ANALYSIS OF AROMA CONSTITUENTS IN CULTIVATED STRAWBERRIES BY GC/MS

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ABSTRACT

In aroma analysis, strawberries have always been the favored fruit because of their relatively high content of typical and pleasant aroma constituents. Esters, aldehydes, alcohols and sulfur compounds have been found to be the main aroma components in strawberry. In recent years, two volatile compounds, 2,5-dimethyl-4-methoxy-3(2H)-furanone (DMF) and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DHF) were reported to contribute heavily to strawberry aroma. These two compounds have been found in all wild strawberries studied, but found only in few cultivated varieties.

In this work, three kinds of cultivated strawberries were sampled and analyzed. The three strawberries all belong to the Camarosa variety. They came from different growing areas: Salinas (California), Orrville (Ohio), and Memphis (Tennessee). The volatile compounds of these three strawberries were separated by Gas Chromatography (GC), and identified by Mass Spectrometer Detector (MSD). Column and experimental conditions were optimized for this particular separation.

Salinas, Orrville and Memphis strawberries have very similar aroma constituents, however, in slightly differing amounts. Several unique peaks were found in each strawberry, which may well account for the differences in the aroma qualities of the three. 2-Furaldehyde was found in both Memphis and Orrville strawberries, but not in Salinas. It is a key odor compound correlated with woody aroma and it has a low odor threshold value. These two properties make it contribute negatively to the pleasant aroma of Memphis and Orrville strawberries.

A compound, 2-furanmethanol, was found only in Salinas strawberries. This compound has a faint burning aroma, however, its high odor threshold value offsets its potentially bad aroma. DMF was found in all three strawberries, but no DHF was detected in any of the three. We propose a possible explanation for the absence of DHF. Ethyl (methylthio) acetate, which is a sulfur-containing compound, was found in both Orrville and Salinas strawberries. This work is the first to report its presence in strawberries of any variety.

An external standard method was employed to quantify seven main aroma components found in the strawberry extracts. Aroma values were introduced and then calculated together with sensory descriptions of these compounds. Salinas strawberry was found to have the best aroma quality of the three. These results indicate that the odors of strawberries of the same variety can be different when grown in different geographical areas.

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I own my greatest thanks to my family. I feel very lucky that in each of my growing steps, I have always had their deepest love, endless support, constant encouragement, sincere care and real understanding. I expect this thesis will be the best gift to partially repay them.

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CHAPTER 1

INTRODUCTION

Background of Strawberry

Strawberry is a small plant of the *Rosaceae* (Rose) family. Most varieties of the strawberry plant, approximately 40 species, belong to the *Fragaria* genus. It grows both as a wild as well as a cultivated plant.[1]

Strawberry is not really a berry or a fruit, but it is instead the enlarged ends of the plant's stamen. It has seeds on the outside skin rather than on the inside, as most berries do. Strawberry plants can be planted in any garden soil, but the richer the soil, the larger the crop. Soil rich in siliceous clayey elements is favored. The plant grows best in a cool, moist climate and does not do well in warm temperatures. The plants may be planted in the spring or fall depending on the temperature. Strawberries grow close to the ground on the stem in groups of three. When the strawberry ripens, the petals of the flower fall off and all that remains is the calyx, a leafy substance is shaped like a star and the greenish white fruits turn to a rich red color. Not every flower produces fruit.

Strawberry is one of the most popular fruits because of their pleasant aroma, palatable taste and attractive color. The strawberry is consumed as fresh fruit and also largely as conserved or as manufactured products like jams, sherbets, ice creams, yogurts, and juices. The food industry determines which strawberries will be used for preserves based for most part on the color, size and firmness of the fruit. Usually better color, medium to large size and moderate firmness are preferred qualities.

The strawberries analyzed in this work were cultivated in Salinas (California), Orrville (Ohio), and Memphis (Tennessee). They all belong to the Camarosa variety. Camarosa is a vigorous strawberry plant, which produces large and firm fruit throughout most of its fruiting cycle. It is one of the commercial short day varieties bearing in June. The strawberries of this variety can maintain huge berry size throughout the season. The interior color of Camarosa is a brilliant red, and the fruit colors are uniform. It is flavorful so that it is favored by food industries and restaurants. The yield potential of it is high to excellent, and it also makes good shipping material.

Historical Work

Because of the popularity of strawberry and its favorable typical aroma, the volatile constituents of cultivated and wild strawberries have been extensively studied since the first results were reported in 1939 by Coppens and Hoejenbos.[2-3] Strawberry aroma is associated with a complex mixture of alcohol, aldehydes, esters and sulfur compounds.[4-8] Both 2,5-diemthyl-4-hydroxy-3(2H)-furanone (DHF) and 2,5-diemthyl-4-methoxy-3(2H)-furanone (DMF), first identified in pineapple, were found in arctic bramble, beef broth, roasted almonds, coffee and soy sauce[9-11]. They were reported by several groups to be the most important aroma compounds in strawberries.[5-7,12-14] Both compounds have strong, sweet and pleasant odors, therefore they contribute greatly to the strawberry typical aroma. DMF and DHF have been found in all wild strawberries studied; however, they have been found in only a few cultivated varieties.[4-8,15] Very few volatile sulfur compounds have been identified in fruits. In strawberries, to my knowledge, only five sulfur compounds have been found. They are hydrogen sulfide, methanethiol, dimethyl disulfide, methylthiol acetate and methylthiol butyrate. Although sulfur compounds are usually present in low amounts, they are still very important contributors to the aroma of strawberries.[5]

With improved analytical instrumentation more and more aroma compounds were found and identified in strawberries. Chemists began to realize that in order to understand more completely and clearly the aroma of a fruit, it is not sufficient to only know the chemical constituents; it also needs to know the relationship between the nature of the constituents and their sensory evaluations. Sensory analysis, in short, is the evaluation of the flavor of a certain food system by human sensory organs. Since this discovery, the study of fruit flavors (including strawberry) is no longer simply studying the chemical constituents. Today both chemical identifications and organoleptic descriptions provide a more complete and accurate description of fruit flavors.

Gas Chromatography in Aroma Analysis

In almost all studies of strawberry aroma constituents, Gas Chromatography (GC) is used to separate the volatile compounds. The Mass Selective Detector (MSD) combined with Gas Chromatography (GC) is often employed to identify those compounds, thus both qualitative and quantitative results are achieved.

The reasons why the GC is ideally suited to aroma studies can be explained based upon the character of the aroma study itself and the main advantages of GC.

Aroma most commonly involves the study of volatile compounds. Gas Chromatography is well known to be the best-suited method for studies of volatile compounds. GC has better resolution and lower detection limits than HPLC in most cases.

In the recent past, capillary GC columns have become the standard in aroma studies. Typically a fused silica column provides about 50,000 to 500,000 theoretical plates depending on the column length. Therefore, a capillary GC column has excellent resolving power. By comparison, the maximum plate count per HPLC column is about 50,000.

The very thin, uniform liquid film (~0.2µm) coated inside the wall of the capillary column permits rapid mass transfer between the gas and liquid phases and thereby very high linear gas velocities are possible, resulting in shorter analysis times.

Fused silica columns have excellent inertness and high tensile strength, which offers exceptional durability. Their flexibility and small diameter make possible the direct insertion of the column into both the injection port and detector of the GC, thus minimizing contact of the aroma compounds with hot metal surfaces. Compound decomposition and discrimination are therefore minimized.

Capillary columns can enhance detection limits by reducing column bleed (background/noise) and by increasing the concentration per unit time of the compound in the detector. Less bleed can be achieved by using the more recently introduced bonded phase columns.

Detectors often used in GC analysis have very good Minimum Detectable Quantities (MDQ). The Flame Ionization Detector (FID) can detect down to 10^{-11}g (~50ppb). The MDQ of the Thermal Conductivity Detector (TCD) is about 10^{-9}g (~10ppm), and that of the Electron Capture Detector (ECD) is10^{-12}g, which is very sensitive. The Mass Selective Detector (MSD) typically has an MDQ of about 10^{-10}g in the scan mode and 10^{-12}g in single ion monitoring (SIM) mode.

Gas Chromatography does have its disadvantages. Capillary columns have limited capacity. Typically only micrograms of sample can be accommodated on the thin films. This problem can be improved by the development of wide bore thick film bonded phase columns. GC can not confirm the identities or structures of the peaks, which can be solved by combining GC with a Mass Selective Detector (MSD).

HPLC has also been applied to aroma studies, however, because of the limitations stated above, it is better suited to those compounds with low vapor pressure or high molecular weight like sugars, proteins, peptides, and amino acids.

Supercritical Fluid Extraction (SFE) is becoming more attractive in food analysis. A primary advantage of SFE is that carbon dioxide (CO_2) replaces large volumes of organic solvents formerly used in sample preparation. Carbon dioxide is non-toxic, cheap, and available in high purity. Only small volumes are needed, and it can be removed very readily from the sample after separation.[16-20]

Aroma Value

Aroma is the most important parameter determining the flavor of foods and beverages. Hundreds of aroma compounds are present in foods and beverages. Not all of these contribute the same amount to the flavor, instead, only a small number of volatile constituents determine the flavor character. These compounds are called " character impact compounds". It is not necessary for chemists to identify all of the aroma compounds, they can focus on the “character impact compounds”. Rothe and Thomas (1963) suggested the term “aroma value” to represent and compare the contributions of these compounds to aroma impression.

Aroma value is the ratio of the concentration of an aroma compound in a food product to its odor threshold value. It is also called “odor value”, “odor unit”, and “flavor activity”. [21] In this thesis, the term “aroma value” is used.

$$AromaValue = \frac{[concentration]}{[odorthreshold]}$$

The concentration is simply the amount of the compound in the food of interest. The threshold, in short, is the lowest concentration of a compound in a particular food system that contributes to the flavor. Thus, compounds with low threshold values are flavorful even at low concentrations. On the other hand, compounds with high threshold value do not affect the overall aroma if they are present in amounts lower than their odor thresholds.[21-23]

CHAPTER 2

EXPERIMENTAL

Instrumentation

The Gas Chromatograph used in this work was a Hewlett Packard (HP) Model 6890 coupled with a Hewlett Packard (HP) Model 5973 Mass Selective Detector (MSD). (See Figure 1)

GC/MS combines the high resolving power of GC with the positive identification capability of MSD. This combination makes possible quantitative trace analysis down to the ppb level. In this work, the GC was equipped with a split/splitless capillary injector. The MSD was controlled by the data system, which featured HP MSD ChemStation software including calibration (tune) of the MSD, acquisition and analysis of data.

The HP 5973 Mass Selective Detector (MSD) is designed for use with the HP 6890 series GC. Figure 2 shows a schematic of the GC/MSD.

