THE ANALYSIS OF THE VOLATILE COMPONENTS IN CANADIAN WINE

To EMJ and the rest of my family

THE ANALYSIS OF THE VOLATILE COMPONENTS IN CANADIAN WINE

By

ALLAN HAROLD JACKSON, B.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

October, 1977

DOCTOR OF PHILOSOPHY (1977) McMASTER UNIVERSITY

Department of Chemistry Hamilton, Ontario

TITLE: The Analysis of the Volatile Components in Canadian Wine

AUTHOR: Allan Harold Jackson, B. Sc. (McMaster University)

SUPERVISOR: Professor R. H. Tomlinson

NUMBER OF PAGES: ix, 139

ABSTRACT

A new technique for the rapid and quantitative analysis of the volatile components in wine has been developed. This technique includes a new design of solvent extractor which is more efficient than any previously described designs and a low-temperature, vacuum concentrator. This technique was applied to the study of the patterns of formation of the volatile components in Concord and Blue Hybrid wines as a function of the fermentation progress. The effect of fermentation temperature on these patterns was also investigated. Identification of a number of volatile components was achieved using both gas chromatographic retention time and mass spectrometric techniques.

ACKNOWLEDGEMENTS

The author wishes to sincerely thank his research director, Dr. R. H. Tomlinson, for all that he had contributed during the last several years. His rare gifts of creativity and enthusiasm have not only been responsible for this research project but also for a lasting friendship and personal respect.

Appreciation is also expressed to Dr. O.E. Hileman, Jr. and Dr. N.H. Werstiuk for their advice, support, and willingness to be associated with an unconventional project.

The complete cooperation of Andre's Wines Limited especially of Mr. E. R. Haynes and Mr. K. Smith deserve recognition.

The contributions of J. Fletcher and D. Bickley with their friendship and sense of humour are acknowledged.

Financial assistance for this research was provided by the National Research Council, the Province of Ontario and McMaster University.

Special thanks to an excellent typist.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER 1

INTRODUCTION

A. History of Wine

There are many reasons for interest in wine. Amongst these reasons are ones that are philosophical, historical, economic, and academic in origin. A brief description of what motivates one to study the chemistry of wine follows.

It has been claimed (1), with some historical justification, that the grape is the only fruit that naturally preserves itself. Any system making such a claim to unique status in nature merits further investigation.

In many ways the history of civilization is reflected in the history of wine. It is believed the first grape-growing civilization was located in a region, which is now northern Iran, several thousand years B.C. 2). There is possibly a geographical link between this civilization and a biblical reference(3) to Noah's cultivation of grapes: "and Noah began to be a husbandman, and he planted a vineyard; and he drank of the wine and was drunken". Noah's Ark is said to have come to rest on Mount Ararat in Armenia.

The first wine industry dates to 3500 B.C. in Egypt from where it spread throughout the Mediterranean(4). It is likely that wine formed an important part of man's diet at this time. It not only represented a safe, healthful, and cheap beverage but also constituted a food source of high caloric value (5).

The importance of wine is richly documented in historical literature. The Code of Hammuràbi (circa 1792 - 1686 B.C.) dealt with the problem of dilution of wine with water (6). Archaeological evidence of covered jars sunken in the ground indicate a knowledge of the effect of both temperature and air on wine during this period. Homer's Iliad and Odyssey contain excellent descriptions of wine (7). The first miracle of

Christ involved the turning of water into wine. Horace and Virgil both praised the quality of wine.

The Greeks succeeded the Egyptians in advancing wine technology and were themselves succeeded by the Romans. The first good classification of grape varieties originated in Rome. The Romans spread vine culture throughout their empire especially into what are now the world's most important wine-growing regions, in northern Europe (8).

The vineyard industry deteriorated along with the Roman Empire. It seems likely that only the need of the Church for sacramental wine preserved the wine industry through the Dark Ages (9). In fact monasteries actually advanced wine technology, apparently introducing widespread aging of wines and regional classification of wine.

The return of political stability accompanied the revival of the wine industry. With the development of wine trading providing the incentive, the quality of wine rapidly improved. Faced with increased demand for wine, the vineyard acreage increased rapidly in the 15th to 17th centuries (10).

Wine production reached a new maximum by 1850 but it is estimated that 25% of the wine spoiled before the fermentation was complete (11). It was to this problem that Louis Pasteur addressed his research and in 1866 he conclusively demonstrated that the spoilage of wines was due to aerobic organisms producing acetic acid (12). This conceivably marked the origins of modern wine chemistry.

In the last century the rapid development of science and industry has resulted in this era being the golden age of wine. It is distinguished by increased quality, availability and lower relative cost of wine.

Studies aimed at the eventual improvement in wine quality have a sound economic motivation. Annual wine sales in the United States alone are about one billion dollars (13).

A reflection of the economic importance of the wine industry in certain countries is the fact that several research institutes, whose main purpose is the study of wine, have been established.

B. Grape Types

While the treatment of the juice in the production of wine is crucial, it is apparent that the single, most basic parameter determining

wine quality is the type of grape juice used.

Grapes belong to the botanical family Vitaceae which includes ten other genera but the grape, genera vitis, is the only food plant (14).

The major commercial wine grape in the world is the species Vitis vinifera. It was native to the area around the Caspian Sea and is now under cultivation throughout most of Europe and South America as well as South Africa, Australia, and California. There are over 5,000 named cultivars of V. vinifera at present (15).

The species which is native to eastern North America is Vitis labrusca which is less suitable for wine making than V. vinifera. Canada and the eastern United States are the only regions where species other than V. vinifera are used extensively for wine making. Attempts to cultivate pure varieties of V. vinifera in Ontario have failed due to the low winter temperatures. In an effort to improve the quality of grapes available for wine production in Ontario, a great deal of effort has been put into creating hybrids of V. vinifera and V. labrusca which incorporate the favourable wine-making characteristics of V. vinifera and the weather resistance of V. labrusca. Hybrids which show promise as wine grapes are often then crossed with each other in an effort to gain further quality characteristics. This process has resulted in the creation of hundreds of different hybrids and significant difficulties in evaluating any single hybrid quickly. Efforts are being made eleswhere (16) to evaluate the wine-producing potential of novel grape hybrids on the basis of the composition of their volatile components. The development of such a scheme of evaluation would be of considerable worth to the Canadian wine industry. In order for such a scheme to be applicable to the Canadian situation, however, the volatile components of both V. labrusca and Canadian hybrid juices and wines must be better characterized than they are presently. This research attempted to initiate this characterization.

C. The Composition of Grape Juice and Wine

The general chemical composition of grape juice, must, and wine is well known. It should be noted that the following definition of wine is assumed throughout this thesis: the beverage resulting from the fermentation by yeasts of grape juice with appropriate processing and additions(17)

Similarly use of the word, must, refers to the prepared grape juice and the fermenting body. This it is the name used to describe the transition from juice to wine (18).

Table 1 shows the relative chemical composition of juice and wine (19).

The primary sugars in juice are dextrose and levulose which are usually present in a 1:1 ratio at maturity. These sugars are converted to ethanol in the presence of yeasts as illustrated by the Guy-Lussac equation (20):

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$$

In Canada sucrose is generally added to increase the total sugar percentage to that desired for fermentation -- about 21%. This procedure is referred to as chaptalisation (21).

Ethanol in wine not only exerts a biochemical influence on the human body but also makes a sensory contribution through its slight odour and being an excellent solvent for other odorous materials (22).

The principal acids of the grape are d-tartaric acid (pK_a= 2.98) and l-malic acid (pK_a= 3.41). The buffering action of these acids ensures wine remains at a relatively low pH which is responsible for the biological stability of wine and the maintenance of typical wine colour (23).

The pigments present in the solution (as well as the pH of that solution) determine the colour of the juice or wine. They are categorized as polyphenols along with the tannins which contribute a bitter or astringent taste to the wine (24).

The nitrogenous constituents of wines include proteins, peptones, polypeptides, amides, amino acids and ammonia. They play an important role in clarification, in bacterial development, and in aroma development (25).

The most abundant of the mineral compounds in wine is potassium. Small amounts or traces of many other cations and anions are present. These inorganic constituents are often of considerable significance to the wine or must in a variety of roles (26).

There is a very small fraction of the chemicals in juice or wine called the volatile components or odorous constituents. They rarely exceed 0.1% of the wine and are even less abundant in the juice. They

TABLE 1

Percentage Major Constituents in Must and Wine

Constituent	Must %	Wine %
Water	70-85	80-90
Carbohydrates	15-25	0.1-0.3
Ethanol	Trace	8-15
Organic Acids	0.3-1.5	0.3-1.1
Polyphenols	0.01-0.10	0.01-0.30
Nitrogenous Compounds	0.03-0.17	0.01-0.09
Mineral Compounds	0.3-0.5	0.15-0.40
Volatile Components	Trace	0.01-0.10

are generally alcohols, esters, organic acids, aldehydes, and ketones in the 50 to 250 molecular weight range (for the higher molecular weight components, 'volatile' is somewhat of a misnomer). They are generally held accountable for the aroma of a wine and, to a varying extent, the flavour of a wine. For example differences in the aroma of various wines are attributed to differences in their volatile composition (27).

The investigation of these volatile components was the general aim of this research.

D. The Volatile Components of Wine

Reports of the analysis of the volatile components in wine appeared in the literature as early as 1921 when Power and Chestnut (28) reported the occurence of methyl anthranilate in the Concord grape which is a cultivar of V. labrusca. Subsequent analyses have since attributed great importance to the presence of methyl anthranilate in Concord grapes (29, 30), concluding that it is the predominant aroma-producing constituent of Concord grapes. These conclusions have resulted in most attempts at improving the quality of grapes cultivated in Ontario being centred around methyl anthranilate.

Early studies of the volatile components in juice and wine up to about 1960 utilized chemical and physical characterization of functional groups as the primary method of identification. By 1957 eight volatile components of Concord juice had been identified (31) and eleven volatile components of V. vinifera species were known (32).

Within the last 15 years the analysis of the volatile components in wine has rapidly expanded due to the coming of age of gas chromatography which provided a quick instrumental means of separating and detecting the volatile components. This technique when teamed with mass spectrometry also provided dependable identifications. A reflection of the rapid expansion of the qualitative analysis of the volatile components is provided by the fact that, at this time, the presence of over 180 volatile components in wine has been reported (33). Over 60 compounds have been reported as being volatile components of Concord wine (34). The unique aroma of certain grape juices is attributed to single volatile components such a methyl anthranilate in Concord grapes, linalool in Muscat grapes,

and 2-phenethanol in V. rotundifolia grapes (35). Recent studies (36) of the volatile composition of wines made from various juices indicate that the characteristic aroma of any one wine is not due to one or more specific components exclusive to other wines. This characteristic aroma is, however, due to the same compounds present in all wines but arranged in individual concentrations relative to one another.

Gas chromatography in particular increased the possibility of doing quantitative analysis of the volatile components. Enologists were thus enabled to make quantitative comparisons of the volatile components of wines produced by varying fermentation parameters which were known or suspected to affect wine quality. One of the first of these parameters that was investigated was the fermentation temperature which had been previously established as having a definite effect on wine quality (37, 38, 39). In 1966 Ough and Amerine thoroughly reviewed the literature on the effects of fermentation temperatures between 10°C and 30°C on wine making (40). They reported the following findings: a decrease in ethanol concentration at higher fermentation temperatures due to the increased partial pressure of ethanol and increased rate of evolution of carbon dioxide carrying ethanol with it; a significant difference in volatile ester concentration as a function of fermentation temperature (with a maxima at 20°C) due to the increased enzyme activity at intermediate fermentation temperatures; a minimum concentration of aldehydes at the intermediate temperatures, although the differences for aldehydes were less significant; and a varying response of higher alcohol concentration with fermentation temperature. Ough, et al. (41) found maximum concentration of 3-methyl-1-butanol and 2-methyl-1-butanol at 24°C as well as a mimimum concentration of 1-propanol. The concentration of 2-methyl-1-propanol ranged erratically with fermentation temperature. Ayrapaa (42) found a maximum yield of higher alcohols at a fermentation temperature of 20°C. Similarly Nordstrom (43) found ester formation to be a maximum at 20°C. Webb (44) in 1973 found when analyzing two replicate fermentations of synthetic mixtures that, of 56 distinguishable components, 25 showed irregular functions of concentration with increasing fermentation temperature. The concentration of 2-phenethanol varied irregularly with fermentation temperature. The fermentation temperatures studied were 5°C,

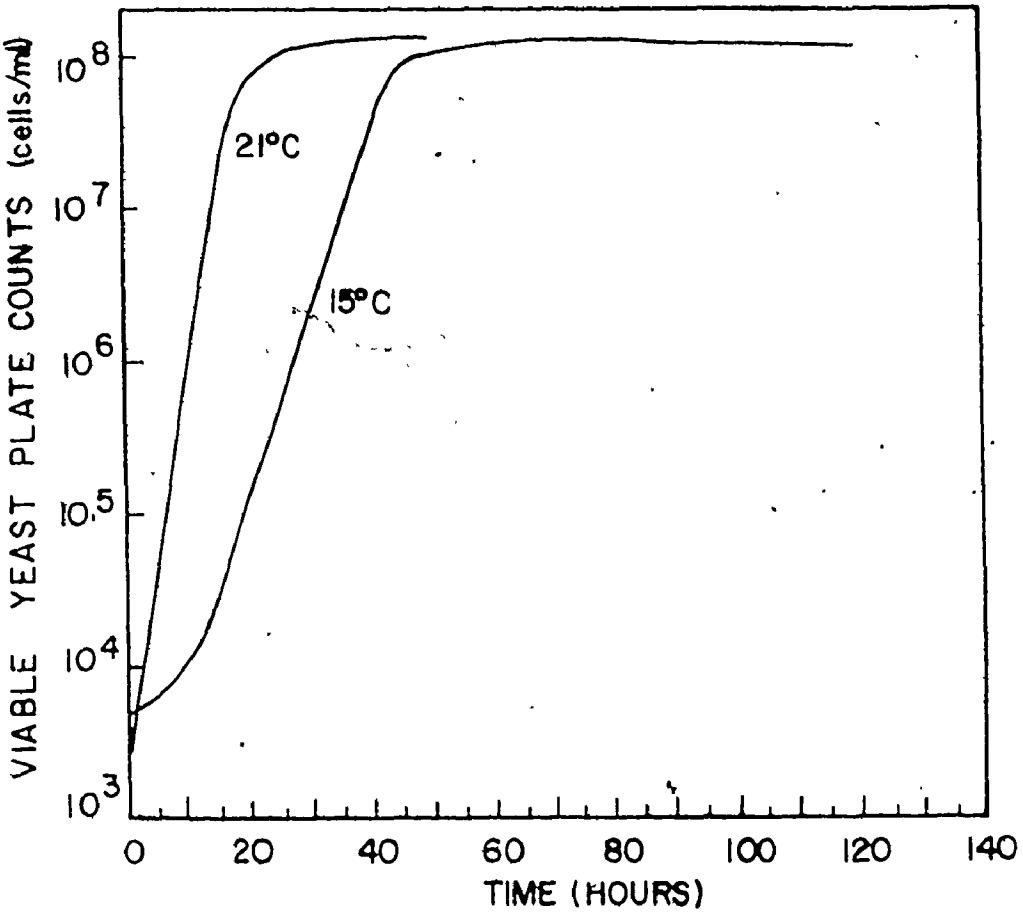
10°C, 15°C, and 20°C. Because of the limited sample sizes available for mass spectral analysis only 17 of the 56 components were identified.

Daudt and Ough in 1973 (45) investigated the effect of various fermentation parameters on the concentration of the volatile acetate esters in the resultant wine. Lower fermentation temperatures generally resulted in higher concentrations of the volatile acetate esters. The yeast strain used also affected the formation of these esters although the amounts of the individual esters formed were not affected equally by the specific yeast. The amount of sulfur dioxide added to the must affected the amount of the volatile acetate esters formed. A large variation in the quantity of these esters was observed with changes in the grape variety used for fermentation. There exists the possibility that the method of pressing the grapes, hot or cold, also affects the volatile components later observed (46). The suggestion was made that the fermentation process plays a central role in the formation of certain volatile components such as the volatile acetate esters because alcoholic beverages from very different sources, including wine and beer, all contain these compounds as major components.

While these forementioned studies examining the effect of various fermentation parameters on the concentration of certain volatile components were being carried out, other studies contributed relevant information. Ough and Amerine (47) studied the effect of fermentation temperature on the fermentation rate and found that for fermentation temperatures in the 20°C to 30°C range there was a linear relationship between the log of the fermentation rate and the reciprocal of the absolute temperature of fermentation. They also found that starting a fermentation with higher temperatures for the first 16 hours resulted in a more rapid yeast growth period but had little effect on the overall rate of fermentation. Further studies by Ough and Amerine (48) considered the effect of the fermentation temperature on the growth of yeast cells. The results of this study are shown in Figure 1. It was also found that a linear relationship existed between the fermentation rate and the fermentation temperature as expressed in degrees Celsius (49). The fact that different juices respond in different ways to changes in the fermentation temperature was also observed (48). These studies determining the effect of

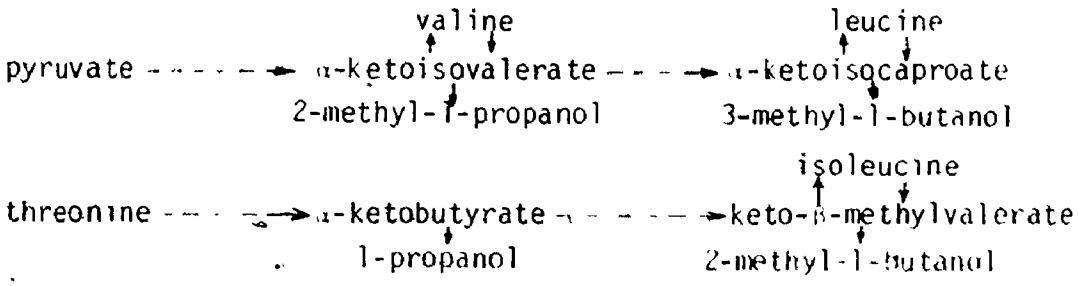
FIGURE 1

Yeast Growth Curves Showing the Effect of Two Fermentation Temperatures, 21°C and 15°C (48)


VIABLE YEAST PLATE COUNTS (cells/ml)
10^8
10^7
10^6
10^5
10^4
10^3
21°C
15°C
0
20
40
60
80
100
120
140
TIME (HOURS)

the fermentation temperature on the rate of fermentation were valuable for comparison with studies determining the effect of the fermentation temperature on the concentration of the volatile components. This comparison supported the suggested link between the formation of certain volatile components and the fermentation process.

Other studies concentrated on proposing mechanisms for the formation of the volatile components. The accepted mechanism (50) for the formation of certain higher alcohols is as follows:



A similar mechanism accounts for the formation of 2-phenethanol. Using such mechanisms, Ough, et al. account for the fact that some higher alcohols are found in highest concentration at the same fermentation temperature that yields maximum yeast growth (50). It is also accepted (51,52) that the formation of some volatile esters during fermentation results from the alcoholysis of the fatty acid moiety of the acyl-CoA compounds following the activation of that moiety. The formation of esters is apparently not the result of direct esterification reactions between alcohols and free acids in the fermenting medium. Consequently the levels of esters may exceed by many times the chemical equilibrium for the corresponding alcohol and acid. The acid function of these esters is expected to have an even number of carbon atoms. It is well known that the even-numbered fatty acids predominate in natural products because the acid precursors are synthesized by successive couplings of the acyl-CoA fragment with acetyl-CoA (a C_2 addition to the molecule). This ester formation mechanism is linked to the fermentation process as was the higher alcohol formation mechanism.

There are apparently no reports in the literature of studies monitoring the concentration of the volatile components as they develop during the fermentation. Such studies would seem likely to complement the types of studies previously described as well as studies currently

being carried out monitoring the concentration of amino acids during the fermentation (53). The latter indicate that most amino acids decrease from substantial levels in the juice to nearly undetectable levels after 6 days of fermentation and then show significant increases in concentration at 21 days after the fermentation commenced.

The reason for the apparent lack of daily analysis of volatile components during the fermentation would seem to be the lack of a method of quick and quantitative analysis of the volatile components. A review of the literature shows most analysts used solvent extraction to isolate the volatile components from the must. Repeated batch extractions are often performed or else conventional continuous downwards (or upwards) displacement extractors are used. A few novel designs are reported (54,55). The common extracting solvents used are methylene chloride (56), isopentane (57), diethyl ether (58), a 2:1 mixture of pentane and methylene chloride (55), and trichlorofluoromethane (59). For most techniques of solvent extraction reported in the literature, there is little information given regarding the efficiency or precision of the extraction of typical volatile components. Such information would seem necessary for any quantitative analysis to be meaningful. The extraction times reported in the literature for quantitative extractions generally exceed 17 hours (60). A 17 hour extraction would make daily analysis difficult. Other methods of isolating the volatile components from wine are reported such as steam distillation but again little data on the quantitative character of the isolation is available (61). Daudt and Ough (62) report a minimum recovery of 84% when using distillation to recover volatile acetate esters.

It is usually necessary to concentrate the volatile components, once they have been isolated, in order that their concentration be sufficient for gas chromatographic analysis. The most common technique of concentration is fractional distillation (58, 63) which can be very time-consuming with respect to the need for daily analysis. Other techniques for enrichment used include rotary evaporation and a few novel procedures (60). As was the case for isolating the volatile components, very little data is available concerning the losses of solutes involved in using these techniques of enrichment. The size of sample to be concentrated determines the final volume of concentrate after sufficient enrichment has

occured. This in turn can affect the choice of concentration technique used.

Gas chromatography is the technique used almost exclusively for separating the individual components of the concentrated extract. Conventional packed columns (64) and open tubular capillary columns (65) are both commonly used for this task. The time of analysis is greater using the capillary column although better separation is usually achieved. A variety of column coatings are used with Carbowax 20M being perhaps the most common (64). Flame ionization detectors are almost exclusively used in the gas chromatographs with an exception being the use of electron capture detectors for methyl anthranilate analysis (66).

The most common means of identification of the volatile components are gas chromatographic retention times (67) or mass spectral analysis(68). The mass spectrometer is usually coupled to the gas chromatograph for combined separation and identification. Recently (67) Brander has used infrared spectroscopy to identify fractions collected from the gas chromatograph manually.

To determine gas chromatographic peak areas, peak height measurement is apparently the most common technique used (69, 70). The peak height times its width at half height is also used (71) to estimate peak area. Reports of actual peak area measurement are uncommon. Few analyses (70) report the calibration of the flame ionization detector in order to convert peak areas to actual amounts of individual components. Once again very little data concerning the accuracy and/or precision of the gas chromatographic analyses is available. Daudt and Ough (62) report the precision of the gas chromatographic analysis of volatile acetate esters to not exceed 7% when using the peak height times its width at half height to estimate peak area.

One quantitative analysis of the volatile components in wine (61) reports precision in the range of 1.1% to 17.0% for the overall analysis of 18 common volatile components in wine using steam distillation to isolate them. Daudt and Ough (72) report a range of precisions of 7.6 to 21.5% for the overall analysis of 5 volatile acetate esters.

E. Statement of the Proposed Research

Whereas many studies of the volatile components of juice and wine have been made, the absence of a relatively fast and quantitative technique of analyzing these components has been very restrictive on the types of research conducted. For example there have been no studies of the rate of formation of the various volatile components during fermentation and there is only minimal knowledge of the differences between the volatile components of V. labrusca and Canadian hybrids. The investigation of the effect of fermentation temperature on the volatile components would also be aided by such a technique.

Examination of the existing techniques shows that the time required for the isolation and concentration of the volatile components must be reduced in order that the overall analysis be relatively fast. It is to this aim that the major thrust of the research was directed.

Once a satisfactory technique of analysis had been developed, subsequent research was designed to evaluate this technique as well as make preliminary studies of the type described above.

CHAPTER 2

EXPERIMENTAL

A. Techniques of Analysis

In order to perform quantitative and qualitative analysis of the volatile components of a wine or must, the following procedure was devised:

(a) sample collection
(b) isolation of the volatile components in the sample
(c) enrichment of the volatile components
(d) separation into individual volatile components
(e) quantitative analysis of the separated components
(f) identification of the separated components

One of the aims of this research was the development of a relatively fast method of routine analysis of the volatile components of wine. It was found that some of the techniques available in the literature were adequate whereas it was necessary to modify others or develop new techiques for certain of the stages of the analysis listed above. A description of the technique adopted and used throughout this project for each stage of the analysis follows.

(a) Sample Collection

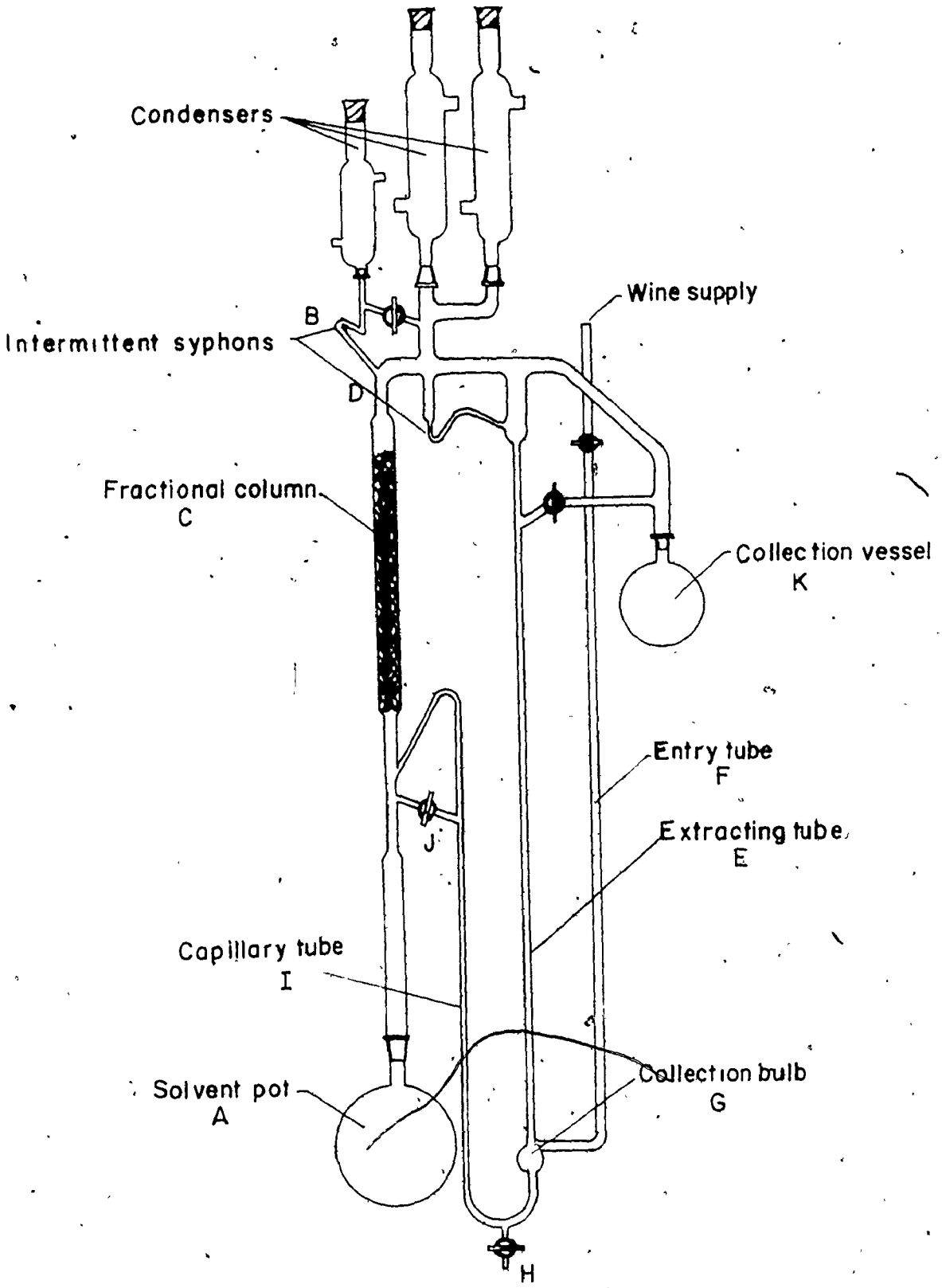
Because it was desirable to obtain a range of samples which were representative of those encountered in the wine industry, grape juice and wine samples were obtained from Andre's Wines Limited in Winona, Ontario. The history of these samples was provided by the winery as were instructions for their transport and handling.

(b) Isolation of the Volatile Components From the Sample

A liquid-liquid extractor of new design was developed specifically for use with the solvent trichlorofluoromethane. This solvent extractor is shown in Figure 2 .The commercial name of trichlorofluoromethane is "Freon 11" and henceforth in this thesis it will be referred to as Freon. All Freon used throughout this research was redistilled by low temperature, vacuum distillation prior to its use.

FIGURE 2

Solvent Extractor


Condensers
Wine supply
B
Intermittent syphons
D
Fractional column
C
Collection vessel
K
Entry tube
F
J
Extracting tube
E
Capillary tube
I
Collection bulb
G
Solvent pot
A
H

The extractor is based on the continuous downwards displacement of Freon beads through the sample performing a liquid-liquid extraction. The extractor was built from Pyrex glass tubing and was operated as follows: a warm water bath (35°C) is placed under the solvent pot A which initially contains Freon (boiling point 24.1°C) and an egg-shaped magnetic stirrer. Vigorous stirring is maintained by means of a heater-stirrer placed underneath the water bath. The resultant Freon vapour then condenses into the intermittent syphons - the reflux intermittent syphon B providing reflux solvent to the fractional distillation column C and the extracting intermittent syphon D providing a volume of Freon sufficient to form a single bead of Freon in the extracting tube E. The latter is filled with the sample (juice or wine) through the entry tube F which leads to the sample supply. The Freon bead falls through the length of the extracting tube into the collection bulb G at its base. The contents of the bulb can be withdrawn through the stopcock H. As consecutive Freon beads fall, the Freon and extracted solutes flow into the solvent pot via the capillary tube I. The stopcock J determines the height of the Freon in the capillary tube and consequently the height of the sample in the extracting tube. The extracted solutes collect in the solvent pot and this warm Freon solution undergoes fractional distillation in the column C enabling distilled Freon to recycle and re-extract the sample. The column is 50 cm long and 2.5 cm in diameter and is packed with 3 mm glass helices. The extracted sample flows out of the top of the extracting tube into the collection vessel K at a rate determined at the sample inlet.

Most design and operational parameters were experimentally determined (being functions of the exact solvent pair and solutes involved) as follows: the extracting tube internal diameter of 10.0 mm; the height of the capillary tube which determines the sample level in the extracting tube being 86 cm for extracting wine and 107 cm for extracting juice; the volume of the extracting intermittent syphon of 1.15 ml and the point of entry of this syphon on the extracting tube so single stable beads of Freon form; and the setting of the reflux ratio for the fractional distillation column.

Note that the operation of the extractor is possible with continuous flow of sample or in a stationary mode with a sample volume equivalent to 95 ml, the volume of the extracting tube, and pressurized air in the entry tube with the stopcock leading to the sample tube closed.

In order to evaluate the efficiency of this extraction technique, standard solutions of certain typical volatile components in 12% ethanol were prepared in the following concentrations: 2.0 ppm, 20.0 ppm, and 200 ppm by dissolving the appropriate amount of each component as measured by a microlitre syringe in 95% ethanol and diluting with water to 12% ethanol.

The components chosen for the standard solutions were: 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octanol, diethyl succinate, and 2-phenethanol. These compounds represent the range of volatilities that were likely to be of interest.

The standard solutions were subjected to solvent extraction with the extractor operating in the stationary mode as follows:
(i) 95.0 ml of the 200 ppm standard solution was introduced to the extracting tube and 400 ml of Freon added to the solvent pot and collection bulb. The solvent pot was then heated to 35°C by a water bath. As consecutive Freon beads descended the extracting tube, the contents of the collection bulb were withdrawn through stopcock K and labelled according to which beads they represented. These bead samples were then submitted to gas chromatographic analysis to measure the concentration of solutes in each. The data thus obtained provided a monitoring of the concentration of solutes with time and an estimate of the completeness of the extraction with time. This procedure was duplicated using the 20.0 ppm standard solution and repeated for both standard solutions. (The results of this study are given in Tables 2 and 3, pages 32 and 33.)
(ii) To determine the amount of solutes carried over by the Freon past fractional distillation column, 250 ml of standard solutions of the same typical volatile components in Freon were placed in the solvent pot and heated at 35°C for three hours with all extracting conditions being duplicated, except no solution occupied the extracting tube. The Freon distillate condensed into the extracting tube and after three hours it

was removed and enriched 100-fold using the low-temperature, vacuum concentrator (described in the next section) and submitted to gas chromatographic analysis. This procedure was carried out for both a 200 ppm and a 20.0 ppm standard solution. Both of these standard solutions were made by dissolving 20.0 µl each of 1-octanol , 2-methyl-1-propanol, 3-methyl-1-butanol, 2-phenethanol, and diethyl succinate in 100.0 ml of Freon (200 ppm) or 1000.0 ml of Freon (20.0 ppm) in volumetric flasks.

For both studies the solvent pot solution was diluted after three hours to its original volume and submitted to gas chromatographic analysis for comparison with a sample of the appropriate standard solution.

For all tests such as this using standard solutions and gas chromatographic analysis, the peak areas were measured using the 'cut and weigh' method. The reproducibility of this measurement procedure from injection of sample through to peak area determination was checked by repeated injections of the same sample. (The results of these studies are shown in Tables 4 and 5, pages 35 and 37.)

