CAPILLARY GC/MS ANALYSIS OF THE VOLATILE FLAVOR COMPONENTS IN WINES

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CAPILLARY GC/MS ANALYSIS OF THE VOLATILE FLAVOR COMPONENTS IN WINES

To Betty, Lloyd and Beth

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ABSTRACT

A new analytical method has been developed for examining flavor compounds in wine that detracts from the traditional method; the isolation-concentration process takes 5½ hours, using only 95 ml of wine. The heart of this method is a new design of a low-temperature, high-vacuum concentrator, which is more efficient than any previously described design of its kind. It was used together with a previously designed solvent extractor to isolate and enrich the volatile fraction of the wine. Capillary GC/MS techniques were used to separate and identify components of the flavor extract.

The method was used to confirm the presence of 2-methoxy-3-isobutylpyrazine, a trace compound previously identified in the grapes but not in the wine, for the first time in Cabernet Sauvignon wine. Ethyl 4-acetyloxybutyrate and 2-hydroxybenzothiazole were also identified for the first time in this wine.

This method was used to analyse the volatile flavor composition of White Riesling, whose vines have recently been successfully cultivated locally. The results of the qualitative and quantitative analyses of the musts and wines of local White Riesling compared quite favorably with results obtained on the same grape variety cultivated in the temperate climate. These results, it is hoped, would enable vintners and viticulturalists anticipate the potentialities of the locally cultivated *vinifera* vines.

It was discovered that the method used to examine the flavor composition of *vinifera* varieties had to be modified for the *labrusca* varieties and, only under inert conditions could the compound tentatively identified as N-(N-methyl,N-hydroxy-γ-aminobutyryl)glycine be detected in all the varieties examined. For the first time, it is being suggested that this compound, rather than methyl anthranilate, may be responsible for the typical *labrusca* flavor. 3-Methyltetrahydrothiophene, 3-methylthiopropanol, phthalide, 2,5-dimethyl-4-hydroxy-3(2H)furanone, dihydrobenzofuran, 2-hydroxybenzothiazole and α-naphthol were identified for the first time in Moulin Rouge and Concord.

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May God Bless You All.

LIST OF ABBREVIATIONS

A^+, $A^{+\cdot}$	Fragment ions
CI	Chemical ionization
CIMS	Chemical ionization mass spectrometry
C_{max}	Maximal concentration
EIMS	Electron impact mass spectrometry
ECD	Electron capture detector
FID	Flame ionization detector
FPD	Flame photometric detector
GC/MS	Combined gas chromatography/mass spectrometry
GC	Gas chromatograph
HPLC	High performance liquid chromatography
HPLC/MS	Combined high performance liquid chromatography/ mass spectrometry
M	Unionized sample molecule
$M^{+\cdot}$	Positive molecular ion
m/z	Mass to charge ratio
N	Neutral particle
pK_a	Negative logarithm of the acid dissociation constant
q	Sample size
n	Number of theoretical plates
R	Unionized reactant gas molecules
$R^{+\cdot}$	Reactant gas ions
%RA	Percentage relative abundance

%RIC	Percentage reconstructed ion current
I	Rentention index
RBF	Round bottomed flask
PFK	Perfluorokerosene
SIR	Selected ion retrieval
SIM	Single ion monitoring
SCOT	Support coated open tubular column
%TIC	Percentage total ion current
TCD	Thermal conductivity detector
TSD	Thermionic sensitive detector also referred to as Nitrogen/Phosphorus detector
u	Atomic mass unit
V_R	Retention volume
WCOT	Wall coated tubular column

TABLE OF QUANTITIES

1 kcal = 4.184 kilojoules (kJ)

1 electron volt (eV) = 1.602×10^{-22} kJ

1 Torr = 133.3 Pa

1 microgram (μg) = 10^{-6} g

1 nanogram (ng) = 10^{-9} g

1 picogram (pg) = 10^{-12} g

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CHAPTER ONE

1.1 GENERAL INTRODUCTION

This thesis presents the development of an analytical methodology for the analysis of trace volatile flavor compounds in grape musts and wines and the application of this technique to studies relating to vitis vinifera and vitis labruscana. The special role of methyl anthranilate in the overall aroma of vitis labruscana varieties has also been examined.

The research involves: (i) the use of a solvent extraction technique that rapidly and efficiently extracts the volatile flavor components from as little as 100 ml of grape musts and wines; (ii) the design and construction of a new low-temperature, high vacuum, 2-stage concentrator apparatus that substantially enhances the concentrations of minor and trace components without significant losses. The first stage of the 2-stage concentration process involves a 2500-fold enrichment of the flavor extract. The gas chromatogram of the concentrated flavor extract obtained at this point is very complicated and may consist of several unresolved chromatographic peaks. Even under "high resolution chromatographic conditions" peaks of major components may overlap those of minor and trace components and thus limit the usefulness and sensitivity of the gas chromatographic determination. In order to provide a more suitable sample for gas

chromatographic (GC) analysis, non-chromatographic methods were applied to selectively remove abundant components in the concentrated flavor extract namely, fatty acids and fusel alcohols, whose identity and analysis need no further investigation, but whose presence interferes substantially in the analysis of minor and trace components. Many components in the sub part per billion (ppb) to part per trillion (ppt) concentration range are barely detectable at this point. The second stage of the concentration process involves approximately a 4-fold enrichment of the simplified flavor extract. This second stage of the concentration process is considered to be a very critical and necessary stage to the success of the detection and identification of sub ppb level volatile flavor components. The whole isolation-concentration procedure takes not more than five and one-half hours to accomplish. Standard gas chromatographic mass spectrometric (GC/MS) methods were used to separate, identify and determine the volatile flavor compounds in musts and wines. The methodology developed was applied to the chemical analysis of the volatile flavor components of musts and wines of the Vitis vinifera cultivars: Cabernet Sauvignon and white Riesling, and the Vitis labruscana cultivars Concord and Moulin Rouge. The sensitivity of the method developed was demonstrated by the detection and identification of 2-methoxy-3-isobutylpyrazine 102 in Cabernet Sauvignon wine for the first time. Although this compound was detected and identified as a trace component by Boidron et al[124] in the grape, its presence in the wine could not be confirmed.[217] In addition, 2-hydroxybenzothiazole 304, and ethyl 4-acetyloxybutanoate 228, were identified as trace components in this wine for the first time. White

Riesling that is now being successfully cultivated on Canadian soil was analyzed to evaluate the effect, if any, the climate, soil, and vinification techniques may have had on the qualitative and quantitative distribution of volatile flavor components. The results of this analysis indicate that the distribution of flavor components in the locally cultivated white Riesling vines compare favorably with those cultivated in the more favorable and cooler climates of Europe. The preliminary results of this analysis should enable winemakers in Canada to anticipate the potentialities of the wines they could produce from the fruits of this vine. The methodology developed was applied to the analysis of the vitis labruscana cultivars: Concord and Moulin Rouge. In this analysis, a method was developed that enabled wines of this variety to be reconstituted for the first time. 3-(methylthio)propanol 155, 3-methyltetrahydrothiophene 173, α-naphthol 311, isobenzofuranone 260, 2,5-dimethyl-4-methoxy-3(2H)-furanone 124, 2,5-dimethyl-4-hydroxy-3(2H)-furanone 213, and 3,5,5-trimethyl-2-cyclohexanone 125 were detected and identified as trace flavor components in these varieties for the first time. A trace compound detected in all the vitis labruscana varieties investigated and tentatively identified as N-(N-methyl,N-hydroxy-γ-aminobutyryl)glycine 223 is being reported as a flavor component in these varieties for the first time. Even more relevant is the observation made that this compound, rather than methyl anthranilate, is believed to be responsible for the typical labrusca flavor.

The utility of an analytical methodology for performing an analysis is a function of its precision and accuracy in relation to

the objectives of the analysis. The procedures developed in this research have been shown to yield accurate and reproducible results. Several factors including large extraction volumes and long extraction periods have mitigated against the adoption of volatile flavor analysis for routine, industrial quality control methods. The small sample sizes, the short extraction periods involved and the wealth of information that could be derived from the methodology developed in this research work, should make it economically attractive and suitable for adoption as a routine analytical method for investigating volatile flavor components in research and industrial establishments.

1.2 GENERAL GOALS OF FLAVOR RESEARCH

The significant role played by the volatile aroma components of wines in determining wine quality, and the complexity of the problem it poses to enologists and psychophysicists in their painstaking efforts to unravel the mystery of natural flavor composition, has prompted a myriad of investigations.[1-16] The goals of these investigations have been broadly classified as the identification and assessment of the contribution of those compounds responsible for imparting the sensations of taste and smell as well as the enhancement of existing natural flavors, and the creation of new synthetic flavor compounds.[17]

1.3 HISTORICAL BACKGROUND

The history of wine is probably as old as the history of civilization. Historical evidence indicates that wine was known to man a thousand years before the coming of Christ. It was during this period that wine was introduced into France and Italy from the Eastern

civilization; the wines of Greece were lavishly praised and generously documented by her poets.[18] With the establishment of the Roman Empire came a diversification in the method of aging wines. Unlike the Greeks, the Romans were not limited to earthenware amphorae, and they aged their wines in wooden barrels and bottles very much like those of today. The Roman method of cultivating the grapevine is still practised, particularly in Southern Italy and Northern Portugal.[19,20] In the period which followed the fall of the Roman Empire, the Church emerged as the repository of the skills of civilization. As dying noblemen and winemakers bequeathed land to it, the Church of the medieval period came to be identified with wine, not only as a symbol of the Blood of Christ, but, as luxury and comfort in this world. For centuries, the Church owned many of the greatest vineyards of Europe. It was within this stable framework that great advances were made in the technology of winemaking. Burgundy, for example, emerged with its delicate, spirity, faintly bubbly white wines and rosés. In the nineteenth century, there was a real revolution in the wine industry. It had become clear that wine stored in a tightly corked bottle lasted much longer than wine kept in a barrel. When stored in a bottle, the wine also acquired a complexity and softness not previously obtained with aging wines in the wooden barrels. As a result, the vin rosés suddenly went out of fashion to be replaced by long-fermented dark-colored wines. It was within this period that the governments of the wine making countries of Europe established several research centers to conduct profitable research into the art of winemaking. Dramatic advances made in the field of winemaking in the last twenty years have enabled wine-

makers to have greater control over what they produce. Most of the winery processes have been automated. With these improvements however, have come temptations to lower the very high standards of the best, to make more wine at the expense of quality, and even worse still, to make neutral, safe wine lacking character.

1.4. COMPOSITION OF GRAPE MUST AND WINE

Must is freshly crushed grape juice. The knowledge of the composition of the must is of prime importance to the winemaker. This information, which usually includes readily measurable properties like those shown in Table 1.1,[21-22] enables the winemaker to determine the

Table 1.1

Composition of Musts and Wines

	Must % by weight	Wine % by weight
Water	70-85	80-90
Ethanol	tr	8-15
Carbohydrate	18-25	0.1-0.3
Polyphenols	0.01-0.10	0.01-0.3
minerals	0.3-0.5	0.15-0.4
volatile compounds	tr	0.01-0.10
nitrogenous compounds	0.03-0.17	0.01-0.09
organic acids	0.3-1.5	0.3-1.1
aldehyde	tr	0.001-0.005

tr = trace

Reprinted from Amerine et al., in "Methods for analysis of musts and wines", J. Wiley and Sons, New York (1980).

most favorable vinification procedure to be adopted - including the possibility of amelioration. Wine is the end product of the conversion of the hexose sugars in the grape-dextrose and levulose - into ethanol by the action of yeasts. This is described by the Gay Lussac[23] equation 1.1. In Canada and the Eastern United States where Chaptalisation

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2 \uparrow \ldots\ldots \qquad 1.1$$

is allowed, sucrose may be added to the must. l-Malic acid (pK_a = 3.41) and d-tartaric acid (pK_a = 2.98), the principal acids of the grape, buffer the wine to a pH low enough to maintain the typical wine color and to confer biological stability. Amino acids, proteins, peptones, amides, peptides and ammonia constitute the nitrogenous compounds in musts and wines. These aid in clarification, bacterial development and in aroma development. The volatile compounds include alcohols, fatty acids, aldehydes, ketones, lactones and esters. In total, they rarely exceed 0.01% by weight of the wine composition but nevertheless, are responsible for the aroma and bouquet of the grape and matured wine respectively. Although several properties, including volatile aroma composition, have been used in assessing and evaluating wine quality,[24-34] it remains true that the final acceptance or rejection of the quality of wine rests firmly with the type of response the human nose has for those volatile flavor compounds. <u>One of the basic aims of this research was to determine the chemical identities of the individual chemical compounds that constitute the volatile flavor fraction of grape musts and wines</u>.

1.5 GRAPE VARIETIES

Of the ten thousand or more grape varieties from which wine can be made, only a few dozen varieties are being used. Of these, only about a dozen types have actually yielded great wines. Why this is so is complicated and puzzling. For example, it is not possible to differentiate a white wine from a red wine merely by looking at the gas chromatogram of the volatile flavor extracts of such wines. It is to such puzzles that enologists have tried to provide answers. While most of the constituent elements of grapes and wines have been subjected to extensive chemical analysis, the components responsible for the characteristic aroma of certain grape varieties which contribute significantly to the bouquet and flavor of the matured wine are believed to be present at such trace levels that they haven't yet been identified. It is well known that it is the distinctive, individual aromas of some grape varieties that enable them to yield great wines. Amerine and Singleton[18] divide wine grape varieties into four categories. The largest (in volume grown) is of grapes which give no special flavor or character to their wine. These are the wines of the grapes of the Mediterranean basin: the Carignan of southern France is a good example. Muscat-flavored grapes such as Muscat d'Alsace, which impart a heavy strong scent and flavor to their wines. Vitis vinifera varieties, such as White Riesling and Cabernet Sauvignon, which impart a characteristically varietal flavor to their wines. These varieties do not only make wine of unmistakably individual character, but wines whose aroma, bouquet and flavor can be extraordinarily complex. They are capable of developing further nuances in the bottle and are the principal grapes

from which the world's greatest wines have been made. The fourth category is American: the native vines of North America, vitis labruscana, and their descendants such as Concord and Kuhlmann 188-2 (Maréchal Foch). It is often argued that the taste of most labrusca wines is extremely simple and offers very little to the sophisticated palate. They lack complexity and subtlety and unless their intense flavors are made more acceptable by dilution, blending and other ameliorating procedures during vinification[35], the end-product becomes simply overpowering. The product is found to be rather tart and unacceptable by those who are familiar with the more subtle sensations that the better vinifera wines offer the nose and the palate. This is one of the main reasons why the extensive viticultural research conducted at the Horticultural Research Institute at Vineland, Ontario and the Agricultural experimental station at Geneva, New York, have focussed on the breeding of new hydrids and the evaluation of the wine-making qualities of the new breeds. Even though the hardiness of these hybrids to the excruciating winter conditions has been achieved, the quality of the wines they produce, although better than the native varieties, are still far from being superior. These varieties however, make sparkling wines and sherries of high quality because the vinification processes remove or mask the labrusca character. The failure to produce superior wines from these hydrids has swayed the emphasis on current viticultural research to the cultivation of vinifera varieties. Unlike the vinifera varieties, the volatile aroma composition of most labrusca and hybrid wines have been poorly characterized. For example, Stevens et al.[51] reported having identified 59 compounds from

the volatile flavor essence of Concord grapes 21 of which were listed as hydrocarbons. A previous study conducted in this laboratory to characterize the volatile aroma composition in Concord and Blue Hybrid identified only 35 volatile flavor compounds.[36] A total of 85 compounds have been identified by Holley et al.[48], Neudoerffer et al.[39] and Stern et al.[51] It appears that there still remains quite a large number of flavor compounds in the native American varieties that have not been identified yet. In order that wine quality evaluation based on volatile aroma composition be of any significant use to the wine industries in Canada and the Eastern United States, it is appropriate to fully characterize the volatile aroma composition of the native American grape and wine varieties as well as those of their hybrids. It was hoped that the analytical methodology that is to be developed in this research would enable further characterization of the volatile aroma composition of these varieties.

A study which would be of considerable value to the local wine industry would be a chemical analysis of the volatile aroma extracts of grape musts and wines derived from vitis vinifera vines successfully cultivated in Canada and the Eastern United States. Climate, grape variety, soil and vinification practices affect the quantitative distribution of the flavor components[37-40] in the fruit of the vine. Since the grape essentially determines the quality of the wine produced, it would be interesting to examine by chemical analysis the effect the local climate and soil may have had on the distribution of the aroma components of the musts and wines derived from these locally cultivated vinifera varieties. A large volume of experimental data is available

on the volatile aroma composition of the grapes and wines of some of the vinifera grape varieties grown in Europe that would permit qualitative and quantitative comparison with the locally grown varieties. For example, Schreier et al.[246] while investigating the volatile constituents of grapes of the varieties Riesling, Traminer, Rulander, Muller-Thurgau, Schereube, Optima and Rieslaner, identified 225 compounds that included 81 hydrocarbons, 48 acids, 31 alcohols, 23 aldehydes, 18 ketones, 11 esters and 13 constituents of miscellaneous structures. Results of such a study, it is hoped, would enable the local winemaker to anticipate the potentialities of these local vinifera vines. One objective of this research will be to use the method of the chemical analysis of the volatile flavor extracts of grape musts and wines of the locally cultivated vitis vinifera vines to determine whether they are potentially capable of producing superior quality wines as their European counterparts.

1.6 METHYL ANTHRANILATE

While the chemical compound(s) that are believed to impart characteristic varietal flavor to most vinifera wines have been identified and characterized, there is still a measure of uncertainty as to the identity of the chemical compound(s) that determine the characteristic aroma of vitis labruscana grape juices and wines. This section reviews the role of methyl anthranilate in the total aroma picture of vitis labruscana grape juices and wines and the experiments to be conducted by the author in an attempt to clear up this uncertainty. Power and Chestnut[41] were the first to identify methyl anthranilate in Concord grapes. This compound has been held responsible for the 'foxiness' associated with the native American

varieties and their hybrids. It has also been considered the single, most important volatile flavor compound in vitis labruscana grapes, grape juices, musts and wines. Its presence in these varieties has been confirmed by Scott[42], Sale and Wilson[43], Robinson et al.[44-47] and by Holley et al.[48].

More recent investigations of the varietal character of the native American grapes and wines have paid particular attention to the role of methyl anthranilate in the overall aroma of these varieties.[49-55] While it has been established that the volatile aroma composition of Concord is dependent significantly on the processing technique[54], it is becoming increasingly clear that the varietal character of these native varieties cannot be explained by the presence or absence of methyl anthranilate alone. Friedman[52] believed that despite the high methyl anthranilate concentration in Concord it was of little significance to the aroma of Concord grapes. Amerine et al.[56] noted in 1959 that the distinctive Catawba aroma was apparently not due to methyl anthranilate. Some varieties like Baco Noir, Elvira, Delaware,[35] Aurore[35,37], Catawba[35,56,60] showed no detectable concentrations of methyl anthranilate, but still possess the distinctive vitis labruscana flavor. The flavor characteristics of white Concord are completely different from those of red Concord. Various authors[52,54,56,59,60] have also concluded that methyl anthranilate alone cannot be the most significant odorous component in vitis labruscana varieties. Even though the presence of one or more compounds other than methyl anthranilate that may be responsible for imparting the "typical labrusca flavor" to these varieties has been speculated upon by previous investigators[54], there

was no evidence in the literature that such compounds have been detected and identified. It is likely that the labrusca flavor may be attributed to one or more organoleptically significant compounds present at trace concentrations in these varieties. The phenomenon of odor perception can be thought of as a process somewhat like that occuring at the exit of a gas chromatographic column; the nerve impulses transmitted to the brain being analogous to the electrometer signals producing peaks on the strip chart recorder. The effluent from the GC column that is comprised of unresolved components is registered by the nose as an intriguing array of odors, not all of which are sharply defined, but which tend to blend and change and blend again within one chromatographic peak produced by the electrometer. The nose provides an "integrated response" to all of the odor-producing substances over a short time span. A typical electrometer response obtained on the analysis of the volatile flavor component of an authentic wine sample is shown in Figure 1.1. The GC response is sometimes too slow and not sufficiently sensitive to duplicate the performance of the nose. Thus, the nose which can detect substances over solution of 1 part-per-10^{12},[217] may register easily minor components like L, M, and N, in Figure 1.1 and those with concentrations much lower, that have significant organoleptic properties. Components like A, B, C, E, G, H, and I which are easily identified by most investigators may not even make any contribution to the total aroma picture. Sensory profiles of wine volatiles obtained by sniffing the GC effluent thus provides a quick and easy method of determining the potential contribution of individual components to a particular flavor character. It may even detect the presence of organoleptically

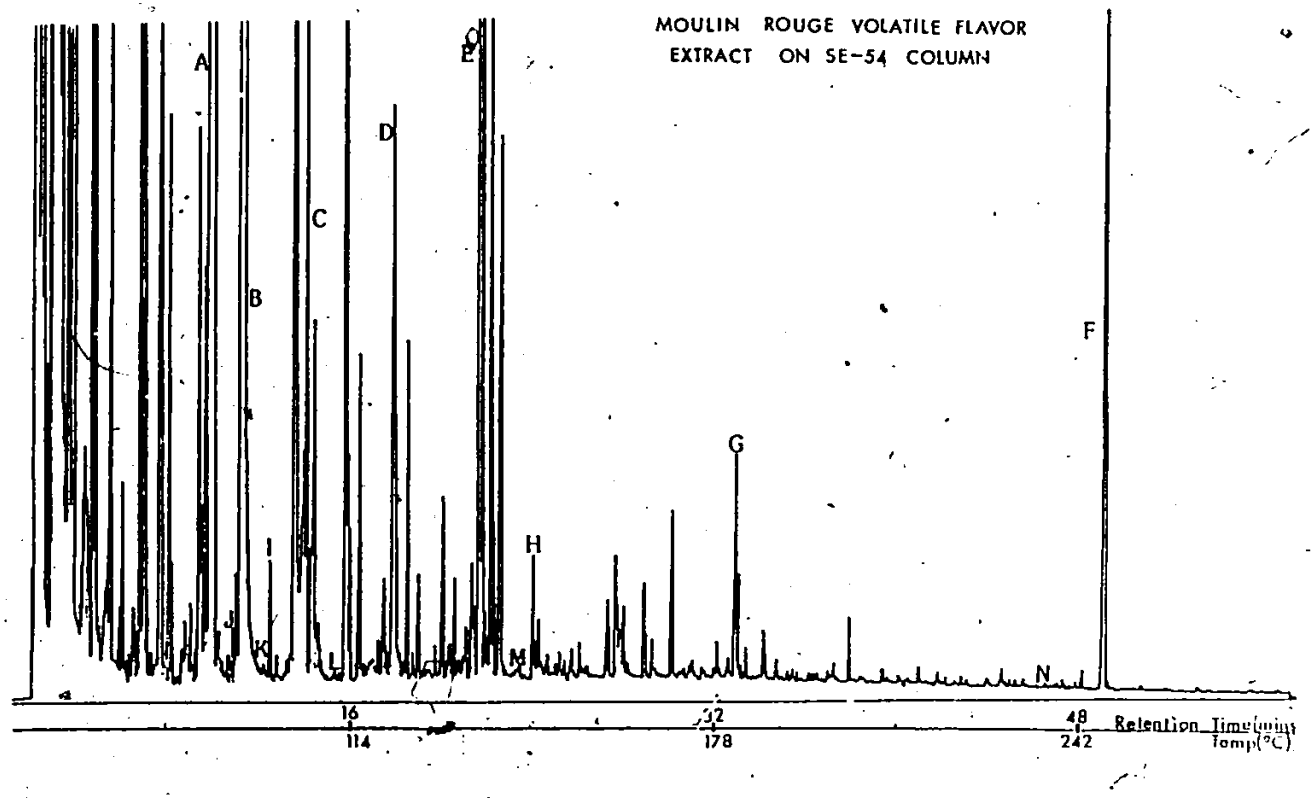

Figure 1.1. Gas chromatogram of an authentic sample of the volatile flavor extract of Moulin Rouge wine on a 50m x .25mm I.D. fused-silica capillary SE-54 column.

significant trace volatile components where the electrometer of the GC fails to give a visible response. This technique of sensory evaluation will form an integral part of this research. It is probable that the compounds responsible for the characteristic labrusca flavor may be ones of very low odor thresholds present at such low concentrations that current analytical procedures for investigating the volatile aroma composition of these varieties are not sensitive enough to detect them for identification. Since the emphasis is on the detection and identification of trace components, one approach that was to be adopted in this research towards the realization of this objective was to design and construct an apparatus that could be used to isolate, and provide sufficient enrichment of volatile flavor components of low odor thresholds, present in alcoholic solutions at concentrations that may be significantly lower than some of those represented in Figure 1.1, to enable their identification.

1.7 ANALYTICAL METHODOLOGY

Our knowledge of the composition of wine has increased tremendously in the last quarter of a century mainly because of extensive improvements in analytical instrumental methodology. The development of liquid chromatography in the early fifties paved the way for the analysis of sugars and non-volatile acids in wines.[62-68] Advances made in high performance liquid chromatography (HPLC) and high performance liquid chromatography mass spectrometry[80] (HPLC/MS) have made available more detailed information on non-volatile sugars and polymeric polyphenols.[69-80] The gate to sophisticated flavor studies was opened in 1955 when Golay[81,82] published his theory on open-tubular columns. Even

more relevant was the description of gas chromatography mass spectrometric (GC/MS) systems by Holmes[83] in 1957 and Gohlke[84,85] in 1958, and the timely invention of the glass capillary drawing machine by Desty[86,87] in 1959. By 1965, several investigators had applied these developments either singly or jointly to the analysis of food flavors taking advantage of the high separating capability of capillary columns and the high sensitivity of the mass spectrometer as a detector for organic compounds.[88-114] Rapp[61] estimates that the concentrations of volatile flavor compounds in grape musts and wines range between 1 g/l and 1 ng/l. Since the concentrations at which volatile compounds influence the flavor of a given food system are extremely low, sometimes at concentrations well below the detection limits of the most sophisticated analytical instruments,[119] it is imperative that the volatile fraction of the grape juice or wine be isolated from the bulk of the food sample and then pre-concentrated. The extremely low odor threshold values of some of the more significant flavor compounds, demand that isolation techniques be aimed at isolating these trace level compounds from several other less organoleptically significant components which may be present at concentrations several orders of magnitude higher. After defining an appropriate sample volume, one or a combination of the following separation techniques can be used to isolate the volatile flavor fraction from the bulk sample: solvent extraction[36,54,57,120-132,223] simple, fractional, steam, atmospheric and vacuum distillation,[133-141] adsorption onto charcoal, silica gel, tenax, Porapak Q and chromosorb[58,142-161] and, headspace techniques.[113,144,162-167] A survey of the literature shows that large sample sizes and long extraction periods have

characterized the isolation stage of the analysis.[57,123-130,169] For example, Sakato et al.,[168] analysed methylene chloride extracts from 197 liters of Carignan wine after 120 hours of extraction, while investigating neutral aroma components in this wine. Results of their analysis indicated that compounds identified were essentially the major ones. Since the solvent extractor previously devised in this laboratory has been shown to extract efficiently volatile flavor components from grape musts and wines in 3 hours, this technique was adopted with only slight modifications in all the experiments conducted. The isolated volatile flavor components is enriched by using rotary evaporation, a gentle gas stream of inert gas,[170] or vacuum techniques to enhance the concentrations of individual components to within the detection range of the detectors to be used. In order to reduce the complexity of the volatile flavor extract at this point prior to the final separation step, one or a combination of the following methods may be used: washing the concentrated extracts with acids or bases to provide acidic and basic fractions, liquid chromatography on alumina or silica gel columns to provide chemical class separation based on polarity[119,171-179] and preparative gas chromatography. Partitioning the volatile flavor extract between freon and propylene glycol has been used to reduce considerably the amounts of higher fusel alcohols produced during fermentation. Heart-cutting from packed to capillary columns has been successfully used in the analysis of complex mixtures since the pioneering work of Deans[181] in the United Kingdom and Schomburg in Germany.[182,183] The major drawbacks of this multidimensional GC technique are increased analysis time, when more than one fraction must be examined.[184-190] In

addition, sample interaction with the precolumn could give rise to missing peaks, while bleed from the column could give rise to artifacts. Most investigators would submit their cleaned-up extracts at this stage to GC analysis. Results of such investigations indicate that major and minor components, at best, are detected and identified. It would appear that an enrichment step could be incorporated here to provide a further enhancement in the concentration of the trace volatile components to within the detection range of the detectors employed. For example with sample sizes of 100 ml, enrichment factors between 10^3 to 10^4 would be required in order that a 0.2 ppb volatile flavor component can be detected by a detector sensitive to at least 0.1 ng of material introduced onto a gas chromatographic column. Very few reports appear in the literature on the simultaneous multiple detection and determination of trace volatile components in the ppb-ppt* concentration range.[179,220-223]

The traditional method of identifying a trace volatile flavor component is to use a large amount of sample and use preparative GC to purify the trace component from major less interesting components.[217-219] In general, where multiple determination of trace volatile components have been reported, the method of overloading the column so that trace components may produce observable chromatographic peaks (detector response) was used. There are however, no reports in the literature on studies conducted on the simultaneous, multiple detection of organoleptically significant volatile flavor components of low odor thresholds,

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* ppb - part per billion; ppt - part per trillion.

present at trace levels in grape musts, and wines. Another thrust of this research project was to be directed toward the design and the use of a system that purports to fulfill the above objectives.

Once preliminary concentration has been achieved, the final separation of the simplified fractions into individual pure components has been conducted on GC columns. The total retention time, t_R, of a solute in a GC column is equal to the time it spends in the mobile phase, t_G, plus the time it spends in the stationary phase, t_R', i.e., $t_R = t_G + t_R'$. The ratio, t_R'/t_G is called the capacity ratio, k. It is a measure of how many times longer the solute spends in the stationary phase than in the mobile phase. If the column is considered to be made up of several individual segments in which equilibrium conditions are instantaneously established between the two phases, it can be visualized that the ratio of the volumes of the mobile and stationary phases in one of these segments/plates will be the same as the ratio of the volume of the phases in the entire column, V_G/V_L. The ratio, V_G/V_L is called the phase ratio, β. It is also equal to the ratio of the inside radius of the column, r, to the average thickness of the liquid-phase film, d_f, i.e., $\beta = V_G/V_L = r/2d_f$. Unlike k and β, there are other terms which have fundamental values because they are dependent only on the stationary phase and the temperature. These are the partition ratio, K, and the relative retention, α. The partition ratio is the ratio of the solute concentrations in the two phases and equals $(\frac{V_G}{V_L})(\frac{W_L}{W_G})$, where W_I is the weight of solute in phase I. The factor, (W_L/W_G) can be shown to be equal to the capacity ratio, k. The partition ratio, K, therefore equals βk and may be rewritten as

$K = \frac{rk}{2d_f}$. Since K is a constant for a given liquid phase at a given temperature, compounds with smaller k will be more difficult to separate than those with larger capacity ratio values. Thus, for low-boiling compounds for example, it is desirable to increase the film thickness, thereby reducing the phase ratio of the column, and so increasing capacity ratios. For high-boiling components, the opposite is true. In this case, it would be better to reduce the capacity ratio in order to reduce the length of time, t_R, the components spend in the column: $t_R = t_G(1+k) = \frac{L}{\bar{u}}(1+k)$, where L = length of column and $\bar{u}$ = average linear carrier gas velocity. The ratio of the adjusted retention times, t'_{R_1}/t'_{R_2}, defines the relative retention, α, for the two peaks 1 and 2. Two peaks are widely separated if α is large, and are completely overlapped if α is 1. The actual resolution obtained between two peaks may be rather poor despite their peak tops being widely spaced, because the width of each peak is a function of kinetic factors such as flow rate, uniformity of column packing, etc. An expression for the overall resolution that takes into account all of the complex kinetic and thermodynamic factors is given by:

$$R = \frac{\sqrt{n}}{4}\left(\frac{\alpha-1}{\alpha}\right)\frac{k}{(k+1)} = \frac{1}{4}\frac{\sqrt{L}}{\sqrt{h}}\frac{(\alpha-1)}{\alpha}\frac{k}{(k+1)} \qquad 1.2$$

where n = number of theoretical plates, α = relative retention, k = partition ratio, h = height equivalent to a theoretical plate, (HETP) and L = column length, Obviously, any attempts to increase n, α and/or k that results in no deleterious effects will enhance the resolution. Columns of greater length and/or smaller diameter will yield more theoretical plates, but changing either parameter leads to an increased

pressure drop through the column, and the van Deemter curves represented by equation 1.3 become sharper. The three terms of the van Deemter equation

$$H = A + \frac{B}{\bar{u}} + C\bar{u} \qquad 1.3$$

where A = eddy diffusion term, B = longitudinal diffusion coefficient, C = resistance to mass transfer coefficient, and $\bar{u}$ = average linear flow rate.

are A for the eddy diffusion of solute into spaces between the packing particles, $\frac{B}{u}$ for the longitudinal diffusion of the solvent along the length of the column, and $C\bar{u}$ for the resistance encountered by the solute to mass transfer between the two phases. In open tubular columns, the absence of large particles of packing material sharply decreases A. The A-term in wall-coated open tubular columns, WCOT is virtually zero. The $C\bar{u}$ term is also reduced in open tubular columns. Because the van Deemter curve is k-specific, the range of solute partition ratios over which maximum resolution can be realized is limited. One method of obtaining very large numbers of theoretical plates by exploiting the increased column lengths in open tubular columns that avoids the limitations imposed by the increased pressure drop is that of recycle chromatography. Lower temperatures and lower temperature programming rates will lead to increased values of k and of α, and permit the system to deliver improved resolution. The disadvantage of this approach is that it leads to longer analysis times and lower sensitivities. Thicker liquid phase films would result in larger partition ratios, but it is observed that beyond 0.4 mm film thickness one of the major advantages of capillary gas chromatography, diffusivity in the liquid phase, no longer becomes inconsequential.

Relative retentions, α, usually vary inversely with column temperature, but are most strongly affected by the choice of liquid phase. In packed column chromatography, the choice of liquid phase is usually the most effective route by which separation efficiency is influenced. In capillary gas chromatography however, there is normally such an abundance of theoretical plates that the choice of liquid phase is a relatively unimportant parameter for many analyses. Fused silica glass capillary columns, now available for gas chromatographic applications, offer high resolution, reproducibility, thermally stable film thickness, excellent chromatographic performance and flexibility in handling. High resolution capillary columns have therefore been used extensively for the qualitative and quantitative analysis[86,87,191-195] of flavor components. Because of the wide range of boiling points represented in a typical flavor extract, capillary columns applied to resolve components in a complex mixture are operated in the temperature programming mode.[156,197] Under linear temperature programmed GC conditions, all eluting components on a column of specific stationary phase can be assigned characteristic retention indices. The retention indices can be calculated by using a linear retention index scale for linear temperature programmed GC suggested by Van Den Dool and Kratz.[196] This retention index scale was applied to several GC analysis[197-203] for calculating the retention index (I) of a substance and is given in eq. 1.4. This linear retention index scale has been found to be superior to Kovats retention index for reporting retention data for flavor components[197,198] with regard to reproducibility and insensitivity to stationary phase film thickness and experimental conditions.

$$I = \frac{T_{R(s)} - T_{R(n\text{-}C_z)}}{T_{R(n\text{-}C_{z+1})} - T_{R(n\text{-}C_z)}} 100 + 100z \qquad 1.4$$

where $T_{R(s)}$ = retention time of substance,

$T_{R(n\text{-}C_z)}$ = retention time of n-paraffin with z carbon atoms,

$T_{R(n\text{-}C_{z+1})}$ = retention time of n-paraffin with z+1 carbon atoms.

The most powerful methods for identifying compounds eluting from a chromatographic column include fourier transform infrared spectroscopy[204-207] and mass spectrometry. Remarkable advances made in recent years in the designing and construction of GC/MS interfaces have enabled capillary column effluents to be transferred directly and quantitatively into the ion source of a mass spectrometer without coming into contact with metal surfaces.[208,209] This has increased the sensitivity of detection of capillary effluents and widened the range of compounds that can successfully be examined by capillary column gas chromatography mass spectrometry. Figure 1.2 shows a schematic of the essential features of a mass spectrometer.

The ion source is the region in the mass spectrometer where sample ionization takes place. The most common method of ionization is by electron impact in which electrons generated from a heated filament in vacuo (13-133 μPa) are accelerated through a voltage, V (usually 70 volts) and directed across the ion chamber. When a volatile sample molecule M is ionized by EI, a molecular ion $M^{+}\cdot$ is produced which may contain sufficient internal energy to fragment by ejection of a neutral particle N or N·, with the formation of a fragment ion A^{+} or $A^{+}\cdot$. If A^{+} has sufficient internal energy, then

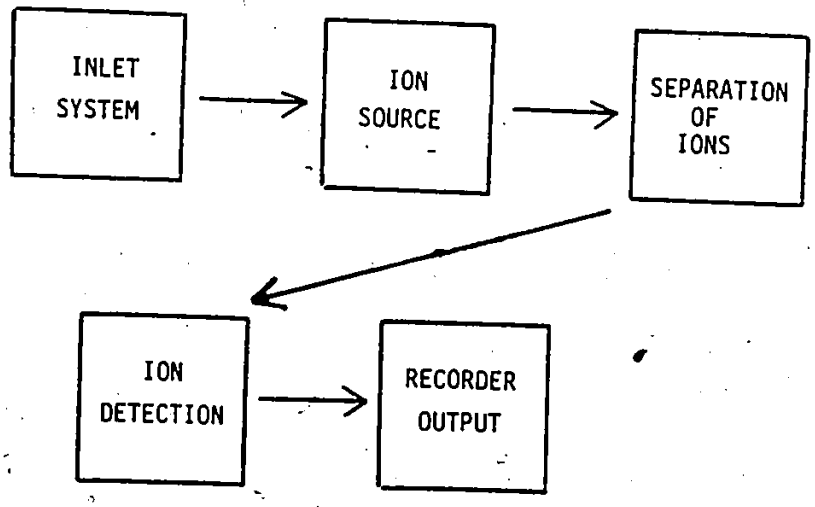


Figure 1.2. Block diagram of the essential features of a mass spectrometer.

further decompositions leading to formation of new fragment ions (B^+, C^+, D^+ etc.) occur till there is insufficient internal energy in any one ion for further decomposition. (Figure 1.3). These fragmentation pathways constitute a fragmentation pattern characteristic for each compound.

Chemical ionization is the ion/molecule reaction between a reactant gas $R^+\cdot$ produced by electron impact and unionized reactant molecules, R, at source pressures of 13 to 133 Pa. This reaction leads to the formation of protonated reactant gas ions $(R+H)^+$ which can ionize other neutral molecules M in the source by proton transfer, proton abstraction or adduct formation (Figure 1.4). At the source pressures employed for chemical ionization, electron capture becomes fairly efficient and the production of negative ions is as likely as for positive ions making both positive ion chemical ionization and negative ion chemical ionization popular complementary ionization techniques. Table 1.2 summarizes the various reactant gases usually employed in chemical ionization mass spectrometry. Note that nitric oxide, Helium, Argon, nitrogen, carbon dioxide ionize sample molecules by charge transfer rather than proton transfer when used as chemical ionization reactant gases. Assuming that the mass spectrometer is tuned to analyse positive ions, the ions produced in the ion source are pushed out of the ion chamber towards the source slit by a low potential applied to the ion repeller plate. Any negative ions produced are discharged at the ion repeller plate and pumped away together with neutral species. The beam of ions is accelerated through a potential (usually 4 kV) after passing through the source slit and the slit

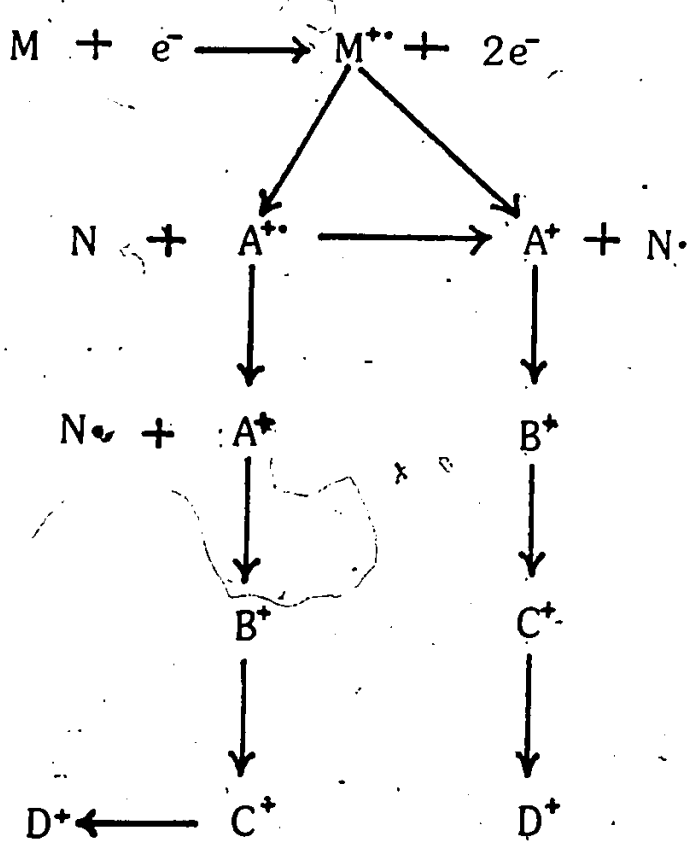


Figure 1.3. Fragmentation pathways for a molecule, M, under electron impact ionization conditions.

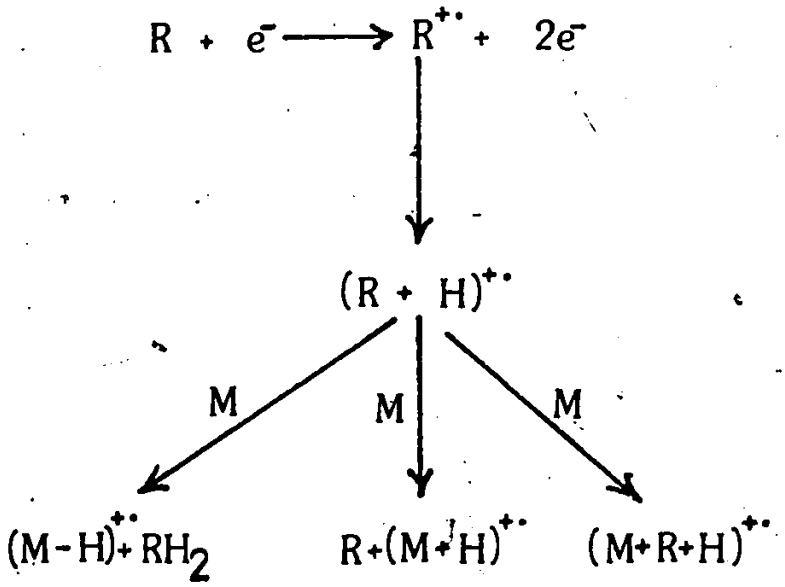


Figure 1.4. Mechanism for the Chemical Ionization of a neutral molecule, M, by CI reactant gas ion $(R+H)^{+\cdot}$.

Table 1.2

Reactant Gases Used in Chemical Ionization Mass Spectrometry

Reactant Gas	Predominant ions at ~ 133 Pa
Methane	CH_5^+, $C_2H_5^+$, $C_3H_5^+$
Propane	$C_3H_7^+$, $C_3H_8^+$
Isobutane	$C_4H_9^+$
Hydrogen	H_3^+
Ammonia	NH_4^+, $(NH_3)_2H^+$, $(NH_3)_3H^+$
Water	H_3O^+
Nitric oxide	NO^+
Helium	He^+
Argon	Ar^+
Nitrogen	N^+
Carbon dioxide	CO_2^+

limiting the angular divergence of the beam. The energy-focussed beam of ions is passed through a magnetic field where mass separation is effected. The magnetic field is varied continuously so that each m/z species is brought to a focus at the same point; this is magnetic scanning of the mass spectrum. Repetitive magnetic scanning over the entire mass range (20-400 u) of interest or over a limited mass range (40-200 u) was the method used in all capillary gas chromatography electron impact induced mass spectrometric studies. Since not much useful information is obtained in the low mass region, gas chromatographic chemical ionization induced mass spectrometric studies were conducted by repetitive magnetic scanning of the mass spectrum above mass 90 u. Isobutane and methane were both employed as CI reactant gases. For sample identification, the chemical ionization approach gives information about (a) the molecular structure from $(M+H)^+$ ion and fragment ions and (b) the molecular weight. The m/z resolved ion beam impinges on the first dynode of an electron multiplier detector and the electrons generated are amplified through a cascading effect on the series of dynodes. The amplified signal from the detector is passed to a recorder.

