9	0.6	nd
15.85	7.46	heptyl acetate	nd	1.3	1.6	nd	1.4	1.3	nd
16.95	7.85	butyl hexanoate	3.1	5.5	4.6	1.7	1.8	0.8	1.1
17.41	8.00	ethyl octanoate	nd	1.7	2.1	1.1	1.5	1.2	1.0
18.33	8.46	octyl acetate	0.6	1.7	1.7	0.9	1.8	1.2	1.1
18.86	8.71	butyl 3-hexenoate	1.2	0.9	0.7	nd	nd	nd	nd
19.46	8.98	4-octen-1-ol acetate	nd	1.0	0.9	nd	0.6	nd	0.4
20.08	9.26	ethyl trans-2 octenoate	nd	0.6	0.8	0.7	0.7	0.7	nd
21.18	9.73	unknown	0.6	nd	nd	nd	nd	nd	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	2.0	2.6	2.3	1.0	0.7	0.6	0.7
21.67	9.93	methyl 4-decenoate	nd	1.1	1.2	0.8	0.7	0.6	0.6
22.07	10.09	ethyl 2,4 (?) octadienoate	nd	1.0	1.4	nd	1.3	0.5	0.4
22.43	10.23	ethyl 4-decenoate	11.0	8.5	6.4	2.7	3.1	1.4	1.5
22.60	10.30	ethyl 2,4 (?) octadienoate	2.6	9.0	8.4	2.7	3.9	3.5	2.7
22.89	10.41	methyl 2,4,6 octatrienoate	nd	nd	0.4	nd	nd	nd	nd
23.79	10.75	methyl 2,4,6 octatrienoate	1.4	2.3	2.5	0.5	1.0	0.7	0.6
23.92	10.80	α-farnesene (E,Z)	9.1	4.6	3.8	1.0	1.8	0.9	1.2
24.40	10.97	α-farnesene (E,E)	473.8	229.0	149.4	38.7	58.8	29.6	31.1
25.11	11.22	methyl trans-2, cis-4-decadienoate	5.7	5.9	10.5	3.4	9.0	8.6	6.9
25.34	11.31	methyl cis-2, trans-4-decadienoate	2.3	2.3	2.3	0.9	1.4	1.1	1.1
25.56	11.38	2-phenylethyl acetate	3.2	3.7	5.2	1.6	2.7	1.0	1.1
25.82	11.47	ethyl cis-2, cis-4 decadienoate	0.9	1.0	2.3	nd	2.3	1.9	2.2
26.07	11.55	ethyl trans-2, cis-4-decadienoate	31.7	40.6	69.3	28.3	56.5	47.1	48.3
27.03	11.87	ethyl trans-2, trans-4 decadienoate	4.3	7.5	10.9	3.5	6.5	4.9	3.5
27.47	12.01	unknown	nd	nd	0.6	nd	0.4	0.4	nd
27.78	12.11	unknown	nd	0.8	1.4	0.5	0.3	0.8	0.6
29.06	12.51	methyl tetradecenoate	nd	0.8	1.8	nd	1.1	1.1	1.0
29.56	12.66	ethyl tetradecenoate	nd	nd	0.4	nd	0.6	nd	nd

(Continued)

Table A.6.17. Continued.

Rt[b] (min.)	I_E^c	Compounds	Days at 20°C 0	2	4	6	8	10	12
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	0.6	nd	0.8	3.5	0.6
31.09	13.10	unknown	nd	0.6	1.1	0.2	1.3	4.5	1.1
33.11	13.66	methyl cis-7 hexadecadecenoate	nd	nd	0.4	nd	nd	nd	nd
33.59	13.78	unknown	nd	nd	0.1	0.7	nd	nd	nd
37.04	14.64	unknown	nd	nd	nd	nd	nd	1.5	nd
Total			679.8	506.8	466.0	241.8	286.4	221.2	207.4

[a] Fruits were harvested at mature unriped stage (flesh firmness 63 N) and stored for 5 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.18. Changes in volatile compounds during ripening of 'Packham's Triumph' pear harvested at late stage of maturity (M3) after storage at 0°C for 6 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	2	4	6	8	10
5.70	1.60	methyl acetate	0.7	nd	nd	nd	nd	1.3
6.07	2.07	ethyl acetate	6.4	4.7	4.8	4.7	11.2	13.8
6.36	2.42	ethanol	0.7	1.8	1.4	0.6	5.8	7.6
6.92	3.04	propyl acetate	nd	0.6	0.6	nd	0.6	0.8
8.55	4.37	butyl acetate	34.5	30.4	29.9	30.2	24.4	19.4
9.61	4.89	isoamyl acetate	0.3	0.3	0.7	0.8	0.9	nd
10.78	5.40	pentyl acetate	2.0	2.7	2.2	3.6	2.9	2.6
11.91	5.85	1-methylpropyl butanoate + butyl butanoate	nd	1.1	0.8	0.2	nd	nd
12.34	6.00	ethyl hexanoate	0.9	1.4	2.0	2.0	1.8	1.1
13.29	6.43	hexyl acetate	53.2	95.2	87.5	98.2	72.9	53.8
14.79	7.06	ethyl heptanoate	0.8	1.2	nd	1.2	0.8	0.8
15.84	7.45	heptyl acetate	0.5	nd	1.6	1.8	1.7	1.3
16.91	7.84	butyl hexanoate	1.1	10.8	8.9	5.8	3.5	nd
17.40	8.00	ethyl octanoate	0.8	1.7	1.8	1.8	1.1	0.8
18.30	8.44	octyl acetate	nd	1.4	1.3	0.5	1.0	0.6
18.85	8.71	butyl 3-hexenoate	0.6	nd	nd	nd	nd	nd
19.41	8.96	4-octen-1-ol acetate	nd	0.7	nd	nd	nd	nd
20.03	9.24	ethyl trans-2 octenoate	nd	0.3	0.4	nd	nd	nd
21.19	9.73	unknown	0.4	nd	nd	nd	nd	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	1.6	3.0	3.5	1.6	1.7	nd
21.67	9.93	methyl 4-decenoate	0.5	1.4	2.5	0.6	1.4	0.5
22.04	10.08	ethyl 2,4 (?) octadienoate	nd	1.2	1.9	0.4	1.0	0.2
22.43	10.23	ethyl 4-decenoate	7.1	6.5	5.1	2.7	3.0	1.9
22.61	10.30	ethyl 2,4 (?) octadienoate	2.5	9.3	9.2	5.6	5.5	2.7
22.89	10.41	methyl 2,4,6 octatrienoate	nd	0.2	0.5	nd	nd	nd
23.76	10.74	methyl 2,4,6 octatrienoate	nd	2.0	1.7	0.4	1.1	0.6
23.93	10.80	α-farnesene (E,Z)	10.5	3.9	2.6	1.1	1.8	1.0
24.40	10.97	α-farnesene (E,E)	452.6	164.0	118.1	78.2	53.4	21.3
25.12	11.23	methyl trans-2, cis-4-decadienoate	6.9	6.0	12.8	14.4	15.0	8.2
25.35	11.31	methyl cis-2, trans-4-decadienoate	1.2	1.3	0.8	0.3	1.0	0.9
25.56	11.38	2-phenylethyl acetate	2.2	3.0	2.5	1.4	2.2	1.5
25.84	11.48	ethyl cis-2, cis-4 decadienoate	2.0	1.2	1.4	1.1	2.5	2.3
26.08	11.56	ethyl trans-2, cis-4-decadienoate	53.4	48.7	64.2	80.7	71.1	50.8
27.04	11.87	ethyl trans-2, trans-4 decadienoate	5.3	6.7	8.2	6.9	6.9	3.8
27.31	11.96	unknown	0.9	0.7	0.2	nd	nd	0.4
27.82	12.12	unknown	1.6	0.9	0.8	0.8	1.2	nd
29.07	12.51	methyl tetradecenoate	1.0	1.1	1.1	1.9	1.8	1.7
29.63	12.68	ethyl tetradecenoate	nd	0.3	nd	0.8	0.5	0.3
30.38	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	0.6	0.6	0.8	0.7
31.07	13.10	unknown	0.5	0.4	1.2	1.1	1.2	1.2
33.39	13.73	unknown	nd	0.2	0.3	nd	nd	nd

(Continued)

Table A.6.18. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	2	4	6	8	10
33.64	13.80	unknown	nd	nd	0.7	0.4	0.8	nd
Total			652.7	416.2	384.1	352.2	302.7	203.8

[a] Fruits were harvested at mature unriped stage (flesh firmness 63 N) and stored for 6 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.19. Changes in volatile compounds during ripening of 'Anjou' pear harvested at early stage of maturity (M1) after storage at 0°C for 3 months[a].