(c) Enrichment of the Volatile Components

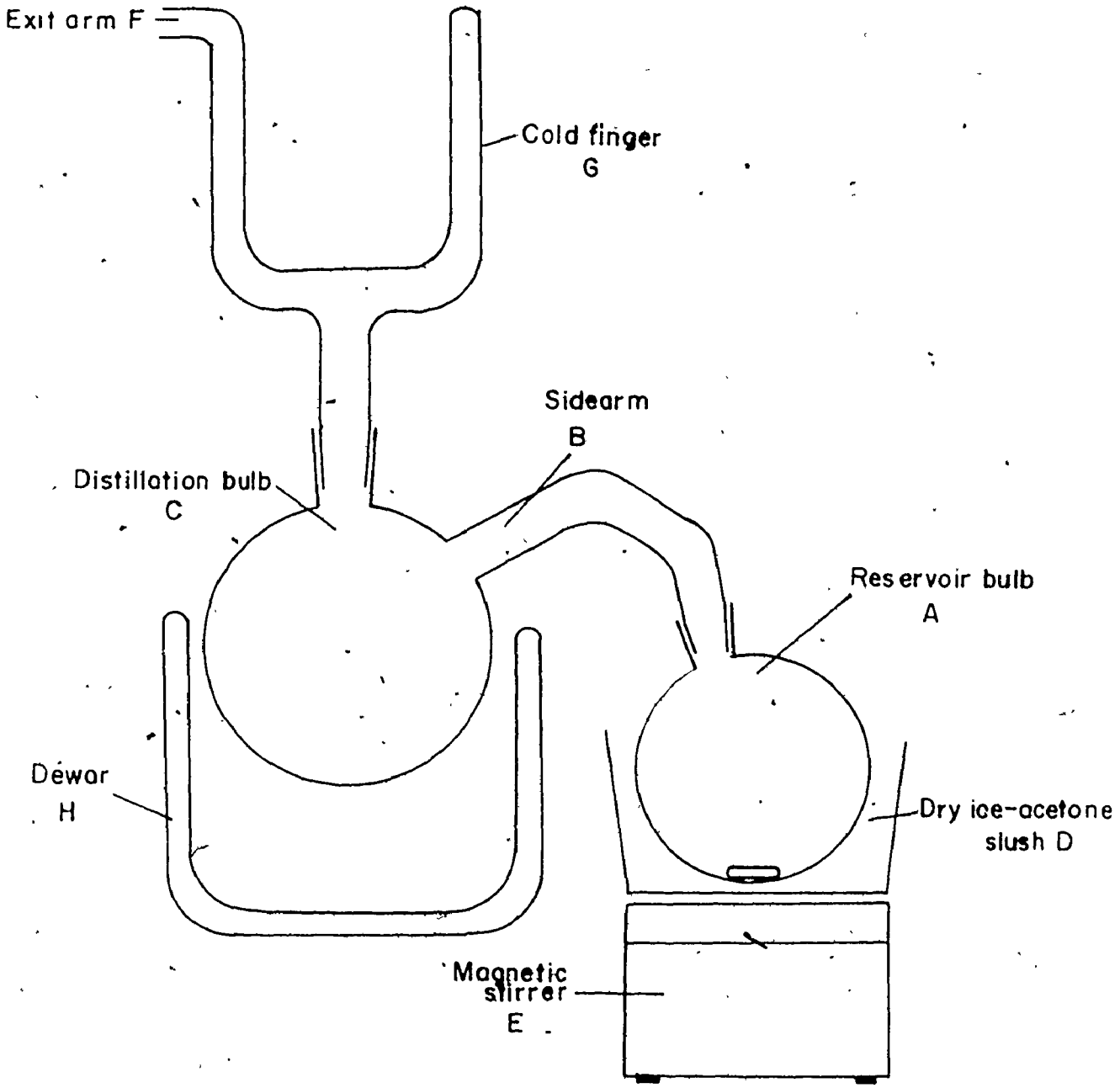
A new design of concentrator was developed which takes advantage of the unusually high vapour pressure of Freon at low temperatures.

The apparatus shown in Figure 3 was designed and built from Pyrex glass tubing for performing low-temperature, vacuum distillations.

The standard procedure for operating this apparatus was as follows: the Freon solution containing the isolated volatile components was placed along with a 2 cm egg-shaped magnetic stirring bar in a 500 ml round-bottomed flask equipped with a B24/40 female, ground glass joint. This reservoir bulb A and its contents were frozen in liquid nitrogen then connected via the ground glass joint to the sidearm B of the distillation bulb C, and cooled from below by a dry ice-acetone slush D (at -78.1°C) which sits atop a magnetic stirring motor E. The entire system was then pumped out via the exit arm F which was connected to a mercury diffusion pump. Once the contents of the reservoir bulb had melted, liquid nitrogen was poured into the cold finger G and the dewar H which surrounded the distillation bulb. This low temperature (-196°C) resulted in the condensation at the glass wall of the Freon vapour which was then boiling out of the reservoir bulb while the Freon solution was

FIGURE 3

Vacuum Distillation Device


Exit arm F
Cold finger
G
Sidearm
B
Distillation bulb
C
Reservoir bulb
A
Dewar
H
Dry ice-acetone
slush D
Magnetic
stirrer
E

being vigorously stirred to prevent the formation of a concentration gradient at the solution's surface. The distillation was continued until the desired final volume in the reservoir bulb was reached. At this time the pumping and all cooling were discontinued and the reservoir bulb was disconnected. Once this bulb and the enriched fraction of interest had warmed to room temperature, the enriched solution was removed by a cooled microlitre syringe, followed by a few Freon rinsings of the bulb to ensure complete transfer of the solution to a calibrated tube allowing accurate measurement of the amount of enrichment. The distilled Freon was allowed to thaw and stored for redistilling.

In order to test the suitability of this technique for enrichment of the isolated volatile components in the range of concentration desired (approximately 100- to 500-fold), 250.0 ml of a standard solution of some typical volatile components in Freon were concentrated to 0.5 ml then diluted back to the original volume with fresh Freon. Then the original standard solution, the diluted concentrate, and distillate were all submitted to gas chromatographic analysis. This procedure was carried out for both the 20.0 ppm and 200 ppm standard solution of ethanol, 1-propanol, 2-methyl-1-propanol, and 3-methyl-1-butanol in Freon. (The results of this study are given in Table 6 , page 43.)

(d) Separation into Individual Volatile Components

Separation of the enriched fraction of interest into individual components in order to allow their identification was performed gas chromatographically using a 15 foot long by 1/8 inch in diameter stainless steel column packed with 80/100 mesh Chromosorb W (AW/DMCS) coated with 5 Carbowax 20 M. This column was used in a Varian 1800 gas chromatograph equipped with twin flame ionization detectors, one being preceded by a 9:1 stream splitter (exit port: detector). A temperature program was utilized going from 40°C initially in 6°C per minute increments until holding at 200°C. The carrier gas used was helium, flowing at 25 ml/min while the flame mixture was hydrogen (25 ml/min) and air (250 ml/min). Both the injector and detector port were kept at 225°C. The standard injection volume was 5.0 µl of sample. Detector output was recorded on a Leeds and Northrup recorder Model Speedomax H operating at a paper speed of 1.0 cm/min.

(e) Quantitative Analysis of the Separated Components

The conventional flame ionization detector was used for quantitative detection operating under the conditions described on page 20.

In order to check the linearity of the response of the flame ionization detector and spectral recorder system with sample size, a series of injections of different volumes of a standard solution was made under the standard operating conditions of the gas chromatograph. A 1000 ppm solution of hexyl acetate, pentyl butyrate, and 1-octanol was prepared by injecting 10.0 µl of each compound into 10.0 ml of Freon. The injection volumes were 0.2, 2.0, 4.0, 8.0, 20.0, 30.0, and 60.0 µl which represented the response range of the detector-recorder system likely to be utilized. (The results of the study are shown in Table 9, page 55.)

The areas of the peaks in the gas chromatographic spectra were measured using the Dupont 310 Curve Resolver. The same four peak envelope was resolved and integrated for all spectra at the same time while the fifth channel was always occupied by a selected reference peak which represented 100% area. Once a single envelope of four peaks was resolved and integrated for all spectra then another four peak envelope was analyzed. Frequent adjustment to the offset integrator drift was made as necessary using the 100% area reference peak.

The relative peak areas provided by this technique were then normalized for all Concord spectra using the peak area of methyl anthranilate as the internal standard.

(f) Reproducibility of the Routine Analysis Technique

In order to obtain a check on the reproducibility of the technique of routine analysis from solvent extraction through quantitative analysis, the following procedure was carried out.

95.0 ml of the 20.0 ppm standard solution of typical solutes in 12% ethanol (refer to page 17) was introduced to the extracting tube and 250 ml of Freon added to the solvent pot and collection bulb. The solvent pot was then heated to 35°C by a water bath. The standard solution was then extracted for three hours at which time the contents of the solvent pot were concentrated to less then 1 ml using the low-temperature, vacuum concentrator and diluted to 10.0 ml with Freon. The resultant

concentrate was then submitted to gas chromatographic analysis and the peak areas measured by 'cut and weigh'. This procedure was repeated twice more for the 20.0 ppm standard solution.

The same procedure was carried out three times (each) for the 200 ppm and the 2.0 ppm standard solutions (12% ethanolic) of 2-methyl 1-propanol, 3-methyl-1-butanol, 1-octanol, 2-phenethanol, and diethyl succinate. In order that the final solution be in the 200 ppm range for gas chromatographic analysis, the concentrate was diluted to 95.0 ml for the 200 ppm solution and to 1.0 ml for the 2.0 ppm solution. (The results of this study are given in Table 7 , page 51.)

(g) Identification of the Separated Components

Identification of the separated volatile components was done by means of gas chromatographic retention times and mass spectral analysis.

The former procedure involved the addition of a standard chemical to the concentrated wine extract. Injection of this combination into the gas chromatograph under standard operating conditions then allowed the observation of whether any peak in the wine extract spectrum matched the standard chemical in retention time. This was repeated for approximately 30 different compounds all of which had previously been reported in wine extracts.

In order to perform mass spectral analysis, manual collection of samples from the exit port of the gas chromatograph's channel equipped with a 9:1 stream splitter (exit port:detector) was employed. These samples were then introduced into a Hitachi RMU-6A mass spectrometer.

The technique used for the mass spectral analysis was a follows: the sample was trapped upon exiting the gas chromatograph in an 8 cm length of 2 mm (inside diameter) glass tubing which fit over the exit port and which was cooled by a tissue soaked with liquid nitrogen to ensure condensation of the gaseous sample on the tube. After warming sufficiently to allow removal of condensed water from the outside of the collection tube, the tube was placed inside another tube containing helium gas at atmospheric pressure and liquid nitrogen temperature ($-196^{o}C$). This outer tube was equipped with a B10/19 ground glass joint which allowed it to be connected to the mass spectrometer. After all gases were pumped out of the system, the tubes were allowed to warm to

room temperature and any water vapour introduced with the sample was pumped off. Then a tissue soaked in liquid nitrogen was wrapped around the glass entry tube leading to the mass spectral source just before the sample leak and the valve leading to the pump was closed to prevent further pumping. Sufficient time was allowed to enable the sample to evaporate from the collection tube and condense at the site of the cooled glass entry tube. All valves preceding this condensation site were then closed to ensure the expansion of the sample as it re-evaporated into a minimum volume. Temperatures throughout the mass spectrometer were then maintained at 90°C including the glass entry tube which was wrapped with heating tape following the evaporation of the sample. For certain components it was necessary to maintain the temperature in the glass entry tube at a higher temperature than 90°C in order to observe a spectrum. The actual temperature required varied with the volatility of the sample but never exceeded 200°C. The ionizing voltage of the source was maintained at 70 eV.

This technique was tested by injecting standard solutions of known compounds in Freon into the gas chromatograph. The separated components were then collected and their mass spectra obtained using the technique described above. These mass spectra were then compared with the corresponding literature values.

A library of mass spectra of compounds which have previously been reported in wine extracts was compiled by consulting the literature. Wherever possible, a spectrum of each compound was obtained using the technique described previously of collection and mass spectral analysis.

The mass spectra of the individual peaks of the concentrated wine extract were obtained by injecting 10.0 µl of concentrate into the gas chromatograph and following the previously described technique of collection and mass spectral analysis. These spectra were then compared with the library of mass spectra for a possible match. (The results of the identification procedures are given in Table 10, page 56a.)

(h) Calibration of Gas Chromatograph

For those components of the wine extract whose identities were known and which were available, 1000 ppm standard solutions of the component in Freon were prepared by injecting 10.0 µl of the component into

10.0 ml of Freon. Then 2.0 µl of the standard solution was injected into the gas chromatograph which was operating under the standard conditions of routine analysis (refer to page 20). The resultant peak's area was measured using the Dupont 310 Curve Resolver, allowing calculation of a ppm in wine per relative peak area calibration factor for each component whose identity was known and which was available. (The results of this study are given in Table 10, page 56a.)

(i) Extraction of Carboxylic Acids

In order to ascertain whether certain carboxylic acids would be extracted from wine by Freon, the following test was conducted. A 50.0 ppm standard solution of acetic acid, 1-butanoic acid, 1-hexanoic acid, 1-octanoic acid, and 1-decanoic acid in 12% ethanol was prepared by injecting 12.5 µl of each acid in 30.0 ml of ethanol and diluting this with distilled water to 250.0 ml in a volumetric flask. 100 ml of this 50.0 ppm solution was then shaken in a 250 ml separatory funnel with 100 ml of Freon for 10 minutes. The Freon solution was then collected and concentrated at 0°C by rotary evaporation to 1 ml. 5 µl of this concentrate was then injected into the gas chromatograph and the resultant spectra checked for the presence of any of the acids found in the standard solution.

(j) Distribution Ratios of Certain Solutes

In order to obtain approximate values of the distribution ratios of some volatile components between 12% ethanol solutions and Freon at 20°C, 100.0 ml of the 200 ppm standard solution of 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octanol, diethyl succinate, and 2-phenethanol in 12 ethanol were shaken vigorously in a 250 ml separatory funnel with 100.0 ml of Freon for 10 minutes. Both solutions were then separated and submitted to gas chromatographic analysis. (The results of this study are shown on page 41.)

B. Application of the Techniques of Analysis

Having developed a satisfactory method of routine analysis of the volatile components in grape juice and wine, it was decided to use this method for a comparison of the change in concentration of these volatile components during fermentation. The first comparison was between the two basic types of wine grapes grown in Ontario: Vitis labrusca and hybrids of Vitis labrusca and Vitis vinifera. Concord represented the former type and Blue Hybrid the latter. The second comparison was between a juice fermented at a relatively low temperature, 20.0°C, and a juice fermented at a relatively high temperature, 28.0°C. These comparisons necessitated four different fermentations to be done and it was decided to do a fifth fermentation which was identical in all respects to one of the others in order to provide an indication of the reproducibility of the entire procedure - fermentation and analysis. The exact proportions of grape juice and additives used were provided by the winery as well as all materials needed. The winery provided complete instructions for conducting the fermentations from start to finish. A description of the procedure followed throughout these studies is found below.

(a) Concord Fermentation Study

On Tuesday, October 12, 1976 (Day 0) approximately 10 gallons of freshly crushed Concord juice were removed from a fermentation tank at the winery prior to any additions. Following immediate transportation to to the laboratory, the juice was divided between two 5 gallon Pyrex glass fermentation vessels, labelled A and B, which both stood in a 20-gallon polyethylene tank filled with water at room temperature (19.5°C). To each fermentation vessel was added 4.0 g of diammonium phosphate, 2.0 g of potassium metabisulfite; 0.40 g of Montrachet Green-Star yeast, and sufficient liquid invert sugar to raise the specific gravity of the must to 21.0°Brix as measured on a Brix hydrometer (with enclosed thermometer and correction scale).

The yeast had previously been softened for 5 hours in a 400 ml portion of juice at 35°C.

The pH of both musts was determined by a pH meter. All pH

measurements were done using a Beckman Zeromatic II pH meter equipped with a Beckman #41623 glass electrode and a silver-silver chloride reference electrode.

A glass fermentation lock containing a 100 ppm aqueous solution of potassium metabisulfite was placed in the neck of both fermentation vessels. A length of glass tubing penetrated this lock in order to allow sampling of the must quickly and easily. During sampling, the glass tubing was joined by Tygon tubing to a 250 ml Erlenmeyer flask which in turn was connected to an aspirator. Aspiration was continued until approximately 150 ml of must had entered the flask at which time a pinch clamp sealed off the Tygon tubing.

The following standard procedure as well as the sampling technique described above was used for all sampling and analyzing for all five fermentations.

The must sample was filtered through a Buchner funnel lined with Whatman No. 1 filter paper which was covered with a 1 cm layer of Kieselguhr (Baker TLC). When the filtration was complete, the clarified must was carefully poured into a glass cylinder and a soluble solids reading taken using the Brix hydrometer after allowing sufficient time for equilibration. The pH of the clarified must was then measured. Then 95.0 ml of the must were then carefully measured using a graduated cylinder and added to the entry tube of the solvent extractor. 20 ml of Freon had previously been added to the collection bulb of the extractor. Compressed air then was used to force the must into the extracting tube. 230 ml of Freon and an egg-shaped magnetic stirring bar were placed inside a 500 ml round bottomed flask equipped with a B24/40 ground glass joint and connected to the solvent extractor as the solvent pot. A 35^{o}C water bath was then placed around the solvent pot supported by a magnetic stirrer-heater. Brisk stirring of the solvent pot was continued throughout the extraction as was the circulation of cold water through all condensers. After 3 hours of solvent extraction, the heating was stopped and the Freon soluble fraction collected in the solvent pot. This flask was disconnected from the extractor and placed in liquid nitrogen in order to freeze the sample. Then, in order to concentrate the isolated volatile components of the sample, the flask was connected to the

low-temperature, vacuum distillation device in the reservoir bulb position and the contents were allowed to melt. A dry ice-acetone slush was placed around this bulb and a liquid nitrogen dewar surrounded the distillation bulb. The cold finger was filled with liquid nitrogen and the exit arm connected to the mercury diffusion pump. Vigorous stirring of the reservoir bulb (still containing the egg-shaped magnetic stirring bar) was maintained throughout the distillation. When visual inspection indicated less than 0.5 ml of concentrate remained in the reservoir bulb, this bulb was disconnected and the cooling discontinued. The concentrate was removed from the bulb by a 100 µl syringe and placed in a calibrated glass tube. The inside of the bulb and the stirring bar were rinsed thoroughly with Freon and the rinsings added to the concentrate. The concentrate was then diluted with Freon to the 0.50 ml mark. The concentrate was then transferred via a cooled 1.0 ml syringe to a gas chromatographic screw cap septum vial. From this vial was removed a 5.0 µl sample for injection into the gas chromatograph using a 5.0 µl syringe. The column used and other conditions of the gas chromatograph are given on page 20. The spectrum was recorded and required manual scale-changing to ensure all peaks were on scale. The spectrum was then stored for later curve resolution and integration while the sample vial was stored at -15°C for future reference.

This procedure was repeated for the Concord fermentations appropriate to the rate of fermentation as determined by the Brix hydrometer readings. For the first 7 days of the fermentation the rate necessitated daily sampling but, as this rate decreased, less frequent sampling was necessary.

The Concord fermentation was allowed to progress without interference except for this sampling procedure and frequent monitoring of temperature. Following winery instructions the two musts were first racked at Day 10 of the Concord fermentation at which time the fining agent, bentonite, was added to 750 ppm as well as an additional 50 ppm of potassium metabisulfite. On Day 21 of the Concord fermentation, the two musts were racked a second time followed by the addition of 400 ppm of bentonite and 20 ppm of potassium metabisulfite. The headspace above the must in both fermentation vessels was purged with carbon dioxide

after each sampling.

The sampling and analysis was discontinued on Day 43 for vessel B and on Day 45 for vessel A.

(b) Blue Hybrid Fermentation Study

On Tuesday, October 19, 1976 (Day 0) approximately 5 gallons of freshly crushed Blue Hybrid juice were removed from the fermentation tank at the winery prior to any additions. Upon transportation to the laboratory the juice was poured into a 5-gallon Pyrex glass vessel, labelled H. This vessel was placed in a 20-gallon polyethylene water bath at room temperature (19.5°C). Identical quantities of the same additives as the Concord musts received were added to the Blue Hybrid must: 4.0 g of diammonium phosphate, 2.0 g of potassium metabisulfite, 0.40 g of Montrachet Green Star yeast, and sufficient liquid invert sugar to raise the specific gravity of the must to 21.0°Brix. Again the yeast had been softened prior to its addition. The volume of the must at this time was 19.5 l and the pH =3.18.

The sampling and analysis procedure was carried out on the Blue Hybrid must twice daily for the first 3 days during the most rapid fermentation period. For the next 14 days daily sampling and analysis was conducted after which time the frequency of sampling and analysis was decreased. The sampling and analysis procedure used for the Blue Hybrid fermentation was identical to that described for the Concord fermentations in all respects. The Blue Hybrid fermentation was allowed to progress without interference as had the Concord fermentations. The temperature was frequently monitored. On Day 16 the Blue Hybrid must was first racked and 750 ppm of bentonite was added along with 75 ppm of potassium metabisulfite. A second racking was done on Day 20. As was done for the Concord fermentations, the headspace above the Blue Hybrid must was purged after sampling with carbon dioxide once this became necessary. The Blue Hybrid sampling and analysis was ceased after Day 42 of its fermentation.

The final addition of the potassium metabisulfite for both the Concord and Blue Hybrid fermentations was based on a free SO_2 analysis by the winery of both musts after both fermentations were virtually complete. The levels of free SO_2 in all musts were then adjusted to 35 ppm.

(c) Fermentation Temperature Study

In order to investigate the effect of fermentation temperature on the growth of the volatile components in the must during fermentation, it was decided to ferment simultaneously two identical volumes of Concord grape juice with the only difference being the fermentation temperature.

On Monday, November 15, 1976 approximately 10 gallons of Concord grape juice were obtained from the winery. These grapes had been crushed about October 14, 1976 and the juice stored in stainless steel tanks at 12°C in order to retard fermentation. This juice was equally divided between two 5-gallon Pyrex glass fermentation vessels and the following additions were made to each vessel: 4.0 g of diammonium phosphate, 2.0 g of potassium metabisulfite, 0.4 g of Montrachet Green Star yeast, and sufficient liquid invert sugar to raise the specific gravity of the must to 21.5°Brix. As had been done previously, the yeast was softened prior to its addition. The volume of both musts at this time was 19.0 l and the pH of each equalled 3.10.

One vessel, marked K, was placed in a 20-gallon water bath at 20.0°C. The other vessel, marked W, was placed in a 20-gallon water bath at 28.0°C. Both water baths were equipped with a thermostat, stirrer, and heater which allowed only a ± 0.1°C variation in water temperature. The contents of both vessels were allowed sufficient time in the baths to equilibrate before the yeast was added. It was necessary to use a 10 foot stainless steel cooling coil with cold water circulating through it to achieve proper temperature stability in the 20.0°C water bath.

The sampling and analyzing procedure used for both of these musts was identical to that described previously on page 26. Because of the faster initial fermentation rate of vessel W it was sampled more frequently until the rate of fermenatation of vessel K surpassed that of vessel W at which time vessel K was more frequently sampled. When the rate of both fermentations became less then 0.5°Brix per day, the sampling frequency was reduced (Day 8). Once the rate of fermentation of vessel W became less then 0.1°Brix per day the temperature of its water bath was reduced to 20.0°C. This was at Day 4 when the specific gravity

of vessel W equalled -0.5°Brix. After this time the temperature of both water baths was the same. On Day 6 must W was first racked and 750 ppm of bentonite added as well as 75 ppm of potassium metabisulfite. The identical treatment was given to must K on Day 10. A second racking was done for both vessels on Day 16 and 400 ppm of bentonite added to each. When necessary, the headspace above both musts was purged with carbon dioxide after sampling. The final sampling and analysis of both must K and must W was done on Day 50.

For all fermentations described in this chapter sampling was carried out at the same time each day in order that the time interval between samples be consistent.

CHAPTER 3

RESULTS AND DISCUSSION

A. Techniques of Analysis

Because of the sensory importance attributed to the volatile components of wine (22, 27, 29), it is of interest to monitor the change in concentration of these components which occurs during the fermentation process. Such monitoring could yield data that might contribute to the understanding of their possible origins. Thus the source of differences in the volatile components between wines made from different grapes or under different fermentation conditions might be better understood. Considering how rapidly the fermentation process can progress in a 24-hour period, the technique used for monitoring the concentration of the volatile components during the fermentation must be quantitatively performed in a few hours. Such a technique is henceforth referred to as a technique for routine, quantitative analysis.

Each stage in the analysis of the volatile components described on page 14 was then considered with respect to the suitability of the available techniques for routine, quantitative analysis. Special attention was focused on the most time-consuming stages in the analysis. These were considered to be the isolation stage and the enrichment stage. The techniques described in the previous chapter that were used for the routine, quantitative analysis of wine and must are discussed in the following sections.

(a) Sample Collection

The acquiring of samples from the winery was beneficial in allowing access to established wine technology and in assuring as much as possible that industrial-type samples were being dealt with.

(b) Isolation of the Volatile Components From the Sample

The new design of the solvent extractor was subjected to certain tests to see if it met the criteria for routine, quantitative analysis.

Tables 2 and 3 show the results of the tests using standard solutions of selected solutes in 12% ethanol to monitor the concentration

TABLE 2

Monitoring of Solute Concentration in Solvent Extraction of 200 ppm Standard Solution

Solute	Relative Concentration[a] of Solute in Bead Number[b]													
	5	10	15	20	25	30	35	50	80	105	150	200	250	300
2-methyl-1-propanol	1.6	1.8	1.9	1.7	1.8	1.8	1.6	1.6	0.8	0.9	0.9	0.4	nd[c]	nd
3-methyl-1-butanol	4.3	4.4	4.5	3.8	3.9	3.8	3.2	2.6	1.1	1.0	0.7	0.3	nd	nd
1-octanol	7.8	10.1	9.5	7.3	7.3	6.3	4.4	2.8	0.7	0.3	0.2	nd	nd	nd
diethyl succinate	6.2	5.7	5.9	4.5	4.6	4.1	3.0	1.9	0.5	0.4	0.3	nd	nd	nd
2-phenethanol	2.9	2.7	2.9	2.7	2.7	2.7	2.5	1.9	0.5	0.3	0.1	nd	nd	nd

[a]Values refer to gas chromatographic peak height (cm) allowing comparison within a given solute only.

[b]Concentration listed for bead n is the average value of the sample which includes beads n-5 to n.

[c]nd=not detectable. The minimum detectable peak height was 0.1 cm.

TABLE 3

Monitoring of Solute Concentration in Solvent Extraction of 20.0 ppm Standard Solution

Solute	Relative Concentration[a] of Solute in Bead Number[b]									
	3	7	11	15	19	50	75	100	125	150
2-methyl-1-propanol	0.3	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.1	nd[c]
3-methyl-1-butanol	0.8	0.6	0.6	0.7	0.8	0.5	0.3	0.2	0.1	nd
1-octanol	1.1	1.0	0.8	1.1	1.0	0.3	0.1	nd	nd	nd
diethyl succinate	0.6	0.6	0.5	0.7	0.6	0.3	0.1	nd	nd	nd
2-phenethanol	0.3	0.3	0.3	0.3	0.3	0.1	nd	nd	nd	nd

[a]Values refer to gas chromatographic peak height (cm) allowing comparison within a given solute only.

[b]Concentration listed for bead n is the average value of the sample which includes beads n-3 to n.

[c]nd=not detectable. The minimum detectable peak height was 0.1 cm.

of solutes as a function of the number of Freon beads descended through the solvent extractor. It is apparent that the concentration of any single solute in the beads remained constant until a time at which a gradual decrease in concentration began and continued until the level of solute in the beads was not detectable. Note that by the time 250 beads had passed through the 200 ppm standard solution, all solutes were no longer detectable. This concentration was chosen for study because it represented the level rarely exceeded by even the most abundant volatile components in wine. For the 20.0 ppm standard solution extraction all solutes were undetectable after 150 beads had descended. 20 ppm is a typical concentration value of many volatile components in wine.

An estimate of the time required for complete extraction of the volatile components from wine using this extractor can be made from this data. It was found that the rate of beads descending the extracting tube was 4 beads per minute (averaged over one hour) when the water bath was at 35°C. This rate was a function of the water bath temperature, the stirring rate in the solvent pot, and the surface area in the solvent pot. Thus the time required for 250 beads to descend through a sample was 63 minutes, at which point even those volatile components originally at 200 ppm in the sample should have been extracted. This figure suggested that the new design of solvent extractor was fast enough for routine analysis and only needed to be proven quantitative.

Table 4 shows the results of mass balance studies using standard solutions of selected solutes in Freon as the solvent pot solution, while measuring the amount of solutes which distilled over with the Freon past the fractional distillation column. The level of solute which distills over determines the limiting concentration at which solutes would no longer be extracted from a sample. Note that the highest concentration of solute in the distillate was 0.60 ppm for 2-methyl-1-propanol with the 200 ppm standard solution in the solvent pot. This corresponds to 0.3% of the solvent pot concentration in the distillate. The concentration of solutes in the solvent pot solution (diluted to its initial volume) after 3 hours of extraction was the same as the concentration of the solutes in the original standard solution within the experimental precision of the analysis technique. This was true for both the 20.0 ppm and the 200 ppm

TABLE 4

Analysis of the Recycled Distillate in the Solvent Extraction Apparatus

Solutes	Concentration (ppm) of Solute in Distillate[a]	
	200[b]	20.0[c]
2-methyl-1-propanol	0.60	0.04
3-methyl-1-butanol	0.40	0.04
1-octanol	0.30	0.03
diethyl succinate	0.20	0.03
2-phenethanol	0.08	0.01

[a] Solvent pot contents showed an undetectable loss of solutes for both studies

[b] 200 ppm standard solution was in solvent pot

[c] 20.0 ppm standard solution was in solvent pot

standard solution. The experimental precision of the gas chromatographic analysis using the 'cut and weigh' method of peak measurement is shown in Table 5.

The levels of solutes which distilled over during the extraction, as measured by this mass balance study, indicated this method of solvent extraction was sufficiently quantitative. Consequently this technique of isolating the volatile components was chosen as a stage in the routine, quantitative analysis. Because it is a new design of solvent extractor, some discussion of its behaviour is appropriate.

This new design of solvent extractor was developed specifically for use with Freon. The extractor is based on the continuous downwards displacement of Freon beads through the sample performing a liquid-liquid extraction. If the sample to be extracted is restricted to a cylindrical length of glass tubing of appropriate internal diameter and an appropriate volume of Freon is introduced at the top of the sample, a single plug or bead of Freon forms of an internal diameter only slightly less than that of the glass tubing and falls through the sample to the bottom. Note that this design of extractor does not resemble conventional downwards displacement extractors which maximize the solvent surface area exposed to the sample by minimizing the solvent drop size. A feature of this design is that the sample is confined to a cylindrical tube through the entire length of which each Freon bead falls. As only a thin film is between the bead and the glass wall, every individual Freon bead can potentially extract the entire volume of sample. This is unlike conventional downwards displacement extractors where each tiny solvent bead interacts with only a very small fraction of the sample. This new design of solvent extraction can be considered in relation to a simple batch extraction for which the expression for the percentage extraction, %E, of a solute from solvent A into solvent B is (73):

$$\%E = 100\, D^{-1}/[D^{-1} + (V_A/V_B)] \qquad (1)$$

Here V represents volume and D is the distribution ratio of the solute between the solvents A and B. The latter is a constant for the specific solute and solvents concerned and equals the ratio at equilibrium of the concentration of the solute in solvent A to the concentration of the solute in solvent B, the extracting solvent.

TABLE 5

Determination of Average Deviation

in Repeated Gas Chromatographic Analyses

Injection	Relative Chromatographic Peak Areas[a]				
	2-methyl-1-propanol	3-methyl-1-butanol	1-octanol	diethyl succinate	2-phenethanol
#1	19.7	21.2	18.1	10.2	13.2
#2	18.0	20.8	17.2	9.7	12.6
#3	19.1	21.1	19.0	10.6	14.5
#4	19.3	22.0	18.9	9.8	14.0
Average	19.0	21.3	18.3	10.1	13.6
Percentage average deviation	2.3	1.8	3.6	3.0	5.0

[a] Repeated injections of 2.0 µl of 200 ppm solutions

One objective of liquid-liquid extraction is to maximize %E. From equation (1) it may be seen that, if the volume of the extracting solvent, V_B, is sufficiently large, this can always be achieved. A further objective however, is to concentrate the extracted solutes and this requires that the volume of the extracting solvent be as small as possible. Recycling the extracting solvent allowed both of these objectives to be achieved and consequently was included in this design of extractor.

For this extractor the total volume of Freon, V_B, is 200 ml. However, due to recycling, the amount of Freon actually exposed to the wine, V'_B, is far in excess of 200 ml (750 ml in a 3 hour extraction) and it is this volume which should be used in equation (1). From equation (1) it can be seen that in principle, if V'_B approaches infinity, then %E approaches 100 . The limiting factor in %E for a given solute approaching 100 is the concentration of that solute in the recycled Freon which must approach zero in order that the recycled extracting solvent be 100 Freon.

Thus, if the limiting concentration of a solute in the recycled Freon, $[solute]^{lim}_{Freon}$, then the limiting concentration of the solute in the wine would be:

$$[solute]^{lim}_{wine} = D \times [solute]^{lim}_{Freon} \qquad (2)$$

where D is the distribution ratio as defined with equation (1). Hence, if $[solute]^{lim}_{Freon}$ goes to zero, $[solute]^{lim}_{wine}$ also goes to zero.

As shown the limit to the efficiency of an extraction such as this, involving recycled solvent, is the purity of the Freon being distilled. This point out the crucial role the fractional distillation column plays in this extractor and why it was included here, in contrast to conventional recycling extractors.

There are two important experimental observations which are apparently important in evaluating this extractor. Firstly it is apparent from studying Table 3, that the concentration of solutes remains constant for at least the first 19 Freon beads which descend and also that the point at which the concentration of solutes in the beads decreases, appears to be approximately the same for four of the five solutes studied. Just as important is the fact all solutes are at undetectable concentrations after

150 beads have extracted the 20.0 ppm standard solution. The fact that the solutes remain at constant levels for the first 19 beads means the beads must be becoming saturated with the solutes during the descent. This saturation could be throughout the entire Freon bead or else be simply in the surface layers. The latter is commonly observed in conventional continuous downwards displacement extractors. Consequently in order to achieve maximum efficiency these devices minimize the drop size. It must be concluded in the new design of extractor that the entire Freon bead is becoming saturated. The evidence for this comes from consideration of a batch extraction equation. If a batch extraction were performed on 95 ml of wine with 1.15 ml of Freon n times in succession, the percentage solute remaining in the wine, %R, is given by:

$$\%R = 100 \left(\frac{95}{\frac{1.15}{D} + 95}\right)^n \qquad (3)$$

where D is the distribution ratio as defined within equation (1) (74). For 3-methyl-1-butanol the value of D is approximately 1.3 as given on page 41. For n = 150 the value of %R is 26%. However Table 3 shows that after 150 beads of Freon (1.15 ml per bead) have descended the solvent extractor that less than 10% of the 3-methyl-1-butanol remains in the wine. The fact that 150 Freon beads in the solvent extractor apparently could extract as efficiently as 150 batch extractions means the entire Freon bead must be becoming saturated. The data for the other solutes in Table 3 and all solutes in Table 2 confirm this conclusion. Visual observation of severe agitation within the Freon beads, as they descend the extracting tube, also suggested stirring within the beads was occuring. This agitation is the result of surface interactions dependent on the diameter of the cylindrical glass tubing. These surface interactions also result in the rate of fall of the Freon bead in the extracting tube being much slower than is observed for drops of this size in the free fall in this medium.