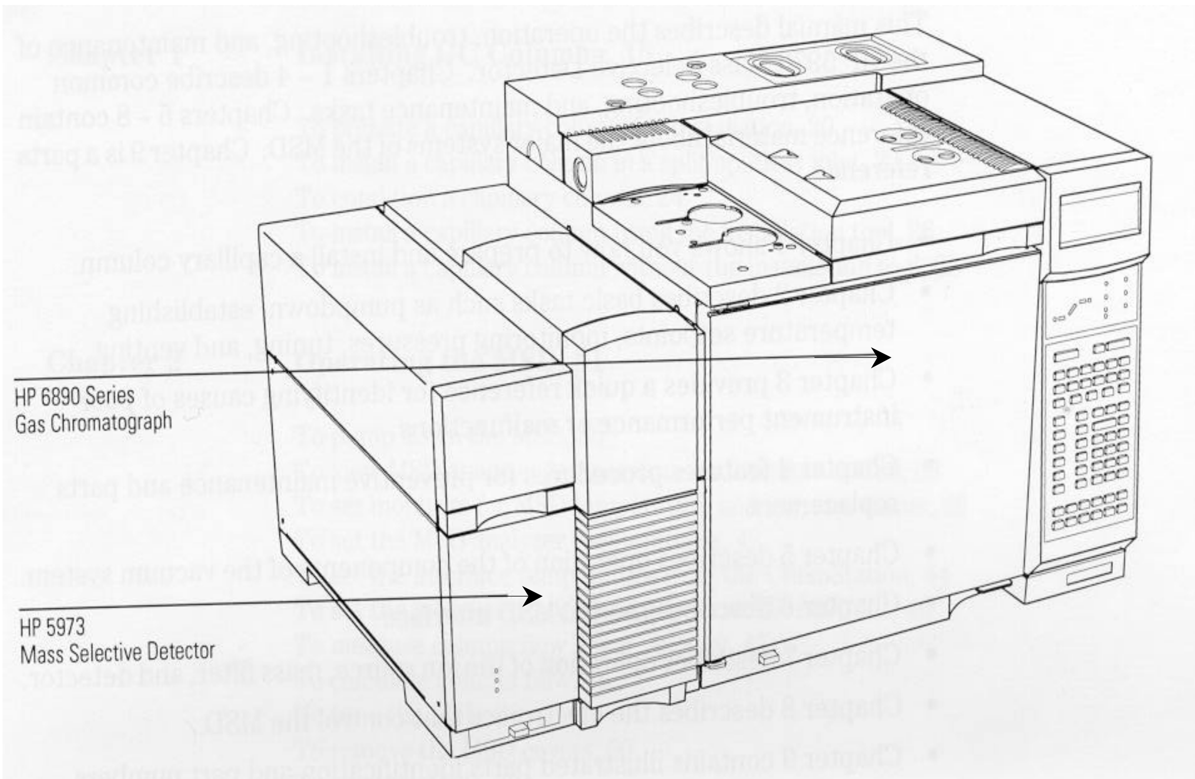


Figure 1 GC/MS HP6890/5973

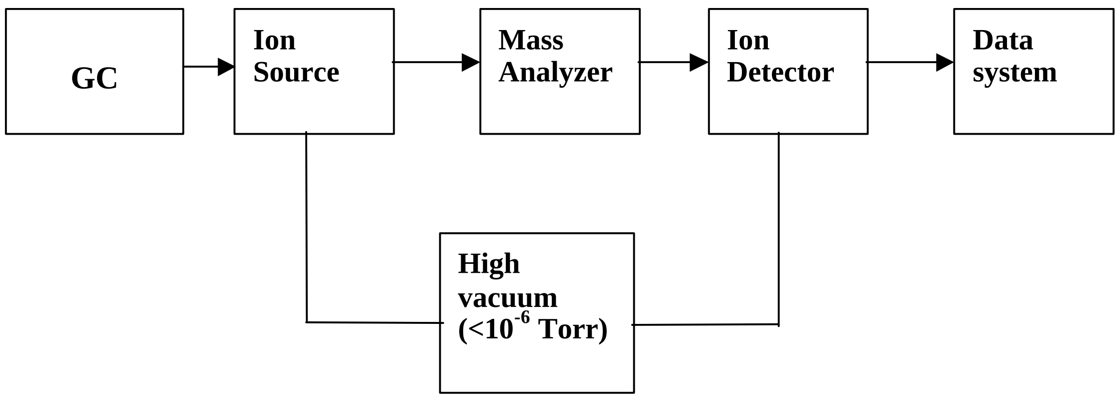


Figure 2   Schematic of a Mass Spectrometer with Sample Inlet from GC

The HP 5973 is a bench-top mass spectrometer. It features a 250L/sec turbomolecular high vacuum pump, rotary vane foreline pump, independently heated Electron-Ionization (EI) ion source, independently heated hyperbolic quadrupole mass filter, high–energy dynode electron multiplier type detector, independently heated GC/MSD interface and power supplies as well as instrument control electronics.

High vacuum, usually lower than 10^{-6} Torr, is imperative for MSD operation. Vacuum avoids or minimizes the collisions of the interested ions with carrier gas or other molecules, thus minimizing the loss of analyte ions.

Packed columns are seldom used in GC/MSD today, because their high carrier gas flow rates, e.g. 50ml/min, are incompatible with the vacuum system required for mass spectrometry. The bulk of the carrier gas needs to be removed preferentially from the sample components prior to their introduction into the mass spectrometer.

Flexible fused silica capillary columns are preferred for GC/MS since they can be fed directly from the GC oven into the ion source of the mass spectrometer. The high vacuum system of the mass spectrometer easily accommodates the low carrier gas flow rates employed in capillary GC, e.g. 0.5-2ml/min.

Electron-Ionization (EI) at 70ev was used as the ion source. Figure 3 is the schematic illustration of EI. The entire source works at high vacuum.

Sample effluents from the GC column are introduced into the ionization source where they collide with a high-energy electron beam produced by a hot filament, usually accelerated through a 70ev potential difference. Sample molecules are ionized to first form molecular ions ($M^{+\cdot}$), which gives the information of molecular weight in mass spectrum. Later dissociation and fragmentation produce other ions characteristic of the analyte. (See Figure 4)

Quadrupole Mass Analyzers are popular and widely used in GC/MS work because of their relatively simple operation. There are four parallel hyperbolic rods in a quadrupole analyzer (Figure 5). Both radio frequency (RF) and alternating DC voltage are applied to opposite pairs of rods. Only those ions controlled by a specific ratio of RF to DC voltage can pass through the rods and reach the detector. Other ions will either collide with the rods or be lost to the vacuum. A mass spectrum is achieved by varying (scanning) the ratio of radio frequency to DC voltage.

GC effluents are at atmospheric pressure; the MSD inlet must operate under vacuum, preferably 10^{-6} Torr. Figure 6 illustrates this problem.

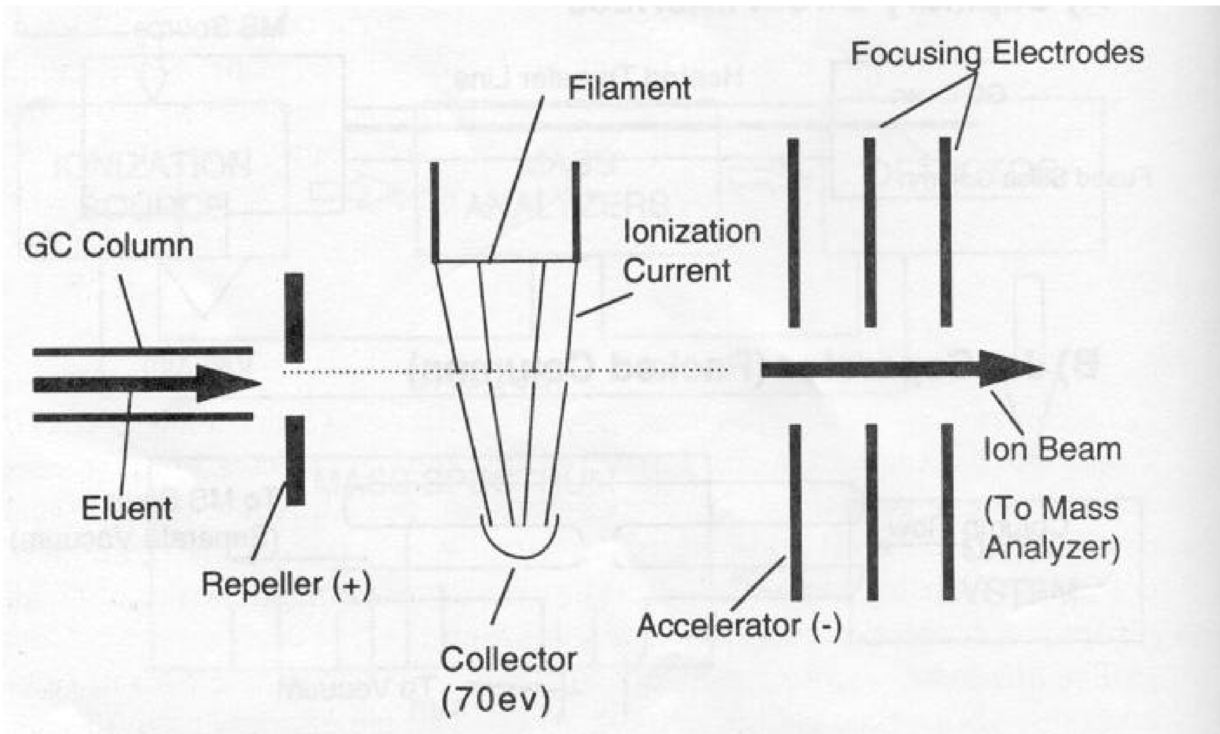


Figure 3 Schematic Illustration of Electron-Ionization

$$\mathbf{M + e = M^{+\cdot} + 2e} \begin{array}{l} \nearrow \mathbf{A^{+} + R^{\cdot}} \textbf{ (radical)} \\ \\ \searrow \mathbf{B^{+\cdot} + N} \textbf{ (neutral)} \end{array}$$

Figure 4 Fragmentation of Molecules in Electron-Ionization Source

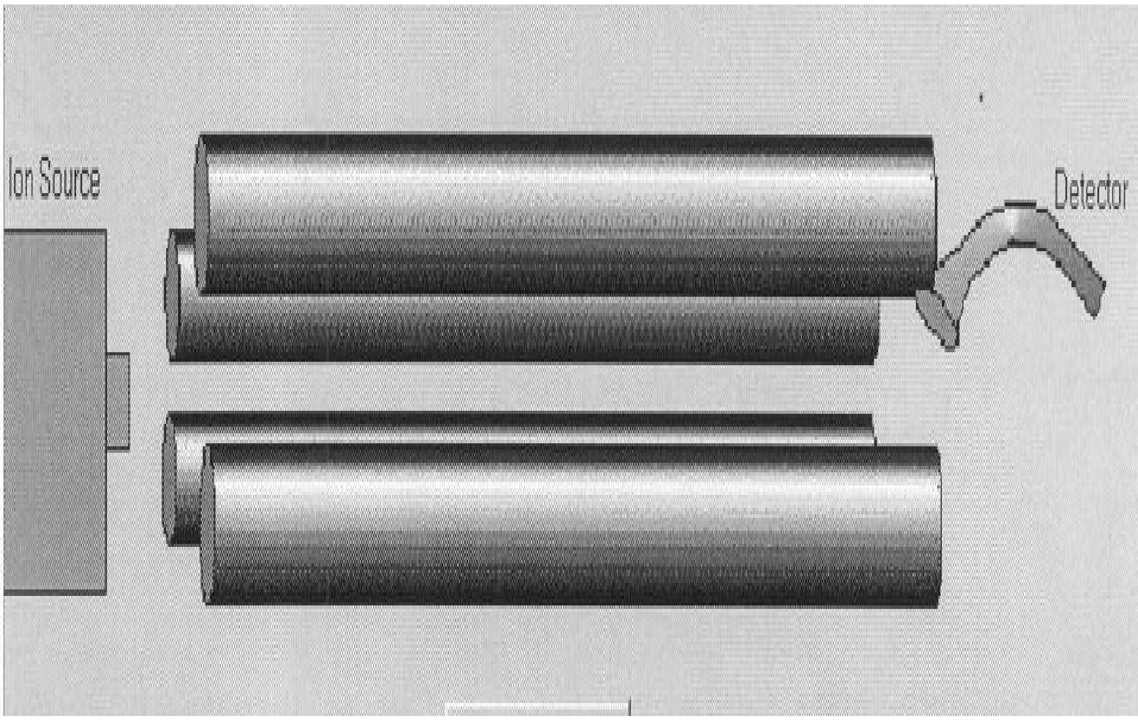


Figure 5   Quadrupole Mass Analyzer in Mass Spectrometer.

