Apple Flavor

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I. INTRODUCTION

Apples are one of the most important fruit crops with about 40 million tonnes produced annually worldwide. Major improvements have been achieved in the last five decades in cultivar selection and improvement, cultural practices, and in developing and improving technologies for fruit preservation. There is a broad range of cultivars produced worldwide, and many of them can be preserved fresh for up to almost a year. Improvement of fruit flavor, however, is still not easily achieved. As is the case for other fruits, apple flavor is not yet well understood despite the fact that apple aroma is one of the best known natural flavors. A great deal of research has been done on apple flavor (mostly on apple aroma) in the last 10 years, and interest in the subject seems to be increasing. A few reviews have been written on apple quality (Fisher and Kitson 1985; Watada and Abbott 1985; Perring 1989) and some on apple odor, which is called apple flavor in most reviews (Williams 1977; Williams et al. 1977a; Paillard 1981; Dimick and Hoskin 1983; Acree and McLellan 1989; Berger 1991). The review written

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by Acree and McLellen considered all components of apple flavor, but was intended more for the flavor of processed rather than fresh fruit.

Flavor is complex in nature and is determined by numerous sensations acting simultaneously on the brain. The components of flavor are interrelated and are difficult to understand, but they can be divided into two major parts: taste and odor. Taste is the perception of chemicals by the taste buds located on the tongue. There are different receptors on the human tongue giving rise to different taste sensations, the five more accepted ones being sweet, sour, salty, bitter, and umami. Umami, which was shown to be a basic taste (Kawamura and Kare 1986), is the perception of body or meatiness. Umami, which means delicious or delightful in Japanese, is characterized by the taste of L-glutamates, short-chain peptides, and 5′nucleotides, and does not seem to play a very important role in the flavor of fresh fruits.

Odor depends on the volatility of the substance and is perceived by receptors located in the mucous layer of the ceiling of the nose. Volatility is not the only prerequisite for a compound to possess odor. It is still not known why a compound is odorous. Unlike taste, which has only five dimensions, there seems to be hundreds of different odors (Acree 1980). Several quality factors, of which texture is the most important one, can affect flavor perception (Moncrieff 1967). All factors affecting fruit development, maturation, ripening, and senescence affect the development and metabolism of flavor.

Because of the lack of understanding of the fundamental nature of flavor perception, objective means of measuring flavor have not been fully developed. Sensory analysis, which is mostly subjective and can be expensive and time consuming, is at present the only way to determine human perception of flavor. Flavor has been commonly characterized by identifying the significant attributes and determining their intensities (Williams and Carter 1977). What is needed now is the understanding and characterizing of human perception and sensory response to flavor, to be able to develop adequate techniques for measurement.

This review presents a comprehensive summary of the components of apple flavor, and how they are affected by various preharvest and postharvest factors.

II. APPLE FLAVOR

Apple flavor is complex and diverse. It is perceived as many sensations such as sweetness, sourness, bitterness, astringency, or fruitiness. Several attempts have been made to identify and understand apple flavor and to evaluate it objectively (Williams et al. 1977a; Watada et al. 1980).
Most studies on the characterization of apple flavor and quality were based on sensory analysis; and those studies generated different attributes, which were recognized by sensory panels. Williams and Carter (1977) developed a vocabulary and assessment procedure for sensory assessment of apples using seven parameters: appearance, feel of the apple in the hand, external and internal apple aroma, texture, taste, and after taste. Various analytical methods have been tried to measure apple flavor components (Williams 1977). However, only soluble solids and acidity measurements are commonly used by the apple industry.

The importance of flavor, especially aroma, to the quality of apples is not clearly defined. Results of several consumer surveys in the United Kingdom suggested that the overall acceptability of apples is principally related to their texture (Smith 1984). However, recent improvements in storage techniques have made it possible to maintain minimal firmness losses in storage. It is very desirable to have better flavor, and especially aroma, in these firm apples. Williams (1979) suggested that certain minimum thresholds for aroma apply when textural requirements are met.

A. Apple Taste

Sweetness and acidity are the dominant taste attributes of dessert apples. Apple taste is mostly related to the balance between sugars and acids. Sweetness in apples is caused by three sugars: sucrose, glucose, and fructose. In most cases 50% of the sugar is fructose. Malic acid (and citric acid in some cultivars) contributes to sourness and acidity. Sugars and acids in apples are easily measured and their synthesis and metabolism are fairly well understood. Therefore, their presence can be easily predicted and manipulated.

Sugar and acid content vary among cultivars. Goodenough and Atkins (1981) stated that high quality dessert apples are in the medium acid category (pH 3.2–3.5) and have a relatively high sugar content (14–16%), whereas most good cooking apples have high acidity (pH 2.8–3.2) and moderate sugar content (11–13%). ‘Delicious’ and ‘Spartan’ apples are in the low acid (pH 3.5–3.7), low sugar (9–11%) category.

Reducing sugar and sucrose increase throughout the growth period. The total sugar content of apples is essentially decided at harvest, and fruit respiration during storage consumes no more than 10% of the initial sugar levels (Fidler and North 1967). Sucrose is slowly hydrolysed during storage to glucose and fructose, but this change probably has little effect on sweetness. The major change after harvest is in the acid level because it may fall by as much as 50%. Sugars and malic acid are the main substrates for respiration.
Phenolics are present in high concentration during the early stages of growth, and then decline to a constant level with little further change during ripening (Hulme and Rhodes 1971). The amount of phenolic compounds are thought to be too low in ripe apples to contribute significantly to astringency or bitterness (Knee and Sharples 1981). Furthermore, astringency and bitterness only occur rarely in fresh apples, and only an empirical relationship has been observed between phenolics and astringency. Bitterness in apple juice has been attributed to the phenolic compound procyanidin (Lea and Timberlake 1974).

B. Apple Aroma

Although taste and texture sensations are very important, it is the presence of trace amounts of volatile components responsible for odor that gives much of the character to the fruit. It has long been recognized that odor in apples is the result of numerous volatiles (Power and Chesnut 1920). Early research was limited by the insensitive analytical techniques then available. Most early studies were done on "total organic volatiles," either by complete combustion or by absorption in sulfuric acid followed by oxidation with ceric sulfate (Smock and Neubert 1950). As early as 1920 (Power and Chesnut 1920) a few apple volatiles were identified, and those responsible for apple aroma were thought to be primarily acetaldehyde and esters of formic, acetic, and hexanoic acids. Geraniol was identified in 1922 (Power and Chesnut 1922), and the important aldehydes hexanal, (E)-2-hexenal, and furfural were identified in 1950 (White 1950).

Detection of volatile compounds at the parts per billion level became possible in the late 1950s due to the introduction of gas chromatography, the use of sensitive detectors such as the flame ionization detector, and the application of more powerful analytical tools, such as the combined gas chromatography–mass spectrometry (GC–MS), infrared, and proton magnetic resonance spectrometry. Since then, the list of volatiles identified in apples has grown at a faster rate (Figure 6.1). More than 350 volatiles are now listed to be produced by apples and apple products (Maarse 1991). The number of compounds occurring in very low concentrations (as low as 1 picogram) is thought to be close to 1,000 (Acree and McLellan 1989). Apple volatiles (Table 6.1) are characterized as being of relatively low molecular weight, belonging to many different chemical classes, and present in trace amounts (Nursten 1970; Salunkhe and Do 1977; Dimick and Hoskin 1983; Acree and McLellan 1989; Maarse 1991).

1. Olfactory Assays. Volatiles do not contribute equally to odor, and in fact, the odor of apple seems to depend on the presence of only a few
Fig. 6.1. Number of volatiles identified to be produced by apples since 1920 (Williams 1978; Maarse 1991).

Volatiles (Cunningham et al. 1986). In the last three decades efforts have been directed to investigate the sensory impact of individual volatile compounds. This was done first by splitting the effluent of the gas chromatograph between the detector and a port outside the oven. Effluent emerging from the outside port was sniffed, and the different peaks emerging from passing the second part of the effluent through the detector were characterized for their odor (Guadagni et al. 1966). By this method Flath et al. (1967) found that ethyl 2-methylbutanoate, hexanal, and (E)-2-hexenal, with threshold concentrations of 0.1, 5.0, and 17.0 ppb (v/v), respectively, were the main volatile components responsible for the odor in the essence of ‘Delicious’ apple juice. Although splitting the GC effluent for sniffing paved the way for some understanding of the sensory significance of volatiles, it had some physical and mechanical problems (Acree et al. 1976). Some of these problems were overcome by the development of a “sniffer,” in which the GC effluent was mixed with a large volume of humid air (Acree et al. 1976). Acree et al. (1984) later developed “Charm,” a bioassay for the measurement of odor intensity. “Charm” is a qualitative and quantitative analysis of gas chromato-
Table 6.1. Volatiles Identified to be Produced by Apple Fruit and Apple Juice to Date.