The fact that 150 Freon beads in the new design of extractor apparently extract more efficently than 150 successive batch extractions relates to a second conclusion. Equation (3) for successive batch extractions is based on the fact that mixing of the solutes in the wine occurs and each successive batch extraction removes less solute than did

the preceding extraction. Since the results of Table 3 show the first 19 beads extract a constant concentration of solute, it is concluded that each of these beads is saturated at the initial concentration of the solute in the wine. This can only occur if the bead is saturated before it reaches the bottom of the extracting tube and if no substantial vertical mixing of the solutes in the wine is taking place.

It is seen that the new design of extractor can be more efficient than successive batch extractions.

From the above discussion it seens that this design of solvent extractor is in some ways analagous to column chromatography. The essential features of the latter are that the compounds of interest are retained in the fixed phase and that a mobile phase, which is capable of eluting the compounds of interest, is in equilibrium with the fixed phase during its descent. These features are similar to those of this design of solvent extractor where the lack of substantial vertical mixing of the wine makes it comparable to a fixed phase.

It is apparent from the preceding discussion that the wine is being stripped of the volatile components from the top first. Thus there is the possibility of a countercurrent system where the wine, which is less dense than the Freon, is removed from the top of the extracting tube and unextracted wine introduced at the base. The extractor was operated in this countercurrent mode and apparently performed successfully although it was not characterized in this mode.

This extractor was designed to take advantage of some physical properties of Freon. Firstly Freon has a very high density of 1.494 g/ml at 17.2^{o}C (75). Conversely a typical density of grape juice is 1.090 g/ml at 17.2^{o}C and wine typically has a density of 0.996 g/ml at 17.2^{o}C (76). This difference in densities between Freon and grape juice and wine makes Freon an excellent solvent for a continuous downwards displacement extractor. The downwards displacement of stable Freon beads through the sample is the basis of this design of extractor. The use of an intermittent syphon, which periodically releases Freon of the minimum volume required for formation of a single, stable bead in the extracting tube, makes a downwards displacement design essential.

Secondly, as illustrated by equation (1), the efficiency of any extraction depends on the distribution ratios of the solutes of interest between the sample and the solvent. The favourable distribution ratios for the volatile components between wine or grape juice and Freon has been previously reported (59) as well as experimentally determined for a few components. Using the procedure described on page 24 the following values of the distribution ratio, D, as defined with equation (1) were determined: for 2-methyl-1-propanol, D = 4.3; for 3-methyl-1-butanol, D = 1.3; for 1-octanol, D = 0.55; for diethyl succinate, D = 0.36; and for 2-phenethanol, D = 0.71.

Thirdly, Freon displays suitable solubility characteristics for use in the solvent extraction of both juice and wine, being effectively insoluble in each (59).

Fourthly, as illustrated earlier, the limiting concentration of solutes, which will be extracted from the wine, depends of the ability to separate the previously extracted solutes from the Freon during solvent recycling. This separation is optimized if the difference in volatilities between the solutes and the solvent is optimized. The unusually low boiling point of Freon, 24.1°C, for a compound which is liquid at room temperature is very advantageous (75). The high volatility of Freon allows the use of a 35°C water bath to heat the solvent pot and achieve both adequate vaporization of the Freon for recycling and adequate separation of solutes and solvent.

This new design of solvent extractor is apparently a highly successful method of isolating the volatile components from wine and grape juice. This technique has been shown both very fast and quantitative for the compounds of interest and is superior to conventional techniques. Other studies have concluded solvent extraction to be the most suitable method for isolating the volatile components from wine or grape juice (59). Of those solvent extraction techniques not using the solvent Freon, the quickest one apparently uses a 2:1 mixture of pentane and methylene chloride and has a 12-hour duration (33). It is not reported how quantitative this technique is. An extraction technique which showed good recoveries of the volatile components using n-pentane as a solvent has been reported but required a 240-hour extraction period (59). Another

study concluded that Freon was the most suitable solvent for isolating the volatile components from wine or grape juice (59). Techniques using Freon in conventional continuous downwards displacement extractors have been described which made semi-quantitative recoveries of the volatile components from 10% ethanol solution in 17 hours (59). Thus it is clear that this new design of continuous downwards displacement extractor, using Freon as the solvent to perform a quantitative extraction in about one hour, compares very well with alternative techniques of isolating the volatile components from wine or grape juice. It is necessary that this isolation technique be coupled with a technique of concentrating the extracted components quickly and quantitatively to a level at which they are easily measured in order that the overall analysis be successful. The alternative to concentrating the extracted volatile components (roughly 100-fold concentration is required) would be to extract larger volumes of sample. This would necessitate a proportionately larger total fermentation volume which would be undesirable for controlled laboratory conditions.

Expanding on this basic design of extractor, modifications are possible which might increase its usefulness for this and other applications. There is no reason why the length of the extracting tube could not be increased substantially, so the descending beads would extract a larger volume of solution. Similarly it is conceivable several extracting tubes could be radially arranged around a single solvent pot-distillation column system. Other non-enological solutions requiring rapid, quantitative solvent extraction might also use this extractor such as for the analysis of drugs in blood. This extractor system also can operate as a countercurrent extractor which is desirable for other types of extraction.

(c) Enrichment of the Volatile Components

The concentrator described in the previous chapter was tested by enriching standard solutions of selected solutes in Freon and analyzing the solute concentration in both the concentrate and distillate solutions. These tests were to determine if this technique of vacuum distillation at -78°C met the criteria for a routine, quantitative analysis. The results of these tests are shown in Table 6. For all solutes the peak areas of the original standard solution and the concentrate, diluted back to its

TABLE 6

Percentage of Solutes in Distillate of Standard Freon Solutions for Vacuum Distillation at -78.1°C

Component	Percentage of Original Concentration in Distillate[a]	
	Original Concentration 200 ppm	Original Concentration 20.0 ppm
1-propanol	1.6	1.9
2-methyl-1-propanol	0.9	1.1
3-methyl-1-butanol	0.5	0.5

[a]The concentrates diluted to their original volume showed an undetectable loss of solutes for both studies

original volume, were the same within the experimental precision of the gas chromatographic technique.

Study of Table 6 indicates that losses of solutes in the 0.5 to 2% range can be expected using this technique of concentration with the loss of solute decreasing with decreasing volatility of the solute.

It was concluded that losses of this magnitude were acceptable especially when compared to alternative techniques. The time required to concentrate 250 ml of Freon solution to 0.5 ml was approximately one hour which was quite adequate for a routine analysis. It was decided that this technique of vacuum distillation of the solvent at -78°C would be used in the routine, quantitative analysis of wine and grape juice.

Note that this technique complements the solvent extraction technique in that it also takes advantage of a physical property of Freon. Freon has an unusually low molar heat of vaporization, ΔH^{o}_{vap} (75). How advantage can be taken of this property of Freon is revealed by the Clausius Clapeyron thermodynamic equation (77):

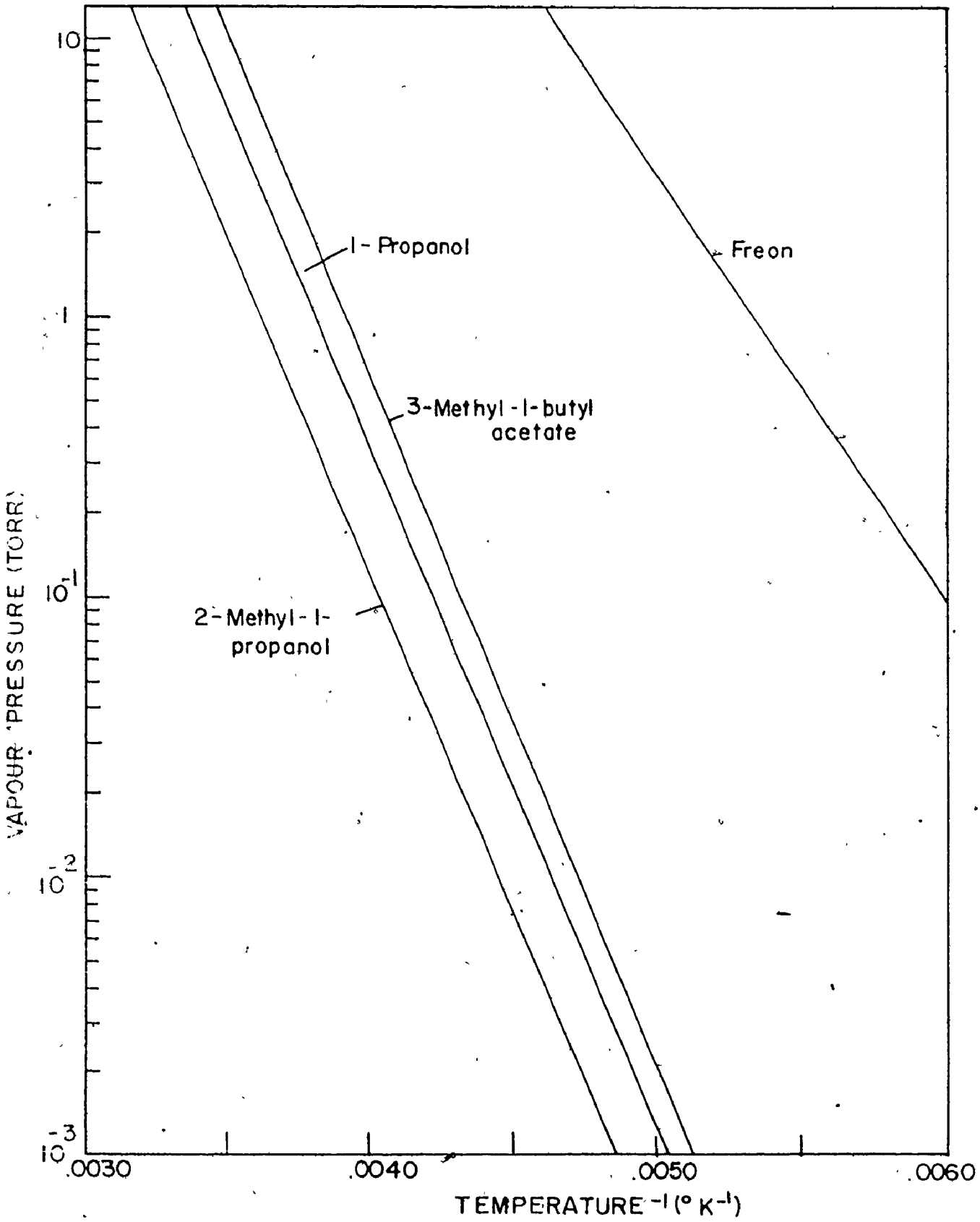
$$\frac{d\ \ell n p}{dT} = \Delta H^{o}_{vap}/RT^{2}$$

where p = vapour pressure, R = gas constant, and T = temperature (°K). This equation shows that, as a result of the low ΔH^{o}_{vap} of Freon, the relative vapour pressure of Freon compared to that of typical volatile components becomes much higher as the temperature decreases. This behaviour is illustrated in Figure 4 .

A few of the operating conditions of the concentrator were experimentally determined. It was found essential that the solution in the reservoir bulb be stirred vigorously, since it was observed that much larger amounts of solutes were distilled over without stirring. This was presumably because, as the Freon molecules entered the vapour phase, the surface layers became depleted of Freon molecules resulting in a concentration gradient with a higher concentration of solutes at the surface of the solution than inside the solution. In accordance with Raoult's Law (78), this would result in a higher vapour pressure of the solutes than would be expected by the average concentration and also result in greater distillation of the solutes. The formation of a surface concentration gradient also determines the limiting volume to which

FIGURE 4

Vapour Pressure Versus Temperature
for Freon and Typical Volatile Components (82)


10
1
10⁻¹
10⁻²
10⁻³
VAPOUR PRESSURE (TORR)
1 - Propanol
Freon
3-Methyl - 1-butyl acetate
2-Methyl - 1-propanol
.0030
.0040
.0050
.0060
TEMPERATURE⁻¹ (° K⁻¹)

the solution could be concentrated. Attempts to use a calibrated tip on the reservoir bulb, which would have indicated the point of sufficient concentration, failed because stirring within the tip seemed unfeasible. The point of sufficient concentration was determined instead by visual inspection. When the concentrate was removed from the reservoir bulb by syringe, it was necessary to cool the syringe with ice in order to prevent loss of the solution through volatilization of the Freon.

This technique seems to compare favourable with alternative techniques of concentrating the isolated volatile components. Fractional disillation techniques require much longer times to accomplish concentrations of this magnitude and the hold-up of any fractional distillation column would need be very small since the final volume of concentrate is only 0.5 ml. Rotary evaporation operated conventionally does not allow low enough pressures to be achieved (10^{-5} atm is adequate for vacuum distillation). There are large losses of volatile components such as 2-methyl-1-propanol and 3-methyl-1-butanol when rotary evaporation is used to concentrate wine extracts at temperatures about $0^{o}C$. There is also substantial difficulty in reducing the concentrate to low volumes such as 0.5 ml without significant losses.

It is quite possible this technique of low-temperature, vacuum distillation could be improved by further modifications. The losses of solutes could be further reduced by using a temperature bath even colder than dry ice-acetone ($-78^{o}C$) but not lower than $-111^{o}C$ which is the freezing point of Freon (75). The time required might conceivably be reduced by expanding the size of the reservoir and distillation bulbs in order to increase the surface area and thus the rate of vaporization If adequately low pressures could be achieved and a reliable procedure developed for concentrating to low volumes, rotary evaporation would likely be preferable to this technique because of increased speed of concentration. Of course it is essential that any such distillation be done at $-78^{o}C$ or lower to minimize losses of solutes. It is conceivable that the principles on which this concentrator is based are applicable to enrichment techniques where solvents other than Freon are used. The requirement for these solvents is that they also have a low molar heat

of vaporization in order to be suitable for low temperature, vacuum distillation. 3-methyl-1-butane is an example of a solvent for which this technique is also applicable.

(d) Separation of the Individual Volatile Components

Unlike the previous two stages of the analysis, there seemed very limited room for improvement of the techniques of separation described in the literature. There was, however, a choice to be made between the two basic types of columns used in gas chromatography which are conventional packed columns or open tubular columns (79). The conventional packed type of column was chosen for the following reasons: (1) because it was decided to collect samples for mass spectrometry from the stream splitter exit port of the gas chromatograph, the packed type of column was favoured since it allowed larger samples to be collected (69); (2) because of the large range of concentrations of solutes in the concentrate that were injected on to the column (roughly 1000-fold), the possibility of the more abundant components flooding the column or the less abundant ones being in undetectable amounts seemed to exist for capillary columns, (3) lacking a recorder with an automatic scale changer, it was necessary to be present for the duration of the spectrum in order to manually change scales as required. Since this stage is one of several in a technique of routine analysis, it could not be too time-consuming relative to the other stages and the use of packed columns promised to be the quicker of the two; and (4) the availability of gas chromatographic facilities was far greater for packed columns than for open tubular ones and this allowed greater flexibility for the overall analysis.

Having chosen to use a packed column, it was then decided to use a 15 foot long by 1/8 inch in diameter stainless steel column packed with 80/100 mesh Chromosorb W (AW/DMCS) coated with 5% Carbowax 20 M. This proved to be suitable when a temperature program of 40°C (initial) to 200°C (final) in 6°C per minute increments was used with a 25 ml per minute flow of helium carrier gas. The resulting spectrum offered an acceptable combination of resolution and time required. A typical gas chromatographic spectrum is shown in Figure 5 along with peak assignments.

A flame ionization detector was used because of its high sensitivity to a wide range of compounds. It also is very well suited for

FIGURE 5

Typical Gas Chromatographic Spectra
(Numbers shown are for subsequent reference to peaks.)

RECORDER RESPONSE

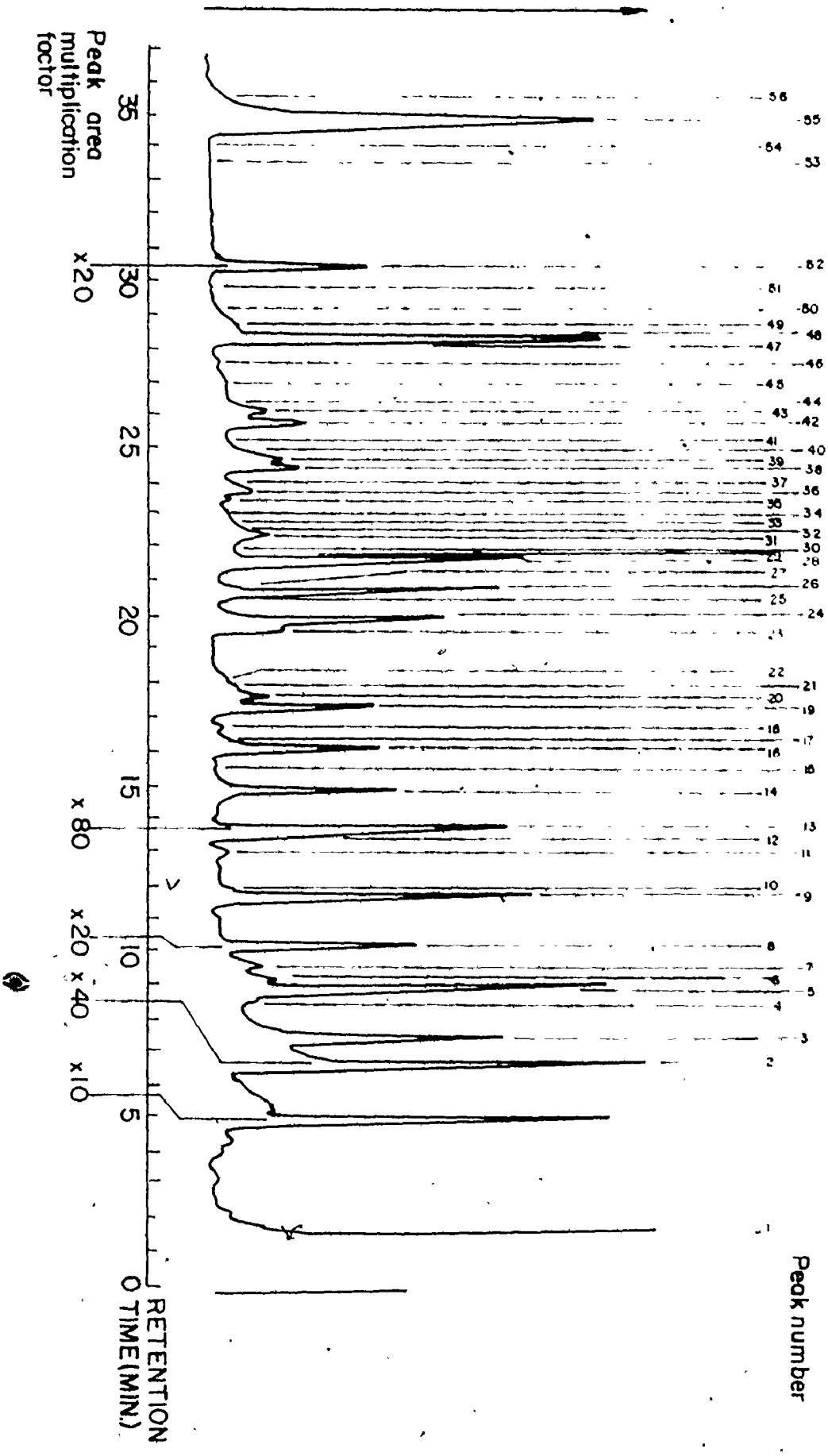
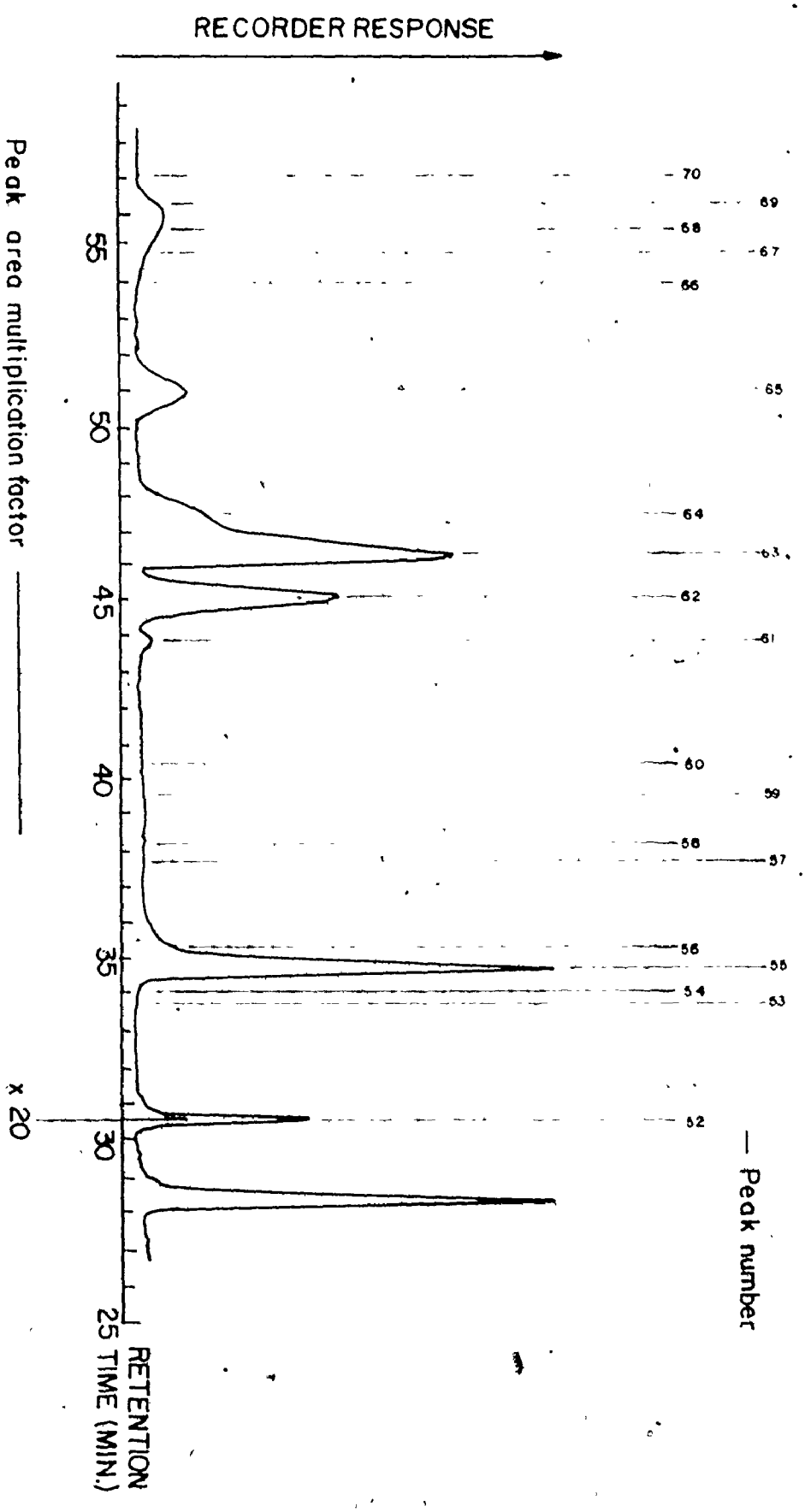


FIGURE 5 (continued)

Typical Gas Chromatographic Spectra


RECORDER RESPONSE
Peak area multiplication factor
x 20
RETENTION TIME (MIN.)
25
30
35
40
45
50
55
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
Peak number

programmed-temperature use (79). It was felt a thermal conductivity detector lacked a wide range of applicability. The 5.0 µl injection volume introduced sufficient solutes without flooding the column with solvent.

(e) Reproducibility of the Routine Analysis Technique

In order to estimate the experimental precision of the results obtained in the technique of routine analysis, standard solutions of selected solutes in 12% ethanol solutions were submitted to repeated extraction, concentration, and gas chromatographic analysis. The results of this study are shown in Table 7 . All concentrated Freon solutions were diluted to a sufficient final volume so that the samples were in the 200 ppm range for gas chromatographic analysis.

The average deviations fall between 1.1% and 7.7%. Thus 8% is felt to be a realistic estimate of the precision involved in any single measurement and to be an acceptable precision for a multi-stage analysis of this type. This precision compares favourably to the precisions reported for other quantitative analyses (72, 61). Error bars of 8% were used in subsequent graphing of data.

Note the less volatile components show higher average deviations in Table 7 in a similar manner to the results of the gas chromatographic reproducibility study shown in Table 5 . This suggests that the gas chromatographic analysis is the major contribution to the average deviation of the overall analysis. The increased tailing observed in the gas chromatographic spectrum apparently results in less precise peak area measurement.

The use of an internal standard in the Concord fermentation studies is likely to improve the precision of those studies. The relatively poor resolution of certain small peaks in the gas chromatographic spectra of the fermentation studies might quite possibly result in poorer precision.

The use of standard deviation instead of average deviation to estimate the precision was considered but too few sets of data were available for actual statistical analysis (or even to estimate the shape of the error curve). These precision values are only estimates of the reproducibility of the technique of routine analysis and any subsequent quantitative comparisons are not made on the basis of single measurements but using several consecutive measurements.

TABLE 7

Routine Analysis Procedure Applied to Standard 12% Ethanol Solutions

		Relative Chromatographic Peak Area				
		2-methyl-1-propanol	3-methyl-1-butanol	1-octanol	diethyl succinate	2-phenethanol
with 2.0 ppm standard solution	#1	210	291	322	224	206
	#2	201	279	284	203	208
	#3	191	259	335	186	247
average		201	276	314	204	220
percentage average deviation		3.0	4.3	6.4	6.4	7.7
with 20.0 ppm standard solution	#1	127	187	185	122	124
	#2	132	175	162	110	119
	#3	133	173	167	101	113
average		131.1	178.2	171.3	111.0	118.5
percentage average deviation		1.9	3.3	5.5	6.9	3.3
with 200 ppm standard solution	#1	179	196	186	113	122
	#2	190	213	183	101	136
	#3	173	208	181	106	136
average		181	206	183	107	131
percentage average deviation		3.3	2.9	1.1	3.7	4.6

In order to determine if the accuracy of the standard solution studies fell within the estimated precision of the routine analysis, the final solution of the Freon and extracted solutes from the extraction of the 200 ppm standard solutions was compared to the 200 ppm standard solution of the same solutes in Freon. The results of this comparision are shown in Table 8 . As expected from the mass balance studies, the standard solution study was accurate within the estimated precision of the routine analysis.

(f) Quantitative Analysis of the Volatile Components

Use of the flame ionization detector without the preceding stream splitter allowed 100% (rather than 10% for the stream splitter detector) of the injected compounds to pass through the detector. For a multicomponent quantitative analysis with substantial experimental uncertainty such as this is, it was preferable to use an internal standard which is of constant concentration throughout the analysis. Because of the complexity of the gas chromatographic spectrum of the must samples, it was unlikely that the samples could be doped with a suitable external standard prior to analysis without having it interfere with the spectrum. Because it has been reported that methyl anthranilate likely remains at constant concentration throughout the fermentation (51), it was decided to use it as an internal standard for the Concord fermentations.

It was decided that the Dupont 310 Curve Resolver was the best available method of resolution and integration of the spectra considering the limitations of time, reliability, and availability of other methods.

The curve resolver utilizes five function generator channels, each capable of generating a single distribution function on a cathode ray tube. Any given number of these functions can be positioned, shaped and summed until a trace is obtained that will match the experimental curve envelope. When the synthesized curve is manipulated into a form identical to the experimental data, the individual components and their relative areas are determined by displaying each individual peak on its respective channel and reading the area from the integration meter (80).

The resolution and integration of the same envelope of four peaks for all spectra at once and the use of the fifth channel as a 100% area

TABLE 8

Comparison of 200 ppm Standard Solution (Freon) with Solution of Extracted Solutes Following the Extraction of 200 ppm Standard Solution (12% Ethanol)

	Relative Chromatographic Peak Area				
	2-methyl-1-propanol	3-methyl-1-butanol	1-octanol	diethyl succinate	2-phenethanol
200 ppm Standard Solution	178	196	197	101	136
Extraction[a] Solution	181	206	183	107	131
percentage deviation	1.7(+)	5.1(+)	7.1(-)	5.9(+)	3.7(-)

[a]Extraction solution refers to solvent pot contents after extraction of 200 ppm standard solution (12% ethanol) following concentration and dilution to 95 ml.

reference peak ensured maximum consistency in this process. The curve resolver was especially meant for use in interpreting spectra which were not completely resolved. Thus it was well suited for this situation. This incomplete resolution of the spectral peaks made 'cut and weigh' techniques and mechanical integrators unsuitable for this task. The full-time use of a digital integrator-processor was not feasible although such a system is probably the best method of resolution and integration.

Table 9 shows the results of the tests to conform the linearity of the response of the flame ionization detector and chart recorder system. This indicates that the detector-recorder system was linear in its response to typical compounds over the range of quantities that were encountered in the gas chromatographic analysis.

(g) Identification of the Volatile Components

An attempt was made to use a coupled gas chromatograph-mass spectrometer system but this was unsuccessful because of insufficient separation of the many components and insufficient sample size. By doing the gas chromatographic collection independently from the mass spectrometry, it was felt both the separation of the components and the amount of sample required could be optimized. It was concluded that the very wide range of concentrations found in these wine extracts would be an obstacle in the use of many coupled gas chromatograph-mass spectrometer systems, even if excellent separation of the components were achieved. The residual background spectrum of the more abundant components would result in the spectrum of the less abundant components being indistinguishable from the background spectrum. Because of the very small amounts of compounds available for mass spectrometry, it was inevitable certain difficulties would be encountered in doing the delicate analysis but it was felt the final mass spectral technique approached the limit of sensitivity available with mass spectrometry.

If the sample tubes were not swept with helium prior to cooling, condensed water entered the mass spectrometer causing occasional decomposition of the sample. A similar result occurred if the sample tubes did not warm sufficiently after sample collection to allow removal of condensation. The maintenance of the gas chromatograph exit port at 200^{o}C

TABLE 9

Linearity of the Response of the Gas Chromatographic Detector-Recorder System With Different Amounts Injected

	Relative Chromatographic Peak Area		
Injection Volume (µl)	hexyl acetate	pentyl butyrate	1-octanol
0.2	21.0	22.0	19.6
2.0	208	215	194
4.0	414	432	378
8.0	820	850	760
20.0	2070	2150	1925
30.0	3140	3220	2880
60.0	6240	6360	5680

was necessary to prevent the eluting samples condensing on it.

The condensation of samples near the source after introduction to the mass spectrometer avoided interactions between the sample and internal adsorbents and contaminants which persisted despite numerous attempts to remove them. This minimized the sample size required. The complete adsorption of the sample on the Pyrex glass walls at ambient temperature was not anticipated and was not apparently due to any contaminant. Varying temperatures up to 200°C were required to desorb the sample. The necessity to purge the system with water vapour after running samples was perhaps indicative of the same phenomenon: strong interactions between the molecules of the sample and the glass. If the temperature of the mass spectrometer was too high, the thermal decomposition of some samples was observed.

Once all the forementioned parameters were determined, the mass spectral technique was very sensitive to even small samples and the spectra compared very favourably with those obtained by conventional mass spectral methods. The preceding observations suggest this technique might be useful for other applications.

The results of the techniques of identification of the volatile component peaks in the gas chromatographic spectra are shown in Table 10. Because each compound has a unique mass spectrum, unlike its retention time, the mass spectral identifications should be regarded as more reliable than the identifications based on gas chromatographic retention times.

For those components which were both identified and available, the gas chromatographic detector-recorder system was calibrated in order to allow conversion of the relative peak areas of Tables 12, 14, and 16 into the original concentration of the component in the must. The calibration factors are listed in Table 10 beside the appropriate component. In order to convert a relative peak area for any component into the concentration of that component, simple multiply the relative peak area by its calibration factor. Note that the relative peak areas are not linearly related to the relative concentrations but these areas are a reasonable approximation for comparing the quantities of various components.

In Table 10, note that certain even numbered acids were identified as being present in the wine extract. Although studies using solvents

TABLE 10

Identification and Calibration of Gas Chromatographic Peaks

Peak[a] #	Identity	M.S.[b]	G.C.[c]	ppm/relative area[d] x10^3
1	Freon	x	x	
2	Ethanol	x	x	
3	Acetone	x	x	
5	1-propanol	x	x	1.33
6	ethyl butyrate		x	0.94
7	butyl acetate		x	1.18
8	2-methyl-1-propanol	x	x	1.00
9	3-methyl-1-butyl acetate	x	x	0.75
12	2-methyl-1-butanol	x	x	0.88
13	3-methyl-1-butanol	x	x	0.88
14	ethyl hexanoate	x	x	0.75
15	butyl butyrate		x	0.92
16	diacetyl	x		
17	3-methyl-1-butyl butyrate		x	0.81
19	ethyl lactate	x	x	1.36
20	1-hexanol		x	0.84
23	acetic acid	x	x	0.92
24	ethyl octanoate	x	x	0.70
25	3-methyl-1-butyl hexanoate		x	0.76
29	1-octanol		x	0.94
30	2-methyl-propanoic acid	x	x	0.77
39	ethyl decanoate		x	0.66
40	diethyl succinate		x	1.00
33	γ-butyrolactone	x		
47	hexanoic acid	x	x	0.88
48	2-phenethyl acetate	x	x	0.80
49	ethyl laurate		x	0.89
52	2-phenethanol	x	x	0.75
54	2-phenethyl butyrate	x	x	
55	1-octanoic acid	x	x	0.82
59	tetradecanol		x	
60	2-phenethyl hexanoate	x	x	0.77
62	methyl anthranillate	x	x	0.95
63	1-decanoic acid	x	x	
66	hexadecanol		x	
68	glycolaldehyde	x		

[a] See Figure 5 for Gas Chromatographic Peak.
[b] Mass spectral identification.
[c] Gas chromatographic identification.
[d] To convert data in Tables 12,14, and 16 from relative peak area to concentration in must as expressed by ppm, multiply the area by this factor. For example for peak #13 multiply the relative area number by 0.88×10^{-3}.

other than Freon had reported the presence of these acids (33, 81), no report of the detection of these acids when using Freon as the extracting solvent could be found. To verify that these acids could be extracted from 12% ethanol into Freon, a simple batch extraction was performed as described on page 25. The gas chromatographic analysis confirmed that Freon can extract these acids from 12% ethanol.

Certain of the components that were identified merit further mention. No previous studies could be found that reported the identification of 2-phenethyl acetate or γ-butyrolactone in Concord must. Some studies have been unable to identify 2-phenethyl acetate as a component although its presence was anticipated (52). Other studies have observed γ-butyrolactone in different varieties of wine (52).

It can be seen from Table 10 that not all components were identified. However, the number that were identified exceed the number identified in a study (44) using similar mass spectral sample sizes, where 17 of 56 components were identified. Another study (34) of Concord wine, using the extract of 13 l of wine, identified 59 components but a minimum of 15 of these were recognized by the authors as being contaminants. This casts doubt on the credibility of many of the other identifications that have not been confirmed by other analyses. It can be concluded that this research, which identified 21 components by both mass spectral and gas chromatographic analysis, 3 by the former only and 12 by the latter only, compares quite well with other Concord analyses. Reproducible mass spectra were obtained for a few peaks but were not matched to available spectra.