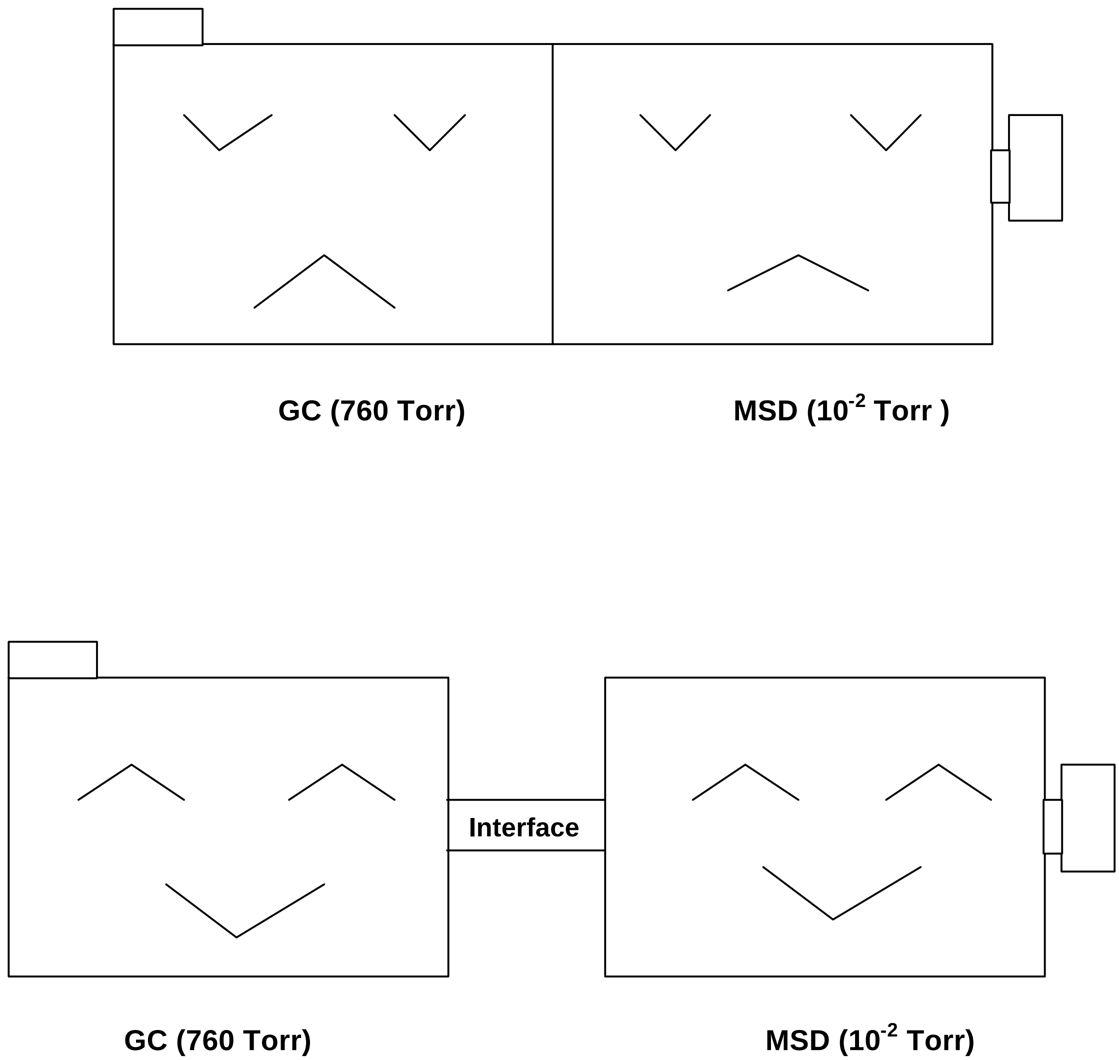


Figure 6 Comical Illustration of Combining GC with MSD

The interface used to connect GC to the MSD is a capillary direct interface. The capillary column of the GC passes directly into the ionization source. If the total flow is less than 5 ml/min, the vacuum system can handle this amount of gas. The interface is heated to avoid adsorption and condensation of the analyte in the interface. Capillary direct interfaces provide higher analysis speed, better detectability, and there are minimal dead volumes, catalytic or adsorption effects.[16, 18-19, 24]

Sample Preparation Method

The three strawberry extracts were provided by the J. M. Smucker Company (Orrville, Ohio). The strawberries were harvested at the same time, in the same ripening stage. Distillation columns and condensing coils were used to distill and condense the volatile constituents that came from the extraction procedures. The samples supplied were concentrates of the essence of strawberries. The exact processing method is proprietary.

Column Selection

In this work, three GC columns were evaluated to optimize the resolution of strawberry aroma. Table 1 compares the performances of these three columns.

Table 1 Comparison of Performances of Three Capillary Columns

*The comparison is limited to these three columns

Column	Composition	Polarity	Bleed	Capacity	Resolution	Disadvantage
DB-5MS (30×0.25×0.25)	5% phenyl 95% methyl-polysiloxane	less polar	Very low	fair	excellent	low capacity
RTX-20 (30×0.32×3.0)	20% phenyl 80% methyl-polysiloxane	semi-polar	low	excellent	good	peak broadening and discrimination of less volatile compounds
Carbowax (50×0.32×0.32)	Poly-ethylene-glycol	polar	maximum temperature of 220°C	good	good	not suitable for non-polar analyte, high bleed

DB-5MS was determined to be the best for this work. DB-5MS is 5% phenyl 95% methyl polysiloxane. “MS” is a symbol to show that the column is more suitable for Mass Spectrometer work due to the higher temperature stability of the stationary phase (i.e. much lower column bleed). The polarity of this 5% phenyl column is between nonpolar (100% polymethyl siloxane column) and polar (polyethylene glycol column), so both polar and nonpolar analytes can be well separated by this column. The column parameters (30m×0.25mm×0.25μm) are commonly used ones.

The other two columns were not as good for this application. The stationary phase Carbowax 20M column is not stable above 200°C; this limits the range of compounds that could be analyzed. The RTX-20 column tested has very good capacity, but its thick liquid film causes peak broadening which results in lower resolution.

GC/MS Operating Conditions

The GC/MS operating conditions are given in Tables 2 and 3. Conditions were optimized in order to get the best resolution, best identification and the least analysis time.

Experimental Procedures

GC/MS Analysis of Samples:

Inject 0.2µl of undiluted strawberry extract into the GC manually using a 10µl syringe. Each of the three samples was analyzed in triplicate under the same optimized GC/MS conditions.

GC/MS Analysis of Standards:

An External Standard Method was employed to quantify seven important aroma constituents found in the three strawberries. Calibration standards included: methyl butyrate, ethyl butyrate, ethyl (methylthio) acetate, linalool, ethyl cinnamate, ethyl acetate and 2-furaldehyde. All of the standard compounds were from Aldrich Chemical Co. (Milwaukee, WI). The first five were prepared as 100, 4 and 2ppm; Ethyl acetate was prepared at 100, 50 and 4ppm and 2-furaldehyde at 100, 20 and 10ppm by serial dilution with methanol. Each standard, 0.2µl, was manually injected and run in triplicate. The three peak areas were averaged for each calibration point. A separate calibration curve was plotted for each standard. The concentration of each aroma constituent was determined using these calibration curve. The Relative Standard Deviation (%RSD) was reported for all data.

Table 2 HP 6890 GC Operating Conditions

OVEN Initial Temperature: 50°C hold 2 min Ramp 1: 15°C to 140°C Ramp 2: 25°C to 280°C Run Time: 13.60min
INLET Mode: split Inlet Temperature: 250°C Pressure: 8.0psi Split Ratio: 20:1 Sample Size: 0.2µl
CAPILLARY COLUMN Mode: DB-5MS Column Length: 30.0m Column Diameter: 0.25mm Column Film Thickness: 0.25µm Pressure: 8.0psi Carrier Gas: Helium Flow Rate: 1.0ml/min Average Linear Velocity: 37cm/sec

Table 3 HP 5973 MSD Operating Conditions

<u>INTERFACE</u> Type: Capillary Direct Interface Temperature: 285°C
<u>TUNE FILE</u> Atune.u
<u>DATA ACQUISITION</u> Mode: scan mode(TIC) Mass Range: Low Mass: 10amu High Mass: 500amu
<u>SOLVENT DELAY</u> 0 min
<u>MS ZONES</u> MS Quadrupole Temperature: 150°C, maximum 200°C MS Source: 250°C, maximum 300°C
<u>EM VOLTAGE</u> 1670.6v

CHAPTER 3

RESULTS AND DISSCUSIONS

Chromatographic Results

Figures 7,8,9 are the total ion chromatograms of Memphis, Orrville and Salinas strawberries. Four interesting compounds, DMF, ethyl (methylthio) acetate, 2-furaldehyde and 2-furanmethanol, are labeled. Figure 9 also gives the extended chromatograms to show the peaks of 2-furanmethanol and ethyl (methylthio) acetate more clearly. Similar profiles in the three chromatograms indicate similar constituents exist in the three strawberries. However, each strawberry still has its unique peak(s), and different peak areas show different amounts of certain aroma compounds in the three strawberries. Comparing the peak areas, the Salinas strawberries have the largest amounts of many aroma compounds.

Tables 4,5,6 list the main constituents found in the strawberries; the Mass Spectrometer match qualities are listed as well, which show how close our spectrum matches the spectrum in the MSD Library. Table 7 shows the comparisons of the compounds found in the three strawberries and their match qualities.

