#### AN ABSTRACT OF THE THESIS OF

<u>Anne Plotto</u> for the degree of <u>Doctor of Philosophy</u> in <u>Plant Physiology</u> presented on <u>March</u> <u>13, 1998.</u> Title: <u>Instrumental and Sensory Analysis of 'Gala Apple'</u> (*Malus Domestica*, <u>Borkh) Aroma.</u>

Abstract approved:

'Gala' is an apple cultivar with a distinctive aroma and flavor. 'Gala' storage season is short in regular atmosphere (RA). Controlled atmosphere (CA) extends 'Gala' storage but volatile production is reduced. 'Gala' odor-active aroma compounds were identified using *Osme*, a gas chromatography and olfactometry technique. Changes in aroma after RA and CA storage were characterized by *Osme* and descriptive sensory analysis (DSA).

Hexyl acetate, butyl acetate and 2-methylbutyl acetate were emitted in the largest amounts and were perceived with the strongest intensities, with "ripe apple", "solvent" and "fruity" descriptors. Production of hexyl acetate and butyl acetate after CA storage decreased significantly compared to apples stored in RA, along with perceived intensities. 2-Methylbutyl acetate only decreased in apples stored 20 weeks in CA. Other esters with an apple odor were butyl 2-methylbutyrate and hexyl 2-methylbutyrate. Methyl 2-methylbutyrate, ethyl 2-methylbutyrate and propyl 2-methylbutyrate had sweet, fruity, and berry-like odors. Ester production after CA storage decreased at different rates. The non-ester compounds 4-allylanisole (anise) and  $\beta$ -damascenone (grape juice) as well as an unidentified compound (watermelon), were perceived mostly from RA stored fruit. Other unidentified peaks had cucumber, mushroom, adhesive tape or skunk odors. Comparison of mixtures of 'Gala' odor-active compounds in water with whole 'Gala' apples revealed that hexyl acetate, hexanal and butyl acetate were necessary to impart an apple odor. 2-Methylbutyl acetate and methyl 2-methylbutyrate also contributed to the least difference between mixture solutions and apples.

DSA of 'Gala' apples stored in RA and CA confirmed the general decrease in fruity aroma following CA storage. A floral descriptor was also significantly affected by CA storage. A musty note appeared in CA stored fruit, which may have corresponded to a garlic odorant peak detected during *Osme*. 'Gala' apples stored 16 weeks in CA followed by 4 weeks in RA emitted more volatiles than fruit stored 20 weeks in CA. The difference in volatile production was perceived by *Osme* analysis, and differences in overall fruity aroma between 16 and 20 weeks CA stored fruit were perceived only for whole fruit. There was no difference between those two types of storage for fruit flavor.

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## INSTRUMENTAL AND SENSORY ANALYSIS OF 'GALA' APPLE (MALUS

## DOMESTICA, BORKH) AROMA

By

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# **CONTRIBUTION OF AUTHORS**

Dr. David S. Lundahl designed the sensory experiment in the second manuscript (Chapter 4).

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# INSTRUMENTAL AND SENSORY ANALYSIS OF 'GALA' APPLE (MALUS DOMESTICA, BORKH) AROMA

# CHAPTER 1 INTRODUCTION

Flavor is a combination of the basic tastes, mouth sensations such as astringency, and aromas (Meilgaard et al., 1991). A few compounds can stimulate the sense of taste, while aroma is due to many more known and unknown volatile molecules. Apple taste is mostly caused by the dominant acids and sugars, malic acid, fructose and glucose (Acree and McLellan, 1993; Visser et al., 1968). Astringency and bitterness are minor components of fresh apples; those attributes are generally due to polyphenols which are desirable in apple cultivars processed for cider (Williams et al., 1977a). Aroma is the perception of volatile compounds in the nose either directly, or retronasally when volatiles are released in the mouth during chewing. An excess of 300 volatile compounds have been identified in apples (Yahia, 1994). However, the odor-activities of only a few are known (Guadagni et al., 1966a; Flath et al., 1967; Williams et al., 1977a; Cunningham et al., 1986). There is generally no direct relationship between compound concentration or volatility and odor-activity (Acree and McLellan, 1993), nor is the relationship between chemical molecular structure, shape, size and odor-activity straightforward (Von Ranson et al., 1992; Takeoka et al., 1995; 1996). Furthermore, odor-active molecules when smelled alone may cause a different response than when interacting with others in mixtures.

Gas-chromatography (GC) is a powerful separation tool for volatile compound analysis. Volatile compounds can be identified using GC combined with mass spectrometry and chemical standards. Sniffing the GC effluent allows the presence of odor-activity during a chromatographic run to be identified. Techniques that evaluate aroma are distinguished by how data are recorded and processed. Techniques in which the sample is successively diluted until no odor is perceived from the GC effluent are called Aroma Extract Dilution Analysis (AEDA, Grosch, 1993) and CharmAnalysis (Acree *et al.*, 1984). These techniques are based on the assumption that the odor response is linear to stimulus concentrations. Using *Osme*, from the Greek word "smell" (McDaniel *et al.*, 1990), the odor of the sample extract is assessed by several panelists during multiple injections. Odor intensity and duration of perception are recorded on a 16-point scale with a linear sliding bar connected to a personal computer. *Osme* is in agreement with Stevens' law of psychophysics which states that the response to a stimulus follows a power function (Stevens, 1957; Da Silva *et al.*, 1994). By using *Osme*, the relative significance of an aroma extract can be established in a reproducible and reliable way (Da Silva *et al.*, 1994). Comparisons between samples can be analyzed using statistical analysis (Da Silva *et al.*, 1993).

'Gala' apple (*Malus domestica*, Borkh) originated in New Zealand from a cross between 'Kidd's Orange' ('Cox's Orange Pippin' X 'Red Delicious') and 'Golden Delicious' (White, 1991). 'Gala' fruit is sweet and subacid, and has a distinct flavor appreciated by most of its consumers (Gordon, 1990). 'Gala' was given high preference ratings by consumer taste panels (Stebbins *et al.*, 1994). 'Gala' is an early ripening cultivar and its eating quality is at its best after harvest. Hedonic ratings, which measure the degree of liking on a 9-point scale, decreased for 'Gala' apples stored in air for more than 60 days (Stebbins *et al.*, 1994; Plotto *et al.*, 1995).

Controlled atmosphere storage (CA) is commercially used to prolong apple shelflife. While low  $O_2$  and high CO<sub>2</sub> controlled atmospheres significantly reduces firmness and acidity losses (Smock, 1979), volatile production is negatively impacted (Patterson *et al.*, 1974). Volatile production after CA storage depends on several factors including apple maturity at harvest (Dirinck *et al.*, 1989; Girard and Lau, 1995), cultivar (Yahia *et al.*, 1990), ratio of  $O_2$  and CO<sub>2</sub> in the atmosphere (Streif and Bangerth, 1988; Fellman *et al.*, 1993; Hansen *et al.*, 1992) and storage duration (Willaert *et al.*, 1983). By alternating high  $O_2$  to low  $O_2$ /high CO<sub>2</sub> atmospheres, Mattheis *et al.* (1998) reduced volatile loss in CA storage without altering firmness loss. However, the effect of the volatile production gain on fruit palatability is unknown. The main objective of this research was to identify compounds contributing to 'Gala' aroma and to characterize the changes in 'Gala' aroma during CA storage. Specific objectives were:

1. To identify compounds contributing to 'Gala' aroma using GC and Osme techniques. Sampling of volatile constituents was optimized for the conditions used for Osme.

2. To identify aroma active compounds most similar to 'Gala' aroma when combined in a mixture. Because GC and *Osme* provide information on individual compounds, mixtures of compounds found in 'Gala' were compared to 'Gala' apples and assessed for difference from whole 'Gala' fruit.

3. To characterize changes in CA storage in 'Gala' aroma using GC and Osme. Observations were performed during two consecutive years, using fruit from the same orchard. Two of the three panelists participated in Osme data collection both years of the study.

4. To characterize changes following CA storage in 'Gala' aroma and flavor using descriptive sensory analysis. Results were compared with *Osme* findings.

# CHAPTER 2 LITERATURE REVIEW

## HISTORY OF THE APPLE, AND ORIGIN OF 'GALA'

Since the beginning of agriculture, humankind has worked toward improving crops. Both cultural practices and species selection have been aimed at increasing yield, removing possible toxicity from wild species and increasing palatability. Extending storage life for fruit and vegetables has also always been a concern because of the perishable nature of those crops as opposed to dry commodities such as cereals and nuts.

Apple (Malus X domestica) has been cultivated since ca. 3500 BC (Morgan and Richards, 1993). The center of origin of the apple species was Central Asia where the greatest diversity can be still found, mostly in Kazakhstan and surrounding countries (Janick et al., 1995). The development of irrigated agriculture, and the rise of great civilizations with long-distance trade contributed to the culture and spread of fruit crops. The Persian Empire (ca. 500 BC), from the Aegean coast of Turkey to the Indus valley, and from Egypt up to the Caucasus and Central Asia, corresponded to a peak of development of agriculture, trade, migration and technology exchanges. Orchards and vineyards flourished extensively, and apple fruit became part of Persian cooking (Morgan and Richards, 1993). In Europe, the cultivation of apples was certainly present from the time of the Romans and possibly much earlier. French fur traders and missionaries introduced apples to Canada during the 16<sup>th</sup> century, and Protestant settlers introduced apples to North-Eastern America during the 17<sup>th</sup> century. Apples were introduced in South Africa by the Dutchman Jan Van Riebeeck in 1654. The first apple trees were planted in Australia in 1788, and in New Zealand in 1814 (Morgan and Richards, 1993). Today, more than 10,000 apple cultivars are known but only a few dozen are grown commercially worldwide. Apple has a wide cultural range: it can be cultivated in Siberia where winter temperatures fall down to -40 °C, and in equatorial countries such as Colombia and Indonesia where two crops can be produced in a single year (Janick et al., 1995).

Apple fruit is popular because of its storability, its tolerance of transport as opposed to soft fruit, and it can be consumed in many different ways. The leading apple producing countries are China (12 million tons) and the United States (5 million tons) (FAO, 1995). Europe produces 13.2 million tons with France and Italy each producing 2.2 million tons (FAO, 1995). The volume produced by Australia and New Zealand is small compared to the former countries (0.3 and 0.5 million tons, respectively). Yet, those countries have diversified the available cultivars on the world market by introducing and promoting 'Granny Smith' (Australia), 'Braeburn' (New Zealand), 'Gala' (New Zealand), and lately 'Pink Lady' (Australia) (Manhart, 1995). European and American markets were until recently dominated by 'Golden Delicious' and 'Delicious'; those cultivars fulfilled the requirements of producing annually high yield of large size and uniform fruit of good storage potential. With the development of controlled atmosphere storage, 'Golden Delicious' and 'Delicious' could be stored for an entire year. Introduction of new cultivars requires that each cultivar's growing behavior and fruit metabolism after harvest be researched. Yet, the effort of diversifying the American apple market has been consistently rewarded; there is always a need to offer the consumers a product they like. Manhart (1995) attributed the low apple consumption of Americans to the market dominated by two or three cultivars only, in contrast with the European market with eight to 10 cultivars. From 1950 to 1980, American apple consumption per capita was one-third to one-half that of Western Europe (Manhart, 1995). When New Zealand marketers introduced 'Gala' and 'Braeburn' in the US in 1981, those apples sold for high prices without promotion, showing a strong potential market for new varieties (Manhart, 1995).

'Gala' apple was bred by J.H. Kidd, a New Zealand fruit grower. Kidd wanted an apple which combined the high yield, attractiveness and sweetness of the American cultivars 'Golden Delicious' and 'Delicious' and the high flavor of English apples such as 'Cox's Orange Pippin' (White, 1991). Kidd first selected a cross from 'Cox's Orange Pippin' and 'Delicious', 'Kidd's Orange' in 1930. He continued to make crosses, and 'Gala' ('Kidd's D-8') was selected in 1962 from the progeny of 'Kidd's Orange' by 'Golden Delicious'. Commercial plantings in New Zealand began in 1965; first commercial shipments of the fruit to the UK and the US were in the 1980s. 'Gala' is a small to medium round to oval apple, and bears bright orange-red stripes on a yellow creamy background (Gordon, 1990). The texture is crisp, with a tendency to become soft after storage. The fruit is sweet and subacid, with a distinct aromatic flavor (Gordon, 1990). 'Gala' is prone to color mutations, and since the first release of 'Kidd's D-8', several strains have been patented (White, 1991). 'Gala' strains are mostly differentiated by the intensity, brightness and percentage covering of the red color; there is no consensus in the literature about the distinction of 'Gala' strains based on taste attributes (Green and Autio, 1993; Kappel *et al.*, 1992).

The success of 'Gala' is mostly due to the work and intuition of its originator, J.H. Kidd. However, there has since been a world effort from horticulturists to improve it, to adapt strains to growing conditions and rootstocks, and to monitor proper harvesting and storage. Once a variety has met horticultural requirements, it has to pass consumer judgments. Sensory science provides tools to measure qualities of foods, in this case, apples.

#### **METHODOLOGY: SENSORY AND INSTRUMENTAL ANALYSES**

#### **Sensory Analysis Methods**

The use of senses in judging food quality is part of our daily action of eating. The need for grading a product has increased with increased trading; professional tasters and consultants found niches in the rising food and beverage industries in the early 1900s (Meilgaard *et al.*, 1991). Currently, sensory science offers several methods to describe and evaluate the quality, or changes in quality of a product. With increasing knowledge of psychology and psychophysics, more precise instrumentation and more powerful statistical techniques, the tests are increasingly sophisticated and useful.

Consumer tests give information on the acceptance of a product. Because people have different perceptions and vary in their judgment of liking or disliking a product, usually large numbers of panelists are needed (Williams, 1981). Stebbins *et al.* (1991;

1994) evaluated the acceptance of new apple cultivars on a 9-point category hedonic scale using 60 to 150 panelists. Daillant-Spinnler *et al.* (1996) tested 12 apple cultivars using a 10 cm line scale with 120 British consumers. The 9-point hedonic scale was also used to evaluate differences in liking of 'Gala' apples stored in air or controlled atmosphere (Boylston *et al.*, 1994), and to evaluate the acceptability of 'Gala', 'Braeburn' and 'Fuji' apples at different maturity stages and storage times (Plotto *et al.*, 1995). The hedonic scale measures the absolute liking of the product presented to the panelists. Acceptance of product attributes can also be evaluated with the hedonic scale, provided attribute descriptors are clearly understood by all consumers. 'Cox's Orange Pippin' and 'Suntan' apples were assessed by 600 visitors on a "just right" scale for eight attributes (Williams and Langron, 1983). When subtle differences are to be tested between treatments, rating against a reference sample (Smith, 1984; Kappel *et al.*, 1992), paired comparison tests (Smith and Stow, 1985) or ranking tests (Paleotti *et al.* 1993) may be more sensitive.

Williams (1981) recommended use of a combination of descriptive sensory analysis and instrumental measurements with consumer data to understand consumer preferences. The variation of one or more attribute intensities due to a storage treatment, or a different cultivar may explain consumer acceptance or rejection of a product. Few descriptive studies are reported for fresh apples. Williams and Carter (1977) developed a lexicon with almost 200 descriptors for external and internal appearance, feel of apple in the hand, external and internal aroma, taste, texture and after-taste. Differences between stored 'Cox's Orange Pippin' apples were evaluated with this lexicon. Dhanaraj et al. (1980) limited the number of descriptors to four, and used a scale anchored with words taking into account degree of ripeness and adjectives specific to each scale; for example: firm, smooth, crisp, soft for texture and mouthfeel. Those researchers' objective was to develop a simple method for routine assessment of apple quality through ripening and storage. Watada et al. (1980) described the characteristic of five apple cultivars using a sensory profile method. They related the aroma and flavor data to chemical measurements (Watada et al., 1981) and the texture attributes to physical firmness measurements (Watada and Abbott, 1985).

Different methods for descriptive analysis have been developed in the last 50 years and the choice of one method over another depends on the research objectives. The first method, Flavor Profile, was developed by Arthur D. Little, Inc. in the 1940s (Meilgaard et al., 1991). Five to eight panelists develop the terminology to describe a product and rate "character notes" on a seven-point intensity scale. Panelists rate the products independently and then discuss the results with the panel leader to arrive at a "consensus" profile for each of the samples. Training and use of references are meant to maximize panelists reproducibility. However, the consensus method was criticized to be prone to bias if the panel leader or one panelist had a strong personality and imposed their views on the other panelists. The Texture Profile method (General Foods Corp.) is specific for description of textural characteristics of foods, and is based on food's rheological properties (Meilgaard et al., 1991). The Texture Profile method has evolved from being an adaptation of the Flavor Profile where the panel verdict may be derived from group consensus, to a more sophisticated technique where data recorded on a line, category or magnitude estimation scale are analyzed statistically. The Quantitative Descriptive Analysis (QDA<sup>®</sup>) method was developed by the Tragon Corporation in collaboration with the Department of Food Science at the University of California at Davis (Meilgaard et al., 1991). This method relies heavily on statistical analysis to evaluate panelists' performances and to judge product differences. Similar to the other descriptive methods, panelists are trained with references, but the panel leader acts as a facilitator rather than as an instructor. Panelists evaluate the samples one at a time in separate booths and do not discuss their data after evaluation. According to Meilgaard et al. (1991), this method is the closest to the ideal of treating human subjects as calibrated instruments. The Spectrum<sup>™</sup> method developed by Gail Civille (Meilgaard et al., 1991) combines the basic principles of descriptive analysis and the knowledge acquired in the field of sensory science to propose a practical approach adapted to the research objectives and to the products being tested (Meilgaard et al., 1991). Unlike the preceding methods where panelists are trained to all use the same terminology, Free Choice Profiling allows panelists to use their own vocabulary (Williams and Arnold, 1984). The data are analyzed by General Procrustes analysis, a multivariate technique

which adjusts for panelists' use of different parts of the scale by normalizing and centering data. Procrustes analysis also finds the best combination of variables (product descriptors) that explain differences between products.

#### **Relating Instrumental to Sensory Measurements**

Instrumental analysis is usually faster, more reproducible and easier to implement than sensory analysis. Ideally, instrumental measurements would be used to estimate fruit sensory characteristics and palatability. However, because of the complexity of the human sensory apparatus, chemical, biochemical and physical interactions of the food constituents occurring in the mouth or nose, and psychological factors that affect rating. relationships between instrumental and sensory data are often imperfect. The Magness-Taylor penetrometer usually correlates positively with sensory evaluation of firmness (Wills et al., 1980; Abbott et al., 1992; Plotto et al., 1997). Crispness, hardness and toughness texture attributes were significantly correlated with firmness measurements of apples with the Instron Universal Testing Instrument (Watada and Abbott, 1985). Nondestructive firmness measurements are also being tested to predict apple texture and firmness (Abbott et al., 1992; 1995). Aroma, taste and flavor attributes are usually explained by the interaction of chemical compounds with taste or olfactory receptors. Most instrumental techniques used to determine chemical compounds related to taste involve wet chemistry and the use of acid titration or liquid chromatography. Aroma is the result of volatile compounds stimulating olfactory receptors, and gas chromatography is therefore the preferred analytical tool.

#### Gas Chromatography and Olfactometry

The development of gas chromatography (GC) in the early 30's coupled with mass spectroscopy (GC-MS) in the 50's allowed food scientists to separate and identify hundreds of volatile components in foods. Additionally, a few groups of researchers have assessed the flavor significance of chemicals analyzed by sniffing effluents at the outlet of the column (Guadagni *et al.*, 1966a; Acree *et al.*, 1984; Cunningham *et al.*, 1986; Grosch, 1993; McDaniel *et al.*, 1990).

Acree and co-workers (1984) and Grosch (1993) inject the aroma extract into the GC after successive dilutions: compounds that are perceived by the human subject at the highest dilution level are believed to be the character impact volatiles of the sample. Those compounds present in the food at concentrations above their odor threshold (odor unit greater than one) contribute to the food aroma. In CharmAnalysis (Acree *et al.*, 1984), data processing considers duration of the perceived compound as well as its dilution value. In aroma extract dilution analysis (AEDA), the dilution level at which compounds are perceived gives the flavor dilution (FD)-factor (Grosch, 1994). The two methods were compared with beer extracts and methods of calculations resulted in different odor activities for the same olfactory data (Abbott *et al.*, 1993). Nevertheless, both CharmAnalysis and AEDA are recognized as useful complements to chemical analysis for screening potent odorants in foods. Results can be graphically represented along the run time of the chromatogram, and compared to the flame ionization (FID) chromatogram (Acree, 1993).

Unlike the former methods based on odor threshold and odor unit, Osme is based on modern concepts of psychophysics which state that odor response to stimulus concentration follows a power function (Stevens, 1957; McDaniel et al., 1990; Sanchez et al., 1992a; Da Silva et al., 1994). Instead of determining thresholds with serial dilutions of the sample, subjects directly record the odor intensity and duration of response for each odor-active component while describing its quality (Da Silva et al., 1994). Unlike CharmAnalysis and AEDA where the panelists give a "yes" or "no" response to the stimulus, Osme panelists rate the intensity of perceived odor on a 16point category scale, where 0 = none and 15 = extreme. The plot of odor intensity of eluted compounds versus retention time is called an Osmegram, and like CharmAnalysis and AEDA, can be compared to the FID chromatogram of the sample run on the same column under the same conditions. As with threshold methods, small peaks on the FID chromatogram may have high odor intensity, and large peaks on the FID may have a low sensory response (Sanchez, 1990; Acree, 1993). By evaluating different solution concentrations, Da Silva et al. (1994) showed that panelists could perceive the concentration changes quite accurately. The sensory response measured as perceived

intensity of the compound was a power function of compound concentration, which is in agreement with Stevens' law (Da Silva *et al.*, 1994). Linear and logarithmic functions also provided a good fit in relating sensory responses to the odorant concentration. Variation between panelists can be expected due to differences in human sensitivity to chemical compounds (Da Silva, 1992; Sanchez, 1990; Abbott *et al.*, 1993; Grosch, 1993). By training panelists to recognize the character of the measured odors, they can come to a consensus on descriptive terms. Likewise, variation within panelists has been observed and attributed to physiological and psychological effects (Da Silva, 1992). Repeated runs are performed to minimize this variability.

The use of the GC-effluent sniffing technique is presently the best available technique to identify odor significance of volatile compounds in a food sample (Da Silva, 1992). The disadvantages or failures of this method are inherent to the GC separation technique such as problems of co-elution, compounds which may not be resolved by the column (Sanchez, 1990). The use of columns coated with different phases, and chromatographic runs using different conditions may partially alleviate the problem (Grosch, 1993). Another problem is presuming a result based on individual compounds rather than an integrated mixture. Last, differences between extraction or headspace trapping methods lead to different products.

## Sampling Methods for Gas Chromatography: Headspace versus Extraction

There are two general approaches used in odor research: one is to study total volatiles in the food sample, and the other considers only food odor and therefore analysis of only the volatiles present in the vapor phase, or "headspace vapor analysis" (Flath *et al.*, 1967; Weurman, 1969). Differences in proportions of classes of volatiles due to differences in vapor pressure, and solubility in water and lipids were found if volatiles were trapped from headspace, distilled, or solvent-extracted in reports from studies on apples (Paillard, 1990). In the "total volatile analysis", the most important step is to isolate the volatiles from the sample, and further concentrate them (Weurman, 1969). Many distillation systems were reviewed by Weurman (1969). However, the heat involved in the procedure may alter some components. When comparing different

extraction techniques for fruit, a cooked aroma was perceived from steam distillate extracts (Güntert et al., 1998). Instead, extraction by organic solvents is often a preferred method employed in volatile analysis. The use of more than one solvent having specific affinities for different groups of components is better than the use of one single "multipurpose" extractant. Cunningham et al. (1986) and Yahia et al. (1990) used Freon 113 (1, 1, 2-trichloro-1, 2, 2-trifluoroethane) to extract apple volatiles. Aroma volatiles from fruit puree were successfully extracted with Freon 12 by using a twochamber glass apparatus and the different freezing temperatures of Freon 12 (-29 °C) and the fruit slurry (0 °C) (Blakesley, 1977). The Freon could be separated from the fruit slurry at -20 °C, similar to a cold distillation. Gunata et al. (1985) developed a method that could extract both free and glycosidically bound volatiles from wine. In the first stage, components from the aqueous food system were adsorbed on the non-ionic resin Amberlite XAD-2 and then eluted with selective solvents. Free volatiles were eluted with pentane and directly analyzed by gas chromatography, while glycosylated forms were enzymatically hydrolyzed to release the aglycone portion. Apple pulp and juice free and bound volatiles were analyzed by Aubert (1997) using this method.

Headspace sampling usually captures low molecular weight low boiling point compounds (Wampler, 1997). High molecular weight esters (above C<sub>10</sub>) are seldom found in headspace extracts of apples (Paillard, 1990). Charcoal was the only adsorbent used before the availability of porous polymeric materials such as Tenax and Porapak Q (Teranishi and Kint, 1993). Tenax is less reactive than charcoal, but has a lower retention volume (Rothweiler *et al.*, 1991). Trap desorption is usually done with organic solvents as in extraction procedures (Teranishi and Kint, 1993). Also, thermal desorption (200 °C for Tenax) was proven to give better recovery than diethyl ether elution (Cole, 1980). Thermal desorption allows near recovery of all trapped compounds while avoiding co-elution of low boiling compounds with the solvent (Wampler, 1997).

In fruit flavor studies, headspace sampling can be done on intact fruit, slices or crushed fruit. Sampling from intact fruit allows for time-course studies (Mattheis *et al.*, 1991b). The amount of volatile compounds in the air depends on the permeability of

fruit tissue (Knee and Hatfield, 1976). Therefore, sampling of crushed tissue might be preferable in aroma studies. However, enzymatic reactions occurring upon crushing or macerating the fruit alter the compounds present in the sample (Drawert, 1975; Paillard and Rouri, 1984). Buttery (1993) suggested addition of a saturated solution of calcium chloride or sodium chloride to deactivate enzymes that synthesize secondary compounds such as those resulting from the lipoxygenase lipid oxidation pathway. Buttery and co-workers found that calcium chloride was more efficient at deactivating enzymes in tomatoes; with this method, Z-3-hexenal concentration remained constant for several hours, which allowed isolation of headspace volatiles (Buttery, 1993). Cunningham *et al.* (1986) crushed apples under methanol as an enzyme denaturing agent. To be as close as possible to the fruit aroma released upon chewing, the enzyme inhibitor should be added after one minute of pulp maceration.

## Odor Units and Odor-Activity of Compounds in Mixtures

Patton and Josephson (1957) introduced the idea of relating a compound's concentration to its odor threshold in order to assess its odor significance. This concept was named "aroma value" by Rothe and Thomas (1963), "unit flavor base" by Keith and Powers (1968), and is now used as the "odor unit" (Teranishi et al., 1991), and the "Odor Activity Value" (OAV) (Grosch, 1994). Odor unit is defined as the ratio of compound concentration to its odor threshold; compounds contributing to the food aroma have odor unit values above one (Teranishi et al., 1991). Guadagni and coworkers studied the flavor significance of pure chemical components and determined their odor thresholds by sniffing diluted series of pure compounds presented in polyethylene wash-bottles (Guadagni et al., 1963). Odor units were then calculated to assess the contribution of chemical compounds to the flavor of apple essence (Guadagni et al., 1966a), hop oil (Guadagni et al., 1966b), and fresh tomato (Buttery et al., 1987). The authors admitted that the odor unit concept does not give any indication of quality, nor does it say anything about stimulus concentration and intensity above the threshold (Guadagni et al., 1966a). However, it gives an indication of the relative importance of components to the food, and comparing between odor units allows ranking of the

components into their most probable order of sensory contribution. Odor units have been criticized because they assume additivity of odor-active chemicals and do not consider synergism or antagonism between compounds (Forss, 1981). Also, they assume linearity between sensory perception and component concentration, ignoring the power relationship between these two variables as postulated by Stevens' law (Frijters, 1978).

In general, binary mixtures of odorants are perceived less intensely than the sum of the intensities of unmixed compounds (Cain, 1975; Laing et al., 1984). The degree of reduction appeared to depend on the relative proportion of each compound in the mixture and on their intensity as individual odorant. Little interaction was observed between two low-intensity odorants at concentrations above the threshold, but suppression of one odorant over the other was strong with a high intensity odorant at high concentration (Laing et al., 1984). Odor suppression or odor masking was studied by Laing and co-workers (Laing et al., 1984), Cain and co-workers (Cain, 1975) and Berglund and co-workers (Berglund et al., 1971). Berglund and co-workers proposed a mathematical model to formalize interactions between two compounds: each odorant was described by a vector with length representing odor intensity. The angle between two vectors is constant for a pair of odorants and depicted the perceptual interaction between compounds. However, this model did not consider asymmetrical interactions. i.e. when one compound reduces the perception of the other and not reciprocally (Laing, 1995). Additionally, the model becomes more complex with more than two components. Berglund et al. (1973) tested mixtures of three compounds at five levels of perceived intensity and found good agreements between theoretical values for the vectors and calculated experimental values.

Laing (1995) and Laing and Livermore (1992) showed human subjects, trained, experts or untrained, could not identify more than three or four odorants in mixtures of eight. Discrimination between complex mixtures of odorants were also difficult to obtain (Laska and Hudson, 1992). When panelists were presented pairs of mixtures of 3, 6 or 12 odorants, 40% of "identical" responses were given to similar pairs presented, and 20% to 40% "identical" responses were given to pairs where one of the mixtures had

one less compound. Based on physiological knowledge of olfactory receptor cells and neural transmission, Laing (1995) summarized the possible mechanisms of odor suppression between two compounds: inhibition could occur through competition for receptor cells or sites. Competition for receptor sites could be also due to allosteric mechanisms where the binding of one odorant to one receptor changes the conformation of the adjacent receptor, preventing binding for other odorant. Also, the binding of one odorant could trigger the release of  $Ca^{2+}$  to fire a neuron, but that excess  $Ca^{2+}$  would act as inhibitor for the next transduction event. Laing (1994) also confirmed the temporal filtering of odorants found by Getchell et al., (1984). Odorants stimulate the receptor cells at different velocities, with differences of several hundred milliseconds. When presenting odorants in series at intervals of several hundred milliseconds with a 6-channel olfactometer, "fast" odorants were perceived first and inhibited the perception of "slow" odorants; the level of inhibition was the same as when odorants were presented simultaneously in mixtures, with the "fast" odorant dominating over the "slow" one. Laing (1994) also mentioned the possible role of transduction pathways in mixture suppressions. Odorants operating via the adenylate cyclase pathway such as carvone would dominate odorants that stimulate cells via the inositol phosphate pathway, such as propionic acid.

At sub-threshold concentrations, odorants were found to have an additive or synergistic effect (Guadagni *et al.*, 1963; Laska and Hudson, 1991). While Guadagni *et al.* (1963) found an additive effect between compounds from the same chemical family, or having a similar chemical structure, Laska and Hudson (1991) measured a decreased threshold of compounds in mixtures as compared to when they were presented alone. Köster (1969) found synergy to occur rarely, while additivity occurred when compounds were mixed in a 1:1 ratio, and suppression occurred when compounds were mixed in 1:6 or 1:8 ratios. Finally, among phenomena occurring in odor mixtures, blending has been used by flavorists and perfumists. Odor blending or fusion occurs when the identity of some or all of the constituents of a mixture is lost, but an olfactory sensation is still perceived (Laing, 1995). The effect of odor mixtures is complex. Psychophysical studies show mostly a suppression of some odorants over others at supra-threshold, while additivity may occur at sub-threshold. The wide array of odorant molecular structures and their odor-activity explains the difficulty of building a model to predict the odor of a mixture of compounds. Odor intensity can be predicted for a mixture containing three or four compounds, but underestimation generally occurs with more complex mixtures (Laing, 1995).

3

#### APPLE FLAVOR

#### **Apple Taste**

Apple flavor is complex and combines taste and flavor attributes. Williams and Carter (1977) used 31 descriptors for apple flavor, including basic tastes (acidity, sweetness, bitterness), astringency and eight aftertastes. Sweetness is mostly due to sugars. Fructose constitutes 50% of the total sugars, which account for 10 to 15% of apple fruit fresh weight (Rouchaud *et al.*, 1985). Glucose and sucrose vary between 2 to 4%, and the sugar alcohol sorbitol, less than 1% (Fourie *et al.*, 1991). Each sugar induces different intensities of sensory response: equimolar solutions of fructose taste sweeter than sucrose, which tastes sweeter than glucose (Shallenberger and Birch, 1975).

Malic acid is the dominant acid in apples and citric is present in lower amounts (10% of malic acid). Malic acid is the primary substrate used in respiration metabolism. Decreases in malic acid during storage in air are usually perceived as decreasing sourness by trained as well as untrained panelists (Williams and Langron, 1983; Gorin, 1973; Plotto *et al.*, 1997; Anderson and Penney, 1973; Visser *et al.*, 1968; Watada *et al.*, 1980). In addition to sourness, malic acid may induce an astringent taste sensation (Straub, 1989).

The major phenolic compounds present in apples are mostly cyanidin-3galactoside, or idaein, and quercetin-3-galactoside (Mazza and Miniati, 1993). Both are anthocyanins and by themselves, do not show specific sensory properties. However, complexed with phenolic compounds, mainly flavonols and phenolic acids, they play a role in bitterness and astringency (Mazza and Miniati, 1993). Bitterness and astringency are desired taste attributes in processed apple products such as cider (Williams *et al.*, 1977a). Phenolic compounds isolated from cider apples were chlorogenic acid, phloretin derivatives, catechins and procyanidins. Only procyanidin derivatives (condensed tannins) contributed to both bitterness and astringency (Williams *et al.*, 1977a).

#### **Volatiles Found in Apple**

Early published methods to determine volatile compounds in apples involved chemical derivatization or ester hydrolysis (Power and Chesnut, 1920), separation was done by paper chromatography and determination by spectrophotometry (Meigh, 1956; 1957). Gas chromatography and mass spectrometry made separation and identification easier, and to date, Yahia (1994) has compiled a list with almost 300 compounds found in apples.

Esters are particularly well represented in analysis of volatiles emitted by apples. In reviews of apple flavor, Paillard (1990) listed 92 esters and Yahia (1994) more than one hundred. Apple esters have straight and branched chains, usually saturated but unsaturated branched chain esters are also found in apples (Yahia, 1994). Esters account for 78 to 92% of the total volatile emission adsorbed by activated charcoal (Paillard, 1967). They are usually emitted in larger quantities by riper fruit (Mattheis et al., 1991b; Dirinck et al., 1989). Esters with even-numbered carbon chains from acetic, butanoic and hexanoic acids and with ethyl, butyl and hexyl alcohols were more frequently found than odd-numbered ones (Paillard, 1967). Differences between apple cultivars were found to be mostly quantitative rather than qualitative. (Hannover, 1991; Paillard, 1990). Headspace analysis of nine cultivars grown in France led to a classification of apple varieties according to the type of predominant esters, acetates, butanoates, propanoates or low ester/high alcohol emitting cultivars (Paillard, 1967). Because of the lower volatility of higher molecular weight esters, hexyl hexanoate and hexyl octanoate are only detected from headspace of cultivars producing large amounts of esters (Kakiuchi et al., 1986). Odor thresholds in water for esters vary from 0.006 ppb for ethyl 2methylbutyrate to 13,500 ppb for ethyl acetate (Takeoka *et al.*, 1995; 1996). Such a wide range of odor activities within one chemical category shows the limitation of chemical analysis alone to explain aroma of a food.

Straight-chain aliphatic and unsaturated alcohols are found in apple headspace and distillate (Paillard, 1990; Yajima *et al.*, 1984). Depending on the cultivar, alcohols (mostly butan-1-ol and hexan-1-ol) represented 6-16% of the total volatile emission (Paillard, 1967). Alcohols are more water soluble and are therefore found in larger proportions in distillate preparations (Kakiuchi *et al.*, 1986). Aliphatic alcohols, some diols and phenylethanol were also found in a glycosylated form in 'Jonathan' apples (Schwab and Schreier, 1988; 1990; Schwab *et al.*, 1989). Those glycosylated forms may play a role as possible precursors or storage alcohols for the formation of esters.

Straight-chain or branched aliphatic aldehydes identified among apple volatiles generally accompany the corresponding alcohol (Paillard, 1990). C<sub>6</sub> aldehydes have been reported by many authors (Drawert, 1975; Paillard, 1979). Hexenal, *E*-2-hexenal and *Z*-3-hexenal are considered as secondary metabolites and are produced by the action of lipoxygenase on polyunsaturated fatty acids after crushing tissue (Drawert *et al.*, 1966; Paillard and Rouri, 1984). Acetaldehyde is a normal constituent of apples but its production increases during anaerobic respiration. In that situation, large amounts of ethanol are also produced (Mattheis *et al.*, 1991a).

Only a few ketones have been reported in apples (Paillard, 1990; Yahia, 1994). They are mostly straight-chain aliphatic ketones including acetone or the hydrocarbon 6methylhept-5-en-2-one.

Carboxylic acids have been reported in apple extracts and volatile emission from headspace (Paillard, 1990). In some extraction procedures, carboxylic acids may be the result of enzymatic hydrolysis of esters (Paillard, 1990). An esterase has been isolated from apple fruit with increasing activity during ripening (Goodenough, 1983).

Two hydrocarbons play a significant role in post harvest apple physiology. Ethylene is the ripening hormone in climacteric fruit. Esters increased concomitantly with ethylene during fruit ripening (Flath *et al.*, 1967; Mattheis *et al.*, 1991b).  $\alpha$ -farnesene is detected in headspace vapor (Kakiuchi *et al.*, 1986) and has been extensively studied for its role in development of scald in apple peel (Huelin and Coggiola, 1968). Neither ethylene nor  $\alpha$ -farnesene are odor-active.