B. Results and Discussion of the Applications of the Technique of Routine Analysis

(a) Discussion of Experimental Procedure

One of the aims of this research aside from the development of a reliable technique of routine analysis was the monitoring of the differences in the volatile components of the two basic types of Canadian grapes during fermentation. Such a study was of interest from the academic point of view since the magnitude of these differences was apparently not well known. Because of the lack of wine research facilities in Canada and of the lack of sufficient interest in Canadian wines at most foreign research centres, there has been very limited analysis of the volatile composition of Canadian wines. From a commercial point of view such a study was of interest because large sums of money are being spent in replacing V. labrusca grapes with recently developed hybrids and, at present, the relative concentrations of a few volatile components are used as the criterion for selecting which hybrids will be cultivated. It is possible that a more sophisticated selection system could be developed as is being attempted elsewhere (16). This would be advantageous to the Canadian wine industry.

It was decided to study red wines because of the larger number of red hybrid grapes, the greater popularity of red wines, and the fact that red wines generally have a higher concentration of volatile components. One of the main constraints in experimental design was the fact that most grape juice is available only in the autumn of each year. Bearing this in mind the most common of all V. labrusca grapes used for wine making, the Concord, was chosen for study. It had the advantages of being available at an appropriate time, of being stored at cool temperatures by the winery and thus available longer, and of being of considerable commercial and enological interest. The hybrid-type grape chosen for study was the Blue Hybrid. Its juice was prepared by hot pressing as was the Concord, making the two compatible for comparative study. It was also available at a suitable interval from the Concord and regarded as a sophisticated Ontario grape in contrast to the Concord. The former was important since another constraint on experimental design was the fact only three routine analyses could be performed daily using this technique. Thus the one week

interval between the arrival of the Concord and the Blue Hybrid was the minimum interval possible.

Originally another type of hybrid grape had been selected for study and, in order that the samples analyzed be as close to industrial samples as possible, it was intended to withdraw several samples weekly from the fermentation tanks at the winery. However, because of several problems, the rapid rate of fermentation and a high fermentation temperature being foremost, this was unsuccessful. Therefore it was decided to duplicate the winery's fermentation procedure on a 5.0 gallon scale in the laboratory (the winery tanks are 20,000 gallons in volume). This initial failure did, however, emphasize interest in the effect of the fermentation temperature on the development of the volatile components. Thus the experimental design now included two basic studies and was set up accordingly.

The choice of the 5.0 gallon laboratory fermentation volume was based on the need for a volume large enough to be not drastically reduced by frequent withdrawal of 150 ml samples but small enough to allow rigorous control of certain parameters especially temperature. Aside from the volume difference, everything else in the fermentation was done identically to the industrial procedure. This was confirmed by frequent communication with the winery regarding the progress of the fermentations which were done simultaneously at the two sites.

It was decided to do two Concord fermentations in order to see if both gave similar results for the development of the volatile components. Fermentation A was a control standard for fermentation B . This would give some idea of the reliability of the entire procedure. It would have been preferable to do 5 or 10 replicate fermentations but this was beyond the limitations of the technique. The use of room temperature (19.5^{0}C) for these fermentations was both convenient to maintain and a typical winery fermentation temperature. The additions to the must were identical in proportion to the winery recipe and had the following purpose the diammonium phosphate, $(NH_4)_2HPO_4$, served as a yeast nutrient; the potassium metabisulphite, $K_2S_2O_5$, served as a source of sulfur dioxide, SO_2, which acted as an antiseptic and antioxidant; the Montrachet Green Star yeast provided a wine yeast for a controlled fermentation; and the liquid

invert sugar provided sufficient sugar for fermentation so the resultant wine would have about 12% ethanol as desired. The yeast was softened or activated in a small portion of must at a slightly elevated temperature in order to ensure the fermentation began promptly before spoilage or other detrimental action could occur. This also allowed time for the temperature equilibration of the must.

Great care was taken at all times to ensure the sampling and analysis procedure was exactly as described for reasons both of reproducibility and of avoiding contamination of the must. The system for removal of samples from the fermentation vessel did not expose the must to air, assured removal from the central body of the must, and resulted in minimal wastage. Filtering assured that insoluble solids did not interfere with the pH or Brix hydrometer determinations. The Kieselguhr acted as a filter aid so that no more than two minutes were required for filtration.

The extraction time of 3 hours was arrived at by tripling the maximum time for complete extraction of any component of the standard solution studies. This time also allowed 3 analyses to be conveniently performed daily as desired. All other conditions of the extraction and concentration duplicated those of the standard solution characterization tests described earlier which defined as much as possible the performance of the system. For the Concord fermentations the frequency of sampling and analysis was adjusted according the rate of fermentation. As planned the need for daily sampling of the Concord terminated as the need for daily sampling of the Blue Hybrid began. All treatments described and their timing followed the explicit instructions of the winery.

Since the purpose of vessel A was to be a control for vessel B, A was sampled and analyzed less frequently than B but sufficiently to compare the two. Any difference in volume the more frequent sampling of B might have caused was eliminated by periodic corrections to the volume of A. Thus it can be assumed fermentations A and B were treated identically in all respects. Very careful monitoring of temperature was maintained throughout the study but it remained at $(19.5 \pm 1)^{o}C$. In order to avoid a large headspace above the must in the fermentation vessel, it is common procedure to add must from another container. This was very undesirable in this case since blending was not

acceptable. As an alternative it was decided to purge the headspace with carbon dioxide in order to prevent the access of air to the must following sampling. This procedure was recommended by the winery.

The fermentation of the Blue Hybrid juice was carried out identically to the Concord fermentations in all respects, in order that the difference in juice be the only difference between them. Note that the various treatment procedures were not applied to both types of fermentation at the same time interval from initiation of fermentation but according to the progress of the fermentation as indicated by the Brix hydrometer reading. For example the first racking took place whenever there were three consecutive steady readings of the Brix hydrometer in the -1.3 to -1.8 region. There was a natural difference in the pH of the juices: 3.10 for the Concord and 3.18 for the Blue Hybrid.

As per the experimental design, the time of infrequent sampling of the Blue Hybrid and the Concord fermentations coincided with the initiation of the fermentation temperature study. It was possible to commence the fermentation temperature study in mid-November only because Concord juice was stored at 12°C by the winery in order to prevent fermentation.

The fact that the fermentation temperature affects the quality of the wine produced has long been (40) recognized and some studies (37) comparing the volatile components of the juice and resultant wine produced at various fermentation temperatures have been made. However it was of interest to actually monitor the development of the volatile components in Concord must at two different fermentation temperatures. Such a study might yield data which, firstly, contribute to understanding the formation of the volatile components and, secondly, contribute to understanding the relative importance of fermentation temperature at the industrial level. The major deterrent to having sophisticated fermentation temperature controls in industry is the cost of this procedure.

It was decided that the two fermentation temperatures would be 20.0°C and 28.0°C. In consultation with the winery, 20.0°C was felt to be a typical and desirable fermentation temperature whereas 28.0°C was a relatively high and undesirable fermentation temperature. More extreme fermentation temperatures could have been chosen but they would not have been

representative of industrial conditions.

Both musts were allowed to come to thermal equilibrium in the water baths before fermentation was initiated. Especially during the early stages of the fermentations, the temperature inside the fermentation vessel was frequently checked to ensure it was the same as the surrounding water bath. Agitation of the must due to evolved CO_2 was quite sufficient to make mechanical stirring unnecessary for thermal equilibrium. The very fast rate of fermentation of the 28.0°C must was anticipated so this must was the subject of frequent early sampling. Once the Brix hydrometer readings fell by less than 0.1°Brix per day, the water bath temperature was lowered to 20.0°C. The choice of when to lower the temperature of the warm fermentation was based on the fact that industrially, when the fermentation rate results in high temperature initially, the must temperature returns to a lower level when the fermentation slows down. It was thus decided that maintaining a high must temperature past this point would not be a realistic reflection of the industrial situation. Since everything else about the treatment of musts K and W was identical, it was assumed any difference in their volatile components were due to this initial temperature difference. Note again the treatment of the musts (racking, etc.) was similar from the standpoint of fermentation progress (Brix hydrometer reading) rather than time.

For all five fermentations the frequent sampling and analysis procedure was ceased about 28 days after fermentation began. This time was chosen because winery treatment of musts for bottling purposes is carried out shortly after this time, rendering any further comparison between laboratory and winery processes meaningless. As well the fermentation process had apparently stopped and a substantial amount of data had already accumulated.

Because of the very small concentration of the methyl anthranilate in the Blue Hybrid must, methyl anthranilate was not suitable for use as an internal standard for this fermentation nor was any other component. Therefore the Blue Hybrid fermentation data are not normalized with respect to each other but it is felt they are still interpretable. The precision estimate of 8% for any single measurement is useful in considering the

TABLE 11

pH of the Musts During the Fermentations

Concord Study Sample	pH	Hybrid Study Sample	pH	Temperature Study Sample	pH
A0-B0	3.10	H0	3.18	K0-W0	3.10
B1	3.10	H1	3.18	W1	3.10
A2	3.10	H3a	3.18	K2	3.10
A3	3.10	H5	3.18	W3	3.10
B3	3.10	H7	3.18	K3	3.10
A5	3.12	H9	3.20	W5	3.12
B5	3.12	H11	3.22	K5	3.12
A7	3.15	H13	3.23	W7	3.15
B7	3.15	H15	3.25	K7	3.15
B11	3.15	H17	3.27	W9	3.18
A13	3.15	H20	3.27	K10	3.18
B14	3.18	H24	3.27	W11	3.18
A16	3.18	H28	3.30	K14	3.20
B17	3.18	H35	3.30	W14	3.20
B21	3.18	H42	3.30	K22	3.20
A23	3.20			W22	3.20
B28	3.20			K29	3.22
A29	3.20			W30	3.22
B43	3.20			K50	3.22
A45	3.20			W50	3.22

significance of the results of hybrid fermentation study.

The values of the pH for each sample of all fermentation studies are given in Table 11. There is nothing apparently unusual about the pH values.

(b) Discussion and Results of Fermentation Studies

(1) Concord Fermentation Studies

For the results of the study monitoring the concentration of the volatile components during two identical fermentations of Concord grape juice see Table 12. For Table12 the peak area data for the same peak in fermentations A and B are displayed side by side for easy visual comparison of the two studies. The first letter of the sample number indicates the fermentation vessel sampled and the following number indicates the day of sampling. The density reading in the column to the right gives the density as measured by a Brix hydrometer for that sample. Thus sample number A16 means the sample was withdrawn from fermentation A on Day 16 of the fermentation. The Brix hydrometer reading for that sample was -1.40^{o}Brix.

The main purposes for doing this study were, firstly, to see if two identical studies gave similar results which would indicate how meaningful such data were and, secondly, to provide some insight into the relative pattern of formation of the various volatile components.

Examining the data from the former perspective, it should be noted that, due to the continuity of sampling up to and including the 28-29 Day samples, serious comparison of fermentations A and B can be made. Restraint should be exercised in comparing the 43-45 Day samples since there were no other samples run at about the same time which might indicate if any trends shown were significant.

It is apparent from studying Table 12 that essentially all components behaved very similarly in both fermentations throughout the investigation. To expedite comparison the peaks were categorized according to their final relative peak area at the 28-29 Day sample. This categorization is shown in Table 13. 'Major' peaks are those with relative peak area exceeding 10,000. 'Medium' peaks have relative peak areas between 1,000 and 10,000. 'Minor' peaks have relative peak areas below 1,000. Because of their magnitude, for the major and medium peaks the comparison of their

TABLE 12

Relative Chromatographic Peak Areas During Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#4		#5		#6		#7		#8	
		B[b] R[c]x10^{-2}	A[a]	B Rx10^{-3}	A$_3$	B Rx10^{-3}	A$_3$	B Rx10^{-2}	A$_2$	B Rx10^{-3}	A$_3$
A0-B0	21.5	nd[d]	nd	nd	nd	.578	.578	nd	nd	nd	nd
B1	19.0	1.30		.253				nd		7.43	
A2	13.5		3.01		.679		1.83		3.02		26.7
B2	11.2	1.66		.088		.840		2.10		25.2	
A3	6.5		2.93		3.72		1.97		6.50		47.4
B3	6.4	3.30		3.31		1.91		4.96		52.1	
B4	1.8	4.30		3.25		2.38		9.64		48.5	
A5	.80		2.98		3.02		1.72		3.70		43.9
B5	-.10	4.43		3.78		1.81		4.78		46.38	
B6	-.60	3.51		3.11		1.22		2.54		40.5	
A7	-1.00		0.88		1.78		1.21		1.89		31.7
B7	-1.10	3.13		3.10		1.49		3.54		40.3	
B9	-1.30	3.53		3.91		1.35		3.78		49.5	
B11	-1.40	3.67		3.86		1.41		3.59		49.2	
A13	-1.40		2.19		4.01		2.31		4.59		47.3
B14	-1.40	3.58		4.97		2.09		5.43		48.9	
A16	-1.40		3.00		5.45		2.38		3.81		53.6
B17	-1.45	4.79		5.68		1.01		5.97		52.2	
B21	-1.45	3.18		4.79		1.42		4.95		49.1	
A23	-1.45		3.47		5.39		1.73		5.37		
B24	-1.50	3.31		5.46		1.47		4.20		53.4	
B28	-1.50	3.47		5.00		1.29		5.04		53.0	
A29	-1.50		3.68		6.07		1.85		5.65		56.6
B43	-1.50	5.12		6.15		1.45		15.0		63.9	
A45	-1.50		5.12		6.63		1.35		13.0		75.1

[a]Relative peak areas in column A are for fermentation A.

[b]Relative peak areas in column B are for fermentation B.

[c]R = Relative peak area

[d]nd = not detectable

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#9		#11		#12		#13		#14	
		B Rx10^{-3}	A_3	B Rx10^{-1}	A_1	B Rx10^{-4}	A_4	B Rx10^{-4}	A_4	B Rx10^{-3}	A_3
A0-B0	21.5	.058	.058	nd	nd	nd	nd	.003	.003	.109	.109
B1	19.0	.043		6.1		1.13		2.08		.112	
A2	13.5		2.53		8.0		5.93		8.83		.810
B2	11.2	3.39		7.1		2.86		4.81		.751	
A3	6.5		12.0		9.3		10.4		16.1		.939
B3	6.4	7.63		12.7		12.2		19.3		.890	
B4	1.8	8.67		10.5		12.9		22.5		.999	
A5	.80		10.5		8.6		12.0		19.3		1.14
B5	-.10	9.38		9.4		13.1		21.8		.994	
B6	-.60	10.6		8.8		12.1		20.2		.884	
A7	-1.00		6.44		10.0		9.99		15.6		.866
B7	-1.10	11.8		8.9		10.9		19.1		1.00	
B9	-1.30	14.0		7.2		12.4		21.4		1.04	
B11	-1.40	13.5		9.0		12.2		21.5		1.14	
A13	-1.40		11.2		9.8		13.8		21.7		1.14
B14	-1.40	13.2		11.4		11.9		21.0		1.30	
A16	-1.40		13.8		9.0		12.7		22.4		1.24
B17	-1.45	14.3		7.2		13.2		23.2		1.41	
B21	-1.45	13.4		8.1		13.3		23.0		1.21	
A23	-1.45				7.7		13.9		22.5		1.37
B24	-1.50	15.6		8.2		13.4		23 4		1.38	
B28	-1.50	14.2		8.4		13.5		23.3		1.47	
A29	-1.50		15.2		6.5		13.9		23.5		1.60
B43	-1.50	16.8		13.5		16.1		28.0		1.87	
A45	-1.50		14.0		14.0		19.6		33.6		1.52

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#15		#16		#17		#18		#19	
		B Rx10^{-1}	A_1	B Rx10^{-3}	A_3	B Rx10^{-2}	A_2	B Rx10^{-2}	A_2	B Rx10^{-3}	A_3
A0-B0	21.5	nd	nd	.013	.013	nd	nd	.36	.36	.602	.602
B1	19.0	6.1		18.8		nd		1.58		.711	
A2	13.5		13.1		6.27		nd		1.20		.566
B2	11.2	10.3		1.77		1.87		.54		.469	
A3	6.5		18.6		.279		1.12		1.61		.743
B3	6.4	15.3		.394		1.27		1.81		.610	
B4	1.8	13.9		.116		1.16		1.92		.755	
A5	.80		15.5		nd		.77		1.63		.930
B5	-.10	19.4		nd		.75		1.55		.919	
B6	-.60	8.8		nd		.79		1.86		.971	
A7	-1.00		11.1		nd		1.00		1.74		.988
B7	-1.10	10.6		nd		1.51		2.23		1.09	
B9	-1.30	9.0		.135		2.52		2.76		1.71	
B11	-1.40	9.0		.664		3.32		2.61		1.91	
A13	-1.40		6.0		.752		5.27		3.37		1.97
B14	-1.40	7.0		1.10		4.20		2.82		2.35	
A16	-1.40		4.0		1.20		6.21		3.55		2.50
B17	-1.45	6.0		1.55		4.54		3.01		2.63	
B21	-1.45	7.1		1.84		4.44		2.94		2.93	
A23	-1.45		7.7		1.37		5.75		3.16		2.86
B24	-1.50	3.2		2.13		4.52		2.48		3.15	
B28	-1.50	6.3		1.66		4.94		2.89		3.46	
A29	-1.50		3.3		1.96		6.52		3.50		3.80
B43	-1.50	4.1		3.13		5.28		3.30		6.80	
A45	-1.50		4.7		2.62		5.89		4.76		6.12

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#20		#21		#22		#23		#24	
		B	A_3	B	A_2	B	A_2	B	A	B	A_3
		Rx10^{-3}		Rx10^{-2}		Rx10^{-2}				Rx10^{-3}	
A0-B0	21.5	.097	.097	nd	nd	.95	.95			.027	.027
B1	19.0	.102		nd		.162				.030	
A2	13.5		.077		nd		2.32				.300
B2	11.2	.152		nd		2.59				.278	
A3	6.5		.251		nd		3.73				.455
B3	6.4	.239		nd		3.34				.445	
B4	1.8	.256		nd		3.19				.558	
A5	.80		.198		nd		2.71				.818
B5	-.10	.291		nd		2.95				.741	
B6	-.60	.306		nd		2.76				.700	
A7	-1.00		.266		nd		2.80				.777
B7	-1.10	.275		nd		3.83				.886	
B9	-1.30	.423		nd		5.45				.908	
B11	-1.40	.530		nd		5.08				1.27	
A13	-1.40		.820		.75		5.50				1.08
B14	-1.40	.788		1.75		6.06				1.31	
A16	-1.40		1.06		2.20		5.91				1.18
B17	-1.45	.740		2.63		5.35				1.33	
B21	-1.45	.969		3.63		7.07				1.21	
A23	-1.45		1.08		2.20		4.45				1.30
B24	-1.50	1.03		3.36		6.11				1.57	
B28	-1.50	1.03		3.68		5.20				1.50	
A29	-1.50		1.44		2.94		5.64				1.69
B43	-1.50	1.50		5.82		9.48				1.48	
A45	-1.50		2.02		3.88		7.56				.899

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	#25 B Rx10^{-2}	#25 A_2 Rx10^{-2}	#26 B Rx10^{-3}	#26 A_3 Rx10^{-3}	#27 B	#27 A	#28 B Rx10^{-3}	#28 A_3 Rx10^{-3}	#29 B Rx10^{-3}	#29 A_3 Rx10^{-3}
		Relative Peak Areas for Spectral Peaks									
A0-B0	21.5	nd	nd	2.00	2.00			2.61	2.61	nd	nd
B1	19.0	nd		3.00				3.25		.117	
A2	13.5		nd		2.74				3.35		nd
B2	11.2	nd		2.65				3.02		.194	
A3	6.5		nd		3.86				4.23		.985
B3	6.4	nd		4.15				4.23		.636	
B4	1.8	nd		2.84				3.47		.383	
A5	.80		nd		3.07				3.93		.904
B5	-.10	nd		2.75				3.54		.469	
B6	-.60	nd		2.49				3.40		.280	
A7	-1.00		nd		3.08				3.83		.866
B7	-1.10	nd		3.15				4.30		.886	
B9	-1.30	1.80		3.53				4.69		.908	
B11	-1.40	2.42		4.17				5.34		1.24	
A13	-1.40		1.50		3.54				4.81		1.16
B14	-1.40	4.73		3.59				5.34		1.18	
A16	-1.40		1.80		3.63				5.09		.982
B17	-1.45	3.94		3.66				5.65		1.06	
B21	-1.45	3.03		3.97				5.97		1.15	
A23	-1.45		2.68		3.04				4.99		.891
B24	-1.50	3.26		3.94				7.33		1.26	
B28	-1.50	1.89		3.22				5.57		.935	
A29	-1.50		5.44		3.77				6.39		1.07
B43	-1.50	4.06		4.77				9.07		1.37	
A45	-1.50		3.10		4.63				8.99		1.40

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#30		#31		#32		#33		#34	
		B Rx10^{-3}	A_3	B Rx10^{-3}	A_3	B Rx10^{-3}	A_3	B Rx10^{-3}	A_3	B Rx10^{-2}	A_2
A0-B0	21.5	nd	nd	.098	.098	.029	.029	nd	nd	1.06	1.06
B1	19.0	nd		.086		.034		nd		1.41	
A2	13.5		.051		.509		.772		.145		1.58
B2	11.2	.089		.193		.295		.111		1.48	
A3	6.5		.532		.571		1.34		.961		2.20
B3	6.4	.503		.566		1.00		.707		2.14	
B4	1.8	.275		.483		.574		.508		1.99	
A5	.80		.577		.418		1.21		1.03		2.32
B5	-.10	.361		.560		.699		.649		2.13	
B6	-.60	.369		.451		.461		.461		1.99	
A7	-1.00		.501		.416		1.44		1.27		2.63
B7	-1.10	.775		.642		1.23		1.21		3.10	
B9	-1.30	.807		.737		1.59		1.34		2.97	
B11	-1.40	1.05		.777		1.92		1.55		3.72	
A13	-1.40		.788		.861		1.98		1.46		2.41
B14	-1.40	.795		.768		2.06		1.75		2.81	
A16	-1.40		.707		.982		1.63		1.41		2.11
B17	-1.45	.735		1.05		2.62		1.37		1.93	
B21	-1.45	.785		1.09		2.30		1.29		1.70	
A23	-1.45		.543		1.07		1.24		.948		2.11
B24	-1.50	.783		1.32		1.48		1.13		1.67	
B28	-1.50	.579		.975		1.30		.951		1.67	
A29	-1.50		.771		1.46		1.65		1.33		2.36
B43	-1.50	.768		1.45		1.86		1.22		2.42	
A45	-1.50		.780		1.49		1.75		1.12		3.01

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#35		#36		#37		#38		#39	
		B	A_2	B	A	B	A_3	B	A_2	B	A_3
		$Rx10^{-2}$				$Rx10^{-3}$		$Rx10^{-2}$		$Rx10^{-3}$	
A0-B0	21.5	nd	nd			.018	.018	nd	nd	nd	nd
B1	19.0	nd				.104		.42		.027	
A2	13.5		.17				.061		1.11		.053
B2	11.2	.22				.012		1.27		.069	
A3	6.5		2.49				.320		1.50		.118
B3	6.4	1.58				.262		1.58		.125	
B4	1.8	1.44				.239		1.44		1.14	
A5	.80		3.08				.393		2.27		.182
B5	-.10	2.28				.286		2.35		.182	
B6	-.60	1.51				.252		1.66		.116	
A7	-1.00		3.34				.507		2.74		.263
B7	-1.10	2.92				.394		2.92		.225	
B9	-1.30	3.89				.671		2.85		.250	
B11	-1.40	4.85				.740		4.99		.436	
A13	-1.40		5.18				.654		3.72		.244
B14	-1.40	6.17				.937		4.80		.428	
A16	-1.40		6.52				.689		2.98		.487
B17	-1.45	5.75				.787		3.44		.627	
B21	-1.45	5.83				.844		2.62		.749	
A23	-1.45		6.24				.473		2.09		.606
B24	-1.50	6.06				.740		2.51		.823	
B28	-1.50	4.89				.692		2.59		.745	
A29	-1.50		8.16				.735		2.24		.739
B43	-1.50	7.36				1.07		nd		1.46	
A45	-1.50		8.31				.645		nd		1.29

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

During Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#40		#41		#42		#43		#44	
		B Rx10^{-3}	A_3	B Rx10^{-3}	A_3	B Rx10^{-3}	A_3	B Rx10^{-3}	A_3	B Rx10^{-3}	A_3
A0-B0	21.5	nd	nd	nd	nd	nd	nd	.190	.190	nd	nd
B1	19.0	.016		.016		.100		.200		.075	
A2	13.5		.057		.051						
B2	11.2	.055		.053		.656		.673			
A3	6.5		.268		.471		1.20		1.05		.746
B3	6.4	.215		.592		1.58		1.16		.864	
B4	1.8	.158		.546		1.34		.790		.790	
A5	.80		.333		.993		1.27		.937		1.50
B5	-.10	.182		.707		1.47		.805		1.28	
B6	-.60	.151		.584		1.44		.681		.956	
A7	-1.00		.388		1.19		1.50		1.08		1.70
B7	-1.10	.321		.948		1.55		.971		1.35	
B9	-1.30	.645		.860		1.63		.993		1.56	
B11	-1.40	.914		1.00		1.80		1.04		1.79	
A13	-1.40		1.03		.911		1.79		1.07		1.73
B14	-1.40	1.07		1.18		1.70		1.04		1.66	
A16	-1.40		1.17		.888		2.06		1.00		1.73
B17	-1.45	.979		.969		1.82		1.04		1.42	
B21	-1.45	1.18		.861		1.75		1.24		1.58	
A23	-1.45		1.09		.679		1.95		.923		1.20
B24	-1.50	1.06		.688		1.82		1.14		1.32	
B28	-1.50	1.00		.576		1.66		1.02		1.23	
A29	-1.50		1.22		.771		2.31		1.03		1.37
B43	-1.50	1.51		.736		.961		.987		1.49	
A45	-1.50		1.37		.913		2.81		1.08		1.33

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#45		#46		#47		#48		#49	
		B Rx10^{-3}	A_3	B Rx10^{-3}	A_2	B Rx10^{-3}	A_3	B Rx10^{-3}	A_3	B Rx10^{-3}	A_3
A0-B0	21.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B1	19.0	nd		nd		nd		.036		.016	
A2	13.5		nd		nd		nd		.954		.029
B2	11.2	nd		.48		nd		.974		.146	
A3	6.5		.153		.76		nd		1.02		.268
B3	6.4	nd		1.04		nd		1.22		.158	
B4	1.8	.296		.95		nd		1.27		.095	
A5	.80		.310		.71		nd		1.12		.319
B5	-.10	.259		.97		nd		1.28		.135	
B6	-.60	.368		.109		nd		1.25		.162	
A7	-1.00		.183		1.74		nd		1.15		.366
B7	-1.10	.566				nd		1.21		.190	
B9	-1.30	.826		3.71		.371		1.97		1.03	
B11	-1.40	.881		5.44		.333		1.76		1.04	
A13	-1.40		.589		6.42		.961		1.48		1.21
B14	-1.40	1.17		9.01		1.29		1.89		1.12	
A16	-1.40		.537		5.28		1.58		1.91		.701
B17	-1.45	.826		5.41		1.66		2.05		.851	
B21	-1.45	.977		4.79		1.72		2.09		.673	
A23	-1.45		.387		3.95		1.71		1.96		.663
B24	-1.50	.835		4.33		1.69		2.10		.636	
B28	-1.50	.649		4.02		1.79		2.20		.598	
A29	-1.50		.507		5.73		1.92		2.25		.560
B43	-1.50	.726		4.97		2.22		2.76		.770	
A45	-1.50		.479		6.71		1.90		2.41		.766

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#50		#51		#52		#53		#54	
		B	A_2	B	A	B	A_4	B	A_2	B	A_2
		Rx10^{-2}				Rx10^{-4}		Rx10^{-2}		Rx10^{-2}	
A0-B0	21.5	nd	nd			.151	.151	nd	nd	nd	nd
B1	19.0	nd				.719		nd		nd	
A2	13.5		nd				2.01		nd		nd
B2	11.2	nd				3.07		nd		nd	
A3	6.5		nd				4.73		nd		nd
B3	6.4	nd				6.03		nd		nd	
B4	1.8	nd				6.30		nd		nd	
A5	.80		.89				5.53		1.07		.84
B5	-.10	nd				6.30		.77		.60	
B6	-.60	nd				6.21		1.62		1.28	
A7	-1.00		1.05				5.32		1.38		1.09
B7	-1.10	nd				6.22		1.64		1.29	
B9	-1.30	1.85				6.44					
B11	-1.40	1.85				5.71		1.03		1.51	
A13	-1.40		2.73				5.42		.87		.54
B14	-1.40	2.81				5.92		.72		1.50	
A16	-1.40		1.85				6.79		1.15		.72
B17	-1.45	1.23				6.30		2.71		2.0[illegible]	
B21	-1.45	.83				6.14		1.53		1.14	
A23	-1.45		1.26				6.52		1.38		.72
B24	-1.50	1.30				6.41		1.43		1.03	
B28	-1.50	.57				6.32		1.30		1.03	
A29	-1.50		.80				6.79		1.71		1.23
B43	-1.50	1.95				8.01		3.07		3.41	
A45	-1.50		1.15				8.68		3.19		2.26

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#55		#56		#57		#58		#59	
		B	A_3	B	A	B	A_3	B	A_3	B	A
		$Rx10^{-3}$				$Rx10^{-3}$		$Rx10^{-3}$			
A0-B0	21.5	nd	nd			nd	nd	nd	nd		
B1	19.0	nd				nd		nd			
A2	13.5		nd				nd		1.04		
B2	11.2	nd				nd		nd			
A3	6.5		nd				nd		.14		
B3	6.4	nd				nd		.76			
B4	1.8	nd				nd		nd			
A5	.80		1.42				nd		.26		
B5	-.10	1.19				nd		.14			
B6	-.60	2.61				.13		nd			
A7	-1.00		1.44				nd		.60		
B7	-1.10	2.12				.27		nd			
B9	-1.30	1.12				.43		nd			
B11	-1.40	2.29				.46		nd			
A13	-1.40		3.20				.52		.07		
B14	-1.40	3.47				.42		nd			
A16	-1.40		4.13				.60		nd		
B17	-1.45	3.92				.43		nd			
B21	-1.45	4.26				.61		nd			
A23	-1.45		4.49				.77		nd		
B24	-1.50	4.13				.69		nd			
B28	-1.50	4.45				.60		nd			
A29	-1.50		5.07				.87		nd		
B43	-1.50	5.44				1.33		nd			
A45	-1.50		4.93				1.16		nd		

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks								
		#60		#61		#62[a]		#63		#64
		B Rx10^{-2}	A_2	B Rx10^{-2}	A_2	B Rx10^{-3}	A_3	B Rx10^{-2}	A_2	B Rx10^{-2}	A_2
A0-B0	21.5	nd	nd	nd	nd	1.57	nd	nd	nd	nd
B1	19.0	nd		nd		1.57	nd		nd	
A2	13.5		.12		nd	1.57		nd		nd
B2	11.2	.21		nd		1.57	nd		nd	
A3	6.5		.48		nd	1.57		nd		nd
B3	6.4	.31		nd		1.57	nd		nd	
B4	1.8	.66		nd		1.57	nd		nd	
A5	.80		.77		nd	1.57		nd		nd
B5	-.10	.72		.14		1.57	nd		nd	
B6	-.60	.66		.13		1.57	nd		nd	
A7	-1.00		1.17		nd	1.57		.33		nd
B7	-1.10	.66		.27		1.57	.17		nd	
B9	-1.30	.63		.27		1.57		.69		
B11	-1.40	.22		.27		1.57	1.54		1.07	
A13	-1.40		nd		.78	1.57		2.26		.85
B14	-1.40	nd				1.57	2.07		2.16	
A16	-1.40		nd		.74	1.57		7.21		5.31
B17	-1.45	nd		.43		1.57	4.19		6.05	
B21	-1.45	nd		.90		1.57	6.80		6.02	
A23	-1.45		nd		1.14	1.57		6.27		5.05
B24	-1.50	nd		1.22		1.57	8.56		5.72	
B28	-1.50	nd		1.19		1.57	8.43		5.72	
A29	-1.50		nd		1.52	1.57		7.13		5.76
B43	-1.50	nd		3.42		1.57	9.61		6.17	
A45	-1.50		nd		3.10	1.57		6.51		6.46

[a]Peak #62 (methyl anthranilate) is internal standard

TABLE 12 (continued)

Relative Chromatographic Peak Areas During

Duplicate Concord Fermentations

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#65		#66		#67		#68		#69	
		B	A_2	B	A	B	A	B	A_3	B	A_2
		Rx10^{-2}						Rx10^{-3}		Rx10^{-2}	
A0-B0	21.5	.55	.55					.005	.005	.27	.27
B1	19.0							.015		nd	
A2	13.5		.65						.317		.77
B2	11.2	.53						.495		1.03	
A3	6.5		.70						1.61		2.35
B3	6.4	.38						1.30		2.59	
B4	1.8	.63						1.82		3.08	
A5	.80		.57						2.13		2.32
B5	-.10	.56						1.91		2.68	
B6	-.60	.73						1.46		2.47	
A7	-1.00		.63						1.46		1.95
B7	-1.10	.71						1.02		2.13	
B9	-1.30	1.85						.397		.65	
B11	-1.40	1.71						.337		.65	
A13	-1.40		1.90						.164		.47
B14	-1.40	1.57						.196		.44	
A16	-1.40		2.41						.110		.40
B17	-1.45	2.32						.092		.45	
B21	-1.45	1.96						.039		.19	
A23	-1.45		2.37						.043		
B24	-1.50	2.29						.010		.20	
B28	-1.50	2.29						nd		.20	
A29	-1.50		2.65						nd		.28
B43	-1.50	3.22						nd		.64	
A45	-1.50		2.87						nd		.43

TABLE 13

Categorization of Gas Chromatographic Peaks

According to Relative Peak Area at Day 28

Major[a]	Medium[b]	Minor[c]
8	5	4
12	6	7
13	9	10
52	14	11
	16	15
	19	17
	20	18
	24	21
	26	22
	28	23
	31	25
	32	27
	39	29
	40	30
	42	33
	43	34
	44	35
	47	36
	48	37
	55	38
	62	41
	63	45
		46
		49
		50
		51
		53
		54
		56
		57
		58
		59
		60
		61
		64
		65
		66
		67
		68
		69
		70

[a]Relative peak area exceeds 10^4

[b]Relative peak area between 10^3 and 10^4

[c]Relative peak area below 10^3

behaviour in fermentations A and B should be easiest and perhaps most meaningful. It is for those peaks that particularly close agreement is observed between fermentations A and B within the experimental precision estimated by standard solution studies. Note that, during the early stages of fermentation, it is necessary to correlate the concentration to the progress of the fermentation in that vessel as indicated by the Brix hydrometer reading rather than to the elapsed time of fermentation. This is because fermentation A initially progresses slightly more slowly than fermentation B until equilization occurs at about 7 days. The similarities of the concentration development patterns of certain peaks as a function of time is shown in Figure 6 for peak #8 (2-methyl-1-propanol), in Figure 7 for peak #13 (3-methyl-1-butanol) and in Figure 8 for peak #24 (ethyl octanoate).