Rt[b] (min.)	I_{ϵ}^{c}	Compounds	Days at 20°C							
			0	1	2	4	6	8	10	12
6.01	1.99	ethyl acetate	nd	nd	nd	nd	nd	nd	1.9	2.8
6.33	2.38	ethanol	nd	nd	nd	nd	nd	nd	4.5	7.1
6.93	3.05	propyl acetate	nd	nd	nd	nd	nd	nd	nd	1.1
8.53	4.35	butyl acetate	nd	4.9	5.5	7.1	18.3	36.8	53.8	50.9
9.78	4.96	1-butanol	nd	nd	nd	nd	1.1	1.1	1.0	0.9
10.76	5.39	pentyl acetate	nd	nd	0.5	0.7	4.1	3.9	4.3	9.3
11.91	5.85	1-methylpropyl butanoate + butyl butanoate	4.8	6.6	12.6	11.9	9.9	2.1	3.7	1.1
13.24	6.41	hexyl acetate	3.1	20.6	10.6	15.8	43.1	135.6	216.6	230.5
15.00	7.14	1-hexanol	nd	3.2	2.1	1.4	3.6	6.7	8.8	8.9
15.44	7.31	2-methylpropyl hexanoate	nd	nd	nd	1.1	1.4	nd	nd	nd
15.83	7.45	heptyl acetate	nd	nd	nd	nd	nd	nd	1.0	2.9
16.25	7.60	methyl octanoate	nd	nd	nd	nd	1.5	1.5	nd	1.1
16.90	7.83	butyl hexanoate+hexyl butanoate	58.7	95.7	143.1	161.7	141.6	90.4	50.9	42.8
17.29	7.96	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	1.1	0.9	1.0	nd	4.6	5.6	3.7
17.41	8.00	ethyl octanoate	nd	nd	nd	nd	5.5	9.7	8.9	5.9
18.04	8.32	2-methylbutyl hexanoate	nd	nd	nd	1.7	0.7	nd	nd	nd
19.20	8.87	pentyl hexanoate	2.6	2.3	8.4	11.4	10.6	6.1	2.8	1.5
19.43	8.97	4-octen-1-ol acetate	nd	nd	nd	nd	0.9	0.8	nd	nd
21.09	9.69	methyl decanoate	nd	nd	nd	nd	nd	nd	nd	1.8
21.25	9.76	unk	nd	nd	1.5	4.3	10.9	7.3	4.3	0.7
21.44	9.84	hexyl hexanoate + butyl octanoate	32.4	65.5	75.5	105.2	111.9	100.3	44.2	29.1
21.70	9.94	methyl 4-decenoate	nd	nd	nd	nd	5.1	6.5	6.3	4.2
22.07	10.09	unknown	nd	nd	nd	4.8	20.3	29.7	18.2	9.6
22.38	10.21	ethyl 4-decenoate	8.1	8.4	8.5	5.0	1.7	nd	nd	nd
22.62	10.31	methyl 2,4,6 octatrienoate	nd	nd	nd	nd	2.9	7.1	7.9	4.8
23.87	10.78	methyl 2-decenoate	nd	nd	nd	nd	nd	0.5	1.4	1.2
23.89	10.79	α-farnesene (E,Z)	20.7	14.5	5.9	14.3	6.5	7.6	6.8	4.0
24.38	10.97	α-farnesene (E,E)	1978.3	1296.8	993.2	651.5	655.3	559.5	362.2	205.3
24.53	11.02	ethyl trans-2 decenoate	nd	nd	nd	nd	nd	3.0	3.8	nd
24.81	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	nd	nd	1.0	nd
25.07	11.21	methyl trans-2, cis-4 decadienoate	2.3	3.4	6.3	22.6	36.3	50.8	41.9	34.8
25.50	11.36	unknown	0.9	1.9	0.6	0.7	1.2	1.2	nd	nd
25.70	11.43	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	3.2	1.1	nd	0.4
26.03	11.54	ethyl trans-2, cis-4 decadienoate	7.0	6.6	9.6	17.6	32.5	71.2	79.8	80.2
26.32	11.64	methyl trans-2, trans-4 decadienoate	nd	nd	nd	nd	2.4	6.7	5.1	1.5
27.04	11.88	ethyl trans-2, trans-4, cis-7 decadienoate	nd	nd	nd	1.7	6.4	14.5	13.8	1nd
27.83	12.13	unknown	nd	nd	nd	nd	4.7	nd	nd	1.3
29.02	12.50	methyl tetradecenoate	0.6	1.5	0.4	3.8	6.7	6.9	4.6	4.2
29.45	12.63	unknown	nd	nd	nd	nd	2.1	0.8	nd	nd
29.56	12.66	ethyl tetradecanoate	nd	nd	0.7	2.5	11.0	20.8	18.6	11.8
30.29	12.87	ethyl cis-5, cis-8 tetradecadienoate	nd	0.7	nd	nd	5.9	17.3	23.3	13.7
31.09	13.10	unknown	nd	nd	nd	nd	nd	5.5	8.1	6.8
31.50	13.22	unknown	nd	nd	nd	nd	0.6	1.7	nd	nd

(Continued)

Table A.6.19. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C							
			0	1	2	4	6	8	10	12
31.99	13.35	unknown	nd	1.4	1.9	3.6	5.1	5.2	3.4	1.5
32.15	13.40	methyl 7-hexadecadecenoate	nd	nd	nd	nd	nd	nd	1.0	1.4
33.11	13.66	unknown	nd	nd	nd	0.5	1.3	nd	2.0	nd
37.04	14.64	unknown	nd	nd	nd	3.7	6.6	4.0	6.3	2.0
Total			2119.4	1535.1	1287.6	1055.8	1182.9	1228.2	1027.9	800.9

[a] Fruits were harvested at mature unriped stage (flesh firmness 68 N) and stored for 3 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.20. Changes in volatile compounds during ripening of 'Anjou' pear harvested at early stage of maturity (M1) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.02	2.01	ethyl acetate	nd	nd	nd	nd	nd	0.8	1.0
6.33	2.38	ethanol	nd	nd	nd	nd	nd	0.7	2.4
6.91	3.03	propyl acetate	nd	nd	nd	nd	0.1	nd	nd
8.55	4.36	butyl acetate	0.2	1.7	2.5	4.4	7.0	10.4	11.0
9.75	4.95	1-butanol	nd	nd	nd	nd	0.3	0.5	0.3
10.76	5.39	pentyl acetate	nd	0.2	nd	0.8	0.4	2.0	2.2
11.96	5.87	1-methylpropyl butanoate + butyl butanoate	1.0	3.1	4.0	2.1	0.2	nd	1.0
12.30	5.99	ethyl hexanoate	nd	nd	nd	nd	0.2	0.1	0.2
13.27	6.43	hexyl acetate	0.4	4.6	4.2	11.8	22.6	34.5	47.3
15.02	7.15	1-hexanol	nd	0.6	0.5	1.6	1.4	3.0	2.7
15.56	7.35	2-methylpropyl hexanoate	nd	nd	0.8	nd	nd	nd	nd
15.83	7.45	heptyl acetate	nd	nd	nd	nd	nd	nd	0.3
16.21	7.59	methyl octanoate	nd	nd	nd	0.2	0.3	nd	0.4
16.95	7.85	butyl hexanoate+hexyl butanoate	9.5	37.3	58.0	43.4	19.3	15.6	14.5
17.29	7.96	butyl 2-methylbutanoate	nd	0.2	0.5	0.2	nd	nd	nd
17.30	7.97	butyl 4-hexenoate	nd	nd	nd	0.6	0.9	1.0	1.2
17.38	7.99	ethyl octanoate	nd	nd	nd	2.8	2.5	2.4	2.1
18.02	8.31	2-methylbutyl hexanoate	nd	nd	nd	0.1	nd	nd	nd
19.23	8.88	pentyl hexanoate	0.1	2.6	4.0	3.6	1.6	0.6	0.8
19.41	8.96	4-octen-1-ol acetate	nd	nd	nd	nd	0.2	nd	nd
21.06	9.68	methyl decanoate	0.3	0.4	2.3	3.5	1.4	0.7	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	5.1	22.2	38.3	42.2	19.2	14.1	10.3
21.65	9.92	methyl 4-decenoate	nd	0.2	1.2	1.1	2.0	1.8	0.7
22.05	10.08	ethyl 2,4 octadienoate	nd	0.7	2.8	8.5	6.3	4.0	1.3
22.43	10.23	ethyl 4-decenoate	3.3	3.1	2.2	0.9	0.5	0.3	0.6
22.60	10.30	methyl 2,4,6 octatrienoate	nd	nd	0.6	2.1	2.6	2.9	3.5
23.79	10.75	methyl 2-decenoate	nd	nd	nd	nd	0.5	0.1	0.1
23.93	10.80	alpha farnesene (E,Z)	4.1	3.4	3.2	3.7	2.3	2.1	1.9
24.41	10.98	alpha farnesene (E,E)	321.8	221.6	204.1	238.1	140.5	122.6	108.6
25.13	11.23	methyl trans-2, cis-4 decadienoate	1.6	4.1	10.5	18.7	13.9	12.4	12.0
25.56	11.38	2-phenylethyl acetate	0.4	0.1	nd	nd	0.6	nd	nd
25.68	11.42	unknown	nd	nd	0.6	0.2	0.4	nd	nd
25.83	11.47	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	0.5	0.4	1.1
26.08	11.56	ethyl trans-2, cis-4 decadienoate	1.0	5.1	10.1	21.2	23.3	21.1	26.9
26.27	11.62	methyl trans-2, trans-4 decadienoate	nd	nd	0.2	2.2	1.4	0.4	0.6
27.04	11.88	ethyl trans-2, trans-4, cis-7 decadienoate	nd	0.6	1.1	3.6	3.7	2.9	3.2
28.62	12.37	unknown	nd	nd	nd	nd	0.3	0.2	0.2
29.05	12.51	methyl tetradecenoate	nd	0.2	1.2	2.0	1.3	1.5	1.5
29.57	12.66	ethyl tetradecanoate	0.2	0.2	0.7	6.1	4.6	4.9	3.8
30.33	12.88	ethyl cis-5, cis-8 tetradecadienoate	nd	0.2	1.0	4.0	4.5	5.4	4.4
31.07	13.10	unknown	nd	nd	nd	0.7	1.7	2.8	2.9
31.47	13.21	unknown	nd	nd	nd	nd	0.3	0.3	nd
31.99	13.35	unknown	nd	0.3	nd	0.4	0.3	nd	0.2

(Continued)

Table A.6.20. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
32.14	13.39	methyl 7-hexadecadecenoate	nd	nd	nd	nd	0.3	nd	0.3
33.11	13.66	unknown	nd	nd	0.2	nd	0.1	0.3	0.3
33.39	13.73	unknown	nd	nd	nd	0.2	0.1	0.6	0.1
37.02	14.64	unknown	nd	nd	0.8	1.5	nd	0.5	0.4
Total			349.2	312.7	355.6	432.6	289.6	273.9	272.4

[a] Fruits were harvested at mature unriped stage (flesh firmness 68 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.21. Changes in volatile compounds during ripening of 'Anjou' pear harvested at early stage of maturity (M1) after storage at 0°C for 5 months[a].