Other compounds are produced by apples, with one or two representatives of a chemical family and present in low or trace amounts (Yahia, 1994). However, they may play important roles in apple aroma when their odor threshold is low or when the odor characteristic is distinct from the fruity note imparted by esters. Examples include  $\beta$ -damascenone, a C<sub>13</sub> nor-isoprenoid compound with a grape juice odor (Cunningham *et al.*, 1986) and 4-methoxyallylbenzene, an allylphenol with an anise character (Williams *et al.*, 1977b).

## **Glycosylated Volatile Compounds in Apples**

Very few studies report analysis of glycosylated volatile compounds in apples. All those studies use the technique of separation with Amberlite XAD-2 column and enzymatic hydrolysis of glycosylated compounds (Gunata *et al.*, 1985). Aliphatic alcohols, diols, C<sub>13</sub> norisoprenoid compounds and fatty acids derivatives were found in 'Jonathan' apples (Schwab and Schreier, 1988; 1990; Schwab *et al.*, 1989). Aubert (1997) additionally reported two terpenols and 14 phenols from 'Golden Delicious' apple juice. Some of those compounds have a low odor threshold such as  $\beta$ damascenone (Buttery *et al.*, 1990b). The knowledge of the presence of bound volatiles is important when the fruit is processed because the bound volatile fraction is released during heating (Schreier *et al.*, 1978; Buttery *et al.*, 1990a). However, more attention should be paid to the presence of those compounds in the fresh fruit, as they may be released in the mouth upon chewing.

#### **VOLATILE METABOLISM**

#### Fatty Acid Metabolism

Fatty acid metabolism is the largest source of substrate for apple volatiles. Volatiles resulting from fatty acids are aliphatic acids, alcohols, carbonyls and esters
(Schreier, 1984). Free fatty acids are degradation products of the membrane phospholipids. In the living cell, there is a constant turnover of phospholipids to maintain the composition and surface charge properties of the membrane. The proposed sequence of events in phospholipid degradation is the following: conversion of phospholipid to phosphatidic acid under the action of phospholipase D (cleavage of the acyl chain at the phosphate ester bond), conversion to diacylglycerol by phosphatidate phosphatase (phosphate removal), and deacylation by acyl hydrolase to free fatty acids (Paliyath and Droillard, 1992). The sequence is more active in senescing cells, explaining the observed increasing ratio of free to esterified fatty acids. Meigh and Hulme (1965) found a decrease of esterified C<sub>18</sub> fatty acids in ripening apples after 160 days after petal fall. In post-climacteric apples, there was a decrease in the lipids associated with plastid (chloroplast) membrane, mostly galactolipids and phosphatidyl glycerol (Gaillard, 1968). Bartley (1985) observed an increase in the rate of degradation of phospholipids in ripening apples. Therefore, there appears to be an increase in substrate for volatile esters in ripening apples.

In fruit, fatty acids are catabolized through two main oxidative pathways:  $\beta$ oxidation and the lipoxygenase (LOX) pathways (Schreier, 1984; Sanz *et al.*, 1997). In
some cases,  $\alpha$ -oxidation may be involved (Tressl and Drawert, 1973) and is considered a
respiration process because CO<sub>2</sub> is released during a decarboxylation step (Paliyath and
Droillard, 1992). The  $\beta$ -oxidation cycle is the same in plants as in animals and microorganisms. Fatty acids, or rather, acyl-CoA derivatives, are metabolized to shorter chain
acyl-CoAs by losing two carbons at every round of the cycle (Goodwin and Mercer,
1983). Apples or apple discs supplied with aliphatic acids (Paillard, 1979) or methyl
esters of C<sub>n</sub> fatty acids (Bartley *et al.*, 1985) produced alcohols or methyl esters with C<sub>n</sub>
or C<sub>n-2</sub>, suggesting active  $\beta$ -oxidation. Acyl-CoA molecules produced by  $\beta$ -oxidation
are used for ester synthesis in ripening fruit (Sanz *et al.*, 1997). The most available acyl
group determines the type of ester. The rate of transformation of butanoate to acetate
was higher in 'Golden Delicious' than in 'Delicious', richer in butanoate esters
(Paillard, 1979). Acyl-CoA molecules from  $\beta$ -oxidation are also reduced to aldehydes

and hydrogenated to the corresponding alcohol. Feeding apple discs (Paillard, 1979) or intact fruit (De Pooter *et al.*, 1981) short chain aliphatic acids resulted in the production of the corresponding alcohols. In banana discs, a proposed pathway for aliphatic ketones such as 2-heptanone and 2-pentanone was octanoate  $\beta$ -oxidation followed by a decarboxylation (Tressl and Drawert, 1973).

The lipoxygenase (LOX) route involves hydroperoxidation of free fatty acids. The peroxide intermediates are highly cytotoxic and unstable, and are rapidly transformed to keto acids, oxo acids and aldehydes by hydroperoxide lyase and isomerase (Schreier, 1984). This pathway is generally activated upon plant cell disruption (Schreier, 1984; Drawert et al., 1966), although it is also active in ripening fruit and senescing tissue (Sanz et al., 1997; Paliyath and Droillard, 1992). LOX purified from 'Golden Delicious' apples was found to be membrane bound (Kim and Grosch, 1979). Depending on the type of LOX and plant tissue, 9- or 13hydroperoxides or a mixture of both are produced; tomato LOX preferentially oxygenates at the 9-position (Gaillard and Matthew, 1977) while apple LOX preferentially oxygenates at the 13-position (Feys et al., 1982). Equally important in the product is the substrate specificity of hydroperoxide lyase. Apple hydroperoxide lyase has a higher affinity for 13-hydroperoxide and, like LOX, is a membrane bound enzyme (Schreier and Lorenz, 1982). Products from hydroperoxide lyase are aldehydes. Hexanal and E-2-hexenal were produced by crushed apples, with a maximum after 5 minutes (Paillard and Rouri, 1984). The LOX pathway in fruit and vegetables is reviewed in detail by Drawert (1975), Schreier (1984) and Sanz et al. (1997) (Figure 2.1).

Hydroperoxide products, aldehydes, are reduced to alcohols by alcohol dehydrogenase, with NADH or NADPH as a cofactor (Rhodes, 1973; Paillard, 1979). Alcohols are then used as a substrate in the formation of esters.

### **Ester Synthesis**

Apple fruit subjected to an atmosphere containing ethanol emit large amounts of all ethyl esters compared to the control (Berger and Drawert, 1984). Applications of



Figure 2.1. Enzymatic activities and products involved in the LOX pathway (from Sanz et al., 1997)

vapors of aldehydes or carboxylic acids (De Pooter *et al.*, 1983), alcohols or short-chain fatty acids (Bartley *et al.*, 1985) resulted in increased ester production by apples. Similar results were obtained from apple discs (Paillard , 1979; Knee and Hatfield, 1981). These feeding experiments demonstrate an active ester forming activity in apples; Paillard (1979) and De Pooter *et al.* (1981) suggested that the substrate available in the fruit was

the limiting factor for ester production. Investigations in other fruit revealed that esterforming activity was related to fruit ripening, since no activity was found in unripe fruit (Yamashita *et al.*, 1977). In fact, significant ester production was only observed at or right after the onset of the climacteric peak in 'Bisbee Delicious' apples (Mattheis *et al.*, 1991b). The lack of ester production during the pre-climacteric stage in apples could be due to lower alcohol dehydrogenase activity, as shown by large aldehyde but low alcohol production (Mattheis *et al.*, 1991b). However in strawberry fruit, pentanal reduction to pentanol occurred at every ripening stage (Yamashita *et al.*, 1977).

The mechanism of ester formation has been well characterized in microorganisms. An acetate-ester transforming enzyme was characterized and identified from Cladosporium cladosporioides (Yamakawa et al., 1978), and alcohol acyltransferase was purified from Neurospora sp. (Yamauchi et al., 1989). Alcohol acyltransferase (AAT) catalyses the transfer of an acyl moiety from an acyl-CoA on to an alcohol (Sanz et al., 1997). Fruit AAT was partially purified from banana (Harada et al., 1985), strawberry (Pérez et al., 1993a), apples (Fellman and Mattheis, 1995) and pears (Suwanagul, 1996). Strawberry AAT showed higher substrate affinity for hexanol and acetyl-CoA than other alcohols and longer chain acyl-CoAs (Pérez et al., 1993a). Assays on several fruit species indicated different affinities for acyl-CoA and alcohol in bananas and strawberries (Olías et al., 1995). Within a fruit species, AAT substrate specificity also varied between cultivars (Pérez et al., 1996). AAT activity decreased when fruit was stored under low oxygen (Fellman and Mattheis, 1995); however, an increase in activity was observed upon retrieval of the fruit to air, which could be either due to enzymatic reactivation or *de novo* synthesis (Fellman and Mattheis, 1995). The ester forming system includes ester turnover: esterase activity also exists in apples (Bartley et al., 1985; Goodenough, 1983). In AAT assays, the measurements of coenzyme A, the acyl-CoA hydrolysis product are more accurate than measurements of esters, as those are also hydrolyzed by esterase (Fellman and Mattheis, 1995).

### Amino Acid Metabolism

Amino acids can act as direct precursors for alcohols, carbonyls, acids and esters. Tressl and Drawert (1973) showed that <sup>14</sup>C-leucine and <sup>14</sup>C-valine are converted into the corresponding methyl-branched esters, alcohols and acids and <sup>14</sup>C-phenylalanine into phenolic esters in banana tissue. The mechanism of conversion involves amino acid transamination, decarboxylation to aldehyde and rapid reduction or oxidation to alcohol or carboxylic acid, respectively (Drawert, 1975). Apples infiltrated with *L*-isoleucine or exposed to vapor phase 2-methylbutyl esters produced increasing amounts of 2- and 3methylbutanol, 2-methylbutyl esters and 2-methylbutyrate esters (Hansen and Poll, 1993; Rowan *et al.*, 1996).

### Shikimic Acid Pathway

As stated above, phenylalanine may be a precursor for some volatile compounds found in banana such as  $\beta$ -phenylethanol,  $\beta$ -phenylethyl acetate and  $\beta$ -phenylethyl butyrate (Tressl and Drawert, 1973). Phenylalanine originates in the shikimic acid pathway, from a condensation reaction between erythrose-4-phosphate and phosphoenol-pyruvate (Goodwin and Mercer, 1983). Phenylalanine was the precursor for allylphenols in plants belonging to the *Labiaceae* family (Manitto *et al.*, 1974). At least one allylphenol was found in apples by Williams *et al.* (1977b), 4-methoxyallylbenzene.

### Mevalonic Acid Pathway

Mevalonic acid (MVA) is considered to be the first precursor of terpenic compounds. Two phosphorylations and a decarboxylation produce isopentenyl pyrophosphate, the base unit of isoprenoid compounds. Of this large family of natural products, carotenoids are the source of the C<sub>13</sub>-norisoprenoid flavor compounds (Winterhalter *et al.*, 1995). The C<sub>13</sub>-norisoprenoid  $\beta$ -damascenone is present in apples in a glycosylated form (Roberts *et al.*, 1994). Roberts and Acree (1995) identified one glycoside of the acetylenic diol precursor of  $\beta$ -damascenone, and detected seven other precursors, possibly triglycosides, diglycosides and polyols.  $\alpha$ -Farnesene is a sesquiterpene and is formed through the mevalonic acid pathway (Jennings and Tressl, 1974). So is 6-methyl-5-hepten-2-one, a degradation product of  $\alpha$ -farnesene (Stanley *et al.*, 1986) and lycopene (Buttery *et al.*, 1988).

# FACTORS AFFECTING VOLATILE PRODUCTION IN APPLES

### **Cultivar Differences**

Taste differences between apple cultivars are due in part to the different amounts of sugars and acids (Watada et al., 1980; 1981; Cliff and Dever, 1990; Rouchaud et al., 1985). Flavor differences are believed to be due to the differences in quantities of volatile compounds (Paillard, 1967). Indeed, with the odor unit theory, only volatile compounds present above their perception threshold contribute to the fruit aroma. However, when comparing 40 cultivars using CharmAnalysis, Cunningham et al. (1986) found that there was no one odor-active peak common to all 40 cultivars. In other words, the odor of the cultivars tested could not be explained by variation in the concentration of a few chemicals. This is because the human olfactory response to compound concentration is not linear (Stevens, 1957), and perception by the nose is more sensitive than the FID detector for some compounds (Cunningham et al., 1986). Paillard (1967) classified eight cultivars according to the predominant esters emitted: 'Calville blanc' and 'Golden Delicious' emitted predominately acetate esters, 'Canada Blanc' and 'Belle de Boskoop' butyrate esters, while other cultivars produced an equal amount of acetate and butyrate esters. 'Canada Gris', a corky peel cultivar, emitted low amounts of all volatiles (Paillard, 1967). Dirinck et al. (1989) classified 25 commercial Belgian cultivars into groups of dominant volatiles using principal components analysis. He also examined the grouping pattern of 17 "acetate-type" cultivars and found similarities between 'Golden Delicious' and 'Jonagold', and between 'Cox's Orange Pippin' and 'Elstar'. Varietal comparisons were also performed by Kakiuchi et al. (1986), while differences between strains of 'Delicious' were found by Fellman et al. (1991). Considering other factors affecting volatile production in the fruit, varietal

differences are only valid if apples are at a comparable physiological stage and grown in the same environment. Brackmann and Streif (1994) measured emission of  $CO_2$ , ethylene and volatile compounds from 28 cultivars; they found a good correlation between  $CO_2$  and ethylene production. However, the correspondence between apples producing large amount of ethylene and aroma volatiles was not true for all cultivars.

### **Pedo-Climatic and Cultural Factors**

Studies in Northern Italy showed differences in quality between 'Golden Delicious' grown in the plains and grown in the mountain areas (Zerbini *et al.*, 1980). Differences were measured for total sugars, soluble solids, and sugar: acid ratio. Regarding the production of volatile compounds, Mattheis *et al.* (1991b) noted that some esters of 'Bisbee Delicious' were absent from apples sampled from one orchard compared to another orchard in the same growing region. Differences between orchards could be due to nutrients availability from soils, fertilization practices or rootstock effect, or different tree age and canopy size, different leaf area: fruit ratio. Fertilization affected the quantity of apple volatile production (Somogyi *et al.*, 1964). N application alone always resulted in lower volatile production as compared to N supplemented with K, P and Ca. The effect of assimilate availability was measured on 'Jonagored' apples by controlling tree crop load (Poll *et al.*, 1996). Apples with the lowest fruit load emitted more butyl acetate, hexyl acetate and butanol.

### **Apple Maturity Stage**

Total volatile production generally increase as fruit ripens (Brown *et al.*, 1965; Shim *et al.*, 1984; Dirinck *et al.*, 1989; Yahia *et al.*, 1990; Song and Bangerth, 1996; Girard and Lau, 1995). Fruit detached from the tree reaches optimum volatile production earlier as it is harvested closer to the climacteric (Dirinck *et al.*, 1989; Song and Bangerth, 1996). Volatiles that follow the general trend are mostly esters. In turn, aldehydes are mostly emitted by pre-climacteric apples (De Pooter *et al.*, 1987; Mattheis *et al.*, 1991b). *E*-2-Hexenal increased during maturation and ripening of 'McIntosh' apples (Yahia *et al.*, 1990). However, this observation could be due to increase in free fatty acids in the cell because E-2-hexenal is usually only present in crushed tissue; those authors used solvent extraction of fruit puree to sample for volatile compounds. Paillard (1986) found a positive correlation between linolenic acid and E-2-hexenal during apple ripening; both decreased as apple lost its green color.

The differences in ester and alcohol production between harvest dates was maintained in air storage for up to six months (Girard and Lau, 1995). 'Golden Delicious' apples harvested at the pre-climacteric stage produced less volatiles than those harvested at the post-climacteric at all times and under any storage condition (Brackmann *et al.*, 1993).

### Storage Effect

Low temperature and high humidity delay senescence and maintain fruit turgidity. Emission of acetate esters by 'Jonathan' apples increased with storage temperature from -1 °C to 10 °C (Wills and McGlasson, 1971). Likewise, low humidity during storage increased emission of hexyl acetate, isopentyl acetate and butyl acetate, while hexanol, isopentanol and butanol were emitted in larger amounts in high humidity atmospheres (Wills and McGlasson, 1970). However, the most significant effect on apple volatile production is due to controlled atmosphere (CA).

Despite its many advantages on preserving fruit quality such as acidity and firmness (Smock, 1979), CA storage inhibits volatile production (Patterson *et al.*, 1974; Streif and Bangerth, 1988; Hatfield and Patterson, 1974; Willaert *et al.*, 1983). The longer the fruit remains in storage, the more pronounced the decrease in volatile production (Streif and Bangerth, 1988; Lidster *et al.*, 1983a; 1983b; Willaert *et al.*, 1983; Yahia *et al.*, 1990). For instance, short term storage of 'McIntosh' apples at 1.5% CO<sub>2</sub> and 1% O<sub>2</sub> at 2.8 °C did not inhibit regeneration of ethyl butanoate and hexanal after subsequent return of the fruit to air (Lidster *et al.*, 1983a; 1983b). However, longterm storage (320 days) under the same conditions resulted in complete loss of the main headspace volatiles, without recovery after return of the fruit to air. Additionally, lower O<sub>2</sub> concentrations in storage resulted in lower volatile production and longer recovery time (Hansen *et al.*, 1992; Streif and Bangerth 1988; Mattheis *et al.*, 198a). The atmosphere composition also affects the total quantity of volatiles (Streif and Bangerth, 1988) as well as the type of esters produced (Brackmann *et al.*, 1993; Fellman *et al.*, 1993; Hansen *et al.*, 1992). The curves of volatile production over days at 20 °C after removal from storage were different at different levels of  $O_2$  in storage; the curves were similar for esters belonging to the same alcohol group (Hansen *et al.*, 1992). Based on the decreased rate of straight-chain acetate esters versus branched-chain acetates after storage, Hansen *et al.* (1992) suggested that the  $O_2$  requirement for  $\beta$ -oxidation was higher than for amino acid transformation. Brackmann *et al.* (1993) observed a higher decrease in straight-chain esters under low  $O_2$  than branched-chain; branched-chains esters decreased significantly under high CO<sub>2</sub> concentrations. Mattheis *et al.*, (1998a) found that while 2-methylbutyl acetate was not negatively affected by low  $O_2$  storage, production of other branched-chain esters decreased. Therefore, it seems that the fatty acid metabolism for ester production is negatively affected by low  $O_2$  and high CO<sub>2</sub>, while the amino acid metabolism is mostly affected by high CO<sub>2</sub> levels (Brackmann *et al.*, 1993).

In the study by Brackmann *et al.* (1993), apples were able to transform exogenous straight-chain alcohols, one acid and one aldehyde to esters. Those results suggested a high turnover of substrate of the later part of fatty acid metabolism, and that alcohol dehydrogenase, esterase and AAT were not irreversibly inhibited by low O<sub>2</sub> (Brackmann *et al.*, 1993). Inhibition could be either at the early steps of  $\beta$ -oxidation, or inhibition of the lipoxygenase activity, which requires O<sub>2</sub>. In turn, CO<sub>2</sub> would suppress amino acid metabolism, but it is not clear which step is affected. Another study from Fellman *et al.* (1993) showed that AAT activity was suppressed at 0.5% O<sub>2</sub>, but was detected at 1% O<sub>2</sub> when measured at the time of storage removal. AAT activity increased to reach a maximum 9 days after removal from storage. Fellman *et al.* (1993) suggested that differences in volatiles affected by CA storage between cultivars could be due to different AAT substrate specificities and differences in substrate availability. It was suggested earlier that low O<sub>2</sub> limits the alcohol availability in the cell (Knee and Hatfield, 1981). De Pooter *et al.* (1987) indicated that high CO<sub>2</sub> concentration might impact alcohol dehydrogenase reducing capability of carboxylic acids to aldehydes. On the other hand, Ke *et al.* (1994) reported low  $O_2$  and/or high  $CO_2$  directly enhanced pyruvate decarboxylase and alcohol dehydrogenase activity of strawberries, but decreased AAT activity. Increased pyruvate decarboxylase and alcohol dehydrogenase activities may result in the accumulation of ethanol which is in turn synthesized to ethyl esters (Mattheis *et al.*, 1991a).

Mechanisms of regulation of volatile production are still unknown. Ethylene certainly plays a role, according to the pattern of volatile production before or after the climacteric peak. Fruit harvested too early (3 - 4 weeks before the optimum) show a delay in production of ripening related volatiles, and the respiratory pattern is strongly altered (Song and Bangerth, 1996). Additionally, 'Golden Delicious' volatile production was reduced upon treatment with the ethylene production inhibitor aminoethoxyvinylglycine (Bangerth and Streif, 1987). A putative effect of ethylene on phospholipase D was suggested with a possible regulatory effect on membrane phospholipid degradation (Paliyath and Droillard, 1992). However, ethylene would not act directly, but a  $Ca^{2+}$  second messenger system would be involved to translate ethylene signal and initiate lipid degradation (Paliyath and Droillard, 1992). This system would be more active in senescing cells, explaining the observed increasing ratio of free to esterified fatty acids, and therefore, increasing substrate for volatile compound formation.

### **POSSIBLE IMPROVEMENT OF APPLE FLAVOR**

### **Precursor Atmospheres**

The capacity of apples to metabolize alcohols, aldehydes and carboxylic acids into esters was explored to improve fruit aroma after CA storage (Kollmannsberger and Berger, 1992). A mixture of aliphatic alcohols in the precursor atmosphere resulted in a better balanced apple aroma than one or two alcohols alone. Panelists could detect a pear-like note in 'Delicious' apples after exposure to precursor atmosphere (Kollmannsberger and Berger, 1992, data not shown). Precursor atmosphere was applied to 'Golden Delicious' apples with aldehydes and carboxylic acids (De Pooter *et*  *al.*, 1983). However, those authors did not observe significant organoleptic improvement of the fruit; additionally, the increase in volatile production did not last more than eight days.

### **Alternate Atmospheres**

Apples exposed to air storage after CA produce more volatiles than had they remained under low  $O_2$  and high  $CO_2$  (Streif and Bangerth, 1988). However, such an increase was not observed with 'Bisbee Delicious' (Mattheis *et al.*, 1995) or with 'McIntosh' (Yahia, 1991) under similar conditions. An increase in ester emission under 1 kPa  $O_2$  was observed after 120 days when fruit was alternatively exposed to ambient air once per week, then returned to CA (Mattheis *et al.*, 1998a). Of all those experiments, only one was confirmed for aroma improvement by a taste panel (Smith, 1984). Panelists could detect an increase in aromaticity of 'Cox's Orange Pippin' when these apples were transferred to 2%  $O_2$  after storage under 1.25%  $O_2$  (Smith, 1984).

### Breeding

Considering the amount of volatiles responsible for apple flavor and the different pathways involved, selection of specific traits is difficult. However, flavor is still considered as one of the most important criteria in apple selection (Janick *et al.*, 1995). Usually, acidity and sweetness are the base of selection for flavor (Janick *et al.*, 1995). Acidity and sweetness are inherited independently. Only a gene for malic acid is known (Janick *et al.*, 1995). Although a single gene controls malic acid in apple, its inheritance is based on a quantitative pattern, with a dominant allele for high acidity. By knowing the sugar and the malic acid concentration in the fruits of a cultivar, parents can be selected to produce progenies that will have the desired sugar and acids contents.

### **CHAPTER 3**

# APPLICATION AND OPTIMIZATION OF GAS CHROMATOGRAPHY AND OLFACTOMETRY TO 'GALA' APPLES (*MALUS DOMESTICA*, BORKH) USING *OSME* ANALYSIS

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### ABSTRACT

The gas chromatography (GC) and olfactometry method Osme records subjects' responses to odorant stimuli by combining intensity and duration of perception. Osme was used to evaluate odor-active volatile compounds emitted by 'Gala' apples (Malus domestica, Borkh). 'Gala' headspace was sampled on either charcoal or Tenax traps in a dynamic flow-through system for 6, 12 and 24 hours and eluted with carbon disulfide (CS<sub>2</sub>) (charcoal traps) or diethyl ether (Tenax traps). Charcoal traps sampled for 24 hours yielded the largest amount of volatile compounds. A total of 44 odor-active peaks were detected by three trained panelists using Osme analysis. Twenty-six of the 39 compounds identified by GC and mass spectrometry were odor-active at the concentration recovered from the traps. Odor-active compounds were mostly esters with a fruity odor. The aromas of hexyl acetate and pentyl acetate were the closest to that emitted by whole 'Gala' fruit. Butyl acetate and 2-methylbutyl acetate were produced in the largest amounts, and had a solvent-like odor. Other esters were perceived as either fruity, apple or berry (strawberry). 4-Allylanisole and βdamascenone were found in 'Gala' headspace and had odors characteristic of anise and grape juice, respectively. Other compounds were found to have watermelon, cucumber, mushroom, adhesive tape and skunk odors, but remain unidentified. Sampling 'Gala' headspace on charcoal for 24 hours with subsequent elution with  $CS_2$  was used in further study of changes of odor-active volatiles in storage.

### INTRODUCTION

Isolation of volatile compounds from a food system is the first important step in aroma analysis. Because of different physical and chemical properties of volatile compounds, their interactions in the food matrix and their affinities with the extracting solvent or trapping system, each method of isolation introduces a bias in the aroma profile (Mistry et al., 1997). Distillation, solvent extraction, cold trapping and headspace techniques are reviewed in most texts on flavor analysis (Leahy and Reineccius, 1984; Reineccius, 1993; Teranishi and Kint, 1993; Parliment, 1997; Wampler, 1997). Headspace of intact fruit is usually preferred for fruit volatile analysis when changes over a period of time are monitored (Rizollo et al., 1992; Mattheis et al., 1991). Sampling an aliquot of headspace without the use of intermediate steps (trapping or solvent extraction) would be the method of choice because the exact food aroma is then analyzed; however, high water vapor content and low amount of volatiles have limited that method's application to fruit (Paillard et al., 1970; Wampler, 1997). The amount of volatiles sampled can be increased in a static headspace by letting fruit produce and accumulate its own volatiles in a closed system. However, in such a system, it becomes difficult to establish whether additional volatiles analyzed are due to increased concentration in the headspace or are new products appearing as a consequence of altered metabolism in a closed system. Therefore, dynamic headspace, where air is flushed through a vessel containing fruit, is preferred as it maintains the fruit in aerobic conditions. Volatiles are entrained and adsorbed on solid materials such as charcoal or porous polymers including Tenax and Poropak Q, then desorbed by heat transfer or with a solvent. Thermal desorption allows near complete recovery of all trapped compounds while avoiding co-elution of low boiling compounds with the solvent (Wampler, 1997). Thermal desorption also limits the possibility of artifact formation from interactions between solute and solvent. However, only solvent desorption allows multiple injections from a single sample.

Once fruit volatiles have been collected, chemical separation by gas chromatography (GC) coupled with either mass spectrometry (MS) or a flame ionization

detector (FID) allows qualitative and quantitative analysis. These analytical techniques do not, however, provide information characterizing aroma activity of individual compounds. Olfactometry techniques where the detector is a human sniffing the GC effluent, are well documented (Acree, 1997; Grosch, 1993; Mistry et al., 1997; Blank, 1997). Acree and co-workers (1984) and Grosch (1993) inject the aroma extract into a GC after successive dilutions: compounds that are perceived by the human subject at the highest dilution level are believed to be the character impact volatiles of the sample. In CharmAnalysis (Acree et al., 1984), data processing evaluates duration of the perceived compound (human response) as well as its dilution value. In aroma extract dilution analysis (AEDA), the dilution level at which compounds are perceived gives the flavor dilution (FD)-factor (Grosch, 1994). Both CharmAnalysis and AEDA are recognized as useful complements to chemical analysis for screening potent odorants in foods. Another GC-olfactometry (GCO) technique, Osme, is based on Stevens' law of psychophysics and combines time and intensity of perception as a response to odorants (McDaniel et al., 1990; Da Silva et al., 1994). Osme gives an odor profile of a food extract and comparison between samples or treatments can be made by either comparing the aroma profiles (Young, 1997; Sanchez et al., 1992a; 1992b), or by statistical analysis (Da Silva et al., 1993). While applications of CharmAnalysis and AEDA usually report one person to have evaluated the GC effluents, Osme has used four (Da Silva et al., 1993, McDaniel et al., 1990; Sanchez et al., 1992a; 1992b; Bazemore, 1995) and three (Young, 1997) panelists, each replicating the sniffing of each sample three or four times. Additionally, Osme panelists are trained to use a time-intensity device with a 16-point intensity scale where 0 =none, and 15 =extreme. Intensity response to odorant concentration was shown to follow the principle psychophysics of Stevens' law given by the equation  $I = k(C-T)^n$ , where I is the reported perception of odor intensity of a compound, C the compound concentration, T the compound's threshold value, n the exponent of the function and k is the constant of proportionality (Da Silva et al., 1994). Those authors also showed that intensity ratings were reproducible with trained panelists using Osme.

Information relative to odor character of compounds found in apples and determined by GCO was first published by Guadagni et al. in 1966. Flath et al. (1967) further determined the relative importance of individual compounds in 'Delicious' apple essence by determining their odor thresholds using sensory methods. Williams et al. (1977a) correlated sensory descriptive analysis data with GCO results for 'Cox's Orange Pippin' apples. In that work and in a subsequent paper (1977b), Williams emphasized the importance of 4-methoxyallylbenzene, a compound with an anise odor that gives a spicy character to that apple cultivar. Nursten and Woolfe (1972) used GC-MS, GCO and sensory difference testing to measure changes in 'Bramley Seedling' apple aroma after processing. GCO has also been used to describe odorous compounds emitted by intact 'Golden Delicious' apples (Pérez et al., 1993; Rizzolo et al., 1989; Rizzolo et al., 1992), and compounds extracted from 'Kogyoku' apple by steam distillation (Yajima et al., 1984). In the latter studies, there was no attempt to quantify the aroma intensities of the odor producing compounds. CharmAnalysis, which determines the potency of odoractive peaks, was applied to Freon extracts from apples to investigate cultivar differences (Cunningham et al., 1986). However, 'Gala' apple was not included in the study. More recently, Young et al. (1996) used CharmAnalysis to investigate the compounds contributing to 'Gala' aroma. They found 2-methylbutyl acetate, butyl acetate, hexyl acetate and butanol to be important contributors to 'Gala' aroma. Those compounds were obtained from vacuum steam distillation and there was no mention of the aroma activity of other compounds.

All the above mentioned GCO studies reported only odor qualities for the compounds found in apples, and no comparisons were made between storage treatments or maturity stages. Only Cunningham *et al.* (1986) reported differences between apple cultivars. 'Gala' apple is a cultivar which originated in New Zealand and has gained worldwide popularity on the European, Asian and American markets because of its unique flavor (White, 1991). However, the storage season of 'Gala' is short, in part due to a decrease in aroma quality after storage (Young *et al.*, 1996). We were interested in quantifying the changes of 'Gala' aroma in storage from an analytical and sensory point of view using GCO. *Osme* was the method of choice because panelists record an

intensity and time-intensity response to compound concentrations, additionally to an odor descriptor, and also data can be analyzed by statistical methods (Da Silva *et al.*, 1993).

Optimization of 'Gala' volatile isolation for both GC and *Osme* applications was the objective of this study. A dynamic headspace technique sampling intact apples was chosen because results could be compared with other studies using methods with intact fruit (Mattheis *et al.*, 1998). Among the many adsorbents available, activated coconut charcoal, Tenax TA and Poropak Q are the most widely used for trapping fruit headspace volatiles. After a few trials, it became obvious that large amounts of volatiles were required for the olfactometric setup. Therefore, charcoal and Tenax GR were compared during the optimization process. Charcoal, with a large adsorbing surface area (1070 m<sup>2</sup>·g<sup>-1</sup> for 20/40 mesh particle size), has the largest capacity for capturing organic compounds. Tenax GR is a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide (Tenax) that contains 30% of graphitized carbon that has been co-precipitated with the polymer. It has the advantage of not being as reactive as charcoal, but with its graphitized surface, presents larger adsorbing capacity than Tenax TA (100 m<sup>2</sup>·g<sup>-1</sup> versus 35 m<sup>2</sup>·g<sup>-1</sup>). In this study, solvent desorption was used for repeated GC injections.

### **MATERIALS AND METHODS**

### Plant Material and Headspace Sampling

'Gala' apples from a commercial orchard near Chelan, WA, were harvested on September 9, 1994, and September 12, 1995. No pre-harvest or pre-storage chemical treatment was applied. Fruit was stored in air at 1 °C for 4 weeks in 1994 and 1995. Fruit was ripened at 22 °C for 5 days prior to volatile collection. Four replicate samples (five apples each, *ca.* 1kg) were placed in 4 L glass jars sealed using Teflon lids with two gas ports. Compressed air purified by flowing through activated charcoal, calcium hydroxide and 5 Å molecular sieve (W.A. Hammond Drierite, Xenia, OH) was passed through the jars at *ca.* 200 mL·min<sup>-1</sup>. Volatiles were collected on activated coconut

charcoal (20/40 mesh, 150 mg, ORBO-32, Supelco, Bellefonte, PA) for 6, 12 and 24 hours (ca. 70, 140 and 280 L, respectively). Trapping for less than 6 hours was first tested but did not yield enough materials for sniffing. Another batch of 20 apples was placed in the same jars as above, and headspace sampled for 24 hours onto Tenax GR (60/80 mesh, 1 g) with an air flow of 100 mL·min<sup>-1</sup> (ca. 150 L). Sampling took place in a ripening chamber maintained at 22 °C. Traps were stored at -25 °C until elution. Volatile compounds were desorbed from charcoal with 300  $\mu$ L of carbon disulfide (HPLC grade, 99.9%+, Sigma-Aldrich, St. Louis, MO) containing 100 mg·L<sup>-1</sup> of tridecane (Sigma, St. Louis, MO) as an internal standard. CS2 was chosen as it was shown to be the most effective solvent at displacing molecules adsorbed on charcoal (Jennings and Nursten, 1967). Furfuryl pentanoate was the internal standard in 1994, but it appeared to contain an odorous impurity not detected by the FID; therefore tridecane was chosen for the following season. Solvent was poured onto the charcoal particles in 1.8 mL vials, then samples were ready for analysis. Tenax traps were eluted with 25 mL of diethyl-ether (HPLC grade, 99.9%, Sigma-Aldrich, St. Louis, MO) containing 30  $\mu$ L of tridecane at 1000 mg·L<sup>-1</sup>. The solvent was concentrated to 300  $\mu$ L with nitrogen at 200 mL·min<sup>-1</sup>, on ice. Both solvents, CS<sub>2</sub> and concentrated diethyl ether with tridecane were checked for the presence of odorous impurities after elution time of five minutes. During the period of the study, samples (sorbent and solvent for charcoal, solvent alone for Tenax) were stored at  $-17 \,^{\circ}$ C.

### Gas Chromatography - Olfactometry

Samples were analyzed on a HP 5890 (Hewlett Packard, Wilmington, DE) gas chromatograph equipped with a 3-way valve (Valco Instruments Co., Inc., Houston, TX) to direct column flow to either a FID or a sniff port. The column was Rtx-5 fused silica coated with crossbonded 5% diphenyl 95% dimethyl polysiloxane, 30 m, 0.53 mm i.d., 1- $\mu$ m film thickness (Restek, Bellefonte, PA). Conditions for chromatography were: splitless injection at 250 °C, initial oven temperature, 40 °C held for 1 min, increased to 165 °C at 5 °C·min<sup>-1</sup>, then to 250 °C at 20 °C·min<sup>-1</sup>, held for 15 min. FID was at 280 °C; H<sub>2</sub>, air and auxilary gas (He) to FID were 30, 390 and 27 mL·min<sup>-1</sup>, respectively. Linear velocity of He carrier gas was  $30.7 \text{ cm} \cdot \text{sec}^{-1}$ . The sniff port was a 40 cm long, 4 mm diameter glass tubing deactivated with 5% dimethyldichlorosilane (Sylon-CT, Supelco) connected with a tee to the outlet of the GC column. Compressed air (breathing quality) was purified and humidified before flow to the sniff port at  $3.5 \text{ L} \cdot \text{min}^{-1}$  (or  $4.64 \text{ m} \cdot \text{sec}^{-1}$ ) through successively: activated charcoal, 5 Å molecular sieve and 2 L distilled water held at 30 °C.

Three panelists were trained to smell and describe the column effluents while rating the perceived intensity on a 16-point intensity scale (0 = none, 15 = extreme). Intensity was rated by moving a linear sliding bar connected to a variable resistor interfaced to a personal computer (Da Silva et al., 1994). The headspace from 'Gala' apple sampled for method development was used for training. Panelists were asked to identify the strongest odor peak and scale the intensity of the rest of the aromagram as to how intense they perceived the peaks. After the panelists had been familiarized with the sample and had developed their own vocabulary, reference standards were provided before each sniffing session so that panelists remained consistent in the naming of odors. Standards were presented in 120 mL jars closed with a Teflon-lined lid, and were: for "sweet, fruity", 107  $\mu$ g·L<sup>-1</sup> of ethyl 2-methylbutyrate, 3.5 mg·L<sup>-1</sup> of butyl acetate and 14  $mg \cdot L^{-1}$  of pentyl acetate in 60 mL odor-free double distilled water (Milli-Q); "green apple", 8.5 mg·L<sup>-1</sup> of hexyl acetate, 3.4 mg·L<sup>-1</sup> of hexanal and 3.4 mg·L<sup>-1</sup> of 2methylbutyl acetate in water; "sweet, bubble gum", 'Bubble Yum' original flavor (Nabisco, East Hanover, NJ); "butterscotch", Werther's original candies (Stork, Chicago, IL); "strawberry", strawberry essential oil (Uncommon Scents, Eugene, OR); "oatmeal", fresh dry oatmeal; "watermelon", fresh cut watermelon; "mushroom", fresh cut mushroom; "grape juice", Welch's 100% grape juice (Welch's Concord, MA); "burnt", burnt matches; "nutty", roasted hazelnuts; "adhesive tape", Scotch<sup>TM</sup> tape (3M, St. Paul, MN). Additionally, 'Gala' apples were presented in the same set-up (5 apples in a 4 L jar) as when they were sampled for volatiles. This was done to familiarize the panelists with the specific odor of 'Gala', and to help panelists identify the compounds having that odor profile. Panelists were allowed to use their own descriptors, as long as they were consistently applied.