There are a few peaks of very small area which were poorly resolved in the gas chromatographic spectrum and show irregular development for both fermentations such as peak #29.

It is concluded that there is sufficient similarity in the concentration development patterns of the volatile components of fermentations A and B to assume that such studies of the concentration development of the volatile components during fermentation do yield meaningful data.

It is also possible that the results shown in Table 12 provide some insight into the relative pattern of formation of the various volatile components. It was anticipated and observed that if the mechanism of formation of these components is regular, then not only should the final quantities of any one component be the same in the two fermentations but also the concentration development pattern of any one component should be the same throughout both fermentations.

Many of the volatile components have concentration development patterns that are quite similar. The general pattern that they resemble is as follows: from an initial concentration in the juice there is a rapid increase in concentration followed by a slow rate of increase in concentration. The development patterns shown in Figures 6, 7, and 8 all fit this general description.

There are certain common variations of this basic development pattern which merit description. Some components such as peak #8

FIGURE 6

Plot of Relative Peak Area vs Time for peak #8 (2-methyl-1-propanol) of Concord fermentation B. Circles represent corresponding data of Concord fermentation A. (For this and all subsequent plots of Relative Peak Area vs Time the curves are drawn through bars which represent the data point ± 8%.)

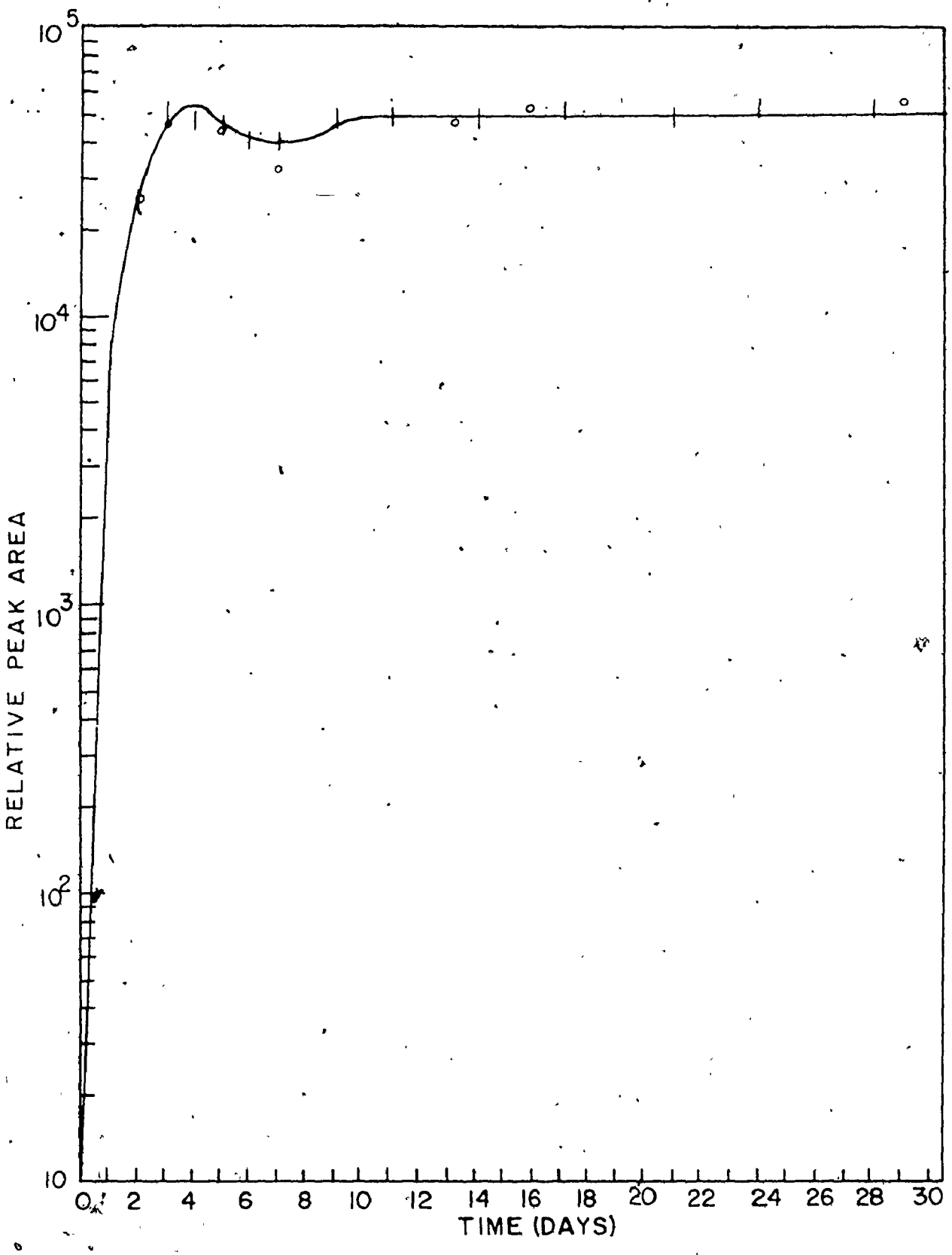

RELATIVE PEAK AREA
10^5
10^4
10^3
10^2
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (DAYS)

FIGURE 7

Plot of Relative Peak Area vs Time for peak #13 (3-methyl-1-butanol) of Concord fermentation B. Circles represent corresponding data of Concord fermentation A.

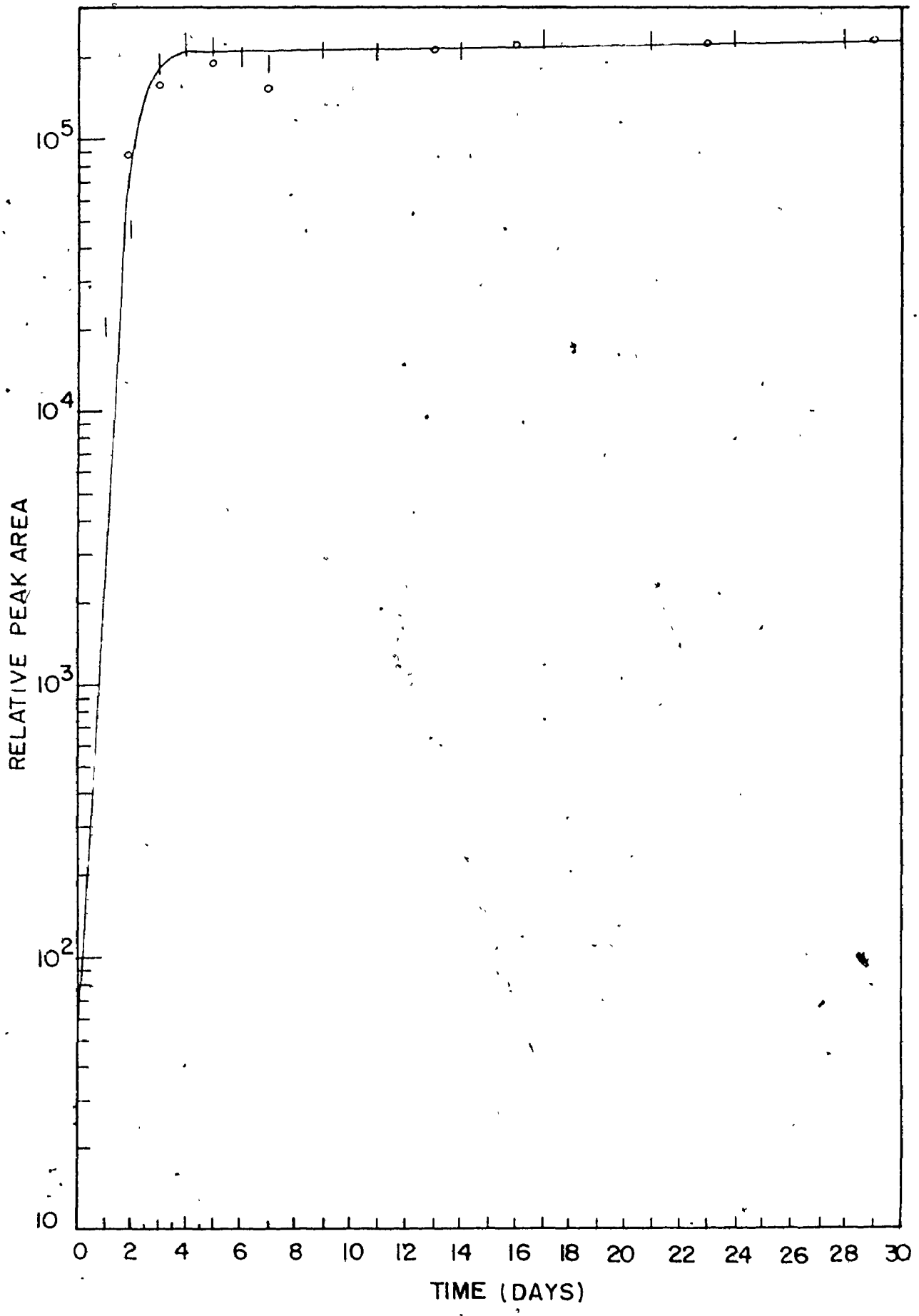

RELATIVE PEAK AREA
10
10^2
10^3
10^4
10^5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

FIGURE 8

Plot of Relative Peak Area vs Time for peak #24 (ethyl octanoate) of Concord fermentation B. Circles represent corresponding data of Concord fermentation A.

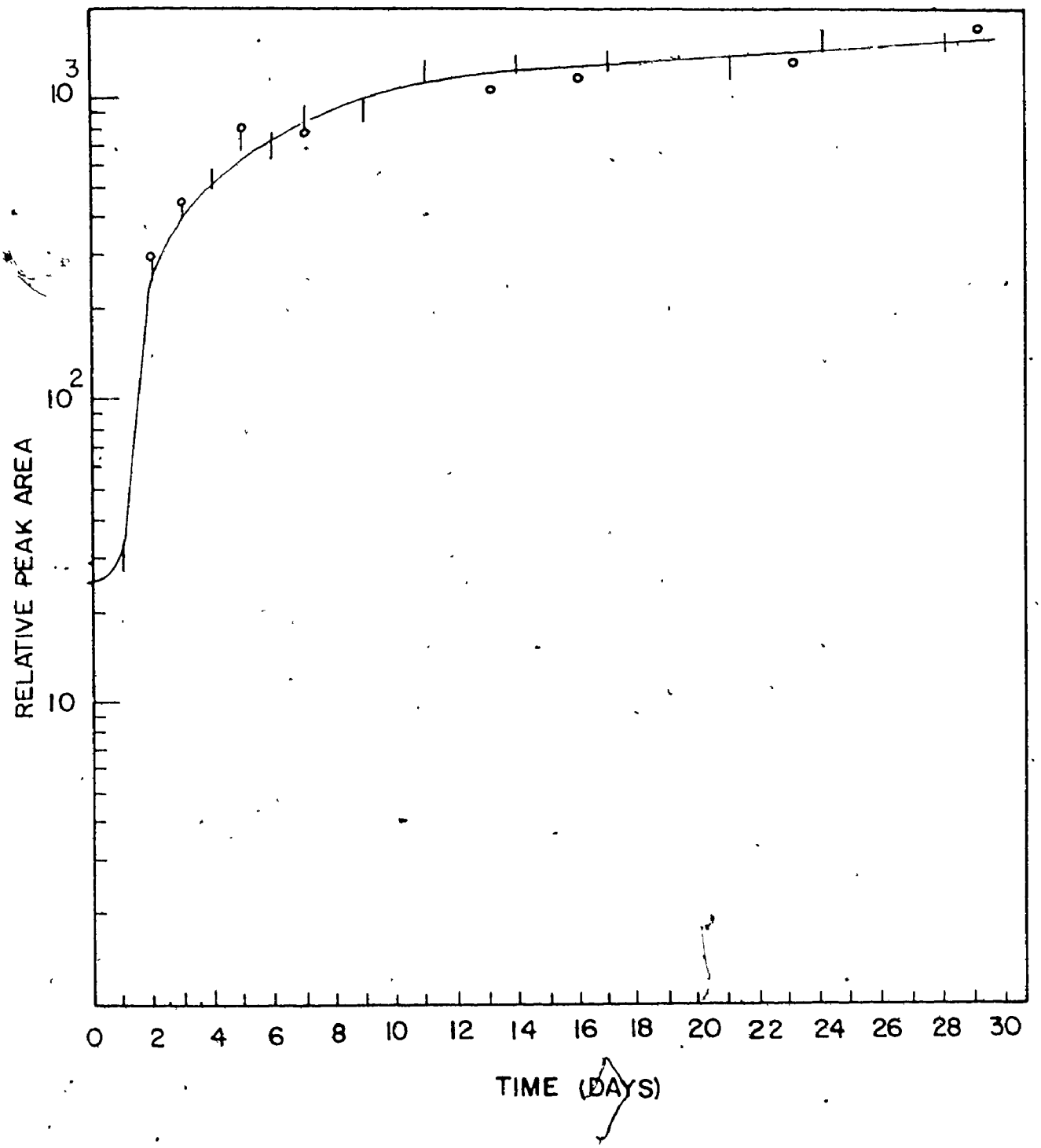

RELATIVE PEAK AREA
10^3
10^2
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (DAYS)

FIGURE 9

Plot of Relative Peak Area vs Time for peak #52 (2-phenethanol) of Concord fermentation B Circles represent corresponding data of Concord fermentation A.

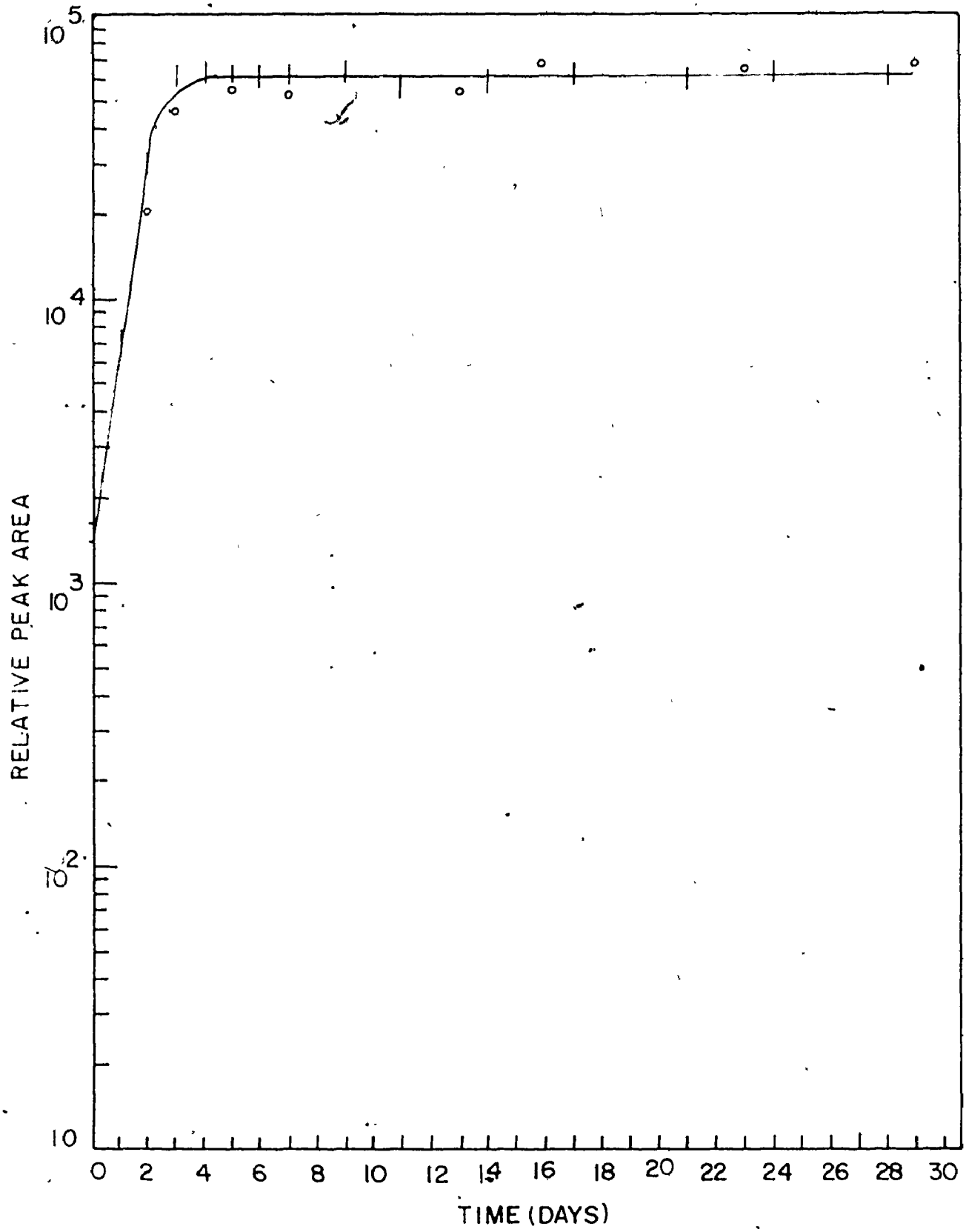

RELATIVE PEAK AREA
10^5
10^4
10^3
10^2
10
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

FIGURE 10

Plot of Relative Peak Area vs Time for peak #55 (1-octanoic acid) of Concord fermentation B. Circles represent corresponding data of Concord fermentation A.

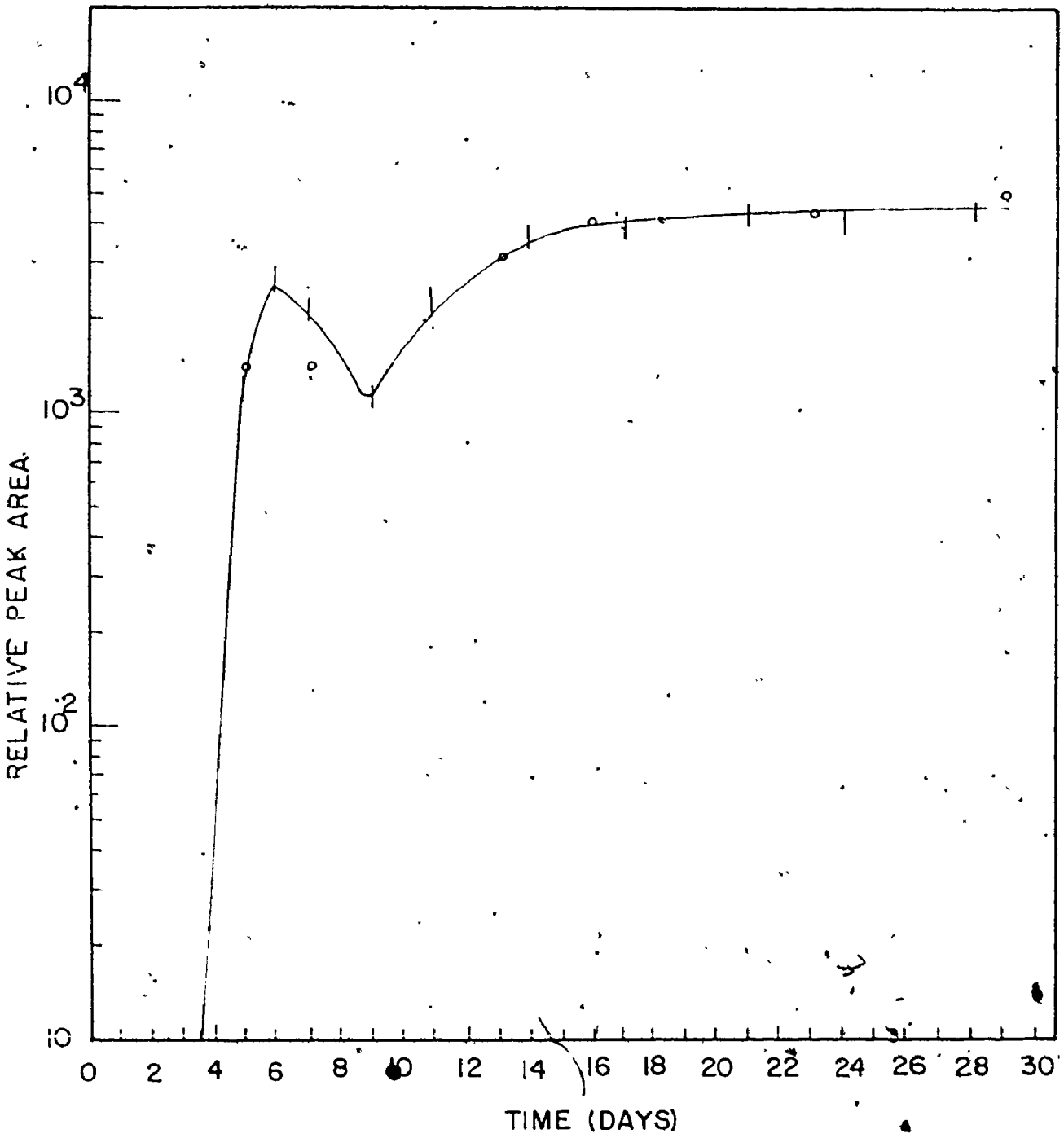

RELATIVE PEAK AREA
10^4
10^3
10^2
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (DAYS)

FIGURE 11

Plot of Relative Fermentation Progress vs Time for Concord fermentation B.

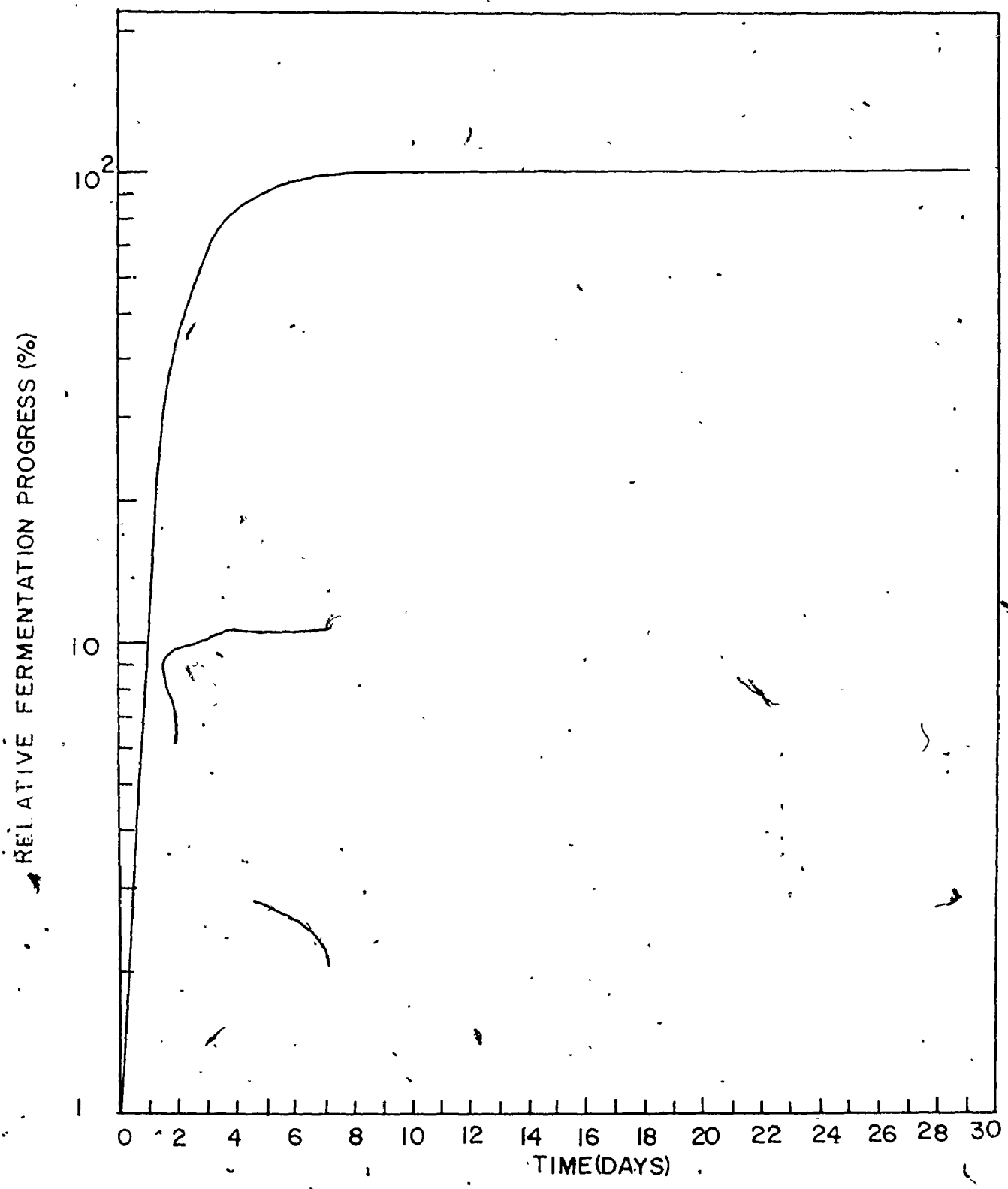
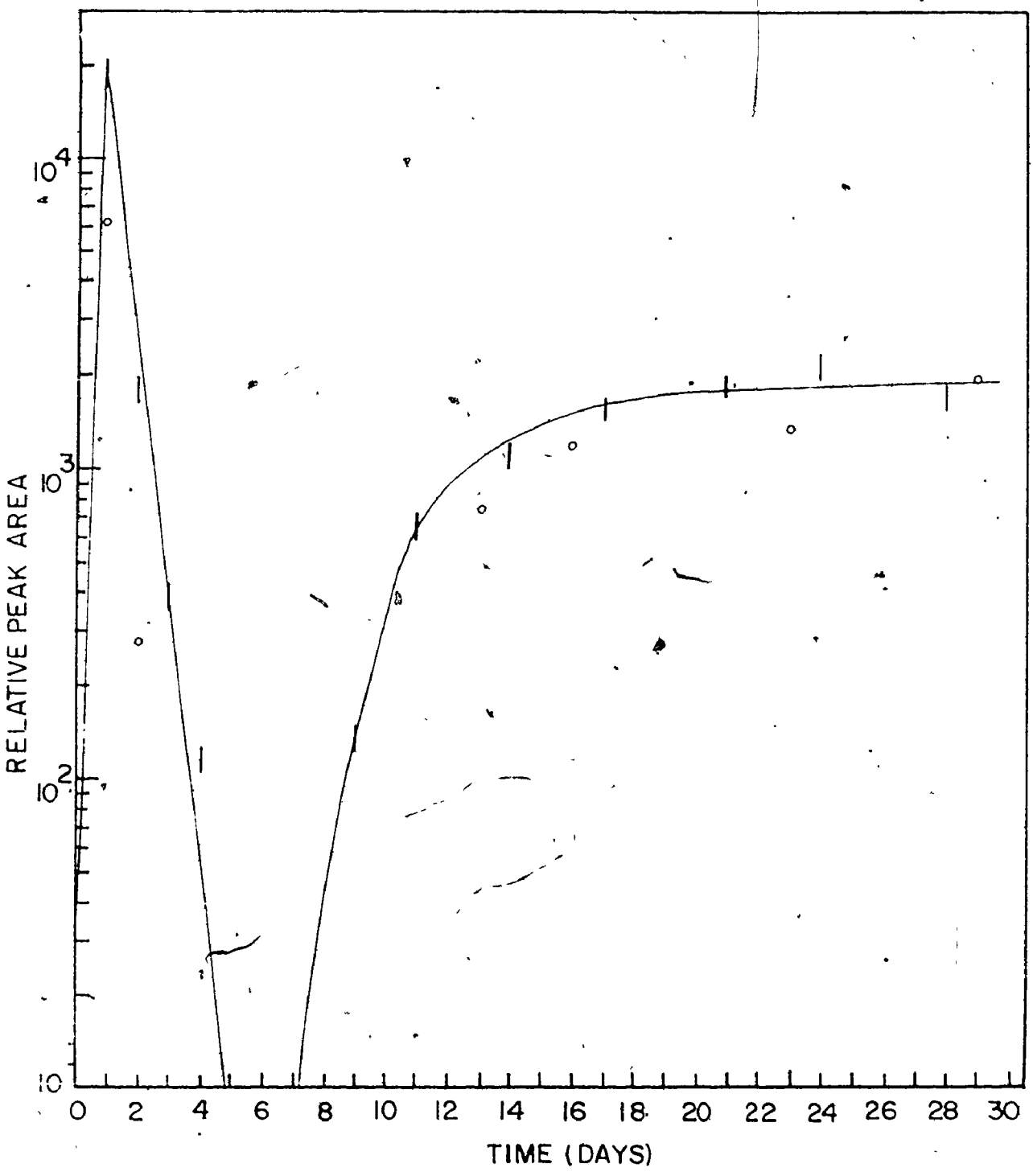

RELATIVE FERMENTATION PROGRESS (%)
10^2
10
1
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME(DAYS)

FIGURE 12

Plot of Relative Peak Area vs Time for peak #16 (diacetyl) of Concord fermentation B. Circles represent corresponding data of Concord fermentation A.


RELATIVE PEAK AREA
10^4
10^3
10^2
10
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

(2-methyl-1-propanol) are at a very low or undetectable level in the juice prior to fermentation whereas other components such as peak #52 (2-phenethanol) have a substantial concentration in the juice. These two peaks are illustrated in Figures 6 and 9 respectively. Certain components such as peak #8 show an increase in concentration which begins immediately with the start of fermentation. Other components such as peak #55 (1-octanoic acid, illustrated in Figure 10) do not begin to increase in concentration until a considerable time (5 days in this example) after the fermentation begins. Once the concentration does begin to increase, the level of some components rises very quickly. Peak #13 (3-methyl-1-butanol) illustrated in Figure 7 exemplifies this. Other components increase in concentration very gradually such as peak #24 (ethyl octanoate shown in Figure 8). The point at which the rate of increase of concentration slows down considerably also varies. Typical points are about 7^{o}Brix as peak #8 illustrates and -1.4^{o}Brix as peak #55 illustrates. Certain components show an apparent local maximum or dip in concentration after the rate of increase slows down. Peak #8 is one of them where the amount of decrease apparently exceeds experimental fluctuation. The fact that the dip appears in both fermentation A and B adds to its credibility.

It is noteworthy that all 4 major peaks, which were all subsequently identified as higher alcohols, behave similarly. Figure 7 illustrates the concentration development pattern for peak #13 (3-methyl-1-butanol). Peak #8 (2-methyl-1-propanol), peak #12 (2-methyl-1-butanol) and peak #52 (2-phenethanol) all resemble this pattern. Mechanisms have been proposed in the literature which associate the formation of certain higher alcohols (50) with particular amino acids. Examples of these mechanisms were shown previously (see page 10). These mechanisms link the rate of formation of certain of these higher alcohols to the rate of yeast growth (50). The distinct similarity between the curve shapes shown in Figures 6 , 7 , and 9 for higher alcohol formation and the curve shape of the yeast growth shown in Figure 1 may be quite significant. The fermentation rate curve for this study is shown in Figure 11. It also resembles the concentration development pattern of the higher alcohols. The falling off of production of the alcohols at about the same time as the fermentation rate drops and the general similarity of the other features of the forementioned curves

tend to confirm the link between higher alcohol formation and the rate of fermentation. The possibility also exists of linking the concentration development pattern of a higher alcohol to that of its corresponding amino acid if both were available (53).

Likewise the results observed for certain esters such as peak #9 (3-methyl-1-butyl acetate), peak #14 (ethyl hexanoate) and peak #24 (ethyl octanoate) would tend to support the acknowledged mechanism of their formation. It has been established that the formation of fatty acid esters occurs during the fermentation as a result of the alcoholysis of the fatty acid moiety of the acyl-CoA compounds in the fermenting medium (51,52). Note for those peaks later identified as fatty acid esters the delay after the initiation of fermentation before significant increases in concentration occur. This delay may correspond to the time required for the appropriate higher alcohols to reach sufficient concentration for the alcoholysis to proceed. The apparent dip in the development pattern of certain higher alcohols could conceivably be related to their role in ester formation. The correspondence between the production of these components and the fermentation rate suggests an ester formation which is linked to the fermentation rate.

A further observation of Table 12 also supports previous findings. Subsequent sample collection identified the following peaks: peak #23 (acetic acid which was found in all other fermentations), peak #29 (2-methyl-1-propanoic acid), peak #47 (1-hexanoic acid), peak #55 (1-octanoic acid) and peak#63 (1-decanoic acid). It is well known that the even-numbered fatty acids predominate in natural products, apparently because the acid precursors are synthesized by successive couplings of the acyl-CoA fragment with the acetyl-CoA (a C_2 addition to the molecule) (51). Only the even-numbered acids are observed and their patterns of concentration development are similar to each other. It is suspected that 1-butyric acid was present but not identified. A typical example is shown in Figure 10 for peak #55 (1-octanoic acid). The rather late appearance of these components is followed by a rapid increase in concentration and levelling off as the rate of fermentation also levels off. This may correspond to a mechanism of formation that is linked to the release of the acid fragment from the yeast cell which occurs as yeast cell growth ceases.

The formation of 2-methyl-1-propanoic acid does not result from this mechanism.

Peak #43 was later identified as γ-butyrolactone and shows a fast increase in concentration which coincides with the most rapid rate of fermentation. This compound has previously been found in non-Concord wines (52) and is reported to be derived from glutamic acid during the fermentation.

There are certain peaks which have unique concentration development patterns that are likely indicative of complex formation and consumption mechanisms. Peak #16 (diacetyl) has a very unusual pattern as illustrated in Figure 12 which features a very sharp increase followed by disappearance then reappearance. This strange behaviour was observed for all five fermentations. Peak #68 also shows an unusual development pattern featuring a quick rise to a maxima at about 1°Brix and followed by a gradual decrease in concentration until an undetectable level is reached by Day 28. Peak #38 behaves similarly to peak #68. These components are apparently intermediates in the fermentation process and are suggestive of unusual mechanisms.

Since the principal purpose of these fermentation studies was to provide comparison of the volatile components of different fermentations, further and more complex mechanistic analysis of the data will be avoided.

The present research has shown, however, that the analytical procedures developed would be useful for obtaining data for such mechanistic analysis.

(2) Hybrid Fermentation Study

Table 14 shows the results of the study monitoring the concentration of the volatile components during the fermentation of the Blue Hybrid juice.