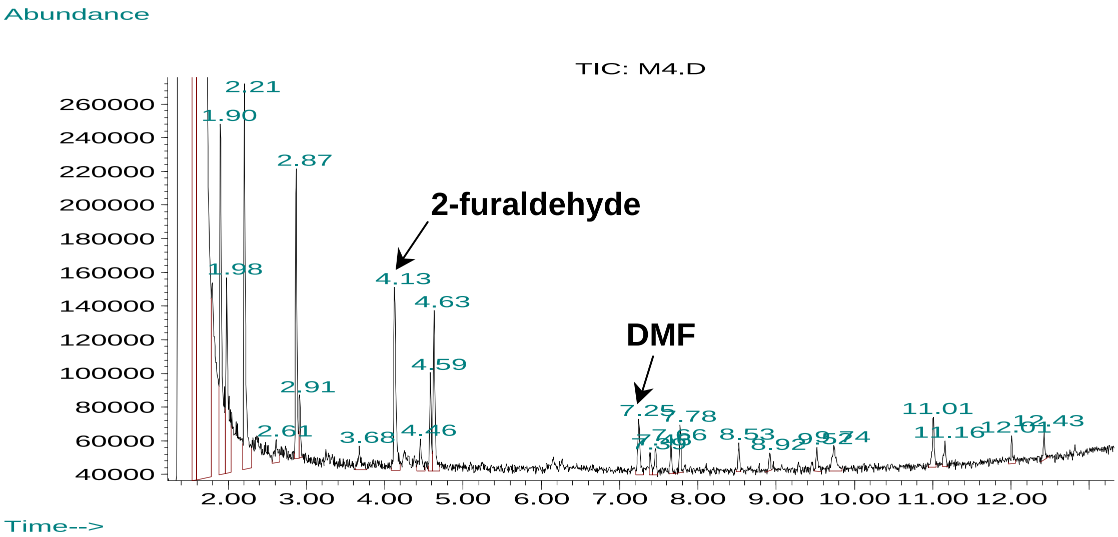


Figure 7 Total Ion Chromatogram of Memphis Strawberry

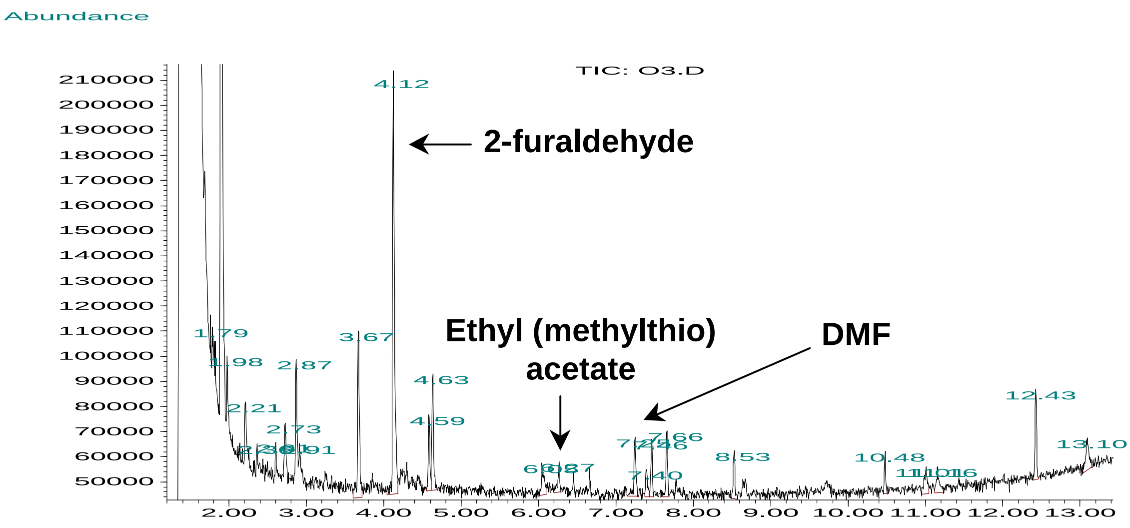


Figure 8   Total Ion Chromatogram of Orrville Strawberry

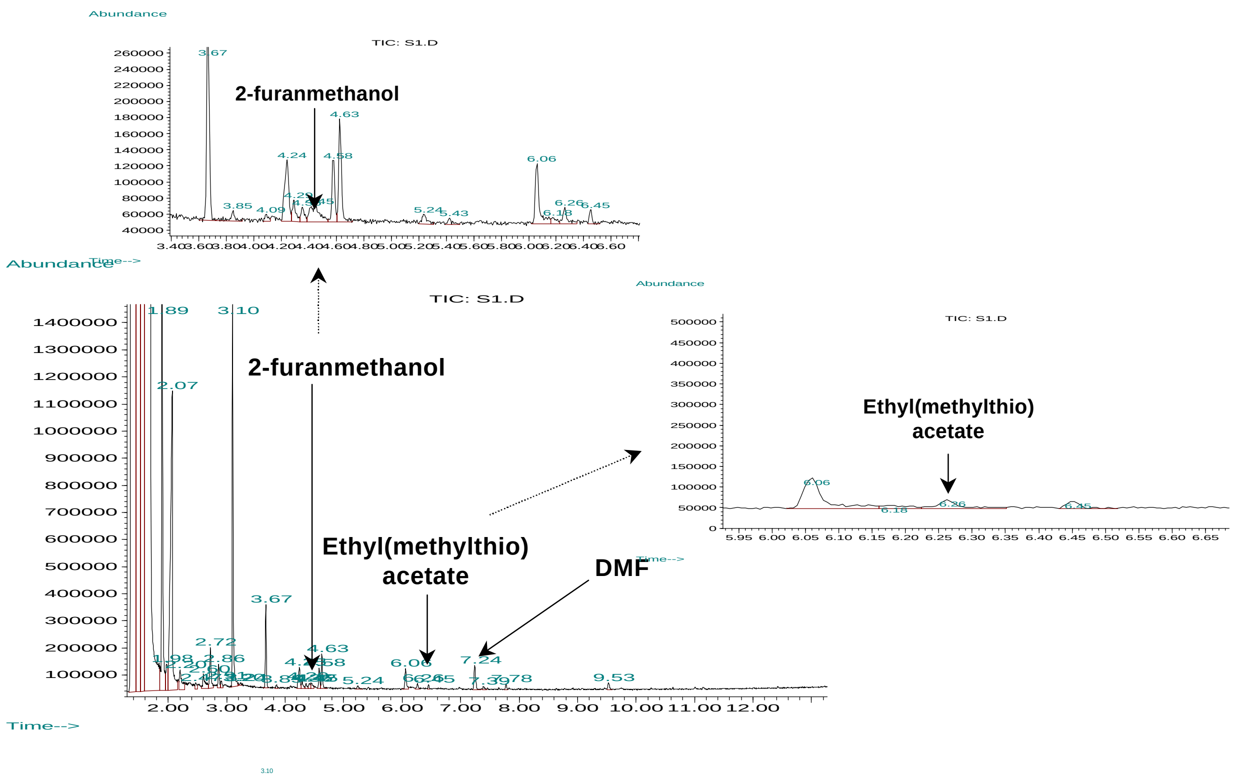


Figure 9 Total Ion Chromatogram of Salinas Strawberry

Table 4 Main Aroma Compounds in Memphis Strawberries

Compounds	Retention Time (min)	Quality Match(%)
Ethanol	1.48	90
Ethyl Acetate	1.90	91
2-Methyl-1-propanol	1.98	91
1-Butanol	2.21	91
Ethyl Butyrate*	3.68	59*
2-Furaldehyde	4.13	95
(Z)-2-Hexen-1-ol	4.59	91
DMF	7.25	81
Linalool	7.78	86
Benzyl Acetate	8.52	95
Ethyl Cinnamate	11.16	87

Table 5 Main Aroma Compounds in Orrville Strawberries

Compounds	Retention Time (min)	Match Quality (%)
Ethanol	1.48	90
Ethyl Acetate	1.90	91
2-Methyl-1-propanol	1.98	86
Methyl Butyrate	2.73	78*
Ethyl Butyrate	3.67	95
2-Furaldehyde	4.12	94
(Z)-2-Hexen-1-ol	4.59	90
Ethyl (methylthio)Acetate	6.26	83
DMF	7.25	81
Benzene Acetate	8.52	91
Ethyl Cinnamate	11.16	96

Table 6 Main Aroma Compounds in Salinas Strawberries

Compounds	Retention Time (min)	Match Quality (%)
Ethanol	1.48	90
Ethyl Acetate	1.90	90
Acetic Acid	2.02	91
Methyl Butyrate	2.72	91
Ethyl Butyrate	3.67	95
2-Furanmethanol	4.42	96
(Z)-2 Hexen-1-ol	4.58	91
Hexanoic Acid	6.06	90
Ethyl (methylthio) Acetate	6.26	87
DMF	7.24	91
Linalool	7.78	94

Table 7 Comparisons of the Compounds Found in the Three Strawberries and their match qualities

	MS Library Quality Match(%)		
Compounds	Memphis	Orrville	Salinas
Ethanol	90	90	90
Ethyl Acetate	91	91	90
Methyl Butyrate	ND	78(?)	91
Ethyl Butyrate	59(?)	95	95
1-Butanol	91	ND	ND
Acetic Acid	ND	ND	91
2-Furaldehyde	95	94	ND
2-Furanmethanol	ND	ND	96
DMF	81	81	91
(Z)-2-hexen-1-ol	91	90	91
Hexanoic Acid	ND	ND	90
Ethyl(methylthio)Acetate	ND	83	87
Linalool	86	ND	94
Ethyl Cinnamate	87	96	ND
Benzyl Acetate	95	91	ND

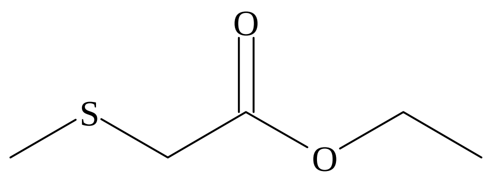
ND= Not Detected

? indicates our spectrum didn't match the MSD library very well

Most volatile compounds listed have high library match qualities except ethyl butyrate in Memphis strawberries and methyl butyrate in Orrville strawberries. This means a lower probability of correct peak identification. Ethyl butyrate and methyl butyrate are two important contributors to strawberry odor besides DHF and DMF.

Methyl butyrate was not found in Memphis strawberries, but it was found in Orrville strawberries that had a low library match quality (78%). In Salinas strawberries it had high match quality (91%). Ethyl butyrate was identified in all three strawberries and had a high library match quality in Salinas strawberries (95%) and in Orrville strawberries (95%), but it had a low library match quality in Memphis strawberries (59%). Low match qualities are probably due to the low concentrations of the compounds present or to the presence of overlapping peaks at low concentration.

Ethyl (methylthio) acetate is a sulfur-containing compound. Only five volatile sulfur compounds, hydrogen sulfide, methylthiol, dimethyl disulfide, methylthiol acetate and methylthio butyrate[5], have been reported to be found in strawberries. This work is the first to report the presence of ethyl (methylthio) acetate in strawberries. Figure 10 shows the structure of this sulfur-containing compound.



$C_5H_{10}O_2S$

Figure 10 Chemical Structure of Ethyl (methylthio) acetate

Ethyl (methylthio) acetate was found only in Salinas and Orrville Strawberries. The concentrations found were low. Sulfur compounds usually exist in relatively low amount in vegetable and fruit flavor; however, they do play an important role because of its very low odor threshold.[5]

Linalool was found in both Salinas and Memphis strawberries, but not in Orrville. The match qualities were 94% and 86% respectively. 2-Furaldehyde of high match quality was identified only in Memphis strawberries (95%) and Orrvillle strawberries (94%). A peak only found in Salinas was identified to be 2-furanmethanol, match quality of it was high (96%)

As expected 2,5-diemthyl-4-methoxy-3(2H)-furanone (DMF) was found in all three strawberries. However, in none of the three strawberries could 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DHF) be found. Under same conditions, standard DHF eluted at 7.15min (Figure 11), in the three chromatograms there are no peaks around 7.15min, which proved that no DHF was found in any strawberry. This result agrees with Ana G. Perez et al.[7] that "DMF and DHF have not been found in all cultivated strawberries, although they have been found in all wild strawberries reported…". There is no good explanation for this result so far [5-7,12-13]. In this work, we propose an explanation for the absence of DHF in most of the cultivated strawberries based on both this work and the historical work [25-27].