Each sniffing session started after solvent elution from the column, and each session lasted 30 min. Data were recorded for time duration and intensity with *Osme* v. 1.0 for Windows 3.1, software developed at Oregon State University. The resulting output was, for each response: a) the odor duration time, b) the maximum odor intensity  $(I_{max})$ , c) the area under the curve generated by the odor stimulus response (time  $\times$  intensity), and d) the retention index (Kovats) at the time of maximum perceived intensity. Kovats indices were calculated after analyzing a series of hydrocarbon standards under the same conditions as the volatile sample. Panelists evaluated each of the four apple-batch replications once. Samples were presented in a complete randomized order blocked by apple batch (replication). Three panelists participated in the testing.

Initial identification of the compounds was made by running the samples under similar conditions on a HP 5890 series II gas chromatograph (Hewlett Packard, Wilmington, DE) equipped with a HP 5971a MS detector (Hewlett Packard, Palo Alto, CA) and a DB-5, 30 m, 0.25 mm i.d., 0.25-µm film thickness capillary column (J&W Scientific, Folsom, CA), and matching spectra using the Wiley/NBS library (1991). Confirmation of identification was made by 1) comparing retention indices of authentic standards from Aldrich Flavors and Fragrances (Milwaukee, WI) and 2) *Osme* evaluation of those standards in the same quantities as in the sample. If the odor of a standard was different from the odor of the sample peak, the compound was not retained for that peak odor identification, even though it was identified by the Wiley library and had the same Kovats index as the sample peak. All standards used for olfactometry were food grade.

### **Statistical Analysis**

Differences between traps and sampling time were analyzed for each chemical compound using ANOVA, with sampling time (and trap) as the main effect. For each perceived odor peak intensity  $(I_{max})$  response variable, panelist was included in the model, and apple batch (replication) was the error being tested. Sampling time (and trap) and panelist were treated as fixed effect and apple batch was treated as random effect. Intensity means were separated with the protected LSD test using apple batch

(replication) as the error term. Additionally, the frequency of perceived odor peaks was examined. All statistical procedures were performed using SAS statistical software v. 6.12 (SAS Institute, Cary, NC).

### **RESULTS AND DISCUSSION**

## Volatile Compounds Produced by 'Gala' Apple

Most of the compounds identified were esters, followed by alcohols, one ketone, one allyl phenol, one hydrocarbon and one  $C_{13}$  nor-isoprenoid compound (Table 3.1). Total esters accounted for 96% to 98% of the volatile compounds eluted from the traps (Table 3.2). Of the total quantity of esters detected, 80% were composed of straightchains with the remaining 20% branched-chains. Those figures fall within the range compiled for headspace analysis of apples (Paillard, 1990). Butyl acetate, hexyl acetate and 2-methylbutyl acetate were present in the largest amounts, representing 21 - 37%, 16 - 23% and 12 - 25% of total volatiles eluted, respectively.

We compared 'Gala' volatile compounds trapped on charcoal or Tenax GR (sampling volume 70 to 280 L) and solvent eluted in this study, with compounds from headspace trapped on Tenax TA (sampling volume 100 mL) and heat desorbed (Mattheis *et al.*, 1998). 'Gala' apples originated in the same orchard and were harvested at the same maturity stage. Heptyl acetate, pentyl propanoate, propyl butyrate, butyl heptanoate, hexyl octanoate, 3-methyl-2-butenyl acetate, butyl 2-methylpropanoate, 3methylbutyl propanoate, hexyl 2-methylpropanoate, 3-methylbutyl hexanoate and hexyl tiglate were detected in samples trapped for 24 hours on charcoal and Tenax GR but were not present in the samples collected on Tenax TA traps and heat desorbed. Conversely, ethyl esters (ethyl acetate, ethyl butyrate, ethyl pentanoate and ethyl hexanoate), 2-methylbutyl 2-methylbutyrate and several aldehydes, were trapped by Tenax TA and heat desorbed but were not present in samples that were trapped on either charcoal or Tenax GR. Some smaller molecular weight compounds (acetic acid, ethanol

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	6 hrs	12 hrs	24 hrs	24 hrs
1-Butanol	13.8 <sup>b</sup>	53.2 <sup>b</sup>	196.0 <sup>a</sup>	54.9 <sup>b</sup>
1-Pentanol	1.1	1.8	4.4	3.6
1-Hexanol	6.8 <sup>b</sup>	28.8 <sup>ab</sup>	82.8 <sup>a</sup>	63.2 <sup>ab</sup>
2-Methyl-1-butanol	39.7	55.2	78.6	64.4
Propyl acetate	68.0 <sup>b</sup>	141.4 <sup>b</sup>	367.5 <sup>a</sup>	98.6 <sup>b</sup>
Butyl acetate <sup>w,x</sup>	1812.5 <sup>b</sup>	2220.8 <sup>b</sup>	3499.4 <sup>a</sup>	2445 2 <sup>b</sup>
Pentyl acetate <sup>w</sup>	58.5 <sup>b</sup>	142.1 <sup>ab</sup>	331.0 <sup>a</sup>	147.9 <sup>ab</sup>
Hexyl acetate <sup>w, x</sup>	1134.1 <sup>b</sup>	1667.2 <sup>b</sup>	2666.3 ª	1724 5 <sup>b</sup>
Heptyl acetate	9.5 <sup>b</sup>	30.9 <sup>b</sup>	60.5 <sup>a</sup>	199 <sup>b</sup>
cis-3-Hexenyl acetate <sup>w,y</sup>	-	-	-	
Propyl propanoate <sup>w</sup>	4.6 <sup>b</sup>	16.1 <sup>ab</sup>	30.3 ª	14 9 <sup>b</sup>
Butyl propanoate <sup>w</sup>	112.6 <sup>b</sup>	344.5 <sup>ab</sup>	629.9 <sup>a</sup>	356 1 <sup>ab</sup>
Pentyl propanoate	11.3	15.0	20.3	10.2
Hexyl propanoate <sup>w</sup>	51.1 <sup>b</sup>	239.5 <sup>b</sup>	491.6 <sup>a</sup>	210.9 <sup>b</sup>
Propyl butyrate <sup>w</sup>	7.7 <sup>b</sup>	17.5 <sup>ab</sup>	36.1 ª	15.2 <sup>b</sup>
Butyl butyrate <sup>w</sup>	100.4 <sup>b</sup>	269.4 <sup>ab</sup>	496 3 <sup>a</sup>	215 7 <sup>b</sup>
Pentyl butyrate	2.9 <sup>b</sup>	9.2 <sup>ab</sup>	$17.6^{ab}$	215.7
Hexyl butyrate <sup>w</sup>	75.1 <sup>b</sup>	243.7 <sup>a</sup>	388 9 <sup>a</sup>	24.4
Propyl hexanoate	32.3 <sup>b</sup>	72.8 <sup>b</sup>	164.2 <sup>a</sup>	43 4 <sup>b</sup>
Butyl hexanoate <sup>w</sup>	281.1 °	734.7 <sup>b</sup>	107.2	532 2 bc
Hexyl hexanoate <sup>w</sup>	117.5 °	619.0 <sup>b</sup>	1470 0 ª	196.1 °
Butyl heptanoate	11.5 <sup>b</sup>	49.5 <sup>b</sup>	126.4 <sup>a</sup>	26.1 <sup>b</sup>
Hexyl octanoate	9.0 °	27.4 <sup>b</sup>	59.0 <sup>a</sup>	110 <sup>bc</sup>
2-Methylpropyl acetate <sup>w</sup>	20.9 <sup>b</sup>	41.1 <sup>b</sup>	91.8 <sup>a</sup>	26.7 <sup>b</sup>
2-Methylbutyl acetate <sup>w, x</sup>	718.6 <sup>b</sup>	1410.4 <sup>ab</sup>	1949 5 <sup>a</sup>	999.6 <sup>b</sup>
2-Methylbutyl butyrate	1.4 <sup>b</sup>	4.3 <sup>ab</sup>	8 1 <sup>a</sup>	$32^{ab}$
3-Methyl-2-butenyl acetate <sup>w</sup>	7.5 <sup>b</sup>	19.4 <sup>b</sup>	$42.0^{a}$	13.8 <sup>b</sup>
3-Methylbutyl propanoate <sup>w</sup>	3.0 <sup>b</sup>	9.5 <sup>b</sup>	$23.9^{a}$	97 <sup>b</sup>
3-Methylbutyl hexanoate	1.6 °	8.4 <sup>b</sup>	$20.8^{a}$	4 0 <sup>bc</sup>
Butyl 2-methylpropanoate	3.7	7.6	12.9	69
Hexyl 2-methylpropanoate	4.3	14.2	23.6	11.9
Methyl 2-methylbutyrate <sup>w</sup>	8.3 <sup>b</sup>	24.6 <sup>b</sup>	55.6 °	29 <sup>b</sup>
Ethyl 2-methylbutyrate <sup>w, y</sup>	2.4	4.5	4.5	1 4
Propyl 2-methylbutyrate <sup>w</sup>	13.6 <sup>b</sup>	41.3 <sup>ab</sup>	104 7 ª	416 <sup>ab</sup>
Butyl 2-methylbutyrate <sup>w</sup>	116.1 <sup>b</sup>	349.5 <sup>ab</sup>	664 9 <sup>a</sup>	355 6 <sup>b</sup>
Hexyl 2-methylbutyrate <sup>w</sup>	35.8 <sup>b</sup>	329.2 <sup>b</sup>	798 9 <sup>a</sup>	340 1 <sup>b</sup>
Hexyl tiglate <sup>w</sup>	016	$49^{ab}$	$Q \Delta^{a}$	۰.۲ ۱ د <sup>۵</sup>
6-Methyl-5-hepten-2-one <sup>w</sup>	4 9 °	27.5 <sup>b</sup>	80 8 <sup>a</sup>	1.0 15 5 <sup>bc</sup>

Table 3.1. Volatile compounds and their quantity  $(ng/\mu L)$  in 'Gala' apple headspace trapped on charcoal for 6, 12 and 24 hours and eluted with  $CS_2$ or on Tenax GR for 24 hours and eluted with ether<sup>2</sup>

# Table 3.1, continued

4-Allylanisole <sup>w</sup>	9.5 <sup>b</sup>	56.8 <sup>b</sup>	202.5 ª	77.0 <sup>b</sup>
α-Farnesene	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	9.7 ª
β-Damascenone <sup>w, y</sup>	-	-	-	-
Total volatiles	4913.0	9353.0	16508.0	8427.0
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<sup>2</sup> Values are means of 4 replicates of dynamic headspace of 1 kg apples. Means followed by the same letter within one row indicate no significant difference by the Waller-Duncan t-test K-ratio, K=100

<sup>w</sup> Odor active compounds at those concentrations

<sup>x</sup> Above the detector linear range

<sup>y</sup> Below the detection limit

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		Charcoal + $CS_2$		Tenax + Ether
Compound group	6 hrs	12 hrs	24 hrs	24 hrs
Alcohols	1.2	1.5	2.2	2.2
Acetates	62.8	44.9	42.0	52.6
Propanoates	3.7	6.6	7.1	7.0
Butyrates	3.8	5.8 15.3	5.7 17.2	5.8
Hexanoates	8.8			9.2
Heptanoate	0.2	0.5	0.8	0.3
Octanoate	0.2	0.3	0.4	0.1
Straight-chain esters	79.4	73.4	73.0	75.1
Branched-chain esters	19.1	24.2	23.0	21.6
Total esters	98.5	97.6	96.1	96.7
6-Methyl-5-hepten-2-one	0.1	0.3	0.5	0.2
4-Allylanisole	0.2	0.6	1.2	0.9

 Table 3.2. Proportion (percent of total) of volatile compounds per sampling method for 'Gala' apple headspace

and ethyl acetate) co-eluted with the solvent peak, which explains their absence in our study.

The absence of either ethyl esters and aldehydes from charcoal and Tenax GR traps indicates that active sites (carbon oxides, Betz *et al.*, 1989) on charcoal or the graphitized Tenax GR might have irreversibly adsorbed those compounds. It is also possible that, in the presence of water vapor (from apples), oxidations or nucleophilic attacks by excess sulfur from CS<sub>2</sub>, and further hydrolysis could occur on the surface of charcoal or graphitized Tenax in a catalytic manner. Another explanation would be that over a long period of sampling, the missing compounds were displaced by the higher molecular weight volatiles. Aldehydes were observed from back up Tenax TA traps connected in series after the charcoal or Tenax GR traps and heat desorbed, but not ethyl esters. 2-Methylbutyl 2-methylbutyrate was not found on either charcoal or Tenax GR traps, but this compound was present on Tenax TA traps heat desorbed.

Alcohols represented a maximum of 2.2% of the total volatile fraction in our samples. We tested alcohol recovery by applying alcohol standards in CS<sub>2</sub> directly onto charcoal traps. Results showed that alcohols were partially adsorbed on charcoal with a 50 to 60% recovery (data not shown). Paillard (1990) reported 6 to 16% alcohols from headspace sampled on charcoal. Production of 4-allylanisole (1-methoxy-4-(2-propenyl)-benzene by 'Gala' apples was confirmed (Young *et al.*, 1996). The relative headspace concentrations of this compound were less than 0.3% when sampled for 16 hours on Poropak Q for all cultivars tested (Williams *et al.*, 1977b), while it accumulated up to 1.23% in our samples (Table 3.2).  $\alpha$ -Farnesene, a compound produced by apple skin and known for its involvement in superficial scald (Huelin and Coggiola, 1968), was present in the samples trapped by Tenax GR and Tenax TA but not by charcoal. It was present in the back-up traps of both Tenax GR and charcoal, indicating that CS<sub>2</sub> did not elute this high molecular weight compound from charcoal.

Using charcoal traps (ORBO 32) to sample 'Golden Delicious' for 4 hours with a nitrogen dynamic headspace and eluting with  $CS_2$ , Pérez *et al.* (1993) found only esters and one alcohol. Kakiuchi *et al.* (1986) also found only traces of aldehydes when sampling apple headspace for 24 hours on Tenax GC. Aldehydes are very reactive

compounds and it possibly explains the difficulty of their recovery after extended sampling duration. Also, aldehydes were not present in 'Calville Blanc' apple direct headspace nor when volatiles were sampled on activated charcoal and vacuum-heat desorbed (Paillard *et al.*, 1970). Desorbed compounds remained in the same proportions in both methods of sampling. Streif (1981) described a method of sampling 2 L of apple headspace on activated charcoal which was heat desorbed in the injection liner of the GC; reported results do not show aldehydes, only acetaldehyde was identified in the samples (Streif, 1981; Brackmann *et al.*, 1993). Young *et al.* (1996) indicated butanol had the largest concentration in distillate prepared from 'Gala' apples. However, n-alcohols have often been found in larger amounts from apple essence: quantitatively 48 to 75% versus 6 to 16% in headspace (Paillard, 1990; Kakiuchi *et al.*, 1986).

### **Olfactometric Significance**

More peaks were perceived in 1995 than in 1994; peaks perceived most frequently and most intensely were perceived both years (Table 3.3). The following discussion refers to the 1995 results unless stated otherwise. Only 26 of 44 odor-active peaks were chemically identified (Table 3.3). Most were esters that had fruity odors. Hexyl acetate (peak 20) and pentyl acetate (peak 11) were perceived as having the closest odor to 'Gala' apples provided as standards. Butyl acetate (peak 4) and 2methylbutyl acetate (peak 7), present in the largest amount with hexyl acetate (Table 3.1), were perceived as solvent and nail polish. Fruity and apple-like descriptors were given to butyl 2-methylbutyrate, hexyl 2-methylbutyrate, butyl hexanoate, hexyl butyrate, hexyl propanoate, butyl propanoate and 3-methylbutyl propanoate. Methyl 2methylbutyrate (peak 2), ethyl 2-methylbutyrate (peak 6) and propyl 2-methylbutyrate (peak 13) had a strong sweet, berry-like (strawberry) distinctive odor. Ethyl 2methylbutyrate has been reported to be the character impact compound of 'Delicious' apple by having a ripe, overripe apple odor (Flath *et al.*, 1967). Butyl butyrate (peak 17) was also recognized with its rotten apple or cheesy descriptors.

GCO illustrates that human olfactory response can differentiate between two compounds having a close but distinct odor: butyl hexanoate and hexyl butyrate (peaks

Category	Peak Kovats <sup>®</sup>		ovats <sup>b</sup> Descriptor Compound		1994	1995	Perceived
	#	Index		-			intensity
Fruity	20	1028	Gala, ripe, pear	hexyl acetate	X	X	12.1
	4	827	nail polish	butyl acetate	Х	Х	10.6
	7	890	solvent	2-methylbutyl acetate	Х	Х	9.9
	6	863	sweet strawberry	ethyl-2-methylbutyrate	х	Х	8.1
	2	787	sweet fruity	methyl-2-methylbutyrate	х	х	8.0
	19	1018	apple	cis-3-hexenyl acetate	no	х	7.2
	19	1018	and toast	+ unknown <sup>f</sup>			
	13	959	very sweet, strawberry	propyl-2-methylbutyrate	Х	х	7.0
	23	1056	fruity, apple	butyl-2-methylbutyrate	Х	х	6.3
	11	926	gala	pentyl acetate	Х	Х	6.1
	43	1437	grape juice	ß-damascenone	Х	Х	5.2
	36	1255	apple, grapefruit	hexyl-2-methylbutyrate	Х	Х	5.2
	31	1205	green apple <sup>e</sup>	butyl hexanoate <sup>e</sup>	Х	Х	4.6
	27	1121	apple <sup>e</sup>	hexyl propanoate	Х	х	3.8
	16	997	fruity, tape	6-methyl-5-hepten-2-one	Х	Х	3.5
	12	933	fruity, sweet, solvent	3-methyl-2-butenyl acetate	Х	Х	2.5
	17	1009	rotten apple, cheesy	butyl butyrate	Х	х	2.2
	18	1010	solvent, gala	unknown <sup>f</sup>	Х	х	2.2
	10	921	fruity, apple	butyl propanoate	Х	Х	2.2
	44	1524	fruity	unknown <sup>f</sup>	Х	х	1.9
	32	1214	apple <sup>e</sup>	hexyl butyrate <sup>e</sup>	Х	х	1.9
	38	1276	grape juice <sup>c</sup>	unknown <sup>f</sup>	х	х	1.3
	42	1418	apple or tape <sup>d</sup>	hexyl hexanoate + unknown	no	х	0.7
	3	813	fruity <sup>d</sup>	propyl propanoate	х	Х	0.0
	15	994	grassy, green apple <sup>c,d</sup>	3-methylbutyl propanoate	х	Х	0.0
	9	910	fruity <sup>d</sup>	propyl butyrate	no	х	0.0

Table 3.3.	Odor active peaks for 'Gala' apple: Kovats indices, odor descriptors, compound identities, presence in 1994 and 1995, and perceived intensities on a 16-point scale ( $0 = \text{none}, 7 = \text{moderate}, 15 = \text{extreme}$ ) through Osme analysis <sup>a</sup>
	identities, presence in 1994 and 1995, and perceived intensities on a 16-point scale ( $0 = \text{none}, 7 = \text{moderate}, 15 = \text{extreme}$ ) through Osme analysis <sup>a</sup>

Floral	26	1118	floral <sup>e</sup>	unknown <sup>t</sup>	X	X	3.7
Anise	33	1222	anise, licorice	4-allylanisole	X	Х	7.7
	22	1041	sweet, anise <sup>d</sup>	unknown <sup>f</sup>	Х	Х	0.4
Cucumber	24	1070	watermelon	unknown <sup>t</sup>	X	X	7.4
	28	1145	cucumber	unknown <sup>f</sup>	no	Х	2.7
	34	1227	cucumber <sup>c, d</sup>	unknown <sup>f</sup>	Х	Х	0.9
Mushroom	14	991	mushroom	1-octen-3-ol	no	Х	4.6
	35	1230	cat urine, mushroom	unknown <sup>f</sup>	Х	Х	4.1
	40	1353	nutty, mushroom <sup>d</sup>	hexyl tiglate	Х	Х	1.7
Spicy,	39	1303	tape or fruity	unknown <sup>r</sup>	Х	X	4.2
adhesive tape	41	1364	tape or musty dirty	unknown <sup>f</sup>	no	Х	2.7
	29	1151	anise, spice or mushroom	unknown <sup>f</sup>	Х	Х	2.3
	30	1197	tape or fruity	unknown <sup>f</sup>	no	Х	2.1
	1	774	tea, garlic, leaves	2-methylpropyl acetate	no	Х	0.6
Rubber,	5	836	skunk, rubber	no peak	Х	X	8.4
skunk	37	1272	strong rubber	no peak	Х	Х	6.9
	8	901	oatmeal, skunk	no peak	no	Х	3.2
	25	1112	dusty, musty	no peak	no	Х	2.3
	21	1038	metallic, skunk	no peak	Х	Х	1.3

# Table 3.3, Continued

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<sup>a</sup> Mean of 3 panelists over 4 replications of 'Gala' apples sampled on charcoal for 24 hours in 1995 <sup>b</sup> Kovats indices on RTX-5 (5% diphenyl 95% dimethyl polysiloxane) column

<sup>c</sup> Perceived by one panelist only <sup>d</sup> At or below odor threshold. Perceived sporadically

<sup>e</sup> Peaks co-elute on the FID, but perceived separately by the panelists (peaks 26 and 27; 31 and 32)

<sup>1</sup> Correspond to peaks detected by FID, but no satisfactory match was found in the Wiley/NBS library

31 and 32) co-eluted on the apolar Rtx-5, but were perceived as green apple and apple, respectively (Table 3.3). Likewise, *cis*-3-hexenyl acetate (peak 19) and hexyl hexanoate (peak 42) (both apple-like) co-eluted with an unidentified compound that had a toast or scotch tape odor, respectively. A floral compound (peak 26) probably co-eluted with hexyl propanoate (apple, peak 27) as their Kovats indices for *Osme* were close but only one peak was detected on the FID. Although these peaks could be perceived distinctly by all three panelists in 1995, butyl hexanoate and hexyl butyrate were perceived as one apple-like peak in 1994. The floral and hexyl propanoate apple odors were also recorded as one peak in 1994, but still both descriptors were used. Two of the three panelists that participated in the sniffing were the same in 1994 and 1995. The ability to discriminate between odors, and the use of the recording device might have improved with the second year of practice.

GCO also allowed identification of some compounds that were not detected using GC-MS but were perceived by the human subjects. For example, ß-damascenone (2,6,6-trimethyl-1-trans-crotonyl-1,3-cyclohexadiene) (peak 43) was present in trace amounts but was identified by having a strong recognizable grape juice odor and by its Kovats index (1437). This compound was reported by Cunningham et al. (1986) to have a high Charm value for some apple cultivars. Another 38% of the compounds remained unidentified or did not correspond to a visible peak on the FID (Table 3.3). The compound with a floral odor mentioned earlier did not correspond to any compound in the Wiley/NBS library. Two anise peaks were perceived: one was unidentified and the other was identified as 4-allylanisole and reported in 'Royal Gala' by Young et al. (1996). Williams et al. (1977b) attributed the distinctive spicy flavor of 'Cox's Orange Pippin' to that compound. One watermelon- and two cucumber-like odors were reported. While the two cucumber peaks were perceived only sporadically and mostly by the most sensitive panelist, the watermelon odor was strong and clearly perceived by all panelists. No satisfying match was found in the Wiley/NBS library for these compounds. Three peaks had a mushroom odor: one was unidentified; hexyl tiglate was identified by GC-MS, Kovats indices and GCO; and 1-octen-3-ol was tentatively identified by matching retention indices and GCO. However, no peak was present on the

sample chromatogram, and the mushroom odor might also be due to 1-octen-3-one coeluting with 1-octen-3-ol but having a lower odor threshold (Blank, 1997). 1-Octen-3one is not available commercially (Blank, 1997) and we could not verify its identity. Hexyl tiglate and 1-octen-3-ol were not reported in apple previously but the ketone was reported in raspberry (Roberts and Acree, 1996) and in apples (Cunningham *et al.*, 1986).

Spicy, adhesive tape, skunk and rubber-like odors were reported from the samples. All of these compounds had a low odor threshold (except peak 30, Figure 3.1) since the chromatographic peaks were small or undetected by the FID. With these odor descriptors, the compounds may be nitrogen or sulfur-containing compounds. It is possible these compounds were artifacts resulting from reactions between entrained compounds and the CS<sub>2</sub> solvent with the charcoal active sites acting as catalyzers. However, all these compounds were also present on the Tenax GR (and also charcoal, data not shown) traps eluted with diethyl ether, although often perceived with a lower intensity. Sulfur compounds have been reported from apple samples. 2-(Methylthio)ethyl acetate and 3-(methylthio)propyl acetate were reported by Schreier et al. (1978), 3-methylthio-1-propanol by Schreier et al. (1978) and by Girard and Lau (1995), and benzothiazole was found in 'Kogyoku' apples (Yajima et al., 1984). Retention time and odor of benzothiazole matched peak 37 in our sample. It is not known at this point whether those compounds are natural compounds emitted by the fruit, or compounds metabolized from sulfur-containing fungicides used on apple trees during fruit development. Panelists that participated in a panel evaluating the same 'Gala' apples as we used in this experiment mentioned a sulfury odor in the background of the fruity apple aroma (Plotto et al., 1998). Because of the high lability of sulfur compounds in stored samples (Hofmann et al., 1996) and their instability at each step of GC run (Block, 1993), the identification and the representativeness of the skunk-, rubber-like peaks perceived by Osme for 'Gala' apples remain to be proven. Descriptors such as rotten, putrid, earthy, mushroom and dry dust were also reported from GCO of 'Golden Delicious' apple sampled by dynamic headspace on activated charcoal and eluted with methylene chloride (Rizzolo et al., 1989).

Figure 3.1. FID chromatogram (top) and *Osme* aromagram (bottom) for 'Gala' apples stored in air (2 °C) for 4 weeks. Samples (1-kg apples) of dynamic headspace for 24 hrs on charcoal traps. Only odor-active peaks are numbered. See Table 3.3 for identity.



Aldehydes that were not present on charcoal or Tenax GR but isolated on Tenax TA heat desorbed traps are probably odor-active in 'Gala' apple because of their low odor threshold, ranging from a high of 16.0  $\mu$ g·L<sup>-1</sup> for butanal to a low of 0.10  $\mu$ g·L<sup>-1</sup> for decanal (Guadagni *et al.*, 1963). Likewise, ethyl butyrate, ethyl pentanoate and ethyl hexanoate odor thresholds are 1, 1.5 and 1  $\mu$ g·L<sup>-1</sup>, respectively (Takeoka *et al.*, 1989). This illustrates the distortions introduced by different methods of volatile isolation and the relevance of comparing more than one method. GCO gives additional information and confirmation of compound identity, provided that authentic standards can be used for comparison between odor characters and sample peaks. Since volatile isolation studies would be necessary (Mistry *et al.*, 1997).

## **Trap Adsorbing Capacities**

The quantity adsorbed by charcoal traps was proportional to the time of sampling duration, or volume sampled (Table 3.1). Sampling on Tenax GR for 24 hours with a lower flow through rate (100 mL·min<sup>-1</sup>) generally yielded quantities comparable to sampling on charcoal for 12 hours at 200 mL·min<sup>-1</sup> (same volume of headspace sampled, *ca.* 140 L). With charcoal, more alcohols were trapped using the longer sampling durations, while more acetate esters were present in samples collected for 6 hours (Table 3.2). It is obvious that higher molecular weight compounds were trapped by longer sampling durations, probably displacing smaller compounds such as acetate esters (Table 3.2). Air flow rate through the traps and long sampling duration times were chosen to optimize collection of the higher molecular weight compounds (Takeoka *et al.*, 1990).

Overall, more odor-active peaks were perceived from the longest sampling durations (Table 3.4). Two panelists out of three perceived more odor-active peaks in the 12 hour charcoal sample than in the 24 hour Tenax, while one panelist (Pan. 2) perceived fewer peaks (Table 3.4). Peaks perceived from charcoal traps had an overall higher intensity, except peaks 1 and 30 (Table 3.5). More fruity peaks were perceived in the charcoal traps, whether sampled for 24 or 12 hours. The floral, a licorice, a watermelon, the adhesive tape and the skunk/rubber peaks were perceived with a higher

	Charcoal + CS <sub>2</sub>										Tenax + Ether		
	6 hrs			12 hrs			24 hrs			24 hrs			
	Pan. 1	Pan. 2	Pan. 3	Pan. 1	Pan. 2	Pan. 3	Pan. 1	Pan. 2	Pan. 3	Pan. 1	Pan. 2	Pan. 3	
Total peaks	28	18	17	36	24	21	42	36	25	29	32	16	
Apple peaks	10	5	5	12	7	8	13	11	8	11	9	2	

 Table 3.4. Total number of odor-active peaks and apple-like peaks perceived by 3 panelists through Osme analysis for each sampling method of 'Gala' apple headspace<sup>a</sup>

<sup>a</sup> Each panelist evaluated four replications per sample

	*		Charcoal + CS <sub>2</sub>							Tenax + Ether		
<b>D I</b> <i>U</i>	Kovats <sup>y</sup>	<b>.</b> .		6 hrs		12 hrs		24 hrs		24 hrs		
Peak #	Index	Descriptor	%	I max	%	I max	%	I max	%	I		
20	1028	Gala, ripe, pear	83	8.54 <sup>b</sup>	92	9.56 <sup>b</sup>	100	12.05 <sup>a</sup>	92	8.40 <sup>b</sup>		
4	827	nail polish	100	8.52 <sup>bc</sup>	100	9.54 <sup>ab</sup>	100	10.60 <sup>a</sup>	100	7 83 °		
7	890	solvent	92	7.37 <sup>b</sup>	92	9.54 <sup>ab</sup>	100	9.91 <sup>a</sup>	100	7.53 <sup>b</sup>		
6	863	sweet strawberry	100	6.28	83	6.32	100	8.06	100	611		
2	787	sweet fruity	100	6.30 <sup>b</sup>	100	7.50 <sup>ab</sup>	100	7.95 <sup>a</sup>	100	8 35 <sup>a</sup>		
19	1018	apple and toast	17	0.88 <sup>b</sup>	50	3.69 <sup>b</sup>	100	$7.17^{a}$	33	1.23 <sup>b</sup>		
13	959	very sweet, strawberry	100	6.49 <sup>ab</sup>	100	7.56 <sup>a</sup>	100	7.04 <sup>ab</sup>	100	5.96 <sup>b</sup>		
23	1056	fruity, apple	67	3.41 <sup>b</sup>	92	5.20 <sup>a</sup>	100	6.30 <sup>a</sup>	67	3.16 <sup>b</sup>		
43	1437	grape juice	33	2.17 <sup>b</sup>	67	4.74 <sup>ab</sup>	75	5.21 <sup>a</sup>	58	3.85 <sup>ab</sup>		
36	1255	apple, grapefruit	17	0.69 <sup>b</sup>	67	3.47 <sup>a</sup>	83	$5.15^{a}$	42	151 <sup>b</sup>		
31	1205	green apple	17	0.99 <sup>b</sup>	50	3.44 <sup>a</sup>	58	4 57 <sup>a</sup>	17	1.31 1.27 <sup>b</sup>		
27	1121	apple	0	0.00 <sup>b</sup>	25	2.66 <sup>a</sup>	50	3.76 <sup>a</sup>	8	0.61 b		
16	997	fruity, tape	17	0.73 <sup>b</sup>	42	2.29 <sup>a</sup>	50	3.51 <sup>a</sup>	0	0.01		
12	933	fruity, sweet, solvent	17	0.72 <sup>b</sup>	33	1.45 <sup>ab</sup>	42	$2.53^{a}$	Ő	0.00		
17	1009	rotten apple, cheesy	8	0.37 <sup>b</sup>	17	1.35 <sup>ab</sup>	25	2.33	0	1 17 <sup>ab</sup>		
18	1009	solvent, gala	17	0.79 <sup>ab</sup>	50	$2.47^{a}$	42	2.22	8	0.25 <sup>b</sup>		
10	921	fruity, apple	0	0.90	25	1.34	58	2.19	33	1.56		
44	1524	fruity	25	1.51	42	1.98	33	1.88	0	0.00		
32	1214	apple	25	1.25	8	1.01	33	1.86	22	0.00		
38	1276	grape juice	17	0.91	8	0.52	17	1.30	50	1.55		
42	1418	apple or tape	8	0.48	25	1.04	17	0.67	50	2.33		
3	813	fruity	8	0.16 <sup>b</sup>	8	0.23 <sup>b</sup>	0	0.07	v Q	0.00		
15	994	grassy, green apple	0	0.00	0 0	0.00	Ő	0.00	0	2.32 0.72		

Table 3.5. Frequency (%) and average intensity  $(I_{max})$  of odor-active peaks trapped on charcoal (eluted with  $CS_2$ ) for 6, 12 and 24 hours and onTenax GR (eluted with ether) for 24 hours (n = 12, 3 panelists with 4 replications each)<sup>z</sup>

26	1118	floral	0	0.00 <sup>b</sup>		 0.49 <sup>b</sup>		3 67 <sup>a</sup>	0	0 00 <sup>B</sup>
33	1222	anise, licorice	33	1.51 °	50	3.76 <sup>bc</sup>	100	7.69 <sup>a</sup>	67	4 71 <sup>b</sup>
	1041	sweet, anise	0	0.00	17	0.68	8	0.37	8	0.37
24	1070	watermelon	25	1.27 °	83	4.92 <sup>6</sup>		7.38 <sup>a</sup>		3 91 8
28	1145	cucumber	0	0.00 <sup>b</sup>	25	1.27 <sup>ab</sup>	50	2.68 <sup>a</sup>	25	1.06 <sup>b</sup>
	1227	cucumber	0	0.00	17	1.07	17	0.93	0	0.00
14	991	mushroom	0	0.00 <sup>c</sup>	25	1.56 <sup>bc</sup>	67	4.62 <sup>a</sup>	58	3 45 <sup>ab</sup>
35	1230	cat urine, mushroom	25	1.47 <sup>b</sup>	25	1.44 <sup>b</sup>	58	4.08 <sup>a</sup>	8	0.41 <sup>b</sup>
	1353	nutty, mushroom	0	0.00 <sup>b</sup>	0	0.00 <sup>b</sup>	33	1.66 <sup>a</sup>	8	0.23 <sup>b</sup>
39	1303	tape or fruity	0	0.00 6	8	0.41 <sup>b</sup>	67	4.15 <sup>a</sup>	 0	0.00
41	1364	tape or musty dirty	17	0.61 <sup>b</sup>	17	0.92 <sup>b</sup>	50	2.68 <sup>a</sup>	8	0.00 <sup>b</sup>
29	1151	anise, spice, or mushroom	8	0.32 <sup>b</sup>	17	0.82 <sup>b</sup>	33	2.33 <sup>a</sup>	25	1 14 <sup>b</sup>
30	1197	tape or fruity	0	0.00 <sup>c</sup>	25	1.15 <sup>bc</sup>	42	2.14 <sup>b</sup>	92	4 98 <sup>a</sup>
1		tea, garlic, leaves	17	0.94 <sup>b</sup>	8	0.51 <sup>b</sup>	8	0.55 <sup>b</sup>	67	4 34 <sup>a</sup>
5	836	skunk, rubber	58	3.08 <sup>b</sup>	100	7.12 <sup>a</sup>	100	8.40 <sup>a</sup>	58	3 50 6
37	1272	strong rubber	33	1.98 °	67	4.91 <sup>ab</sup>	67	6.88 <sup>a</sup>	50	2 98 <sup>bc</sup>
8	901	oatmeal, skunk	8	0.41 <sup>b</sup>	25	1.92 <sup>a</sup>	58	3.21 <sup>a</sup>	67	4.61 <sup>a</sup>
25	1112	dusty, musty	42	2.08 <sup>ab</sup>	25	0.95 <sup>b</sup>	33	2.31 <sup>ab</sup>	67	4 10 <sup>a</sup>
21	1038	metallic, skunk	0	0.00 <sup>b</sup>	0	0.00 <sup>b</sup>	25	1.25 <sup>a</sup>	8	0.22 <sup>b</sup>

<sup>2</sup> Means for peak height with a different letter superscript within a row significantly different by the LSD test, P < 0.05<sup>y</sup> Kovats indices on RTX-5 (5% diphenyl 95% dimethyl polysiloxane) column <sup>x</sup> Intensity on a 16-point scale: 0 = none, 7 = moderate, 15 = extreme

intensity from the charcoal traps sampled for 24 hours. Frequency analysis is another way of evaluating GCO data without necessarily using dilution techniques. Recently, Pollien and co-workers (1997) showed that reproducible aromagrams could be generated from the frequency of perceived odor peaks with 6 to 8 panelists. The advantage of this GCO method is that panelists do not require any training because no scale is used. The lowest coefficient of variation was achieved by using at least 8 panelists, as opposed to one, two or three panelists as usually reported in the GCO literature (Pollien *et al.*, 1997). Those authors showed that frequency values increased with compound concentration and could therefore be used in sample differentiation, frequency being then equivalent to odor intensity. However, they did not mention that when more than one peak is perceived 100% of the time, there is no measurement for differences between peaks. As an example, 10 peaks were perceived by all panelists at all sniffing runs (100% of the time) for 'Gala' apples sampled on charcoal for 24 hours, but the average intensities ranged from 12.0 to 6.3 (Table 3.5).