The main purpose for doing this study was to obtain a qualitative and quantitative comparison of the concentration development patterns of the hybrid and the Concord volatile components during the fermentation.

With respect to the qualitative comparison, it was uncertain prior to the study whether the volatile components of the hybrid would be the same ones observed in the Concord and, if they were substantially

TABLE 14

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#4 $R \times 10^{-2}$	#5 $R \times 10^{-3}$	#6 $R \times 10^{-3}$	#7 $R \times 10^{-2}$	#8 $R \times 10^{-4}$	#9 $R \times 10^{-3}$	#10 $R \times 10^{-3}$
H0	21.0	.17	.025	.010	.14	.018	.140	.030
H1	20.7	.55	.155	.036	1.06	.212	.585	.049
H2a	19.9	.70	.147	.237	1.61	.565	.322	.103
H2p	16.0	.55	.628	.677	.84	1.03	.197	.151
H3a	11.9	.55	2.79	2.01	2.37	3.85	.907	.628
H3p	9.0	1.10	4.54	1.82	2.79	5.32	1.35	.872
H4	7.0	1.64	4.75	1.82	2.86	6.32	1.55	1.12
H5	4.0	2.19	6.12	2.32	3.35	7.59	2.22	1.79
H6	2.8	2.19	4.78	1.89	2.79	7.80	2.89	1.75
H7	2.0	1.92	4.96	1.91	2.79	7.63	2.85	1.71
H8	1.85	3.46	5.46	1.77	3.35	10.2	4.19	1.19
H9	1.15	3.19	4.70	1.87	4.05	8.19	3.49	1.03
H10	.50	4.66	5.85	1.88	4.05	8.96	4.56	1.12
H11	-.15	4.50	4.61	1.88	3.42	9.07	5.16	.984
H12	-.70	2.80	5.02	1.55	3.35	8.51	3.74	.907
H13	-1.05	3.41	5.22	1.58	4.12	7.63	6.66	.837
H14	-1.40	3.95	5.14	1.73	3.56	8.40	4.76	.893
H15	-1.75	2.74	5.99	1.74	3.56	9.77	4.72	.963
H16	-1.75	1.70	5.47	1.84	3.49	8.67	5.43	.907
H17	-1.75	1.70	6.13	1.68	3.49	10.4	5.01	.879
H20	-1.75	2.69	6.57	1.26	3.42	8.51	4.51	.754
H24	-1.80	1.21	6.14	1.18	3.42	7.47	4.62	.705
H28	-1.80		6.90	1.38	7.89	7.59	4.54	.999
H35	-1.80	3.30	6.70	1.40	9.49	7.59	4.51	1.01
H42	-1.80	1.31	5.86	.670	5.02	7.08	3.85	.837

[a]R - Relative Peak Area

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#12 $Rx10^{-5}$	#13 $Rx10^{-5}$	#14 $Rx10^{-2}$	#15 $Rx10^{-2}$	#16 $Rx10^{-3}$	#17 $Rx10^{-2}$	#18 $Rx10^{-2}$
H0	21.0	nd[a]	.013	2.23	nd	nd	nd	nd
H1	20.7	nd	.096	.42	.07	nd	nd	.10
H2a	19.9	nd	.177	1.40	1.29	4.40	1.54	.94
H2p	16.0	nd	.461	1.19	.70	4.50	2.23	.70
H3a	11.9	.620	1.22	6.21	2.79	2.32	1.95	.56
H3p	9.0	1.03	2.04	8.23	3.56	.789	1.74	1.40
H4	7.0	1.11	2.21	8.23	2.93	.370	1.95	1.40
H5	4.0	1.45	2.85	8.65	2.93	.356	2.37	2.58
H6	2.8	1.57	3.08	8.65	2.44	.188	2.09	2.37
H7	2.0	1.51	2.96	8.79	1.40	.070	1.40	2.30
H8	1.85	1.84	3.65	8.58	.77			
H9	1.15	1.41	2.80	7.47	nd	.023	1.26	2.37
H10	.50	1.86	3.65	9.07	nd			
H11	-.15	1.78	3.51	9.14	nd	.070	1.61	2.93
H12	-.70	1.79	3.52	9.07	nd			
H13	-1.05	1.57	3.11	7.89	nd	.049	1.40	2.86
H14	-1.40	1.75	3.48	8.65	nd			
H15	-1.75	1.84	3.60	9.07	nd	.056	1.40	2.86
H16	-1.75	1.62	3.24	8.86	nd			
H17	-1.75	1.85	3.64	8.44	nd	.063	1.75	2.79
H20	-1.75	1.81	3.51	9.07	nd	.063	1.74	2.72
H24	-1.80	1.57	3.19	7.54	nd	.265	2.16	2.09
H28	-1.80	1.78	3.46	9.07	nd	.482	3.28	2.79
H35	-1.80	1.64	3.25	8.37	nd	.488	3.84	3.00
H42	-1.80	1.58	2.99	7.26	nd	.419	2.44	2.58

[a]nd - not detectable

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#19 $Rx10^{-3}$	#20 $Rx10^{-2}$	#21 $Rx10^{-2}$	#22 $Rx10^{-2}$	#23 $Rx10^{-2}$	#24 $Rx10^{-3}$	#25 $Rx10^{-2}$
H0	21.0	.338	.77	nd	.27	nd	.032	nd
H1	20.7	3.14	2.30	.66	1.22	nd	.033	nd
H2a	19.9	3.93	2.57	.84	1.22	nd	.023	nd
H2p	16.0	3.77	2.14	.21	2.58	nd	.057	nd
H3a	11.9	3.78	3.41		3.70	nd	.237	nd
H3p	9.0	4.48	3.35		3.70	nd	.419	nd
H4	7.0	4.13	2.69		3.49	nd	.593	nd
H5	4.0	5.12	3.74	.35	4.68	nd	.635	nd
H6	2.8	4.61	3.79	1.47	3.49	nd	.803	nd
H7	2.0	4.55	3.13		3.00	nd	.803	1.10
H8	1.85							
H9	1.15	4.24	3.19		2.79	nd	.761	2.08
H10	.50	5.06	3.19		4.05	nd		
H11	-.15	4.98	3.24		3.00	nd	1.49	4.39
H12	-.70							
H13	-1.05	4.47	3.30		2.44	nd	1.22	3.84
H14	-1.40	4.97	3.30		2.79	nd	1.33	4.50
H15	-1.75	5.53	3.30		3.35	4.05	1.61	5.49
H16	-1.75							
H17	-1.75	5.61	3.89		3.00	3.21	1.40	5.43
H20	-1.75	5.60	2.91	.21	2.58	1.67	1.32	4.44
H24	-1.80	5.83	4.94	.77	2.09	3.84	1.33	5.32
H28	-1.80	6.87	4.88	.77	3.35	6.07	1.75	5.54
H35	-1.80	6.94	5.54	1.67	3.56	3.49	1.55	4.77
H42	-1.80	5.83	5.71	1.54	2.65	3.49	1.40	3.74

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#26 $Rx10^{-2}$	#27 $Rx10^{-1}$	#28 $Rx10^{-3}$	#29 $Rx10^{-3}$	#30 $Rx10^{-3}$	#31 $Rx10^{-3}$	#32 $Rx10^{-3}$
B0	21.0	.33	.2	.045	.074	.010	.005	.010
B1	20.7	.98	.9	.161	.016	.016	.020	.016
B2a	19.9	.77	1.4	.216	.016	.028	.016	.017
B2p	16.0	.68	.7	.114		.035	.145	.042
B3a	11.9	.77	nd	.209	.088	.161	.405	.293
B3p	9.0	.91	nd	.349	.181	.300	.419	.628
B4	7.0	.70	nd	.698	.368	.468	.537	.768
B5	4.0	1.40	nd	1.17	.549	.712	.803	1.61
B6	2.8	1.40	nd	1.26	.477	.705	.705	1.61
B7	2.0	2.09	nd	1.39	.466	.558	.649	1.47
B8	1.85							
B9	1.15	1.40	nd	1.40	.466	.502	.698	1.38
B10	.50		nd	2.27	.807	.914	.977	
B11	-.15	2.58	nd	2.72	.993	1.10	1.12	2.30
B12	-.70							
B13	-1.05	1.88	nd	1.59	.477	.530	.621	1.58
B14	-1.40	1.95	nd	2.09	.658	.719	.837	1.68
B15	-1.75	2.44	nd	3.15	1.04	1.13	1.26	2.21
B16	-1.75							
B17	-1.75	2.16	nd	2.96	1.00	1.08	1.19	2.20
B20	-1.75	1.95	nd	1.95	.483	.593	.844	1.41
B24	-1.80	2.23	nd	2.67	.971	1.04	1.23	2.05
B28	-1.80	3.00	nd	5.46	1.37	1.41	1.79	2.44
B35	-1.80	2.44	nd	2.80	.807	.949	1.12	1.63
B42	-1.80	1.95	nd	2.42	.658	.768	.796	1.54

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#33 $Rx10^{-3}$	#34 $Rx10^{-3}$	#35 $Rx10^{-2}$	#36 $Rx10^{-2}$	#37 $Rx10^{-2}$	#38 $Rx10^{-2}$	#39 $Rx10^{-3}$
H0	21.0	nd	.012	.04	.02	.09	.11	.024
H1	20.7	.011	.020	.07	.06	.13	nd	.028
H2a	19.9	.013	.070	.20	.18	.35	.17	.091
H2p	16.0	.040	.105	.27	.33	.64	.76	.098
H3a	11.9	.346	.482	.84	1.33	2.37	2.74	.426
H3p	9.0	.466	.482	.91	1.54	2.79	3.41	.349
H4	7.0	.565	.551	1.26	1.54	3.07	4.11	.488
H5	4.0	.933	.830	1.47	2.16	4.40	5.77	1.03
H6	2.8	.922	.816	1.40	2.23	4.68	6.04	1.12
H7	2.0	.862	.775	1.40	2.30	4.33	5.54	.977
H8	1.85							
H9	1.15	.686	.635	1.40	2.23	4.19	4.66	1.25
H10	.50							
H11	-.15	1.21	1.03	2.86	3.49	8.23	6.21	2.30
H12	-.70							
H13	-1.05	.779	.670	2.72	2.51	5.37	5.38	1.81
H14	-1.40	.868	.789					
H15	-1.75	1.08	1.07	5.65	4.68	8.37	4.11	2.91
H16	-1.75							
H17	-1.75	1.10	1.10	4.68	4.68	7.54	4.11	2.95
H20	-1.75	.610	.621	3.28	3.21	4.95	3.84	2.00
H24	-1.80	1.06	1.11	3.84	6.21	11.9	5.10	3.06
H28	-1.80	1.22	1.12	4.47	5.37	9.70	1.21	4.64
H35	-1.80	.894	.844	4.19	4.68	8.65	1.21	4.66
H42	-1.80	.669	.642	3.49	3.56	6.77	1.27	4.26

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#40 $Rx10^{-3}$	#41a $Rx10^{-3}$	#41b $Rx10^{-3}$	#42 $Rx10^{-3}$	#43 $Rx10^{-3}$	#44 $Rx10^{-3}$	#45 $Rx10^{-3}$
H0	21.0	.016	.028	.028	.007	.021	.029	.003
H1	20.7	.007	.033	.033	.027	.056	.058	.014
H2a	19.9	.015	.071	.190	.084	.179	.106	.135
H2p	16.0	.084	.158	.187	.258	.733	.109	.061
H3a	11.9	.216	.286	.698	1.22	.977	.543	.272
H3p	9.0	.279	.516	.419	1.51	1.31	.768	.349
H4	7.0	.426	.761	.698	1.78	1.29	.988	.398
H5	4.0	.551	1.13	.789	2.62	1.56	1.64	.621
H6	2.8	.600	1.21	1.05	2.65	1.54	2.02	.816
H7	2.0	.747	1.25	1.10	2.44	1.49	1.92	.809
H8	1.85							
H9	1.15	.768	1.12	1.40	2.00	1.75	2.26	.907
H10	.50							
H11	-.15	1.01	1.61	2.23	3.15	3.10	4.34	2.23
H12	-.70							
H13	-1.05	.893	1.31	1.31	2.34	1.88	2.98	1.72
H14	-1.40							
H15	-1.75	1.23	1.81	1.81	3.77	3.15	5.04	3.02
H16	-1.75							
H17	-1.75	1.41	1.96	1.74	4.33	3.28	4.71	2.67
H20	-1.75	1.49	.858	.907	3.37	2.30	3.05	1.61
H24	-1.80	2.62	1.13	1.12	3.94	2.77	4.21	3.33
H28	-1.80	2.70	1.17	1.34	5.16	3.94	5.23	3.30
H35	-1.80	2.30	1.03	1.12	4.38	2.88	3.83	2.38
H42	-1.80	1.61	.733	.754	3.18	2.12	2.79	2.30

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#46 $Rx10^{-3}$	#47 $Rx10^{-2}$	#48 $Rx10^{-3}$	#49 $Rx10^{-2}$	#50 $Rx10^{-2}$	#51 $Rx10^{-2}$	#52 $Rx10^{-4}$
H0	21.0	.059	nd	.042	.22	.54	.66	.062
H1	20.7	.033	nd	.042	.21	.58	.68	.101
H2a	19.9	.063	nd	.048	.57	.98	.69	.223
H2p	16.0	.022	nd	.070	1.27	.98	.69	.588
H3a	11.9	.035	nd	.258	2.74	2.58	1.54	1.84
H3p	9.0	.154	nd	.349	3.30	2.65	1.61	3.61
H4	7.0	.181	nd	.488	5.43	3.91	2.09	3.94
H5	4.0	.230	nd	.949	7.35	3.84	2.09	5.86
H6	2.8	.244	nd	1.05	6.04	2.09	4.19	5.85
H7	2.0	.279	2.79	1.06	4.44	.70	nd	6.10
H8	1.85							6.82
9	1.15	.216	2.65	1.26	4.39	.70	nd	5.75
10	.50							7.56
H11	-.15	.579	5.58	1.66	5.32	.77	nd	7.65
H12	-.70							7.29
H13	-1.05	.468	4.54	1.40	4.44	.77	nd	6.00
H14	-1.40							6.74
H15	-1.75	1.10	5.58	1.93	5.49	1.26	nd	7.40
16	-1.75							6.55
H17	-1.75	1.13	5.58	1.93	5.32	1.05	nd	7.40
H20	-1.75	.614	4.40	1.73	3.19	.70	nd	6.76
H24	-1.80	1.56	4.88	1.91	3.19	.91	nd	6.70
H28	-1.80	1.31	5.58	1.94	4.11	.70	nd	7.23
35	-1.80	1.05	4.95	1.80	3.84	.70	nd	7.02
H42	-1.80	.977	4.12	1.61	3.41	.35	nd	5.86

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks						
		#53 $Rx10^{-2}$	#54	#55 $Rx10^{-3}$	#56	#57 $Rx10^{-2}$	#58 $Rx10^{-2}$	#59
H0	21.0	nd		nd		nd	1.24	
H1	20.7	nd		nd		nd	.42	
H2a	19.9	.33		nd		nd	2.16	
H2p	16.0	.35		nd		nd	.31	
H3a	11.9	2.04		.094		nd		
H3p	9.0	.17		.195		nd	.28	
H4	7.0	2.09		.216		nd	nd	
H5	4.0	1.74		.957		nd	nd	
H6	2.8	1.47		1.15		nd	nd	
H7	2.0	nd		1.25		nd	nd	
H8	1.85	nd		1.83		nd	nd	
H9	1.15	nd		1.59		.19	nd	
H10	.50							
H11	-.15	nd		2.59		.33	nd	
H12	-.70							
H13	-1.05	nd		2.22		.15	nd	
H14	-1.40							
H15	-1.75	nd		2.86		.42	nd	
H16	-1.75							
H17	-1.75	nd		2.81		.58	nd	
H20	-1.75	nd		2.72		.59	nd	
H24	-1.80	nd		2.76			nd	
H28	-1.80	nd		3.06		1.19	nd	
H35	-1.80	nd		2.96		1.67	nd	
H42	-1.80	nd		2.25		1.19	nd	

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks					
		#60	#61 $Rx10^{-2}$	#62 $Rx10^{-1}$	#63 $Rx10^{-2}$	#64 $Rx10^{-2}$	#65	#66 $Rx10^{-1}$
H0	21.0		nd	3.5	nd	nd		
H1	20.7		nd	4.2	nd	nd		
H2a	19.9		nd	4.2	nd	nd		
H2p	16.0		nd	4.2	nd	nd		
H3a	11.9		nd		nd	nd		
H3p	9.0		nd	3.8	nd	nd		
H4	7.0		nd	2.6	.14	.07		
H5	4.0		nd	2.8	.59	.90		
H6	2.8		nd	2.4	.59	.90		
H7	2.0		nd	4.9	.76	1.15		
H8	1.85		nd		2.29	4.03		
H9	1.15		nd		.97	3.01		
H10	.50		nd		1.86	2.19		
H11	-.15		nd		5.74	4.31		
H12	-.70							
H13	-1.05		.07		3.33	4.24		
H14	-1.40				5.18	4.35		
H15	-1.75		.38	2.6	6.93	4.45		
H16	-1.75							
H17	-1.75		.57	2.5	6.15	4.48		
H20	-1.75		.64	3.5	5.73	4.21		2.1
H24	-1.80							
H28	-1.80		2.42	5.1	6.97	4.06		9.8
H35	-1.80		3.22	4.2	6.98	4.05		8.5
H42	-1.80		3.55	2.2	6.03	4.03		8.4

TABLE 14 (continued)

Relative Chromatographic Peak Areas During

Blue Hybrid Fermentation

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks			
		#67 Rx10^{-2}	#68 Rx10^{-2}	#69	#70 Rx10^{-1}
H0	21.0		nd		
H1	20.7		nd		
H2a	19.9		2.30		
H2p	16.0		2.80		
H3a	11.9		5.02		
H3p	9.0		2.93		
H4	7.0		3.42		
H5	4.0		4.40		
H6	2.8		2.51		
H7	2.0		1.71		
H8	1.85		1.19		
H9	1.15		.56		5.1
H10	.50		nd		7.7
H11	-.15		nd		
H12	-.70		nd		
H13	-1.05		nd		
H14	-1.40		nd		
H15	-1.75		nd		
H16	-1.75		nd		
H17	-1.75		nd		
H20	-1.75		nd		
H24	-1.80		nd		
H28	-1.80	1.14	nd		
H35	-1.80	1.27	nd		
H42	-1.80	1.55	nd		

the same, whether the pattern of development would be similar suggesting similar mechanisms of formation. Study of the data suggests that, to a large extent, the volatile components in the hybrid must are the same as those in the Concord must. There are a few exceptions to this general observation such as peaks #36, #41b, and #51 all of which are apparently unique to the hybrid fermentation whereas peak #10 is unique to the Concord fermentation. It should be noted that these peaks are all 'minor' peaks. In fact the small size of these peaks was a contributing factor to their remaining unidentified. All of the 'major' and 'medium' peaks as categorized in Table 13, are common to both fermentations.

For the vast majority of these common components the concentration development pattern is very similar in the hybrid and Concord fermentations. The basic pattern of a fast increase followed by a slow or zero rate of increase in concentration is repeated for the hybrid fermentation. Especially for the large peaks where the greatest diversity in development patterns would be observable, development patterns are similar. Comparisons of the concentration development patterns of peak #13 (3-methyl-1-butanol), peak #24 (ethyl octanoate), and peak #52 (2-phenethanol) are shown in Figures 13, 14, and 15 respectively. Note that components such as peak #13 and peak #52 which showed a levelling off of the concentration at 7°Brix in the Concord fermentation, do so at about 4°Brix in the hybrid fermentation. This is perhaps accounted for by the fact that the hybrid fermentation progressed more slowly than did the Concord fermentation, taking 10 days to reach 0.0°Brix rather than 5 days as did the Concord fermentation. The relative fermentation rates are compared in Figure 16. The formation of some volatile components exhibiting this type of behaviour was associated with the rate of fermentation in the previous section. Thus it is expected that the volatile component formation curves would be somewhat displaced. The observation of a slower rate of fermentation than that which occurred under identical fermentation conditions with a different juice agrees with previous conclusions (48) that the fermentation rate is a function of the juice used.

The peaks which were associated with the levelling off of the Brix values as the Concord fermentation slowed in rate, show a similar association for the hybrid fermentation. Peak #47 (1-hexanoic acid) and peak #55 (1-octanoic acid) exemplify this.

FIGURE 13

Plot of Relative Peak Area vs Time for peak #13 (3-methyl-1-butanol) of hybrid fermentation. Circles represent corresponding data of Concord fermentation B.

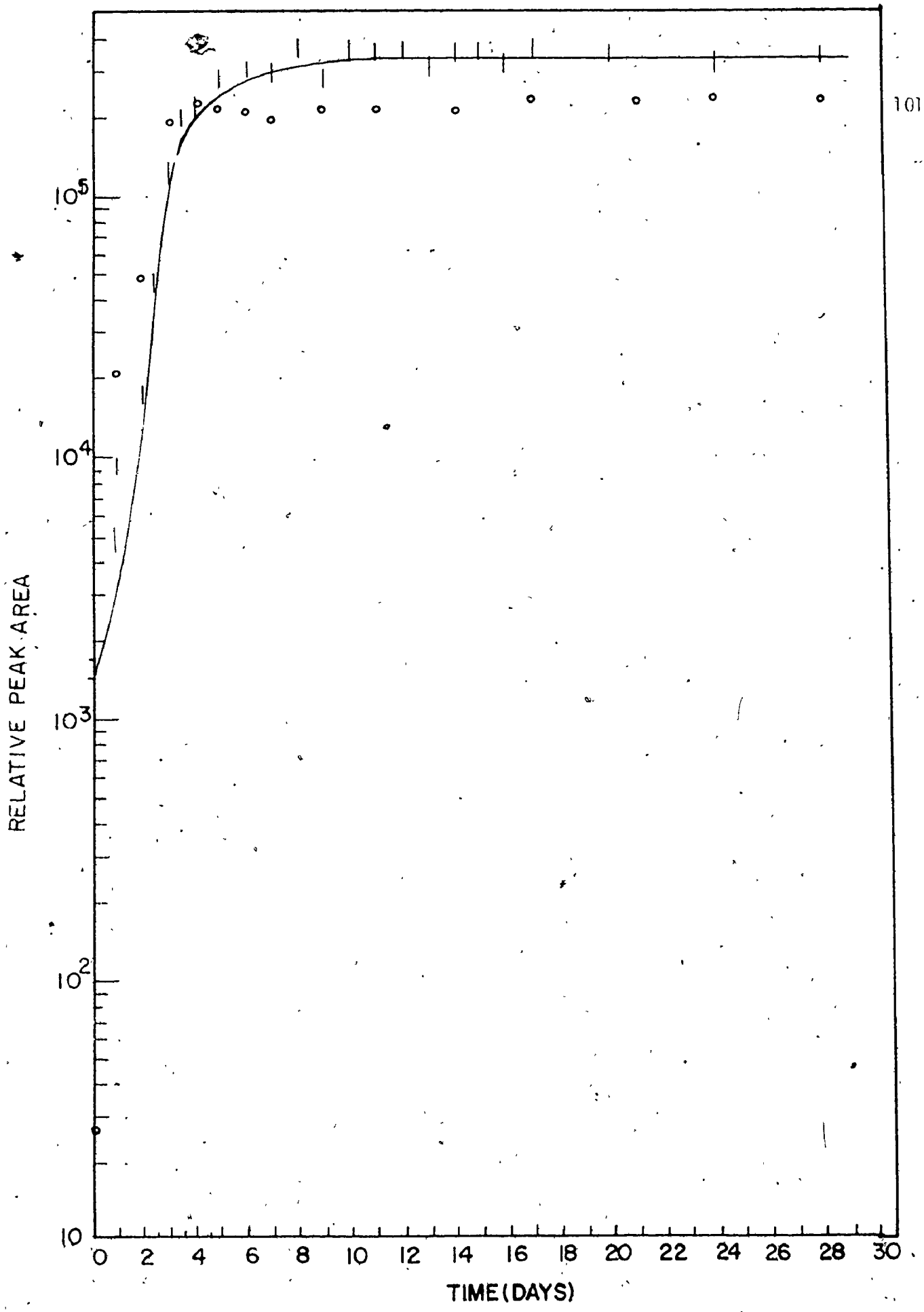

RELATIVE PEAK AREA
10^5
10^4
10^3
10^2
10
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

FIGURE 14

Plot of Relative Peak Area vs Time for peak #24 (ethyl octanoate) of hybrid fermentation. Circles represent corresponding data of Concord fermentation B.

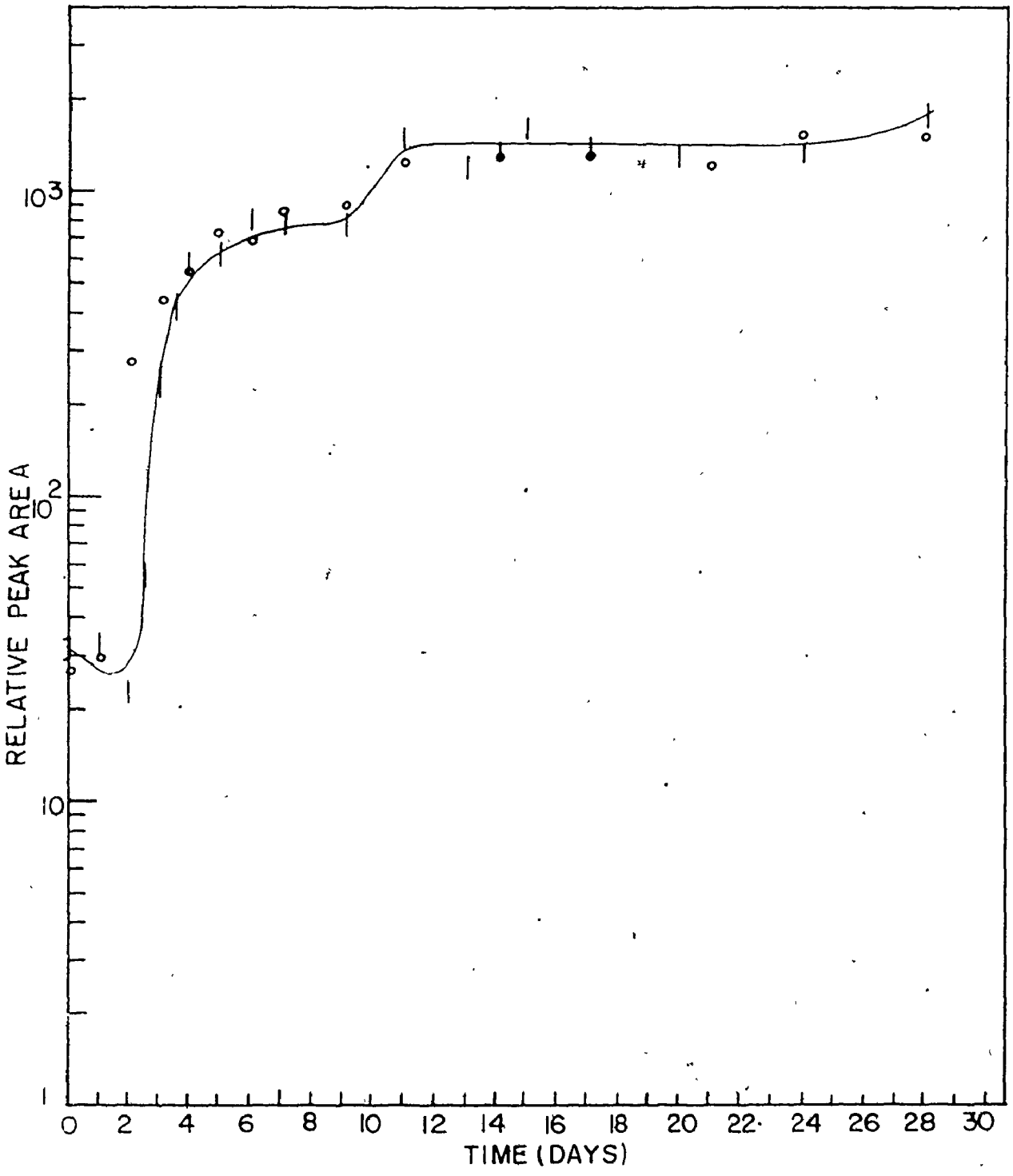

RELATIVE PEAK AREA
10³
10²
10
1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

FIGURE 15

Plot of Relative Peak Area vs Time for peak #52 (2-phenethanol) of hybrid fermentation. Circles represent corresponding data of Concord fermentation B.

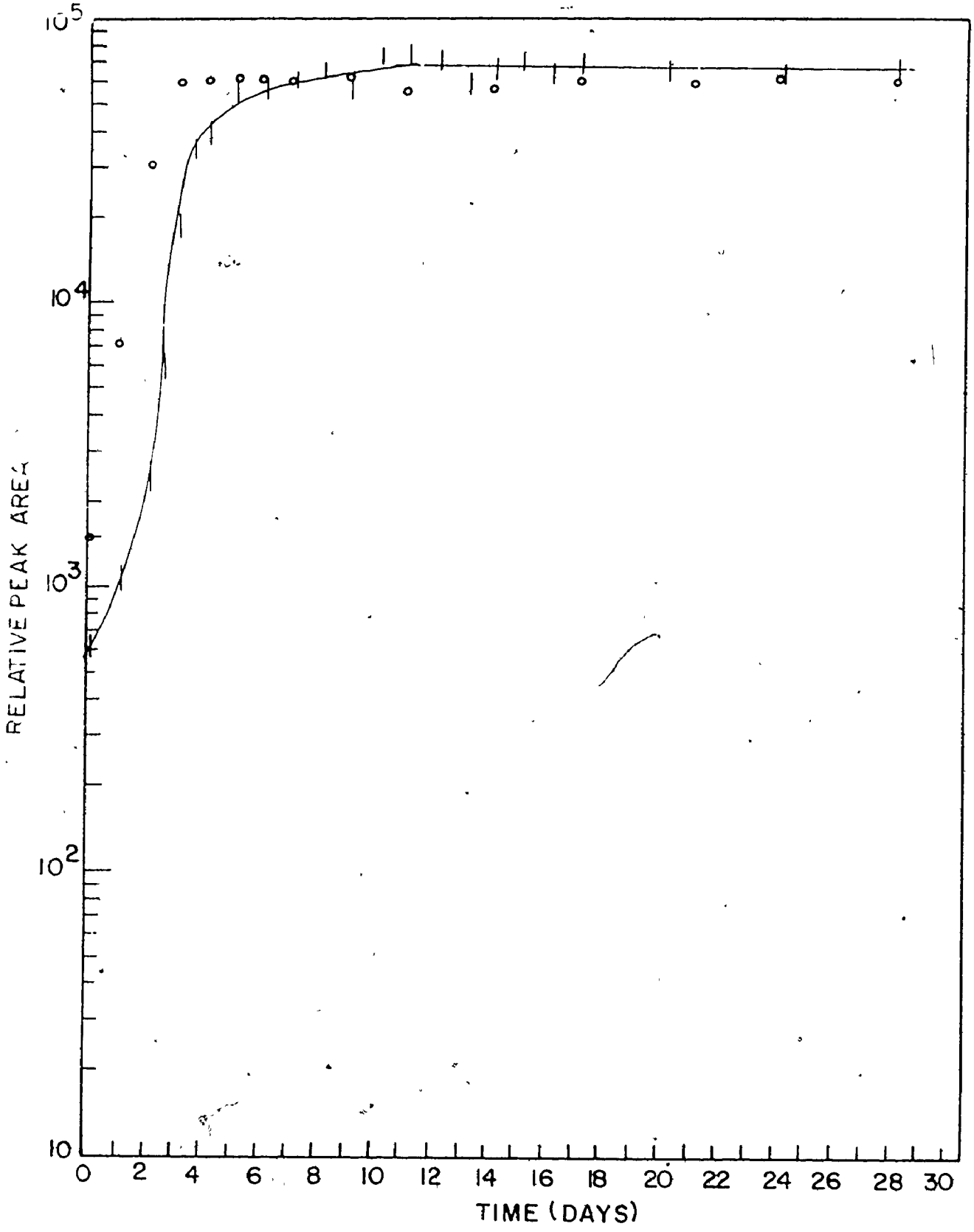

RELATIVE PEAK AREA
10^5
10^4
10^3
10^2
10
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

FIGURE 16

Plot of Relative Fermentation Rate vs Time for hybrid fermentation and Concord fermentation B.