**ALCOHOLS**
methanol, ethanol, n-propanol, i-propanol, isopropanol, n-butanol, 2-butanol, pentanol, 2-methyl butanol, 2-methylbutan-1-ol, 2-methyl-2-butanol, 3-methyl butanol, 2-pentanol, 3-pentanol, 4-pentanol, 2-methyl-2-pentanol, 3-methyl pentanol, 4-methyl pentanol, hexanol, (E)-2-hexanol, (E)-3-hexanol, (E)-2-hexanol, (E)-3-hexanol, 1-hexen-3-ol, 5-hexenol, (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, (E)-2-hexen-1-ol, (E)-2-hexan-2-yl, heptanol, 2-heptanol, 6-methyl-5-heptanol, octanol, 2-octanol, 3-octanol, (Z)-3-octen-1-yl, (Z)-5-octenol, nonanol, 2-nonanol, 6-methyl-5-heptanol, decanol, 3-octenol, a-terpinol, terpen-4-ol, benzyl alcohol, 2-phenethanol, terpinen-4-ol, isoboreol, citral, geraniol, eugenol

**ALDEHYDES**
formaldehyde, acetaldehyde, propanal, 2-oxopropanal, 2-propenal, butanal, isobutanal, 2-methyl butanal, (E)-2-butanal, pentanal, isopentanal, hexanal, (E)-2-hexenal, (Z)-3-hexenal, (E)-3-hexenal, octanal, nonanal, decanal, undecanal, dodecanal, benzaldehyde, phenyacetaldehyde

**KETONES**
2-propanone, 2-butanone, 3-hydroxybutan-2-one, 2,3-butanedione, 2-pentanone, 3-pentanone, 4-methylpentane-2-one, 2-hexanone, 2-heptanone, 3-heptanone, 2-octanone, 7-methyloctan-4-one, acetophenone, gamma-undecalactone

**ACIDS**
formic acid, acetic acid, propanoic acid, butanoic acid, isobutanoic acid, 2-methyl butanoic acid, 3-methyl butanoic acid, pentanoic acid, 4-methyl pentanoic, hexanoic acid, (E)-2-hexanoic acid, heptanoic acid, (E)-3-heptanoic acid, octanoic acid, (Z)-octenoic acid, nonanoic acid, (Z)-3-nonenolic acid, decanoic acid, decenoic acid, undecanoic acid, undecenoic acid, dodecanoic acid, dodecenoic acid, tridecanoic acid, tridecenoic acid, tetradecanoic acid, tetradecenoic acid, pentadecanoic acid, hexadecanoic acid, hexadecenoic acid, heptadecanoic acid, heptadecenoic acid, octadecanoic acid, 9-octadecenoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, nonanoic acid, decanoic acid, decenoic acid, nonadecanoic acid, nonadecanoic acid, eicosanoic acid, benzoic acid

**ESTERS**
methyl formate, ethyl formate, propyl formate, butyl formate, 2-methyl butyl formate, 3-methyl butyl formate, pentyl formate, i-pentyl formate, hexyl formate, methyl acetate, ethyl acetate, propyl acetate, butyl acetate, isobutyl acetate, t-butyl acetate, pentyl acetate, 2-methyl butyl acetate, 3-methyl butyl acetate, hexyl acetate, heptyl acetate, octyl acetate, benzyl acetate, (Z)-3-hexenyll acetate, (E)-hex-3-enyl acetate, (E)-2-hexenyl acetate, 2-phenyl ethyl acetate, n-octyl acetate, nonyl acetate, decyl acetate, methyl n-propionate, ethyl propionate, ethyl 2-methyl propionate, ethyl hydroxy propionate, propyl propionate, butyl propionate, isobutyl propionate, 2-methyl butyl propionate, 3-methyl butyl propionate, hexyl propionate, methyl butyrate, ethyl butyrate, propyl butyrate, isobutyrate, isobutyrate, butyrate, isobutyrate, pentyl butyrate, hexyl butyrate, cinnamyl butyrate, ethyl crotonate, methyl isobutyrate, ethyl isobutyrate, butyl isobutyrate, pentyl isobutyrate, hexyl isobutyrate, methyl 2-methylbutyrate, ethyl 2-methylbutyrate, propyl 2-methylbutyrate, butyl 2-methylbutyrate, isobutyl 2-methylbutyrate, pentyl 2-methylbutyrate, hexyl 2-methylbutyrate, 2-methylbutyl butyrate, ethyl 2-methylbutyrate, propyl 2-methylbutyrate, butyl 2-methylbutyrate, isobutyl 2-methylbutyrate, pentyl 2-methylbutyrate, hexyl 2-methylbutyrate, 2-methylbutyl butyrate,
Table 6.1. *Continued*

3-methylbutyl butyrate, 2-methylbutyl octanoate, methyl pentanoate, ethyl pentanoate, propyl pentanoate, butyl pentanoate, amyl pentanoate, isoamyl pentanoate, hexyl pentanoate, methyl isopentanoate, ethyl isopentanoate, isopentenyl isopentanoate, methyl hexanoate, ethyl hexanoate, propylhexanoate, butyl hexanoate, isobutyl hexanoate, butyl-(E)-hex-2-enoate, pentyl hexanoate, 2-methyl butyl hexanoate, 3-methyl butyl hexanoate, butyl trans-2-hexanoate, ethyl heptanoate, hexyl heptanoate, propyl heptanoate, butyl heptanoate, ethyl octanoate, propyl octanoate, butyl octanoate, isobutyl octanoate, pentyl octanoate, isopentyl octanoate, hexyl octanoate, ethyl nonoate, ethyl decanoate, butyl decanoate, iso-butyl decanoate, pentyl decanoate, isopentyl decanoate, hexyl decanoate, ethyl dodecanoate, butyl dodecanoate, hexyl dodecanoate, diethyl succinate, ethyl 2-phenylacetate, dimethylphthalate, diethylphthalate, dipropylphthalate

**MISCELLANEOUS**

diethyl ether, methyl propyl ether, dibutyl ether, 2-methyl butylether, methyl propyl ether, 3-methyl butyl ether, dihexyl ether, methyl phenyl ether, 4-methoxyallyl benzene, (Z)-linalool oxide, (E)-linalool oxidoeethylamine, butylamine, isoamylamine, hexylamine, diethoxymethane, dibutoxyethane, dihexoxymethane, dihexoxyethane, 1-ethoxy-1-propoxyethane, 1-butoxy-1-ethoxyethane, 1-etoxy-1-hexoxyethane, 1-ethoxy-1-octoxyethane, 1,1-diethox-yethane, 1,1-dibutoxyethane, 1-butoxy-1-2-methylbutoxy ethane, 1,1-diisobutoxyethane, 1-butoxy-1-hexoxyethane, 1,1-di-2-methylbutoxyethane, 1,2-methyl butoxy-1-hexoxy ethane, 1,1-di-hexoxyethane, 1,1-dithoxypentane, 4-methoxyallyl benzene, furan, furfural, 5-hydroxymethylfurfural, 2,4,5-trimethyl-1,3-dioxolane, ethane, ethylene, a-farnesene, B-farnesene, benzene, ethyl benzene, 1-methylnaphthalene, 2-methylnaphthalene, B-damascenone, a-pinene


graphic effluents. It combines high resolution gas chromatography with the use of n-paraffin standards, computerized data collection, and a sensory procedure based on the odor detection thresholds.

2. **Character Impact Volatiles.** Fruits are divided into four categories, on the basis of their flavor volatiles (Burger 1991):

1. Fruits whose aroma is largely produced by one compound, a “character impact volatile.”
2. Fruits whose aroma is produced by a small number of volatiles.
3. Fruits whose aroma is caused by a large number of volatiles.
4. Fruits whose aroma cannot be reproduced even by a large number of volatiles.

It has been believed that the aroma in ‘Delicious’ apples is given by a “character impact compound” plus a number of other contributary volatiles (Dimick and Hoskin 1993). Several individual volatiles have
been reported to contribute (character-impact volatiles) to the aroma of apples (Table 6.2).

The large number of “character-impact compounds” reported are due to several factors. Authors differ greatly in the way of determining the sensory significance of different volatiles. The existence of a large number of apple cultivars makes it difficult to define only one or a few “character-impact compounds.” Furthermore, there is a frequent lack of separation between primary (natural) and secondary volatiles. “Primary or natural” volatiles are produced by controlled enzymatic reactions in the intact tissue. “Secondary” volatiles are formed by various uncontrolled enzymatic reactions when plant tissues are disrupted, such as by cutting, chewing, homogenization, or heating (Schreier 1984). Although primary volatiles are important to the flavor of the intact fresh fruit, secondary volatiles are expected to play a significant role in the flavor of the juice and of the fresh fruit after it is cut or chewed. The suppression or elimination of the formation of secondary volatiles, by using inhibitors such as methanol, is important when investigating whole fruit natural volatiles. Several of the volatiles listed in Table 6.2, such as (E)-2-hexenal, (E)-2-hexenol, (Z)-3-hexenal, and (Z)-3-hexenol, are secondary volatiles (Drawert et al. 1968; Croft et al. 1993) and may not contribute significantly to the aroma of ripe fresh fruit, but may occur in processed fruit.