Rt[b] (min.)	I_E^c	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.05	2.04	ethyl acetate	nd	nd	nd	0.8	0.5	nd	1.6
6.34	2.38	ethanol	nd	nd	nd	0.2	0.7	nd	2.0
7.03	3.15	propyl acetate	nd	nd	nd	0.3	nd	nd	0.2
8.57	4.37	butyl acetate	0.5	2.5	2.1	3.1	5.6	6.8	7.9
9.81	4.98	1-butanol	nd	0.3	nd	0.1	0.7	1.0	1.1
10.79	5.40	pentyl acetate	nd	nd	0.5	nd	1.1	1.2	1.5
11.97	5.87	1-methylpropyl butanoate + butyl butanoate	1.3	3.1	2.7	1.6	0.9	0.8	0.9
12.33	6.00	ethyl hexanoate	nd	nd	nd	nd	0.6	0.4	nd
13.30	6.44	hexyl acetate	2.0	nd	4.1	6.3	17.4	23.0	22.8
15.06	7.16	1-hexanol	0.8	0.9	nd	0.9	1.8	2.2	1.7
15.84	7.45	heptyl acetate	nd	nd	nd	nd	0.3	nd	0.7
16.29	7.62	methyl octanoate	0.4	nd	nd	0.8	nd	nd	0.5
16.98	7.86	butyl hexanoate+hexyl butanoate	10.8	41.0	40.2	21.4	23.9	15.1	11.9
17.32	7.97	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	0.4	0.6	0.6	1.4	nd	0.9
17.41	8.00	ethyl octanoate	nd	nd	nd	1.3	2.0	2.4	1.3
18.03	8.31	2-methylbutyl hexanoate	nd	nd	nd	0.4	nd	nd	nd
19.29	8.91	pentyl hexanoate	0.8	3.3	3.2	2.5	2.2	nd	1.1
21.09	9.69	methyl decanoate	0.3	0.8	1.8	2.5	1.2	1.0	0.7
21.51	9.86	hexyl hexanoate + butyl octanoate	6.0	22.4	27.3	24.4	22.0	11.3	9.0
21.69	9.94	methyl 4-decenoate	0.4	0.5	1.4	2.3	2.7	2.2	1.8
22.07	10.09	unknown	nd	1.4	2.5	3.8	4.7	3.1	2.1
22.46	10.24	ethyl 4-decenoate	2.8	2.6	1.5	0.6	0.8	0.8	0.4
22.61	10.30	methyl 2,4,6 octatrienoate	nd	nd	0.7	1.6	3.5	4.1	3.4
22.91	10.42	unknown	nd	nd	0.4	nd	0.5	0.7	nd
23.80	10.75	methyl 2-decenoate	nd	nd	nd	nd	0.7	0.8	0.5
23.95	10.81	α-farnesene (E,Z)	4.5	3.5	3.1	nd	2.4	1.6	1.7
24.43	10.98	α-farnesene (E,E)	282.8	211.8	182.0	129.1	145.6	96.6	81.3
24.58	11.04	ethyl trans-2 decenoate	nd	nd	nd	nd	nd	5.3	nd
24.82	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	0.6	nd	0.8
25.15	11.24	methyl trans-2, cis-4 decadienoate	3.2	7.8	11.2	15.3	19.3	13.1	9.2
25.68	11.42	unknown	nd	0.4	0.7	0.5	0.4	nd	nd
25.84	11.48	ethyl cis-2, cis-4 decadienoate	nd	0.4	0.4	nd	0.7	0.9	1.0
26.10	11.56	ethyl trans-2, cis-4 decadienoate	1.6	5.9	10.7	14.0	23.9	25.7	23.1
26.32	11.64	methyl trans-2, trans-4 decadienoate	nd	nd	1.0	1.3	1.5	1.0	0.6
27.05	11.88	ethyl trans-2, trans-4, cis-7 decadienoate	nd	0.9	1.7	2.5	4.0	3.5	2.7
27.85	12.13	unknown	0.7	nd	nd	0.4	0.7	0.8	0.5
28.65	12.38	unknown	nd	nd	nd	nd	nd	0.8	0.3
29.07	12.51	methyl tetradecenoate	nd	0.7	1.0	1.2	1.5	1.5	1.4
29.63	12.68	ethyl tetradecanoate	nd	nd	1.6	3.4	4.5	4.1	2.5
30.39	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	0.7	1.3	2.1	3.9	4.3	3.6
31.10	13.11	unknown	nd	nd	0.3	0.6	1.3	2.0	2.5
32.01	13.36	unknown	nd	nd	0.4	0.6	nd	nd	0.4

(Continued)

Table A.6.21.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
33.15	13.67	unknown	0.4	nd	nd	0.7	0.4	0.9	0.3
Total			319.4	311.1	304.3	247.1	306.0	238.9	205.9

[a] Fruits were harvested at mature unriped stage (flesh firmness 68 N) and stored for 5 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.22. Changes in volatile compounds during ripening of 'Anjou' pear harvested at early stage of maturity (M1) after storage at 0°C for 6 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.32	1.09	unknown	nd	nd	nd	nd	0.7	0.5	nd
5.59	1.45	unknown	nd	nd	nd	nd	2.7	0.6	nd
5.67	1.57	methyl acetate	nd	nd	nd	nd	1.2	0.8	0.3
6.04	2.03	ethyl acetate	nd	nd	nd	nd	6.3	1.4	2.8
6.36	2.41	ethanol	nd	nd	0.6	nd	2.5	2.9	2.4
8.55	4.36	butyl acetate	1.3	2.0	2.9	2.2	4.0	4.2	5.5
9.74	4.95	1-butanol	nd	nd	0.4	0.6	0.9	0.3	0.1
10.79	5.40	pentyl acetate	nd	0.3	nd	0.7	1.5	1.1	0.6
11.96	5.86	1-methylpropyl butanoate + butyl butanoate	0.7	nd	1.6	1.5	1.1	0.6	0.3
13.28	6.43	hexyl acetate	2.8	nd	3.0	4.6	13.0	14.5	16.6
15.04	7.15	1-hexanol	0.7	nd	1.2	1.3	1.9	1.5	1.5
16.27	7.61	methyl octanoate	0.5	nd	nd	nd	nd	nd	nd
16.96	7.85	butyl hexanoate+hexyl butanoate	10.6	24.6	29.9	17.0	18.5	8.7	8.0
17.33	7.98	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	0.7	0.8	0.7	nd	0.5	0.2
17.40	8.00	ethyl octanoate	nd	nd	nd	1.3	2.2	0.6	0.9
19.26	8.89	pentyl hexanoate	nd	1.3	2.4	1.9	1.7	0.8	0.7
21.07	9.68	methyl decanoate	nd	0.7	1.8	0.9	1.1	0.5	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	4.7	12.7	23.7	12.3	12.5	4.9	3.9
21.68	9.93	methyl 4-decenoate	0.8	1.0	2.4	2.2	2.5	1.2	1.0
22.08	10.09	ethyl 2,4 octadienoate	nd	1.0	3.4	3.4	2.9	1.4	1.4
22.44	10.23	ethyl 4-decenoate	3.0	2.0	1.8	1.6	0.9	0.7	0.6
22.57	10.29	methyl 2,4,6 octatrienoate	nd	0.1	2.0	2.7	3.2	2.1	2.5
22.93	10.42	unknown	nd	0.2	nd	0.6	nd	nd	nd
23.78	10.74	methyl 2-decenoate	nd	nd	0.6	nd	nd	0.5	nd
23.93	10.80	α-farnesene (E,Z)	5.2	2.9	4.7	nd	2.9	1.3	0.9
24.41	10.97	α-farnesene (E,E)	299.1	184.6	272.9	123.0	166.2	74.6	71.2
24.81	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	0.5	0.6	nd	0.5
25.12	11.23	methyl trans-2, cis-4 decadienoate	4.8	10.3	22.1	11.6	14.7	6.7	6.4
25.56	11.38	unknown	0.5	nd	0.4	nd	nd	nd	nd
25.83	11.47	ethyl cis-2, cis-4 decadienoate	nd	nd	0.7	0.6	1.1	0.5	0.7
26.07	11.55	ethyl trans-2, cis-4 decadienoate	2.3	6.6	24.9	15.3	24.4	13.3	15.0
26.31	11.63	methyl trans-2, trans-4 decadienoate	nd	0.2	1.3	1.1	1.1	0.6	0.3
27.05	11.88	ethyl trans-2, trans-4, cis-7 decadienoate	nd	0.9	3.4	2.7	3.0	1.7	1.8
27.83	12.13	unknown	0.6	nd	nd	2.1	1.2	1.0	0.8
29.08	12.51	methyl tetradecenoate	0.4	0.7	4.0	2.0	2.1	1.2	1.2
29.63	12.68	ethyl tetradecanoate	nd	0.7	3.5	3.9	3.6	2.0	1.6
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	0.6	1.0	2.5	2.8	3.1	2.0	1.6
31.08	13.10	unknown	nd	0.4	1.0	0.8	1.6	1.0	1.4
31.99	13.35	unknown	0.3	0.2	0.5	0.4	nd	nd	nd
32.25	13.43	methyl 7-hexadecadecenoate	nd	0.3	0.5	0.4	nd	0.4	0.3
33.04	13.64	unknown	nd	0.1	0.5	1.0	nd	0.3	0.5
34.11	13.92	unknown	0.3	0.3	nd	1.3	0.7	0.4	0.8

(Continued)

Table A.6.22.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
37.02	14.64	unknown	nd	nd	0.7	nd	nd	1.1	1.0
Total			339.0	255.8	422.0	224.8	307.4	158.4	155.5