Recording the Mass Spectrum

Having determined the ion intensities for a set of ion masses, it is usual to record the mass spectrum either in a normalized form (% RA, percentage relative abundance) or as percentage of the total ion current (% TIC). If the mass spectrum is processed to remove 'background' ions of constant unwanted impurities, such as chemical ionization reactant gases, or decomposition products of the stationary

phases of gas chromatographic columns in GC/MS, then the % TIC method becomes percentage reconstructed ion current (% RIC). Table 1.3 shows a computer output listing of the mass spectrum of 2-phenethyl alcohol in both % RA and % TIC forms. In the analysis of a pure sample, it is a simple matter to record one scan when the TIC for example reaches a suitable level. When multiple components are involved, as in GC/MS, use of repetitive scanning of the mass spectrometer is preferred. As the scans are taken at regular and frequent intervals in time, the % TIC, or % RA provides a reasonable sample intensity profile equivalent to a normal gas chromatogram. A mass resolved chromatogram is a plot of the abundance of ions of a specified mass against time or scan number. When an effluent from a gas chromatographic column enters the ion source, the ions which comprise its mass spectrum will all increase in abundance to a maximum simultaneously, at the retention time. If a chromatographic peak contains more than one compound, provided that each component has in its mass spectrum at least one distinguishing mass peak, mass resolved chromatograms will reveal the true peak profiles of the individual components despite being unresolved chromatographically. Such a situation is illustrated in Figure 1.5 in which a peak, (scan number 450) apparently homogeneous by inspection of the total ion current chromatogram, is shown by selected ion retrieval to be composite (see Figure 1.6 for respective mass spectra). Figure 1.5 shows part of an analysis of a mixture of known volatile flavor standards by capillary GC electron impact induced mass spectrometry. To be resolved by this technique, chromatographic peaks must maximize at least two scans apart.

Table 1.3

Ion abundances in the mass spectrum of 2-phenethyl alcohol

($C_8H_{10}O$, mol. wt = 122)

Peak No.	Mass	%HT. RA	% TIC
1	27.2	1.8	0.69
2	28.1	0.1	0.05
3	29.0	1.3	0.48
4	30.9	4.7	1.81
5	38.1	1.4	0.53
6	39.0	8.9	3.40
7	41.0	2.1	0.80
8	49.9	2.9	1.12
9	51.0	6.8	2.60
10	52.1	1.7	0.64
11	62.1	1.5	0.58
12	63.1	4.3	1.65
13	65.1	15.9	6.06
14	77.1	4.6	1.75
15	78.1	3.8	1.43
16	89.0	2.7	1.01
17	90.0	1.7	0.64
18	91.0	100.0	37.94
19	92.1	59.2	22.53
20	93.1	4.2	1.59
21	103.1	2.7	1.01
22	104.2	2.0	0.74
23	122.1	26.4	10.04
24	123.1	2.4	0.90

Data was obtained on the McMaster University VG7070 GC/MS system operating under low resolution EI conditions.

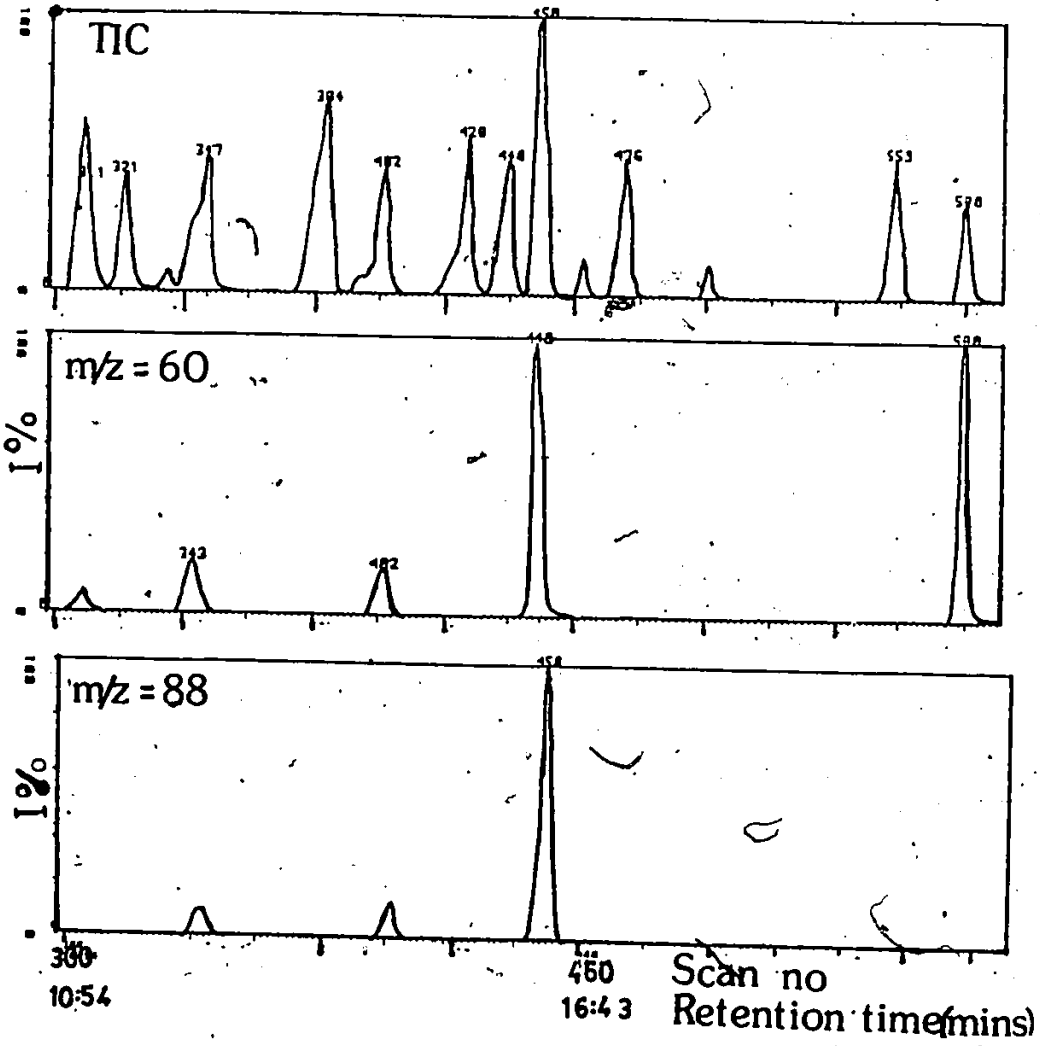


Figure 1.5. Part of the total ion current chromatogram of an analysis of volatile flavor standards by fused silica capillary. GC/MS (top). Mass resolved chromatograms of ions at m/z 60 (middle) and 88 (bottom). Note that the mass chromatograms maximize at different scans.

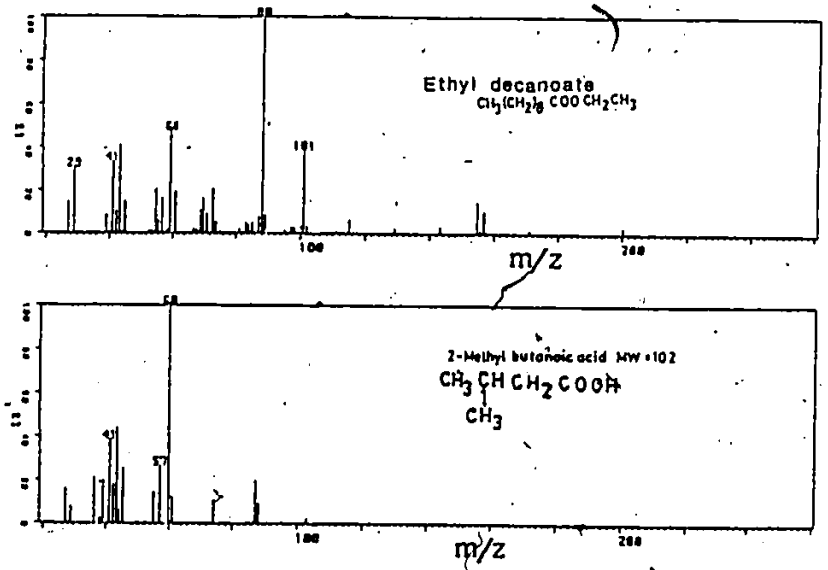


Figure 1.6. Mass spectrum of component at scan 450 (Ethyl decanoate) and mass spectrum of component at scan 448 (2-methyl-butanoic acid).

At the fast scanning rates employed in this study, (1 second/decade) this technique provided a very reliable method for determining reproducible retention indices for flavor components eluting from the gas chromatographic column. Structural identification can be made through accurate mass measurements of molecular and fragment ions, comparison of acquired mass spectra with library files (international data bases and home-made mass spectral compilations) and studies of metastable ions.

Often, the very small quantities of material involved exclude the use of other, more confirmatory physical methods. Using various designs of GC effluent splitters, it is possible to split the capillary column effluent with simultaneous recording of GC traces by a non-selective detector such as FID or TCD, and a selective detector such as FPD, ECD or TSD.[210-216] A more common approach is to split the chromatographic effluent directing one part into a mass spectrometer source and the other part to a "sniffing" port. Olfactograms so generated can be used to locate areas of organoleptic interest in the chromatogram. The FID allows a reproduction of the % TIC obtained by GC/MS. The TSD, ECD, and FPD allow the enhanced sensitivity and selectivity required to detect a specific compound present in trace amounts. Figure 1.7 summarizes the protocol usually followed in the analysis of a natural flavor extract that has been described in this section. Compounds identified in such a study must be quantificated by an appropriate quantitative method.

1.8 THE RESEARCH PROBLEM

1. Methyl anthranilate has long been considered to be responsible for

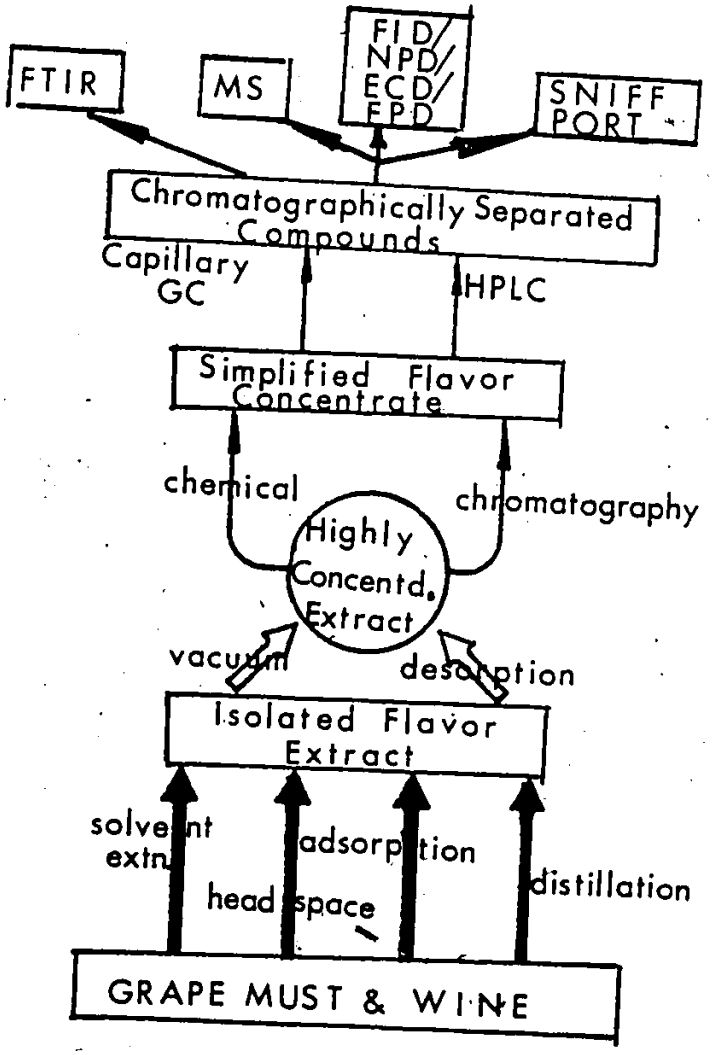


Figure 1.7. Analytical methodology usually followed in the examination of a natural flavor extract.

the "typical labrusca flavor" associated with the vitis labruscana varieties of grapes and wines. More recent investigations into the role played by methyl anthranilate in the overall aroma of the native varieties indicate that other compounds or compound, probably of very low odor thresholds and presumably present at very low concentrations, may rather be responsible for imparting this typical flavor. Current analytical methods for investigating volatile flavor components have failed to detect and identify what these other compound(s) could be. There is no evidence in the literature that such compound(s) have as yet been detected and identified in the vitis labruscana varieties.

2. Until recently, vitis vinifera vines could not be cultivated successfully on Canadian soil. The current breakthrough, it is anticipated, would help boost up the production of superior quality wines in Canada. Climate, soil and vinification practices are known to influence the composition and quality of grapes grown and therefore the wines produced. Although commercial wineries in Canada use the usual indicators to determine and control the quality of wine produced from these "new varieties", there is no evidence in the literature that the composition, the qualitative and quantitative distribution of the volatile flavor fraction of the musts and wines of these "new varieties" are being analysed. There is a large volume of published qualitative and quantitative analytical data on the composition and distribution of volatile aroma compounds of the vitis vinifera varieties grown in the more favourable, cooler climate of Europe. The question which vintners and viticulturalists would like to be able to answer is whether these "new varieties" which have produced superior wines in

Europe can also produce superior wines in Canada and Eastern United States?

1.9 The Research Objectives

1. To determine the identity or identities of the compound(s) believed to be responsible for the typical labrusca flavor.
2. To determine whether the vines of vitis vinifera varieties such as White Riesling now being cultivated successfully in Canada and the Eastern United States are potentially suitable for producing superior quality wines as those grown on the more favorable, cooler climate of Europe.

1.10 Experimental Design Considerations for the Realization of the Research Objectives

1. Develop a new high-vacuum, low-temperature concentrator apparatus that substantially enhances the concentration of especially trace level volatile flavor compounds to within the detection range of our analytical instruments. Eliminate limitations on trace analysis presented by major component overloading of capillary columns and mass spectrometer.
2. Test the sensitivity and suitability of the new apparatus to the detection of trace compounds of very low odor thresholds known to be present in Cabernet Sauvignon.
3. Use the developed apparatus for the analysis of several vitis labruscana varieties of grape musts and wines to determine if any trace level compounds of significant organoleptic importance may be detected that are common to all these varieties.

4. Determine the chemical/structural identities of any such compounds that may be detected.

5. Use the apparatus to analyse locally cultivated vitis vinifera musts and wines and to monitor the qualitative and quantitative distribution of the volatile flavor compounds as they develop from grape must to the aged wine.

CHAPTER TWO

EXPERIMENTAL DETAILS

2.1.1 Volatile Flavor Standards and Solvents

All flavor compounds used in this investigation were of Analar grade purchased from one of the following chemical companies: British Drug House (England), Eastman Kodak (USA), Fisher (Canada), Sigma (USA), Pyrazine Specialities (USA), Aldrich (USA) and McArthur (Canada). All compounds were checked for chromatographic purity before use. Acetone (British Drug House) and Freon (Canadian Liquid Air) were purified by vacuum distillation.

2.1.2 Gas Chromatographs

Two types of gas chromatographic equipment were used in this study. One of these was a Varian Aerograph model 1800 dual column, dual flame ionization detector gas chromatograph that could accommodate 1/4-inch O.D and 1/8-inch O.D columns. A dual differential electrometer enabled independent and simultaneous operation of the two flame ionization detectors. A microsample splitter (Figure 2.2a) provided an approximately 1:10 split ratio of the chromatographic effluent to one of the two detectors and exit port. The exit port effluent was either directed to a fraction collector or a receptacle where the nose of the experimenter could evaluate its significance. A 15' x 1/8-inch O.D stainless steel 10% Carbowax 20 M on 80/100 mesh Chromosorb W

(AW/DMCS, Chromatographic specialities, Canada) column, a 6' x 1/4-inch O.D x 2mm I.D glass column packed with Superpak 20M (Analabs, Connecticut U.S.A.) and a 6' x 1/4-inch O.D x 2mm I.D. glass column packed with 10% SE-30 on 80/100 mesh Chromosorb W (AW/DMCS, Chromatographic Specialities, Canada) were the principal columns used for packed column gas chromatography. Because of prior experience with the severely limited separation ability of packed columns for the complicated wine flavor extract, it was found necessary to modify one of the channels of this instrument to accommodate capillary columns. Figures 2.1a and 2.1b show the injector port assembly before and after modification to accommodate capillary columns. The main objective was to effectively minimize dead volume in the system. This splitter/splittless system incorporated a 10 cm long, 1/4-inch x 2mm I.D demountable sample vaporization tube connected to an adjustable needle valve (SGE, Australia) by a 5' x 1/4-inch O.D stainless steel buffer volume. The split point consisted of a 1/16-inch stainless steel tube silver-soldered through a 1/16-inch hole drilled in the hexagonal body of a 1/4-inch to 1/16-inch swagelok union. A 1/16-inch O.D x 0.75 mm I.D glass lined transfer tube (GLT) was installed such that the end of the tube in the vaporization tube was at least 7 cm above the split exit point to ensure correct operation of the splitter. Normal splitter flow was set at 50 ml/min of prepurified nitrogen gas (Canadian Liquid Air). A septum purge (approx. 2 ml/min) connected to an adjustable SGE needle valve was included in this design to eliminate peak broadening due to sample and solvent flashback during injection and also septum bleed usually produced during temperature programming. Figure 2.3 illustrates

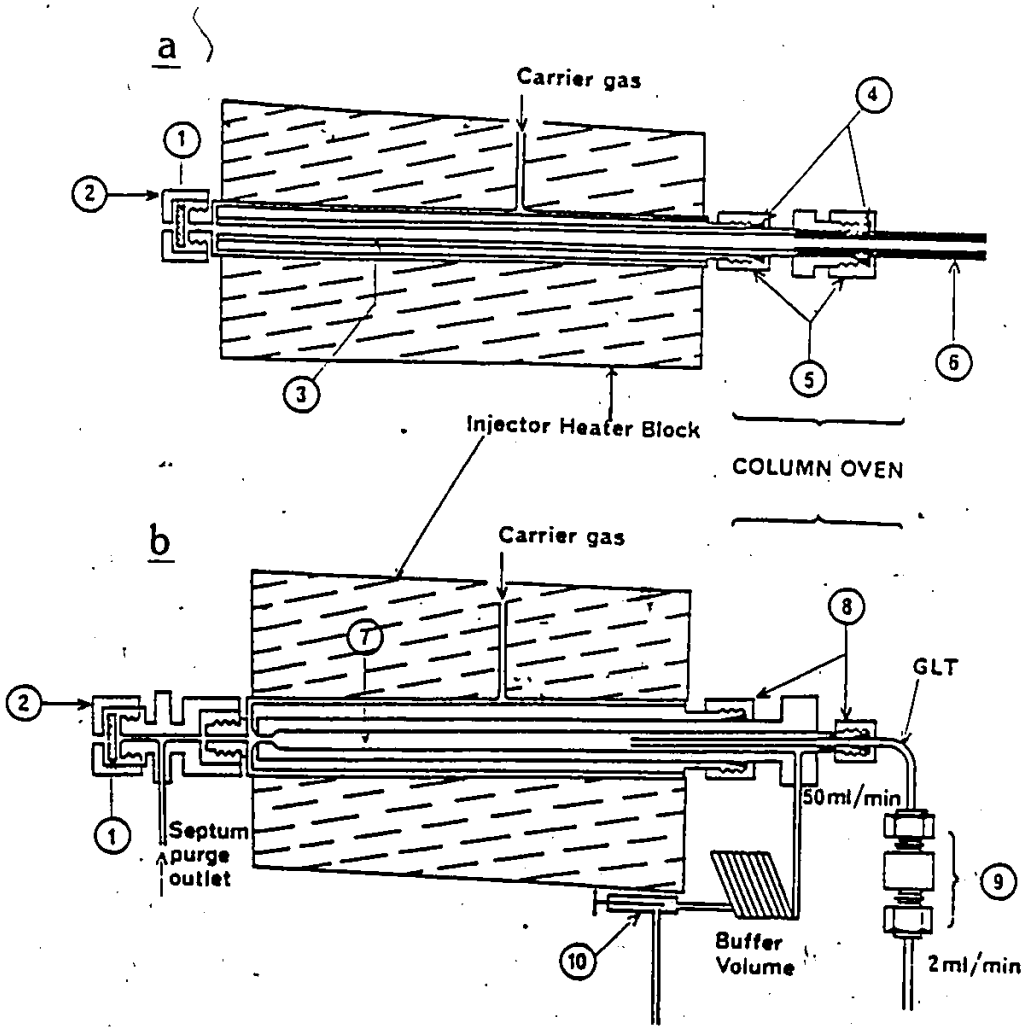
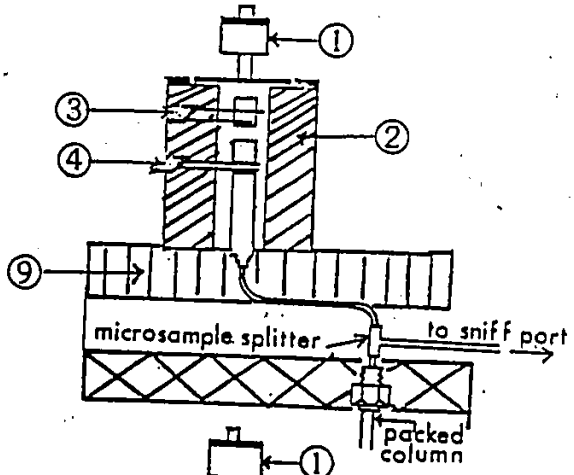


Figure 2.1a. Varian Aerograph model 1800 GC injector port assembly before modification.

Figure 2.1b. Varian Aerograph model 1800 injector port assembly after modification to accommodate capillary columns. (1) teflon-coated silicone rubber septum; (2) septum retainer; (3) stainless steel 1/4" O.D. insert; (4) stainless steel terrules; (5) 1/4" to 1/4" swagelock union; (6) 1/4" O.D. column; (7) 1/4" O.D. x 2mm ID glass; (8) 1/4" to 1/16" swagelock union; (9) zero dead volume 1/16" to 1/16" swagelock union; (10) SGE needle valve.

2.2



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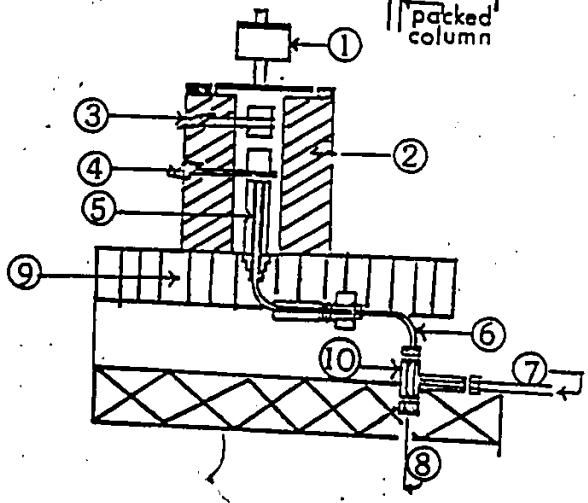


Figure 2.2. The Standard Varian 1800 FID assembly before modification.

Figure 2.3. The FID assembly after modification to accommodate capillary columns. (1) FID tower cap; (2) detector oven; (3) collector electrode; (4) polarizer electrode; (5) glass insert; (6) glass lined transfer tube; (7) make up gas inlet; (8) capillary column; (9) detector oven base; (10) make up gas tee.

modifications made on the FID assembly (Figure 2.2) to ensure low dead volume in the detector. It incorporated a 1 mm to 1/16-inch to 1/16-inch make-up gas tee (19) and a glass capillary insert (5) in the flame tip assembly. The output from the packed column detector was recorded on a Leeds and Northrup model Speedomax recorder operated at a fixed chart speed of 1.0 cm/min. The output from the capillary column detector was recorded on a two-pen Linear (Canada) recorder. The performance of this modified capillary system was evaluated with a 45 m x 0.5 mm I.D glass SCOT Carbowax 20 M (Chromatographic Specialities, Canada). Figure 3.1 (Chapter 3) shows the test chromatogram for this capillary system. The other instrument was a Varian model 3700 gas chromatograph coupled to a double-focussing magnetic sector mass spectrometer (MM70-70F, VG Analytical Ltd., Altrincham, England) interfaced with a VG Data system model 2035 equipped with a 32K PDP8A processor, a VT55 display unit and a Bryans X-Y plotter.

2.2 TECHNIQUES OF ANALYSIS

The following scheme was devised in order to perform qualitative and quantitative analysis of the volatile flavor components of musts and/or wines:

(i) sample collection

(ii) solvent extraction of the volatile flavor components from the sample

(iii) enrichment of the isolated flavor components

(iv) gas chromatographic separation of the enriched volatile flavor extract

(v) identification of the separated components, and,

(vi) quantitative analysis of the separated and identified components.

In order that one of the basic aims of this project (the development of an analytical methodology for routine analysis of flavor volatile components) be realized, the underlying principle adopted in the development of the analytical methodology was to modify and minimize, where possible, stages in existing analytical procedures that required extremely long periods to accomplish. This section describes the technique adopted for each stage of the analysis and used throughout this research project.

2.2.1 Sample Collection

In order that the analysis performed may reflect directly on what obtains in the wine industries, samples for analysis were obtained directly from the following wineries: Chateau-Gai Wineries, St. Catharines, Canada; Andrés Wines Limited, Winona, Canada. The 1972 Chateau Pichon Lalande wine was obtained from Professor Tomlinson's wine cellar. The wineries usually provided the history of the samples, as well as instructions for their storage.

2.2.2 Isolation of the volatile flavor components from the sample

A solvent extraction methodology, first developed in this laboratory[36] and shown to perform efficient, rapid, and reproducible extraction of flavor volatile components from alcoholic solutions in three hours, was adopted with only a slight modification. Figure 2.4 illustrates the all-glass solvent extraction apparatus designed for use with Freon, used in some sections of this project. It was operated in a batch mode as follows: the solvent pot, T, (RBF 500 ml)

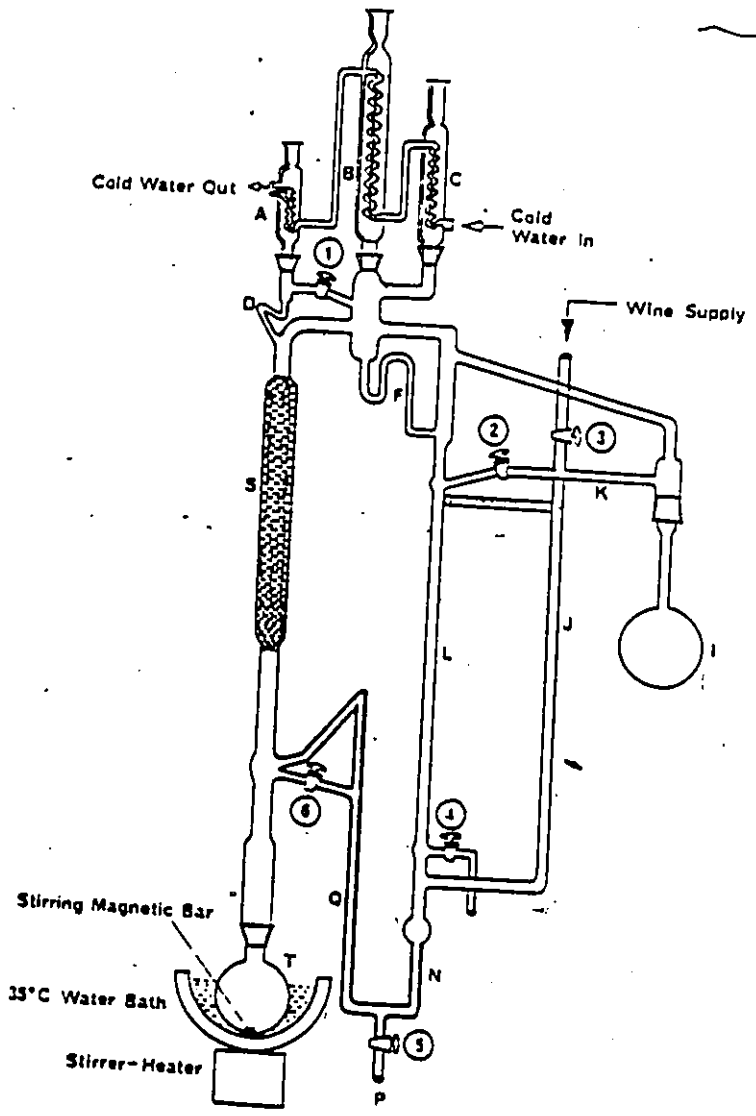


Figure 2.4. Solvent extraction apparatus

D: reflux syphon F: intermittent syphon
J: sample entry tube L: extracting tube
N: collection bulb S: fractional distillation column
T: solvent pot

initially containing 250 ml of Freon (bp 24.1°C) and a 7mm x 12 mm egg-shaped stirring bar magnet (teflon coated) sits in a warm water bath held at 35°C by a heater-stirrer. Vigorous stirring is maintained in T. The resulting freon vapor condenses into the reflux syphon D which provides reflux solvent to the fractionating column S, (50 cm x 2.5cm O.D packed with 3 mm glass helices) and the intermittent syphon, F, which provides a volume of Freon (1.15 ml) sufficient to form a single bead in the extracting tube, L. L is filled with approximately 100 ml of sample through the entry tube, J, and held stationary in L with pressurized air in the entry tube with stopcock 3 closed. The freon bead falls through the length of L into the collection bulb, N. The contents of N may be withdrawn through stopcock 5. As the consecutive freon beads fall, the Freon and extracted volatile flavor components flow into T through the capillary tube, Q. Stopcock 6 determines the height of freon in the extracting tube and therefore the height of the sample in L. (95 ml for wine and 100 ml for grape juice). The extracted volatile flavor components collect in T and the warm, stirred, Freon solution undergoes fractional distillation in S. This enables purified freon to recycle and re-extract the sample in L. When the extractor was operated in a continuous mode, the extracted sample (sample, less flavor volatiles) flows out of the top of L through stopcock 2 into a collection flask, I, at a rate determined the sample inlet. Since the device has been shown to provide efficient, reproducible extraction of volatile flavor components at concentrations higher than 2 ppm in 12% ethanol solutions, it was sufficient to check the validity of the claim for concentrations lower than those in this

previous study.

In order to evaluate the efficienty of the solvent extraction technique, a stock solution consisting of approximately 400 ppm of each of the listed components, previously identified as grape juice and/or wine components, was made by dissolving appropriate amounts of each component by a microliter syringe into 95% ethanol solution and diluting with water to 12% in a calibrated volumetric flask. Appropriate dilutions of this stock solution were made with 12% ethanol solutions to obtain standard solutions of the following concentrations 2.0, 0.02, 0.002 ppm. 95.0 ml of the 2.0 ppm solution of standard volatile components was introduced into the extracting tube L and 250 ml of freon added to the solvent pot T and collecting bulb, N. Extraction of the volatile components was continued for a period of three hours; the contents of T were removed and enriched 500-fold, using the low temperature, vacuum concentrator (Figure 2.5) used in a previous study, and submitted to gas chromatographic mass spectrometric analysis. This procedure was repeated in triplicate for each of the solutions at the specified concentrations. Percentage recoveries for each component obtained by this technique of solvent extraction were calculated and the precisions involved in repeated sample extractions also calculated. The results of this study are shown in Table 3.2.1.

To evaluate the efficiency of the fraction distillation column, S, in purifying refluxing freon solvent required for the re-extraction of volatile components, 250 ml of a 2 ppm solution of the same volatile flavor components in Freon were placed in T and heated at 35°C for 3 hours duplicating all extracting conditions except that no solution

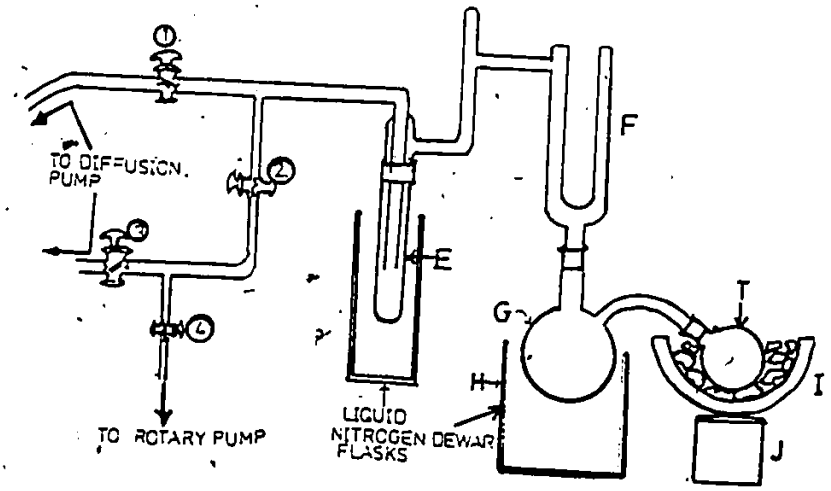


Figure 2.5. Low temperature, vacuum concentrator used in a previous study.[36]

E: cold trap F: cold finger of a cold trap
G: distillation bulb H: liquid nitrogen dewar flask
I: dry ice-acetone slush bath J: magnetic stirrer
T: solvent pot

occupied the extracting tube, L. After 3 hours the freon distillate that was collected in L was removed, enriched and submitted to capillary GC/MS analysis. The results of this study are shown in Table 3.2.2. When the enrichment higher than that provided by the apparatus shown in Figure 2.5 was required, the solvent pot, T, (Figure 2.4) was replaced by a modified solvent pot, N, shown in Figure 2.6.

2.2.3 Enrichment of the Isolated Volatile Flavor Components

Freon has an unusually high vapor pressure at low temperatures. This property of freon was taken advantage of in the design and development of the low-temperature, high vacuum concentrator. Figure 2.6 shows the front view of the apparatus constructed from pyrex glass tubing and used in the first stage of the two-step enrichment process. Operational parameters for this new design of low temperature, high vacuum concentrator, were experimentally determined as follows: the extruding bulb, S, blown onto N had an internal volume of 700 µl and outer dimensions of 2.5cm x 8.0mm O.D. In order to determine by visual inspection the point of sufficient distillation, calibration marks corresponding to 100 µl and 500 µl of freon in S were engraved on the glass. Stirring in S was achieved with a 7mm x 2mm micro magnetic bar, Q, which spun about a horizontal axis. An egg-shaped magnetic bar P was selected for stirring solutions in N in its initial configuration such that its dimensions (20mm x 12mm; 10mm at mid-point section) prevented it from dropping into the extruding bulb, S.

This new design of concentrator was operated as follows: the freon solution containing the isolated flavor components in the reservoir bulb, N, is frozen in liquid nitrogen and then cooled from underneath

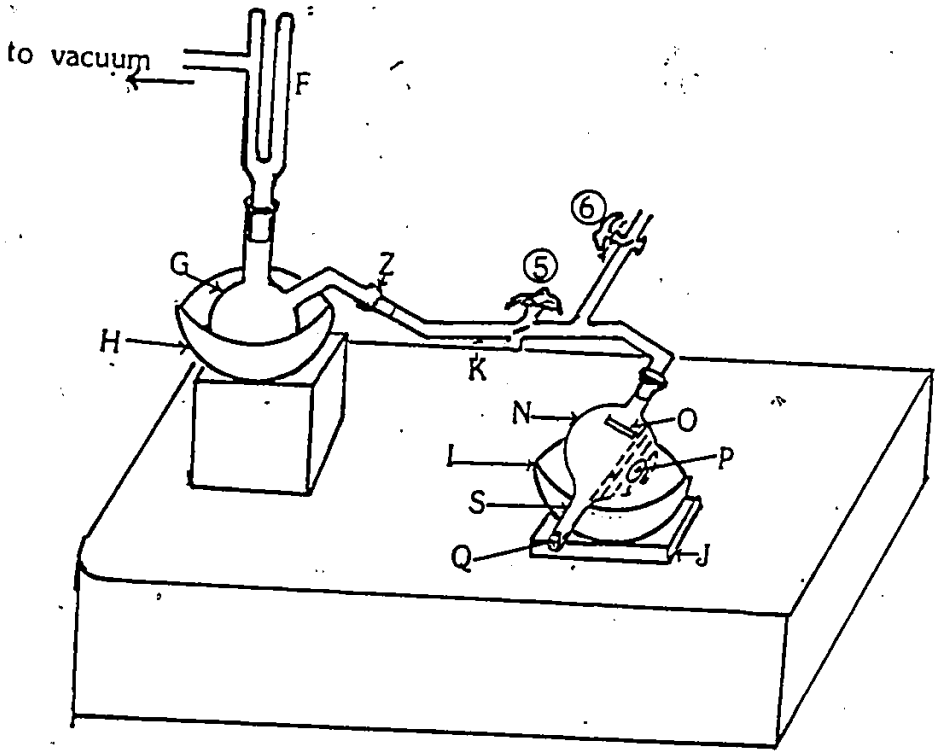


Figure 2.6. New design of low temperature, high vacuum concentrator in its initial operating configuration used in the first stage of the enrichment procedure.

by a dry-ice acetone slush bath, I, (-78.1°C). The reservoir bulb, N, was connected to the distillation bulb, G, through the side arm by way of the transfer tube, K, designed to provide the desired configuration required for performing the necessary distillation procedures. In its initial configuration, K allows N to rest in I at an angle of approximately 30° to the horizontal. When the contents of N had melted, stopcock 5 was opened with stopcock 6 closed and liquid nitrogen was poured into the cold finger, F, and the dewar, H, surrounding the distillation bulb, G, while maintaining vigorous stirring in N to prevent any concentration gradient build-up at the solution surface. The temperature gradient set up, (-78.1°C to -196°C) resulted in the condensation of freon vapor on the cold finger, F, and the glass walls of G. Distillation was continued until visual inspection indicated that the volume of solution left in N was approximately 2 ml at which time the apparatus was slowly rotated downward about joint Z while lowering the adjustable laboratory jack J, so that the solution in N was transferred into S as shown in Figure 2.7. The magnetic bar P was retrieved with a horseshoe magnet, R, and harnessed in position by O. When the remaining solution in N has been completely transferred into S, vigorous stirring was maintained in S with the micro magnetic bar, Q. Distillation was discontinued when the desired volume in S was reached. At that point, all stirring, pumping and cooling were discontinued. N was disconnected from the vacuum line by closing stopcock 5 and opening stopcock 6.

Once the bulb and its contents had warmed up to near room temperature, the contents were transferred into either a cooled 3 ml

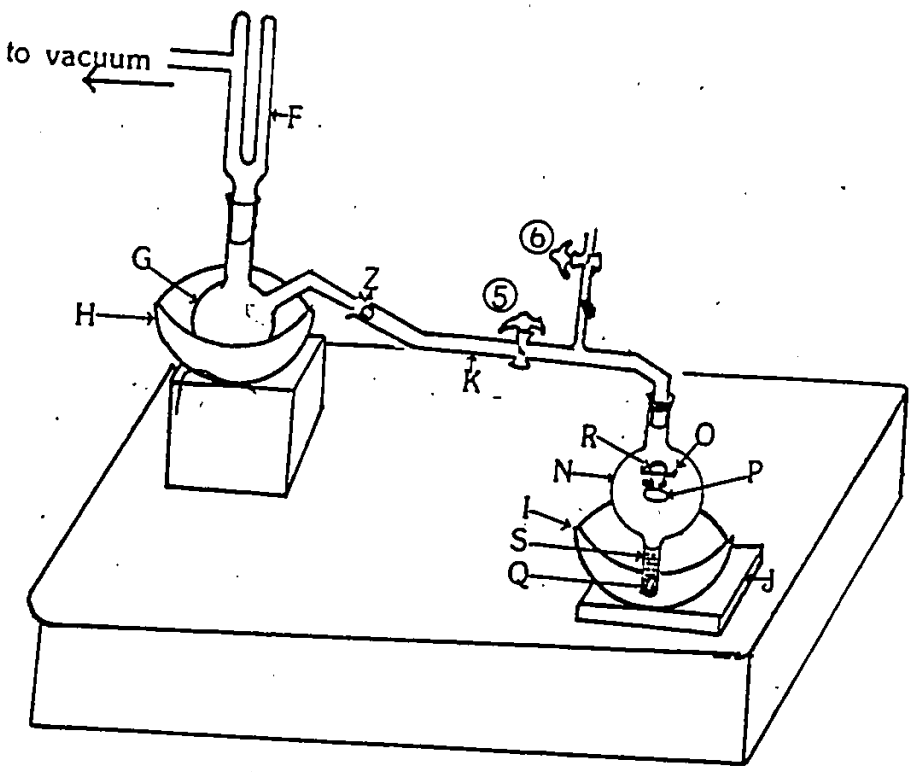


Figure 2.7. Final configuration of concentrator used in the first stage of the enrichment procedure.

K: transfer tube N: modified solvent pot or reservoir bulb O: harness for horseshoe magnet P: stirring bar magnet Q: micro stirring bar magnet R: horseshoe magnet S: extruding bulb.

teflon septum sealed screw-capped reaction vial (Chromatographic Specialities, Ontario, Canada) or into vial T which replaces bulb N in Figure 2.7 as shown in Figure 2.8.

In order to evaluate the efficiency and the reproducibility of the new design of concentrator shown in Figure 2.7, 250 ml of the 2 ppm standard solution of volatile components in freon was subjected to the first stage of the concentration procedure and concentrated as described above. It was transferred with a 500 microliter syringe into a cooled teflon septum sealed screw-capped sample vial for capillary gas chromatography mass spectrometric analysis. This procedure was repeated twice more for the 2 ppm solution and in triplicate for a 0.02 ppm and 0.002 ppm standard solutions of flavor components. The results of these experiments are shown in Table 3.2.3. (Chapter 3).

2.2.4 Extraction of Fatty Acids from the Partially Concentrated Volatile Flavor Extracts

In order to investigate the effect of an aqueous bicarbonate solution on the extraction of fatty acids in Freon, 100 µl of a 5% aqueous $NaHCO_3$ solution was added to 100 µl of a 5000 ppm (equivalent of 2500-fold enrichment of the 2 ppm solution) of the same volatile flavor standards in Freon in a 3 ml teflon septum sealed screw-capped reaction vials. The contents of the reaction vial were stirred vigorously for 10 mins with the aid of a 7mm x 5mm egg-shaped magnetic bar. The two phases were allowed to separate and the freon layer was transferred by means of a cooled 500 µl syringe into either a cooled 1 ml teflon septum sealed screw-capped vial and analysed by capillary

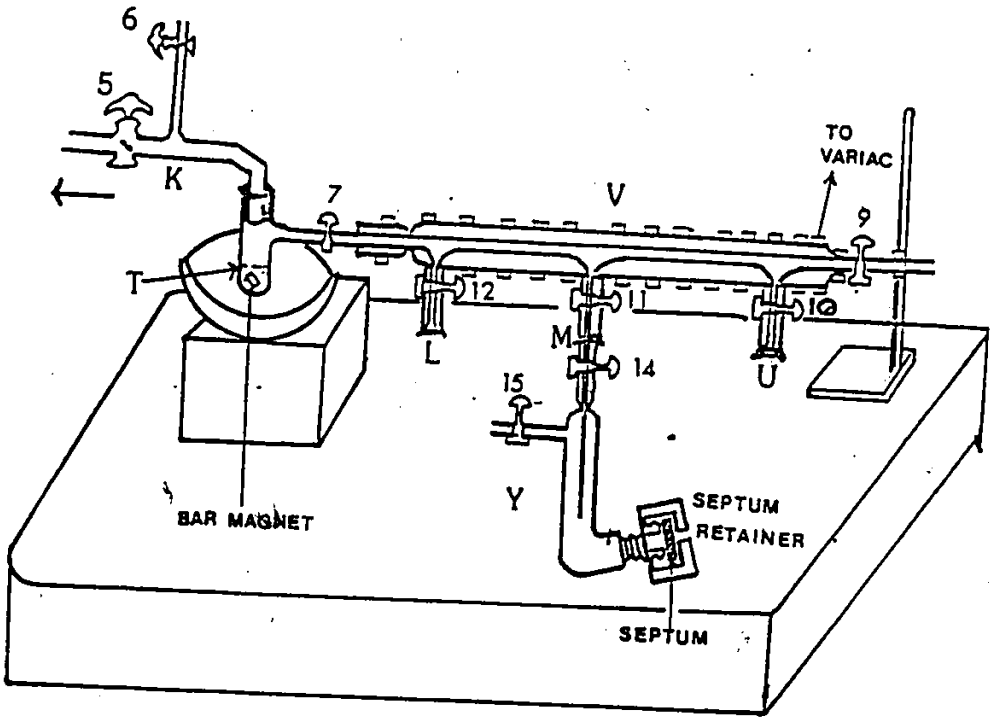


Figure 2.8. New design of low temperature, high vacuum distillation/ concentration apparatus used in the final step of the two-step enrichment procedure.