Peaks that were not perceived were given a zero value as in the Da Silva et al. (1993) method of Osme data treatment. Missing peaks in GCO have been subject to discussion since it is unclear if they are not perceived because they originate from compounds at threshold concentration, or if they are missed due to panelist inattention, fatigue or exhaling. In GCO with dilution methods such as CharmAnalysis and AEDA, the missing peaks or "gaps" are attributed to the fact that threshold values are not absolute but represent a range of concentrations at which the presence of a compound may be perceived (Abbott et al., 1993). In the Osme method, since panelists evaluate four replications of the same sample, peaks that are perceived one and two times out of four may be considered to be from compounds at near threshold concentrations. This was confirmed by plotting the response  $(I_{max})$  against compound concentration for the quantified compounds (Appendix 5, Figures A.1 and A.2). By giving a zero value to the non-perceived peaks instead of treating them as missing data, the mean intensity  $I_{max}$  of those peaks is lowered. Compounds that are in the threshold range for all three panelists are perceived sporadically, and resulting in mean intensities below 2 (between "just detectable" and "very slight intensity"), when individually, ratings may be 3 - 4 ("slight"
and "slight to moderate"). These peaks then constitute the "noise" of the aromagram, similar to compounds that are present at the FID detection limit represent the noise of a chromatogram. These compounds were usually perceived 1, 2, 3 or 4 times out of 12 (8, 17, 25 and 33%, respectively). More peaks were present at threshold or below threshold for the sampling on charcoal for 6 hours and on Tenax GR compared to the 12 and 24 hours sampling on charcoal (Table 3.5). Peaks 44, 32, 38, 42, 3, 15, 22, 34, 40 and 21 can be considered as noise as they were all perceived less than 50% of the time with an average  $I_{max}$  below 2.0. It is unclear whether these peaks were a response to a true stimulus, or due to other psychological factors especially when they were perceived less than 50% of the time. Additionally, at threshold, some peaks were not clearly recognized with one unique descriptor, such as peaks 41, 29, 30 and 1 (Table 3.5). All fruity-like peaks in doubt were identified as esters by GC-MS and GCO of authentic standards and therefore were not artifacts. However, the identity and trueness of the adhesive tape-like peaks remain unknown. The importance of peaks with low  $I_{max}$  in apple aroma should be evaluated in model mixture validation experiments. In mixtures, compounds below threshold concentrations were found to interact in an additive manner (Guadagni et al., 1963)

A problem not addressed in earlier *Osme* studies was co-eluting peaks. When compounds had a distinct odor, it was possible to calculate the frequency for each. However, there was not enough elution time between the two peaks for panelists to record  $I_{max}$  for both odors. Also, one compound might be dominant over the other, leading to odor suppression of the other compound as might have occurred for peak 19. Peak 19 was perceived like apple 8 and 17% of the time, and like toast 50 and 100% of the time in the 12 and 24 hours sampling on charcoal, respectively (Table 3.5). Therefore, it is likely  $I_{max}$  was recorded for the toast odor. Problems of co-elution are generally solved by the use of two columns with different polarities, but it is also necessary to use olfactometry as well as an FID or MS detector during the GC optimization process. In practice, the availability and reproducibility of a panelist may limit the feasibility of the process. The method development could include the time for panelist training to *Osme* analysis.

### CONCLUSION

Charcoal was chosen because of its high adsorbing surface area. However, because of presence of active sites, irreversible binding of some compounds and artifacts were expected. Indeed, aldehydes were not eluted from charcoal traps, and alcohols were only partially eluted. To elute solutes from the charcoal, a strong solvent was required, and CS<sub>2</sub> was used (Weurman, 1969; Pérez et al., 1993; Tang and Jennings, 1967). Active sites present on charcoal might have catalyzed the production of artifacts derived from solute-solute, or solute-solvent reactions. The presence of sulfur-like odoractive compounds led to the second hypothesis. However, samples eluted with diethyl ether from either charcoal (data not shown) or Tenax GR traps yielded identical odoractive analytes. It is not known at this point whether sulfur-like odor-active compounds are natural compounds emitted by the fruit, or fungicide residues. Sampling large volumes of headspace was achieved with charcoal and Tenax GR sampled for 12 and 24 hours. Overall, less odor-active compounds were perceived from Tenax GR than from charcoal traps. Because esters are the major compounds with an apple odor and it is unlikely that artifacts were formed with the chemically stable esters, and because getting large amounts of odor-active compounds was of interest, charcoal traps with a 24 hour sampling time were chosen for further study of the changes in storage of odor-active compounds of 'Gala' apple.

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# **CHAPTER 4**

# VALIDATION OF GAS CHROMATOGRAPHY OLFACTOMETRY RESULTS FOR 'GALA' APPLES BY EVALUATION OF AROMA-ACTIVE COMPOUND MIXTURES

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# ABSTRACT

'Gala' is an early maturing apple variety with a distinctive aroma and flavor. Previous research has determined 'Gala's aroma-active compounds by using *Osme*, a gas chromatography olfactometry method that records subjects' olfactory response on a time-intensity scale. Sixteen of those compounds were combined in mixtures in water solutions at concentrations determined by analyzing apple headspace. Sixteen panelists compared aromas of the solutions with fresh apples and rated degree of difference for aroma. In a pilot study, mixture solutions were prepared by combining compounds based on their intensities as perceived by *Osme*; results showed a large variability between panelists for perception of the solutions. Another experiment used a statistical screening design. Hexyl acetate, hexanal, butyl acetate, 2-methylbutyl acetate, and methyl 2-methylbutyrate contributed to the least difference between mixtures and apples; while pentyl acetate, hexyl 2-methylbutyrate, butyl hexanoate, and 4-allylanisole contributed to the largest difference. Further experiments using statistical designs will be necessary to determine interactions between compounds.

#### INTRODUCTION

Smelling gas chromatograph effluents to determine the odor characteristic of a compound has been practiced in flavor research chemistry since the development of gas chromatography in the 1950's; it has been formalized and is now known as gas chromatography olfactometry (GCO) (Acree, 1997; Mistry et al., 1997). However, without any quantification of the chemical stimuli and of the subjects' responses, GCO is limited to screening odor-active volatiles among those present in a complex sample. Potent odorants are often near or beyond the limit of detectability by GC analysis (Guadagni et al., 1966; Cunningham et al., 1986). Patton and Josephson (1957) introduced the idea of relating a compound's concentration to its odor threshold in order to assess its odor significance. This concept was named "aroma value" by Rothe and Thomas (1963), "unit flavor base" by Keith and Powers (1968), and is now used as the "odor unit" (Teranishi et al., 1991), and the "Odor Activity Value" (OAV) (Grosch, 1994). The concepts of odor activity, odor potency, and odor threshold of a compound have been further developed with the use of dilution techniques in GCO analysis and named CharmAnalysis (Acree et al., 1984) and Aroma Extract Dilution Analysis (AEDA) (Grosch, 1993). Using these techniques, the compounds that are perceived at the highest dilution level are deemed the most potent in the sample. In other words, the odor potency of a compound is determined by the quantity necessary to give a response: the smaller the concentration, the more potent the compound. Both CharmAnalysis and AEDA assume that the response to an odorous stimulus is linear and that all compounds have identical response slopes with increasing concentration. In contrast, psychophysical events are based on the principles of Stevens' law, which states that the response to a stimulus follows a power function, and that the exponent of the function is between 0.3 and 0.8 for odorants (Stevens, 1957; Cain, 1969). Another GCO technique, Osme, is based on Stevens' law of psychophysics and combines time and intensity of perception as a response to odorants (McDaniel et al., 1990; Da Silva et al., 1994). Osme produces an odor profile, and comparisons between samples can be made by either comparing

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sample profiles (Young, 1997; Sanchez et al., 1992a; 1992b), or by statistical analysis (Da Silva et al., 1993).

All GCO techniques are useful for determining the odor activity, quality, and potency of compounds in foods, and thus allow for sample comparisons. However, the limitation inherent to GC techniques is that the information is obtained for individual compounds, which are presented to the nose outside of the food matrix. Also, the different GCO methods and their data analyses may lead to different conclusions as to which compounds are most important in a sample (Abbott et al., 1993; Young, 1997). Validations of GCO by aroma reconstitution are required. Confirmation of GCO results by sensory comparison of mixtures with the original samples has been demonstrated for strawberry juice (Schieberle, 1994; Schieberle and Hofmann, 1997), cheddar cheese (Dacremont and Vickers, 1994), and apple (Young et al., 1996). The first four authors combined AEDA results with the OAV concept to compare reconstituted aroma mixtures with the original samples. Schieberle and colleagues added odor-active compounds in pectin, sugars, and acids for strawberry juice (Schieberle and Hofmann, 1997). They determined the sensory importance for each compound by omitting them from the model solution one by one, and they compared the mixtures to the original samples. Mixtures most similar to the sample were those containing all the compounds with an OAV above one. Dacremont and Vickers (1994) combined 15 compounds in two fractionated factorial designs and matched the resulting odors with cheddar cheeses. They narrowed the number of optimum compounds to six, and matched the mixtures' odors to 15 cheeses to determine which of the cheeses had a cheddar note. Young and co-workers (1996) used the four most potent compounds found by CharmAnalysis, combined them in four concentration levels, and used sensory descriptive analysis to measure differences for attributes generated by the mixture's aroma and flavor. They found that the combination of 2-methylbutyl acetate, hexyl acetate, and butanol approached most closely the "Red apple" attribute associated with 'Gala' apple flavor. Synthetic tomato aroma was also made by using the odor unit values concept (but without previous determination of compounds odor potency by GCO) and reported to have a tomato odor by a sensory panel (Buttery et al., 1987; 1990).

'Gala' apple (Malus domestica Borkh) is an early ripening cultivar that resulted from a cross between 'Kidd's Orange' ('Cox's Orange Pippin' X 'Red Delicious') and 'Golden Delicious' (White, 1991). 'Gala' has a sweet and perfumey aroma and flavor, which distinguishes it from other cultivars (Green and Autio, 1990). Storage techniques such as controlled atmosphere (CA) maintain the apple fruit firmness and acidity for up to seven to eight months, but a significant aroma decrease is generally observed (Patterson et al., 1974). Determination of compounds contributing to the aroma of fresh harvested 'Gala' aroma would assist further research aimed at maintaining 'Gala' flavor in storage. The odorants used in this study were previously determined by GCO and Osme to contribute to 'Gala' aroma (Plotto et al., 1995). This paper explores two methods to validate Osme data by comparing 'Gala' apples with model solutions. One method is based on the results found by Osme on compounds' perceived intensities. This approach is similar to the odor unit concept, and results are discussed by comparing Osme and odor unit values. The other method explored model solutions prepared with compounds at the same concentrations as found in apples. The solutions were prepared following a statistical screening design, but ignored odor units or odor intensities.

#### MATERIALS AND METHODS

# Materials

Volatiles emitted by 'Gala' apples previously stored in air at 1 °C were analyzed one week before sensory analysis as previously described (Mattheis *et al.*, 1991). Briefly, headspace of ca. 1 kg apples was trapped onto 50 mg of Tenax TA traps by using a dynamic flowthrough system with purified air at 100 mL/min. Samples consisted of 100 mL of headspace, and traps were thermally desorbed. Compounds were analyzed on a HP 5890A-5971A GC-MSD system. Previous work on 'Gala' using *Osme* had identified 26 compounds which had various levels of odor activity (Plotto *et al.*, 1995). Fifteen chemically identified compounds that were perceived consistently by all three panelists using *Osme* were used to construct model solutions (Table 4.1). Hexanal was

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	Apple Usadanaaa <sup>a</sup>	Partition	Theoretical	Pilot Study	Screening Design				
Compound in Apple	rieadspace	Coefficient	Concentration	Solution	Solution				
Compound in Apple	(µg/L)	<u> </u>	(µg/L)	(mg/L)	(mg/L)				
Butyl acetate	7.044	0.0133	529.60	30.00	50.00				
Hexyl acetate	1.160	0.0241	48.12	2.73	4.54				
2-Methylbutyl acetate	0.567	0.025	22.66	1.28	2.14				
Butyl hexanoate	0.549	0.04	13.72	0.78	1.29				
Hexyl 2-methylbutyrate	0.531	0.05	10.62	0.60	1.00				
Butyl butyrate	0.234	0.029	8.07	0.46	0.76				
Butyl 2-methylbutyrate	0.228	0.04	5.71	0.32	0.54				
Hexyl butyrate	0.218	0.0394	5.53	0.31	0.52				
Butyl propanoate	0.184	0.0197	9.34	0.53	0.88				
Methyl 2-methylbutyrate	0.093	0.028	3.30	0.19	0.31				
Pentyl acetate	0.088	0.0211	4.18	0.24	0.39				
2-Methylpropyl acetate	0.049	0.0221	2.22	0.13	0.21				
Hexyl propanoate	0.045	0.0277	1.64	0.09	0.15				
Ethyl 2-methylbutyrate	0.014	0.030	0.46	0.03	0.04				
Hexanal	0.011	0.0087	1.26	0.07	0.12				
4-Allylanisole	0.010	n.a. <sup>d</sup>	n.a.	0.042 <sup>e</sup>	0.07 <sup>e</sup>				

Table 4.1. Concentration of apple headspace compounds, air/water partition coefficient, theoretical concentration in water, and compound concentrations used in the pilot study and in the screening experiment

<sup>a</sup> 100 mL of dynamic headspace of 1 kg 'Gala' apples
 <sup>b</sup> Henry's law air/water partition coefficient; from Buttery et al., 1971; Jordan, 1954; Lyman et al., 1982; Pierotti et al., 1959

<sup>c</sup> Calculated by dividing column 1 by column 2 <sup>d</sup> No published vapor pressure found for this compound at 25 °C. <sup>e</sup> Added in the same proportions as found in apple headspace

also used in the experimental mixtures, even though it was not present in the Osme analysis of samples prepared from charcoal traps and eluted with CS2. It was reported as present in the samples heat desorbed from Tenax traps, and was previously found to contribute to apple odor with a green apple descriptor (Flath et al., 1967). The headspace concentrations measured in the 100 mL sample used in this study were converted to concentrations in water by using Henry's law to calculate K, the air-water partition coefficient (Buttery et al., 1971; Jordan, 1954; Lyman et al., 1982; Pierotti et al., 1959) (Table 4.1). The low solubility of 4-allylanisole did not follow the ideality assumptions necessary to calculate its air-water partition coefficient K at 25 °C (Table 4.1). Therefore, 4-allylanisole was used in the mixtures in the same proportions as found in the apple headspace. Experimental solutions were first prepared by mixing the compounds as calculated for the theoretical concentration in water (Table 4.1). However, the odor intensities of these solutions were too weak to be compared with apples; therefore, concentrations in water were increased until the overall aroma could be compared with apples while keeping the same relative ratios between compounds. All compounds were purchased from Aldrich Flavors and Fragrances (Milwaukee, WI) and were food grade. Compound purity was verified by GC-FID and by sniffing the GC effluent of a preparation of standards in the same concentrations as found in apple headspace. Compounds were mixed in odor-free double distilled water (Milli-Q) according to the designs described below.

## **Experimental Designs**

**Pilot Study.** Based on the compounds' odor intensities from *Osme* analysis (Table 4.2), sixteen mixtures were prepared by sequentially adding compounds in an incremental manner. The first solution was only hexyl acetate in water, the second solution was butyl acetate added to hexyl acetate, the third solution was made by adding 2-methylbutyl acetate to butyl acetate and hexyl acetate, and so on; the final solution was the mixture of the 16 compounds in water. Two replicates of each mixture were prepared in 50 mL water at the concentrations shown in Table 4.1 and presented in 120 mL glass jars with Teflon-lined screw caps. Means separation between mixtures for

Table 4.2. A) Apple compounds sorted by decreasing Osme intensity<sup>a</sup>, corresponding descriptors and perceived Osme intensity B) Apple compounds sorted by decreasing odor units, concentrations in water calculated from headspace, published odor thresholds, and calculated odor units

A)			B)	Theoretical	Odor	Odor
Apple compounds sorted		Osme	Apple compounds sorted	Concentration	Threshold	Unit <sup>b</sup>
by decreasing Osme intensity	Descriptor	Intensity <sup>a</sup>	by decreasing odor unit	(ug/L)		$(\mathbf{C}/\mathbf{T})$
Hexyl acetate	gala, ripe, pear	12.26	Hexyl acetate	48.12	2.00 <sup>c,d</sup>	24.06
Butyl acetate	nail polish	9.72	Methyl 2-methylbutyrate	3.30	$0.25^{\circ}$	13 20
2-Methylbutyl acetate	solvent	8.56	Butyl acetate	529.60	66.00 <sup>c,d</sup>	8.02
Methyl 2-methylbutyrate	sweet fruity	7.36	Ethyl 2-methylbutyrate	0.46	0 10 <sup>d</sup>	4 60
Ethyl 2-methylbutyrate	sweet strawberry	7.28	2-Methylbutyl acetate	22.66	5.00 <sup>d</sup>	4.00
4-Allylanisole	anise, licorice	6.48	Pentyl acetate	4.18	5.00 <sup>d</sup>	0.84
Pentyl acetate	gala	5.82	Hexyl 2-methylbutyrate	10.62	22.00 °	0.01
Hexyl 2-methylbutyrate	apple, grapefruit	5.74	2-Methylpropyl acetate	2.22	$5.00^{d}$	0.10
Butyl 2-methylbutyrate	fruity, apple	5.64	Butyl propanoate	9.34	25.00 <sup>d</sup>	0.37
Butyl propanoate	fruity, apple	5.24	Butyl 2-methylbutyrate	5.71	$17.00^{\circ}$	0.34
2-Methylpropyl acetate	tea, leaves	4.22	Hexanal	1.26	4 50 <sup>c,d</sup>	0.24
Hexanal	n.a.	n.a.	Hexyl propanoate	1.64	8.00°	0.20
Hexyl propanoate	apple	2.97	Butyl butyrate	8.07	100 00 °	0.08
Butyl butyrate	rotten apple	2.43	Hexyl butyrate	5 53	250.00 °	0.00
Hexyl butyrate	apple	2.02	Butyl hexanoate	13 72	200.00 °	0.02
Butyl hexanoate	green apple	2.02	4-Allylanisole	0.74	35.00 <sup>f</sup>	0.02

<sup>a</sup> From Plotto et al., 1995; intensity on a 16-point scale: 0 = none, 7 = moderate, 15 = extreme <sup>b</sup> Teranishi et al., 1991. <sup>c</sup> Takeoka et al., 1990. <sup>d</sup> Flath et al., 1967. <sup>e</sup> Takeoka et al., 1989. <sup>l</sup> Williams et al., 1977.

n.a.: non applicable. Hexanal was not present in the samples used for Osme analysis

degree of difference from apple was performed with the least significant difference (LSD) test, with panelist as a random effect.

Screening Design. Because all possible combinations of the 16 compounds would generate too many samples to evaluate, a screening design was used. These designs are often used by food developers to identify which among many ingredients in a sample are the most important to achieve a product characteristic; for example, which sugars and acids are necessary to combine in a fruit beverage to have a determined level of sweetness. The 16 compounds tested in the pilot study were mixed in 30 mL water following combinations computed by the ECHIP v.6.1.2 (Hockessin, DE) statistical package. A linear D-optimal screening design was used, with 16 variables (the 16 compounds) and 8 replicates (Table 4.3). The design resulted in 25 combinations containing 6 to 10 compounds (and 16 for the combination containing all compounds). Eight combinations were replicated, as the design dictated. Therefore, a total of 33 samples were prepared for each panelist in the same jars as described above. Concentrations and sample headspace used for this experiment were adjusted based on panelists' comments during the pilot study, without altering their relative proportions (Table 4.1). This experiment was repeated once. Each experiment, the pilot study and the two replications of the screening design, was conducted on a different day.

# **Sensory Analysis Procedure**

Sixteen panelists participated in the testing. Procedures were discussed with the panelists for one hour before the beginning of the study. For both pilot study and screening design, the 33 samples were evaluated in three sets of 11 jars containing the compound mixtures according to a complete randomized block design across sets. The first experiment had 32 jars (two replicates of 16 samples), but the first jar was triplicated to give the total of 33 samples and was not used in the statistical analysis. Twenty 'Gala' apples were used for each testing day. They were put in four 4 L glass jars (5 apples, ca. 1 kg, per jar) and presented randomly to the 16 panelists, one jar for 4 panelists. When the testing began, one apple jar was covered with aluminum foil and presented to the panelist with the sample mixtures. The apple jar remained covered

	<u> </u>	Solution Number																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Hexyl acetate	Χ			Χ	Χ		X			X				X		X		X	X	X	X			 X	$\frac{-x}{X}$
Butyl acetate	Х		X			Х	Х		Х						Х		Х		х	Х	x	x			x
2-Methylbutyl acetate		Х	Х		Х			Х	Х		Х					х		х		X	x	x	x		x
Methyl 2-methylbutyrate		Х		Х	Х		X			Х	Х	Х					x		x		x	x	x	x	x
Ethyl 2-methylbutyrate	X			Х		Х	Х		Х			Х	X					x		x		x	x	x	x
4-Allylanisole	Х		Х			Х		Х	X		Х		х	х					x		x		x	x	x
Pentyl acetate	X		Х		Х			х		Х	Х	х		X	х			4	~ •	x		x		x	x
Hexyl 2-methylbutyrate	Х		Х		Х		Х			Х		Х	Х		X	х					x	21	x	21	x
Butyl 2-methylbutyrate		Х	Х		Х		Х		Х				Х	Х		X	x				1	x		x	x
Butyl propanoate	Х			Х	Х		Х		Х		Х			Х	х		x	x				21	x	1	x
2-Methylpropyl acetate		Х	Х			Х	Х		Х		Х	х			X	x		x	x				~	x	x
Hexanal	Х			Х	Х			Х	Х		Х	х	x			x	x	••	x	x				Λ	x x
Hexyl propanoate		Х	Х			Х	Х			Х	X	X	X	x		••	x	x	2 K	x	x				л V
Butyl butyrate		Х		Х	Х			х	х			x	x	x	x			x	x	71	x	v			л v
Hexyl butyrate		Х		Х		х	х			х	x		x	x	x	x		71	x	x	Λ	A Y	$\mathbf{v}$		л v
Butyl hexanoate		Х		Х		X		х	х			x	- <b>-</b>	X	x	x	x		11	x	x	Λ	л V	v	л v
Total compounds	8	8	8	8	9	7	10	6	10	6	9	9	8	9	8	8	7	7	8	9	<u>7</u> 9	8	<u>л</u> 8	<u>^</u> 8	$\frac{\Lambda}{16}$

 Table 4.3. Combinations of compounds for the solutions used in the sceening experiment as computed by ECHIP statistical software<sup>a</sup>

<sup>a</sup> "X" indicates presence

during the testing. Panelists were asked to lift the 4 L jar cover, smell the apples, close the lid, open the sample containing the mixture solution, smell it, and rate degree of difference between the mixture and the apples on a 16-point category scale (0 = nodifference, 15 = extremely different). Panelists could also comment on the quality of the mixture. Panelists were asked to rest one minute after the first five samples, and take a 10 minute break between sets. They were only allowed to smell the samples once. All samples and apples were presented at room temperature. Panelists were seated in individual testing booths equipped with PCs and Compusense Five, v. 2.2 (Guelph, Ontario) software for data recording.

#### **RESULTS AND DISCUSSION**

# Pilot Study: Mixtures Prepared from Osme Odor Intensity Values

The differences in degree of difference ratings between solutions were small (Table 4.4). Average difference ratings ranged from 5.03 to 7.56 (slightly to moderately different). The largest average difference ratings was given to the solution containing hexyl acetate alone and the solutions containing four, five, six, and seven compounds; the least differences were found for the solutions containing 13 and 14 compounds. Based on previous research (Schieberle and Hofmann, 1997), a decrease in the degree of differences from apples as more compounds were present in the solutions was expected for the first five compounds with an odor unit above one, but this trend was not observed. Variability between panelists' perception of the sample aromas and their comparison with apples was considerable. Some mixtures were found to be very close to the apples by some panelists, and rather different for others. Some of this variability may have been due to variation in apples used as reference. A variation of 20% is not uncommon in apple headspace (Poll and Hansen, 1990) and was observed by sampling 'Gala' headspace with Tenax traps (unpublished results). Additionally, apples produce volatile compounds continuously, and it is possible that, within the few hours in which the experiment took place, headspaces were different from jar to jar when presented to

panelists. Another source of variability was the lack of training for this specific task although panelists had been trained for other types of sensory analysis. Finally, different perceptual response between panelists is usually expected.

Number of Compounds in Solution <sup>y</sup>	Average Difference from Apples <sup>2</sup>
1	7.56 ª
2	5.81 <sup>bc</sup>
3	5.84 <sup>bc</sup>
4	6.44 <sup>ab</sup>
5	6.41 <sup>ab</sup>
6	6.29 <sup>ab</sup>
7	6.69 <sup>ab</sup>
8	5.81 <sup>bc</sup>
9	5.59 <sup>bc</sup>
10	6.06 <sup>bc</sup>
11	6.22 <sup>bc</sup>
12	6.13 <sup>bc</sup>
13	5.03 °
14	5.06 °
15	5.56 <sup>bc</sup>
16	5.81 <sup>bc</sup>

# Table 4.4. Degree of difference between odorant mixtures and apples(n = 32 observations)

<sup>y</sup> Compounds were added incrementally in the order shown in Table 4.1

<sup>z</sup> Difference from apple: 0 = no difference, 15 = extremely different. Means followed by the same letter were not significantly different by the LSD test (P < 0.05) with panelist as random variable

The experimental design in this pilot study was based on assumptions about the relative odor activity of the 16 compounds. The combination of aroma-active compounds was determined from the data obtained by GCO of 'Gala' apples where odorant peak intensities were rated on a 16-point category scale (Plotto et al., 1995). To relate Osme data to the odor unit concept, ranking of compounds by decreasing odor intensity was compared to the ranking of compounds by decreasing calculated odor units (Table 4.2). Odor units were calculated by using odor threshold values published by the U.S.D.A. Western Regional lab (Flath et al., 1967; Takeoka et al., 1990; 1989) except for 4-allylanisole (Williams et al., 1977). Hexanal, not present in the 'Gala' sample analyzed by using Osme, was ranked at the 12th position, similar to the ranking based on calculated odor units. Comparison of odor intensity and odor unit data for 'Gala' apple headspace indicated that the first five compounds with an odor unit above one were also those with the highest odor intensity (Table 4.2). Except for compounds 13 to 16 that had the exact same ranking order and 4-allylanisole that was ranked last by the odor unit value, there were inversions in the ranking of some of the compounds, but the inversions did not exceed two positions. For example, methyl 2-methylbutyrate was ranked in the fourth position by Osme intensity and in the second position according to the odor unit value. The odor threshold value of 4-allylanisole was obtained from a different group of researchers (Williams et al., 1977), and this may explain the discrepancy with values obtained from the U.S.D.A. Western Regional laboratory. Headspace samples used to obtain Osme data and calculate odor units were taken from different groups of apples (same orchard, same storage type but stored for one versus five months) which might account for the slight discrepancy between the two ranking methods. The ranking obtained from the perceived odor peak areas, which combine odor intensity and time during the perception of the odorous stimulus (Da Silva et al., 1994), also resulted in a few inversions from odor intensities (data not shown).

Overall, the ranking of aroma-active esters present in 'Gala' apple according to the information obtained from the GCO technique *Osme* resulted in an order comparable with ranking based on odor units. However, we found limitations to both approaches in determining which compounds contributed most significantly to 'Gala' headspace aroma.

The use of odor units requires the knowledge of an odor threshold value. Odor threshold determination is very time consuming, and threshold values were found to vary considerably between laboratories and methods used (Pangborn et al., 1964; Larsen and Poll, 1990; Guadagni et al., 1963; Takeoka et al., 1996). For example, the method of presenting compounds (100 mL glass jars with lids or in Teflon squeeze bottles) was found to significantly affect thresholds and reproducibilities (Guadagni et al., 1963). Compounds presented in squeeze bottles had 100 fold lower threshold values than glass jars with lid. Odor thresholds used in this study were generated with the method using squeeze bottles (Teranishi et al., 1991; Flath et al., 1967; Takeoka et al., 1989; 1990); this may explain the discrepancy between the concentrations analyzed from apple headspace (in µg/L) and the concentration (in mg/L) necessary to attain a similar level of odor in the experimental solutions presented in glass jars with lids (Table 4.1). Additionally, odor units or OAV, like Charm values, ignore the power function of the response to stimulus concentrations and slope differences between different odorants (Dravnieks, 1977). The limitation in using GCO data to prepare mixtures of the odoractive compounds stands in the fact that unidentified odor-active compounds are not accounted for (Dacremont and Vickers, 1994). In 'Gala' apple, 19 of the 44 odor-active compounds were unknown (Plotto et al., 1995). Among those, compounds that had mushroom, earthy, or skunk descriptors had high odor intensities and probably contributed to the apple aroma. One comment from a panelist confirmed that hypothesis: this panelist rated some solution mixtures similar to the apples, but commented the mixtures were missing a sulfury component perceived in the fruit. The lack of duplication of apple aroma by combining apple-like odor-active compounds in a decreasing order of odor activities led to the use of an experimental screening design to identify those odor-active compounds contributing most to 'Gala' apple aroma. The volume of headspace and concentration of compounds in the jars were adjusted after some panelists mentioned that some solutions were "too weak" in the first experiment (Table 4.1).

### **Screening Design**

The first replicate test indicated that hexyl acetate, butyl acetate, and hexanal were necessary to impart the least difference between the solutions and apples. Pentyl acetate and hexyl 2-methylbutyrate contributed the most to differences between solutions and apples. In the second replicate test performed one week later, hexyl acetate and hexanal were found again to contribute to the least difference from apples, as did 2methylbutyl acetate and methyl 2-methylbutyrate. Similarly, pentyl acetate and hexyl 2methylbutyrate contributed to the largest difference, along with butyl hexanoate and 4allylanisole. The difference between the two replicate tests again may be due to variation between apples on the same day of the experiment as mentioned above, and differences in ripening between apples from one week to the other. Nevertheless, common results from both replicate tests indicated that hexyl acetate and hexanal contributed to 'Gala' aroma. The combination of results of both tests showed that the four esters having the highest Osme value and an odor unit greater than one (Table 4.2) contributed the most to 'Gala' aroma. Unfortunately, odor intensity of hexanal was not available from the samples sniffed by Osme. Published threshold values for hexanal gave an odor unit of less than one for our apples; however, the screening design experiment showed that hexanal in mixtures contributed significantly to 'Gala' aroma. This confirms that no definite conclusion can be drawn from the odor activity of compounds alone. Results regarding pentyl acetate and hexyl 2-methylbutyrate led to the same conclusion. Both compounds individually have a definite apple odor, but it seems that when present in the combinations of mixtures, they enhanced the difference from the apple control. 4-Allylanisole imparted a similar effect to the mixtures. This could be the effect of the chemical aromaticity of that compound or the result of a miscalculation of the concentration used, because the air/water partition coefficient K could not be theoretically calculated (see materials and methods).

# **About Odor Mixtures**

It is generally admitted and has been experimentally demonstrated that odoractive compounds with a certain odor characteristic do not create a novel odor when

mixed, and the perceived intensity of the mixture is less than the sum of intensities of individual compounds (Laing and Panhuber, 1979). All compounds in the mixtures in this study were esters with fruity, apple-like odors, and one aldehyde with a green apple odor (hexanal), and an allyl phenol compound with the odor of anise (4-allylanisole). Comments that were generated from some mixtures were either fruity, pear, banana, apple-like, or tended towards descriptors like "artificial apple", "bubble gum", "solvent, nail polish". At the concentrations tested, butyl acetate and 2-methylbutyl acetate alone had those qualities of descriptors (Table 4.2). It was expected that adding other compounds would attenuate the solvent note to give a descriptor closer to apple, but there was no agreement between panelists as to which combination was closer to the apples. Part of the variation between panelists might be an effect of the carryover from one solution to the next, because the instructions did not specify resting time between jars within a subset of five. Olfactory adaptation occurs between odorants having a similar aroma (Moncrieff, 1956; Cain and Polack, 1992) and similar chemical structure (Pierce et al., 1995). 4-Allylanisole, a compound structurally different from the esters, was occasionally perceived in some but not all solutions containing it. It was believed to contribute to the unique aromatic character of 'Cox's Orange Pippin' apple (Williams et al., 1977), and we also hypothesized that it might contribute to 'Gala' aroma. However, 4-allylanisole enhanced the difference from apples at the concentration used in this study. Butyl acetate was present in the largest amount in 'Gala' apple headspace, followed by hexyl acetate and 2-methylbutyl acetate. Those same compounds were chosen by Young et al. (1996) as having the highest Charm value for 'Gala' apple. Those authors also included butanol, present in the largest proportion (Young et al., 1996). However, they used vacuum steam distillation to isolate flavor volatiles. Butanol was not included in our study. We did not believe it would contribute significantly to 'Gala' aroma because it has a high odor threshold: 500 ppb (Flath et al., 1967), and the concentration found in whole 'Gala' headspace was 0.698  $\mu$ g/L. Young and co-workers measured the effect of compound interactions on a few sensory descriptors that were used to describe 'Gala' apple flavor and aroma. They found negative interactions between hexyl acetate and butanol, and between 2-methylbutyl acetate and butanol; the former affected "Red apple

aroma" and the latter "characteristic apple flavour". However, their method did not compare the mixtures with whole apples. Dacremont and Vickers (1994) used a concept matching technique with partial factorial designs to screen for the compounds contributing to cheddar cheese odor. Similar to the design we used, they questioned the reliability of the information obtained for the main effects when the main effects (compounds) were included in interactions with other compounds. Nevertheless, their technique optimized mixtures of compounds whose odor matched the concept of Cheddar and other cheeses (Dacremont and Vickers, 1994). In the end, whichever method and design is used, the making of mixtures relies on the previous step of GC analysis. Different recoveries observed in methods used for flavor isolation are welldocumented (Reineccius, 1993; Weurman, 1969). We used a headspace technique with purge and trap on Tenax because this technique captured the volatile profile of the sample with good recovery and without artifacts. However, the method used for Osme previously revealed that low odor threshold sulfur compounds were present in the samples but were not identified, therefore these compounds could not be included in the mixture experiments.

### CONCLUSION

Mixing 'Gala' odor-active compounds in proportions found in apple headspace and in combinations selected by a screening design has confirmed results obtained by the *Osme* GC-olfactometry technique. Hexyl acetate, hexanal, butyl acetate, 2-methylbutyl acetate and methyl 2-methylbutyrate were found to contribute to overall 'Gala' aroma. The use of a D-optimal linear screening design gave interesting information. The advantage of this design was that it was easily implemented since the number of compound combinations were limited, and there was no need to train panelists. Further experiments using response surface methodology will be necessary to determine 1) the level of interactions between compounds, and 2) how the odor mixtures change when compounds vary in different proportions. The latter determination would be very useful to post harvest physiologists because volatiles produced by apples vary in different proportions when stored in CA as opposed to air. Reduced oxygen and high  $CO_2$  in CA affect straight-chain acetate esters more than branched-chain esters and aldehydes (Brackmann *et al.*, 1993).

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# CHAPTER 5

# CHARACTERIZATION OF CHANGES IN 'GALA' APPLE AROMA DURING STORAGE USING OSME ANALYSIS, A GAS CHROMATOGRAPHY-OLFACTOMETRY TECHNIQUE

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#### ABSTRACT

'Gala' is an early ripening apple cultivar with a distinct aroma and flavor. Its storage season is short and volatile production is reduced following controlled atmosphere (CA) storage. Changes in odor-active volatiles for 'Gala' apples were measured after 5, 10, and 19 weeks storage at 1 °C in regular atmosphere (RA) and CA in 1994-95, and 4, 10 and 20 weeks in RA and CA in 1995-96. In 1995-96, apples were also stored 16 weeks in CA followed by 4 weeks in RA. 'Gala' apple dynamic headspace was collected on charcoal traps for 24 hours, then eluted with CS2. Aroma was evaluated using Osme, a method that combines gas chromatography and olfactometry (GCO). Three panelists recorded intensity and duration of odor-active compounds eluting from the gas chromatograph via a sniff port. Data were analyzed using analysis of variance and multivariate factor analysis (FA). Production of volatile esters decreased along with corresponding fruity aromas during CA storage. Hexyl acetate, butyl acetate and 2-methylbutyl acetate were emitted in the largest amounts and perceived with the strongest intensities from RA stored fruit. While hexyl acetate and butyl acetate concentrations and aromas intensities decreased significantly during CA storage, 2-methylbutyl acetate remained at RA concentration until 16 weeks in CA. Butyl 2-methylbutyrate and hexyl 2-methylbutyrate contributed to the apple, fruity aroma of RA stored fruit. Methyl 2-methylbutyrate, ethyl 2-methylbutyrate and propyl 2methylbutyrate had strong sweet and berry-like odors. These compounds were perceived less intensely than hexyl acetate and butyl acetate in RA stored fruit, but did not decrease as much in CA stored fruit. 4-Allylanisole, B-damascenone and 1-octen-3ol, as well as an unknown compound with a watermelon descriptor, were perceived in RA stored fruit more than CA stored apples; these compounds had high loadings on factor 1 in FA, indicating an important contribution to aroma of 'Gala' apples stored 4 weeks in RA in 1995-96. Even though these compounds do not have an apple odor, they may act synergistically or antagonistically when present with the fruity esters .

# **INTRODUCTION**

'Gala' apple originated in New Zealand in the early 1960s from a cross between 'Kidd's Orange' ('Cox's Orange Pippin' X 'Delicious') and 'Golden Delicious' (White, 1991). This apple has become popular for its distinct flavor and taste. Consumer taste panels have almost always given 'Gala' high to very high ratings on a 9-point hedonic scale (Stebbins *et al.*, 1994). Stebbins and co-workers (1994) also noted a loss of flavor after 60 days in regular air storage (RA), and hedonic ratings were low when apples were tasted after 4 months in air (Plotto *et al.*, 1995).