Ethyl 2-methylbutanoate was first described as the character-impact odor in ‘Delicious’ apples by Flath et al. (1967) and then later by several other authors. However, this compound seems to be a postharvest-induced volatile, since it was present in very low concentration (2–6 ng/g) in ‘McIntosh’ and ‘Cortland’ apples ripened on the tree (Yahia et al. 1990b), and in very high concentration (up to 80 ng/g) in apples ripened off the tree and after long periods of cold storage (Yahia 1989a; Yahia et al. 1990a; 1991). In addition, sensory studies of the odor of 40 apple cultivars ripened on the tree showed that ethyl 2-methylbutanoate contributed less than 1% to their odor (Cunningham et al. 1986). Therefore, this volatile is probably not as important to the flavor of fruit ripened on the tree as it is to the flavor of postharvest-ripened and postharvest-processed apples. The postharvest formation of this volatile seems to be induced and/or accelerated by low temperature.

Table 6.2. Volatiles Reported to Contribute to Apple Aroma.


Experiments that determined the relative odor activity of natural or primary volatiles in apples have found that between 20 and 40 volatiles are responsible for apple aroma (Cunningham et al. 1986). These volatiles fall into three classes: the "apple-peel smelling" esters (hexyl hexanoate), the lipid oxidation products [(E)-2-hexenal], and the terpeneoid β-damascenone. Cunningham et al. (1986) made a thorough Charm study of 40 apple cultivars and found that most volatiles display little or no odor activity at the concentration at which they occur in the fruit. They ranked the "natural" aroma volatiles according to their odor intensity (Table 6.3).

The top-ranked and most odor-active compound in Table 6.3, β-damascenone, is one of the least concentrated in apple fruit (Acree and McLellan 1989). β-Damascenone is present in fresh apple and freshly extracted juice in very low concentrations (1 μl/liter), but present in very high concentration in heated apple products. This volatile is thought to be generated during thermal processing from a nonvolatile precursor (Acree and McLellan 1989).

### III. APPLES OFF-FLAVOR

Off-flavors have been described for apples kept in atmospheres with very high levels of CO₂ and/or, especially, with very low levels of O₂. Aeration removes some of the off-flavor and off-odor if the injury is not too severe. Considerable off-flavors were present in 'Delicious' apples stored in 3% CO₂ at 0°C for 1 month (Mattheis and Olson 1989). However, raising the storage temperature to 1.1°C alleviated the fruit from off-flavor and development of any long-term damage. 'Granny Smith' and 'Yellow Newtown' apples maintained in an atmosphere containing 0.02–0.25% O₂ at 0, 4 or 10°C for 3–35 days developed alcoholic off-flavor (Ke et al.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Odor Volatile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Damascenone</td>
</tr>
<tr>
<td>2</td>
<td>Unknown</td>
</tr>
<tr>
<td>3</td>
<td>Hexyl hexanoate and butyl octanoate</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl butanoate</td>
</tr>
<tr>
<td>5</td>
<td>Butyl hexanoate and hexyl butyrate</td>
</tr>
<tr>
<td>6</td>
<td>Unknown</td>
</tr>
<tr>
<td>7</td>
<td>6-Methyl-hep-5-en-2-one</td>
</tr>
<tr>
<td>8</td>
<td>Unknown</td>
</tr>
<tr>
<td>9</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>Propyl butyrate and ethyl valerate</td>
</tr>
</tbody>
</table>

Source: Cunningham et al. (1986).
`Delicious` apples stored in 0.05% O₂ and 0.2% CO₂ at 1°C for 30 days produced large concentrations of ethanol and acetaldehyde (Mattheis et al. 1991a).

Ethanol and acetaldehyde accumulation have been implicated as the cause for off-flavor (Fidler and North 1971; Ke et al. 1991). As early as 1925 (Thomas 1925), ethanol accumulation in apples stored in an O₂ free atmosphere was related positively to the length of exposure and temperature of the fruit. Ethanol accumulation in the flesh was found to be related to the apple cultivar, the season, the level of O₂ in storage, and to other treatments that influence the physiological age of the fruit (Blanpied et al. 1968). After 14 weeks in an atmosphere containing 0% O₂, `Delicious` apples had 10 times more ethanol than fruit stored in 0.5–1.5% O₂ (Nicholas and Patterson 1987). Upon transfer to ambient air for 7 days, up to 50% of the ethanol accumulated was lost. Nicholas and Patterson (1987) also found that late-harvested fruit accumulated significantly higher tissue ethanol concentrations than fruit from early harvest dates at all O₂ levels tested. Upon removal from low O₂ into ambient air, fruit from the early harvest lost more ethanol than those from the later harvest. Ke et al. (1991) estimated the ethanol content that causes off-flavor in ripe fruits of various commodities including apples. A concentration of more than 1,000 μl ethanol/liter was reported by these authors to be necessary to cause off-flavor in `Yellow Newtown` apples. Ke et al. (1991) also reported that soluble solids content (SSC) plays an important role in determining the ethanol level that causes off-flavor; the higher the SSC, the higher the ethanol content to cause off-flavor in the fruit.

The tainted taste associated with anaerobiosis in `Cox`s Orange Pippin` apples was reported to be accompanied by the accumulation of ethanol and ethyl acetate in the flesh (Blanpied 1983). Taste panel evaluation of these apples artificially treated with ethanol and ethyl acetate indicated that the taint flavor is caused by ethyl acetate rather than the ethanol, and the threshold for ethyl acetate taint was 5 mg/100 g of flesh. The critical O₂ level for `Cox`s Orange Pippin` apples was suggested to be between 0.45 and 0.55 kPa at 3°C (Knee 1991). The lower O₂ limit was reported to range from 0.7 to 1.5 kPa at 0°C and from 0.8 to 6.4 kPa at 25°C for `Delicious` and `Marshall McIntosh`, respectively. Below the critical O₂ level, ethanol can accumulate at a rate of up to 100 mg/kg • day. Ethanol is then further metabolized to ethyl acetate, which gives off-flavors when it reaches about 50 mg/kg. If the oxygen concentration is raised, the fruit can metabolize ethanol at up to 20 mg/kg • day at 10°C. Fidler and North (1971) investigated the effect of periods of anaerobiosis and found that the highest alcohol concentration tolerated (for salvageable apples) is 120 mg/100 g fruit.

Off-flavors in apples (as is the case for other fruits) have never been chemically identified and characterized. Olfactory assays can be helpful in identifying and structurally characterizing these flavors.
It is doubtful that ethanol and acetaldehyde (which are produced naturally by all fruits) are the cause of off-flavors, but rather an index to anaerobiosis. Ethanol typically accumulates in overripe and senescent apples even without shortage of O₂ (Thomas and Fidler 1933). Ethanol concentration in the head space of immature intact ‘Bisbee Delicious’ apple fruit was higher (4,866 pl/kg • hr) than the sum of all other 36 volatiles analyzed including alcohols, aldehydes, and esters (Mattheis et al. 1991a). It decreased as the fruit matured, but was still one of the most concentrated volatile in mature fruit. Blanpied and Jozwiak (1993) showed that ‘McIntosh’ and ‘Delicious’ apples stored in air accumulated ethanol throughout the storage period. These fruits accumulated ethanol very rapidly when moved from 2.2° to 21°C, and had higher O₂ thresholds for ethanol accumulation than CA stored apples. ‘Delicious’ apples stored in CA with 3% O₂ had higher O₂ thresholds for ethanol accumulation than comparable apples stored at 1.5% O₂. Unpublished data by G. D. Blanpied (Cornell University) showed that visible low O₂ injury was not caused by the accumulation of ethanol. Senescing air-stored apples held at 20°C accumulate more ethanol than comparable apples with high incidences of low O₂ injury.

IV. BIOGENESIS OF APPLE FLAVOR

Flavor research has shifted lately from the isolation, identification, and characterization of compounds to the investigation of their origins and fates. The complexity of apple flavor becomes more obvious after realizing the diverse origins and mechanisms of biogenesis of the flavor compounds (Figure 6.2). The origin of most taste components of flavor such as sugars, acids, and phenolic compounds, and the changes in these components during fruit maturation, ripening, and senescence are fairly well understood. The origin, formation, and metabolism of odor-causing volatiles, however, is much less documented and understood.

A. Biogenesis of Primary Volatiles

Primary or natural volatiles in fruits are thought to be derived from the enzymatically controlled lipid, terpene, amino acid, and phenyl propane metabolism (Schreier 1984).