Y: sample receiver V: air-jacketed tube
L,M,U: side arms of V.

GC/MS (see Table 3.2.4 for results of this analysis) or a 3 ml teflon septum sealed screw-capped reaction vial for further experimentation. The aqueous fraction was acidified with 5% HCl solution. The acidified solution was added to 95 ml of a 12% ethanol solution and the acids isolated and concentrated by the methods outlined in sections 2.2.2 and 2.2.3. Table 3.2.5 shows the results of a capillary GC/MS analysis of the isolated, and concentrated fatty acids.

2.2.5 Extraction of Fusel Alcohols into Propylene Glycol

To evaluate the extent to which fusel alcohols in freon would be extracted, a 100 µl of propylene glycol was added to 100 µl of a 5000 ppm solution of the same volatile flavor standards in freon in a teflon septum sealed screw-capped reaction vial. The contents of the vial were stirred vigorously for 10 mins. The separated propylene glycol fraction was removed and another 100 µl portion of propylene glycol was added and the mixture was stirred again for 10 mins. The separated freon fraction was transferred with a cooled 500 µl syringe into either a 1 ml teflon septum sealed screw-capped vial for capillary GC/MS analysis (see Table 3.2.6 for results of this analysis) or into the reservoir tube, T, (see Figure 2.8) for the final stage of the enrichment procedure.

2.2.6 Final Stage of the Enrichment Procedure

Figure 2.8 shows the apparatus designed and constructed from pyrex glass tubes and used for the final stage of the two-step enrichment procedure. The reservoir bulb, T, containing 100 µl of the 5000 ppm solution of volatile flavor standards in freon was cooled in a

liquid nitrogen dewar and connected to the main vacuum line through the adaptor K with stopcocks 5, 6 and 7 closed. T was connected to the air-jacketed tube, V, (20 cm long and 4 mm I.D) which was provided with three side arms L, M, U. These side arms were connected to sample receivers like Y through a B14/23 ground glass joints. Y was designed such that stopcocks 14 and 15 enabled its isolation and detachment from the system while the teflon septum sealed screw-capped design enabled samples to be withdrawn from Y by means of syringes. The air jacket was wound with nichrome heating wire connected to a Variable voltage supply (variac). The outside of this jacket was coated with asbestos paper which provided both electrical and heating insulation.

The concentrator was operated as follows: with the contents of T still frozen in liquid nitrogen, the sample receiving trap, Y, was connected to V through joint M and stopcocks 5, 7, 11 and 14 were opened with stopcocks 6, 9, 10, 12 and 15 closed, and the system was evacuated. When a suitable vacuum was attained in this system (13-130 mPa) stopcock 7 was closed and the contents of T were degassed. The concentrator was then isolated from the pumping system by closing stopcock 5. The receiving trap Y was cooled in liquid nitrogen and the contents of T were allowed to warm up to 40°C (40°C water bath) and stirred vigorously with the micromagnetic bar. The temperature gradient set up between Y and T results in the condensation of volatile components in Y. Approximately 40 minutes after the commencement of this process, stopcock 14 was closed, all warming and stirring discontinued and any uncondensed vapors in V condensed back into T

(∿ 20 mins). Stopcock 7 was closed and Y was disconnected from the system by opening stopcock 9. A temperature of 55°C was maintained in V during the distillation. The contents of the reservoir bulb, T. (25 µl) and the receiving trap Y (70 µl) were analysed by capillary GC/MS. The results of these analyses are shown in Tables 3.2.7 and 3.2.8.

2.2.7 Gas Chromatographic Separation of the Enriched Volatile Flavor Extract

Gas chromatographic columns were used to separate the enriched volatile flavor extracts into individual components for subsequent identification. For packed column chromatography, the injector port temperature was held at 200°C, the flame ionization detector temperature was held at 250°C, while the column temperature was programmed from 20°C to 210°C at 6°C/min for the Carbowax 20 M columns. Helium (25 ml/min) was the carrier gas used. Hydrogen and air flow rates through the detector were set at 25 ml/min and 250 ml/min respectively. Volumes of 5 to 10 µl of samples were injected onto these packed columns which were used in preliminary experiments to evaluate areas of the gas chromatograms produced by injection of samples of natural volatile flavor extracts which were of organoleptic interest. SCOT columns (45m x 0.5mm I.D glass Carbowax 20 M) were operated as follows: with the split valve closed, a given volume of sample (1-4 µl) was injected onto the column with the injector port temperature and flame ionization detector held at 200°C and 250°C respectively. Where the mass spectrometer served as the detector for the eluting gas chromatographic effluent, the ion source temperature was held at

220°C. After 20 seconds, the split valve was opened to provide the desired flow rate of helium into the detector. The column was temperature programmed from 50°C to 210°C at 4°C/min and held for 20 mins at the final temperature. WCOT columns were operated similarly except that there was no need to close the split valve at the time of injection.

2.2.8 Identification of the Separated Components

Tentative identification of the separated components in the isolated fractions was made by (a) matching retention indices of unknown components in the natural flavor extract with those of known volatile flavor standards and, (b) mass spectral analysis. In order to calculate retention indices for all the known volatile flavor standards available in our laboratory, a solution of a mixture of these standards in freon was co-injected with a fixed number of n-alkanes onto a Carbowax 20 M or a SE-54 capillary fused silica column. The same number of n-alkanes was co-injected with isolated volatile flavor extracts of grape juices, musts and wines and gas chromatograms obtained by GC/FID technique and/or % TIC chromatograms by GC/MS technique. In order to provide mass spectral data for the tentative identification of the separated volatile flavor components, electron impact mass spectra, EIMS, were obtained for all available volatile flavor standards eluting from the capillary column into the ion source of the mass spectrometer which was operated as follows: ionizing voltage, 70 eV; accelerating voltage, 4 kV; trap current, 100 μA; ion source temperature, 220°C; electron multiplier amplifier

gain was set at 1 and a sensitivity of 10^{-7} amps with a response time of 0.1 ms. Mass range of interest was scanned in an exponential down-scan mode at 1 second/decade at resolving powers of ≥ 1000 (10% valley definition). The same procedure was adopted for obtaining mass spectra of compounds in the natural flavor extracts.

Mass spectra obtained for components in the natural flavor extract are matched against known compounds whose mass spectra are listed in a reference library file. A fit is found for such a compound if its mass spectrum bears some similarity to one or more listed reference compounds. For those compounds whose mass spectra are not matched to any in the library file, manual interpretation of the mass spectral data was used.

In order to confirm the molecular weights of components whose EI mass spectra had been obtained as above, and to establish the molecular weights for those compounds whose EI mass spectra showed no detectable molecular ions, the eluting components from the capillary column into the ion source were subjected to chemical ionization mass spectrometry. To achieve this the mass spectrometer was operated as follows: the chemical ionization reactant gas manifold was evacuated and CI reactant gas was admitted into the manifold. It was allowed to purge to waste for a few seconds after which time the reactant gas was allowed to enter the ion source. The source current was set to 500 μA and the electron energy at 50 eV; source temperature was held at 150°C and the CI ion exit slit was selected. Optimum sensitivity was obtained for m/z 17 and 29 for methane and 43, 57 for isobutane. n-Eicosane was introduced into the ion source via the solid probe and

its $(M-H)^+$ ion at 281 (base peak) was used to optimise the source current setting. Figure 2.9 shows the chemical ionization mass spectrum of n-eicosane when the mass spectrometer is operating properly under chemical ionization conditions. Using standard GC operating procedures and with the mass spectrometer operating under chemical ionization conditions, chemical ionization mass spectra were obtained for all components in the mixture of standards as well as in the natural flavor extracts.

In order to provide elemental composition analysis for the molecular ions and fragment ions in the mass spectra of individual components as an aid to their identification, accurate mass measurements were made at low resolution setting of the mass spectrometer. This was achieved with the mass spectrometer operating under standard EI conditions but at a resolving power of 1500 (10% valley definition). The mass spectrometer was first calibrated using PFK as primary reference compound at a scan rate of 1 second/decade. The PFK was removed from the ion source and tetraiodoethylene, C_2I_4 (Pfaltz and Bauer, Inc., Conn. U.S.A.) was introduced into the ion source with the solid probe. Secondary calibration was performed using the 2 reference ions at m/z = 277.8080 and 126.9047 and the air peak at m/z = 31.9898. Samples are introduced into the mass spectrometer ion source under standard GC operating procedures and mass spectra were acquired as before with C_2I_4 still held in the ion source. Accurate mass measurements were thus obtained on molecular ions (where possible) and on fragment ions. Despite the success obtained with this technique in this work it must be emphasized that under these low resolution conditions the

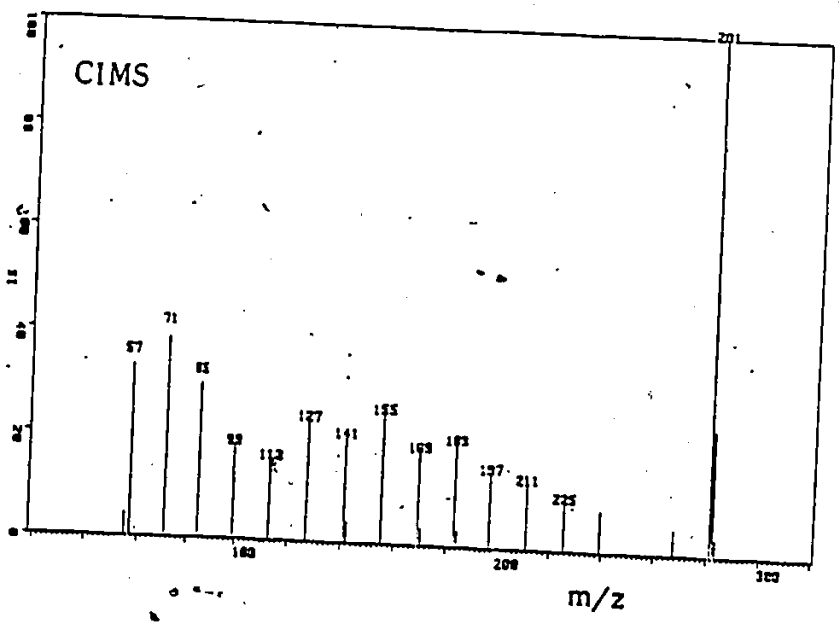


Figure 2.9. Chemical ionization mass spectrum of n-eicosane MW = 282.

accurate mass measurements data on fragment ions in particular should be used with caution.

In order to establish the presence of nitrogen and/or phosphorus compounds in the volatile flavor extracts, the components separated by gas chromatography were subjected to simultaneous detection by FID/TSD.

2.2.9 Quantitative Analysis of the Separated and Identified Components

Mass-intensity data were obtained and stored in the computer memory from repetitive magnetic scans taken on samples of volatile flavor extracts separated by fused silica capillary gas chromatpgraphy. The data obtained by this technique was used for quantitative analysis of the volatile flavor components identified in this study.

In order to perform quantitative analysis of the compounds identified in this study, a mixture of volatile flavor standards was prepared by adding 9.5 µl ethyl lactate, 8.5 µl γ-Butyrolactone, 8.5 µl 2-phenethyl alcohol, 9.5 µl diethyl succinate and 14.48 mg decanoic acid to 25 ml of freon in a 25.0 ml calibrated volumetric flask. 1 µl portions of this sample were injected onto the GC column and detector responses (peak areas) were measured for each eluting component. This procedure was repeated three times more on the same solution. This solution was labelled A for clarity. Three more solutions B, C, D, were prepared by adding approximately the same amounts of standards as in A and the detector responses were measured as before for each solution. From this data variances in the GC/MS analysis and in the preparation of solutions were calculated. The results of this analysis and the analysis of variance carried out on this data are shown in Table 3.2.12 and Table 3.2.13. This enabled the proper calibration curves

to be constructed.

In order to estimate relative response factors of the identified components so that they may be quantificated, 1 µl samples of the known volatile components in freon at the following concentrations: 0.5, 2.0, 5.0, 20.0 and 50.0 ppm, each containing a fixed amount of acetophenone (20 ppm) as internal standard were injected onto the capillary gas chromatographic column. The different mass spectrometric responses (peak areas) to various amounts of substance were measured against the response to the internal standard. Detector responses were obtained on a second solution containing approximately the same amounts of flavor components. The results from these measurements were used to plot a calibration curve of response ratio against concentration. Figure 3.3 shows the calibration curves for ethyl hexanoate, 1-pentanol and ethyl lactate. Slopes of these curves were calculated and relative weight response factors (RWR) for each component were calculated.

In order to provide an estimate of the reproducibility of the whole analytical procedure - from sample isolation to peak area measurements - a second sample of the 0.02 ppm solution of volatile flavor standards (section 2.2.2) was submitted to the total analytical procedure. It must be emphasized that in almost all cases, appropriate dilutions of the concentrated extracts were made in order that the responses measured were within the linear working range adopted in this study.

2.3 APPLICATION OF THE ANALYTICAL TECHNIQUES DEVELOPED

Once a satisfactory method was developed for the isolation, concentration and analysis of the volatile flavor components in synthetic alcoholic solutions on a routine basis, it was decided to

evaluate the effectiveness of this technique to the simultaneous detection and identification of organoleptically significant volatile aroma components of low odor thresholds present at trace level concentrations in grape juices and wines. A 1972 Cabernet Sauvignon wine made and bottled by Chateau Pichon Lalande de Pauillac was selected for this analysis. Having demonstrated the effectiveness of the techniques developed to the detection and identification of trace level volatile flavor compounds of organoleptic importance in wine, it was decided to use the method to attempt to isolate, detect and identify those chemical compound(s) other than methyl anthranilate, believed to impart the characteristic flavor of V. labruscana and hybrid grape varieties. It is conceivable that these are trace compound(s) whose identity or identities are not yet known. Concord wine and Moulin Rouge wine which represent the product of the two basic types of wine grapes grown in Canada and the Eastern United States were selected for this analysis. Moulin Rouge wine is the commercial name given to a blend of three hybrid grapes namely, de Chaunac, Vincent, and Marechal Foch and bottled by Andrés Wineries at Winona, Canada. This analysis was also conducted in order to provide further characterization of the volatile aroma composition of V. labruscana and hybrid grape juices and wines which has already been initiated in this laboratory and elsewhere.

It was also decided to use the technique developed to provide analytical data on the composition of volatile flavor extracts of grape juices and wines made from the grapes of locally grown v. vinifera grapevines. Such data could be used for predicting and/or evaluating the potential of these grape types in making good wines. The following sections describe the procedures adopted in these analyses.

2.3.1 Analysis of a 1972 Red Wine Bottled by Chateau Pichon Lalande, France

95.0 ml of this red wine was introduced into the extracting tube, L, of the solvent extraction apparatus. The modified solvent pot contained 250.0 ml of freon. The standard method for isolating volatile components from samples with the solvent extractor operated in a batch mode was followed. After three hours, the contents of the solvent pot were enriched 2500-fold using the new design of low temperature, high vacuum concentrator. The concentrated volatile flavor extract was treated with a 5% $NaHCO_3$ solution, then propylene glycol and the freon fraction was concentrated further before analysis by capillary GC/MS, capillary GC/FID or Capillary GC/FID/TDS. Sensory analysis was conducted on separated components eluting from analytical columns. The column effluent was split such that one fraction was directed to a sniff port and the other to a flame ionization detector.

2.3.2 Analysis of Moulin Rouge and Concord Wines

In order that samples used for analysis would be representative of industrial samples, all the crushing of grapes, fermentation and aging of the wines involved were conducted by the wineries involved. Samples for analysis were taken from holding tanks or barrels at the winery and transported to McMaster University in corked bottles.

Volatile flavor components were isolated from 757 ml of Moulin Rouge wine into 700 ml of freon with the solvent extractor operated in the continuous flow mode. Pressurized air was used to drive the wine through the system at a flow rate of 4 ml/min. The isolated volatile fraction was removed and concentrated 7000-fold

using the new design of low temperature high vacuum concentrator. The residual wine which had been stripped of its volatile flavor contents collected in bulb I. This bland wine will henceforth be referred to as wine base in this thesis. The wine base was split into five equal portions and the following experiments were carried out on them. It was decided to reconstitute Moulin Rouge wine by merely redissolving the isolated fraction back into the wine base. 20 µl portion of the concentrated volatile flavor extract was added to one aliquot of the wine base. It was observed that the two fractions would not dissolve each other. Even ultrasonication did not aid in producing a homogeneous solution of the two fractions. The volatile fraction merely formed a kerosene type film on the surface of the wine base. The mixture had a strong burned odor associated with it in addition to a foul off odor completely unlike the aroma of the original wine. The isolated volatile fraction itself had an aroma reminiscent of the original wine.

100 µl of freon was added to another aliquot of wine base to assess whether the presence of small amounts of the extracting solvent had any contribution to the above observations. To investigate this phenomenon further, 757 ml of Concord wine (Chateau-Gai Wineries), Similkameen Red (Andrés Winery) and Pichon Lalande were extracted to isolate their volatile flavor components. The extracts were concentrated 7000-fold and the following experiments were conducted on their wine bases and volatile fractions. 20 µl portions of each of Concord, Similkameen red, and Pichon Lalande were added to 150 ml portion of their respective wine bases. 20 µl portions of the volatile extract of Pichon

Lalonde were added to 150 ml each of the wine bases of Moulin Rouge, Concord and Similkameen Red. 20 µl portions of Concord extract were added to each aliquot of Moulin Rouge, Similkameen Red and Pichon Lalande wine bases. The same experiment was repeated for both Moulin Rouge and Similkameen Red. In all the experiments involving the addition of Pichon Lalande concentrate to wine bases, homogeneous solutions were formed and the reconstituted wines had aroma very much like that of Pichon Lalande. All other additions produced results similar to the addition of Moulin Rouge concentrate to its wine base.

In an attempt to resolve this discrepancy, 95.0 ml of Moulin Rouge was extracted under more carefully controlled conditions with the solvent extractor operated in the batch mode. Compressed nitrogen was used to pressurize the wine sample instead of air and the final venting of the system to air in order to isolate and remove the concentrated extract was made to a stream of nitrogen gas instead. The concentrated extract obtained under these controlled experimental conditions was found to redissolve in its wine base producing an aroma very similar to that of the original wine.

Having found a method to reconstitute Moulin Rouge it was decided to conduct a series of experiments to evaluate the organoleptic significance of methyl anthranilate to the overall aroma of v. labrusca grapes and wines. Preliminary gas chromatographic experiments conducted to evaluate the importance of separated components of Moulin Rouge, Concord wine and grape juice eluting from a packed column and directed towards a sniffing port, showed that the most important region of the GC/FID gas chromatogram was that around octanoic acid where sensory

analysis indicated the presence of a compound or compounds coeluting with octanoic acid whose aroma almost completely mimicked that of the original wine. 757.0 ml of Moulin Rouge wine was extracted under carefully controlled conditions using pressurized nitrogen to provide a wine flow rate of 4 ml/min into the extracting tube. After concentrating the volatile extract 7000-fold, the wine base was split into five equal parts. Components eluting from a Superpak 20 M column in defined regions of the gas chromatogram were trapped into each aliquot of wine base until a total injection volume of 20 µl of concentrated extract had been dispensed onto the GC column as follows: all components eluting up to and including phenethanol were trapped into wine base aliquot I; all components eluting up to but excluding methyl anthranilate were trapped into aliquot II; all components eluting after phenethanol up to but excluding methyl anthranilate were trapped into aliquot III; all components eluting after methyl anthranilate (methyl anthranilate inclusive) were trapped into aliquot IV; all components eluting off the column excluding freon solvent were trapped into aliquot V. This also acted as a control for comparative sensory evaluation. In all cases of trapping fractions eluting from gas chromatographic columns, the solvent was allowed to go to waste.

95.0 ml each of Moulin Rouge and Concord wines was extracted under carefully controlled conditions to isolate their volatile flavor components. The extracts were then concentrated 2500-fold, treated with 5% $NaHCO_3$, then with 200 µl of propylene glycol, concentrated further and then subjected to capillary GC/MS analysis.

2.3.3 Analysis of Grape Juices and Musts Made from the Grapes of Locally Cultivated V. Vinifera Grapevines

On September 19, 1980 white Riesling grapes were prematurely harvested from Chateau Gai vineyards, Ontario. These grapes were processed for grape juice on the 20th of September. Grape pH was determined as 2.80 and the must had a specific gravity of 15.6°Brix. This was fermented for a period of 17 days by the winery using Fermivin dried yeast flakes (G.B. Fermentation Ltd.). Samples for anlaysis were sent to McMaster University in properly corked bottles held at between 15° to 20°C shortly after extracting the grape juice, immediately after fermentation had ceased, and after two years of bottle storage.

100.0 ml of Riesling grape juice was extracted for its volatile flavor components. The isolated fraction was concentrated 2500-fold. The concentrated extract was treated with 5% $NaHCO_3$ and then propylene glycol, concentrated further and subjected to capillary GC/MS analysis. This procedure was repeated for the samples taken shortly after fermentation and the two year old wine except that 95.0 ml of sample was used.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1.1 Instrumental Design

Figure 3.1 shows a test chromatogram of an activity test mixture (see Table 3.1.1 for composition) on a glass capillary SCOT Carbowax 20M column mounted in the modified Varian 1800 GC instrument. The composition of the test mixture was designed to test operating features of both the capillary column and the GC instrument as follows:

Table 3.1.1

Composition of the activity test mixture

Component	
n-decane n-hexadecane n-octadecane naphthalene	These hydrocarbons are used to determine "dead volume effects" (tailing) due to improper instrument conditions
2-octanone	It is used to determine reactivity with Lewis acid sites derived from residual metal oxides on the column surface
1-octanol	This will show excessive peak tailing in the presence of unreacted sites due to hydrogen bonding
2,4-dimethylaniline	It is used to determine the tendency toward column acidity
2,6-dimethylphenol	It is used to determine the tendency toward column basicity

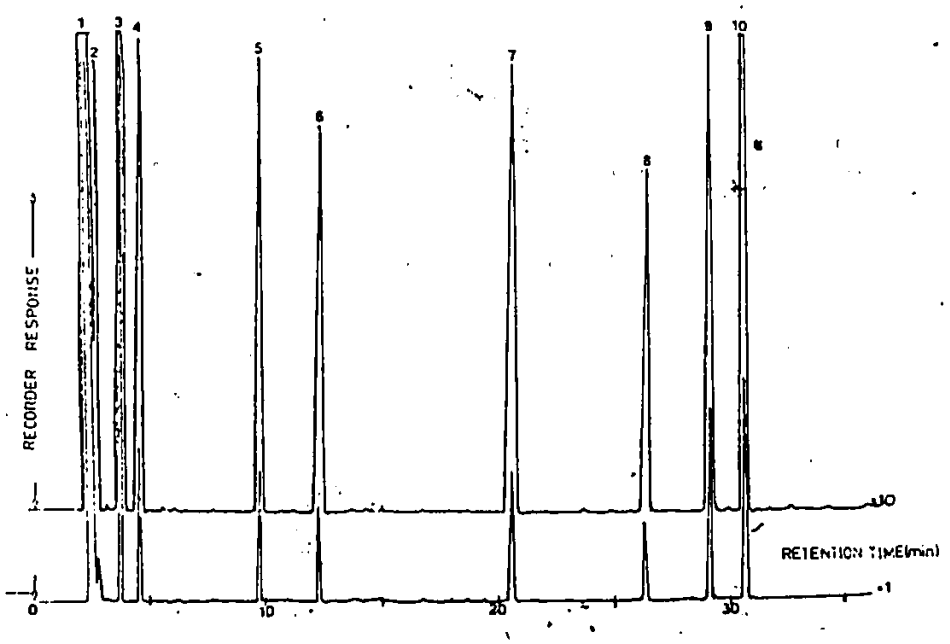


Figure 3.1. Gas chromatogram of activity test mixture on a 45m x 0.5mm I.D. glass SCOT Carbowax 20M column mounted in a Varian 1800 GC modified to accommodate capillary columns.

(1) Freon; (2) 2-butanone; (3) n-decane; (4) 2-octanone; (5) 1-Octanol; (6) n-Hexadecane; (7) Naphthalene; (8) n-Octadecane; (9) 2,4-dimethylaniline; (10) 2,6-dimethylphenol

The test chromatogram (Figure 3.1) shows that both the column and GC instrument were in a satisfactory operating condition. This GC instrument, with a modified injection port and detector assembly was used for all subsequent analysis involving both packed column/FID and/or capillary column/FID applications.

3.2 TECHNIQUES OF ANALYSIS

One of the main objectives of this research was the analysis of trace (ppb-ppt concentration levels) volatile flavor components in grape juices and wines. A review of the literature on existing isolation and analysis techniques revealed that while some methods have been developed for the determination of trace components,[217-223] none could be used in their entirety for the analysis at hand. The techniques developed and described in Chapter Two for the analysis of trace volatile flavor components are now discussed in the sections that follow.

3.2.1 Sample Collection

In order that the analytical data generated in this research work be representative of samples produced for commercial purposes, samples of wine, musts and juices meant for this analysis were obtained from wineries in the region - Chateau Gai Winery and Andres Winery, and abroad - Chateau Pichon Lalande, France.

3.2.2 Isolation of the Volatile Flavor Components

The technique of solvent extraction was used in this research project to isolate volatile flavor components from their alcoholic solutions for the following reasons: firstly, a new design of a

solvent extraction apparatus which uses the principle of downward displacement by freon beads to extract volatile components in alcoholic solutions held in either a stationary or mobile state in a cylindrical glass tube had been constructed in this laboratory. Secondly, this device was shown in a previous study[36] to perform rapid, quantitative isolation of flavor volatiles at concentrations of 20 ppm or more in alcoholic solutions. Thirdly, freon as an extracting solvent is less prone to artifact formation and is easily purified. Fourthly, the insolubility of freon in aqueous and alcoholic solutions, its non-toxicity and non-flammability make it highly attractive as an extracting solvent. Lastly its low boiling point (24.1°C at 101 kPa) allows freon to be used for the extraction of volatile compounds at temperatures comparable to those at which the wines are held hence reducing the incidence of artifact formation.

Since the emphasis in this thesis was on trace component analysis, the device had to be evaluated with respect to its efficiency in isolating components at trace level concentrations in alcoholic solutions. Table 3.2.1 shows the results of the analysis conducted on 12% alcoholic solutions of selected volatile flavor components at approximate concentrations of 2, 20 and 2000 ppb. The volatile flavor components selected represented the types of compounds likely to be encountered in an analysis of this kind. All of these components have been previously identified as wine or grape juice constituents. The data shows that within a period of 3 hours this device quantitatively and reproducibly extracts flavor components from alcoholic solutions.

Since this device takes three hours to perform quantitative

Table 3.2.1

Efficiency of recovery and reproducibility of the solvent extraction method for volatile flavor compounds in 12% ethanol solution

Compound Name	% recovery ± (s,3)		
	2 ppm	0.02 ppm	0.002 ppm
Ethyl hexanoate	99 ± 3	97 ± 3	96 ± 4
Ethyl 3-hydroxybutyrate	98 ± 7	97 ± 5	98 ± 6
Methyl furoate	98 ± 5	99 ± 4	95 ± 5
Diethyl succinate	99 ± 5	99 ± 7	97 ± 5
Hexanoic acid	97 ± 7	96 ± 4	96 ± 4
2-Phenethyl alcohol	100 ± 4	100 ± 6	98 ± 4
Octanoic acid	98 ± 3	99 ± 3	96 ± 4
Diethyl malate	100 ± 4	98 ± 4	98 ± 6
Methyl anthranilate	100 ± 3	99 ± 7	97 ± 4
Decanoic acid	96 ± 7	97 ± 4	97 ± 7

s = standard deviation

and reproducible isolation of flavor volatiles from dilute alcoholic solutions, this procedure was adopted as a stage in the analytical scheme being developed for trace components. Table 3.2.2 shows the data obtained on the evaluation of the efficiency of the fractional distillation column, S, incorporated into the design of this solvent extractor in providing purified freon solvent for re-extraction. This Table shows that the recycled freon distillate is pure and that the distillation column S is highly efficient in the solvent recycling scheme. Note that components like methyl anthranilate that were detected in the distillate amounted to less than 1% of their initial concentration in the solvent pot. The efficiency with which recycled freon can re-extract a given component is a function of the distribution ratio of the solute between freon and the alcoholic solution, and the purity of the recycled freon. For a component of a given distribution ratio, the maximum amount of this component will be extracted into freon if the amount of this component in the distillate is zero.

The isolated volatile flavor components still constitute a very dilute solution and must be preconcentrated in order that their concentrations may be sufficiently enhanced in order to enable their detection by the analytical instruments available to us. An enrichment step was therefore a necessary requirement in the development of a suitable analytical scheme.

3.2.3 Enrichment of the Isolated Volatile Components

Table 3.2.3 shows the results of the recoveries and reproducibilities obtained on various concentrations of flavor components in alcoholic solutions. It can be seen that good recoveries and reproduci-

Table 3.2.2

Evaluation of the efficiency of the fractional distillation column, S, in purifying freon solvent for re-extraction*

Compound Name	% by weight in distillate
Ethyl hexanoate	0.2
Ethyl 3-hydroxybutyrate	nd
Methyl furoate	nd
Diethyl succinate	0.5
Hexanoic acid	nd
2-Phenethyl alcohol	nd
Octanoic acid	nd
Diethyl malate	nd
Methyl anthranilate	0.8
Decanoic acid	nd

nd: not detected

*Each component had an approximate concentration of 2 ppm in this solution.

Table 3.2.3

Efficiency of recovery and reproducibility of the first stage of the 2-stage low temperature high vacuum concentration technique in providing the desired enrichment of volatile flavor components in dilute solutions

Compound Name	% recovery ± (s,3) approximate concentration of solutions		
	2 ppm	0.02 ppm	0.002 ppm
Ethyl hexanoate	98 ± 8	96 ± 7	97 ± 8
Ethyl 3-hydroxybutyrate	99 ± 8	93 ± 7	92 ± 4
Methyl furoate	98 ± 2	98 ± 3	97 ± 5
Diethyl succinate	98 ± 3	96 ± 4	98 ± 6
Hexanoic acid	99 ± 9	98 ± 3	98 ± 4
2-Phenethyl alcohol	100 ± 2	103 ± 5	101 ± 4
Octanoic acid	98 ± 8	96 ± 4	95 ± 5
Diethyl malate	100 ± 3	98 ± 4	97 ± 5
Methyl anthranilate	99 ± 8	99 ± 5	100 ± 6
Decanoic acid	97 ± 8	99 ± 6	93 ± 6

bilities were obtained for practically all of the volatile components that were examined especially when it is compared with other systems that use similar techniques to achieve the enrichment of flavor extracts prior to analysis.[170] It may be concluded from Table 3.2.3 that this stage of the analysis is efficient and reproducible. Even more relevant and attractive is the fact that this stage of the preconcentration process takes only one hour to accomplish. It must be emphasized that this process takes a considerably longer period to accomplish if the desired pressures in the vacuum lines are not attained. It is conceivable that under these conditions a plug of air of non-condensable gases forms in either flask N or the distillation bulb, G, which prevents the escape of solvent vapor molecules from the stirred solution in the extruding bulb, S. (See Figures 2.6 and 2.7.) Since this is a new design of solvent concentrator, some discussion of its operational features is appropriate at this point. The apparatus was designed to take advantage of the unusually high vapor pressure of freon. Figure 3.1.1 is a plot of $\ln p/p^\circ$ against $1/T$ for A (freon) and typical volatile flavor components: B (isobutyric acid), C (ethyl propanoate), and, D (ethyl furoate), where p is the vapor pressure of the volatile component in pascals and, T is the temperature of the vapor in degrees Kelvin.

Figure 3.1.1 shows that the vapor pressure of freon decreases much less than that of typical volatile flavor components as the temperature is lowered. The vertical line (-78°C) in Figure 3.1.1 represents the temperature at which this concentrator was operated. At this temperature, freon has a vapor pressure of 1 kPa while the other components have vapor pressures several orders of magnitude lower. 5 Pa for isobutyric acid, 62 mPa for ethyl propanoate and 7 mPa for ethyl

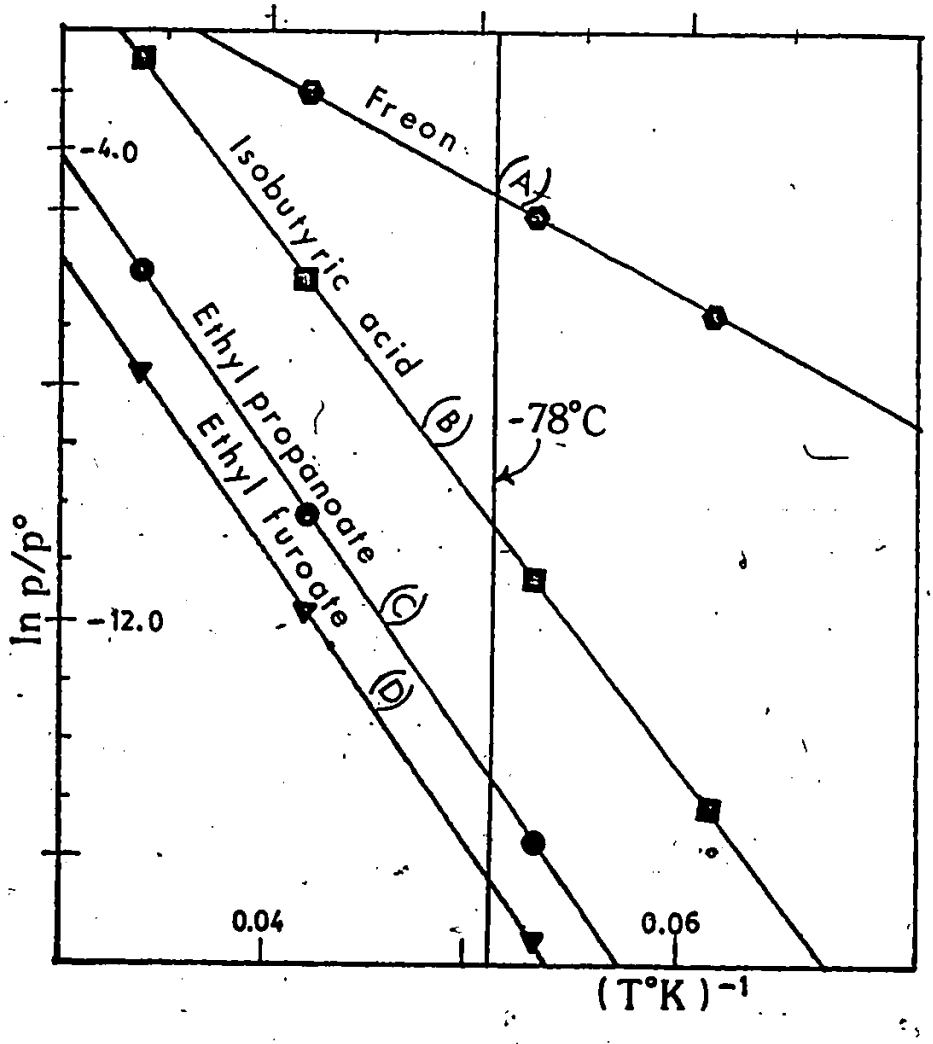


Figure 3.1.1. A plot of ln p/p° versus 1/T for freon (A) and selected volatile flavor compounds B, C and D, where p is the pressure measured in pascals, p° is the atmospheric pressure, 101kPa, and T is the temperature in degrees Kelvin. Lines were plotted from data in Ref. 224.

furoate. This rationale, plus the fact that a steady temperature of -78°C could readily be established (dry ice-acetone bath), was the basis for operating under the indicated experimental conditions. There is no reason why this device could not be operated at lower temperatures than those used in this study in an effort to further reduce any losses if they were desired, providing temperatures selected are above the normal freezing point of freon (-111°C). This concentrator assumes a 2-angled configuration during the concentration process. This is made possible by the connecting adaptor, K, (see Figures 2.6 and 2.7 for the two configurations). The adaptor, K, also allows the bulb, N to be isolated from the pumping system by closing stopcock 5 and vented to air or a stream of nitrogen gas by opening stopcock 6. This adaptor played a very useful role in those experiments described in section 2.3.2. The reason for the two configurations was to enable a smooth transfer of concentrated material from the large 500 ml flask (where it could be spread over a wider surface area) to a smaller diameter, smaller volume vessel (where it is confined to a much smaller surface area and volume) in a continuous process. Sample sputtering and the development of a concentration gradient in the solution being concentrated, were the two major causes of sample losses, and hence, poor recoveries associated with the enrichment of volatile flavor components. To prevent the former necessitated that the extruding bulb, S be brought to the same temperature as that of the larger bulb, N prior to the complete transfer of material from N to S. This is why the concentrator was slowly rotated downward from its initial 30° inclination to the horizontal to 90° in the dry ice-acetone slush bath. To prevent the

latter required that vigorous stirring be maintained throughout the concentration process. This was achieved by sequentially stirring the solution in N with the magnetic stirrer bar, P, and that in S with the micro stirring bar magnet Q. To prevent Q from being pulled magnetically towards P in the final configuration of the concentrator during the concentration process, a mechanism was devised which allowed P to be retrieved from the flask, N and harnessed in position with the horseshoe magnet, R. The success of the concentration process was found to be highly dependent on the shape and geometry of the extruding bulb, S. Note the very gentle curvature at the extruding point of the concentrator bulb designed and used throughout this experiment. (Figure 3.1.2) Other bulb designs B and C were found to be unsatisfactory and showed considerable losses of volatile flavor components. This observation seems to suggest that the geometry of the extruding position of this bulb, A, allowed the desired reflux ratio of freon solvent to be set up within S, thus enabling volatile components to condense on the gently curved portions and flow back into the bulb while freon vapor distilled off into the distillation bulb, G. It can be understood that the steep edges of the extruding points in vessel C may have prevented the establishment of the proper reflux ratio resulting in considerable loss of volatile component molecules by entrainement and sputtering. It is not certain why vessel B was found unsuitable. It is conceivable that not enough glass surface was available at the extruding points in this design to enable sufficient material to condense to provide the needed reflux ratio. It is conceivable also that the vacuum in the system at the time this vessel was being evaluated may not have been

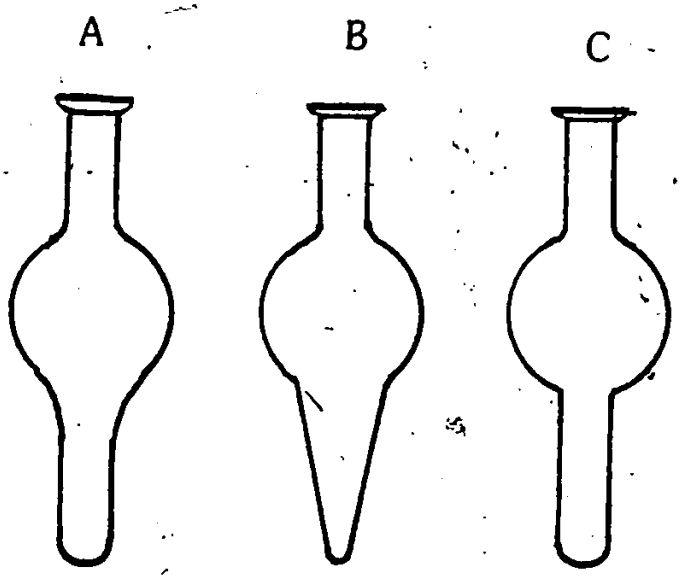


Figure 3.1.2. Various designs of concentrator vessels. A: this work, B and C were found to be unsatisfactory.

up to the required levels and may have been the reason why this particular design was associated with considerable sputtering of material and long distillation periods. Whatever the explanation is for the observations made on design B, it would be appropriate to evaluate its behaviour again before a final statement on its rejection or acceptability can be made. These features, incorporated into this new design of concentrator, make this low temperature, high vacuum concentrator a highly successful method for the enrichment of the isolated volatile flavor components in freon, without significant losses. The observations made and the experiences gained on the use of various designs of glass vessels to concentrate volatile components make it difficult to believe the conclusions arrived at by Junk et al.[170] who used similar systems to obtain enrichment of their flavor volatiles prior to analysis from non-stirred solutions. While their report does not indicate how long it took to carry out the concentration process, it is quite possible that their process took a long time to accomplish.

3.2.4 Extraction of fatty acids from the partially concentrated volatile flavor extracts

The usefulness of fatty acids to the preservation of wine and the stabilization of the wine color cannot be overemphasized. These acids, however, are usually characterized by pronounced undesirable odors. Preliminary experiments conducted on gas chromatographically separated components of isolated and concentrated wine extracts on a Superpak 20M column revealed that most of the fatty acids showed retention characteristics similar to those of ethyl esters and acetates.

For example, the following acid-ester pairs showed similar retention behavior on this column: acetic acid-ethyl octanoate; 3-methylbutyric acid-ethyl decanoate; and, hexanoic acid-2-Phenethyl acetate. The components eluting from this column and producing electrometer signals at the retention temperatures of these fatty acids were shown by sensory analysis to be usually associated with very desirable and intense flavors. In the special case of extracts obtained from labrusca products, a seemingly "homogeneous electrometer peak" produced by compound(s) co-eluting at the retention temperature of octanoic acid was found to be completely dominated by this acid. This peak was later shown by sensory analysis to be the total electrometer response from a multiple of unresolved components, some of which were characterized by highly desirable and potent flavors, quite distinct from that of octanoic acid. These flavors were quite reminiscent of the typical labrusca flavor. These observations prompted the greater part of the research related to the characterization of the volatile aroma composition of labrusca grape juices and wines to be focussed on this region of the gas chromatograms obtained on these varieties. If the electrometer responses of the other flavor components co-eluting with octanoic acid on this column were being masked as a result of a high concentration disparity, then removal of the electrometer response due to octanoic acid alone would at least enable the other components to produce visible electrometer responses, if their concentrations lie above the detection limit of the detector. This is why it was found necessary to remove octanoic acid from the flavor extracts. Table 3.2.4 shows the results of the treatment of a synthetic solution of volatile flavor components

Table 3.2.4

Evaluation of the efficiency of the $NaHCO_3$ treatment in eliminating fatty acids from the concentrated volatile flavor extract in freon

Compound Name	% recovery obtained on the freon fraction enriched 2500-fold*
Ethyl hexanoate	98
Ethyl 3-hydroxybutyrate	96
Methyl furoate	99
Diethyl succinate	97
Hexanoic acid	nd
2-Phenethyl alcohol	96
Octanoic acid	nd
Diethyl malate	97
Methyl anthranilate	98
Decanoic acid	nd

nd: not detected

*1 analysis only

in freon with a 5% aqueous $NaHCO_3$ solution. The Table illustrates that the fatty acids are quantitatively removed from the extracts by this method. In order to verify the extent to which these acids were back extracted into the aqueous phase merely by virtue of their distribution ratios, the acidified, aqueous fraction was dissolved in 95 ml of 12% ethanol and the resulting ethanolic solution was extracted with freon. Table 3.2.5 shows the results of recoveries made on this concentrated flavor extract. The results of this experiment indicate that most of the fatty acids were recovered in the aqueous phase. The amounts of other flavor components also extracted into the aqueous phase are sufficiently low to allow this technique to be used as a method for the quantitative removal of fatty acids from freon solutions of volatile flavor components.

3.2.5 Extraction of fusel alcohols into propylene glycol

The percentage recoveries of various flavor components in freon on treatment with propylene glycol are given in Table 3.2.6. The results of the experiments described in section 2.2.5 show that treating freon solutions of volatile flavor components with propylene glycol results in the removal of most of the alochols, but relatively little of the esters, ketones and acids. These observations agree very well with similar observations made by Hardy,[225] Suffis and Dean.[226] Fusel alochols are generally the major components of freon extracts of wines and grape juices. Their selective removal by treatment with propylene glycol, therefore permits esters and other minor components remaining in freon to be further concentrated. This method was therefore adopted as a stage in the analytical methodology that was being developed.

Table 3.2.5

Recoveries obtained on fatty acids after treating the concentrated flavor extract in freon (approx. 5000 ppm) with 5% $NaHCO_3$ solution*

Compound Name	% recovered
Ethyl hexanoate	3
Ethyl 3-hydroxybutyrate	8
Methyl furoate	3
Diethyl succinate	2
Hexanoic acid	99
2-Phenethyl alcohol	5
Octanoic acid	98
Diethyl malate	5
Methyl anthranilate	3
Decanoic acid	98

*This refers to the acidified aqueous fraction.