Controlled atmosphere storage (CA) is a common practice to prolong apple shelf-life. Reduced O2 and increased CO2 levels minimize firmness, acidity and chlorophyll losses as well as delay the appearance of some storage disorders in apples (Smock, 1979). However, reduced production of volatile compounds is also usually observed following CA storage (Guadagni et al., 1971; Patterson et al., 1974; Girard and Lau, 1995; Streif and Bangerth, 1988; Yahia et al., 1990). Apple maturity stage at harvest (Dirinck et al., 1989; Mattheis et al., 1991; Girard and Lau, 1995; Song and Bangerth, 1996), ratio of O<sub>2</sub> and CO<sub>2</sub> in the atmosphere (Streif and Bangerth, 1988; Brackmann et al., 1993; Fellman et al., 1993; Hansen et al., 1992) as well as storage duration (Willaert et al., 1983) affect the recovery of volatile production after CA storage. Lower O<sub>2</sub> concentration in storage resulted in lower volatile production and longer recovery time (Hansen et al., 1992; Streif and Bangerth, 1988; Mattheis et al., 1998a). By raising oxygen levels to 2% (Smith, 1984) or to 21% (Streif and Bangerth, 1988; Brackmann et al., 1993) after a 1.25% or 3% O2 storage, respectively, apples were able to produce more volatiles than had they remained under low O2 during all the storage period. However, the amount produced was less than for air stored fruit. Nevertheless, panelists could detect an increase in aromaticity of 'Cox's Orange Pippin' when these apples were stored in higher O<sub>2</sub> atmosphere (Smith, 1984). In similar experiments with 'Bisbee Delicious' (Mattheis et al., 1995) or with 'McIntosh' apples (Yahia, 1991), no such volatile increase was observed.

Odor-active volatiles play a significant role in flavor perception. By using regression analysis to correlate sensory data with instrumental measurements, Watada et al. (1981) found some volatile compounds partly explained the variation in sweetness and acidity descriptors in 'Golden Delicious' and 'York Imperial' apples. Williams and Knee (1977) reported direct correlations between the 'Cox's Orange Pippin' apple character and hexyl acetate and butyl acetate, and between the banana-like attribute and 2- or 3-methylbutyl acetate when apples were rated by expert panelists, and volatile compounds measured after removal from CA storage. Esters with a molecular weight between 100 and 130 were necessary for apple aroma (Dimick and Hoskin, 1983). Moreover, when using gas chromatography combined with olfactometry (GCO), other classes of compounds and also unknown compounds produced by apples had significant odor activity (Flath et al., 1967; Williams et al., 1977a; Cunningham et al., 1986). Flath et al. (1967) reported green apple descriptors for hexanal and trans-2-hexenal, 4methoxyallylbenzene was found to impart a spicy note to the 'Cox's Orange Pippin' apple (Williams et al., 1977a), while B-damascenone had an intense odor activity in CharmAnalysis (Cunningham et al., 1986). 2-Methylbutyl acetate, butyl acetate, hexyl acetate and butanol were found to be important aroma contributors to 'Gala' aroma using CharmAnalysis (Young et al., 1996). However, that study analyzed 'Gala' essence, which can result in higher proportions of alcohols and less esters (Paillard, 1990; Kakiuchi et al., 1986).

The objective of the present study was to identify volatile compounds contributing to the aroma of 'Gala' apples and evaluate the impact of CA storage on aroma production. A combination of instrumental and sensory analyses were used. *Osme*, a gas chromatography-olfactometry technique based on the modern laws of psychophysics, measures panelists' response to odorants on a time-intensity scale (McDaniel *et al.*, 1990). *Osme* has been shown to be a reproducible method with trained panelists (Da Silva *et al.*, 1994), and data can be analyzed using parametric statistical techniques (Da Silva *et al.*, 1993). Optimization of 'Gala' headspace sampling for the *Osme* method was presented in an earlier paper (Chapter 3). Forty-four compounds were odor-active in 'Gala' apples stored in air at 1 °C for 4 weeks. In the present study, changes in production of volatile compounds and perception of odor-active peaks over five months in RA and CA storage were quantified. Data were analyzed using univariate statistics with analysis of variance (ANOVA). Multivariate factor analysis (FA) was then used to establish relationships between odor-active peaks and the impacts of storage treatments.

# **MATERIALS AND METHODS**

## **Plant Material and Storage**

A preliminary study was conducted using 'Gala' apples harvested in a commercial orchard near Chelan, WA, on September 12, 1994. Apple maturity stage and homogeneity of the lots was assessed through the ground color; ground color was found earlier to be a good indicator of 'Gala' maturity (Plotto *et al.*, 1995). No preharvest or pre-storage chemical treatment was applied to the fruit. Fruit was stored at the USDA-ARS Tree Fruit Research Laboratory in Wenatchee, WA, at 1 °C for 5, 10 and 19 weeks in regular atmosphere (RA) and 9 and 18 weeks in controlled atmosphere (CA) with  $O_2$  and  $CO_2$  at 1% and 1%, respectively. After removal from storage, fruit was shipped to Corvallis, OR, and ripened at room temperature for 5 days prior to volatile collection. In 1995, 'Gala' apples from the same orchard were harvested on September 12 using the same maturity criteria as in 1994. Fruit was stored for 4, 10 and 20 weeks at 1 °C in either RA or CA. In 1995-96, one additional storage treatment was 16 weeks in CA followed by 4 weeks in RA. After removal from storage, apples were shipped to Corvallis and stored at 2 °C for 5 days upon receipt. Apples were then ripened at 22 °C for 5 days prior to volatile collection.

# **Headspace Sampling**

Four replicate samples (five apples each, ca. 1 kg) were placed in 4 L glass jars sealed using Teflon lids with two gas ports. Compressed air purified by flowing through activated charcoal, calcium hydroxide and 5 Å molecular sieve (W.A. Hammond

Drierite, Xenia, OH) was passed through the jars at ca. 200 mL·min<sup>-1</sup>. Volatiles were collected on activated coconut charcoal (20/40 mesh, 150 mg, ORBO-32, Supelco, Bellefonte, PA) for 24 hours. Sampling took place in a ripening chamber maintained at 22 °C. Traps were stored at -25 °C until elution. Volatile compounds were desorbed from charcoal with 300  $\mu$ L CS<sub>2</sub> (HPLC grade, 99.9%+, Sigma-Aldrich, St. Louis, MO) containing 100 mg·L<sup>-1</sup> tridecane (Sigma, St. Louis, MO) as an internal standard. CS<sub>2</sub> was poured onto the charcoal particles in 1.8 mL vials, then samples were ready for analysis. Throughout the study, samples (sorbent and solvent for charcoal) were stored at -17 °C.

# Gas Chromatography - Olfactometry

Samples were analyzed using an HP 5890 (Hewlett Packard, Wilmington, DE) gas chromatograph equipped with a 3-way valve (Valco Instruments Co., Inc., Houston, TX) to direct the column flow to either a flame-ionization detector or a sniff port. The analytical column was a Rtx-5 fused silica column coated with crossbond 5% diphenyl -95% dimethyl polysiloxane, 30 m, 0.53 mm i.d., 1-µm film thickness (Restek, Bellefonte, PA). Conditions for chromatography were as follows: splitless injection at 250 °C, initial oven temperature, 40 °C held for 1 min, increased to 165 °C at 5 °C·min<sup>-1</sup>, then to 250 °C at 20 °C min<sup>-1</sup>, held for 15 min. FID was at 280 °C; H<sub>2</sub>, air and auxilary gas (He) to FID were 30, 390 and 27 mL·min<sup>-1</sup>, respectively. Linear velocity of He carrier gas was 30.7 cm·sec<sup>-1</sup>. The sniff port was a 40 cm long, 4 mm diameter glass tubing deactivated with 5% dimethyldichlorosilane (Sylon-CT, Supelco) connected with a tee to the outlet of the GC column. Compressed air (breathing quality) was purified and humidified by flowing to the sniff port at 3.5 L·min<sup>-1</sup> (or 4.64 m·sec<sup>-1</sup>) through activated charcoal, 5 Å molecular sieve and 2 L distilled water held at 30 °C. Three panelists were trained to smell and describe the column effluents while rating the perceived intensity on a 16-point intensity scale (0 = none, 15 = extreme). Panelists recorded intensity by moving a linear sliding bar connected to a variable resistor interfaced to a personal computer (Da Silva et al., 1994). The procedure for panelist training was as described (Chapter 3). Each sniffing session started after the solvent had completely eluted on the
column and lasted 30 min. Response duration and intensity for individual compounds were recorded with *Osme* v.1.0 for Windows 3.1 software developed at Oregon State University. The resulting output for each response was: a) duration of odor perception, b) maximum odor intensity  $(I_{max})$ , c) area under the curve generated by the odor stimulus response (duration × intensity), and d) retention index (Kovats) at the time of maximum perceived intensity. Kovats indices were calculated after GC analysis of a series of hydrocarbon standards under the same conditions as the volatile samples.

Initial identification of the compounds was made by running the samples under similar analytical conditions on a HP 5890 series II gas chromatograph (Hewlett Packard, Wilmington, DE) equipped with a HP 5971a MS detector (Hewlett Packard, Palo Alto, CA) and a DB-5, 30 m, 0.25 mm i.d., 0.25-µm film thickness capillary column (J&W Scientific, Folsom, CA). Spectra of individual compounds were compared with those in the Wiley/NBS library (1991). Confirmation of identification was made by 1) comparing retention indices of authentic standards (Aldrich Flavors and Fragrances, Milwaukee, WI) and 2) *Osme* evaluation of those standards in the same quantities as in the sample. If the odor of a standard was different from the odor of the sample peak, the compound was not retained for that peak odor identification; even though it was identified by the Wiley library and had the same Kovats index as the sample peak. All standards used for olfactometry were food grade.

## Statistical Design and Analysis

In 1994-95, headspace was initially analyzed for volatile quantity. For each storage treatment, the sample of the four replicates with the median amount of volatiles was chosen for further *Osme* analysis. That sample was evaluated four times by all three panelists. This design measured panelist variation.

The following year, each panelist evaluated once each of the four replicate samples for each storage treatment. This design evaluated apple variability; panelist variability was included in the experimental error. Volatile quantity was measured later. Samples were presented completely randomized within a block, where block was sample replication. Three panelists participated in the testing each year, two of them participated in both years of the study.

For both years, storage type (RA or CA) and duration were treated independently and will be referred to as "storage treatment" in further discussions. Differences between storage treatments were analyzed for each compound quantity using a one-way ANOVA, with storage as the main effect.

For each set of peak odor intensity  $(I_{max})$  or peak area response variables, differences between storage treatments were analyzed using a 4-way ANOVA. For the 1994-95 data-set, the model was: *Response Variable = Storage Panelist Storage\*Panelist Replication Storage\*Replication Panelist\*Replication*, where replication was the panelist evaluation of the sample. The error term in the denominator for the F-ratio and used for the LSD test for means separation was (*Storage\*Panelist*). For the 1995-96 data-set, sample replicate was nested within storage treatment [*Sample(Storage)*] because panelists evaluated each sample only once. The model was: *Response Variable = Storage Sample(Storage) Panelist Panelist\*Storage. Storage* and *Panelist* were treated as fixed effects while *Sample* and therefore *Sample(Storage)* was treated as a random effect. *Sample(Storage)* was used as the error term for the Fratio; by doing so, apple variability was tested, and panelist was included in the experimental error.

Data were also examined using multivariate analysis. Factor analysis (FA) was performed on the FID relative peak area of identified odor-active volatile compounds, on the peak intensities  $(I_{max})$  and peak areas of odors perceived by *Osme*.

For FID peak areas, FA using the principal component method was performed on the data correlation matrix to account for differences in peak scaling (Johnson and Wichern, 1992).

For each set of *Osme* peak intensity and peak area variables, FA using principal component method was performed on the covariance matrix of the residuals of a general linear model (GLM) where panelist was the main effect. The GLM residuals were used to remove the variability due to panelists using different parts of the scale (Piggott and

Sharman, 1986). It was then possible to use the residuals covariance matrix to maximize differences between storage treatments. Based on the eigenvalues and the scree tests for each analysis (Tabachnick and Fidel, 1989), two factors were extracted from the principal component initial analysis, and rotated with the Varimax method. The orthogonal rotation Varimax maximizes high and minimizes low correlations, and maximizes the variance explained by the new factors (Tabachnick and Fidel, 1989). It was performed to determine how peaks correlated with each other. The plots of storage treatments in the two-factor coordinate system allowed determination of the direction variation due to peak intensities (or peak area, or FID area) pulled the storage factor scores. In other words, the graphical representation allowed compounds with the most weight in specific storage treatments to be identified.

All statistical analyses were performed using SAS statistical software v.6.12 (SAS Institute Inc., Cary, NC).

## **RESULTS AND DISCUSSION**

## **Volatile Production in Storage**

In 1995-96, 'Gala' apples emitted the largest amount of volatiles after storage in RA for 4 weeks (Table 5.1). Although apples used in this study were post-climacteric at harvest (ethylene production above 1 ppm – Table A.1), they kept ripening and may have reached the highest point of the respiration climacteric after 4 weeks in storage. Brackmann and Streif (1994) noted that higher volatile production was observed from apples stored 10 to 60 days in RA than from apples sampled at harvest. Fellman and Mattheis (1995) observed a close association between ester formation and climacteric status. A decrease in volatile production for most compounds was observed after 10 weeks in RA and then the amount increased again after 20 weeks in RA (Table 5.1). This result is contradictory with other findings where less volatiles were produced after the longest time in RA (Streif and Bangerth, 1988; Fellman and Mattheis, 1995; Mattheis *et al.*, 1998a). Our results could be due to experimental error, or the apples produced more volatiles at later stages of senescence because the same trend was observed in

1 kg apples <sup>z</sup> . To	otal volatiles by chemic	cal group are a	lso presented	epicates of uy	mannic neadsp	ace of			
			Storage T	уре					
		RA				СА			
-		Storage Duration (Weeks)							
Compound	4	10	20	10	16 <sup>w</sup>	20			
Propyl acetate	409.0 <sup>a</sup>	261.4 <sup>a</sup>	271.0 <sup>a</sup>	53.8 b	26.0 b	20 26 <sup>b</sup>			
Butyl acetate <sup>x,y</sup>	3863.2 <sup>a</sup>	4001.2 <sup>a</sup>	4258 0 <sup>a</sup>	1076 2 b	600 0 bc	2.0 71.0 <sup>c</sup>			
Pentyl acetate <sup>x</sup>	353 1 <sup>a</sup>	193.4 bc	$261.8^{ab}$	1370.2	009.9	/1.9 <sup>-</sup>			
Hexyl acetate <sup>x,y</sup>	2032 8 <sup>a</sup>	2520.1 ab	201.0 2725 0 ab	120.2	45.3	10.9 °			
Hentyl acetate	2752.0 (5.0 <sup>a</sup>	2330.1	2125.0	1758.7	662.9 °	141.6 °			
Total actate		23.0 °		23.3 °	9.3 <sup>bc</sup>	2.6 °			
i otal acetate esters	7624.0	7009.1	7540.3	3940.2	1354.3	229.6			

Table 5.1. Volatile compounds emitted by 'Gala' apples after regular (RA) or controlled atmosphere (CA) storage(1% O2, 1% CO2) in 1995-96. Values (ng.kg<sup>-1</sup>.L<sup>-1</sup>) are means of 4 replicates of dynamic headspace of

Propyl propanoate <sup>x</sup>	34.1 <sup>a</sup>	18.0 <sup>b</sup>	14 4 bc	4 2 bc	20°	0.0 °
Butyl propanoate <sup>x</sup>	691.0 <sup>a</sup>	428.7 <sup>a</sup>	533.9 <sup>a</sup>	100 3 <sup>b</sup>	2.9 41.3 <sup>b</sup>	0.8
Pentyl propanoate	22.2 <sup>a</sup>	91 <sup>bc</sup>	13.6 <sup>b</sup>	100.5	41.5	4.2 °
Hexyl propanoate <sup>x</sup>	536.5 <sup>a</sup>	264.2 bc	332.5 <sup>b</sup>	125 7 <sup>cd</sup>	4.7 57.6 <sup>d</sup>	0.9 *
Total propanoate esters	1283.9	720.0	894.5	241.0		9.0 14.9
Propyl butyrate	39.5 <sup>a</sup>	17.3 <sup>bc</sup>	22.5 <sup>b</sup>	10.4 bcd	g n cd	1 1 d
Butyl butyrate <sup>x</sup>	536.0 <sup>a</sup>	255.9 <sup>b</sup>	483.6 <sup>a</sup>	153.1 <sup>bc</sup>	50.2 °	1.1 11.5 °
Pentyl butyrate	$20.8^{a}$	10.8 bc	15.3 abc	133.1 32.6 <sup>a</sup>	39.2	11.5
Hexyl butyrate <sup>x</sup>	428.7 <sup>ab</sup>	327.6 <sup>b</sup>	568 7 <sup>a</sup>	25.0		7.2 °
Total butyrate esters	1025.0	611.5	1090.1	441.9	108.9 <b>193.8</b>	<u> </u>
Propyl hexanoate	179.1 <sup>a</sup>	40.8 <sup>bc</sup>	54.2 <sup>b</sup>	26.7 <sup>bc</sup>	15 0 <sup>bc</sup>	15 <sup>c</sup>
Butyl hexanoate <sup>x</sup>	1313.2 <sup>a</sup>	631.9 <sup>b</sup>	1063 9 <sup>a</sup>	526.7 bc	725 5 cd	4.5
Hexyl hexanoate	1605.7 <sup>a</sup>	277 1 °	500 9 <sup>b</sup>	214 1 cd	233.5 109.0 <sup>cd</sup>	03.0
Total hexanoate esters	3098.0	949.8	1619.0		359.4	32.8 <sup>-</sup>
Butyl heptanoate	134.8 <sup>a</sup>	27.4 <sup>bc</sup>	42.8 <sup>b</sup>	30.6 <sup>bc</sup>	14.6 <sup>bc</sup>	4.7 °

Propyl propanoate<sup>x</sup>

# Table 5.1, Continued

Hexyl octanoate	63.3 <sup>a</sup>	14.6 bc	20.6 <sup>b</sup>	9.9 bcd	4.6 <sup>cd</sup>	1.4 <sup>d</sup>
2-Methylpropyl acetate	99.3 <sup>b</sup>	75.6 <sup>bc</sup>	173 3 <sup>a</sup>	1113 <sup>b</sup>	710 <sup>bc</sup>	25 5 <sup>c</sup>
2-Methylbutyl acetate <sup>x,y</sup>	2099.4 <sup>a</sup>	1089.0 bc	1456.2 <sup>ab</sup>	1974 4 <sup>a</sup>	1432 8 <sup>ab</sup>	23.5 ۲ م م
3-Methyl-2-butenyl acetate <sup>x</sup>	45.0 <sup>b</sup>	10.0 °	9.2 °	$110.2^{a}$	122.0 <sup>a</sup>	437.7 20.9 b
3-Methylbutyl propanoate	25.8 <sup>a</sup>	6.8 <sup>cd</sup>	7.4 <sup>cd</sup>	19.8 <sup>ab</sup>	$14.0^{bc}$	39.0 31 <sup>d</sup>
2-Methylbutyl butyrate	8.7 <sup>ab</sup>	5.0 <sup>bc</sup>	7.1 <sup>ab</sup>	10.3 <sup>a</sup>	75 <sup>ab</sup>	2.1 2.0°
3-Methylbutyl hexanoate	22.5 <sup>a</sup>	4.7 <sup>c</sup>	7.0 <sup>c</sup>	16.4 <sup>b</sup>	14.5 <sup>b</sup>	5.3 °
Butyl 2-methylpropanoate	13.7 <sup>b</sup>	12.0 <sup>b</sup>	23.4 <sup>a</sup>	5.6 <sup>bc</sup>	2.5 °	02°
Hexyl 2-methylpropanoate	25.0 <sup>ab</sup>	18.9 <sup>bc</sup>	40.7 <sup>a</sup>	15.4 bcd	7.0 <sup>cd</sup>	$0.3^{d}$
Total methyl propanoate esters	38.7	30.9	64.1	21.0	9.4	0.5
Methyl 2-methylbutyrate <sup>x</sup>	59.7 <sup>b</sup>	45.9 <sup>bc</sup>	100.0 <sup>a</sup>	10.7 <sup>d</sup>	18.5 <sup>cd</sup>	0 0 <sup>d</sup>
Ethyl 2-methylbutyrate <sup>x</sup>	4.7 <sup>ab</sup>	3.1 <sup>bc</sup>	7.6 <sup>a</sup>	0.9 <sup>c</sup>	0.1 °	0.0 °
Propyl 2-methylbutyrate <sup>x</sup>	113.5 <sup>a</sup>	55.8 <sup>b</sup>	46.2 <sup>bc</sup>	12.4 bcd	9.7 <sup>cd</sup>	0.0 0.2 <sup>d</sup>
Butyl 2-methylbutyrate <sup>x</sup>	724.1 <sup>a</sup>	528.0 <sup>a</sup>	730.4 <sup>a</sup>	158.0 <sup>b</sup>	62.0 <sup>b</sup>	6.0 <sup>b</sup>
Hexyl 2-methylbutyrate <sup>x</sup>	872.8 <sup>a</sup>	477.4 <sup>bc</sup>	768.3 <sup>ab</sup>	156.2 <sup>cd</sup>	45.5 <sup>d</sup>	10.7 <sup>d</sup>
Total methyl butyrate esters	1774.8	1110.2	1652.4	338.0	135.8	17.0
Hexyl tiglate <sup>x</sup>	10.0 <sup>a</sup>	1.5 <sup>b</sup>	1.5 <sup>b</sup>	1.2 <sup>b</sup>	2.2 <sup>b</sup>	0.2 <sup>b</sup>
6-Methyl-5-hepten-2-one <sup>x</sup>	86.8 <sup>a</sup>	12.5 <sup>b</sup>	9.8 <sup>b</sup>	4.8 <sup>b</sup>	2.6 <sup>b</sup>	0.0 <sup>b</sup>
4-Allylanisole <sup>x</sup>	216.2 <sup>a</sup>	35.9 <sup>b</sup>	48.4 <sup>b</sup>	43.8 <sup>b</sup>	24.6 <sup>b</sup>	9.2 <sup>b</sup>
1-Butanol	211.4 <sup>a</sup>	57.0 <sup>bc</sup>	119.5 <sup>b</sup>	115°	10.8 °	81 <sup>c</sup>
1-Pentanol	4.5 <sup>a</sup>	1.3 <sup>bc</sup>	3.9 <sup>ab</sup>	1.5 bc	13 <sup>bc</sup>	0.1 0 9 °
1-Hexanol	88.2 <sup>a</sup>	28.3 <sup>b</sup>	78.1 <sup>a</sup>	16.8 <sup>b</sup>	6.0 <sup>b</sup>	17 <sup>b</sup>
2 Methyl-1-butanol	78.6 <sup>a</sup>	27.0 <sup>bc</sup>	48.1 <sup>b</sup>	49.1 <sup>ab</sup>	35.5 bc	10.8 °
Total alcohols	382.7	113.5	249.6	78.9	53.5	21.4

<sup>2</sup> Means followed by the same letter are not significantly different within the same line by the Fisher protected LSD test, alpha = 0.05<sup>9</sup> Above the linear range of the FID detection <sup>\*</sup> Odor active compounds at those concentrations <sup>w</sup> 16 weeks in CA was followed by 4 weeks in RA

1994-95 (Table A.2). Fellman (1997) observed a correlation between fruit softening and increasing ester synthesis in some apple cultivars, speculating that some glycosidically bound volatile compounds might be liberated during cell wall degradation. Dettweiler *et al.* (1990) suggested that the formation of volatile compounds as well as conjugation of hydroxy compounds to glycosides was similar to a detoxification process during cell senescence. Those authors found an increasing amount of free and bound C<sub>8</sub> diols during the course of ripening of 'Purpurroter Cousinot' apples. Straight-chain esters are products of lipid degradation (Paillard, 1979); fatty acids oxidized during senescence may be the source of substrates for ester formation.

Overall, total volatile production decreased during CA storage in both 1994-95 and 1995-96 (Table 5.1 and Table A.2). In 1995-96, 'Gala' apples exposed to air for 4 weeks after 16 weeks in CA storage produced more volatiles than fruit stored 20 weeks in CA, without reaching the level produced after 10 weeks in CA (Table 5.1). A similar effect was reported in earlier work with 'Cox's Orange Pippin' (Smith, 1984), and 'Golden Delicious' (Streif and Bangerth, 1988) after exposing apples to higher O<sub>2</sub> levels after CA storage.

Acetate esters comprised the largest proportion of volatiles emitted by 'Gala' apples; butyl acetate, hexyl acetate and 2-methylbutyl acetate were produced in the largest amounts (Table 5.1). While all straight-chain esters showed a significant drop in production after CA storage, 2-methylbutyl acetate was still produced in high amounts after 16 weeks in CA (Table 5.1). Similar results were observed by Mattheis *et al.* (1998a) for 'Gala' apples. Production of straight-chain esters decreased more than branched-chain esters under low  $O_2$ , and branched-chain esters decreased under high levels of  $CO_2$  (Brackmann *et al.*, 1993). Both straight and branched-chain esters decreased under high acetate and 2-methyl-1-butanol (Table 5.1).

3-Methyl-2-butenyl acetate was the only ester increasing significantly during CA storage, especially after 10 weeks CA or 16 weeks in CA followed by 4 weeks in RA (Table 5.1). This unsaturated ester and its different behavior in CA storage has also been reported in 'Jonagold' apples (Hansen *et al.*, 1990).

Other non-ester compounds detected included several alcohols, one allyl phenol and one ketone. With the exception of 2-methyl-1-butanol, all these compounds significantly decreased in both storage atmospheres after 10 weeks (Table 5.1).

In 1994-95, volatile production after 19 weeks in RA was significantly higher compared to fruit stored in CA for most compounds (Table A.2). Unlike 1995-96, total volatile emission was similar after 5 and 10 weeks in RA. Similar to results in 1995-96, volatile production decreased after CA storage.

#### **Volatile Perception After Storage**

Osme results are presented for peak height or maximum peak intensity  $(I_{max})$  as panelists were trained to use the scale for that measurement, and peak area results are presented in appendices (Tables A.3 and A.4). Peak aroma intensity gives an indication of compound potency in the samples (Da Silva *et al.*, 1993). However, the area under the curve gives additional information about the psychophysical response as it integrates the duration of perceived odor activity.

Panelist variability was significant both years for most compounds (data not shown). Regression curves relating perceived intensity or peak area to odorant concentrations showed differences in panelist sensitivities for some compounds, and different responses to increasing concentrations (Appendix 5, Figures A.1 and A.2). Nevertheless, panelists were consistent in rating concentration changes with storage treatments, resulting in little or no significant panelist by treatment interaction.

Compounds with fruity odors were esters and one ketone (Table 5.2). The decrease in ester production following CA storage was quantified: 1) by GC-FID analysis; and 2) as reduced perception of fruity odors perceived using *Osme*. CA storage resulted in a loss of fruity peaks, with different amounts of loss of perceived intensities.

Hexyl acetate, butyl acetate and 2-methylbutyl acetate, emitted by 'Gala' apples in the largest amounts of all compounds detected, were also perceived with the highest intensities until 10 weeks in CA storage (Table 5.2). While hexyl acetate and butyl

Table 5.2.	Peak aroma intensity ( $I_{max}$ Osme analysis in 1995-96. replicates for 3 panelists <sup>2</sup> .	the control of the termination of te
	replicates for 3 panelists <sup>2</sup> .	

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					Storage T	уре		
	<b>T</b> Z (1	v		RA			CA	
<b>D</b> • "	Kovats			Sto	rage Duratio	n (Weeks)		
Peak #	Index	Descriptor (Compound)	4	10	20	10	16 <sup>x</sup>	20
20	1028	Gala, ripe, pear (Hexyl acetate)	11.4 <sup>a</sup>	9.3 6	10.6 <sup>ab</sup>	9.3 <sup>b</sup>	4.8 <sup>c</sup>	3.9 °
4	827	Nail polish, gala (Butyl acetate)	10.3 <sup>a</sup>	8.9 <sup>a</sup>	9.6 <sup>a</sup>	8.1 <sup>ab</sup>	5.2 °	1.7 °
7	890	Solvent, gala (2-Methylbutyl acetate)	11.0 <sup>a</sup>	8.6 <sup>c</sup>	8.4 <sup>cd</sup>	10.4 <sup>ab</sup>	9.2 bc	6 9 <sup>d</sup>
2	787	Sweet, fruity (Methyl 2-methylbutyrate)	8.9 <sup>a</sup>	8.0 <sup>a</sup>	7.9 <sup>a</sup>	7.3 <sup>a</sup>	7.2 <sup>a</sup>	126
13	959	Very sweet, strawberry (Propyl 2-methylbutyrate)	6.8 <sup>a</sup>	6.8 <sup>a</sup>	5.7 <sup>a</sup>	65 <sup>a</sup>	57 <sup>a</sup>	7.2 7.2 <sup>b</sup>
6	863	Sweet, strawberry (Ethyl 2-methylbutyrate)	6.2 <sup>ab</sup>	6.7 <sup>ab</sup>	7.4 <sup>a</sup>	6.1 <sup>ab</sup>	49 <sup>b</sup>	2.5 27°
23	1056	Fruity, apple (Butyl 2-methylbutyrate)	5.6 <sup>a</sup>	3.7 <sup>b</sup>	5.3 <sup>a</sup>	2.6 <sup>b</sup>	4.9 1.0 °	2.7 0.2 °
36	1255	Apple, grapefruit (Hexyl 2-methylbutyrate)	5.1 <sup>a</sup>	2.4 bc	3.8 <sup>ab</sup>	1.3 <sup>cd</sup>	0.0 <sup>cd</sup>	0.2
31	1205	Green apple (Butyl hexanoate + hexyl butyrate)	4.7 <sup>ab</sup>	2.8 <sup>b</sup>	63 <sup>a</sup>	3.4 <sup>b</sup>	0.9 °	0.2 0.0 °
19	1018	Apple and toast (Unknown)	7.0 <sup>a</sup>	30 <sup>b</sup>	2.2 <sup>bc</sup>	2 0 <sup>bc</sup>	2.5 <sup>bc</sup>	0.0
16	997	Fruity, tape (6-Methyl-5-hepten-2-one)	3.3 <sup>a</sup>	10 <sup>b</sup>	0.3 <sup>b</sup>	0.3 <sup>b</sup>	2.5 0.6 <sup>b</sup>	0.5
18	1010	Solvent, gala (Unknown)	31 <sup>a</sup>	0.4 <sup>cd</sup>	3.1 <sup>a</sup>	176	1.2 bc	0.5 0.0 <sup>d</sup>
27	1121	Apple (Hexyl propanoate)	3.0 <sup>a</sup>	1.3 <sup>abc</sup>	2.1	0.5 <sup>bc</sup>	1.5 0.0 °	0.0
17	1009	Rotten apple (Butyl butyrate)	23 <sup>ab</sup>	7.3 ab	$2.0^{a}$	0.5	0.0	0.0
44	1524	Fruity (Unknown)	$1.6^{a}$	1.0 <sup>a</sup>	1.2 <sup>ab</sup>	1.5	0.0	0.0
10	921	Fruity, apple (Butyl propanoate)	1.0 <sup>a</sup>	1.9 1.7 <sup>a</sup>		0.7 0.0 <sup>b</sup>	0.0	0.0
3	813	Fruity (Propyl propanoate)	0.0 <sup>b</sup>		O.7	0.0	0.0	0.0 °
12	933	Fruity, sweet, solvent (3-Methyl-2-butenyl acetate)	0.0 °	0.2	0.8	1.4	0.6	0.4 **
••••••		Total fruity	0.4	0.0	0.6	5.2	4.8 -	2.7 °
			91.0	69.1	78.3	63.1	44.4	20.3
43	1437	Grape juice (B-Damascenone)	6.2 <sup>a</sup>	2.2 <sup>bc</sup>	2.4 <sup>b</sup>	2.2. <sup>bc</sup>	14 <sup>c</sup>	0.0 d
38	1276	Grape juice (Unknown)	1.7 <sup>a</sup>	0.0 <sup>b</sup>	0.5 <sup>b</sup>	0.0 <sup>b</sup>	006	0.0
		Total grapejuice	7.9	2.2	2.9	2.2	1.4	0.0

# Table 5.2, Continued

	1118	Floral (Unknown)	<b>1.9</b> <sup>a</sup>	<b>0.0</b> <sup>b</sup>	<b>0.0</b> <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>
24	1070	Watermelon (Unknown)	6.9 <sup>a</sup>	1.0 <sup>b</sup>	0.0 <sup>c</sup>	0.0 °	0.0 °	0.1 <sup>c</sup>
	1227	Cucumber (Unknown)	2.2 <sup>a</sup>	0.4 <sup>b</sup>	0.8 <sup>b</sup>	0.0 <sup>b</sup>	0.0 *	0.1
		Total watermelon, cucumber	9.1	1.4	0.8	0.0	0.0	0.1
33	1222	Anise, licorice (4-Allylanisole)	7.6 <sup>a</sup>	3.0 <sup>bc</sup>	2.3 <sup>bcd</sup>	3.5 <sup>b</sup>	1.0 <sup>cd</sup>	0 5 <sup>cd</sup>
22	1041	Sweet, anise (Unknown)	1.3 <sup>ab</sup>	0.0 <sup>c</sup>	0.0 <sup>c</sup>	1.6 <sup>a</sup>	0.3 bc	0.0°
	1151	Anise, spice, perfumey (Unknown)	2.6 <sup>a</sup>	0.6 <sup>bc</sup>	0.0 <sup>c</sup>	2.1 <sup>ab</sup>	0.4 <sup>c</sup>	0.9 <sup>bc</sup>
		Total anise	11.5	3.7	2.3	7.2	1.7	1.4
35	1230	Mushroom, cat urine (Unknown)	5.2 <sup>ab</sup>	3.1 <sup>cd</sup>	6.9 <sup>a</sup>	4.6 bc	1.4 <sup>d</sup>	1.4 <sup>d</sup>
14	991	Mushroom (1-Octen-3-ol)	2.9 <sup>a</sup>	0.3 <sup>b</sup>	0.3 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>
		Total mushroom	8.1	3.4	7.2	4.6	1.4	1.4
5	836	Skunk (Unknown)	9.0 <sup>a</sup>	4.0 <sup>b</sup>	4.0 <sup>b</sup>	5.2 <sup>b</sup>	4.7 <sup>b</sup>	3.4 <sup>b</sup>
25	1112	Dusty, musty (Unknown)	6.4 <sup>a</sup>	5.3 <sup>a</sup>	3.8 <sup>a</sup>	4.3 <sup>a</sup>	2.4 <sup>b</sup>	3.9 <sup>a</sup>
37	1272	Rubber (Unknown)	5.4 <sup>a</sup>	5.2 <sup>a</sup>	1.6 bc	2.0 <sup>b</sup>	0.4 <sup>bc</sup>	0.0 °
8	901	Oatmeal, skunk (Unknown)	2.1	0.0	0.0	0.0	0.0	0.0
	1038	Metallic, skunk (Unknown)	1.6	0.0	0.0	0.0	0.0	0.0
		Total skunk, rubber	24.5	14.5	9.4	11.5	7.5	7.3
1	774	Tea, garlic, leaves (Unknown)	2.2	15	3.0	47	3.0	0.0
30	1197	Tape or fruity (Unknown)	$1.7^{a}$	0.0 6	0.0 6	4.7 0.2 <sup>b</sup>	3.0 0.0 <sup>b</sup>	0.8
41	1364	Tape or musty, dirty (Unknown)	1.6 <sup>b</sup>	0.5 <sup>bc</sup>	33 <sup>a</sup>	0.2 0.3 °	1.1 <sup>bc</sup>	0.7
		Total tape, others	5.5	2.1	6.4	5.3	<b>4.1</b>	<u> </u>
9	920	Garlic (Unknown)	0.7 <sup>b</sup>	0.0 <sup>b</sup>	0.3 <sup>b</sup>	<b>3.5</b> <sup>a</sup>	3.0 <sup>a</sup>	1.2 <sup>b</sup>

<sup>2</sup> Means followed by the same letter are not significantly different within the same line by the Fisher protected LSD test, alpha = 0.05<sup>y</sup> Kovats indices on RTX-5 (5% diphenyl 95% dimethyl polysiloxane) column

<sup>x</sup> 16 weeks in CA was followed by 4 weeks in RA

acetate odor intensities decreased after 10 weeks in CA, 2-methylbutyl acetate had the highest perceived intensity of all fruity compounds throughout CA storage.

While there was a drop in other fruity peak intensities after CA storage, methyl 2methylbutyrate, propyl 2-methylbutyrate and ethyl 2-methylbutyrate were still rated at or above 5.0 (slight to moderate) after 16 weeks in CA storage (Table 5.2). These three compounds along with hexyl acetate, butyl acetate, 2-methylbutyl acetate and 3-methyl-2-butenyl acetate were all rated above 1.5 (just detectable) after 20 weeks in CA, while other compounds were not perceived. 3-Methyl-2-butenyl acetate was the only ester that increased during CA compared to RA storage; its odor was also perceived higher after CA storage (Table 5.2).

A general decrease in the perceived intensity of the fruity peaks was also observed during 1994-95 for fruit stored in CA (Table 5.3). Odor-active compounds were the same as in 1995-96, except two fruity peaks were perceived in 1994-95 that were not perceived in 1995-96 (peak 36b and 39, unknown); also one peak, peak 19 (unknown), was perceived in 1995-96 and not perceived in 1994-95. Hexyl acetate and 2-methylbutyl acetate were still rated above 5.0 after 18 weeks in CA, while the intensity for butyl acetate decreased significantly to 1.9 (Table 5.3). Methyl-, propyl-, and ethyl 2-methylbutyrate were also rated above 2.0 after 18 weeks in CA; however, they were less potent as indicated by lower ratings for the RA treatments than hexyl acetate, butyl acetate and 2-methylbutyl acetate (average of 6.0 for the former versus 9.0 for the later) (Table 5.3).