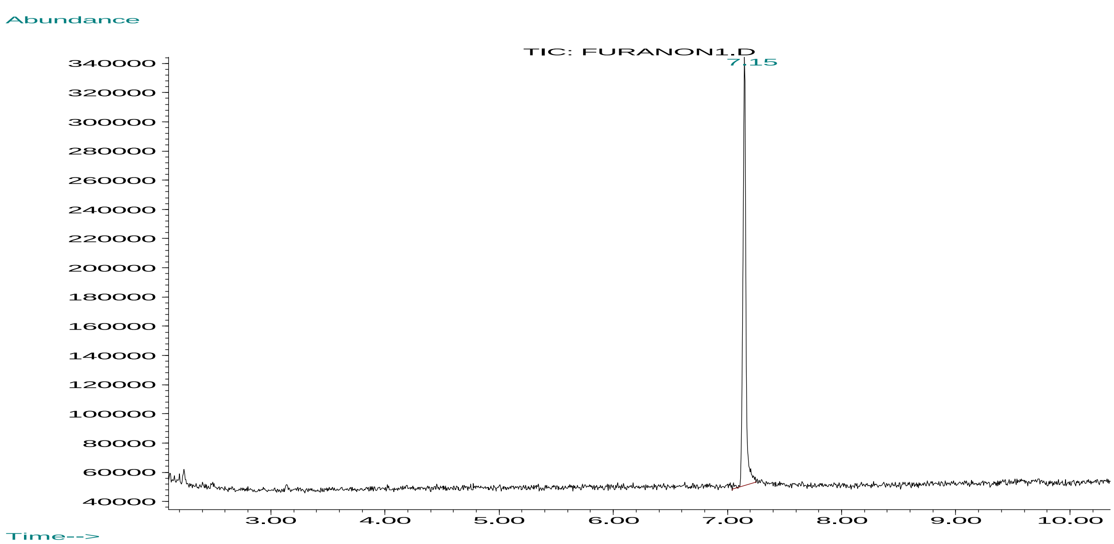


Figure 11 Total Ion Chromatogram of DHF Standard

First, DHF could be lost during sample preparation. The strawberry extracts were concentrated through distillation and condensation. Wilhelm et al.[27] proposed that DHF be highly oxygenated so that it does not steam distil.

Second, two groups[26-27] reported that DHF could be irreversibly adsorbed on the glass chromatographic column. Only fused silica or aqueous acid leached soft glass columns make detection possible. In this work, column DB-5 (5% phenyl 95%methyl polysiloxane) was employed, which minimized the possibility of adsorption according to these two groups' conclusion. Thus this explanation is probably not the reason for the absence of DHF.

Another possible reason can be based on the physical and chemical properties of DHF. DHF is very unstable in air and in aqueous solutions at 25°C[25], so degradation of DHF during sample preparation or separation might be the explanation for no detection of DHF. Figure 12 shows the molecular structure of DHF. Hiryl et al. [25]observed that DHF is most stable at pH4 at room temperature. A study of the degradation products from DHF has not been found in the literature. A possible degradation mechanism and the degradation products were reported by Hiryl et al. in 1980. Figure 13,14,15 schematically illustrate the proposed products formed by DHF degradation.[25]

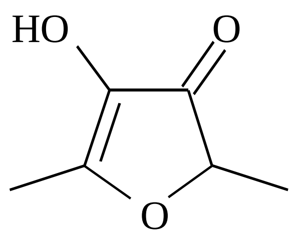


Figure 12 The Molecular Structure of DHF

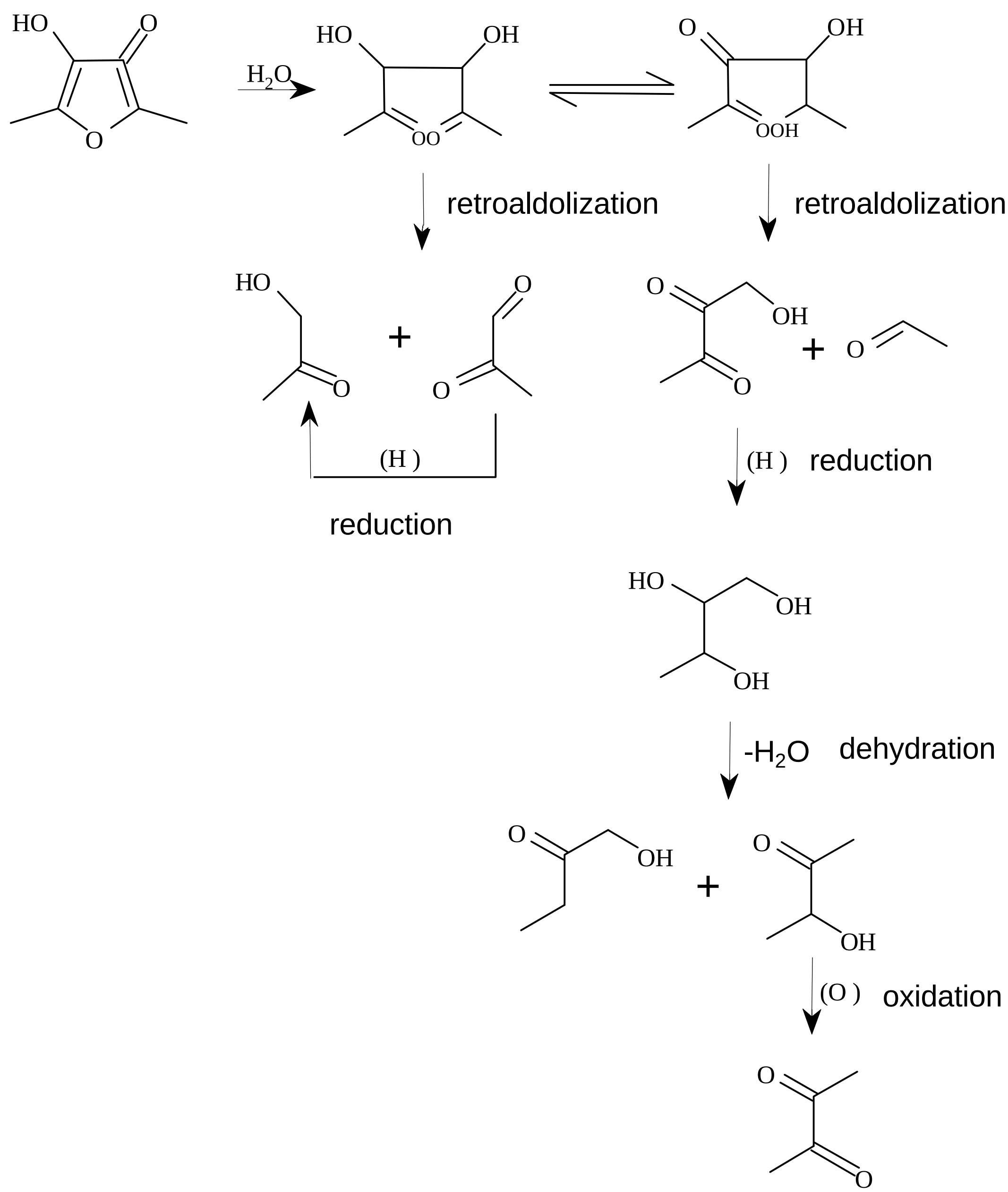


Figure 13 Schematic Illustration of Proposed Primary Degradation of DHF.

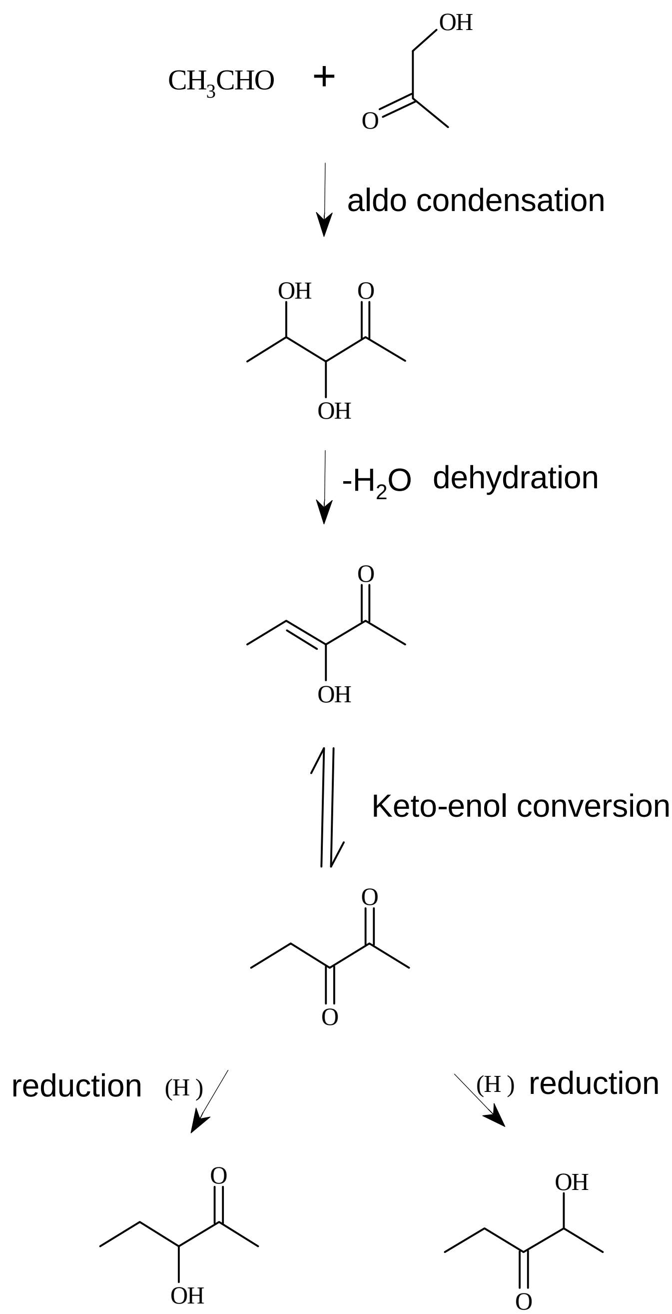


Figure 14 Possible Scheme for the Formation of Secondary Breakdown Products from DHF Degradation