Table 3.2.6

Evaluation of the efficiency of the propylene glycol treatment in reducing the amounts of fusel alcohols present in the concentrated freon extract (≈ 5000 ppm) of the volatile flavor components*

Compound Name	% recovered
Ethyl hexanoate	98
1-Pentanol	1
1-Hexanol	1
Isoamyl hexanoate	97
1-Octamol	2
γ-Butyrolactone	95
Diethyl succinate	98
2-Phenethyl alcohol	1
Octanoic acid	96
Methyl anthranilate	98
Decanoic acid	98

*solutions were diluted a 100-fold prior to GC/MS analysis. Only 1 analysis was performed.

3.2.6 Final stage of the enrichment procedure

The results of the analysis conducted on the concentrator described in Chapter Two and used for the final enrichment of the isolated volatile flavor components are shown in Tables 3.2.7 and 3.2.8. These analysis were conducted to establish whether (i) any further enrichment in the concentration of trace volatile components that are barely detectable after the first enrichment can be achieved, and (ii) this concentrator could be incorporated as a suitable enrichment apparatus in the development of a routine analytical method for the analysis of trace volatile components.

Tables 3.2.7 and 3.2.8 show that a mild enrichment in the concentration of the volatile flavor components was obtained by this technique of sample enrichment. The Tables also show that good recoveries were obtained on most of the components investigated. They also show that generally, components with normal boiling points greater than approximately 190°C remain undistilled in the solvent pot (reservoir T) while those of lower polarity and boiling points lower than 190°C are preferentially distilled over into trap Y. The components are arranged according to their order of elution on a Carbowax 20M column. The only exception to the above observation is the behaviour of the fatty acids under the operating conditions. All the fatty acids seem to distill over into trap Y together with the low boiling fraction. While there seems to be no definite explanation for the behaviour of the fatty acids, it is conceivable that under the operating conditions, intermolecular association of the acid vapor

Table 3.2.7

Percentage recoveries* and distribution of volatile flavor components in trap Y.

Compound (Mol. Wt.)	B. Pt. °C	% recovered ± (s,4)
Ethyl hexanoate (144)	168	95 ± 4
1-Pentanol (88)	137	97 ± 3
Ethyl 2-hydroxyisopropionate (132)	150	96 ± 4
Amyl butyrate (158)	186	97 ± 6
Ethyl lactate (118)	160	95 ± 3
1-Hexanol (102)	158	93 ± 4
cis-3-Hexen-1-ol (100)	156	97 ± 5
cis-2-Hexen-1-ol (100)	158	93 ± 4
Acetic acid (60)	118	97 ± 6
Ethyl octanoate (172)	208	2.0 ± 0.1
Isoamyl hexanoate (186)	225	3.0 ± 0.4
2-methylbutanoic acid (102)	176	95 ± 7
Hexanoic acid (130)	223	96 ± 3
Octanoic acid (144)	265	95 ± 4
Decanoic acid (172)	270	95 ± 6

*These values have been corrected for the degree of enrichment (= 1.5-fold).

Table 3.2.8

Percentage recoveries and distribution of volatile flavor components in reservoir bulb, T

Compound (Mol. Wt.)	B. Pt. °C	% recovered ± (s,4)
Ethyl octanoate (172)	208	96 ± 4
Isoamyl hexanoate (186)	225	96 ± 7
Benzaldehyde (106)	178	89 ± 4
Ethyl 2-hydroxyisopropionate (132)	185	91 ± 5
l-Butyl lactate (146)	200	95 ± 3
l-Octanol (130)	195	89 ± 2
Methyl furoate (126)	181	92 ± 4
Isophorone (138)	215	95 ± 6
γ-Butyrolactone (86)	206	93 ± 4
Ethyl decanoate (200)	241	97 ± 6
Neral (152)	230	91 ± 4
Isoamyl octanoate (214)	270	97 ± 5
Diethyl succinate (174)	217	90 ± 4
Geranial (152)	229	92 ± 7
2-Phenethyl acetate (164)	-	98 ± 1
Ethyl laurate (228)	273	98 ± 6
2-Phenethyl alcohol (122)	219	89 ± 7
trans-Cinnamaldehyde (132)	253	88 ± 3
Diethyl malate (190)	253	91 ± 3

..... continued

Table 3.2.8 (continued)

Compound (Mol. wt.)	B. Pt. °C	% recovered ± (s,4)
Methyl anthranilate (151)	256	96 ± 4
Ethyl anthranilate (165)	268	97 ± 3
Phthalide (134)	290	98 ± 7
2-Phenethyl octanoate (248)	-	95 ± 4

molecules occurs through hydrogen-bonding (a well known behaviour of acetic acid molecules) making these acids less polar and characterised by lower boiling points. The behaviour of the fatty acids during this enrichment stage provided an added advantage in the analysis of V. labrusca products. Any fatty acids which were not completely removed after the $NaHCO_3$ treatment, would then be distilled off into trap Y, leaving the residual fraction in T free from any free fatty acids, which usually interferred with the analysis of the more interesting components. The distillation process therefore offers two distinct advantages: (i) Analysis is performed on a more simplified sample and (ii) If the components of interest are associated with any one fraction, then only that fraction needs to be analysed, thereby reducing the length of time required for analyses. The distillation time of forty minutes was estimated as the time required to achieve a quantitative transfer of the fatty acids into trap Y. The mild enrichment obtained with this design of concentrator, and the relatively short time (approx. sixty minutes) required to achieve this enrichment, were considered to be two contributing factors in favor of adopting this technique of enrichment as a suitable stage in the development of the analytical methodology.

3.2.7 Gas Chromatographic separation of the enriched volatile flavor extract

Figure 1.1 shows a gas chromatogram of a 1 µl injection of an isolated, enriched volatile flavor extract of Moulin Rouge wine on a 50m x 0.25 mm I.D. SE-54 fused silica capillary column. The wide range of concentrations encountered in an analysis of this nature and the

complexity of the mixture involved, necessitated the use of high resolution capillary columns. Even under high resolution capillary chromatographic conditions, certain peaks which appear homogeneous have been shown by the use of multiple detector systems to be composites of unresolved component peaks. As a result of this experience, all chromatographic separations for GC/MS analysis were conducted on high resolution glass and/or fused silica columns, except in the case of the studies related to vitis labruscana products, where some separations were conducted on packed columns in order to take advantage of their large holding capacities.

3.2.8 Identification of the Separated Components

One approach that was taken in determining the identity and structure of a component whose mass spectral data had been obtained and stored in the memory of a computer, was to search through a library of low-resolution mass spectra (compiled by the National Bureau of Standards as well as those compiled in this laboratory) for a match between its mass spectrum and that of a compound in the library. The matching routine involves calculating a similarity index (MIX), a match factor or purity (PUR) between the unknown mass spectrum and the library (reference) spectra. Usually, a value of zero represents a complete mismatch while a value of 1000 represents a perfect match. The data system also calculates a reverse fit factor by comparing only those peaks in the reference spectrum with those in the unknown spectrum. This means that extraneous peaks due to impurities or unresolved components will not prevent a correct match if the compound is in the library.

Table 3.2.9 shows the output of a typical library search conducted on a mixture of flavor components whose mass spectra were acquired by capillary GC/MS data system. FLSTD is the file name of this TIC chromatogram and the number following the file name is the scan number. For most of these listings (scan 111, 257, 276, 466 and 510), the first entries are usually the correct fits and give the correct identification of the compounds 1-pentanol, 2(3H)-dihydrofuranone, hexanoic acid, benzeneethanol and butanedioic acid, diethyl ester, whose mass spectrum was stored for that scan number. In others, for example, FLSTD.408 several fits were assigned to a component which showed the retention characteristics of 1-octanol on this column. It is being emphasized here that the results of these searches were treated with extreme caution. Usually, a fit obtained by library searching, together with a match with the retention index of a known compound on Carbowax 20M and/or SE-54 column were considered sufficient prerequisites for assigning a "tentative" identification to an unknown, subject to further proof. In most cases, mass spectra of compounds obtained by repetitive magnetic scanning by capillary GC/MS techniques could not be matched with compounds in the library. Under these circumstances, more experimental data were obtained to help deduce the identity and structure of the component by manual interpretation of the mass spectral data. In this regard, chemical ionisation mass spectra of the chromatographically separated components were obtained using either methane or isobutane as reactant gas in order: (i) to confirm the molecular weights of those components whose molecular weights had been deduced from their EI mass spectra and, (ii) to provide the

Table 3.2.9

Typical Computer Output of a mass spectral library search

FLSTD 111 2 FITS													
PUR	MIX	REV	S.REF	BASE	M1:%I	M2:%I	M3:%I	M4:%I	C	H	N	O	F	S	CN
958	958	991	P 236	42	31:68	29:67	41:60	55:50	5	12	0	1	0	0	1-Pentanol
805	888	864	P2776	42	70:98	41:99	55:90	27:70	5	11	0	0	0	0	Pentane, 1-chloro-
FLSTD 149 0 FITS															
FLSTD 257 1 FITS															
PUR	MIX	REV	S.REF	BASE	M1:%I	M2:%I	M3:%I	M4:%I	C	H	N	O	F	S	CN
903	917	969	P 767	42	29:46	27:46	41:46	56:31	4	6	0	2	0	0	2(3H)-Furanone, Dihydro-
FLSTD 329 0 FITS															
FLSTD 376 2 FITS															
PUR	MIX	REV	S.REF	BASE	M1:%I	M2:%I	M3:%I	M4:%I	C	H	N	O	F	S	CN
899	899	983	P1725	60	73:12	27:35	41:32	43:26	6	12	0	2	0	0	Hexanoic acid
930	891	906	P1211	60	73:34	27:32	29:28	41:21	5	10	0	2	0	0	Pentanoic acid

..... continued

Table 3.2.9 (continued)

FLSTD 408 · 19 FITS

PUR	MIX	REV	S.REF	BASE	M1:%I	M2:%I	M3:%I	M4:%I	C	H	N	O	F	S	CN
922	922	973	P5337	55	69:93	43:89	57:82	56:82	8	18	0	1	0	0	1-Heptanol, 6-methyl-
916	916	976	05003	43	55:98	41:96	69:87	57:79	8	18	0	1	0	0	Isooctanol
901	901	983	P1375	43	55:98	41:95	56:76	69:73	11	24	0	1	0	0	1-Undecanol
887	887	986	P1346	44	56:85	43:82	55:81	27:70	8	18	0	1	0	0	1-octanol
888	886	987	P1734	56	41:95	55:93	43:90	70:68	9	20	0	1	0	0	1-Nonanol
877	877	974	P1366	41	43:98	35:84	29:71	56:65	10	22	0	1	0	0	1-Decanol
960	960	976	P1381	43	55:98	41:96	69:76	56:73	12	26	0	1	0	0	1-Oodecanol
857	877	965	P6379	41	43:87	55:82	69:78	56:70	10	17	0	2	3	0	Acetic acid, trifluoro, octyl ester
956	903	937	P6344	56	55:79	43:57	42:57	41:54	9	16	0	0	0	0	Cyclopropane- pentyl-
852	872	940	P8077	43	55:95	56:90	70:78	41:78	10	20	0	0	0	0	1-Octene, 3,7-dimethyl-

FLSTD 2 FITS

PUR	MIX	REV	S.REF	BASE	M1:%I	M2:%I	M3:%I	M4:%I	C	H	N	O	F	S	CN
951	951	994	P 150	91	92:62	122:32	65:14	39: 6	8	10	0	1	0	0	Benzeneethanol
857	890	905	P2850	91	92:53	65:14	51:12	63:12	7	10	2	0	0	0	Hydrazine, (Phenylmethyl)-

..... continued

Table 3.2.9 (continued)

FLSTD 510 1 FITS

PUR	MIX	REV	S.REF	BASE	M1:%I	M2:%I	M3:%I	M4:%I	C	H	N	O	F	S	CN
961	961	986	P1542	101	29:82	129:64	27:46	55:28	8	14	0	4	0	0	Butanedioic acid, diethyl ester

molecular weights of those components whose EI mass spectra showed no observable molecular ion peak, from $(M+1)^+$ and occasionally $(2M+1)^+$ data. For example, the EI mass spectrum of trans-5-Butyl-4-methyl-2(3H)-dihydrofuranone 203, a flavor component of Cabernet Sauvignon showed no observable molecular ion peak (Figure 3.1.3). The isobutane CI mass spectrum however, showed a highly intense $(M+1)^+$ peak at m/z = 157 thus enabling the molecular weight of a compound whose EI mass spectrum gave no molecular weight information to be deduced. In general, CI mass spectra using methane as reactant gas showed more fragmentation than those obtained using isobutane and aided in the elucidation of the fragmentation pattern of some of the volatile flavor components.

Very often, it was necessary to provide elemental composition data on $M^+\cdot$ to confirm the molecular formula of an identified component as well as the elucidation of the mechanisms of mass spectral fragmentation patterns of individual flavor components. Since most of the components that were being investigated were at such concentrations as to preclude their being obtained as pure individual components to enable high resolution mass spectral data to be obtained on them, it was decided to use the technique developed by Haddon et al.,[227] for obtaining accurate mass measurement data at low resolution setting of the mass spectrometer on gas chromatographically separated components in a mixture Figure 3.1.4 illustrates the total ion current chromatogram of a flavor mixture after background subtraction. Figures 3.1.5 and 3.1.6 are representative mass spectra taken from the peaks labelled A and C in Figure 3.1.4. Tables 3.2.10 and 3.2.11 illustrate the partial low resolution accurate mass measurement data acquired for the two components 2-Phenethyl alcohol and Octanoic acid.

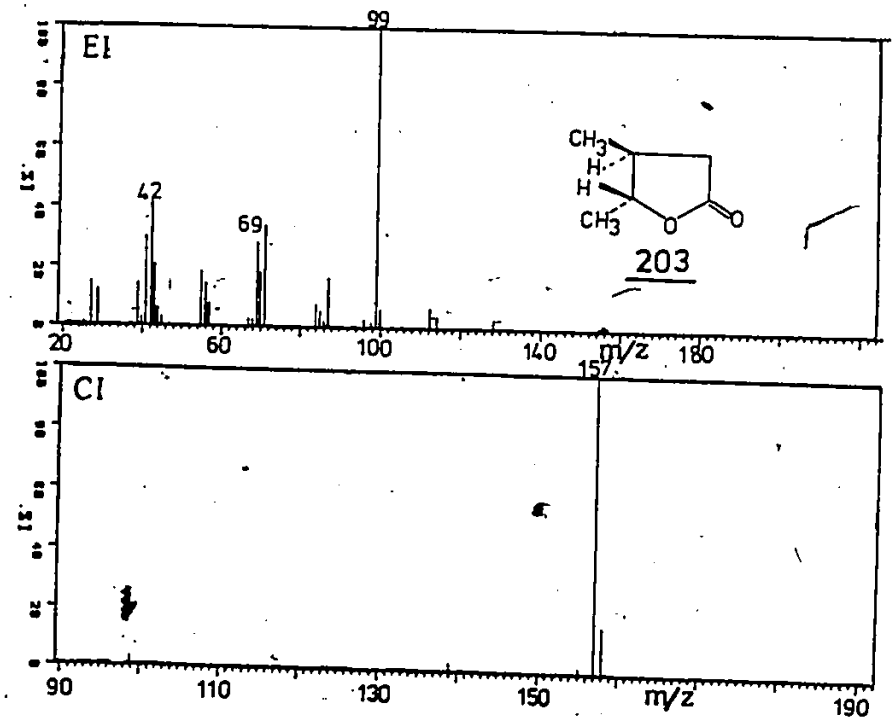


Figure 3.1.3. EI and CI mass spectra of trans-5-Butyl-4-methyl-2(3H)dihydrofuranone, 203 obtained by capillary GC/MS in this investigation.

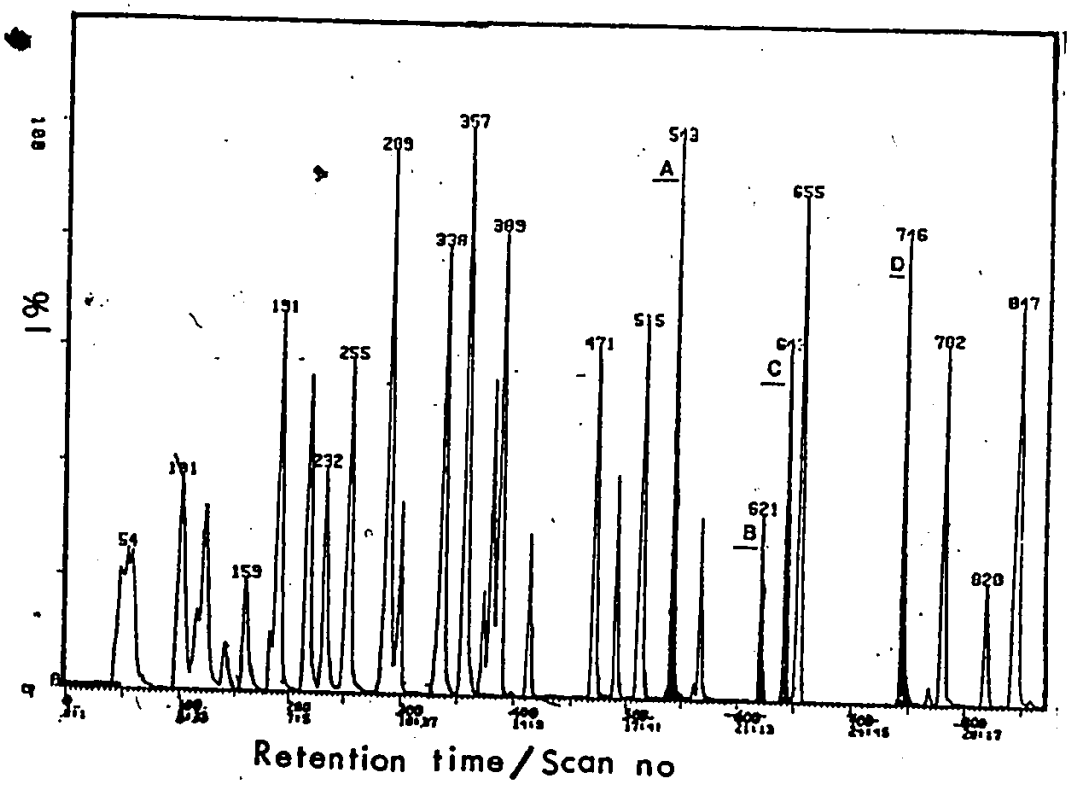


Figure 3.1.4. Reconstructed ion chromatogram of a standard mixture of flavor compounds.

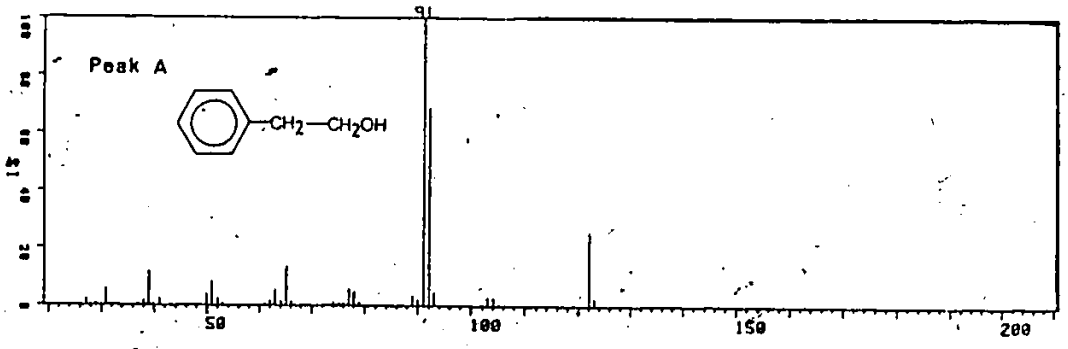


Figure 3.1.5. EI mass spectrum of component peak A in Figure 3.1.4.

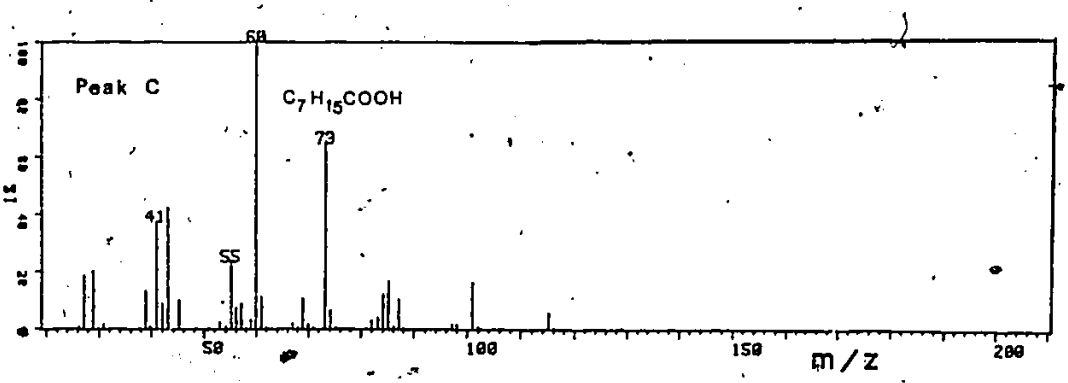


Figure 3.1.6. EI mass spectrum of component peak C in Figure 3.1.4.

Table 3.2.10

Partial low resolution accurate mass measurement data for

2-phenethyl alcohol [$C_8H_{10}O$]

m/z	C	H	O	N	S	I	MMU	OBS.MASS	%HT. MDD
			16	14	32				
122	8	10	1	0	0	0	-1.3	122.0743	25.40
	*8	9	1	0	0	0	-5.8		
91	7	7	0	0	0	0	-2.2	91.0570	100.00 B
	*7	6	0	0	0	0	-6.7		
65	5	5	0	0	0	0	3.2	65.0359	24.03
	*5	4	0	0	0	0	-1.3		

The experimentally determined accurate mass of the molecular ion m/z = 122 showed a deviation of only 1.3 millimass units (mmu) from the theoretical value. The base peak at m/z = 91 also showed a deviation of 2.2 mmu, while the medium intensity peak at m/z = 65 showed a deviation of 3.2 mmu. Figure 3.1.6 shows the EI mass spectrum of Octanoic acid, (molecular weight = 144) which shows no observable molecular ion peak. The experimentally determined accurate mass of the fragment ion at m/z = 115 showed a deviation of 5.5 mmu from the theoretical value (see Table 3.2.11). The fragment ion at m/z = 73 and the base peak at m/z = 60 showed deviations of 4.1 and 5.1 mmu respectively.

As can be seen, the mass measurement accuracy on these GC peaks containing between 500 ng and 10 ng of components is very good, being generally better than 10 mmu. At this point, it is worthwhile to point out that the mass resolution of the instrument did not deteriorate

Table 3.2.11

Partial low resolution accurate mass measurement data for

Octanoic acid ($C_7H_{15}COOH$)

m/z	C	H	O	N	S	I	MMU	OBS.MASS	%HT. MOD
			16	14	32				
115	6	11	2	0	0	0	-5.5	115.0814	6.75
	*6	10	2	0	0	0	-10.0		
73	3	5	2	0	0	0	4.1	73.0209	64.39
	*3	4	2	0	0	0	2.7		
60	2	4	2	0	0	0	5.1	60.0160	100.00 B
	*2	3	2	0	0	0	4.5		

markedly with increasing source pressure. For example, it was possible to observe two well-separated peaks (less than 10% valley) at m/z = 57, $C_4H_9^+$ and $C_2H_5CO^+\cdot$; Δm_{exptl} = 0.038; Δm_{theo} = 0.0364; m/Δm = 1500. Gas chromatograms obtained on a wine sample such as that shown in Figure 3.2 by dual detection (FID/TDS) showed that a typical wine sample may contain several N/P containing components. Retention data from such a chromatogram, which generally showed precision of ± 5 retention index units, were used to support or reject the inclusion of nitrogen in the assignment of elemental composition to various molecular ions and their fragments. For example, the component, E, appearing as a shoulder on the peak labelled F, largely due to 2-phenethyl alcohol gave the EI spectrum shown in Figure 3.3. The highest mass recorded in the EI spectrum of this compound at m/z = 129 was shown by accurate mass measurement to have a composition of $C_{16}H_{13}NO$. This was confirmed as

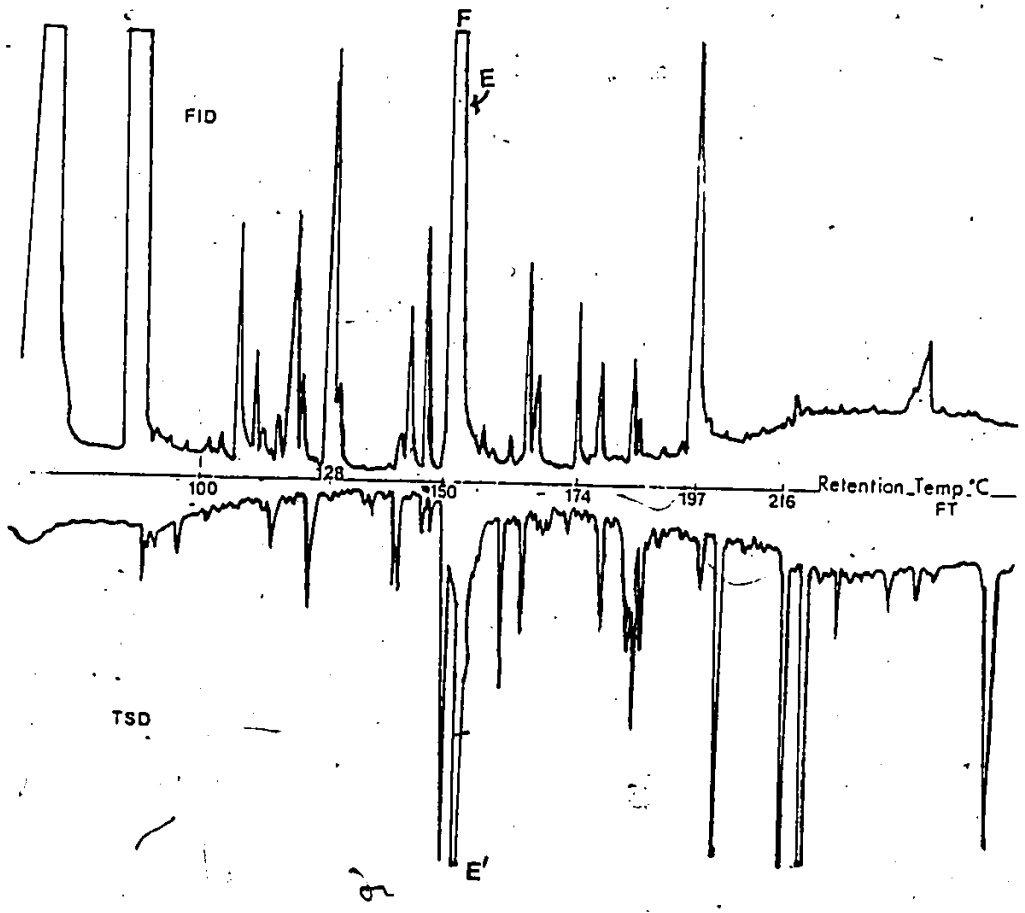
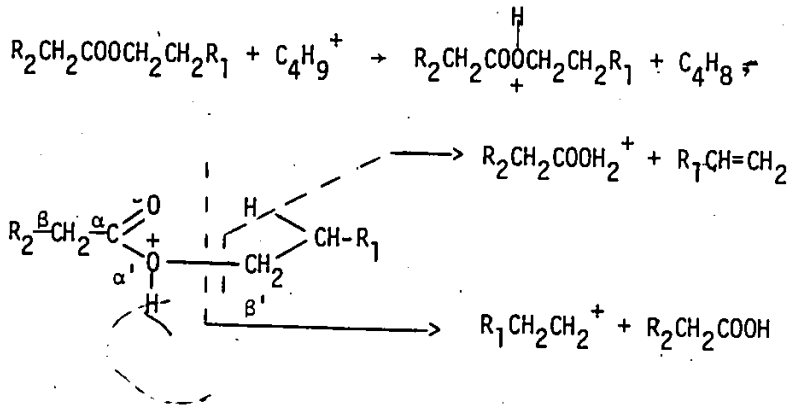


Figure 3.2. Partial gas chromatogram of a concentrated extract of Moulin Rouge wine showing simultaneous recording by TSD and FID. Column was a 30m x 0.25mm I.D. fused silica Carbowax 20M capillary column temperature programmed from 70°C to 220°C at 4 deg/min.

the molecular weight from the $(M+1)^{+\bullet}$ ion at m/z = 130 obtained by chemical ionisation using isobutane as reactant gas (see Figure 3.3). The presence of the N/P containing component at E' on the TSD chromatogram was used to substantiate the presence of nitrogen in the component of interest which was later identified as N-(3-methylbutyl)acetamide 195. These complementary techniques enabled the presence of several compounds that had previously been identified from their EI mass spectra alone to be confirmed. Most of these compounds were generally those that showed very complex EI mass spectra with little or no ionisation in the molecular weight region. These compounds included high molecular weight esters, esters of hydroxy acids, esters of dibasic acids and nitrogen-containing compounds.

The high molecular weight esters showed isobutane CI mass spectra which could be explained by simple β' cleavage at the alcohol moiety with or without hydrogen rearrangement, yielding the protonated acid, $R_2CH_2COOH_2^+$ and the alkyl fragment ion $R_1CH_2CH_2^+$ as shown in Scheme 3.1.



Scheme 3.1

Postulated mechanism for the isobutane-CI mass spectral fragmentation of higher molecular weight esters.

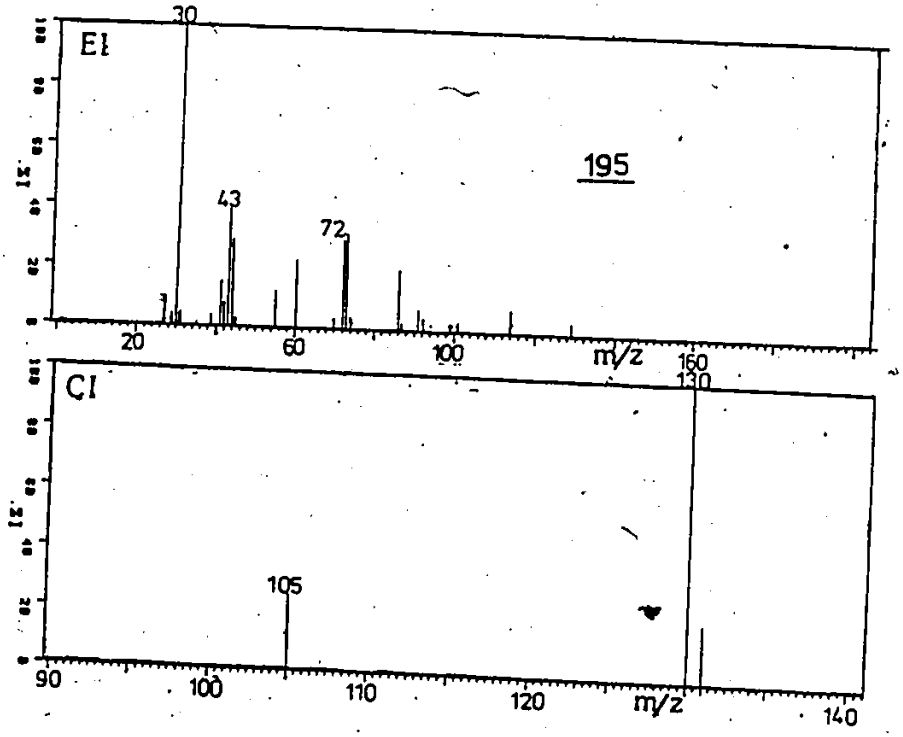
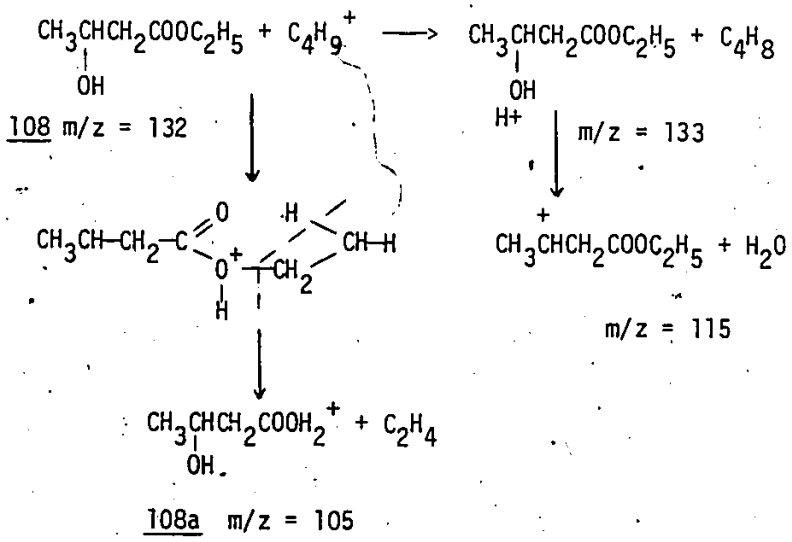


Figure 3.3. EI and CI mass spectra of N-(3-methylbutyl-)acetamide 195 (labelled E in Figure 3.2).

Most ions in the EI mass spectra of these high molecular weight esters could very easily be accounted for by either α or β cleavage with or without hydrogen rearrangement. Similarly, CI mass spectrometry aided the confirmation of the structural identities of several esters of hydroxy acids. For example, the isobutane CI mass spectrum of ethyl 3-hydroxybutyrate 108, (Figure 3.4) shows appreciable ionisation at the protonated molecular ion, and major fragments corresponding to the protonated 3-hydroxybutyric acid 108a, and, loss of one molecule of water from the protonated molecular ion (Scheme 3.2). It may be postulated that under these conditions the following mechanism operates:



Scheme 3.2

Postulated mechanism for the isobutane CI mass spectral fragmentation of ethyl 3-hydroxybutyrate 108

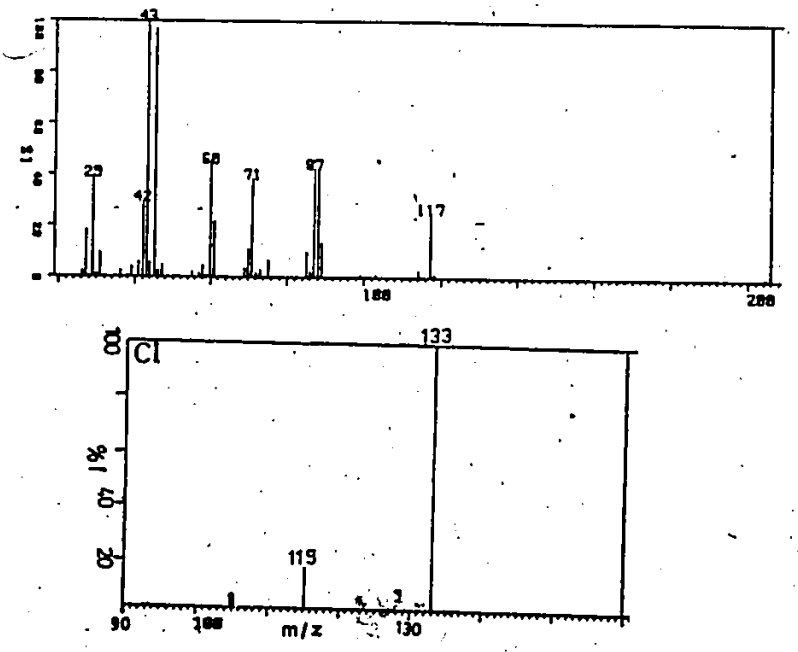


Figure 3.4. EI and CI mass spectra of ethyl 3-hydroxybutyrate 108.

The EI mass spectrum of ethyl 3-hydroxybutyrate 108, however, shows a distrubtion of ions heavily weighted toward the low mass range (see Figure 3.4). Accurate mass measurements on these fragment ions gave the following composition: m/z = 60 $C_2H_4O_2$, m/z = 87 $C_4H_7O_2$, m/z = 45 C_2H_5O, m/z = 43 C_2H_3O. Such data allowed the ions of m/z 45 and 43 to be assigned structures such as $CH_3\underset{OH}{CH}$ / $CH_3CH_2O^+$ and $CH_2{-}CH^+$ (with O) respectively, thereby making it possible to explain the EI mass fragmentation patterns of hydroxy acids based on simple α, α', β, and β' cleavages with or without gamma hydrogen rearrangement.

It was observed that certain compounds were characterized by specific fragment ions. For example, m/z = 71 and 89 seemed to be characteristically intense ions for all the malates, while all the succinates showed fragment ions at m/z = 55 and 101.

Most aliphatic alcohols showed a protonated molecular ion peak in their CI mass spectra, unlike their EI mass spectra as well as an $(M+1-H_2O)^+$ ion. The only exceptions to this generalization being the low molecular weight alcohols (C_6 and lower) which gave only the $(M+1-H_2O)^+$ ion. The aromatic alcohols, such as 2-phenethyl alcohol, showed no $(M+1)^+$ peak in their CI mass spectra. Rather, they showed an intense molecular ion and m/z = 105 as base peak, probably due to loss of a molecule of water from a "protonated" molecular ion (see Figure 3.5).

3.2.9 Quantitative analysis of the separated and identified components

It was hoped during the course of this study that a qualitative and quantitative knowledge of the composition of the wines studied

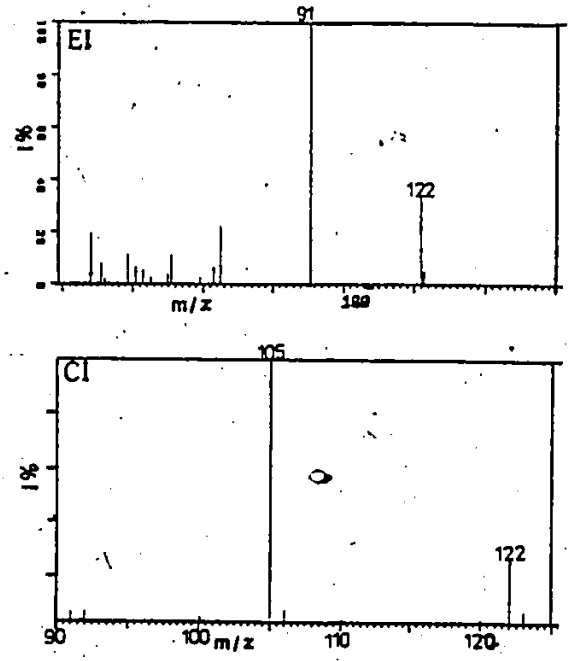


Figure 3.5. EI and CI mass spectra of 2-phenethyl alcohol 194 obtained by capillary GC/MS.

would furnish us with a method to make a synthetically constituted wine or flavors characteristic of the various wines studied. In order to quantificate the various flavor components of wine separated by capillary GC, it was decided to adopt the technique of selected ion retrieval in preference to the technique of selected ion monitoring for the following reasons:

(i) the amounts of material introduced onto the GC into the Mass spectrometer source were of the order of nanograms and more,

(ii) the large number of components that were to be quantificated would have rendered the use of the method of SIM cumbersome and hardly worth the effort,

(iii) the availability of a dedicated high speed computer interfaced to the GC/MS system for acquiring and processing GC/MS data and,

(iv) the availability of computer software for converting repetitively scanned data to SIR mode.

Although this method generally offers less sensitivity and precision compared to SIM, it yields maximum amount of information. For example, the method offered the advantage of allowing any ion or ions of choice to be retrieved from acquired and stored data in a single GC/MS run, so that the most useful ions for the analysis were determined without the need for further sample injections. An inspection of the mass spectrum of an authentic sample of a compound allowed the ratio of intensities of its ions to be estimated. In order to determine which of the ions had the lowest interference from ions of interfering substances in order that only such ions would be used for quantification, each ion intended to be monitored was assigned its current intensity in turn. If the

other ions showed either the correct intensities or intensities that were too high, then it was inferred that interference was least at this m/z value. If however, the other ions showed intensities which appear lower than they should then, it was inferred that there was interference at this m/z value. In most cases in this work, three carefully selected ions were sufficient for the assessment of the most suitable ion for quantification. For the actual analysis itself an internal standard was selected whose m/z value for quantification was selected following the above guidelines. In addition, any ions selected for quantification accounted for greater than 40% of the total ion current. Since an internal standard was used for the analysis by SIR, the intensity of only one of the ions being monitored from the compound itself was required in order to construct a calibration curve. To construct a proper calibration curve, it was decided to investigate which of the two variables, response ratio and weight (concentration) response had the greater variance. Table 3.2.12 illustrates the results of the GC/MS response ratios determined on the five components for 1 µl injections of the solutions labelled A, B, C and D. The variance of the GC/MS determination includes the variances of the injection onto the gas chromatographic column, the variance of the mass spectrometric response and the variance of the measurements of peak areas (response) by the SIR method. This was obtained from replicate injections (4) of each of the solutions. ($\nu_{gcms} = \nu_{within\ solution}$). Any mass spectrometric responses determined for the independently prepared solutions, A, B, C, D will be a measure of the sum of the variances of the gravimetric and volumetric procedures in making up the solutions, $\nu_{solution}$, and

Table 3.2.12

Average response ratios determined for the independently prepared solutions A, B, C and D.

	Ethyl Lactate	γ-Butyrolactone	2-phenethyl alcohol	Diethyl malate	Decanoic acid
Solution	Average response ratios (± s,4)				
A	1.84±0.21	1.61±0.17	1.47±0.15	2.00±0.25	2.70±0.16
B	2.05±0.31	1.77±0.28	1.68±0.25	2.17±0.32	2.89±0.43
C	1.40±0.16	1.22±0.14	1.15±0.13	1.48±0.17	2.05±0.37
D	1.78±0.23	1.55±0.19	1.47±0.19	1.88±0.23	2.51±0.30

the variance of the GC/MS analysis. The variance in the preparation of solution is given by ν_{soln}. For γ-butyrolactone for example, $\nu_{soln} = 0.020$ and $\nu_{gcms} = 0.040$. Application of the F-test to these two variances gives an F-ratio of 2.0, which is insignificant at the 0.95 probability level (see Table 3.2.13). It may be concluded from this analysis that the errors involved in the preparation of the standard solutions are approximately equal to the errors involved in the GC/MS determinations. This suggests that a calibration curve of response ratio versus weight per µl (concentration) or vice versa can be constructed. Figure 3.6 shows typical calibration curves plotted in the study for selected flavor compounds: ethyl hexanoate 45, 1-pentanol 44, and ethyl lactate 56.

The concentration C_x of a component x in the aqueous alcoholic or wine solution was estimated from equation 3.1 using relative weight response factors (RWR) deduced from calibration curves.

$$C_x = \frac{A_x}{A_{is}} \cdot C_{is} \cdot \frac{M_x}{M_{is}} \cdot \frac{1}{RWR} \cdot \frac{V_f}{V_i} \qquad 3.1$$

where:

C_x = concentration in ng/µl of sample

C_{is} = concentration in ng/µl of internal standard (20 ng/µl).

M_x, M_{is} = Molecular weights of x and internal standard

RWR = relative weight response factor

V_f = final volume after enrichment

V_i = initial volume of solution taken

A_x = area under the total ion current chromatogram for the identifying m/z value for component x

A_{is} = area under the total ion current chromatogram for the identifying m/z value for internal standard.

Table 3.2.14 shows the results of the analysis procedure applied to a 20 ppb standard solution of volatile flavor compounds in 12% ethanol solution. Standard deviations obtained vary between 4% and 11%. A precision of 11% was therefore felt to be a reasonable estimate in any single determination in a multistage analysis of this kind.