Other compounds with grape juice, floral, watermelon, cucumber and anise odors, were perceived with a higher intensity after 4-weeks in RA (Table 5.2). βdamascenone (2,6,6-trimethyl-1-trans-crotonyl-1,3-cyclohexadiene) and 4-allylanisole (1-methoxy-4-(2-propenyl)-benzene) were responsible for the most important of the two and three grape juice and anise odors, respectively, and were rated between 2.0 and 3.0 until 10 weeks in CA. In the 1994-95 season, grape juice, watermelon and anise peaks were perceived with equal intensities for all RA treatments (Table 5.3).

Two mushroom-like peaks were perceived. The unknown peak 35 was perceived both years, and was rated with a higher intensity in 1995-96. Peak 14,

Table 5.3. Peak aroma intensity $(I_{max})$ in 'Gala' headspace after regular (RA) or controlle by <i>Osme</i> analysis in 1994-95. Values on a 16-point intensity scale (0 = none, 15 4 replicates for 3 panelists <sup>z</sup> .	ed atmosphere (CA) storage 5 = extreme) are means of
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			······		Storage T	уре	
	<b>1</b> 7 ( )	,		RA		СА	
/	Kovats				Storage Durat	tion (Weeks)	
Peak #	Index	Descriptor (Compound)	5	10	19	9	18
20	1028	Gala, ripe, pear (Hexyl acetate)	10.4 <sup>a</sup>	11.3 <sup>a</sup>	11.2 <sup>a</sup>	8.8 <sup>b</sup>	5.9 °
4	827	Nail polish, gala (Butyl acetate)	9.4 <sup>a</sup>	10.0 <sup>a</sup>	8.8 <sup>ab</sup>	6.3 <sup>b</sup>	1.9 <sup>c</sup>
7	890	Solvent, gala (2-Methylbutyl acetate)	9.7 <sup>a</sup>	8.9 <sup>a</sup>	8.4 <sup>a</sup>	8.5 <sup>a</sup>	64 <sup>b</sup>
2	787	Sweet, fruity (Methyl 2-methylbutyrate)	6.2 <sup>a</sup>	6.0 <sup>a</sup>	5.5 <sup>a</sup>	5.1 <sup>a</sup>	27 <sup>b</sup>
13	959	Very sweet, strawberry (Propyl 2-methylbutyrate)	6.2 <sup>a</sup>	6.2 <sup>a</sup>	6.0 <sup>a</sup>	4.8 <sup>b</sup>	3.2 °
6	863	Sweet, strawberry (Ethyl 2-methylbutyrate)	6.2 <sup>a</sup>	6.1 <sup>a</sup>	6.1 <sup>a</sup>	3.8 <sup>b</sup>	3.6 <sup>b</sup>
23	1056	Fruity, apple (Butyl 2-methylbutyrate)	5.3 <sup>a</sup>	5.5 <sup>a</sup>	4.3 <sup>ab</sup>	2.3 <sup>bc</sup>	0.0 °
36	1255	Apple (Hexyl 2-methylbutyrate)	1.9 <sup>ab</sup>	4.5 <sup>a</sup>	1.9 <sup>ab</sup>	1.9 <sup>ab</sup>	0.0 0.4 <sup>b</sup>
36b	1260	Grapefruit (Unknown)	2.3	1.8	1.5	0.0	0.4
31	1205	Green apple (Butyl hexanoate + hexyl butyrate)	1.6 <sup>ab</sup>	4.6 <sup>a</sup>	4.4 <sup>a</sup>	1.6 <sup>ab</sup>	0.0 0.3 <sup>b</sup>
16	997	Fruity, tape (6-Methyl-5-hepten-2-one)	2.7	0.6	14	0.8	0.5
18	1010	Solvent, gala (Unknown)	2.6 <sup>ab</sup>	4.4 <sup>a</sup>	2.5 <sup>ab</sup>	0.0 0.6 <sup>b</sup>	0.4
27	1121	Apple (Hexyl propanoate)	4.4 <sup>a</sup>	4.3 <sup>a</sup>	$4.6^{a}$	0.0	0.2 0.2 <sup>b</sup>
17	1009	Rotten apple (Butyl butyrate)	5.2 <sup>a</sup>	2.8 <sup>ab</sup>	37 <sup>b</sup>	0.0	0.2 0.5 <sup>b</sup>
44	1524	Fruity (Unknown)	1.7	19	0.3	1.4	0.5
10	921	Fruity, apple (Butyl propanoate)	3.0 <sup>a</sup>	$31^{a}$	3.0 <sup>a</sup>	1.4 0.0 <sup>b</sup>	0.0
39	1290	Fruity (Unknown)	2.1	24	07	0.0	0.0
3	813	Fruity (Propyl propanoate)	$0.8^{ab}$	2.7 28 <sup>a</sup>	1.6 <sup>ab</sup>	2.2 0.0 <sup>b</sup>	0.9
12	933	Fruity, sweet, solvent (3-Methyl-2-butenyl acetate)	11	2.8	1.0	0.0	0.0
		Total fruity	82.6			1.U 49.6	1./
43	1437	Grape juice (B-Damascenone)	2.6	2.5	1.7	3.2	47.0 1.9

# Table 5.3, Continued

9	920	Garlic (Unknown)	0.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	3.7 <sup>a</sup>	1.5 <sup>a</sup>
		Total skunk, rubber	11.4	9.5	7.4	6.4	3.9
37	1272	Rubber (Unknown)	3.3 <sup>a</sup>	3.0 <sup>a</sup>	2.0 <sup>ab</sup>	0.0 <sup>b</sup>	0.4 <sup>b</sup>
5	836	Skunk (Unknown)	8.1 <sup>a</sup>	6.5 <sup>ab</sup>	5.4 <sup>b</sup>	6.4 <sup>ab</sup>	3.5 °
-	0.0.6	Total mushroom	9.0	6.2	4.7	3.1	0.3
40	1353	Mushroom (Hexyl tiglate)	2.6 <sup>a</sup>	3.4 <sup>a</sup>	1.9 <sup>a</sup>	2.1 <sup>a</sup>	0.0 <sup>b</sup>
35	1230	Mushroom, cat urine (Unknow)	4.3 <sup>a</sup>	2.9 <sup>ab</sup>	2.8 <sup>ab</sup>	1.0 <sup>b</sup>	0.3 <sup>b</sup>
		Total anise	9.4	8.4	10.6	4.6	3.4
22	1041	Perfumey, anise (Unknown)	3.4 <sup>a</sup>	3.9 <sup>a</sup>	4.1 <sup>a</sup>	0.5 <sup>b</sup>	0.0 <sup>b</sup>
33	1222	Anise, licorice (4-Allylanisole)	6.0 <sup>a</sup>	4.5 <sup>b</sup>	6.5 <sup>a</sup>	4.1 <sup>b</sup>	3.4 <sup>b</sup>
		Total watermelon, cucumber	6.9	8.7	6.7	0.9	0.0
34	1227	Cucumber (Unknown)	0.8 <sup>ab</sup>	2.4 <sup>a</sup>	1.5 <sup>ab</sup>	$0.9^{ab}$	0.0 <sup>b</sup>
24	1070	Watermelon (Unknown)	6.1 <sup>a</sup>	6.3 <sup>a</sup>	5.2 <sup>a</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>

<sup>2</sup> Means followed by the same letter are not significantly different within the same line by the Fisher protected LSD test, alpha = 0.05<sup>y</sup> Kovats indices on RTX-5 (5% diphenyl 95% dimethyl polysiloxane) column

perceived in 1995-96, was identified as 1-octen-3-ol by matching Kovats index and odor quality with standard (Table 5.2), while hexyl tiglate (peak 40) was perceived only in 1994-95 (Table 5.3). The total mushroom peak intensities were higher at the beginning of the storage season (4 weeks in RA) both years of the study (Table 5.2 and 5.3).

More skunk-, rubber-like peaks were perceived in 1995-96 than in 1994-95. Overall, they followed the same trends as the fruity peaks, higher for apples stored 4 weeks in RA in 1995-96, and higher throughout RA storage in 1994-95. The ratings given to peak 5 were very close for each storage treatment both years (Table 5.2 and Table 5.3). Two of the skunk-like peaks (peak 8 and 21) were perceived only in the 4 weeks RA stored fruit in 1995-96. Because of uncertainty as to the origin of these compounds, skunk- and rubber-like peaks were not included in the FA analysis of *Osme* peak intensities and peak areas.

Peaks with multiple descriptors (i.e. peaks 1, 30 and 41) and with low intensity ratings were below the odor threshold, and considered as noise in the aromagram (Chapter 3). These peaks were not perceived in 1994-95.

One compound with a garlic odor (peak 9) was perceived only from CA stored fruit both years of the study (Table 5.2 and 5.3). It did not correspond to any peak on the FID chromatogram.

## **Correlative Relationships Between Odor-Active Volatiles**

Factor analysis (FA) of FID peak areas in 1994-95 indicated factors 1 and 2 explained 78% and 10% of the total variation in the dataset, respectively (Figure 5.1-A). In 1995-96, 69% of the total variation was explained by factor 1, and 11% by factor 2 (Figure 5.2-A). The plots of factor scores for FID relative peak areas reflected the decrease of all odor-active compounds during CA storage except 3-methyl-2-butenyl acetate (peak 12) (Figures 5.1-A and 5.2-A). In the factor plots, each peak is represented by its vector: vector angles reflect peak correlations with each other, and the vector magnitude [which is actually the variable (peak) loading] reflects the relative contribution of the peak to each factor. In 1994-95, the 19 week RA treatment was Figure 5.1. 1994-95 factor plots of FID peak area (A), Osme peak intensity (B) and Osme peak area (C) of 'Gala' apples stored in regular (RA) and controlled atmosphere (CA) (1% O<sub>2</sub>, 1% CO<sub>2</sub>).

Scores for treatments are in the 2-factor space. 5 RA, 10 RA, 19 RA are 5, 10 and 19 weeks in RA, and 9 CA, 18 CA are 9 and 18 weeks in CA. Factor loadings are determined by the vector lengths for each peak. All vectors start at the origin. Their directions and magnitudes (loadings) are represented by the figure diamonds. Peak numbers: 2: methyl 2-methylbutyrate, 3: propyl propanoate, 4: butyl acetate, 6: ethyl 2-methylbutyrate, 7: 2-methylbutyl acetate, 9: unknown garlic, 10: butyl propanoate, 12: 3-methyl-2-butenyl acetate, 13: propyl 2-methylbutyrate, 16: 6-methyl-5-hepten-2-one, 17: butyl butyrate, 18: unknown solvent, 20: hexyl acetate, 23: butyl 2-methylbutyrate, 24: unknown watermelon, 27: hexyl propanoate, 29: unknown perfumey, anise, 31: butyl hexanoate + hexyl butyrate, 33: 4-allylanisole, 34: unknown cucumber, 35: unknown fruity, 40: hexyl tiglate, 43: ß-damascenone, 44: unknown fruity. Diamonds without numbers in the *Osme* factor plots represent odor-active peaks that contribute less significantly to the variation due to 'Gala' storage.

# Figure 5.2. 1995-96 factor plots of FID peak area (A), Osme peak intensity (B) and Osme peak area (C) of 'Gala' apples stored in regular (RA) and controlled atmosphere (CA) (1% O<sub>2</sub>, 1% CO<sub>2</sub>).

Scores for treatments are in the 2-factor space. 4 RA, 10 RA, 20 RA, 10 CA, 20 CA are 4, 10 and 20 weeks in RA and CA, respectively. 16 CA is 16 weeks in CA followed by 4 weeks in RA. Factor loadings are determined by the vector lengths for each peak. All vectors start at the origin. Their directions and magnitudes (loadings) are represented by the figure diamonds. Peak numbers: 1: unknown tea, leaves, 2: methyl 2-methylbutyrate, 3: propyl propanoate, 4: butyl acetate, 6: ethyl 2-methylbutyrate, 7: 2-methylbutyl acetate, 8: unknown oatmeal, 9: unknown garlic, 10: butyl propanoate, 12: 3-methyl-2-butenyl acetate, 13: propyl 2-methylbutyrate, 14: 1-octen-3-ol, 16: 6-methyl-5-hepten-2-one, 17: butyl butyrate, 18: unknown apple solvent, 20: hexyl acetate, 23: butyl 2methylbutyrate, 24: unknown watermelon, 26: unknown floral, 27: hexyl propanoate, 29: unknown perfumey, anise, 31: butyl hexanoate + hexyl butyrate, 33: 4-allylanisole, 35: unknown mushroom, 36: hexyl 2-methylbutyrate, 43: ßdamascenone. Diamonds without numbers in the Osme factor plots represent odor-active peaks that contribute less significantly to the variation due to 'Gala' storage.







characterized by the vectors with high loadings for 4-allylanisole (peak 33), and all the branched-chain esters (Figure 5.1-A). The 5 and 10 week RA treatments had high positive scores for factor 2, characterized by the vectors for straight-chain esters. The vector directions for branched-chain esters and 4-allylanisole indicates those compounds were correlated with each other and decreased at a similar rate in storage. Likewise, straight-chain esters decreased at similar rates in storage, but the rates were different from those for branched-chain esters. Hexyl acetate (peak 20), butyl acetate (peak 4) and 6-methyl-5-hepten-2-one (peak 16) had the highest loadings on factor 2 indicating high levels of those compounds in 5 weeks RA and 10 weeks RA stored fruit . CA storage treatments were positioned in the opposite quadrant from the vectors for all volatile peaks, reflecting the decrease in volatile production during CA storage. In 1995-96, fruit stored 20 weeks in RA also had the highest score on factor 1 (Figure 5.2-A). However, there was no grouping by class of volatiles as occurred in 1994-95. Fruit stored 4 or 10 weeks in RA had similar scores for factor 1 while the 4 week RA treatment had a high score for factor 2 and was located in the same quadrant as all the vectors for FID peak area. Fruit stored 10 weeks in RA had a negative factor 2 score, suggesting that the rate of decrease of hexyl propanoate (peak 27), 2-methylbutyl acetate (peak 7), hexyl 2-methylbutyrate (peak 36) and 6-methyl-5-hepten-2-one (peak 16) was more important relative to other compounds in fruit stored 10 weeks. The 10 and 16 week CA treatments, as well as the vector for 3-methyl-2-butenyl acetate, had high negative scores for factor 1 and positive scores for factor 2. The 20 week CA treatment was located, as in 1994-95, in the opposite quadrant from the FID peak vectors, indicating that fruit stored 20 weeks in CA produced the least amount of volatiles.

# Odor-Active Peaks and Storage Treatments in the Two-Factor Space

The distribution of storage treatments in the space of the *Osme* peak intensities and peak areas reflect the higher ratings given to fruit from the RA storage treatments. Factor 1 represented 40% and 36% of the variation in peak intensities (Figures 5.1-B and 5.2-B) and 57% and 58% of the variation in peak areas (Figures 5.1-C and 5.2-C) in 1994-95 and 1995-96, respectively. The larger amount of variation explained by factor 1 for peak areas indicates larger co-variation between peak areas than between peak intensity ratings. This could be the result of panelists being more consistent rating peak area than peak intensity (Da Silva *et al.*, 1994). Factor 2 represented 10% and 11% of the variation in peak intensities, and 7% and 12% of the variation in peak areas in 1994-95 and 1995-96, respectively.

In 1994-95, there was no significant difference between RA treatments which all had positive scores on factor 1 (Figures 5.1-B and -C). The position of CA treatments with negative scores for factor 1 (9 week CA) and factors 1 and 2 (18 week CA) is related to the low intensity ratings given to all odor peaks for these treatments. Only peak 9 (garlic), peak 12 (fruity, 3-methyl-2-butenyl acetate) and peak 43 (grape juice, ßdamascenone) had negative loadings on factor 1. Peak 9 was perceived after CA storage only, and peaks 12 and 43 were perceived with higher intensities after CA compared to RA storage (Table 5.3). Vectors with high loadings on factor 1 and near zero on factor 2 were, for peak intensities, peaks corresponding to the watermelon odor (peak 24), anise, perfumey odor (peak 29), apple (butyl propanoate, peak 10), solvent (2methylbutyl acetate, peak 7) and mushroom odor (peak 35). Vectors representing odoractive peaks due to hexyl acetate (peak 20), butyl acetate (peak 4), methyl-, ethyl-, propyl- and butyl 2-methylbutyrate (peak 2, peak 6, peak 13 and peak 23) were positively correlated with each other, with high loadings on factor 1 and 2 (Figure 5.1-B). Vectors for 4-allylanisole (peak 33) and butyl butyrate (peak 17) were in the same direction as the peaks listed above, but with lower loadings, indicating their correlations with the former peaks, but a lesser contribution to overall variability.

Factor 2 for peak intensity in 1994-95 was explained by mushroom (hexyl tiglate, peak 40), grape juice (B-damascenone, peak 43), apple (hexyl 2-methylbutyrate, peak 36), cucumber (peak 34) and adhesive tape (peak 39) (Figure 5.1-B). The 10 week RA treatment had high factor 1 and 2 scores, with the vectors for "Gala" (unknown, peak 18), green apple (butyl hexanoate, peak 31) and apple (hexyl propanoate, peak 27) highly correlated in that same direction. In other words, fruit stored 10 weeks in RA had developed a stronger "apple" component for peak intensity than other treatments.

Correlations between *Osme* peak areas were different from peak intensities, resulting in a different distribution of the corresponding vectors in the factor space (Figure 5.1-C). Peaks corresponding to hexyl and butyl acetate (peak 20 and peak 4) had the highest loadings on factor 1. 2-Methylbutyl acetate (peak 7) and the mushroom odor (peak 35) had high loadings on factor 1, but negative loadings on factor 2, indicating less contribution to the perception duration for fruit from RA treatments. Vectors for the peaks corresponding to methyl-, ethyl-, propyl-, butyl- and hexyl 2-methylbutyrate odors (peaks 2, 6, 13, 23 and 36), butyl butyrate (peak 17), and peak 29 (anise, perfumey), were in the direction of all RA treatments indicating a similar rate of decrease in the perception (duration × intensity) of those compounds from RA to CA storage.

Factor 2 for peak area in 1994-95 was explained by the same odors as for peak intensity, except peak 18 (Gala, unknown), peak 31 (green apple, butyl hexanoate) and peak 36 (apple, hexyl 2-methylbutyrate). The later peaks were correlated with the peaks mentioned above (peaks 2, 23, 29). The difference between peak area and peak intensity for the loadings of some odor peaks on factor 2 resulted in a better clustering of the RA treatments on one side, and the CA treatments on the other side (Figure 5.1-C). This confirms the observation by Da Silva *et al.* (1994) that panelists discriminate better differences between treatments using peak area than peak intensity. This also explains why the variation explained by factor 1 is larger for peak area (57%) than for peak intensity (40%).

In 1995-96, the 4 week RA treatment had a high factor 1 score in both peak intensity and peak area spaces (Figures 5.2-B and 5.2-C). Vectors with high loadings on factor 1 in the peak intensity space were watermelon (peak 24), apple (butyl 2methylbutyrate, peak 23), apple (hexyl 2-methylbutyrate, peak 36), anise (4-allylanisole, peak 33), apple (hexyl propanoate, peak 27) and mushroom (peak 14) (Figure 5.2-B). Peaks due to methyl-, ethyl- and propyl 2-methylbutyrate with a sweet, fruity, berry odor (peaks 2, 6 and 13) and 2-methylbutyl acetate (solvent, fruity, peak 7) were correlated and had high loadings on factor 2. The vectors for hexyl acetate ('Gala', peak 20) and butyl acetate ('Gala', nail polish, peak 4) had high loadings on both factors 1 and 2. All RA treatments were in the positive quadrant; scores on factors 1 and 2 were lower for fruit stored 10 weeks in RA compared to fruit stored 4 and 20 weeks in RA, indicating lower ratings for peak intensity. As in 1994-95, the negative scores for CA treatments reflect the decrease in intensity ratings of odor-active peaks. The vectors for peak 9 (garlic), peak 12 (fruity, 3-methyl-2-butenyl acetate), peak 3 (apple, propyl propanoate) and peak 1 (tea, leaves) had negative scores for factor 1 (Figure 5.2-B).

The plot of factor scores for odor peak areas confirmed correlations between some of the peaks, and the differences between the 4 week RA and other storage treatments was emphasized (Figure 5.2-C). Peak 43 (grape juice) had the highest score on factor 1, indicating the duration × intensity (peak area) of perception of Bdamascenone was more important than intensity alone. This result explains why ßdamascenone had the highest Charm value in apples (Cunningham et al., 1986) because Charm values integrate the dilution factor of the sample injected in the GC and duration of odor perception. B-damascenone had longer perception duration compared to other compounds using Osme analysis, possibly due to a different interaction to the olfactory receptor, or a different transduction mechanism. Also, because B-damascenone has a high boiling point, it eluted late in the chromatographic run, with some peak broadening; this may explain why it was perceived for a longer duration than other compounds. 4-Allylanisole (peak 33), watermelon (peak 24), and 1-octen-3-ol (mushroom, peak 14) also had high loadings on factor 1; perception of these compounds was highest after 4 weeks in RA, then perception decreased later in storage (Table 5.2). Similar to FA of peak intensities, hexyl acetate (peak 20) and butyl acetate (peak 4) were correlated and had high loadings on both factors. However, 2-methylbutyl acetate (solvent, peak 7) was correlated with, butyl 2-methylbutyrate (apple, peak 23), hexyl 2-methylbutyrate (apple, peak 36) and propyl 2-methylbutyrate (sweet, berry, peak 13) (Figure 5.2-C). This shows different interrelationships between odor intensities and between duration  $\times$ intensity for those compounds. Unidentified peaks (peak 18, Gala, solvent), peak 26 (floral) and peak 29 (anise, perfumey) and peak 16 (5-methyl-5-hepten-2-one, fruity) were also correlated to peak 23, 7, 36, 4 and 20, but had lower loadings, indicating a lesser contribution to 4 week RA treatment. Similar to peak intensities, methyl 2methylbutyrate (peak 2) and ethyl 2-methylbutyrate (peak 6) had high loadings on factor 2, both having a sweet fruity odor.

## Significance of Odor Activities After Storage

Results in 1994-95 illustrated the aroma differences between RA and CA stored fruit. There was no compound that alone was responsible for fresh 'Gala' aroma, all RA treatments had high factor 1 scores. RA-stored fruit emitted volatiles that had fruity, apple-like, mushroom, rubber and skunk odors. Factor analysis in 1994-95 showed the importance and correlations between odor intensities and areas of hexyl acetate and butyl acetate, and between odors perceived from methyl-, ethyl-, propyl- and butyl 2methylbutyrate. In other words, the perception recorded for peak intensity and intensity × duration for hexyl acetate and butyl acetate, as well as the butyrate esters decreased at the same rate.

In 1995-96, compounds contributing to 'Gala' aroma (those with vectors pointing in the same direction as 4 weeks RA and highly perceived in fruit from that treatment) were 4-allylanisole (anise), ß-damascenone (grape juice), compounds perceived as watermelon (unknown), and mushroom (1-octen-3-ol). None of these compounds have an apple odor. Their contribution to 'Gala' aroma may be by interactions with the other fruity, apple-like compounds in whole apples. Because these compounds are strongly perceived from fruit stored 4 weeks in RA, they may contribute significantly to the characteristic aroma of 'Gala' apple. 4-Allylanisole was found in 'Cox's Orange Pippin' and was responsible for the spicy character determined by a taste panel (Williams et al., 1977a). This compound was perceived as anise, licorice by a trained panel in a descriptive analysis of the external aroma of 'Gala' apples, although with very low intensity (Chapter 6). Identification of 4-allylanisole by Williams et al. (1977a) was important because it showed compounds other than esters and aliphatic aldehydes and alcohols contributed to apple aroma. Straight-chain esters and their corresponding alcohols and aldehydes are known to originate from fatty acid metabolism via lipoxygenase activity or  $\alpha$ - and  $\beta$ -oxidations (Paillard, 1990). In turn, 4-allylanisole is a product of the shikimic acid pathway (Williams et al., 1977b), in which the primary

precursors of shikimate, erythrose-4-phosphate and phospho-enol-pyruvate, derive from glycolytic metabolism (Jensen, 1985). The shikimic acid derived *L*-phenylalanine was shown to be the precursor for allylphenols in plants belonging to the *Labiaceae* family (Manitto *et al.*, 1974). The transformation of the side chain of *L*-phenylalanine into the allyl group involves the loss of the  $C_1$  carboxylic acid carbon atom, which remains to be demonstrated. 4-Allylanisole was also found in 'Golden Delicious' and 'Delicious' apples, but at lower concentrations than in 'Cox's Orange Pippin' (William *et al.*, 1977b). These three cultivars are all parents of 'Gala' apple; the relative proportion of 4-allylanisole found in 'Gala' in our study was 4 times higher than found in 'Cox's Orange Pippin' with a different sampling method (Chapter 3). The isomer of 4-allylanisole, anethole (1-methoxy-4-(1-propenyl)-benzene), was also found in trace amounts in 1995-96, and in quantifiable amounts in 1994-95 (Table A.2), but the amount was too low to be odor-active.

β-damascenone usually occurs in a glycosidically bound form and is primarily found in processed products after hydrolysis (Roberts and Acree, 1995; Nursten and Woolfe, 1972; Braell et al., 1986; Buttery et al., 1990). It has a very low odor threshold with a sweet fruity, floral odor and non-typical of a particular fruit. It is believed to be an important aroma component of the products in which it was found. It had the highest Charm value of compounds extracted from 40 apple cultivars analyzed by Cunningham et al. (1986). In spite of low volatility and its bound form, it was captured on charcoal (and also Tenax) traps from 'Gala' headspace during long sampling times, and was perceived as a grape juice odor (Chapter 3). In 1994-95, ß-damascenone was perceived with equally low intensity throughout storage (Table 5.3). However, in 1995-96, its odor perception was more intense after 4 weeks in RA, and it decreased significantly in CA storage (Table 5.2). B-damascenone is a C13 nor-isoprenoid compound believed to be derived from the xanthophyll neoxanthin (Williams et al., 1992). Its more intense perception in 1995-96 implies emission from 'Gala' apples in higher amounts compared to 1994-95. Environmental as well as cultural factors may explain the differences between years. As an example of a cultural factor, Aubert (1997) found an increase in

glycosylated compounds and a decrease in ester production in 'Golden Delicious' apples sprayed with sterol inhibitor fungicides.

The compound perceived as watermelon had high loadings in FA of peak intensities and areas both years of the study, indicating its importance in fresh 'Gala' aroma as determined by GCO. Watermelon was chosen as a descriptor by panel consensus in the descriptive analysis of 'Gala' aroma, but it was never given high ratings (Chapter 6). Nevertheless, the compound responsible for the watermelon odor probably contributes in a subtle way to 'Gala' aroma. Identifying this compound and testing it in mixtures with other known components would clarify its contribution to 'Gala' aroma. Nona-*Z*,*Z*-3,6-dienol, with an odor threshold of 10 ppb and a clear watermelon aroma, was found in muskmelon (Buttery, 1981; Kemp *et al.*, 1974).

Compounds contributing to the overall fruitiness of 'Gala' aroma in all RA treatments in 1995-96 were hexyl acetate and butyl acetate described as ripe 'Gala' and 'Gala, nail polish', respectively, and butyl 2-methylbutyrate and hexyl 2-methylbutyrate, both described as apple, fruity. A positive correlation was observed between hexyl acetate and butyl acetate in perceived intensities and peak areas both years of the study, indicating production of these two compounds decreased similarly in response to CA conditions, and their olfactory perception was highly correlated. The enzyme responsible for ester production is alcohol acyl transferase (AAT) (Pérez et al., 1993; Fellman et al., 1993). The quantitative predominance of butyl and hexyl esters indicated that 'Gala' AAT had larger affinity for  $C_4$  and  $C_6$  alcohols, and/or that  $C_4$  and  $C_6$  alcohols are readily available as substrates (Paillard, 1990). Olías et al. (1995) have shown AAT substrate specificity varies between fruit species. Within fruit species such as strawberry, AAT substrate specificity also varies between cultivars (Pérez et al., 1996). The larger amount of acetate esters produced indicates acetyl-CoA was the preferred carboxy acid for AAT, or that acetyl-CoA is more available in the cell. A higher affinity for acetyl-CoA was demonstrated for strawberry AAT (Pérez et al., 1993).

Methyl 2-methylbutyrate, ethyl 2-methylbutyrate and propyl 2-methylbutyrate contributed significantly to the aroma of RA fruit in 1994-95, and fruit stored 20 weeks in RA in 1995-96, as plotted by the factor scores of both *Osme* peak intensities and peak

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areas (Figures 5.1 and 5.2, B and C). These compounds have distinctive sweet, fruity and strawberry, berry odors. Perceptions of their intensities and peak areas were positively correlated both years of the study. Methyl 2-methylbutyrate and ethyl 2methylbutyrate have low odor thresholds: 0.25  $\mu$ g·L<sup>-1</sup> and 0.1  $\mu$ g·L<sup>-1</sup>, respectively (Flath et al., 1967; Takeoka et al., 1989). 2-Methylbutyl acetate, with a nail polish odor, was also a significant 'Gala' odor volatile. Using banana disks, Myers and co-workers (1970) demonstrated valine, L-leucine and L-isoleucine were the precursors for branched-chain ester synthesis. Infiltration of whole apple or loading apple peels with Lisoleucine resulted in production of 2-methylbutyric acid, 2-methyl butyrate esters and 2methyl butanol (Hansen and Poll., 1993; Rowan et al., 1996). In 'Golden Delicious' apples, branched-chain esters were less affected than straight-chain esters by storage in low O2, but branched-chain esters were negatively affected by high CO2 (Brackmann et al., 1993). This was confirmed for 2-methylbutyl acetate in our study and by Mattheis et al. (1998a). However, production of other branched-chain esters was reduced by the CA conditions in this study and that reported by Mattheis et al. (1998a). High production of 2-methylbutyl acetate and 2-methyl-1-butanol after CA storage (Table 5.1) may be due to larger substrate availability because amino acid metabolism is less affected after CA at 1% CO<sub>2</sub> (Brackmann et al., 1993).

Other odor-active esters such as butyl and hexyl propanoate, butyl and hexyl butyrate, and hexyl hexanoate, as well as the ketone 6-methyl-5-hepten-2-one, had fruity, apple-like aromas. These compounds had lower intensities after RA storage, and usually were not perceived after CA storage (Table 5.2). They also had lower loadings in the FA, indicating they contributed less to the variability of odor-active peaks due to storage treatments.

The fruity peak corresponding to 3-methyl-2-butenyl acetate and the unknown garlic peak were only perceived after CA storage both years of the study. 3-Methyl-2-butenyl acetate was identified in 'Jonagold' by Hansen *et al.* (1990). This and other 2-methyl but-2-enyl esters increased under low  $O_2$  storage (Hansen *et al.*, 1992). The same group of authors showed L-isoleucine was a possible precursor of these unsaturated branched-chain esters as well as branched-chain alcohols (Hansen and Poll,

1993). They suggested a glycosidic intermediary of L-isoleucine metabolism accumulated during CA storage. Schawb and Schreier (1990) found aliphatic glycosylated alcohols in apples. Some of those alcohols could be metabolized to 2-methyl-but-2-enol which then is esterified upon exposing apples to air (Hansen *et al.*, 1992).

The garlic peak remains unidentified because it was present below FID detection threshold. It might contribute to a musty note found in CA stored 'Gala' by a trained panel (Chapter 6).

Rubber, skunk, mushroom and oatmeal odors were perceived more intensely after 4 weeks in RA. Two of the mushroom peaks were hexyl tiglate (1994-95) and 1octen-3-ol (1995-96); the other mushroom odors remain unidentified and also undetected by FID (Chapter 3). The rubber and skunk compounds may be sulfur containing compounds with a very low odor threshold. They are usually not perceived from whole apple aroma. The presence of sulfur may indicate synthesis from breakdown products of sulfur-containing amino acids (Sanz *et al.*, 1997). Protein turnover was shown in apples after harvest (Gorin, 1973).

## CONCLUSIONS

This study confirmed the contribution of hexyl acetate, butyl acetate and 2methylbutyl acetate to 'Gala' aroma (Young *et al.*, 1996). While hexyl acetate and butyl acetate concentrations and perceived aromas decreased after CA storage, 2-methylbutyl acetate (and its perceived odor intensity) was less affected by the low  $O_2$  and high  $CO_2$ regime. Butyl 2-methylbutyrate and hexyl 2-methylbutyrate contributed to the apple, fruity aroma in RA stored fruit. The lower molecular weight compounds methyl 2methylbutyrate, ethyl 2-methylbutyrate and propyl 2-methylbutyrate had stronger sweet and berry-like odors, and because they were still rated high after CA storage, they may contribute significantly to fruit aroma. 4-Allylanisole,  $\beta$ -damascenone and 1-octen-3-ol, as well as a compound with watermelon descriptor were important contributors to 'Gala' aroma after 4 week RA storage in 1995-96. They were also perceived with a higher intensity in all RA fruit in 1994-95. Even though these compounds do not have an apple odor, they may act synergistically or antagonistically when present with the fruity esters. The differences between the two years of the study in the magnitude of odor activity perception illustrates the effect of environmental and possibly cultural factors that affect apple aroma production.

The GCO technique, *Osme*, allowed measurement of the odor activity of known and unknown volatile compounds in 'Gala' apple. Important compounds contributing to fresh 'Gala' apple aroma were deduced by comparing fruit stored in CA and RA. However, results were limited by the sampling method. Aldehydes are produced by 'Gala' apples (Mattheis *et al.*, 1998b) but were not eluted by the sampling method used in this study. The odor activity of aldehydes in the 'Gala' apple matrix remains to be studied. On the other hand, more compounds were found to be important to 'Gala' aroma in our study than in another study where apple distillate were used to determine odor-active compounds in 'Gala' apple (Young *et al.*, 1996). While the GCO technique is a powerful tool for the study and screening of odor-active compounds among many volatiles analyzed by GC, confirmation of the results with model mixtures is necessary to confirm the importance of individual compounds.

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## **CHAPTER 6**

# DESCRIPTIVE SENSORY ANALYSIS OF 'GALA' APPLE AROMA AND FLAVOR IN STORAGE

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## ABSTRACT

'Gala' apple has a distinctive aroma and flavor, but its storage season is short. Controlled atmosphere (CA) storage maintains apple firmness and acidity, but volatile production is reduced. In this study, the aroma and flavor characters of 'Gala' apples were identified by ten trained panelists. A vocabulary of 13 descriptors for the aroma of whole and cut fruit and 16 descriptors for flavor were used to characterize the changes of apples stored in CA and/or regular atmosphere (RA) over 20 weeks. When compared to RA storage, intensity of fruity (pear, banana and strawberry) and floral descriptors decreased after 10 weeks in CA for whole and cut fruit aroma and flavor. During the entire storage period under CA, aroma of cut apples retained high vegetative and citrus characters but had a less intense anise aroma. Sourness, starchiness and astringency were significantly higher; however, sweetness was significantly lower. A musty note was perceived in whole apples stored in CA for 20 weeks. Differences between fruit stored for 16 weeks in CA followed by 4 weeks in RA and fruit stored 20 weeks in CA were only found for fruitiness (lower in 20 weeks CA) of whole fruit and sourness of cut fruit (higher in 20 weeks CA). The changes in descriptor ratings during storage are discussed in relation to gas chromatography and olfactometry data obtained with the Osme method.

#### INTRODUCTION

Consumer opinion often determines the success or failure of a product on the market (Williams, 1981). However, when able to express an emotion of acceptance or rejection, consumers are usually vague and not consistent with the vocabulary they use to explain why they prefer one product to another (Williams and Knee, 1977). Panelists can be trained to use consistent terminology and to quantify their response, a technique referred to as descriptive sensory analysis (DSA). DSA can identify characteristics perceived by the human senses that affect product variations. Changes in individual attributes may explain consumer acceptance or preference of a product (Williams and Knee, 1977). Instrumental measurements may then be used to understand the physicochemical stimuli necessary to induce a response from the sensory receptors.

Assessing horticultural products through DSA, instrumental measurements and consumer panels has been extensively used to understand 'Cox's Orange Pippin' apple quality (Williams and Carter, 1977; Williams and Knee, 1977; Williams and Langron, 1983; Williams et al., 1977). Williams and Carter developed a lexicon with 95 terms to describe 'Cox's Orange Pippin'. Descriptors were classified by external and internal appearance, feel of apple in the hand, external and internal aroma, taste, texture and after-taste. 'Cox's Orange Pippin' apples were rated one, two and six weeks after harvest for the 95 descriptors on a 0 to 5 scale where each scale number was explained by a phrase pertaining to the attribute rated (Williams and Carter, 1977). Reference standards were also provided to the panelists. Aroma descriptors were correlated with gas chromatographic data (Williams and Knee, 1977). Descriptive analysis data for 'Cox's Orange Pippin' were used to interpret a consumer survey where panelists were asked to rate acceptability of apples that had been stored under different atmospheres and temperatures (Williams and Langron, 1983). Other DSA studies were used to classify apple cultivars by aroma, flavor and texture characteristics (Daillant-Spinnler et al., 1996; Watada et al., 1980). By using preference mapping combined with multivariate statistics, Daillant-Spinnler and collaborators (1996) attempted to determine the apple attributes consumers used to make quality judgements. Despite thorough

descriptive analysis and the large sample size used in the study (12 apple cultivars representing a wide range of characteristics were tested, unpeeled and peeled), the authors did not determine a clear relationship between apple sensory properties and consumer preferences. They did confirm the importance of texture and taste for consumer acceptance stated earlier by Williams (1979). When optimum texture was present, Williams (1979) suggested that only aroma and flavor could improve apple quality.