Fatty acid metabolism was implicated in the formation of a range of natural plant volatiles such as the aliphatic esters, alcohols, acids, and carbonyls (Drawert 1975; Paillard 1979). Most fruits produce a variety of fatty acids ranging from C-1 to C-20 and traces of primary and secondary alcohols in the immature stage. Galliard (1968) studied the identity,
Fig. 6.2. Some possible routes for the synthesis of flavor compounds in apples.

composition, and concentration of lipids in the apple pulp during pre- and postclimacteric stages of fruit development. The distribution of fatty acids of both pre- and postclimacteric apples was similar. The proportion of linoleic acid, which was the major component of fatty acids in apples, was lower in the postclimacteric than in the preclimacteric fruit. Meigh
et al. (1967) found an initial build-up of fatty esters and free fatty acids, both saturated and unsaturated, in the apple peel about 120 days after petal fall. After 150 days from petal fall, the breakdown of fatty acids proceeds almost as rapidly as their synthesis. The fall in free fatty acids was initially thought to be due to continued esterification, but subsequently, a loss of esterified acids was also noticed. The rise in respiratory activity was accompanied by rapid accumulation of free and esterified fatty acids in apple peels, and subsequently free fatty acids begin to disappear, followed by the loss of esterified acids (Meigh et al. 1967). During fruit ripening, some fatty acids were converted into esters, ketones, and alcohols, as revealed by labeling (Tressel and Drawert 1973) and non-labeling experiments (Yamashita et al. 1977). Bartley (1985) found that the rate of degradation of phospholipids increases in apples during ripening, and this could supply free-fatty acids for the synthesis of volatiles.

The biosynthesis of apple volatiles was also studied by adding fatty acids and alcohols to discs of aged apple tissues (Paillard 1979). Alcohols were formed from the β-oxidation of aliphatic acids having either the same number of carbon atoms, or higher homologues. Fatty acids with an even carbon number produced butanol and hexanol, and those with an odd carbon number formed propanol and pentanol. The origin of the “impact” decadienoate esters in pears were also suggested to be originated from β-oxidation of linoleic acid (Jennings and Tressl 1974).

The synthesis of esters in apples is also often studied by supplying the fruit with short chain alcohols and/or carboxylic acids. This is usually done by exposing the fruit to a stream of air containing the vapors of one or a combination of more than one chemical. Whole apples supplied with hexanol vapor (Table 6.4) produced hexyl acetate (Knee and Hatfield 1981). When alcohols (C-2–C-8) and methyl esters of short chain fatty acids (C-4–C-8) were supplied as vapors to whole fruits stored in 2% O₂ at 3°C, the alcohols were converted to the corresponding acetate esters, whereas the methyl esters of short chain fatty acids were converted to esters with an alkyl group of C-2 or C-4 (Bartley et al. 1985). These results

### Table 6.4. Production of Hexyl Acetate In ‘Cox’s Orange Pippin’ Apples Supplied with Hexanol.

<table>
<thead>
<tr>
<th>Storage Atmosphere</th>
<th>Control</th>
<th>Hexanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>1.85</td>
<td>6.93</td>
</tr>
<tr>
<td>2% O₂ + 98% N₂</td>
<td>0.00</td>
<td>6.95</td>
</tr>
</tbody>
</table>

Source: Knee and Hatfield (1981).
indicated to the authors the presence of an active β-oxidation pathway for fatty acids in whole fruits. The precursors of alcohols for ester synthesis were thought to be derived from the oxidation of long chain fatty acids through a number of cycles of the β-oxidation pathway (Paillard 1974, 1979). The site of β-oxidation activity was suggested to be localized in the peroxisomes (Gerhardt 1983). Short-chain acyl CoA is reduced by acyl CoA reductase to aldehyde which in turn can be reduced by alcohol dehydrogenase (Bartley 1980). Apple fruit was reported to reduce aldehydes to alcohols, which were subsequently esterified with carboxylic acids (De Pooter et al. 1987). The enzyme systems ester synthetase and the short-chain acyl CoA reductases are yet to be characterized, and the mechanism for the transport of β-oxidation products to the site of reduction is still not known. De Pooter et al. (1981) did not find any evidence for extensive β-oxidation activity in ‘Golden delicious’ apples. However, the advantage of methods that utilize the addition to the storage atmosphere of volatile precursors, such as alcohols, carboxylic, and fatty acids (Knee and Hatfield 1981; De Pooter et al. 1983; Berger and Drawert 1984; Bartley et al. 1985), in enhancing the flavor content of apples has been illustrated by Drawert (1988).

A comparison of the rates of ester production among apple skin, peeled apples, and whole apples indicated that the skin is the major site of ester synthesis (Guadagni et al. 1971). Microwave blanching of apple peels, or storing apples in an inert atmosphere, stopped the production of esters. This indicates that oxygen as well as a viable enzymatic system are required for ester synthesis.

Fatty acids seem to be the most important source of primary or natural odor volatiles in apples. However, other compounds have been shown to produce odor volatiles in other fruits. Amino acids were found to be the source of some of the branched chain esters. Myers et al. (1970) incubated ripe banana discs with l-leucine-U-14C, and found that up to 81% of the volatile radioactivity was in iso amyl alcohol, and relatively little (less than 10%) in iso amyl acetate. Labeling experiments using postclimacteric banana tissue slices showed that 14C-leucine was converted to isovalerate and isovaleryl esters and that 14C-valine was converted to isobutyrate and isobutyl esters (Tressl and Drawert 1973). This conversion is thought to be accomplished via the transamination and subsequent oxidative decarboxylation of the amino acid.

**B. Biogenesis of Secondary Volatiles**

Drawert et al. (1968) identified several compounds, including the aldehydes (Z)-3-hexenal and (E)-3-hexenal, and the alcohols (Z)-3-hexanol and phenyl ethyl alcohol, after homogenizing apples. These and some
other C-6 compounds are usually found in apple juice, but are rarely found or only found in very low levels in the head space of intact apples (Schreier 1984). These compounds are formed by the enzymatic oxidation of linoleic and linolenic acids after the fruit is crushed and exposed to oxygen (Drawert et al. 1973). Within 10 min after apples are crushed, hydrolytic and oxidative enzymes catalyze reactions of lipids and oxygen to form these volatiles. Drawert et al. found that production of (E)-2-hexenal reaches a maximum of 15 mg/liter in less than 20 min after crushing in a pilot plant operation. Feys et al. (1980b) found an increase of 302% and 104% of hexanal and (E)-2-hexenal, respectively, when 0.1% linoleic acid was added, and increases of 102% and 435%, respectively, when 0.1% linolenic acid was added during homogenization of apple tissues.

The production of secondary aroma volatiles from free fatty acids is common in plant tissues and food systems (Erikson 1975; Galliard et al. 1976; Hatanaka 1983; Croft et al. 1993). The process is catalysed by lipoxygenases (LOX), which catalyse the peroxidation of polyunsaturated fatty acids with a Cis,Cis,1,4-pentadiene structure to form conjugated hydroperoxides (Gardner 1980). The fatty acid hydroperoxides are unstable and cytotoxic, particularly to proteins and membrane structures. Depending on the type of LOX and plant tissue, the reaction produces either 9- or 13-hydroperoxides or a mixture of both. Hydroproxide lyase can catalyse the breakdown of 13-hydroperoxides to a C-6 product, (Z)-3-hexenal, and a C-12 product, 12-oxo-cis-9-dodecenoic acid. (Z)-3-Hexenal can then form (Z)-3-hexenol, (E)-3-hexenol, or (E)-2-hexenal. The C-12 product is a precursor of the plant wound hormone traumatic acid. The breakdown of hydroperoxides contributed significantly to the development of secondary odor volatiles in tomato (Galliard et al. 1977), grape (Crouzet et al. 1984), and cucumber fruits (Galliard et al. 1976).

The importance of hexanal and (E)-2-hexenal to the odor of apples was demonstrated by Flath et al. (1967). These two volatiles were formed quickly after apple tissues were crushed and homogenized (Feys et al. 1980b). They might be also of importance during cutting and chewing of fresh apples.

Kim and Grosch (1979) partially purified LOX in apples. The enzyme formed hydroperoxides and therefore could be important for the formation of apple odor. Meigh et al. (1967) found a rise in LOX activity before the increase in respiration and ethylene production during apple ripening. Meigh and Hulme (1965) found an increase in LOX activity in the peel of apples ripened on and off the tree, and they suggested that LOX might be responsible for the decrease of linoleic and linolenic acids during ripening. This has been associated, then, with the possible role of
LOX in the biogenesis of ethylene (Wooltorton et al. 1965). A survey of LOX contents in various fruits and vegetables indicated that crude extracts of ‘Golden Delicious’ and ‘Delicious’ apples contained relatively little activity on a fresh-weight basis, but one of the highest activities on the basis of protein (Rhee and Watts 1966). A similar survey made by Pinsky et al. (1971) also indicated a relatively small LOX activity on the basis of fresh weight of apple extracts in the presence of the nonionic detergent Triton x-100. Triton x-100 acts as a solubilizing agent, indicating that LOX in apples may be associated with membranes. The precise localization of LOX in plant cells remains unclear; however, it has been shown in mammalian cells that a soluble form of the enzyme is translocated to membranes upon the influx of extracellular Ca²⁺ and can then metabolize substrates released by membrane phospholipases (Rouzer and Kargman 1988; Wong et al. 1990). A membrane associated LOX was found in tomato fruit (Todd et al. 1990) and senescing carnation (Rouet-Mayer et al. 1992).