In order to verify whether the accuracy of the measurements fell within the estimated precision of the developed analysis procedure, the analysis of 2000 ppb and 2 ppb standard solutions of volatile flavor components in 12% ethanol solutions after solvent extraction and enrichment were compared to the same concentrations of volatile flavor components in freon. Table 3.2.15 shows the results of this comparative mass balance study. It can be seen that the measurements are accurate to within the precision estimated for the analysis procedure. A response ratio of 0.01 was the minimum value that could be detected under the conditions of the GC/MS analysis. This response ratio translates into a concentration value of 0.15 ng for a component such as ethyl hexanoate. The limit of detectability for the developed analysis procedure was therefore estimated as approximately equal to 0.15 ng

Table 3.2.13

Analysis of Variance (ANOVA) for the data in Table 3.2.12

(a) Ethyl lactate:

Source of variance	Degrees of freedom	Sum of Squares	Mean Square	Variance given by	F-ratio
total	15	1.5163	0.101	ν_{soln}	
between soln.	3	0.8646	0.288		
within soln.	12	0.6517	0.054	ν_{gcms}	5.3

(b) γ-Butyrolactone:

total	15	1.1380	0.076	ν_{soln}	
between soln.	3	0.6588	0.020		
within soln.	12	0.4812	0.040	ν_{gcms}	2.0

(c) 2-Phenethyl alcohol:

total	15	0.9769	0.065	ν_{soln}	
between soln.	3	0.5649	0.188		
within soln.	12	0.4120	0.034	ν_{gcms}	5.5

(d) Diethyl malate:

total	15	1.7706	0.118	ν_{soln}	
between soln.	3	1.0235	0.341		
within soln.	12	0.7471	0.062	ν_{gcms}	5.5

(e) Decanoic acid

total	15	2.8520	0.190	ν_{soln}	
between soln.	3	1.6321	0.544		
within soln.	12	1.2199	0.102	ν_{gcms}	5.3

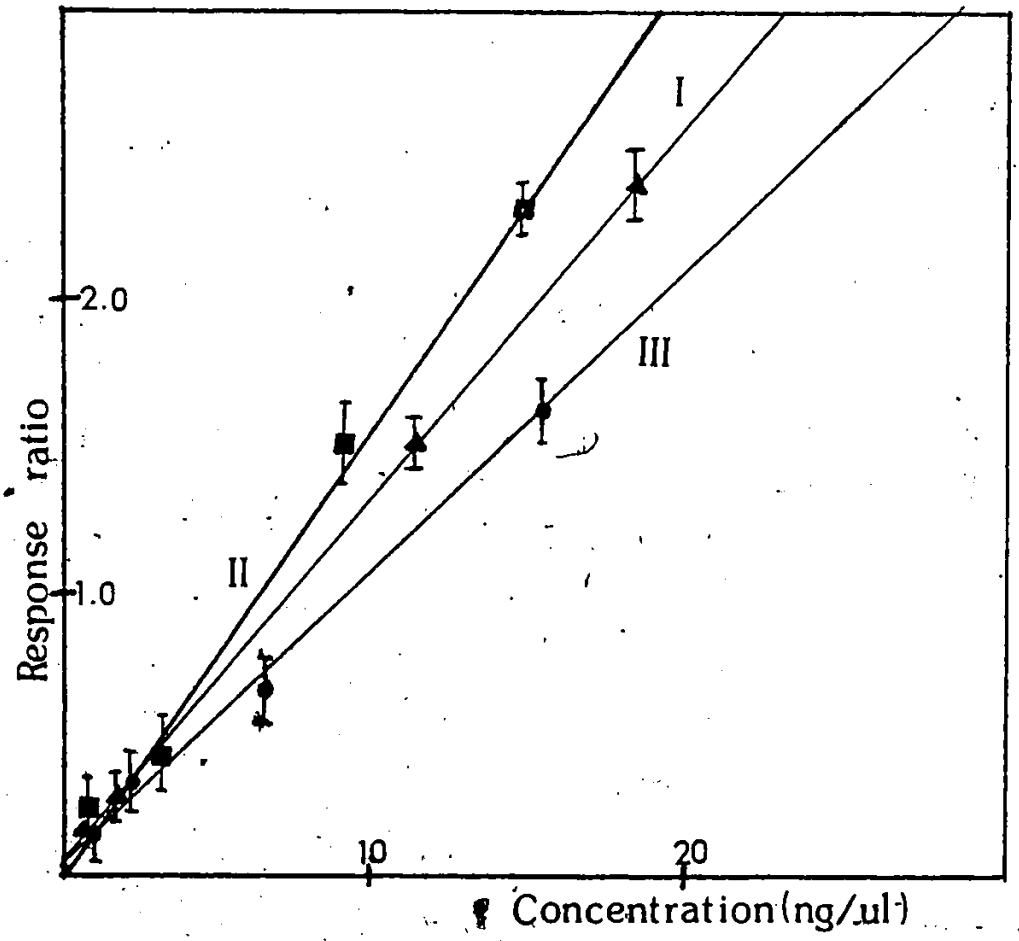


Figure 3.6. Calibration curves for:

I ethyl hexanoate 45,
II 1-pentanol 44 and
III ethyl lactate 56.

Regression equations for the three compounds are:

$y_I = 1.2702x + 0.0525$ r = 0.9995
$y_{II} = 1.5405x + 0.002$ r = 0.9993
$y_{III} = 1.0263x + 0.0359$ r = 0.9994

Table 3.2.14

Replicate analysis of a 20 ppb standard solution of volatile flavor compounds in 12% Ethanol using the developed analytical method

Compound	Average concentration ± (s,3) $(pg/\mu l)x10^{-1}$	% Standard Deviation
Ethyl hexanoate	1.57 ± 0.15	9.5
Ethyl 3-hydroxybutyrate	1.81 ± 0.11	6.1
Methyl furoate	2.07 ± 0.20	9.7
Diethyl succinate	1.71 ± 0.19	11.0
Hexanoic acid*	1.71 ± 0.06	3.5
2-Phenethyl alcohol	nd	nd
Octanoic acid*	1.54 ± 0.16	10.4
Diethyl malate	1.95 ± 0.16	8.2
Methyl anthranilate	2.16 ± 0.14	6.5
Decanoic acid*	1.74 ± 0.12	6.9

*Acidic fraction obtained after treatment with aq $NaHCO_3$, acidification, and extraction with freon.

nd - propylene glycol fraction was not analysed.

Table 3.2.15

Estimation of the accuracy of the developed analytical procedure at various concentration levels

Concentration of Soln.	2000 ppb		2 ppb	
Compound	average concn. ± (s,3)* $(pg/\mu l)x10^{-3}$	% deviation from true concentration	average concn. ± (s,3)* $(pg/\mu l)$	% deviation from true concentration
Ethyl hexanoate	1.74 ± 0.14	5.2	1.7 ± 0.1	8.2
Ethyl 3-hydroxybutyrate	2.03 ± 0.12	7.9	2.0 ± 0.1	7.5
Methyl furoate	2.36 ± 0.22	9.3	2.4 ± 0.3	8.8
Diethyl succinate	2.08 ± 0.22	10.1	2.1 ± 0.2	11.0
Hexanoic acid	1.85 ± 0.06	9.7	1.9 ± 0.1	10.5
2-Phenethyl alcohol	nd	nd	nd	nd
Octanoic acid	1.72 ± 0.16	7.0	1.7 ± 0.2	6.4
Diethyl malate	2.26 ± 0.19	10.2	2.3 ± 0.2	9.1
Methyl anthranilate	2.34 ± 0.15	9.8	2.3 ± 0.2	10.0
Decanoic acid	2.00 ± 0.14	8.0	2.0 ± 0.1	10.0

*This refers to the experimentally determined concentration of volatile flavor component in standard 12% aqueous ethanolic solutions, following solvent extraction, enrichment and GC/MS analysis after correcting for the extent of enrichment.

of component injected onto the GC column.

3.3 APPLICATIONS OF THE TECHNIQUES OF ANALYSIS

RESULTS AND DISCUSSION

One of the approaches adopted for the realization of the objectives of this research was to develop an analytical methodology capable of isolating volatile flavor components of low odor thresholds present in alcoholic solutions at trace level concentrations for detection and identification. This is because it is believed that flavor components at their suprathreshold, near threshold or subthreshold concentrations will determine if odor suppression, odor addition or synergism will occur.[228-232]

Extensive experimental research conducted on the grapes of v. vinifera cultivar Cabernet Sauvignon, led to the identification of 2-methoxy-3-isobutyl pyrazine (odor threshold, 0.002 ppb) as the single most important contributor of the green bell-pepper aroma that characterizes this variety.[217] It was estimated that the cis form of the oak lactone, 3-methyl-1,4-octalactone, (odor threshold 75 ppb)[233] was an important contributor to the flavor of French wines. Similarly, 1,1,6-trimethyl-1,2-dihydronaphthalene is considered a very important contributor to the flavor of bottle-aged Riesling (odor threshold approximately 20 ppb).[234] Most of these highly significant flavor components were estimated to have concentrations in these wines that were near their threshold values. Because most of these highly significant flavor compounds had been isolated, identified and quantificated by extracting very large volumes of wines in previous studies, it was felt that a real test of the analytical method developed

in this research would lie in the success or failure with which such compounds could be isolated, detected and quantificated in actual wine samples. It was for this reason that the 1972 Cabernet Sauvignon wine made by the Chateau Pichon Lalande, France was selected for analysis. In addition to possessing all the characteristics of a high quality wine, there is a large volume of qualitative and quantitative data available on this cultivar to enable a meaningful evaluation of the developed techniques to be conducted. A discussion of the results obtained on the analysis of the volatile flavor components of Cabernet Sauvignon wine now follows.

3.3.1 Qualitative Analysis of a 1972 Cabernet Sauvignon Wine Bottled by Chateau Pichon Lalande, France

The objectives for which this analysis was conducted were met by using the analytical methodology developed to isolate, detect and identify (qualitatively) as many volatile flavor components of Cabernet Sauvignon as possible. Table 3.2.16 summarizes the results of the number of compounds identified in this study. For Cabernet Sauvignon, 253 compounds were detected. This included 16 compounds that still remain to be identified (see Appendix 1 for mass spectra and retention index data), 9 compounds that have been assigned tentative identities, (Table 3.2.17) that had not been previously reported as flavor components in this wine, and 226 compounds (Table 3.2.18) previously reported as flavor components in this wine and confirmed in this analysis. Three new compounds were identified in this wine for the first time, namely, ethyl 4-acetyloxybutyrate 228, 2-hydroxybenzothiazole 304, and 2-methoxy-3-isobutylpyrazine 102 (Table 3.2.19). Figure 3.3.1 illustrates

Table 3.2.16

Summary of Qualitative Analysis Data Presented in Tables 3.2.17, 3.2.18 and 3.2.19

	Cabernet Sauvignon	White Riesling	Moulin Rouge	Concord
Number of compounds previously identified and also identified POSITIVELY* in this research	226	204	123	110
Number of compounds assigned TENTATIVE** identification in this research and have not been previously identified	9	-	9	2
Number of compounds POSITIVELY identified in this research and are being reported for the first time	3	-	3	9
Number of compounds detected in this research that remain to be identified	16	1	3	7

* EI, CI mass spectra, accurate mass measurements of ions in EI spectra and retention characteristics on a C20M and SE-54 column matched those of an authentic compound run on the same instrument under similar conditions.

** EI mass spectra and retention characteristics on C20M were similar to published data. CI mass spectra and accurate mass measurement data appear to support the assignment. Nevertheless, there was no authentic sample available in our collection to confirm this assignment.

Table 3.2.17

Compounds assigned tentative identification in this investigation but have not been previously reported as flavor components in the indicated varieties

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)	Extpal. I on SE-54	Cabernet Sauvignon	Wine Variety: White Riesling	Wine Variety: Moulin Rouge	Wine Variety: Concord
82	Hexyl isothiocyanate	143	1405		1228	*		*	
94	Hexyl thiobutyrate	188	1447		1310			*	
131	2-methanol-1,3-dioxolane	104	1572		1109			*	
171	Valeraldehyde,2,2-dimethyl oxime	129	1762		1502			*	
184	Phenylpyrrole	143	1815		1288	*			
199	Diisobutoxymethane	160	1890		1380	*		*	
223	N-(N-hydroxy,N-methyl-γ-aminobutyryl)-glycine	190	2035		1645			*	*
231	2-amino-4-hydroxy-3-formylthiophen	143	2077		1385	*			
239	1-methyl-3-phenyl-2,5-pyrrolidinedione	189	2140		1720	*			
245	Benzyloxy 2-butanol	180	2202		1695	*			

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Table 3.2.17 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)	Extpal. I on SE-54	Cabernet Sauvignon	Wine Variety: White Riesling	Wine Variety: Moulin Rouge	Wine Variety: Concord
277	3-Hydroxy-β-damascone	208	2580		1755	*		*	
280	5-Butyl isobutylthioacetate	160	2590		1702			*	
300	1-Undecen-2-ol	155	-		1610	*			
322	3-Phenyldihydrobenzofuran-2-one	210	-		1725	*			

Table 3.2.18

Flavor Compounds Positively Identified in this Study thathave also been previously identified

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
1	Acetone	58	815		548			*	*
2	1,1-Diethoxyethane	118	850		728	*	*	*	*
3	Ethyl acetate	88	870	872	615	*	*	*	*
4	Isopropyl acetate	102	872		665	*	*	*	*
5	Ethanol	46	901	900	518	*	*	*	*
6	2-Butanone	72	906	908	599	*	*[d]		
7	Ethyl propionate	102	950	944	720	*	*	*	*
8	Isopropyl propionate	116	954	945	758	*	*		
9	Ethyl isobutyrate	116	959	956	766	*	*		
10	Diacetyl	86	961		621	*	*[d]		*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
11	Isobutyl acetate	116	1005	1000	761	*	*	*	*
12	1-propanol	60	1009	1002	555	*	*[d]	*	*
13	1-propyl propionate	116	1013	1010	805	*	*	*	*
14	Ethyl butyrate	116	1020	1025	803	*	*	*	*
15	Isopropyl butyrate	130	1030	1030	845	*	*	*	*
16	Ethyl 2-methylbutyrate	130	1050	1049	857	*	*		
17	Isobutyl alcohol	74	1056	1054	634	*	*[d]	*	*
18	1-Butyl acetate	116	1060	1059	824	*	*		
19	Ethyl 3-methylbutyrate	130	1062	1060	858	*	*		
20	Isobutyl propionate	130	1071	1071	883	*	*		
21	Isobutyl isobutyrate	144	1084		918	*	*	*	*
22	Propyl n-butyrate	130	1110	1110	909	*	*	*	*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
23	2-Methylbutyl acetate	130	1110		889	*	*	*	*
24	Propyl isobutyrate	130	1110		901	*			
25	n-Butanol	74	1121		673	*	*[d]	*	*
26	Ethyl valerate	130	1124	1124	902	*	*	*	*
27	Butyl propionate	130	1130		920	*	*	*	*
28	Isopropyl valerate	144	1130		951	*	*		
29	1-Propyl-2-methylbutyrate	144	1134		964	*			
30	1-Butylisobutyrate	144	1139		957	*	*		
31	Isobutyl butyrate	144	1152		959	*			
32	Myercene	136	1156	1156	1004		*[d]		
33	Amyl acetate	130	1169	1161	913	*	*	*	*
34	Isobutyl 2-methylbutyrate	158	1171	1171	1008	*			
35	Amyl propionate	144	1180		973	*	*		

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
36	Isoamyl propionate	144	1181		972	*	*	*	*
37	2-Methyl-1-butanol	88	1182	1184	737	*	*[d]	*	*
38	3-Methyl-1-butanol	88	1182	1197	737	*	*[d]	*	*
39	Ethyl 3-methylbutyrate	158	1184		1012	*	*		
40	2-Methylbutyl isobutyrate	158	1189	1187	1028	*			
41	1-Propylvalerate	144	1200		1012	*	*	*	*
42	Limonene	136	1205	1206	1129	*	*		
43	1-Butylbutyrate	144	1208		997	*			
44	1-Pentanol	88	1210	1213	778	*	*[d]	*	*
45	Ethyl hexanoate	144	1220	1223	1001	*	*	*	*
46	1-Hexen-3-ol	100	1222		798		*[d]		
47	1-Butyl-2-methylbutyrate	158	1224		1047	*	*	*	*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
48	1-Amyl isobutyrate	158	1230		1053	*	*	*	*
49	Diethyleneglycol monoethyl ether	120	1265		865	*			
50	Isoamyl 2-methylbutyrate	158	1271		1105	*	*	*	
51	Acetoin	88	1272	1270	791	*	*[d]	*	*
52	4-Methyl-1-pentanol	102	1279		856	*	*[d]		
53	Isoamyl isovalerate	172	1284		1110	*	*		
54	3-Methyl-1-pentanol	102	1290		870	*	*[d]		
55	cis-3-Hexenyl acetate	142	1295		1005	*	*		
56	Ethyl lactate	118	1305	1309	935	*	*[d]	*	*
57	1-Amylbutyrate	158	1305		1096	*	*	*	*
58	1-Hexyl acetate	144	1307		1030	*	*	*	*
59	trans-2-Hexenyl acetate	142	1312		1015	*	*		

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit)[c] White Riesling[d]	Moulin Rouge	Concord[i]
60	Hexanol	102	1315	1316	876	*	*[d]	*	*
61	Isopropyl lactate	132	1317		952	*	*	*	*
62	2-Hydroxyethyl acetate	104	1319		655	*	*		
63	trans-3-Hexen-1-ol	100	1322	1345	815	*	*[d]	*	
64	Diacetone alcohol	116	1325		902	*	*[d]		*
65	Neroloxide	152	1328		1098		*		
66	Isopropyl hexanoate	158	1331		1052	*	*	*	*
67	3-Ethoxy-1-propanol	104	1334		881	*		*	*
68	cis-3-Hexen-1-ol	100	1337	1351	865	*	*[d]	*	*
69	1-Amyl isovalerate	172	1339		1153	*	*	*	*
70	3-Octanol	130	1352		950	*	*[d]		
71	Isoamyl isovalerate	170	1352		1156	*	*		

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
72	cis-Roseoxide	154	1354		1105		*		
73	Ethyl 2-hydroxyisobutyrate	132	1359		963	*	*		
74	trans-2-Hexen-1-ol	100	1365	1368	872	*	*[d]		
75	2-Butoxyethanol	118	1368		940	*			
76	trans-Roseoxide	154	1370		1118		*		
77	2-Hydroxyethyl propionate	130	1375		710			*	
78	Ethyl 2-hydroxy-3-methylbutyrate	146	1395		991			*	*
79	Methyl 3-(methylthio)propionate	134	1400				*		
80	Acetic acid	60	1402	1400		*	*[e]	*	*
81	1-Butyl hexanoate	172	1403		1195	*	*	*	*
83	2-Octanol	130	1407		980	*			
84	3-(Methylthio)propyl acetate	148	1408			*	*		
85	Ethyl 3-(methylthio)propionate	148	1408			*	*		

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
86	Ethyl octanoate	172	1410	1423	1198	*	*	*	*
87	1-Heptanol	116	1414		975	*	*[d]	*	*
88	Isobutyl lactate	146	1420			*	*[d]		
89	cis-Linalool oxide	170	1428	1423	1069		*[d]		
90	1-Butyl lactate	146	1430		1015		*[d]	*	*
91	3-Methylbutyl hexanoate	186	1435		1210			*	*
92	3-Methyl-1,4-octalactone	156	1438		1125	*			
93	1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)	172	1440		1462		*[f]		
95	Furfural	96	1449		833	*	*[d]		*
96	trans-Linalool oxide	170	1451	1432	1113		*[d]		
97	2-Methylbutyl hexanoate	186	1455		1256	*	*	*	*
98	2-Acetyl furan	110	1458	1472	910	*	*[d]	*	*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
99	Ho-trienol	152	1462			*	*		
100	trans-1-Octen-3-ol	128	1469		986	*	*[d]		
101	Dimethyl malonate	132	1470		914	*			
103	Propionic acid	74	1480			*	*[e]	*	*
104	Ethyl furoate	140	1485		1019	*			
105	Benzaldehyde	106	1495	1502	965	*	*	*	
106	Vitispirane	192	1499			*	*[g]		
107	Isobutyl octanoate	200	1500		1310	*	*	*	*
108	Ethyl 3-hydroxybutyrate	132	1505			*	*	*	*
109	5-Nonanol	144	1508		997	*	*		
110	1-Amyl lactate	160	1508		1051	*			
111	2,3-Butanediol	90	1509		875	*	*[d]	*	

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
112	Linalool	154	1510	1541	1110	*[h]	*		*
113	2,3-Butanediol monoacetate	132	1511		995	*	*[d]	*	
114	trans-3-Octen-1-ol	128	1511		1054	*	*[d]		
115	Isopropyl 2-butenoate	128	1511			*			
116	Isobutyric acid	88	1513			*	*[e]	*	*
117	1-Octanol	130	1515	1519	1079	*	*[d]	*	*
118	α-Copaene	204	1519		1416		*		
119	3-Methylbutyl octanoate	214	1520		1338	*	*		
120	Ethyl 3-ethoxypropionate	146	1526					*	*
121	2-Methylbutyl lactate	160	1535		1059	*	*	*	*
122	3-Methyl 2-hexanol	116	1537		870			*	*
123	2-Ethylbutanol	102	1538		1040				*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit)[c] White Riesling	Moulin Rouge	Concord[i]
126	Diethyl malonate	160	1544	1542	1061	*	*		
127	β-Bourbonene	204	1544		1424		*		
128	1,2-Propanediol	76	1559		974	*	*[d]		
129	5-Methylfurfural	110	1563		960		*		
130	4-Terpineol	154	1570	1587	1193	*[h]	*		*
132	cis-3-Octen-1-ol	128	1580		1061	*			
133	5-Methyl-2(3H)dihydrofuranone	100	1585		957			*	
134	3-Methylthiopropyl acetate	148	1590				*		
135	β-cyclocitral	152	1600		1190	*[h]			
136	Butyric acid	88	1600			*	*	*	*
137	γ-Butyrolactone	86	1610		903	*	*[d]	*	*
138	1-Decanol	158	1610		1022	*	*[d]	*	*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
139	3-Methylpentanoic acid	116	1620			*	*[d]	*	*
140	Phenylacetaldehyde	120	1624	1605	1042		*		
141	Methyl-6-nonenoate	170	1625		1265	*			*
142	1-Butyl octanoate	200	1630		1361	*	*	*	*
143	β-Farnesene	204	1630				*		
144	β-Caryophyllene	204	1632	1642	1446		*		
145	3-Methyl 3-hydroxybutanol	104	1635		1105			*	
146	trans-2-Hexen-1-ol	100	1636	1634		*	*[d]		*
147	Ethyl decanoate	200	1640	1647	1125	*	*	*	
148	Ethyl benzoate	150	1645		1170		*		
149	3-Methylbutyric acid	102	1645			*	*	*	*
150	Isopropyl succinate	160	1650		1107	*	*	*	*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
151	γ-Muurolene	204	1655	1655			*		
152	3-Methylbutyl octanoate	214	1658	1648	1451	*	*	*	*
153	α-Terpineol	154	1661	1677	1205		*		*
154	Diethyl succinate	174	1665		1165	*	*	*	*
156	1,3-Propanediol diacetate	110	1671			*	*[d]		*
157	2,7-Dimethyl octanol	158	1673					*	
158	Benzyl acetate	150	1680		1162	*			
159	α-Humulene	204	1682	1682	1483		*		
160	3-Ethylthiopropanol	120	1682		1018		*[d]		
161	1,3-Butanediol	90	1692		958	*	*[d]	*	*
162	Hexyl lactate	174	1695			*	*[d]	*	*
163	Propyl decanoate	214	1700		1485	*	*	*	*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
164	Citronellol	156	1722	1755	1233		*		
165	1-Undecanol	172	1723		1281	*	*[d]	*	*
166	α-Muurolene	204	1730	1740	1518		*		
167	1-Butylbutyryl lactate	216	1732		1349	*	*		
168	Valeric acid	102	1745			*	*[e]	*	*
169	2-Methylpentyl-2-propenoate	176	1750			*			
170	Nerol	154	1757		1236		*		
172	γ-Cadinene	204	1764	1774	1540		*		
174	5-Nonanol	144	1770		1092	*			
175	Ethylphenyl acetate	164	1780			*	*		
176	2-Phenethyl acetate	164	1789	1785	1251	*	*	*	*
177	1-Phenethyl alcohol	122	1792		1092	*	*[d]		
178	Geraniol	154	1797	1833	1262		*[d]		

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
179	Butyl decanoate	228	1798		1593	*	*	*	*
180	β-Damascenone	190	1798	1801	1425	*[h]	*	*	*
181	Hexanoic acid	116	1803			*	*[e]	*	*
182	Hexyl octanoate	228	1806		1582	*	*	*	
183	Ethyl 2-methylpropyl succinate	202	1810			*	*		
185	Benzyl alcohol	108	1824	1822	1051	*	*	*	*
186	Ethyl laurate	228	1826	1833	1610	*	*	*	*
187	Di-n-amylamine	157	1832		998	*	*		
188	α-Ionone	192	1833		1434	*[h]	*		
189	N-(3-methylthiopropyl)acetamide	147	1840		1207	*	*[d]	*	
191	2-Phenethyl propionate	178	1845	1855	1346	*	*	*	*
192	2-Phenethyl isobutyrate	192	1846		1392	*	*		
193	3-Methylbutyl decanoate	242	1857		1633	*	*	*	

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
194	2-Phenethanol	122	1861		1122	*	*[d]	*	*
195	N-(3-methylbutyl)acetamide	129	1877			*	*[d]	*	*
196	Ethyl isobutyl glutarate	216	1882			*	*		
197	2-Phenethyl isovalerate	206	1886		1505	*	*		
198	2-Phenethyl butyrate	192	1888	1881	1438	*			
200	2-Phenethyl valerate	206	1892		1525	*	*	*	
201	trans 4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	192	1892		1440	*			
203	trans-5-Butyl-4-methyl-2(3H)dihydrofuranone	156	1896			*	*		
204	2-Pyrrolidine-5-carboxylic acid	129	1896			*	*	*	*
205	Dipropyl glutarate	216	1896			*	*		
206	Diethyl tartrate	206	1899			*			
207	n-Butyl n-undecanoate	242	1901		1674	*			

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Wine Variety(Lit) Cabernet Sauvignon[b]	White Riesling[c]	Moulin Rouge	Concord[i]
208	Heptanoic acid	130	1903			*	*[e]	*	*
209	β-Ionone	192	1912	1918	1492		*		
210	2-Phenethyl isohexanoate	220	1930			*			
211	Methyl furoate	126	1937		1565			*	*
212	cis-cinnamaldehyde	132	1942		1850			*	
213	Isoamyl n-undecanoate	242	1945	1948	1730	*	*	*	*
215	4,4'-Dimethyl-3-hydroxy-2(3H)dihydrofuranone	130	1957			*	*[d]		
216	α-Nerolidol	222	1961		1542		*[d]		
217	trans-Cinnamaldehyde	132	1993	1996	1269	*	*	*	*
218	n-Butyl laurate	252	2000		1775	*	*		
219	Di-isobutyl succinate	230	2000		1605	*			
220	para-Cresol	108	2011		1069	*			
221	4-Ethyl guaiacol	152	2011		1283	*			*

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Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
222	Octanoic acid	144	2035			*	*[e]	*	*
224	Ethyl-4-phenyl-3-butenoate	190	2036			*	*	*	*
225	Diethyl malate	190	2046			*	*[d]	*	*
226	Isoamyl laurate	266	2048		1847	*	*		
229	Di-n-butyl succinate	230	2059		1619	*	*		
230	Ethyl 2-acetyloxy-4-methylvalerate	202	2072			*			
232	4-Ethylphenol	122	2091		1131	*	*		
235	4-(4-Hydroxyphenyl)-2-butanone	164	2118			*	*		
236	Di-3-methylbutyl succinate	268	2128			*	*		
238	2-Phenethyl hexanoate	220	2136	2136	1637	*	*	*	*
240	Diethyl 2-hydroxyglutarate	204	2159			*	*	*	*
241	3,5-Dimethylbenzoic acid	150	2170					*	

..... continued

Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on[a] C20M (Lit.)	Extpal. I on SE-54	Cabernet[b] Sauvignon	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
242	Benzothiazole	135	2180		1198		*		
243	Methyl anthranilate	151	2181	2183	1349			*	*
244	2-Pentyl furan	138	2182			*			
246	5-Carboethoxy-2(3H)dihydrofuranone	158	2229			*	*[d]	*	*
247	2-Phenethyl heptanoate	234	2233		1736	*	*		
248	Ethyl anthranilate	165	2234	2232	1414			*	*
249	Ethyl propyl malate	204	2240		1692	*		*	
250	Ethyl 2-hydroxy-3-phenylpropionate	194	2256			*			
251	Dihydroactinidiolide	180	2262	2258	1310		*		
252	4-Hydroxy-5-phenyl-2-penten-3-one	176	2263			*			*
253	Decanoic acid	172	2270			*	*[e]	*	*
254	Isoamyl isopropyl malate	246	2285		1781	*	*	*	
255	Cinnamyl acetate	176	2290	1475				*	*

..... continued

Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moutin Rouge	Concord[i]
256	1-Methoxy-2-phenethyl acetate	194	2296			*	*	*	*
258	Diethyl phthalate	222	2303		1580	*		*	*
259	Ethyl isoamyl malate	232	2308		1708	*	*		
261	2-Phenethyl octanoate	248	2341		1837	*	*		
263	Benzoic acid	122	2365			*	*[e]	*	*
265	Ethyl 4-hydroxyvalerate	146	2370			*	*		
268	Decyl acetate	200	2373			*	*	*	
270	Theaspirane	194	2420				*		
271	2-Phenethyl nonanoate	262	2439		1940	*	*		
272	Di-amyl succinate	258	2445			*			
274	2-Phenethyl decanoate	276	2541		2040	*	*		
275	Phenylacetic acid	136	2558			*		*	

..... continued

Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
276	Vanillin	152	2577		1938	*			
279	Benzyl acetoacetate	192	2584			*			
281	Lauric acid	200	2610			*			
282	2-Phenethyl undecanoate	290	2643			*			
283	N-(2-Phenethyl)acetamide	163	2649			*	*	*	*
286	L-Glutamic acid	147	2651					*	
287	Methyl vanillate	182	2653			* *	* *	*	*
288	2-Phenethyl laurate	304	2657			*			
289	Ethyl vanillate	196	2669			*	*	*	*
292	N-(phenyl-4-ethoxy)acetamide	179	2695			*	*		
293	Phenacetin	179	2710			*			
295	4-(3-Hydroxy-1-)-2-cyclohexen-1-one	210	2715					*	

..... continued

Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)[a]	Extpal. I on SE-54	Cabernet Sauvignon[b]	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
295	2-Phenethyl tridecanoate	332	2735			*	*	*	*
297	Dibutyl Phthalate	278					*	*	*
299	3,4-Diethoxybenzoic acid	210				*			
301	2-Phenethyl tetradecanoate	346				*	*	*	
302	2,2-Dimethyldibutyl succanate	258				*			
303	Ethyl-3-phenyl(4-hydroxy-3-methoxy)-propionate	224				*			
305	trans-Cinnamic acid	148				*	*		
307	Methyl-3-(4-hydroxyphenyl)propionate	180				*	*		
309	Methyl α,β-dihydrocoumarate	180				*			
310	Methyl 4-hydroxybenzoate	152				*	*		
312	β-(para-Carboethoxyphenyl)ethanol	196				*		*	
313	trans-Ethyl-4-hydroxy-3-phenyl-2-propenoate	192				*			

..... continued

Table 3.2.18 (continued)

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal: I on Carbowax 20M	Kovats I on[a] C20M (Lit.)	Extpal. I on SE-54	Cabernet[b] Sauvignon	Wine Variety(Lit) White Riesling[c]	Moulin Rouge	Concord[i]
314	2-Furanacrylate	166				*			
316	Ethyl 2-hydroxybenzoate	182				*			
317	Propyl vanillate	226				*	*		
323	Methyl 4-phenylmethoxybutyrate	208				*	*		
324	Diisobutyl phthalate	278				*	*	*	*
325	trans-Ethyl coumarate	192				*			
326	2,6-Dimethylphenol	154			1128	*	*	*	*
327	cis-Ethyl coumarate	192				*			

* detected a. ref 260, b. ref 129, c. ref 261, d. ref 249, e. ref 134, f. ref 234, g. ref 219, h. ref 262.

Table 3.2.19

Compounds identified in this research and being reported as flavor components in the indicated variety for the first time

Compd. Ref. Number	Flavor Compound	Molecular Weight Confirmed by CIMS	Exptal. I on Carbowax 20M	Kovats I on C20M (Lit.)	Extpal. I on SE-54	Cabernet Sauvignon	White Riesling	Moulin Rouge	Concord
102	2-Methoxy-3-isobutylpyrazine	165	1473		895	*			
124	2,5-Dimethyl-4-methoxy-3(2H)furanone	142	1539		1495				*
125	Isophorone	138	1540		1472				*
155	3-Methylthiopropanol	106	1670		1505				*
173	3-Methyltetrahydrothiophene	102	1765		1412			*	*
213	2,5-Dimethyl-4-hydroxy-3(2H)furanone	128	1948		1458				*
260	Phthalide	134	2310		1465			*	*
262	Dihydrobenzofuran	120	2350		1400				*
304	2-Hydroxybenzothiazole	151			1755	*		*	*
228	Ethyl-4-acetyloxybutyrate	174	2049		1591	*			
311	α-Naphthol	144			1557				*

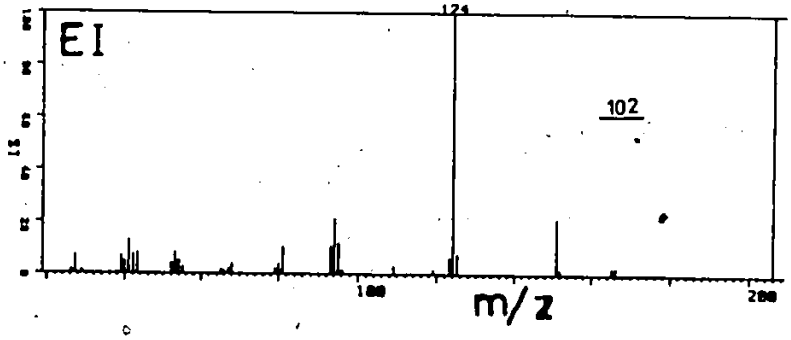
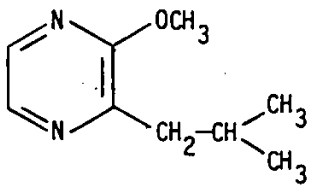


Figure 3.3.1. EI mass spectrum of 2-methoxy-3-isobutyl pyrazine, 102



the EI mass spectrum of 2-methoxy-3-isobutylpyrazine 102 obtained by capillary GC/MS. The mass spectrum and retention indeces measured for component 102 on both Carbowax 20M and SE-54 matched that of an authentic sample of this compound that was obtained from Pyrazine Specialities. Even though this compound was previously detected and identified as a trace component in Cabernet Sauvignon grapes,[127,217] its presence in the wine was not confirmed. This identification therefore confirms the presence of 2-methoxy-3-isobutylpyrazine 102, in Cabernet Sauvignon wine for the first time. Figure 3.3.2 shows the EI mass spectrum of 2-hydroxybenzothiazole 304, identified for the first time in Cabernet Sauvignon. Accurate mass measurements gave the following composition for the prominent ions in the mass spectrum of 2-hydroxybenzothiazole: 304, m/z = 151 (C_7H_5NOS), 304b, m/z = 123 (C_6H_5NS), 304a, m/z = 108 (C_6H_4S), and 304c, m/z = 96 (C_5H_4S). The loss of CO appears to be a significant process in the fragmentation of the molecular ion. It is conceivable that the ionized molecular ion exists in equilibrium with its Keto tautomer, which then looses a molecule of CO to form 304b which subsequently looses HCN to form 304c. Thus, the mass spectrum of 2-hydroxybenzothiazole may be explained according to Scheme 3.3. Figure 3.3.3 shows the EI mass spectrum of ethyl 4-acetyloxybutyrate 228 identified in Cabernet Sauvignon wine for the first time in this investigation. Retention parameters and the EI mass spectra matched those of an authentic sample of 228. The presence of another acetyloxy was established in this wine as ethyl 2-acetyloxy-4-methylpentanoate 230. It was not certain whether it had been previously reported as a flavor constituent of Cabernet Sauvignon wine. These acetyloxy

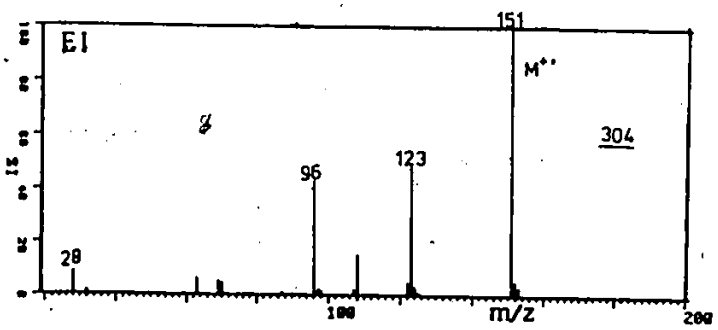
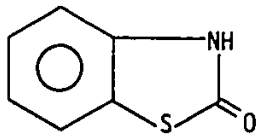


Figure 3.3.2. EI mass spectrum of 2-hydroxybenzothiazole, 304.



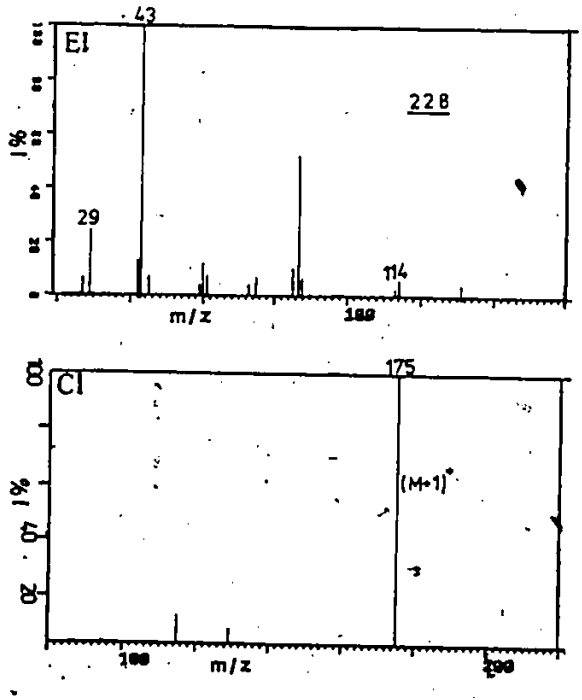
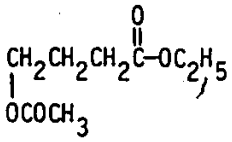
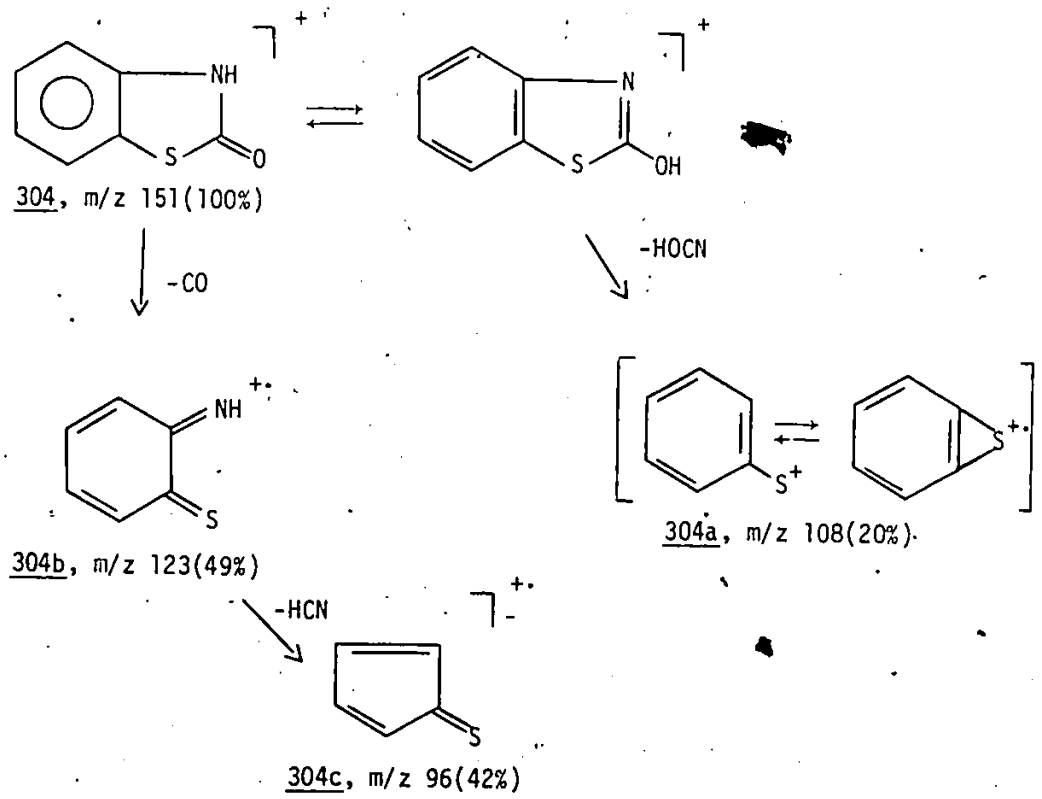


Figure 3.3.3. EI and CI mass spectra of ethyl 4-acetyloxybutyrate, 228.





Scheme 3.3. Postulated mechanism for the EI fragmentation pattern of 2-hydroxybenzothiazole, 304.

esters may be considered to be derivatives of the corresponding hydroxy esters arising through fermentation processes. The occurrence of 228 in Cabernet Sauvignon is of particular interest because it is widely believed to exhibit a much stronger analgesic effect than acetylsalicylic acid.[235] The anaesthetic effect of the precursor of this ethyl ester, 4-hydroxybutanoic acid has been discussed by Makoto and Hoshino.[236] The presence of a wide variety of flavor components that had been

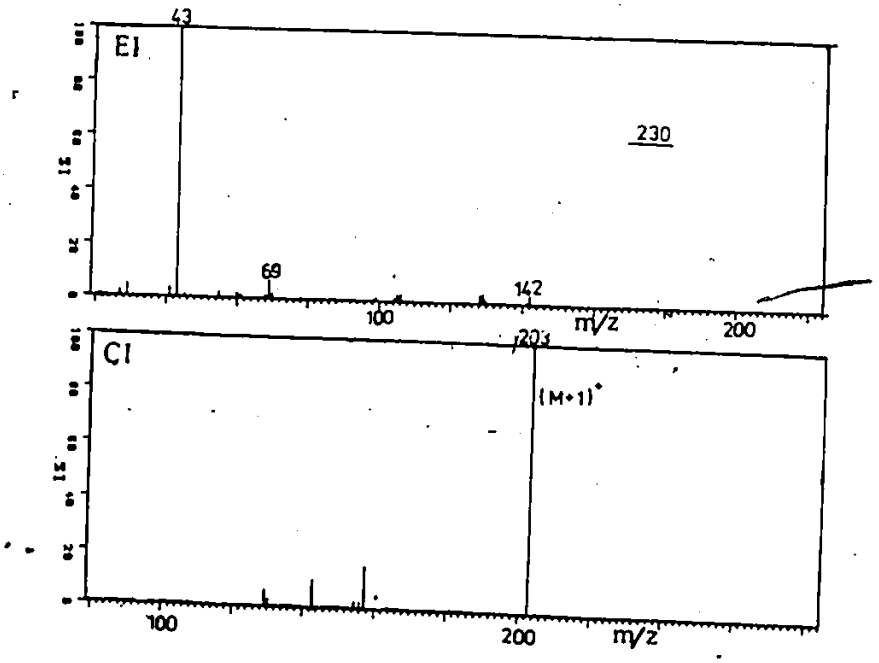
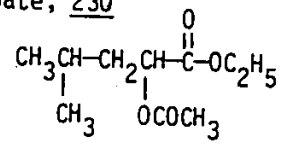


Figure 3.3.4. EI and CI mass spectra of ethyl 2-acetyloxy-4-methylpentanoate, 230



previously identified in this wine was confirmed in this investigation. Figure 3.3.5 shows a partial TIC chromatogram of Cabernet Sauvignon wine (Pichon Lalande) following solvent extraction and treatment of the concentrated extract with $NaHCO_3$. The carboxylic acids, components 80, 139, 149, 168, 181, 208 and 222 could not be detected in this fraction. The phenolic compounds, 220, 221, 232, 303, 326 and the phenolic esters 303, 307, 309, 310, 313, 316, 319, 325 and 327 were retained in this fraction. The EI mass spectra of most of these compounds may be found in Appendix 1. Using the techniques described in detail in Section 2.3.2 enabled the identification of a large number of esters and hydroxy esters. For example, component 240 which showed a retention index of 2159 units on Carbowax 20M was identified as ethyl 2-hydroxyglutarate. The CI mass spectrum (see Figure 3.3.6) showed ions at m/z = 205 (100%), 187, 159 and 131 suggesting a compound of molecular weight 204 and containing a hydroxy functional group $[(M+1 - H_2O)^+$ m/z = 187], and an ethyl ester function $[(M+1 - 74)^+$ m/z = 131]. The EI mass spectrum of 240 is also presented in Figure 3.3.6. Low resolution accurate mass measurement of the ions in the EI spectrum gave the following composition: m/z = 159 [$C_7H_{11}O_4$, 240a], m/z = 131 [$C_6H_{13}O_3$, 240b], m/z = 102 [$C_4H_8O_3$, 240c], m/z = 85 [$C_4H_6O_2$, 240d], and m/z = 29 [C_2H_5, 240e]. It is postulated that under electron impact conditions, 240 looses an ethoxy group by α-cleavage to form 240a the highest ion observed in the EI mass spectrum, which readily looses a molecule of CO to form the reasonably stable fragment ion $[C_2H_5O-\overset{\overset{O}{\|}}{C}-CH_2CH_2\overset{\overset{OH}{|}}{C}H]^+$ 240b. This ion then looses an ethyl group followed by cyclization (these two processes may be occurring simultaneously) with loss of a molecule of H_2O to

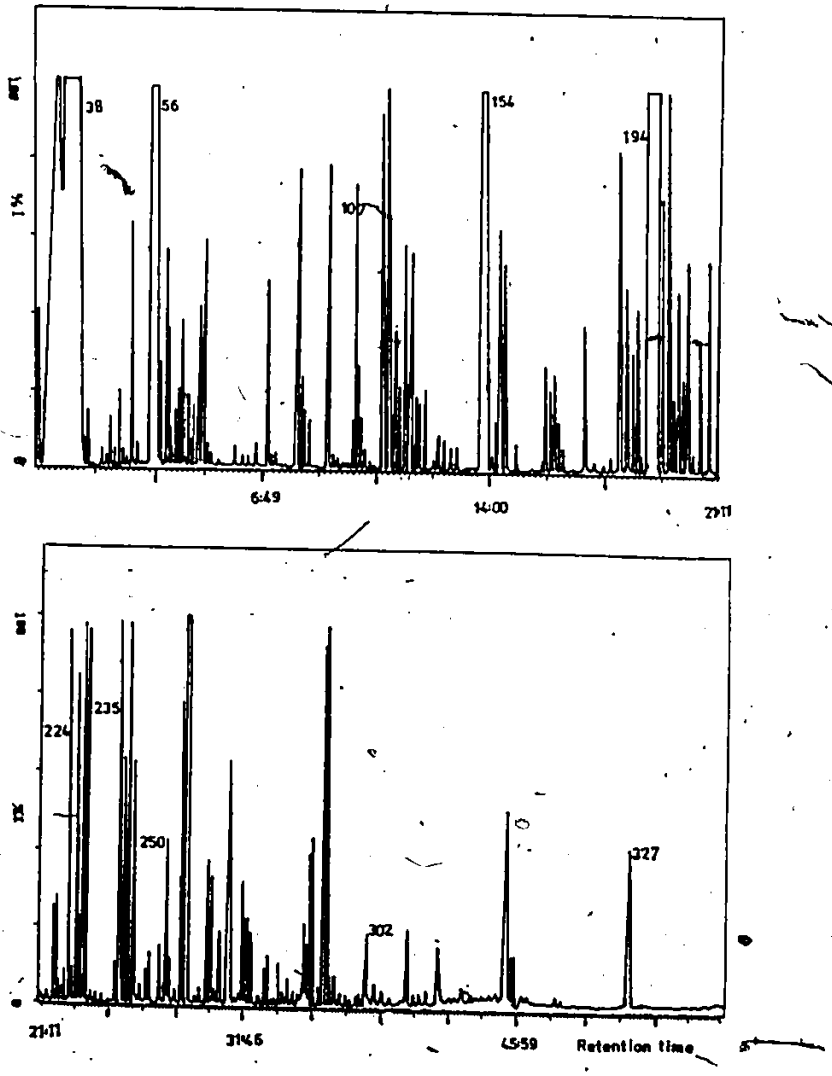


Figure 3.3.5. Partial TIC chromatogram of a 1 µl sample of concentrated and simplified volatile flavor extract of Cabernet Sauvignon wine injected onto a 50m x 0.25mm ID fused silica capillary C20M column coupled directly to a VG7070 mass spectrometer. [Numbers in Figure 3.3.5 represent compound numbers as they appear in Tables 3.2.17-3.2.19.]