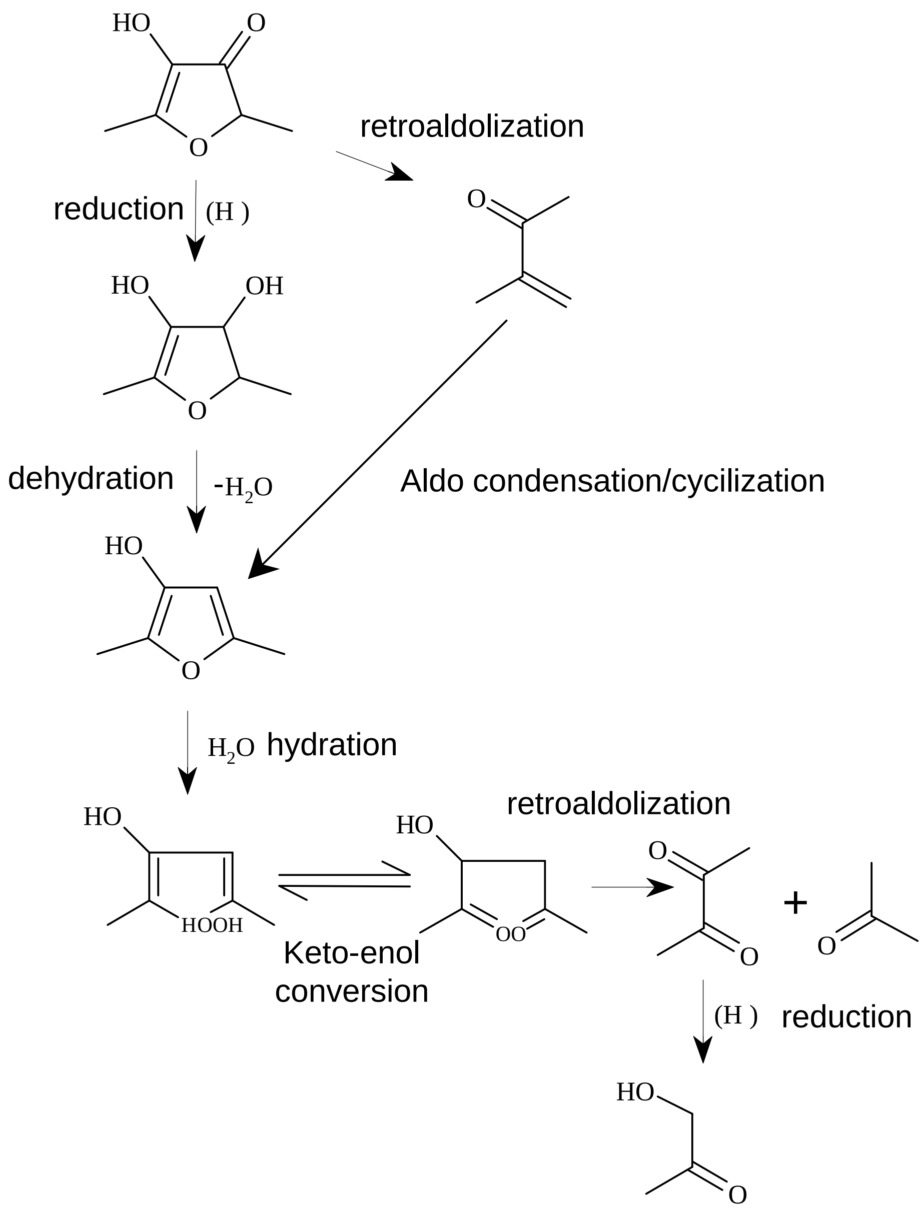


Figure 15 Tendency of Formation of 2,5-dimethyl-3(2H)-furanone and other Breakdown Products from DHF Degradation

According to the schemes given in these figures, the ring structure of DHF opens first to an intermediate when the degradation starts. The intermediate is not stable and then undergoes a retroaldolization process to produce the primary products, which include acetaldehyde, hydroxyacetone, 2,3-butanedione etc. The primary products continue to react in an intermolecular fashion and form secondary products, e.g. 2-hydroxy-2-pentanone, etc. DHF also has a tendency to form 2,5-dimethyl-3(2H)-furanone via reduction/dehydration or/and aldol condensation/cyclizations illustrated by Figure 15.[25]

Table 8 lists the volatile compounds found by Hiryl's group from the degradation of DHF. To determine if the degradation played the decisive role in the detection of DHF in this work, all the volatile constituents found in this work from the MSD report were checked and compared to the degradation products. If all or parts of primary and/or secondary degradation products were found, it could be concluded that degradation is the main reason of not detecting DHF. Unfortunately, in our results, only acetic acid and ethyl acetate were found, which indicates the low probability of DHF degradation pathway in our study.

The last and also the simplest reason may be that there is no DHF in these three strawberries, or the concentration of it is lower than the detection limit of the MSD (100 parts per billion).

Table 8 The volatile compounds formed by the degradation of DHF.

1. Acetaldehyde	8. Acetoin	15. 3-acetoxy-2-butanone
2. Acetone	9. 1-hydroxy-2-butanene	16. 2,5-dimethyl-3(2H)-furanone
3. Methyl Ethyl Ketone	10. 3-hydroxy-2-butanone	17. 2,4,5-trimethyl-3(2H)-furanone
4. 2,3-butanedione	11. 2,4-pentanedione	18. 2-ethyl-5-methyl-3(2H)-furanone
5*. Ethyl Acetate	12. 3-hydroxy-2-pentanone	19. unreacted DHF
6. Hydroxyacetone	13. 2-hydroxy-3-pentanone	20. 2,4-dimethyl-5-ethyl-3(2H)-furanone
7. 2,3-pentanedione	14. Acetoxyacetone	21*. Acetic acid

*indicates the compounds found in our study

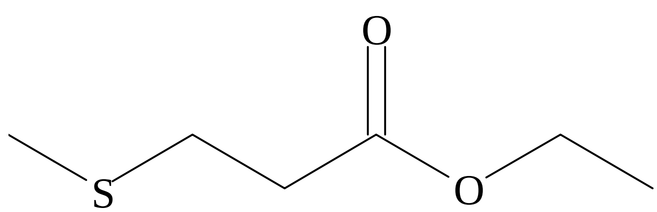
Sensory Analysis

Table 9 lists the published odor threshold values and the sensory descriptions of the important aroma compounds in strawberries. [28-30]

DHF and DMF have the lowest odor threshold values, 0.04ppm and 0.03ppm respectively. So even at low concentrations they can still give strong aroma impressions. With their pleasant, fruity, sweet sensory odor, it is assumed that these two compounds are the most important aroma constituents in strawberry.

Ethyl butyrate (0.13ppb), linalool (6ppb), 2-furaldehyde (6ppb), ethyl acetate (11ppb), ethyl cinnamate (16ppb), methyl butyrate (59ppb) are also major odor contributors due to their low odor threshold values. All the aroma constituents listed in the table 8 are correlated well to the overall fruity, sweet, ester-like strawberry odor except 2-furaldehyde and 2-furanmethanol. Both 2-furaldehyde and 2-furanmethanol are positively correlated with a "woody", "dusty", "overcooked" aroma. 2-Furaldehyde, was found only in Memphis and Orville strawberries. It has a low odor threshold value of 6ppb, so it gives the intense woody negative aroma impression in both Memphis and Orville strawberries. 2-Furanmethanol, found only in Salinas strawberries, has an odor threshold value of 30,000ppb, which is too high to affect the overall strawberry aroma. A very small peak in the total ion chromatogram (Figure 9) indicates that only a small amount of 2-furanmethanol exists in Salinas strawberries. This low level would have a minimal effect on the Salinas aroma.

Ethyl (methylthio) acetate is reported here for the first time in strawberries. There is no sensory descriptions or odor threshold value of it available. One of its homologous compound, ethyl 3-(methylthio) propionate (Figure 16), has been reported to be an aroma chemical, which is pineapple-like, “tropical”. [5,31] Ethyl (methylthio) acetate is supposed to have similar physical property to its homologue, thus to have pleasant sensory impression.



$C_6H_{12}O_2S$

Figure 16 Chemical Structure of Ethyl 3-(methylthio) Propionate

Table 9 Threshold values and the sensory descriptions of the important aroma compounds in strawberries

Aroma Compounds	Threshold Value in Water(ppb)	Sensory Description
Ethyl Acetate	11	Fruity, Pleasant
Methyl Butyrate	59	Fruity, ester-like, green
Ethyl Butyrate	0.13	Fruity, ester-like, sweet
Linalool	6	Flowery, sweet
DMF	0.03	Fruity, caramel, green
DHF	0.04	Caramel, sweet, sherry-like
Ethyl Cinnamate	16	Cinnamate-like
2-Furaldehyde	6	**Peculiar, woody, dusty**
2-Furanmethanol	3×10^4	**Faint burning**
(Z)-2-hexene-1-ol	70	Grassy, green
1-mehtyl-butanoic acid	180	Fruity, buttery

Quantification Results

Figure 17 is the total ion chromatogram of six standards, ethyl acetate, ethyl butyrate, methyl butyrate, ethyl (methylthio) acetate, linalool and ethyl cinnamate. Figure 18 is the total ion chromatograms of the 2-furaldehyde standard solution. Figures 19-25 are the calibration curves of area counts versus concentration and the calibration equations for seven standard odor compounds. These were done by using linear regression function of MathCad software. The closer the linear regression coefficient is to 1, the better the fit. The concentrations of these compounds were calculated and listed in Table 10, the relative standard deviations (%RSD), which ranged from 0.5 to 9.1, were calculated and listed as well.