Controlled atmosphere (CA) storage is commercially used to prolong apple shelf life. While low O2 and high CO2 significantly reduce acidity and firmness loss (Smock, 1979), volatile production is negatively impacted (Patterson et al., 1974). A consumer panel preferred the texture of CA-stored 'Cox's Orange Pippin' apples but found those apples had poor aroma and flavor compared to apples stored in air (RA) (Williams and Langron, 1983). Panelists judged the apples stored in 1%  $O_2$  as "too crisp and too low in taste intensity", but those stored in 2% O2 and ripened at 17 °C following storage, "too sweet and too soft". Firmer fruit was preferred (Knee and Sharple, 1981). Other studies comparing CA with RA-stored fruit found similar differences in firmness, tartness, aroma and flavor descriptors (Gorin et al., 1975; Smith, 1984; Anderson and Penney, 1973; Frijters, 1979). CA-stored apples were generally preferred to RA-stored fruit. However, storage atmosphere, apple cultivar and maturity stage at harvest affect differentially the changes in firmness and the amount of volatiles produced in and after CA storage (Mattheis et al., 1995; Yahia et al., 1990). 'Gala' apples stored in air were preferred to CA fruit (Boylston et al. 1994). In that study, the CA atmosphere was implemented after one month of air storage, and the usual benefits of CA for firmness and acidity retention were absent. Nevertheless, air-stored fruit emitted more volatiles than CA stored fruit. The preference of RA stored 'Gala's was therefore due to a higher aroma (Boylston et al., 1994). Another sensory study showed a decrease in flavor was perceived before decreases in soluble solids concentration (SSC), malic acid and firmness, and decreased apple acceptance (Gorin et al., 1975).

'Gala' apple has increased in popularity since its introduction from New Zealand in the late 60s (White, 1991; Green and Autio, 1990). Consumer taste panels showed
strong preferences for 'Gala' over other cultivars (Green and Autio, 1990; Stebbins *et al.*, 1994). However, hedonic ratings from consumer taste panels decreased for 'Gala' apples stored in air for more than 60 days (Stebbins *et al.*, 1994; Plotto *et al.*, 1997). 'Gala' apples stored in CA maintain high levels of firmness and acidity (Drake, 1996), but the reduction of volatile production reported for other apples also occurs for 'Gala' (Mattheis *et al.*, 1998; Boylston *et al.*, 1994; Chapter 5). The odor significance and relative olfactory power of volatile compounds emitted by 'Gala' was determined using the gas chromatography and olfactometry technique, *Osme* (Chapter 5). Compounds with a fruity and apple-like odor decreased significantly in CA and CA followed by RA storage. The *Osme* technique measures odor activity of individual compounds when presented directly to the nose. However, it gives an incomplete picture of the aroma perceived from the whole fruit. The objectives of this study were to measure the changes in storage of aroma from whole 'Gala' apples using DSA, and to relate DSA results to *Osme* analysis. We also determined changes in aroma and flavor perception due to cutting apples.

#### **MATERIALS AND METHODS**

#### **Plant Material and Storage Conditions**

'Gala' apples from a commercial orchard near Chelan, WA, were harvested on September 12, 1995. Apple maturity stage and homogeneity of the lots was assessed through the ground color; ground color was found earlier to be a good indicator of 'Gala' maturity (Plotto *et al.*, 1995). No pre-harvest or pre-storage chemical treatment was applied to the fruit. A batch of apples was stored at 2 °C for two weeks until the first tests, and was considered the control. The remaining fruit was stored at 1 °C for 10 and 20 weeks in either regular (RA) or controlled atmosphere (CA) with both O<sub>2</sub> and  $CO_2$  at 1% at the USDA-ARS Tree Fruit Research Laboratory in Wenatchee, WA. One additional storage treatment was 16 weeks in CA followed by 4 weeks in RA (CA/RA). After removal from storage, apples were shipped to Corvallis and stored at 2 °C for 5 days upon receipt. Apples were then ripened at  $22 \pm 2$  °C for 5 days prior to testing; the control fruit was ripened for 5 and 10 days.

#### **Panel Selection and Training**

Twelve volunteer panelists were screened for their capacity to perceive and describe odors by nose and flavors by mouth. For odor recognition, matching tests were used, with tasks of increasing difficulty (Meilgaard et al., 1991). Panelists were presented with two sets of samples in wine glasses covered with aluminum lids and perforated aluminum foil to hide the contents. Samples were, for the first two tests, cut fruits (banana, apple, strawberry), cut vegetables (cucumber, mushroom, grass) and floral extracts from Uncommon Scents, Eugene, OR (rose, lily, violet), and for the next four tests, cut apples ('Golden Delicious', 'Red Delicious', 'Granny Smith', 'Braeburn', 'Fuji', 'Gala', 'Gravenstein'). Panelists were asked to smell the content of the glasses in one set, and match aromas with the glasses of the second set. When apples were used, panelists were asked to describe the perceived odor in their own words. These descriptors were later discussed during ballot development. For taste discrimination, triangle tests were used. Panelists were instructed to choose, based on taste, the odd sample between three cut pieces (a quarter of fruit) of apple. Apple varieties tested were, by pair: 'Fuji' and 'Braeburn', 'Fuji' and 'Red Delicious', 'Fuji' and 'Gala', and 'Gala' from different orchards and/or at different maturity stages.

Ten panelists of the twelve screened participated in eight one-hour training sessions where descriptors, reference standards and a standardized method of smelling and presenting the fruit were developed. All panelists were students and staff from Oregon State University, and 80% of them were from the Department of Food Science and had previously participated in descriptive panels. Of the 13 descriptors chosen for aroma and 16 for flavor, 11 were identical. Flavor was defined as the combined effect in the mouth of aromatics, basic tastes and mouthfeel (Meilgaard *et al.*, 1991). Descriptors and references used for the panel are listed in Table 6.1. Each descriptor was rated using a 16-point intensity scale where 0 = "none", 7 = "moderate" and 15 = "extreme".

Table 6.1.	Attribute descriptors, reference standards and their intensities for descriptive sensory analysis of 'Gala' apple aroma and flavor. Intensity rated on a 16-point category scale ( $0 = $ none, $7 = $ moderate and $15 = $ extreme)
	extreme)

Descriptor	Reference Standards	Intensity
Aroma and Flavor		
Overall fruity	- 3.5 ppm of butyl acetate, 0.8 ppm of pentyl acetate	3
	and 0.07 ppm of ethyl 2 methyl butyrate in water <sup>a</sup>	
	- very ripe Gala <sup>b</sup>	13
Pear	'Bartlett' pear <sup>b</sup>	13
Banana	banana <sup>b</sup>	13
Watermelon	watermelon <sup>b</sup>	8
Strawberry	strawberry essential oil (Uncommon Scents, Eugene,	8
	OR) diluted to 100 ppm <sup>a</sup>	
Citrus	citral: 25 ppm <sup>a</sup>	10
Floral	'Hawaiian blossom' (Uncommon Scents, Eugene, OR)	6
	diluted to 0.1 ppm <sup>a</sup>	
Anise	anethole: 7.5 ppm <sup>a</sup>	10
Overall vegetative <sup>c</sup>		
Grassy <sup>c</sup>	cis-3-hexen-1-ol: 50 ppm <sup>a</sup>	6
Green <sup>c</sup>	trans-2- hexenal : 25 ppm <sup>a</sup>	8
Woody/stemmy <sup>c</sup>	hexanal: 9 ppm <sup>a</sup>	8
Musty/dirty	terpinen-4-ol: 25 ppm <sup>a</sup>	10
Fermented	apples fermented for one week in a tightly closed jar	14
Anise flavor	fennel	14
Cooked fruit flavor	'Welsh' grape juice	14
<u>Taste</u>		
Sweet	sucrose in water: 2, 5 and 10% <sup>d</sup>	3, 8, 13
Sour	malic acid in water: $0.025$ , $0.05$ and $0.08\%^{d}$	2, 6, 12
Starchy	jicama root	13
Astringent	alum in water: 0.05% <sup>d</sup>	14

<sup>a</sup> All chemical standards were from Aldrich (Milwaukee, WI) except anethole which was from Fluka Chemika (Milwaukee, WI). Standards were in 50 mL of odor-free double distilled water (Milli-Q)

presented in 150 mL glass vials closed with Teflon lids. <sup>b</sup> All fruit samples were cut in pieces (peeled and cored) and placed in wine glasses covered with aluminum lids.

<sup>c</sup> "Grassy", "green" and "stemmy" were combined into "overall vegetative" for flavor attributes. <sup>d</sup> Taste standards diluted in drinking water (Aqua-Cool, Portland, OR)

'Gala', 'Braeburn' and 'Fuji' apples were used to develop the descriptors as these varieties represent a wide range of aromas and flavors. Chemical standards were obtained from Aldrich Flavors and Fragrances (Milwaukee, WI) and Fluka (Milwaukee, WI) and were food grade. They were diluted in odor-free double distilled water (Milli-Q) to a concentration comparable to what was perceived in apples. The training was finalized with four pre-testing sessions where 'Gala' apples at different maturity stages and growing origins were compared. Before testing after each two-month interruption, a review session was organized where panelists compared 'Gala', 'Braeburn' and 'Fuji'.

Testing took place in individual booths set in a well ventilated room under daylight illumination. Panelists were provided with drinking water (Aqua-Cool, Portland, OR) and expectoration cups to cleanse their palates between samples.

#### **Sample Presentation**

Six sets of five 'Gala' apples per treatment were placed in single 4 L wide mouth glass jars and left uncovered at room temperature for one to six hours prior to testing. Two jars representing each storage treatment (or ripening time for the control) were presented to each panelist during each test session. Upon their arrival, panelists covered the jars with aluminum lids to allow volatile compounds to accumulate for 5 to 10 minutes while they reviewed reference standards. Panelists then opened the jar lids, smelled and rated external aroma (EA) of the apples. All the aroma descriptors were rated at once for each jar. After rating EA, panelists took one fruit of each treatment, cut it in half, cut one half into two-cubic cm pieces with peel, placed the pieces in a wine glass labeled with the same three-digit number as the corresponding jar, and covered the glass with an aluminum lid. Cut apples were allowed to stay in the glass at least one minute to accumulate volatile compounds. The other half of the apple was set aside to evaluate flavor by mouth. Panelists then rated cut fruit for internal aroma (IA) by using the same descriptors as for EA. Finally, panelists reviewed the taste standards and rated the remaining half apple for flavor. Of the half apple set aside, approximately 1 cm was removed on each side, eliminating any possible oxidized tissue. The top, bottom and the core of the wedge were also removed, since there was a clear taste difference between

those portions of the fruit as demonstrated by Dever and Cliff (1995). Panelists tasted the middle  $2 \text{ cm}^3$  section of apple with peel.

Panelists evaluated each storage treatment for a total of 6 times (6 replications) over a period of 3 days, with two replications per day.

#### **Instrumental Measurements**

Instrumental measurements were performed using fruit from the same batch as DSA. Apple headspace was sampled from whole apples (5 fruit, approximately 1 kg) placed in 4 L wide mouth glass jars with a dynamic flow-through system. Volatile compound analysis and *Osme* are fully described in Chapters 3 and 5.

Approximately two quarter sections cut longitudinally were taken on opposite sides of 20 'Gala' apples. Samples were juiced and soluble solids concentration was measured with an Auto Abbe electronic refractometer (Leica Inc., Buffalo, NY). Ten mL juice was titrated with 0.1 N NaOH to a malic acid end-point of pH 8.2 for titratable acidity (TA) measurements (Metrohm AG, Herissau, Switzerland). Juice pH was recorded before titration.

#### **Statistical Analysis**

Panelists' performances were evaluated through individual analyses of variance (ANOVA) after each storage pull-out. Differences between storage treatments (RA versus CA) were analyzed for each storage time with the compound F-test (Anderson and Bancroft, 1952) for each descriptor, using appropriate transformation when necessary. Panelists and batch replications were included in the model as random effects. Pair-wise comparisons were performed between the two types of storage within each storage time. When the ratings of one or two panelists did not agree with the panel for one descriptor (rating in the opposite direction), that panelist data were deleted for that specific analysis.

Principal components analysis (PCA) was performed on pooled taste panels. PCA was performed using a factor analysis with the principal component method (SAS Institute Inc., Cary, NC) on the covariance matrix of the residuals of a general linear model (GLM) where panelist was the main effect. The GLM residuals were used as a standardizing method to remove the variability due to panelists using different parts of the scale. Storage scores for each principal component were analyzed using ANOVA. Storage means were plotted in the dimensions of the first two principal components (PC). In this plot, descriptors were represented as vectors. Vector angles reflected descriptor correlations with each other, and vector magnitude reflected the relative contribution to each PC.

Instrumental data were analyzed for storage effect using one-way ANOVA. Sample means were separated with the LSD test (P < 0.05).

All statistical analyses were performed using SAS statistical software, version 6.12 (SAS Institute Inc., Cary, NC).

#### RESULTS

#### **Descriptive Sensory Analysis**

The differences are presented pair-wise between RA and CA stored fruit for 10 and 20 weeks in storage, and between CA and CA/RA after 20 weeks in storage (Tables 6.2, 6.3 and 6.4). There were no significant differences between descriptors for the control apples (2 weeks in RA) ripened 5 or 10 days, therefore only the results from the 5 days of ripening are presented because apples from other storage times were also ripened for 5 days.

Differences between RA and CA storage increased with time in storage for overall fruity, pear, banana, strawberry and floral EA descriptors (Table 6.2). All fruity and floral characters decreased in CA storage. For the same descriptors, differences between CA and the combination of CA/RA storage were not as significant as differences between RA and CA storage. A slight musty note was perceived in CA stored fruit after 20 weeks.

Table 6.2. Descriptive profile of external aroma (EA) of 'Gala' apples stored for 2, 10 and 20 weeks in regular atmosphere (RA), controlled atmosphere (CA)  $(1\% O_2, 1\% CO_2)$  and 16 weeks in CA followed by 4 weeks in RA (CA/RA). Ratings are on a 16-point category scale  $(0 = \text{none}, 15 = \text{extreme})^c$ 

Time (weeks) and type of storage										
	2 <sup>a</sup>	10		20			20			
Attribute	RA	RA	CA		RA	CA		CA/RA	CA	
Overall fruity	8.27	9.02	6.92	***	9.48	6.25	***	7.25	6.38	*
Pear	5.17	5.70	3.95	***	6.03	3.62	***	4.38	3.80	*
Banana	3.02	2.82	1.72	*	2.80	1.30	*	2.22	1.62	*
Watermelon	147	1.58	1.50		1.48	1.28		1.32	1.17	
Strawberry	1.65	2.32	1.68	*	2.33	1.47	**p	1.70	1.70	
Citrus	1.90	2.17	1.70		1.92	1.62		1.85	1.57	
Floral	3.70	4.32	3.10	**	4.27	2.23	***	3.22	2.41	*p
Anise	0.43	0.88	0.68		1.48	0.50		1.08	0.68	
Grassy	0.90	1.35	1.43		1.35	1.52		1.27	1 22	
Green	2.90	2.22	1.85		1.73	1.92		1.83	1.50	
Stemmy	1.73	1.62	1.85		1.57	2.13		1.52	1 73	
Musty	0.90	0.83	0.73		0.50	1.13	*p	1.02	1.75	
Fermented	0.23	0.43	0.21		1.07	0.98		0.25	0.47	

<sup>a</sup> Apples stored for 2 weeks were considered as the control. Only results from 5-day ripening are presented

<sup>b</sup> Numbers in italics were data analyzed after deletion of outliers

<sup>c</sup> Significance between two types of storage (RA versus CA) within one storage time: \*, \*\*, \*\*\*: significant at P < 0.05, P < 0.01 and P < 0.001, respectively

Table 6.3. Descriptive profile of internal aroma (IA) of 'Gala' apples stored for 2, 10 and 20 weeks in regular atmosphere (RA), controlled atmosphere (CA)  $(1\% O_2, 1\% CO_2)$  and 16 weeks in CA followed by 4 weeks in RA (CA/RA). Ratings are on a 16-point category scale  $(0 = \text{none}, 15 = \text{extreme})^c$ 

Time (weeks) and type of storage									
	2ª		10		20			20	
Attribute	RA	RA	CA		RA	CA	. <u> </u>	CA/RA	CA
Overall fruity	7.65	8.30	5.93	***	8.63	5.10	***	5.13	5.35
Pear	4.87	5.57	3.67	**	5.80	2.68	***	2.82	3.02
Banana	2.63	2.52	1.00	**	3.10	1.02	**	1.10	1.13
Watermelon	1.45	1.77	1.90		1.57	1.50		1.30	1.30
Strawberry	1.22	1.50	1.06	**	1.61	0.98	*p	1.03	0.98
Citrus	1.95	2.10	2.58		1.50	2.85	*p	2.45	2.50
Floral	3.55	3.48	2.10	**	3.52	1.53	**	1.95	2.02
Anise	0.27	0.76	0.24	*	1.07	0.38	**	0.55	0.52
Grassy	1.43	1.30	1.96	*	0.90	2.25	*p	1.75	1.65
Green	3.72	2.25	3.13	*p	1.40	2.93	*p	2.47	2.70
Stemmy	2.27	1.31	2.58	*p	1.52	2.43	**	2.57	2.10
Musty	0.78	0.88	1.17		0.92	1.38		2.10	2.10
Fermented	0.32	0.52	0.98		0.97	1.35		0.77	0.97

<sup>a</sup> Apples stored for 2 weeks were considered as the control. Only results from 5-day ripening are presented

<sup>b</sup> Numbers in italics were data analyzed after deletion of outliers

<sup>c</sup> Significance between two types of storage (RA versus CA) within one storage time: \*, \*\*, \*\*\*: significant at P < 0.05, P < 0.01 and P < 0.001, respectively

		Time	(weeks	) and typ	e of stora	ge				
	2 <sup>a</sup>	10		20			20			
Attribute	RA	RA	CA		RA	CA		CA/RA	CA	
Sweet	8.05	8.80	6.88	***	8.85	6.15	**	7.35	7.22	
Sour	5.18	3.83	5.28	* <sup>0</sup>	3.58	6.75	***	4.52	5.04	* <sup>D</sup>
Starchy	1.93	1.02	3.06	*0	1.00	3.45	**	3.02	3.02	
Astringent	1.07	0.60	1.55	*	0.81	2.16	**	1.80	1.87	
Overall fruity	7.50	8.46	6.12	**	8.37	5.3I	***	6.42	6.30	
Pear	4.67	4.97	3.17	*	5.90	3.10	**	297	3 22	
Banana	2.30	2.22	0.87	**	2.72	0.61	**	1.20	0.93	
Watermelon	1.75	1.37	1.73		1.57	1.75		1.57	1.83	
Strawberry	1.00	1.25	0.70		1.27	1.20		1.20	1.05	
Citrus	2.38	1.93	2.93	*	1.48	3.37	**	2.63	2.80	
Floral	3.58	3.74	2.65	*	4.29	2.17	** <sup>0</sup>	2.75	2.45	
Anise	1.28	0.87	0.62		1.27	0.50		0.50	0.75	
Cooked	1.33	2.90	1.15	*	3.17	1.33	**	1 57	1 55	
Vegetative	3.62	2.57	4.58	***	2.68	4.90	***	4 23	4 18	
Musty	0.38	0.67	0.58		0.92	1.00		0.77	0.57	
Fermented	0.20	0.50	0.13		0.92	1.83		0.12	0.17	

Table 6.4. Descriptive profile of flavor of 'Gala' apples stored for 2, 10 and 20 weeks in regular atmosphere (RA), controlled atmosphere (CA)  $(1\% O_2, 1\% CO_2)$  and 16 weeks in CA followed by 4 weeks in RA (CA/RA). Ratings are on a 16-point category scale (0 = none,  $15 = \text{extreme})^c$ 

<sup>a</sup> Apples stored for 2 weeks were considered as the control. Only results from 5-day ripening are presented

<sup>•</sup> Numbers in italics were data analyzed after deletion of outliers

<sup>c</sup> Significance between two types of storage (RA versus CA) within one storage time: \*, \*\*, \*\*\*: significant at P < 0.05, P < 0.01 and P < 0.001, respectively

Differences between CA and RA storage intensity ratings were within the same ranges or slightly less for IA compared to EA for the overall fruity, pear, banana, strawberry and floral characters (Table 6.3). The grassy, green and stemmy characters were perceived lower and anise higher for RA-stored fruit. The rating for citrus was lower for fruit stored 20 weeks in RA. There were no significant differences for any IA descriptors between CA and CA/RA stored fruit.

Sweetness ratings decreased for CA-stored fruit, and sourness decreased in RA (Table 6.4). Starchiness and astringency were rated higher for CA-stored apples. Similar to the aroma characters, flavor ratings for overall fruity, pear, banana and floral decreased after CA storage. The ratings given to citrus and overall vegetative flavor notes were higher in CA-stored fruit, and the cooked fruit character was higher in RA-stored fruit. Similar to internal aroma, there were no differences between CA and CA/RA stored fruit after 20 weeks in storage for any flavor descriptor except for sourness which was rated higher for CA-stored fruit.

#### Effect of CA Storage on 'Gala' Apple Ratings

The first principal component (PC 1) explained 38, 42 and 38% of the dataset variation for EA, IA and flavor, respectively (Figure 6.1). PC 1 was explained by overall fruity, pear, banana and floral descriptors for EA, IA and flavor, and also by sweet for flavor. Storage types were separated (P < 0.001) on the first principle component axis with positive scores for RA stored fruit (including the control) and negative scores for CA stored fruit. Treatment scores for PC 1 showed differences for fruity descriptors between RA and CA fruit increased with storage time.

The second principal component (PC 2) explained 11, 13 and 12% of the total variation for EA, IA and flavor, respectively (Figure 6.1). Treatment differences in the second principal component axis were only significant (P < 0.001) for IA. PC 2 was explained on the positive side by the descriptors green, grassy, stemmy and citrus for EA and IA, and sour, starch, citrus and overall vegetative for flavor. The fermented descriptor had a high negative loading on PC 2 for EA and IA, and banana also had a

# Figure 6.1. Principal components analysis plots for external aroma, internal aroma and flavor of 'Gala' apples stored for 10 and 20 weeks in regular atmosphere (RA), controlled atmosphere (CA) (1% O<sub>2</sub>, 1% CO<sub>2</sub>) and 16 weeks in CA followed by 4 weeks in RA (CA/RA). Principal components loadings are determined by the vector lengths for each sensory attribute. All vectors start at the origin. Their directions and magnitudes (loadings) are represented by the figure diamonds. Scores for storage treatments are drawn in circles. 20 CA<sub>1</sub> and 20 CA<sub>2</sub> were 20 weeks in CA compared to 20 weeks in RA and CA/RA, respectively. The control was stored 4 weeks in RA.

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high negative loading on PC 2 of EA (Figure 6.1). The control sample had the highest PC 2 score for IA evaluation. Also, IA of CA fruit had slightly higher scores than IA of RA fruit when compared within a storage time. Fruit stored for 20 weeks in CA (20  $CA_1$ ) had the highest PC 2 score for flavor when compared with RA-stored fruit.

PCA was performed on the pooled taste panel results with the assumption that panelists were calibrated by training and by using reference standards during each panel. It nevertheless appears that panelists rated by comparing and contrasting the two treatments that were presented to them in the two jars. Principal component scores for the same treatment (20 weeks in CA storage) were lower on PC 1 when fruit stored 20 weeks in CA (20 CA<sub>1</sub> and 20 CA<sub>2</sub>) was compared with RA fruit (20 RA versus 20 CA<sub>1</sub>) than compared with CA/RA fruit (CA/RA versus 20 CA<sub>2</sub>), especially on flavor ratings (Figure 6.1). Therefore, PCA plots should be interpreted by considering the pair-wise contrast between storage treatments within a storage time.

#### DISCUSSION

## Relationship Between Aroma Descriptors, *Osme* Data, and Volatiles Emitted by 'Gala' Apples

The general decrease of perceived fruitiness in CA stored apples confirmed gas chromatography and olfactometry (*Osme*) results (Chapter 5). GC analysis showed the highest production of volatile esters by fruit stored 4 weeks in RA, which was the same fruit as the control in the descriptive analysis experiment (Table 6.5 and Chapter 5). Accordingly, *Osme* data for peak intensities were higher for that batch of apples (Table 6.5 and Chapter 5). Nevertheless, descriptive analysis showed ratings for the control that were equal to or lower than 10 and 20 storage weeks in RA for fruity characters. PCA scores for the control were also lower than scores for 10 and 20 weeks in RA on the fruity , pear, banana and floral component (PC 1, Figure 6.1). Because of time constraints, we could not test all storage treatments at one panel session. Therefore, it appears that we measured a contrast effect (Meilgaard *et al.*, 1991) in the descriptive

Table 6.5. Total odor-active esters (first row) emitted by 'Gala' apples after regular (RA) or controlled<br/>atmosphere (CA) storage<sup>a</sup>. Odor-active peak intensities measured by Osme analysis (2<sup>nd</sup> row<br/>and below). Total fruity odor peaks is the sum of intensities of 18 peaks due to esters.<br/>Individual odor-active peaks (compounds in parenthesis) are means of 12 data-points<sup>b, c</sup>

	Storage Type									
		RA			CA					
	Storage Duration (Weeks)									
	4	10	20	10	<b>16<sup>d</sup></b>	20				
Total esters $(ng.kg^{-1}.L^{-1})$	14608	10860	13360	7450	3514	837				
Total fruity odor peaks	91.6	69.1	78.3	63.1	44 4	20.3				
Grapejuice (B-Damascenone)	6.2	2.2	2.4	2.2	1.4	0.0				
Floral (Unknown)	1.9	0.0	0.0	0.0	0.0	0.0				
Watermelon (Unknown)	6.9	1.0	0.0	0.0	0.0	0.1				
Anise (4-Allylanisole)	7.6	3.0	2.3	3.5	1.0	0.1				
Garlic (Unknown)	0.7	0.0	0.3	3.5	3.0	1.2				

<sup>a</sup> Values (ng.kg<sup>-1</sup>.L<sup>-1</sup>) are means of 4 1-kg replications of dynamic headspace sampling

<sup>b</sup> 3 panelists x 4 replications each. Values are on a 16-point intensity scale (0 = none, 15 = extreme)

<sup>c</sup> Adapted from Chapter 5

<sup>d</sup> 16 weeks in CA followed by 4 weeks in RA

analysis between RA and CA stored fruit rather than the real difference between treatments along storage time. The PCA plots for DSA were nevertheless similar to volatile analysis PCA plots; CA and RA stored fruit were separated on the first principal component with high loadings on fruity descriptors and volatile esters (Chapter 5 and Figure 6.1).

The overall fruity attribute could be explained by volatile esters that had fruity and apple-like odors according to *Osme* analysis (Chapter 5). Hexyl acetate, butyl acetate and 2-methylbutyl acetate have been identified to be primarily responsible for apple aroma in several cultivars (Williams and Knee, 1977; Paillard, 1975) including 'Gala' (Young *et al.*, 1996; Chapter 4). Hexyl acetate is also an important contributor to pear aroma (Suwanagul, 1996). The descriptor given to hexyl acetate odor in *Osme* analysis was "Gala, ripe apple, pear" (Chapter 5). When presented to panelists diluted in distilled water, it had an aroma similar to apple (Chapter 4). Therefore, hexyl acetate was probably the volatile contributing the most to the overall fruity and pear attributes. 2-Methylbutyl acetate was suggested to be related to banana descriptors in DSA of 'Cox's Orange Pippin' (Williams and Knee, 1977). However, it did not have a banana descriptor in *Osme* analysis , and no other compound had that descriptor (Chapter 5). It is probably the combination of some esters that generated the banana aroma term.

Methyl 2-methylbutyrate, ethyl 2-methylbutyrate and propyl 2-methylbutyrate had a characteristic sweet and strawberry-like aroma using gas-chromatography and olfactometry analysis (Chapter 5). There were smaller differences between storage treatments for the perception of those compounds by *Osme* (Chapter 5). The strawberry descriptor in the 'Gala' DSA was not given a high rating, nor did it contribute significantly to PC loadings. These methylbutyrate esters may be important for the general fruity and sweet aroma of 'Gala', without having a character impact.

The floral descriptor contributed significantly to separate CA and RA-stored apples on the PCA plot. However, only one unknown peak had a floral descriptor in *Osme* analysis; it was only perceived in the control fruit with a low intensity (Chapter 5 and Table 6.5). Therefore, more compounds than this floral peak perceived through *Osme* analysis contribute to the floral note of 'Gala' apple. It would be interesting to

determine through mixtures of volatile compounds found in 'Gala' which combination produces this specific aroma.

Using *Osme*, a compound with a watermelon odor was strongly perceived in the control fruit (Chapter 5 and Table 6.5). However, the watermelon descriptor was not rated high, or was not perceived by some panelists in the descriptive analysis. The watermelon compound perceived in *Osme* analysis was probably blended in other fruity notes, or it might also contribute to the floral character. Floral and watermelon share a fresh and sweet type of fragrance.

The musty note perceived in CA stored fruit (Table 6.2) could be due to a compound with a garlic odor (Chapter 5 and Table 6.5). This unknown compound perceived only in fruit stored in CA was also given low ratings in *Osme* analysis. However, because the compound had a distinct odor compared to the fruity esters, it could be perceived by the descriptive panel.

#### Effect of Combined Atmospheres on Aroma Perception

The inhibiting effect of CA storage on volatile production by apples is well documented (Yahia, 1994). A partial recovery of volatile production may occur when apples are placed in air or higher  $O_2$  levels for some weeks before removal from storage (Smith, 1984; Streif and Bangerth, 1988; Brackmann *et al.*, 1993). Volatile compounds were emitted in larger amounts when 'Gala' apples stored in CA for 16 weeks were then placed in air for 4 weeks (Chapter 5). Differences were also perceived by *Osme* analysis (Chapter 5). The differences were only perceived for the EA fruity and floral descriptors by DSA (Table 6.2). A slight decrease from CA was also perceived in CA/RA stored fruit for the sour descriptor (Table 6.4). Smith (1984) reported an increase in perceived aromaticity of 'Cox's Orange Pippin' by a panel of 12 to 16 experts after apples were stored in 1.25%  $O_2$  then in 2%  $O_2$  for 2 months before testing. He used a paired comparison test between treatments, which might be a more sensitive test than intensity ratings to perceive differences. An increase in volatile production was not always observed in similar experiments (Mattheis *et al.*, 1995; Yahia, 1991). The response may therefore depend on the cultivar, maturity stage at harvest, storage atmosphere combinations and other cultural factors.

#### Cut Fruit Aroma and Flavor

The descriptors green, grassy and stemmy were perceived with higher intensities in cut than in whole apples, with significant differences between CA and RA stored fruit (Table 6.3). Those descriptors were rated higher in CA than in RA stored fruit. Compounds known to possess green apple-like odors are hexanal and *trans*-2-hexenal (Flath *et al.*, 1967). Those compounds result from enzymatic reactions occurring upon cutting or crushing the fruit cells (Drawert *et al.*, 1966). Therefore, we could expect higher ratings for green-like attributes in cut apple aroma and flavor. Fellman *et al.* (1993) found more hexanal in flesh of 'Rome' apples stored in 1% O<sub>2</sub> than stored in air. However, Yahia *et al.* (1990), Mattheis *et al.* (1995) and Mattheis *et al.* (1998) found a negative effect of CA storage on hexanal from 'Cortland' apple juice, 'Bisbee Delicious' and 'Gala' apple headspaces of whole fruit, respectively. Hexanal was not found in the samples used for *Osme* analysis. It is possible the green-like attributes were perceived with higher intensities in 'Gala' apples stored in CA because the fruity attributes were not as strong.

Green, grassy and stemmy EA and IA descriptors were combined into overall vegetative in the flavor DSA descriptors. As in cut fruit aroma, the vegetative flavor descriptor decreased in RA stored apples; however, the difference between RA and CA-stored fruit was larger for the flavor than for the IA descriptor (Table 6.4). Hexanal, *trans*-2-hexenal, and possibly other compounds such as alcohols deriving from enzymatic reaction upon chewing the fruit may be present in larger amounts in CA-stored fruit because those fruit do not use their reserves by respiration. In our study, the headspace analysis that we used to sample free volatiles emitted by whole apples could not reflect the amount actually present in the fruit.

Anise descriptor ratings for IA increased in RA-stored fruit (Table 6.3). 4-Allylanisole, with an anise character, was emitted in the largest amount and perceived most intensely in apples stored 4 weeks in RA by *Osme* analysis (Table 6.5 and Chapter

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5). As mentioned for other descriptors, the difference in rating due to storage might be due to a contrast between RA and CA stored fruit because 4-allylanisole was not present in perceivable amounts in CA fruit.

The citrus attribute was correlated with green, grassy and stemmy descriptors in the PCA of EA and IA (Figure 6.1). A slight drop was observed from CA in the citrus ratings for IA after 20 weeks in RA (Table 6.3), and a more significant decrease was perceived by flavor (Table 6.4). Odor of aldehydes such as citral, octanal, and decanal are part of citrus aroma (Young, 1997; Bazemore, 1995). Citral has not been reported in apples (Paillard, 1990), but octanal and decanal were present in 'Gala' apple headspace (Mattheis *et al.*, 1998). Those two aldehydes have low odor thresholds: 0.70  $\mu$ g·L<sup>-1</sup> and 0.10  $\mu$ g·L<sup>-1</sup> for octanal and decanal, respectively (Guadagni *et al.*, 1963). However, they were not found in samples analyzed by *Osme* because of the volatile isolation technique used (Chapter 3). Therefore, their contribution to apple aroma is not clear. A decrease in citrus aroma flavor in RA fruit could be due to the lower perceived sourness from decreasing total acidity.

Cooked fruit flavor was rated higher in RA stored fruit (Table 6.4). ß-Damascenone may contribute to this descriptor. ß-Damascenone is a glycosidically bound compound (Buttery *et al.*, 1990) and it is released in the mouth upon chewing apple flesh. It was found in apples (Chapter 5; Schreier *et al.*, 1978; Cunningham *et al.*, 1986) with a low odor threshold. It was perceived with a higher intensity in RA stored 'Gala' apples using *Osme* analysis (Chapter 5).

## **Relation Between Taste Descriptors and Instrumental Measurements**

Sourness differences between CA and RA stored fruit corresponded to the measured differences in pH and in titratable acidity (TA) (Table 6.6). The decrease of malic acid from fruit respiration in air storage and a reduced acid loss in CA is well documented (Smock, 1979; Chen *et al.*, 1985; Anderson and Penney, 1973). Changes in titratable acidity in apples are usually perceived by trained and untrained panelists (Williams and Langron, 1983; Gorin, 1973; Plotto *et al.*, 1997; Anderson and Penney, 1973; Visser *et al.*, 1968; Watada *et al.*, 1980).

	Storage Type									
	<u> </u>	CA								
	Storage Duration (Weeks)									
	4	10	20	10	16 <sup>y</sup>	20				
pH	3.88 <sup>b</sup>	3.85 °	4.00 <sup>a</sup>	3.83 <sup>c</sup>	3.79 <sup>d</sup>	3.78 <sup>d</sup>				
TA (% malic acid)	0.371 <sup>b</sup>	0.328 <sup>c</sup>	$0.264^{\text{ d}}$	0.394 <sup>a</sup>	0.364 <sup>b</sup>	0.359 <sup>b</sup>				
SSC (°Brix)	14.0 <sup>b</sup>	13.3 <sup>c</sup>	13.1 <sup>c</sup>	14.5 <sup>a</sup>	13.8 <sup>b</sup>	13.2 °				

# Table 6.6. 'Gala' pH, titratable acidity (TA) and soluble solid content (SSC) in regular (RA) and controlled<br/>atmosphere storage (CA). Values are means of 20 apples z

<sup>z</sup> Means followed by the same letter are not significantly different within the same line by the Fisher protected LSD test, alpha = 0.05<sup>y</sup> 16 weeks in CA was followed by 4 weeks in RA

Sweetness ratings were significantly lower for CA than RA stored fruit (Table 6.4). However, the sensory difference between treatments was not reflected by the refractometer measurements (Table 6.6). Refractive index alone is usually a poor predictor for perceived sweetness in apples (Dever and Cliff, 1995; Knee and Smith, 1989; Plotto *et al.*, 1997). Rouchaud *et al.* (1985) did not find good correlative values between total sugar content in 'Jonagold' or 'Cox's Orange Pippin' perceived sweetness by a panel of experts. The difference of 1 °Brix or 10% total sugars between two levels of SSC may just not be enough to be perceived as sweetness differences. Dever and Cliff (1995) found better predictive values for sensory data with instrumental measurements by looking at the underlying dimensions of the combined variables using canonical correlations. The differences between CA and RA in perceived sweetness could be due to the differences in perceived acidity; sweetness perception could also be the result of both soluble sugars and volatile compounds to the sweetness and tartness ratings of 'Golden Delicious' and 'York Imperial'.

Descriptors for starchiness and astringency were significantly higher in CA stored apples (Table 6.4). Panelists might have perceived some starch that remained from harvest in CA storage. Starch index with the iodine test (Bartram *et al.*, 1993) at harvest was 3.9 on a 1 - 6 scale (1 = non starch breakdown, 6 = all starch hydrolyzed). The magnitude of the difference between RA and CA stored apples could be a contrast effect between no starch in RA stored fruit, and a little starch present in CA stored fruit. Ratings for the astringent descriptor decreased in RA storage in all cultivars tested (Watada *et al.*, 1980). The feeling of astringency has long been accepted to be due to the binding between polyphenolic compounds with saliva proteins (Bate-Smith, 1973). Some acids also induce astringency: malic acid at 0.037% or 0.075% (w/v) in water induced stronger intensity responses for astringency than for sourness (Straub, 1989). Therefore, the higher astringent ratings for CA stored fruit may be due to the higher malic acid content of the fruit. Finally, it is not clear whether all panelists could distinguish clearly the difference between the feeling left in the mouth by starch coating and the puckering sensation characteristic of astringency.