[a] Fruits were harvested at mature unriped stage (flesh firmness 68 N) and stored for 6 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.23. Changes in volatile compounds during ripening of 'Anjou' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 2 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C											
			0	1	2	3	4	5	6	7	8	9	10	12
6.35	2.40	ethanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.5	4.8	2.0
6.94	3.06	propyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.2
7.19	3.32	2-methylpropyl acetate	nd	nd	6.4	nd	nd	nd	nd	nd	nd	nd	nd	nd
8.52	4.35	butyl acetate	0.6	1.5	7.0	10.5	9.3	12.7	14.0	8.0	27.9	30.7	35.0	41.9
9.59	4.88	Isoamyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.6
9.77	4.96	1-butanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.9
10.24	5.17	butyl propanoate	nd	nd	nd	nd	nd	0.9	nd	nd	nd	nd	nd	nd
10.75	5.39	pentyl acetate	nd	nd	nd	nd	nd	1.6	3.6	5.1	17.1	3.1	8.4	8.4
11.96	5.86	1-methylpropyl butanoate + butyl butanoate	3.4	7.6	14.7	14.3	11.3	9.1	7.2	5.8	4.2	0.7	0.9	nd
12.41	6.03	ethyl hexanoate	nd	nd	1.6	nd	nd	nd	nd	nd	nd	nd	nd	nd
13.28	6.43	hexyl acetate	6.2	24.7	18.8	18.4	18.4	30.4	32.1	55.4	89.5	134.1	151.9	205.6
14.53	6.95	propyl tiglate	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
15.01	7.14	1-hexanol	0.7	4.1	3.1	0.6	1.6	0.6	nd	2.6	4.6	5.3	6.3	6.5
15.45	7.31	2-methylpropyl hexanoate	nd	nd	nd	nd	0.6	nd	nd	nd	nd	nd	nd	nd
15.84	7.45	heptyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	1.5	0.9	3.4	3.6
16.20	7.59	methyl octanoate	nd	nd	nd	nd	nd	0.3	1.7	1.7	3.4	2.2	0.5	0.9
16.96	7.85	butyl hexanoate+hexyl butanoate	89.6	106.7	177.1	182.7	154.5	155.9	108.3	101.9	86.0	52.7	39.4	11.5
17.33	7.98	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.1	0.5	1.4
17.39	8.00	ethyl octanoate	nd	nd	nd	nd	nd	1.8	4.6	6.8	11.2	9.5	7.3	5.6
18.03	8.31	2-methylbutyl hexanoate	nd	nd	nd	nd	1.8	2.1	1.2	0.5	nd	nd	nd	nd
19.25	8.89	pentyl hexanoate	3.8	5.0	10.4	12.1	1nd	12.1	9.1	8.5	9.2	1.7	3.0	nd
21.03	9.67	methyl decanoate	nd	nd	nd	0.5	2.8	6.8	6.4	8.5	9.8	4.5	5.2	1.9
21.49	9.86	hexyl hexanoate + butyl octanoate	57.5	70.8	100.1	112.6	89.6	112.3	77.5	89.6	110.2	54.5	40.9	21.2
21.67	9.93	methyl 4-decenoate	nd	nd	nd	nd	nd	4.8	3.6	5.8	7.2	5.4	5.6	4.1
22.05	10.08	ethyl 2,4 octadienoate	nd	nd	nd	1.0	1.8	8.6	11.5	13.9	30.1	22.4	17.2	8.4
22.44	10.24	ethyl 4-decenoate	11.0	8.0	8.3	2.7	0.6	2.6	0.6	nd	nd	nd	nd	nd
22.59	10.29	methyl 2,4,6 octatrienoate	nd	nd	nd	nd	nd	2.1	3.0	3.7	7.2	9.5	12.4	4.9

(Continued)

Table A.6.23. Continued

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C											
			0	1	2	3	4	5	6	7	8	9	10	12
23.81	10.76	methyl 2-decenoate	nd	nd	nd	nd	nd	nd	nd	0.9	nd	1.4	nd	nd
23.94	10.80	α-farnesene (E,Z)	28.6	13.9	13.3	11.8	6.4	6.3	1.3	5.2	5.4	6.8	4.7	nd
24.43	10.98	α-farnesene (E,E)	2780.5	1359.8	1187.0	935.2	556.5	460.2	347.9	348.5	436.3	449.8	275.4	276.4
25.13	11.23	methyl trans-2, cis-4 decadienoate	nd	2.6	4.1	9.6	13.1	23.1	19.6	29.1	40.8	32.9	31.5	24.2
25.58	11.39	unknown	7.0	1.1	1.4	1.4	nd	nd	nd	0.4	1.6	0.9	nd	nd
25.67	11.42	ethyl cis-2, cis-4 decadienoate	nd	nd	nd	1.5	1.9	1.9	2.0	2.2	4.3	nd	nd	nd
26.08	11.56	ethyl trans-2, cis-4 decadienoate	2.6	4.6	6.1	6.5	7.6	13.5	12.9	17.5	38.2	44.7	35.4	30.7
26.47	11.69	methyl trans-2, trans-4 decadienoate	nd	nd	nd	1.1	nd	1.1	2.5	2.8	7.1	6.1	4.4	4.0
27.03	11.87	ethyl trans-2, trans-4, cis-7 decadienoate	nd	nd	nd	nd	nd	1.9	2.6	4.3	5.5	1nd	8.1	6.6
28.64	12.38	unknown	nd	nd	nd	nd	nd	nd	nd	nd	1.6	nd	nd	nd
29.09	12.52	methyl tetradecenoate	1.0	1.2	2.0	1.7	2.0	1.4	2.5	2.7	4.2	nd	nd	nd
29.57	12.66	ethyl tetradecanoate	1.1	1.5	1.3	1.7	2.3	5.2	6.7	9.7	24.1	15.9	18.0	17.2
30.36	12.89	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	nd	nd	nd	1.0	3.4	4.8	17.5	17.1	17.4	14.7
31.09	13.10	unknown	nd	nd	nd	nd	nd	nd	nd	nd	4.7	6.1	6.8	5.0
32.02	13.36	unknown	1.6	2.1	3.0	4.9	5.5	2.2	4.0	1.0	3.8	4.1	2.1	1.3
33.12	13.66	methyl 7-hexadecadecenoate	nd	nd	nd	nd	0.8	1.2	nd	1.4	1.6	nd	nd	nd
35.03	14.15	unknown	nd	nd	nd	nd	2.2	nd	nd	nd	nd	nd	1.7	0.5
37.05	14.64	unknown	nd	nd	nd	1.5	nd	1.2	0.4	3.2	4.9	2.1	3.3	2.5
Total			3017.7	1637.7	1588.1	1354.8	923.5	907.3	713.6	774.1	1044.9	963.1	776.1	741.0

[a] Fruits were harvested at mature unriped stage (flesh firmness 62 N) and stored for 2 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 μg propyl tiglate used as internal standard (response factor = 1) and expressed in μg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 μg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2μm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.24. Changes in volatile compounds during ripening of 'Anjou' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 3 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.03	2.02	ethyl acetate	nd	nd	nd	nd	nd	2.0	3.7
6.32	2.37	ethanol	nd	nd	nd	nd	0.6	2.8	4.1
6.93	3.05	propyl acetate	nd	nd	nd	nd	0.4	1.4	1.6
8.52	4.35	butyl acetate	1.2	14.4	23.1	27.5	39.7	44.5	34.1
9.78	4.96	1-butanol	nd	nd	nd	2.1	nd	1.6	0.9
10.76	5.39	pentyl acetate	nd	2.3	3.9	4.6	8.6	9.3	4.4
11.96	5.86	1-methylpropyl butanoate + butyl butanoate	4.9	12.9	11.8	4.0	nd	0.6	nd
12.31	5.99	ethyl hexanoate	nd	nd	nd	nd	1.2	1.5	nd
13.27	6.43	hexyl acetate	11.1	51.0	57.8	72.5	143.2	178.6	158.1
13.41	6.49	methyl heptanoate	2.6	nd	nd	nd	nd	nd	nd
15.01	7.14	1-hexanol	nd	4.6	2.2	4.8	5.8	3.6	5.3
15.80	7.44	heptyl acetate	nd	nd	nd	nd	0.3	3.6	1.3
16.23	7.60	methyl octanoate	nd	nd	nd	2.0	1.7	0.8	nd
16.95	7.85	butyl hexanoate+hexyl butanoate	57.4	165.8	192.6	136.0	74.2	24.4	9.0
17.32	7.97	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	1.3	nd	1.4	3.9	3.5	3.0
17.40	8.00	ethyl octanoate	nd	nd	2.1	4.3	11.8	6.4	4.4
18.02	8.31	2-methylbutyl hexanoate	nd	nd	nd	0.9	nd	nd	nd
18.30	8.44	octyl acetate	1.1	2.3	nd	nd	nd	nd	nd
19.23	8.88	pentyl hexanoate	0.5	8.2	11.0	11.3	6.6	nd	0.9
19.43	8.97	4-octen-1-ol acetate	nd	nd	1.1	1.6	nd	nd	1.1
21.19	9.73	unknown	0.8	nd	4.0	7.8	9.2	1.2	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	35.8	110.3	136.9	130.4	101.1	27.6	17.1
21.66	9.93	methyl 4-decenoate	nd	nd	3.6	6.0	6.6	3.6	2.4
22.06	10.09	ethyl 2,4 octadienoate	nd	1.9	7.9	23.4	31.9	12.1	7.9
22.43	10.23	ethyl 4-decenoate	11.7	9.1	7.0	3.3	1.5	nd	nd
22.60	10.30	methyl 2,4,6 octatrienoate	nd	nd	2.2	5.3	9.4	5.5	7.6
23.08	10.48	unknown	nd	nd	nd	0.8	0.9	0.4	1.0
23.93	10.80	α-farnesene (E,Z)	17.8	13.1	9.4	10.3	11.7	6.8	6.4
24.14	10.88	unknown	nd	nd	1.1	1.4	nd	nd	nd
24.42	10.98	α-farnesene (E,E)	1701.3	1143.3	850.7	737.6	828.9	425.4	334.0
25.13	11.23	methyl trans-2, cis-4 decadienoate	2.2	8.4	23.3	35.8	48.2	27.5	26.0
25.56	11.38	unknown	4.5	2.8	nd	nd	1.1	nd	nd
25.67	11.42	unknown	nd	1.8	2.5	6.1	2.7	nd	nd
25.82	11.47	ethyl cis-2, cis-4 decadienoate	nd	nd	0.7	0.4	1.4	nd	0.3
26.07	11.56	ethyl trans-2, cis-4 decadienoate	3.3	13.8	26.9	53.7	71.2	44.4	48.7
26.47	11.69	methyl trans-2, trans-4 decadienoate	nd	1.6	0.6	6.9	6.2	3.3	2.1
27.03	11.87	ethyl trans-2, trans-4, cis-7 decadienoate	nd	1.0	3.7	7.2	14.5	8.2	7.1
27.79	12.12	unknown	1.0	0.4	nd	nd	0.3	1.7	nd
28.63	12.38	unknown	nd	nd	nd	nd	1.3	nd	nd
29.07	12.51	methyl tetradecenoate	nd	2.4	1.7	4.9	7.1	4.0	5.0
29.63	12.68	ethyl tetradecenoate	nd	nd	2.0	22.3	17.7	13.9	9.7
29.78	12.72	methyl cis-5, cis-8 tetradecadienoate	nd	nd	nd	5.9	nd	nd	nd