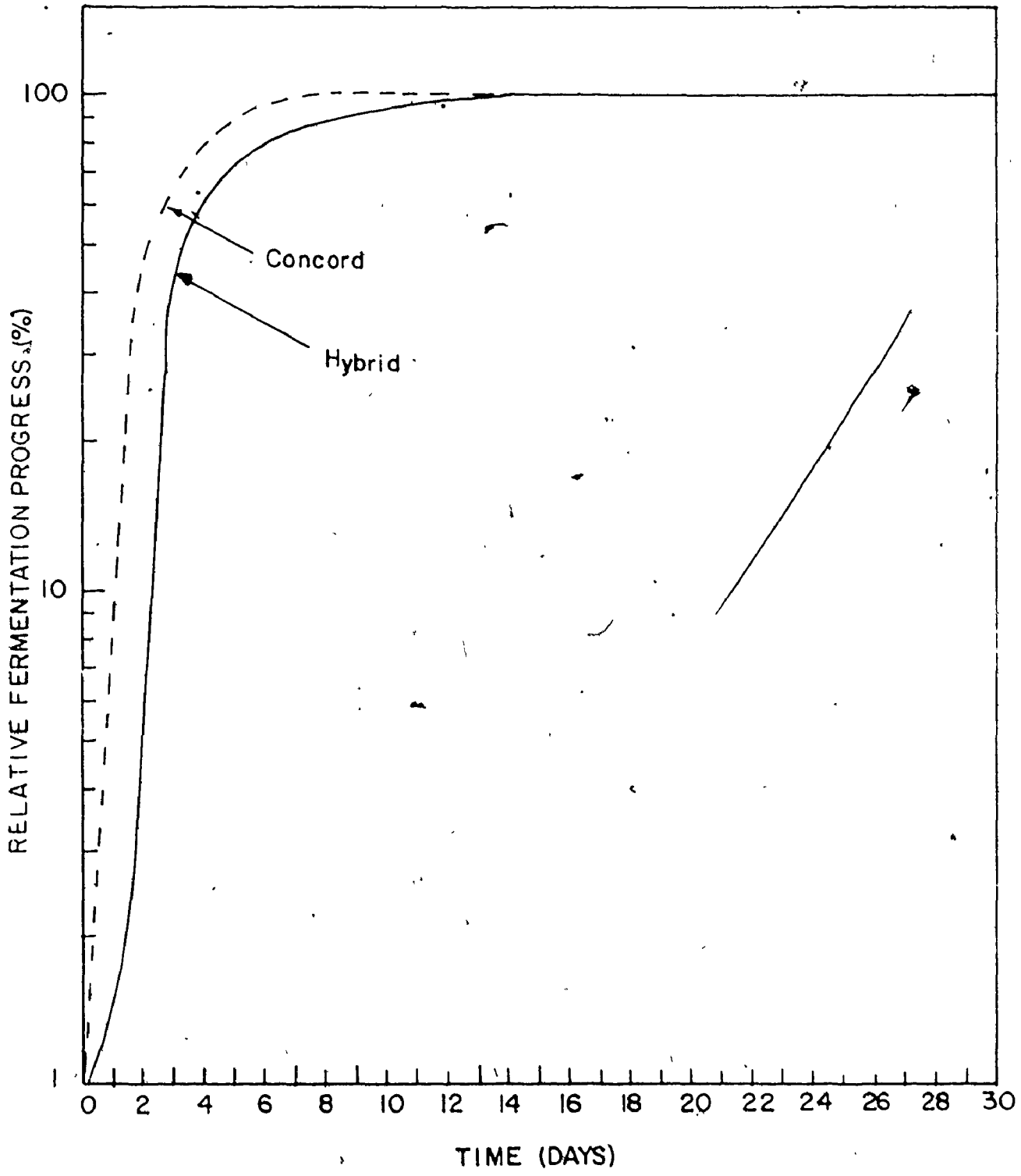
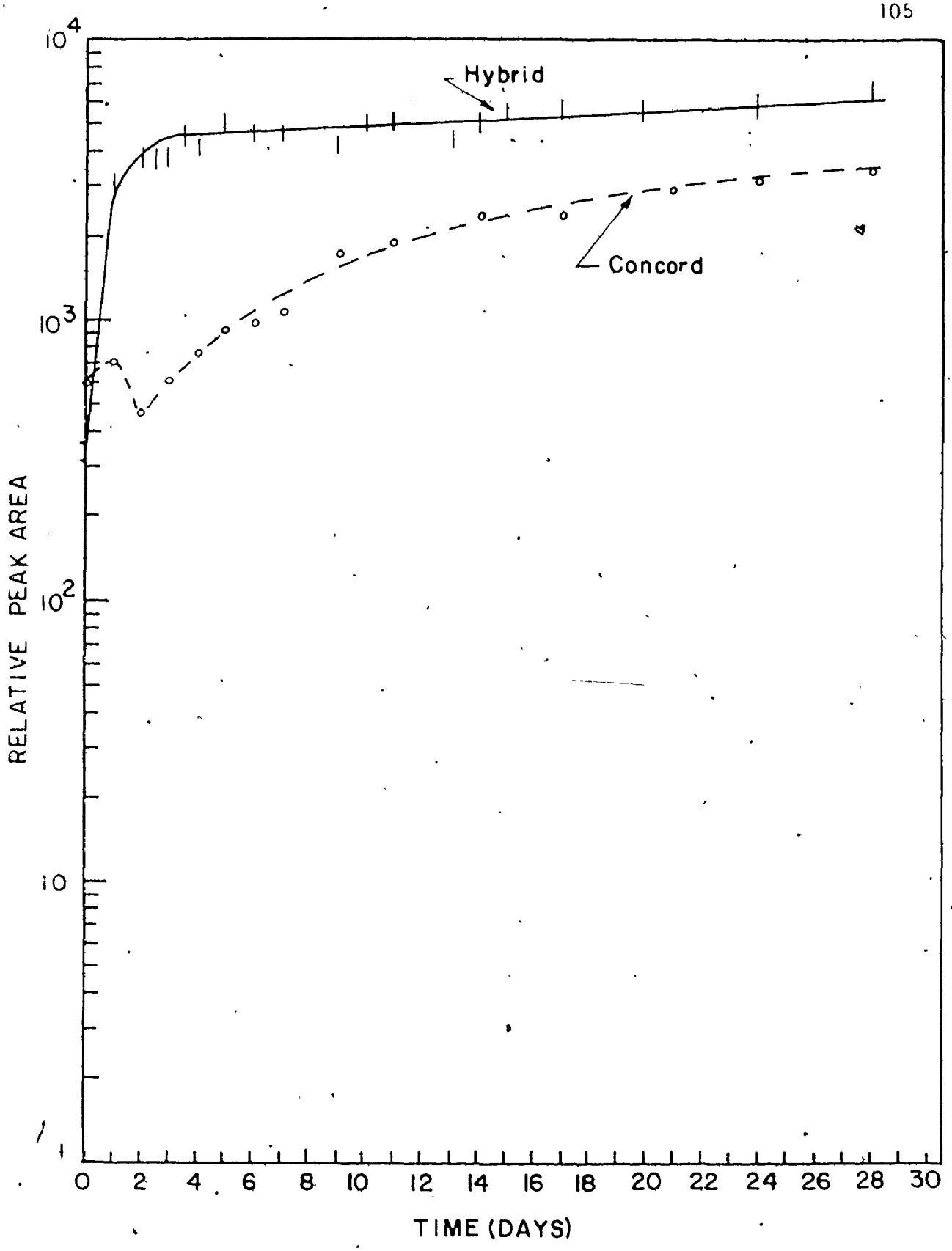

100
10
1
RELATIVE FERMENTATION PROGRESS (%)
Concord
Hybrid
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (DAYS)

FIGURE 17

Plot of Relative Peak Area vs Time for peak #19 (ethyl lactate) of hybrid fermentation. Circles represent corresponding data of Concord fermentation B.


Hybrid
Concord
RELATIVE PEAK AREA
TIME (DAYS)
10^4
10^3
10^2
10
1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

Note that peak #16 (diacetyl) and peak #68 show very similar patterns of concentration development in both the Concord and the hybrid fermentations despite the complexity of these patterns.

While the similarity of the development patterns of the volatile components in both the Concord and hybrid fermentation suggest the mechanisms of formation are the same, it is expected that, if these mechanisms were different, the difference in development patterns would be immediately apparent. A possible example of this is peak #19 (ethyl lactate) which showed a distinctive development pattern in the Concord fermentation but whose development pattern in the hybrid fermentation appears very different, as illustrated in Figure 17. Peaks #26 and #28 are other 'medium' peaks that develop quite differently in the two types of fermentation. Other smaller peaks show less distinct differences that are of questionable significance.

The qualitative comparison of the concentration development patterns of the volatile components of the hybrid fermentation with those of the Concord fermentation suggest that the volatile components are mostly the same and have similar development patterns in both fermentations.

Considering the quantitative comparison of the volatile components of the two fermentations, the differences are more apparent. For example the relative concentrations throughout both types of fermentation can be seen for two of the 'major' peaks, #13 and #52, in Figures 13 and 15. The two other 'major' peaks #8 (2-methyl-1-propanol) and #12 (2-methyl-1-butanol) show that the concentration in the hybrid must is significantly higher than in the Concord must after 28 days. Similar analysis by comparing Table 12 with Table 14 shows for the 'medium' components that there are roughly the same number of peaks where the hybrid concentration is higher, where the hybrid concentration is lower, and where the hybrid concentration is insignificantly different with respect to the Concord concentration. This comparison is based on the last 3 samples up to and including the 28 Day sample. This categorization is shown in Table 15 with differences exceeding 20% being considered significant.

The results shown in Table 15 are not surprising since it was expected that certain components would be higher in concentration in the hybrid must, others would be higher in concentration in the Concord must

TABLE 15

Comparison of Relative Areas of Major and Medium Peaks at Day 28 Between Hybrid and Concord Fermentations

Hybrid[a]	Equal	Concord[b]
5	6	9
8	24	14
12	31	16
13	32	26
19	52	28
23	63	47
39		48
40		62
42		
43		
44		
55		

[a] Final relative area of peak in hybrid must is significantly greater

[b] Final relative area of peak in Concord must is significantly greater

and some would be about equal in both. What is of greater interest is the magnitude of these differences. It was uncertain prior to the study whether the differences would be orders of magnitude or less than 100%. Of the 'major' components peak #13 (3-methyl-1-butanol) shows the greatest difference about 49%. For the 'medium' components a greater range is shown. Peak #62 (methyl anthranilate) has a ratio of 31:1 (Concord to hybrid) which is the greatest difference. With the exception of a few peaks however, the ratio is less than 5:1. This fact coupled with the fact that there are few peaks unique to one type of must illustrates the point that the difference between the hybrid's volatile component content and that of the Concord is rather subtle and certainly not gross. This could be regarded as a very important observation since it indicates very careful scrutiny of the balance of the volatile components in a juice or wine must be made before speculating about its probable sensory quality. It is generally conceded that the hybrid juice produces a qualitatively superior product compared to the Concord but the difference in the volatile composition of the two, which apparently accounts in part for this difference, is rather subtle in nature. While the large difference in the methyl anthranilate contents of the two types of must is traditionally held accountable for much of the sensory differences, it should be pointed out that the ratio of 3-methyl-1-butanol to methyl anthranilate in the Concord must is about 150:1. Although the sensory contribution of any component is not linearly related to concentration, it seems unlikely that the methyl anthranilate would dominate the 3-methyl-1-butanol in sensory effect in view of the vast excess of the latter and its distinctive aroma which is easily sensed.

This study suggests the conclusion that the characteristic aroma of a wine is derived from the same compounds, but having different concentrations, as are present in other wines. It seems unlikely that one or more specific compounds exclusive to other wines dominate the aroma. Other studies have also reached this conclusion (52).

(3) Fermentation Temperature Study

The results of the study comparing the development of the volatile components in Concord juice fermented at 20.0°C with Concord juice fermented at 28.0°C are shown in Table 16.

TABLE 16

Relative Chromatographic Peak Areas During Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	#4 K[a] R[c]x10	#4 W[b] R[c]x10	#5 K Rx10^{-3}	#5 W$_3$ Rx10^{-3}	#6 K Rx10^{-3}	#6 W$_3$ Rx10^{-3}	#7 K Rx10^{-2}	#7 W$_2$ Rx10^{-2}	#8 K Rx10^{-4}	#8 W$_4$ Rx10^{-4}
K0-W0	21.5	.18	.18	.157	.157	nd[d]	nd	1.07	1.07	.136	.136
W1	19.8		nd		.106		.085		1.05		.137
K2	18.0	.10		.122		nd		1.18		.122	
K3a	15.0	.86		.925		.622		3.48		.670	
W2a	12.6		.78		1.05		.852		1.23		1.07
K3p	10.0	2.01		2.07		k.19		5.29		1.17	
K4a	7.4	1.56		2.11		1.10		3.97		1.34	
W2p	7.0		1.26		1.89		.946		2.62		1.77
K4p	4.5	.94		1.92		.808		2.66		1.44	
K5	1.5	1.85		1.91		.767		3.14		1.41	
W3	.80		1.99		1.40		.458		4.58		2.54
K6	-.10	2.31		2.50		.840		3.57		1.60	
W4	-.40		1.30		2.43		.964		2.73		2.60
W5	-.50		1.82		2.66		1.06		3.48		2.73
W7	-.50		3.07		3.31		.886		3.89		2.75
K7	-.60	2.23		2.48		1.06		3.63		1.53	
K8	-.60	1.53		2.11		.837		4.37		1.40	
W9	-.60		1.84		3.11		1.00		2.82		2.66
K10	-.60	2.38		2.81		1.10		5.31		1.93	
W10	-.60		2.54		2.90		1.24		3.47		2.99
K12	-.90	2.46		2.74		6.78		5.21		1.89	
W14	-.70		2.87		3.20		.945		3.93		3.21
K14	-.90	2.87		3.26		.757		5.38		2.23	
W17	-.80		3.30		3.86		1.07		4.63		3.52
K16	-.90	2.06		3.31		.886		3.99		2.14	
W20	-.85		1.25		2.76		.859		2.86		2.63
K18	-1.00	3.11		3.68		.876		5.65		2.30	
W21	-.90		1.62		3.24		.826		3.83		3.47
K29	-1.05	2.57		3.71		.896		6.32		2.40	
W30	-.90		2.13		3.92		1.06		4.54		3.59
W50	-.90		1.50		4.37		.427		4.49		3.36
K50	-1.05	2.21		3.75		.951		7.90		2.53	

[a] Relative peak areas in column K are for 20.0°C fermentation.

[b] Relative peak areas in column W are for 28.0°C fermentation.

[c] R = Relative peak area

[d] nd = not detectable

TABLE 16 (continued)

Relative Chromatographic Peak Areas During Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#9		#11		#12		#13		#14	
		K Rx10^{-3}	W_3	K Rx10^{-2}	W_2	K Rx10^{-4}	W_4	K Rx10^{-5}	W_5	K Rx10^{-3}	W_3
K0-W0	21.5	.474	.474	1.33	1.33	.201	.201	.041	.041	.073	.073
W1	19.8		.393		1.38		.320		.058		.078
K2	18.0	1.22		1.02		.246		.050		.091	
K3a	15.0	1.18		nd		1.89		.390		.842	
W2a	12.6		1.32		.37		2.16		.405		.740
K3p	10.0	3.14		.91		2.92		.606		1.74	
K4a	7.4	4.87		.79		3.83		.807		2.37	
W2p	7.0		2.79		.28		3.15		.637		1.29
K4p	4.5	6.21		.92		4.04		.815		2.59	
K5	1.5	7.81		1.05		4.43		9.38		2.78	
W3	.80						4.40		.898		1.79
K6	-.10	7.91		.84		4.20		.866		2.37	
W4	-.40		5.35		.88		4.28		.864		1.37
W5	-.50		5.49		.58		4.45		.933		1.33
W7	-.50		5.35		.88		4.48		.903		1.29
K7	-.60	7.98		1.15		4.28		.892		2.39	
K8	-.60	7.05		.93		4.35		.889		2.31	
W9	-.60		5.94		.97		4.36		.880		1.46
K10	-.60	8.01		1.21		5.36		1.10		2.27	
W11	-.60		5.34		.83		4.20		.850		1.32
K12	-.90	8.30		1.04		5.34		1.18		2.17	
W14	-.70		5.78		.94		4.90		.999		1.30
K15	-.90	8.53		1.10		5.62		1.16		2.17	
W17	-.80		6.39		1.10		6.04		1.23		1.46
K18	-.90	7.90		1.07		5.53		1.12		2.15	
W22	-.85		4.58		.72		5.15		1.04		1.15
K22	-1.00	8.46		1.22		6.59		1.27		2.20	
W25	-.90		5.37		.88		5.11		1.02		1.16
K29	-1.05	9.11		1.26		6.66		1.31		2.34	
W30	-.90		5.58		.97		5.32		1.08		1.28
W50	-.90		5.20		1.01		5.52		1.11		1.22
K50	-1.05	7.26		1.34		7.77		1.53		2.00	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#15		#16		#17		#18		#19	
		K Rx10^{-2}	W_2	K Rx10^{-3}	W_3	K Rx10^{-2}	W_2	K Rx10^{-2}	W_2	K Rx10^{-3}	W_3
K0-W0	21.5	nd	nd	.064	.064	nd	nd	.35	.35	.144	.144
W1	19.8		.05		.260		.48		.65		.328
K2	18.0	.26		.852		.85		.51		.231	
K3a	15.0	.73		3.75		2.75		1.58		.265	
W2a	12.6		.74		4.36		3.46		1.84		.247
K3p	10.0	.55		3.51		1.64		.86		.183	
K4a	7.4	.79		1.38		1.69		.93		.199	
W2p	7.0		.56		1.30		2.62		1.18		.187
K4p	4.5	.92		.569		1.47		.73		.184	
K5	1.5	.96		.436		1.05		1.03		.288	
W3	.80				.458		1.37		.89		.224
K6	-.10	.70		.210		.84		.55		.294	
W4	-.40		.58		.127		1.27		.85		.419
W5	-.50		1.04		.151		1.28		1.01		.568
W7	-.50		.88		.204		1.65		1.46		.808
K7	-.60	.88		.071		.88		.88		.372	
K8	-.60	1.93		.074		.93		1.25		.474	
W9	-.60		.78		.516		2.43		1.53		1.15
K10	-.50	.97		.386		1.57		1.62		.760	
W11	-.60		.66		.662		1.90		.98		1.13
K12	-.90	.73		.646		.271		1.40		.730	
W13	-.70		.56		.851		2.15		1.10		1.31
K14	-.90	.73		.913		2.37		1.43		.849	
W17	-.80		.44		1.07		2.53		1.39		1.74
K18	-.90	.78		1.28		2.43		1.53		1.12	
W22	-.85		.43		.887		3.29		1.80		1.85
K23	-1.00	.75		1.59		2.26		1.34		1.34	
W25	-.90		.60		1.56		2.95		1.40		2.14
K29	-1.05	.53		1.90		2.85		1.41		1.90	
W30	-.90		.43		1.53		3.03		1.36		2.49
W50	-.90		.45		1.56		3.14		1.59		3.92
K50	-1.05	.40		1.90		2.41		1.37		3.19	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#20		#21		#22		#23		#24	
		K Rx10^{-3}	W_3	K Rx10^{-2}	W_2	K Rx10^{-2}	W_2	K Rx10^{-2}	W_2	K Rx10^{-3}	W_3
K0-W0	21.5	.039	.039	.31	.31	.53	.53	nd	nd	.022	.022
W1	19.8		.054		.25		.58		nd		.023
K2	18.0	.37		.85		.61		nd		.021	
K3a	15.0	.091		1.10		1.66		nd		.302	
W2a	12.6		.123		.99		1.94		nd		.321
K3p	10.0	.091		.91		1.43		nd		.502	
K4a	7.4	.099		.99		1.56		nd		1.09	
W2p	7.0		.131				1.85		nd		.553
K4p	4.5	.092		.28		1.44		nd		1.32	
K5	1.5	.157		.26		1.92		3.05		1.85	
W3	.80				4.58				nd		.916
K6	-.10	.119		nd		1.48		3.85		1.90	
W4	-.40		.136		nd		1.68		nd		1.34
W5	-.50		.116		nd		1.91		nd		1.51
W7	-.50		.243		nd		2.30		3.11		1.55
K7	-.60	.88		nd		1.74		5.13		2.08	
K8	-.60	.93		.19		1.61		4.84		2.09	
W9	-.60		.389		2.24		2.47		5.06		1.70
K10	-.60	.253		1.21		2.09		6.28		2.10	
W11	-.60		.397		.91		2.54		4.96		1.58
K12	-.90	.386		1.67		2.46		5.21		1.91	
W13	-.70		.449		1.22		2.21		2.53		1.53
K14	-.90	.465		1.92		2.15		6.39		1.98	
W15	-.80		.628		3.09		2.94		2.98		1.72
K16	-.90	.603		2.63		2.22		6.62		1.97	
W17	-.85		.615		2.72		2.82				1.72
K22	-1.00	.753		3.30		2.22		6.50		2.08	
W23	-.90		.688		2.75		2.86		2.56		1.65
K29	-1.05	.980		4.01		2.90		7.27		2.33	
W30	-.90		.746		2.49		2.73		2.60		1.62
W50	-.90		.820		2.69		2.65		4.27		1.63
K50	-1.05	1.05		3.88		2.32		6.83		2.05	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#25		#26		#27		#28		#29	
		K Rx10^{-2}	W_2	K Rx10^{-3}	W_3	K Rx10^{-2}	W_2	K Rx10^{-3}	W_3	K Rx10^{-2}	W_2
K0-W0	21.5	nd	nd	1.97	1.97	.52	.52	1.70	1.70	.65	.65
W1	19.8		nd		2.52		.23		2.12		.27
K2	18.0	nd		1.80		.67		1.57		.55	
K3a	15.0	nd		2.66		.91		2.09		.91	
W2a	12.6		nd		2.43		1.36		2.00		1.23
K3p	10.0	2.46		2.53		2.19		2.53		2.56	
K4a	7.4	3.48		2.64		1.59		2.28		3.48	
W2p	7.0		3.28		2.30		1.87		2.16		2.81
K4b	4.5	3.12		2.24		1.29		2.06		2.85	
K5	1.5	5.05		2.97		2.09		2.79		6.36	
W3	.80		nd		1.93		2.52		1.97		
K6	-.10	5.04		2.47		1.89		2.34		6.16	
W4	-.40		3.21		2.46		3.02		2.38		2.92
W5	-.50		4.87		2.66		3.60		2.54		4.76
W7	-.50		4.28		2.83		2.92		2.98		5.84
K7	-.60	7.08		2.44		1.77		2.32		4.25	
K8	-.60	5.58		2.63		2.05		2.43		4.46	
W9	-.60		4.87		3.28		2.92		3.21		9.44
K9	-.60	8.69		3.06		2.65		2.78		6.03	
W10	-.60		4.96		2.86		2.48		2.90		6.62
K10	-.90	7.30		2.99		1.88		2.83		5.42	
W11	-.70		3.84		2.75		2.15		2.94		5.52
K11	-.90	5.75		2.64		1.10		2.67		4.84	
W12	-.80		3.86		3.10		2.87		3.31		6.28
K12	-.90	4.77		2.92		.97		3.03		4.38	
W13	-.65		5.30		3.08		4.01		3.42		5.72
K13	-1.00	6.03		2.98		1.70		3.25		5.18	
W25	-.90		3.74		3.41		2.95		3.96		6.88
K29	-1.05	6.75		3.33		1.90		3.68		5.80	
W30	-.90		3.68		3.31		3.57		3.87		6.49
W50	-.90		3.14		3.31		3.59		4.12		5.84
K50	-1.05	5.09		3.50		2.41		3.99		5.36	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#30		#31		#32		#33		#34	
		K	W_2	K	W_3	K	W_3	K	W_2	K	W_2
		$Rx10^{-2}$		$Rx10^{-3}$		$Rx10^{-3}$		$Rx10^{-2}$		$Rx10^{-2}$	
K0-W0	21.5	nd	nd	.009	.009	.018	.018	nd	nd	.91	.91
W1	19.8		.04		.026		.023		nd		1.00
K2	18.0	nd		.008		.010		nd		.82	
K2a	15.0	nd		.174		.087		nd		1.13	
W2a	12.6		nd		.194		.163		nd		1.21
K3p	10.0	nd		.169		.162		nd		.66	
K4a	7.4	1.32		.237		.328		1.27		1.15	
W4p	7.0		3.06		.390		.432		2.04		1.35
K4p	4.5	1.22		.174		.412		1.89		1.17	
K5	1.5	3.39		.496		.644		3.84		1.69	
W5	.80				.188						1.12
K6	-.10	3.18		.404		.829		3.46		1.36	
W4	-.40		1.70		.454		.730		2.53		2.28
W5	-.50		2.67		.593		.707		3.25		2.03
W7	-.50		4.54		.706		1.18		5.09		2.68
K7	-.60	2.87		.394		.674		3.35		1.72	
K8	-.60	2.56		.421		.723		2.30		1.59	
W9	-.60		6.08		.934		1.38		6.17		3.07
K10	-.60	3.25		.512		.746		2.68		2.20	
W11	-.60		5.25		.879		1.18		4.19		2.68
K11	-.90	3.44		.451		.855		2.92		1.89	
W12	-.70		3.27		.733		.871		3.85		2.33
K12	-.90	3.55		.507		.805		2.59		1.51	
W13	-.80		3.54		.908		.876		3.27		2.22
K13	-.90	3.24		.598		.710		2.53		1.58	
W14	-.85		3.81		.879		.873		4.25		2.50
K14	-1.00	3.02		.593		.640		2.40		1.55	
W25	-.90		4.78		1.10		.932		3.73		2.10
K25	-1.05	3.72		.738		.743		2.65		1.71	
W50	-.90		4.21		1.00		.883		3.17		2.10
W50A	-.90		4.81		1.16		.949		3.23		2.29
K50	-1.05	3.48		.844		.624		2.10		1.65	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#35		#36		#37		#38		#39	
		K Rx10	W_2	K	W	K Rx10	W_2	K Rx10	W_2	K Rx10	W_3
K0-W0	21.5	nd	nd			.20	.20	nd	nd	.064	.064
W1	19.8		nd				.13		.09		.080
K2	18.0	nd				.34		nd		nd	
K3a	15.0	nd				1.14		1.14		.196	
W2a	12.6		nd				.77		.77		.064
K3p	10.0	nd						2.33		.148	
K4a	7.4	1.23				1.76		4.71		.200	
W2p	7.0		nd				.85		1.82		.122
K4p	4.5	1.89				2.57		4.92		.143	
K5	1.5	4.24				3.88		7.22		.305	
W3	.80		nd				2.08		2.84		.165
K6	-.10	3.87				4.24		7.93		.336	
W4	-.40		1.61				3.21		5.58		.253
W5	-.50		2.15				3.02		5.97		.275
W7	-.50		2.37				4.90		6.34		.411
K7	-.60	3.61				4.78		7.94		.315	
K8	-.60	3.56				4.59		8.35		.353	
W9	-.60		3.37				6.01		5.58		.625
K10	-.60	4.62				5.47		8.35		.422	
W11	-.60		3.00				4.39		5.18		.689
K12	-.90	4.64				4.72		6.02		.507	
W14	-.70		2.31				3.78		4.36		.667
K15	-.90	4.17				4.47		6.54		.570	
W17	-.80		2.27				4.27		4.41		.794
K18	-.90	4.41				4.33		5.09		.719	
W22	-.85		2.36				4.72		4.12		.779
K22	-1.00	4.27				5.08		5.71		.708	
W25	-.90		3.08				4.05		7.30		.823
K29	-1.05	4.12				4.51		8.69		.953	
W30	-.90		2.63				4.41		5.80		.904
W50	-.90		3.29				4.08		nd		2.05
K50	-1.05	4.69				3.42		nd		1.87	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	#40		#41		#42		#43		#44	
		K Rx10^{-2}	W_2	K Rx10^{-2}	W_2	K Rx10^{-2}	W_2	K Rx10^{-2}	W_2	K Rx10^{-3}	W_3
[illegible]	21.5	.43	.43	nd	nd	nd	nd	nd	nd	nd	nd
W1	19.8		.94		.55		.87		.46		.019
K2	18.0	.86		.86		1.38		.31		.051	
K3a	15.0	1.14		.75		3.40		3.95		.132	
W2a	12.6		1.94		nd		2.34		4.53		nd
K3p	10.0	1.13				2.37		3.64		.131	
K4a	7.4	4.01		3.61		2.74		3.73		.266	
W2p	7.0		2.04		nd		2.13		4.85		.155
K4p	4.5	3.93		1.63		3.33		3.93		.246	
K5	1.4	4.78		5.03		5.03		5.79		.586	
W3	.80				nd		2.08		3.92		
[illegible]	-.10	5.13		6.11		4.10		5.04		.577	
W4	-.40		6.41		7.10		6.01		4.79		.855
W5	-.60		7.55		8.03		5.88		6.01		.832
W7	-.80		8.94		9.95		6.54		7.26		1.04
[illegible]	-.60	5.10		5.43		4.92		5.07		.638	
	-.60	5.37		5.29		5.75		5.22		.721	
[illegible]	-.60		9.63		7.94		6.41		7.88		1.06
[illegible]	-.60	6.91		6.96		5.96		5.79		.755	
[illegible]	-.60		6.30		6.54		7.12		6.91		.879
	-.90	5.15		5.97		5.29		4.73		.730	
W13	-.70		5.98		6.90		5.59		6.06		.887
K14	-.90	4.89		5.98		5.34		4.43		.733	
W17	-.80		7.58		7.09		6.68		6.68		.940
[illegible]	-.90	5.22		6.34		5.09		4.60		.774	
[illegible]	-.85		8.84		8.84		8.14		7.41		1.06
[illegible]	-1.00	6.33		6.68		5.74		4.49		.745	
[illegible]	-.90		6.28		5.96		6.40		7.74		.802
[illegible]	-1.05	6.86		6.60		5.43		4.79		.738	
W50	-.90		6.24		6.51		5.80		7.19		.803
W50	-.90		5.00		4.63		6.57		6.98		.694
K50	-1.05	5.96		4.74		5.52		5.08		.635	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#45		#46		#47		#48		#49	
		K Rx10	W_2	K Rx10	W_2	K Rx10	W_3	K Rx10	W_3	K Rx10	W_2
[illegible]	21.5	.19	.19	nd	nd	nd	nd	nd	nd	nd	nd
[illegible]	[illegible]		.19		.19		nd		.012		.33
[illegible]	[illegible]	.21		.27		nd		.008		.43	
[illegible]	15.0	.38		nd		.189		.890		4.07	
[illegible]	[illegible]		nd		nd		nd		.753		2.44
[illegible]	10.0			nd		.519		1.40		4.81	
[illegible]	7.4	1.35		.74		1.09		1.57		3.88	
[illegible]	7.0		.38		nd		.309		1.23		3.46
[illegible]	4.5	1.25		.68		1.29		1.76		3.78	
[illegible]	1.5	3.77		1.32		1.54		2.11		6.75	
[illegible]	.80		2.08		nd		.595		1.08		4.90
[illegible]	-.10	4.97		2.22		1.55		2.43		6.20	
[illegible]	-.40		5.49		4.01		.902		1.57		5.41
[illegible]	-.50		5.16		3.45		1.00		1.73		5.12
[illegible]	-.50		6.62		5.26		1.01		1.73		5.62
[illegible]	-.60	4.63		2.19		1.81		2.42		5.90	
[illegible]	-.60	6.05		2.68		1.91		2.70		5.21	
[illegible]	-.60		8.70		9.14		1.11		1.84		6.62
[illegible]	-.60	5.96		2.84		1.89		2.89		5.47	
[illegible]	-.60		5.62		4.66		1.10		1.51		4.77
[illegible]	-.90	5.93		3.00		1.97		3.08		5.49	
[illegible]	-.70		4.82		4.20		1.10		1.83		5.09
[illegible]	-.90	5.53		3.01		1.79		2.77		4.25	
[illegible]	-.80		5.44		4.82		1.24		2.02		6.49
[illegible]	-.90	5.94		3.53		2.07		3.28		5.94	
[illegible]	-.85		6.36		3.25		1.15		2.03		7.07
[illegible]	-1.00	5.24		2.91		2.13		3.51		5.74	
[illegible]	-.90		4.98		2.63		1.18		1.88		5.67
[illegible]	-1.05	5.65		3.69		2.30		3.59		5.65	
[illegible]	-.90		4.68		4.33		1.19		1.94		3.47
[illegible]	-.90		4.63		4.86		1.32		2.11		5.46
[illegible]	-1.05	4.14		3.04		2.14		3.44		5.96	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brax)	Relative Peak Areas for Spectral Peaks									
		#50		#51		#52		#53		#54	
		K	W_2	K	W	K	W_4	K	W_2	K	W_2
		Rx10^{-2}				Rx10^{-4}		Rx10^{-2}		Rx10^{-2}	
[illegible]-W9	21.5	.26	.26			.120	.120	nd	nd	nd	nd
[illegible]	19.8		.27				.285		nd		nd
[illegible]	18.0	.27				.418		.39		nd	
[illegible]	15.0	.68				.989		1.37		nd	
[illegible]	12.6						1.49		nd		nd
K3p	10.0	.37				1.08				nd	
K4a	7.4					1.32				nd	
W2	7.0		.38				1.78		nd		nd
K4p	4.5	.28				1.63				nd	
K5	1.5	.36				1.92		.87		.48	
W3	.80		2.36				2.29		1.88		nd
K6	-.10					2.03		.70		.55	
W4	-.40		.72				2.63				nd
W5	-.50		.72				2.55		1.44		nd
W7	-.50		.80				2.72		1.21		.79
[illegible]	-.60	.36				2.14		.88		.70	
[illegible]	-.60	.38				2.33		93		.73	
[illegible]	-.60		.80				2.75		2.00		1.27
[illegible]	-.60	.49				2.47		1.21		.95	
[illegible]	-.60		1.03				2.50		1.19		
[illegible]	-.90	.77				2.61		1.04		.82	
[illegible]	-.70		1.16				2.71		1.27		.92
[illegible]	-.90	1.51				2.77		.91		.72	
[illegible]	-.80		1.37				3.15		.90		.71
[illegible]	-.90	1.61				2.82		.97		.77	
W22	-.85		1.47				2.86		1.59		1.03
[illegible]	-1.00	1.17				3.03		.94		.74	
W35	-.90		1.22				2.67		1.62		1.28
K29	-1.05	.65				3.15		nd		.83	
W30	-.90		.89				2.95		.89		1.06
W50	-.90		1.16				3.15		.92		2.55
K30	-1.05	3.04				3.58		nd		2.62	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#55		#56		#57		#58		#59	
		K	W_4	K	W	K	W_1	K	W	K	W
		Rx10				Rx10				Rx10	
K0-W0	21.5	nd	nd			nd	nd			nd	nd
W1	19.8		nd				nd				
K2	18.0	.65				nd				.39	
K3a	15.0	.320				nd				.66	
W2a	12.6		.175				nd				.56
K3p	10.0	.414				nd					
K4a	7.4	.474				nd				1.06	
W3p	7.0		.293				nd				
K4p	4.5	.542				nd				.98	
K5	1.5	.605				nd				.93	
W	.80		.332				nd				3.27
K6	-.10	.654				nd				.75	
W4	-.40		.420				nd				nd
W5	-.50		.441				nd				nd
W6	-.56		.448				nd				nd
K7	-.60	.711				nd				.84	
K8	-.66	.764				nd				.61	
W7	-.60		.530				nd				nd
K9	-.60	.810				nd				.18	
W8	-.60		.533				nd				nd
K10	-.90	.807				nd				.31	
W9	-.70		.558				nd				nd
K11	-.90	.830				nd				.27	
W10	-.80		.583				2.0				nd
K12	-.90	.868				nd				.29	
W11	-.85		.584								nd
K22	-1.00	.911				2.8				.28	
W25	-.90		.536				4.7				nd
K29	-1.05	.960				1.9				.32	
W30	-.90		.556				7.8				nd
W50	-.90		.561				6.7				nd
K50	-1.05	1.05				6.4				nd	

TABLE 16 (continued)

Relative Chromatographic Peak Areas During

Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks							
		#60		#61		#62[a]	#63	#64	
		K	W	K	W_2	K W_3	K W_3	K	W_2
					Rx10^{-2}	Rx10^{-3}	Rx10^{-3}		Rx10^{-2}
K0-W0	21.5			nd	nd	1.74	nd nd	nd	nd
W1	19.8				nd	1.74	nd		nd
K2	18.0			nd		1.74	nd	nd	
K3	15.0			nd		1.74	.842	nd	
W2a	12.6				nd	1.74	.370		1.88
K3p	10.0			nd		1.74	1.09	5.74	
K4a	7.4			nd		1.74	1.70	6.16	
W2p	7.0				nd	1.74	1.25		4.75
K4p	4.5			nd		1.74	1.94	7.01	
K5	1.5			nd		1.74	2.28	6.34	
W3	.80				nd	1.74	1.45		4.03
K6	-.10			nd		1.74	1.92	5.47	
W4	-.40				nd	1.74	1.64		4.64
W5	-.50				nd	1.74	1.66		5.53
W7	-.50				nd	1.74	1.78		4.93
K7	-.60			nd		1.74	1.76	5.80	
K8	-.60			nd		1.74	1.84	6.40	
W9	-.60				nd	1.74	2.01		5.10
K10	-.60			nd		1.74	2.48	7.26	
W11	-.60				.30	1.74	1.84		4.38
K12	-.90			nd		1.74	2.23	6.53	
W14	-.70				.34	1.74	1.86		5.41
K14	-.90			.22		1.74	2.37	6.96	
W17	-.80				.33	1.74	1.96		5.72
K18	-.90			.58		1.74	2.36	6.33	
W22	-.85				.69	1.74	1.81		5.97
K22	-1.00			.57		1.74	2.54	7.30	
W25	-.90				.76	1.74	1.75		5.80
K29	-1.05			.63		1.74	2.70	8.17	
W30	-.90				.83	1.74	1.67		5.55
W50	-.90				2.60	1.74	1.72		5.02
K50	-1.05			2.40		1.74	2.59	8.63	

[a]Peak #62 (metnyl anthranilate) is internal standard

TABLE 16 (continued)

Relative Chromatographic Peak Areas During Fermentations for Fermentation Temperature Study

Sample	Density (°Brix)	Relative Peak Areas for Spectral Peaks									
		#65		#66		#68		#69		#70	
		K	W_2	K	W	K	W_2	K	W_1	K	W_2
		Rx10^{-2}				Rx10^{-2}		Rx10^{-1}		Rx10^{-2}	
K0-W0	21.5	nd	nd			nd	nd	nd	nd	nd	nd
W1	19.8		nd				nd		nd		nd
K2	18.0	nd				nd		nd		nd	
K3a	15.0	nd				2.05		nd		1.36	
W2a	12.6		.37				.44		nd		.59
K3p	10.0	nd				2.23		nd		2.12	
K4a	7.4	.60				3.93		2.6		3.35	
W2p	7.0		.56				2.30		2.2		.65
K4p	4.5	.61				5.72		5.4		2.93	
K5	1.5	.52				7.42		5.9			
W3	.80		.69				1.76		6.0		1.37
K6	-.10	.42				7.93		4.8		1.83	
W4	-.40						3.40		4.2		.53
W5	-.50		.70						nd		
W7	-.50		.58				1.04		nd		.47
K7	-.60	.53				4.77		3.5		1.32	
K8	-.60	1.11				2.77		2.2		1.11	
W9	-.60		.58				1.16		nd		3.25
K10	-.60	1.26				1.00		nd		.72	
W11	-.60		.64				nd		nd		nd
K12	-.90	.44				.47		nd		.63	
W14	-.70		.72				nd		nd		nd
K13	-.90	.55				nd		nd		.55	
W17	-.80		.66				nd		nd		nd
K18	-.90	.58				nd		nd		nd	
W22	-.85		.86				nd		nd		nd
K22	-1.00	.57				nd		nd		nd	
W25	-.90		.60				nd		nd		nd
K29	-1.05					nd		nd		nd	
W30	-.90		.65				nd		nd		nd
W50	-.90		.77				nd		nd		nd
K50	-1.05	.80				nd		nd		nd	

The main purposes for doing this study were to obtain a qualitative and quantitative comparison of the concentration development patterns of the volatile components produced at the two fermentation temperatures.