V. FACTORS AFFECTING FLAVOR

A. Preharvest Factors

1. Fruit Maturity and Ripening. Apple is a climacteric fruit characterized by a sudden rise in respiration and ethylene production during ripening (Hulme and Rhodes 1971). The climacteric rise has long been recognized as the onset of fruit ripening. Ripening in apples is characterized by an array of physical and chemical changes, including changes in color, texture, and flavor. Just before visible changes occur, the rates of respiration and ethylene production of the fruit start to rise. Ethylene, which is recognized as the “ripening hormone,” is assumed to initiate these physical and chemical changes (Abeles et al. 1992; Blanpied et al. 1984). It appears that ethylene initiates the synthesis of enzymes responsible for manifold biochemical processes. For example, the softening of cell walls, loss of chlorophyll, decrease in acidity, loss of starch, and increase in flavor, are all associated with the increase in ethylene production by the fruit (Hulme and Rhodes 1971).

Ethylene is suggested to promote changes that are important to flavor development. The changes include conversion of starch to sugars, loss of acidity, and formation of aroma volatiles (Blanpied et al. 1984). The ethylene concentration was tested as a method of determining varietal flavor in ‘Delicious’ apples (Blanpied and Black 1976). Fruit lacking varietal flavor had significantly lower ethylene concentrations; however, no clear relationships were found. Studies on several climacteric
fruits such as apples (Brown et al. 1966; Yahia 1986; Yahia et al. 1990b), pears (Heintz et al. 1965; Jennings and Tressl 1974), and bananas (Tressl and Drawert 1973) revealed that the formation of most flavor-related volatiles are initiated at or after the climacteric rise in respiration, and reach a maximum in the postclimacteric ripening phase. Aldehydes are reported to be the dominant volatiles detectable in intact immature apple fruit (De Pooter et al. 1987), whereas maturing and ripening fruits produce primarily esters and aliphatic alcohols (Flath et al. 1967). Mattheis et al. (1991b) found that as ‘Bisbee Delicious’ apple development progressed, concentrations of butanal, pentanal, (E)-hexenal, and heptanal in the head space declined. Yahia et al. (1990b) studied the evolution of 22 odor-active volatiles during the maturation and ripening of ‘McIntosh’ and ‘Cortland’ apples on the tree. Only 12 volatiles were produced on the tree and showed four patterns of change (Table 6.5). Most of the important odor-active volatiles were not produced until later stages of ripening, and their production followed the autocatalytic evolution of ethylene.

Fruit maturity at harvest is a critical factor, which affects ripening and flavor development after harvest. If a fruit is picked too early, it is likely to be small in size, lack the proper color, and may never develop full flavor (Brown et al. 1966; Paillard 1981). The onset of volatile production was delayed in early picked ‘Jonagold’ apples, and the production was lower compared to late picked fruits (Hansen et al. 1992a). Fruits picked very late had a tendency to produce fewer volatiles compared to those picked at the optimum harvest date. Dirinck and Schamp (1989) reported the prediction of optimal harvest date using butyl acetate concentrations in the head space of ‘Golden Delicious’ apples.

2. Fruit Internal Factors. Some internal factors such as fruit structure and metabolic changes also influence flavor formation. Apple peels that had been stored for 2 days produced more volatiles than the flesh or whole unpeeled apples (Guadagni et al. 1971). This was suggested to be due to higher enzyme activities, the abundance of fatty acids in the peel and outer flesh, and to metabolic changes that occurred in the excised fruit tissues. Secondary aroma volatiles, such as hexanal and (E)-2-hexenal, are released in higher amounts in the peel and the outer flesh after crushing and disruption of the cells (Paillard 1981). The activity of LOX is highest in the peel compared to other parts of the fruit (Feys et al. 1980a). Peels of apples with waxy cuticle (such as ‘Caville Blanc’ and ‘Starking’) produce alcohols and esters, whereas those with corky coating (such as ‘Canada Gris’) produce alcohols and high concentrations of ketones, but no esters (Paillard 1981).
Table 6.5. Patterns of Production of Some Odor-Active Volatiles During the Maturati

on and Ripening of ‘McIntosh’ and ‘Cortland’ Apples on the Tree.

<table>
<thead>
<tr>
<th>General Behavior</th>
<th>Volatiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>High in immature apples and decreased gradually during ripening</td>
<td>hexyl acetate, methyl butanoate</td>
</tr>
<tr>
<td>Continuous increase during maturation and ripening</td>
<td>(E)-2-hexenal in ‘McIntosh’</td>
</tr>
<tr>
<td>Produced at low level before ripening and suddenly increased after ripening</td>
<td>hexanal, ethyl 2-methylbutanoate</td>
</tr>
<tr>
<td>Not detected or very low before ripening then suddenly increased after ripening</td>
<td>ethyl butanoate, propyl butanoate, methyl hexanoate butyl hexanoate, hexyl hexanoate, ethyl 2-methylbutanoate, hexyl 2-methylbutanoate</td>
</tr>
</tbody>
</table>

Source: Yahia et al. (1990b).

3. Cultivar. Different cultivars possess different flavors. The sugar:acid ratio varies significantly among cultivars. Several studies have indicated that cultivars differ in the kind and amount of volatiles produced (Smock and Neubert 1950; Brown et al. 1968; Cunningham et al. 1986; Yahia 1986; 1989a; Yahia et al. 1990b; 1991). ‘McIntosh’ apples produced higher amounts of ethyl butanoate, propyl butanoate, ethyl 2-methylbutanoate, propyl 2-methylbutanoate, ethyl pentanoate, butyl pentanoate, and butyl hexanoate, whereas ‘Cortland’ apples produced slightly higher amounts of (E)-2-hexenal (Yahia 1986, 1989a; Yahia et al. 1990a, b, 1991). Butyl-, pentyl-, and isopentyl pentanoate were found in various apple cultivars (Dimick and Hoskin 1983), but they were absent from ‘McIntosh’ and ‘Cortland’ apples (Yahia 1989a; Yahia et al. 1990a, b, 1991; Yahia 1991a). Total amount of volatiles detected using the head space method were dominantly esters and were highest in the cultivar ‘Hatsuaki’ followed in descending order by the cultivars ‘Kogyoku’, ‘Golden Delicious’, ‘Mutsu’, and ‘Fuji’ (Kakiuishi et al. 1986). However, using the vacuum distillation method, the total volatiles were predominantly aldehydes and were highest in the cultivar ‘Kogyoku’ followed by ‘Hatsuaki’, ‘Golden Delicious’, ‘Mutsu’, and ‘Fuji’. ‘Kogyoku’ and ‘Hatsuaki’ apples had the highest total volatiles and showed higher aroma intensity than other cultivars. Aroma patterns were used by Dirinck and Schamp (1989) to classify apple cultivars. ‘Golden Delicious’, ‘Jonagold’, and ‘Jubilé Delbar’ are closely related and characterized by high concentrations of butyl and hexyl acetate. ‘Elstar’ and ‘Cox’s
Orange Pippin' have similar aroma patterns. 'Nico', 'Granny Smith', 'Paulared', and 'Summerred' are characterized by high levels of ethyl butanoate and hexanol. 'Boskoop' and 'Jacques Lebel' are characterized by high concentrations of a-farnesene and hexyl 2-methylbutanoate, respectively. Therefore, there are qualitative and quantitative differences between different apple cultivars.

4. Climate During Growth and Development. Zerbini et al. (1980) observed that stored 'Golden Delicious' apples from the plains had higher specific weight, higher soluble solids, lower total sugars, and higher reducing sugars and greater sugar:acid ratio than those from the mountain area in Italy. Warmer weather seem to favor the development of attributes associated with apple quality, but also may favor the development of physiological disorders.

5. Fertilization. Guelfat-Reich et al. (1982) found that different levels of nitrogen (800 or 1,600 kg NH₄ NO₃/ha) had no effect on total soluble solids (TSS) of 'Golden Delicious' apples stored for 5–6 months. TSS content was lower in fruits from trees with higher yields (Zeiger 1978). Gormley et al. (1982) found that soil management practices had an influence on the TSS content of 'Cox's Orange Pippin' apples. Somogyi et al. (1964) showed that 'McIntosh' apples from high nitrogen treatment produced more volatile flavor components than those from low nitrogen treatments. Phosphorous fertilization increased the production of three volatiles (tentatively identified as esters) in freshly harvested 'Golden delicious' apples (Brown et al. 1968). On the other hand, higher levels of phosphorous fertilization depressed the production of ethyl esters, acetaldehyde, and hexanal in 'McIntosh' apples after cold storage (Forsyth and Webster 1971).