#### CONCLUSION

A descriptive sensory study of 'Gala' apples stored in air and controlled atmosphere showed a significant decrease in fruity and floral descriptors (pear-, bananaand strawberry-like) for EA, IA and flavor of CA stored fruit. Descriptor differences were due to a decrease in volatile ester production, mostly hexyl acetate, butyl acetate and 2-methylbutyl acetate. Green, vegetative and citrus descriptors were perceived with higher intensities in cut fruit aroma and flavor. Volatiles responsible for those odors are released by the cells upon cutting. The higher ratings for those descriptors in CA stored fruit was probably due to the decrease in fruity aromas, because volatile aldehydes responsible for green-like odors decreased in CA stored fruit.

The combination of DSA, GC and *Osme* analyses explained earlier findings from a consumer panel with 'Gala' apples (Boylston *et al.*, 1994). In that study, the decrease in total volatiles resulted in a lower acceptance of delayed CA stored 'Gala' apples with the same firmness values as RA stored fruit. On the other hand, firmer apples are usually preferred (Williams, 1979; Daillant-Spinnler *et al.*, 1996). Transferring apples to air storage for 4 weeks following 16 weeks CA improved volatile production. However, the increase in volatile esters was not perceived as increased fruity flavor in our panel.

The use of CA technology allows maintenance of quality and freshness of apples from harvest compared to air storage. However, the complexity of the effects of physical and chemical stimuli on human taste buds and olfactory receptors require sensory analysis following CA experiments to evaluate fruit eating quality.

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## CHAPTER 7 SUMMARY AND CONCLUSION

'Gala' apple aroma was evaluated by using Osme, a gas chromatography and olfactometry (GCO) technique. During the optimization phase, headspace sampling time and trap type were compared, and panelists' performances evaluated. Of the volatile compounds identified by gas chromatography and mass spectrometry, esters were odoractive with different fruity odors. The aroma of hexyl acetate and pentyl acetate most closely approximated that of emitted by 'Gala' apples. Butyl acetate and 2-methylbutyl acetate were produced in the largest amounts, and were described as solvent and fruity. Butyl 2-methylbutyrate, hexyl 2-methylbutyrate, butyl hexanoate and hexyl butyrate were perceived as apple or green apple. Methyl 2-methylbutyrate, ethyl 2-methylbutyrate and propyl 2-methylbutyrate had sweet, fruity, berry-like odors. Butyl butyrate was perceived as cheesy. Non ester compounds with characteristic odors were 4-allylanisole (anise) and  $\beta$ -damascenone (grape juice). Unidentified peaks had cucumber or watermelon, mushroom, adhesive tape or skunk odors. Analysis of variance for peak intensities and peak areas showed a sampling time effect. Peaks perceived 100% of the time from the 24 hour charcoal traps could be ranked by decreasing order of perceived intensity. 'Gala' headspace sampled with activated charcoal for 24 hours yielded the largest amount of volatile compounds and the largest number of odor-active peaks. Therefore this sampling method was used for the storage study.

GCO provided information for individual compounds presented to the nose via an olfactometer. Compounds that had intense odor activity were mixed in water then the mixture odors were compared with 'Gala' apples. Compounds at concentrations found in apple headspace were mixed based on the odor unit theory (Teranishi *et al.*, 1991), or following a statistical screening design. The design based on odor units theory did not give information as to which mixture most closely resembled 'Gala' odor, because all the sample combinations had an odor close to 'Gala'. The design based on statistical screening revealed that hexyl acetate, hexanal, butyl acetate, 2-methylbutyl acetate and methyl 2-methylbutyrate contributed to the least differences between mixtures and apples. Pentyl acetate, hexyl 2-methylbutyrate, butyl hexanoate and 4-allylanisole contributed to the largest differences. Solutions were prepared based on analytical results from 'Gala' headspace, and headspace sampling tends to be more representative of lower molecular weight volatile compounds. Although *Osme* analysis showed that compounds other than those used in the mixtures were present in 'Gala' headspace, similarities to 'Gala' aroma were still apparent. Validation of GCO with model solutions, although a necessary step to understand compound interactions, is limited by the information obtained from the previous GC analysis.

Changes in 'Gala' aroma during storage were characterized using Osme analysis and by descriptive sensory analysis (DSA). During the first year of the study, apples were stored 5, 10 and 19 weeks at 1 °C in regular (RA) and controlled atmosphere (CA). In the second year of the study, apples were stored 4, 10 and 20 weeks in RA and CA; one treatment was 16 weeks in CA followed by 4 weeks in RA (CA/RA). DSA was only performed during the second year. Production of volatile esters decreased along with fruity aroma during CA storage. Hexyl acetate and butyl acetate were emitted in the largest amounts in RA but decreased significantly in CA. 2-Methylbutyl acetate, also produced in large quantities by fruit stored in RA, did not decrease as much in CA as butyl acetate and hexyl acetate. 2-Methylbutyl acetate remained at RA concentrations until 16 weeks CA, and was perceived accordingly with high intensity. 2-Methylbutan-1ol, the acyl moiety of 2-methylbutyl acetate, was also less affected by CA storage. Butyl 2-methylbutyrate and hexyl 2-methylbutyrate contributed to the apple, fruity aroma of RA stored fruit. Methyl 2-methylbutyrate, ethyl 2-methylbutyrate and propyl 2methylbutyrate were perceived less intensely than hexyl acetate and butyl acetate in RA stored fruit, but did not decrease as much in CA. A multivariate factor analysis of peaks perceived using Osme revealed 4-allylanisole (anise), β-damascenone (grape juice), 1octen-3-ol (mushroom) and an unknown compound with a watermelon odor were perceived mostly in RA stored fruit.

DSA of 'Gala' apples confirmed the significant decrease in fruit aroma following CA. Specific descriptors from DSA and *Osme* analysis did not necessarily correspond.

For instance, a floral descriptor contributed to 'Gala' aroma in DSA, but was not perceived intensely during *Osme* analysis. The watermelon compound strongly perceived in RA stored fruit during *Osme* did not have an important weight in DSA. A musty note appeared in CA stored fruit, which could be the same as a garlic odor peak during *Osme*. Green and grassy descriptors in DSA of cut apples were not explained by any peak in *Osme* analysis. Those attributes are usually due to aldehydes released upon cutting the cells; this explain their absence from samples used for *Osme* analysis. 'Gala' apples stored 16 weeks in CA followed by 4 weeks in RA emitted more volatiles than fruit stored 20 weeks in CA. The difference in volatile production was perceived by *Osme* analysis, and differences in overall fruity aroma between 16 and 20 weeks CA stored fruit were perceived only for whole fruit external aroma. There was no difference between those two types of storage for fruit flavor.

Relationship between compound quantity and odor intensity obtained with *Osme* has allowed determination of the key compounds contributing to 'Gala' aroma. However, the exact reproduction of 'Gala' aroma through model mixtures was not achieved. The sampling technique chosen, although representative of the overall 'Gala' headspace, still gave a distorted image of 'Gala' headspace. Preferential adsorptions and desorptions, interactions between solute-solute and solute-solvent certainly occurred. Additionally, headspace sampling could not give information on volatiles in the matrix such as those glycosidically bound; those compounds no doubt contribute to the specific 'Gala' flavor. Finally, GC-MS showed some limitation at identifying some compounds below the detection limit of MS, but perceived by the nose. Compounds with watermelon, mushroom, cucumber and skunk odors yet remain to be identified. Predictive models to determine aroma when compounds vary in different proportions is necessary. The later determination would be useful to postharvest physiologists because volatiles produced by apples vary in different proportions when stored in CA as opposed to RA.

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**APPENDICES** 

F

1994-95	Date	Weight	Fthylene	Firmpose	SSC	_ 11	
	measured	(g)	(npm)	(N)	(°Briv)	рн	Intratable
Harvest	12-Sep		<u> </u>	70.7	<u>( DI IX)</u> 12 7		Acidity (% malic)
				, .,,	12.7	-	0.304
5 RA	26-Oct	192.78	86.89	57.20	12.87	3.84	0.307
		(19.77)	(45.64)	(4.56)	(0.34)	(0.06)	(0.02)
10 RA	29-Nov	179.01	163.45	51.54	12.74	4.02	0.295
		(14.19)	(49.99)	(2.05)	(0.53)	(0.05)	(0.023)
19 RA	28-Jan	180.20	358.24	46.51	12.65	4 17	0.213
		(18.03)	(101.28)	(4.14)	(0.48)	(0.10)	(0.030)
9 C A	19-Nov	187.80	34 66	67 22	12.16	2.76	( , , , , , , , , , , , , , ,
	17-1404	(10 / 6)	J4.00	07.33	13.10	3.76	0.338
		(18.40)	(29.06)	(5.91)	(0.74)	(0.05)	(0.032)
18 CA	22-Jan	197.49	23.53	65.12	12.87	3.87	0.303
		(22.35)	(24.78)	(6.43)	(0.58)	(0.06)	(0.026)
1005-06							
harvest	12-Sen	217.01	2.46	80.82	12.74		0.400
nui (Ost	12.000	(28.05)	2.40	60.65	13.74	-	0.498
		(28.03)	(0.93)	(8.25)	(0.73)		(0.053)
4 RA	17-Oct	214.4	267.6	55.07	13.99	3.88	0.371
		(23.86)	(96.24)	(5.66)	(0.84)	(0.06)	(0.042)
10 RA	1-Dec	219.4	758.7	49.06	13.27	3 85	0 328
		(27.99)	(200.57)	(2.37)	(0.65)	(0.05)	(0.025)
20 D A	0.5-1	014.0	<b>5</b> 01 <b>5</b>		. ,	(0.02)	(0.023)
20 KA	9-Feb	214.2	731.7	43.63	13.14	4.00	0.264
		(30.16)	(266.20)	(4.42)	(0.67)	(0.07)	(0.022)
10 CA	29-Nov	232.1	52.7	60.14	14.54	3.83	0.394
		(24.87)	(31.89)	(4.89)	(0.68)	(0.04)	(0.038)
16 CA	14-Feb	220.8	179.8	55 58	13.83	3 70	0.264
		(26.42)	(82.38)	(5 20)	(0.57)	(0.02)	0.304
<b>.</b>		/	(0=:00)	(5.20)	(0.57)	(0.03)	(0.028)
20 CA	7-Feb	222.1	26.0	60.46	13.18	3.78	0.359
		(22.78)	(23.54)	(7.85)	(0.89)	(0.07)	(0.036)

Table A.1. 'Gala' maturity<sup>a</sup> and ripening indices after storage.
Storage was: 5, 9, 10, 18, 19 weeks in regular (RA) or controlled (CA) atmosphere in 1994-95; 4, 10 and 20 weeks in RA or CA in 1995-96.
16 CA is 16 weeks in CA followed by 4 weeks in RA in 1995-96.
Values are means (standard deviation) of 20 individual fruit.

<sup>a</sup>: Starch index on a 1 - 6 scale (1 = no starch breakdown, 6 = all starch hydrolyzed) with iodine test at harvest: 2.4 in 1994-95, 3.9 in 1995-96

			Storage T	ype	
		RA		CA	
			Storage Duration	n (Weeks)	7.4 <del>8</del>
Compound	5	10	19	9	18
Propyl acetate	160.2 <sup>ab</sup>	98.4 bc	270.6 <sup>a</sup>	13.3 °	3.2 °
Butyl acetate <sup>x, y</sup>	2821.8 <sup>a</sup>	4879.3 <sup>a</sup>	4738.7 <sup>a</sup>	769.1 <sup>b</sup>	101.1 6
Pentyl acetate <sup>x</sup>	221.9 <sup>ab</sup>	271.1 <sup>a</sup>	395.1 <sup>a</sup>	71.6 <sup>b</sup>	19.1 <sup>b</sup>
Hexyl acetate <sup>x, y</sup>	2722.6 <sup>ab</sup>	4430.6 <sup>a</sup>	4021.3 <sup>ab</sup>	1397.4 bc	350 1 °
Heptyl acetate	22.8 <sup>ab</sup>	30.6 <sup>a</sup>	33.1 <sup>a</sup>	11.9 <sup>ab</sup>	53 <sup>b</sup>
Total acetate esters	5949.3	9710.1	9458.8	2263.3	478.8
Propyl propanoate <sup>x</sup>	29.2 <sup>ab</sup>	14.7 <sup>bc</sup>	48.6 <sup>a</sup>	0.0 °	0 0 °
Butyl propanoate <sup>x</sup>	536.5 <sup>b</sup>	582.8 <sup>b</sup>	1098.6 <sup>a</sup>	33 5 °	8.0°
Pentyl propanoate	15.8 <sup>a</sup>	14.6 <sup>a</sup>	24.7 <sup>a</sup>	34 <sup>b</sup>	33 <sup>b</sup>
Hexyl propanoate <sup>x</sup>	428.9 <sup>b</sup>	459.1 ab	804.6 <sup>a</sup>	75.8 °	34 8 °
Total propanoate esters	1010.2	1071.2	1976.5	112.8	46.3
Propyl butyrate	23.1 <sup>b</sup>	13.0 <sup>bc</sup>	39.9 <sup>a</sup>	0.0 °	٥٥،
Butyl butyrate <sup>x</sup>	466.1 <sup>b</sup>	528.0 <sup>b</sup>	959.7 <sup>a</sup>	81.7 °	22.3 °
Total butyrate esters	489.2	540.9	999.6	81.7	22.3
Propyl hexanoate + pentyl butyrate	116.0 <sup>ab</sup>	77.5 <sup>bc</sup>	179.0 <sup>a</sup>	17.0 °	10.5 °
Butyl hexanoate <sup><math>x</math></sup> + hexyl butyrate <sup><math>x</math></sup>	2103.1 <sup>a</sup>	2568.4 <sup>a</sup>	3882.2 <sup>a</sup>	620.2 <sup>b</sup>	208 0 <sup>b</sup>
Hexyl hexanoate	983.1 abc	1705.8 ab	2072.5 <sup>a</sup>	$643.9^{bc}$	200.0 231.5 °
Total hexanoate esters	3202.2	4351.7	6133.6	1281.0	450.0
Butyl heptanoate	22.3 <sup>b</sup>	18.1 <sup>bc</sup>	42.5 <sup>a</sup>	6.6 <sup>c</sup>	23.8 <sup>b</sup>
Hexyl octanoate	28.2 <sup>ab</sup>	61.5 <sup>a</sup>	53.9 <sup>a</sup>	1.0 <sup>b</sup>	· 9.2 <sup>b</sup>

Table A.2. Volatile compounds emitted by 'Gala' apples after regular (RA) or controlled atmosphere<br/>(CA) storage (1% O2, 1% CO2) in 1994-95. Values (relative FID peak area) are means of 4<br/>replicates of dynamic headspace of 1 kg apples<sup>z</sup>. Total volatiles by chemical group are presented

## Table A.2, Continued

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2-Methylpropyl acetate	57.5 <sup>b</sup>	50.0 <sup>b</sup>	160.8 <sup>a</sup>	25.8 <sup>b</sup>	21 2 <sup>b</sup>
2-Methylbutyl acetate <sup>x, y</sup>	1963.2	1564.2	2667.1	1555.5	1138 1
3-Methyl-2-butenyl acetate <sup>x</sup>	14.6 <sup>b</sup>	11.3 <sup>b</sup>	24.1 <sup>b</sup>	28.3 <sup>b</sup>	49 9 <sup>a</sup>
3-Methylbutyl propanoate	29.6 <sup>a</sup>	13.5 <sup>ab</sup>	22.1 <sup>ab</sup>	89 <sup>b</sup>	12 4 <sup>b</sup>
2-Methylbutyl butyrate	0.1	0.1	0.1	0.1	0.1
3-Methylbutyl hexanoate	24.6 <sup>b</sup>	18.3 <sup>b</sup>	27.7 <sup>ab</sup>	32.1 <sup>ab</sup>	49.0 <sup>a</sup>
Butyl 2-methylpropanoate	16.2 <sup>a</sup>	14.9 <sup>a</sup>	28.2 <sup>a</sup>	1.4 <sup>b</sup>	0 0 <sup>b</sup>
Hexyl 2-methylpropanoate	38.7 <sup>ab</sup>	33.9 <sup>abc</sup>	70.1 <sup>a</sup>	14.2 bc	5.0°
Total methyl propanoate esters	54.9	48.8	98.3	15.6	5.2
Methyl 2-methylbutyrate <sup>x</sup>	16.9 <sup>b</sup>	9.1 <sup>b</sup>	147.9 <sup>a</sup>	1.5 <sup>b</sup>	0 0 <sup>b</sup>
Ethyl 2-methylbutyrate <sup>x</sup>	1.7 <sup>b</sup>	0.0 <sup>b</sup>	6.9 <sup>a</sup>	0.0 <sup>b</sup>	0.0 b
Propyl 2-methylbutyrate <sup>x</sup>	46.4 <sup>ab</sup>	16.4 bc	66.9 <sup>a</sup>	0.8 °	0.0 °
Butyl 2-methylbutyrate <sup>x</sup>	859.1 <sup>b</sup>	612.7 <sup>b</sup>	1639.9 <sup>a</sup>	64.1 °	13.1 °
Hexyl 2-methylbutyrate <sup>x</sup>	1531.9 <sup>b</sup>	1243.4 bc	3075.0 <sup>a</sup>	337.1 <sup>cd</sup>	87.2 <sup>d</sup>
Total methyl butyrate esters	2455.9	1881.6	4936.7	403.5	100.4
Hexyl tiglate <sup>x</sup>	24.7 <sup>ab</sup>	27.9 <sup>ab</sup>	41.7 <sup>a</sup>	19.7 <sup>ab</sup>	5.9 <sup>b</sup>
6-Methyl-5-hepten-2-one <sup>x</sup>	25.2 <sup>a</sup>	30.0 <sup>a</sup>	17.2 <sup>ab</sup>	4.2 <sup>b</sup>	4.9 <sup>b</sup>
4-Allylanisole <sup>x</sup>	102.1 <sup>b</sup>	70.0 <sup>b</sup>	218.1 <sup>a</sup>	54.2 <sup>b</sup>	69 0 <sup>b</sup>
Anethole <sup>x</sup>	98.4 <sup>bc</sup>	132.9 <sup>ab</sup>	201.6 <sup>a</sup>	49.0 <sup>bc</sup>	23.6 °
1-Butanol	386.0 <sup>b</sup>	1393.5 <sup>a</sup>	989.5 <sup>a</sup>	68.7 <sup>b</sup>	41.0 <sup>b</sup>
1-Pentanol	4.6 <sup>b</sup>	7.0 <sup>ab</sup>	10.8 <sup>a</sup>	0.0 °	0.0 °
1-Hexanol	63.1 <sup>bc</sup>	122.7 <sup>ab</sup>	158.4 <sup>a</sup>	20.8 °	7.5 °
2-Methyl-1-butanol	96.8 <sup>ab</sup>	83.4 <sup>ab</sup>	153.4 <sup>a</sup>	51.6 <sup>b</sup>	48.5 <sup>b</sup>
Total alcohols	550.5	1606.5	1312.1	141.0	96.9

<sup>2</sup> Means followed by the same letter are not significantly different within the same line by the Fisher protected LSD test, alpha = 0.05<sup>x</sup> Odor active compounds at those concentrations <sup>y</sup> Above the linear range of the FID detection

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Table A.3. Perceived aroma peak area in 'Gala' headspace after regular (RA) or controlled atmosphere (CA)<br/>storage by Osme analysis in 1995-96. Values (I max\*duration of odorant perception) are means of<br/>4 replicates for 3 panelists<sup>z</sup>.

					Storage T	уре			
				RA			CA		
	Kovats		Storage Duration (Weeks)						
Peak #	Index	Descriptor (Compound)	4	10	20	10	16 <sup>x</sup>	20	
20	1028	Gala, ripe, pear (Hexyl acetate)	1.79 °	0.99 <sup>bc</sup>	1.25 <sup>b</sup>	0.85 °	0.35 <sup>d</sup>	0.23 <sup>d</sup>	
4	827	Nail polish, gala (Butyl acetate)	1.70 <sup>a</sup>	0.94 °	1.25 <sup>b</sup>	0.64 <sup>d</sup>	0.29 <sup>e</sup>	0.06 <sup>e</sup>	
7	890	Solvent, gala (2-Methylbutyl acetate)	1.26 <sup>a</sup>	0.65 <sup>b</sup>	0.67 <sup>b</sup>	1.03 <sup>a</sup>	0.67 <sup>b</sup>	0.43 <sup>b</sup>	
2	787	Sweet, fruity (Methyl 2-methylbutyrate)	0.78 <sup>a</sup>	0.86 <sup>a</sup>	0.85 <sup>a</sup>	0.72 <sup>a</sup>	0.78 <sup>a</sup>	0.08 <sup>b</sup>	
13	959	Very sweet, strawberry (Propyl 2-methylbutyrate)	0.54 <sup>a</sup>	0.43 <sup>ab</sup>	0.42 <sup>ab</sup>	0.42 <sup>ab</sup>	0.33 <sup>b</sup>	0.07 °	
6	863	Sweet, strawberry (Ethyl 2-methylbutyrate)	0.47 <sup>b</sup>	0.49 <sup>ab</sup>	0.69 <sup>a</sup>	0.43 <sup>b</sup>	0.29 <sup>bc</sup>	0.12 °	
23	1056	Fruity, apple (Butyl 2-methylbutyrate)	0.32 <sup>a</sup>	0.16 <sup>b</sup>	0.32 <sup>a</sup>	0.12 bc	0.03 <sup>cd</sup>	0.01 <sup>d</sup>	
36	1255	Apple, grapefruit (Hexyl 2-methylbutyrate)	0.32 <sup>a</sup>	0.10 bc	0.21 <sup>ab</sup>	0.07 °	0.04 °	0.01 °	
31	1205	Green apple (Butyl hexanoate + Hexyl butyrate)	0.35 <sup>b</sup>	0.17 °	0.51 <sup>a</sup>	0.18 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	
19	1018	Apple and toast (Unknown)	0.78 <sup>a</sup>	0.24 <sup>b</sup>	0.15 <sup>bc</sup>	0.15 bc	0.14 bc	0.01 °	
16	997	Fruity, tape (6-Methyl-5-hepten-2-one)	0.17 <sup>a</sup>	0.03 <sup>b</sup>	0.01 <sup>b</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.01 b	
18	1010	Solvent, gala (Unknown)	0.17 <sup>a</sup>	0.02 <sup>cd</sup>	0.13 <sup>ab</sup>	0.09 bc	$0.04^{cd}$	0.00 <sup>d</sup>	
27	1121	Apple (Hexyl propanoate)	0.17 <sup>a</sup>	0.06 bc	0.10 <sup>ab</sup>	0.01 bc	0.00 °	0.00 °	
17	1009	Rotten apple (Butyl butyrate)	0.08 <sup>a</sup>	0.11 <sup>a</sup>	0.13 <sup>a</sup>	0.06 <sup>ab</sup>	0.00 <sup>b</sup>	0.00	
44	1524	Fruity (Unknown)	0.07 <sup>ab</sup>	0.10 <sup>a</sup>	0.03 <sup>ab</sup>	$0.03^{ab}$	0.00 b	0.00 b	
10	921	Fruity, apple (Butyl propanoate)	0.05	0.05	0.04	0.00	0.00	0.00	
3	813	Fruity (Propyl propanoate)	0.00	0.01	0.04	0.06	0.00	0.00	
12	933	Fruity, sweet, solvent (3-Methyl-2-butenyl acetate)	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>	0.31 a	0.02	0.02	
		Total fruity	9.01	5.40	6.80	4 86	2 00	1.05	
42	1.405					7.00	4,77	1.05	
43	1437	Grape juice (B-Damascenone)	1.49 <sup>a</sup>	0.24 <sup>b</sup>	0.30 <sup>b</sup>	0.25 <sup>b</sup>	0.11 <sup>b</sup>	0.00 <sup>b</sup>	
38	1276	Grape juice (Unknown)	0.08 <sup>a</sup>	0.00 <sup>b</sup>	0.02 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	
		Total grape juice	1.57	0.24	0.32	0.25	0.11	0.00	

## Table A.3, Continued

26	1118	Floral (Unknown)	0.09 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>
24	1070	Watermelon (Unknown)	0.48 <sup>a</sup>	0.04 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0 00 <sup>b</sup>
	1227	Cucumber (Unknown)	0.02	0.04	0.00	0.00	0.00	0.00
		Total watermelon, cucumber	0.50	0.08	0.00	0.00	0.00	0.00
33	1222	Anise, licorice (4-Allylanisole)	0.61 <sup>a</sup>	0.11 <sup>b</sup>	0.10 <sup>b</sup>	0.15 <sup>b</sup>	0.03 <sup>b</sup>	0.01 <sup>b</sup>
22	1041	Sweet, anise (Unknown)	0.06 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.06 <sup>a</sup>	$0.01^{a}$	0.00 6
29	1151	Anise, spice, perfumey (Unknown)	0.12 <sup>a</sup>	$0.02^{ab}$	0.00 <sup>b</sup>	0.12 <sup>a</sup>	$0.02^{ab}$	0.00 <sup>ab</sup>
		Total anise	0.79	0.13	0.10	0.34	0.07	0.04
35	1230	Mushroom, cat urine (Unknown)	0.35 <sup>b</sup>	0.15 <sup>cd</sup>	0.58 <sup>a</sup>	0.29 <sup>bc</sup>	0.07 <sup>d</sup>	0.04 <sup>d</sup>
14	991	Mushroom (1-Octen-3-ol)	0.16 <sup>a</sup>	0.01 <sup>b</sup>	0.02 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>
		Total mushroom	0.51	0.16	0.60	0.29	0.07	0.04
5	836	Skunk (Unknown)	0.69 <sup>a</sup>	0.22 <sup>b</sup>	0.23 <sup>b</sup>	0.29 <sup>b</sup>	0.28 <sup>b</sup>	0 22 <sup>b</sup>
25	1112	Dusty, musty (Unknown)	0.56 <sup>a</sup>	0.39 <sup>ab</sup>	0.27 <sup>b</sup>	0.31 <sup>ab</sup>	0.12 <sup>b</sup>	0.26 <sup>b</sup>
37	1272	Rubber (Unknown)	0.51 <sup>a</sup>	0.24 <sup>b</sup>	0.09 <sup>c</sup>	0.05 °	0.02 °	0.20°
8	901	Oatmeal, skunk (Unknown)	0.08 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 b	0.00 b
21	1038	Metallic, skunk (Unknown)	0.06 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 b
		Total skunk, rubber	1.90	0.85	0.59	0.64	0.42	0.48
1	774	Tea, garlic, leaves (Unknown)	0.15 <sup>bc</sup>	0.12 bc	0.23 <sup>ab</sup>	$0.41^{a}$	0.20 <sup>bc</sup>	0.04 °
30	1197	Tape or fruity (Unknown)	0.09 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.01 b	0.00 6	0.04 0.02 <sup>b</sup>
41	1364	Tape or musty, dirty (Unknown)	0.07 <sup>b</sup>	0.01 °	0.18 <sup>a</sup>	0.01 °	0.03 60	0.02
		Total tape, others	0.30	0.13	0.42	0.44	0.03	0.05
9	920	Garlic (Unknown)	0.04 <sup>b</sup>	0.00 <sup>b</sup>	0.02 <sup>b</sup>	<b>0.21</b> <sup>a</sup>	0.18 <sup>a</sup>	0.07 <sup>b</sup>

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<sup>2</sup> Means followed by the same letter are not significantly different within a row by the Fisher protected LSD test, alpha = 0.05

<sup>y</sup> Kovats indices on RTX-5 (5% diphenyl 95% dimethyl polysiloxane) column

<sup>x</sup> 16 weeks in CA was followed by 4 weeks in RA

Table A.4.	Perceived aroma peak area in 'Gala' headspace after regular (RA) or controlled atmosphere (CA) storage by <i>Osme</i> analysis in 1994-95. Values ( $I_{max}$ * duration of odorant perception) are means of $A$ replicates for 3 penalists <sup>2</sup>
	4 replicates for 3 panelists <sup>2</sup> .

			Storage Type				
	V			RA	<u>-</u> - <u>-</u> <u>-</u>	CA	
	Kovats'			S	storage Durat	ion (Weeks)	·····
Peak #	Index	Descriptor (Compound)	5	10	19	9	18
20	1028	Gala, ripe, pear (Hexyl acetate)	1.98 <sup>ab</sup>	2.46 <sup>a</sup>	2.31 <sup>a</sup>	1.03 bc	0.48 <sup>c</sup>
4	827	Nail polish, gala (Butyl acetate)	1.70 <sup>a</sup>	1.80 <sup>a</sup>	1.64 <sup>a</sup>	0.45 <sup>b</sup>	0.10 <sup>b</sup>
7	890	Solvent, gala (2-Methylbutyl acetate)	1.34 <sup>a</sup>	1.05 <sup>ab</sup>	1.09 <sup>ab</sup>	0.90 <sup>ab</sup>	0.61 <sup>b</sup>
2	787	Sweet, fruity (Methyl 2-methylbutyrate)	0.78 <sup>a</sup>	0.75 <sup>a</sup>	$0.50^{ab}$	0.35 <sup>ab</sup>	0.19 <sup>b</sup>
13	959	Very sweet, strawberry (Propyl 2-methylbutyrate)	0.59 <sup>a</sup>	0.68 <sup>a</sup>	0.50 <sup>ab</sup>	0.36 <sup>ab</sup>	0.20 <sup>b</sup>
6	863	Sweet, strawberry (Ethyl 2-methylbutyrate)	0.62 <sup>a</sup>	0.62 <sup>a</sup>	0.57 <sup>a</sup>	0.24 <sup>b</sup>	0.23 <sup>b</sup>
23	1056	Fruity, apple (Butyl 2-methylbutyrate)	0.50 <sup>a</sup>	0.53 <sup>a</sup>	0.34 <sup>ab</sup>	0.10 <sup>b</sup>	0.00 <sup>b</sup>
36	1255	Apple (Hexyl 2-methylbutyrate)	0.20 <sup>b</sup>	0.56 <sup>a</sup>	0.12 <sup>b</sup>	0.13 <sup>b</sup>	0.02 <sup>b</sup>
36b	1260	Grapefruit (Unknown)	0.36	0.12	0.21	0.00	0.00
31	1205	Green apple (Butyl hexanoate + Hexyl butyrate)	0.18 bc	0.55 <sup>a</sup>	0.47 <sup>ab</sup>	0.09 <sup>c</sup>	0.01 °
16	997	Fruity, tape (6-Methyl-5-hepten-2-one)	0.23	0.04	0.12	0.05	0.02
18	1010	Solvent, gala (Unknown)	0.23 <sup>ab</sup>	0.27 <sup>a</sup>	0.18 <sup>ab</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>
27	1121	Apple (Hexyl propanoate)	0.40 <sup>a</sup>	0.36 <sup>a</sup>	0.34 <sup>a</sup>	0.00 <sup>b</sup>	0.02 <sup>b</sup>
17	1009	Rotten apple (Butyl butyrate)	0.35 <sup>a</sup>	0.19 <sup>ab</sup>	0.28 <sup>a</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>
44	1524	Fruity (Unknown)	0.13	0.13	0.02	0.06	0.03
10	921	Fruity, apple (Butyl propanoate)	0.20	0.36	0.30	0.00	0.03
39	1290	Fruity (Unknown)	0.14	0.15	0.04	0.16	0.05
3	813	Fruity (Propyl propanoate)	$0.07^{ab}$	0.25 <sup>a</sup>	0.10 <sup>ab</sup>	0.00 <sup>b</sup>	0.00 b
12	933	Fruity, sweet, solvent (3-Methyl-2-butenyl acetate)	0.08 <sup>ab</sup>	0.00 <sup>b</sup>	0.07 <sup>ab</sup>	$0.06^{ab}$	$0.00^{\circ}$
		Total fruity	10.08	10.87	9.19	4.04	2.20
43	1437	Grape juice (B-Damascenone)	0.37	0.31	0.23	0.41	0.18

## Table A.4, Continued

24	1070	Watermelon (Unknown)	0.48 <sup>a</sup>	0.63 <sup>a</sup>	0.40 <sup>ab</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>
34	1227	Cucumber (Unknown)	0.05 <sup>ab</sup>	0.22 <sup>a</sup>	0.11 <sup>ab</sup>	0.08 <sup>ab</sup>	0.00 <sup>b</sup>
		Total watermelon, cucumber	0.53	0.85	0.51	0.08	0.00
33	1222	Anise, licorice (4-Allylanisole)	0.47 <sup>b</sup>	0.31 bc	0.71 <sup>a</sup>	0.22 <sup>c</sup>	0.21 °
22	1041	Perfumey, anise (Unknown)	0.31 <sup>ab</sup>	0.33 <sup>a</sup>	0.29 <sup>abc</sup>	0.03 <sup>bc</sup>	0.00 <sup>c</sup>
		Total anise	0.78	0.64	1.00	0.25	0.21
35	1230	Mushroom, cat urine (Unknown)	0.36 <sup>a</sup>	0.36 <sup>a</sup>	0.29 <sup>ab</sup>	0.06 <sup>b</sup>	0.02 <sup>b</sup>
40	1353	Mushroom (Hexyl tiglate)	0.17 <sup>a</sup>	0.25 <sup>a</sup>	0.13 <sup>ab</sup>	0.10 <sup>ab</sup>	0.00 <sup>b</sup>
		Total mushroom	0.53	0.62	0.41	0.16	0.02
5	836	Skunk (Unknown)	0.86 <sup>a</sup>	0.52 <sup>ab</sup>	0.45 <sup>b</sup>	0.56 <sup>ab</sup>	0.35 <sup>b</sup>
	1272	Rubber (Unknown)	0.26 <sup>a</sup>	0.25 <sup>a</sup>	0.15 <sup>ab</sup>	• 0.00 <sup>b</sup>	0.02 <sup>ab</sup>
		Total skunk, rubber	1.11	0.76	0.60	0.56	0.37
9	920	Garlic (Unknown)	0.00 <sup>b</sup>	<b>0.00</b> <sup>b</sup>	0.00 <sup>b</sup>	0.27 <sup>a</sup>	0.09 <sup>b</sup>

<sup>2</sup> Means followed by the same letter are not significantly different within a row by the Fisher protected LSD test, alpha = 0.05<sup>y</sup> Kovats indices on RTX-5 (5% diphenyl 95% dimethyl polysiloxane) column

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## Appendix 5: Relation between Osme response and compound concentrations

During the development of the *Osme* method, Da Silva *et al.* (1994) found that in general, a logarithmic or power function was generated between the response for peak intensity and stimulus concentration, while peak area produced a linear response. In our study, as the design was not planned for that purpose, we could not validate the response curves with the lack of fit test (Neter *et al.*, 1989). However, because there was a large range of concentrations being analyzed for some of the compounds, response curves relating peak intensity and peak area to compound concentration were generated (Figure A.1 and A.2). The data showed that: 1) panelists do not all have the same sensitivity and/or do not use the category scale in the same manner, 2) intensity responses tended to flatten out with a logarithmic fit and area responses were more often of a power type, and 3) response curves are relevant only if the range of stimuli induce responses that are in the range covered by the extremes of the category scale.

Butyl acetate (Gala, solvent, fruity) and hexyl acetate (Gala, ripe apple, pear) concentrations covered the whole range of intensity responses (Figure A.1). All panelist curves had a good fit, with R<sup>2</sup> above 0.65. Intensity response curves followed a logarithmic function for all three panelists for butyl acetate, and a power function for hexyl acetate. There was a good fit for power and linear functions for peak area responses of butyl acetate and hexyl acetate. No good fit was found for 2-methylbutyl acetate (solvent) intensity responses; all ratings were high, indicating concentrations were at the plateau of perception for that compound. However, a slightly better fit with higher slopes for the linear functions were found for the peak area response. The difference between peak intensity and peak area response curves for butyl acetate, hexyl acetate and 2-methylbutyl acetate could indicate the psychological upper limits imposed to panelists with a category scale. Peak area is not constrained over time, therefore the curves could follow power functions with higher slopes.

No good fit was found for ethyl 2-methylbutyrate (strawberry) (Figure A.1). This compound has a very low odor threshold and was present at the lower limit of detection for the FID; however, intensity ratings ranged from 2.0 to 10.0. Logarithmic and linear functions were found between methyl 2-methylbutyrate (sweet fruity), propyl 2-methylbutyrate (very sweet, strawberry), butyl 2-methylbutyrate (fruity, apple) and hexyl 2-methylbutyrate (apple, grapefruit) concentrations and peak intensities (Figure A.2). Panelists had different sensitivities and reproducibilities, as was shown by the curve slopes and R-squares. The poor fits found for methyl 2-methylbutyrate and propyl 2-methylbutyrate indicated those compounds were not presented over a wide range of concentrations, and more dilute solutions would have clarified the psychophysical response to those compounds. The best curve fits for the series of methyl butyrate esters were found for butyl 2-methylbutyrate peak intensities and peak areas.

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- Figure A.1. Response curves for butyl acetate, hexyl acetate, 2-methylbutyl acetate and ethyl 2-methylbutyrate perceived intensity and peak area by *Osme* versus compound concentration. Each point is one panelist response. Best curve fits are shown with corresponding R<sup>2</sup>.
- Figure A.2. Response curves for methyl 2-methylbutyrate, butyl 2-methylbutyrate, propyl 2-methylbutyrate and hexyl 2-methylbutyrate perceived intensity and peak area by Osme versus compound concentration. Each point is one panelist response. Best curve fits are shown with corresponding R<sup>2</sup>.

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Appendix 5 - Figure A.1

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Appendix 5 - Figure A.1 (continued)

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Appendix 5 - Figure A.2



Appendix 5 - Figure A.2 (continued)

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