(Continued)

Table A.6.24. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
30.32	12.88	ethyl cis-5, cis-8 tetradecadienoate	0.7	nd	0.9	6.7	14.9	13.9	12.3
31.09	13.10	unknown	nd	nd	nd	2.1	4.7	4.7	5.9
31.47	13.21	unknown	nd	nd	nd	nd	1.8	1.2	nd
32.01	13.36	unknown	0.9	1.7	6.5	6.2	7.0	1.9	2.5
33.13	13.66	methyl 7-hexadecadecenoate	nd	nd	0.8	0.4	1.4	nd	0.5
36.52	14.52	unknown	nd	nd	nd	0.6	1.0	nd	nd
37.02	14.64	unknown	nd	nd	1.1	3.6	5.5	0.6	nd
Total			1858.6	1574.5	1399.3	1362.2	1507.4	892.6	728.4

[a] Fruits were harvested at mature unriped stage (flesh firmness 62 N) and stored for 3 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.25. Changes in volatile compounds during ripening of 'Anjou' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.03	2.02	ethyl acetate	nd	nd	nd	nd	1.0	0.5	0.7
6.30	2.34	ethanol	nd	nd	nd	0.1	0.2	1.0	1.9
6.94	3.06	propyl acetate	nd	nd	nd	nd	nd	0.4	0.4
8.54	4.36	butyl acetate	3.8	9.7	10.9	14.4	14.5	15.3	12.9
9.75	4.95	1-butanol	nd	0.5	nd	0.4	0.8	nd	0.3
10.76	5.39	pentyl acetate	nd	1.6	2.1	2.4	2.8	2.9	2.0
11.95	5.86	1-methylpropyl butanoate + butyl butanoate	0.9	4.9	3.5	0.6	0.2	0.4	0.8
13.28	6.43	hexyl acetate	11.5	36.9	27.7	44.7	47.7	53.2	52.2
15.01	7.14	1-hexanol	0.3	2.3	1.8	1.1	1.9	2.4	2.8
15.84	7.45	heptyl acetate	nd	nd	nd	nd	0.9	nd	0.3
16.95	7.85	butyl hexanoate+hexyl butanoate	12.9	84.7	63.0	37.2	20.1	11.6	15.1
17.28	7.96	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	0.2	1.1	1.3	1.2	0.8	1.1
17.40	8.00	ethyl octanoate	nd	nd	1.8	3.4	3.8	1.3	1.3
19.25	8.89	pentyl hexanoate	nd	4.6	3.8	2.4	1.5	nd	1.3
21.06	9.68	methyl decanoate	nd	0.7	1.9	1.6	3.7	nd	0.4
21.48	9.85	hexyl hexanoate	7.5	64.4	43.0	33.5	20.7	7.2	12.1
21.66	9.92	methyl 4-decenoate	0.1	0.4	1.4	1.8	3.1	1.1	2.2
22.06	10.09	ethyl 2,4 octadienotae	nd	2.8	6.2	8.6	7.6	2.6	3.6
22.43	10.23	ethyl 4-decenoate	3.1	5.7	2.9	0.6	0.2	0.2	nd
22.60	10.30	methyl 2,4,6 octatrienoate	nd	0.6	1.1	3.0	3.3	2.9	5.2
23.93	10.80	α-farnesene (E,Z)	4.9	7.7	6.0	6.6	5.0	2.2	5.6
24.41	10.97	α-farnesene (E,E)	415.6	580.3	402.7	351.1	230.4	141.5	202.9
25.12	11.23	methyl trans-2, cis-4 decadienoate	3.1	8.6	11.3	14.4	16.0	8.8	14.2
25.55	11.38	unknown	0.5	0.9	nd	0.2	0.5	nd	0.3
25.66	11.42	ethyl cis-2, cis-4 decadienoate	nd	0.6	0.3	0.4	0.3	nd	nd
25.78	11.46	unknown	nd	nd	0.4	0.4	0.3	0.2	0.8
26.08	11.56	ethyl trans-2, cis-4 decadienoate	1.6	9.6	15.3	22.4	26.4	19.0	32.2
26.30	11.63	unknown	nd	nd	1.2	0.6	1.4	nd	0.5
27.03	11.87	ethyl trans-2, trans-4, cis-7 decadienoate	nd	1.6	2.5	4.1	4.1	2.6	3.7
29.06	12.51	methyl tetradecenoate	nd	2.0	1.9	2.2	0.9	0.7	2.9
29.63	12.68	unknown	nd	nd	1.4	2.8	5.1	2.8	4.1
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	0.7	1.2	3.5	7.1	4.1	7.0
31.08	13.10	unknown	nd	0.4	0.3	1.7	3.8	2.4	6.5
31.98	13.35	unknown	nd	0.4	1.2	1.4	1.1	0.2	0.7
Total			465.9	832.8	617.8	569.0	437.6	288.2	398.1

[a] Fruits were harvested at mature unriped stage (flesh firmness 62 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 μg propyl tiglate used as internal standard (response factor = 1) and expressed in μg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 μg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2μm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.26. Changes in volatile compounds during ripening of 'Anjou' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 5 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.07	2.06	ethyl acetate	nd	nd	nd	1.1	1.7	1.4	nd
6.39	2.44	ethanol	nd	nd	0.7	0.5	2.3	2.6	1.7
6.98	3.10	propyl acetate	nd	nd	0.2	0.4	0.6	0.5	0.6
7.52	3.65	2-methylpropyl acetate	nd	nd	nd	nd	nd	nd	0.4
8.57	4.38	butyl acetate	3.8	10.4	10.1	11.9	12.0	10.3	10.1
9.57	4.87	isoamyl acetate	nd	0.6	nd	0.5	nd	nd	nd
9.80	4.98	1-butanol	0.6	1.2	1.2	1.2	1.3	1.1	nd
10.79	5.40	pentyl acetate	0.6	2.3	2.2	2.2	3.0	2.5	1.0
11.98	5.87	1-methylpropyl butanoate + butyl butanoate	2.2	2.0	1.6	1.1	0.8	0.4	0.6
12.35	6.01	ethyl hexanoate	nd	nd	nd	0.6	0.9	0.7	0.9
13.30	6.44	hexyl acetate	12.7	51.0	27.4	29.3	33.4	32.7	31.1
15.03	7.15	1-hexanol	0.5	2.5	1.9	1.7	2.0	1.9	nd
15.87	7.47	heptyl acetate	nd	nd	nd	nd	0.6	nd	0.9
16.25	7.60	methyl octanoate	nd	nd	0.1	0.2	0.4	nd	nd
16.96	7.85	butyl hexanoate+hexyl butanoate	19.0	47.1	30.1	22.0	14.2	9.0	9.7
17.33	7.98	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	1.3	1.2	1.0	nd	nd	0.5
17.41	8.00	ethyl octanoate	nd	nd	1.7	2.6	3.3	2.2	1.5
18.34	8.46	octyl acetate	nd	nd	nd	nd	nd	0.4	nd
19.25	8.89	pentyl hexanoate	1.0	2.3	2.1	1.7	1.1	0.8	0.6
19.46	8.98	4-octen-1-ol acetate	nd	1.1	0.6	nd	nd	0.3	nd
21.07	9.68	methyl decanoate	0.7	1.1	0.7	1.2	0.7	nd	nd
21.19	9.73	unknown	0.4	nd	nd	nd	nd	nd	nd
21.49	9.86	hexyl hexanoate + butyl octanoate	11.3	38.5	22.4	18.0	10.9	6.7	8.0
21.68	9.93	methyl 4-decenoate	1.0	1.6	1.7	1.9	1.8	1.6	1.5
22.07	10.09	ethyl 2,4 octadienoate	0.9	2.8	4.8	6.0	nd	3.0	2.3
22.44	10.24	ethyl 4-decenoate	5.1	4.0	2.4	1.4	0.6	1.8	nd
22.61	10.30	methyl 2,4,6 octatrienoate	0.5	2.1	2.9	3.8	4.1	5.0	5.1
22.89	10.41	unknown	nd	nd	nd	0.2	0.6	2.0	nd
23.80	10.75	methyl 2-decenoate	nd	0.4	0.6	nd	nd	nd	nd
23.94	10.81	α-farnesene (E,Z)	7.4	6.4	5.2	4.5	3.6	5.0	4.3
24.42	10.98	α-farnesene (E,E)	557.2	441.6	281.2	211.1	135.1	135.2	157.0
24.82	11.12	methyl cis-2, cis-4 decadienoate	nd	nd	nd	nd	0.6	0.8	nd
25.13	11.23	methyl trans-2, cis-4 decadienoate	6.9	12.8	11.3	11.6	9.1	9.1	7.0
25.58	11.39	unknown	1.4	0.7	0.8	0.5	nd	0.6	nd
25.67	11.42	unknown	nd	0.4	0.8	0.6	0.7	2.6	1.2
26.09	11.56	ethyl trans-2, cis-4 decadienoate	5.2	18.4	21.6	23.1	20.3	21.7	26.6
26.31	11.64	unknown	0.6	0.9	0.9	1.2	0.9	1.6	0.7
26.48	11.69	methyl trans-2, trans-4 decadienoate	nd	0.5	nd	nd	nd	nd	nd
27.05	11.88	ethyl trans-2, trans-4, cis-7 decadienoate	0.3	3.6	3.7	4.2	3.0	2.8	3.1
27.84	12.13	unknown	1.9	0.7	0.8	0.9	0.7	0.8	0.7
28.65	12.38	unknown	nd	nd	nd	0.3	0.5	nd	0.5
29.09	12.52	methyl tetradecenoate	0.8	0.9	2.9	3.1	2.8	2.6	2.8
29.56	12.66	ethyl tetradecenoate	0.2	1.7	1.8	2.9	3.0	2.4	2.3