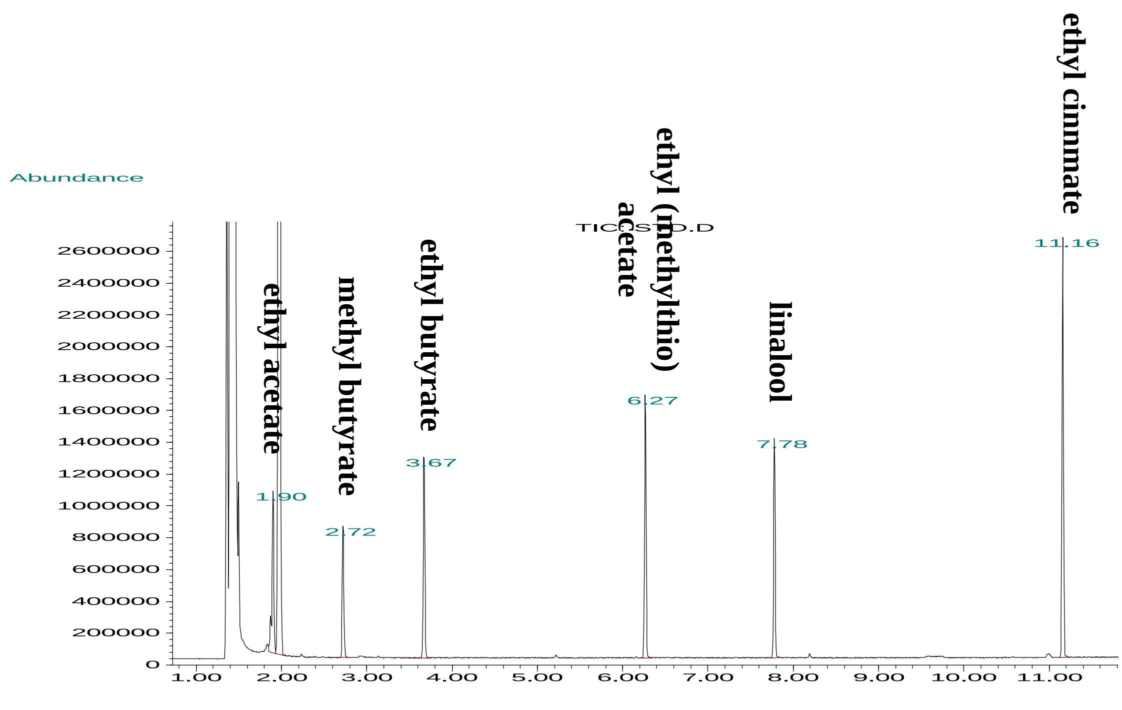


Figure 17 Total Ion Chromatogram of Six Standards

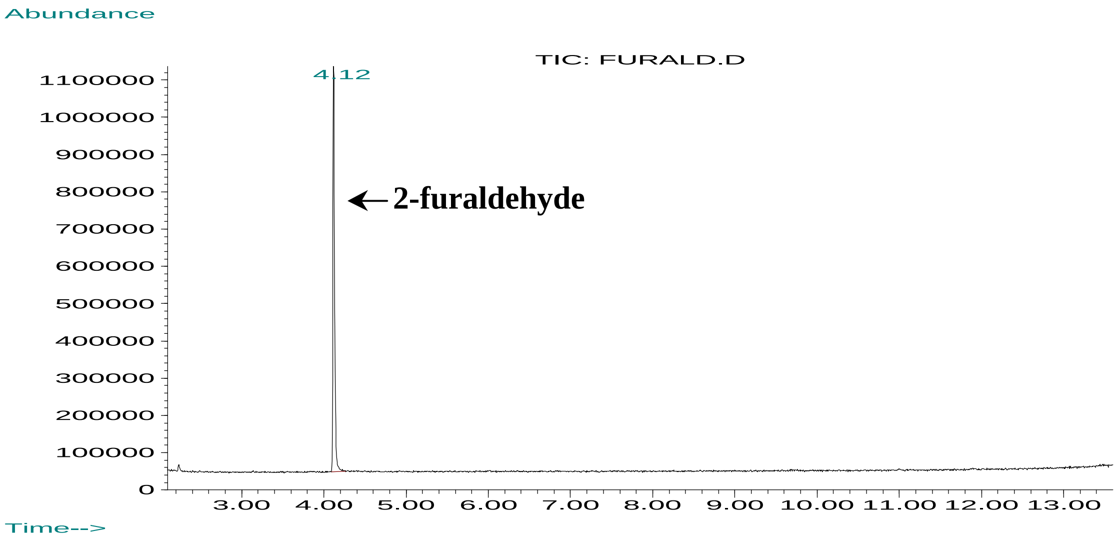


Figure 18 Total Ion Chromatogram of Standard 2-fualdehyde

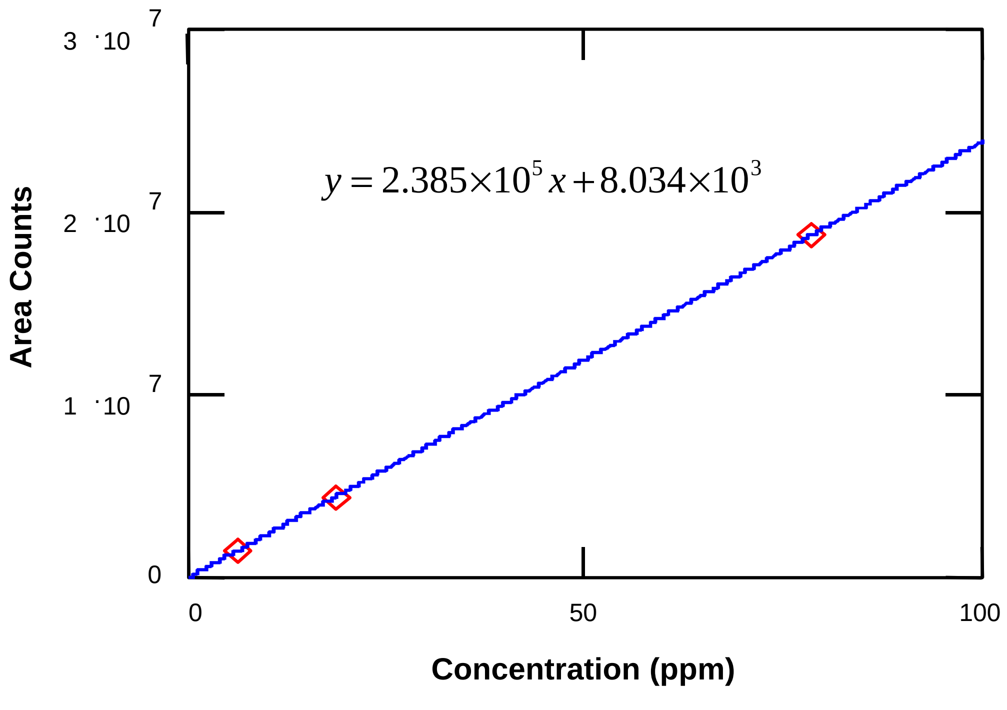


Figure 19 Calibration Curve for 2- Furaldehyde

(Linear Regression Coefficient is 1.00)

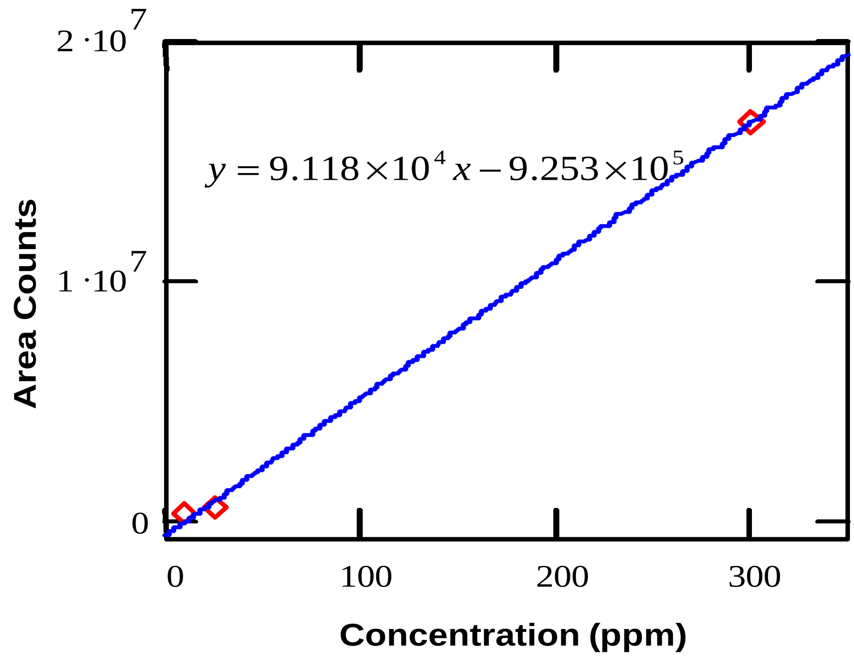


Figure 20 Calibration Curve for Ethyl Butyrate

(Linear Regression Coefficient is 1.00)

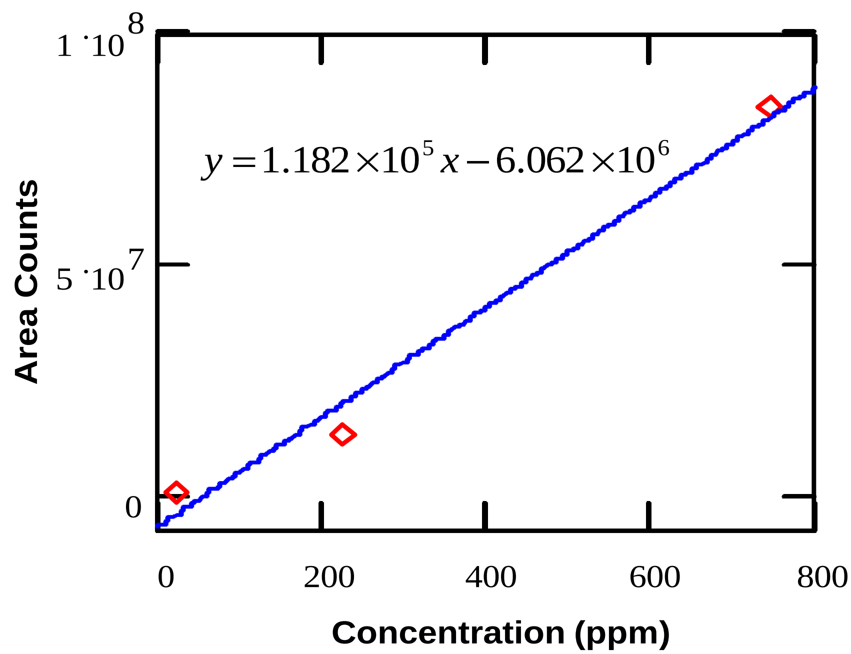


Figure 21 Calibration Curve for Ethyl Acetate

(Linear Regression Coefficient is 0.992)

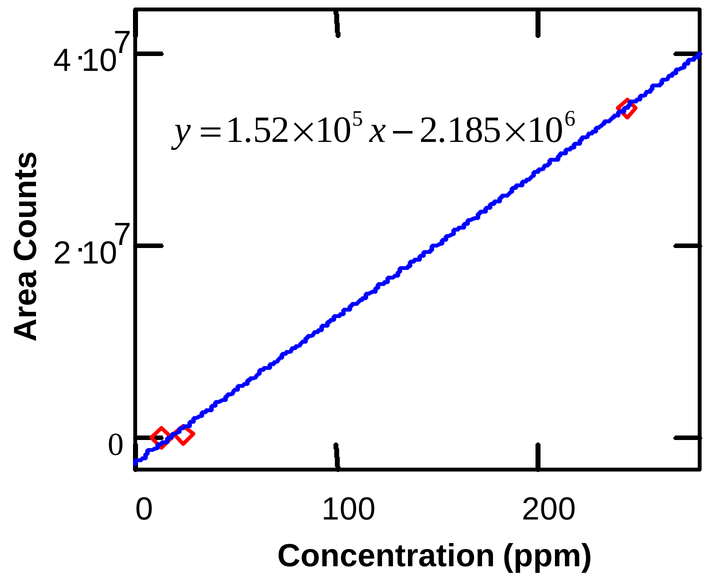


Figure 22 Calibration Curve for Ethyl Cinnmate

(Linear Regression Coefficient is 0.999)