6. Irrigation. Irrigation methods affect the availability of soil nutrients and water to the tree and, therefore, affect apple quality. Apples from trickle-irrigated trees were more mature and higher in yellow color, soluble solids, and pH than apples from sprinkle-irrigated trees (Drake et al. 1981). The fruits from the trickle-irrigated trees resulted in applesauce superior in consistency and firmer apple products. Guelfat-Reich et al. (1982) found that the acidity was higher in 'Golden Delicious' apples from sprinkler-irrigated trees than in those from trickle-irrigated trees. 'Delicious' apples from trees that received regulated deficit irrigation (applying less water through irrigation than trees would have used) early in the growing season had higher soluble solids content and lower acids (Ebel et al. 1993). The soluble solids of these fruits remained higher during storage.
7. **Growth Regulators.** Butanedioic acid mono 2,2-dimethylhydrazide (daminozide) is a growth regulator, which was used in apples to inhibit ethylene production, delay apple ripening, and reduce preharvest fruit drop and loss of firmness after harvest (Looney 1983). Its use is now prohibited because breakdown products were thought to be carcinogenic. Preharvest application of daminozide greatly increased the effect of low ethylene controlled atmosphere storage for ‘McIntosh’ apples (Liu and Samelson 1986). Sensory tests indicated that ‘McIntosh’ apples sprayed with 2,000 ppm daminozide had poorer quality than apples not sprayed with daminozide, but a lower rate of application (500 ppm) had no adverse effects on flavor (Murphy et al. 1971). Preharvest application of daminozide at 1,000 ppm delayed the production of five odor-active volatiles in ‘McIntosh’ and ‘Cortland’ apples in the same manner it delayed fruit ripening (Yahia et al. 1990b). These volatiles were (E)-2-hexenal, methyl hexanoate, butyl hexanoate, hexyl hexanoate, and hexyl 2-methylbutanoate. However, daminozide did not suppress the production of any other volatiles. It is probable that it only delayed the onset of ripening rather than having any abnormal effect. Since all five volatiles delayed by daminozide are six carbon derivatives, daminozide may have a specific and selective effect on the metabolism of odor-active volatiles in apples. Aminoethoxy vinyl glycoside (AVG), an effective inhibitor of ethylene synthesis and fruit ripening, had adverse effects on the volatile development in pears (Romani et al. 1983).

Ethylene has a major influence on flavor development. During ripening, apples show a loss in acidity and an increase in sweetness and aroma. Ethylene stimulates the ripening of the fruit and the changes in acids, sugars, and aromatic volatiles. Ethylene stimulates the degradation of starch to sugars and the degradation of organic acids thus increasing sweetness (Kader 1985). Ethylene (100 ppm) stimulated the production of esters in muskmelon (Bliss and Pratt 1979). However, ethylene generally does not have an effect on soluble solids or titratable acids in apples (Forsyth et al. 1969; Liu 1977). Low ethylene (1–3.8 ppm), in comparison to high levels (10 and 500 ppm), in controlled atmosphere, did not significantly effect the soluble solids or titratable acidity of ‘Delicious’, ‘Golden Delicious’, ‘Idared’, ‘Cortland’, and ‘McIntosh’ apples. It is possible that ethylene affects the biosynthesis and degradation of individual sugars, but not the total content of either.

**B. Postharvest Factors**

1. **Temperature.** The lowest possible storage temperature delays fruit ripening and senescence, retains fruit quality, and prolongs storage life.
Therefore, low temperature delays starch and acids breakdown, and development of aroma volatiles. A study of ‘Jonathan’ apples stored between -1°C and 10°C showed an increase in the emission of acetic esters with an increase of storage temperature (Wills and McGlasson 1971). Intermittent warming of apples increased volatile production (Wills and McGlasson 1970). Transfer to a temperature of 20°C increased the production of some odor-active volatiles in controlled atmosphere stored ‘McIntosh’ and ‘Cortland’ apples, but there was no significant increase in low (3.3°C) temperature (Yahia et al. 1985a; Yahia 1989a; Yahia et al. 1990a, b; Yahia 1991b; Yahia et al. 1991).

2. Humidity. Low relative humidity in CA storage reduced the production of acetaldehyde, ethyl acetate, ethyl propanoate, and caproaldehyde, but increased the production of ethyl n-butanoate in apples compared to those stored with high relative humidity (ca. 92%) (Forsyth and Eaves, 1974). However, increasing water loss by storage in low humidity atmosphere increased the production of butyl-, isopentyl-, and hexyl acetate, and decreased the production of the corresponding alcohols butanol, iso pentyl alcohol, and hexanol, in ‘Jonathan’ apples stored at -1°C (Wills 1968; Wills and McGlasson 1970). The water loss was considered to enhance the production of acetate esters and to provide a “carrier” for the removal of esters from the fruit.

3. Controlled Atmosphere Storage (CA). CA storage, in which the atmosphere is altered by reducing the O₂ and/or raising the CO₂ levels, is a well-established method to prolong the storage life of apples (Smock 1979). Although the physiological and biochemical basis of the CA effect on apples is not well understood, it is known that CA storage decreases the rate of ethylene synthesis and action, and prevents or delays the appearance of some storage disorders (Smock 1979). Normal ripening patterns in apples, which include the rise in respiration, degradation of cell wall, and pigment changes, were disrupted by CA storage (Knee 1971).

Several CA technologies were developed to prolong the storage life of apples. These include “conventional CA (CCA)” (Bartsch and Blanpied 1984), “rapid CA (RCA)” (Lau 1983), “ultra-low O₂ CA (ULO)” (Sharples et al. 1977, Dewey and Bourne 1982; Lidster 1982; Sharples 1982), “high CO₂ CA (HCCA)” (Couey and Olson 1977), “low ethylene CA (LCA)” (Blanpied 1990, Liu 1985a). In CCA, the fruit is used to establish the initial modification of the atmosphere, after which active control of the atmosphere takes place. In RCA, the atmosphere is actively controlled immediately after sealing the CA room. In ULO, oxygen levels are
lowered to 0.5% without increasing the CO₂ levels. In HCCA, the CA room is enriched with 10–20% CO₂ for 10–15 days before normal CA atmosphere is implemented. In LCA, the ethylene level in the CA room is controlled at or below 1 ppm during storage. Apples stored in RCA, HCCA, ULO, or LCA are usually firmer, juicier, have higher acid content, and store longer than comparable apples stored in CCA.

The proper harvest time for apples intended for CCA, especially for ULO and LCA, is critical. It has been recommended that apples be picked near the onset of the climacteric for CCA (Smock 1948), and shortly before the onset of the climacteric for ULO and LCA (Liu and Samelson 1986), to preserve the keeping quality of the fruit.

A number of studies have demonstrated that apples ripened after CA storage do not develop full characteristic flavor (Knee and Sharples 1981; Liu 1985; Streif and Bangerth 1988; Yahia et al. 1990a; 1991). However, early studies (Kidd and West 1936) had indicated that CA-stored apples developed their characteristic flavor after a short conditioning period in air at room temperature.

The levels of acids in apples may fall by as much as 50% during conventional refrigerated storage (Knee and Sharples 1981). CA storage reduces the normal acidity losses in apples (Fidler and North 1967; Porritt and Meheriuk 1968; Murata and Minamide 1970); however, Knee (1975) reported that 8% CO₂ without any control of O₂ had no effect on acidity losses in ‘Bramley Seedling’ apples.

Gorin (1973) and Gorin et al. (1975) evaluated ‘Golden Delicious’ apples after CA storage (8% CO₂ + 4% O₂ at 4°C for 7 months) with sensory and analytical methods. They found that the L-malate concentration decreased continuously during CA storage. The sucrose content decreased up to 120 days of storage and then became constant, whereas glucose and fructose remained practically constant. Citrate increased initially and became constant after 90 days of storage. The inflection point of the decreasing sucrose coincided with taste panel evaluation of the fruit as having 40–50% ripe flavor and 40–50% overripe flavor, which occurred after about 130 days of storage. Sensory evaluation indicated that the odor deterioration preceded the deterioration of the sweet–sour relation and texture in ‘Golden Delicious’ apples stored in 4% O₂ + 8% CO₂ at 4°C (Gorin et al. 1975). These authors suggested that odor is the limiting factor of the quality of ‘Golden Delicious’ apples, and since odor is the first quality attribute to deteriorate, CA storage for a long period “produces apples with a pleasant appearance but with an unpleasant flavor.”

Apples stored in CA were reported to fail to produce normal quantities of odor volatiles during subsequent ripening (Hatfield and Patterson
Patterson et al. (1974) reported a “residual effect of CA” on the production of volatiles in ‘Cox’s Orange Pippin’ and ‘Golden Delicious’ apples. Hatfield and Patterson (1974) reported a 75% reduction in the production of some volatile esters in ‘Cox’s Orange Pippin’ apples after a 5-month storage in 2% O₂ with or without 5% CO₂. CA storage has also been reported to arrest the ability of the peel of ‘Golden Delicious’ apples to develop aroma (Guadagni et al. 1971). It has been concluded that the severity of suppression of apple flavor by CA storage depends on the atmospheric composition and the length of storage period. The higher the CO₂, the lower the O₂ concentration, and the longer the fruit is kept under CA storage, the greater the suppression of flavor (Lidster et al. 1983; Yahia et al. 1990a; Yahia 1991a, b; Yahia et al. 1991). LCA storage caused a greater suppression of flavor than CCA storage (Yahia 1989a; Yahia et al. 1990a; 1991). Therefore, any valid conclusion about CA effects, especially on fruit flavor, must take in consideration the exact gas composition, temperature, and storage duration.