(Continued)

Table A.6.26. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
30.21	12.85	unknown	nd	nd	nd	nd	nd	0.3	0.7
30.39	12.90	ethyl cis-5, cis-8 tetradecadienoate	1.5	1.7	2.4	3.7	5.5	4.5	5.6
31.09	13.10	unknown	0.9	1.5	1.6	1.8	4.2	4.1	5.7
31.48	13.21	unknown	nd	nd	nd	nd	nd	nd	0.2
32.01	13.36	unknown	0.8	1.5	nd	0.8	0.7	0.7	0.9
32.16	13.40	unknown	nd	nd	0.3	0.7	0.8	0.7	1.2
33.14	13.66	methyl 7-hexadecadecenoate	nd	nd	nd	nd	0.5	nd	0.6
Total			645.3	665.5	452.0	381.9	288.7	282.1	297.7

[a] Fruits were harvested at mature unriped stage (flesh firmness 62 N) and stored for 5 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.27. Changes in volatile compounds during ripening of 'Anjou' pear harvested at optimum stage of maturity (M2) after storage at 0°C for 6 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	2	4	6	8	10
5.37	1.16	acetaldehyde	0.5	nd	nd	0.7	nd	nd
5.82	1.76	unknown	1.8	nd	nd	1.1	1.1	nd
6.33	2.38	ethanol	nd	0.6	0.7	1.7	1.9	2.9
6.96	3.08	propyl acetate	nd	nd	nd	nd	0.8	0.7
7.97	4.05	ethyl butanoate	0.4	nd	nd	0.1	nd	0.3
8.56	4.37	butyl acetate	5.8	9.8	11.2	12.7	15.1	11.7
9.62	4.89	isoamyl acetate	nd	nd	nd	nd	1.6	0.5
9.81	4.98	1-butanol	1.4	2.5	1.2	1.6	2.3	0.9
10.80	5.41	pentyl acetate	0.7	3.5	2.6	3.1	3.2	2.7
11.96	5.86	1-methylpropyl butanoate + butyl butanoate	2.7	2.2	1.8	1.0	0.5	1.3
12.30	5.99	ethyl hexanoate	nd	0.4	nd	0.5	nd	0.7
13.29	6.44	hexyl acetate	13.8	43.2	23.5	23.4	21.6	27.0
15.04	7.15	1-hexanol	1.3	2.6	1.6	1.6	1.6	2.1
15.86	7.46	heptyl acetate	nd	nd	nd	nd	0.3	0.9
16.95	7.85	butyl hexanoate+hexyl butanoate	23.0	36.7	20.8	14.8	7.4	7.0
17.33	7.97	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	0.9	0.7	0.7	1.0	0.2
17.41	8.00	ethyl octanoate	nd	nd	1.3	1.6	nd	nd
18.30	8.45	octyl acetate	nd	0.5	nd	nd	nd	nd
19.24	8.88	pentyl hexanoate	nd	2.2	1.7	1.6	0.7	0.5
21.06	9.68	methyl decanoate	0.8	nd	0.7	nd	nd	nd
21.49	9.86	hexyl hexanoate + butyl octanoate	12.3	22.9	10.9	9.6	4.1	4.0
21.68	9.93	methyl 4-decenoate	1.3	1.9	1.7	2.0	1.3	1.2
22.07	10.09	ethyl 2,4 octadienoate	1.5	1.9	2.9	3.3	1.9	1.7
22.44	10.24	ethyl 4-decenoate	4.5	1.9	1.3	1.2	0.7	0.6
22.60	10.30	unknown	1.2	2.1	3.1	4.3	2.8	3.3
22.92	10.42	methyl 2,4,6 octatrienoate	nd	nd	0.5	nd	nd	nd
23.80	10.75	methyl 2-decenoate	nd	0.5	0.5	0.8	0.6	0.6
23.93	10.80	α-farnesene (E,Z)	9.1	3.7	2.7	3.3	2.1	2.1
24.41	10.98	α-farnesene (E,E)	552.5	193.7	129.1	135.0	75.6	75.7
25.12	11.23	methyl trans-2, cis-4 decadienoate	7.7	9.3	7.6	9.5	5.2	5.3
25.58	11.39	unknown	1.1	nd	nd	nd	nd	nd
25.84	11.48	ethyl cis-2, cis-4 decadienoate	0.5	0.7	0.6	0.3	0.6	0.5
26.08	11.56	ethyl trans-2, cis-4 decadienoate	10.7	14.3	19.7	26.0	14.9	15.0
26.26	11.62	unknown	nd	0.7	0.8	1.0	0.7	0.9
26.40	11.66	methyl trans-2, trans-4 decadienoate	nd	nd	nd	nd	nd	0.2
26.83	11.81	ethyl trans-2, trans-4, cis-7 decatrienoate	nd	nd	nd	nd	nd	0.9
27.04	11.87	ethyl trans-2, trans-4, cis-7 decadienoate	0.9	2.3	2.9	3.3	1.9	2.7
27.77	12.11	unknown	nd	1.5	1.0	0.7	1.1	0.8
29.03	12.50	methyl tetradecenoate	0.2	1.6	2.1	1.9	nd	1.5
29.63	12.68	ethyl tetradecenoate	0.9	1.2	1.7	2.4	1.7	1.1
30.38	12.90	ethyl cis-5, cis-8 tetradecadienoate	1.8	nd	2.8	3.5	1.1	3.1
31.08	13.10	unknown	1.3	0.9	2.4	2.3	2.2	3.1

(Continued)

Table A.6.27. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	2	4	6	8	10
32.00	13.36	unknown	1.1	0.6	0.8	0.6	nd	nd
32.13	13.39	unknown	nd	0.2	0.5	0.4	0.4	0.5
33.10	13.65	methyl 7-hexadecadecenoate	nd	nd	nd	0.4	nd	0.6
34.13	13.92	unknown	nd	nd	0.5	0.6	nd	2.1
37.04	14.64	unknown	nd	nd	1.1	0.8	nd	0.5
Total			661.0	367.3	265.1	279.3	177.9	187.3