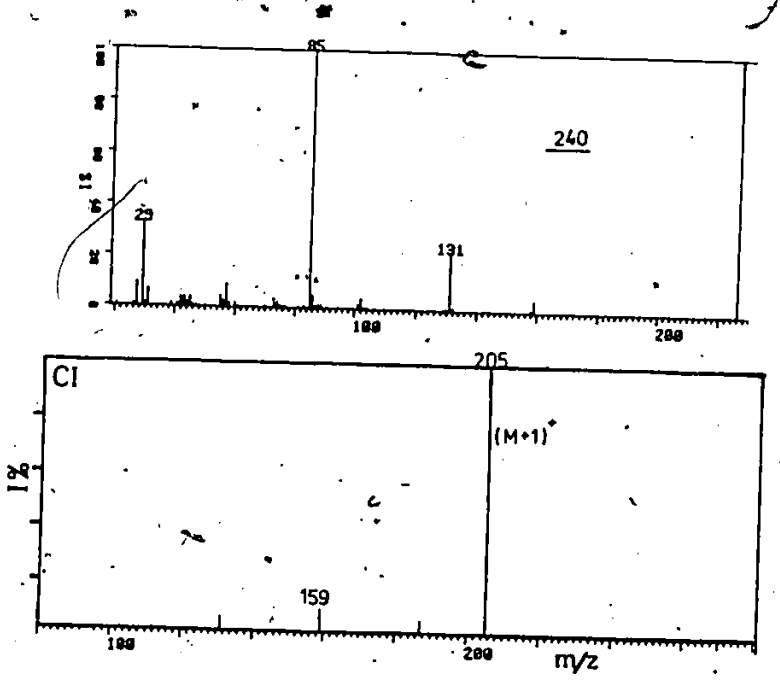
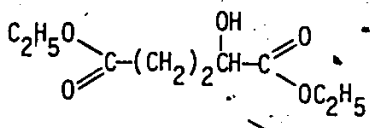
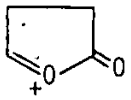


Figure 3.3.6. EI and CI mass spectra of ethyl 2-hydroxyglutarate, 240.



form the highly stabilized ion  m/z = 85, the base peak in the EI mass spectrum. Trans-cinnamic acid, 305, 4-ethyl guaiacol 221, 4-ethylphenol 232, and several phenolic derivatives were also identified. Almost without exception, they showed detectable molecular ions in their EI mass spectra, thus making it possible to deduce their chemical identities from accurate mass data. Esters of para-hydroxybenzoic acid (310, 316), vanillic acid (287, 289, 317, 320) syringic acid (319), ferulic acid (313), coumaric acid (309, 325, 327) and α,β-dihydroferulic acid (303) were all identified in this investigation. Several compounds with a 2,6,6,10-tetramethyl-1-oxaspiro[4,5]-decane skeleton have been reported as volatile flavor components of raspberries,[237] tea,[238] grape juice, wine and distilled grape spirits.[239] One of these spiroethers, vitispirane 106, was identified in this wine. Figure 3.3.7 shows the EI mass spectrum of 106 of molecular weight 192 and shown to have an elemental composition of $C_{13}H_{20}O$. The presence of other C_{13} compounds in this wine was confirmed with the identification of the structurally related compounds α-ionone, 188, and β-damascenone, 180 (see Figures 3.3.8 and 3.3.9 for their respective EI mass spectra). Figure 3.3.10 shows the EI mass spectrum of component 277 detected in this wine and assigned a tentative identity of 3-hydroxy-β-damascone. Low resolution accurate mass measurement data on the molecular ion gave a composition of $C_{13}H_{20}O_2$. The mass spectrum shown in Figure 3.3.10 matched the published mass spectrum of 3-hydroxy-β-damascone identified as a volatile flavor component in tobacco leaves[240] and Kudzu oil.[241] There was no report in the literature that this compound had been previously isolated and identified as a volatile component in grape

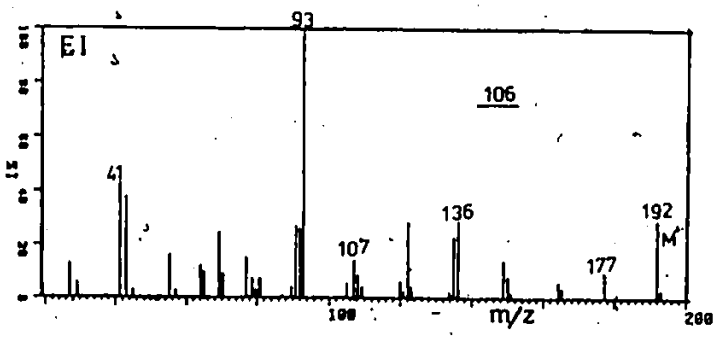
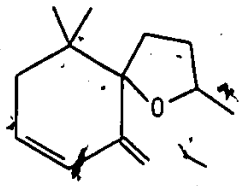


Figure 3.3.7. EI mass spectrum of vitispirane, 106.



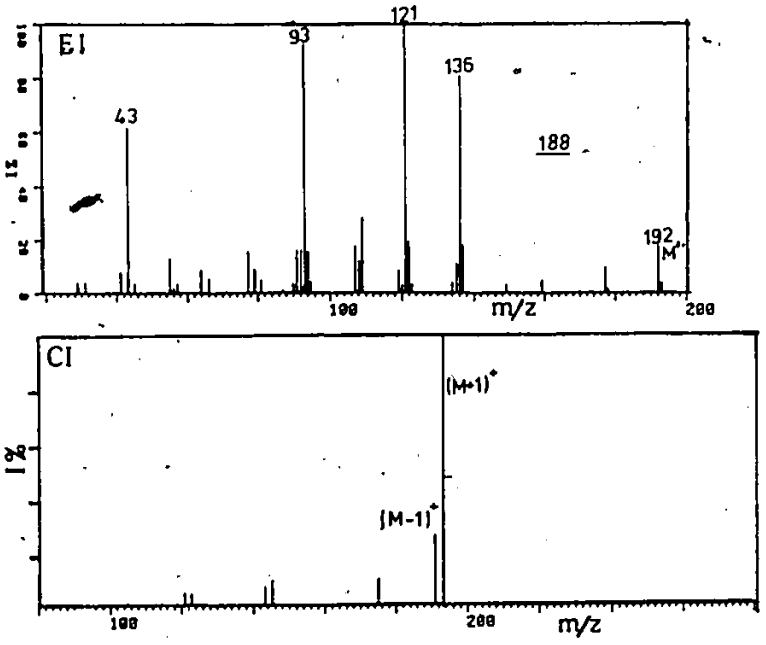
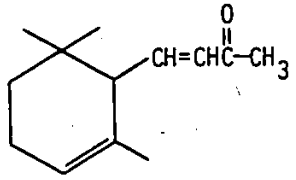


Figure 3.3.8. EI and CI mass spectra of α-Ionone 188



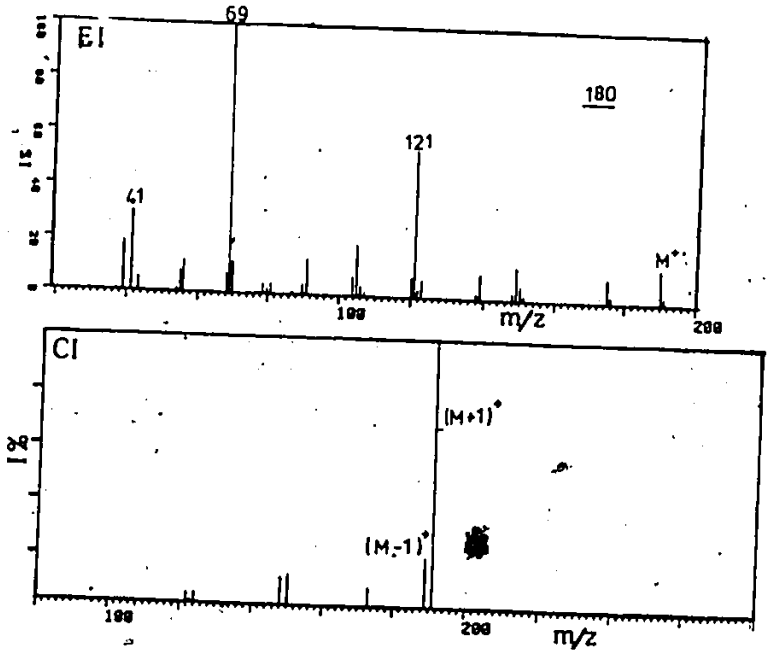
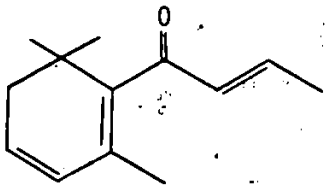


Figure 3.3.9. EI and CI mass spectra of β-damascenone 180.



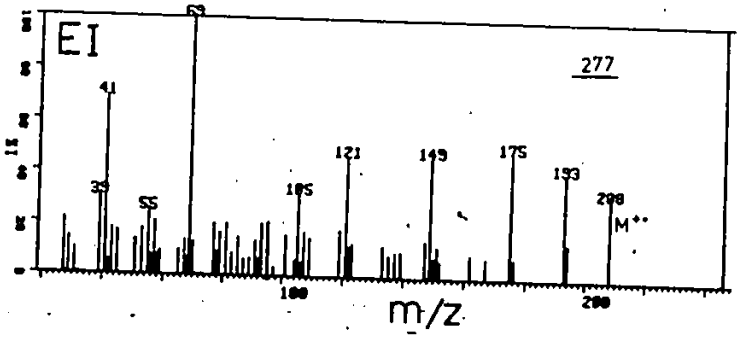
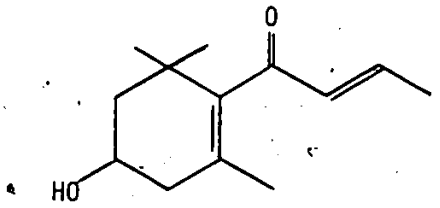
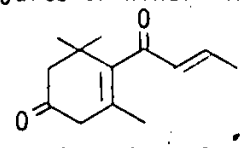


Figure 3.3.70. EI mass spectrum of component 277 tentatively identified as 3-hydroxy β-damascone.



juice or wine. There is an attempt to synthesise this compound from  , using L-selectride.[242] It is believed that once the identity of compound 277 is proven, it will be the first time it would have been identified as a flavor component in Cabernet Sauvignon wine. Figure 3.3.11 shows the EI mass spectrum of component 231 tentatively identified in this wine as 2-amino-4-hydroxy-3-formyl-thiophen. Figures 3.3.12 to 3.3.16 represent the EI mass spectra of compounds detected in this analysis and assigned tentative identification only, because there was no authentic sample to prove the identification, after a mass spectral match was obtained by a library search routine of NIH/EPA mass spectral data bank. All of these compounds had not been previously reported as flavor components in Cabernet Sauvignon wine.

Nitrogen and/or sulphur containing flavor components were also identified in this wine. These included 3-methylthiopropanol 155, 3-methylthiopropyl acetate 84, and N-(3-methylthio)propyl acetamide 189. N-(3-methylbutyl)acetamide 195, N-(2-phenethyl)acetamide 283 (see Figure 3.3.17 for EI mass spectrum) and N-(phenyl-4-ethoxy)-acetamide, 292. The identification of para-phenacetin, 293 is of interest because of the analgesic effect of this compound. It was not detected in the white Riesling or any of the labrusca varieties investigated. Like phenacetin, the acetyloxy esters were also detected in Cabernet Sauvignon and not in White Riesling or the labruscas. Since the objective for which this stage of the experiment was set up had been met, attention is now going to be focussed on the quantitative aspects of this analysis. A discussion of the results of the experiments conducted to determine quantitatively the concentrations of

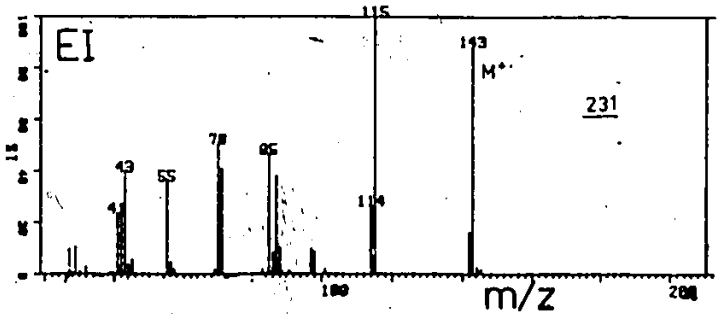
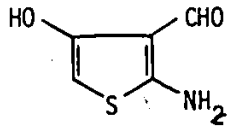


Figure 3.3.11. EI mass spectrum of component 231 tentatively identified as 2-amino-4-hydroxy-3-formylthiophen



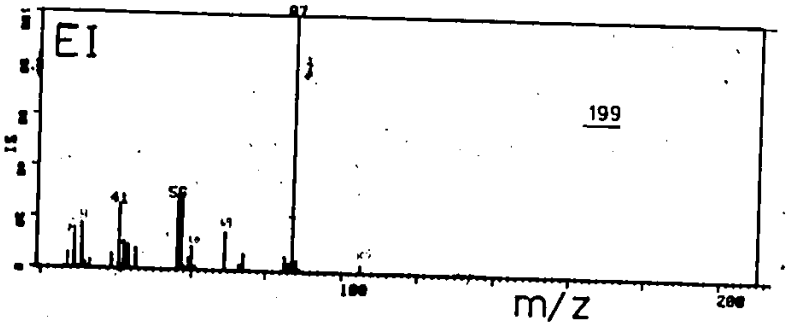
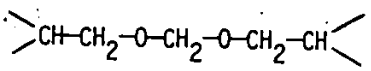


Figure 3.3.12. EI mass spectrum of component peak 199 tentatively identified as diisobutoxymethane



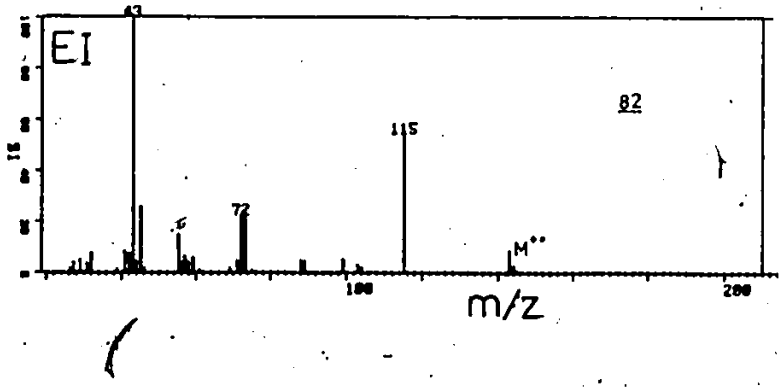


Figure 3.3.13. EI mass spectrum of component 82 tentatively identified as hexyl isothiocyanate $CH_3(CH_2)_5NCS$.

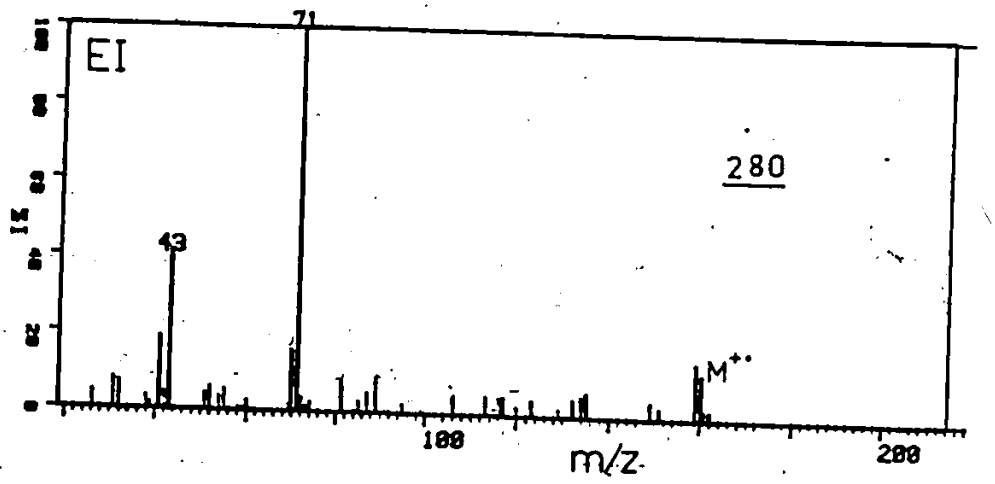
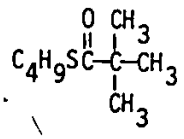


Figure 3.3.14. EI mass spectrum of component 280 tentatively identified as 5-butyl isobutylthioacetate



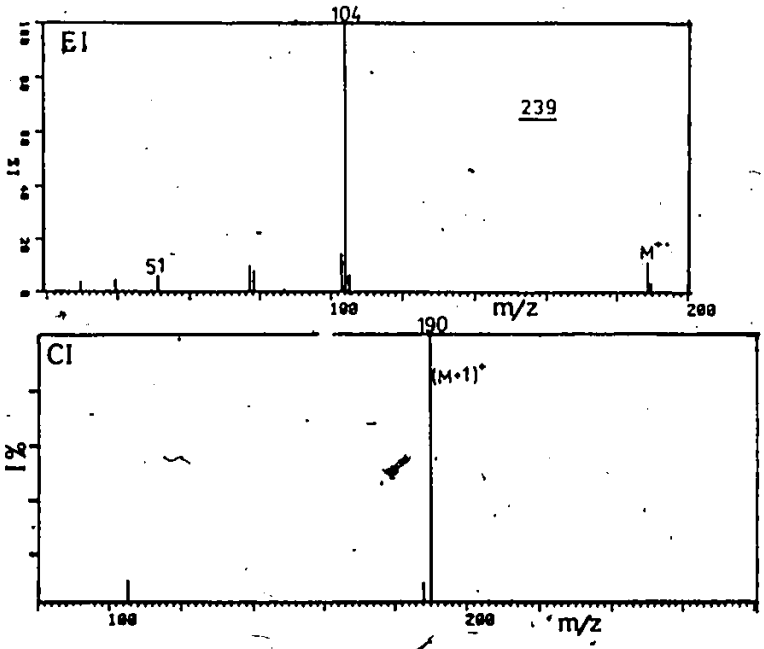
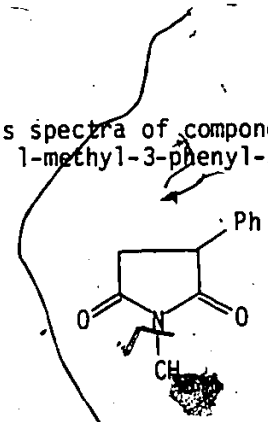


Figure 3.3.15. EI and CI mass spectra of component 239 tentatively identified as 1-methyl-3-phenyl-2,5-pyrrolidinedione.



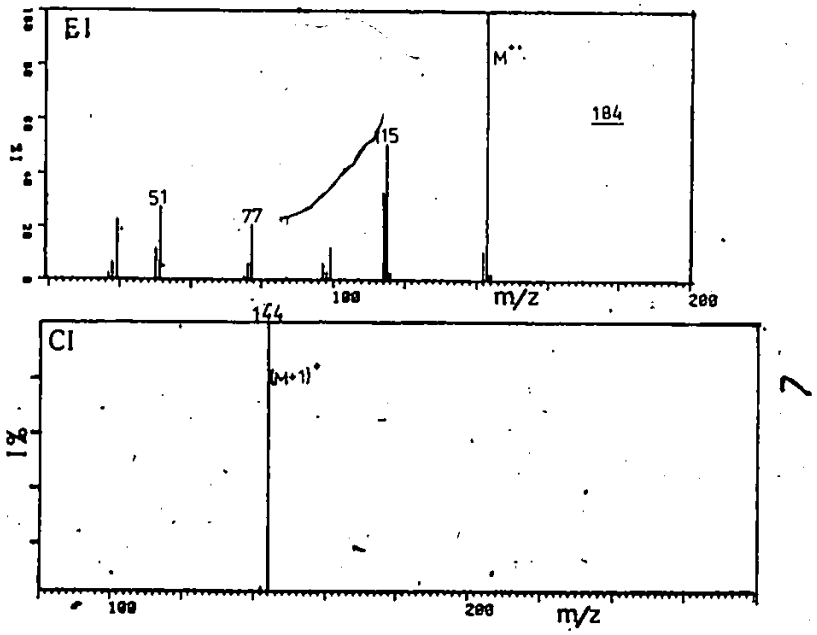
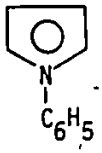


Figure 3.3.16. EI and CI mass spectra of component 184 tentatively identified as phenylpyrrole.



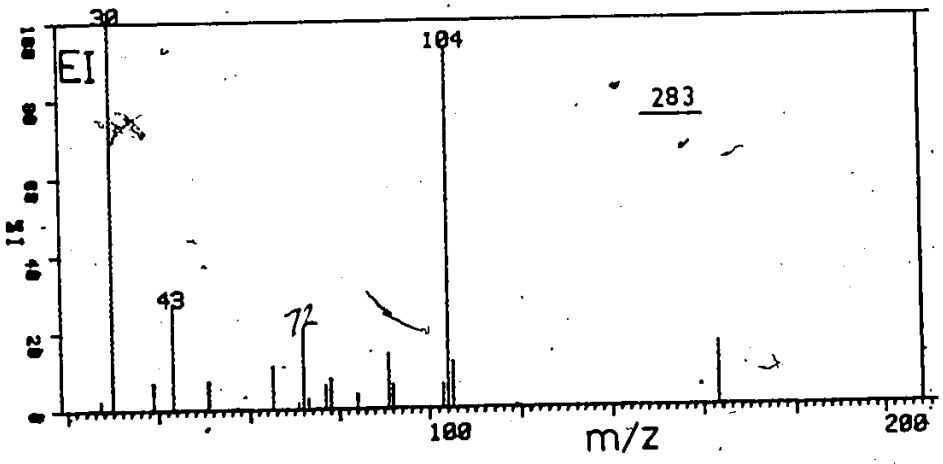
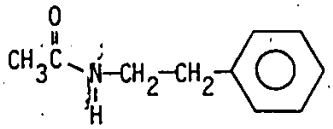


Figure 3.3.17. EI mass spectrum of N-(2-phenethyl)acetamide 283



selected volatile flavor components identified in this wine is presented in Section 3.4.

3.3.2 Qualitative Analysis of the Volatile Flavor Components of Native North American Grape Varieties - Concord and Moulin Rouge

In Canada and the Eastern United States where the native North American vines constitute the backbone of the wine industry, extensive research has been conducted over the years into the development of hybrid grapevines. These hybrids it was hoped, would combine the winter resistance characteristics of the native varieties with the superior grape-producing quality of the vinifera vines, to produce very high quality wines. The failure of these hybrid grapes to produce the anticipated high quality wines, has presumably discouraged enologists from spending considerable research time and effort into the chemical analysis of the volatile flavor composition of the grapes and wines of native and hybrid varieties, as has been done extensively for the wines of Europe and California. A fair amount of experimental data has however been obtained on the volatile aroma composition of these varieties. They are poorly characterized and it is generally believed that the analytical methodology for investigating these varieties are not sufficiently sensitive to detect and identify those compounds, other than methyl anthranilate, believed to impart the floral and fruity flavor to these varieties. Such components are suspected to be ones of low odor thresholds present at trace levels in these varieties. Since the analytical methodology that was developed has been shown to successfully detect and identify compounds of low odor thresholds present at trace levels in a model wine solution and a real wine, it was decided

to use it in an attempt to detect and identify such volatile flavor compounds in the vitis labruscana varieties. Such an identification, it was hoped, would greatly facilitate the reconstitution of the labrusca flavor and improve our understanding of what is described as the "typical labrusca character". Also, because these varieties are rather poorly characterized, this analysis would provide further characterization of the volatile flavor composition. Concord, was therefore selected as a variety for study because of its ease of availability and also because it is one of the best known of the native varieties. Moulin Rouge was also selected for the hybrid study because it portrays the qualities of hybrid wines. Analytical data were also obtained for Dutchess, Elvira and Vidal grape musts and wines but time constraints would not allow the results on these varieties to be presented here in detail.

In order to eliminate any bias due to laboratory scale pilot plant processes, grape musts and wines were all obtained from commercial wineries. These wineries also conducted all the experimentation involved - from maceration to aging. This section presents the results of the experimental studies designed to furnish analytical data on the chemical composition of the volatile flavor extracts of wines made from native American grapes and their hybrids in a continuing effort to provide further characterization of the volatile flavor components of these varieties. Figure 3.4.1 shows a partial reconstructed TIC chromatogram of a concentrated flavor extract of Moulin Rouge. As can be seen, (Table 3.2.16), most of the components detected were positively identified. Of the 138 components detected in the flavor extract of

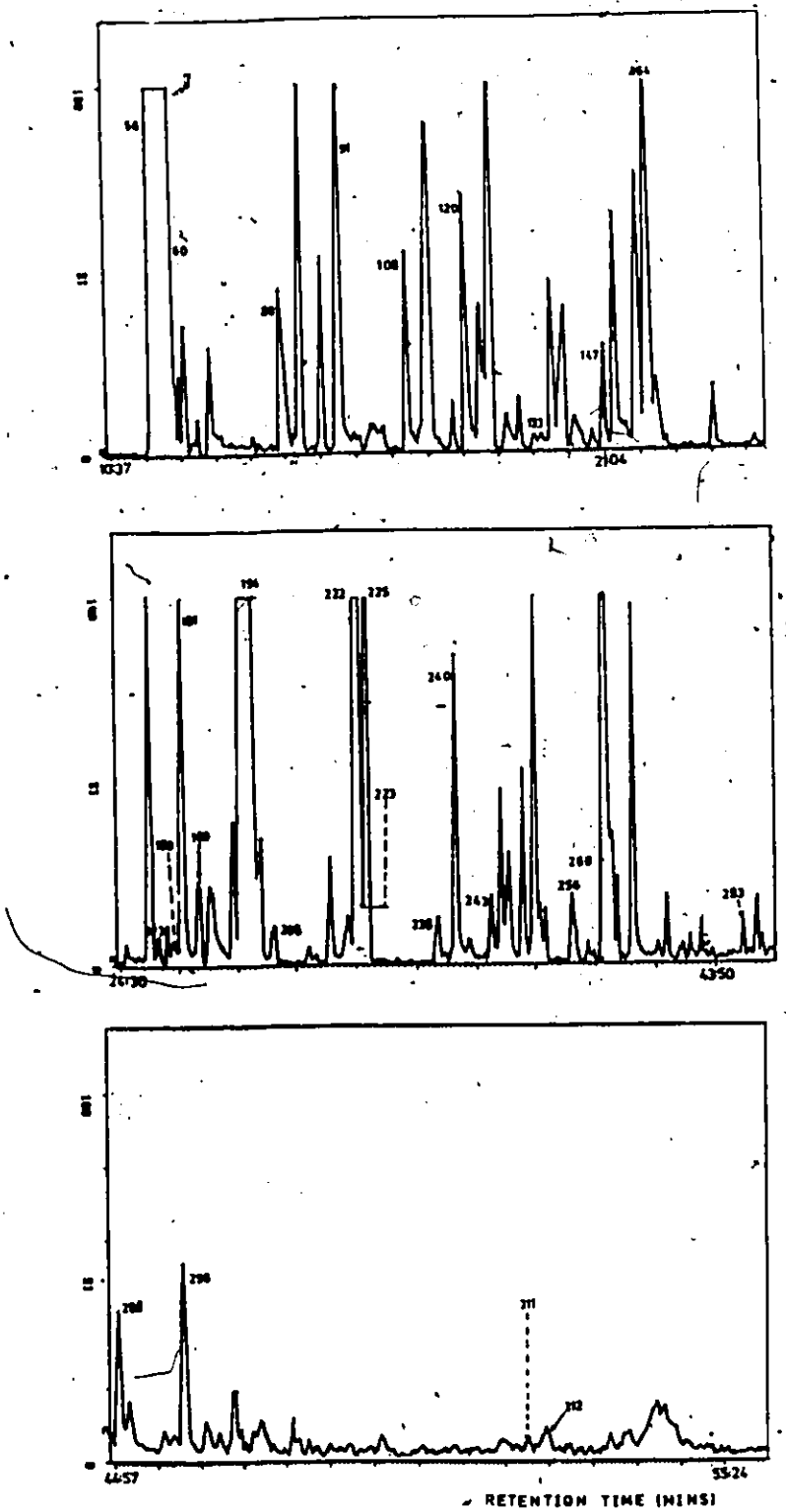


Figure 3.4.1. Partial reconstructed TIC chromagram of a volatile flavor extract of Moulin Rouge, following solvent extraction and enrichment.

Moulin Rouge, 123 components were positively identified. This number confirms the collective efforts of several authors over several years of research. Nine compounds namely, hexyl isothiocyanate 82, hexyl thiobutyrate 94, 2-methanol-1,3-dioxolane 131, valeraldehyde,2,2-dimethyl oxime 171, diisobutoxymethane 199, 3-hydroxy-β-damascone 277; s-butyl isobutylthioacetate, 280, and N-(N-hydroxy,N-methyl-γ-aminobutyryl)-glycine 223 were assigned tentative identification based on their mass spectral data only. Their identities remain to be confirmed once authentic samples become available. All of these components had not been previously reported as flavor components in these varieties. Only two of the nine components, 173 and 223, assigned tentative identities in Moulin Rouge were detected in Concord.

Three new compounds were identified in Moulin Rouge for the first time (see Table 3.2.19). By contrast, nine new compounds were identified in Concord for the first time. Component 173 was identified as 3-methyltetrahydrothiophene based on the following mass spectral data. Accurate mass measurements on the ions in the EI mass spectrum of 173 (see Figure 3.4.2) gave the following elemental composition:

m/z	102	101	87	74	60	47	45
% RI	36	5	100	35	42	29	33
Composition	$C_5H_{10}S$	C_5H_9S	C_4H_7S	C_3H_6S	C_2H_4S	CH_3S	CHS

An M+2 ion at m/z 104 of relative intensity of 4% suggested the presence of a Sulphur atom in the compound of molecular weight 102 confirmed by CH_4 and Isobutane Chemical ionization mass spectrometry. The identity was confirmed with an authentic sample of 3-methyltetrahydrothiophene

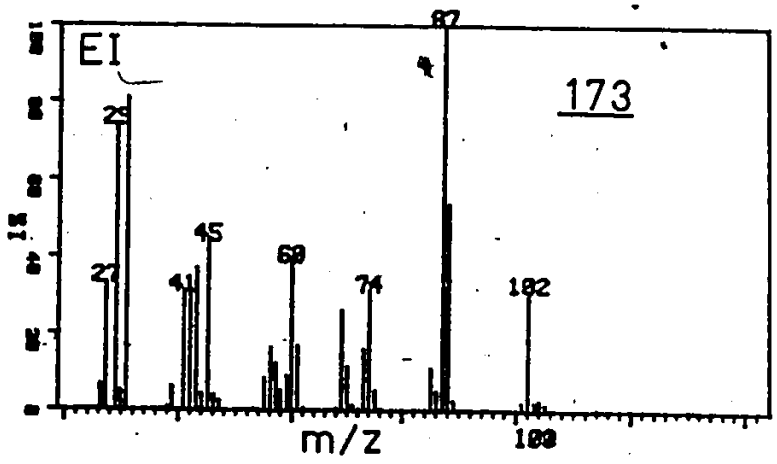
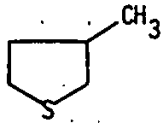
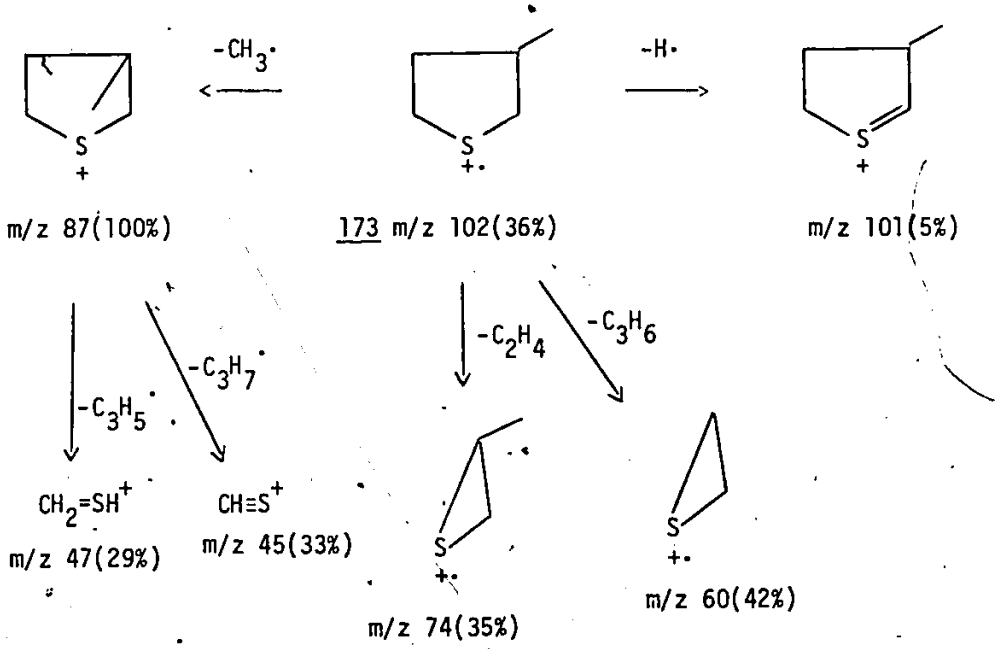


Figure 3.4.2. EI mass spectrum of 3-methyltetrahydrothiophene 173



which gave identical mass spectrum and retention index on the C20M and SE-54 columns. The fragmentation pattern for this compound is rationalized as shown in Scheme 3.4.



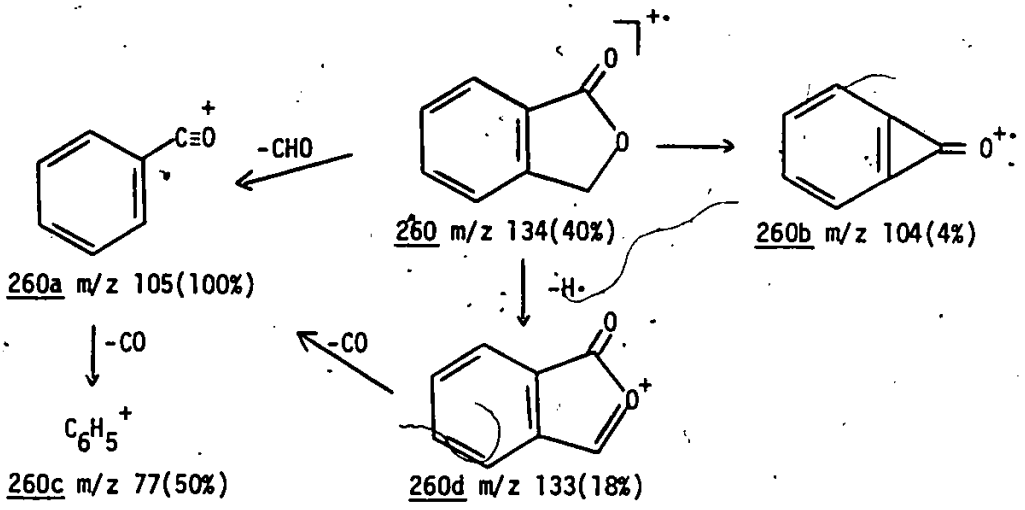
Scheme 3.4. Postulated mechanism for the EI fragmentation pattern of 3-methyltetrahydrothiophene, 173.

The thiol compound, 3-methylthiopropanol 155 was detected in Concord but not in Moulin Rouge. There was no evidence in the literature that this compound has been reported as a flavor component of Concord. It is therefore being reported here for the first time as a flavor component of Concord. N-(3-methylthiopropyl)acetamide 189 was detected in Moulin Rouge but not in Concord. Two benzofuranone compounds were detected and identified. Phthalide [1-(3H)-Isobenzofuranone] 260

was detected in both Moulin Rouge and Concord while dihydrobenzofuranone 262 was detected in Concord only. The EI and CI mass spectra of 260 are shown in Figure 3.4.3. Accurate mass measurements of the ions in the EI mass spectrum of 260 gave the following composition:

m/z	136	133	105	104	77
composition	$C_8H_6O_2$	$C_8H_5O_2$	C_7H_5O	C_7H_4O	C_6H_5

It appears that the molecular ion of 260, confirmed by isobutane chemical ionisation mass spectrometry (see Figure 3.4.3) looses a formyl radical upon electron impact to form the highly stabilized benzoyl ion 260a, the base peak in the spectrum. 260a may loose a molecule of CO to form 260c. 260 may also form 260b through the loss of a molecule of formaldehyde. The fragmentation pattern of Phthalide may be adequately explained by the mechanism postulated in Scheme 3:5.



Scheme 3.5. Postulated mechanism for the EI mass spectral fragmentation pattern of Phthalide, 260.

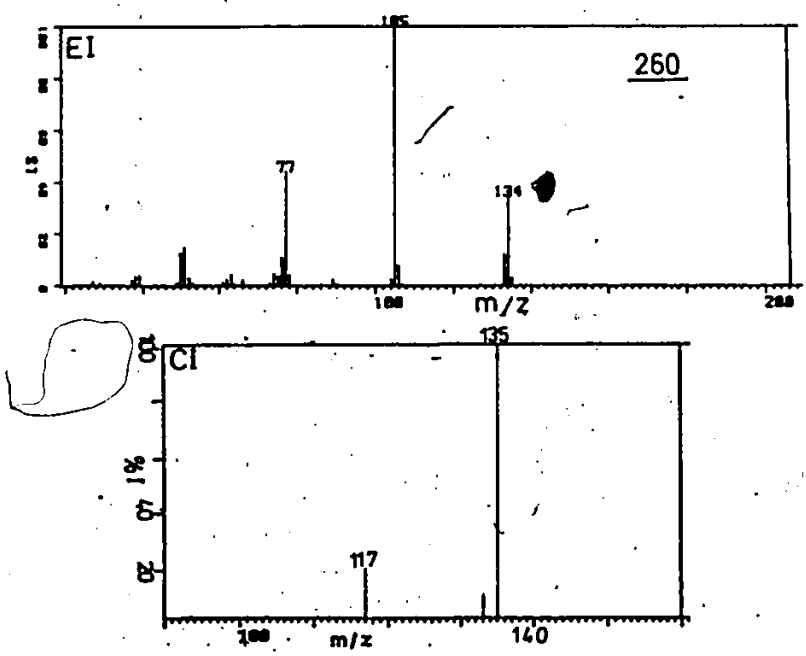
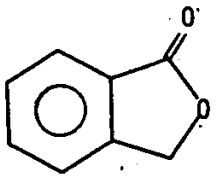


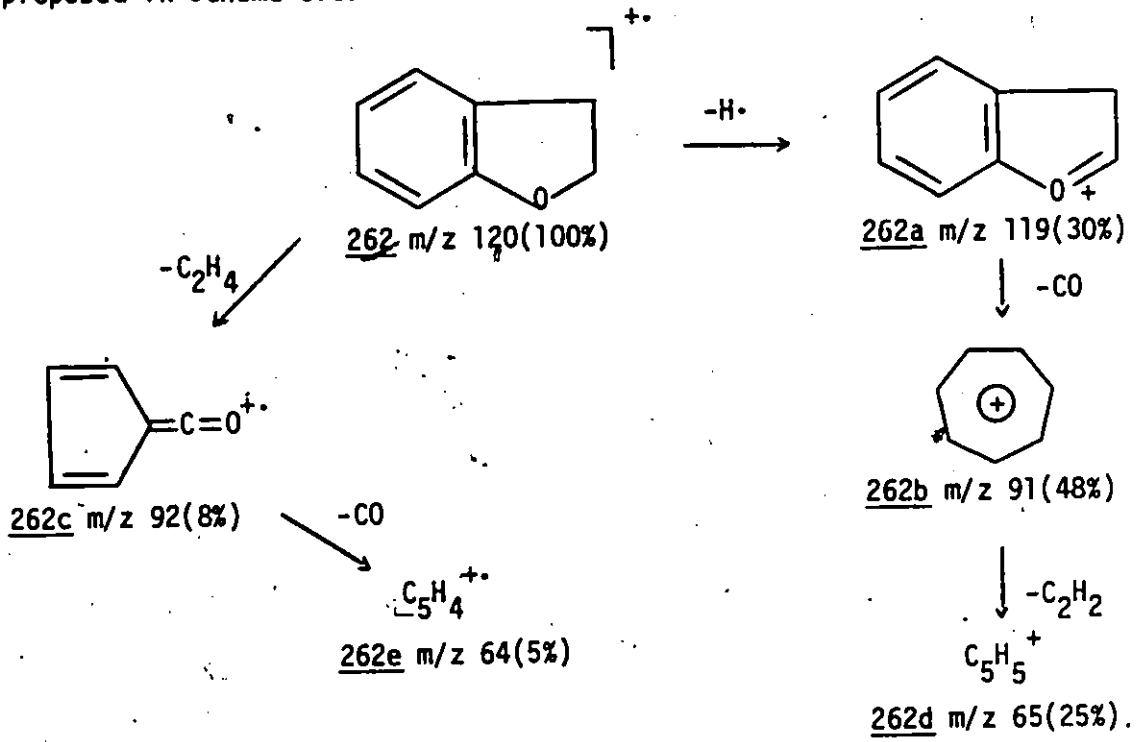
Figure 3.4.3. EI and CI mass spectra of Phthalide 260.