Considering the qualitative appearance of the development patterns of the volatile components, it is apparent that most of them resemble the same basic shape described earlier, of a fast increase in concentration after a certain induction time, followed by a slow or zero rate of increase in concentration. Because both the previous Concord and hybrid studies showed this type of behaviour, it was not surprising that the 20.0°C fermentation showed this behaviour since it should be very similar to the previous Concord studies. That the 28.0°C fermentation also showed this basic development pattern was more significant as will be discussed.

The comparison of the 20.0°C and 28.0°C development patterns suggests an immediate observation. There are no detectable peaks that are unique to one fermentation. All components are common to both fermentations. This rules out, at least for fermentation temperatures not exceeding 28.0°C, the possibility that the precursors of these components have two (or more) kinetically-controlled alternative pathways leading to different products. This agrees with previous studies (37).

With regard to the similarity of the concentration development patterns of any single component produced at 20.0°C and at 28.0°C, it seems there are four general categories into one of which virtually all comparisons of patterns fit. In making these comparisons it need be noted that, because of the higher fermentation temperature, the rate of the 28.0°C fermentation is initially faster than that of the 20.0°C fermentation as illustrated in Figure 18. Thus the peak area of any component must be correlated to the fermentation progress as indicated by the Brix hydrometer reading rather than the elapsed time of fermentation for the first few days of the study. The progress of the 20.0°C fermentation equalizes that of the 28.0°C fermentation by Day 6. This observation agrees with a previous study's conclusion that a higher initial fermentation temperature results in faster yeast growth originally but makes no difference in the overall fermentation rate (47). The period of higher temperature was 84 hours as opposed to 16 hours for the previous study.

The observation of a higher final Brix hydrometer reading is indicative of a lower ethanol content in the 28.0°C fermentation. This also agrees with previous findings (40) which attribute this occurence to the higher partial pressure of ethanol at 28.0°C than at 20.0°C and greater loss of ethanol by entrapment with escaping carbon dioxide which is evolved more quickly at 28.0°C.

The four categories of comparison are as follows:

(1) The concentration development pattern of the volatile component is very similar both qualitatively and quantitatively throughout both of these fermentations. From this it must be concluded that the entire formation process for these components is not affected by fermentation temperature. It can also be concluded that these components do not contribute to the sensory difference observed in wines produced at different fermentation temperatures. Peak #5 (1-propanol) is exemplary of this type of comparison and is illustrated in Figure 19. Peak #52 (2-phenethanol - a 'major' peak), peak #26 and peak#28 are other notable components unaffected by the fermentation temperature.

(2) From the first sampling after the initiation of the fermentation the 20.0°C fermentation shows a higher concentration of the volatile component that does the 28.0°C fermentation. This difference apparently does not become greater throughout the entire study but remains relatively constant after only a few days of the fermentations. The basic shapes of the two development curves are not too dissimilar, only the 20.0°C curve is displaced vertically above the 28.0°C curve. Peaks #9 (3-methyl-1-butyl acetate), #14 (ethyl hexanoate), #47 (1-hexanoic acid) and #55 (1-octanoic acid) all yield this type of comparison which is illustrated in Figure 20 for peak #14 (ethyl hexanoate).

(3) From the first sampling after the initiation of fermentation the 28.0°C fermentation shows a higher concentration of the volatile component than does the 20.0°C fermentation. This difference remains relatively constant after the first several days of the fermentations (after the fermentation rate slows). Peak #8 (2-methyl-1-propanol) belongs to this category as do peak #19 (ethyl lactate) and peak #31. No other 'major' or 'medium' peaks (see Table 13) belong to this category.

FIGURE 18

Plot of Relative Fermentation Rate vs Time for 20.0°C fermentation and 28.0°C fermentation.

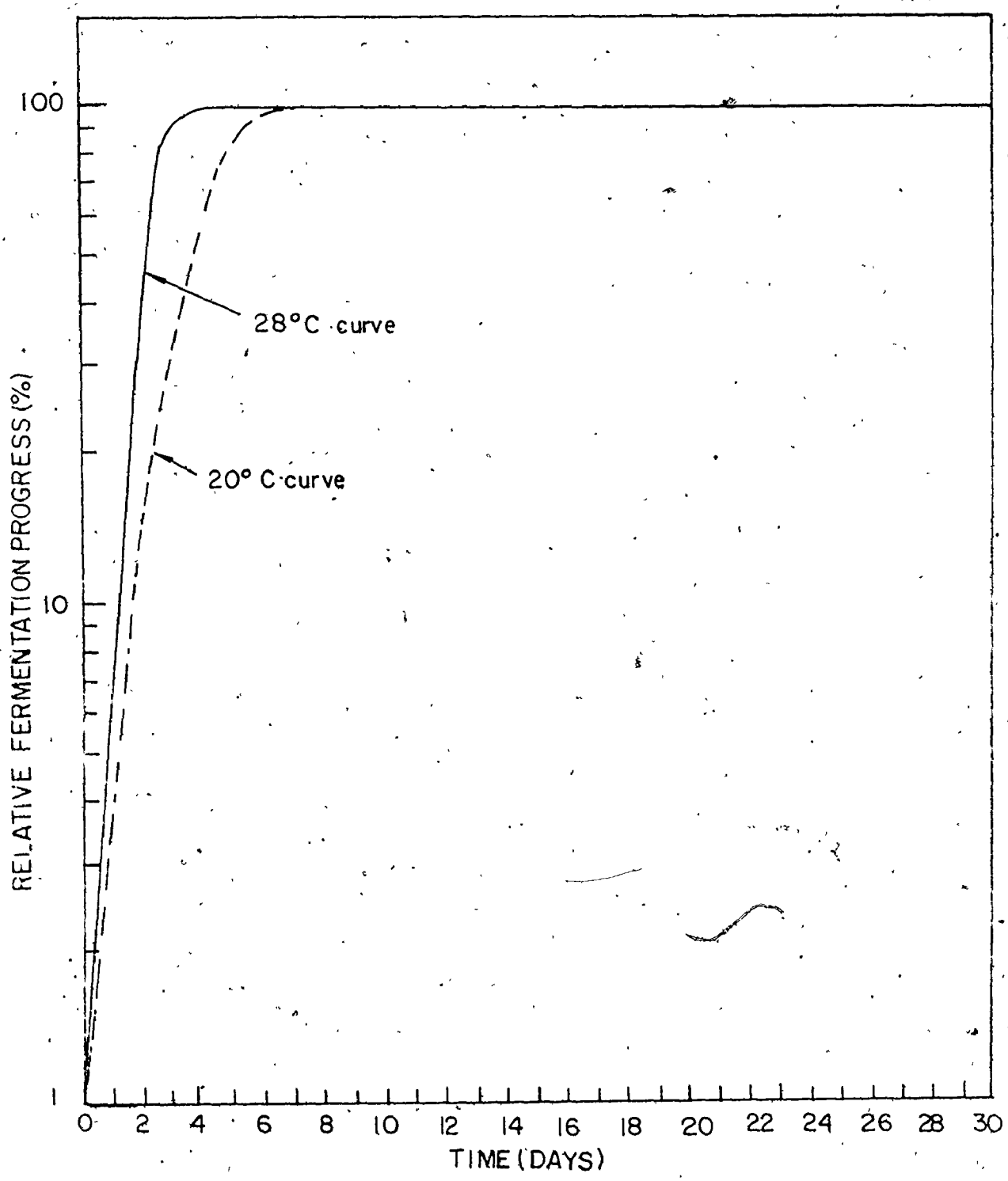

100
10
1
RELATIVE FERMENTATION PROGRESS (%)
28°C curve
20° C curve
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
TIME (DAYS)

FIGURE 19

Plot of Relative Peak Area vs Time for peak #5 (1-propanol) of 20.0°C fermentation. Circles represent corresponding data of 28.0°C fermentation.

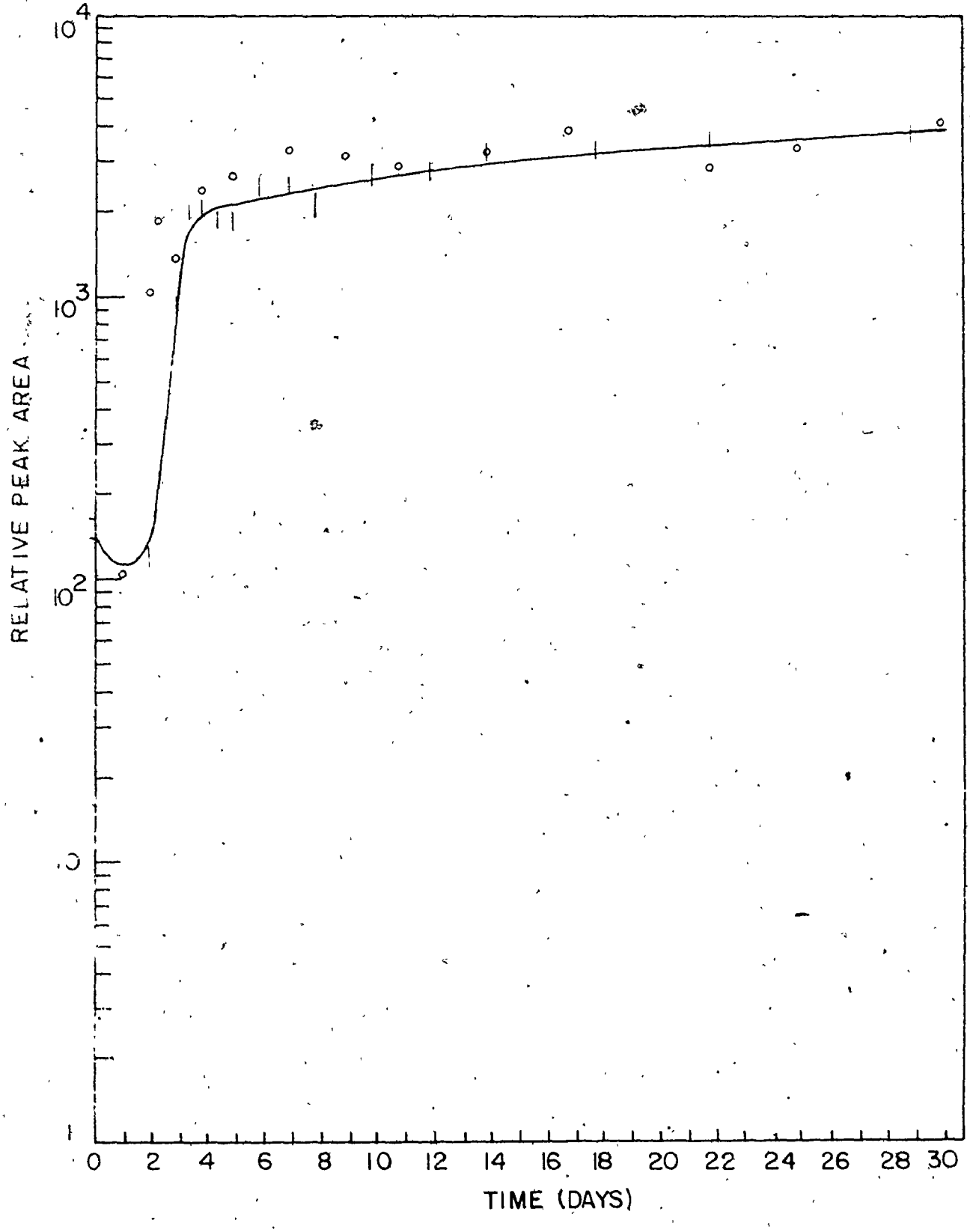
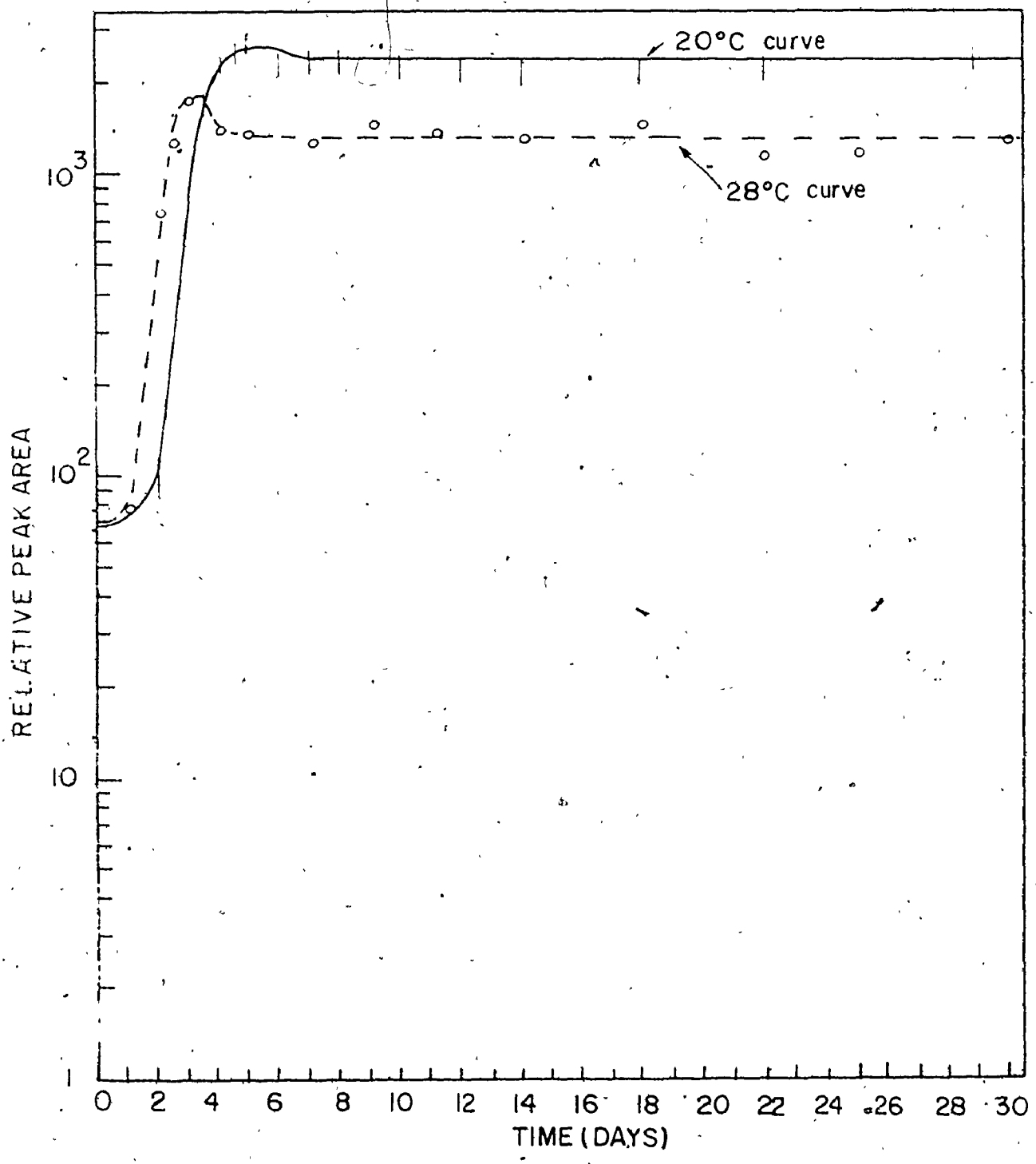

RELATIVE PEAK AREA
10^4
10^3
10^2
1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

FIGURE 20

Plot of Relative Peak Area vs Time for peak #14 (ethyl hexanoate) of 20.0°C fermentation and 28.0°C fermentation.


20°C curve
28°C curve
RELATIVE PEAK AREA
10^3
10^2
10
1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (DAYS)

4) The concentration development pattern of the volatile component in the 20.0°C fermentation is very similar qualitatively and quantitatively to that in the 28.0°C fermentation until the fermentation rate levels off (about Day 9). The concentration of the component in the 20.0°C fermentation then steadily becomes higher than that in the 28.0°C fermentation up to Day 28. Peak # 12 (2-methyl-1-butanol) and peak #13 (3-methyl-1-butanol) are examples of this type of comparison. Note that this divergence occurs after the temperature of the two fermentations were equalized. This implies that any change in the chemistry of the musts which would account for this divergence must have occured several days earlier.

It would seem unlikely that, for a component belonging to any of the last three categories, there exists an alternate mechanism that accounts for the difference in concentration observed between the two fermentations. It is more likely that the same mechanism occurs in both fermentations but proceeds more efficiently at one of the two temperatures. For example, if for peaks belonging to category (4), the 28.0°C fermentation showed no production of the component at all after Day 9, a second mechanism would be suspected in order to account for the production of more of that component in the 20.0°C fermentation. This, however, is not the case and previous studies (50) have accounted for the difference in the amount of both 3-methyl-1-butanol and 2-methyl-1-butanol produced at different fermentation temperatures using a single mechanism. The similar shapes of the concentration development patterns (except for the simple vertical displacement) of components belonging to categories (2) and (3) also do not suggest the existence of an alternate mechanism. The exact effect of the temperature difference on the amount of loss due to volatility and on the enzyme activity is not known (56). Both of these factors would contribute to determining the final concentration of any one component.

Even peaks displaying unusual concentration development patterns, which are perhaps indicative of complex mechanisms, such as peak #16 (diacetyl) and peak #68 behave very similarly at both fermentation temperatures.

On the basis of the preceding qualitative comparison of the results shown in Table 16, it is concluded that not only are the volatile components formed at the different fermentation temperatures the same but

also the mechanism of formation of these components does not change with fermentation temperature. This means that most probably any differences observed in the concentrations of the volatile components during the fermentations are due to the influence of the temperature difference on the same mechanism.

Considering the quantitative aspects of this fermentation temperature study, it is apparent from Table 16 that the lower fermentation temperature generally favours higher concentrations of the volatile components to be formed. Of all 'major' and 'medium' peaks only peaks #8 (2-methyl-1-propanol), #19 (ethyl lactate), and peaks #31, #32, and #43 appear in a significantly higher concentration in the 28.0°C fermentation. Of the remaining 'major' and 'medium' peaks there are very nearly the same number of components where the peak areas are insignificantly different in the two fermentations and where the peak area in the 20.0°C fermentation is greater than that in the 28.0°C fermentation. Table 17 gives a breakdown of the effect of fermentation temperature on the relative peak areas for 'major' and 'medium' peaks. It should be stressed that the greater formation of volatile components at the lower fermentation temperature is for the fermentation of Concord juice. Similar results would not necessarily be expected with other varieties of juice (40).

It can be seen that, while the formation of 3-methyl-1-butanol and 2-methyl-1-butanol is favoured and that of 2-methyl-1-propanol is not favoured at the lower fermentation temperature, the formation of 1-propanol and 2-phenethanol is unaffected by the fermentation temperature. This is similar to previous studies of higher alcohols in wines (41, 44).

The components later identified as acids, peaks #23 (acetic), #47 (1-hexanoic), #55 (1-octanoic), and #63 (1-decanoic) had higher concentrations in the 20.0°C fermentation. The mechanism of their formation is apparently favoured by the lower fermentation temperature.

Certain components later identified as esters had higher levels in the lower temperature fermentation such as peaks #9 (3-methyl-1-butyl acetate), #14 (ethyl hexanoate), #24 (ethyl octanoate) and #48 (phenethyl acetate). This observation also agrees with previous studies (43). Most of the 'minor' peaks, which are quite likely esters, however show little difference.

TABLE 17

Comparison of Relative Areas of 'Major' and 'Medium' Peaks at Day 29 Between 20.0°C and 28.0°C Fermentations

20.0°C[a]	Equal	28.0°C[b]
9	5	8
12	6	19
13	16	31
14	20	32
24	24	43
47	26	
48	28	
55	39	
63	40	
	42	
	44	
	52	
	62	

[a]Final relative area of peak in 20.0°C fermentation is significantly higher

[b]Final relative area of peak in 28.0°C fermentation is significantly higher

It is apparent that the most significant observation of the quantitative aspects of this study of the effect of fermentation temperature on the development of the volatile components in Concord must is the small magnitude of the differences in the concentration of the volatile components: for example, at the 29-30 day sampling peak #23 (acetic acid) is the only peak where the difference in concentrations between the 20.0°C and the 28.0°C fermentations exceeds 100%.

In view of the generally acknowledged effect of the fermentation temperature on the quality of wine produced (37, 39), these quantitative differences in the volatile components resulting from different fermentation temperatures must be considered significant although subtle.

It is noteworthy that the magnitude of the differences in quantities of the volatile components observed in the fermentation temperature studies are significantly less than the magnitude of differences observed in fermentations of different juices. This observation again stresses the basic importance of the juice fermented which apparently exceeds that of the fermentation temperature.

Possibly these findings will be useful to the Canadian wine industry in determining whether research funds would be better spent on hybrid development or improved fermentation technology.

CHAPTER 4

SUMMARY

A new design of solvent extractor was developed which was capable of achieving fast and quantitative isolation of the volatile components from juice or wine. The efficiency of this extractor suggests it may be very useful for other applications.

A technique for enriching the extracted volatile components quantitatively and quickly was developed which complemented the method of solvent extraction.

A sensitive procedure for performing mass spectral analysis of separated volatile components following the manual collection of the samples from the gas chromatograph was used to identify a number of compounds.

Using the forementioned techniques and certain established procedures, a method of routine, quantitative analysis of the volatile components in juice or wine was established. This method allows several analyses to be performed daily.

The new technique of routine quantitative analysis was applied to the study of two replicate fermentations of Concord grape juice. The results of this study indicate the technique yields meaningful and reproducible data.

A comparison of the concentration development patterns of the volatile components during the fermentation of Blue Hybrid juice with those during the fermentation of Concord juice revealed that the volatile components of both are the same with a few exceptions. The shapes of the concentration development curves of common components were generally similar. Quantitative differences between the concentrations of common components in the two types of fermentations after 28 days did not usually exceed a 5:1 ratio.

The concentration development patterns of the volatile components which appeared in the fermentation of Concord juice at 20.0°C were

compared to those which appeared in the fermentation of Concord juice at 28.0°C. All components were common to both fermentations and the shapes of the concentration development curves were quite similar. Quantitative differences did not exceed 100% (with one exception) and higher levels of many components appeared in the 20.0°C fermentation.

CHAPTER 5

FUTURE WORK

Because of the apparent potential of the new design of solvent extractor for various applications, further investigation of it would be useful. Such work might include: various tests leading to the optimization of all parameters; especially the length of the extracting tube; tests with different solvent-solute systems in order to determine its general applicability; further tests with standard solutions in order to further elucidate the mechanism of the extraction process; and investigation of certain modifications such as several extracting tubes arranged radially about a central solvent recycling system.

Identification of further components should be possible using the adopted mass spectral technique, although the use of open tubular capillary columns in the gas chromatograph is recommended in order to achieve better separation of the components. It is believed the mass spectrometric technique is sufficiently sensitive to tolerate the smaller samples obtained. For routine analysis time constraints may prevent this.

Using the technique of routine, quantitative analysis, the effect of various fermentation parameters on the concentration development patterns of the volatile components found in the resultant must could be investigated. Some of the fermentation parameters to investigate might be a greater range of fermentation temperatures than examined in this research; various yeast strains; several different juices (both red and white); the concentration of sulfur dioxide; chaptalisation; aging; the method of obtaining the juice (hot press or cold press); the fermentation volume; and the type of fermentation vessel. Studies of this type could be of considerable interest to the wine industry.

The technique of analysis could also be applied to study the mechanisms of formation of the volatile components. An example of this would

be the simultaneous monitoring of the concentration of the volatile components and the amino acids in the same fermentation. The monitoring of certain radioactively-labelled compounds during the fermentation would also be of interest. Studies could be conducted to investigate the parameter to which the concentration of the volatile components is best correlated. This study confirms the formation of these components is closely linked to the fermentation rate which suggests it is a likely parameter for such a correlation. Study of correlating two parameters simultaneously to the concentration (such as time and soluble solids) also a possibility. Studies such as these would not only be of interest to the industry but might contribute to the fundamental understanding of the volatile components.

BIBLIOGRAPHY

1. Amerine, M. A. and Singleton, V. L. "Wine: An Introduction for Americans", University of California Press, Los Angeles (1965), p. 1.

2. Ibid, p. 9.

3. Genesis 9, v. 20.

4. Amerine, M. A., Berg, H. W., and Cruess, W. V. "Technology of Wine Making", Avi Publishing, Westport Conn. (1972), p. 1.

5. Amerine, M. A. and Singleton, V. L. "Wine: An Introduction for Americans", p. 14.

6. Ibid, p. 10.

7. Ibid, p. 11.

8. Ibid, p. 15.

9. Ibid, p. 16.

10. Ibid, p. 21.

11. Ibid, p. 22.

12. Amerine, M. A., Berg, H. W., and Cruess, W. V. "Technology of Wine Making", p. vii.

13. Amerine, M. A. and Singleton, V. L. "Wine: An Introduction for Americans", p. 8.

14. Ibid, p. 26.

15. Amerine, M. A., Berg, H. W., and Cruess, W. V. "Technology of Wine Making", p. 123.

16. Chemical and Engineering News 53 (June 16, 1975), p. 34.

17. Amerine, M. A. and Singleton, V. L. "Wine: An Introduction for Americans", p. 3.

18. Ibid, p. 56.

19. Amerine, M. A., Berg, H. W., and Cruess, W. V. "Technology of Wine Making", p. 114.

20. Ibid, p. 177.

21. Lichine, A. "New Enclyclopedia of Wines and Spirits", Alfred A. Knopf, New York (1976), p. 161.

22. Amerine, M. A., Berg, H. W., and Cruess, W. V. "Technology of Wine Making", p. 201.

23. Ibid, p. 98.

24. Ibid, p. 220.

25. Ibid, p. 217.

26. Ibid, p. 225.

27. Webb, A. D. and Kepner, R. E. J. Food Science 22, 384 (1957).

28. Power, F. B. and Chestnut, V. K. J. Am. Chem. Soc. 43, 1741 (1921).

29. Webb, A. D. Die Wynboer, Series 468, 62 (1970).

30. Amerine, M. A., Berg, H. W., and Cruess, W. V. "Technology of Wine Making", p. 112.

31. Holley, R. W., Stoyla, B., and Holley, A. D. J. Food Science 20, 30 (1955).

32. Webb, A. D. and Kepner, R. E. J. Food Science 22, 394 (1957).

33. Drawert, F. and Rapp, A. Chromatographia 1, 453 (1968).

34. Stern, D. J., Lee, A., McFadden, W. H., and Stevens, K. L. J. Agr. Food Chem. 15, 1100 (1967).

35. Amerine, M. A., Berg, H. W., and Cruess, W. V. "Technology of Wine Making", p. 113.

36. Brander, C. F. Amer. J. Enol. Viticult. 25 (1), 13 (1974).

37. [illegible], Proc. of Third Spec. Symp. on Yeasts, Helsinki, Part II, 297 (1973).

38. Kondakis-Kourakou, V. (1966) ref. 5 of Webb, loc. cit. ref. 37.

39. Singh, C. S. and Amerine, M. A. Amer. J. Enol. Viticult. 12 (3), 117 (1961).

40. Singh, C. S. and Amerine, M. A. Calif. Agr. Expt. Sta. Bull. 827, 8 (1966).

41. Dough, C. S., Guymon, J. F., and Crowell, E. A. J. Food Science 31, 620 (1966).

42. Ayrapaa, T. Brauwissensnaft 23 (2), 48 (1970).

43. Nordstrom, K. (1965) ref. 13 of Daudt, C. E. and Ough, C. S., loc. cit. ref. 45.

44. Webb, A. D. Proc. of Third Spec. Symp. on Yeasts, Helsinki, Part II, 305 (1973).

45. Daudt, C. E. and Ough, C. S. Amer. J. Enol. Viticult. 24 (3), 130 (1973).

[illegible] Rice, A. C. "Chemistry of Winemaking", A. D. Webb, ed., American Chemical Society, Washington (1974), p. 103.

[illegible] Ough, C. S. and Amerine, M. A. Amer. J. Enol. Viticult. 12 (3), 126 (1961).

48. Ough, C. S. and Amerine, M. A. Calif. Agr. Expt. Sta. Bull. 827, 9 (1964).

49 Ough, C. S. Amer. J. Enol. Viticult. 15, 167 (1964).

50. Ough, C. S., Guymon, J. F., and Crowell, E. A. J. Food Science 31, 623 (1966).

[illegible] Guymon, J. F. and Crowell, E. A. Amer. J. Enol. Viticult. 20, 77 (1969).

52 Brander, C. F. Amer. J. Enol. Viticult. 25 (1), 15 (1974).

[illegible] Private communication from Dr. L. R. Mattick on "Amino Acids of New York State Grapes".

54. Stevens, K. L., Flath, R. A., Lee, A., and Stern, D. J. J. Agr. Food Chem. 17, 1102 (1969).

[illegible] Crayon, [illegible] and Rapp, A. Chromatographia 1, 447 (1968).

56. Webb, A. D. Proc. of Third Spec. Symp. on Yeasts, Helsinki, Part II, 299 (1973).

57. Stern, D. J., Let, A., McFadden, W. H., and Stevens, K. L. J. Agr. Food Chem. 15, 1100 (1967).

58. Webb, A. D. and Kepner, R. E. Amer. J. Enol. Viticult. 13 (1), 1 (1962).

59. Hardy, P. J. J. Agr. Food Chem. 17, 656 (1969).

60. Hardy, P. J. and Ramshaw, E. H. J. Sci. Food Agric. 21, 39 (1970).

61. Postel, W., Drawert, F., and Adam, L. Chem. Mikrobiol. Technol. Lebensm. 1, 228 (1972).

62. Caudle, C. E. and Ough, C. S. Amer. J. Enol. Viticult. 24 (2), 128 (1973).

63. Stevens, K. L., Flath, R. A., Lee, A., and Stern, D. J. J. Agr. Food Chem. 17, 1103 (1969).

64. Postel, W., Drawert, F., and Adam, L. Chem. Mikrobiol. Technol. Lebensm. 1, 225 (1972).

65. Hardy, P. J. and Ramshaw, E. H. J. Sci. Food Agric. 21, 40 (1970).

66. Mattick, L. R., Robinson, W. B., Wiers, L. D., and Barry, D. L. J. Agr. Food Chem. 11, 334 (1963).

67. Brander, C. F. Amer. J. Enol. Viticult. 25 (1), 14 (1974).

68. Hoogden, W. H. and Teramishi R. Nature 200, 19 (1963).

69. Webb, A. D. Proc. of Third Spec. Symp. on Yeasts, Helsinki, Part II, 301 (1973).

70. Hardy, P. J. J. Agr. Food Chem. 17, 657 (1969).

71. Ough, C. E. and Ough, C. S. Amer. J. Enol. Viticult. 24 (3), 127 (1973).

72. Ibid, p. 129.

73. Morrison, G. H. and Freiser, H. "Solvent Extraction in Analytical Chemistry", John Wiley, New York (1957), p. 12.

74. Ibid, p. 81.

75. "Handbook of Chemistry and Physics", Chemical Rubber Co., Cleveland (1948), p. 884.

76. Amerine, M. A. "Laboratory Procedures for Enologists", Associated Students Store, Davis, California (1965), p. 101.

77. Daniels, F. W. "Physical Chemistry", Addison-Wesley, Toronto (1964), p. 219.

78. Ibid, p. 269.

79. Willard, H. H., Merritt, L. L., and Dean, J. A. "Instrumental Methods of Analysis", Van Nostrand, New York (1974), p. 524.

80. "Dupont 310 Curve Resolver Instruction Manual", Dupont Instrument Products Division, Wilmington, Delaware (1968), p. 1.

81. Crowell, E. A. and Guymon, J. F. Amer. J. Enol. Viticult. 20, 155 (1969).

82. "The Handbook of Chemistry and Physics", Chemical Rubber Co., Cleveland (1968), p. D121.