[a] Fruits were harvested at mature unriped stage (flesh firmness 62 N) and stored for 6 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.28. Changes in volatile compounds during ripening of 'Anjou' pear harvested at late stage of maturity (M3) after storage at 0°C for 3 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.01	1.99	ethyl acetate	nd	nd	nd	nd	nd	0.3	2.9
6.34	2.39	ethanol	nd	nd	nd	0.3	0.7	0.8	2.3
6.90	3.02	propyl acetate	nd	nd	nd	nd	nd	0.1	0.4
8.53	4.35	butyl acetate	0.9	4.7	7.9	10.5	14.4	18.1	14.6
9.56	4.86	isoamyl acetate	nd	nd	nd	nd	0.2	0.4	nd
9.77	4.96	1-butanol	nd	0.2	0.5	0.5	0.6	0.8	0.6
10.78	5.40	pentyl acetate	nd	1.0	0.3	1.6	2.2	2.9	2.2
11.93	5.85	1-methylpropyl butanoate + butyl butanoate	0.5	3.8	3.3	1.8	0.3	0.1	0.7
12.34	6.00	ethyl hexanoate	nd	nd	nd	0.4	nd	0.2	0.2
13.26	6.42	hexyl acetate	2.8	18.6	23.4	39.4	58.8	77.9	63.8
15.02	7.14	1-hexanol	0.4	1.3	1.5	1.7	2.2	2.4	2.4
15.86	7.46	heptyl acetate	nd	nd	nd	0.2	0.3	1.2	1.1
16.22	7.59	methyl octanoate	nd	nd	nd	0.5	nd	nd	0.4
16.94	7.84	butyl hexanoate+hexyl butanoate	12.7	50.8	52.4	36.6	17.0	9.9	10.7
17.32	7.97	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	0.3	0.3	1.3	1.5	1.3	0.9
17.40	8.00	ethyl octanoate	nd	nd	0.8	3.1	3.1	2.4	1.2
19.22	8.88	pentyl hexanoate	0.1	2.0	3.0	2.4	1.3	0.3	0.3
19.43	8.97	4-octen-1-ol acetate	nd	nd	nd	0.4	0.3	0.5	nd
21.06	9.68	methyl decanoate	nd	nd	1.0	1.8	0.6	0.3	0.4
21.47	9.85	hexyl hexanoate + butyl octanoate	7.1	33.8	41.7	36.8	19.0	7.4	10.1
21.68	9.93	methyl 4-decenoate	nd	nd	1.1	1.5	1.5	0.3	1.3
22.05	1nd8	ethyl 2,4 octadienoate	nd	0.7	4.6	8.5	6.6	3.1	3.7
22.43	10.23	ethyl 4-decenoate	3.5	3.5	2.3	1.1	0.6	nd	nd
22.58	10.29	methyl 2,4,6 octatrienoate	nd	0.1	1.3	2.4	2.3	1.5	2.9
23.79	10.75	methyl 2-decenoate	nd	nd	nd	0.8	0.6	nd	0.5
23.93	10.80	alpha farnesene (E,Z)	3.2	3.2	3.3	3.0	2.2	1.9	2.3
24.40	10.97	alpha farnesene (E,E)	334.8	298.1	199.8	161.3	121.8	76.0	89.7
24.53	11.02	ethyl trans-2 decenoate	nd	nd	nd	0.7	0.5	nd	0.5
25.10	11.22	methyl trans-2, cis-4 decadienoate	0.2	3.2	7.8	11.0	11.8	7.8	10.7
25.56	11.38	unknown	0.9	0.8	0.5	nd	nd	nd	nd
25.67	11.42	ethyl cis-2, cis-4 decadienoate	nd	nd	0.4	0.7	nd	nd	nd
26.06	11.55	ethyl trans-2, cis-4 decadienoate	0.5	7.2	14.2	18.2	18.5	12.1	19.6
26.47	11.69	methyl trans-2, trans-4 decadienoate	nd	0.3	0.4	1.3	1.2	0.3	0.4
27.04	11.88	ethyl trans-2, trans-4, cis-7 decadienoate	nd	0.7	2.3	4.0	3.5	1.7	2.7
27.78	12.11	unknown	0.2	nd	0.3	nd	nd	nd	nd
27.82	12.12	unknown	0.8	nd	nd	nd	nd	nd	nd
29.07	12.51	methyl tetradecenoate	nd	0.8	1.5	1.5	1.8	0.7	1.5
29.58	12.66	ethyl tetradecanoate	nd	nd	nd	0.1	nd	nd	nd
29.63	12.68	unknown	nd	nd	nd	1.9	3.2	2.0	3.2
29.82	12.74	unknown	nd	nd	nd	nd	nd	1.1	0.2
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	nd	nd	0.8	2.6	4.2	3.2	5.2
31.08	13.10	unknown	nd	nd	nd	1.2	1.8	1.8	3.5
31.98	13.35	unknown	0.2	0.4	1.1	1.1	0.7	nd	0.2

(Continued)

Table A.6.28. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
32.15	13.40	unknown	nd	nd	nd	0.4	0.6	0.2	0.6
33.12	13.66	methyl 7-hexadecadecenoate	nd	nd	nd	nd	nd	nd	0.5
37.05	14.64	unknown	nd	nd	nd	0.6	0.7	nd	nd
Total			369.0	435.4	377.8	363.0	306.3	241.3	264.4

[a] Fruits were harvested at mature unriped stage (flesh firmness 58 N) and stored for 3 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.29. Changes in volatile compounds during ripening of 'Anjou' pear harvested at late stage of maturity (M3) after storage at 0°C for 4 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
5.34	1.12	acetaldehyde	nd	nd	nd	nd	nd	0.3	1.4
6.05	2.04	ethyl acetate	nd	nd	nd	0.3	nd	0.7	1.9
6.33	2.38	ethanol	nd	nd	0.1	0.5	0.7	1.1	1.0
7.07	3.20	propyl acetate	nd	nd	0.4	0.3	0.3	0.3	nd
7.84	3.96	ethyl butanoate	nd	0.2	nd	nd	nd	nd	nd
8.54	4.36	butyl acetate	5.2	4.8	8.1	7.4	7.3	5.9	7.6
9.69	4.92	isoamyl acetate	nd	nd	nd	nd	nd	nd	0.2
9.75	4.95	1-butanol	nd	0.3	0.3	0.7	0.4	0.5	1.1
10.69	5.36	pentyl acetate	nd	nd	nd	nd	nd	0.3	nd
11.94	5.86	1-methylpropyl butanoate	3.0	0.6	1.3	1.2	1.1	1.0	1.4
11.95	5.86	butyl butanoate	nd	2.1	2.0	0.7	0.4	0.3	0.3
13.27	6.43	hexyl acetate	13.1	nd	0.2	0.4	0.3	0.3	0.2
14.76	7.05	unknown	0.8	10.7	12.7	9.4	10.4	7.0	9.8
15.01	7.14	1-hexanol	0.9	1.0	1.3	0.9	0.8	0.9	1.0
15.85	7.46	heptyl acetate	nd	nd	0.4	0.4	0.6	0.5	0.6
16.91	7.83	butyl hexanoate+hexyl butanoate	26.4	31.6	27.1	11.8	6.0	4.0	4.2
17.31	7.97	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	0.6	0.8	0.9	0.8	0.7	0.7
17.40	8.00	ethyl octanoate	nd	nd	1.4	1.3	1.2	0.8	0.8
18.33	8.46	octyl acetate	nd	nd	nd	0.2	0.3	0.2	0.3
19.22	8.87	pentyl hexanoate	0.1	1.2	1.6	0.7	0.4	0.3	0.1
19.44	8.97	4-octen-1-ol acetate	nd	0.5	0.6	0.4	0.3	0.3	0.1
21.05	9.67	methyl decanoate	nd	0.2	0.7	0.5	0.3	0.2	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	18.2	27.2	26.3	10.2	5.8	2.9	3.2
21.67	9.93	methyl 4-decenoate	nd	0.6	1.0	0.7	0.7	0.5	0.6
22.06	10.09	ethyl 2,4 octadienoate	1.1	1.5	4.3	3.1	2.6	nd	1.2
22.42	10.23	ethyl 4-decenoate	6.5	2.5	1.9	0.6	0.5	nd	0.2
22.60	10.30	methyl 2,4,6 octatrienoate	nd	1.1	2.1	1.4	1.6	1.2	1.6
23.80	10.75	methyl 2-decenoate	nd	0.3	0.4	0.3	0.3	0.2	0.3
23.92	10.80	α-farnesene (E,Z)	8.6	2.9	2.8	1.5	2.0	1.2	1.6
24.41	10.98	α-farnesene (E,E)	854.2	293.4	229.7	90.3	105.0	49.3	56.8
25.11	11.22	methyl trans-2, cis-4 decadienoate	5.0	4.9	6.8	5.1	4.8	3.4	3.7
25.56	11.38	unknown	1.5	0.3	0.4	0.2	nd	nd	nd
25.67	11.42	ethyl cis-2, cis-4 decadienoate	nd	0.4	0.6	0.3	nd	nd	nd
25.82	11.47	unknown	nd	0.2	0.4	0.3	0.3	0.3	0.5
26.07	11.55	ethyl trans-2, cis-4 decadienoate	6.2	8.4	14.1	9.7	11.4	7.7	9.7
26.28	11.62	unknown	nd	0.3	0.8	0.6	0.7	0.4	0.3
26.46	11.68	methyl trans-2, trans-4 decadienoate	nd	0.2	0.3	nd	nd	nd	nd
27.04	11.88	ethyl trans-2, trans-4, cis-7 decadienoate	nd	1.5	2.6	1.8	1.7	1.1	1.0
27.80	12.12	unknown	nd	nd	nd	0.3	0.2	0.1	0.7
29.05	12.51	methyl tetradecenoate	0.8	0.8	1.2	0.9	1.2	0.8	nd
29.55	12.66	ethyl tetradecanoate	0.7	0.4	0.2	nd	nd	nd	nd
29.63	12.68	unknown	nd	nd	0.9	1.0	1.3	0.8	0.7
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	1.1	0.7	1.1	1.4	2.0	1.5	1.1

(Continued)

Table A.6.29. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
31.08	13.10	unknown	0.7	0.5	1.0	1.0	1.4	1.2	1.1
31.99	13.35	unknown	2.0	1.1	1.5	0.9	1.1	0.5	0.5
32.15	13.40	unknown	nd	nd	nd	0.3	0.5	0.4	0.2
33.11	13.66	methyl 7-hexadecadecenoate	nd	nd	nd	0.2	0.3	0.2	nd
34.12	13.92	unknown	nd	0.2	0.9	nd	nd	0.1	nd
Total			956.1	403.4	360.5	170.2	177.3	99.4	117.4