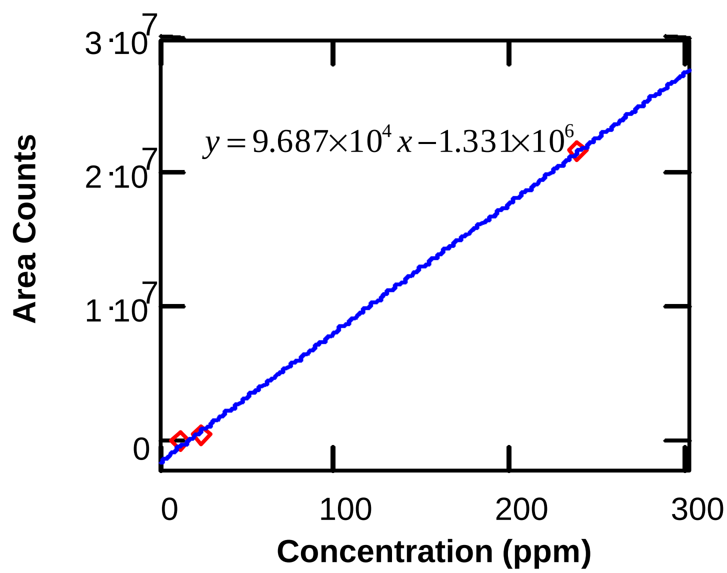


Figure 23 Calibration Curve for Ethyl (methylthio) Acetate

(Linear Regression Coefficient is 0.999)

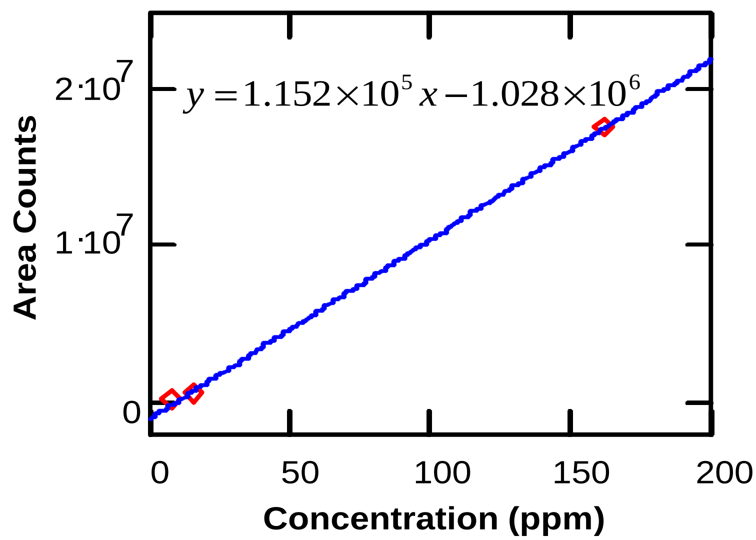


Figure 24 Calibration Curve for Linalool

(Linear Regression Coefficient is 0.999)

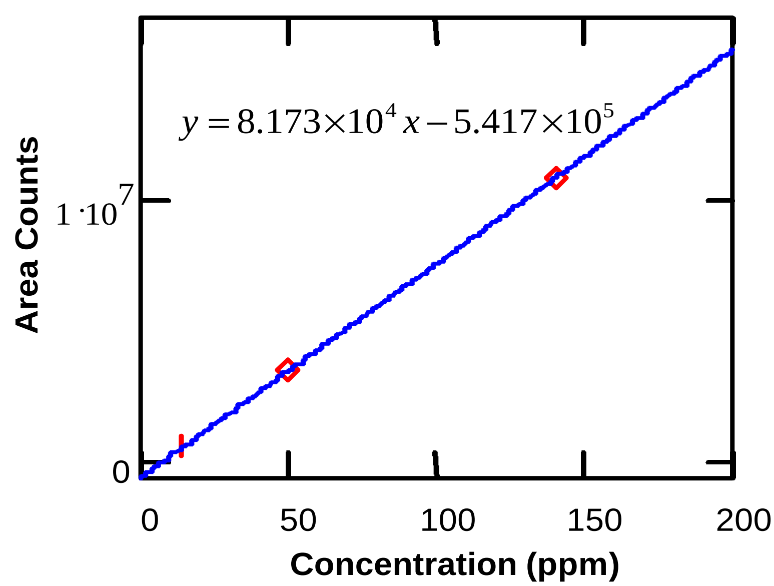


Figure 25 Calibration Curve for Methyl Butyrate

(Linear Regression Coefficient is 1.00)

The major odor compounds identified in Salinas strawberry have higher concentrations than those in Memphis and Orrville strawberries. Figure 26 compares the key aroma constituents quantitatively. Compounds, ethyl acetate, ethyl butyrate, methyl butyrate, and ethyl (methylthio) acetate, have the highest amounts in Salinas strawberries. 2-Furaldehyde, which contributes a woody odor to the overall pleasant aroma, was not detected in Salinas strawberries. Higher amounts of the key pleasant aroma compounds and no negative odor make the Salinas strawberries have the best aroma.

To determine more convincingly the aroma qualities of these three strawberries, aroma values were calculated using the equation of aroma value. The results were listed in Table 11. Ethyl butyrate, having low odor threshold value of 0.13ppb, has the highest aroma values (~10^5) among the key aroma compounds in this study. So this ester-like, sweet character impact compound contributes greatly to the overall pleasant odor. Most of the key aroma compounds in Salinas strawberries have the highest aroma values, which again confirms the aroma quality of the Salinas strawberries.

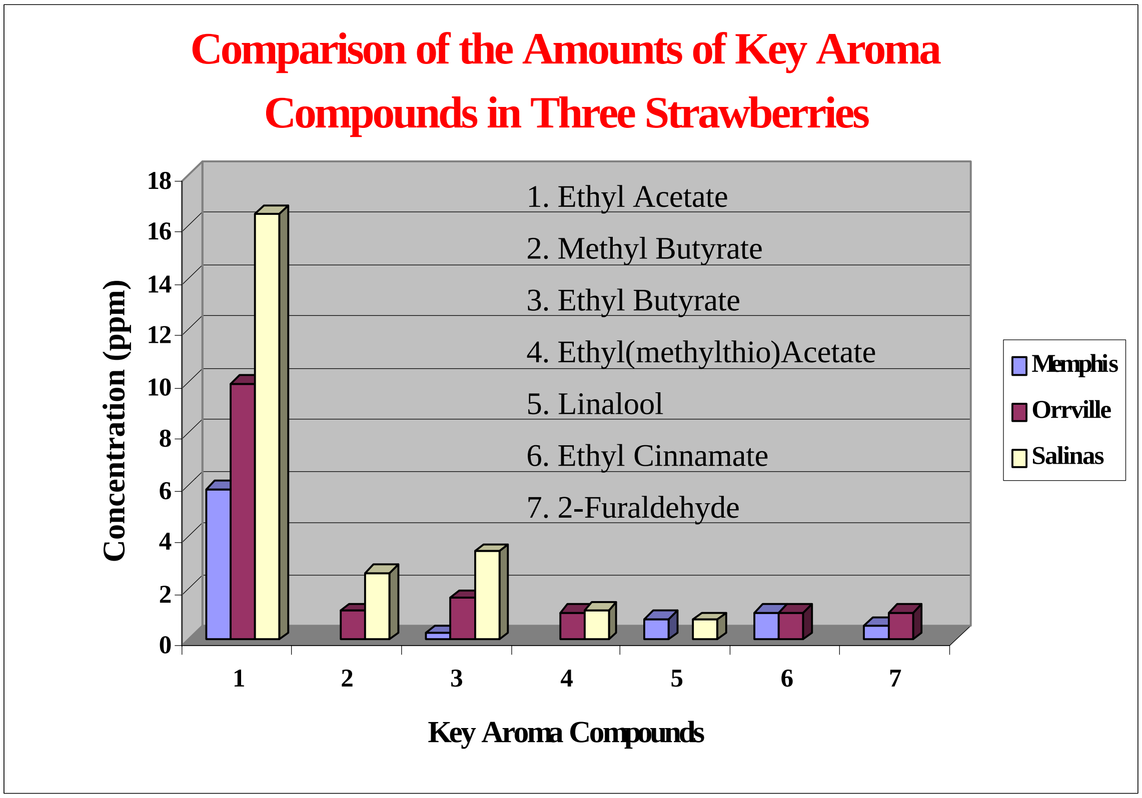


Figure 26 Comparison of the amounts of the key aroma constituents in three strawberries

Table 10 Concentrations of Key Aroma Compounds and Relative Standard Deviation in Memphis, Orrville, Salinas Strawberries

Compounds	Memphis		Orrville		Salinas	
	concentration (ppm)	%RSD (n=3)	concentration (ppm)	%RSD (n=3)	concentration (ppm)	%RSD (n=3)
Ethyl Acetate	87.6	5.7	149	8.3	247	8.3
Methyl Butyrate	ND *		16.8	6.3	39.0	8.5
Ethyl Butyrate	12.0	3.0	24.2	5.3	51.2	1.3
Ethyl (methylthio) Acetate	ND*		16.3	7.9	17.4	9.1
Linalool	12.1	8.3	ND*		11.7	3.3
Ethyl Cinnamate	16.0	0.5	15.9	16.4	ND*	
2-Furaldehyde	18.6	6.7	29.5	7.7	ND*	

*ND= Not Detected

Table 11 Aroma Values of Key Odor Components in Memphis, Orrville and Salinas Strawberries

Aroma Values	Memphis	Orrville	Salinas
Ethyl Acetate	7.9×10^3	1.4×10^4	2.3×10^4
Methyl Butyrate	0	2.9×10^2	4.8×10^2
Ethyl Butyrate	2.8×10^4	1.9×10^4	3.9×10^5
Linalool	2.0×10^3	0	2.0×10^3
2-Furaldehyde	3.1×10^3*	4.9×10^3*	0
Ethyl Cinnamate	1.0×10^3	9.9×10^2	0

** indicates the negative contribution to the overall pleasant strawberry aroma*

CHAPTER 4

CONCLUSIONS

The application of GC/MS techniques for the measurement of aroma quality has generated many applications in the past 20 years. GC/MS is well suited for aroma (volatile) compounds; it has excellent resolving power, high reliability (precision) and low detection limits (100ppb). GC/MS is also fast, and can confirm the identities or molecular structures of most peaks. Capillary GC columns are more and more used in aroma analysis. Their small diameter, thin film, and high flexibility make them more attractive in this analytical field.

Aroma value is a useful and convenient parameter used to determine the aroma quality of a food system. The higher the value, the greater the compound’s contribution to the aroma quality.

DMF and DHF are two important aroma compounds frequently reported in wild strawberries. They have rarely been found in cultivated strawberries. The reasons for this are not well known. DMF was detected in all three cultivated Camarosa strawberries, Memphis, Orville, and Salinas. Its very low odor threshold value and fruity sensory description make it one of the most important contributors to the overall pleasant aroma quality. However, DHF was not detected in any of these three strawberries. If present in our cultivated Camarosa, it was below our detection limit.

Ethyl butyrate was calculated to have the highest aroma value for our strawberries. Because of its ester-like, sweet impression, it is another important contributor to the overall pleasant aroma of strawberry. Ethyl (methylthio) acetate, a sulfur-containing compound, is reported here for the first time in strawberries. No sensory descriptions and odor threshold value have been published. It is expected to be pineapple-like, “tropical” in odor similar to its homologous compound ethyl 3-(methylthio) propionate.

Similar aroma constituents were found in Memphis, Orrville, and Salinas strawberries, but differing in their amounts. Overall, most of the key aroma compounds in Salinas strawberries have the highest aroma values, and 2-furaldehyde, the major contributor to a bad woody odor, was not found in Salinas. This confirms that Salinas strawberries have the best aroma quality among these three Camarosa variety. The results also indicate that the strawberries of the same variety can have different aroma qualities when they are grown in different places.

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VITA

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