Storage of apples in CA (0.5–1.0% CO₂ added to air at 3°C) decreased the production of alcohols, aldehydes, ketones, and esters compared to apples stored in air (Meigh 1957). Head space volatile analysis indicated that ‘Cox’s Orange Pippin’ apples transferred from CA storage into air at 20°C produced lower rates of butanol, butyl-, and hexyl acetate compared to similar apples ripened after harvest (Hatfield and Patterson 1974). The atmosphere of 5% CO₂ + 2% O₂ for 5.5 months almost completely inhibited the production of these volatiles. Lidster et al. (1983) found that CA storage (3% O₂ + 5% CO₂ at 2.8°C) suppressed the head space ethanol and acetaldehyde in ‘McIntosh’ apples compared to similar apples stored in air at 0°C. Acetaldehyde, ethanol, ethyl butyrate, and hexanal were further suppressed when the apples were stored in a more strict CA atmosphere (1.5% O₂ + 1.5% CO₂ or 1.0% O₂ + 1.5% CO₂ at 2.8°C). Low oxygen storage for a long period (1.0% O₂ + 1.5% CO₂ for 320 days) completely suppressed the formation of these volatiles even after the apples were moved into air.

The criterion used in most studies that determined the CA effect on flavor components (Patterson et al. 1974; Hatfield and Patterson 1975; Knee and Sharples 1981; Lidster et al. 1983; Streif and Bangerth 1988; Hansen et al. 1992b) was to compare the production of these components during ripening of the fruit in air after harvest and after CA storage. This comparison usually ignores their behavior while the fruit is under CA storage. The criteria used by Yahia et al. (1990a; 1991) was to follow the changes of odor-active volatiles in the following conditions: (1) during ripening in air at 20°C immediately after harvest, (2) during storage at the
optimum low temperature, (3) during storage in CA at the optimum temperature, and (4) during ripening in air at 20°C after CA storage. The behavior of each odor-active volatile under all four storage conditions was used to determine the residual suppression effect of CA. On the basis of these criteria, out of the 16 volatiles evaluated, CCA (3% O₂ + 3% CO₂ + 94% N₂ at 0°C) for 19 weeks caused a “residual suppression” effect on the production of propyl butanoate, butyl butanoate, and hexyl hexanoate. CA “residual suppression” effect was defined as the suppression effect, which lasts during ripening of the fruit after CA and is clearly due to the action of CA.” Most volatiles behaved differently under CCA than after CCA storage. Some volatiles were severely or completely suppressed during CCA storage, but when ripened after storage, some were suppressed, some were produced normally, while a few were produced in higher amounts. Several volatiles were produced in lower concentration during ripening after CCA than during ripening after harvest. However, this cannot be attributed entirely to a “CA residual suppression” effect. The loss in some volatiles after CCA compared to before CCA was due to a gradual loss of these volatiles and/or their precursors during long-term CCA storage as a result of natural senescence, rather than to an inhibitory action of CCA. Propyl butanoate, butyl hexanoate, and hexyl hexanoate were severely or completely suppressed during CCA storage, and were produced in less quantities during ripening after CCA than after harvest. These three volatiles are important to apple flavor. For example, hexyl hexanoate was found to be among the five most intense odor compounds in ‘McIntosh’ and ‘Cortland’ apples (Table 6.3). 

Apples stored in “lower ethylene CA” conditions (3% O₂ + 5% CO₂ and <5 ppm ethylene) produced less acetaldehyde, ethyl alcohol, ethyl acetate, and ethyl butanoate than apples stored in higher ethylene CA (>500 ppm), which in turn produced lower amounts of these volatiles than apples held in air (Forsyth and Eaves 1974). Apples are generally accepted by consumers after prolonged storage periods in LCA (Liu 1985). However, Yahia (1991b) found that the contents of some important flavor components in these apples was very low. The content of these volatiles in LCA was lower than in fruits stored in simulated LCA (Yahia et al. 1985a, b; 1991), possibly because of the ethylene removal method. Potassium permanganate used for ethylene removal in LCA storage (Yahia 1991b) oxidizes hydrocarbons other than ethylene. Further studies are needed to evaluate the effect of different ethylene removal methods on flavor development. Yahia (1989a; 1991a) and Yahia et al. (1991) found that some aldehydes and acetates were unaffected, while butanoates, 2-methylbutanoates, pentanoates, and hexanoates were either severely or completely suppressed during storage of ‘McIntosh’ and ‘Cortland’ apples in simulated
LCA (3% O₂ + 3% CO₂ + 94% N₂ at 3.3°C, 1-6ppm ethylene, for up to 8 months). However, most volatiles were produced normally during ripening after simulated LCA (Yahia 1989b; Yahia et al. 1991). Volatiles that were found to be suppressed during ripening after simulated LCA were methyl butanoate, propyl butanoate, butyl 2-methylbutanoate, hexyl 2-methylbutanoate, butyl hexanoate, and hexyl hexanoate. There is a similarity between the suppression effects of CCA (Yahia et al. 1990a) and LCA (Yahia et al. 1991), except that the LCA effect is more severe. This suggests a common basis for the CA effect.

Some odor-active volatiles increased during ripening after CA storage included methyl- and ethyl 2-methylbutanoate and ethyl- and butyl pentanoate (Yahia 1989a; Yahia et al. 1990a; 1991). The increases in these volatiles, especially that of ethyl 2-methylbutanoate, is significant because of their importance to apple odor.

Williams and Knee (1977) reported that the esterifying enzymes operate at similar rates in apples stored in air or in 2% O₂. Therefore, the suppression of odor volatiles by CA was suggested to be due to the limited supply and further metabolism of the volatile precursors (Knee and Sharples 1981). Since whole apples kept in low O₂ atmospheres were able to esterify added alcohols as rapidly as apples kept in air (Table 6.4), Knee and Hatfield (1981) concluded that the low levels of esters in apples kept in low O₂ were a consequence of low rates of alcohol synthesis. This might be only partially true since several esters were unaffected by CA (Yahia et al. 1990a; 1991a). Furthermore, the effect of CA on the members of the same alcohol group was not the same (Yahia 1989a; Yahia et al. 1990a; 1991; Yahia 1991a). The differences in the CA effect on the various volatiles in apples are probably due to the diverse origins and pathways, which lead to their production (Figure 6.2). The fact that some odor-active volatiles were produced normally during ripening in air after CA while others were either suppressed or stimulated (Yahia 1989a; Yahia et al. 1990a; 1991) indicate that CA storage has altered the normal metabolism of the fruit. This may suggest that some pathways might not have been affected, while others might have been blocked either temporarily or permanently and either partially or completely. It may also suggest that some pathways or mechanisms were diverted to different directions, or new synthetic pathways became operative. The increase of ethyl 2-methylbutanoate might be due to the operation of new pathways.

Concerns regarding the lack of full flavor in apples after CA storage prompted several studies to find ways to improve flavor while maintaining the benefits of CA storage in terms of texture. Hatfield (1975) reported that much of the loss in flavor in CA storage could be regenerated if the apples were kept at 5–15°C after storage before being transferred to 20°C.
Maintaining CCA and LCA stored ‘McIntosh’ and ‘Cortland’ apples in air at 3.3°C for up to 6 weeks was not sufficient to enhance the production of odor-active volatiles (Yahia 1986; 1989a; 1991a). Air at 20°C significantly increased the production of some odor-active volatiles. However, this temperature accelerates softening and acidity losses of apples. High ethylene might enhance flavor development after storage in CA, especially LCA and ULO. At an appropriate time, ethylene removal in the LCA room can be stopped and the gas is allowed to reaccumulate in storage, or the store room can be purged with ethylene. However, the addition of 500 ppm ethylene into LCA chambers for 6 weeks, after 8 months’ storage (Yahia 1986; Yahia 1989b; Yahia et al. 1991) enhanced the production of odor-active volatiles only slightly. This might be because of the extended storage period. Further studies are necessary to test the effect of high ethylene treatments after shorter periods of LCA storage. Smith (1984) found that the suppression of aroma by ULO (1.25% O₂) was slightly relieved by raising the O₂ level after about 3.5 months of storage. Light treatment slightly stimulated the production of some odor-active volatiles and the sensory quality of LCA ‘McIntosh’ and ‘Cortland’ apples (Yahia 1986; 1989a; 1991b). Knee et al. (1979) found an increase in the head space concentration of esters such as butyl- and hexyl acetate by illuminating ‘Cox’s Orange Pippin’, ‘Lady Sudely’, and ‘Golden Delicious’ apples with fluorescent light tubes during storage in low O₂ atmospheres (2% O₂ + 98% N₂) at 5°C. The increase in volatiles was suggested to be caused by membrane lipid peroxidation leading to further metabolism to alcohol and esters. Frenkel (1978) and Frenkel and Garrison (1976) used higher concentrations of O₂ (60–100%) in the presence of 10 ppm ethylene to accelerate ripening in several fruits and storage organs. The formation of lycopene and the evolution of ethylene in tomatoes, and the evolution of ethylene and flesh softening in pears were accompanied by a rise in H₂O₂ and lipid peroxides. High O₂ (100%) treatment (without the addition of ethylene) caused only a very slight stimulation in ripening and in the production of odor-active volatiles in ‘McIntosh’ and ‘Cortland’ apples (Yahia 1986; 1989a; 1991b). The use of high O₂ in combination with ethylene might be useful in stimulating ripening and flavor development of CA-stored apples.