[a] Fruits were harvested at mature unriped stage (flesh firmness 58 N) and stored for 4 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.30. Changes in volatile compounds during ripening of 'Anjou' pear harvested at late stage of maturity (M3) after storage at 0°C for 5 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C						
			0	2	4	6	8	10	12
6.05	2.04	ethyl acetate	nd	nd	0.4	0.3	nd	1.2	nd
6.35	2.40	ethanol	nd	0.4	1.2	2.7	nd	3.5	3.0
6.96	3.08	propyl acetate	nd	nd	0.4	0.4	nd	1.2	0.8
8.56	4.37	butyl acetate	5.5	10.9	13.9	22.9	13.9	17.1	15.2
9.58	4.87	isoamyl acetate	nd	nd	nd	4.3	nd	nd	0.8
9.78	4.96	1-butanol	nd	1.3	0.8	4.9	1.6	1.7	2.0
10.77	5.40	pentyl acetate	nd	2.3	2.3	5.3	2.8	2.8	2.6
11.97	5.87	1-methylpropyl butanoate + butyl butanoate	2.4	3.2	2.8	2.3	1.0	0.7	nd
12.33	6.00	ethyl hexanoate	nd	nd	nd	0.5	0.7	1.3	1.3
13.29	6.44	hexyl acetate	17.6	61.2	35.7	40.2	45.2	54.8	53.6
15.03	7.15	1-hexanol	1.4	2.9	2.2	2.5	2.7	2.7	2.5
15.86	7.46	heptyl acetate	nd	nd	nd	0.8	0.6	1.0	1.0
16.95	7.85	butyl hexanoate+hexyl butanoate	17.6	52.9	40.8	26.2	15.4	nd	9.1
17.28	7.96	butyl 2-methylbutanoate	nd	nd	nd	nd	nd	nd	0.1
17.31	7.97	butyl 4-hexenoate	nd	0.8	1.3	1.4	0.8	nd	0.8
17.39	8.00	ethyl octanoate	nd	nd	3.1	3.1	2.0	3.2	2.2
19.26	8.89	pentyl hexanoate	0.7	2.2	2.5	1.6	nd	1.0	nd
19.44	8.97	4-octen-1-ol acetate	nd	1.0	nd	nd	nd	nd	nd
21.06	9.68	methyl decanoate	nd	0.7	nd	1.4	nd	nd	nd
21.49	9.86	hexyl hexanoate + butyl octanoate	9.3	38.6	30.7	20.1	9.5	7.7	5.7
21.68	9.93	methyl 4-decenoate	0.6	1.2	2.3	2.4	nd	1.2	1.2
22.05	10.08	ethyl 2,4 octadienoate	nd	2.9	7.5	7.8	2.3	2.9	2.3
22.44	10.24	ethyl 4-decenoate	5.6	4.5	3.2	1.6	nd	0.8	4.7
22.60	10.30	methyl 2,4,6 octatrienoate	nd	1.3	4.4	5.1	2.9	3.8	0.7
23.61	10.68	methyl 2-decenoate	nd	0.1	1.0	1.2	nd	nd	nd
23.93	10.80	α-farnesene (E,Z)	7.3	7.4	6.9	6.7	3.9	4.4	4.6
24.41	10.97	α-farnesene (E,E)	520.1	512.4	403.9	274.3	255.6	156.0	142.6
25.13	11.23	methyl trans-2, cis-4 decadienoate	4.5	9.8	12.8	13.3	8.2	8.3	7.8
25.56	11.38	unknown	1.2	1.0	0.9	nd	nd	nd	nd
25.66	11.42	ethyl cis-2, cis-4 decadienoate	nd	1.1	0.9	nd	nd	nd	nd
25.82	11.47	unknown	nd	0.9	1.1	1.0	nd	1.3	1.9
26.08	11.56	ethyl trans-2, cis-4 decadienoate	4.3	15.9	29.4	34.2	30.7	27.2	28.9
26.28	11.62	unknown	nd	nd	1.3	2.1	nd	0.9	1.0
26.45	11.68	methyl trans-2, trans-4 decadienoate	nd	0.7	nd	nd	nd	nd	nd
27.02	11.87	ethyl trans-2, trans-4, cis-7 decadienoate	nd	2.9	5.7	6.1	2.6	3.6	3.0
27.82	12.12	unknown	1.0	0.6	1.6	1.3	nd	0.3	1.3
28.64	12.38	unknown	nd	nd	nd	0.6	nd	0.7	0.5
29.06	12.51	methyl tetradecenoate	nd	2.5	3.3	4.2	3.0	2.7	0.1
29.55	12.66	ethyl tetradecanoate	nd	1.1	1.6	4.0	2.4	2.0	2.2
30.38	12.90	ethyl cis-5, cis-8 tetradecadienoate	1.2	1.1	4.0	6.5	5.0	5.0	4.7
31.08	13.10	unknown	nd	nd	2.8	4.2	4.7	4.4	4.4
31.48	13.21	unknown	nd	nd	0.7	0.8	0.7	nd	nd
32.01	13.36	unknown	1.0	2.0	2.7	2.5	1.9	1.2	0.9
32.13	13.39	methyl 7-hexadecadecenoate	nd	nd	1.0	1.2	0.9	1.1	0.9

(Continued)

Table A.6.30. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C 0	2	4	6	8	10	12
33.11	13.66	unknown	2.5	0.5	0.4	0.3	0.5	nd	0.5
33.61	13.79	unknown	nd	nd	0.4	nd	0.3	0.8	0.7
34.12	13.92	unknown	nd	0.3	0.6	0.7	nd	nd	nd
Total			603.9	748.2	638.6	523.0	421.8	328.5	315.7

[a] Fruits were harvested at mature unriped stage (flesh firmness 58 N) and stored for 5 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).

Table A.6.31. Changes in volatile compounds during ripening of 'Anjou' pear harvested at late stage of maturity (M3) after storage at 0°C for 6 months[a].

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C					
			0	2	4	6	8	10
6.08	2.08	ethyl acetate	nd	nd	nd	2.0	1.0	nd
6.38	2.43	ethanol	nd	0.6	0.9	2.2	3.0	2.5
7.06	3.19	propyl acetate	0.3	nd	0.1	0.5	0.3	nd
8.55	4.37	butyl acetate	5.1	7.7	9.7	15.4	10.9	7.4
9.77	4.96	1-butanol	0.4	1.0	2.7	1.6	1.5	1.2
10.77	5.40	pentyl acetate	0.4	2.0	4.3	2.9	2.5	2.0
11.96	5.86	1-methylpropyl butanoate + butyl butanoate	1.8	1.3	1.7	1.7	1.0	0.7
12.37	6.01	ethyl hexanoate	nd	nd	0.8	0.7	0.3	0.3
13.28	6.43	hexyl acetate	15.3	56.3	34.1	36.0	32.1	23.0
14.77	7.05	unknown	0.9	nd	nd	nd	nd	nd
15.02	7.15	1-hexanol	1.2	3.1	nd	3.0	2.5	1.6
15.48	7.32	2-methylpropyl hexanoate	nd	nd	nd	0.1	0.5	0.3
15.85	7.46	heptyl acetate	nd	1.0	0.1	0.8	0.6	nd
16.94	7.84	butyl hexanoate+hexyl butanoate	15.1	31.3	19.4	24.5	17.2	9.9
17.32	7.97	butyl 2-methylbutanoate+butyl 4-hexenoate	nd	2.0	2.2	2.5	1.4	0.9
17.40	8.00	ethyl octanoate	nd	nd	nd	nd	1.4	nd
18.34	8.46	octyl acetate	nd	0.5	nd	nd	nd	nd
19.25	8.89	pentyl hexanoate	0.8	nd	1.5	2.3	1.6	0.9
19.45	8.98	4-octen-1-ol acetate	nd	0.8	0.2	0.5	0.2	nd
21.05	9.68	methyl decanoate	0.4	nd	0.4	0.6	0.4	nd
21.48	9.85	hexyl hexanoate + butyl octanoate	9.3	26.4	15.7	18.5	11.1	6.0
21.67	9.93	methyl 4-decenoate	0.9	1.9	1.3	1.8	1.6	1.1
22.05	10.08	ethyl 2,4 octadienoate	1.2	1.7	2.1	3.7	2.6	1.7
22.42	10.23	ethyl 4-decenoate	3.9	3.2	1.6	1.6	1.1	nd
22.61	10.30	methyl 2,4,6 octatrienoate	1.5	3.4	3.8	5.5	5.5	3.7
23.80	10.75	methyl 2-decenoate	nd	0.6	0.8	0.8	0.7	0.5
23.92	10.80	alpha farnesene (E,Z)	9.9	7.1	4.2	5.1	3.7	2.7
24.40	10.97	alpha farnesene (E,E)	515.7	298.7	152.7	158.9	121.1	76.3
25.12	11.23	methyl trans-2, cis-4 decadienoate	7.0	10.0	7.5	8.5	7.9	3.6
25.56	11.38	2-phenylethyl acetate	1.1	0.8	0.3	nd	nd	nd
25.82	11.47	unknown	0.7	0.8	0.7	1.0	1.0	0.8
26.07	11.55	ethyl trans-2, cis-4 decadienoate	11.3	17.8	20.4	24.7	27.1	14.3
26.28	11.63	methyl trans-2, trans-4 decadienoate	0.5	0.3	0.7	0.9	0.9	0.6
27.03	11.87	ethyl trans-2, trans-4, cis-7 decadienoate	1.0	2.1	2.5	3.5	2.8	1.8
27.82	12.12	unknown	1.3	0.5	1.1	1.5	0.2	nd
29.05	12.51	methyl tetradecenoate	1.4	2.6	3.1	4.6	3.4	2.5
29.56	12.66	ethyl tetradecanoate	0.4	0.4	0.7	1.4	1.5	0.9
30.37	12.90	ethyl cis-5, cis-8 tetradecadienoate	1.8	1.2	1.5	3.1	3.9	2.1
31.07	13.10	unknown	1.8	1.5	2.1	3.7	4.1	3.3

(Continued)

Table A.6.31. Continued.

Rt[b] (min.)	I_E[c]	Compounds	Days at 20°C 0	2	4	6	8	10
31.99	13.35	unknown	1.4	1.6	1.3	1.4	1.1	0.7
32.13	13.39	unknown	nd	nd	0.4	0.7	0.7	0.6
Total			614.0	490.2	302.7	348.3	280.4	173.8

[a] Fruits were harvested at mature unriped stage (flesh firmness 58 N) and stored for 6 months at 0°C before ripening at 20°C. Compounds were trapped from static headspace of fruits sample and analysed using SPME technique. Units are calculated based on 180.8 µg propyl tiglate used as internal standard (response factor = 1) and expressed in µg compound kg^{-1} hr^{-1}. nd referred to not detected or detected below 0.05 µg kg^{-1} hr^{-1}.

[b] Retention times on 25m x 0.2mm i.d. x 0.2µm Carbowax 20M column (temperature program: initial hold at 60°C for 5min then increased to 220°C at rate of 5°C/min).

[c] Ethyl ester retention indices (Van den Dool and Kratz, 1963).