Accurate mass measurements gave the following composition for the ions in the EI mass spectrum of dihydrobenzofuran 262 (see Figure 3.4.4).

m/z	120	119	92	91	65
composition	C_8H_8O	C_8H_7O	C_6H_4O	C_7H_7	C_5H_5

The molecular ion of 262 was confirmed by isobutane CIMS to be m/z = 120 (see Figure 3.4.4). This presumably looses either a molecule of ethylene to form 262c which may in turn loose a molecule of CO to form the ion 262e of m/z = 64, or a hydrogen radical to form 262a which subsequently looses CO to form 262b. It is postulated that the EI fragmentation pattern of 262 may be adequately explained by the mechanism proposed in Scheme 3.6.



Scheme 3.6. Postulated mechanism for the EI mass spectral fragmentation pattern of dihydrobenzofuran, 262.

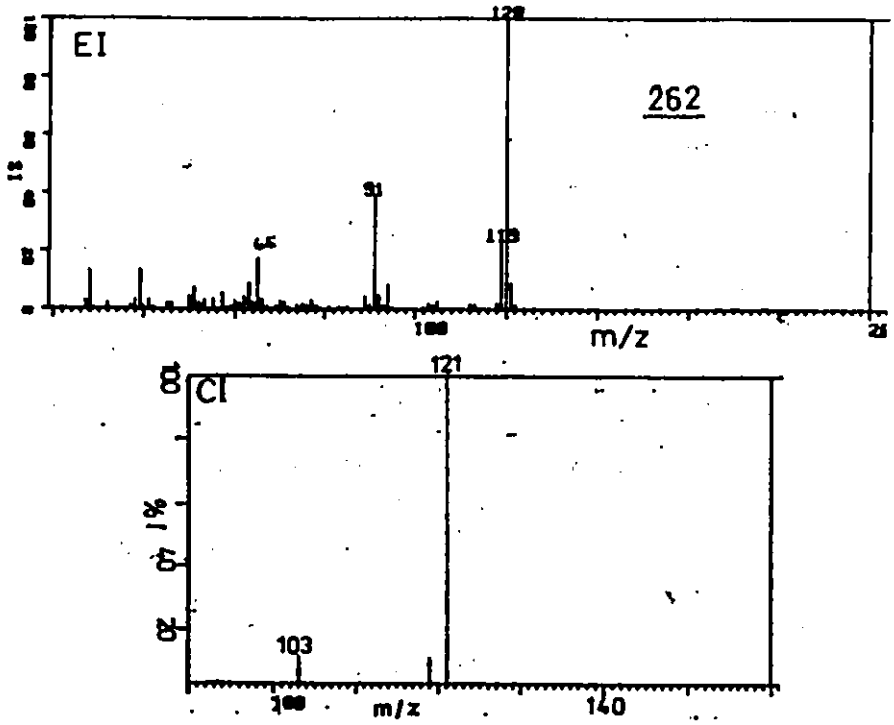


Figure 3.4.4. EI and CI mass spectra of dihydrobenzofuran 262

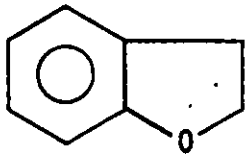


Figure 3.4.5 illustrates the EI and CI mass spectra of component 124 positively identified in Concord for the first time as 2,5-dimethyl-4-methoxy-3(2H)furanone. This compound was perceived to have a potent herbacious aroma with a sweet candy-like undernote. It has been previously identified as a flavor component of strawberries.[252] The molecular ion at m/z 142 was confirmed by isobutane CIMS and shown by accurate mass measurements to have a composition, $C_7H_{10}O_3$. The ion at m/z 127 of composition $C_6H_7O_3$, is formed by the loss of a methyl radical from the molecular ion. The ion at m/z 99 determined to have a composition of $C_5H_7O_2$, is probably formed by a loss of an acetyl radical from the molecular ion or a molecule of CO from the ion at m/z 127. The base peak in the EI mass spectrum of 124 of composition, C_2H_3O, may be due to the acetyl ion, CH_3CO^+ and/or the protonated ketene $CH_2{=}CH{=}OH^+$. The other prominent ions in the mass spectrum at m/z 69, 71 and 112 were shown by accurate mass measurements to have been formed from the molecular ion by losses of $C_3H_5O_2$, $C_3H_3O_2$, and CH_2O respectively. The EI fragmentation pattern of 2,5-dimethyl-4-methoxy-3(2H)furanone may be explained by the scheme postulated in Scheme 3.7.

Figure 3.4.6 shows the EI and CI mass spectra of 2,5-dimethyl-4-hydroxy-3(2H)furanone, 214 positively identified as a flavor component in Concord for the first time. This compound was perceived to have a caramel-like aroma varying from sweet caramel to a fruity character at low concentrations in 10% ethanol and, a burnt-sugar aroma at high concentrations. This compound was previously identified in beef broth,[253] pineapples and strawberries.[254] The molecular ion at m/z 128 was determined to have a composition of $C_6H_8O_3$. The base peak in the spectrum

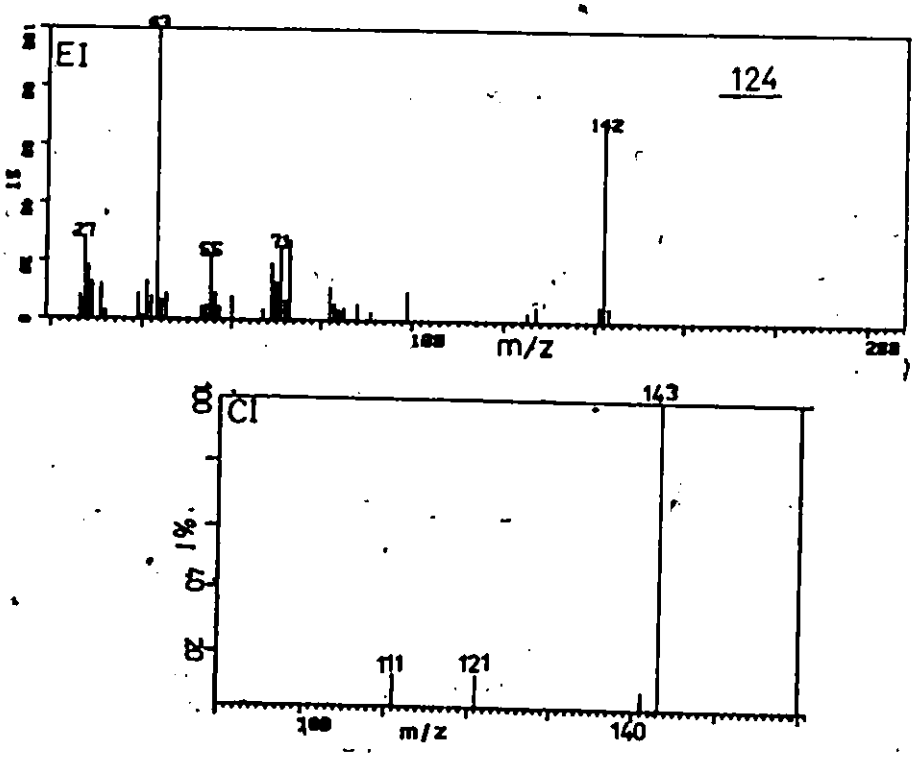
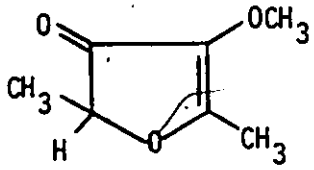


Figure 3.4.5. EI and CI mass spectra of 2,5-dimethyl-4-methoxy-3(2H)furanone 124



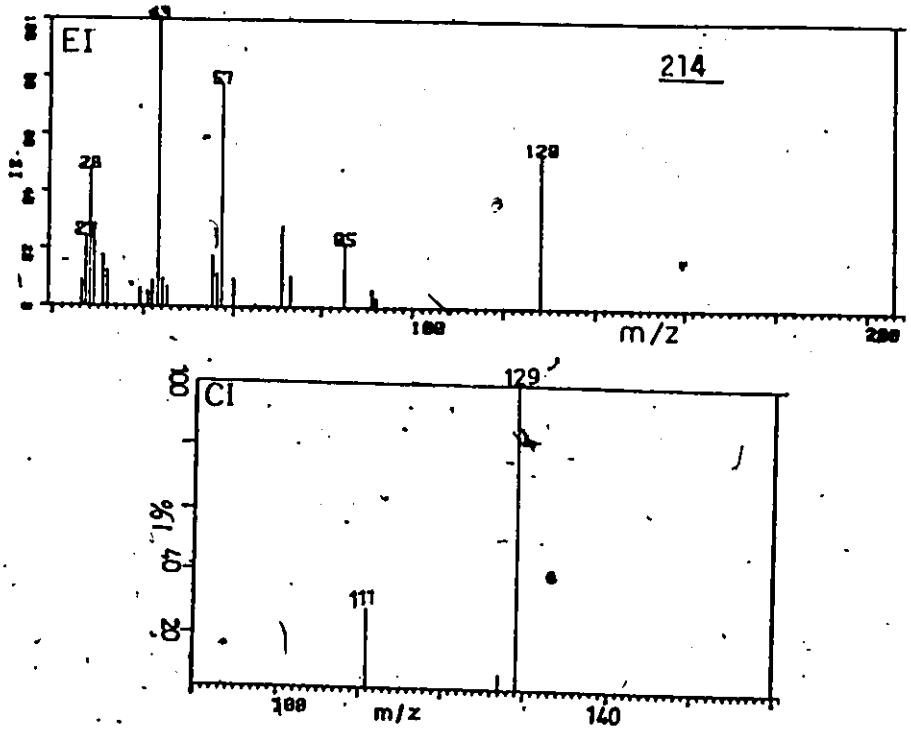
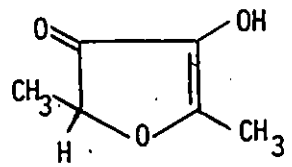
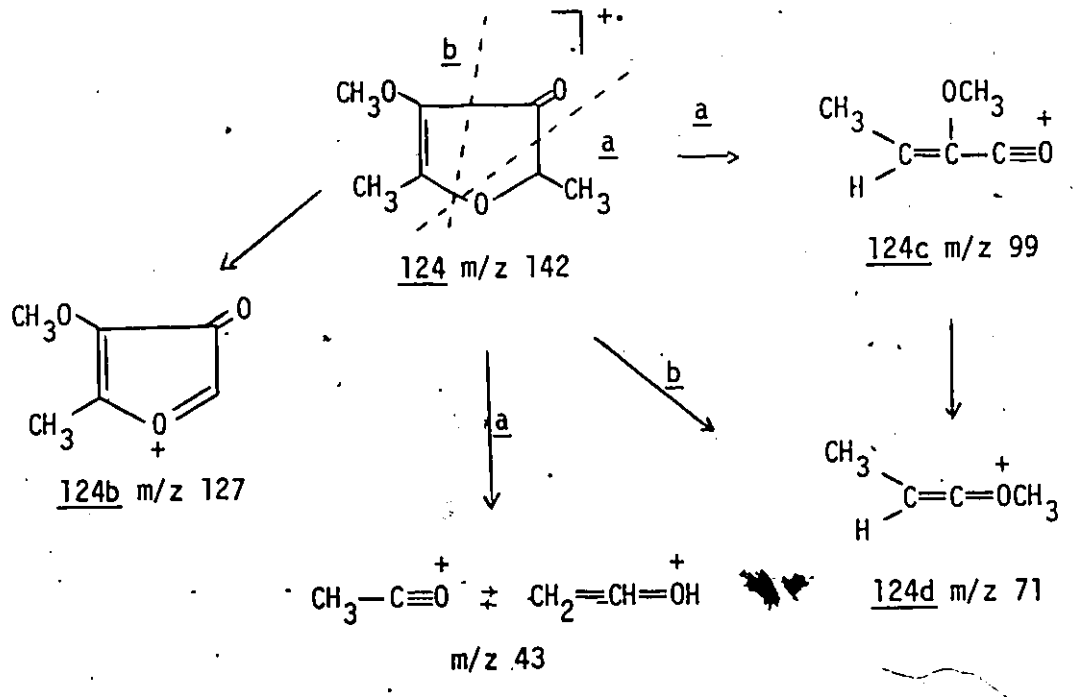


Figure 3.4.6. EI and CI mass spectra of 2,5-dimethyl-4-hydroxy-3(2H)furanone 214





Scheme 3.7. Postulated mechanism for the EI mass spectral fragmentation pattern of 2,5-dimethyl-4-methoxy-3(2H)furanone, 124.

was found to have composition C_2H_3O due to the acetyl ion, CH_3CO^+. The ion at m/z 85 of composition $C_4H_5O_2$ is probably formed by the loss of an acetyl radical from the molecular ion. The EI fragmentation pattern of component 213 may be explained according to the scheme presented in Scheme 3.8. Isophorone(3,5,5-trimethyl-2-cyclohexen-1-one) 125 was also positively identified in Concord. It was not detected in Moulin Rouge. It is being reported as a flavor component of Concord for the first time. This compound was previously identified in cranberries,[255] roasted filberts,[256] saffron and mushroom.[257] Figure 3.4.7 shows the EI mass spectrum of isophorone 125. 2-Hydroxybenzothiazole 304 is the only new compound being reported in this study that was found common

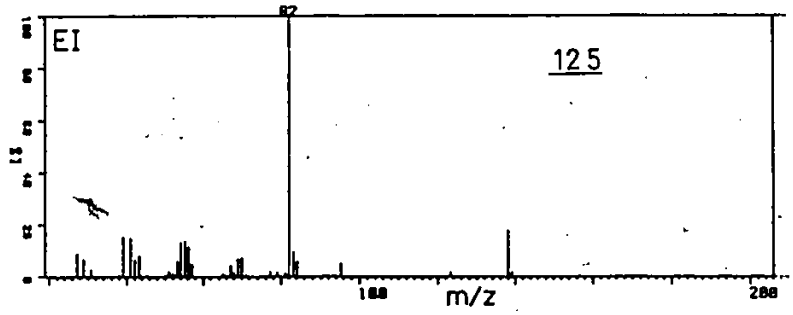
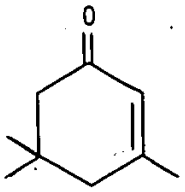
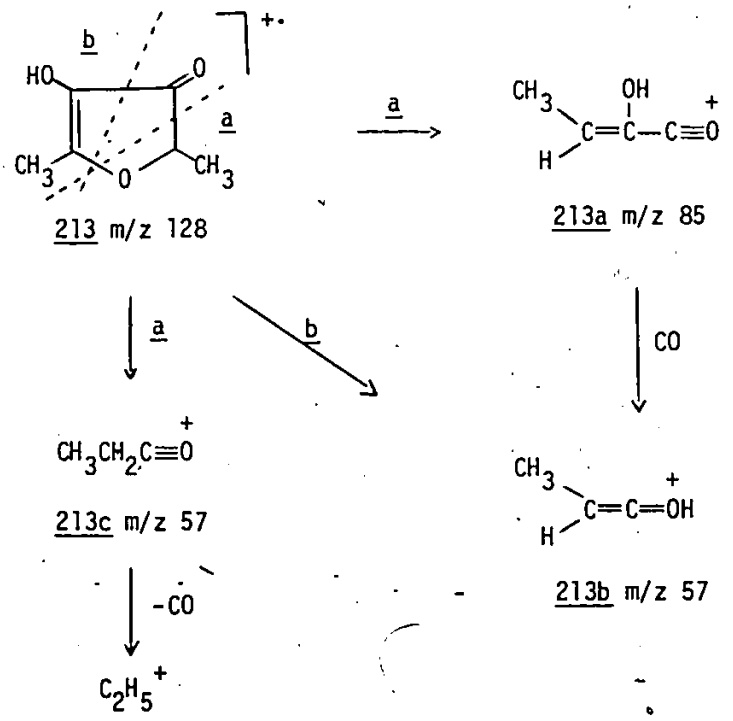


Figure 3.4.7. EI mass spectrum of isophorone 125





Scheme 3.8. Postulated mechanism for the EI mass spectral fragmentation pattern for 2,5-dimethyl-4-hydroxy-3(2H)furanone 213.

to Cabernet Sauvignon, Concord and Moulin Rouge. The mass spectrum of this compound has already been presented in Figure 3.3.2. α-Naphthol 311 was positively identified in Concord for the first time. Like phthalide 260, this compound has been previously identified by Schreier et al[249] in Rülander, Traminer and Scheurebe. The EI mass spectrum of α-naphthol is shown in Figure 3.4.8. Qualitatively, it may be concluded that the volatile flavor composition of Cabernet Sauvignon is far more complex than those of Concord and Moulin Rouge. Between Concord and Moulin Rouge it appears that most of the components that were found to be present in both wines were essentially the major components. These components that were detected in one wine and not in the other were

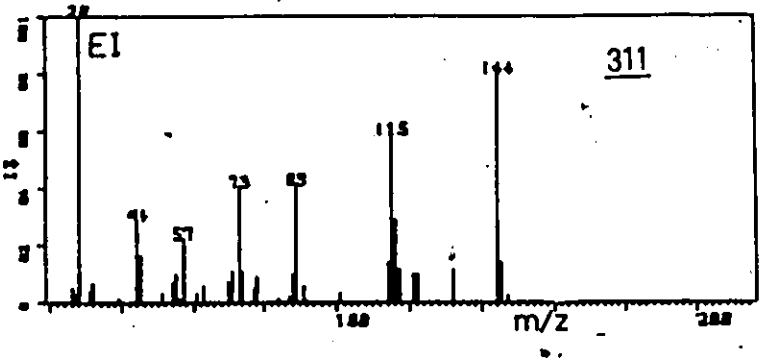
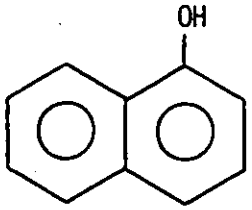


Figure 3.4.8. EI mass spectrum of α-naphthol 311



usually those present at relatively low concentrations. It may therefore be concluded from these observations that the distribution of major volatile flavor components of Concord and Moulin Rouge is similar and that distinct differences in the aroma, flavor and quality of these wines may be due to significant variation in the composition of minor and trace level components. This conclusion agrees with those made in a previous study conducted in this laboratory on Concord and Blue hybrid wines.[36]

This study in which 119 components in Concord and 126 in Moulin Rouge were identified in one single analysis is the most sensitive of any reported studies in the literature thus far on the volatile flavor composition of native North American varieties. Compare with 35 compounds identified in Concord and Blue Hybrid earlier in this laboratory.[36] The results of this study also conclusively demonstrates that the 21 hydrocarbons previously identified as flavor components of Concord,[51] none of which were detected and identified in this analysis, could not have been original components of Concord essence but merely artefacts in the extracting solvent. The results of this study should improve substantially the characterization and understanding of the volatile flavor composition of vitis labruscana grape musts and wines. New components that were identified for the first time in these varieties ought to be taken into account in any experiments that purport to formulate or control the flavor of these wines.

Possibly, the results of this study may be useful to vintners, viticulturalists and enologists in Canada and the Eastern United States.

The next section presents the results and discussion of the

experiments conducted to provide data to meet the principal objective of this analysis - namely, the detection and identification of those chemical compound(s), other than methyl anthranilate (Benzoic acid, 2-amino, methyl ester) 243 currently suspected to be responsible for the characteristic labrusca flavor. Figure 3.4.9 shows the EI mass spectrum of component 243. Table 3.2.20 summarizes the results of the experiments conducted on the reconstitution of several wines, after isolating their volatile flavor fractions. Results of these experiments indicate that reconstitution of all wines was impossible except for those involving Pichon Lalande (a vitis vinifera variety). None of the isolated volatile flavor essence of Labrusca-based wines would form a homogeneous solution with their own or other labrusca wine bases. The volatile flavor extract of Pichon Lalande however, redissolved readily in the labrusca wine bases to give a product reminiscent of the original Pichon Lalande flavor and without the slightest note of the labrusca flavor. It became obvious from these experiments that (i) the isolation-concentration procedure used for Pichon Lalande (vitis vinifera variety) produces a flavor fraction that is compatible with its wine base and even those of labrusca wines. (ii) The same isolation-concentration procedure when used for labrusca and hybrid varieties appeared to produce two incompatible fractions thus making reconstitution impossible. The burnt flavor associated with the mixture of the two incompatible labrusca fractions may be ascribed to a build up of lactones, since lactones are generally held responsible for the aroma of smoked meats. It is also conceivable that unlike their vinifera counterparts, the volatile flavor composition of vitis labruscana wines may consist of easily oxidizable components

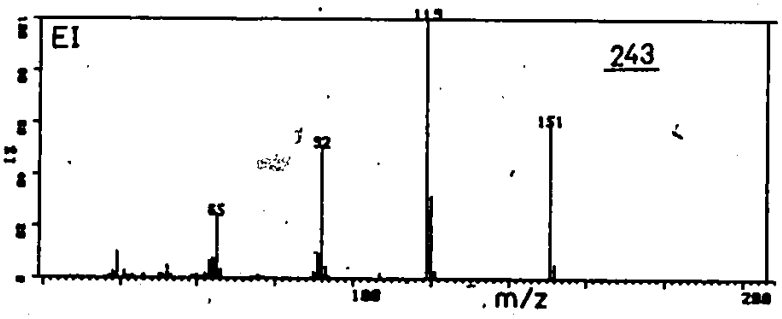


Figure 3.4.9. EI mass spectrum of methyl anthranilate 243

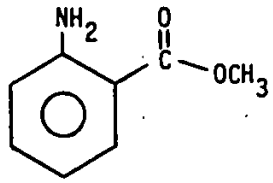


Table 3.2.20

Experiment to reconstitute several wines after isolating their volatile flavor fractions

Volatile flavor fraction (A)	Wine base (B)	Observation after adding (A) to (B)
20 µl Concord	1. 150 ml Concord 2. 150 ml Similkameen Red 3. 150 ml Moulin Rouge 4. 150 ml Pichon Lalande	(a) Surface film (kerosene-like formed (b) Non-homogeneous mixture results (c) Strong burned odor develops
20 µl Similkameen Red	1. 150 ml Concord 2. 150 ml Similkameen Red 3. 150 ml Moulin Rouge 4. 150 ml Pichon Lalande	(a), (b), (c)
20 µl Moulin Rouge	1. 150 ml Concord 2. 150 ml Similkameen Red 3. 150 ml Moulin Rouge 4. 150 ml Pichon Lalande	(a), (b), (c)
20 µl Pichon Lalande	1. 150 ml Concord 2. 150 ml Similkameen Red 3. 150 ml Moulin Rouge 4. 150 ml Pichon Lalande	(i) Homogeneous solution formed (ii) Flavor of Pichon Lalande regenerated (iii) Mixture had no detectable labrusca flavor
100 µl of Freon-11	1. 150 ml Concord 2. 150 ml Similkameen Red 3. 150 ml Moulin Rouge 4. 150 ml Pichon Lalande	A. Two distinct liquid phases observed B. Mixture retains the bland aroma of wine base

whose nature could very easily be altered by the choice of the isolation method. Also, repetitive isolation-concentration of labrusca wines yielded flavor extracts of varying golden hue. These suspicions and observations led to the investigation of a solvent extraction procedure for labrusca and hybrid varieties conducted under a controlled inert atmosphere condition (i.e., a modified version of the method used to isolate v. vinifera volatile flavor fraction). When the experiments described in Table 3.2.20 were repeated using the modified isolation-concentration method, it was succeeded for the first time in reconstituting labrusca and hybrid wines. This finding is quite significant and must be taken into account in any analysis that purports to isolate the volatile flavor fraction of labrusca and hybrid grape musts and wines. This modified solvent extraction-concentration procedure that was shown to preserve the nature and composition of the flavor extract was used in all experiments involving labrusca varieties.

Once a satisfactory method was developed for reconstituting labrusca wines, it was decided to conduct the experiments detailed in Chapter 2 and summarized in Table 3.3.21 to evaluate the role of methyl anthranilate in the total flavor picture of these varieties. Figure 3.4.10 is a gas chromatogram of a simplified standard volatile flavor mixture obtained on a 6'x1/4" O.D. glass C20M column that pictorially represents the experiments summarized in Table 3.2.21. The results of these experiments show that the resulting mixture obtained under II, III and V gave products that could be described as typically labrusca. I showed no noticeable labrusca character whilst IV had only a faint almost unnoticeable labrusca character. These experiments demonstrate

Table 3.2.21

Experiments conducted to evaluate the significance of Methyl Anthranilate (Benzoic acid, 2-amino, methyl ester) 243 to the total aroma of v. labrusca and hybrid wines*

wine base aliquot designation	Total of 20 l of flavor extract injected and trapped into 150 ml of cooled wine base (fraction collected)	Flavor description of resulting solution
I	All components eluting up to and including 2-Phenylethyl alcohol	No discernible labrusca flavor-mainly roses
II	All components eluting up to but excluding methyl anthranilate	Definite labrusca flavor
III	All components eluting from Phenylethyl alcohol (inclusive) up to but excluding methyl anthranilate	Characteristic labrusca flavor exemplified
IV	All components eluting from methyl anthranilate (inclusive) and thereafter	Faint to almost imperceptible labrusca flavor
V	All components eluting excepting freon solvent	Labrusca flavor retained

* Wines tested included Vidal, Dutchess, Moulin Rouge, Elvira and Concord.

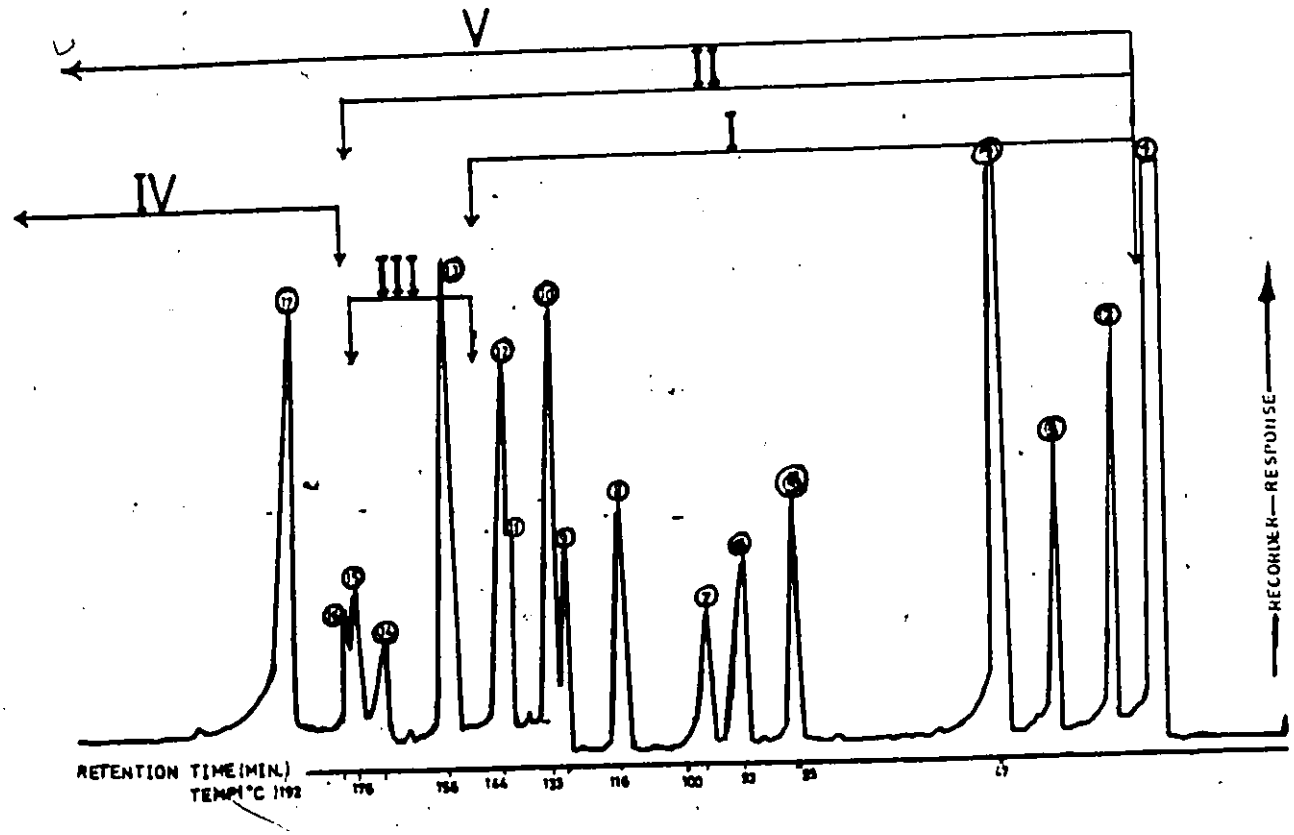


Figure 3.4.10. Gas chromatogram of a simplified standard volatile flavor mixture depicting the experiments conducted to evaluate the role of methyl anthranilate in the total aroma picture of v. labrusca and hybrid wines.
1. Freon 2. Isoamyl acetate 3. Ethyl hexanoate 4. Ethyl lactate + hexanol 5. Ethyl octanoate 6. γ-Butyrolactone 7. 1-Octanol 8. Diethyl succinate 9. Ethyl decanoate 10. 2-Phenethyl acetate 11. Hexanoic acid 12. 2-Phenethanol 13. trans-cinnamaldehyde 14. Octanoic acid 16. Methyl anthranilate 17. Decanoic acid.

that the labrusca character is exemplified by components other than methyl anthranilate. Notably, the fraction of components eluting between 2-phenylethyl alcohol and octanoic acid (III) up to but excluding methyl anthranilate. This observation affirms the suspicions of other previous investigators of the flavor composition of these labrusca varieties. Any further examination of organoleptically significant volatile flavor components of labrusca wine was thereby confined to region III.

A series of analysis was therefore conducted in which grape musts and wines of Concord, Moulin Rouge, Elvira, Dutchess, Vidal and Similkameen were examined. After simplification of the flavor extract by removal of the fusel alcohols and fatty acids, a component peak 223 was detected in all of these varieties using the modified isolation-concentration procedure. Its presence could however not be detected in those experiments using the same isolation-concentration procedure used for the v. vinifera varieties. It is relevant to mention at this point that Elvira, Dutchess and Vidal showed no detectable concentrations of methyl anthranilate.

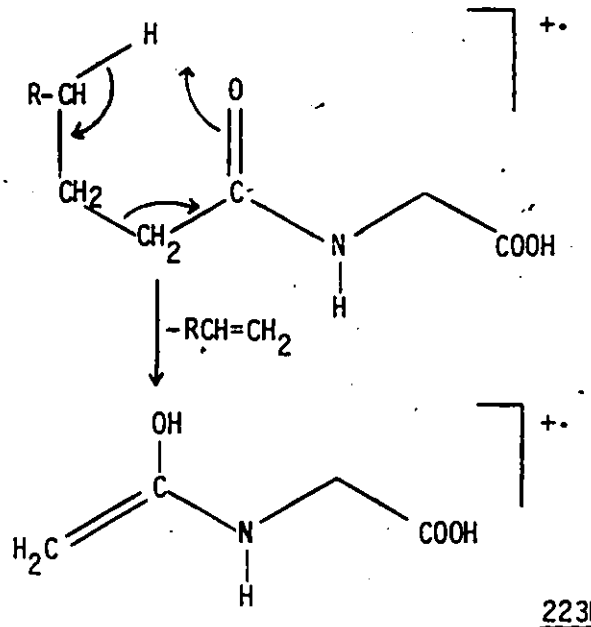
The EI mass spectrum of 223 gave the following fragment ions whose composition were determined by low resolution accurate mass measurements:

m/z	30	43	71	72	75	89	99
% RI	85	40	100	18	18	37	8
Composition	CH_4N	C_3H_7	C_3H_5NO C_4H_7O	C_3H_6NO	$C_2H_5NO_2$	$C_3H_7NO_2$	$C_4H_5NO_2$

m/z	117	145
% RI	80	8
Composition	$C_4H_7NO_3$	$C_6H_{11}NO_3$

A pressure-dependent peak at m/z = 191 was observed under EI conditions. The same mass spectrum was obtained when the components of the flavor extracts were chromatographically resolved on an SE-54 column, thus establishing the purity of the mass spectrum of the compound 223. Isobutane-CIMS established the molecular weight of this component as 190. As a nitrogen containing compound (also confirmed by dual-detection techniques - NPD/FID) 223 must contain an even number of Nitrogens.

The presence of the intense peak at m/z = 117 suggested that this fragment ion was probably formed by a β-cleavage with gamma hydrogen rearrangement in the acyl moiety of the precursor ion (I), a scheme which is only possible with the presence of at least a butyryl group.

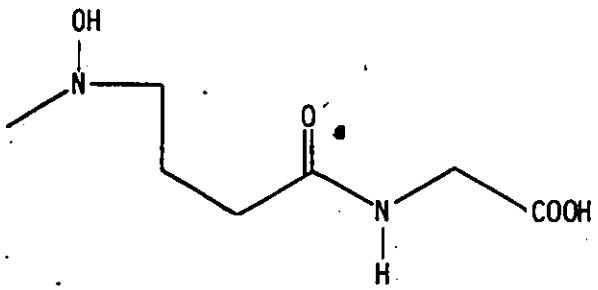


(I)

223b m/z 117

The composition of the fragment ion at m/z = 145 suggests that R is equivalent to a hydrogen atom, thus establishing the identity of the precursor ion I as the N-butyryglycine molecular ion. The rest of the molecule must be accounted for by a fragment of mass 45 with the probable composition of CH_3NO. The loss of mass 45 from the molecular ion to give the ion $C_6H_{11}NO_3^{+\cdot}$ at m/z = 145 readily, appears to suggest the presence of a labile hydrogen on this end of the molecule that is readily transferred to form the ion I. It was therefore postulated that the best arrangement of this end of the molecule would be an N-substituted methyl, N-substituted hydroxy group, where the hydrogen of the hydroxyl group is easily lost through a four-centred bond cleavage mechanism to form the fragment ion I. Compound 223 is then tentatively assigned to structure II.

(II)



As a prelude to the substantiation of the chemical identity of the postulated molecule II, a series of N-acylglycine compounds were synthesized according to the method of Vernstern and Moore.[251] Figures 3.4.11 and 3.4.12 illustrate the mass spectra obtained for the synthesized N-Butyrylglycine (mol. wt. = 145) and N-Hexanoyl glycine (mol. wt. = 173). These mass spectra compared quite well with those obtained for component 223. Table 3.2.22 shows the high resolution data obtained on the synthesized compounds. The elemental composition assigned to fragment

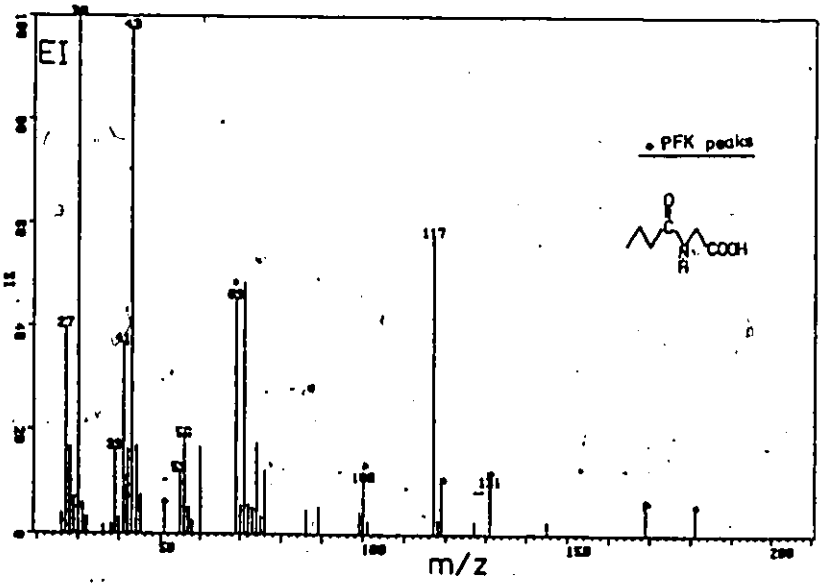


Figure 3.4.11. EI mass spectrum of N-Butyrylglycine.

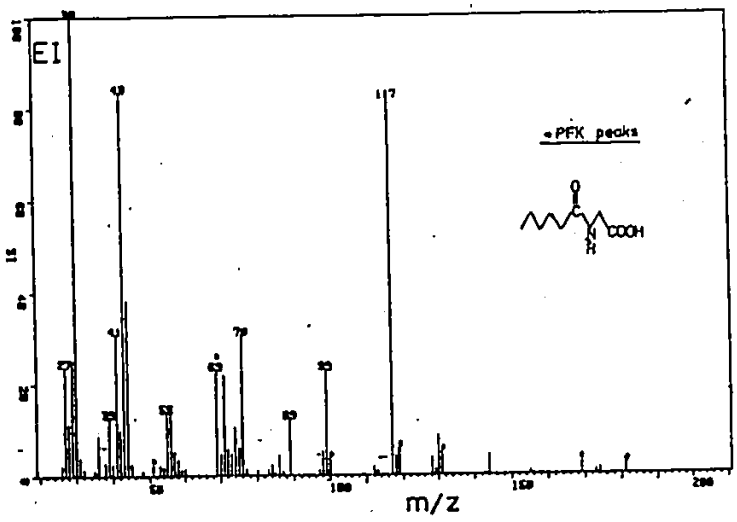


Figure 3.4.12. EI mass spectrum of N-Hexanoylglycine.

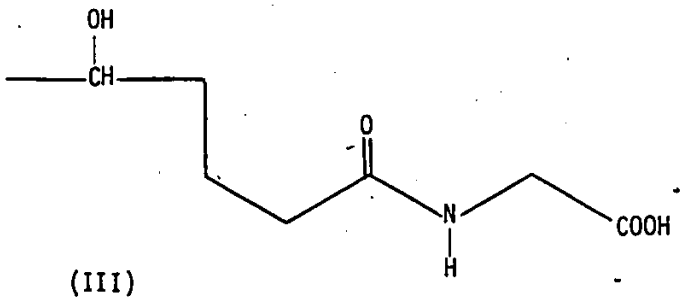
Table 3.2.22

High resolution mass spectral data on the ions of N-acylglycines

Calculated mass	Observed mass	Elemental Composition
	N-Butyrylglycine	
145.0705	145.0708	$C_6H_{11}NO_3$
117.0393	117.0391	$C_4H_7NO_3$
71.0495	71.0491	C_4H_7O
71.0339	71.0338	C_3H_5NO
43.0456	43.0458	C_3H_7
30.0312	30.0316	CH_4N
	N-Hexanoylglycine	
173.1017	173.1019	$C_8H_{15}NO_3$
144.0627	144.0619	$C_6H_{10}NO_3$
117.0393	117.0389	$C_4H_7NO_3$
99.0288	99.0290	$C_4H_5NO_2$
71.0339	71.0341	C_3H_5NO
71.0495	71.0498	C_4H_7O
43.0456	43.0459	C_3H_7
30.0312	30.0313	CH_4N

ions in these compounds under high resolution MS techniques parallel those obtained for 223 under low resolution accurate mass measurement conditions.

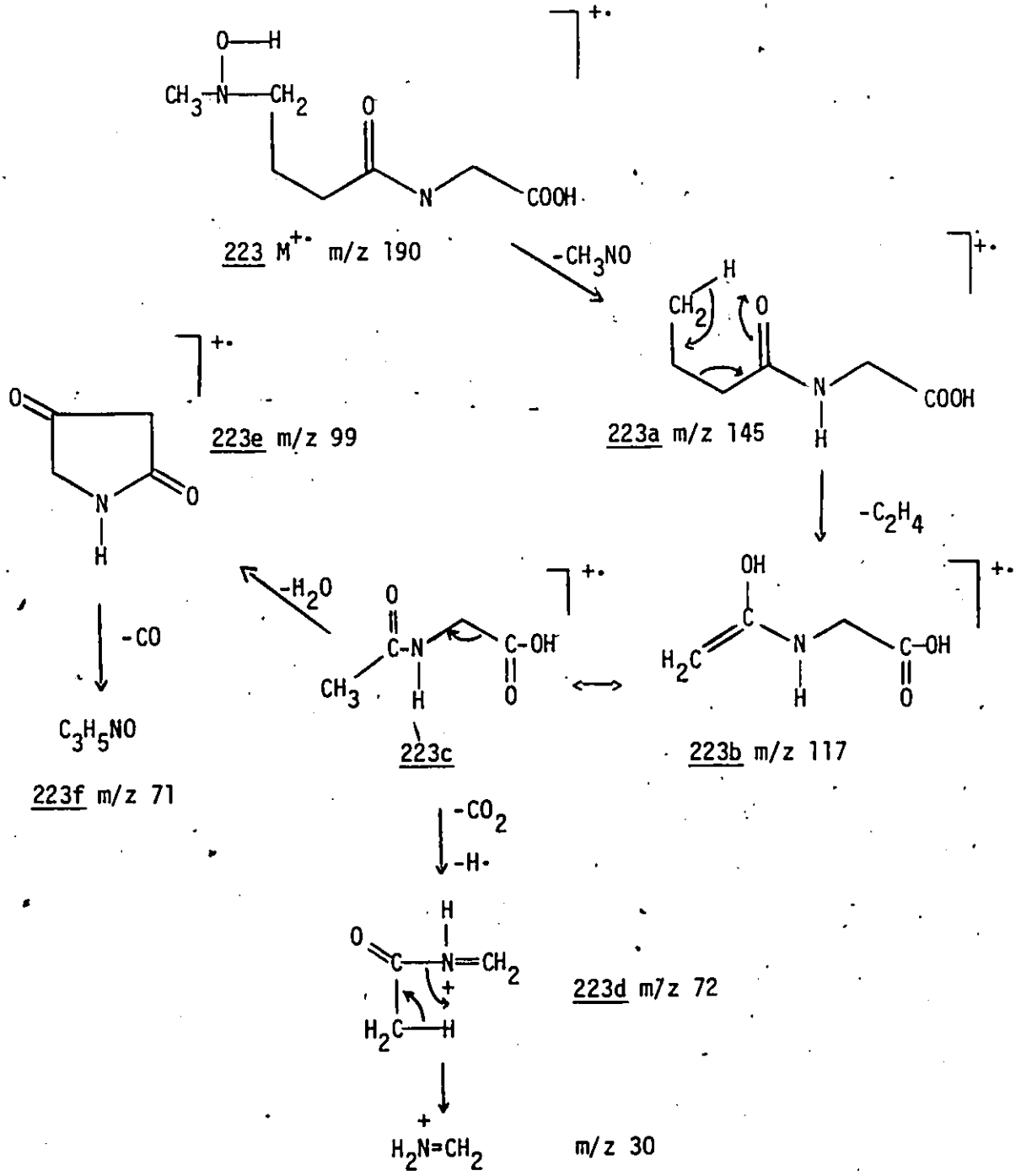
It may be concluded that the proposal of the presence of a N-butyryglycine moiety in compound II is justified. Alcoholic solutions of these N-butyryglycine moiety in compound II is justified. Alcoholic solutions of these N-acylglycine compounds did not possess any significant organoleptic properties. Compound III was synthesised from 5-ketohexanoic acid and glycine after reduction of the keto group to the alcohol. The mass spectrum of this product showed similar fragmentation pattern except that a highly intense peak was observable at m/z = 45 which is hardly noticeable in the other compounds; probably due to the CH_3CHOH^+ ion. In addition, alcohol solutions of this compound had a flavor quite reminiscent of the vanilla flavor.



(III)

The substitution of a γ-hydrogen atom in N-butyrylglycine or a δ-methylene group in N-hexanoyl glycine imparts flavor characteristics to an otherwise bland compound. It is possible that the substitution of a (N) instead of a (C-H) group in the molecule III may lead to a flavor modification of that which is anticipated. Research is still in progress in an attempt to synthesize completely compound 223

believed to be the most significant aroma contributing component in labrusca and hybrid musts and wines. The mass spectral fragmentation pattern of 223 may be rationalized as shown in Scheme 3.9.



Scheme 3.9. Postulated mechanism for the EI mass spectral fragmentation pattern of N-(N-methyl,N-hydroxy-γ-aminobutyryl)glycine, 223.

It has been demonstrated vividly in this thesis that other flavor components, yet unidentified, and present in labrusca wines at trace level concentrations may be responsible for the typical labrusca flavor of these varieties. It is being postulated in this thesis that a compound detected as a trace compound in these varieties and tentatively identified as N-(N-methyl, N-hydroxy-γ-aminobutyryl)glycine, may be primarily responsible for this characteristic labrusca flavor. It has also been demonstrated that unlike their vinifera counterparts the isolation-concentration procedures for native North American varieties must be conducted under controlled inert atmosphere conditions. The results of the quantitative analysis on the volatile flavor extracts of Moulin Rouge and Concord are presented in Section 3.4.