Application of vapors of aldehydes, alcohols, or carboxylic acids to intact apples contributed to an increase in volatile contents, but there was no significant reproducible organoleptic effect (De Pooter et al. 1983).

Although CA is a very well-established method for apple storage, its effects on flavor (and especially on odor) is not yet fully understood. Apple flavor is not usually considered when CA technology is developed
or improved. Most CA advancement was made on the basis of fruit texture retention. Therefore, no treatment or strategy has yet developed to reduce flavor deterioration during CA storage, or to enhance flavor after storage.

VI. CORRELATION BETWEEN INSTRUMENTAL AND SENSORY ANALYSIS OF APPLE FLAVOR

The great differences in human perception of flavor and the very limited knowledge in understanding them are the major difficulties facing the study of flavor at present. In addition, there are difficulties in understanding the interaction among the different quality factors. A person’s reaction to a flavor stimulus can be influenced by other sensory properties such as color, appearance, and texture (Maga 1974; Christensen 1977). Aroma volatiles can have tastes, and nonvolatile flavor compounds can have considerable influence on the release of volatiles and hence on the aroma and flavor sensations experienced (Ahmed et al. 1978).

A major challenge in flavor research is to correlate sensory quality and chemical identity. Objective analytical and chemical identity determination of critical flavor components must be coupled with objective evaluations by a taste panel to yield useful and meaningful information about flavor. Human subjects were used with analytical tools to determine contribution of identified compounds. Human subjects were also used to describe the intensity and quality of compounds identified by individual GC peaks or groups of peaks, and to determine their relationships to the aroma of the whole isolate. Minor or trace components may have far more sensory importance than other volatile compounds present in much higher concentrations, due to the difference in their detection threshold.

Several methods have been used to evaluate the contribution of volatile components to flavor, including threshold measurements, difference tests, and descriptive tests. It is assumed that the substance has a significant sensory importance when it is present above its threshold concentration. Some compounds, however, can modify an odor even if they are present at below threshold level. A mixture of two or more components, each below its threshold level, may produce a detectable odor.

In a study of odor intensities, Guadagni et al. (1966) employed the concept of odor unit (OU). The ratio of the actual concentration of a given compound in a given sample to the threshold concentration of that
compound represents the number of OU contributed by that compound. One advantage of this approach is that one can estimate the contribution of a compound to the overall aroma in terms of odor rather than in terms of concentration. An example of the practical usefulness of the OU concept has been shown by Guadagni et al. (1966). Components of the essence of ‘Delicious’ apples were separated by GC. The relative importance of the various peaks in terms of characteristic apple odor was determined by a trained panel. The panel described the odor of compounds corresponding to individual peaks as each compound was eluted from the column, after 20 μl of essence had been injected. A high percentage of “apple” or “apple-like” descriptions was assigned to three peaks, indicating their importance to apple odor. Triangle tests comparisons made for these three compounds individually after they were trapped indicated that the smallest peak on the chromatogram accounted for most of the desirable aroma, and the largest peak was the least important. Subsequent work (Flath et al. 1967) using GC-MS, infrared, and proton magnetic resonance spectrometry revealed that ethyl 2-methylbutanoate, (E)-2-hexenal, and hexanal are the most important components of the essence of ‘Delicious’ apple aroma, respectively. The Charm analysis technique (Acree et al. 1984) measures the intensity of detected constituents, in Charm units, without the necessity of knowing the concentration or the threshold values of these constituents.

Several attempts have been made to correlate volatile constituents in apples with maturity or ripeness indices. The head space volatile composition of ‘McIntosh’ apples has been correlated with several maturity and ripeness indices such as acidity, internal chlorophyll (nondestructive spectrophotometric absorbance), firmness measurements, soluble solids content, and stiffness coefficient calculated from sonic resonance data (Sapers et al. 1977). Potentially useful correlations were found between volatile levels and fruit firmness or stiffness coefficient. Watada et al. (1981) correlated sensory attributes, which were selected by a trained panel, and the concentration of head space volatiles, soluble solids, and titratable acidity of ‘Golden Delicious’ and ‘York Imperial’ apples. Stepwise regression analysis indicated that the variation of sweetness of ‘York Imperial’ and the acidity of both cultivars can be accounted for, in part, by the measurable soluble solids, titratable acidity, and head space volatiles. Astringency, mustiness, starchiness, and floral fruitiness correlated poorly with the volatiles, soluble solids, and titratable acids.

Panasuik et al. (1980) attempted to correlate aroma, which was determined organoleptically by a trained panel, and volatile composition for ‘McIntosh’ apples. A correlation was found between the overall
aroma intensity and the sum of two components, which were tentatively identified as hexanal and 2-hexenal. The ripe aroma correlated with C-6 aldehydes for stored and unstored apples and with total peak areas for stored apples. The unripe quality was negatively correlated with total peak area. Overripeness in unstored apples correlated with total peak area and the sum of volatile esters, which were tentatively identified as ethyl propanoate, ethyl 2-methylpropanoate, and ethyl pentanoate. No significant correlation was found between overripeness and volatiles produced by the stored ‘McIntosh’ apples.

Although human judgment is always the ultimate test of sensory quality, particularly in terms of pleasantness, unpleasantness, and overall acceptability, it is very desirable that objective analysis be developed that can take their place. It is much easier to calibrate and learn the accuracy, precision, sensitivity, long-term reproducibility, and reliability of instruments than it is to establish these same characteristics with people. More work is needed before relationships between chemical structures, concentrations, and sensory effects will be fully understood.

VII. FUTURE RESEARCH NEEDS

1. More attention should be paid to flavor quality when breeding and selecting new apple cultivars.

2. Apple cultivars vary greatly in their flavor. Therefore, studies are still needed to identify and characterize the flavor profile of different cultivars. Olfactory (sniffing) assays coupled with adequate analytical techniques are of great importance and should be used more often. In addition, it is necessary to understand more about the relationship between chemical structure, concentration, and sensory effects. It is very important to differentiate between “primary or natural” odors (those important for intact fresh fruit) and “secondary” odors (those important for processed fruit).

3. Studies on the biosynthesis and metabolism of apple odor components are important in order to manipulate the flavor of the fruit, including manipulation with molecular biology techniques. Due to the very large number of volatiles produced by apples, only odor-active volatiles should be considered and studied in detail.

4. Further studies are still needed on the effects of various preharvest and postharvest treatments on flavor, and especially on odor-active volatiles. It is still not yet fully understood how odor-active volatiles are affected by factors such as cultural practices, climatic conditions, and postharvest treatments. Studies are still needed to
investigate the nature of flavor (especially odor) suppression by CA, and whether this suppression can be reversed.

5. Studies on the physiological and biochemical basis of human perception to flavor are of great importance. There is still a strong need to develop adequate objective analytical techniques to measure flavor that are based on the understanding of human perception.

6. Research is needed to identify and characterize off-odors and off-flavors, and to determine the mechanism of their generation.

LITERATURE CITED


Sharples, R. O. 1982. Effects of ultra-low oxygen conditions on the storage quality of English
Controlled atmospheres for storage and transport of perishable agricultural commodi-
ties. Timber Press, Beaverton, OR.
commercial scale storage of Cox’s Orange Pippin apples in 1.25% oxygen. E. Malling Res.
Sta. Rpt. 147.
Smith, S. M. 1984. Improvement of aroma of Cox’s Orange Pippin apples stored in low
52:176–182.
New York.
Delicious apple fruits after storage for various times in different CO₂ and O₂ concen-
Thomas, M. 1925. A quantitative study of the production of ethyl alcohol and acetaldehyde
by cells of the higher plants in relation to concentrations of oxygen and carbon dioxide.
Thomas, M., and J. C. Fidler. 1933. Studies to zymasis: IV. Zymasis by apples in relation to
of quality of fruits and vegetables. AVI Publ. Co., Westport, CT.
106:130–132.
Williams, A. A. 1977. Measurement of flavor quality in apples, apple juices and fermented
Washington, D. C.
Williams, A. A. 1978. Flavor research in horticulture: What it can offer the producer,
processor and consumer. Report of Long Ashton Research Station, University of Bristol,
LTD, London.