3.3.3 Qualitative Analysis of the Volatile Flavor Components of Grape Must and Wine made from the Grapes of Locally Cultivated Vitis Vinifera Vine, White Riesling

The failure to produce the anticipated high quality and/or great wines from native North American grape varieties and their hybrids has presumably swayed the direction of viticultural and vinification practices in Canada and the Eastern United States towards the cultivation of v. vinifera vines locally for wine making instead of importing v. vinifera grapes or concentrates from Europe. The objective of this experiment was to analyse the volatile flavor composition of White Riesling musts and wine to determine if any significant changes may have occurred in the qualitative and quantitative distribution of flavor compounds as a result of the local climatic and agrotechnological

conditions. This section presents the results of the analysis of the volatile flavor components of the grape must, freshly fermenting must, and young wine of the vitis vinifera variety, White Riesling. Table 3.2.16 summarizes the number of components identified in White Riesling. Five esters were detected and identified in the grape must. All the other esters were detected in the freshly fermented must and the young wine. This observation appears to confirm the conclusion that the esters are the end products of the fermentation process. A large number of carbonyl compounds was identified. With the exception of furfural 95, 5-methylfurfural 129, benzaldehyde 105, and trans-cinnamaldehyde 217 which were identified in only the freshly fermented must and the young wine, all other carbonyl compounds were present in all three phases of this analysis. This observation, confirms the conception that these named aldehydes are developed only under oxidative conditions.[61] The propylene glycol fraction of the concentrated volatile flavor extract contained several terpene alcohols, including geraniol 178, citronellol 164, α-terpineol 153, 4-terpineol 130, and nerol 170. These alcohols are believed to contribute significantly to the fruity aroma that characterizes this grape and its young wines. It is of interest to note that of the 81 hydrocarbons reported previously by Schreier et al as flavor components of White Riesling, only the following were detected and identified in this analysis: myercene 32, limonene 42, α-copaene 118, β-bourbonene 127, β-farnesene 143, β-caryophyllene 144, γ-muurolene 151, α-humulene 159, α-muurolene 166, and γ-cadinene. There was no evidence found in this study for the presence of C_{10}-C_{32} n-alkanes, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}-C_{32} n-alkenes, toluene, ethylbenzene,

meta, ortho and para xylenes, 3-alkylbenzenes, 5-alkylbenzenes, diphenyl and 3-methyldiphenyl as reported. It is suspected that these may have been contaminants of the extracting solvent rather than authentic flavor components. Table 3.2.23 shows the EI mass spectra of terpenes/sesquiterpenoids identified in White Riesling. The chemical structures of these compounds are presented in Figure 3.5.1. In general, the terpenes and components such as α-ionone 188, β-ionone 209, cis-linalool oxide 89, trans-linalool oxide 96, neroloxide 65, and cis-reseoxide 72, decreased in concentration from the must to the young wine (see Table 3.2.24). In contrast, compounds like TDN 93, theaspirane 270, and vitispirane 106 which were not detected in the must, were found to increase with bottle storage. It is pertinent to mention at this point that the aroma of this young wine was quite pleasant and had a considerably diminished fruitiness to it, much unlike the aroma of the grape must. It is conceivable that the observed decrease in the Riesling bouquet with bottle aging may be explained by decreases in the amounts of terpenoids which strongly influence the aroma of the must, and by an increase in the content of those volatile components like vitispirane 106, theaspirane 270, and TDN 93 believed to be formed by slow oxidative processes.[218,219] Figure 3.5.2 shows a reconstructed TIC chromatogram of a flavor extract of freshly fermented White Riesling grape must. The peak numbers in Figure 3.5.2 refer to the compound numbers used in Table 3.2.17 to 3.2.19. The results of this qualitative analysis of the volatile flavor components of the must, freshly fermented must and young wine demonstrate that the qualitative distribution of flavor components of White Riesling grape musts and wines made from locally

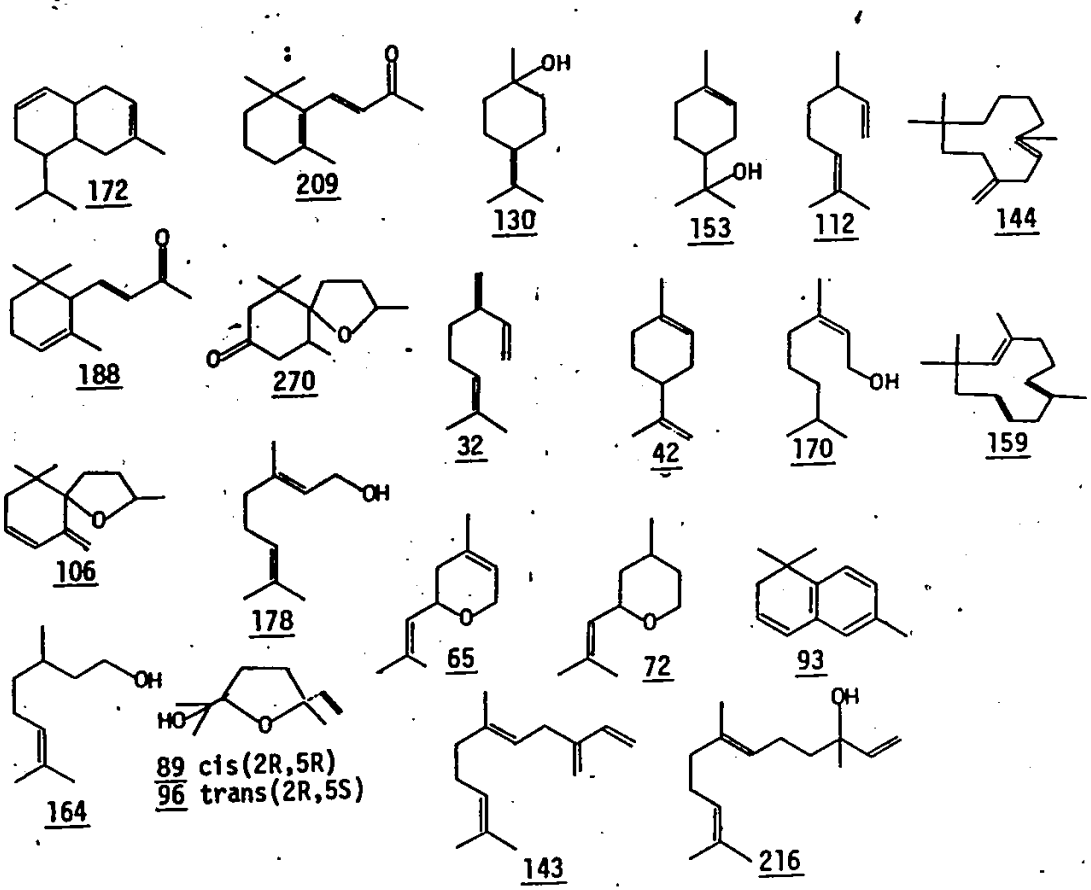


Figure 3.5.1. Terpenes and Sesquiterpenoids characterized in White Riesling wine made from grapes of locally cultivated vine. [Numbers refer to peak numbers in Table 3.2.16.]

Table 3.2.23

Mass spectral characteristics of some of the terpenoid flavor compounds identified in White Riesling

Compound reference number	Flavor compound	Mol. Wt. (% rel. int.)	m/z (% rel. intensity)
32	Myrcene	136(0.2)	41 93(96) 69(70) 91(25) 79(18)
65	Neroloxide	152	68 83(72) 67(66) 41(18) 55(13) 69(13)
99	Ho trienol	152(0.02)	71 82(70) 43(51) 67(48) 41(14)
118	α-Copaene	204	161 119(95) 105(83) 93(55) 81(25)
127	β-Bourbonene	204(0.05)	81 80(59) 123(52) 79(27) 161(25) 41(20) 91(17) 77(10)
143	β-Farnesene	204(1)	93 41(98) 69(76) 55(70) 107(44) 119(35)
144	β-Caryophyllene	204(0.4)	41 133(98) 93(92) 91(69) 69(69) 79(58) 105(42)
151	γ-Muurolene	204(29)	161 105(38) 93(35) 119(32) 91(30)
159	α-Humulene	204(23)	93 121(80) 80(45) 41(44) 53(17)
164	Citronellol	156(12)	69 82(60) 41(47) 67(45) 81(45) 95(39)
166	α-Muurolene	204(35)	105 161(65) 93(47) 81(27) 119(25)
172	γ-Cadinene	204(48)	161 134(60) 119(56) 105(50) 91(36)
178	Geraniol	154	169 41(95) 68(65) 93(49) 43(40) 55(35)
209	β-Ionone	192(25)	43 177(65) 134(40) 93(37) 95(35) 107(32)

..... continued

Table 3.2.23 (continued)

Compound reference number	Flavor compound	Mol. Wt. (% rel. int.)	m/z (% rel. intensity)
216	Nerolidol	204(2)	69 93(55) 61(54) 43(38) 107(25)
251	Dihydroactinidiolide	180(27)	111 109(60) 137(45) 67(30) 43(50) 69(15) 152(14)
270	Theaspirane	194	138 82(40) 96(22) 83(19) 55(14)

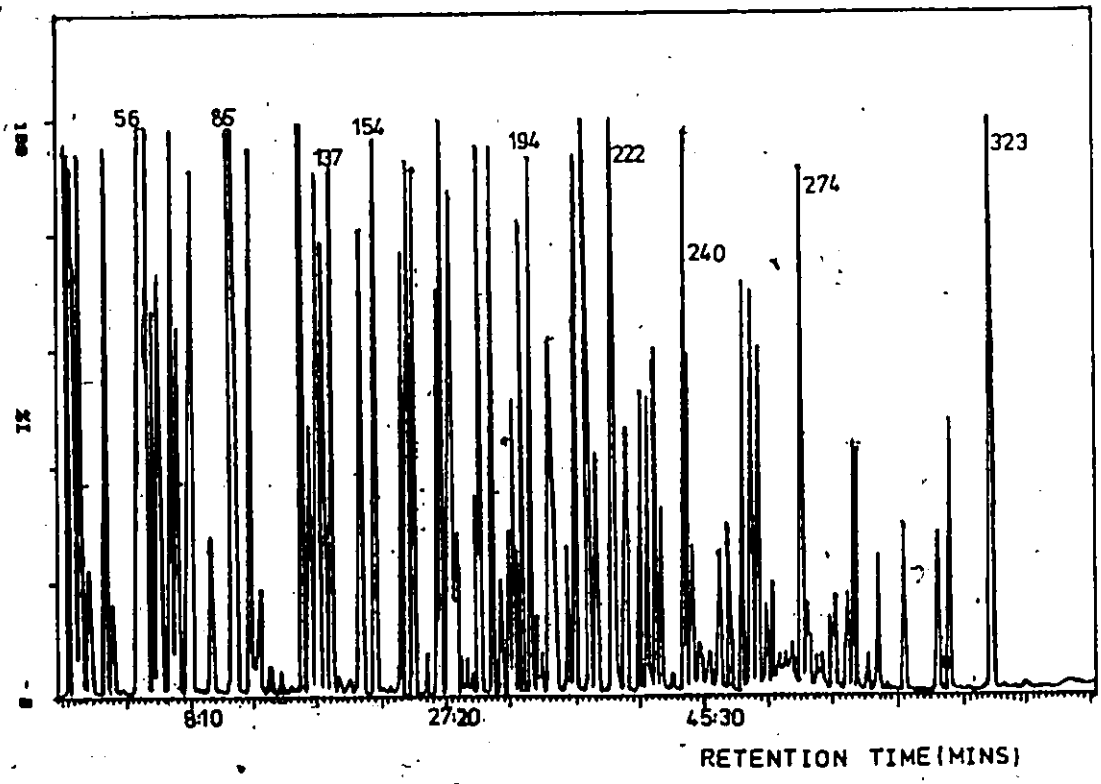


Figure 3.5.2. Reconstructed TIC chromatogram of a freshly fermented volatile flavor extract of White Riesling following solvent extraction and enrichment.

cultivated Riesling vines is very similar to those cultivated in the much cooler and more favorable European climate.[222,243-249]

3.4 Quantitative analysis of the volatile flavor components of the wines identified in this study

One of the objectives of this research project was to develop an analytical method capable of detecting and identifying organoleptically significant volatile flavor components of low odor thresholds present at trace level concentrations in wines, with the hope of identifying those flavor components responsible for the typical v. labruscana flavor. The results of the quantitative analysis conducted to determine the concentrations of the volatile flavor components identified in Cabernet Sauvignon, Concord, Moulin Rouge (a hybrid wine) and White Riesling are presented in Table 3.2.24.

3.4.1 Cabernet Sauvignon Wine

As previously explained, this wine was selected for analysis in order that the success and/or failure with which the developed analytical method could be used for the analysis of real life wine samples, may be evaluated. The results of the qualitative analysis presented earlier in Section 3.3 establish that the method developed is highly successful in the detection and identification of volatile flavor components in Cabernet Sauvignon wine. Diacetyl(2,3-butanedione) 10 with an odor threshold of 2.5 ppb and acetoin(3-hydroxy-2-butanone) 51 were previously reported to be present in Cabernet Sauvignon wine at concentrations ranging between 400 - 1800 ppb and 2000 - 84,000 ppb respectively.[259] Acetoin 51 was determined to be present at a concentration of 5000 ppb

Table 3.2.24

Quantitative Analysis of Selected Volatile Flavor Components of the Wines Investigated in this Study

Compound Reference Number	odor threshold (Lit.) (ppb)	Fragment ion** m/z	Experimentally determined concentrations of volatile flavor components Mean ± (s,3) (ppb)						Previously reported concn. (Literature)(ppb)			odor description
			Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling			Cabernet Sauvignon[f]	Concord[k]	White Riesling[l]	
						Must	Freshly Fermented Must	Young Wine				
3	160,000[a]	61	71,000±2500	100,000±5,000	112,000±6,720	800±400	10,000±425	46,000±1,850		510	3,500-285,000	sweet, peardrops
6		43	195±12	86±6	15±2	10±1	75±5	175±8				ethereal, sweet[m]
7		102	25±2	80±4	550±42	-	70±6	200±16	400-3,000		0-1,200	burnt toffee, rum
10	2.5[f]	86	1,590±310	-	385±40	120±18	400±50	290±52	400-1,800[n]		80-3,400	buttery
11	65[b]	73	207±11	225±14	350±21	15±0.9	100±7	185±12		200		
14	1.0[b]	71	75±4	107±9	295±16	-	16±1	40±4		170		estery, pineapple
23	11,000[b]	70	895±15	595±8	5875±120	8±1	48±2	300±15		6200	0-4,800	estery, fragrant
26	5[a]	88	85±3	157±6	172±7	-	25±1	188±7				estery, pineapple
32	15[g]	93	-	-	-	12.0±0.8	5.9±0.6	0.080±0.004				wall flowers[m]
40	100[f]	70	198±8	185±9	56±3	-	100±4	375±22				apple-like
42		136	97±3	-	-	24.9±0.9	9.8±0.5	0.060±0.002				
45	1[b]	88	145±9	664±58	950±82	5.5±0.4	135±9	355±28		630	100-2,000	estery, apples, pineapple
51		43	5,000±100	1,613±81	1,775±71	1,950±97	6,500±260	200±10	2,000-84,000[n]		1,900-31,700	sweet sugary

.....continued

Table 3.2.24 (continued)

Compound Reference Number	odor threshold (Lit.) (ppb)	Fragment ion** m/z	Experimentally determined concentrations of volatile flavor components Mean ± (s,3) (ppb)						Previously reported concn. (Literature)(ppb)			odor description
			Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling			Cabernet Sauvignon	Concord[k]	White Riesling[l]	
						Must	Freshly Fermented Must	Young Wine				
65		68	-	-	-	18.1±0.7	5.3±0.4	5.0±0.2				floral, fruity
68	70[b]	67	785±90	751±92	105±9	-	198±27	95±10		41		green, creamy, sherry-l
72		139	-	-	-	14.9±0.9	12.2±0.5	8.9±0.5				floral, fruity
84	25[b]	148	8.0±0.5	-	-	-	29.5±0.9	8,1±0.5			0-15[h]	
85		74	3.0±0.2	-	-	-	48±3	8±1			0-6[h]	potent meat-like flavor
89		69	20±2	-	-	257±30	98±10	55±3				floral, nutty
93		172	-	-	-	-	1.9±0.6	10±2			<40[d]	sweet, caramellike
95	5,800[e]	95	57±3	-	150±9	-	500±22	597±30				sweet, caramellike
96	6[b]	69	-	-	-	10.3±0.9	6.8±0.8	5.1±0.5				floral, piney
99		71	-	-	-	959±48	712±22	251±8				floral
102***	0.002	124	0,50±0.07	-	-	-	-	-				green pepper
104		95	3.0±0.4	-	-	-	-	-				
105	350[a]	106	3.6±0.3	105±3	98±4	-	9.5±0.4	17.9±0.7				almonds
106		192	19.0±0.6	-	-	-	4.9±0:3	19.8±0.9				hydrocarbon, kerosene

..... continued

Table 3.2.24 (continued)

Compound Reference Number	odor threshold (Lit.) (ppb)	Fragment ion** m/z	Experimentally determined concentrations of volatile flavor components Mean ± (s,3) (ppb)						Previously reported concn. (Literature)(ppb)			odor description
			Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling			Cabernet Sauvignon	Concord[k]	White Riesling[l]	
						Must	Freshly Fermented Must	Young Wine				
112	100[g]	93	470±24	57±8	-	353±21	223±18	178±17.	400[n]			lavender like, lemon peel
118		161	-	-	-	8.0±0.9	2.4±0.4	0.49±0.006				fruity, carrots[m]
124***		142	-	-	978±15	-	-	-				herbacious, sweet candy
125***		82	-	-	2.6±0.5	-	-	-				
126		115	987±30	2,015±101	1,925±58	-	259±10	757±23				
127		81	-	-	-	188±24	107±21	21.5±0.8				
130		71	15.0±0.9	-	9.5±0.7	215±10	122±9	18±2				floral, estery
134		148	-	-	-	-	4.9±0.7	8.1±0.9				fresh mushroom
137		86	1,870±66	2,270±45	1,958±42	455±21	1,100±35	1,810±40	+++[c]			sweet, fragrant
143		93	-	-	-	30.4±1.5	8.1±0.9	1.5±0.2	o			sweet, floral
144		133	-	-	-	8.9±0.8	4.0±0.4	1.1±0.2				
147		88	100±5	995±40	697±41	-	170±8	397±27	+[c]	640	<300	fermented apples
148	2[b]	105	-	-	-	-	0.50±0.08	1.9±0.3	-			sweet, floral
151		161	-	-	-	95±3	21±5	5.0±0.9				

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Table 3.2.24 (continued)

Compound Reference Number	odor threshold (Lit.) (ppb)	Fragment ion** m/z	Experimentally determined concentrations of volatile flavor components Mean ± (s,3) (ppb)						Previously reported concn. (Literature)(ppb)			odor description
			Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling			Cabernet Sauvignon	Concord[k]	White Riesling[l]	
						Must	Freshly Fermented Must	Young Wine				
153		121	40±1	-	18±1	162±1	104±2	89±2				fruity
155***		106	-	-	6.5±0.7	-	177±1	410±2			350-550[g]	sweet soup or meatlike
158		108	10.8±0.9	-	-	-	-	-			-	
159	160[g]	121	-	-	-	6.9±0.7	2.9±0.2	1.80±0.04				
164		82	-	-	-	37.4±2.1	32±2	27±1				roses[m]
166		105	-	-	-	22±1	15±1	3.8±0.2				
170	400[g]	69	-	-	-	10±1	95±3	112±3				
172		161	-	-	-	5.1±0.6	2.6±0.2	0.75±0.08				fruity, sweet[m]
173***		87	-	1,575±210	1,610±195	-	-	-				
176	650[f]	104	218±7	395±10	400±13	-	95±9	200±10	+++[c]	860	<800	roses
178	130[g]	69	-	-	-	780±12	567±17	354±20				sweet, fruity, geranium
180	0.009	121	9.5±0.6	8.8±0.6	1.00±0.06	-	0.28±0.04	0.68±0.04		1.58[j]	0.7-0.9[j]	sweet, floral
185	5,500[f]	108	685±30	603±22	8.9±0.4	-	0.90±0.07	2.7±0.2	+[c]			floral
186		88	156±11	760±42	152±11	-	158±9	378±12			<600	

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Table 3.2.24 (continued)

Compound Reference Number	odor threshold (Lit.) (ppb)	Fragment ion** m/z	Experimentally determined concentrations of volatile flavor components Mean ± (s,3) (ppb)						Previously reported concn. (Literature)(ppb)			odor description
			Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling			Cabernet Sauvignon	Concord[k]	White Riesling[l]	
						Must	Freshly Fermented Must	Young Wine				
187		99	145±10	-	-	-	57±6	150±11				
188		121	72±0	-	-	11.8±0.6	5.0±0.4	0.95±0.08				violets
189		100	1.62±0.11	75.5±0.9	98.0±0.9	2.9±0.5	19±2	33±2				
195		73	1,052±63	1.017±70	1.061±59							
203	75[e]	99	785±23	-	-	-						
209	0.008	177	-	-	-	17.9±0.9	6.9±0.6	2.6±0.2				violets
214***		128	-	-	571±29	-	-	-				burnt flavor, sweet caramel
215	50[e]	71	88±5	-	-	-						fruity, sweet
216		93	-	-	-	124.1±0.9	95±2	83.1±0.8				
217		131	8.5±0.8	865±21	927±29	-	4.8±0.5	19.9±0.7				cinnamon
224		117	1,146±42	519±46	197±8	-						roses
225		71	1,515±91	2,100±107	2,250±121	-	498±27	1,782±110	+[c]			
228***		87	476±21	-	-	-	-	-				
230		43	12±1	-	-	-	-	-				

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Table 3.2.24 (continued)

Compound Reference Number	odor threshold (Lit.) (ppb)	Fragment ion** m/z	Experimentally determined concentrations of volatile flavor components Mean ± (s,3) (ppb)						Previously reported concn. (Literature)(ppb)			odor description
			Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling			Cabernet Sauvignon	Concord[k]	White Riesling[l]	
						Must	Freshly Fermented Must	Young Wine				
232		107	58±7	377±20	480±11	70±2	190±6	390±18	++[c]			
240		85	985±80	2,591±	2,962±	-	755±	1285±				
242		135	-	-	-	-	1.9±0.2	2.5±0.3				green carrots[m]
243		119	-	1,280±	1,675±	-	-	-		560		oil of wintergreen
248		119	-	107±5	111±5	-	-	-				fruity, wintergreen
251		111	-	-	-	-	5.1±0.4	15.1±0.7				tea leaves
260***		105	-	10.2±0.9	15±2	-	37±3	26±3				
262***		120	-	-	250±8	-	-	-				
270		138	15.2±0.5	-	-	-	47.9±0.9	91.5±0.9				tea leaves
283		104	8.2±0.8	29±1	26.5±0.9	-	7.2±0.7	19±2				
287		152	5.8±0.9	98±12	157±14	-	0.9±0.1	8.5±0.7				
289		152	125±8	210±12	272±12	-	75±4	187±5				
293		108	8.9±0.7	-	-	-	-	-				
304***		151	7.7±0.6	2.5±0.8	0.90±0.05	-	-	-				

..... continued

Table 3.2.24 (continued)

Compound Reference Number	odor threshold (Lit.) (ppb)	Fragment ion** m/z	Experimentally determined concentrations of volatile flavor components Mean ± (s,3) (ppb)						Previously reported concn. (Literature)(ppb)			odor description
			Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling			Cabernet Sauvignon	Concord[k]	White Riesling[l]	
						Must	Freshly Fermented Must	Young Wine				
309		137	12±1	-	-	-	-	-				phenolic, medicinal
311***		144	-	-	8.5±0.4	-	-	-				
325		147	1,975±40	-	-	-	-	-				dry, smoky, phenolic
326		154	385±12	55±4	110±6	-	-	-				
327		147	1,507±90	-	-	-	-	-				

** Fragment ion retrieved from TIC chromagram.

*** New compounds identified for the first time in this study.

a. ref. 11; b. ref. 139; c. ref. 168; d. ref. 218; e. ref. 233; f. ref. 235; g. ref. 243; h. ref. 246; i. ref. 248; j. ref. 250; k. ref. 54; m. ref. 258; n. ref. 259.

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while diacetyl 10 was present at 1590 ppb in Cabernet Sauvignon. Ethyl butyrate 14, ethyl valerate 26, 3-methylthiopropyl acetate 84 and damascenone 180, all of which are volatile flavor components of known low odor thresholds, were determined to be present in this wine at concentrations in the low ppb range, i.e. 75, 85, 8.0 and 9.5 ppb respectively. Ethyl 4-acetyloxybutyrate 228, identified in this wine for the first time was found to be present at a concentration of 476 ppb. The structurally related ethyl 2-acetyloxy-4-methylvalerate 230 was found to be present at a concentration of 12 ppb. 2-Hydroxybenzothiazole 304, also identified in Cabernet Sauvignon for the first time, was present at a concentration of 7.7 ppb. The confirmation of the presence of 2-methoxy 3-isobutylpyrazine 102 in Cabernet Sauvignon wine for the first time in this study is noteworthy. Although Bayonove et al[217] identified this compound in the grapes, they could not establish its presence in the wine. Nor could they provide any quantitative data on the amounts present in the grapes. It was however predicted that the concentration in grapes would be about 5 ppb. Kepner et al[127] also failed in their attempt to confirm its presence in Cabernet Sauvignon wine. This organoleptically significant volatile flavor compound of an odor threshold of 0.002 ppb was determined to be present in this wine at a concentration of 0.5 ppb. This lower concentration of component 102 in the wine may have been the reason why its presence could not be deteeted by Kepner et al. Despite the extensive research that has been conducted into the analysis of Cabernet Sauvignon grapes and wines over the years, it is noteworthy that the methods developed in this research project have enabled the presence of previously suspected volatile flavor

compounds to be confirmed for the first time, and to detect and identify new components. Confirmation of the identities of the nine components currently assigned tentative identities and the sixteen components that have not been assigned any identities yet may add to the number of new compounds identified that were previously not known as flavor components of Cabernet Sauvignon.

In conclusion, the evaluation conducted on the developed analytical method shows that the method is highly successful and sensitive to the isolation, detection and quantification of organoleptically significant volatile flavor components at trace level concentrations in wines.

3.4.2 Concord and Moulin Rouge

Linalool 112, the only sesquiterpene alcohol found in Moulin Rouge, was present at a concentration of 57 ppb. It was not found in Concord. α-Terpineol 153 and 4-Terpineol 130 were found to be present at a concentration of 18 ppb and 9.5 ppb respectively in Concord. These alcohols were not found in Moulin Rouge. Isobutyl acetate 11 (odor threshold 65 ppb) was found to be present at a concentration of 350 ppb and 225 ppb in Concord and Moulin Rouge respectively. Acree et al[54] reported its concentration as 200 ppb in Concord using Freon 113 as the solvent extractant. Ethyl butyrate 14 (odor threshold 1 ppb) was determined to be present in Moulin Rouge at a concentration of 107 ppb and in Concord at 295 ppb. The previously reported concentration in Concord was 170 ppb.[54] Ethyl hexanoate 45 (odor threshold 1 ppb) was determined to be present at a concentration of 950 ppb in Concord and 664 ppb in Moulin Rouge. The previously reported concentration in

Concord was 630 ppb.[54] Similarly, cis-3-hexen-1-ol 68 (odor threshold 70 ppb) was determined to be present in Concord at a concentration of 105 ppb, about three times the previously reported concentration.[54] Ethyl lactate 56, one of the most abundant components in Concord, was determined to be present at a concentration of 19,000 ppb in Concord and 18,750 ppb in Moulin Rouge. Its concentration in Concord has been previously reported as only 230 ppb.[54] Ethyl decanoate 147, previously reported to be present in Concord at a concentration of 640 ppb was found to be present at a concentration of 697 ppb in Concord and 995 ppb in Moulin Rouge. At this point, it is worth mentioning that preliminary experiments that were conducted at the initial stages of this project in a search for a suitable extracting solvent showed that using Freon 113 resulted in considerable losses of low boilers such as ethyl acetate 3, ethyl propionate 7, isobutyl acetate 11, ethyl butyrate 14, ethyl hexanoate 45, ethyl lactate 56 etc. and may explain the discrepancies being observed here with the previously reported values. These losses of low boilers in Freon 113 may be due to its relatively higher boiling point and the most unfavorable vapour pressure under vacuum and reduced temperature with respect to the enrichment of such volatile flavor components. On the basis of the accuracies and precision involved in the developed analytical method it would be reasonable to conclude that the concentrations of the volatile flavor components of Concord determined in this research work more accurately represent the concentrations in Concord than as previously reported. Methyl anthranilate was found to be present at a concentration of 1675 ppb in Concord and 1280 ppb in Moulin Rouge. 3-Methylthiopropanol 155, identified for the first time in Concord, was present at a concen-

tration of 6.5 ppb. 3-Methyltetrahydrothiophene 173, identified for the first time in Moulin Rouge and Concord was present at concentrations of 1575 ppb and 1610 ppb respectively. Damascenone 180 was present at a concentration of 1 ppb in Concord and 8.8 ppb in Moulin Rouge. At a concentration that is 100 times in Concord and 1000 times in Moulin Rouge greater than the odor threshold, it is likely that damascenone may play a greater role in influencing the aroma of Concord more than in Moulin Rouge. 2,5-Dimethyl 4-hydroxy-3(2H)furanone 214, also identified for the first time in Concord, was present at a concentration of 571 ppb. Phthalide 260 was found as a trace component in both Moulin Rouge and Concord at concentrations of 10.2 and 15 ppb respectively. Dihydrobenzofuran 262, identified only in Concord was found to be present at a concentration of 250 ppb. 2-Hydroxybenzothiazole 304 was found to be present in Moulin Rouge at a concentration of 2.5 ppb and in Concord at 0.9 ppb. α-Naphthol 311 identified in Concord for the first time was found to be present at a concentration of 8.5 ppb. In conclusion, the results of the analyses of the volatile flavor composition of Moulin Rouge and Concord reveal the identities of several new compounds that had previously been unknown as flavor components of vitis labruscana wine varieties. While the results of this analyses improve considerably the characterization of and quantification of the volatile flavor components of v. labruscana varieties, the characterization process is far from complete yet, considering the number of components that have only been assigned tentative identities as well as those that have been detected but have not even been identified yet. Unless special precautions are taken to exclude air during the isolation-preconcentration

stages of the analysis of vitis labruscana grape musts and wines, components like N-(N-methyl, N-hydroxy-γ-aminobutyryl)glycine 223, suspected to be responsible for the typical labrusca flavor easily escapes detection. This precaution which appears to have been ignored in previous analysis of v. labruscana varieties may be one reason why the presence of a compound such as 223 has defied detection for so long.

3.4.3 White Riesling

Unlike Cabernet Sauvignon, Moulin Rouge and Concord, a large number of sesquiterpenes and sesquiterpene alcohols were identified in White Riesling grape must, freshly fermented must and the two-year old bottle aged wine. Without exception, all of them were found to be present in the must, the freshly fermented must and the young wine; their concentrations generally decreasing from the grape must to the aged wine. For example, myrcene 32 was present at a concentration of 12 ppb in the grape must, 5.9 ppb in the freshly fermented grape must and 0.08 ppb in the young wine. Limonene 42 decreased in concentration from 24.9 ppb in the grape must to 0.06 ppb in the young wine. Similarly, Neroloxide 65 decreased in concentration from 18.1 ppb in the must to 5 ppb in the wine. Ho-trienol(3,7-dimethyl-1,5,7-octatrien-3-ol) 99 decreased in concentration from 959 ppb in the must to 251 ppb in the young wine. Components 68, 72, 89, 96, 112, 118, 127, 130, 143, 144, 151, 159, 164, 166, 172, 188, 209, and 216 all showed a similar trend of decreasing concentration from the grape must, to the young wine (see Table 3.2.24 for numerical details). Rapp et al[68] have done extensive quantitative analyses of the volatile flavor composition of White Riesling grapes and wines from various locations in Germany and have reported that the concentrations of

citronellol, geraniol, and linallol which influence the aroma of the grapes considerably, generally follow a linalool/citronellol ratio of 2.0 to 30.8 and a geraniol/citronellol ratio of 13.7 to 23.7. The ratio of linalool 112 to citronellol 164 concentration in the grape must determined in this analysis is 9.4 and 20.9 for the geraniol 178 to citronellol 164 ratio. These comparative values suggest that some of the important properties of the most important indicators of the quality of White Riesling grapes grown in Europe are also retained by those that have been cultivated locally. It would be expected that the presence of these organoleptically significant sesquiterpenes and sesquiterpene alcohols in White Riesling at concentrations at or near the odor threshold values in the grape must would contribute significantly to the flavor and aroma of the must. This indeed appears to be the case since the grape must was characterized by a strong fruity flavor which diminished considerably after fermentation. While the concentrations of the sesquiterpenes decreased with fermentation and bottle aging, other compounds usually absent in the must were observed to develop at fermentation and increase in concentration with aging. For example, benzothiazole 242 increased in concentration from 1.9 ppb after fermentation to 2.5 ppb in the wine. Methyl vanillate 287 and ethyl vanillate 287 both increased in concentration from 0.9 to 8.5 ppb and 75 to 187 ppb respectively. Theaspirane 270 almost doubled its concentration after fermentation over the two year period. Dihydroactinidiolide-(2-hydroxy-2,6,6-trimethyl cyclohexylidene-1-acetic acid lactone) 251 concentration tripled over the same period, while the concentration of vitispirane 106 quadrupled. The concentration of 1,1,6-trimethyl-1,2-

dihydronaphthalene (TDN) 93 also increased from 1.9 ppb in the freshly fermented must to 10 ppb in the wine. Simpson[218] reported in 1977 that the TDN concentrations in White Riesling grapes of the following vintages: 1977, 1973, 1968 and 1959 were 0, 15, 25 and 36 ppb respectively. The concentration determined in this analysis is lower than the odor threshold of ≈ 20 ppb and would therefore not be expected to influence the aroma of the young wine significantly. Nevertheless, if the anticipated trend of increasing concentration with aging continues, it is very likely that its concentration will exceed the flavor threshold and contribute the familiar "bottle-aged character" to the wine. Schreir et al[246] reported the concentrations of 3-methylthiopropylacetate in a 1971 and 1972 vintage Riesling wines to be 5.5 and 12.5 ppb respectively and also concluded from the analysis of several Riesling wines from various locations that the concentration of 3-methylthiopropylacetate 84 generally ranged between 0 - 15 ppb. This compound was determined to be present in the young wine at a concentration of 8.1 ppb. Ethyl 3-methylthio-propanoate 85 previously reported to be at a concentration of < 6 ppb,[246] was found to be present in the locally produced White Riesling wine at a concentration of 8 ppb. 3-Methylthiopropanol 155 was found to be present at a concentration of 410 ppb in the young wine. The previously reported concentration in White Riesling wine ranges between 350 to 550 ppb.[243]

The results of the quantitative analysis of the volatile flavor composition of the must and wine made from the fruits of locally cultivated White Riesling vines show that the quantitative distribution of the flavor components compare quite favorably with those of the more favorable temperature climates. Preliminary though these results may be,

it may be inferred that sufficient data has already been generated on the qualitative and quantitative distribution of volatile flavor components in the local *vitis vinifera* cultivar, White Riesling, that will at least help influence the direction of viticultural research and vinification practices in Canada and the Eastern United States.

CHAPTER FOUR

SUMMARY

A new design of a concentrator apparatus was developed and used in conjunction with a slightly modified version of a previously designed solvent extraction apparatus to study the volatile flavor composition of vitis vinifera and vitis labrusca musts and wines. This concentrator, which is probably the most efficient and sensitive of its kind to be designed, was shown to provide sufficient enrichment of organoleptically significant volatile flavor components present at trace levels, without significant losses, to enable their detection, identification and quantification.

Standard analytical methods (high resolution capillary GC/MS, ancilliary mass spectrometric techniques such as chemical ionisation, high resolution, accurate mass measurements at low resolution, selected ion retrieval of selected m/z values from previously acquired and stored TIC data) were used to separate, detect, identify and quantify the volatile flavor components present in the must and wine samples. The methods used enabled the detection and identification of several trace components from as little as 100 ml of sample, without resorting to the traditional method of starting with several liters of sample and using preparative GC to isolate one trace component of interest from a host of other uninteresting but major components. The analytical method developed was

evaluated using Cabernet Sauvignon wine. Trace compounds of low odor thresholds and/or are organoleptically significant, previously reported to be present in this wine, were detected and identified. In addition, 2-methoxy-3-isobutylpyrazine, an organoleptically significant flavor component whose presence in the wine was suspected but was never confirmed, was detected and identified in this wine for the first time. Ethyl 4-acetyloxybutyrate (concn. 476 ppb), 2-hydroxybenzothiazole (concn. 7.7 ppb) were also detected and identified in Cabernet Sauvignon wine for the first time.

These findings seem to suggest that despite the extensive work that has been done already with regards to the analysis of the flavor composition of this wine, the system developed in this research project may probably be the most sensitive one currently available for the examination of the volatile flavor components of wines.

The solvent extraction-preconcentration procedure used for the analysis of Cabernet Sauvignon wine, was found to be unsuitable for the examination of the flavor components of vitis labruscana varieties. A modified solvent extraction-preconcentration procedure, conducted under carefully controlled inert atmosphere conditions, was developed specially for these varieties because, it was discovered that only under these carefully controlled conditions could the isolated volatile flavor extract and the residual wine be made compatible with each other. It was also discovered that only under these controlled experimental conditions could the compound, tentatively identified as N-(N-methyl,N-hydroxy-γ-aminobutyryl)glycine and evaluated by sniffing experiments conducted on the GC effluents to be probably the most significant component in v.

labruscana varieties, be detected in all the labruscana varieties investigated (Concord, vidal, elvira, dutchess and Moulin Rouge). Elvira, vidal and dutchess showed no detectable concentrations of methyl anthranilate which has hitherto been held responsible for imparting the 'typical labrusca character' to these varieties. 3-Methylthiopropanol (6.5 ppb in Concord), 3-methyltetrahydrothiphene (1575 ppb in Moulin Rouge and 1610 ppb in Concord), 2,5-dimethyl 4-hydroxy-3(2H)furanone (571 ppb in Concord), phthalide (10.2 ppb in Moulin Rouge, and 15 ppb in Concord), dihydrobenzofuran (250 ppb in Concord), 2-hydroxybenzothiazole (2.5 ppb in Moulin Rouge, 0.9 ppb in Concord) and α-naphthol (8.5 ppb in Concord) were all identified in these varieties for the first time.

The results of this analysis should improve the characterization of the volatile flavor composition of the labruscana varieties. The method used should provide the needed fillip to provide a more complete characterization of the volatile flavor composition of v. labruscana varieties.

Vitis vinifera vines have only recently been successfully cultivated on the previously considered 'hostile climate and soils' of Canada and the eastern United States. One of these varieties, White Riesling, was selected for analysis of its volatile flavor composition, using the methods described for Cabernet Sauvignon wine, in order to establish whether the different local climate and soil have significantly changed the qualitative and quantitative distribution of the volatile flavor components from what it should have been had it been cultivated in the temperate climate. The results of the analysis of the grape must, freshly fermented must and the young bottle-aged wine indicate that no

significant changes have occurred in the qualitative and quantitative distribution of the volatile flavor composition of the local varieties. While it is realized that the final acceptance or rejection of the quality of the wine made from the grapes of the locally cultivated White Riesling grapevine will eventually be based on a taste panel evaluation, it may be concluded from the results of the chemical analysis presented in this thesis that a high quality wine may be expected to be made from this variety.

CHAPTER FIVE

FUTURE WORK

A large number of flavor components still remain unidentified in this study. It is believed that the mass spectral data acquired already in this study on these unidentified components may be enough to identify them. Failing that, however, other ancilliary mass spectrometric methods such as, linked scan metastable ion techniques, may have to be used to elucidate the fragmentation mechanisms and thereby lead to their ultimate identification. The identification of these components, most of which were detected at trace levels, would enable enologists to better appreciate and rationalize some of the features of these complex natural flavor mixtures for which there are still no reasonable explanation. The compound reported in this study as that likely to be responsible for imparting the typical labrusca flavor to the v. labruscana varieties will need to be synthesized and the identity of the detected flavor compound proven. Its presence in several other v. labruscana varieties not analysed in this study must be confirmed and the quantitative amounts of this compound in these v. labruscana varieties determined.

The interesting preliminary results obtained on the volatile flavor composition of the musts and young wines made from the grapes of locally cultivated White Riesling vines, make it attractive to repeat

the analysis on all other vinifera varieties also currently being cultivated locally and the aged wines. It would be interesting to use the analytical method developed to conduct time-profile studies to monitor the effect factors such as, period of maturation of the grapes, method of juice extraction, fermentation temperature and yeasts, chaptalisation, oak-wood and/or bottle cooperage would have on the concentration-developmental pattern of these volatile flavor compounds. The results of such studies may provide adequate and sensitive indicators that may be used to predict at an early stage of the vinification process the quality of the wine that might be produced. For the enologist, such studies conducted using isotopically labelled components may help elucidate the various biosynthetic pathways, origin and fate of some of these trace level components.

It has been observed that acutely schizophrenic patients usually give out a characteristic but peculiar mouth odor. While some work has already been done on the volatile composition of the mouth odor taken from such patients, there is no evidence in the literature that the compound(s) responsible for the typical mouth odor have been detected and identified. Since the mouth odor will essentially be a mixture of volatile components, it is suspected that the method developed in this study for alcoholic beverages may be used either in its entirety, or with modification to study the volatile composition of samples taken from such patients. Such a study, conducted in collaboration with those involved in drug metabolic profiling studies, may lead to a correlation not previously observed. It may also lead to the identification of those compound(s) suspected to impart the characteristic odor.

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APPENDIX

Mass spectral characteristics of flavor components detected in various wine varieties in this study but have not yet been assigned any chemical identities

Compound reference number	Mass spectral characteristics of unknown flavor compound m/z (% relative intensity)	Mol. wt. deduced from CIMS	Experimentally determined I on C20M	Source of Detection					
				Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling		
							Grape Must	Freshly Fermented Must	Young Wine
190	70 71(45) 55(40) 91(30) 155(25) 92(22) 173(10)	192				*			
202	43 87(65) 70(57) 55(18) 99(10) 111(10) 129(10)	129		*					
227	87 85(75) 57(42) 45(40) 41(38) 56(30) 29(30)	188		*	*	*		*	*
233	85 71(50) 43(40) 29(35) 55(20) 102(20) 159(19)	200				*			
234	111 97(60) 81(40) 156(40) 83(30) 55(30)	178				*			
237	151 123(85) 121(60) 77(30) 95(27) 166(18) 192(3)	210				*			
257	87 70(68) 43(60) 45(52) 71(45) 85(33)	188		*					
264	85 43(45) 71(38) 29(35) 70(33) 199(30) 171(20)	199		*					
266	71 85(97) 43(80) 103(40) 131(30) 55(30) 29(30) 173(24)	191		*					
267	91 121(40) 148(35) 43(35) 103(27)	218		*		*			
269	107 120(58) 123(30) 194(28) 77(18)	226		*					
273	84 104(30) 108(27) 137(25) 230(5) 41(15)	230		*					
284	137 102(55) 176(50) 120(48) 43(40) 160(25) 91(30)	176		*					
285	44 45(63) 55(20) 61(24) 57(18) 103(10)	170			*				
290	84 85(25) 41(18) 177(15) 222(15) 27(10)	222							

..... continued

Mass spectral characteristics of flavor components (continued)

Compound reference number	Mass spectral characteristics of unknown flavor compound m/z (% relative intensity)	Mol. wt. deduced from CIMS	Experimentally determined I on C20M	Source of Detection					
				Cabernet Sauvignon	Moulin Rouge	Concord	White Riesling		
							Grape Must	Freshly Fermented Must	Young Wine
291	224 105(50) 77(30) 225(27) 179(25) 208(20)	239			*				
294	107 138(30) 196(28) 77(20) 87(19) 108(17)	263		*					
298	104 105(63) 45(38) 73(20) 189(8)	189		*					
306	107 181(30) 226(20) 191(7) 151(6)	226		*					
308	181 212(99) 136(40) 45(37)	212		*					
315	167 45(30) 210(28) 168(20) 151(10) 134(9)	210		*					
318	137 194(60) 151(30) 104(31) 45(33) 117(30)	194				*			
321	91 73(30) 89(15) 99(10) 101(10) 168(10)	232		*					

* detected